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Eucalyptus camaldulensis Impact on arbuscular mycorrhizal fungi abundance and density

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Abstract
Sahel is the transition zone between the wooded savannas of the south and the true Sahara Desert. It is dominated by the *Acacia* genus (*A. albida, A. senegal, A. seyal*). Most of them yield natural gum of different types, but only those varieties produced by *A. senegal* and *A. seyal* are regarded to be of commercial interest. Sahelian zone is also a zone of cultivation of several other leguminous food crops or cash crops including groundnuts. These legumes are largely dependent on symbiotic microorganisms, especially arbuscular mycorrhizal fungi (AMF or AM fungi). Diversity and / or abundance of these fungi are a good indicator of soil fertility. In addition, arbuscular mycorrhizal fungal determine the structure and composition of plant communities and productivity especially herbaceous vegetation. Introduction of *Eucalyptus* for massive reforestation in the Sahelian zone has caused a great deal of ecological concern, in particular their impact on the mycorrhizal potential in new area. The objective of this work is to evaluate this impact on diversity and abundance of AMF in soils under *Eucalyptus* plantation in Senegal.

Keywords: *eucalyptus*, AMF, impact, diversity, TTGE

Introduction

Arbuscular Mycorrhizal fungi (AMF or AM fungi) by using their hyphae network, allowing greater absorption of water and nutrients, especially those of low soil mobility, such as phosphorus, zinc and copper (Moreira & Siqueira, 2006) [1]. So, AMF provide a large number of other benefits to host plants such as pathogen and herbivory protection, alleviation of water stress, enhanced tolerance of salinity, low pH and heavy metals (Soumare *et al.* 2015, Manga 2017) [2, 3]. Van der Heijden *et al*., (2006) [4] show that diversity and productivity of plants in ecosystems are significantly influenced by the diversity of arbuscular mycorrhizal fungi. Similarly, Argüello *et al.* (2016) [5] found that diversity of AMF promotes cooperation between plants and AMF. In view of this ecological and agronomic importance, it is therefore important to evaluate impact of agricultural and silvicultural practices on these fungi. In the Sahelian zone specially, AM fungi are the most functional symbiotic that would facilitate the adaptation of Sahelian species to this arid zone (Ducousso 1991, Juniper and Abbott 1993, Diop *et al*. 1994) [6, 7, 8]. Assessing Mycorrhizal soil infectivity is potential Indicator of soil Health and fertility indicator of fertility. In fact, soil quality is closely linked to its biological properties, particularly its richness in mycorrhizal propagules (Duponnois *et al*., 2010) [9]. Thus, diversity and / or abundance of microorganisms may be a good indicator of soil quality (Ohl *et al.*, 2011) [10]. Some mycotrophic plants improve this potential while other plants may have a selective, inhibitory or neutral effect on AM fungi. In Senegal, since the 1980s, the exotic species belonging in *Eucalyptus* genus, have been planted on a large scale to deal with desertification and to meet the wood demand of the populations. However, the impact of *E. camaldulensis* plantations on AMF (density, diversity) has not been studied extensively, especially in Senegal. Indeed, *Eucalyptus* by their acidifying effect (Couto and Betters et Betters, 1995) [11] and their litter (rich in inhibitory chemical compounds) may modify inhibitors, modify the physicochemical properties of the soil (Boyden *et al*., 2005, Soumare *et al.*, 2016) [12, 13]. According to Wilson and Tommerup (1992) [14], these modifications can have a negative impact on telluric microbial communities including AMF.

The objective of this work is to evaluate the density and diversity of AM fungi in soils under *Eucalyptus*. In this study, first, we have analyzed density and diversity of spores in soils sampled under eucalyptus plantations (SC) and in control soils (HC). In a second step we have analyzed and compared the diversities of the AMF colonizing the roots of five species (*A. seyal, A. senegal, A. albida, A. hypogea* and *Zea mays*) grown on SC and on HC using Temporal Temperature Gradient Gel Electrophoresis (PCR-TTGE) molecular fingerprinting technique. Achieving this objective can provide insight into the strategies for conservation of the diversity in semiarid

ecosystems.

Material and Methods

Soil sampling: The soils were sampled in 6 plantations located in different regions of Senegal. The GPS data of the different sites are presented in Table 1. From each site, five soil samples were collected under five *Eucalyptus* trees from 0 to 30 cm soil depth and mixed to form one composite sample (noted SC). And three composite samples were collected per plantation. This soil was considered to be influenced by *Eucalyptus*. At each site, three other composite soil sample was formed with soil collected in areas free of *Eucalyptus* and located at a distance of 30 m from *Eucalyptus* plantings (control sample or HC). These soil samples were supposedly uninfluenced by *Eucalyptus.*

Table 1: GPS data

Sampling place	GPS data
Lompoul	17°07 N; 03°69 W
Sinthiou Garba (abbreviated by Sinthiou)	17° 15N; 06° 82W
Matam	17°30N; 06° 87W
Tambacounda (abbreviated by Tamba)	15°23 N; 06°40W
Kolda	14° 27 N; 05°77W
Nioro	15°19N; 04°17W

Soil analysis

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 (Olsen *et al*., 1954; Aubert, 1978) [15, 16]. Analyses were performed at LAMA (Laboratoire des Moyens Analytiques IRD/ISRA, laboratory certified by International Organization for Standardization ISO 9001 version 2008).

Spores extraction and identification

AMF spores were extracted from soil sample by wet-sieving and decanting technique according to the method of Gerdemann and Nicolson, (1963) [17]. Spores were counted for each soil pair (HC / SC) and the count was repeated 3 times for each sample. AMF spores were isolated and examined under a stereomicroscope (AmScope SE306-PZ-P), and grouped according to their morphotypes. Spores of each morphotype were mounted in polyvinylalcohol–lactic acid–glycerol (PVLG) (Omar *et al.*, 1979) [18] and PVLG mixed with Melzer's reagent (1 : 1, v : v). The taxonomic identification of spores to species and genus level was based on spore morphological characteristics as size, colour, pigmentation, ornamentation, characteristics of their wall and by comparison with herbarium specimens of the Canadian National Mycological Herbarium (DAOM) Ottawa Canada, reference type-specimens, species material provided by the International Collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu), and species descriptions (Blaszkowski, 2012) [19]. Species diversity between HC and SC soils was analyzed by the Shannon-Weaver Index. The Shannon index is given by the formula: $H = - \Sigma p_i \log_2 p_i$ or p_i corresponds to the proportions of the genotypes. A high value of this index means a significant number of genotypes of the population studied.

Diversity of MA fungi in legume roots traps

DNA extraction from roots: Root samples of each species were washed thoroughly with tap and sterile water, dried with clean tissue, randomly assembled. Root samples were ground in liquid nitrogen and extracted using a DNeasy plant mini kit (Qiagen), following the manufacturer recommendations.

Pre-amplification of the small 18S subunit of ribosomal rDNA

The 18S small subunit (SSU-rDNA) of the mycorrhizal fungi was amplified by the AM1 / NS31 primer pair. Primer NS31 (Simon *et al*. 1992) [20] and an AMF-specific primer AM1, was designed to amplify the 550 bp fragment of SSU-rDNA from Glomerales and Diversisporales from colonized roots. Amplification were carried out in a final volume of 25 µl, using, 5 µl of 10X PCR buffer (100mMTris–HCl, pH 8.3 at 25°C; 500mM KCl; 15mM MgCl2; 0.01% gelatine), 0.2 mM of dNTP, 0.4 µM of primers, 2.5 U of Taq polymerase (all Sigma) and 2 µl of DNA extract. The following cycle conditions: 95°C for 2 min, followed by 33 cycles at 94°C for 1 min, 56°C for 30, 72°C for 1 min and final extension at 72°C for 8 min were used.

Nested PCR amplification

Smaller fragment (350 bp) was amplified from the NS31-AM1 SSU-rDNA PCR product (1 µl) with GC-NS31 (5′-CGC CCG CCGCGC GCG GCG GGC GGG GCG GGG GCA CGGGGG GTT GGA GGG CAA GTC TGG TGC C-3′) and the TTGE2: 5'-ATCCTAGAAACCAACAAAATA-3' primer developed by Norini *et al*. submitted for publication). The GC-clamp stabilize the melting behaviour of the PCR product for TTGE analysis. Conditions for the PCR were as follows: initial denaturation at 95°C for 2 min, followed by 30 cycles at 94°C for 1 min, 56°C for 30 s, 72°C for 30 s and a final extension step at 72°C for 8 min.

TTGE analysis

TTGE analysis was carried out with a Dcode Universal Mutation Detection System (Bio-Rad). Ten microliters of GC-NS31-TGGE2 PCR products were double diluted with loading dye (Bio-Rad) and applied directly on to a 6% (w/v) polyacrylamide/bisacrylamide (37.5:1; 30%) (Sigma) gel containing 6 M urea with 1.25X TAE buffer (50 mM Tris base, 25 mM acetic acid, glacial, 1.25 mM EDTA, pH 8.0), 0.1% (v/v) of TEMED and 0.1% (w/v) of ammonium persulfate. The electrophoresis was performed at a constant voltage of 130 V, with a temperature gradient from 54.5°C to 59°C and a ramp temperature of 0.6°C h−1.

After electrophoresis, the gel was incubated for 15 min in dH2O containing 0.5 mg l−1 of ethidium bromide (Bio-Rad), rinsed for 10 min with dH2O and photographed with UV transillumination using Kodak DC 290 and an UV filter.

Statistical analyzes

The data were processed with the XLSTAT 2015 statistical software. An analysis of variance (ANOVA) was performed. The means of the variables were compared using the Newman Keuls test at the probability threshold p = 5%. The Shannon index was used as an index to appreciate diversity. Correlation tests between the chemical composition of the soil and the

number of spores have also been made.

Results

Soil analysis and spore number and correlation

Soils of the different sites were slightly acidic but SC soils were more acidic with a pH of about 5.6 whereas the HC soils had an average pH of about 6.5 (Table 2). Phosphorus levels were higher overall in HC than in SC, particularly for soil sampled in Matam. Spore number per 100 g of soil were significantly higher in free *Eucalyptus* soil for Lompoul and Sinthiou (Table 3), contrasting with the results obtained from Nioro and Kolda. For the Matam and Tambacounda soils, the differences were not significant between HC and SC. Soil from Matam showed very low spores compared to other sites. Spore density were negatively correlated with mineral content. This correlation was statistically significant at the 5% significance level ($P < 0.05$) for carbon and nitrogen; it is highly significant ($P < 0.01$) for P. total and P. assimilable (Table 4). The pH was also negatively correlated with spore density ($P < 0.05$, Table 4).

Table 2: Compostions chimique des sols des différents sites d'étude.

Sols	pH		N total (%)	Carbone total (%)	C/N	P total (mg/kg)	P assimilable (mg/kg)
	H2O	KCl					
T HC	6,41	5,62	0,069	0,992	17	82	6,11
T SC	5,87	5,30	0,056	0,807	17	58	8,73
K HC	5,87	5,28	0,045	0,599	16	49	3,93
K SC	5,55	4,95	0,049	0,671	16	53	4,80
L HC	6,34	5,37	0,028	0,295	12	28	3,49
L SC	5,12	4,46	0,056	0,688	14	44	11,35
MHC	7,44	6,86	0,093	0,950	12	351	141,40
M SC	6,67	6,00	0,062	0,699	13	237	47,13
N HC	6,83	6,32	0,050	0,629	15	98	20,08
N SC	5,53	4,66	0,044	0,543	14	56	9,16
S HC	6,17	5,04	0,024	0,240	12	70	4,36
S SC	5,35	4,28	0,022	0,224	12	48	6,11

Abbreviations for sampling site L: Lompoul, M: Matam, S: Sinthiou, T: Tamba, K: Kolda, and N: Nioro; HC : control samples and SC : Under Eucalyptus samples.

Table 3: Number of spores per 100 g of soil in HC and SC

Traitements	Lompoul	Matam	Sinthiou	Tamba	Kolda	Nioro
Hors couvert	340,5b	3,66 a	146,33b	272,66a	97.66 a	138,66 b
Sous couvert	42,00 a	5,00 a	88,00a	134,00a	205, 33a	442,00a

For each column, the values followed by the same letter are not significantly different according to the Fischer test at the 5%

Table 4: Pearson's correlation between soil chemical data, pH and AMF number of spore (Arbuscular mycorrhizal fungi)

Paramètres sol	pH	N(%)	C (%)	P total	P. assim.
Spores	-0,344*	-0,351*	-0,21*	-0,533**	-0,476**
P assim.	0,724*	0,744*	0,47*	0,951**	
P total	0,777*	0,746*	0,492*		
C (%)	0,544*	0,935**			
N(%)	0,681*				

***p < 0.0001; **p < 0.001; *p < 0.05.

Morphological identification of AMF spore

The total number of AMF species found was 11, two species belonging to the genus *Glomus*, five to *Scutellospora*, and two to *Gigaspora* and to *Acaulospora*. The AMF most frequently found were members of the genus *Scutellospora spp* especially in HC soils (Figure 1; Table 5). However, the species of the genus *Acaulospora* and *Gigaspora* are mainly found in SC soils. *Acaulospora* spp. consist of three walls: a colourless or coloured outer wall forming the spore surface, and two colourless inner walls. Spore are ornamented while spores of the genus *Gigaspora* are yellow in color, globose with a diameter between 260-400µm (Figure 1a and b). Shannon's diversity index (H) does not show a significant difference between HC soils (H = 5.86) and SC (H = 5.87). Spore populations of AMF generally were more abundant and diverse in HC soils.

Table 5: Percentage of spores identified (at the genus or species scale) based on morphological criteria

	Glomus sp 1	*Glomus sp 2*	*Scutellospora greagaria*	*Sclerospora sp1*	*Sclerospora sp2*	*Sclerospora sp3*	*Sclerospora sp4*	*Gigaspora margarita*	*Gigaspora rosea*	*Acaulospora sp1*	*Acaulospora sp2*
LHC	10	40						50			
LSC	13	30,75				56,25		56,25			
MHC				20		80					
MSC						100					
SHC				23,8	14,28	9,5	61,9				
SSC	86,2			13,72							
THC			44,11			55,8					
TSC	33,3		22,2							44	
KHC			33,3	6,7		50					10
KSC								10,5	10,5	89,5	
NHC	65,7	20				14,28					
NSC	50							30		20	

Spaces without values without values mean that the species has not been found. Abbreviations for sampling site L: Lompoul, M: Matam, S: Sinthiou, T:

Tamba, K: Kolda, and N: Nioro; HC : control samples and SC : Under Eucalyptus samples.

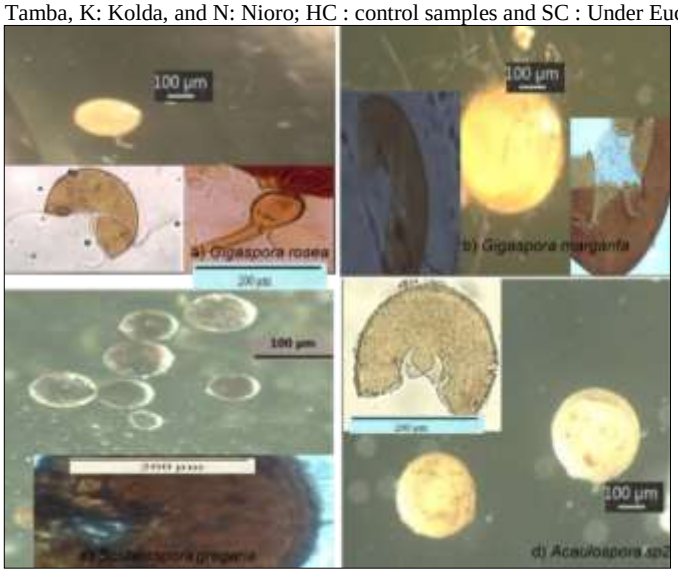

Fig 1: Morpho-anatomical aspects of some spores isolated from soils *Gigaspora* (a, b); *Sculetllospora* (c) *Acaulospora* (d).

TGGE analysis

A total of 26 bands (each band represents one genotype) were obtained from the roots of all species. The difference between HC and SC depends on the sampling site and the trap plant. In the sites of Lompoul and Nioro, there are more genotypes in HC compared to SC whereas in Kolda, the results had an opposite trend. For Matam, Sinthiou and Tamba, there was no difference in genotypes distribution between HC and SC. There were a strong similarity of AMF patterns in the roots of plants grown in HC compare to SC (Figure 2).

PCR-TTGE analysis show a classification of the communities according to their host plants (Figure 3). The first axis of principal components analysis discriminates *Arachis hypogea* from species (*A. seyal*, *A. albida*, *A. senegal*, and *Zea mays*) by the higher number of bands and little difference HC / SC diversity. The second axis separates *A. senegal* from *A. seyal*, *A. albida* and *Z. mays*. In fact, the AM fungi in these three species were closer and HC / SC discrimination very poor (Figure 3). In addition, result shows that ***Zea mays*** plants are associated with a greater diversity of MA fungi.

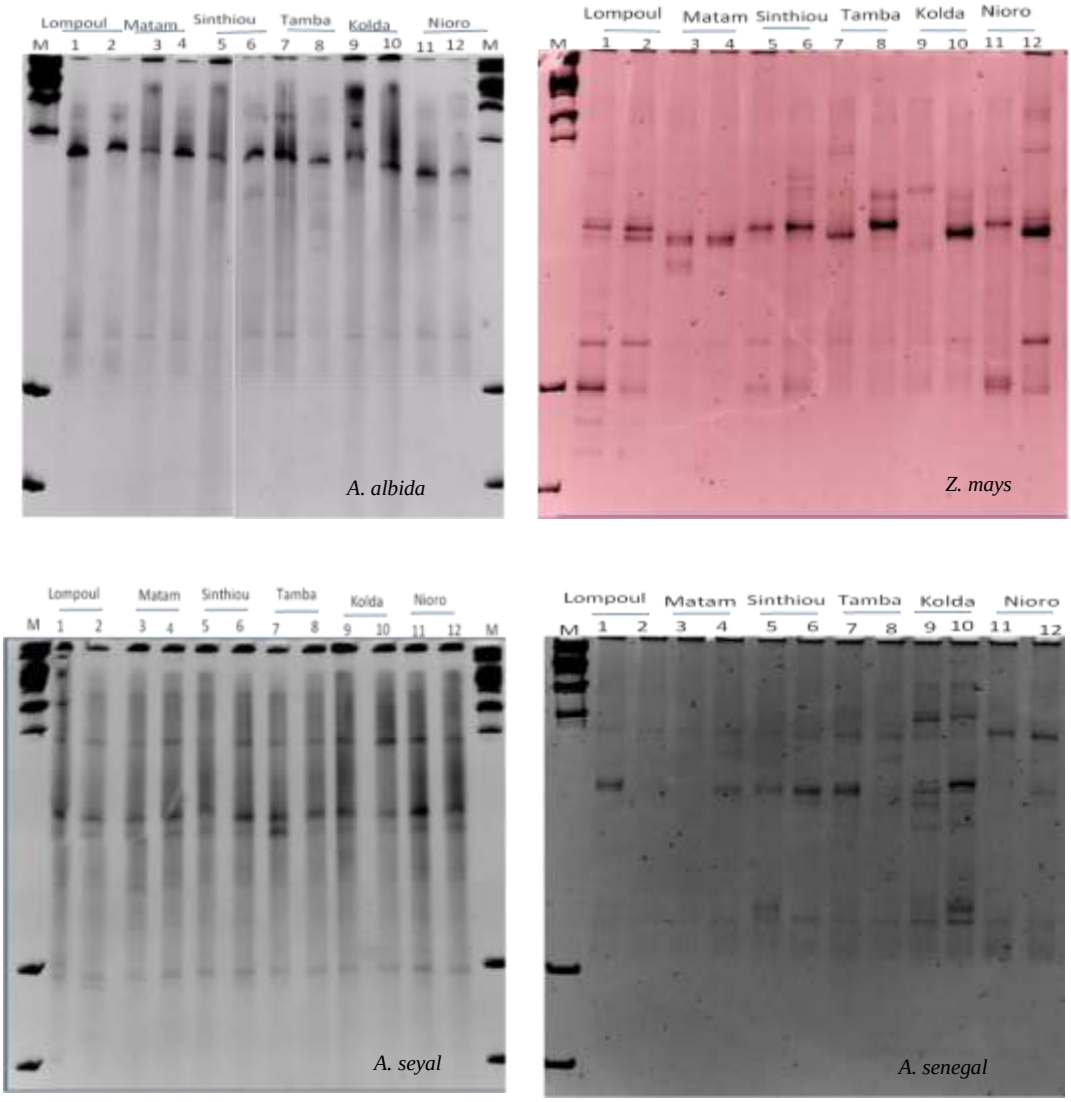

Fig 2: TTGE profiles from roots of different species. Samples 1, 3,5,7,9 and 11 correspond HC while 2,4,6,8, 10 and 12 correspond SC samples

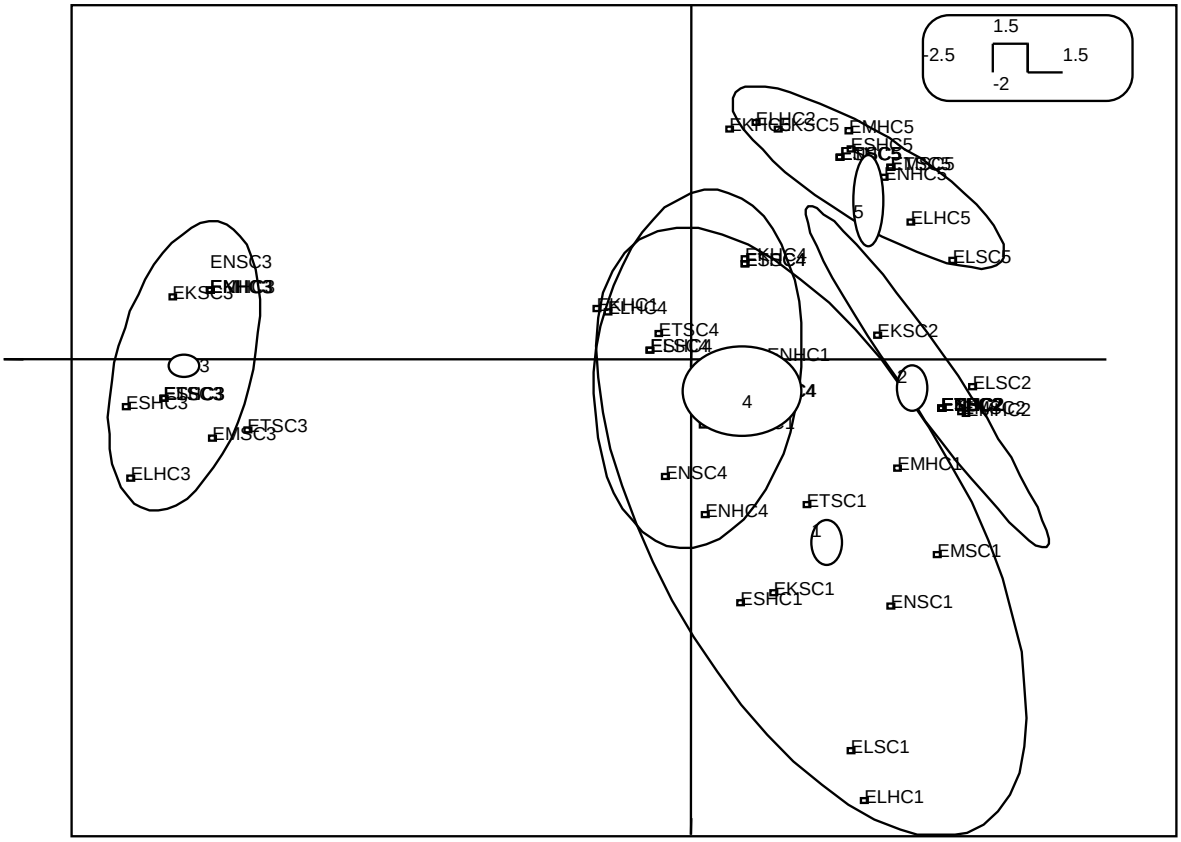

Fig 3: PCA of TTGE profiles of different species. Each ellipse groups the AMF genotypes associated with each species in the different sites. The number correspondent respectively 1 = *Zea mays* ; 2 = *A. seyal*; 3 = *Arachis hypogea*; 4 = *A. albida*; 5 = *A. senegal*. ELSC ELHC, EMHC, EMSC, ESC. ect. followed by the numbers correspond to the different genotypes

Discussion

Spores of *Scullelospora* were found in abundance in almost every soil sample. This was similar to earlier reports (Diagne *et al*. 2011) [21] in their studies on AM fungal in soils from Niayes (Senegal). According to Houngnandan *et al*., 2009 [22], the genus *Glomus* and *Scutellospora* appear to be tolerant to a wide range of ecological niches which would explain their predominance in our sites. The distribution of spores between HC and SC shows that the genus *Acaulospora* was mostly found in SC soils from *Eucalyptus* to Tamba and Kolda and is dominant compared to *Glomus* genus. This result confirm the hypothesis of Pagano *et al*., 2008 [23], according to which *Eucalyptus* favors species of the genus *Acaulospora* with respect to the genus *Glomus*. This selective effect is due to the acidification of soils under *Eucalyptus* noted in all sampling sites.

The authors Ohl *et al*. (2011) [10], showed that *Acaulospora paulinae* were only found in acid soils. Thus, the higher spore count in SC in this Kolda site is explained by this selective effect of acidity on AM fungi. However, in our previous study (Soumare *et al*., 2013) [24], we showed that this large amount of AMF spores was not correlated with the inffectiveness of the tested trap plants. On the contrary, mycorhization intensity was higher for species grown on this HC soil.

The site of Lompoul is however dominated by the genera *Gigaspora* and *Glomus* with equivalent percentages between HC and SC. However, spore density is higher in HC, as mycorrhization (Soumare *et al*., 2013) [24]. In this site, *Eucalyptus* decreases the number of spores. This result corroborates that of Kisa *et al*., 2009 [25], which observed the same effect under *Eucalyptus* in Burkina Faso. The result on diversity by the TTGE approach reinforces the morphological identifications. Indeed, the HC / SC profiles obtained on the roots for each species and at each site show a great resemblance of diversity (band at the same position between HC and SC profiles). On the other hand, the comparison of the profiles obtained from the different trap plants shows that there was a preferential mycorhization. Our result is supported by those from Manga *et al*. 2007 [26], which showed that *A. seyal* preferentially associates with two MA fungi whose sequences have a homology with *Glomus claroideum* and *Glomus uncinatum*. More recently, Chifflot *et al*., 2009 [27], have shown that the quality of litter brought to the soil modifies the development of AM fungi. Negative correlations between the number of spores and the chemical composition of SC soils show Eucalyptus litter negatively impacting AM fungi. Indeed, AM fungi, as Casazza *et al*. (2017) [28] have shown, are largely dependent on the chemical composition of the soil.

Matam soil showed a lower spore density probably related to high levels of phosphorus in this soil. In fact, soil phosphorus is negatively correlated with spore density. Several studies have already shown that phosphorus inhibits the functioning of mycorrhizal symbiosis, especially sporulation (Nelsen *et*

al., 1981, Nagahashi *et al*., 1996) [29, 30]. Phosphorus acts directly on AM fungi by inhibiting their development or indirectly on host plant tissues.

Conclusion

Eucalyptus plantations do not alter the specific diversity of AM fungi. However, they negatively impact the distribution, density and ability of AMF to form symbioses. These negative changes are related to acid pH and phosphorus content in SC soils. AMF diversity seems varied according to the trap plant. This study suggest that species of the genus ***Acaulospora*** could be a good inoculum for acid soils in the restoration programs of degraded sites

References

1. Moreira FMS. Siqueira JO. Microbiologia e bioquímica do solo. 2 ed. Lavras: UFLA, 2006, 729:1.
2. Soumare A, Diop T, Manga A, Ndoye I. Role of arbuscular mycorrhizal fungi and nitrogen fixing bacteria on legume growth under various environmental stresses. Int. J. Biosci. 2015; 7:31-46.
3. Manga A, Dalpé Y, Soumaré A, Diop AT. Diversity of Arbuscular mycorrhizal fungi associated to *Acacia seyal* (Delile) in semi-arid zone of Senegal. World Journal of Microbiology Vol. 3(2), 120-127. © www.premierpublishers.org.
4. van der Heijden MGA, Bakker R, Verwaal J, Scheublin TR, Rutten M, van Logtestijn R, Staehelin C. Symbiotic bacteria as a determinant of plant community structure and plant productivity in dune glassland. FE MS Microbiology Ecology. 2006; **56**:178-187.
5. Argüello A, O'Brien MJ, van der Heijden MGA, Wiemken A, Schmid B, Niklaus PA. Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. Ecology Letters. 2016; 19(6):648-656.
6. Ducousso M. Importance des symbioses racinaires pour l'utilisation des Acacias d'Afrique de l'Ouest. Thèse d'université Claude-Bernard de Lyon, 1991.
7. Juniper S, Abbott L. Vesicular-arbuscular mycorrhizas and soil salinity. *Mycorrhiza.* 1993 ; 4:45-57.
8. Diop TA, Guèye M, Dreyfus BL, Plenchette C, Strullu DG. Indigenous arbuscular mycorrhizal fungi associated with *Acacia albida* Del. in different areas of Senegal. Applied and Environmental Microbiology. 1994; 60:3433-6,
9. Duponnois R, Prin Y, Baudoin E, Galiana A, Dreyfus B. Les champignons mycorhiziens : une composante majeure dans les processus biologiques régissant la stabilité et la productivité des écosystèmes forestiers tropicaux. Le projet majeur africain de la Grande Muraille Verte, 2010, 442.
10. Oehl F, Jansa J, Ineichen K, Mäder P, van der Heijden M**.** Champignons mycorhiziens arbusculaires, bioindicateurs dans les sols agricoles suisses. Recherche Agronomique Suisse. 2011; 2 (7–8):304-311.
11. Couto L, Betters DR. Short Rotation Eucalypt Plantations in Brazil: Social and Environmental Issues. Oak Ridge National Laboratory, Oak Ridge, TN, 1995.
12. Boyden S, Binkley D, Senock R. Competition and facilitation between eucalyptus and nitrogen-fixing falcataria in relation to soil fertility. Ecology. 2005; 86:992-1001.
13. Soumare A, Sall SN, Sanon A, Cissoko M, Hafidi M, Ndoye I, Duponnois R. Changes in soil pH, polyphenols content and microbial community mediated by Eucalyptus camaldulensis Applied Ecology and Environmental Research. 2016; 14(3):1-19.
14. Wilsona JM, Tommerup IC. Interactions between fungal symbionts: VA mycorrhizae. In Allen MF, ed. Mycorrhizal functioning: an integrative plant-fungal process. New York: Chapman and Hall, 1992, 992-1001,
15. Olsen SR, Cole CV, Watanabe FS, Dean LA. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Department of Agriculture circular, vol. 939. U.S. Department of Agriculture, Washington, DC, 1954, 19.
16. Aubert G. Méthodes d'Analyse des sols. - Edition CRDP, Marseille, 1978, 360.
17. Gerdemann JW, Nicolson TH. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Transtions of British Mycological Society. 1963; 46:235.
18. Omar MB, Bolland L, Heather WA. A permanent mounting medium for fungi. Bull. Br. Mycol. Soc. 1979; 13:31-32.
19. Błaszkowski J. Glomeromycota. W. Szafer Institute of Botany, Polish Academy of Sciences, Krakóv, Poland, 2012.
20. Simon L, Lalonde M, Bruns TD. Specific simplification of 18S fungal ribosomal genes from vesicular–arbuscular endomycorrhizal fungi colonizing roots. Appl Environ Microbiol. 1992; 58:291-295.
21. Diagne N. Diversité, fonctionnement et incidence sur la symbiose actinorhizienne des champignons mycorhiziens associés aux Casuarinaceae dans leur zone endémique et dans leur zone d'introduction, 2011, 261.
22. Houngnandan P, Yemadje RGH, Kane A, Boeckx P, Van Cleemput O. Les glomales indigènes de la forêt claire à Isoberlinia doka (*Craib et Stapf*) à Wari-Maro au centre du Bénin. Tropicultura, 2009, 83-87.
23. Pagano MC, Cabello MN, Bellote AF, Sá NM, Scotti MRM. Intercropping system of tropical leguminous species and Eucalyptus camaldulensis, inoculated with rhizobia and/or mycorrhizal fungi in semiarid Brazil. Agroforestry systems. 2008; 74231=-242.
24. Soumare A, Kane A, Justin PNG, Naré A, Thiao M, Ndoye I. Influence of soil under *E. camaldulensis* on *Acacia* seedlings growth and their symbiotic status. Current Research in Microbiology and Biotechnology. 2013; 1(5):218-223,
25. Kisa M, Sanon A, Thioulouse J, Assigbetse K, Sylla S, Spichiger R, *et al*. Arbuscular mycorrhizal symbiosis can counterbalance the negative in£uence ofthe exotic tree species *Eucalyptus camaldulensis* on the structure and functioning of soil microbial communities in a sahelian soil. FEMS Microbiology Ecology. 2007; 62:32-44.
26. Manga A, Diop TA, van Tuinen D, Neyra M. Variabilité génétique des champignons mycorhiziens associés à *Acacia seyal* en zone semi-aride du Sénégal. Sécheresse.

2007; 18(2):129-33.
27. Chifflot V, Rivest D, Olivier A, Cogliastro A, Khasa D. Molecular analysis of arbuscular mycorrhizal community structure and spores distribution in treebased intercropping and forest systems. Agriculture, Ecosystems & Environment. 2009; 131:32-39.
28. Casazza G, Lumini E, Ercole E, Dovana F, Guerrina M, Arnulfo A, *et al*. The abundance and diversity of arbuscular mycorrhizal fungi are linked to the soil chemistry of screes and to slope in the Alpic paleo-endemic Berardia subacaulis. *PLoS ONE*. 2017; 12(2):e0171866. https://doi.org/10.1371/journal.pone. 017 1866
29. Nelsen CE, Bolgiano NC, Furutani SC, Safir GR, Zandstra BH. The effect of soil phosphorus levels on mycorrhizal infection of field-grown onion plants and on mycorrhizal reproduction. Journal of the Americani Society for Horticultural Science. 1981; 106:786-788.
30. Nagahashi G, Douds DD, Abney GD. Phosphorus amendment inhibits hyphal branching of the VAM fungus *Gigaspora margarita* directly and indirectly through its effect on root exudation. Mycorrhiza. 1996 ; 6 :403-408.