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Arbuscular Mycorrhizal Fungi and Soil Enzyme Activities in Different Fonio Millet (*Digitaria exilis* Stapf.) Agroecosystems in Senegal

Fatou Ndoye[1,2]*, Ibou Diop[2,3], Mame Codou Gueye[4], Oumar Sadio[5], Mame Oureye Sy[3], Diegane Diouf[2,6], Aboubacry Kane[2,3] and Abdala Gamby Diedhiou[2,3]

[1]Department of Agronomy and Plant Production, RTU SAEPAN, University of Sine Saloum El Hadj Ibrahima Niass, PB 55, Kaolack, Senegal.
[2]Common Laboratory of Microbiology (IRD/ISRA/UCAD), PB 1386, PC 18524, Dakar, Senegal.
[3]Department of Plant Biology, Faculty of Sciences and Techniques, Cheikh Anta Diop University, PB 5005, Dakar-Fann, Senegal.
[4]Regional Study Center for Drought Enhancement and Adaptation (CERAAS), Senegalese Agricultural Research Center, PB 3320, Thies, Senegal.
[5]IRD, University of Brest, CNRS, Ifremer, Lemar, PB 1386, PC 18524, Dakar, Senegal.
[6]Department of Environment, Biodiversity and Sustainable Development, RTU SSE, University of Sine Saloum El Hadj Ibrahima Niass, PB 55, Kaolack, Senegal.

Abstract

In plant roots, arbuscular mycorrhizal fungi (AMF) are the most prevalent microsymbionts, and thereby provide many key ecosystem services to natural and agricultural ecosystems. Despite AMF's significance for the environment and the economy, little is known about the mycorrhizal inoculum potential and diversity of AMF associated with orphan African cereal crops, specially fonio millet (*Digitaria exilis* stapf.) under field conditions. We hypothesized that the type of fonio millet agroecosystem influences the AMF density and distribution in soils. We therefore, assessed the inoculum potential, density and diversity of AMF spores and soil enzyme activities in five fonio millet agroecosystems belonging to three climatic zones (Sudanian, Sudano-Sahelian and Sudano-Guinean). By combining AMF spore identification from field-collected soils and trap culture, 20 species belonging to 8 genera (*Acaulospora, Ambispora*, *Dendiscutata, Gigaspora, Glomus*, *Racocetra, Sclerocystis* and *Scutellospora*) were identified. *Glomus* was the most represented genus with 8 species, followed by *Gigaspora* (5 species) and *Acaulospora* (2 species); the remaining genera were each represented by one species. Except for *Ambispora* which was not found in the Sudanian area, all genera occurred in the three climatic zones. The abundance and diversity of AMF species and FDA-hydrolytic and phosphatase activities varied between fonio millet agroecosystems as well as between climatic zones. Soil pH and soil texture were the variables that best explained the density and distribution of AMF spores. Our results contribute to paving the way towards the development of microbial engineering approaches for agronomic improvement of fonio millet.

Keywords: AMF Spore Diversity, Soil Properties, *Digitaria exilis*, Orphan Crop, Species, Trap Culture

***Correspondence:** fatou.ndoye@ussein.edu.sn

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INTRODUCTION

Fonio millet (*Digitaria exilis,* stapf), also called "Acha", is one of the oldest cereal crops originated in West Africa.[1] It has very good prospects for semi-arid and upland areas as it tolerates poor soils and drought conditions, and matures very quickly (6-8 weeks).[2] Moreover, Fonio grains contain higher amounts of amino acids (e.g., methionine and cystine),[3] iron, potassium, calcium and phosphorus.[4,5]

However, fonio consumption is still low, particularly in urban areas where it has long been considered as an orphan crop.[6,7] In recent years, this crop has attracted considerable research interest.[8-10] So, it has been reported as a priority crop for West Africa due to its organoleptic qualities, nutritional and health benefits, and potential contribution to crop diversification and food security.[11-13]

In the other hand, arbuscular mycorrhizal fungi (AMF) form the most prevalent microbial symbiotic association with the majority of terrestrial plant species.[14,15] These beneficial soil microorganisms have a great potential for contributing to crop production and thereby helping to achieve sustainable global food security.[16,17] Indeed, AMF can provide to their host plants multiple benefits, including increase of nutrient uptake, stimulation of phytohormones production, tolerance to drought stress, as well as protection against pathogens.[18-20] Furthermore, AMF play a crucial role in enhancing the physicochemical and biological characteristics of soil.[21-23] Hence, harnessing the potential of AMF is considered as a potential less costly solution to increase crop yields.[16,24,25] Meanwhile, abiotic and biotic factors influence the effects of AMF taxa on plant development and production.[24,26,27] In addition, it has been reported that AMF abundance and diversity varied depending on the ecological zone,[28,29] soil properties,[30-32] vegetation type[33] and agricultural management pratice.[34]

In Senegal, fonio millet is cultivated under various agricultural management practices across different climatic zones.[35] However, little is known about the AMF density, diversity and distribution across the fonio millet agroecosystems. We hypothesized that the type of fonio millet agroecosystem and pedoclimatic conditions might influence the AMF density and distribution in soils. We therefore, assessed in this study the inoculum potential, density and diversity of AMF spores, and soil enzyme activities in five fonio millet agroecosystems belonging to three climatic zones (Sudanian, Sudano-Sahelian and Sudano-Guinean).

MATERIALS AND METHODS

Study site and soil sampling

Rhizosphere soils were collected after the growing season from fonio millet fields located in five sites (Koumbidia Soce, Missirah, Togue, Sare Yoba and Mandina Findifeto) (Figure 1). Koumbidia Soce (13°54'22''N and 14°52'0''W) and Missirah (13°31'34''N and 13°30'53''W) are located in Kaffrine and Tambacounda region, respectively. They belong to the Sudano-Sahelian zone characterized by mean annual rainfall varying between 600 and 800 mm, temperatures between 30°C and 35°C and leached soil, without ferruginous spots or weakly stained on sandy-clay sandstones. The vegetation is a savannah in Missirah. Togue (12°30'0''N and 12°1'0''W) is located in the region of Kedougou in the Sudano-Guinean zone with mean annual rainfall of 1103 mm and temperatures varying between 30°C and 33°C. Sare Yoba (12°55'59''N and 14°07'59''W) and Mandina Findifeto (12°48'32''N and 15°31'53''W), are located in Sedhiou and Kolda region, respectively, in the Sudanian zone. In this zone, the mean annual rainfall is 1048 mm, the temperatures fluctuate between 27°C and 38°C and the soil is leached with pseudo-gley and ferruginous concretions on shale or weakly ferralitic on sandy-clay sandstones.

In each fonio millet agroecosystem, a sampling area of 100 × 100 m was delimited, soil was collected from 6 points at a depth of 0 to 25 cm and then the soil samples were pooled together in plastic bags and brought to the lab. The soil samples were sieved to <4 mm and kept at 4°C.

Characterization of soil properties

Physical (sand, silt, clay) and chemical (C, N, P, P_2O_5, C/N) characteristics of the five soils were analyzed at the Laboratory of Soil, Water and Plant of ISRA/CNRA at Bambey (Senegal) using standard methods. The soil physical characterization was

Figure 1. Map of Senegal showing the soil collection sites

carried out as described in Disale *et al.*[36] The soil samples were placed in a mechanical shaker and sieved for 5 min through a series of sieves to determine the size of different soil particles. The combustion system Thermo-Finnigan Flash EA 1112 (Thermo Finnigan, France) was used to quantify the total amount of soil carbon and nitrogen.[37] The amount of soil total and available phosphorus was evaluated as described by Bibi and colleagues.[38] Soil organic matter (OM) was determined from organic carbon as follows:

OM (%) = organic carbon (%) × 1724. Soil pH was measured in soil-water (1:2,5) suspensions.[37]

Determination of mycorrhizal inoculum potential

The mycorrhizal inoculum potential in each soil sample was evaluated by the dilution technique.[39] Briefly, a quarter-fold dilution series (1, 1/4, 1/16, 1/64, 1/256 and 1/1024) was prepared by thoroughly mixing defined proportions of non-sterilized and sterilized soil. Then, 50 g of each diluted soil sample were placed in 5 pots, and 3 seeds of *Zea mays* L. (a highly mycotrophic plant) were sown per pot. The seedlings were thinned to one per pot and all plants were kept in glasshouse and watered with demineralized water. After 45 days, roots of all plants from the dilution ratios were harvested and stained with Trypan blue as described in Founoune-Mboup *et al.*[40] The presence of mycorrhizal infection in stained root segments was observed by light microscope at a magnification of 100X. The most probable number (MPN) of AMF propagules that can colonize plant roots was calculated as follows: Log MPN = (x log a)–K, where x represents mean of mycorrhized plants for all dilution ratios, a (factor of dilution) = 4, K=constant given by the table of Fisher & Yates.[41,42]

Identification and enumeration of AMF spores from field-collected soils

The extraction of AMF spores from each field-collected soil was carried out using the wet sieving and decanting method.[43] Briefly, 100 g of soil were mixed with 1 L of water and decanted in a series of 400-50 µm sieves. Then, the material of 200, 100 and 50 µm pore-sieves was re-suspended in water and collected in tubes. Two solutions of sucrose at 20% and 60% were successively added and centrifugation was done at 3000 g/min for 3 minutes. After that, the supernatant containing

AMF spores was poured in a 50 µm mesh and rinsed with tap water. AMF spores were grouped and counted according to their morphological characters and using a dissecting microscope. The International Culture Collection of Arbuscular and Vesicular-Mycorrhizal Fungi was used for AM fungi description (https://invam.wvu.edu/methods/spores/enumeration-of-spores). The AMF spore density and abundance of each AMF species were expressed per 100 g of soil. Three replicates were made for each composite soil from each sampled site.

Determination of AMF species composition from trap culture

The trap culture method allows to confirm AMF spore identification (spores are sometimes damaged) from the field-collected soils. On the other hand, this method induces emergence of AMF that would not naturally sporulate.[44] The trap culture was performed with field-collected soils using mays (*Zea mays* L.) for 3 months under glasshouse conditions. For this purpose, each field-collected soil was mixed with an autoclaved nutrient-poor sandy soil from Sangalkam (1:2 v/v) to serve as culture substrate. For each agroecosystem, 9 pots of 1 kg were filled with the culture substrate and 3 fonio seeds were sown per pot (9 replicates x 5 soil sites). Plants were watered every two days for three months. At the end of experiment, plants were harvested and AMF spores were isolated from soils, enumerated and identified as previously described.[43]

Soil enzymatic activities

Enzymatic activities were determined from field-collected soils as described in Ndoye *et al.*[28,45] The activity of FDA (3'.6'-diacetylfluorescein) hydrolysis was measured according to Patle *et al*.[46] For this test, to 1 g of the soil, 15 ml of 60 mM potassium phosphate buffer (pH 7.6) and 0.2 ml of 1000 µg FDA ml^1 were added (with 3 replicates per soil origin). A blank enzyme without FDA and a blank substrate without soil were included. After 1h of shaking on an orbital incubator at 30°C, the flasks were removed and 1 ml of each suspension were transferred into Eppendorf tube and mixed with 1 ml of acetone to stop the reaction. After centrifugation (10000 rpm/min for 5 min), 1 ml of the supernatant was measured at 490 nm on a spectrophotometer (Ultrospec 3000 Pharmacia Biotech). The concentration of fluorescein was calculated using the calibration graph standard and expressed as µg FDA/g of soil/h.

Acid and alkaline phosphatase activities were quantified using a colorimetric determination of p-nitrophenol released after soil incubation with p-nitrophenyl phosphate as substrate (pNPP, 5 mM).[47,48] Briefly, 25 mg of soil sample was mixed with 400 µl of buffered sodium p-nitrophenyl phosphate solution (pH 6 and pH 11 for acid and alkaline phosphatase, respectively) and 100 µl pNPP (p-Nitrophenyl Phosphate, 5 mM). A blank enzyme without pNPP and a blank substrate without soil were included. After incubation at 37°C for 1 h, the reaction was complexed with 100 µl of $CaCl_2$ (0,5 M) then stopped by adding 400 µl of NaOH (0,5 M) solution. After centrifugation (10000 rpm/min for 5 min), 1 ml of the supernatant was measured at 400 nm on a spectrophotometer (Ultrospec 3000 Pharmacia Biotech). The amount of released p-nitrophenol was determined at 400 nm and expressed as µg pNPP/g of soil/h.

Statistical analysis

The Shapiro–Wilk and Levene tests were used to checked the normality and homogeneity of variance, respectively. So, comparisons of means were performed by Kruskal-Wallis test instead of one-way ANOVA when the test was significant. Statistical analyses were performed using rcompanion, FSA, TH.data, pgirmess packages in R software.[49,50] The probability threshold "p value" was set at 0.05 in order to establish statistically significant differences between groups.

For each agroecosystem, AMF species richness, Shannon and Wiener diversity index (H') and Simpson Dominance index (D) were determined. The Shannon and Wiener diversity index was calculated as H' = -pi (ln pi) where *pi* represents the proportion of individuals found in the *i*th species, estimated as *ni /N*, *ni* being the number of individuals in the *i*th species and *N,* the total number of individuals. The inverse of the Simpson dominance index was evaluated using the following formula: 1-D = 1-[ni (ni-1)/N (N-1)]; where ni represents the number of the *i*th types and N the number of individuals in the population.

Pearson correlation coefficients were determined to investigate the relation between

AMF spore diversity and density, soil enzyme activities, and soil physicochemical characteristics. All statistical analyses were conducted in R v4.3.1.

RESULTS

Soil physicochemical characteristics

Our results showed that the sampled soils were sandy silt clay in Missirah and Mandina Findifeto and sandy clay silt in the other three sites with pH ranging from 5.84 to 6.98 (Table 1). For soil C, N, P, P_2O_5, and organic matter (OM) contents, the highest values were obtained in Togue, and the lowest values in Mandina Findifeto. On the contrary, soil pH follows an opposite trend, showing the lowest value in Togue and the highest in Mandina Findifeto. Considering the climatic zones, the Sudanian zone had the lowest C, N, P, P_2O_5 and OM contents in soil and the highest value of soil pH, whereas the Sudano-Sahelian zone had intermediate values, as compared to the Sudano-Guinean zone (Table 1).

Inoculum potential, AMF species diversity and spore density in field-collected soils

Mycorrhizal soil infectivity of the 5 field-collected soils ranged from 5 to 71 propagules in 50 g of dry soil (Table 2). Mandina Findifeto soil showed higher MPN value (70.90 propagules per 50 g of soil) as compared to those of other field-collected soils (ranging from 5.20 to 12.60 propagules per 50 g of soil). Those latter soils did not differ significantly in terms of MPN values. The lowest MPN was obtained in Sare Yoba located in the same climatic zone with Mandina Findifeto.

In addition, a total of 12 morphotypes of AMF belonging to 7 genera (*Scutellospora, Gigaspora, Racocetra, Dendiscutata, Acaulospora, Glomus* and *Sclerocystis*) was recorded from the 5 field-collected soils (Figure 2 and 3). Nine (*Scutellospora* sp*. aff. dipurpurascens, Gigaspora* sp.1, *Gigaspora* sp.2 aff. *gigantea, Racocetra gregaria, Dendiscutata* sp *aff. heterogama, Acaulospora* sp.1*, Glomus* sp.2, *Glomus* sp.3, *Glomus* sp.4) out of the 12 AMF species were common to the 5 sites, two (*Gigaspora* sp.3

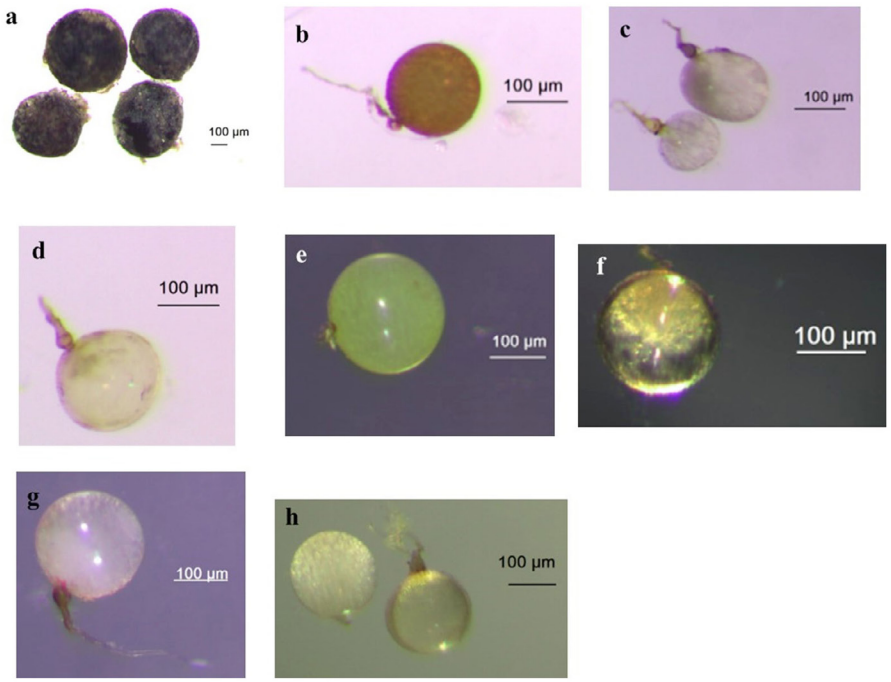

Figure 2. Some AMF species found from field-collected soils and trap culture
(a) *Racocetra* sp aff. *gregaria* (b) *Dendiscutata* sp. (c) *Scutellospora* sp. (d) *Gigaspora* sp.1 (e) *Gigaspora* sp.2 aff. *gigantea* (f) *Gigaspora* sp.3 aff. *albida* (g) *Gigaspora* sp.4 aff. *rosea* (h) *Gigaspora* sp.5

and *Glomus* sp.1) were found only in two sites (Koumbidia Soce and Sare Yoba) and one (*Sclerocystis* sp) in three sites (Koumbidia Soce, Missirah and Togue).

The spore density of AMF species varied depending on soil origin (Table 3). For instance, *Glomus* sp.2 and *Glomus* sp.3 displayed their highest density in Koumbidia Soce (1017.33 and 770.67 spores/100 g of soil, respectively), while *Scutellospora* sp. *aff. dipurpurascens* and *Dendiscutata* sp. *aff. heterogama* had their highest density in Mandina Findifeto (97.67 and 920.67

Table 1. Physicochemical characteristics of the field-collected soils

Sites (climatic zones)	Soil properties									
	Clay (%)	Silt (%)	Sand (%)	C (%)	N (%)	P (‰)	P_2O_5 (‰)	C/N	OM (%)	pH (H_2O)
Koumbidia Soce (Sudano-Sahelian zone)	5.13	4.87	90.00	0.66	0.05	1.08	2.46	12.10	1.13	6.15
Missirah (Sudano-Sahelian zone)	5.42	10.58	84.00	0.77	0.06	0.61	1.34	12.45	1.33	6.09
Togue (Sudano-Guinean zone)	3.86	5.66	**90.48**	**1.71**	**0.14**	**3.374**	**7.72**	12.58	**2.95**	5.84
Sare Yoba (Sudanian zone)	**5.56**	5.56	88.89	0.55	0.05	0.538	1.23	12.01	0.94	6.45
Mandina Findifeto (Sudanian zone)	3.74	**10.73**	85.53	0.49	0.04	0.410	1.19	12.17	0.84	**6.98**

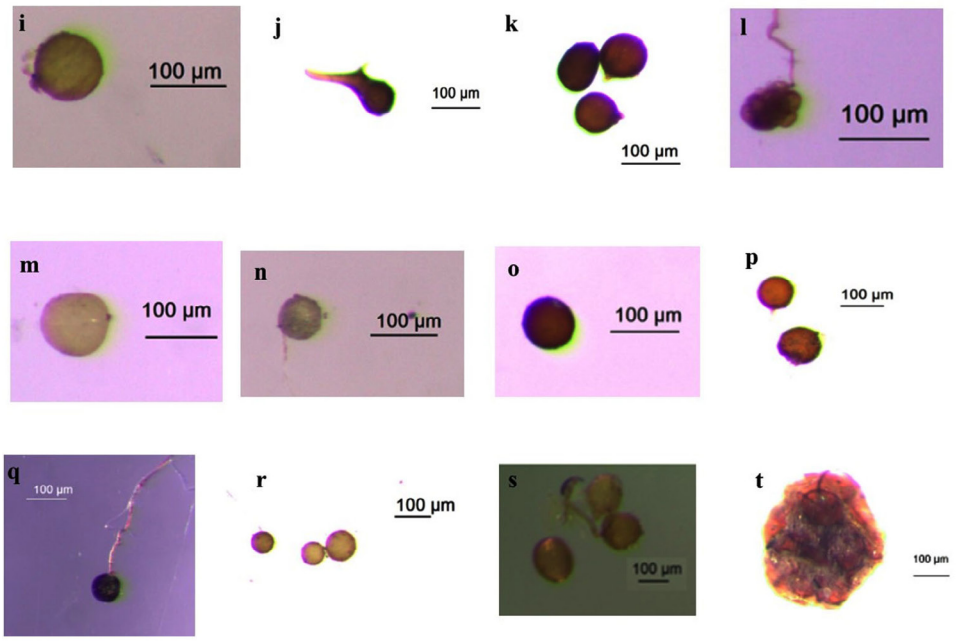

Figure 3. Some AMF species found from field-collected soils and trap culture
(i) *Acaulospora* sp.1 (j) *Acaulospora* sp.2 (k) *Ambispora* sp. (l) *Sclerocystis* sp. (m) *Glomus* sp.1 (n) *Glomus* sp.2 (o) *Glomus* sp.3 (p) *Glomus* sp.4 (q) *Glomus* sp.5 (r) *Glomus* sp.6 (s) *Glomus* sp.7 (t) *Sporocarpe* of *Glomus* sp.8

spores/100 g of soil, respectively). The lowest AMF spore densities were observed in Koumbidia with *Scutellospora* sp, *Gigaspora* sp.1 and *Gigaspora* sp.2; in Missirah with *Dendiscutata* sp and *Glomus* sp.2; in Togue with *Racocetra* sp and *Glomus* sp.3; in Sare Yoba with *Glomus* sp.4; and in Mandina Findifeto with *Acaulospora* sp.1.

Table 2. Mycorrhizal inoculum potential (MPN*) in the field-collected soils

Climatic zone	Sites	MPN	MPNi	MPNs
Sudano-Sahelian	Koumbidia Soce	12.59b	4.66b	25.84b
	Missirah	9.14b	4.28b	19.53b
Sudano-Guinean	Togue	12.10b	5.89b	26.90b
Sudanian	Sare Yoba	5.20b	2.43b	11.10b
	Mandina Findifeto	**70.90a**	**33.18a**	**151.47a**

*MPN= Most probable number. MPNi: MPN minimal. MPNs: MPN maximal
In column, values followed by the same letter are not significantly different according to the Kruskal-Wallis test (P < 0.05)

The total spore density of AMF also differed significantly between the five sites, ranging from 957 to 3166 spores per 100 g of dry soil (Table 3). The density of AMF spores was significantly higher in Mandina Findifeto (3165.33 spores/100 g of soil) than in other sites. It was followed by those of Koumbidia Soce, then Togue (2320.33 and 1379.67 spores/100 g of soil, respectively). The lowest AMF density was recorded in Sare Yoba (957.67 spores/100 g of soil).

Shannon index ranged from 1.63 to 1.29, while Simpson index varied from 0.77 to 0.67 and Hill index from 0.75 to 0.59. The highest diversity indices were observed in Sare Yoba in the Sudanian zone, whereas the soil from Koumbidia Soce in the Sudano-Sahelian zone showed the lowest diversity indices (Table 3).

Table 3. Density of arbuscular mycorrhizal fungal spores in the field-collected soils

	Sites				
	Sudano-Sahelian zone		Sudano-Guinean zone	Sudanian zone	
AMF species	Koumbidia Soce	Missirah	Togue	Sare Yoba	Mandina Findifeto
Scutellospora sp. aff. *dipurpurascens*	4.67d	51.33b	19.33c	55.00b	**97.67a**
Gigaspora sp.1	8.67b	41.67a	10.67b	**46.33a**	**42.33a**
Gigaspora sp.2 aff. *gigantea*	12.67b	**20.67a**	12.00b	5.67c	10.67b
Gigaspora sp.3 aff. *albida*	8.33b	0.00c	0.00c	**12.00a**	0.00c
Dendiscutata sp. aff. *heterogama*	59.67b	15.67c	18.33c	67.00b	**920.67a**
Racocetra sp. aff. *gregaria*	3.33a	3.33a	2.00a	4.00a	**4.67a**
Acaulospora sp.1	**31.67a**	18.33b	31.33a	15.00b	8.00c
Sclerocystis sp.	**1.67a**	1.00a	1.00a	0.00a	0.00a
Glomus sp.1	4.67b	0.00c	0.00c	**12.00a**	0.00c
Glomus sp.2	**1017.00a**	145.67e	663.67c	226.33d	824.33b
Glomus sp.3	**770.33a**	557.33b	312.67c	350.67c	709.33a
Glomus sp.4	397.67b	305.67c	308.67c	163.67d	**548.00a**
Total density of AMF spores	**2320.33b**	**1160.67d**	**1379.67c**	**957.67e**	**3165.33a**
AMF species richness	**12**	**10**	**10**	**11**	**9**
Diversity index					
Shannon-Weiver (H')	**1.29b**	**1.44ab**	**1.32ab**	**1.63a**	**1.56ab**
Simpson (1-D)	**0.67a**	**0.68a**	**0.67a**	**0.77a**	**0.77a**
Hill (1-Hill)	**0.59a**	**0.65a**	**0.60a**	**0.75a**	**0.73a**

In row, values followed by the same letter are not significantly different according to the Kruskal-Wallis test (P < 0.05)

Table 4. Species of AMF associated with *Digitaria exilis* stapf in trap culture

Families	Genera	Species	Sites				
			Sudano-Sahelian zone		Sudano-Guinean zone	Sudanian zone	
			Koumbidia Soce	Missirah	Togue	Sare Yoba	Mandina Findifeto
Gigasporaceae	*Racocetra*	*Racocetra* sp. aff. *gregaria*	+	+	+	+	+
	Dendiscutata	*Dentiscutata* sp. aff. *heterogama*	+	+	+	+	+
	Scutellospora	*Scutellospora* sp. aff. *dipurpurascens*	+	+	+	+	+
	Gigaspora	*Gigaspora* sp.1	+	+	+	+	+
		Gigaspora sp.2 aff. *gigantea*	+	+	+	+	+
		Gigaspora sp.3 aff. *albida*	+	-	+	+	-
		Gigaspora sp.4 aff. *rosea*	+	+	-	-	-
		Gigaspora sp.5	-	+	+	+	-
Acaulosporaceae	*Acaulospora*	*Acaulospora* sp.1	+	+	+	+	+
		Acaulospora sp.2	+	+	-	-	-
Ambisporaceae	*Ambispora*	*Ambispora* sp.	+	+	+	-	-
		Glomus sp.1	+	+	+	+	-
		Glomus sp.2	+	+	+	+	+
Glomeraceae	*Glomus*	*Glomus* sp.3	+	+	+	+	+
		Glomus sp.4	+	+	+	+	+
		Glomus sp.5	-	-	-	+	-
		Glomus sp.6	-	-	-	+	-
		Glomus sp.7	+	+	-	-	-
		Glomus sp.8	+	-	+	-	+
	Sclerocystis	*Sclerocystis* sp.	+	+	+	+	+
4 families	**8 genera**	***20 AMF species***	**17**	**16**	**15**	**15**	**11**

Composition of AMF species from trap culture

A total of 20 AMF species belonging to 8 genera (*Racocetra, Dendiscutata, Scutellospora, Gigaspora, Acaulospora, Ambispora, Glomus* and *Sclerocystis*) were recorded from trap culture (Table 4). Of the 20 AMF species, 17 were found in Koumbidia Soce, 16 in Missirah, 15 in Togue, 15 in Sare Yoba and 11 in Mandina Findifeto. Only 10 out of the 20 AMF species were shared by the 5 sites, while two AMF species, *Glomus* sp.5 and *Glomus* sp.6, were recorded exclusively in Sare Yoba. Besides, 8 of the 20 AMF species revealed by trap culture were not detected by spore identification from field-collected soils (Tables 3 & 4).

The abundance of AMF genera in each soil was presented in Figure 4 (a, b, c, d, e). *Glomus* was the most diverse genus with 8 AMF species, and accounted for around 35%, 35%, 31%, 33%, 40% and 36% of total abundance in Koumbidia Soce (Figure 4a), Missirah (Figure 4b), Togue (Figure 4c), Sare Yoba (Figure 4d) and Mandina Findifeto (Figure 4e), respectively. It was followed by *Gigaspora* with 5 AMF species accounting for 18.18% to 26.65% of total abundance across sites. *Acaulospora* was represented by 2 AMF species accounting for 6.67% to 12.5% of total abundance across sites. Although being each represented by one AMF species, the genera *Dendiscutata*,

Racocetra, *Scutellospora* and *Sclerocystis* were found in all sites. *Ambispora* represented by one AMF species was not found in Sare Yoba and Mandina Findifeto, the two sites located in the Sudanian zone (Table 4). Meanwhile, it contributed from 6% to 7% of total abundance in each site where it was found (Figures 4d and 4e).

Thus, the trapping culture revealed 8 more AMF species (*Gigaspora* sp.4 aff. *rosea, Gigaspora* sp.5, *Acaulospora* sp.2, *Ambispora* sp., *Glomus* sp.5, *Glomus* sp.6, *Glomus* sp7, *Glomus* sp.8) than direct observation from field-collected soils.

Soil enzyme activities

Soil FDA-hydrolytic activity in Koumbidia Soce (0.53 *µ*g FDA/g of soil/h) was significantly higher than those in Togue, Sare Yoba and Mandina Findifeto. The lowest FDA-hydrolytic activity was obtained in the Mandina Findifeto soil with 0.27 µg FDA/g of soil/h (Figure 5A). The activity of acid phosphatase was significantly higher in soils from

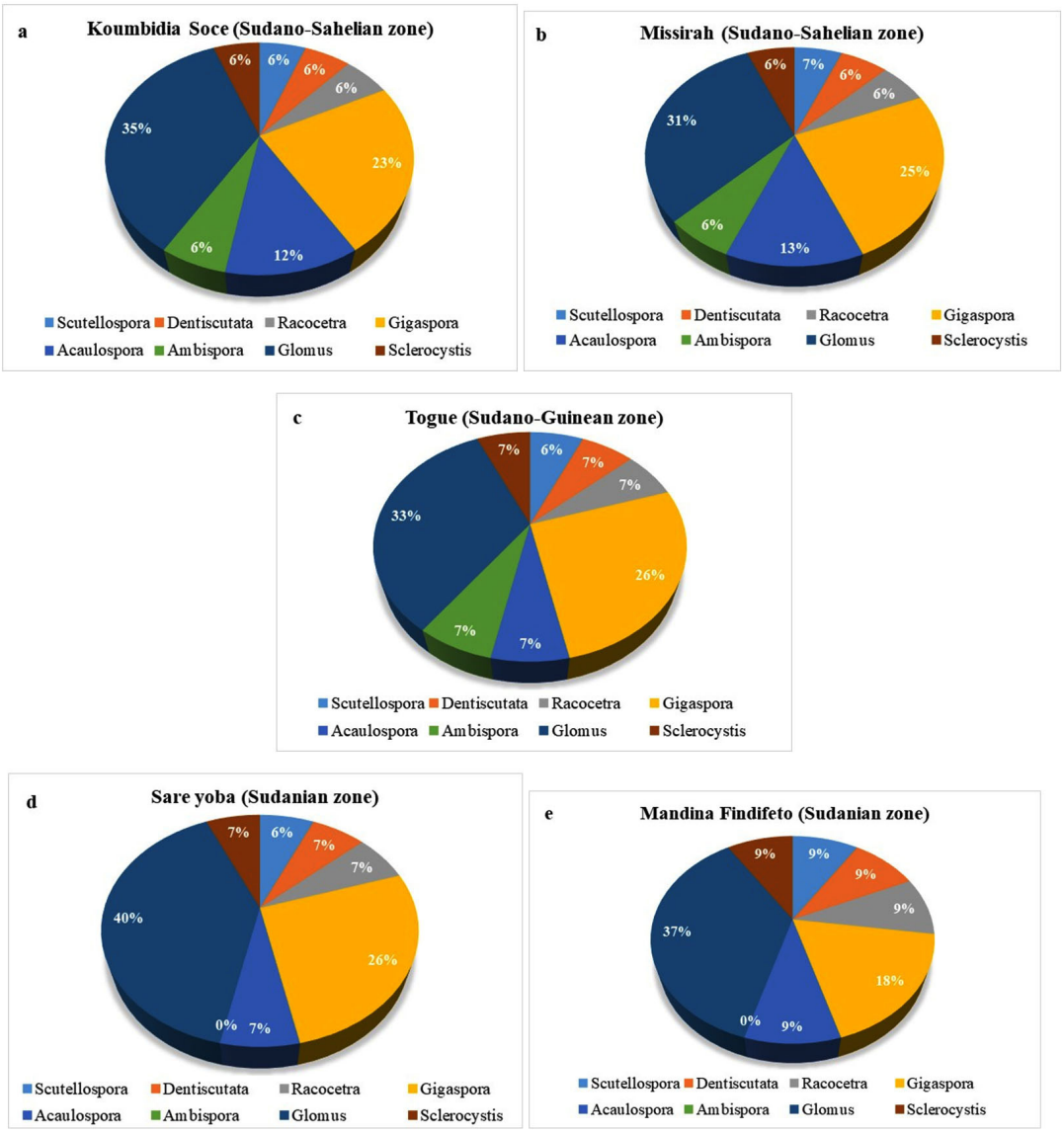

Figure 4. Relative abundance of AMF genera found in the rhizosphere of *Digitaria exilis* stapf

Koumbidia Soce (188.98 µg pNPP/g of soil/h) and Missirah (187.14 µg pNPP /g of soil/h), the two sites located in the Sudano-Sahelian zone, as compared to other sites (Figure 5B). There were no statistically significant differences in acid phosphatase activity between soils collected from Togue, Sare Yoba and Mandina Findifeto. However, the greatest alkaline phosphatase activity was obtained in soil collected from Togue (276.67 µg pNPP /g of soil/h), followed by that from Missirah,

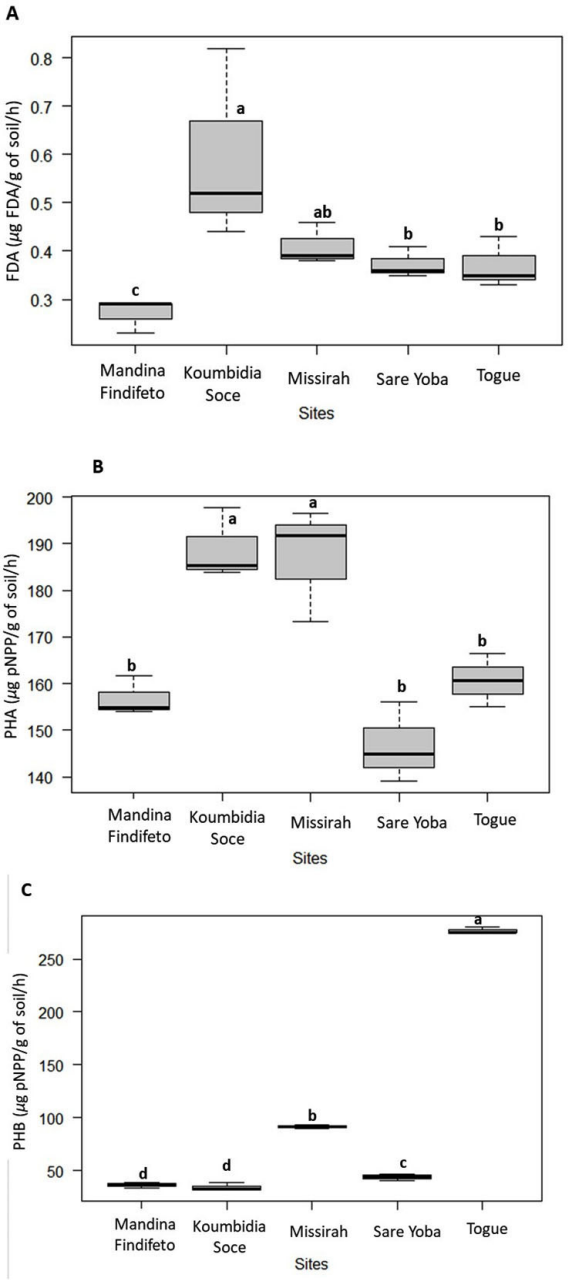

Figure 5. Enzyme activities of field-collected soils
(A) FDA (Fluorescein diacetate. µg FDA/g of soil/h). (B) PHA and (C) PHB (Phospatasis acid and Basis. µg pNPP/g of soil/h)
Boxes followed by the same letter are not significantly different according to the Kruskal-Wallis test ($P < 0.05$)

Sare Yoba, Mandina Findifeto and Koumbidia Soce (Figure 5C).

Correlation matrix between density and diversity of AMF, soil physicochemical properties and soil enzyme activities

Soil N, P, P_2O_5, C and OM had significant positive correlations between them (Figure 6). Soil pH was strongly positively correlated with AMF spore density (r^2 = 0.650, P-value = 0.235) and soil mycorrhizal potential (r^2 = 0.820, P-value = 0.089); and negatively correlated with the diversity of AMF from field-collected soil (r^2 = -0.419, P-value = 0.235) and the diversity of AMF from trap culture (r^2 = -0.810, P-value = 0.096). Soil C, N, P and available P were negatively correlated with soil MPN, spore density and the diversity of AMF from field-collected soils; and positively correlated with the diversity of AMF from trap culture although those correlations were not significant.

In addition, soil alkaline phosphatase was positively and significantly correlated with total C (r^2 = 0.989, P-value = 0.001), total N (r^2 = 0.988, P-value = 0.002), total P (r^2 = 0.945, P-value = 0.015), available P (r^2 = 0.989, P-value = 0.015), and OM (r^2 = 0.990, P-value = 0.01). A positive and significant relationships between soil FDA activity and the diversity of AMF from field-collected soils in one hand (r^2 = 0.893, P-value = 0.041); and the diversity of AMF from trap culture (r^2 = 0.918, P-value = 0.028) were noted. The correlation between the diversity of AMF from trap culture and MPN was significantly negative (r^2 = -0.906, P-value = 0.034).

DISCUSSION

Understanding the microbial community diversity and structure in the soil-plant continuum is essential to harness beneficial plant-microbe interactions in agricultural ecosystems.[51-53] Indeed, it would help in developing efficient inoculants and sustainable strategies for the successful manipulation of microbial communities to improve crop yields and soil resilience.[54-56]

Here, we analyzed the density and diversity of AMF; and the enzyme activities in soils from 5 fonio millet agroecosystems in Senegal. Our

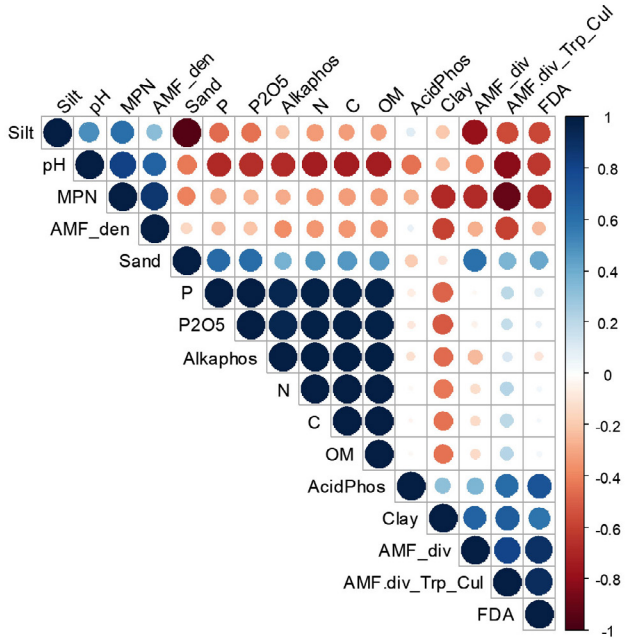

Figure 6. Correlation matrix of the different variables (AMF density and diversity. soil physicochemical properties and soil enzyme activities)
Positive correlations are displayed in blue and negative correlations in red color. Color intensity and size of circle are proportional to correlation coefficients. In the right side of the correlogram. the legend color shows the correlation coefficients and the corresponding colors

results revealed that agroecological conditions influence AMF spore density and diversity. These findings might be partially explained by differences in physicochemical characteristics of soils and rainfall. Previous study from Ndoye *et al*.[28] reported the influence of environmental factors on soil AMF spore density. Also, a significant difference in AMF spore density between three agroecological zones of the Central African Republic was observed by Djasbe and colleagues.[57] This is consistent with the results of Maffo and coworkers[58] obtained from two agroecological zones in Cameroon.

On the other hand, it has been reported that AMF inoculum potential has a major influence on mycorrhizal effectiveness and early root infection.[59] In this study, the observed high AMF spore density and mycorrhizal inoculum potential in Mandina Findifeto might be partially attributed to its lower clay, nutrient and organic matter contents; and higher pH and silt content as compared to other sites. Similarly, Swarnalatha and colleagues[60] had obtained a higher AMF spore density in a silty sandy loam soil compared to a silty clay loam soil. Moreover, the presence of clay might reduce the production of AMF spores.[60] These findings indicate the influence of soil type on AMF density.

One of the objectives of the present work was to determine the AMF diversity in soils from five fonio millet agrosystems. A total of 12 species from field-collected soils and 20 species from trap culture was recorded with differences between sites. Those site effects could be linked to soil physicochemical properties and environmental conditions.[60,61] The negative correlations obtained between soil nutrient contents and AMF spore density and diversity collaborate other findings.[61,62] In fact, it is reported that soil mineral nutrients, specially P might influence AMF diversity and density.[63] For example, in North China, study of Lang and colleagues[64] in a long-term field experiment revealed that AMF alpha diversity gradually decreased as the P fertilizer rate increased. On the other hand, Delroy and colleagues[51] found that the diversity of AMF tends to expand at optimal P. However, evidence points out that P supply does not necessarily have a detrimental effect on AMF diversity.[65] Those results suggested that besides nutrient contents, other parameters (organic matter, humidity, pH, etc) might influence soil AMF parameters.[63] Previous studies have shown the influence of soil pH and rainfall on AMF sporulation,[66,67] spore density and richness.[68-70] In this respect, Zhao and colleagues[71] reported that increase in temperature and precipitation can promote mycelia and spore development by allowing the plant to supply more photosynthetic products to AMF.

Furthermore, several studies have focused on how geographic distance and the local environment affect the structure of AMF communities.[72-74] Congruently, our results as well as those obtained from other agroecosystems in Senegal[75,76] showed site effects on AMF diversity. In the present work, AM fungi species richness in field-collected soils and trap culture was higher in Koumbidia Soce site which contains higher amounts of N, P, C, OM, sand and clay than Mandina Findifeto site. It is apparent that abiotic factors, particularly soil chemical properties, can influence the AMF community structure and abundance.[76,77] Results of Song *et al*.[78] on *Sephora flavescens* Ait in China also supports the hypothesis that soil chemicals exert a selective effect of soil AMF population.

On the other hand, due to their effects on several ecosystem processes such as soil geochemical cycles, plant diversity and productivity, and soil composition, *Glomeromycota* communities have a wide environmental impact.[19] *Glomus* was the dominant genus in our sites as observed in various environments.[74-76,79] This might be related to their greater environmental adaptability and capacity to colonize plant roots more widely because of their efficient production of mycelia and spores.[80] Moreover, *Glomus* species have been reported to promote fonio growth and yield under glasshouse conditions.[9] In the present study, the low spore density and diversity of *Scutellospora* and *Racocetra* might be explained by their huge spores which take longer to mature than small spores[81] and/or by the ability to grow only from an intact mycelium or with live spores.[82,83]

In addition, *Glomus, Dendiscutata* and *Scutellospora* dominated in Mandina Findifeto site which contained the lowest amounts of nutrient contents and OM compared to other sites. In contrast, *Acaulospora* has greater abundance in Togue site and lower abundance in Mandina Findifeto. Songachan and Kayang[83]

noted that *Glomus* species dominated in natural sites and *Acaulospora* species in cultivated ones, due probably to the failure of hyphal network disturbance in environments that might have benefited Glomus species.

In this work, some AMF species (belonging to *Ambispora*, *Glomus* and *Gigaspora*) revealed by trap culture were not detected by spore identification from field-collected soils. Similar findings were reported by Leal *et al.*,[84] Chairul *et al*.[85] and Rodriguez-Morelos *et al*.[86] This demonstrated that cryptic AMF spores that are invisible during sampling or in field conditions can be encouraged to germinate through trap culture.[44,87] This shows the importance of the combination of spore identification from trap culture and field-collected soils in AMF analysis.[86]

Furthermore, it was shown that soil pH might affect directly or indirectly AMF community composition by impacting P availability.[80,88] Our results revealed positive relationships between soil mycorrhizal inoculum potential, AMF density and soil pH even if that was not significant. Bainard et colleagues[77] found a negative correlation between some AMF species and phosphate concentrations in the soil. Thus, the main factors influencing the spatial variation in the AMF community across the site appeared to be soil pH or pH-driven changes in soil chemistry and Electrical conductivity.[70,89]

The lower AMF diversity observed in Mandina Findifeto compared to Togue and other sites might partially be related to soil OM and carbon contents as reported by Zhang *et al.*[90]

Moreover, many studies have shown correlations between soil nutrients and enzymatic activities,[91,92] as confirmed by our study. Also, we found that soil FDA activity was positively and significantly correlated with AMF diversity and negatively with soil MPN and soil pH. This is consistent with the study of Cheng and coworkers[93] showing a positive correlation between AMF diversity and soil enzyme activities. However, the correlation between soil alkaline phosphatase and soil nutrient contents was positive except for soil pH. In this way, Moradi *et al*.[94] observed a positive correlation between acid and alkaline phosphatase activity, soil OM and N.

CONCLUSION

Our study shows an appreciable AMF density and diversity in the five tropical soils in Senegal. The results in field-collected and trap culture samples, respectively, revealed 12 and 20 species of AMF belonging to 8 genera and 4 families from 5 fonio millet agroecosystems into three climatic zones (Sudanian, Sudano-Sahelian and Sudano-Guinean). The AMF diversity increases with the trap culture. In both field-collected and trap culture soils, *Glomus* was the dominant genus in term of spore density and diversity in the five agroecosystems. The abundance and diversity of AMF species and FDA-hydrolytic and phosphatase activities varied between fonio agroecosystems as well as between climatic zones. Thus, abiotic factors like soil physicochemical properties might influence AMF spore density and diversity. Furthermore, Soil pH and texture were the variables that best explained the distribution of AMF spores.

This work contributes to our understanding of diversity and ecology of AMF in fonio millet agroecosystems. It therefore contributes to paving the way towards the development of microbial engineering approaches for agronomic improvement of fonio millet. However, more studies are necessary to better identify and explain the main driving factors of AMF community at different locations.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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