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Density and diversity of arbuscular mycorrhizal fungi in *Anacardium occidentale* L. plantations in Senegal

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Cashew nut cultivation plays an important socio-economic role in Senegal. However, it suffers from several problems, including declining soil fertility which leads to low productivity. To overcome these constraints, the use of arbuscular mycorrhizal fungi (AMF) could be a sustainable alternative. However, the positive effects of AMFs may depend on their infectious potential, density, and diversity. So far, little attention has been given to exploring these aspects in cashew plantations. The proposal of this study is to determine the infectious propagules, density and diversity of AMF spores in cashew agro-systems. Soil samples were collected from four cashew production areas in Senegal (Ziguinchor, Sédhiou, Kolda and Fatick). The soil samples were previously treated by wet sieving and decantation technique and the spores were isolated by centrifugation; thereafter, a morphological identification of the extracted spores was carried out. To compare AMF propagule numbers between sites, a most probable number (MPN) bioassay was performed under greenhouse conditions using *Zea mays* as the host plant. The average AMF spore density was significantly higher in Ziguinchor (610 spores/100 g soil), Sédhiou (586 spores/100 g soil), and Fatick (445 spores/100 g soil) compared to Kolda (211 spores/100 g soil). However, no significant difference was noted between Ziguinchor, Sédhiou, and Fatick. Spore and propagule densities show opposite results, MPN was high in sites with low spore density. The identification of the spores showed 6 genera belonging to *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, *Entrophospora,* and Racocetra. Identified AMFs could be isolated and multiplied to produce bioinoculants for cashew trees.

Key words: Abundance, Arbuscular mycorrhizal fungi, morphological diversity, most probable number, *Anacardium occidentale,* Senegal.

INTRODUCTION

Cashew is a tropical nut crop and an extremely important source of income for thousands of people in Senegal. It is cultivated widely in the South of the country, especially in Casamance and in the Groundnut Basin of Senegal with a national production estimated at around 28 900 tons per year (Hien, 2019). In Casamance, cashew is mainly grown in the administrative regions of Kolda, Sedhiou, and Ziguinchor, while in the Groundnut Basin it concerns

the Factick region. Cashew constitutes a major source of income in these regions by supporting more than 22500 households which represent a total population of around 352600 inhabitants (ASEPEX and IRD, 2017). In spite of the importance of this crop, cashew nuts yields gradually declined during these past years (Ndiaye et al., 2017). Currently, the yield per tree fluctuates from 3.2 to 5.81 kg and the yield per hectare is around 542 kg/h (Samb et al., 2018b). This production remains low compared to other producing countries such as Brazil, Vietnam, India, Indonesia, Philippines, Ivory Coast, Nigeria, and Benin (Samb et al., 2018b). Of the several factors associated with low productivity, poor soil fertility especially in nitrogen and phosphorus is a major factor limiting production. In addition, very little attention, in term of research has been accorded to this crop. There is therefore the need to step up research to improve cashew production, yields and quality as well as standardize effective propagation techniques to increase the productivity of this crop. In this context, the use of Abuscular Mycorrhizal Fungi (AMF) could promote plant growth and provide protection against phytopathogens (Sadhana, 2014; Barrow, 2012). In fact, under natural conditions, plants maintain symbiotic relationships with microorganisms as a strategy to withstand adversities by increasing plant resilience to water deficit and soil nutrient scarcity (Cius, 2017; Manga et al., 2022; Soumare et al., 2023). So, it is important to know which AMF are present in rhizosphere and in roots of naturally occurring plants in order to identify the most frequent ones and those showing high adaptability to environmental conditions. In fact, variations in AMF diversity can influence the stability and population dynamics of an ecosystem by changing plant competiveness and persistence. However, until recently, there was little focus on AM fungal diversity and ecosystem variability and productivity. This study aims to assess the MPN of infective propagules, density and diversity of AMF associated to *Anacardium occidentale* L. in different cashew agro-ecological zones in Senegal.

MATERIALS AND METHODS

Presentation of the study area

This study was conducted in two agro-ecological zones: the Casamance and the Groundnut Basin favorable to cashew cultivation (Figure 1). In Casamance, the sampling sites corresponding to the administrative regions of Kolda (Sanankoro 12°53'00'' N, 14°57'00'' W), Sédhiou (Colane 12°42'29'' N, 15°33'25'' W) and Ziguinchor (Boutoupa Camaracounda 12°33'40'' N, 16°17'00'' W) while in groundnut Basin, the sampling have been taken in Fatick region (Ndiémou 14°19'00'' N, 16°25'00 '' O).

In Casamance, the annual rainfall varies between 600 and 1775

mm distributed from June to October with an annual average temperature of 26.7°C (Camara, 2018). The soils are tropical ferruginous and/or ferralitic, clayey-loamy, hydromorphic types (ANSD, 2013). By contrast, Fatick region belongs to semi-arid tropical climate with an average annual temperature between 21 and 37°C and an average annual rainfall of 611 mm (Samb et al., 2018a). The soils are saline and acidified saline halomorphic soils (Samb et al., 2018a).

Soil sampling

At each site, five composite soil samples were obtained from under five *A. occidentale* trees. The sampling was carried out at a distance of 3/4 of the crown radius at a soil depth of 0 to 20 cm, following the East, West, North, and South directions of each tree. The soils that have been taken from each tree are mixed together to form a composite sample. Five composite samples were collected per site.

Characterization physicochemical of soils

A physical and chemical characterization of the soil was carried out in the Laboratory of Soil, Water and Plant of ISRA/CNRA in Bambey following the method of Bremner and Mulvaney (1982). The samples were placed in a mechanical shaker and sieved for 5 min through a series of sieves with 50, 100, 250, 500, and 1000 µm diameter to determine the size of different soil particles. All soil samples were characterized by measuring pH, total soil C and N after dry combustion in Elemental Analyzer (LECO Corporation, St. Joseph, MI, USA). Total and available P were analyzed by Olsen-Dabin method (Aubert, 1978). Other components were also determined such as Ca^{2+}, Mg^{2+}, and K^+ the Atomic Absorption Spectrophotometry method and exchangeable aluminium was used (David, 1960).

Quantification and taxonomic identification of AM fungi spores

For each composite sample, the AM fungi spores were isolated from *A. occidentale* rhizosphere by using a wet sieving and decanting technique according to Gerdemann and Nicolson (1963). Three subsamples (100 g) from each sample were independently suspended in 500 mL of water in a beaker. After the settlement of heavier particles, the suspension was carefully poured on a stack of sieves (500, 200, 100 and 50 µm, arranged µm mesh size) arranged in descending order. The processes were repeated almost three times to trap all spores of AM fungi. The AM fungal spores on the bottom sieves, to know, 100 and 50 µm, were collected. Spores were purified by re-suspending the sieving in 60% of sucrose solution and centrifuged at 1000 rpm for 5 min at 4°C. Afterwards, the AM fungal spores were rinsed in tap water and were counted under a microscope. Result was expressed as the spore number per 100 g of dry.

The AM spores of different genera have been described by spore morphology and as per the standard key referring to Souza (2015) and the INVAM (https://invam.ku.edu/spores). The identification of each spore type was done on the basis of color, shape, size, surface ornamentation, spore wall structure and type of hyphal attachment (Redecker et al., 2013). Identification of AM spores was done after trap culturing as propagation of field collected AM

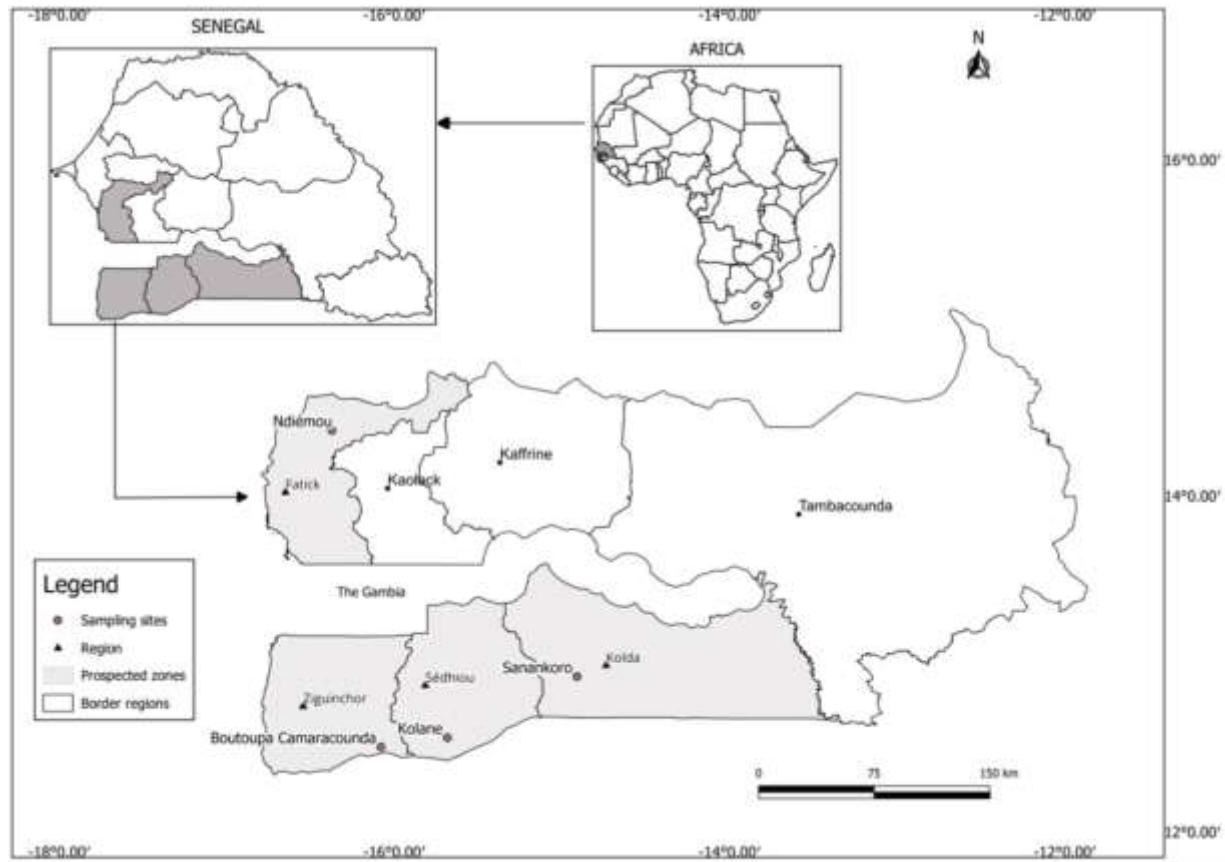*Corresponding author. E-mail: k.thiocone5328@zig.univ.sn. Tel: +221773863152.

Figure 1. Map of Senegal indicating the culture area of cashew in Senegal and the soils sampling zone.

species sometimes require controlled environment.

The most probable number (MPN) of AM fungi

This has been used to determine total propagules based on colonization of roots of the assay host. MPN is based on the use of a series of successive soil dilutions form 10^{-1} to 10^{-6} (Mosse, 1986). The dilutions were prepared by mixing the original soil with the same soil autoclaved at 120°C for 1 h (Gianinazzi and Vosátka, 2004). The soil mixture is divided into five replicates of 50 g/pot. One pregerminated *Zea mays* seeds were planted per pot under greenhouse conditions. After 45 days, the plants were uprooted and root system was stained according to the method of Phillips and Hayman (1970) in order to visualize the structures' mycorrhizal. Using non mycorrhized and mycorrhized roots obtained for each level of dilutions, the MPN of propagules present in soil was calculated with the formula of Fisher and Yates (1963):

log = (x × log a) − K, which is the estimated number of propagules;

where 'x' is the mean of positive pots; 'a' is the dilution factor and 'K' is a constant on Fisher and Yates' statistical. Results were expressed as number of propagules per unit soil.

Data analysis

All the data obtained were entered using the spreadsheet excel version 2013. The data were statistically analyzed using the XLSTAT software version 2016. A comparison between the treatments was carried out using an analysis of one-way variance (ANOVA). Significant differences between spore abundance and between soil physical and chemical properties were tested using Fisher's least significant difference (LSD) for $P < 0.05$. A principal component analysis (PCA) was used to determine the multiple correlations between the physicochemical properties of the soils and the types of AMF identified in the sampling sites. Some data were previously transformed by the formula Arsin$\sqrt{\ }$ x to normalize them. The MPN of arbuscular mycorrhizal fungi was calculated using the excel table.

RESULTS

Physico-chemical soil analysis

The grain size analysis and the soil texture triangle showed a textural variability of soils between the two agro-ecological zones. The soils sampled from the three areas of the agro-ecological zone of Casamance (Ziguinchor, Kolda and Sedhiou) are clays sand, while the soil of the agro-ecological zone of Groundnut Basin (Fatick) is sandy. The analysis of soils' chemical parameters revealed a significant difference (p-value <0.05) in their content in nitrogen, Ca^{2+} cation, and pH

Table 1. Physico-chemical characteristics of soils.

Physicochemical property	Localities			
	Ziguinchor	**Sédhiou**	**Kolda**	**Fatick**
Physical				
Sands (%)	87.64[b]	86.48[b]	89.77[ab]	94.82[a]
Stringers (%)	4.814[a]	5.67[a]	5.49[a]	2.81[ab]
Clays (%)	7.546[a]	7.86[a]	4.75[ab]	2.38[b]
Chemical				
pH	5.11[b]	5.18[b]	5.41[b]	6.07[a]
C (%)	0.514[a]	0.71[a]	0.48[a]	0.63[a]
N (%)	0.056[b]	0.05[b]	0.04[b]	0.09[a]
MO (%)	0.886[a]	1.22[a]	0.84[a]	1.08[a]
C/N	9.542[ab]	14.08[a]	12.63[ab]	6.78[b]
P (ppm)	11.877[a]	9.73[a]	8.71[a]	14.74[a]
Ca^{2+}	1.829[a]	1.87[a]	2.20[a]	0.64[b]
Mg^{2+}	0.22[b]	0.29[ab]	0.25[ab]	0.46[a]
K^+	0.072[a]	0.07[a]	0.08[a]	0.13[a]

In the same line, the values followed by the same letter are not significantly different at 5% threshold according to the Newman-Keuls test. pH: potential of the hydronium ion, C%: proportion of total organic carbon, N%: proportion of total nitrogen, C/N: the ratio between proportion of total organic carbon and proportion of total nitrogen, OM%: the organic matter content, P. assimilable: assimilable phosphorus, Ca^{2+}: calcium ion, Mg^{2+}: magnesium, K^+: the potassium ion.

values (Table 1). The soils pH of Casamance agro-ecological areas is slightly acidic and show similar values in the three sites. These soils are also characterized by low values of N (%) and high value of Ca^{2+} elements. However, the soils from Fatick agro-ecological were less acidic and closer to neutral pH (6). The soils as opposed to Casamance soils are characterized by high values of N (%), lowest Ca^{2+} cation content, and C/N ratio.

However, no significant differences (p-value >0.05) were observed in organic carbon matter, assimilable phosphorus, and K^+ cation, between sampling sites.

Density of AMF spores in the different sites studied

The results indicate a variation of the density of the spores according to the sites (Figure 2). The density is significantly higher in Ziguinchor (610 spores/100 g of soil), Sédhiou (445 spores/100 g of soil) and Fatick (445 spores/100 g of soil) compared to Kolda (211 spores/100 g of soil). Variance analysis did not show significant (P >.0. 05) difference in the number of spores between Ziguinchor, Sédhiou and Fatick sites. However, the latter were significantly lower in Kolda.

Morphological diversity of arbuscular mycorrhizal fungi

Six genera of AMF morphotypes were recorded from the different sites based on morpho-anatomical features of

the spores (Figure 3). The observed genera are *Glomus*, *Gigaspora*, *Racocetra*, *Entrophospora*, *Scutellospora* and *Acaulospora*. These genera belong to Glomerales and Diversisporales orders.

The analysis of variance shows that the genera *Glomus* showed significantly the highest frequencies (66.75%) followed by *Acaulospora* (23.87%), while *Gigaspora, Scutellospora, Entrophospora* and *Racocetra* genera recorded the lowest frequencies (4.8, 2.17, 1.52, and 0.87% respectively). In addition, there were no significant differences between these genera

AMF spore abundance according to the different sites

The analysis of variance (Table 2) revealed that the genera of *Scutellospora*, *Glomus*, *Gigaspora*, and *Entrophospora* have a significantly different abundance depending on the sites. However, the genera of *Acaulospora* and *Racocetra* do not show any significant difference between sites.

Abundance of AMFs infective propagules (MPN)

The number of infective propagules of the different site is presented in Table 3. This number varied between from the lowest mean value of 141. 6 recorded in Ziguinchor and to the highest mean value of 992.05 recorded in Fatick and Kolda. The MPN of infective propagules in 100
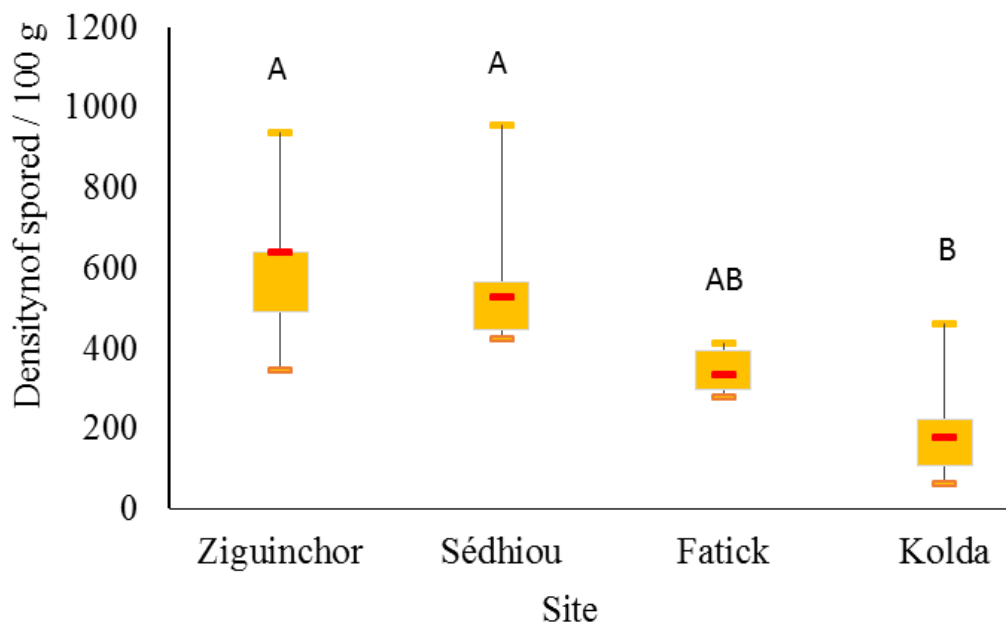
Figure 2. AMF spore abundance according to sites. The values followed by the same letter are not significantly different at the 5% level according to the Fisher test.

Table 2. Distribution of AMFs isolated from soils of cashew sites.

Site	Gender						
	Scutellospora	*Acaulospora*	*Racocetra*	*Glomus*	*Gigaspora*	*Entrophospora*	**Total abundance**
Fatick	22[a]	48[a]	8[a]	267[ab]	62[a]	21[a]	344[ab]
Sédhiou	6[b]	100[a]	4[a]	456[a]	17[ab]	4[a]	565[a]
Ziguinchor	4[b]	131[a]	1[a]	469[a]	4[b]	2[a]	604[a]
Kolda	4[b]	97[a]	2[a]	105[b]	3[b]	1[a]	208[b]
Pr > F	0.00	0.32	0.13	0.002	0.013	0.044	0.007
Significant	Yes	No	No	Yes	Yes	Yes	Yes

The values followed by the same letter are not significantly different at the 5% level according to the Fisher test.

g of dry soil for Sédhiou was 524.95 propagules/100 g of dry soil.

Relationship between soil physico-chemical properties and AMF spores abundance

Figure 4 presents the correlation between the abundance of AMF spores and the physicochemical properties of soils. The contribution of axis F1 is 74.15% collecting *Gigaspora*, *Scutellospora*, *Entrophospora* and *Racocetra.* This axis is related to high total N and assimilable P and characterize Fatick site which is opposed to the site of Ziguinchor. The axis 2 account for 16.67% and is related to high C% and MO% content in soil and abundance of *Glomus* characterize Sédhiou site which is opposed to Kolda site and that of axis 2 is 16.67%.

Analysis of Table 4 shows that there is a positive correlation between spore abundance and available phosphorus, carbon, nitrogen and organic matter. On the other hand, a negative correlation was observed between the abundance of spores and the pH, but also between the abundance of spores and the C/N ratio. It was also noted that the MPN of the soils studied are positively correlated with pH, phosphorus, carbon, nitrogen and organic matter.

DISCUSSION

The abundance of morphotype spores (spore density) and MPN estimates of AMF infective propagules were significantly variable depending on the exploited site. Our results showed that soil properties played important roles
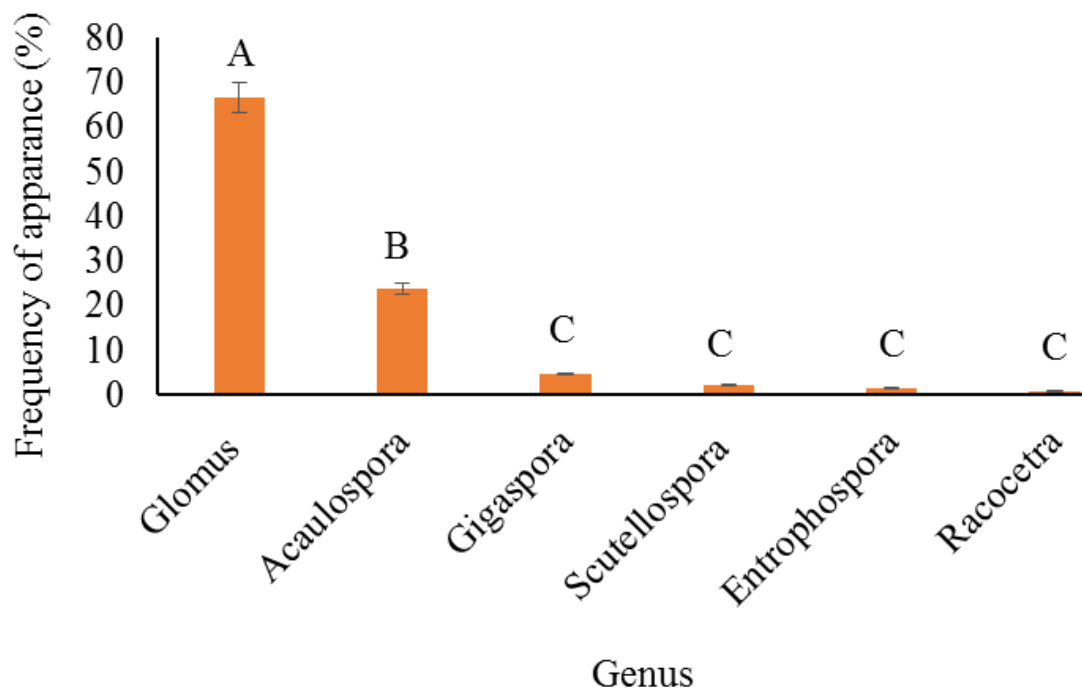
Figure 3. Frequency of appearance of spores according to genera. The values followed by the same letter are not significantly different at the 5% level according to the Fisher test.

Table 3. Most probable number of propagules in 100 g of dry soil from each site.

Feature	Sites			
	Ziguinchor	Kolda	Sédhiou	Fatick
Number of propagules/100 g of dry soil	141.60	992.05	524.95	992.05
Confidence interval at the threshold of P<5%	302.59< 141.60 < 62.26	2119.97< 992.05 < 464.23	1121.80< 524.95 < 245.65	2119.97< 992.05 < 464.23

in the global variation of mycorrhizal fungal diversity and abundance. As reviewed by many authors (Kachkouch et al., 2012; Soumare et al., 2015) several factors influence arbuscular mycorrhizal fungal spores distribution, such as soil types, age of planting, cultural practices, etc. According to our results, consistent chemical drivers of AMF communities were available P, Total N, Total C, and OM soil contents. Previous studies (Nyawira et al., 2012; Edlinger et al., 2022) have shown that soil total carbon and total nitrogen contents positively influence the abundance of AMF spores. For instance, Ba et al. (2012), have reported that the allocation of C to the roots and exudation from roots to soil could
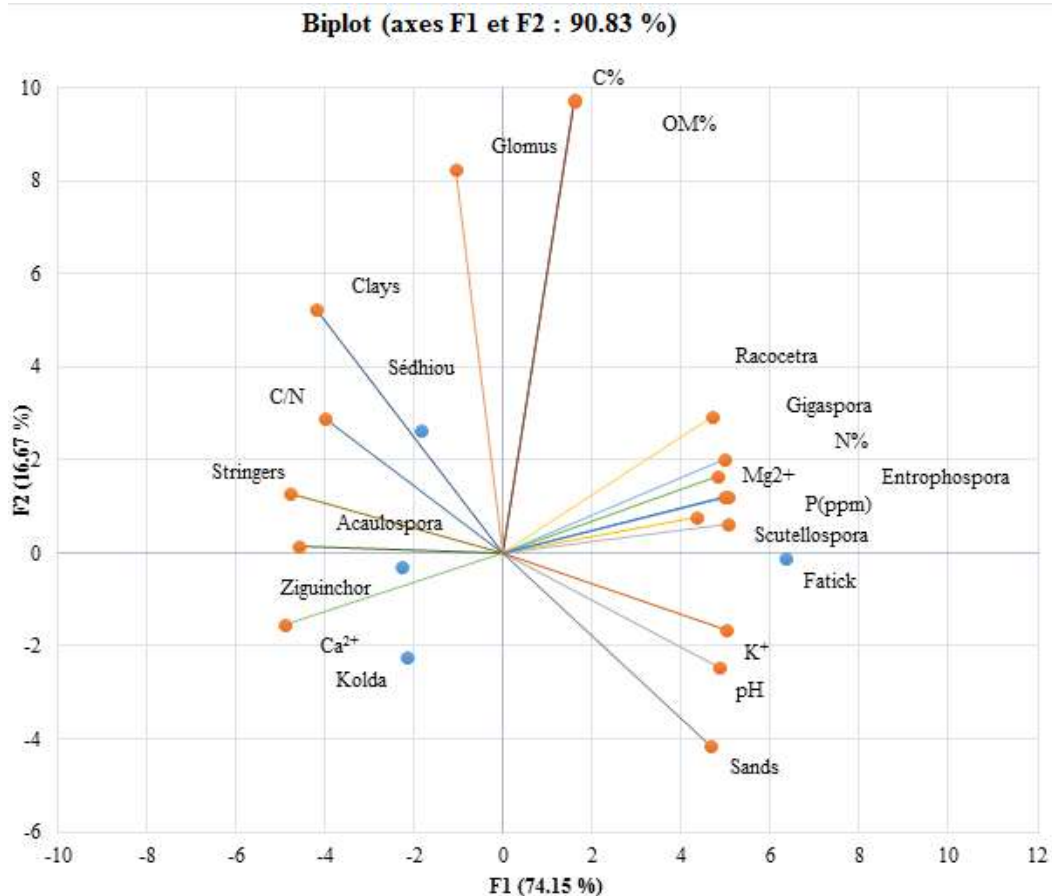
Figure 1. Principal component analysis of the diversity of arbuscular mycorrhizal fungi and physicochemical parameters of soils.

be beneficial for the sporulation of AM fungi. The present finding is in contrast to Datta and Kulkarni (2012) and Ma et al. (2023) who found negative correlation between AMF and available phosphorus. Ma et al. (2023) have shown that AMF richness and Shannon index decreased with increasing soil available P and increased with soil pH. These contradictions may be due climatic factors, and the phosphorus status of the plant.

Soil pH is another important predictor of global AMF abundance, in this study, there was an inverse relation between soil pH and spore density. This result is in line with earlier report of Cibichakravarthy et al. (2015) which have shown that pH, negatively impact on the AMF spore abundance and infective propagules. This indicates that certain AMF species are induced to sporulate abundantly under low pH as it is the case of Ziguinchor and Sedhiou in this study. In addition, soil texture and C allocation to mycorrhizae may result in low mycorrhizal fungal diversity and abundance.

In the current study, the difference in spore count and MPN, that is, high MPN versus low spore count can be explained by the fact that many AMFs, spread more with

other types of propagules such as hyphae and extra-root mycelia fragments than by spores (Brito et al., 2012). The majority of spores found in soils of the present study belong to the Glomeraceae family (Driai, 2016). The dominance of the number of propagules in Kolda and Fatick could be due to the fact that in these two sites the cashew trees are associated with vegetable crops.

Regarding diversity, our study showed that spores of the genera *Glomus* and *Acaulospora* were more abundant. This result is in agreement with previous research which explains this fact by a developmental difference. Spores of *Glomus* spp. have different adaptive capacity and high plasticity to biotic and abiotic factors (Dare et al., 2013). According to Oehl et al. (2003) and Zhao et al. (2003), these genera have an outward distribution in all types of agroecosystems of the world. Fabre et al. (2021) explain this dominance by a higher spore production capacity in a shorter time compared to other genera. In addition, species of arbuscular mycorrhizal fungi belonging to the genera *Glomus* and *Acaulospora* are resistant to soil disturbances and altered ecosystems (Welemariam et al., 2018). The large

Table 4. Pearson correlation coefficients between soil chemical parameters, AMF spore abundance, and soil MPN.

Soil parameter	Sands (%)	Stringers (%)	Clays (%)	pH	P (ppm)	C (%)	N (%)	C/N	MO (%)	Ca^{2+}	Mg^{2+}	K$^+$	Total abundance	MPN of soil
Sands (%)	**1.00**													
Stringers (%)	-0.90	**1.00**												
Clays (%)	-0.97	**0.77**	**1.00**											
pH	**0.98**	-0.87	-0.96	**1.00**										
P (ppm)	**0.72**	-0.95	-0.55	**0.70**										
C (%)	-0.04	-0.11	**0.12**	**0.14**	0.20	**1.00**								
N (%)	**0.79**	-0.96	-0.64	**0.81**	0.97	0.37	**1.00**							
C/N (%)	-0.79	**0.94**	**0.65**	-0.71	-0.94	**0.14**	-0.86	**1.00**						
MO (%)	-0.04	-0.12	**0.12**	**0.15**	0.20	0.99	0.37	0.14	**1.00**					
Ca^{2+}	-0.82	**0.96**	**0.69**	-0.86	-0.94	-0.39	-1.00	**0.83**	-0.39	**1.00**				
Mg^{2+}	**0.87**	-0.86	-0.80	**0.94**	0.76	0.46	0.90	-0.65	0.46	-0.93	**1.00**			
K$^+$	**0.96**	-0.97	-0.88	**0.97**	0.86	0.15	0.93	-0.85	0.16	-0.95	**0.94**	**1.00**		
Total abundance	-0.45	**0.01**	**0.63**	-0.40	**0.28**	0.50	0.19	-0.05	0.50	-0.12	-0.13	-0.19	**1.00**	
MPN of soil	**0.71**	-0.35	-0.84	**0.75**	0.06	0.00	0.25	-0.13	0.00	-0.33	**0.61**	**0.56**	-0.83	**1.00**

Values in bold are significant at the threshold p-value= 0.05.

variations in AMF abundance and frequency between sites could also be due to deferential survival strategies depending on soil properties. According to Datta and Kulkarni (2012), fluctuations in the number of spores could be associated with the process of germination, formation, and degradation of spores.

Conclusion

In the present study, we have investigated the MPN, AMF spore density and diversity in soils of different *A. occidentale* L. plantations in Senegal. From the results obtained, it appeared that soil physical and chemical properties affect AMF diversity and abundance. Six AMF morphotypes belonging to *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora*, *Entrophospora* and *Racocetra* were found. *Glomus* and *Acaulospora* genera were the predominant in all soil samples. These findings could be a starting point for the development of well performing and inocula suitable for the application in field. In future study, we will highlight if the distribution patterns differ between diversity and abundance of AMF.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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