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Assessment of Agricultural Practices Impact on the Development of Arbuscular Mycorrhizal Fungi

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OB, SS and KD designed the study, wrote the protocol, wrote the first draft of the manuscript. Author KN'A managed the analyses of the study. Author SN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In natural environments, the development of plants depends on the interactions they maintain with their environment, in particular with soil microorganisms such as arbuscular mycorrhizal fungi, especially since the used of inputs is scarce by the majority of farmers in Côte d'Ivoire. The present study was carried out to study the endomycorrhizogenic potential of the soils on which tomato,

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cashew and banana are grown. Place and Duration of Study: Soil sampling in bananas, cashews and tomatoes fields (July and August 2016), spore trapping in WASCAL (West African Science Service Center on Climate Change and Adapted Land Use) greenhouse in the city of Bingerville and spore extraction and identification in Laboratory of Biotechnology, Agriculture and Development of Biological Resources (September 2016 to March 2017). Material and Methods: Soil samples were taken away from the same depth, 10 to 20 cm, in cashew, tomato or banana fields. They were then used for trap pot culture by sorghum [*Sorghum bicolor* (L.)] and cowpea [*Vigna ungugulata* (L.) Walp.]. The wet sieving revealed spore density and morphological diversity. Results: Spores were more abundant in the soils sampled under cashew (22 spores / gram of soil) in comparison to banana (9 spores / gram of soil) and tomato (3 spores / gram of soil). There were a real diversity of glomeromycete in the different agrosystems. There is a great richness and diversity of AMF spore form under cashew (18 morphotypes) in Côte d'Ivoire compared to tomato (2 morphotypes) and banana (4 morphotypes). Conclusion: Land use system impact spore richness and diversity. Controlled mycorrhization of cashew, tomato and banana could be considered.

Keywords: Arbuscular mycorrhizal fungi; inputs; endomycorrhizogenic potential; spore density; morphotypes; agrosystems.

1. INTRODUCTION

Since Independence, the States of sub-Saharan Africa have been keen to boost the development of their rural territories, in particular by increasing and diversification of agricultural production. Sixty years later, Côte d'Ivoire is one of the word leading countrie in many agricultural domains. For example, Côte d'Ivoire produced 13,000 tonnes of cashew nuts in 1990, but is now the world largest producer with 688,000 tonnes in 2018 [1,2]. The performances are implemented in great majority by small producers with little or no training, and maintenance and harvesting remain family activities [3].

The fertility and productivity of tropical soils are highly determined by their biological activity as fertilizers are scarce for the majority of farmers [4]. Therefore, the development of plants depends on the interactions they maintain with the environment, in particular with soil microorganisms. This is the case with mycorrhizae which are mutualistic symbionts between plant roots and mycorrhizogenic telluric fungi [5]. These fungi are an important component in the functioning and diversity of terrestrial ecosystems. The importance of mycorrhizae is explained by their ubiquity and their direct involvement in the essential processes that take place at the soil/plant interface [6]. Among these symbiotic fungi, the arbuscular mycorrhizal fungi (AMF) constitute the most commonly encountered group whose beneficial effects on the growth and stress tolerance of the majority of economically important plants are admited. Despite their multiple benefits, AMF are untapped and almost ignored by Ivorian farmers.

Indeed, the increase in production will only have real value if it takes into account the now unavoidable criteria of production that meets the requirements of sustainable development and internationally recognized quality standards. Consequently, in this 21st century, agriculture must produce more, but above all, produce better. Notwithstanding this, in developing countries, very few studies on the main ecological and agronomic functions of AMF in soils have been carried out. Yet, the efficient use of these fungi in agriculture requires first, understanding the diversity and dynamics of these fungi in their natural environment.

The objectives of this study are to study the abundance and diversity of AMF associated with different crops and to assess the effect of the land use system on the abundance and diversity of these symbionts in the soil.

2. MATERIASLS AND METHODS

2.1 Materials

2.1.1 Soil samples

The soil samples were taken from plantations at the base of cashew trees, banana and tomato plants. These samples were used for direct extraction of the arbuscular mycorrhizal fungi spores present therein.

2.1.2 Trap plants

Cowpea [*Vigna ungugulata* (L.) Walp.] and sorghum [*Sorghum bicolor* (L.)] were used as trap plants for AMF. The goal was to trap and identify spores that direct extraction would not have revealed.

2.1.3 Spores of AMF

Spores were used for fungi morphological characterization.

2.1.4 Field characteristics and weeding method

A questionnaire addressed to farmers made it possible to determine the characteristics of their field and the weeding method.

Table 1. Questionnaire addressed to farmers

N°	Questions	Answers
1	How old is your cashew field ?	
2	What is the densty of plants in cashew fields ?	
3	What is the weeding method in your cashew field	

2.2 Methods

2.2.1 Soil sampling

On each cultivated plot, three main sampling points were chosen at random in the direction of the diagonal (Fig. 1). Around each of these three points were defined 12 other secondary points from which the samples were taken. These points are arranged as shown in Fig. 2 showing the example of the main point 1 of plot 1.

Indeed, 4 and 8 equidistant sampling points are placed on two concentric circles with respective radii of 3 m and 6 m (Fig. 2). The soil was sampled from the 12 secondary points thus determined.

To do this, the auger was driven to a depth of 10 to 20 cm and the soil was collected. The 12 soil samples thus obtained were mixed well in the tank then reduced to about 1 kg and kept in a polyethylene plastic bag. The sample thus obtained is that of point 1 of plot 1 (Fig. 2).

The operation was repeated using the same method for the 3 points chosen at random in the diagonal of each farm. However, before moving from one farm to another, the auger was cleaned with 95% alcohol, flared, then disinfected with 12% sodium hypochlorite.

2.2.2 Direct estimate of spore density

The presence of AMF spores is the usual method of estimating the number of species and their abundance in the community [7, 8]. To this end, two methods were applied to extract and enumerate the spores.

Extraction by the wet sieve method is performed directly from soil samples collected in the field [7]. For this purpose, 50 g of each soil sample was diluted in 500 ml of tap water. Each mixture was shaken for a long time for homogenization, then left to stand for 1 min. Then, it is filtered through a series of sieves of decreasing mesh (2 mm, 710 µm, 500 µm, 90 µm, and 45 µm). The fractions retained in the last 4 sieves (Fig. 3) were each transferred to a beaker. Each suspension, by successive aliquots, was observed with a binocular magnifying glass (Gx40). The aliquots were transferred to tissue paper spread in a grid Petri dish (Fig. 4). Extraction and direct enumeration of spores were performed twice with the same amount of soil (50 g) for each point sampled. The total number of spores obtained by this method is denoted by M1.

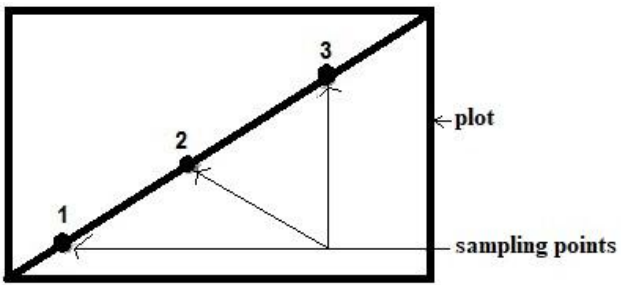

Fig. 1. Sampling points of a plot

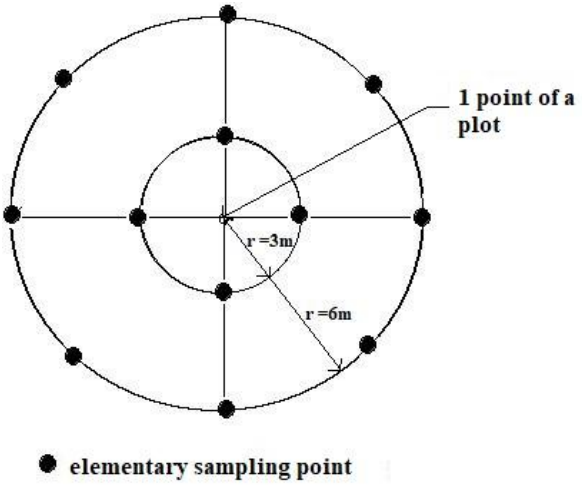

Fig. 2. Soil sampling point

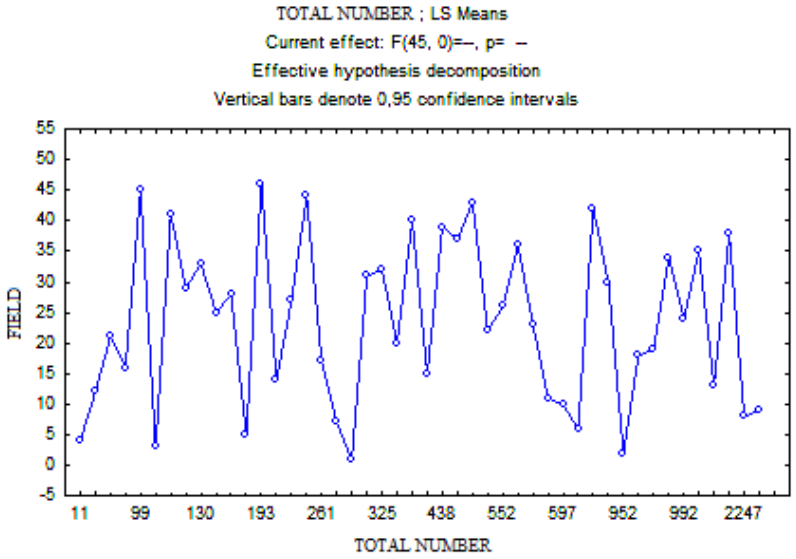

Fig. 3. Mean number of spores per field

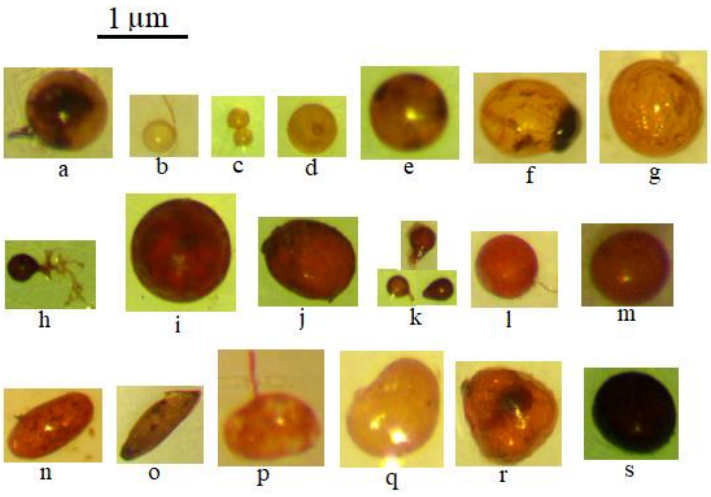

Fig. 4. Different morphotypes of spores isolated in soils

2.2.3 Trapping of AMF from soils samples

Soils sampled were used as an inoculum to allow the eventual hatching of all spores and their developpement [9]. Thus, cowpea [*Vigna ungugulata* (L.) Walp.] and sorghum [*Sorghum bicolor* (L.)] seeds were sown in polyethylene bags containing 500 g of soil sampled from each field (2 seeds per pot and 2 pots per soil sample).

The seedlings were kept in a greenhouse where the plants were exposed to daylight. These plants were only watered with tap water every 3 days without any amendment.

Two controls were made in jars containing a mixture sterilized in an oven at 121 ° C for 15 min, synthetic soil, and sand in the proportions 2/1.

Two controls were made in polyethylene bags containing 500 g of soil sterilized in an oven at 121 ° C for 15 min.

2.2.4 Estimation of spore density after trapping

After 60 days of cultivation, the plants were delicately dug up, and their rhizosphere, taken and supplemented to 100 g by the soil just around. Then, 50 g of this 100 g mass of soil was treated according to the wet sieving method [7].

Microscopic observation made it possible to re-count the spores and verifying the presence of morphotypes which might not have been observed during direct extraction. The total number of spores obtained by this method is denoted by M2.

2.2.5 Identification of AMF morphotypes

Morphotyping of spores was done based on the assumption that morphologically similar spores are phylogenetically related [10,11]. For this purpose, the extracted spores were collected in Petri dishes and then mounted for observation. Observation under an optical microscope made it possible to note: shape, size, color, and suspensory bulb, sporocarp, ornamentation [12,10,11].

2.2.6 Statistical analyzes

The data collected were subjected to an analysis of variance with one classification criterion (ANOVA I) using Statistica version 8. software. A post ANOVA analysis was performed using the Newman-Keuls test for the comparison of means *(P = .05)*.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Spore abundance in tomato, cashew and banana plantations

Analysis of the soil samples showed the presence of spores at all the sites studied. Analysis of the relative abundance of these spores also showed variability from one site to another (Fig. 3).

The majority of cashew farms are over 10 years old (Table 2), their density, in general, is greater than 100 plants per hectare (Table 3), and annual chemical weed control has been the most used by the farmers (Table 4). However, neither the density of cashew trees (*P = .42*) nor the weeding process used (*P = .99*), let alone the age of the farm (*P = .92*), affected soil spore density.

Table 2. Age of cashew fields (years)

	Numbers	Percentages
≤ 10	12	26.10
]10 - 20[	23	50.00
≥ 20	09	19.56

NB : data were not obtained for 7 fields (04.34 %)

Table 3. Densty of plants (plants/ha) in cashew fields

	Numbers	Percentages
< 100	00	00.00
= 100	13	28.26
> 100	26	56.52

NB : data were not obtained for 7 fields (15.22 %)

Table 4. Weeding method in cashew fields

		Numbers	Percentages
Chemical (Weedkiller)		23	50.00
Physical	Machete	11	23,91
	Plough	01	02.17
Mixed (Weedkiller / Machete)		04	08.70

NB: data were not obtained for 7 fields (15.22 %)

Small spores (diameter <500 µm) are very abundant and constitute more than 99 % of the total number of spores extracted in the soils where the three crops are grown (Table 5, 6, and 7). Also, they were less abundant in the soils sampled under banana (9 spores / gram of soil) and tomato (3 spores / gram of soil) in comparison to soils under cashew (22 spores / gram of soil).

3.1.2. Spore diversity

Microscopic observation of the spores revealed the presence of 19 different morphotypes depending on color, shape, attachment hypha (suspensory bulb) and ornamentation (Fig. 4). This diversity was very important in soils under cashew trees, unlike soils under banana and tomatoes where it is rather low (Table 7).

3.2 DISCUSSION

The life cycle of AMF is completed by the formation of spores within the extraracinar mycelium. These spores can remain viable in the soil for a very long time and then enter another process of colonization of host plants [13, 14]. They are often regarded as the main reserve of propagules in soils [15].

The age of the cashew plantation did not have a significant impact on spore density. This result agrees with those obtained in an area reforested by *Gmelina arborea* and *Tectona grandis* [16]. Indeed, it has also been shown that after disturbances, few types of spores increase their quantitative presence [17].

The identification of spores revealed the presence of 19 morphotypes. This exploratory study was limited to the morphological identification. It, therefore, does not make it possible to assess the real diversity in terms of fungal species even if certain morphotypes are related to the genus Glomus. Indeed, the majority of the spores were small. It has been shown that fungi of Glomus genus are small [5] and the most abundant in tropical forests [18] as well as in arid environments [19, 20]. Furthermore, it was reported that *Glomus* appears to be the most ubiquitous genus of endomycorrhizal champions [21]. This means that most of the spores extracted could be *Glomus*. However, this remains to be confirmed by further taxonomic analyzes by molecular biology [22, 18, 23].

Table 5. Average number of spores in the different cashew fields

Fields N°	Stitch of sieves (µm)					Density (sp.g^{-1})
	710	500	90	45	$\frac{M1+M2}{2}$	
01	00	10	200	60	270	10.80
02	00	01	131	820	952	38.08
03	00	00	42	71	113	04.52
04	00	00	07	04	11	00.44
05	00	00	58	120	178	07.12
06	00	01	81	560	642	25.68
07	00	00	61	206	267	10.68
08	00	00	101	2146	2247	89.88
09	00	02	79	2277	2358	94.32
10	00	00	261	336	597	23.88
11	00	01	69	515	585	23.40
12	00	02	11	54	67	02.68
13	00	04	79	1373	1456	58.24
14	00	05	49	151	205	08.20
15	00	03	44	360	407	16.28
16	00	01	30	63	94	03.76
17	00	00	62	199	261	10.44
18	00	02	189	776	967	38.68
19	00	01	111	871	983	39.32
20	00	00	18	325	343	13.72
21	00	01	27	61	89	03.56
22	00	03	30	514	547	21.88
23	00	00	42	529	571	22.84

Fields N°	Stitch of sieves (µm)					Density (sp.g^{-1})
	710	500	90	45	$\frac{M1+M2}{2}$	
24	00	00	54	938	992	39.68
25	00	07	33	115	155	06.20
26	00	00	84	468	552	22.08
27	00	01	23	194	218	08.72
28	00	00	24	153	177	07.08
29	00	05	50	68	123	04.92
30	00	02	247	540	789	31.56
31	00	00	88	192	280	11.20
32	00	00	48	277	325	13.00
33	00	00	71	59	130	05.20
34	00	00	339	647	986	39.44
35	00	02	88	1003	1093	43.72
36	00	00	120	434	554	22.16
37	00	00	99	363	462	18.48
38	00	00	161	1399	1560	62.40
39	00	00	100	338	438	17.52
40	00	04	61	291	356	14.24
41	00	05	40	75	120	04.80
42	00	03	114	603	720	28.80
43	00	01	63	428	492	19.68
44	00	05	56	163	224	08.96
45	00	00	27	72	99	03.96
46	00	00	58	135	193	07.72
Total	00	72	3830	21346	25248	
Percentage (%)	0	0,3	15,2	84,5	100	

M1: total number of spores obtained by direct extraction; M2: total number of spores obtained by extraction after trapping; sp.g^{-1}: spore per gram of soil

Table 6. Mean number of spores extracted from the soil of banana plantations

Fields N°	Stitch of sieves (µm)				$\frac{M1+M2}{2}$	Density (sp.g^{-1})
	710	500	90	45		
01	00	01	46	119	166	6,64
02	00	01	55	247	303	12,14
03	00	00	43	239	282	11,28
04	00	02	21	72	95	3,8
05	00	03	46	230	279	11,16
Total	00	07	211	907	1125	
Percentage (%)	00	0,62	18,75	80,62		

M1: total number of spores obtained by direct extraction; M2: total number of spores obtained by extraction after trapping; sp.g^{-1}: spore per gram of soil

Table 7. Mean number of spores extracted from the soil under the tomato

Fields N°	Stitch of sieves (µm)				$\frac{M1+M2}{2}$	Density (sp.g^{-1})
	710	500	90	45		
1	00	00	07	29	36	01.44
2	00	01	06	48	55	02.20
3	00	00	17	86	103	04.12
4	00	00	25	51	76	03.04
Total	00	01	55	214	270	
Percentage (%)	00	00.37	20.37	79.26	100	

M1: total number of spores obtained by direct extraction; M2: total number of spores obtained by extraction after trapping; sp.g^{-1}: spore per gram of soil

Table 8. Description of spore morphotypes

Morphotypes	Farm	Couleur et forme	Hyphe de suspension
a	Cashew	Orange with dark content	Cylindrical
b	Cashew, banana, tomato	Pale yellow, spherical	Cylindrical
c	Cashew, banana	Beige, spherical	cylindrical and straight
d	Cashew	Orange yellow, sphérique	Cylindrical
e	Cashew	Orange, spherical	Cylindrical
f	Cashew	Orange yellow, alongated	Bulbous
g	Cashew	Orange yellow, irregular	Cylindrical
h	Banana	Black, spherical to piriformis	Cylindrical, branched
i	Cashew	Red, spherical	cylindrical and straight
j	Cashew	Red, oval	Bulbous
k	Cashew	Red, spherical to piriformis	Cylindrical
l	Cashew	Brown, spherical	Cylindrical
m	Cashew	Brown, adorned with black dots, spherical	Cylindrical
n	Cashew	Brown, alongated	Cylindrical
o	Cashew	Pale yellow, alongated	Cylindrical
p	Cashew	Orange yellow, bulb	Cylindrical
q	Cashew	ocher, ovoid	Cylindrical
r	Cashew	Brown, irregular	Bulbous
s	Cashew, tomato, banana	Black, spherical	Cylindrical

In cashew plantations, a higher number of AMF was observed than in other crops (tomato, banana). This could be explained by various reasons, including the absence of vegetation at certain times in tomato and banana fields. Indeed, unlike the cashew tree which is a perennial plant, banana and tomato are plants with short cycles. As AMF are obligate symbionts, they require host plants for their maintenance in soils. Soils poor in roots or in continuous maintenance (hoeing, weedkilling), such as those where tomatoes and bananas are cultivated, are therefore poorer in spores since they remain longer in the soil without encountering a potential root host. Such a situation leads to spores degradation and their dead. On the other hand, under continuous plant cover, the spores germinate and quickly infect new roots and the mycorrhizae are in continuous sporulation [24]. Besides, organic or mineral fertilization carried out during the cultivation of tomatoes and bananas is a well-known cultural practice. This practice contributes to enriching the soil, in particular with phosphorus. However, a high level of phosphorus in the soil can reduce root colonization and spore density [25, 26]. A difference in AMF composition between conventional cropping systems with high input use and organic systems without fertilizer application was reported [27].

The rhizosphere of the cashew tree is richer in spores compared to tomatoes and bananas. This shows that certain species can promote the development of fungal propagules in their rhizosphere [28, 29, 30] and thus ensure and maintain a high endomycorrhizogenic potential of the soil.

4. CONCLUSION

The AM symbiosis is recognized as being one of the major microbial components in the development of the main biogeochemical cycles of soils (C, P, and N) and consequently in the development of plants by improving their mineral nutrition, but also water and their health status.
The results of this study highlight the richness and diversity of the spore form of AMF in Côte d'Ivoire. They revealed that, the longer the plant cycle, the more abundant the spores are in the soil. Conversely, the shorter the cycle is, the less abundant the spores are. So, the land use system impact spore richness and diversity.

By taking into account the poverty of our soils in assimilable phosphorus, these fungi could compensate for these deficiencies and thus contribute to maintaining and restoring soil fertility. Due to their non-specificity, AMF can be associated with crops, thus contributing to an increase in yield. In a context of reduced

chemical input in agriculture and the development of ecologically intensive agriculture, mycorrhization should be encouraged. In fact, to the well-known advantages of mycorrhizae on the vegetal growth of plants, there are several benefits, in particular for the resistance induced to biotic and abiotic stresses. The "plant-mycorrhiza-parasite-environment" complex is for this purpose the standard to be maintained or restored to ensure environmental sustainability.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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