

Full Length Research Paper

Impact of previous legumes on millet mycorrhization and yields in sandy soil of West African Sahel

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A preliminary study was conducted during the raining seasons 2012 through 2015 to assess the status of arbuscular mycorrhiza fungi associated with 4 legumes and millet on the sandy soil of Sador  , Niger. A factorial completely randomized block design was used for the layout. Crop roots parameters of mycorrhization and soil fungi spore density and biodiversity were investigated as responses to varied planting densities, rates of rock phosphate, and of urea application on millet monoculture. Spores of *Glomus* were present in 100% of plots with respectively 96%, 47%, 77%, and 13% as relative frequency during rainy seasons 2012, 2013, 2014, and 2015; but spores of *Gigaspora* were present in 70% of plots and with 3.63%, 4%, 1.38%, and 1% as relative frequency the same years. Spore density/100g of root zone soil varied with crop species and rate of applied crop residue. The maximum intensity of mycorrhization was 78% while the arbuscular had a rate of 48% as maximum. The parameters of mycorrhization were influenced by the crop species but not by the rates of applied rock phosphate and the rate of returned crop residues as well. Millet yield in monoculture was affected by residual effects of previous basis legume crop.

Key words: Preceding crops, millet mycorrhiza, residue, sandy soil, Niger.

INTRODUCTION

Pearl millet is an important staple food crop in African and Asian regions (Vidushi and Yassir, 2013). In the semi-arid countries of West Africa Sahel (WAS) such as Niger, millet is a well-adapted warm and dry land cereal crop. It is grown primarily on sandy soils, mostly characterized by a low organic content, poor water and nutrient holding capacities, and high acidic pH (Bado et al., 1997; Bationo and NTare, 2000; Adamou et al., 2007; Salou et al., 2011). However, millet production is affected by many biotic and abiotic constraints including poor

income capacity of farmers (Bationo et al., 2004), low soil fertility, inappropriate soil management practices, monocropping, poor application of organic and mineral fertilizers (Adamou et al., 2007; Salou et al., 2011). Drought and pests can also affect yield. Soil phosphorus and nitrogen deficiencies are also a major concern (Salou et al., 2011; Bado et al., 2012). Although, the use of bio-fertilizer such as arbuscular mycorrhizal fungi (AMF) are not well known by farmers in WAS, these are used to significantly increase crop yields in Asian and

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Mediterranean countries of North Africa (Knopf et al., 2016). Some studies have shown how crop yield could be increased through the implementation of appropriate soil management techniques, which integrate biodiversity and soil fertility management (Garbaye, 2013; Fortin and Taktek, 2016). Many efforts have been developed to improve soil fertility management practices for Sub-Saharan regions (especially, in Niger). These include legume/cereal crop rotations and intercropping (Bationo and NTare, 2000), Zaï techniques, fertilizers and manure micro dosing (Tabo et al., 2007; Ibrahim et al., 2014). Many results of experiments advocated the importance of examining the relationship between host plants on the soils mycorrhizal fungi composition, richness, their relative density and diversity (Johnson et al., 1992). Some others highlighted the effects of cultural practices such as crop rotation and monoculture on indigenous soil mycorrhizal arbuscular fungi distribution within crop species growing area (Schenck and Kinloch, 1980). Plant root architectural characteristics have been said to influence their relationship with AMF fungi and also water and nutrient uptake (Passot et al., 2016). Therefore, the observations of crop root architecture could provide solid arguments while selecting crop species to be included in a crop rotation system. The mono cropping of millet is the most common practice for farmers in Niger. In the traditional system, crop residues are exported as livestock feeds, fencing, and also for diverse domestic uses. Power et al. (1998) and Bationo et al. (2004) reported that two nights of nocturne settlement of cattle over the field could produce 5 to 13% increase in millet yield compared to those without settlement. Available cheaper fertilizer (such as natural rock phosphate of Tahoua and restitution of crop residue), affordable cultural technique (such as crop rotation, using local species), and taking in account bio-fertilizers (such as mycorrhizal arbuscular fungi) could help improve soil fertility. The beneficial effects of legume crops as preceding crop to millet and soil natural bio-fertilizers could be exploited coupled with the agronomic advantages of the restitution of crop residues as a sustainable integrated strategy of soil fertility management for smallholder farmers of the Sahel.

These affordable technologies could be a reliable alternative to improving both soil fertility and crop yields. Therefore, there is a need to investigate the effect of cropping systems, such as crop rotations with legume crops on mycorrhizal arbuscular presence and abundancy. Three hypotheses were postulated; which are: (i) Legume based crop rotation with restitution of crop residue to the field have a positive impact on the mycorrhization and yield of millet crop; (ii) The parameters of mycorrhization and the fungal biodiversity are influenced by crop species (legumes/cereal) and (iii) The returned crop residue enhance the effect of rock phosphate on mycorrhization rate.

The specific objectives were to: (i) Evaluate the fungal

biodiversity associated with legume crop and millet in Sadore sandy soils; (ii) Determine the effects of the restitution of dry residues of the preceding crops on the mycorrhization and the yield of pearl millet, and (iii) Identify the best preceding crop that permits the highest rate of mycorrhization parameters of millet crop in monoculture.

MATERIALS AND METHODS

Experiment site

This study was conducted at the ICRISAT Sahelian Research Centre in the village of Sadore. Geographically, Sadore research centre is located at 45 km Southwest of the capital Niamey in the bioclimatic zone of Southern-Sahel (latitude 13° 14' N and longitude 2° 17' E, altitude 235 m).

Climate and precipitations

In most of the Sahel regions, the rainy season is short and limited to the period between May/June and September/October. The average normal rainfall (period 1920-1980) is about 560 mm (Sivakumar, 1989). The rainfall data from the last 4 years (2012-2015) showed that there is an inter-annual irregularity (Figure 1) with a peak of 740 mm in 2014. The mean normal rainfall is exceeded during the 4 years of the trial, which indicates a general irregular trend of rainfall in the area of Sadore. Average monthly maximum temperatures during the four years of trial fluctuated between 30.7 and 42.0°C, with the highest in May and the lowest in August. The highest maximum temperature (Figure 2) was recorded in May 2015 as 42.4°C; while the lowest was recorded the same year in May as 30.7°C. The highest evaporation value was recorded in May 2015 and August 2013 as 10.7 and 2.9 mm, respectively (Figure 3).

Soil properties

The soil is tropical, yellowish red, friable (Subbarao et al., 1999), sandy ferruginous, strongly acidic (pH = 4-5.2), low in fertility, and poor in organic matter (0.22%) (Saminou, 2003). The field capacity reaches 16.5 mm at 0 to 17 cm soil depth, while the wilting point is 1.7 mm at the same depth. But between 17 and 32 cm depth, field water capacity is limited to 7.4 mm; whereas, the permanent wilting point raises up to 2.5 mm (ICRISAT, 1990). The particle size analysis (Table 1) shows a coarse texture enriched with 90 to 95% of wind sands on 20 cm depth; the fine fraction comprised of 3 silt and 2.9% clay.

2012 Agronomic experiment

The experiment was carried out on a sandy soil, which had mucuna as the previously grown crop in 2011 growing season. A factorial randomized complete block (RCBD) was used in three replications with 30 plots treatments: $5 \times 2 \times 3 = 30$ experimental units. Main treatments included five species of crops, that is, four legumes (cowpea, dolichos, voandzou and sesbania) and one cereal (Pearl millet); main plots sizes were: $40 \text{ m} \times 8 \text{ m} = 320 \text{ m}^2$. Secondary treatments included two planting densities D1 and D2 of the crop species (Table 3). Two blocks of plots were used as controls 1 and 2; the control 1 was a block of natural fallow (J) with a natural vegetation cover; no crop was planted on the block. The vegetation

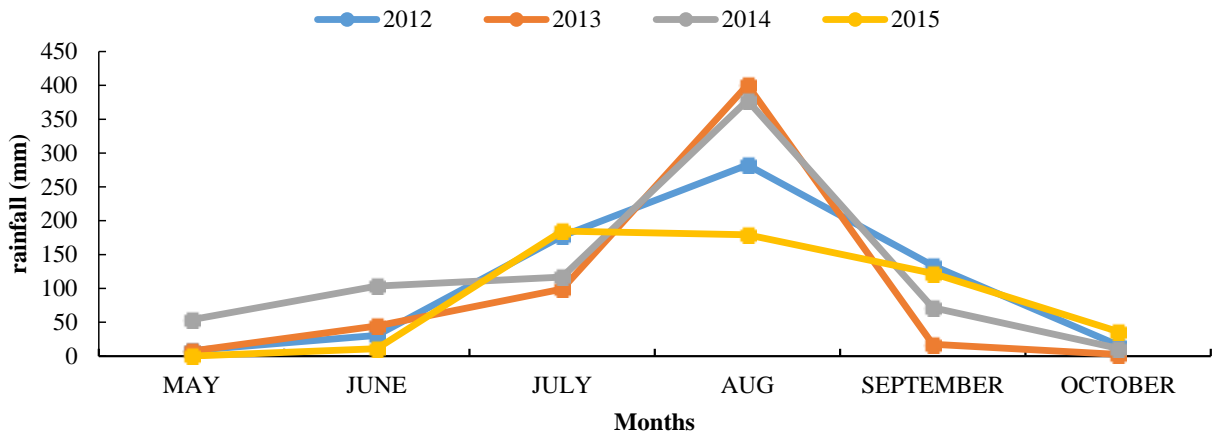


Figure 1. Monthly rainfall (mm) during crop growth (2012 - 2015).

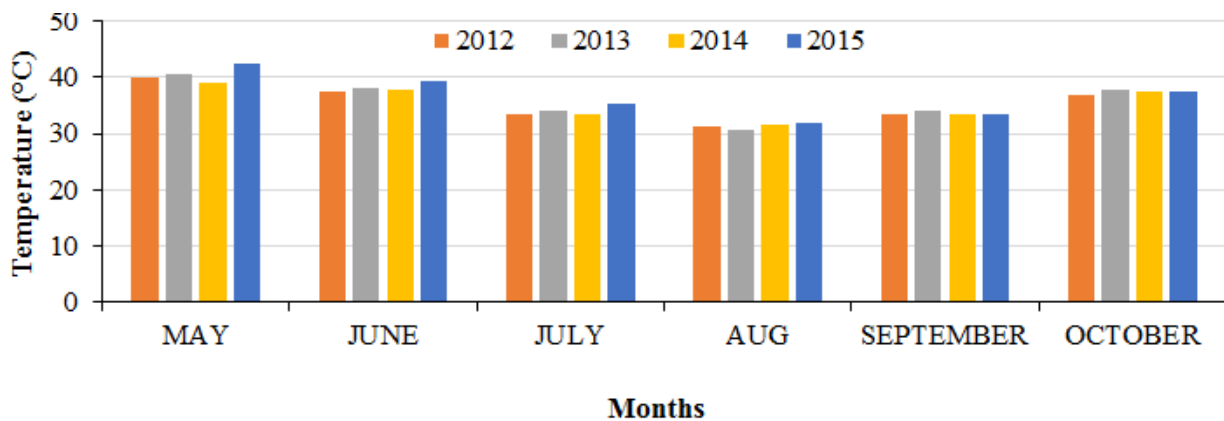


Figure 2. Mean maximum temperature during the six months of crop growth for the period of 2012 through 2015 at Sadore measured in degrees Celsius (°C).

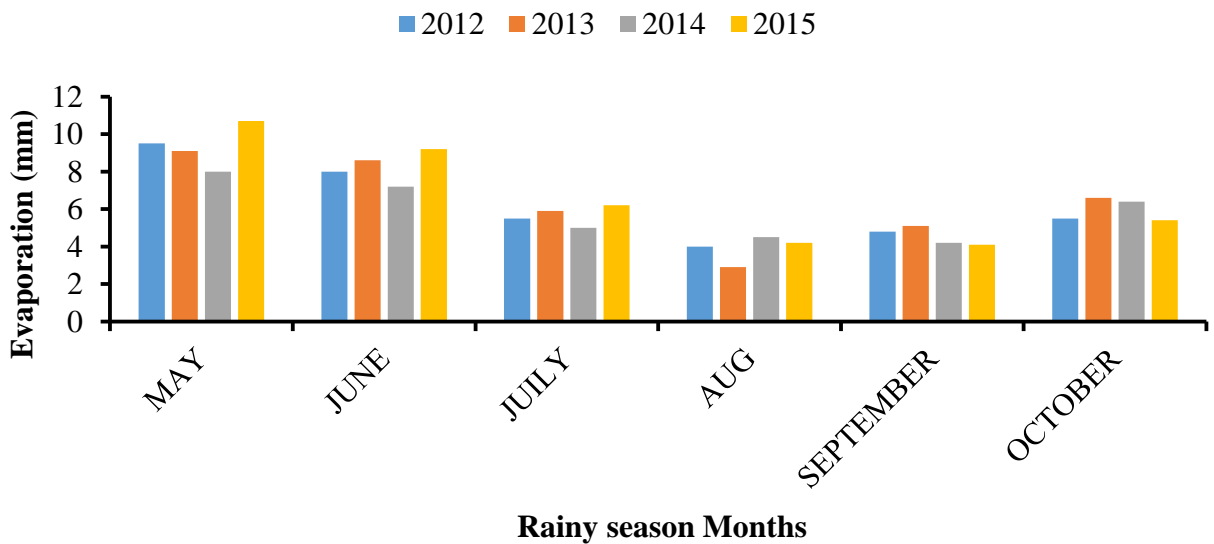


Figure 3. Mean maximum evaporation during the six months of crop growth for the period of 2012 through 2015 at Sadore (mm).

Table 1. Soil initial textural properties in 5 fractions at 0-10, 10-20 and 20-30 cm depth.

Depth (cm)	Coarse sand (%)	Fine sand (%)	Coarse silt (%)	Fine silt (%)	Clay (%)
0-10	43.73	52.42	1.89	1.23	0.74
10-20	41.18	53.82	1.59	1.13	2.28
20-30	43.42	48.6	1.48	1.13	5.36
Standard deviation	1.39	2.7	0.21	0.06	2.35

cover was harvested and weighed at the end of season. Control 2 was a bare plot (Pnue) block that was regularly weeded at the same time as the plots under cultivation; the plot had no culture during the 2012 season. All residues including that of control 1 and crops were returned to their respective plots in February 2013.

Data were analysed with GenStat Release 14.1 program using a completely randomized block in unbalanced design. The lsd test (5%) was used to compare pairs of means whenever a significant difference was found among treatments.

Data on the mycorrhizal arbuscular fungi diversity and variation were analysed with Minitab 16 program and the test of Tukey (95% interval of confidence) was used for multiple comparison of mean values.

Evaluation of soil properties

Field layout

The replications of the trial were subdivided into parcels of 40 m × 8 m or 320 m²; crops were then sown in accordance with the experimental protocol. Each crop species was sown with two densities D1 (farmers usual spacing) and D2 (research recommended spacing) as presented in Table 3. Although the natural fallow plots (J) remained under natural cover with neither input nor other intervention. The sampling plots were delineated by leaving two border lines in the directions of the length and the width. All measurements, observations, and sampling were conducted inside the sampling plot areas. It should be noted that for fallow blocks, there was no density of seedling because no culture were sown on them in 2012; only natural weeds were allowed to grow on J plots.

Table 3 is a description of crop species plant spaces with D1 representing traditional planting spaces utilized by farmers and D2 that represent planting spaces recommended by researchers.

2013 Experiment

Each 2012 plot during 2012/13 included cropped, fallow and bare which were subdivided into 3 parcels (10 m × 8 m = 80 m²). Three doses of phosphorus in the form of rock Phosphate of Tahoua (PNT): P0 (no PNT), P1 (60 kg/ha de PNT, that is, 6.5 kg de P2O₅), and P2 (120 kg / ha of PNT, that is, 13 kg P₂O₅) were separately applied. The 2012 trial crop residues were returned to their respective 2013 plots. No return of residue was made on the plot Pnue, as it did not bear any vegetal cover in 2012. Just before planting, PNT doses were applied to plots according to assigned treatments. The application was made in the following manner:

1. For P1 treatment (60 kg PNT/ha), the quantity of 0.48 kg of PNT was thoroughly mixed in a 10 l plastic container with 5 g of soil taken from 80 m² targeted plot. The mixture was uniformly dispatched all over the plot. Then, a hoe was used to incorporate it into the plot soil.
2. For P2 treatment (120 kg/ha of PNT), 0.96 kg of PNT was mixed

in 10 l container with 5 kg of soil taken inside the targeted plot, and then dispatched all over the 80 m² plot. The mixture was incorporated as aforementioned.

3. For P0 treatment, no PNT was applied.

2014 experiment

The crop residues from 2013 trial treatments were returned to their respective 2014 plots; no fertilizer was applied. For all treatment plots, a single millet variety was grown at density of 0.8 m × 0.8 m; the same procedures were then repeated for the subsequent years.

2015 experiment

The plots used for the trial conducted in 2014 were subdivided into two parcels (8 m × 5 m = 40 m²), corresponding to the two doses of nitrogen namely, U0 (no input) and U1 (applied in micro dose of 3.36 g of urea/planting hill at panicle initiation and at panicle exertion stage) of about 45 kg/ha. Unlike in other years, no restitution of the previous crop residue from the 2014 trial was used on the plots of the 2015 trial. Since there was a great disparity among the plots in terms of dry straw production due to bad and heterogeneous plants stand and development, it would not be possible to get enough amounts of crop residues for some of the treatment plots. Therefore, it was decided that crop residue should not be applied.

Sampling of soils and roots

Soils

After the delimitation of replications or blocks, a sampling of soil was made before any disturbance of profile at depths of 0 - 10, 10-20 and 20-30 cm, at the level of each replication, on the diagonals and medians. Samples were mixed by depth to get three composite samples, totalling 9 composite samples (Keith, 2006). The samples were analysed to determine the soil initial physical (Table 1) and chemical properties (Table 2) of the experimental plots.

The evaluation focused on some initial physical and chemical properties of soil and comprised of the following parameters: pH KCl, aluminum (Al), organic carbon (C org), total phosphorus (Tot P), Olsen phosphorus (P Olsen), Bray1 phosphorus (P-bray1), sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), cation exchange capacity (CEC), and total nitrogen (Tot N). The analysis were done in the ICRISAT/Sadore soil and plant analytical laboratory. The analytical methods used are based on the manual published by Reeuvijk (1993) and Houba et al. (1995). The results of chemical properties analysis are shown in Table 2.

Particle size distribution was made by sieving particles > 50 µm and pipetting for particulate matter < 50 µm. The results of textural analysis comprised of the rates of sand, silt, and clay and are presented in Table 1.

Table 2. Initial chemical properties of sample of the experiment plots.

Parameter	pH-H ₂ O	pH-KCl	H ⁺	Al ³⁺	C. O	Total-P	P-Bray1	P-Olsen	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	CEC
	1:2.5	1:2.5	cmol+/kg	cmol+/kg	%-C.Org	mg-P/kg	mg-P/kg	mg-P/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg	cmol+/kg
Sampling depth: 0-10 cm													
se	0.06	0.23	0.01	0.00	0.02	9.88	1.47	1.78	0.04	0.03	0.22	0.01	0.37
M	5.39	4.59	0.06	0.04	0.23	75.08	7.30	9.05	0.13	0.19	1.30	0.19	1.85
Sampling depth: 10-20 cm													
se	1.89	1.50	0.03	0.10	0.07	26.79	2.23	2.81	0.04	0.06	0.39	0.06	0.58
M	5.39	4.16	0.08	0.23	0.15	84.02	5.21	6.18	0.12	0.13	1.15	0.14	1.88
Sampling depth: 20-30 cm													
se	1.69	1.33	0.03	0.19	0.06	26.73	2.18	2.51	0.04	0.05	0.38	0.06	0.65
M	5.27	4.00	0.11	0.49	0.13	53.64	3.34	5.26	0.09	0.14	1.13	0.10	2.20

se: Standard error; M, mean.

Table 3. Traditional plants spacing (D1) and recommended plants spacing (D2).

Crop species	D1		D2	
	Lines (m)	Pockets (m)	Lines (m)	Pockets (m)
Dolichos	1.00	0.75	0.50	0.75
Sesbania	0.40	0.50	0.75	0.75
Cowpea	0.75	0.75	0.50	0.50
Voandzou	0.20	0.20	0.15	0.15
Millet	1.00	1.50	1.00	1.00

Roots

The samplings were made according to the following procedure: For the roots and soil sampling, plants were taken randomly in each plot.

To achieve this, at the level of each parcel, 5 lines were taken in drawing; on each line, a planted hill was taken at random; in each pocket, a plant was taken at random and numbered 1 to 5. In the rooting zone of each plant, fine rootlets were collected and put into conservation in a solution of glycerol-ethanol-water (GEW 1 / 1: 1 V/V). At the same time, 500 g of soil were collected at the same place between 0 and 25 cm depth, and then put in plastic

bags and labelled with the date of collection, treatment name, replication number, and depth. Samples were spread on paper sheets to dry at ambient room temperature and then stored in a refrigerator at 4°C.

Roots architectural and morpho-physiological observations

In order to compare the root architectures of crop species, a pot test was conducted during 2016 in which seeds of crop (cowpea, dolichos, voandzou, sesbania and millet) were planted in PVC tubes (1.50 m depth × 0.40 m

diameter); these pots were filled with a mixture of sand/compost at the ratio of 5 sand/1 compost; then 5 seeds of each crop species were planted in 3 replications that is 3 pots per crop specie. The seeds and the soil were not treated with chemical nor analysed. After germination, only three seedlings were kept in each pot and allowed to grow. One month later, the plants were cautiously washed to remove the soil. Naked roots were scanned in order to examine their architectural characteristics. The equipment used for this work was a WIN RHIZO 2009 a,b,c Basic, Reg, Pro and Arabidopsis for Root Measurement (Regent Instrument CANADA Inc). The results of scanning are shown in Photo 2. The measurement of root

characteristics are shown in Figure 14.

Extraction of arbuscular mycorrhizal fungal spores

Extraction of spores was conducted according to the method of Walker (1982) using 100 g of sample soil that were poured in a container filled with water to 3/4. The mixture was hand-stirred vigorously for 5 to 10 s. After resting for 10 to 20 s, the supernatant was transferred in a second container and was stirred again then left to rest for 10 to 30 s. The suspension went through five sieves superposed from the bottom to the top respectively of 63, 160, 250, 315 and 630 μm of diameters. The refusal of the different sieves was collected in Petri dishes. The content of the later was then mixed. A sample of 1 ml was taken from mixed solution and used for the description of the genus and the estimation of the number of spores.

Estimate of number of spores in the soil of the rooting zone and assessment of AM fungal biodiversity variations

Estimation of the number of spores: The number of spores contained in 1 ml of supernatant was recorded under a binocular microscope and extrapolated according to the total volume of the supernatant. The identification of the genus of spores observed was conducted using a binocular microscope at magnification of 100 and 400 times according to the criteria proposed by Schenck and Smith (1982). To do that, each 1 ml sample was thoroughly examined and the individual spores were regrouped by categories using a dissecting nail. The discrimination among categories was based on observable differences; the number of spores was counted while the descriptions were done based on a reference descriptive sheet provided by the mycological laboratory. This method allowed to distinguish and describe the morphology of the spores (color, shape, size, the appearance and hyphal structure) and to classify them according to the genus on the basis of the type of mycelium attachment. Spores without hyphal attachment and very small ones could not be identified but were counted and classified as non-identified spores.

Assessing fungal biodiversity variation: After counting the number of spores their categorization was done based on the discrimination criteria of appearance, size, color and hyphal attachment. This was done by examining each soil sample. The arbuscular mycorrhizal fungus diversity was evaluated using the method of Shannon - Weiner index calculation table. The category studied was the AM fungal genera. This method allowed computing the following measures value:

1. The percentage frequency of individual genus PF% was calculated by the: number of plots in which individual spore genus *100/ Total number of AMF genera in all plots. The number of plot was 30 in 2012 trial; 108 in 2013 and 2014 trials, and 216 in 2015 trial.
2. The occurrence of AMF spore genus in a plot = occurrence in a plot*100/total number of plots
3. Relative frequency of *Glomus* (RF *Glomus*) = number of plots with *Glomus**100/number total of plots
4. Relative frequency of *Gigaspora* spore (RF *Gigaspora*) = number of plot with *Gigaspora**100/number total of plot
5. Relative frequency of non-identified spore genus (RF non identified Spores) = number of plot with non-identified spore*100/number total of plot
6. Relative density of spore genus (RD) = Density of spore of a genus*100/total density of spore
7. The richness (S): which is the number of species within the study area.

8. The Shannon - Weiner Index general of diversity (H') describe the relationship of individuals of varying categories (here, AMF spore genera) $H' = \sum (ni/N.\ln ni/N)$ where ni is the number of individuals of each category (that is, AMF genera), N is the total number in that location (Choudhary et al., 2014).

Preparation of the roots

Preparation was done according to the method of Philips and Hayman as amended (1970), which consisted in the steps from digestion, whitening, and acidification with HCl 1% to coloring in trypan blue and lacto-phenol solution for 24 h at least. The root fragments were subsequently mounted between blades and slats with 10 fragments per blade with a thin layer of glycerine.

Assessment of mycorrhizal colonization

Assessment was made according to the method of Trouvelot et al. (1986) and comprised of the reading of the parameters of mycorrhization under microscope at magnification 100 X and 400 X, on 30 root fragments for each sample plant. The parameters evaluated included: Mycorrhizal frequency (F%, percentage of root fragments with endomycorhizes); intensity of mycorrhizal colonization of the cortex infection developed in part of the endomycorrhized root system (M%, proportion of colonized cortex, expressed in percentage); rate of arbuscular of the entire root system (A%, proportion of root cortex containing arbuscular, expressed in percentage).

Crop yields

2012 Experiment

Crop species was harvested at maturity. The legume crop cowpea was harvested two times based on the percentage of matured pods. The first harvesting occurred when 50% of the pods were dried; followed by a second harvest 10 days thereafter. The sample plants were cut, weighed and sun-dried. A measurement of weight was taken every seven days. The last weight was recorded when no variation of weight was observed between two consecutive weightings (third and fourth). Voandzou was harvested after seed maturity and when the pods were hard *Sesbania* was harvested at pod maturity and when in midst of a drought. Millet was harvested after checking that the grains were hardened. The checking was done by randomly removing 2 to 3 grains from panicles selected randomly and trying to break them with the teeth. If the grain breaks with resistance, then it is dried. Grain and biomass harvested within the sampling area were weighed and the yields were calculated in kilogram per hectare (kg/ha). *Dolichos* have a growth period of 6 months and was therefore harvested before flowering, therefore no grain were present.

RESULTS

2012 Experiment

Fungal diversity and density of spores

Soil samples collected from the rooting zone of the cultures were treated and observed in the laboratory using binocular loupes for the description and identification of the genus of spores of AMF (Photo 1).

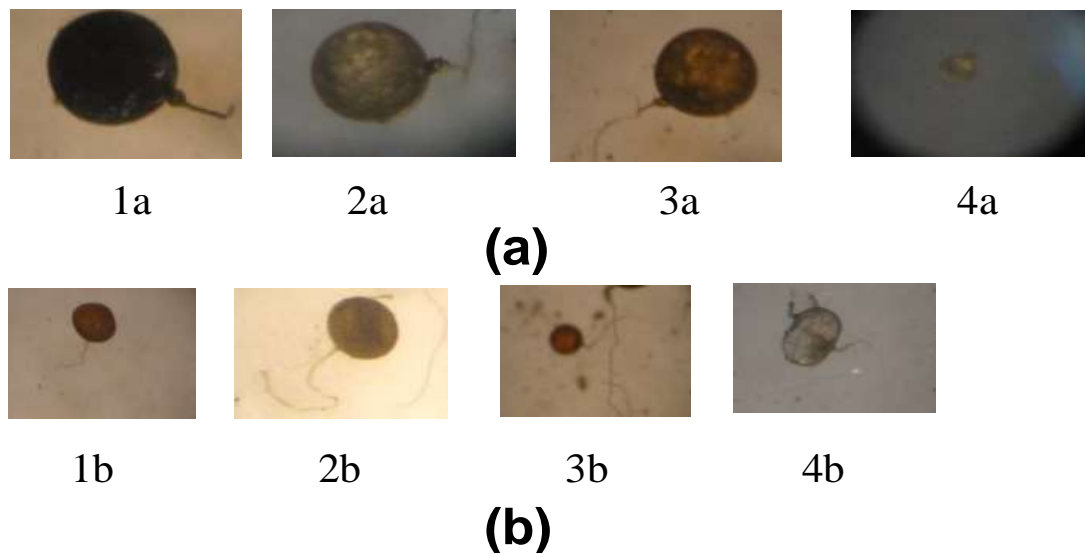


Photo 1. Fungal diversity in Sadore soil. (a) Diversity of spores of *Gigaspora* genus based on the color and the shape. 1a: Black *Gigaspora* spore; 2a: Grey *Gigaspora* spore; 3a: Ochre *Gigaspora* spore; 4a: Minuscule white transparent *Gigaspora* spore. (b) Diversity of spores of *Glomus* genus based on the color and the shape. 1b: Grey *Glomus* spore; 2b: Ochre *Glomus* spore; 3b: Ochre *Glomus* spore attached to 2 minuscule spores; 4b: Chlamydospore *Glomus multicaule* with 3 filaments attached.

Table 4. Analysis of variance for the *Glomus* spores.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	6627	3314	0.03	0.973
Culture	4	869642	217411	1.82	0.170
Density	1	816174	816174	6.82	0.018
Culture.Density	4	4170579	1042645	8.71	<.001
Residual	18	2154569	119698		
Total	29	8017592			
s.e.			346		
Cv%			45		

Variate: *Glomus*.

Two genera of spores were found in the soil samples: The *Gigaspora* genus and the *Glomus* genus. The spores were examined to distinguish an intra-specific diversity of both types of genus on the basis of the form, color, and appearance (as shown in the Photo 1 black, grey, ochre and yellow transparent spores were observed). The *Gigaspora* genus is distinguished from the others by the presence of bulb bearing filaments. The spores of the *Glomus* genus are distinguished by the absence of bulb bearing filaments and the presence of sessile filaments in the spores. The size of the spores varied from 63-125-160 to 315 μm based on the mesh sizes of the sieves; it should be noted that no specific measuring of spores was done in this experiment. The proportion of the two spores of fungal genera, which were identified in the soil samples varied depending on the crop species (Table 4).

The results of ANOVA table indicated that the proportion of the *Glomus* genus was higher (2321 spores/100 g of soil), 90.91% of the total number of spores; while the proportion of the *Gigaspora* genus was lower (232 spores/100 g), 9.09% of total number of spores. Cowpea had the highest density of spores with 721 spores /100 g of soil, followed by sesbania (578 spore / 100 g soil) and pearl millet (556 spores / 100 g soil).

Table 5 is an illustration of the genus and the number of arbuscular mycorrhizal fungal spores associated with the legumes crop species as well as millet crop.

Table 6 shows the ANOVA table of *Gigaspora* spore.

Diversity of AMF spores

Photo 1 indicates the arbuscular mycorrhiza fungal

Table 5. Analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	12037	6019	0.05	0.948
Culture	4	853153	213288	1.89	0.156
Density	1	861586	861586	7.63	0.013
Culture.Density	4	4245685	1061421	9.40	<.001
Residual	18	2032307	112906		
Total	29	8004767			
s.e.			336		
Cv%			42.1		

Variate: Total spore.

Table 6. Analysis of variance.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep	2	3380.	1690.	0.87	0.437
Culture	4	2107.	527.	0.27	0.893
Density	1	615.	615.	0.32	0.581
Culture.Density	4	5972.	1493.	0.77	0.561
Residual	18	35040.	1947.		
Total	29	47114.			
s.e.			336		
Cv%			42.1		

Variate: Gigaspora.

biodiversity in Sadore sandy soils.

Impact of crop planting density on the total number of spores /100 g of soil

The impact of crops planting density on the number of spores /100 g of soil in the root zone is illustrated in Figure 4. The analysis of variance showed a significant difference ($P = 0.010$) between the effects of the crop species *plant density on the total number of spores. According to ANOVA a significant interaction between culture and density ($P < 0.001$) effect on the total number of spores. It was observed that the rate of spores was higher with the D2 plant density (scientifically recommended plant density) compared to that of D1 (traditional, lower planting density) for millet, dolichos and voandzou. In the case of cowpeas and sesbania, the number of spores in D1 plots overpassed ($P = 0.013$) the number in the D2 plots.

Impact of the crop plant density on the number of spores/100 g of soil as a function of genus

Number of Gigaspora/100 g of soil: The impact of

crop planting density on the number of spore of Gigaspora/100 g of soil is illustrated in Figure 5. The analysis of variance showed no statistical difference between the effect of the two planting densities D1 and D2 on the number of Gigaspora; the number of *Gigaspora* spore/100 g of soil was neither influenced by the crop species nor by the density of seedlings (Table 6). However, considering the numerical values for the D1, sesbania recorded the highest number of spores (73 spores of *Gigaspora*/100 g soil) follow by the voandzou (35 spores of *Gigaspora*/100 g soil). For the D2 density, the highest number was recorded for dolichos (52 spores of *Gigaspora* / 100 g soil), followed by cowpea and millet with 36 spores of *Gigaspora*/100 g soil and 32 spores/100 g of soil, respectively.

The lowest number of spores of *Gigaspora* was recorded for voandzou at the D2 density with 2 spores/100 g of soil.

Number of spore of *Glomus* / 100 g of soil: Figure 6 describes the impact of crops seedling density on the number of the *Glomus* spore/100 g of soil sampled in the root zone. There was, on the one hand, a significant difference ($P=0.013$) between the effects of crop planting densities on the number of spores of the genus *Glomus*; however, a highly significant difference ($P < 0.001$) of

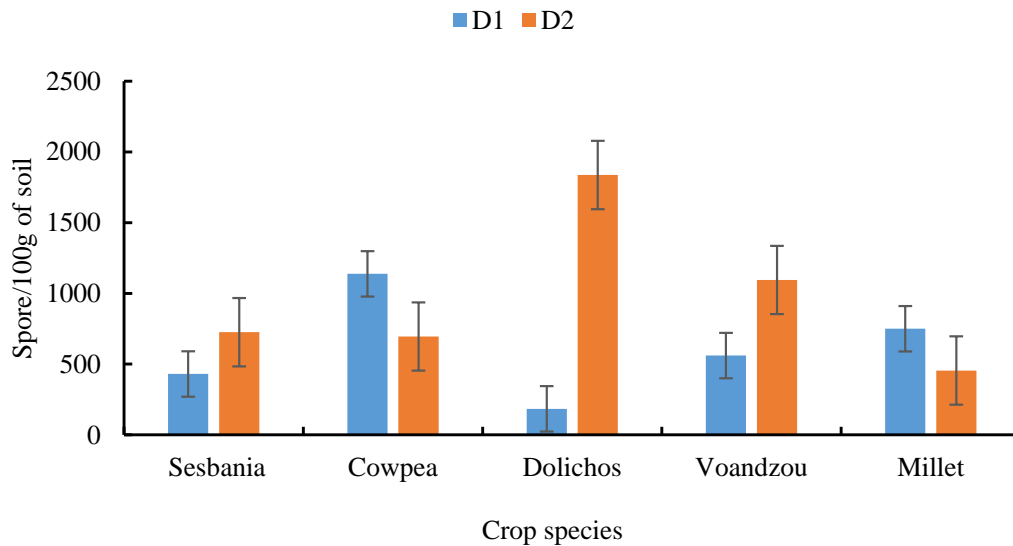


Figure 4. Impact of crop and plant density on the number of AMF spore/100 g of soil.

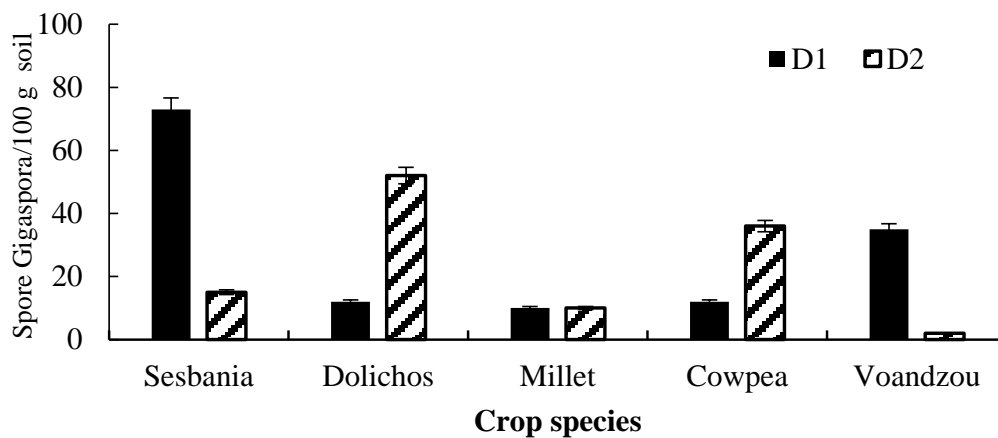


Figure 5. Impact of crop planting density on the number of Gigaspora spore/100 g of soil in 2012.

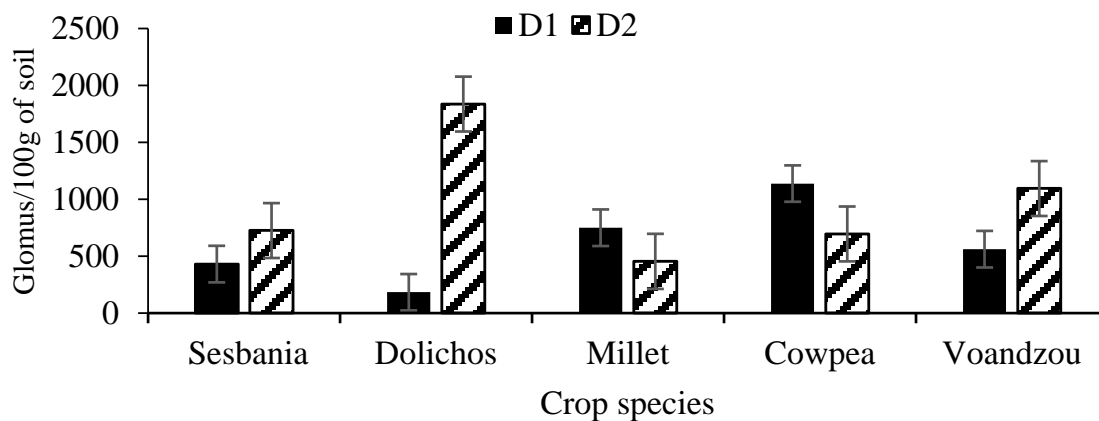


Figure 6. Impact of crop planting density on the number of Glomus spore/100 g of soil in 2012. D1 = farmer traditional planting spaces; D2 = improved recommended planting spaces.

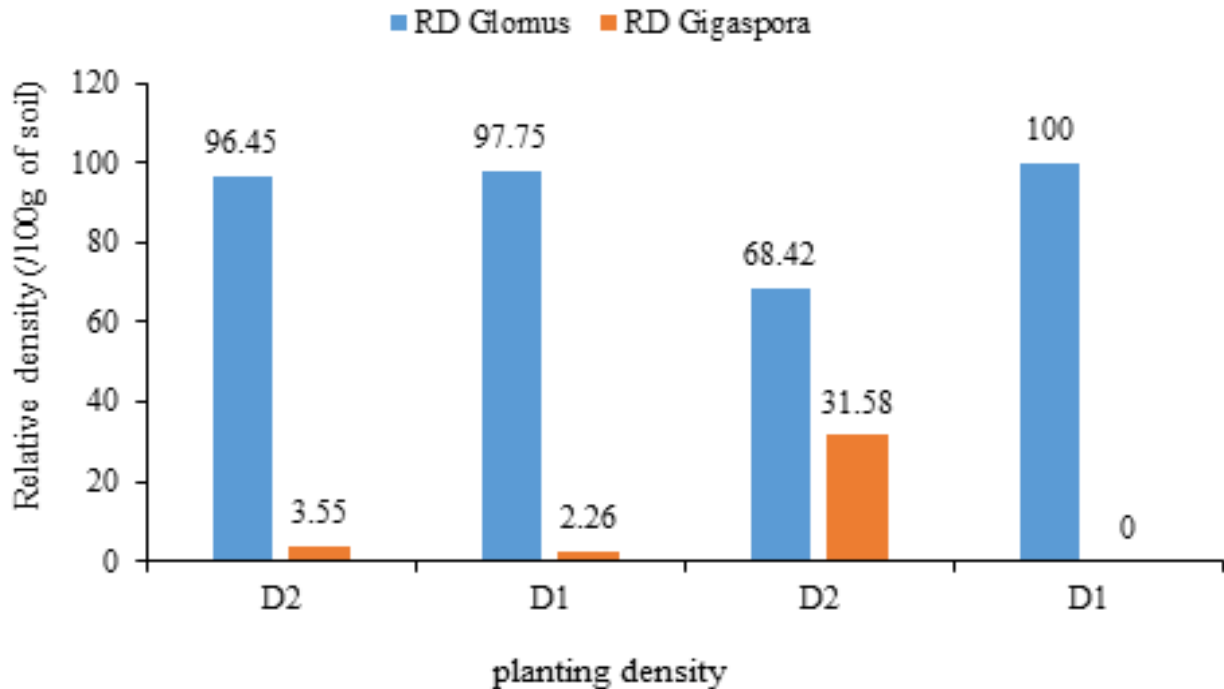


Figure 7. Relative density of *Glomus* vs *Gigaspora* as function of crop density in 2012. RD is relative density of spores; D: dolichos; M: millet; N: cowpea; S: sesbania; V: voandzou; D1: plant density 1 (traditional); D2: plant density 2 (improved).

the effects of the interaction Crop × Planting density on the number of spore *Glomus*/100 g of soil was found; this indicates that the influence of planting density on the number of spore *Glomus* genus/100 g of soil is linked to the crop species. Figure 6 shows that cowpea at density D1 recorded the highest number of spores of *Glomus* spores / 100 g of soil, while voandzou was the second culture at the D2 density with 1095 spores/100 g of soil.

Arbuscular mycorrhiza fungus spore diversity variations

Richness: Results of relative frequency calculations showed the richness was 3 including non-identified spores. In 2012 these non-identified spore were not estimated. The richness of the plots area was made of 2 genera of arbuscular mycorrhizal fungi : *Glomus* and *Gigaspora*; *Glomus* was the genus of spores that was present in all the 30 plots with 96.35% as relative frequency and had a very high density as 1953 spore/100 g of soil. But, *Gigaspora* was present in 21 plots with only 3.63% as relative frequency and had a very low density as 5 spores/100 g of soil. It was observed that diversity in crop species (legumes and cereal) did not make differences in fungal biodiversity. Not only AM fungal spore genus density was not related to crop species but also was not to plant density. In terms of relative importance

with 1138 spores/100 g of soil; while millet was the second ranked culture with 751 spores/100 g of soil. Dolichos had the lowest number of spores *Glomus* with 184 spores/100 g of soil at planting density D1; however, this same dolichos crop, when planted at the D2 density, was ranked first and highest with 1837 of value, the *Glomus* genus was far above the *Gigaspora* genus across all the 30 plots with an average of 98.64% against 4.75%. The calculated value of Simpson's index was 0.942 while that of the Simpson's index of diversity (1-D) reached 0.058.

Figure 7 shows the relative density of *Glomus* vs *Gigaspora* as function of crop density in 2012.

Figure 8 shows the Index of general diversity of the two AM fungi spores as function of crop plant density. The general index of AM fungus diversity value was very high with 0.97 *Glomus* under the treatment DD2 compared to *Gigaspora* for the same species of crops. Among crop species, the highest value of *Glomus* index of diversity was exceptional 0 and out of average range compare to average value obtained under other crops.

The Figure 9 is showing the calculated value of the relative importance of the two spores' genera *Glomus* and *Gigaspora* as function of crop at two plant densities in 2012 trial. Significant difference was observed between RIV *Glomus* and RIV *Gigaspora*. Crop Density for *Glomus* genus. The levels of relative importance value of *Glomus* fluctuated between 89.78 to 104.48/100 g of sol

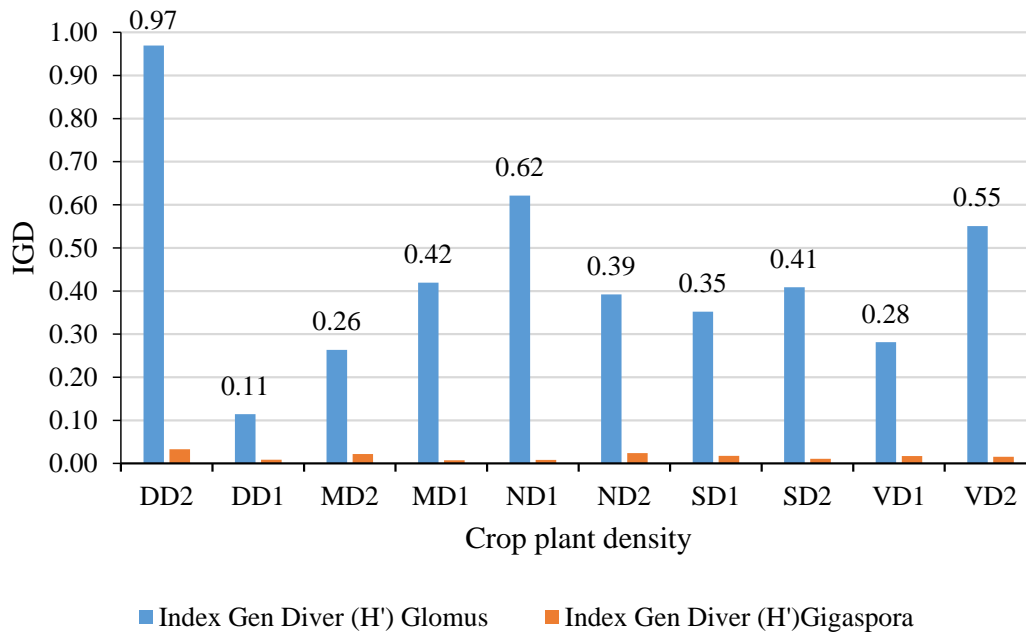


Figure 8. Index of general diversity of Glomus vs Gigaspora as function crop plant density according to Shannon and Weaver index in 2012. S, Sesbania; D, Dolichos; N, cowpea; V, Voandzou; Pnue, bare soil; J, natural fallows; D1= farmer traditional plant density; D2= recommended plant density. Index Gen Diver: Index of general diversity.

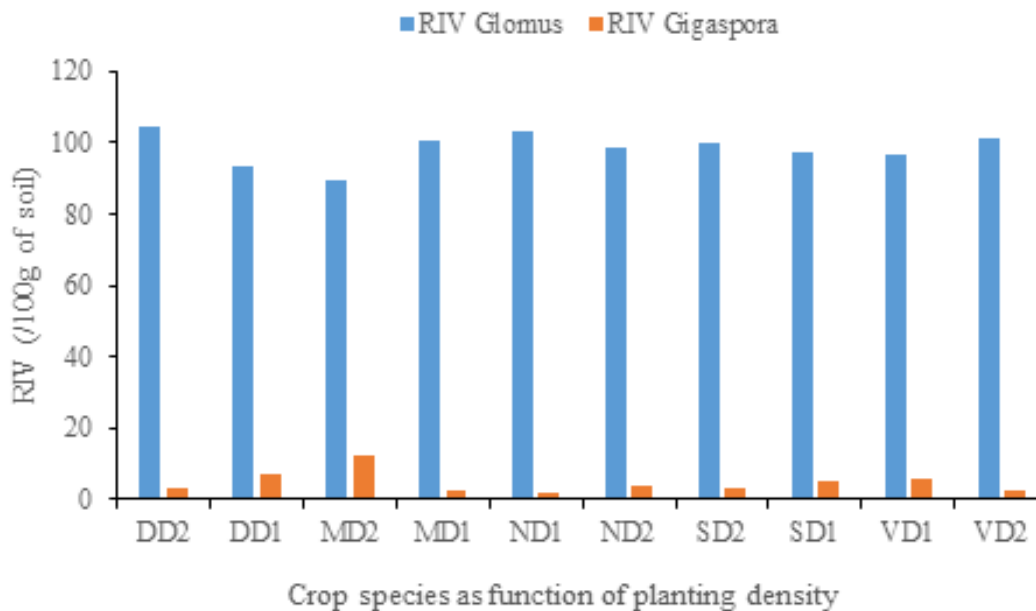


Figure 9. Relative importance value of spores Glomus vs Gigaspora as function of crop plant density in 2012. S (Sesbania), D (Dolichos); N (cowpea), V (Voandzou), Pnue (bare soil), J (natural fallows), D1= farmer traditional plant density, D2= recommended plant density.

while that of Gigaspora were between 1.72 and 12.25spores/100g of soil. It was observed that the drop in RIV value for Glomus corresponded to a rising for RIV of

Gigaspora.

Figure 10 presents the evolution of Simpson's and Shannon-Weiner Index of diversity of AM fungal spores

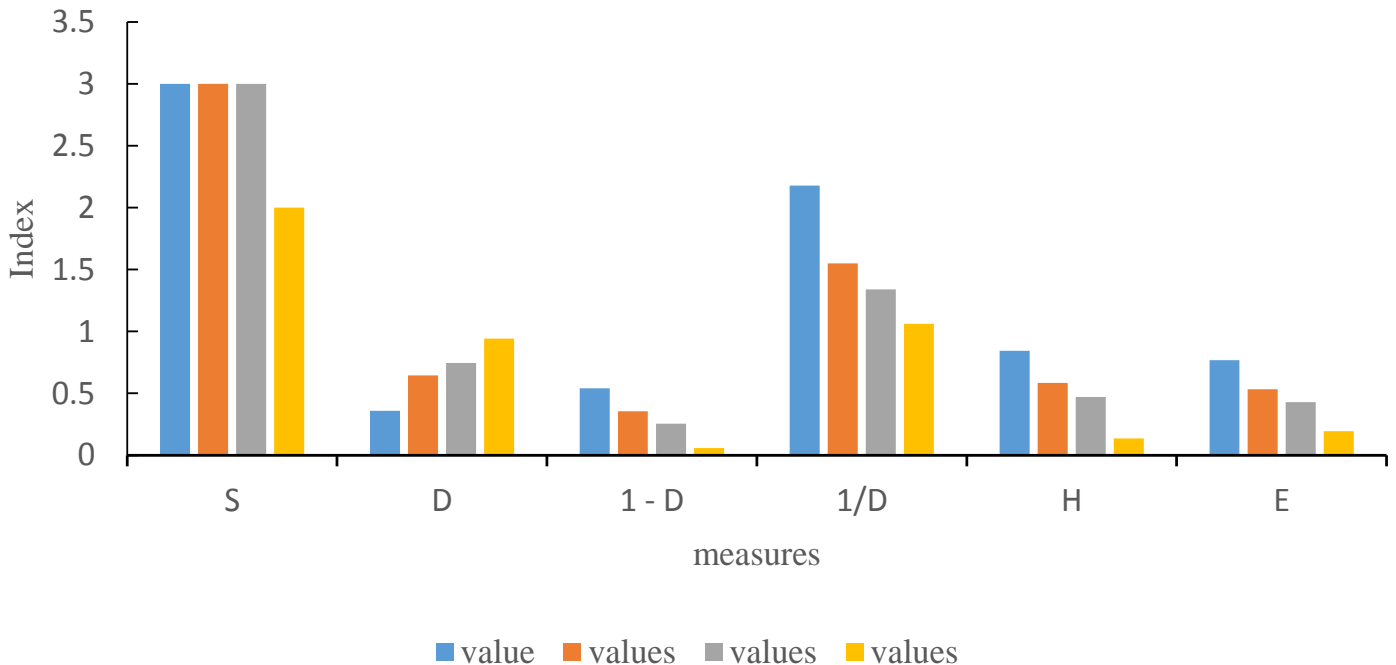


Figure 10. Evolution of Shannon and Weiner Index values of diversity from year 2012 through 2015. S: Richness indicates the number of AM fungal genera observed in the study area; D: Simpson's index indicating the probability that two randomly selected individuals in the community belong to the same category (AMF genus); 1-D: Simpson's index of diversity: the probability that two randomly selected individuals in a community belong to different category (AMF genus); 1/D: Simpson's reciprocal index: the number of equally common categories (AMF genus); H: Shannon and Wiener Index of diversity indicates the degree of uncertainty of predicting that a randomly selected individual spore will belong to *Glomus* or *Gigaspora* genus; E: Equitability; Soil richness in diversity category AM fungus genera): Soil richness (S) in AM fungus was limited to 2 genera (2012) but was 3 in from 2013 to 2015.

under legume and cereal crops species mostly at the treatment locations of DD1 and MD2. The same tendency was seen at the treatment locations SD1 and VD1.

Figure 10 is a representation of Simpson's and Shannon-Weiner index which indicate the level of diversity of AM fungus genera within the trial area from 2012 through 2015.

Simpson's index of diversity: The probability that two randomly selected individual spores in the spore community belong to exclusively *Glomus* genus or exclusively to *Gigaspora* genus (Simpson's index, D) was higher in 2012 compared to other years with a ranking as follows: 2012 > 2015 > 2014 > 2013 with respectively 0.92, 0.75, 0.65, and 0.36. But the situation for the probability that these individual spores belong to *Glomus* and *Gigaspora* (Simpson's Index of diversity, 1-D) was just the opposite of former situation with the following ranking: 2012 < 2015 < 2014 < 2013 and values recorded were respectively 0.06, 0.25, 0.36 and 0.54.

As illustrated in Figure 10, the Simpson's reciprocal index, that represents the number individual spore of common category (AMF genera *Glomus* and *Gigaspora*), showed yearly fluctuations that follow the same trend as observed with the preceding index; the highest value

obtained was in 2013 reaching 2.178 against the lowest value of 1.062 obtained in 2012.

Shannon-Weiner index of category diversity (H): Inter annual values comparison of Shannon-Weiner index revealed also yearly fluctuations in the same way as it happened with Simpson's reciprocal index with the order of 2012 < 2015 < 2014 < 2013. The highest value was 0.83 in 2013 against 0.34 in 2012.

Figure 11 shows the evolution of percentage of frequency AM fungal genera over 3 years of monoculturing of millet 2013 through 2015.

The frequency percentage for *Glomus* genus remained at the same level of 100% during the three consecutive years while yearly fluctuations occurred with *Gigaspora* genus (29.63 to 78.7%) with a rate increase of 2.65 times the percentage of year 2015 over that of 2014 and 1.35 times over that of 2013. The frequency percentage of non-identified spores genus were very high with a slight fluctuation between years going from 82.4 in 2013 to 100% in 2014 and 2015.

Relative frequency of AM fungal genera

Figure 12 is a representation of the evolution of relative

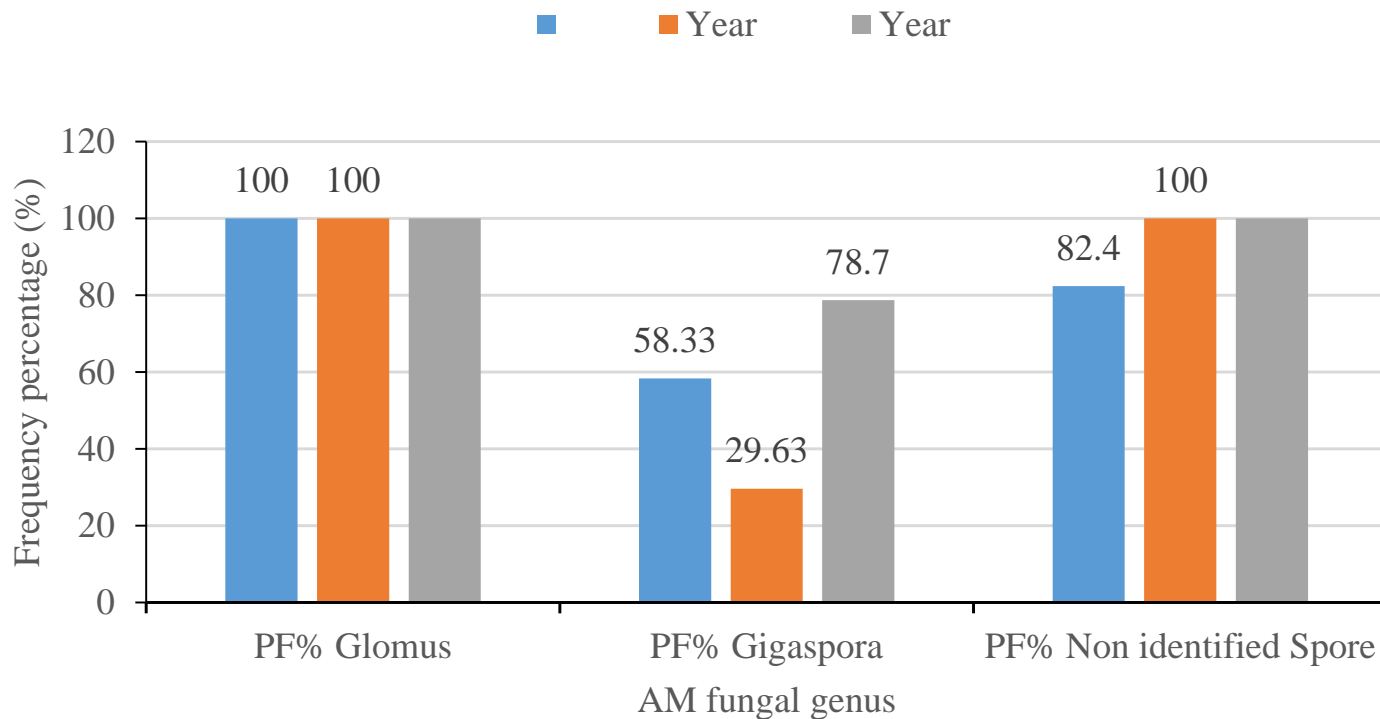


Figure 11. Evolution of percentage of frequency AM fungal genera over 3 years of monoculturing of millet from 2013 to 2015. PF: Percentage of frequency; AM: Arbuscular mycorrhizae.

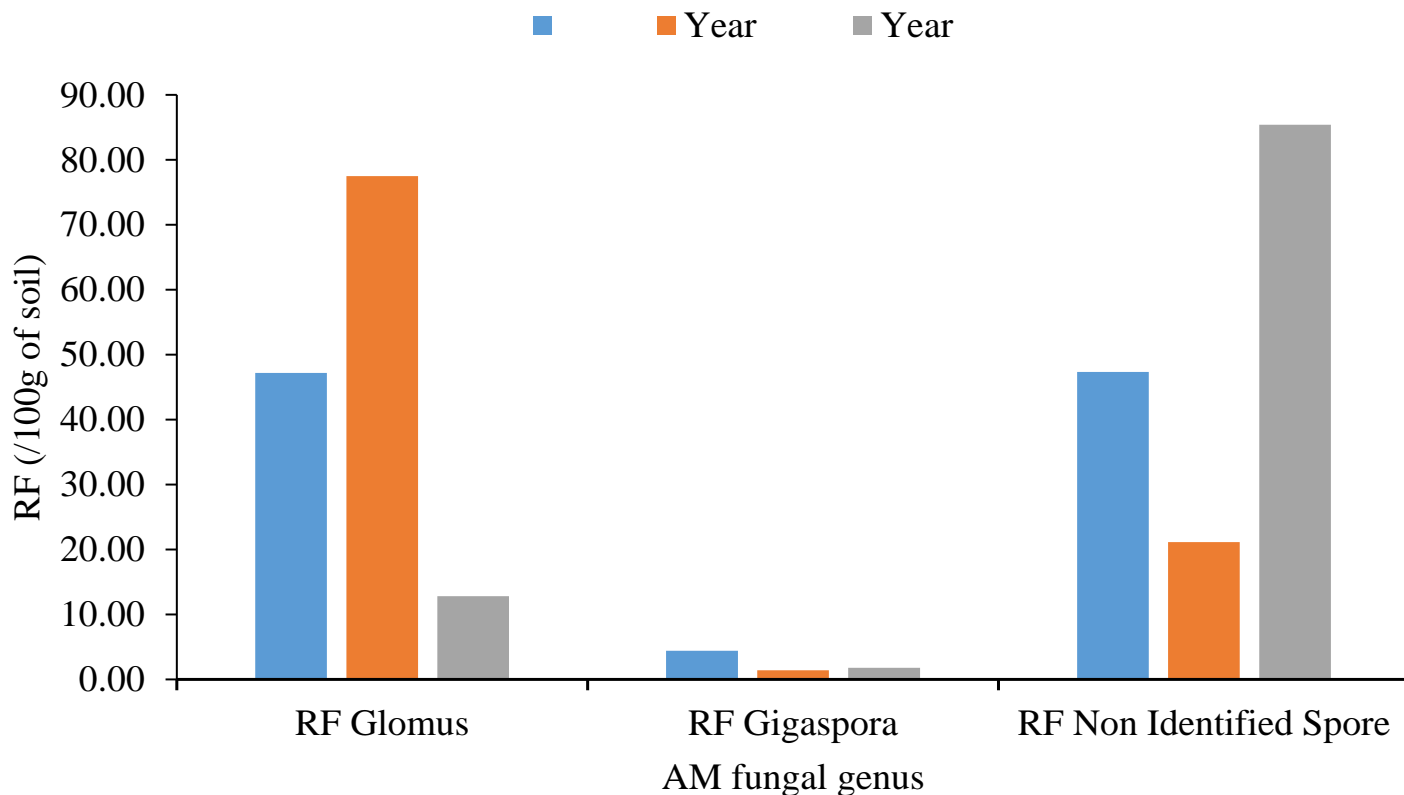


Figure 12. Evolution of relative frequency of AM fungal genera over 3 years of monoculturing of millet from 2013-2015. RF: Relative frequency; AM, arbuscular mycorrhizae.

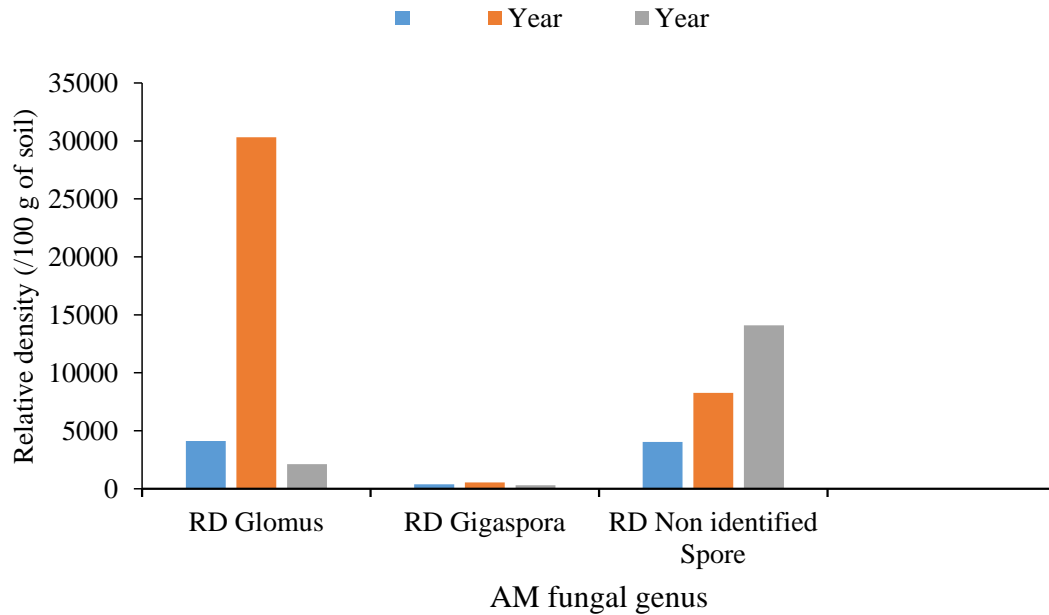


Figure 13. Evolution of relative density of AM fungal genera over 3 years of monoculturing of millet from 2013-2015. RD: Relative density of spore; AM: Arbuscular mycorrhizae.

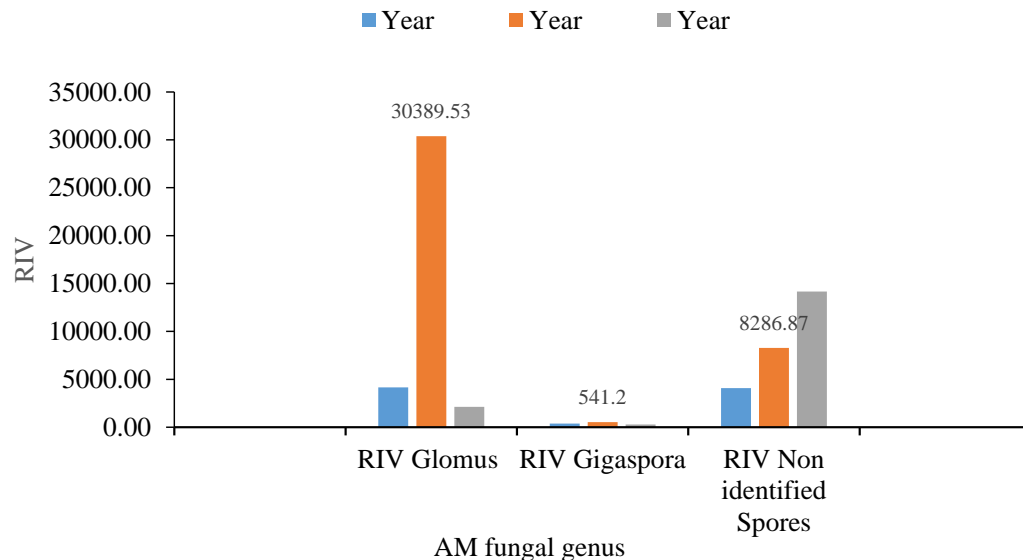


Figure 14a. Evolution of relative importance of AM fungal genera over 3 years of monoculturing of millet. RIV: Relative importance value; AM: Arbuscular mycorrhizae.

frequency of AM fungal genera over 3 years of monoculturing of millet.

Figure 13 is the evolution of relative density of AM fungal genera over 3 years of monoculturing of millet.

It could be observed that the relative density of Gigaspora is the lowest during the 3 years of millet monocropping.

A significant difference ($P=0.05$) was observed

between the effects of preceding crops on the relative density of Gigaspora and that Glomus genus. Glomus and non-identified spores had higher RD compared to Gigaspora. It was also observed that for Glomus the RD in 2014 was the highest compared to that of 2013 and 2015 while more non-identified spores were found in 2015. For these latter spores, the ranking of RD values was 2015 > 2014 > 2013. Figure 14a exhibits the evolution

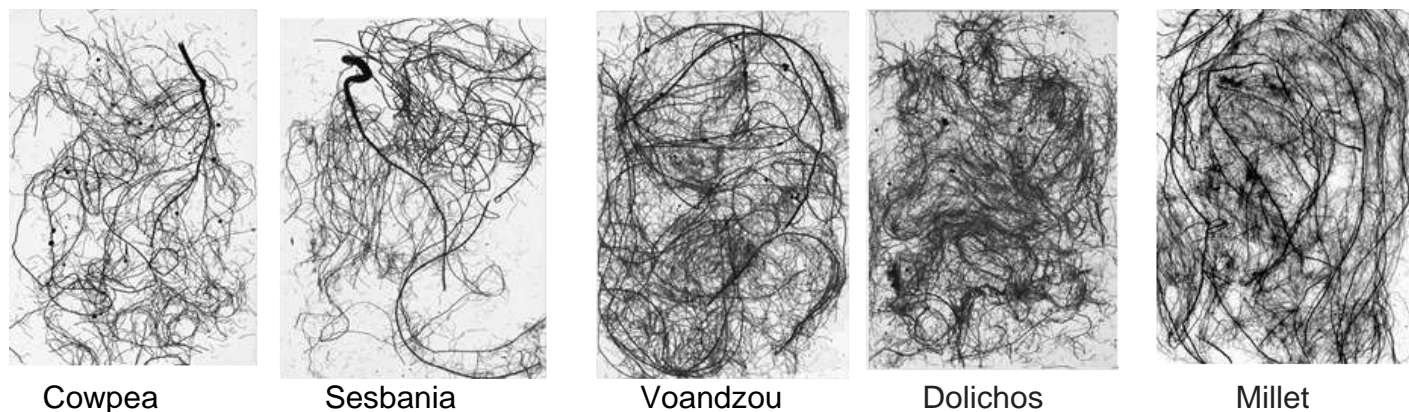


Photo 2. Architecture of 30 days seedlings roots of crop species (Photo pot).
Source: Sangare (2016).

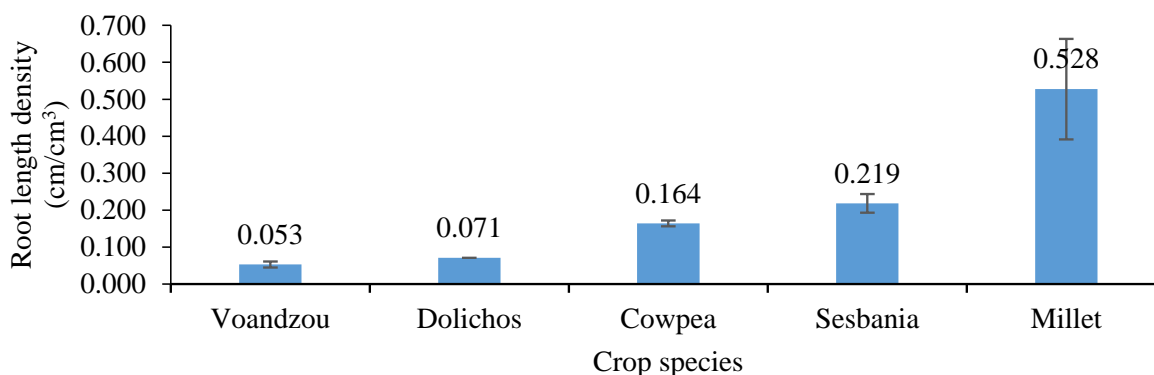


Figure 14b. Root length density as function of crop species.

of relative importance of AM fungal genera over 3 years of monoculturing of millet. In the same way that the relative density of spore's genus happened across years, the RIV showed a similar trend with *Glomus* being the most important genus compared to non-identified spores and *Gigaspora*. The relative importance value was the highest in 2014 with *Glomus* genus (30389.53) while it was the lowest for *Gigaspora* genus (541.2 the same year). Non identified spores also took over *Gigaspora* spores over the three consecutive years 2013-2014-2015 and over *Glomus* genus in 2013 and 2015. All the above figures indicated a very low performance of *Gigaspora* genus compared to *Glomus* genus in terms of AM fungal genus biodiversity characteristics measures. Variability of crop root architecture and morpho-physiological characteristics in link with AM fungus colonisation. Photo 2 is an exposition of crop roots architecture 30 days after planting in PVC tubes. The comparison of 30 days old crop root architectures indicated that dolichos and voandzou have very less fibrous roots compared to cowpea sesbania and millet. These could be ranked, based on root length and density, as follows: Voandzou < dolique < cowpea < millet < Sesbania. Figure 14b is a

comparison of crop species root length density values of 30 days old plantlets. As shown in both Figure 14b and Photo 2, low values of root length density were associated with voandzou (0.053 cm/cm³ and dolichos 0.071 cm/cm³) while the highest values were associated with millet followed by sesbania and cowpea with respectively: 0.528, 0.219 and 0.164 cm/cm³.

Figure 15 is a representation of the Shannon's index of diversity indicating the degree of uncertainty that a randomly selection of a spore will always belong to *Glomus* or *Gigaspora*.

The Shannon's index for *Gigaspora* spore genus was the highest with millet plot which root length density (RDL) was the highest value too (0.53 cm/cm³). For the Shannon's index of *Glomus* genus in was observed a value of 0.51 in dolichos which RLD was only 0.07 cm/cm³.

Figure 16 show the correlation between the Shannon's index of diversity and the crop root length diameter (cm/cm³) 2012 millet grain and legumes biomasses yields as function of plant density and crop species.

Figure 17 shows the grain yield of millet and the legume biomasses. The yields of millet dry residue and

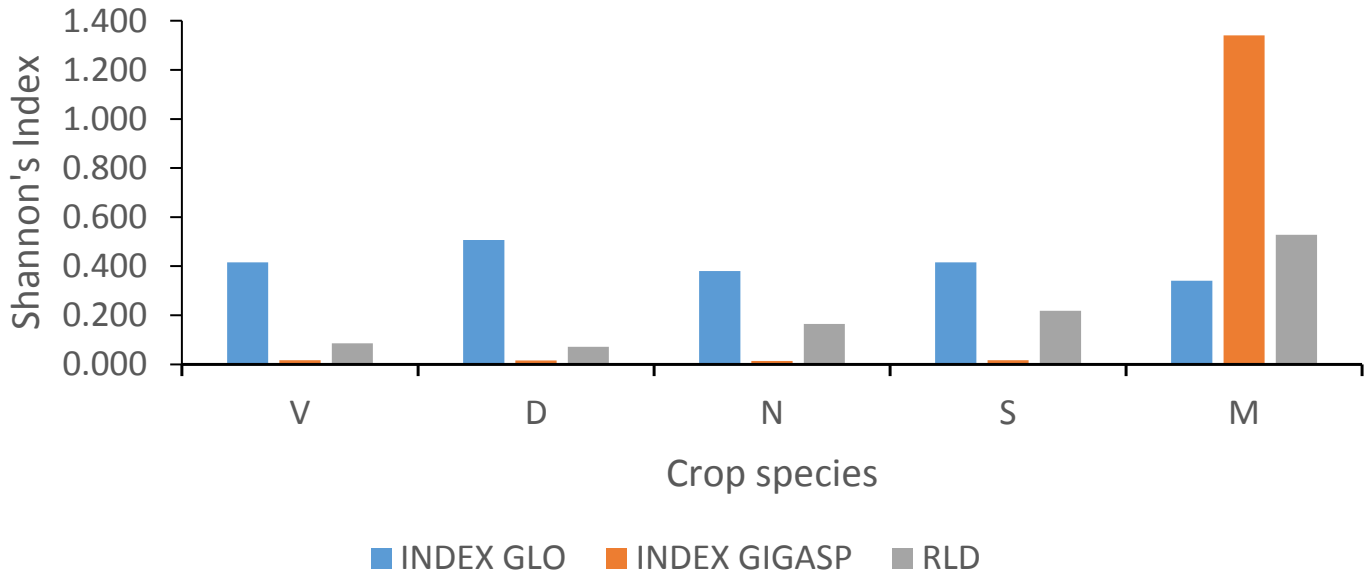


Figure 15. Shannon's index of diversity as function of crop species and root length density. INDEX GLO: Shannon's index of diversity for Glomus spore; INDEX GIGASP: Shannon's index of diversity for Gigaspora spore; RLD: Root length density.

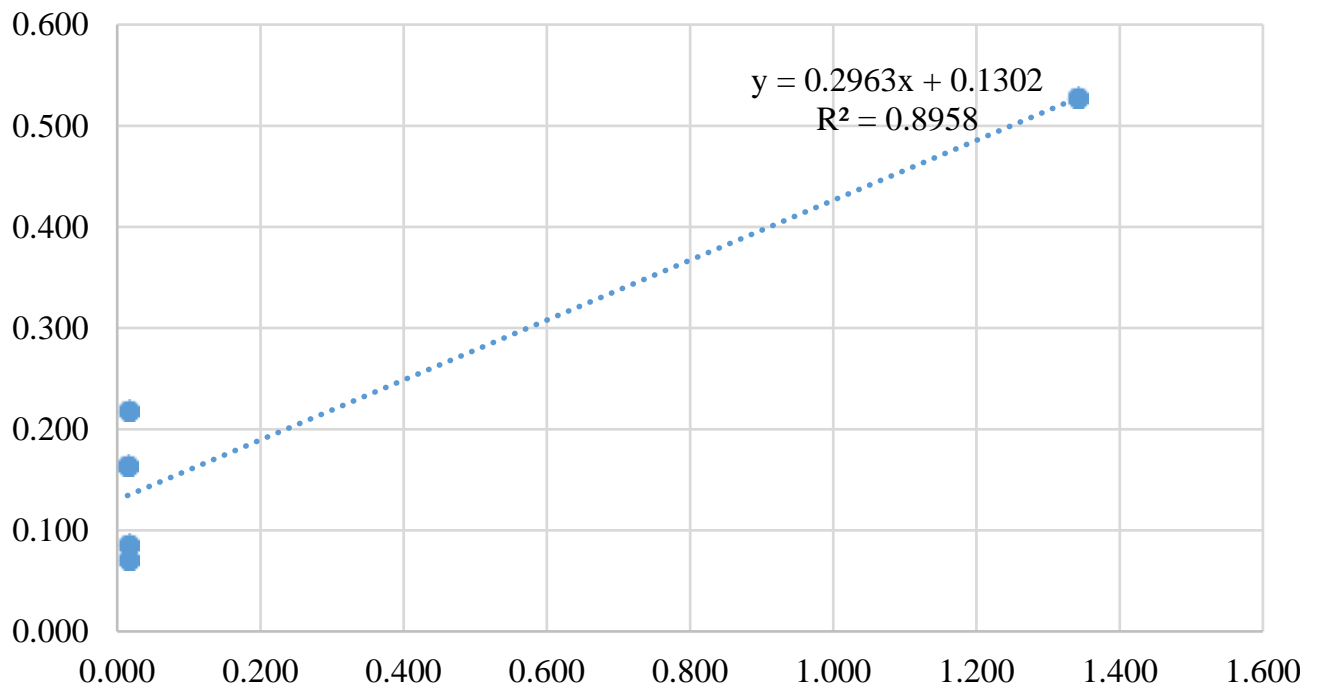


Figure 16. Correlation between the Shannon's index for Gigaspora spore as a function of root length density and crop species. A strong correlation with $R^2 = 0.896$ as coefficient of determination was found.

grain as well were better with recommended plant density D2 compared to low and traditional millet plant density D1. The dry residue obtained with plant density D1 was 798 kg/ha against 1215.98 kg/ha with plant density D2. In the same way treatment D1 yielded 358.44 kh/ha compare to 459.54 kg/ha for D2 treatment.

Years 2013, 2014 and 2015 experiments

Evolution of the number of spores from 2013 to 2015 as a function of the previous crop

From 2013 to 2015 millet was the only grown at a unique

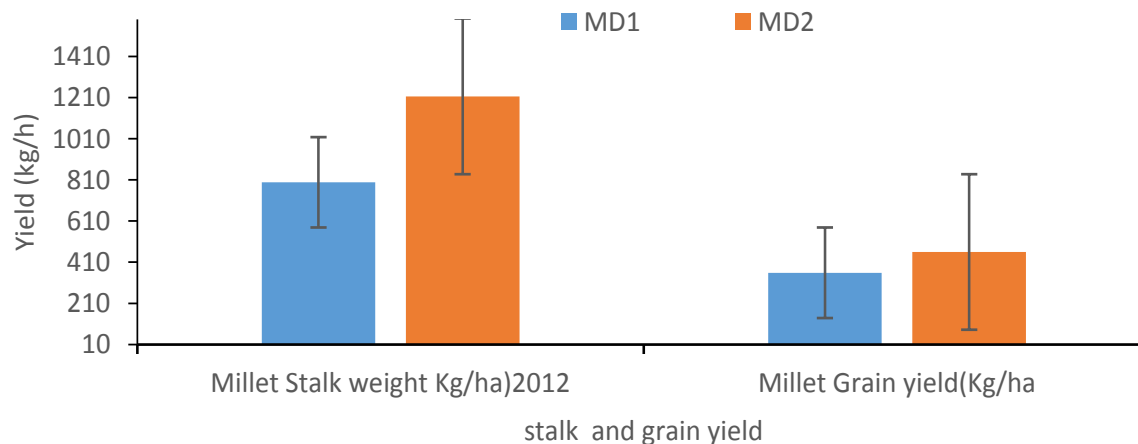


Figure 17. Millet grain and biomasse as fonction of plant density en 2012. M, millet; **D1** = farmer traditional plant density; **D2** = recommended plant density.

Table 7. Genus and number of AMF spore associated with crop species.

Culture	Glomus	Gigaspora	Total
Dolichos	1010 ^a	32 ^a	1042 ^a
Millet	603 ^b	21 ^a	624 ^b
Voandzou	739 ^{ab}	24 ^a	763 ^{ab}
Cowpea	917 ^{ab}	24 ^a	941 ^{ab}
Sesbania	578 ^b	44 ^a	622 ^b
Total	3847	145	3992
Total (%)	91	9	
Probability	**	NS	**

Probability: ** = 0.001; NS = non-significant.

plant density of 0.8 × 0.8 m. The residual effects of returned previous crops residues and that of rock phosphate application on AM fungus biodiversity and distribution were assessed.

In Table 8, it was that the found the effect of 'Year' on the evolution of the average number of spores as function of the previous crop species and the dose of crop residue returned to the soil.

With the successive legume/millet and the restitution of the minimum dose (D1's) of crop dry residues, the highest total number of 207 spores/100 g of soil was obtained in the soil sample collected in the root zone of the millet grown after the previous sesbania. In the case of maximum dose of dry crop residues (D2's), the highest number of spores 202 spores/100 g of soil was obtained from samples taken in millet root zone having cowpea as previous crop. On the other hand, for minimum dose D1's and maximum dose D2's of dry residue of culture, the lowest numbers of spores were obtained from treatment plots with millet, dolichos, and voandzou as preceding crops.

The analysis of variance showed a highly significant

difference ($P < 0.001$) between the effects of years for the x difference ($P < 0.001$) between the effects of years for the average number of total spores. The highest density of spores was obtained in year 2014 for Glomus. The DUNCAN test for the means comparison is depicted in Table 9.

The comparison of the impact of previous crop species on the basis of the year showed that the number of spores varied with previous crop species and depending on the year with the following classification:

2013: S > Pnue > J > D = N > M = V

2014: J = Pnue > N > S > V > M

2015: J > S > N = V = M = D

There was an interaction of the effects of year × dose of residue × Previous crop on the number of Glomus for the minimum dose of crop biomass D1 as indicated in Table 5. Similarly, there was a strong interaction of the maximum crop Biomass dose D2 * Glomus number/100 g of soil ($P > 0.001$). In the case of the genus Gigaspora, there was no such statistically significant interaction ($P = 0.005$) as shown in Table 7.

Table 8. Effects of interaction year × dose of previous residue on the number of spore per genus/100 g of soil.

Year	Glomus		Gigaspora	
	D1	D2	D1	D2
2014	321 ^a	279 ^a	4 ^a	7 ^a
2013	40 ^b	43 ^b	4 ^a	4 ^a
2015	22 ^b	20 ^b	3 ^a	2 ^a

The alphabetic letters (a, b, c,) indicate whether two treatments are similar or not in the same column. The same letters indicate that in the same column treatments have similar effects on a given variable D1 = Minimum biomass Dose; D2 = Maximum biomass dose.

Table 9. The average number of spores/100 g of soil as a function of applied dose of crop residue from to 2013 to 2015.

Year	Spore total/100 g of soil	
	D1	D2
2014	259 ^a	238 ^a
2013	80 ^c	86 ^c
2015	171 ^b	157 ^b

Impact of urea application on the total number of spores as a function of previous crop species

No statistically significant interaction was observed in the number of spores of Gigaspora/100 g of soil sampled in the root zone of the millet regardless the years and doses of returned dry residues of previous crop.

Impact of crop planting density on parameters of mycorrhization of the millet

Table 6 describes the effects of the interaction between seeding density and the mycorrhizal parameters such as the frequency, the intensity of mycorrhizal colonization, and the arbuscular rate; these parameters were not influenced by crop planting densities.

Intensity of mycorrhization and the arbuscular rate as a function of crop species and plant density

No significant difference was obtained between the effects of crop species on the intensity of mycorrhization and the arbuscular rate; in the same way, no significant difference was seen not only between the effects of crop planting densities but also the effects of the interaction (*Crop species*Plant density*) on the intensity of mycorrhizal colonization and arbuscular rate. However, in terms of numerical value, there was a tendency for the intensity of mycorrhization (73.35%) and the arbuscular rates (39.58%) to have higher values with the dolichos sown at density D1 compared to D2; the lowest values were observed in sesbania at planting density D1 for the

mycorrhizal intensity (52%) and the arbuscular rate (20.03%). It was also noted that the frequency of mycorrhization was about 100% for all cultures, regardless of the planting density.

Evolution of the arbuscular rate of crops between 2012 and 2014

In 2012, the arbuscular rate was the highest using dolichos (39.58%) as treatment and the lowest for the natural fallow treatment (11.37%). In 2013 and 2014, the highest arbuscular rate was 25% for millet plot with sesbania and cowpeas as previous crops; while the minimum rate was 4% for millet plots having had previously millet at plant density D1 and voandzou at plant density D2. Rotations with legumes increased the arbuscular rate. The arbuscular rate in the monoculture of millet without additional nitrogen reduced the soil mycorrhizal activities.

Frequency of mycorrhization from 2012 to 2014

The millet roots showed mycorrhizal colonization for all treatments. The frequency of mycorrhizal colonization declined for all treatments in the 2014 trial contrary to the total number of spores. Frequency is lowest in the millet plot with voandzou and dolichos as previous crops.

Figure 7 shows the curve of the evolution of the intensity of mycorrhization depending on the dose of the previous crop biomass.

The intensity of mycorrhization was almost the same for the years 2012 and 2013. No significant difference

was found between the intensity of mycorrhizal colonization in 2012 and 2013. In 2014, there was a significant decrease in the values of the intensity of mycorrhizal compared with the values in previous years.

Impact of previous crop and the return of dry straw on millet grain yield

The analysis of variance showed a variation of grain yield in millet as a function of years and the dose of dry biomass returned to the soil. In 2013, for the minimum biomass dose D1 returned to the soil, the residual effects of the previous crops, sesbania and dolichos, on the grain yield of millet are 341 and 305 kg/ha represented in the same order, 5.6 and 5 times, respectively; for those obtained on parcels having had the millet as of previous crop. Similarly, millet yields obtained on the plots previously left under fallow (J) and bare parcel (P_{new}) were 2 and 4 times, respectively of those having had millet previously. For the dose of maximum biomass D2 returned to the soil, the yields of millet obtained on plots having had sesbania and dolichos crops previously are 5.7 and 4.8 times, respectively higher than those having had previously millet crop. Similar results were obtained by Power et al. (1998) who explained this by the positive effects crop residues on soil physical and chemical properties.

A high significant difference was found between the effect of urea application and no urea application on millet grain yield in 2015 ($P < 0.001$; s.e. 7.662)

DISCUSSION

The objective of these experiments was to study the effects of the preceding crop residues on the AM fungal biodiversity associated with crop species and the effects of planting density that has the most effective impact on the parameters of mycorrhization and yield of millet.

The initial soil was dominated by high level of sand (more than 90%) conferring it a very low water and minerals retention capacity, that is, low exchangeable bases and soluble phosphorus, which is easily adsorbed by Fe and Al hydroxide compounds (Mateete et al., 2010). Soil total P (53.64-84.02 mg/kg of soil), Bray1-P (3.34-7.30 mg/kg of soil), and Olsen-P (5, 26-9, 05 mg/kg of soil) were very low. The sandy texture, the low clay and the organic matter content contribute to other nutrient losses by leaching. However, with regard to phosphate, it was found that the acidic pH condition favours its solubilisation, which is beneficial to phosphate nutrition of the millet crop (Bationo et al., 2006; Mateete et al., 2010).

Fungal diversity and density

In 2012, legumes crop recorded the highest number of

spores compared to the millet, thus confirming that the mycorrhizal dependence of millet crop is less than other legume crops in genus. The highest density of spores was obtained in the rooting zone of dolichos, followed by that of cowpea and voandzou. These correlate to the results found by Plenchette et al. (2000). Many studies also convened that factors that result in maximum plant growth will lead into maximum sporulation (Daft and Nicolson, 1972, Hetrick et al., 1992). In this respect, plant root architecture or morpho-physiological characteristics, that is, root length density, play a determinant role in their link with AM fungus. The main reasons are that plants with fibrous roots can explore and uptake nutrients and water more efficiently in the rhizosphere than the ones with less fibrous roots (Hetrick et al., 1992). These results corroborate with the findings in our study where we observed that plants with less fibrous absorbing roots (Photo 2) such as dolichos voandzou were the most associated with high spore density. Lower spore density were associated with millet, sesbania which had more fibrous absorbing roots. The richness was only 3 categories including *Glomus*, *Gigaspora* genera and non-identified spores genus. The values Shannon's index of diversity varied was mostly linked with the crop species. Except for millet plots, where the value was exceptionally high, the Shannon's index was very low for *Gigaspora*. But for *Glomus*, the index was almost the same value with *Glomus* spore in all crop specie locations (Figure 15). The index was strongly correlated with the root length density with a value of determination reaching $R^2 = 0.896$. This is an indication that the uncertain that we can have in getting a *Gigaspora* spore genus while randomly selecting sampling soil. This was an evidence in our study as the values of relative frequency (Figure 12) and relative density (RD) of *Gigaspora* genus were very low compared to that of *Glomus* genus and non-identified spore genus (Figure 13). One of the reason why such differences exist in the distribution of AM fungus genus within the study area may be the influence of abiotic factors such as soil pH, aeration, temperature, content if total C and N as suggested by Trappe et al. (1984) and Garbaye (2013). Some biotic factors might have influenced spore genus distribution such as host plants species affinity with AMF. Other reason for the uneven distribution among AM fungus spore could be that some of the non-identified spore genus might belong to *Gigaspora* genus.

It has been demonstrated in many studies that mono-cropping can narrow down AM fungus diversity index (Maiti, 2011). Mono cropping being the most practiced cultural system in the zone of Sadore, poor native AM fungal diversity might be a consequence of such practice. Crop rotation with diversification and the practice of improved fallow with selected AMF dependant plant species could be alternatives to reduce declining AM fungus diversity index (Maiti, 2011). In 2014, the overall millet yields were the lowest even with the highest

recorded rainfall at the beginning of the season compared to the rainfall of the other years with 273.9 mm cumulating from May, June and July against 207.7, 151.9 and 195.6 mm, respectively for 2012, 2013 and 2015 cumulating for the same months. Another reason for poor millet growth may be attributed to nitrogen deficiency in the soil (Bado et al., 2012). It was observed that the soil has a sandy texture with very low proportions of fine silts and clay thus having little or no water retention capabilities and essential minerals (such as phosphorus and nitrogen). This may partly explain the poor performance of millet in the absence of nitrogen in 2014 trial and its positive response to the application of nitrogen in 2015.

However, the highest density of spores was obtained in year 2014. This suggests that the proliferation of spores increased to adapt to a harsh environmental condition (such as high soil water content). These results are similar to those found by Füzy et al. (2013) who observed that an increase in soil moisture increase the proliferation of spores of AMF but reduced AMF colonization of root.

The spores of *Glomus* were the most abundant (90%) compared to the spores of *Gigaspora* (9%) in the soil of Sadore in the root zone of both legumes and millet crops as observed in the 2012 trial. Over the following three years 2013, 2014 and 2015, the predominance of the spores of *Glomus* on the *Gigaspora* persisted with the monoculture of millet on the plots of previous legume crops. The reason could be linked with many factors as it has been already well documented. Firstly, *Gigaspora* might not be as performant as *Glomus* in sandy soils due to its incapability to produce sufficient spores and germinate fast and efficiently to extend mycelium within host plant roots. This was demonstrated by the studies of Johnson et al. (1992) in which it was found that some fungal species were influenced by both soil and host plant while some others were influenced by either soil types only or either host solely. Similar results indicating the predominance of *Glomus* over other 12 AMF genus, were found by Lakshman and Channabasava (2015) from their study on *Guizotia abyssinica*. Other studies conducted in West African sahelian zones have confirmed the same trends between *Glomus* and *Gigaspora* genera. It might happen also that *Gigaspora* did not find appropriate or compatible host plants to get well established. Soil chemical properties such as low pH (4-5.6) might also be of concern (Warner and Mosse, 1982).

Our results are consistent with those of Dahiratou (1994) and Ambouta et al. (2009) on some ligneous shrubs species in the dune of Goure area, in Northern Niger; their studies showed the same trend of dominance of *Glomus* over *Gigaspora*; but, the rate of sporulation and colonization were lower compared to those found in this study. The works of Dalpé et al. (2000), Sanon (2005) and Raya et al. (2014) also outlined that the genus *Glomus* predominate the *Gigaspora* and that *Glomus* is the most common genus spore in different

localities of Africa, thus confirming its better adaptation to the different edaphic conditions; this agrees with the results found by Füzy et al. (2013). The predominance of *Glomus* over *Gigaspora* is probably due to its ability to germinate quickly, develop a network of interconnected mycelia, and sporulate extensively to deal with the various constraints of its environment (Voets et al., 2006). It was observed that the number of spores was higher in plots previously with natural fallow. This is consistent with the results of Kabir and Koide (2000), showing that weeds can not only serve as intermediate hosts for the AMF but also can enrich the soil carbon content. Our results showed that the total number of spores (and also *Glomus*) counted in millet plots with precedent as bare fallow (Pnue), surpassed that obtained in millet plots previously with Cowpea, Dolichos or millet crops. This contradicts with the work of Troeh and Loynachan (2003), who found that the bare fallow reduced the number of viable propagules in the soil. In the case of *Gigaspora* spores, the proportion remains low compared to *Glomus* but does not justify its low importance at fortiori; two assumptions could be possible: (1) It could be that the *Gigaspora* spores are very efficient and have the same status as the minor elements or micro-elements from macronutrients in soil nutrients; although the plants do not need large amount of micro- elements, the deficiency in these elements can have serious consequences, thus affecting the development of the plants; similarly, *Gigaspora* at low proportion in the soil could germinate and play an essential role for the nutrition of the host plant via symbiotic nutrients exchange momentarily in the life of the host plant. More investigations are necessary to justify this statement; (2) It is also possible that either edaphic conditions such as temperature, pH, moisture or other stimuli (Koske and *Gigaspora*, 1981) as cited by Barbara (1984). Ross and Ruttencutter (1977), comparing the performance of colonization of a same plant by *Glomus macrocarpum* and *Gigaspora gigantea*, suggested that the same plant host is not favourable to the proliferation of the spores of *Gigaspora*. The performance of *G. macrocarpum* was better than that of the *G. gigantea* because the latter did not form vesicles. As suggested by Schenck and Kinch (1975), the affinity between the genus of fungus and crop species can influence the number of spores germinating in the soil. In their studies, the number of spores of genus *Gigaspora* spp. was higher around the roots of soybeans, while the genus *Glomus* and *Acaulospora* were the most numerous around the cultures of monocotyledonous. Further studies on the number of crop species either in plots or full fields are needed to elucidate this difference in performance between the two genera of spores, *Glomus* and *Gigaspora*.

Sporulation of the following millet

In this study, it appeared that in the legume/millet

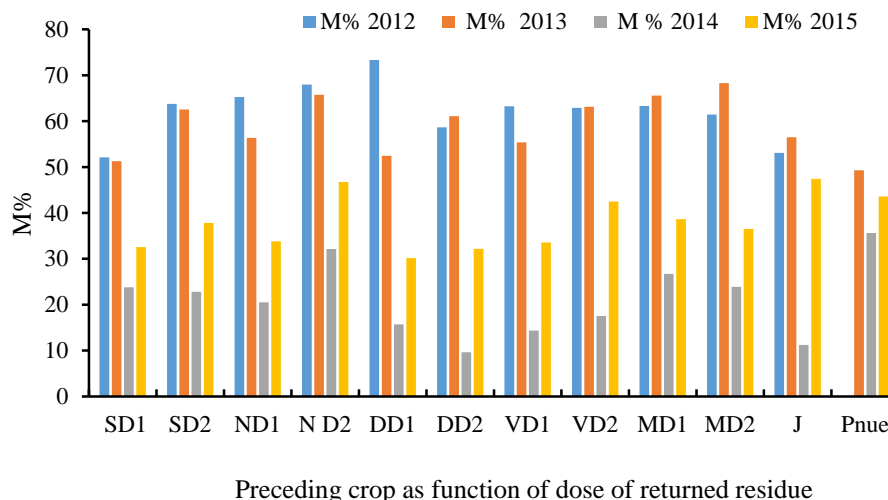


Figure 18. Evolution of the intensity of mycorrhization from 2012 to 2015. S, Sesbania; D, Dolichos; N, cowpea; V, Voandzou; Pnue, bare soil; J, natural fallows; D1 = minimum dose of crop residue; D2 = maximum dose of crop residue; M% = intensity of mycorrhization indicate the percentage of root that bears fungus.

rotation, the legume crops influenced the mycorrhizal formation of millet root and that millet following cowpea as previously grown crop had the highest density of total spores in the root zone. This is consistent with the results found by Bagayoko (1999). The percentage of sporulation is dependent of the crop species that host the mycorrhizal fungus. Some species favour the proliferation of the spores of fungus than others depending on their influence on the edaphic conditions including pH, which may vary with the root secretions.

Legumes facilitate sporulation in their root zone with regards to the relationship plant-rhizobia root - AMF that develops with legumes, this relationship is very beneficial to the plant mineral nutrition. Nodules are the site of activity of rhizobia for the synthesis of nitrogen, while through their intra/inter and extra cellular matrix hyphae, arbuscular mycorrhizal fungi supply host plant roots with minerals (such as phosphorus, nitrogen, zinc, calcium, water and others).

Mycorrhizal parameters

In the case of the mycorrhizal parameters such as the mycorrhizal frequency (F%), the intensity of mycorrhizal (M%) and the arbuscular rate (%), our results showed that the roots of all crop species are colonized by the AMF with varying degrees. These are similar to the results found by Dahiratou (1994), Dalpé et al. (2000) and Ambouta et al. (2009).

Mycorrhizal frequency (F%)

The frequency of mycorrhization is at peak (99 to 100%)

across cultures (Figure 19); this demonstrates symbiotic association with AMF. These results are consistent with those obtained by Issoufou (2013) who conducted studies on the mycorrhization of millet and Cowpea. Furthermore, Ambouta et al. (2009) work on ten shrubs in Niger and obtained varied frequency rates. Planting density of cultures had an influence on frequency. The frequency of mycorrhizal colonization had the same value in 2013 for millet in rotation with legumes.

Intensity of mycorrhizal colonization (M%)

A high intensity of mycorrhization between 50 and 75% was obtained in the results. From 2012 and 2013, the intensity of mycorrhizal colonization has not shown any statistical difference. The 2013 grown millet benefits from the residual positive effects of previous 2012 legumes crops with arbuscular mycorrhizal fungus propagating parts (such as roots, spores and mycelium). In 2014, a significant decrease in the intensity of mycorrhizal colonization was observed (Figure 18) due to high humidity of the soil, which reduces the root colonization by the AMF as a result of high rainfall (740 mm) compared to the other years (Figure 1). These results are in agreement with the results found by Bagayoko (2000b) and Deepika and Kothamasi (2015). On the other hand, since the soil is very poor in mineral elements and mostly soluble P, the mycorrhization becomes obviously the only way to ensure phosphate nutrition from the stocks of non-soluble phosphorus, which is well known to maximize colonization and sporulation (Daniels and Trappe, 1986) and that any abundance of phosphorus in the soil solution around the plant roots could reduce the intensity of the mycorrhizal activity. The pH has a significant

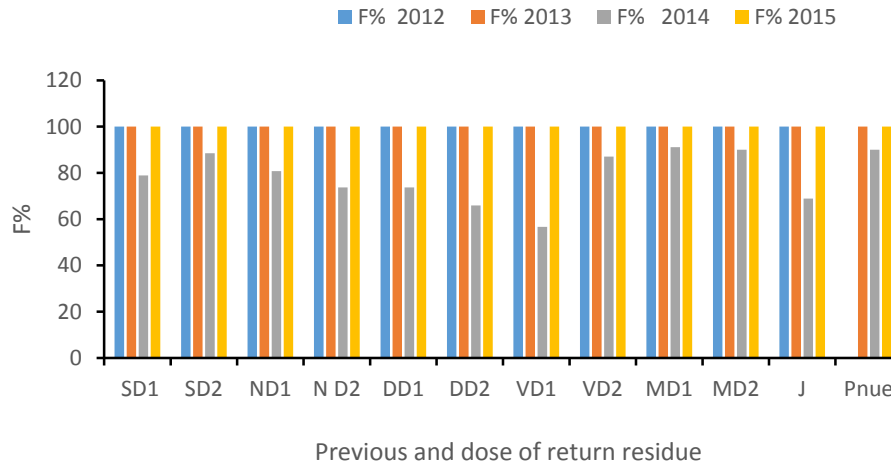


Figure 19. Evolution of the frequency of mycorrhization of millet root as a function of previous crop from 2012 to 2015. S, Sesbania; D, Dolichos; N, cowpea; V, Voandzou; Pnue, bare soil; J, natural fallows; D1 = minimum dose of crop residue; D2 = maximum dose of crop residue; F% = Frequency of mycorrhization indicate the percentage of root that bears any portion of fungus.

influence on the ability of infection and reproduction of the fungus (Hepper, 1984). This optimal pH, however, allows a maximum development of the fungus and strongly favours the growth of the host plant. For example, Trappe et al. (1984) suggested that the germination of *Glomus* is optimal at the neutral pH, while the performance of the Gigasporaceae is maximal at a slightly acid pH between 4 and 6. This contradicts with the results of the present study though the pH was less than 6, the predominance of *Glomus* spores over *Gigaspora* spores was observed. There may be other factors other than the pH limiting the proliferation of *Gigaspora*. Guissou (2001) showed that *Glomus manihotis* is a species of AMF tolerant to significant changes in pH. According to some research findings, other factors controlled by the plant (such as speed of growth, thickness of the roots hairs, efficiency of the root uptake, mycorrhizal colonization and resistance to pathogens resistance) could affect the plant mycorrhizal dependency (Hetrick et al., 1992) and may vary depending on the morphology of roots. Plants with large roots and little hairs, without the mycorrhizal association, are theoretically less effective in the exploration of soil and absorption of mineral elements than plants equipped with a root system branched with many hairs.

Arbuscular rate (A%)

The effectiveness of AMF is summed up in the formation of arbuscular structures privileged with metabolic/mineral exchange sites between the plant and the fungus (Abbot, 1982; Lagrange, 2009). The arbuscular rate in this study varied depending on the crop species and did not

correlate with their planting density. This is consistent with the results found by Bagayoko et al. (2000) pointing out the positive impact of legumes on the parameters of mycorrhization of crops in rotation. It was observed that crop species differ based on their impacts on mycorrhizal parameters; this correlates with the results found by Karanja et al. (2011) and Guissou (2001), showing that legume species differently influence the development of mycorrhizal colonization. Also, inter- and intra-specific differences between strains of AMF exist and affect the mycorrhizal dependency (Plenchette et al., 2000). In the present study, low rates of arbuscular ranging from 20% to less than 40% were obtained. Although between the crop species as well as the densities of seedlings, no significant differences were found in the arbuscular rate (Figure 20). This result showed that a high frequency of mycorrhizal colonization and a high intensity of mycorrhization of crops are not linked a fortiori with high efficiency of colonization, which is translated into a high arbuscular rate.

The inter-annual variation of arbuscular rates observed between 2013 and 2014, depending on previous cropping, are consistent with the results found by Füzy et al. (2013); Knopf et al. (2016) highlights the impact of the humidity of the soil on the sporulation and mycorrhizal parameters. Rotation with legumes improved the arbuscular rate compared with monoculture (this is similar to the results found by Li et al. (2009)). On the other hand, Vincent (2008) suggested that the inoculum left by a culture has a positive or negative influence on the settlement of the following crop. The monoculture of millet without additional nitrogen reduced the rate of sporulation in the soil. This is consistent with the work of Oehl et al. (2003) who justified this by the fact that

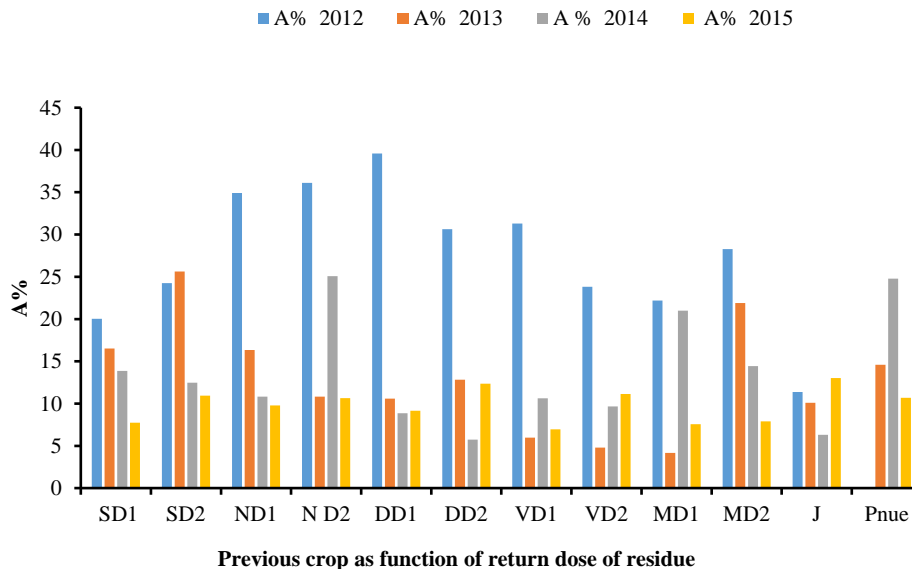


Figure 20. Evolution of arbuscular rate on millet crop roots as a function of the dose of returned crop residue 2012-2013-2015. **S**, Sesbania; **D**, Dolichos; **N**, cowpea; **V**, Voandzou; **Pnue**, bare soil; **J**, natural fallows; **D1** = minimum dose of crop residue; **D2** = maximum dose of crop residue; A% = arbuscular rate indicating the percentage of root with mycorrhizae root that bears arbuscular formation.

the monoculture of a crop species could, not only, alter the fungal biodiversity of a medium but also limit it to species that are very weak and not beneficial to the symbiotic association (Xiao et al., 2010).

Impact of millet grain yield

The millet yields obtained in 2012 were far superior to those of other successive years from 2013 to 2015 (Figure 22). Firstly, the previous crop, which was the *Mucuna* sp. certainly produced the organic substrate and the minerals that were favourable to millet growth; on the other hand, rainfall had been pretty good and favourable to the cultivation of millet. In 2014, the millet yield declined at its lowest level because of the irregularity in the patterns of rainfall.

The performance of millet in 2015 was wiped out by red mite attacks, thus causing enormous damage on the millet after the heading stage. However, the effect of applying urea in micro dosing significantly increased millet grain yield (Figure 21). This shows the importance of urea in enhancing the effect of phosphorus and crop residue on soil fertility relief (Tabo et al., 2007, Adamou et al., 2007; Bado et al., 2014).

Production of dry crop biomass

The study showed that the production of crop dry biomass varied according to crop species and their

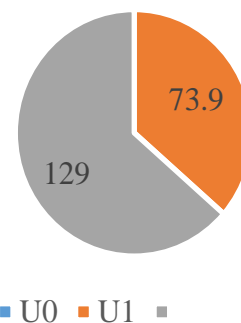


Figure 21. Effect of Urea application on millet grain yield in 2015 (kg/ha).

densities of seedlings. Nevertheless, in this study, it must be noted that due to the damages caused by squirrels, rats and hedgehogs, crop species (such as voandzou and dolichos) seedling densities did not affect the production of the dry biomass. The proliferation of arbuscular mycorrhizal fungi in the soils is much influenced by the level of soil fertility in the particular soil solution content of mineral elements and available phosphorus and nitrogen, as well as the level of acidity of the (Garbaye, 2013). The pH level of our test plot varies from 4 to 4.6, which represents a condition of high acidity risk, which may lead to aluminium toxicity according to Arvalis Institut du végétal, the Chamber of Agriculture Tarn (2005).

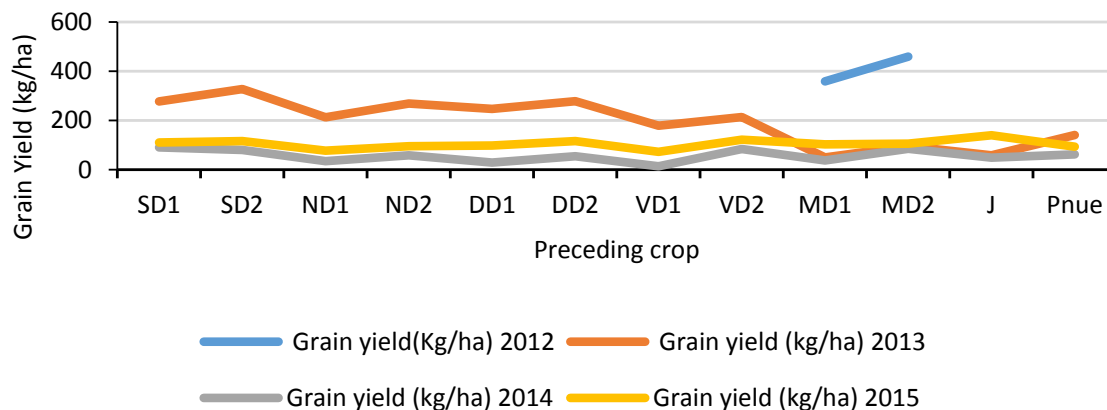


Figure 22. Millet grain yield as compared to the yields of 2013, 2014 and 2015.

Conclusion

The results of this study have shown that the sandy soils of Sadore contained spores of arbuscular mycorrhizal fungi. The two most important types of spores found in this study are the genus *Glomus* and the genus *Gigaspora*. *Glomus* was more abundant compared to *Gigaspora*. Non-identified spore genus was also found in the soil. The legumes and cereal showed strong mycorrhizal links. The crops with the highest density of total spore/100 g of sample soil in the rooting zone were dolichos, followed by cowpea, voandzou, millet and sesbania. This was in link with their rooting system architectural characteristics. The Shannon's index of diversity was influenced by crop species and varied across the year of monoculturing of millet. The parameters of mycorrhization were neither influenced by the planting density of legume crops nor by the dose of crop residue returned to the soil across years. The residual effects of the previous crop residue on millet yield persisted over years mostly for sesbania, dolichos and cowpea as base crop. However, the performance and the durability of the residual effects of Natural fallows (J) and bare parcel (Pnue) on the mycorrhizal (mycorrhizal parameters and fungus spores number) were observed during the three years of monoculture of millet. It was recommended that fallow is still a way/option of restoration of the soil fertility and should be introduced as an alternative in the cycle of monoculture. Alternative solution to soil the increased Shannon's index of diversity could be the practice of crop rotation, improved fallows and crop diversification.

In the case of the Sadore sandy soil, the results obtained after the application of urea showed that the parameter 'Nitrogen' also played a decisive role in the mycorrhizal colonization and yield of the millet. Therefore, appropriate time and application rate such as micro dosing at tiller development and panicle initiation stages could tremendously favour mycorrhizal activities and millet crop productivity.

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

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