

## Research Article

# Changes in Land Use System and Environmental Factors Affect Arbuscular Mycorrhizal Fungal Density and Diversity, and Enzyme Activities in Rhizospheric Soils of *Acacia senegal* (L.) Willd.

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The responses of the soil microbial community features associated to the legume tree *Acacia senegal* (L.) Willd. including both arbuscular mycorrhizal fungal (AMF) diversity and soil bacterial functions, were investigated under contrasting environmental conditions. Soil samples were collected during dry and rainy seasons in two contrasting rainfall sites of Senegal (Dahra and Goudiry, in arid and semiarid zone, resp.). Soils were taken from the rhizosphere of *A. senegal* both in plantation and natural stands in comparison to bulk soil. A multiple analysis revealed positive correlations between soil physicochemical properties, mycorrhizal potential and enzyme activities variables. The positive effects of *A. senegal* trees on soil mycorrhizal potential and enzyme activities indicates that in sahelian regions, AMF spore density and diversity as well as soil microbial functions can be influenced by land-use systems (plantation versus natural population of *A. senegal*) and environmental conditions such as moisture and soil nutrient contents. Our study underlines the importance of prior natural AMF screening for better combinations of *A. senegal* seedlings with AMF species to achieve optimum plant growth improvement, and for restoration and reforestation of degraded lands.

## 1. Introduction

Soil microorganisms and their enzymatic activities play key roles in the biochemical functioning of soils, including soil organic matter formation and degradation and nutrient cycling [1]. However, much less is known on the status of

enzyme activities in semiarid regions as a function of land-use and management systems [2]. Additionally, the study of several enzyme activities together can provide information on the influence of soils, vegetation, and climatic factors on soil ecosystem functioning and quality [3]. This information would allow the selection of more sustainable and

economically feasible cropping systems that guarantee the viability of agricultural activities in semiarid soils [2].

Arbuscular mycorrhizal fungi (AMF) are one of the most widespread and important components of the soil microbiota in natural and agricultural systems [4]. They form symbiotic associations with their host plants and improve their water and nutrient uptake like phosphorus (P), nitrogen (N), and micronutrients, and act as biocontrol agents against plant pathogens [5]. Furthermore, species composition and productivity of plant communities were shown to be conditioned by AMF species richness and diversity [6]. In Senegal, a few studies were done on the diversity of AMF [7–9]. For instance, Duponnois et al. [8] studied the diversity of AMF in soils from different aged fallows, and Manga et al. [9] studied that from rhizosphere of *Acacia seyal*. Although the mycorrhizal symbiosis is considered as a key factor to sustain vegetation cover in natural habitats, there are little information about AMF community assembly associated with *Acacia senegal* trees, particularly when considering land-use systems (natural population versus plantation) and the geographical rainfall gradient (north-south transect) in Senegal.

Likewise, the above- and below-ground environment is directly and indirectly influenced by trees. Distinct tree species affect chemical and microbial properties of soil to different extents [10]. Moreover, soil microbiological properties can be differently affected by communities of the same tree species depending on its management system (e.g., natural stands versus plantations) [11].

*Acacia senegal* (L.) Willd. is a multifunctional leguminous tree widely distributed in arid and semiarid zones of Africa and the Middle East. It has long been used for gum and fuelwood production and medicinal products [12]. This legume tree has a remarkable adaptability to drought and substantially contributes to soil fertility replenishment owing to its ability to symbiotically fix nitrogen. This tree is also known to host AMF symbionts and can sustainably improve soil mycorrhizal potential. Thus, *A. senegal* is used in agroforestry systems and reforestation programs [13]. Connecting vegetation characteristics and key soil community features could be useful to better understand natural forest ecosystems functioning and impact of forest conversion to plantation [14]. Thus, the objectives of this study are (i) to evaluate whether soil mycorrhizal infectivity and diversity as well as overall soil microbial community activities are influenced by land-use systems and environmental conditions and (ii) to investigate how soil enzyme activities, nutrient contents, and AMF community distribution are interrelated in our contrasted situations.

## 2. Materials and Methods

**2.1. Study Sites and Samplings.** The study sites (Dahra and Goudiry) were situated along a northern-southern region transect and concomitantly along a rainfall gradient in Senegal. Dahra, an arid zone, was located in the region of Louga (northern part of Senegal and at 300 km from Dakar, Lat. 15° 21 N; Long. 15° 29 W). Goudiry, a semiarid zone, was situated in the region of Tambacounda (southern part of Senegal and at 650 km from Dakar, Lat. 14° 11 N, Long.

12° 43 W). The average annual rainfall ranges from 300 to 400 mm and from 600 to 1000 mm, respectively, at Dahra and Goudiry. On each site, a natural population (naturally occurring plants) and a plantation of *A. senegal* (installed by humans by transplanting saplings of *A. senegal*) were selected. Each stand of *A. senegal* plantation is constituted of 4 plots and each plot comprised 25 trees. Stands of *A. senegal* natural population were constituted by trees of *A. senegal* irregularly distributed together with other species (*Ziziphus* spp., *Combretum* spp., *Acacia seyal*, etc.).

On each plot of plantation or natural stand, 5 trees were randomly chosen, soil samples were collected in the rhizosphere of the selected trees from the 0–25 cm layer and also in bulk soils. The soil samples from each origin were then mixed to obtain a composite soil sample. In each site (Dahra and Goudiry), soils were taken in dry (February 2008) and rainy (August 2008) seasons. A total of 12 composite soil samples (3 types x 2 sites x 2 seasons) were obtained for all sites and seasons. The composite samples were sieved (2 mm) and stored at 4°C.

In each plot of plantation or natural stand, 5 trees were randomly chosen and fine roots of *A. senegal* plants were collected in rainy season and pooled to get a composite sample.

**2.2. Physical and Chemical Characterization of Soils.** The analysis of physicochemical characteristics of soils (Table 1) was performed at LAMA (Laboratoire des Moyens Analytiques, certified ISO 9001 version 2000, IRD, Dakar, Senegal). The soil was sandy and sandy silt, respectively, at Dahra and Goudiry. Soil total C, N, and P contents were higher in *A. senegal* natural population than in *A. senegal* plantation and bulk soils. Hence, these mineral contents were greater in Goudiry than in Dahra. In contrast, soil available P content was higher in soils from Dahra than those from Goudiry. Soil pH was slightly acid in both sites.

**2.3. Assessment of *A. senegal* Root Colonization Rate.** Root systems of *A. senegal* trees were cleared in a 10% KOH solution for 1 h at 90°C and stained with Trypan blue (0.05%) for 30 min [15]. Root segments (1 cm) were mounted on a microscope slide and observed under a compound microscope. The proportion of root length colonized by AMF was estimated [16].

**2.4. AM Fungal Spore Extraction and Enumeration.** In each site, as well as in plantation and in natural population of *A. senegal* and bulk soil, spores were recovered from a 100 g subsample soil (with 10 replicates per soil type) by wet sieving, decanting, and sucrose gradient centrifugation methods [17]. Then, the supernatant was poured successively through 50, 100, 200, and 400 µm pore-size sieves and rinsed with running tap water. Spore density (total number of spores in 100 g of dry soil) was determined by counting spores with a normal appearance under a compound microscope (40X).

**2.5. Determination of AM Fungal Species Composition**

**2.5.1. AM Fungal Trap Culture and Spore Isolation.** Trap culture was achieved for 5 months under glasshouse conditions.

TABLE 1: Physicochemical characteristics of soils from Dahra and Goudiry (in Senegal).

	Sites and soil origins					
	PL	Dahra NP	BS	PL	Goudiry NP	BS
Clay (%)	3.5b	5.53c	2.60a	6.86d	6.46d	6.76d
Silt (%)	10.3a	11.00a	10.25a	19.1c	18.23c	15.2b
Sand (%)	85.3cd	83.10c	85.97d	73.87a	75.46a	79.32b
Total carbon (%)	0.52b	0.83c	0.41a	0.77c	1.71d	0.74c
Total nitrogen (%)	0.05a	0.08c	0.05a	0.07c	0.16d	0.06b
C/N ratio	10	10	9	11	11	12
Total phosphorus (mg/kg)	49.33b	83.03c	33.12a	79.33c	166.00d	53.00b
Available phosphorus (mg/kg)	8.00a	29.68c	12.66b	8.29a	9.16a	7.47a
Ca (%)	0.92ab	1.11b	0.56a	1.33bc	6.44d	1.69c
Mg (%)	0.42a	0.56a	0.29a	0.42a	3.58b	0.53a
Na (%)	0.11ab	0.15b	0.09ab	0.15b	0.03a	0.14b
K (%)	0.20a	0.29b	0.19a	0.28b	0.90c	0.27b
pH (H <sub>2</sub> O)	6.05	6.11	5.71	6.13	6.53	6.23

PL: plantation of *Acacia senegal*; NP: natural population of *Acacia senegal*; BS: bulk soil. In the same row, values followed by the same letter are not significantly different according to the Student-Newman-Keuls test ( $P < 0.05$ ).

For each composite soil, 250 g of it were placed into 2 kg pots, containing 1 kg of sterilized (121°C, 2 hours) sand. *Zea mays* (L.) was sown as a trap plant with a density of 5 seeds per pot, with 4 replicates per soil origin. Pots were watered every two days with deionized water. Once a month, pots were fertilized with 100 ml of Long Ashton's nutritive solution [18]. After 5 months, spores of AMF were isolated from the trap culture by wet sieving and decanting method. On the basis of differences in spore morphology (color under transmitted light, shape, size, and wall ornamentation), various taxa were recognized [19].

**2.5.2. DNA Extraction from Spores and Amplification.** Ten spore types were identified and DNA was extracted from each spore type using the Purelink Plant Total DNA Purification Kit (Invitrogen, France) following the manufacturer's instructions.

The large subunit (LSU) region of the nuclear rDNA was used as target region for the PCR experiment. DNA was amplified in a 25  $\mu$ L reaction volume containing 5  $\mu$ L of 5X PCR reaction buffer, 0.3  $\mu$ L GoTaq polymerase (0.5 U), 2  $\mu$ L dNTPs (2.5 mM), 0.625  $\mu$ L each primer (20  $\mu$ M), 0.5  $\mu$ L BSA (10 mg/mL), with 3  $\mu$ L DNA template. The first fungal DNA amplification was performed using the fungal primers LR1/NDL22 [20]. The PCR was carried out as follows: 5 min at 93°C, followed by 35 cycles of 1 min at 93°C, 1 min at 58°C, 1 min at 72°C, and a final elongation of 10 min at 72°C. A 1  $\mu$ L aliquot of the first PCR product was directly used as template for the second PCR amplification using the specific primers FLR3/FLR4 [21] under conditions described above. The nested PCR products were analyzed in electrophoresis using a 1% agarose gel (Sigma, France) in a Tris-Acetate-EDTA buffer with a DNA size standard (Eurogentec SmartLadder). The amplified fragments of about 400 pb were excised under UV light and purified with the Purelink Gel Extraction Kit (Invitrogen, France) according to the manufacturer's instructions.

**2.5.3. Cloning, Sequencing, and Phylogenetic Analysis.** Two-round PCR products of the two AMF spore types (FNSP2 and FNSP8) were subcloned into XL-2 blue using the pGEM-T Easy Vector (Promega/Catalys, Wallisellen, Switzerland) following the manufacturer's instructions. Ten positive clones for each spore type were reamplified using the primers pair FLR3/FLR4 and the products were revealed on agarose gel. Bands obtained were excised and purified as described above.

The purified PCR products were sequenced using primers FLR3/FLR4. Sequencing reactions were analyzed on a 3730 XL (Applied Biosystems) 96 capillary sequencers using a BigDye 3.1 Sequencing Kit (Genoscreen, France). Sequences were corrected using ChromasPro v1.33 (Technelysium Pty) and sequence similarities were determined by using the BLAST sequence similarity search tool [22] provided by GenBank. Corrected sequences were submitted to GenBank and were allocated with accession numbers. Multiple alignments and maximum likelihood tree were performed using the programs ClustalX [23] and MEGA version 5 [24], respectively.

**2.6. Enzymatic Activities Assessment.** The activity of 4 enzymes (fluorescein diacetate, dehydrogenase, and acid and alkaline phosphatases) was measured on the rhizospheric soil samples. For each enzymatic determination, controls with twice-autoclaved soil samples were included for nonenzymatic decomposition of the soil solution. The concentrations of enzymatic hydrolysis products were determined by spectrophotometer and compared with a standard curve.

**2.6.1. FDA Activity.** Total soil microbial activity potential was measured through fluorescein diacetate (3, 6'-diacetylfluorescein) hydrolysis assay, according to Alef et al. [25]. After 1 h of incubation on a rotary shaker, the fluorescein released from FDA was measured in the supernatant at  $\lambda = 490$  nm and expressed as  $\mu$ g FDA/h/g of soil.

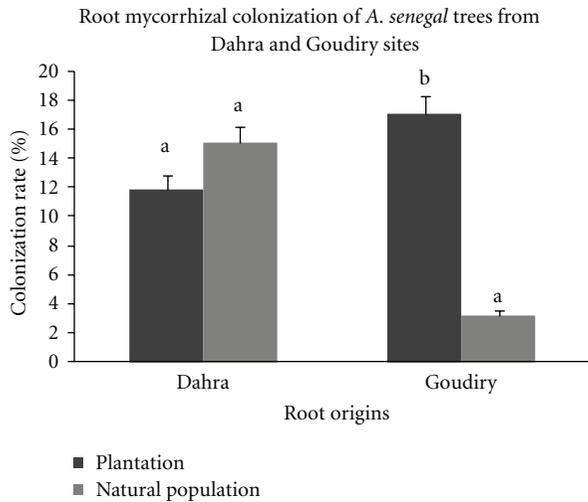


FIGURE 1: Mycorrhizal colonization of roots from *A. senegal* plantations and natural stands in Dahra and Goudiry (in Senegal). For each site, bars with the same letter are not significantly different according to the Student-Newman-Keuls test ( $P < 0.05$ ).

**2.6.2. Dehydrogenase Activity.** Dehydrogenase activity was measured following the method of Skujiņš [26]. After 24 h of soil incubation in a triphenyl tetrazolium chloride (TTC) solution and a Tris-HCl buffer in darkness, followed by a second incubation at 37°C for 2 h on a rotary shaker, the extracted formazan was estimated at  $\lambda = 546$  nm. Its concentration was calculated from a standard curve and dehydrogenase activity was expressed as  $\mu\text{g}$  formazan/h/g of soil.

**2.6.3. Acid and Alkaline Phosphatase Activities.** Acid and alkaline phosphatase activities were assayed using a colorimetric determination of p-nitrophenol released when soil was incubated (during 1 h at 37°C) with p-nitrophenyl phosphate as substrate (pNPP, 5 mM) in pH 6 and pH 11 buffers, respectively [27]. The amount of p-nitrophenol released was determined by reading the optical density at  $\lambda = 400$  nm and expressed as  $\mu\text{g}$  pNPP/h/g of soil.

**2.7. Statistical Analyses.** Data were subjected to a one-way analysis of variance (ANOVA) using the SPSS software version 13. Mean values were compared using the Student-Newman-Keuls range test ( $P < 0.05$ ). Percentage data of root colonization were arcsine transformed prior to analysis. Three-factor analysis of variance was performed using the Student-Newman-Keuls range test ( $P < 0.05, 0.001$ , and  $0.0001$ ) of the XLSTAT version 13, in order to determine the main effects of sites, zones, and seasons and their interactions on soil enzyme activities.

The biochemical variables were grouped into 3 classes: (1) soil physicochemical properties including total organic C, N, P, and available P, soil pH and physical properties; (2) soil mycorrhizal parameters including AMF spore density and richness and root colonization rates; (3) enzymes variables including FDA hydrolysis, dehydrogenase, and acid and alkaline phosphatases. STATIS method was conducted on the

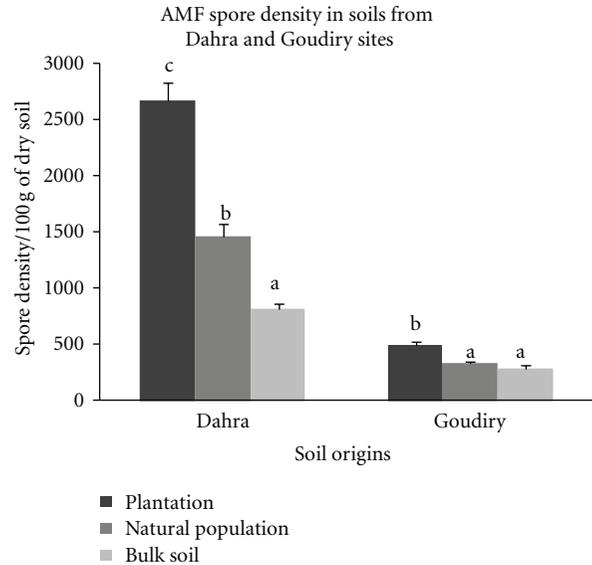


FIGURE 2: AM fungal spore density in soils from Dahra and Goudiry (in Senegal). For each site, bars with the same letter are not significantly different according to the Student-Newman-Keuls test ( $P < 0.05$ ).

soil physicochemical variables, as well as on the vegetation cover and soil microbial properties, to determine how these variables were interrelated. The STATIS method is introduced by Escoufier [28] and is used to analyze multiple data tables, each with information from the same set of individuals. It identifies what tables are alike, provides a summary table of all and describes the differences and similarities between the tables in relation to this summary table. These differences and similarities between said tables are analyzed by means of a structure called the compromise. The main steps of the STATIS method are interstructure, compromise, and intrastructure. A standardized principal component analysis (PCA) was used to balance the influence of different parameters. The implementation of this method was performed in the R environment using the software ADE4.

### 3. Results

**3.1. Mycorrhizal Colonization of *A. senegal* Roots and Soil Spore Density.** In Dahra, the natural occurrence of arbuscular mycorrhizas in *A. senegal* roots was not significantly different between natural population and plantation. However, in Goudiry, colonization rate was significantly lower in the natural stand compared to the plantation that presented value of root colonization not significantly different from those of the Dahra site (Figure 1).

The density of AMF spores varied highly among the treatments from 290.71 spores per 100 g of soil (in bulk soil of Goudiry) to 2673.29 spores per 100 g of soil (in soil collected from *A. senegal* plantation in Dahra). In both sites, AMF spore abundance was significantly higher in soil samples from *A. senegal* plantations than those from natural populations and bulk soils (Figure 2).

TABLE 2: AMF species richness in soils from plantations, natural stands of *Acacia senegal*, and bulk soils in Dahra and Goudiry (Senegal).

Morphotypes of AMF spores	Plantation of <i>A. senegal</i>		Natural stand of <i>A. senegal</i>		Bulk soil	
	Dahra	Goudiry	Dahra	Goudiry	Dahra	Goudiry
FNSP1 ( <i>Glomus sp1</i> )	+	+	+	+	+	+
FNSP2 ( <i>Glomus sp2</i> )	+				+	
FNSP3 ( <i>Glomus sp3</i> )	+	+	+	+	+	+
FNSP4 ( <i>Gigaspora sp1</i> )	+	+	+	+	+	+
FNSP5 ( <i>Scutellospora sp1</i> )	+					
FNSP6 ( <i>Glomus sp4</i> )	+	+	+	+	+	
FNSP7 ( <i>Glomus sp5</i> )	+					
FNSP8 ( <i>Acaulospora sp1</i> )	+					
FNSP9 ( <i>Acaulospora sp2</i> )			+		+	
FNSP10 ( <i>Acaulospora sp3</i> )						+
AMF species richness	8	4	5	4	6	4

TABLE 3: Enzyme activities of soils from Dahra and Goudiry sampled during the dry season.

Sites	Soil origins	Enzyme activities			
		FDA ( $\mu\text{g FDA/h/g}$ of soil)	Dehydrogenase ( $\mu\text{g INTF/h/g}$ of soil)	Acid phosphatase ( $\mu\text{g pNPP/h/g}$ of soil)	Alkaline phosphatase ( $\mu\text{g pNPP/h/g}$ of soil)
Dahra	Plantation	0.344a	0.040a	67.937a	1.905ab
	Natural population	0.388a	0.131b	146.349b	2.381b
	Bulk soil	0.290a	0.032a	86.667a	1.111a
Goudiry	Plantation	0.757a	0.319b	208.254a	13.860a
	Natural population	1.710c	0.099a	416.190b	132.857b
	Bulk soil	1.179b	0.094a	427.619b	137.460b

For each site and in each column, values followed by the same letter are not significantly different according to the Student-Newman-Keuls test ( $P < 0.05$ ).

**3.2. AMF Species Richness and Community Composition.** Ten AM fungal morphotypes belonging to 4 genera (*Glomus*, *Scutellospora*, *Acaulospora*, and *Gigaspora*) were isolated and identified as well in the rhizosphere of *A. senegal* in plantations and natural populations as in bulk soils (Table 2, Figure 3). In Dahra, 5 and 8 morphotypes representing 4 genera were found in *A. senegal* natural population and plantation, respectively, compared to 6 morphotypes within 3 genera in bulk soil (*Glomus*, *Gigaspora*, and *Acaulospora*). In Goudiry, rhizosphere of *A. senegal* plantation and natural population had the same number of AMF species (4) representing 2 genera (*Glomus* and *Gigaspora*). Finally, the bulk soil displayed 4 morphotypes within 3 genera (*Glomus*, *Gigaspora*, and *Acaulospora*). Three AMF spore morphotypes (FNSP1, FNSP2, and FNSP4) were found in all sites, while FNSP2 and FNSP9 were detected only at Dahra site. The AMF spore types FNSP5, FNSP7, and FNSP8 were only observed at the *A. senegal* plantation of Dahra and FNSP10 only at the bulk soil of Goudiry.

BLAST search results of large subunit (LSU) sequences obtained from these AMF spores isolated from trap culture confirmed that the species belonged to the Glomeromycota. Results of the phylogenetic analysis of the LSU sequences (Figure 4) indicated the presence of 4 main groups: the first, *Acaulospora* group included FNSP9 and FNSP10 species which presented 92% of similarity with *Acaulospora longula* and the species FNSP8 which had 100% of homology with

*Acaulospora tuberculata*. The species FNSP4 belonged to the *Gigaspora* group and had 100% of similarity with *Gigaspora gigantea*. The third, *Glomus* group B included FNSP2 and FNSP3 species which presented 100% of homology with *Glomus globiferum*. The last, *Glomus* group A encompassed the species FNSP1 and FNSP7 which presented 82% and 98% of homology with *Glomus* sp. isolate HE577801 and *Glomus intraradices*, respectively.

Morphological and anatomical identification indicated that the morphotype FNSP5 belonged to *Scutellospora gregaria* and the morphotype FNSP6 to *Glomus* sp.

### 3.3. Effect of Land Use and Moisture on Soil Enzyme Activities

**3.3.1. Soils Collected in Dry Season.** In Dahra, FDA hydrolysis activity was not significantly different between soils from the rhizosphere of *A. senegal* plantation, natural population, and bulk soil. By contrast, in Goudiry, soils from *A. senegal* natural population and bulk soil had the highest FDA hydrolysis activity. Furthermore, dehydrogenase activity was higher in soil from *A. senegal* natural population in Dahra, whereas in Goudiry, the greatest activity was found in soil from *A. senegal* plantation. In Dahra, the activity of acid and alkaline phosphatases was significantly greater in soil from natural population, whereas in Goudiry, the best responses were observed in soils from rhizosphere of *A. senegal* natural population and bulk soil (Table 3).

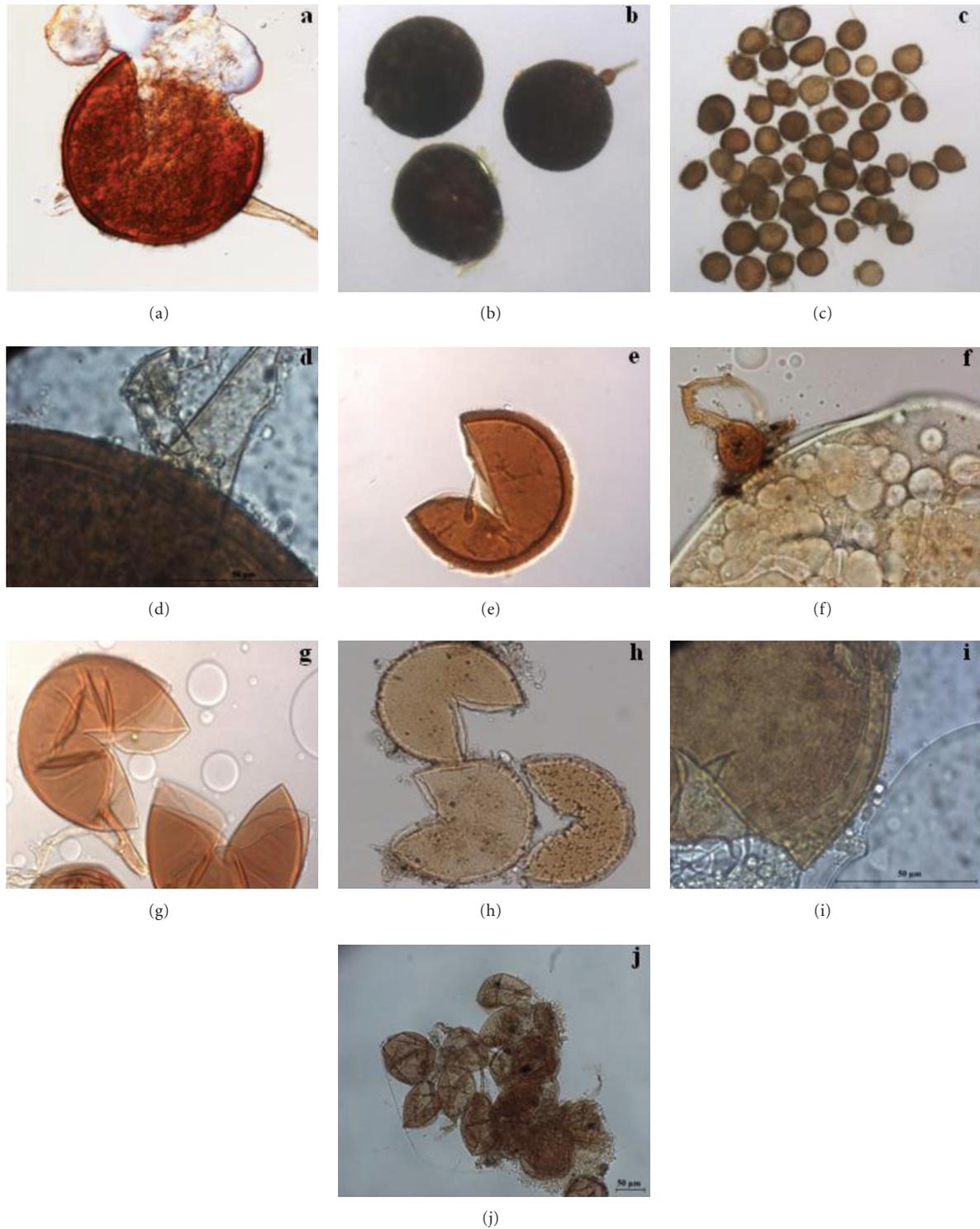


FIGURE 3: Spores of arbuscular mycorrhizal fungi isolated in soils from Dahra and Goudiry sites after trap culture. (a) *Glomus* sp. isolate FNSP1 in PVLG reagent, (b) *Scutellospora* sp. aff *gregaria* isolate FNSP5, (c) *Glomus* sp. isolate FNSP6, (d) *Glomus globiferum* isolate FNSP2 in PVLG + Melzer reagent, (e) *Glomus globiferum* isolate FNSP3 in PVLG + Melzer reagent, (f) *Gigaspora gigantea* isolate FNSP4 in PVLG reagent, (g) *Glomus intraradices* isolate FNSP7 in PVLG + Melzer reagent, (h) *Acaulospora tuberculata* isolate FNSP8 in PVLG + Melzer reagent, (i) *Acaulospora longula* isolate FNSP9 in PVLG + Melzer reagent, (j) *Acaulospora longula* isolate FNSP10 in PVLG + Melzer reagent.

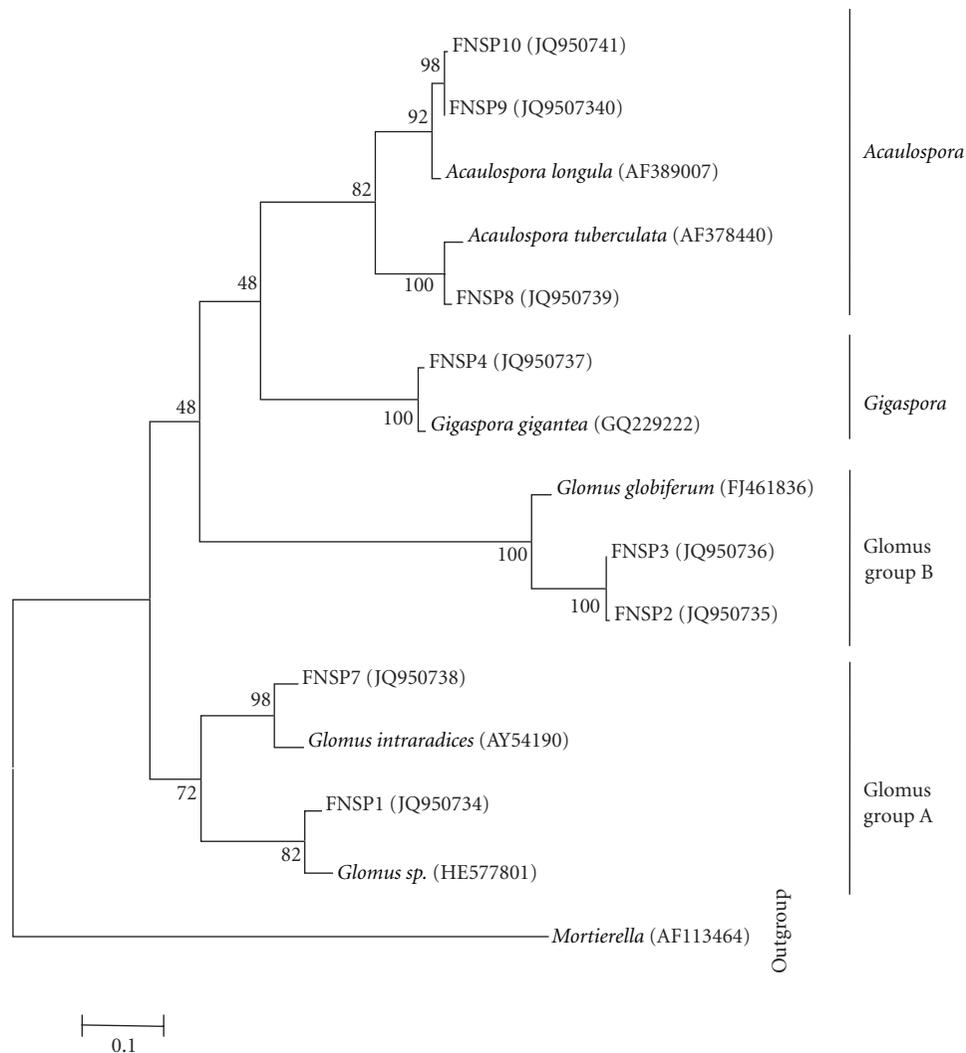


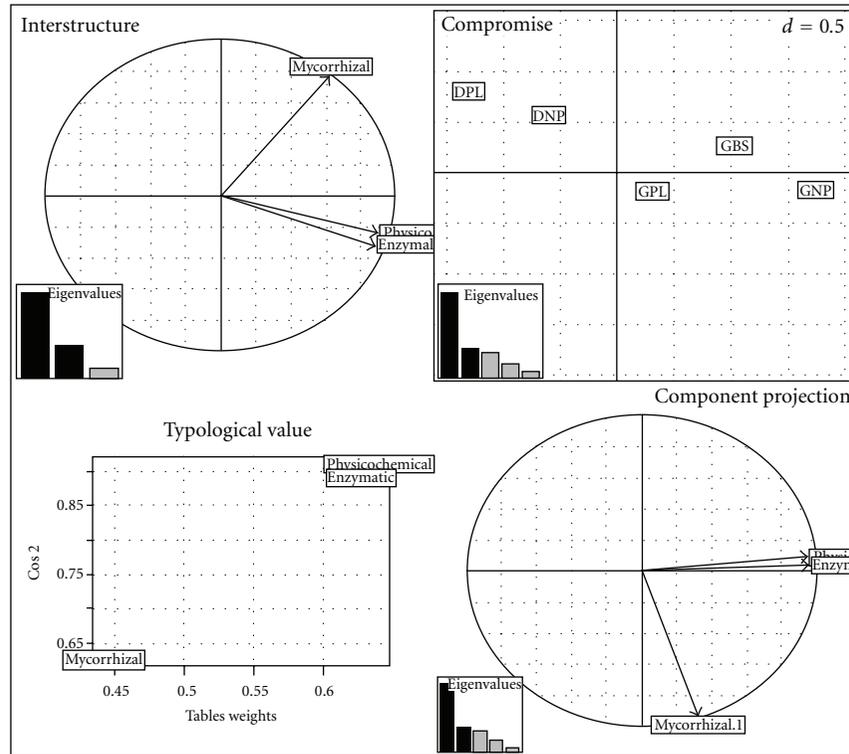
FIGURE 4: Maximum likelihood tree of partial LSU rDNA sequences from AMF spores from trap culture under soil samples collected in different origins in Senegal. Known AMF sequences from the NCBI database are included. Bootstrap values were estimated from 100 replicates and *Mortierella polycephala* was used as outgroup.

**3.3.2. Soils Collected in Rainy Season.** Table 4 presents the results obtained on the activity of soil enzymes in rainy season. FDA, dehydrogenase, and acid phosphatase activities were significantly higher in soils from *A. senegal* natural population and lower in those from bulk soils, in both sites. In Dahra, the highest alkaline phosphatase activity was observed in soil from *A. senegal* plantation and the lowest in bulk soil. In Goudiry, the greatest alkaline phosphatase activity was recorded in *A. senegal* natural population and the lowest in that from *A. senegal* plantation.

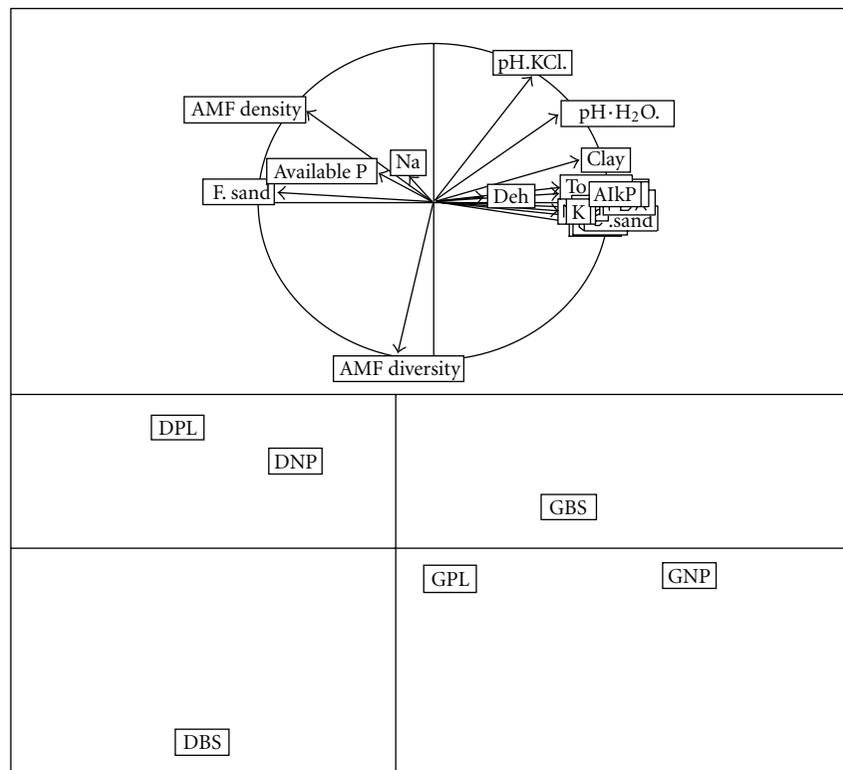
**3.4. Effects of Factors and their Interactions on Soil Enzyme Activities.** The effects of different factors and their interactions on soil enzyme activities are summarized in Table 5. The effects of factors (sites, zones, and seasons) were highly significant ( $P < 0.0001$ ) on the activities of all enzymes except that of sites for acid phosphatase activity. The dual ((sites x zones), (sites x seasons), and (zones x seasons)), and

triple (sites x zones x seasons) interactions were highly significant ( $P < 0.0001$ ) for FDA, dehydrogenase, and alkaline phosphatase activities. For acid phosphatase activity, the effects of (sites x seasons) and (zones x seasons) were highly significant and those of (sites x zones) and (sites x zones x seasons) significant at  $P < 0.05$ .

**3.5. Relations between All Datasets.** The plan of the first two axes of the interstructure (Figure 5(a)) explained 92.43% of the total variability and showed the isolation of mycorrhizal parameters from the physicochemical and enzymes tables. The results of the intrastructure gave a matrix vector correlation between the 3 tables. They showed a strong positive correlation between soil physicochemical properties and enzyme activities datasets (coefficient of variation  $RV = 0.77$ ). In contrast, mycorrhizal parameters values were weakly correlated with soil physicochemical characteristics (coefficient of variation  $RV = 0.38$ ) and enzyme activities variables (coefficient of variation  $RV = 0.33$ ).



(a)



(b)

FIGURE 5: Interstructure and compromise from STATIS analysis: Interstructure and compromise (a) and intrastructure (b) of variables and sites. DPL: Dahra plantation of *Acacia senegal*, DNP: Dahra natural population of *Acacia senegal*, DBS: Dahra bulk soil, GPL: Goudiry plantation of *Acacia senegal*, GNP: Goudiry natural population of *Acacia senegal*, and GBS: Goudiry bulk soil.

TABLE 4: Enzyme activities of soils from Dahra and Goudiry sampled during the rainy season.

Sites	Soil origins	Enzyme activities			
		FDA ( $\mu\text{g FDA/h/g}$ of soil)	Dehydrogenase ( $\mu\text{g INTF/h/g}$ of soil)	Acid phosphatase ( $\mu\text{g pNPP/h/g}$ of soil)	Alkaline phosphatase ( $\mu\text{g pNPP/h/g}$ of soil)
Dahra	Plantation	0.633b	0.000a	1488.095b	299.191c
	Natural population	1.971c	0.069b	1994.921c	219.937b
	Bulk soil	0.322a	0.000a	816.825a	21.365a
Goudiry	Plantation	0.883a	0.010b	1173.651b	22.349a
	Natural population	1.411b	0.047c	1607.937c	291.341c
	Bulk soil	0.786a	0.000a	591.429a	155.885b

For each site and in each column, values followed by the same letter are not significantly different according to the Student-Newman-Keuls test ( $P < 0.05$ ).

TABLE 5: Significance level ( $F$ -values) of effects of different factors and their interactions on soil enzyme activities based on three-way analysis of variance (ANOVA).

Enzyme activities	Sites	Zones	Seasons	Zones $\times$ sites	Zones $\times$ seasons	Sites $\times$ seasons	Sites $\times$ zones $\times$ seasons
FDA ( $\mu\text{g FDA/h/g}$ of soil)	205.42***	221.13***	47.53***	11.09**	53.99***	162.52***	68.42***
Dehydrogenase ( $\mu\text{g INTF/h/g}$ of soil)	433.18***	261.46***	1701.91***	445.22***	256.83***	507.92***	308.43***
Acid phosphatase ( $\mu\text{g pNPP/h/g}$ of soil)	1.11 <sup>ns</sup>	266.08***	2754.66***	3.91*	251.62***	185.71***	3.43*
Alkaline phosphatase ( $\mu\text{g pNPP/h/g}$ of soil)	55.5***	422.14***	1307.31***	674.78***	457.23***	564.61***	153.71***

\*, \*\*, \*\*\*, and ns indicate the level of significance at  $P < 0.05$ , 0.001, 0.0001, and the absence of significance, respectively.

For intrastructure of variables, the plan 1-2 of the compromise explained 73.92% of the total variability (Figure 5(b)). The first source of variability between groups of variables appeared on the axis 1 and concerned the physicochemical characteristics of soils and enzyme activities parameters. Soils rich in clay and silt were also rich in total C, N, and P and in base elements. In contrast, soils rich in sand and available P were rich in AMF spore density and species number. For intrastructure of sites, the analysis of the Figure 5(b) showed a classification of sites into 3 main groups. The first group was formed by the 3 soils from Dahra (DPL, DNP, and DBS), which were sandy and characterized by high amount of available P, AMF density and diversity, and low enzyme activities. The group 2 was composed by the soil from *A. senegal* plantation of Goudiry (GPL) rich in clay, silt, and Na and characterized by high dehydrogenase activity. Finally, the group 3 encompassed the soil from *A. senegal* natural population and bulk soil of Goudiry (GNP and GBS) which were rich in clay and silt with high amounts of total C, N, and P, correlated to high FDA and phosphatases activities.

#### 4. Discussion

The present study clearly underlined that AMF density and diversity, and soil enzyme activities were strongly influenced by land-use systems, soil nutrient contents, and moisture. It also indicated that interrelations exist between the above parameters along our northern-southern transect in Senegal.

*4.1. Effects of Tree Plantation and Soil Moisture on AM Fungal Spore Abundance and Diversity and on Root Colonization.* Arbuscular mycorrhizal fungi (AMF) play an essential role

in ecosystem functioning by influencing nutrient fluxes and organism interactions [29]. A set of reports has underlined the effect of vegetation on AMF formation, density, and diversity [30]. In this present work, the total number of spores per 100 g of dry soil was higher in rhizospheric soil from *A. senegal* than in bulk soil for all studied sites. This was in accordance with results of Karthikeyan and Selvaraj [31] on rhizospheric and nonrhizospheric soils of *Ipomoea pes-caprae* and *Phyla nodiflora* and also with observations made by Namenasrullah and coworkers [32] on wheat and maize crops under soils from different localities. These results suggested that AMF spore abundance varies depending on soil physicochemical properties and moisture as well as on vegetation cover. The presence of vegetation cover improves soil microbial biomass and activity by facilitating a vast network of AM hyphae and spores interconnecting the roots of the plant cover [33]. It was reported that lower plant density and vegetation supply less carbohydrates to the soil than needed by AMF [34], significantly reducing spores number and thus limiting AM infection. Importantly, soil texture may influence spore density [35]. Moreover, it can be inferred from our results that AMF spore density was greater in plantation than in natural population of *A. senegal*. Variations observed in soil physicochemical properties and plant density may encourage such AMF spores distribution [8, 36]. This present study reveals that AMF spore density and diversity were greater in Dahra (dry region) than in Goudiry (wet region). In wet soil, water availability would increase fungal mycelium growth for root colonization, leading to a decrease on spore germination [5]. The study also illustrates the diversity of AMF spores under soils from different land

uses and climatic zones. Ten AMF morphotypes belonging to 4 genera (*Glomus*, *Acaulospora*, *Scutellospora*, and *Gigaspora*) were detected in the soils. These results were comparable to the diversity of AMF described in Senegal [7, 8] and in other countries [37]. In contrast, Opik et al. [38] described 34 different AMF taxa in a single habitat (a boreal herb-rich coniferous forest). Among the 10 AMF spores described here, the FNSP5 species closed to *Scutellospora gregaria* and the FNSP7 species closed to *Glomus intraradices* were previously described in Senegal by Duponnois et al. [8] and Manga et al. [9], respectively. The genus *Glomus* is the most abundant in these investigated sites and in many ecologically different environments from natural stands to managed agroecosystems in Senegal [7, 9] and other countries [39, 40]. Its dominance in arid and semiarid regions might be due to their remarkable adaptation to drastic conditions such as drought and salinity [41]. Our findings indicated that AMF species richness varied between sites (Dahra, Goudiry) and sampling areas (plantation, natural sand, and bulk soil). AMF species richness was more important in Dahra than in Goudiry. Klironomos and Hart [42] described large between- and within-site variations in the composition of AMF communities. Physical and chemical properties of soil and climatic factors such as soil moisture might explain differences observed between Dahra and Goudiry sites [43, 44]. The effect of land uses (plantation versus natural population) on the diversity of AMF was noticed in the site of Dahra, but not in that of Goudiry. Opik and coworkers [38] found in a boreal herb-rich coniferous forest that management practices had no adverse impact on the AMF biodiversity. These results suggested that the relationship between management practice and AMF richness is complex and may be positive [45] or negative [46]. Thus, AMF spore density and diversity can vary depending on edapho-climatic conditions including rainfall, soil texture, and management practices [30].

In order to ensure plants efficient growth, especially under semiarid regions which are often characterized by low levels of P availability in soil [47], it has been suggested to boost the native soil mycorrhizal potential through the culture of highly mycotrophic plants or through the controlled mycorrhization [48]. However, as it was noticed by Kumar et al. [49] on *Sida cardifolia* in different locations, variations were observed in the rates of AM colonization between root sampling sites. These variations could be attributed to factors such as soil nutrient content and fertility, soil moisture, changes in microhabitat, and acclimatization of a particular AMF genus/species to a particular location [49].

#### 4.2. Spatial and Seasonal Variations of Soil Enzyme Activities.

Soil enzyme activities in a forest floor are influenced by several variables such as nutrient availability and input of leaf litter [50]. Results from this work confirmed previous reports indicating that enzyme activities could vary with land-use systems and environmental factors [51, 52]. In Dahra, soil enzyme activities were higher in the rhizosphere of *A. senegal* and lower in bulk soil, whereas in Goudiry, the highest values (except dehydrogenase activity) were obtained in the

rhizosphere of *A. senegal* natural population. These findings could be related to the differences in soil nutrient contents between soil under *A. senegal* and bulk soil. Importantly, increased enzyme activities could be attributed to increased organic matter and litter quality and quantity as well as improved soil physical parameters [53]. Particularly, legumes (by their rhizodeposition rich in amino acids and soluble sugars) help in solubilizing insoluble P in soil, improving the soil physical environment, and raising soil microbial activity [54].

When a between-sites comparison was performed, we noted that enzyme activities were greater in Goudiry than in Dahra. For instance, soils from Goudiry contained low available P but the highest total P and phosphatases activity. Similar results have been obtained by Gianfreda et al. [55] and recently by Wang et al. [56]. It is known that when available P is deficient in soil, soil biota can stimulate the production of extracellular phosphatases to increase the supply of inorganic P in soil [56]. As the two sites differ in their ecological parameters including soil moisture and soil fertility, it could be assumed that these parameters, which constitute key factors controlling the growth and survival of soil microbes [57], might have encouraged such spatial variation in enzyme activities.

The results indicated that soil enzyme activities studied here (except dehydrogenase activity) were greater in rainy season than in dry season. This enhancement could be attributed to an increase in water availability. Soil drying is known to reduce microbial activity and mineralization of organic C, N, and P [58] to decrease microbial mobility [59] and to restrict substrate and nutrient availability [60]. Interestingly, Fall et al. [61] in their study implemented in Senegal recorded a stimulation of microbial biomass in rhizosphere of *A. senegal* during the wet season. Finally, changes in the chemical composition of litter during its decomposition might also contribute to seasonality in enzyme activities [62].

**4.3. Relationships between All Datasets.** Results reported here showed that a set of relationships exists between soil enzyme activities, mycorrhizal variables, and physicochemical properties. Soil physicochemical properties are strongly related to the activities of studied enzymes ( $RV = 0.77$ ). Enzyme activities are in certain soils (GNP, GBS) positively correlated with total C and total N, as previously found by Acosta-Martinez et al. [2]. The positive correlation between enzyme activities and total N may be related to the low organic matter content of semiarid soils. However, a weak correlation between soil physicochemical properties and mycorrhizal datasets was noticed ( $RV = 0.33$ ). Several studies have indicated that soil nutrient content mainly phosphorus may impact AMF [7]. For instance, Grant et al. [63] has found that when the available P increases in soil, the amount of P also increases in the plant, and carbon drain on the plant by the AMF symbiosis becomes nonbeneficial to the plant. Our findings converged towards this affirmation when we considered soil samples from Goudiry, which had low number of AM spores concurrently with high level of total N, P, and C contents and phosphatase activity (which

may further result in greater mobilization of P for plant nutrition). Nevertheless, differences in AMF spore density and species richness were observed in some cases and were not directly related to soil physicochemical characteristics. Other factors such as abiotic conditions (soil moisture and temperature, temporal and spatial variation in organic nutrient availability, etc.) and biotic constraints including the level of microbial degradation of enzyme molecules in soil might further be taken into consideration to fully address the question.

## 5. Conclusion

The present study suggests a positive effect of *A. senegal* trees on soil mycorrhizal potential and enzyme activities. It also indicates that in sahelian regions, AM fungal spore density and diversity as well as soil microbial functions can be influenced by land-use systems (plantation versus natural population of *A. senegal*) and environmental conditions such as moisture and soil nutrient contents. Strong relationships between enzyme activities and soil physicochemical properties have been noticed. This underlines the importance of prior natural AMF screening for better combinations of *A. senegal* seedlings with AMF species to achieve optimum plant growth improvement and environment protection.

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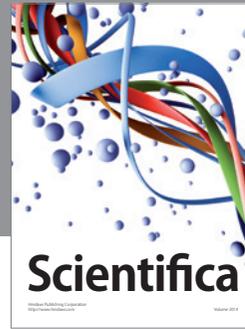
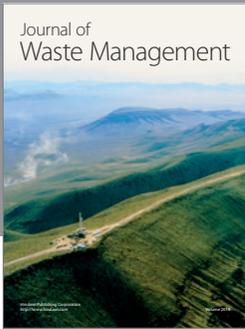
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