Mycorrhizal interaction between companion trees and cocoa trees (*Theobroma cacao* L.) in traditional agroforestry systems in Côte d'Ivoire

ABSTRACT

Aims: The effective use of companion trees in agroforestry systems could improve the health and productivity of cocoa trees by understanding the interactions they promote.This study aims to decipher the characteristics of arbuscular mycorrhizal communities in the roots and soil of cocoa trees and their companion trees to understand the interaction between these two types of trees.
Methodology: Five mycorrhization parameters measured in cocoa trees and nearby companion trees were compared. These were, shared species diversity, number of spores, mycorrhization frequency, mycorrhization intensity and colonization intensity in the mycorrhized part of the root system. correlation tests between these parameters were also used to establish the link between the data.
Results: The study identified 49 AMF species involved in the interactions between companion and cocoa trees. *T. superba* (64.28%) and *B. mannii* (62.5%) recorded the highest species diversity and spore numbers. The highest mycorrhization rates were found between *T. ivorensis* and cocoa. Statistical analysis revealed strong positive correlations for species diversity and spore numbers with specific companion trees. Based on the results, *T. superba*, *B. mannii*, and *R. heudelotii* are recommended for promoting mycorrhizal spore production, while *T. ivorensis*is suggested for high mycorrhization levels.
Conclusion: The study of interactions thus disclosed strong links between all cocoa trees and companion trees on the basis of the 5 types of variables used to establish correlations.But*, A. boonei, T. superba, T. ivorensis, T. heckelii, M excelsa, B. mannii, B. mannii*and *G. kola* can be recommended in cocoa agroforestry systems as they have the most important mycorrhizal interactions with cocoa trees.

Keywords:*cocoa; mycorrhiza, companion trees, interaction*

1. INTRODUCTION

In Côte d'Ivoire, cacao tree is seen as a stable source of income for the local population, contributing to the development of the regional and national economy. It is also perceived as a key model for developing community programs. However, the sustainability of cocoa agrosystems is undermined by the preponderance of climatic and anthropogenic pressures (Cramer et al., 2018; Thiébault and Moatti, 2016). Over time, these pressures are expected to induce significant changes in plant community dynamics (Bussotti et al., 2014), making this ecosystem vulnerable to the onset of various biotic and abiotic stresses. To mitigate the damaging effects on cocoa farming, several strategies have been implemented to improve the response of cocoa trees. Input use, selection and genetic improvement remain favored and promising avenues (Gupta et al., 2020; Khan et al., 2018). However, breeding and genetic improvement are long and costly processes that may prove insufficient in the face of rapid environmental change. Also, the use of inputs involves risks to human and soil health.

To find innovative and sustainable solutions, stakeholders in Côte d'Ivoire's cocoa industry are promoting the use of agroforestry (Adou et al., 2016), because of its ability to encourage the cohabitation of trees and cocoa trees to promote environmentally-friendly, economically and socially viable agriculture. A more recent, but highly promising, field of investigation that could perfectly benefit cocoa agroforestry systems concerns the improvement of interactions between plants and beneficial microorganisms, particularly at rhizosphere level (de la Fuente Cantó et al., 2020).Indeed, plant roots host a wide diversity of microorganisms (microbiota) playing a crucial role in plant growth and health, as well as in plant community dynamics (de la Fuente Cantó et al., 2020; Hardoim et al., 2015).Among these microbiota communities are arbuscular mycorrhizal fungi (AMF), highly important organisms in plant growth and health (Jha et al., 2011), establishing complex interactions with roots to cope with a wide range of abiotic and biotic stresses. Given that arbuscular mycorrhizal communities colonize around 90% of vascular plants (Coyne, 2000), and that in cocoa agroforestry systems a large number of plant species coexist in the same soil space, optimizing plant response to these stresses can be enhanced by in-depth knowledge of the interactions established between different plants within the ecosystem, and the identification of key players within these interactions. To achieve this, biodiversity analyses are a prerequisite. However, they are generally limited to above-ground biodiversity, underestimating biodiversity in the soil, which is a crucial element in the proper functioning of the plant stratum.The present study proposes to decipher the characteristics of mycorrhizal arbuscular communities in the roots and soil of cocoa trees and companion trees, in order to understand the interaction between the companion tree and the cocoa tree.

2. MATERIAL AND METHODS

2.1 Survey area

Cocoa farms from 32 villages in Côte d'Ivoire were sampled. These plantations come from the 4 main cocoa-producing zones, located from east to west along the pioneer cocoa-growing front (Figure 1). The first agroecological zone (ZAE) is located in the east and comprises the departments of Agnibilekro and Abengourou. a hot, humid, sub-equatorial Attieen facies climate (Eldin, 1971). Average annual temperature ranges from 24.6°C to 28.4°C. Average annual rainfall is between 1006 and 1068 mm. The second ZAE is in the center-west and includes the Divo and Lakota departments. Temperatures in this zone range from 24.9°C to 25.3°C. Average annual rainfall is 1388 mm and 1457 mm. The third South-West ZAE covers the Soubré and Méagui departments. Average annual temperatures range from 25°C to 25.4°C. Average annual rainfall is between 1384 and 1563 mm. Finally, the fourth ZAE is located in the west, covering the

UNDER PEER REVIEW

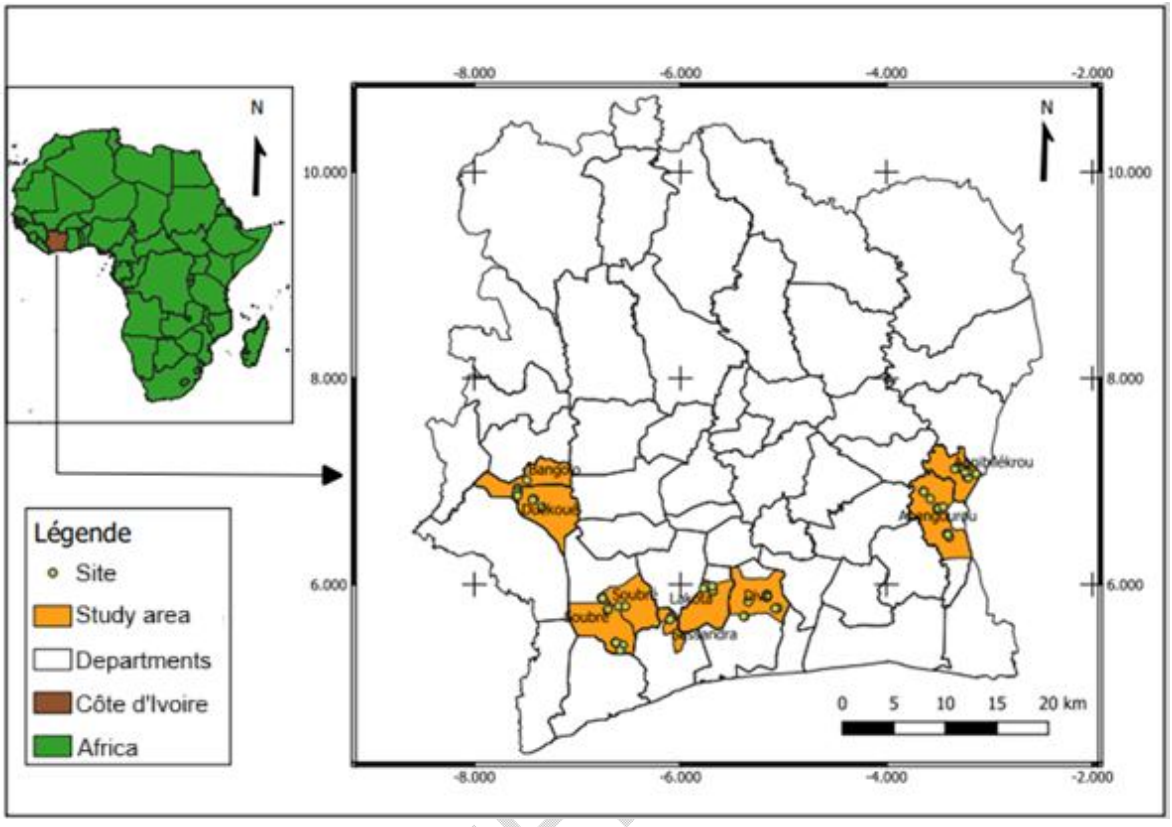

Fig. 1: Location of study sites

UNDER PEER

Duekoué and Bangolo departments. The average temperature is between 25.7°C and 25.8°C. Average rainfall is 1302 mm and 1320 mm. The last 3 ZAEs benefit from a tropical climate. All the cocoa plantations studied were established under shade trees.

2.2 Selection of companion trees and collection of mycorrhizal inoculum

Thirteen companion trees were used for this study. They are as follows*Ricinodendronheudelotii* (Akpi), *Perseaamericana* (Avocatier), *Ceiba pentandra* (Fromager), *Alstoniaboonei* (Emien), *Terminalia superba*(Fraké), *Cola nitida* (Colatier), *Miliciaexcelsa* (Iroko), *Irvingiagabonensis* (Kplé), *Garcinia kola* (Petit cola), *Gliricidiasepium*(*G. sepium*), *Terminalia ivorensis* (Framiré), *Tieghemellaheckelii* (Makoré), and *Beilschmiediamannii* (Bitéi). They were selected on the basis of a survey of farmers' perceptions of agroforestry (Amani et al., 2020). Sampling was carried out on cocoa trees and companion trees. Sampling of mycorrhizal inoculum was started by locating the companion tree in the field. Around each companion tree identified, three nearest cocoa trees in three different directions were also sampled(Figure 2).
The soil sample consisted of 5 cores (approx. 5 kg) from the 0 - 20 cm depth horizon (Rincón et al., 2021; Anguiby et al., 2019), within a radius of 100 cm around the base of the trunk (Dahiratou et al., 2013) of each cocoa and companion tree. A total of 2810 soil samples were taken from cocoa trees, for 936 samples from cocoa companion trees.
In addition, the root systems of cocoa and companion trees were carefully excavated, and fine roots (<3 mm in diameter) were collected (Anguiby et al., 2019). Around 200 g of roots were collected from each tree, including cocoa and companion trees (Ramírez et al., 2016).
All samples were assigned with a code corresponding to the name of the tree, the locality and the serial number of the sample. The samples were then packed in plastic bags and transported to the lab for analysis. After drying the soils at 24°C for 7 days, some of thelabelled and repackaged samples were stored at 4°C. Root samples were also split, with one part stored in a distilled ethanol/glycerol/water solution (1:1:1) (Ducousso, 1991) until use. Unpreserved samples were used as mycorrhizal inoculum for the present work.

2.3 Determination of the physical and chemical properties of soils under cocoa trees and companion trees

A subsample of each soil was air-dried, sieved (2 mm) and pH measured in water (I:5) and KCl (I:5). Organic matter was assessed using the Walkey-Black method according to Jackson (1976) and total nitrogen was measured by the Kjeldahl method. Extractable P was assessed according to Olsen et al. (1952) using sodium bicarbonate. In this extract, P was measured by the method of Murphy and Riley (1962) and exchangeable Ca, K and Mg were measured by atomic absorption spectrophotometry in the same extract.

2.4 AMF trapping and mycorrhizal inoculum multiplication methods

The cocoa tree roots and those of companion trees taken from each plantation were cut into 2 cm fragments and mixed with the corresponding mycorrhizal soils. The mixture was used as mycorrhizal inoculum for trap cultures (Morton and Walker, 1992) to induce AMF spore multiplication and assess the rate of root colonization. Three pre-germinated cowpea seeds (previously disinfected with 2% bleach for 10 minutes) were sown in pots containing 500 g of mycorrhizal inoculum., 250 g of soil from the same source but sterilized (autoclaved 3 times at 121°C for 1h) and 250g of sterilized beach sand used as a nutrient-poor substrate. Under the same experimental conditions, control pots were also made with autoclaved inoculum to check that the culture conditions were free of contaminants. After 60 days of greenhouse

UNDER PEER REVIEW

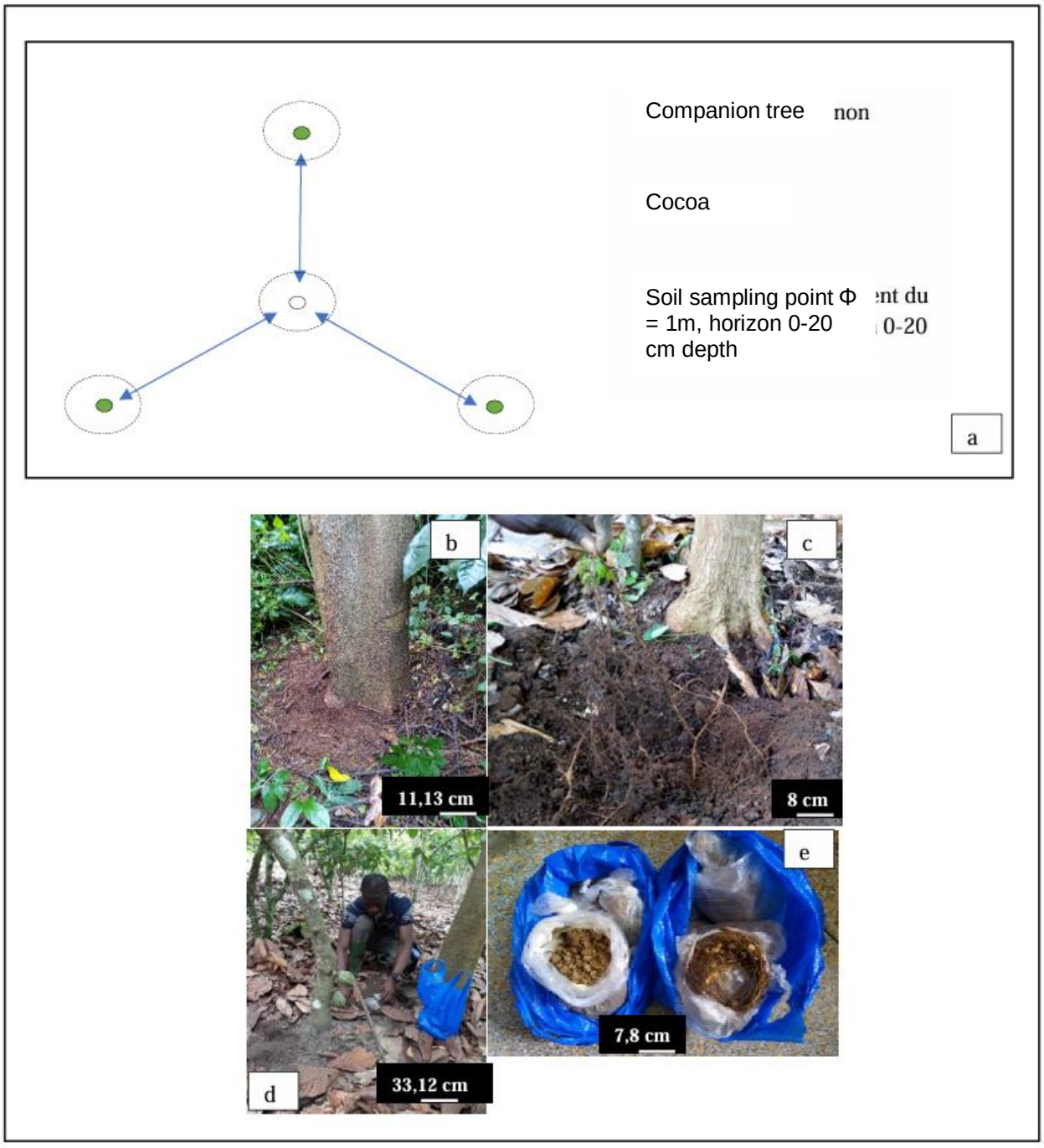

Fig. 2.Schematic diagram of the sampling system (a) and the various steps (b,c,d,e) for taking soil and root samples in cocoa farms

a : Diagram of the soil and root sampling system.
b : Identification of a cocoa companion tree (*Alstoniaboonei*) and clearing of the surrounding areabefore taking soil and root samples.
c : Excavation of *Perseaamericana* roots.
d : Soil and cocoa root sampling.

e : Packing of samples in plastic bags and labelling.
culture, watering was stopped until day 70. Cowpea roots and culture substrate are collected for further study.

2.5. Methods for enumerating and identifying AMF in soil samples

Soil samples were carefully homogenized and AMF spores isolated from 50 g of soil using the Gerdemann and Nicolson (1963) wet sieving method and decantation followed by sucrose centrifugation (Sieverding, 1991). Isolated spores were counted and separated using a binocular magnifier (60 x). Then, for each sample, 100 spores were mounted on slides using polyvinyl lactoglycerol alcohol (PVLG) and Melzer's reagent as mounting medium (Brundrett et al., 1996) and observed at 100X, 400X and 1000X magnification. The AMFs were then identified on the basis of the identification keys available in the scientific literature.(Schenck et Perez (1987), Morton et Benny (1990) ; Agere (2001) ; Becerra et Cabello (2008) ; INVAM (http://invam.caf.wvu.Edu), la collection de Blaszkowski (www.agro.ar.szczecin.pl/,jblaszkowski/)).

2.6. Methods for determining the rate of mycorrhization in samples of cocoa tree roots and companion trees

The presence of AMFs in roots was determined using the staining method of Phillips and Hayman (1970). Root colonization rates were assessed using the Trouvelot et al. (1986) method, based on 100 stained root fragments. Mycorrhization was observed under a light microscope (GX40) by dark-blue staining of fungal structures in the roots. For each root fragment, the presence or absence of vesicles, arbuscules and hyphae is noted at each millimetre and expressed as a percentage of infection. Infection percentages are rated from 0 to 5, with each rating corresponding to an infection percentage class. Rating 0: 0%; rating 1: 0 to 1%; rating 2: 1% to 10%; rating 3: 10% to 50%; rating 4: 50% to 90%; rating 5: > 90%. This rating method enabled us to calculate three parameters of mycorrhizal colonization: frequency of mycorrhizal colonization (F%), intensity of colonization of the root cortex (M%) and intensity of colonization in the mycorrhizal part of the root system (m%).

$$F(\%) = \frac{\text{Number of mycorhyzed fragments}}{\text{Total number of fragments observed}} \times 100$$

$$M(\%) = \frac{(95N5 + 70N4 + 30N3 + 5N2 + N1)}{\text{Total number of fragments observed}} \times 100$$

Where Ni = number of fragments corresponding to dimension i.

$$m(\%) = \frac{\text{total number of fragments observed}}{\text{number of mycorhyzed fragments}} \times M$$

2.7. Study of the mycorrhizal interaction between the cocoa tree and its 13 companion trees

The interaction between the cocoa tree and each of the 13 companion trees was measured using Pearson's correlation test (Tomassone et al., 1993) by comparing 5 mycorrhization parameters. These are shared species diversity, spore number, mycorrhization frequency, mycorrhization intensity and colonization intensity in the mycorrhized part of the root system.

UNDER PEER REVIEW

The correlation ranged from -1 to 1 (Kendall, 1955). Data were interpreted according to thefollowing scale: a correlation between 0 and 0.1 means that there is no relationship between the data. A correlation between 0.1 and 0.39 is weak. It is moderate if it is between 0.4 and 0.69. The correlation is strong if it is between 0.7 and 1.

2.8. Statistical analysis

Data relating to mycorrhization parameters and physico-chemical variables in soils sampled under target tree species were subjected to descriptive analysis followed by one-way analysis of variance (ANOVA 1). If the hypothesis of equality of the means was rejected, they were compared using the Newman-Keuls test with a threshold of 5%.

3. RESULTS

3.1. Soil physical and chemical properties

The analysis revealed three types of texture. Clay texture for soils under *R. heudelotii*, *B.mannii*, *C. nitida*, *A. boonei*, *T. superba*, *T. ivorensis* (Table 1). Sandy-clay texture for soils containing *P. americana*, *C. pentandra*, *G. sepium*, *M. excelsa*, *I. gabonensis*, *T. heckelii*. Finally, *G. kola* has a sandy loam texture. Soil pH varies from very acidic to acidic (4.1 in *T. superba*) to acidic (5.5 in *C. nitida*). Analysis of soil chemical fertility discloses phosphorus levels ranging from 41 ppm in *G. kola* (poor) to 61 ppm in *T. heckelii* (average). CEC values,.ranging from 1 to 6, distinguish between very poor and poor soils. support of the findings. The results and discussion part can also be described as separate, if appropriate

3.2. AMF species diversity

An analysis of mycorrhizal diversity in companion trees revealed 49 AMF species (Table 2) in common between cocoa and companion trees. *T. superba* and *B. mannii* share the highest number of species in common with cocoa trees, estimated at 64.28% and 62.5% respectively (Table 3). *C. etunicatum* (Figure 3) is the main species within this interaction for all companion trees studied. This species is followed by *A. myriocarpa* and *R. fasciculatus* in *T. superba* and by A. tuberculata and *A. bireticulata* in *B. mannii*. On the other hand, *C.pentendra* has the lowest ratio of AMF species in common with cocoa (16.12%). Apart from *C. etunicatum*, the main species involved are *P. scintillans* and *T. nevadensis*. Furthermore, the companion trees with the greatest diversity of AMF species in their rhizospheres are *T. superba*, *P. americana* and *M. excelsa*, with 42, 37 and 35 species respectively, compared with 49 species in cocoa trees.

3.3. Sporulation capacity of cocoa and companion tree AMFs

The numbers of spores from the cocoa rhizosphere and those from companion trees, except for*T. ivorensis*, *C. pentendra* and *T. heckelii*, showed no significant differences where significant differences were observed (P <.05). The number of AMF spores highlights *T. superba* (23.57 to 25.65 spores/g soil), *B. mannii* (19.9 to 21.86 spores/g soil) and *R. heudelotii* (16.77 to 19.13 spores/g soil) as the companion trees with the highest spore production in interaction with cocoa trees (Figure 4). *I. gabonensis*, on the other hand, recorded the lowest levels of spore production in this interaction (13.71 and 17.8 spores/g soil).

UNDER PEER REVIEW

Table 1.Physicochemical characteristics of rhizosphere soils of cocoa companion tree species in surveyed fields

Type of soilsamples	Soil texture				Soil Ph		Soilchemicalfertility							
	Clay %	Sand%	Limon %	Texture	pH (KCl)	pH (eau)	C %	C/N	N%	P. ass (ppm)	K	Ca	Mg	C.E.C
R. heudelotii	41.9	37.9	20.2	Clay	4.3	5.3	1.1	12.9	0.11	42	0.2	0.5	0.6	2
P. americana	35.2	46.6	18.1	Sandy-clay	4.4	5	1.6	13.4	0.13	54	0.2	0.6	0.9	1
B. mannii	42.8	44.4	12.8	Clay	4.5	5.5	1.9	12.1	0.18	58	0.3	2.1	2.1	1
C. nitida	40.7	40.9	18.4	Clay	5.5	5.9	2	10.1	0.19	55	0.3	5.2	2.2	6
A. boonei	42.7	39.4	17.9	Clay	4.3	5.5	1	14.3	0.08	43	0.2	0.9	0.4	1
T. superba	43	42.2	14.9	Clay	4.1	5.3	0.8	14	0.06	55	0.1	0.5	0.1	2
T. ivorensis	49.4	36.8	13.8	Clay	4.5	5.3	1.3	9.6	0.14	51	0.1	2.6	0.7	1
C. pentandra	36.8	46.1	17.1	Sandy clay	5	5.6	1.5	10.2	0.14	53	0.4	2.3	0.7	2
G. sepium	36.6	46.4	17	Sandy clay	4.3	5.2	1.1	11.3	0.11	60	0.1	1.4	0.6	1
M. excelsa	36.8	48.7	14.5	Sandy clay	4.6	5.6	1.2	9.3	0.11	54	0.1	1	0.5	1
I. gabonensis	46.8	30.6	22.5	Clay	5	5.9	1.7	10.3	0.19	56	0.5	4.6	1.9	6
T. heckelii	36.4	47	16.6	Sandy clay	4.6	5.8	0.8	8.7	0.08	61	0.3	2.7	0.4	4
G. kola	42.4	39.7	17.9	Silty-clay	4.6	5.7	1.7	10.7	0.17	41	0.1	1.7	1.5	2

C: Carbon content
N: Nitrogen content
P ass : Assimilable phosphorus content
C/N: Carbon/nitrogen ratio
CEC: Cation exchange capacity
K: Potassium
Ca: Calcium
Mg: Magnesium

UNDER PEER REVIEW

Table 2. Main species of arbuscular mycorrhizal fungi and their relative abundance in the rhizosphere of cocoa companion trees in the fields surveyed

AMFspecies	Cocoa companiontreespecies												Average
	R. heu	*P. ame*	*B. man*	*C. nit*	*A. boo*	*T. sup*	*T. ivo*	*C. pen*	*M. exc*	*I. gab*	*T. he*	*G. col*	
Acaulosporabireticulata	0,70	0,38	8,11	-	-	1,10	-	-	2,52	-	25,00	11,11	4,08
Acaulosporadelicata	-	0,38	-	1,37	3,17	1,75	-	0,57	1,68	-	-	-	0,74
Acaulosporaexcavata	0,70	-	-	-	-	0,44	-	1,71	-	2,38	-	-	0,44
Acaulosporagedanensis	1,40	1,15	-	1,37	-	1,10	-	4,00	0,42	7,14	-	-	1,38
Acaulosporarehmii	-	0,38	-	-	-	-	-	-	-	-	-	-	0,03
Acaulosporascrobiculata	5,59	2,29	-	0,46	-	5,04	8,33	4,00	4,20	4,76	16,67	-	4,28
Acaulosporathomii	-	0,38	-	-	-	-	-	-	-	-	-	-	0,03
Acaulosporatuberculata	-	0,38	16,22	1,37	9,52	5,26	-	5,71	3,36	4,76	-	5,56	4,35
*Acaulospora*sp 1	0,70	0,38	-	-	1,59	0,88	8,33	1,14	0,84	-	-	-	1,16
Ambisporafecundispora	2,10	2,67	2,70	0,46	4,76	2,19	-	0,57	1,68	-	-	-	1,43
Archaeosporaundulata	-	-	-	0,91	-	0,66	-	-	7,14	-	-	-	0,73
Archaeosporamyriocarpa	0,70	-	-	0,46	4,76	4,17	-	0,57	-	-	-	-	0,89
Archaeosporatrappei	1,40	0,38	-	4,11	-	-	-	-	-	-	-	-	0,49
Claroideoglomusetunicatum	45,45	35,88	45,95	50,23	31,75	19,74	16,67	25,71	33,61	26,19	41,67	33,33	33,85
Corymbiglomustortuosum	1,40	2,29	-	-	-	4,61	8,33	2,29	3,36	14,29	-	-	3,05
Dentiscutataerythropa	1,40	2,67	5,41	-	1,59	1,97	-	4,57	0,84	2,38	-	-	1,74
Funneliformisgeosporum	0,70	4,58	-	0,91	-	0,44	25,00	4,00	0,84	-	-	-	3,04
Gigasporagigantea	-	-	-	-	-	0,22	-	0,57	-	-	-	-	0,07
*Gigaspora*sp 1	-	-	-	0,46	3,17	0,66	-	-	1,68	2,38	-	-	0,70
*Gigaspora*sp 2	-	-	-	-	-	0,22	-	-	-	-	-	-	0,02
*Gigaspora*sp 4	0,70	1,15	-	-	-	0,22	-	0,57	0,84	-	-	-	0,29
*Gigaspora*sp 5	1,40	0,38	-	1,37	1,59	1,32	-	0,57	2,10	-	-	-	0,73
Glomus hoi	3,50	4,58	-	-	-	0,88	-	2,29	2,52	9,52	-	11,11	2,87
Glomus macrocarpum	-	0,38	-	-	1,59	0,66	-	-	0,42	-	-	-	0,25
Glomus multicaule	-	0,76	-	-	-	-	-	-	0,42	-	-	-	0,10
Glomus sterilum	0,70	0,76	-	1,37	1,59	0,88	-	-	2,94	-	-	-	0,69
Glomus sp 1	-	1,53	-	1,83	4,76	4,61	-	2,86	3,78	-	-	-	1,61
Glomus sp 2	-	1,15	-	1,37	-	1,10	-	4,57	0,84	-	8,33	-	1,45
Glomus sp 3	-	0,38	-	-	-	0,22	-	-	-	-	-	-	0,05
Glomus sp 4	-	0,38	2,70	1,37	-	2,19	-	-	-	-	-	5,56	0,94
Glomus sp 6	-	-	-	-	-	0,22	8,33	-	-	-	-	-	0,66

R. heu =*Ricinodendronheudelotii* ; P. ame = *Persea americana ;* B. man = *Beilschmiediamannii*; C. nit = *Cola nitida ;* A. boo = *Alstonia boonei ;* T. sup = *Terminaliasuperba ;* T. ivo = *Terminaliaivorensis ;* C. pen = *Ceibapentandra* ; M. exc = *Miliciaexcelsa ;* I. gab = *Irvingiagabonensis* ; T. he = *Tieghemellaheckelii* ; G. col = *Garcinia kola*

Table 2Main species of arbuscularmycorrhizal fungi and their relative abundance in the rhizosphere of cocoacompaniontrees in the fieldssurveyed(Continue)

AMFspecies	Cocoa companiontreespecies												Average
	R. heu	*P. ame*	*B. man*	*C. nit*	*A. boo*	*T. sup*	*T. ivo*	*C. pen*	*M. exc*	*I. gab*	*T. afr*	*G. col*	
Glomus sp 5	-	0,38	-	-	-	0,88	-	0,57	-	-	-	-	0,14
Pacisporascintillans	-	-	-	3,20	-	-	-	3,43	0,84	-	8,33	-	1,22
Paraglomusoccultum	-	-	-	0,46	-	0,66	-	-	-	-	-	-	0,09
Rhizophagusintraradice	1,40	3,05	-	-	-	1,97	-	0,57	-	-	-	-	0,58
Rhizophagusfasciculatus	2,10	3,44	-	4,57	3,17	4,17	-	-	2,10	2,38	-	5,56	2,29
Sacculosporabaltica	2,10	1,91	-	0,91	-	3,07	-	2,29	-	2,38	-	-	1,05
Sclerocystisrubiformis	2,80	1,91	-	-	4,76	3,29	-	1,71	1,26	-	-	-	1,31
Sclerocystissinuosa	-	-	-	-	-	0,88	-	-	0,42	-	-	-	0,11
Scutellosporaovalis	0,70	0,38	-	0,91	-	-	-	0,57	-	2,38	-	-	0,41
*Scutellospora*sp 1	-	1,15	-	0,46	-	-	-	-	-	-	-	-	0,13
*Scutellospora*sp 2	-	-	5,41	-	-	0,44	-	-	0,84	-	-	-	0,56
*Scutellospora*sp 3	0,70	1,53	-	-	-	0,44	-	-	1,26	-	-	-	0,33
*Scutellospora*sp 4	0,70	-	-	-	-	0,22	-	-	0,42	2,38	-	-	0,31
*Scutellospora*sp 5	0,70	-	-	-	-	0,44	-	2,86	-	-	-	-	0,33
Septoglomusconstrictum	-	1,53	-	11,42	-	0,66	-	0,57	2,10	2,38	-	11,11	2,48
Septoglomusdeserticola	0,70	0,38	-	-	-	1,10	-	-	-	-	-	5,56	0,64
Tricisporanevadensis	1,40	5,34	-	0,46	1,59	3,73	8,33	1,71	2,94	2,38	-	-	2,32
P-value	0,49	0,07	0,73	0,20	0,03	0,01	0,03	0,055	0,11	0,25	0,66	0,80	

UNDER PEER REVIEW

Table 3. Summary of species rates involved in mycorrhizal symbiosis between cocoa and companion trees

Soil samples from companion trees	Number of AMFs involved in symbiosis with the cocoa tree	Species in common with neighbouring cocoa trees	Ration (%)
C. pentandra	31	5	16.13
I. gabonensis	17	4	23.53
A. boonei	16	4	25.00
T. heckelii	16	4	25.00
T. ivorensis	14	4	28.57
G. kola	13	4	30.77
R. heudelotii	31	11	35.48
P. americana	37	17	45.95
C. nitida	32	15	46.88
M. excelsa	35	18	51.43
B. mannii	16	10	62.50
T. superba	42	27	64.29
G. sepium	16	4	25.5

UNDER PEER REVIEW

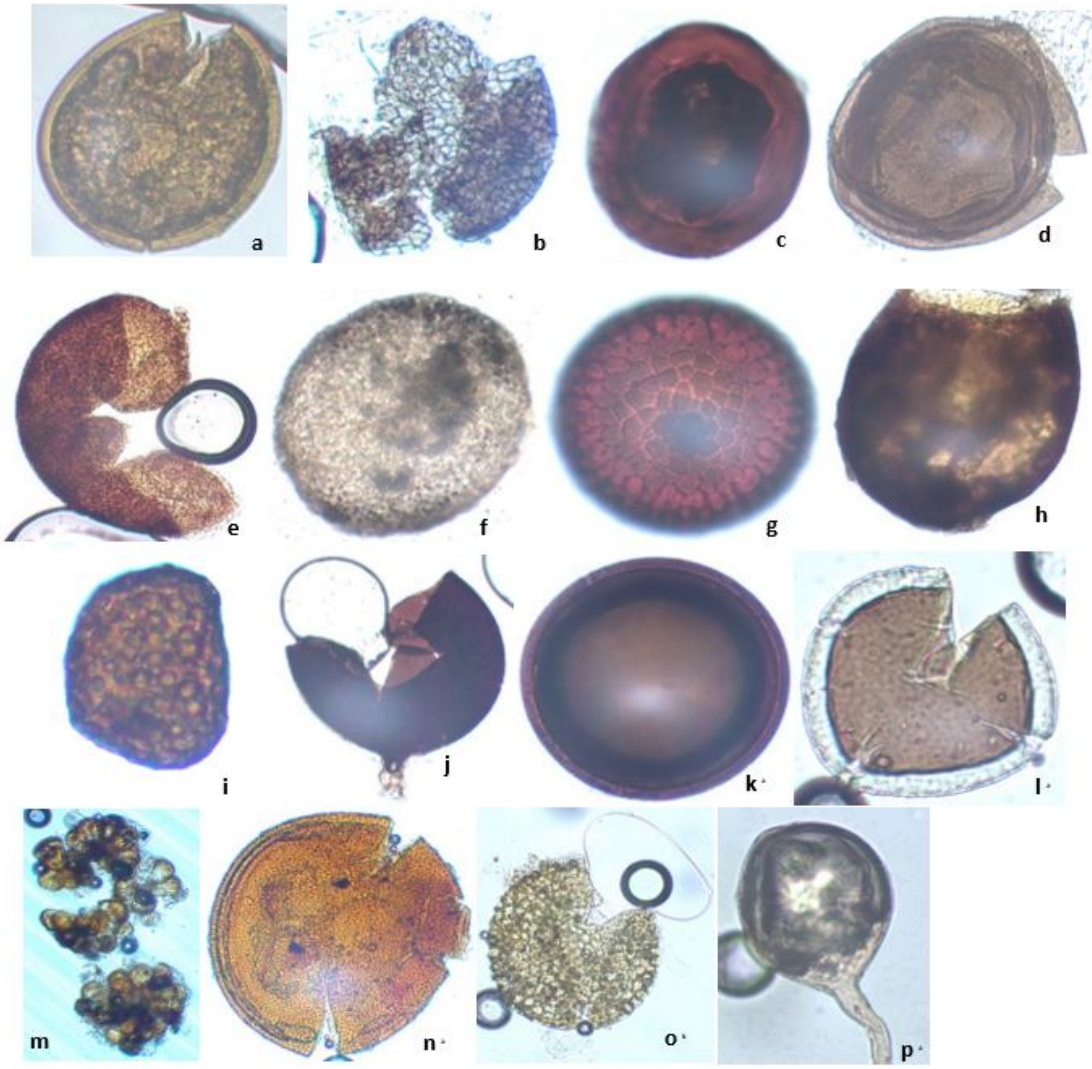

Fig. 3. AMF species involved in cocoa and companion-tree interaction on farm
(a)*Claroideoglomusetunicatum,* **(b)***Archaeosporamyriocarpa,* **(c)***Rhizophagusfasciculatus*, **(d)***Acaulosporatuberculata*, **(e)***Acaulosporabireticulata,* **(f)***Pacispora scintillans*, **(g)***Tricisporanevadensis,* **(h)** *Glomus intraradice,* **(i)***Glomus macrocarpum,* **(j)***Septoglomusconstrictum,* **(k)** *Glomus fecondisporum,* **(l)***Sacculosporabaltica,* **(m)***Sclerocystissinuosa,* **(n)***Acaulosporascrobiculata,* **(o)***Acaulosporaexcavata,* **(p)***Sclerocystisrubiformis.*

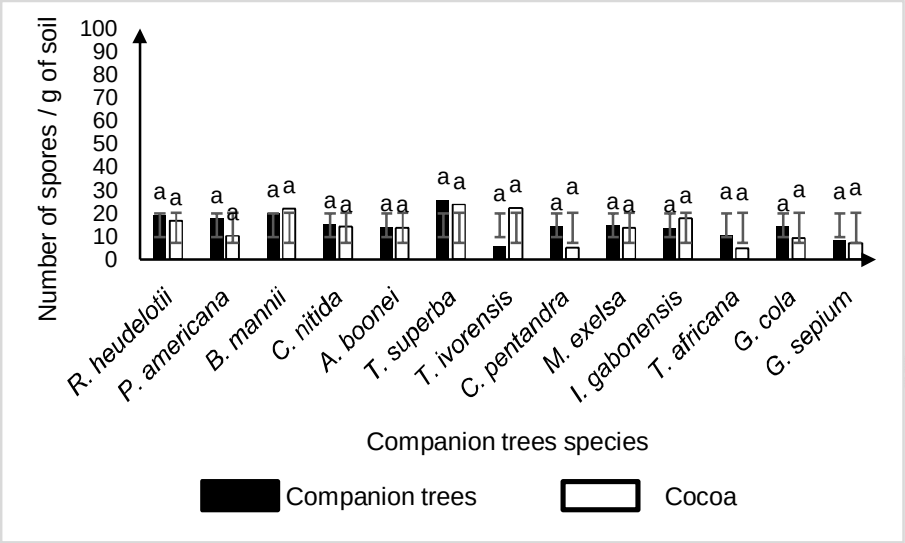

Fig. 4. Average number of spores of arbuscular mycorrhizal fungi per gram of soil in the rhizosphere of cocoa trees and each of the companion tree species

For each pair formed by the cocoa tree and a companion tree species, the numbers of spores per gram of soil followed by the same letter are not significantly different between the rhizospheres, according to the Kruskal Walis test at the 5% threshold.

UNDER PEER REVIEW

3.4. Root mycorrhization rate

Observation of the roots revealed the presence of mycorrhizal structures such as hyphae, arbuscular vesicles and spores (Figure 5). Statistical analysis did not show significant difference ($p>0.05$) between the companion tree and the cocoa tree in terms of mycorrhization intensity and frequency. The highest mycorrhization intensities were observed in the interaction between *T. ivorensis* and the cocoa tree, where the intensity was around 40% (Figure 6; Figure 7). On the other hand, the lowest intensity, below 15%, is observed between *R. heudelotii* and cocoa. Overall, mycorrhization frequency remained high (over 70%). *T. ivorensis* generated the highest frequencies, reaching 100%. The lowest frequencies were observed in *R. heudelotii*, where they ranged from 71.56% to 82.12% (Figure 8).

3.5. Mycorrhizal interaction between companion trees and cocoa trees

The study of interactions displayed strong links between cocoa trees and companion trees on the basis of the 5 types of variable used to establish correlations (Table 4).
In addition to *G. kola*, correlation analysis indicated a strong and significant positive correlation between all companion trees and cocoa trees in terms of species diversity ($0.75<r<0.97$; $P = .001$). Spore number showed a moderate non-significant positive correlation in *T. heckelii* ($r = 0.56$) and *G. kola* ($r = 0.63$) and a strong one in *P. americana* ($r = 0.87$). Mycorrhization intensity was positively correlated with *A. boonei* ($r = .74$), *T. superba* ($r = 0.94$), *T. ivorensis* ($r = 0.86$), *G. kola* ($r = 0.91$) and *T.heckelii* ($r = 0.77$). Mycorrhization frequency showed a positive correlation for interaction betweencocoa trees and *A. boonei* ($r = 0.82$) and *T. superba* ($r = 0.82$). This was significant for *M excelsa* ($r = 0.99$; $P = .02$). Root cortex mycorrhization intensity was significantly negatively correlated between cocoa and *B. mannii* ($r = -0.95$; $P = .04$) and positively correlated, but not significantly, for *B. mannii* ($r = 0.84$) and *G. kola* ($r = 0.91$).

3.6. Characterization of companion tree mycorrhizal inocula based on their interactions with cocoa trees

A CAH was used to analyze the interaction between cocoa trees and companion trees. The dendrogram of the CAH, based on parameters such as AMF diversity, spore numbers and mycorrhization rates of mycorrhizal inoculums from cocoa and cocoa companion trees, highlights two main classes (Figure 9). Substrates belonging to the first class are characterized by strong, positive correlation coefficients for the mycorrhization intensity parameter. It is composed of 2 sub-classes. The first sub-class includes *T. superba* and *M. excelsa* substrates, which show a strong positive correlation for mycorrhization frequency (0.82 and 0.99 respectively) and a moderate, non-significant negative correlation for spore number (-0.63 and -0.42 respectively). The second sub-class is characterized by a strong positive correlation for the intensity of mycorrhization developed in the root cortex. It is made up of the *T.ivorensis* substrate, and the *T. heckelii* and *G. kola* substrates, which also show a moderate positive correlation with spore quantity (0.56; 0.63).
The second class is characterized by negative correlations for the mycorrhization intensity parameter. It is subdivided into 3 subclasses. The first sub-class contains *A.boonei* substrate. This is positively correlated with spore number (0.74) and mycorrhization frequency (0.82). The second sub-class is made up of *C. nitida* and *I. gabonensis* substrates, positively correlated with mycorrhization frequency, and *P. americana*, *C. pentandra* and *R. heudelotii* substrates, moderately positively correlated with spore number. The third subclass, made up of 2 subclasses, is made up of inoculum with intermediate spore counts (between 956 and 1093 spores per 50 g of soil). The first subclass is divided into 3 blocks.The first sub-class includes *C. nitida*inocula with mycorrhization frequencies of over 95%, *A. boonei*, *G. kola*, *I. gabonensis*, *C. pentandra* and *T. heckelii*inocula with the highest mycorrhization

UNDER PEER REVIEW

intensities (40.89%) and the lowest number of species.The second sub-class includes *B. manni*inocula with

UNDER PEER REVIEW

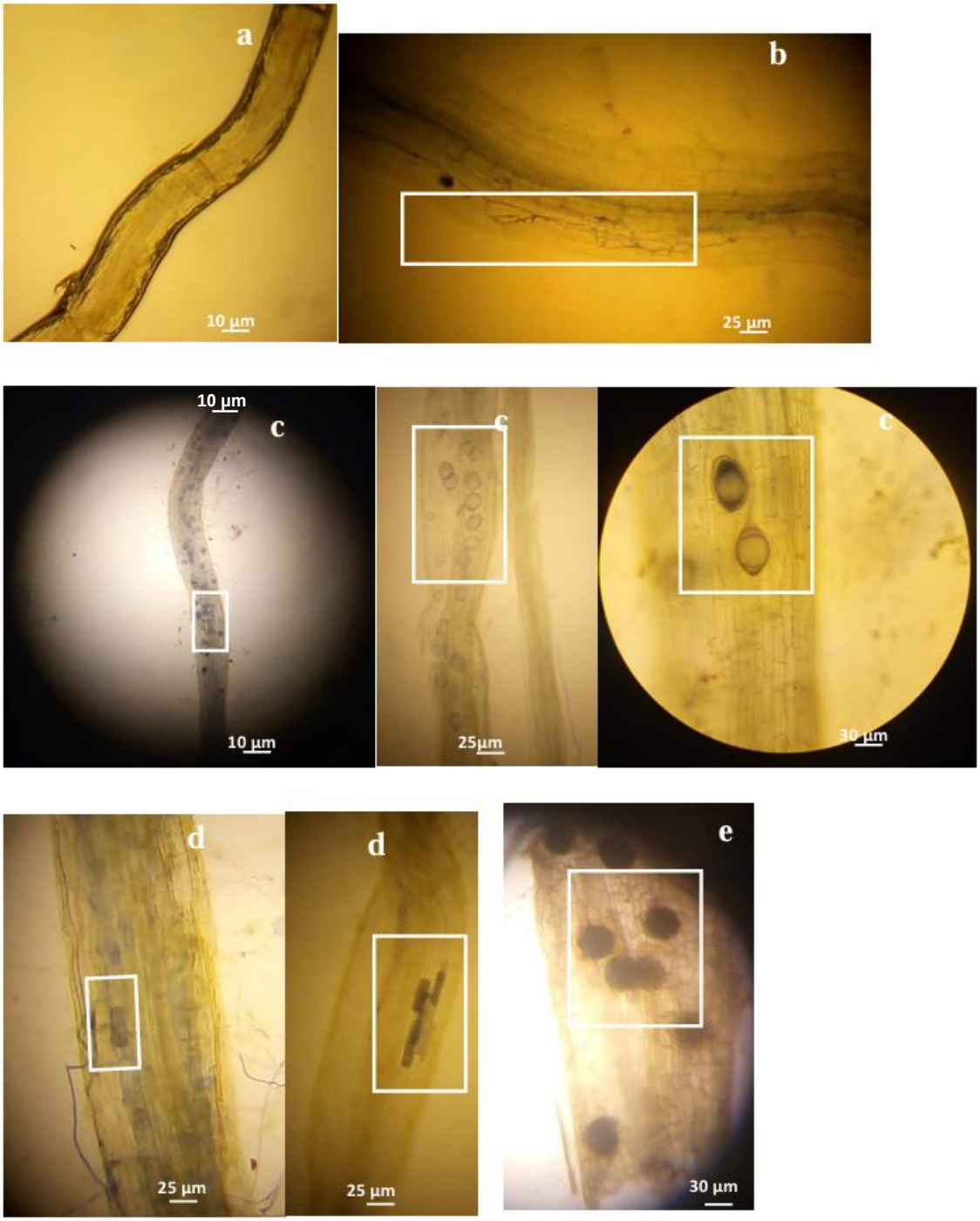

Fig. 5.Appearance of non-mycorrhizalroots (a) and mycorrhizal structures present in mycorrhizalroots (b, c, d and e) of cocoatrees and cocoacompanionspecies.

a: non-mycorrhizal root
b : intra-root hyphae
c:vesicle
d: arbuscules
e: intra-root spore

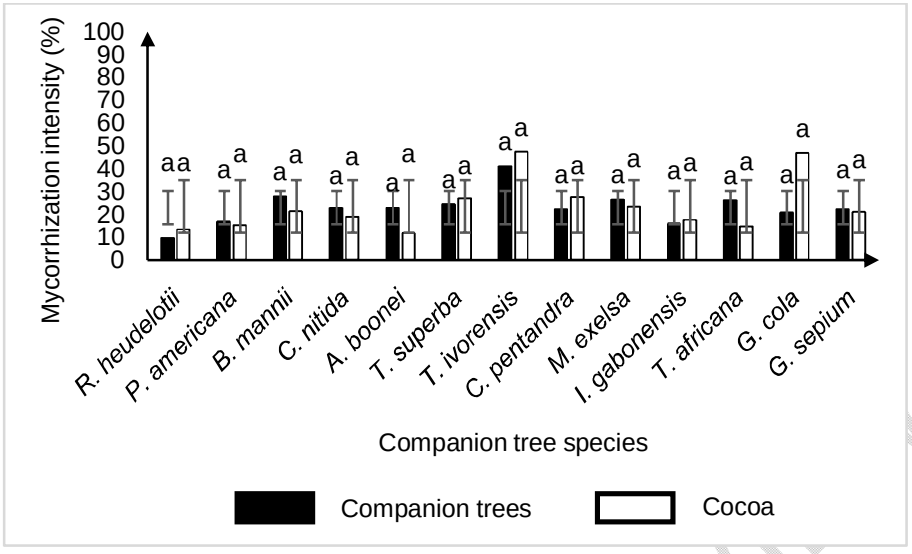

Fig. 6. Mycorrhization intensity of cocoa tree roots and each companion tree species

For each pair formed by the cocoa tree and a companion tree species, the mycorrhization intensities of the roots followed by the same letter are not significantly different between the rhizospheres, according to the Kruskal Walis test at the 5% threshold.

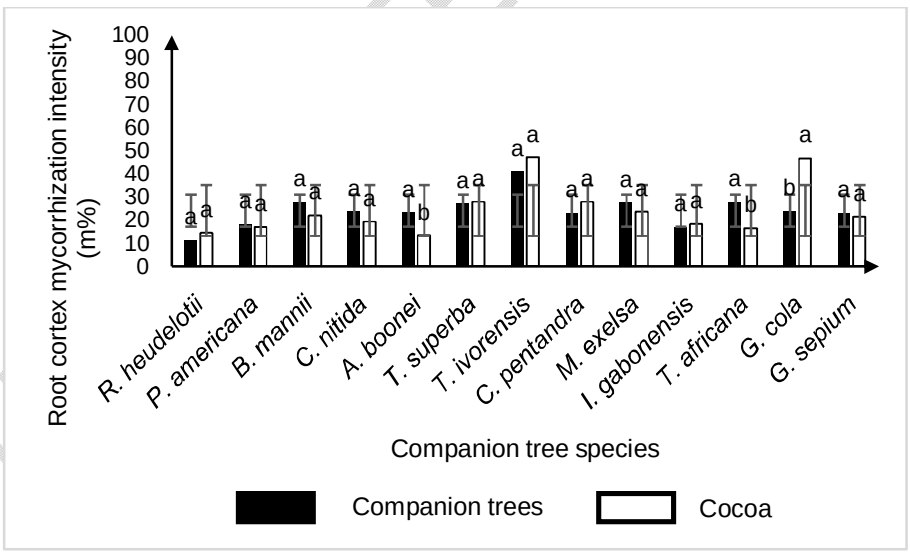

Fig.7. Intensities of mycorrhizal colonization of the root cortex of cocoa trees and companion tree species

For each pair formed by the cocoa tree and a companion tree species, he mycorrhization intensities of the root cortexfollowed by the same letter are not significantly different between the rhizospheres, according to the Kruskal test with a threshold of 5%.

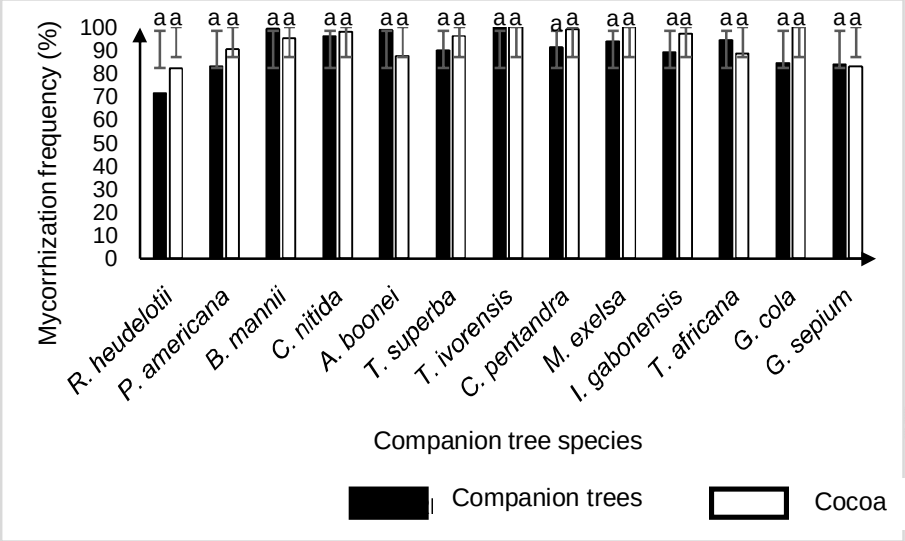

Fig. 8. Mycorrhization frequencies of cocoa tree roots and cocoa tree companion species in the plantations surveyed

For each pair formed by the cocoa tree and a companion tree species, the mycorrhization frequencies of the roots followed by the same letter are not significantly different between the rhizospheres, according to the Kruskal Walis test with a threshold of 5%.

UNDER PEER REVIEW

Table 4. Correlation coefficients between the values of mycorrhization parameters obtained in the rhizosphere of cocoa trees and in each cocoa companion plant species in the plantations investigated

Cocoa companion trees	Pearson correlation coefficient (r)									
	NE	P value	NS	P value	M%	P value	F%	P value	m%	P value
Ricinodendronheudelotii	**0,972***	**0,00***	0,01	0,98	- 0,12	0,85	- 0,08	0,90	- 0,02	0,98
Perseaamericana	**0,929***	**0,00***	0,81	0,39	- 0,11	0,93	- 0,14	0,91	- 0,22	0,86
Beilschmiediamannii	**0,933***	**0,00***	- 0,13	0,87	- 0,56	0,62	- 0,58	0,42	**- 0,95***	**0,04***
Cola nitida	**0,925***	**0,00***	- 0,14	0,86	- 0,42	0,58	0,63	0,37	- 0,39	0,61
Alstoniaboonei	**0,722***	**0,00***	0,74	0,26	0,71	0,29	0,83	0,17	- 0,11	0,89
Terminalia superba	**0,785***	**0,00***	- 0,63	0,18	0,95	0,21	0,83	0,17	0,95	0,21
Terminalia ivorensis	**0,912***	**0,00***	- 0,58	0,42	0,86	0,14	-0,33	0,67	0,86	0,15
Ceiba pentandra	**0,921***	**0,00***	0,51	0,49	- 0,15	0,85	0,57	0,43	- 0,13	0,87
Gliricidiasepium	**0,902***	**0,00***	- 0,58	0,41	0,75	0,14	- 0,42	0,56	0,60	0,30
Miliciaexcelsa	**0,966***	**0,00***	- 0,42	0,34	0,36	0,77	**0,99***	**0,02***	0,30	0,81
Irvingiagabonensis	**0,797***	**0,00***	0,54	0,55	- 0,47	0,53	0,54	0,46	- 0,44	0,56
Tieghemellaheckelii	**0,751***	**0,03***	0,56	0,44	0,78	0,43	- 0,35	0,77	0,84	0,36
Garcinia kola	0,183	0,64	0,64	0,56	0,92	0,26	0,50	0,67	0,92	0,26

UNDER PEER REVIEW

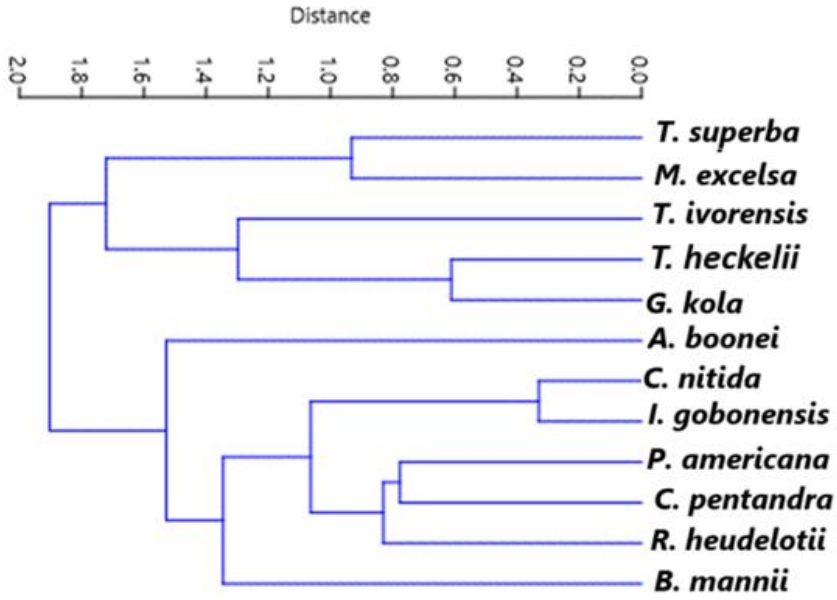

Fig. 9. Classification of AMF of companion tree inocula based on interactions with the cocoa

UNDER PEER REVIEW

high mycorrhization rates and *P. americana*and *R. heudelotii* with the lowest mycorrhization rates (M% = 9.64, m = 11.36; M% = 16.6, m% = 11.36). The third sub-class consists of *B. mannii* inoculum. This is characterized by a negative correlation for all parameters except species diversity. It is important to note that all substrates are positively correlated for species diversity.

4. DISCUSSION

This study identified 49 species of AMFs present in the interaction between trees and cocoa trees. Indeed, AMFs colonize around 90% of vascular plants (Coyne, 2000) and have already been recorded in the Ivorian cocoa orchard (Zako et al., 2012; Rincón et al., 2021). In agroforestry systems, organization is heterogeneous.This promotes the coexistence of several plant species in the same soil space, and increases the possibility of finding fruitful associations with various AMF species. This coexistence between species is due to the degree of mycorrhizal dependence of the plants (Plenchette et al., 1983; van der Heijden et al., 1998). AMFs will promote coexistence between species by increasing the ability of less competitive species to access nutrients (Moora and Zobel, 1996). In this case, *C. etunicatum* is the dominant species within the interaction between the cocoa tree and all the companion trees studied. This could be explained by the fact that this species colonizes the orchard and extends its mycelial network, linking the other plants in smaller numbers, enabling them to better exploit nutritive resources (Chiarello et al., 1982). Indeed, Grime et al. (1987) hypothesized that AMFs would enhance the floristic diversity of plant communities by creating mycelial networks between plants, from dominant species to less abundant ones. Furthermore, the wide diversity of AMFs observed stimulates coexistence between plants, by increasing the possibility for each plant species to associate with a compatible and efficient fungal partner (Hart et al., 2003).
In addition, this study revealed that the number of spores is higher in the soil under *T. superba* (23.57 to 25.65 spores/g soil), *B. mannii* (19.9 to 21.86 spores/g soil) and *R. heudelotii* (16.77 to 19.13 spores/g soil). The large number of spores in the soil under these companion trees could indeed explain the high mycorrhizogenic potential of these soils. This is significantly higher than that observed in the rhizosphere of *C. pentandra* (3.58 spores/g soil) and *T. heckelii* (5.35 spores/g soil) by Anguiby et al. (2019) and in primary, secondary and reforested forests by Zézé et al. 2007 (0.15, 0.16 and 0.21 respectively). The presence of trees of this floristic diversity significantly enriched the cocoa rhizosphere in spores, promoting massive spore production in the cocoa ecosystem. These results confirm that certain plant species have the capacity to promote the development of fungal propagules in their rhizosphere (Eom et al., 2000; Azcon-Aguilar et al*.,* 2003; Lovelock et al., 2003). Mycorrhizal fungi spore counts showed a predominance of the Glomus genus in all interactions with companion trees. This abundance of the genus Glomus has also been found in various studies carried out in Côte d'Ivoire (Touré et al*.,* 2021; Amaniet al., 2023) and in the West African sub-region (Johnson et al. (2013), in Benin). The predominance of species of the genus Glomus in most ecosystems suggests a better adaptation of this genus either to the most hostile conditions such as drought, salinity and other environmental stresses, or to a wide range of ecological niches (Houngnandan et al., 2009). Indeed, the Glomus genera propagate much more by spores, which are forms of AMF resistance to harsh conditions, while other genera, such as Gigaspora and Scutellospora, propagate more by other types of propagules, such as hyphae and extra-root mycelial fragments. In addition, the study showed low mycorhozation intensities of less than 50%. These results are in line with those of Sidibe et al. 2015 carried out on banana and Anguiby et al. 2019 carried out on makoré and fromager, who highlighted low endomycorrhization (< 50%) in studies carried out in cote d'Ivoire. This low level of mycorrhization could be due to the disturbed soils in the study areas. According to Dickie et al, (2004), degradation of natural plant communities (population structure and species diversity) generally leads to a loss or reduction of

UNDER PEER REVIEW

mycorrhizal propagules in the soil and, consequently, decreases mycorrhizal potential in degraded areas. On the other hand, mycorrhization frequencies are highly variable, ranging from 71 to 100%. Anguiby et al., 2019 obtained similar results for *C. pentandra* and *T. heckelii*. According to Diagne and Ingleby (2003), above 12% mycorrhization intensity, the benefits derived by the plant symbiont are not negligible. The results obtained therefore suggest that the species concerned can contribute to improving cocoa production through mycorrhization.

The mycorrhizal interaction between cocoa and companion trees was assessed using Pearson's correlation test for the variables of diversity, mycorrhization rate and spore number. The study also revealed a strong interaction between companion trees and cocoa trees. It shows the role of mycorrhizal isolates in spore production, or in the intensity of root mycorrhization. This interaction is even stronger in terms of AMF species diversity, for all mycorrhizal inocula. At species level, a strong positive and significant correlation was recorded between cocoa and companion trees, with the exception of *G. kola*. The various results obtained under controlled conditions make it possible to propose companion trees based on their mycorrhizal characteristics, for cocoa cultivation in agroforestry systems.

CONCLUSION

This study is part of a research program aimed at improving cocoa production in Côte d'Ivoire by optimizing mineral nutrition and plant resistance to biotic and abiotic factors. Its main objective was to decipher the characteristics of AMFs in the roots and soil of cocoa trees and companion trees, in order to understand the interaction between the companion tree and the cocoa plant. Morphological characteristics of the spores showed a high diversity of AMFs, with 49 species present within the interaction**.** The various results obtained under controlled conditions make it possible to propose companion trees based on their mycorrhizal characteristics, for cocoa cultivation in agroforestry programs. Thus, *T. superba, B. mannii* and *R. heudelotii* can be recommended to promote the production of mycorrhizal spores in interaction with cocoa trees, and *T. ivoriensis* to generate high levels of mycorrhization.*A. boonei, T. superba, T. ivorensis, T. heckelii, M excelsa, B. mannii, B. mannii*and *G. kola*can be recommended in cocoa agroforestry systems as they have the most important mycorrhizal interactions with cocoa trees.Overall, all the companion trees studied favoured high mycorrhization frequencies when in close proximity to cocoa trees, thus revealing the importance of companion trees in cocoa agroforestry systems. Further studies, integrating the role of companion tree substrates in their ability to generate AMF, effective against biotic and abiotic stress should be conducted to strengthen the explanation of mycorrhizal interaction.

DISCLAIMER(Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

Adou Yao, C.Y., Kpangui, K. B., Vroh, B. T., &Ouattara, D., (2016). Farming practices, use values and farmers' perception of cocoa companion species in traditional agroforests in

UNDER PEER REVIEW

central Côte d'Ivoire. Revue d'ethnoécologie, 9, published online July 1, 2016. https://doi.org/10.4000/ethnoecologie.2474.

Amani, Y. F. C., M'bo, K. A. A., Koné, D., &Kouamé, C. (2020). Ivorian cocoa farmers' perception of agroforestry: support tools for decision making. Agricultural Science Research Journal, (12) :321 – 331.

Amani, Y. F. C., M'bo, K. A. A., Cherif, M., Koné, D., &Kouamé, C. (2023). Diversité des champignons mycorhiziens à arbusculeassociés aux cacaoyers (*Theobroma cacao* L.) en Côte d'Ivoire. European Scientific Journal, ESJ, 19 (27), 179. https://doi.org/10.19044/esj.2023.v19n27p179.

Anguiby, B. L., Ouattara, G., Bomisso, E. L., N'goran, B., Ouattara, B., Coulibaly, S. A., & Ake, S. (2019). Evaluation du statutmycorhiziend'arbres de *Ceiba pentandra* (L), Gaertn et *Tieghemellaheckelii* (A.Chev), Pierre, du jardinbotanique de Bingervilleen Côte d'Ivoire. Journal of Applied Biosciences, 138 : 14092-14105. https://dx.doi.org/10.4314/jab.v138i1.9.

Azcon-Aguilar, C., Palenzuela, J., Roldan, A., Bautsta, S., Vallejo, R., & Barea, J. M. (2003). Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened mediterranean shrublands. Applied Soil Ecology, 22, 29-37. https://doi.org/10.1016/S0929-1393(02)00107-5.

Brundrett, M. C., Ashwath, N., & Jasper, D. A. (1996). Mycorrhizas in the Kakadu region of tropical Australia. Propagules of mycorrhizal fungi and soil properties in natural habitats. Plant and Soil, 184, 173–184. https://doi.org/10.1007/BF00029286.

Bussotti, F., Ferrini, F., Pollastrini, M., &Fini, A. (2014). The challenge of Mediterranean sclerophyllous vegetation under climate change: from acclimation to adaptation. Environ. Exp. Bot., 103, 80–98. https://doi.org/10.1016/j.envexpbot.2013.09.013.

Chiarello, N., Hickman, J. C., & Mooney, H. A. (1982). Endomycorrhizal role for interspecific transfer of phosphorus in a community of annual plants. Science, 217(4563):941-3. DOI: 10.1126/science.217.4563.941.

Cramer, W., Guiot, J., Fader, M., Garrabou, J., Gattuso, J.-P., Iglesias, A., Lange, M. A., Lionello, P., Llasat, M. C., & Paz, S. (2018). Climate change and interconnected risks to sustainable development in the Mediterranean. Nat. Clim. Chang., 8, 972–980. https://doi.org/10.1038/s41558-018-0299-2.

Coyne, M. S. (2000). Soil microbiology : an exploratory approach. Delmar Publisher, New York uneapprocheexploratoire (1ère édition). Delmar Publishers. Current Opinion in Chemical Biology, 4 (5) : 559 – 566.

De la Fuente Cantó, C., Simonin, M., King, E., Moulin, L., Bennett, M. J., Castrillo, G., &Laplaze, L. (2020). An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. Plant J., 103(3), 951-964. DOI: 10.1111/tpj.14781.

Diagne, O. & Ingleby, K. (2003). Ecology of arbuscular mycorrhizal fungi infectious to *Acacia raddiana*. In: Un arbre au desert, IRD Editions, Paris, 205-228.

Dickie, I. A., Guza, R. C., Krazewski, S. E. & Reich, P. B. (2004). Shared ectomycorrhizal fungi between a herbaceous perennial (*Helianthemum bicknellii*) and oak (Quercus)

UNDER PEER REVIEW

seedlings. New Phytologist, 164(2), 375-382. https://doi.org/10.1111/j.1469-8137.2004.01177.x.

Ducousso, M. (1991). Importance of root symbioses for acacia utilization in West Africa. Thesis, Université Claude Bernard, Lyon I (CIRAD-ISRA), Nogent sur Marne, France and Dakar, Senegal, 216 p.

Eldin, M. (1971). Le climat de la Côte d'Ivoire. In Le milieu naturel de Côte d'Ivoire, Mémoire ORSTOM, Paris, ORSTOM, 73-108. http://www.documentation.ird.fr/hor/fdi:16372.

Eom, A. H., Hartnett, D. C., & Wilson, G. W. T. (2000). Host plant effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. Oecologia, 122, 435-444. https://doi.org/10.1007/s004420050050.

Gerdemann, J. W., & Nicolson, T. H. (1963). Spores of endogone species from soil by wet sieving and decanting. Trans. Br. Myc. Soc., 46, 235-244. https://doi.org/10.1016/S0007-1536(63)80079-0.

Grime, J. P., Mackey, J. M. L., Hillier, S. H., & Read, D. J., (1987). Floristic diversity in a model system using experimental microcosms. Nature, 328, 420-422. https://doi.org/10.1038/328420a0.

Gupta, A., Rico-Medina, A.,&Caño-Delgado, A. I. (2020). The physiology of plant responses to drought. Science, 368, 266–269. https://doi.org/10.1126/science.aaz7614.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., &Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol. Mol. Biol. Rev. 79, 293–320. https://doi.org/10.1128/mmbr.00050-14.

Hart, M. M., Reader, R. J. &Klironomos, J. N. (2003). Plant coexistence mediated by arbuscular mycorrhizal fungi. TRENDS in Ecology and Evolution, 18(8), 418-423. https://doi.org/10.1016/S0169-5347(03)00127-7.

Houngnandan, P., Yemadje, R. G. H., Kane, A., Boeckx, P., &Van Cleemput, O. (2009). Les glomalesindigènes de la forêtclaire à *Isoberliniadoka* (Craib et Stapf) à Wari-Maro au centre du Bénin. TROPICULTURA, 27(2), 83-87. https://popups.uliege.be/2295-8010/.

INVAM. (2021). International culture collection of VA Mycorrhizal fungi. Consulted on July 15, 2021. http://www.invam.caf.wvu.edu.

Jha, A., Kumar, A., Saxena, R. K., Kamalvanshi, M., Chakravarty, N. (2011). Effect of arbuscular mycorrhizal inoculations on seedling growth and biomass productivity of two bamboo species. Indian J. Microbiol., 52(2), 281-285. DOI: 10.1007/s12088-011-0213-3.

Kendall, M. G. (1955). Further contributions to the theory of paired comparisons. Biometrics. 11 (1) : 43 – 62. DOI:10.2307/3001479.

Khan, A., Pan, X., Najeeb, U., Tan, D. K. Y., Fahad, S., Zahoor, R., & Luo, H. (2018). Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives in cotton as vital constituents for plant stress resilience and fitness. Biol. Res., 51(1), 47. https://doi.org/10.1186/s40659-018-0198-z.

UNDER PEER REVIEW

Lovelock, C. E., Andersen, K., & Morton, J. B. (2003). Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. Oecologia, 135, 268-297. https://doi.org/10.1007/s00442-002-1166-3.

Moora, M., & Zobel, M. (1996). Effect of arbuscular mycorrhiza and inter- and intraspecific competition of two grassland species. Oecologia, 108, 79-84. https://doi.org/10.1007/BF00333217.

Morton, J. B., & Benny, J. (1990). Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new soborders, Glominae and Gigasporinae, and two new families, Acaulosporaceae and Gigasporaceae, with an amediation of Glomaceae. Mycotaxon, 37, 471-491.

Morton, J. B.(1993). Problems and solutions for Integration of glomalean taxonomy, systematic biology, and the study of endomycorrhizal phenomena. Mycorrhiza, 2, 97-109. https://doi.org/10.1007/BF00203855.

Olsen, S. B. (1952). Measurement of surface phosphore on hydroxylapatite and phosphate rock with radiophosphorus. Journal of Physical Chemistry, 56(5), 630-632. https://doi.org/10.1021/j150497a016.

Phillips, J. M. & Hayman, D. A. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society, 55, 158-161. http://dx.doi.org/10.1016/S0007-1536(70)80110-3.

Plenchette, C, Fortin, J. A., &Furlan, V. (1983). Growth response of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant & Soil, 70, 199-209. https://doi.org/10.1007/BF02374780.

Ramírez, J. G., Osorno, L., &Osorio, N. W. (2016). Presence of mycorrhizal fungi and a fluorescent *Pseudomonas* sp. in the rhizosphere of cacao in two agroecosystems and their effects on cacao seedling growth. AgronColomb., 34(3), 385–392. https://doi.org/10.15446/agron.colomb.v34n3.57950.

Rincón, C., Droh, G., Villard, L., Masclaux, F. G., N'guetta, A., Zeze, A., &Sanders, I. R. (2021). Hierarchical spatial sampling reveals factors influencing arbuscular mycorrhizal fungus diversity in Côte d'Ivoire cocoa plantations. Mycorrhiza, 31, 289–300. https://doi.org/10.1007/s00572-020-01019-w.

Schenck, N. C., & Pérez-Collins, Y. (1987). Manual for the identification of VA mycorrhizal fungi (First Edition Synergetic Publicotions). Gainesville, Florida, U.S.A, University of Florida, 245 p.

Sidibe, D., Fofana, I. J., Silue, S., Diarrassouba, N., Zeze, A. S., &Nguetta, P. A. (2015). Evaluation of symbiosis effect of some arbuscular mycorrhizal fungi on growth of yams (*DioscoreaAlata*) on experimental conditions. J Pharm Chem Biol Sci; 3(3), 346- 357.

Sieverding, E. (1991). Vesicular-arbuscular mycorrhiza management in tropical agrosystems. Eschborn: GTZ.

Thiébault, S., &Moatti, J.-P. (2016). The mediterranean region under climate change: a scientific update. IRD Editions, Marseille, 736 p. DOI:10.4000/books.irdeditions.22908.

UNDER PEER REVIEW

Tomassone, R., Dervin, C. & Masson J. P. (1993). Biométrie. modélisation de phénomènesbiologiques. International System for Agricultural Science and Technology, Elsevier, 1: 53 p.

Touré, G.-P. T., Nandjui, J., Koné, A. W., Kouadjo, A. G. Z., Ebou, A., Tiho, S. &Zézé A. (2021). Diversité des champignons mycorhiziens à arbuscules et interactions avec le système sol-litière dans un écotoneforêt-savane, Côte d'Ivoire. Étude et Gestion des Sols, 28, 93-104.

Van der Heijden, M. G. A., Boller, T., Wiemken, A., & Sanders, I. R. (1998). Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology, 79, 2082-2091.

Zako, B. I. M. S., Tié, B. T., Zirihi, G. N., Kouadjo, Z. C. G., Fossou K. R. & Adolphe Z. (2012). Arbuscular mycorrizal fungi associated with *Theobroma cacao* L. in the region of Yamoussoukro (Côte d'Ivoire). African Journal of Agricultural Research, 7(6), 993-1001. DOI:10.5897/AJAR11.2057.

UNDER PEER REVIEW