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Research Article

Influence of biofertilizers on the diversity of indigenous arbuscular mycorrhizal fungi associated with the rhizosphere of plants during crop rotation between *Sorghum bicolor* L. Moench and *Glycine max* L. Merrill

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Abstract

This study aimed to evaluate the influence of biofertilizers applied during crop rotation between *Sorghum bicolor* and *Glycine max* on the diversity of native arbuscular mycorrhizal fungi in the plant rhizosphere. The plant materials consisted of seeds of *S. bicolor and G. max*. The experimental design was a split-plot design. Four treatments were applied: control, *Bradyrhizobium japonicum, Trichoderma asperellum*, and *Bradyrhizobium japonicum* + *Trichoderma asperellum*. Two growing seasons were conducted, interspersed with a three-month fallow period. Crop rotation was implemented during the second growing season. Soil samples from the rhizosphere of *S. bicolor* and *G. max* plants, as well as plant roots, were collected randomly. The physicochemical characteristics of the soils, mycorrhization indices, and morphological characterization of arbuscular mycorrhizal fungi associated with the plant rhizosphere were carried out according to the applied treatments. After three months of culture, growth parameters were evaluated. The results showed that the soil pH was acidic (5.8) and had a clayey texture. The highest mycorrhization frequency was 100% in *S. bicolor* and *G. max* plants at month 3, for all treatments under non-rotation conditions. The relative abundance of AMF spores appeared to be 6.25% under rotation conditions in *B. japonicum* and 4.9% under non-rotation conditions in the presence of *T. asperellum*. Seven genera of arbuscular mycorrhizal fungi were identified in the soils using https://invam.ku.edu; *Acaulospora* sp. *Claroideoglomus* sp., *Entrophospora* sp., *Funneliformis* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora sp*. The growth parameters varied significantly depending on the treatment applied. The *Bradyrhizobium japonicum* + *Trichoderma asperellum* treatment had the greatest influence on growth but resulted in the lowest yields of *S. bicolor* and *G. max*. Crop rotation and fertilization significantly influenced the presence of indigenous arbuscular mycorrhizal fungi in the rhizosphere.

1. Introduction

Arable soils have become vulnerable due to climate change, degradation, intensive agriculture, and biodiversity loss [1]. Consequently, many farmers, primarily in developing countries, struggle to produce, market, and feed their families [1,2] while facing increasingly unpredictable climates, low and unpredictable crop yields, and chronic food insecurity [2-4]. These challenges are particularly acute in sub-Saharan Africa, where approximately 65% of the agricultural land is degraded [5]. Of the approximately 350 million hectares, representing 20 to 25% of the total land area, at least 100 million hectares are estimated to be severely degraded, primarily due to agricultural activities, the major consequence of which is the loss of soil fertility [5, 6]. Numerous techniques have been proposed to address soil fertility problems has become increasingly common. Chemical fertilization has thus far been the most widespread practice in agricultural production [7]. However, the large quantities of chemical fertilizers applied over the past few decades to feed a growing world have led to a series of environmental problems, such as soil degradation, nitrogen (N) leaching, and a drastic reduction in soil organic matter content and microbial activity [8]. The urgent need to use environmentally friendly strategies, such as crop rotation and cultivation using microorganisms, would help to mitigate the long-term effects of chemical fertilizer application.

Crop rotation is a strategy used in organic farming to maintain soil fertility, with particular attention paid to nitrogen nutrition through the inclusion of legumes [9]. One of the key benefits of crop rotation is the prevention of plant diseases and pest infestations. Changing crops from one season to the next on the same plot reduces the duration of the disease or pest proliferation. Crop rotation is advantageous for farmers because it improves tolerance to drought conditions [10], increases the replenishment of nitrogen residues, providing more nitrogen for the following harvest [11], and consequently leads to improved or more stable yields [12,13]. Furthermore, numerous studies have reported that crop rotation increases in soil organic carbon (SOC) content and improves soil porosity compared to intensive cropping systems [14]. Some authors have reached mixed conclusions regarding the impact of the experimental duration of crop rotation on soil properties [15]. However, it is worth noting that research has highlighted that crop rotations improve soil fertility while increasing microbial biomass, diversity, function, and activity, one consequence of which can be the stimulation of nutrient cycles, such as the nitrogen cycle [16–18]. Nevertheless, according to [16], soil properties and soil microbial communities evolve under the influence of crop rotation diversification and the type of crop used. However, the impact of crop rotation on the mycorrhizal types present in the soil is poorly understood. Arbuscular mycorrhizal fungi are microorganisms that live in symbiosis with the roots of many plant species [19]. They are ubiquitous in soil. They help plants obtain improved hydromineral nutrition and consequently, contribute to optimizing their growth [19]. Therefore, the effectiveness of this symbiosis could be improved by practicing crop rotation [20]. Indeed, crop rotations affect the composition of arbuscular mycorrhizal fungi spore communities in the soil, with greater AMF diversity under crop rotation compared to monoculture [20–22]. However, a concern remains, because some farmers apply chemical products during crop rotation to address soil infertility. However, what impact can the application of biofertilizers have on the diversity of soil microorganisms during crop rotation? Does this cultivation practice influence the diversity of native arbuscular mycorrhizal fungi? However, the variability among the species of arbuscular mycorrhizal fungi present in the rhizosphere depends on the plant's response to applied fertilizers. Identifying the native AMF in the rhizosphere under fertilization conditions would allow us to understand whether they are also capable of establishing symbiosis when the plant has another source of nutrition. Thus, this study aimed to evaluate the influence of biofertilizers applied during crop rotation between *Sorghum bicolor* and *Glycine max* on the diversity of native arbuscular mycorrhizal fungi in the soil.

More specifically, it will: characterize the soil structure of the cultivation site, determine the mycorrhizal status of *S. bicolor* and *G. max* in the first and second campaigns of cultivation under no-rotation and rotation conditions, depending on the treatments applied to characterize morphologically the different mycorrhizal types present in the soils of the experimental site, before and after treatment, and to assess the impact of the applied fertilizer on the growth and yield of *S. bicolor* and *G. max* plants.

2. Materials and methods

2.1. Experimental site and characterization of the physicochemical structure of the planting site before sowing

The work was conducted in the Centre Region, Mfoundi Department, and more specifically at the Higher Teacher Training College of Yaoundé 1. The field was prepared just outside the boundary between the Teacher Training College and the National School of Administration and Magistracy (ENAM), with the geographical coordinates 03°51′36′′ North latitude and 11°30′37.5′′ East latitude. This sampling site is located in the humid forest zone with bimodal rainfall (Zone V). The analysis of the soil's physicochemical characteristics was carried out at the University of Dschang, Faculty of Agronomy and Agricultural Sciences, Soil Analysis and Environmental Chemistry Research Unit (URASCE), following the current methods recommended by [23] and complying with ISO, AFNOR, NF, and EN standards. The analyses carried out in the surface soil samples (0–20 cm) mainly included the sand, clay and silt content, the pH in aqueous (pH - H2O) and saline (pH KCl) media, the organic carbon (CO) content, the total nitrogen (Ntot) content, the exchangeable base (Ca, Mg, K and Na) content, the cation exchange capacity (CEC), the available phosphorus (P Bray II) content, the sum of exchangeable bases (SBE) and the saturation rate (V).

2.2. Plant, fungal, and bacterial material

The plant material used consisted of a variety of *S. bicolor* (S35), characterized by a semi-maturity cycle of 90 days, a potential yield of 3 t/ha, with very small, white seeds; and a variety of *G. max* (TGX-1835-10E), characterized by a sowing-to-maturity cycle of 110-120 days, a dwarf/erect growth habit, a potential yield of 1.5 to 2 t/ha, and small, round, cream-colored grains. These varieties were obtained from the IRAD station in Maroua (variety S35) and the IRAD station in Foumbot (variety TGX-1835-10E). The biofertilizer used consisted of *Trichoderma asperellum*, obtained from the Biological Control Laboratory of IRAD in Yaoundé, and a strain of *Rhizobium* bacteria (*Bradyrhizobium japonicum*), obtained in Nigeria, to coat the soybean seeds before sowing.

2.3. Experimental design

The design was a split-plot consisting of 32 plot units, 16 for *S. bicolor* and 16 for *G. max*. The treatments applied were as follows: control plants; plants fertilized with *Bradyrhizobium japonicum* (RH), *Trichoderma asperellum* (TR), and *Bradyrhizobium japonicum* + *Trichoderma asperellum* (RH+TR). Each treatment was repeated three times. Two sowing campaigns were conducted in this study. Plant growth was monitored for three months for each campaign. A three-month fallow period was observed between the two campaigns. In the first growing campaign, *S. bicolor* and/or *G. max* plants were sown in 16 plot units, following the treatments applied. In the second growing campaign, each of the 16 plot units allocated to *S. bicolor* and/or *G. max* was divided into two units of 8 plots each. The plants were sown separately. In the first 8 plot units, *S. bicolor* and/or *G. max* were sown in the same plots as in the first campaign (no-rotation condition). However, in the second 8 units, the *S. bicolor* and *G. max* plants were rotated between the *S. bicolor* and/or *G. max* plots from the first campaign (rotation condition).

2.4. Sowing, fertilization and plot maintenance

The seeds of the *G. max* and *S. bicolor* varieties were sown in rows according to their technical specifications within the plot units according to the treatments applied. The distance between two the adjacent plots was 1 m. Each plot contained four rows of *G. max* or *S. bicolor*, with two successive rows spaced 20 cm apart. Each planting hole received an average of three seeds, with a spacing of approximately 25 cm between the holes. A total of 30 g of dried brewer's grain powder containing the *T. asperellum* strain was applied to each hole in the plots fertilized with *Trichoderma* on the day of sowing. However, for fertilization with the bacterium, 30 g of *Bradyrhizobium japonicum* was used to coat the grains. After sowing, the plots were watered daily, in the morning and evening. Site maintenance was carried out monthly during the three-month growing season by manual weeding.

2.5. Determination of the mycorrhizal status of S. bicolor and G. max under non-rotation and rotation conditions, according to applied treatments

Mycorrhizal status was evaluated according to the protocol of [24] on *S. bicolor* and *G. max* plants in the first and second trimesters of cultivation under non-rotation and rotation conditions, according to the applied treatments. Root hairs from *S. bicolor* and *G. max*, harvested in the field and following treatments every month, were cleaned, sectioned to a length of 1 to 1.5 cm, and successively soaked in 10 $g.L^{-1}$ of KOH for 15 min at 90 °C in a water bath, then blanched with 10% H_2O_2 for 3 to 6 h, and rinsed with 10% HCl. These root hair fragments were stained directly with 0.15% acid fuchsin (0.15 g fuchsin + 50 mL glycerol + 30 mL lactic acid + 20 mL distilled water). Microscopic observations were performed using a YVMEN optical microscope at 400X magnification. The root fragments were observed per treatment, and the mycorrhizal status (F% and M%) was calculated according to the scale proposed by [24].

2.6. Morphological characterization of the different AMF present in the soils of the experimental site, before and after treatment, according to growing conditions

To determine the AMF present in the soils, soil samples were collected in three stages: before sowing the plots, after the first campaign, and after the second campaign, following non-rotation and rotation conditions. In the laboratory, the soil samples collected after each plant treatment were labeled and dried at room temperature for further analysis. The wet sieving method [25] was used for spore extraction and enumeration in the soil. This method is based on sieving soil through a series of stacked sieves with different mesh sizes: 0.5, 0.25, 0.125, and 0.0625 µm. Spores extracted and enumerated from the soil before and after treatment were morphologically described according to the IVAM identification key [26]. Identification was performed using a light microscope. The basic element used for the identification of AMF is spore morphology (shape, size, color, number of separating membranes and cell walls, etc.). Spores were mounted by type and according to the plant species between slides and coverslips in PVLG (polyvinyl lactoglycerol) composed of 100 mL distilled water, 100 mL lactic acid, 10 mL glycerol, and 16.6 g polyvinyl alcohol. The distinction and description of the extracted species were carried out using the identification key of [27], the European Bank of Glomales (BEG) key, and the International Culture Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi [26]. The classification method [28] was used to classify the species. The spore density was determined for each soil sample. It is defined as the number of spores in 1 g. of soil. Species diversity was assessed using species richness, the Shannon and Wiener diversity indices [29]. The Shannon index (H), which ranges from 0 (a single species, or one species that is far more dominant than all others) to log S (when all species have the same abundance), is based on the concept of entropy. Biodiversity can be measured according to the following formula.

$$H = -\sum_{i=1}^{S} ni/\,N\ Log(\ ni\,/N\ )$$

S = total number of species, Ni = number of individuals of genus, i in a given category, N = total number of individuals of all genera in a given category, log2 = base-2 logarithm, H = Shannon diversity index.

2.7. Evaluation of growth parameters in S. bicolor and G. max according to the different fertilizers applied during each growing season

To assess the impact of applied fertilizers on growth, agronomic growth parameters were measured in *S. bicolor* and *G. max* during the first and second (under rotation and no-rotation conditions) growing seasons after 90 days of cultivation, following the protocols of Bengono [30]. These parameters were: average plant height, average stem diameter, average number of leaves, average leaf area, average number of roots, average number of ears or pods, and yield (kg/m^2).

2.8. Statistical analyses

The results were subjected to descriptive analysis (mean ± standard deviation). The results are presented in the form of graphs and tables (Microsoft Excel 2013). IBM SPSS version 20.0 was used to perform statistical analyses and compare means using analysis of variance (ANOVA) with the Student-

Table 1. Physico-chemical analyses of the soils at the cultivation site before sowing.

Physical analyses			Chemical analyses												pH	
C (%)	S (%)	S (%)	TBE	CEC	V (%)	OM (%)	P (%)	N (%)	C (%)	C/N	K^+ (%)	Ca^{2+}	Mg^{2+}	Na^+	H_2O	KCl
46,25	16	37,75	5,05	18,25	27,67	1,77	19,89	0,231	1,03	4,44	0,98	3,01	1,05	0,01	5,8	4,8

Sand (S%), clay (C%), and silt (S%) content. Organic carbon (CO), total nitrogen (N) Exchangeable bases (Ca^{2+}, Mg^{2+}, $K^{+,}$ and Na^+), Cation exchange capacity (CEC), Available phosphorus content (P Bray II), Total exchangeable base (TBE), and Saturation rate (V).

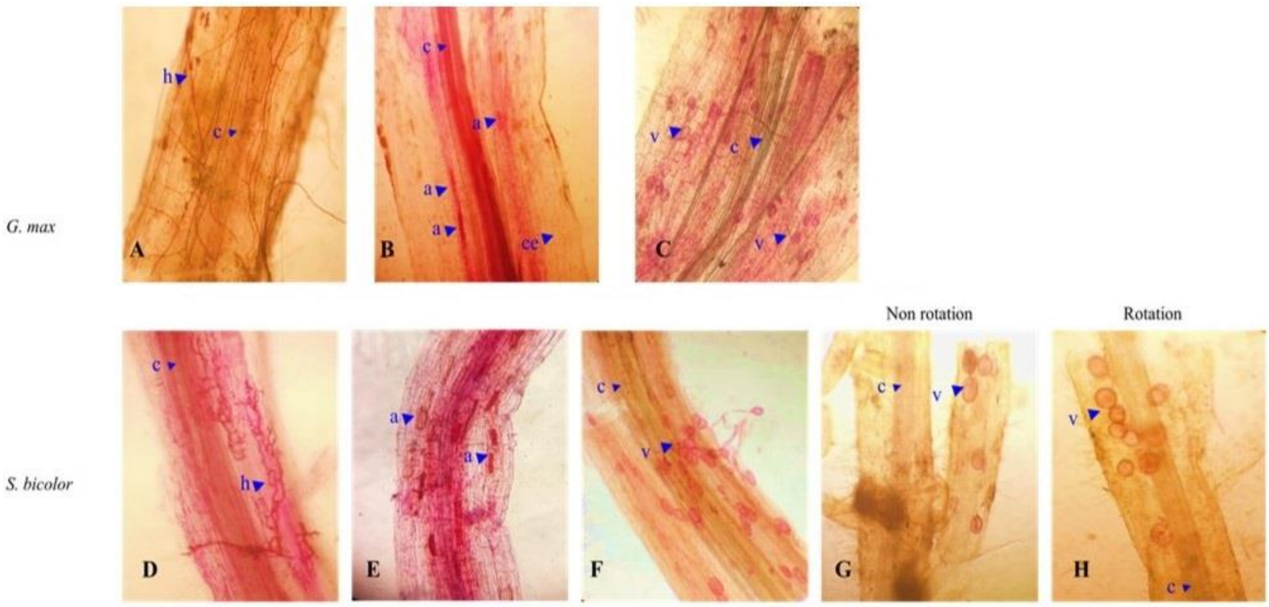

Figure 1. Mycorrhizal structures observed in root hair fragments of *S. bicolor* and *G. max* plants stained with acid fuchsin at 400X. Roots showing hyphae (A and D), arbuscules (B and E), and vesicles (C, F, G, and H). Large vesicles observed under non-rotation and rotation conditions in *S. bicolor* plants with *B. japonicum* + *T. asperellum* (G and H). Arbuscules (a), hypodermal plant cells (Ce), cortex (c), and hyphae (h).

Newman-Keuls test at the 5% significance level. Correlation analyses of the studied parameters were performed using R software version 4.2.3. Furthermore, a pairwise matrix was established to examine the linearity between each pair of variables, which is an essential condition for the validity of Pearson's test.

3. Results

3.1. Physicochemical analysis of the soil

The soil substrate analysis of the cultivated site (Table 1) revealed that, the soil was composed of 46.25% clay, 16% silt, and 37.75% sand, classifying it as clayey. Chemically, the pH was acidic, with a pH of 4.8 (HCl). The soil was very poor in organic matter (OM = 1.77%). The results showed that the soil was rich in mineral nitrogen (N = 0.231%) according to the Kjeldahl method (NFISO 11261 standard). We also observed very low organic matter mineralization with a C/N ratio of 4.44 (C/N < 6), a high cation exchange capacity (CEC) (18.25%), and a normal level of exchangeable bases (EBS) (EBS = 5.05%). It should be noted that the soil at the cultivation site is very poor in terms of available phosphorus (Table 1).

3.2. Cytological analysis of roots

The cytological organization of root hair fragments observed in *S. bicolor* and *G. max* plants revealed endophytic structures such as intra and inter-root hyphae traversing the root cortex, vesicles, and arbuscules (Fig. 1). These structures, stained dark or light red with fuchsin, are characterized by their high chitin content. All *S. bicolor* and *G. max* plants exhibited root colonization by intra and inter-root hyphae (Figs. 1 A and D). The structures of the arbuscules (Figs. 1 B and E) and vesicles (Figs. 1 C, F, G, and H) were also recorded. It is noted that some vesicles appeared larger, as observed in the root hair fragments of *S. bicolor* plants fertilized with *B.*

Table 2. Evaluation of mycorrhizal status during the first and second campaign of culture in *S. bicolor* and *G. max.*

Plants	**Time (Months)**	**Treatments**	**Mycorrhizal status**					
			First campaign		**Second campaign**			
					Non-rotation		**Rotation**	
			F (%)	**M (%)**	**F (%)**	**M (%)**	**F (%)**	**M (%)**
S. bicolor (S35)	1	Control	88.00d	8.35d	93.00b	14.68de	98.00b	14.00c
		RH	70.00d	4.88b	100.00b	17.10f	90.00b	4.15a
		TR	67.90c	5.60c	97.00b	19.64f	87.30b	4.77a
		RH+TR	40.00b	3.98b	95.00b	6.05b	95.00b	2.35a
	2	Control	94.00e	19.58g	98.00b	7.97c	90.00b	14.80c
		RH	62.00c	2.00a	100.00b	26.00h	95.00b	4.60a
		TR	62.00c	0.91a	100.00b	11.90d	95.00b	2.10a
		RH+TR	42.50b	1.14a	100.00b	20.05	90.00b	16.45d
	3	Control	92.00e	11.30e	100.00b	26.55h	100.00b	15.50c
		RH	95.00e	15.9f	100.00b	36.80i	100.00b	21.43e
		TR	95.00e	7.04d	100.00b	16.75f	100.00b	9.75b
		RH+TR	97.50e	16.10f	100.00b	22.78g	98.00b	14.00c
G. max (TGX-1835-10E)	1	Control	29.00a	0.61a	92.00b	11.50d	92.00b	11.50b
		RH	37.00a	1.80a	92.00b	2.74a	93.00b	13.80c
		TR	34.18a	8.39d	85.00ab	12.77d	64.31a	34.18f
		RH+TR	26.00a	0.65a	80.00ab	3.01a	100.00b	25.44e
	2	Control	89.00d	9.32d	100.00b	12.35d	100.00b	15.12c
		RH	69.50c	6.91c	97.00b	7.31c	100.00b	13.81c
		TR	67.35c	5.31b	94.00b	5.62b	96.91b	10.62b
		RH+TR	50.50c	1.46a	93.00b	4.67b	97.00b	15.28c
	3	ControlT	71.00d	1.81a	100.00b	14.84de	100.00b	14.40c
		RH	74.50d	4.28b	100.00b	12.07d	100.00b	11.72b
		TR	74.50d	3.87b	100.00b	13.42d	100.00b	13.02c
		RH+TR	61.00c	7.35d	100.00b	13.01d	100.00b	20.72e

Student-Newman-Keuls test (P<0.05). Means with the same letter in the same column are not significantly different. Treatment: Control; *Bradyrhizobium japonicum* (RH); *Trichoderma asperellum* (TR) and *Bradyrhizobium japonicum* + *Trichoderma asperellum* (RH+TR).
F%: Frequency of mycorrhizal colonization and M%: Intensity of mycorrhizal colonization.

japonicum + *T. asperellum* (Figs. 1G and 1H).

3.3. Mycorrhizal status

Analyses showed that the frequency (F%) and intensity (M%) of mycorrhization increased over time and varied according to the treatments applied, depending on the growing conditions (Table 2). *S. bicolor* plants exhibited high frequencies of 88.00% and 94.00% in months 1 and 2, respectively, during the first campaign. In month 3, the frequency was 97.50% in plants fertilized with *B. japonicum* + *T. asperellum*. In the second campaign, under non-rotation conditions, the frequency was 100% in all treatments applied from month 2 onward (Table 2). *B. japonicum* treatment showed a 100% frequency of mycorrhization in Months 1, 2, and 3. Under rotation conditions, the frequency was high at 98.00%, in the control plants. A 100% mycorrhization frequency was recorded in month 3 in both control plants and plants fertilized with *B. japonicum* and *T. asperellum*. The plants fertilized with *B. japonicum* + *T. asperellum* showed the lowest frequencies, at 95.00%, 90.00%, and 98.00% in months 1, 2, and 3, respectively. Similarly, the mycorrhizal intensity obtained was less than 40%. The maxima observed in plants fertilized with *B. japonicum*, in month 3, were 15.49 in the first quarter, then 36.80 and 21.43% in the second quarter, respectively.

In *G. max*, during the first cultivation campaign, the

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results showed that mycorrhization frequency and intensity were below 90% and 10%, respectively, for all treatments (Table 2). During the second campaign, under both non-rotation and rotation conditions, all the applied treatments showed a frequency of 100. High intensities were 34.18% and 25.44% in plants fertilized with *T. asperellum and B. japonicum + T. asperellum*, respectively (Table 2).

3.4. Determination of the relative abundance of AMF spores associated with the rhizosphere of S. bicolor and G. max

The graphs of the relative abundance of AMF spores associated with the rhizospheres of S. *bicolor* and *G. max* plants showed variations in the number of spores per gram of soil, depending on the treatments applied and the growing conditions (Figs. 2A and B).

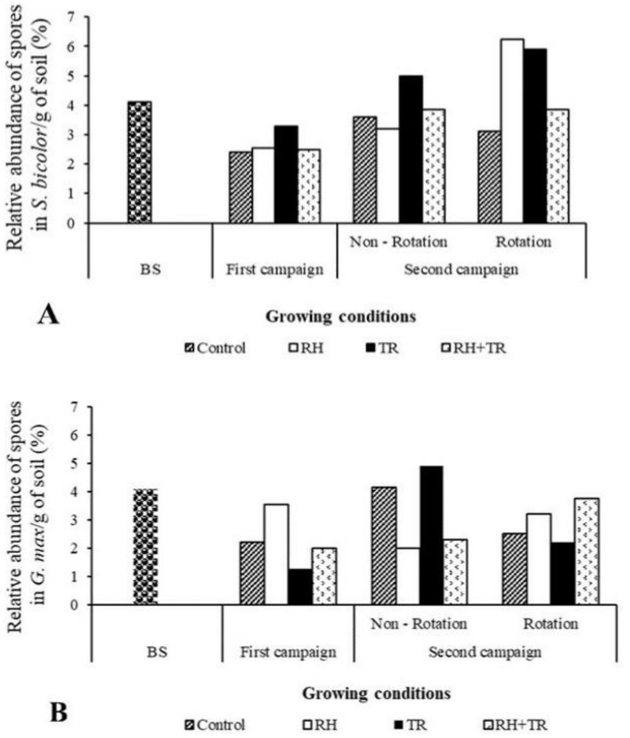

Figure 2. Relative abundance of arbuscular mycorrhizal fungal spores/g of soil under the applied culture conditions. In *S. bicolor* (A) and *G. max* (B). Control, treatment with *Bradyrhizobium japonicum* (RH), *Trichoderma asperellum* (TR), and *Bradyrhizobium japonicum + Trichoderma asperellum* (RH + TR) complex. BS: before sowing.

Before sowing, the relative abundance of spores in one gram of soil at the growing site was 4.1% (Figs. 2A and B). Spores were more abundant in the rhizosphere of *S. bicolor* plants compared to those of *G. max*. In *S. bicolor* plants, during the first campaign, the relative abundance of spores/g of soil was lower than the pre-sowing 4.1%. Under non-rotation conditions, the highest abundance was 5% per 1g of soil in the rhizosphere of plants fertilized with *T. asperellum* (Fig. 2A). Under rotation conditions, higher values of 6.25% and 5.9% were observed in plants fertilized with *B. japonicum* and *T. asperellum*, respectively. In *G. max* plants, the relative abundance obtained for the applied treatments was also lower than 4.1%, despite the 3.55% value recorded in plants fertilized with *B. japonicum*. However, in the second campaign, the values for this abundance were 4.1% and 4.9% in the control and *T. asperellum*-fertilized plants, respectively. This relative abundance was lower at 4.1% under all conditions, in *S. bicolor* and *G. max* plants fertilized with *B. japonicum + T. asperellum* (Figs. 2A and B).

3.5. Determination of the Shannon index according to growing conditions

The results of the evaluated Shannon-Wiener index analyses showed that this index was 1.05 for the pre-sowing growing site (Table 3).

In *S. bicolor*, the maximum values of this index were observed in the first and second campaigns under non-rotation and rotation conditions, with 1.10 in the control plants and plants fertilized with *B. japonicum* and *T. asperellum*, respectively. In *G. max*, higher values were recorded in the first campaign than those obtained before sowing, with a value of 1.10 in the plants fertilized with *B. japonicum* (Table 3). In the second campaign, the maximum value was 1.00 for the control plants under non-rotation conditions. However, it was also 0.87 in plants fertilized with *B. japonicum + T. asperellum*. Furthermore, under rotation conditions, a Shannon index of 0.00 was observed in both control plants and those fertilized with *T. asperellum*.

3.6. Morphological descriptions of the spores of AMF species associated with the rhizosphere of S. bicolor and G. max plants

The spores of the different AMF species in *S. bicolor* and *G. max* were described according to the INVAM identification key (2025) of [24]. Significant variations were observed between spores of the same species or different species, particularly in terms of shape, color, wall shape, and spore cleft. Microscopic observations of AMF spores isolated from the rhizosphere of *S.*

Table 3. Evaluation of the Shannon-Wiener index during the first and second campaigns in *S. bicolor* and *G. max*.

Plants	Treatments	Shannon index			
		BS	First campaign	Second campaign	
				Non-rotation	Rotation
	Contol	01.05	01.10	00.69	00.50
S. bicolor (S35)	RH		00.63	01.10	01.04
	TR		01.04	01.01	01.10
	RH+TR		00.00	00.95	00.64
	Control		00.64	01.00	00.69
G. max	RH		01.10	00.56	00.64
(TGX-1835 10E)	TR		00.00	00.00	00.00
	RH+TR		00.64	00.87	00.69

BS: before sowing. Treatment: Control; Bradyrhizobim japonicum (RH); Trichoderma asperellum (TR) et Bradyrhizobim japonicum + Trichoderma asperellum (RH+TR)

bicolor and *G. max* plants, according to the treatments applied, and under non-rotation and rotation conditions, allowed the distinction of 7 genera: *Acaulospora* sp. *Claroideoglomus* sp., *Entrophospora* sp., *Funneliformis* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. These seven genera belong to four families: Acaulosporaceae, Claroideoglomeraceae, Gigasporaceae, and Glomeraceae. Spores of the genus *Glomus* were the most abundant (Figs. 3 and 4, and Table 4). *Acaulospora* sp. spores were spherical to ovoid, hyaline to pale yellow, with a glossy appearance due to the presence of a hyaline globular body. *Claroideoglomus* spores are characterized by shapes ranging from globose to subglobose, and are cream to light yellow in color. Some are orange to reddish-brown. Spores are composed of two layers (L1 and L2) that differentiate sequentially as the spores develop. Furthermore, the genus *Funneliformis* sp. is characterized by pigmented spores formed individually in the soil or in clusters of approximately 1–20 spores surrounded by a coarse mycelial mantle, either complete or partial. The spore wall normally consists of two or three layers, the outermost layer of which is often hyaline and frequently desquamates as the spore matures. The genus *Gigaspora* exhibits solitary spores of highly variable shapes including globular, subglobular, ovoid, subovoid, ellipsoid, pyriform, irregular, oblong, reniform, fusiform, and elongated. These spores have diameters ranging from 168 to 385 µm and are whitish when young and yellow when mature. The genus *Glomus* sp. is characterized by solitary spores, which are often spherical and rarely ovoid, ranging in color from pale yellow to dark yellow, yellow to brownish-yellow, or even black. They consist of two distinct cell walls. Similarly, the genus *Scutellospora* sp. has solitary spores, often of variable color, ranging from creamy white to pale yellow to black, and measuring 260–400 µm in diameter.

Before sowing, arbuscular mycorrhizal fungal spores of *Acaulospora* sp., *Glomus* sp., and *Scutellospora* sp. were recorded at the culture site (Fig. 3). In the *S. bicolor* rhizosphere, during the first campaign, three genera of arbuscular mycorrhizal fungi were recorded: *Acaulospora* sp., *Gigaspora* sp., and *Glomus* sp. (Fig. 4). In the second campaign, under non-rotation conditions, five genera were identified in the rhizosphere soils: *Acaulospora* sp., *Claroideoglomus* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. (Fig. 4), compared to the rotation conditions, where four genera were observed: *Acaulospora* sp., *Claroideoglomus* sp., *Glomus* sp., and *Scutellospora* sp. (Fig. 5).

In the *G. max* rhizosphere, four genera of arbuscular mycorrhizal fungi were recorded: *Acaulospora* sp. *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. (Fig. 4) were observed in the first campaign. However, during the second campaign, under non-rotation conditions, five genera were recorded: *Acaulospora* sp., *Entrophospora* sp., *Funneliformis* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp. (Fig. 5). Similarly, under rotation conditions, three genera were identified: *Acaulospora* sp., *Gigaspora* sp., and *Glomus* sp. (Fig. 5).

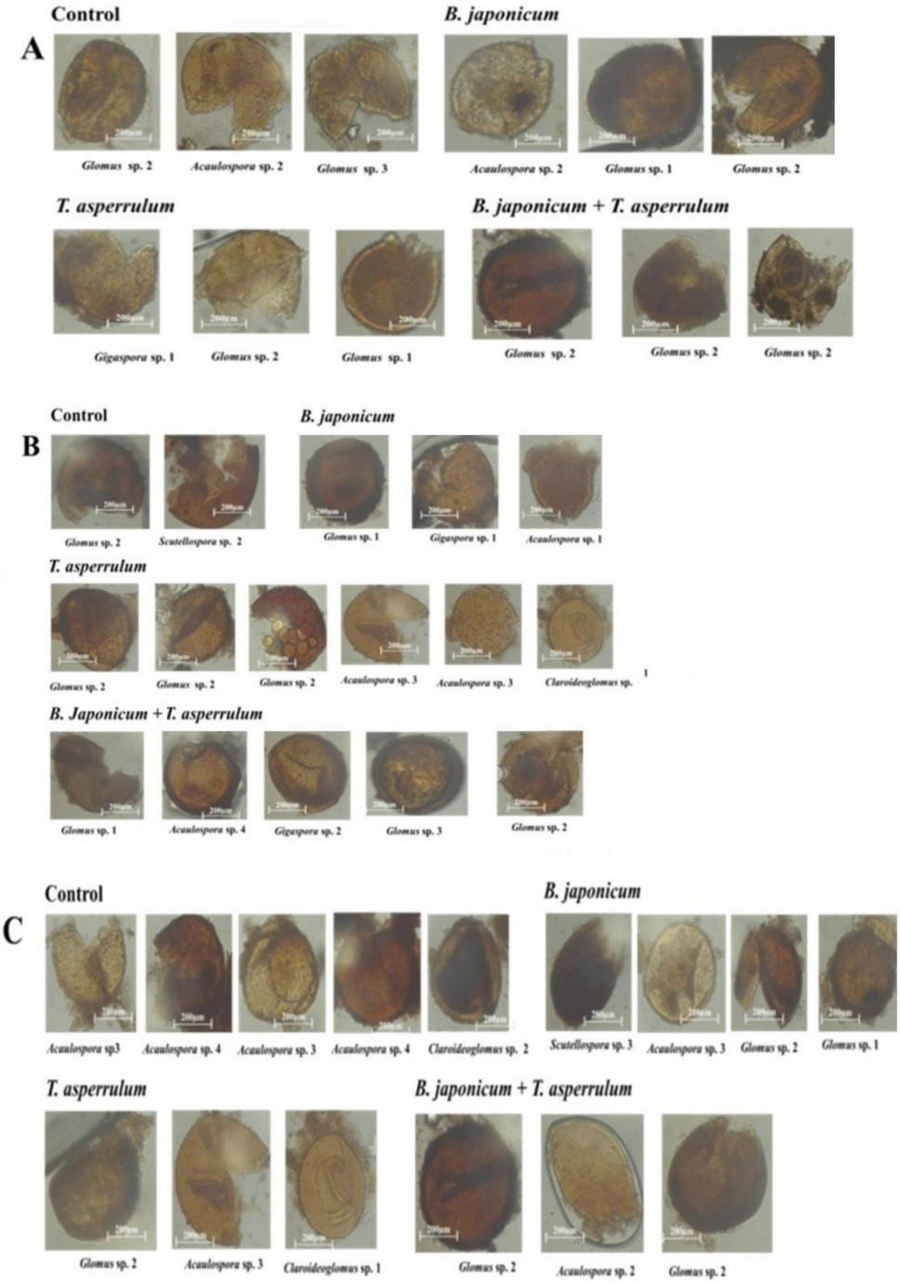

Figure 3. Morphological aspects of arbuscular mycorrhizal fungal spores associated with the rhizosphere of *S. bicolor* plants following the treatments applied. First campaign of growth (A), and the second campaign: non-rotation condition (B) and rotation condition (C).

3.7. Growth pattern in response to fertilization according to growing conditions and correlation analysis between the parameters studied

The growth parameters evaluated differed significantly among the treatments in the Student-Newman-Keuls test at the 5% level (Tables 5–7). In the first growing campaign, *Trichoderma asperellum* + *Bradyrhizobium japonicum* treatments significantly influenced the growth of both *S. bicolor* and *G. max* (Table 5). However, at the end of the growing season, the yields obtained according to the applied fertilizers did not vary significantly. Regarding the second growing campaign, under both no-rotation and rotation conditions, it should be noted that, for the no-rotation condition (Table 6), although growth was significant with the *Trichoderma asperellum* + *Bradyrhizobium japonicum* treatment, the recorded growth values were lower compared to those of the

Table 4. Distribution of different AMF strains according to the treatments applied following the different campaigns.

Plants	**BS**	**Treatments**	**First campaign**	**Second campaign**	
				Non-rotation	**rotation**
S. bicolor (S35)	*Scutellospora* sp.1 *Glomus* sp., 1 *Acaulospora* sp. 1 *Claroideoglomus* sp., 1	Control	*Glomus* sp., 2 *Acaulospora* sp.2 *Glomus* sp., 3	*Glomus* sp., 2 *Scutellospora* sp.1	*Acaulospora* sp. 3 *Acaulospora* sp. 4 *Claroideoglomus* sp., 2
		RH	*Acaulospora* sp. 2 *Glomus* sp., 1 *Glomus* sp., 2	*Glomus* sp., 1 *Gigaspora* sp., 1	*Glomus* sp., 1; *Glomus* sp., 2 *Acaulospora* sp. 3 *Scutellospora* sp. 3
		TR	*Gigaspora* sp.,1 *Glomus* sp., 1 *Glomus* sp., 2	*Glomus* sp., 2 *Acaulospora* sp. 3 *Claroideoglomus* sp., 1	*Acaulospora* sp. 3 *Claroideoglomus* sp.,1 *Glomus* sp., 2
		RH+TR	*Glomus* sp., 2	*Acaulospora* sp. 4 *Glomus* sp., 1; *Glomus* sp., 2 *Glomus* sp., 3; *Gigaspora* sp.,2	*Acaulospora* sp. 2 *Glomus* sp., 2
G. max (TGX-1835 10E)	*Scutellospora* sp.1 *Glomus* sp., 1 *Acaulospora* sp. 1 *Claroideoglomus* sp., 1	Control	*Acaulospora* sp. 2 *Acaulospora* sp. 3 *Scutellospora* sp. 3	*Acaulospora* sp. 4 *Acaulospora* sp. 5; *Glomus* sp., 3 *Gigaspora* sp.,3; *Gigaspora* sp.,4 *Funneliformis sp.*, 1	*Acaulospora* sp. 2 *Glomus* sp., 2 *Gigaspora* sp.,5
		RH	*Acaulospora* sp. 4 *Gigaspora* sp., 1 *Glomus* sp., 2	*Glomus* sp., 7 *Glomus* sp., 9	*Acaulospora* sp. 3 *Gigaspora* sp.,4
		TR	*Glomus* sp.,4 *Glomus* sp., 5	*Glomus* sp., 5 *Glomus* sp., 6; *Glomus* sp., 8	*Glomus* sp., 5
		RH+TR	*Glomus* sp., 6 *Glomus* sp., 7 *Glomus* sp., 8	*Acaulospora* sp. 5 *Acaulospora* sp. 6 *Claroideoglomus* sp.,1 *Entrophospora* sp., 1 *Gigaspora* sp., 4	*Gigaspora* sp.,4 *Gigaspora* sp.,5

Treatments: Control; *Bradirhizobim japonicum* (RH); *Trichoderma asperellum* (TR); and *Trichoderma asperellum* + *Bradirhizobim japonicum* (RH+TR)

first growing campaign. However, a 45% increase in yield was observed in *S. bicolor* with *Bradyrhizobium japonicum* treatment and approximately 29% in *G. max* with *Trichoderma asperellum* treatment, respectively (Table 6). However, under rotation conditions, the obtained yield was appeared almost similar to that of the first growing campaign and growth was weak despite the application of fertilizers compared to the first campaign (Table 7).

Analysis of the correlation matrix between the evaluated growth and mycorrhization parameters showed both negative and positive correlations between these parameters in both S. *bicolor* and *G. max* (Figs. 6A and B). Furthermore, in *S. bicolor*, a significant positive correlation was observed between the average number of ears/pods (ANE/P) and the mean number of leaves (P=0.001) and leaf area (P=0.004) (Fig. 6A). In *G. max*, this correlation is significant and positive between the average number of ears/pods (ANE/P) and the average plant height (APH) (P=0.033), average stem diameter (ASD) (P<0.001), average leaf area (P=0.010), and the average number of roots (ANR) (P=0.002) (Fig. 6B). However, a positive correlation was observed between the mycorrhization frequency (F%) and the average plant height (APH) and average number of roots (ANR) in

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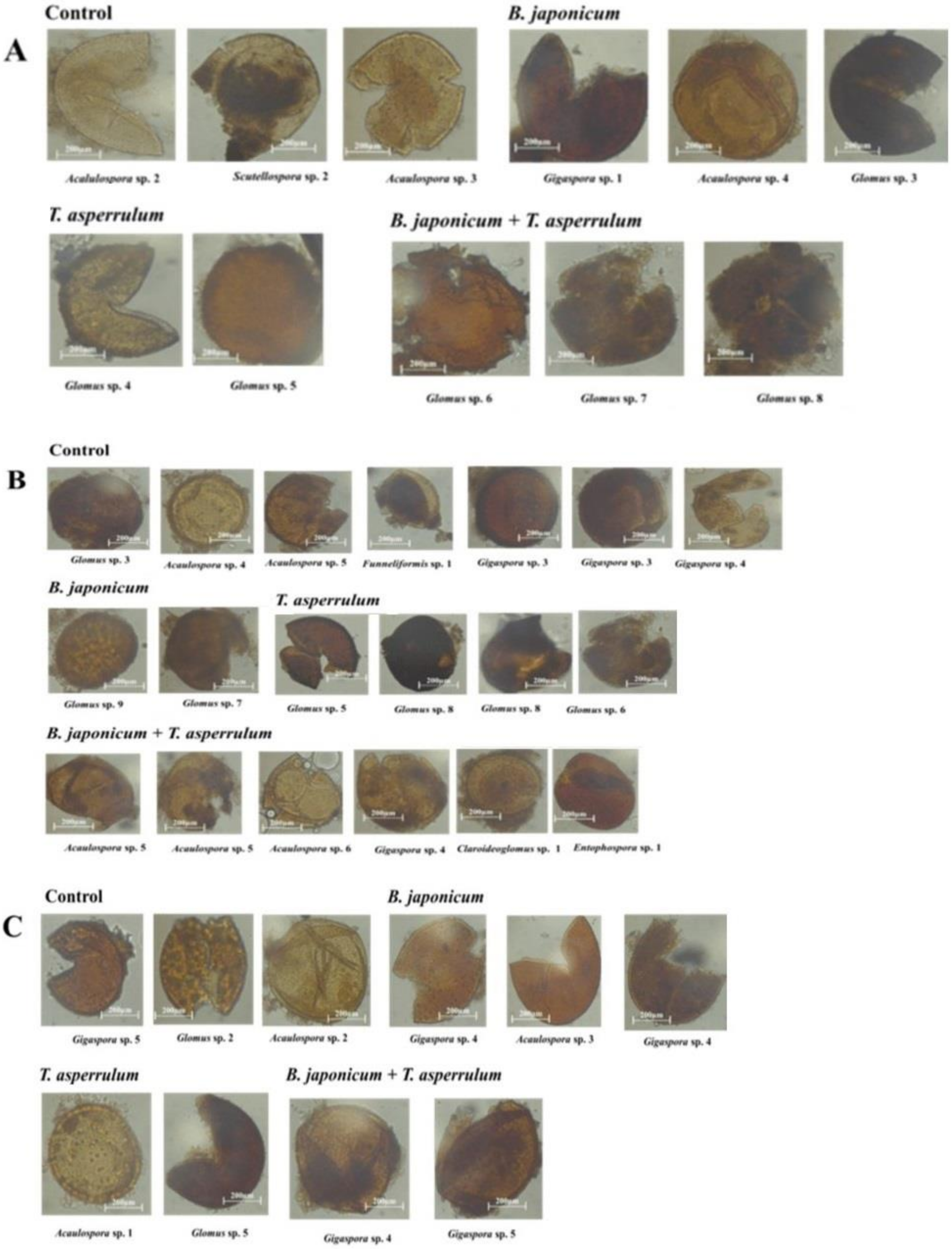

Figure 4: Morphological aspects of arbuscular mycorrhizal fungal spores associated with the rhizosphere of *G. max* plants following the treatments applied. First campaign of growth (A), and the second campaign: non-rotation condition (B) and rotation condition (C).

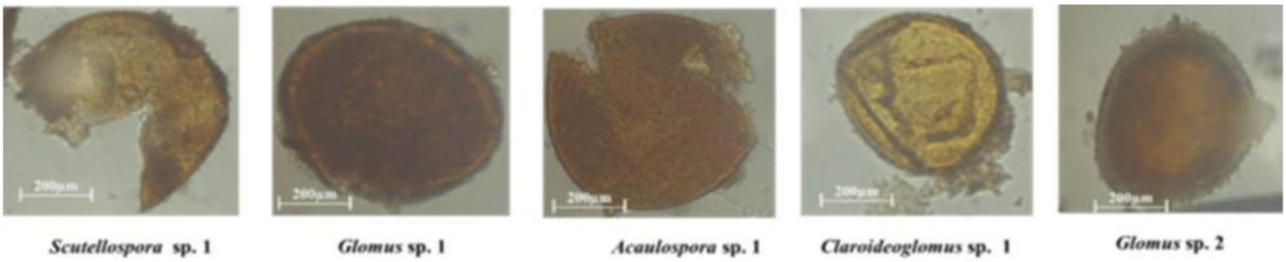

Figure 5. Morphological aspects of arbuscular mycorrhizal fungal spores from the culture site before sowing.

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Table 5. Agronomic parameters evaluated in *S. bicolor* and *G. max* after three months of growth, in the first growing campaign.

Plants	Treatments	Agronomic growth parameters assessed						
		Average plant height (cm)	Average stem diameter (cm)	Average number of leaves	Average leaf area (cm^2)	Average number of roots	Average number of ears/pods	Yield (kg/m^2)
S. bicolor (S35)	Contol	180.00±06.12c	01.06±00.13a	08.40±01.67a	111.45±76.58c	030.00±00.00c	002.30±01.34a	1.67±0.33a
	RH	182.00±06.32c	01.54±00.11a	14.80±02.05c	314.60±42.48e	031.50±13.43c	003.10±00.72a	1.33±0.11a
	TR	185.84±07.61c	01.81±00.12a	07.00±01.29a	220.60±47.92d	033.50±00.71c	001.93±00.95a	1.38±0.45a
	RH+TR	189.80±06.14c	01.86±00.54a	17.40±04.22d	345.95±45.08e	035.50±00.35c	003.50±01.67a	1.38±0.13a
G. max (TGX-1835-10E)	Contol	063.92±09.17a	00.76±00.11a	09.33±08.44a	052.55±03.88a	019.00±00.00a	047.60±06.02b	1.48±0.78a
	RH	052.60±05.90a	00.70±00.21a	07.00±06.04a	041.66±05.90a	020.00±01.41ab	130.70±10.85d	1.77±0.12a
	TR	074.20±17.01b	01.10±00.30a	08.80±02.59a	060.00±03.96b	015.78±00.85a	123.31±47.89c	1.62±0.22a
	RH+TR	074.40±15.66b	01.14±00.26a	10.65±08.25ab	067.47±03.92b	021.50±01.77ab	149.00±30.90e	1.53±0.12a

Student-Newman-Keuls test ($P<0.05$). Means with the same letter in the same column are not significantly different. Treatments: Control; *Bradirhizobim japonicum* (RH); *Trichoderma asperellum* (TR); and *Trichoderma asperellum* + *Bradirhizobim japonicum* (RH+TR)

Table 6. Agronomic parameters evaluated in *S. bicolor* and *G. max* after three months of growth, in the second growing season under non-rotation conditions.

Plants	Treatments	Agronomic growth parameters assessed						
		Average plant height (cm)	Average stem diameter (cm)	Average number of leaves	Average leaf area (cm^2)	Average number of roots	Average number of ears/pods	Yield (kg/m^2)
S. bicolor (S35)	Contol	125.60±06.62b	00.88±00.07a	06.80±01.17b	186.75±17.45e	11.00±00.00a	02.20±00.99a	2.28±0.42b
	RH	134.60±11.17b	01.14±00.15a	08.60±02.19c	165.12±48.96d	20.00±00.00c	02.80±01.48a	2.43±0.08b
	TR	138.20±16.18b	01.16±00.11a	05.60±00.55b	197.45±42.05e	15.00±00.00ab	01.80±00.84a	1.92±0.11a
	RH+TR	150.20±15.66c	01.48±00.43a	08.60±02.97c	220.57±30.43f	22.00±00.00c	03.00±01.41a	1.46±0.12a
G. max (TGX-1835-10E)	Contol	059.20±06.20a	00.34±00.05a	02.60±01.50a	010.60±06.94a	10.00±00.00a	21.00±03.16b	2.21±0.23b
	RH	056.80±12.28a	00.44±00.05a	02.80±03.11a	028.50±19.70b	15.00±00.00ab	35.40±05,.90c	1.17±0.11a
	TR	051.00±07.35a	00.42±00.08a	12.00±05.48d	040.47±12.16c	13.00±00.00a	32.60±07.73c	2.27±0.05b
	RH+TR	059.40±05.50a	00.65±00.07a	03.40±01.95a	020.46±10.47b	17.00±00.00b	56.60±15.79d	1.15±0.03a

Student-Newman-Keuls test ($P<0.05$). Means with the same letter in the same column are not significantly different. Treatments: Control; *Bradirhizobim japonicum* (RH); *Trichoderma asperellum* (TR); and *Trichoderma asperellum* + *Bradirhizobim japonicum* (RH+TR)

S. bicolor, whereas in *G. max*, this mycorrhization frequency appeared to significantly and positively influence all agronomic growth parameters (Figs. 6 A and B). In general, there was no correlation between the Shannon index and yield, or between these two parameters and all the growth parameters evaluated.

4. Discussion

This study aimed to evaluate the influence of biofertilizers applied during crop rotation between *Sorghum bicolor* and *Glycine max* on the diversity of native arbuscular mycorrhizal fungi in the plant rhizosphere. The results varied significantly depending on the fertilizers applied under non-rotation and rotation conditions.

Chemical analyses of the soils at the cultivation site showed that these soils had an acidic pH, with values of 5.8 for pH(H_2O) and 4.8 for pH(HCl). This result is consistent with those of Yerima et al. [30] and Bengono et al. [31], who demonstrated that Cameroonian soils are acidic. This acidity range obtained at our experimental site is favorable for agriculture, as Neina et al. [32], highlighted that pH levels of 5.8 to 6.3 are satisfactory for nutrient exchange in the soil and for good biological activity. The applied treatments would help *S. bicolor* and *G. max* plants to adapt the soil acidity. The C/N ratio < 6(very low) suggests that the soils at this site have organic matter that is rich in nitrogen relative to carbon (Table 1). The rapid decomposition of the existing organic matter results in low organic matter stability and rapid nitrogen release.

This soil was rich in total nitrogen with a value of 0.23 (between 0.15 and 0.25, according to the NFISO 11261

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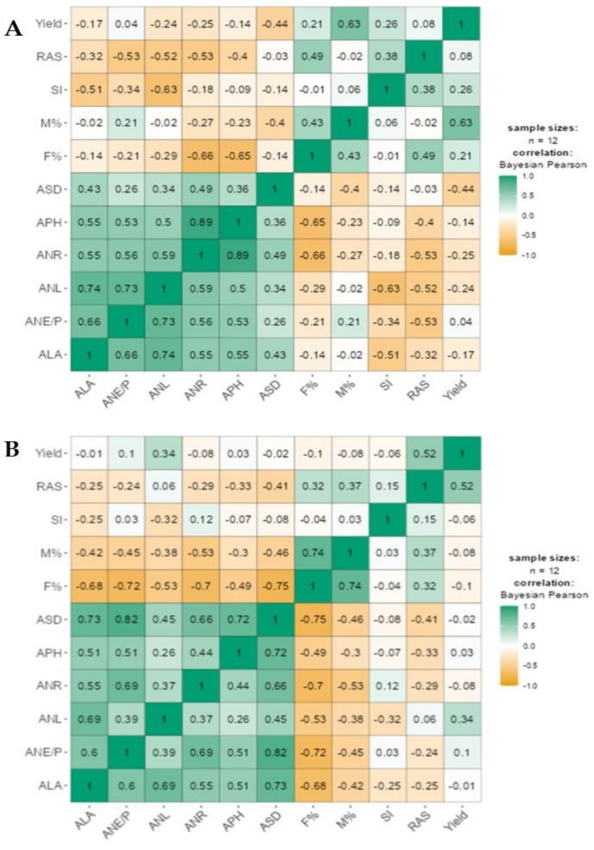
Table 7. Agronomic parameters evaluated in *S. bicolor* and *G. max* after three months of growth, in the second growing season under rotation conditions.

Plants	Treatments	Agronomic growth parameters assessed						
		Average plant height (cm)	**Average stem diameter (cm)**	**Average number of leaves**	**Average leaf area (cm²)**	**Average number of roots**	**Average number of ears/pods**	**Yield (kg/m²)**
S. bicolor (S35)	Contol	090.33±06.01c	01.13±00.10a	07.33±00.97b	118.07±15.03b	11.00±00.00a	01.33±00.37a	1.11±0.06a
	RH	127.80±15.06d	00.96±00.09a	05.80±01.48a	133.17±44.10b	11.00±00.00a	01.20±00.45a	1.67±0.45a
	TR	122.96±21.53d	01.96±00.74a	06.11±00.92ab	140.22±28.86c	16.00±00.00d	01.67±01.60a	1.44±0.12a
	RH+TR	128.40±08.62d	01.22±00.19a	08.40±01.14c	158.59±74.77c	18.00±00.00e	02.20±00.84a	1.69±0.27a
G. max (TGX-1835-10E)	Contol	041.40±01.50a	00.34±00.05a	04.80±00.75a	029.53±07.98a	10.00±00.00a	10.60±01.02b	1.10±0.08a
	RH	062.80±03.11b	00.44±00.05a	04.80±00.84a	039.87±21.73a	14.00±00.00bcd	27.20±03.77c	1.15±0.33a
	TR	052.78±09.85a	00.46±00.09a	04.40±01.82a	031.43±18.33a	14.00±00.00bcd	37.60±20.48d	1.30±0.24a
	RH+TR	062.80±03.39b	00.73±00.11a	05.75±04.14a	046.94±73.82a	15.00±00.00d	65.16±25.02e	1.33±0.03a

Student-Newman-Keuls test ($P<0.05$). Means with the same letter in the same column are not significantly different. Treatments: Control; *Bradirhizobim japonicum* (RH); *Trichoderma asperellum* (TR); and *Trichoderma asperellum* + *Bradirhizobim japonicum* (RH+TR).



A

	ALA	ANE/P	ANL	ANR	APH	ASD	F%	M%	SI	RAS	Yield
Yield	-0.17	0.04	-0.24	-0.25	-0.14	-0.44	0.21	0.63	0.26	0.08	1
RAS	-0.32	-0.53	-0.52	-0.53	-0.4	-0.03	0.49	-0.02	0.38	1	0.08
SI	-0.51	-0.34	-0.63	-0.18	-0.09	-0.14	-0.01	0.06	1	0.38	0.26
M%	-0.02	0.21	-0.02	-0.27	-0.23	-0.4	0.43	1	0.06	-0.02	0.63
F%	-0.14	-0.21	-0.29	-0.66	-0.65	-0.14	1	0.43	-0.01	0.49	0.21
ASD	0.43	0.26	0.34	0.49	0.36	1	-0.14	-0.4	-0.14	-0.03	-0.44
APH	0.55	0.53	0.5	0.89	1	0.36	-0.65	-0.23	-0.09	-0.4	-0.14
ANR	0.55	0.56	0.59	1	0.89	0.49	-0.66	-0.27	-0.18	-0.53	-0.25
ANL	0.74	0.73	1	0.59	0.5	0.34	-0.29	-0.02	-0.63	-0.52	-0.24
ANE/P	0.66	1	0.73	0.56	0.53	0.26	-0.21	0.21	-0.34	-0.53	0.04
ALA	1	0.66	0.74	0.55	0.55	0.43	-0.14	-0.02	-0.51	-0.32	-0.17

B

	ALA	ANE/P	ANL	ANR	APH	ASD	F%	M%	SI	RAS	Yield
Yield	-0.01	0.1	0.34	-0.08	0.03	-0.02	-0.1	-0.08	-0.06	0.52	1
RAS	-0.25	-0.24	0.06	-0.29	-0.33	-0.41	0.32	0.37	0.15	1	0.52
SI	-0.25	0.03	-0.32	0.12	-0.07	-0.08	-0.04	0.03	1	0.15	-0.06
M%	-0.42	-0.45	-0.38	-0.53	-0.3	-0.46	0.74	1	0.03	0.37	-0.08
F%	-0.68	-0.72	-0.53	-0.7	-0.49	-0.75	1	0.74	-0.04	0.32	-0.1
ASD	0.73	0.82	0.45	0.66	0.72	1	-0.75	-0.46	-0.08	-0.41	-0.02
APH	0.51	0.51	0.26	0.44	1	0.72	-0.49	-0.3	-0.07	-0.33	0.03
ANR	0.55	0.69	0.37	1	0.44	0.66	-0.7	-0.53	0.12	-0.29	-0.08
ANL	0.69	0.39	1	0.37	0.26	0.45	-0.53	-0.38	-0.32	0.06	0.34
ANE/P	0.6	1	0.39	0.69	0.51	0.82	-0.72	-0.45	0.03	-0.24	0.1
ALA	1	0.6	0.69	0.55	0.51	0.73	-0.68	-0.42	-0.25	-0.25	-0.01

Figure 6. Matrice de corrélation des rangs de Pearson entre les différents paramètres étudiés. S. bicolor (A) et G. max (B). La couleur orange indique une corrélation négative et le vert une corrélation positive. Average leaf area (ALA), Average number of ears/pods (ANE/P), Average number of leaves (ANL), Average number of roots (ANR), Average plant height (APH), Average stem diameter (ASD), Frequency of mycorrhizal colonization (F%), Intensity of mycorrhizal colonization (M%), Shannon-Wiener index (SI), Relative abundance of arbuscular mycorrhizal fungal spores (RAS) and Yield. Significance levels: p ≤ 0.05, p ≤ 0.01, and p ≤ 0.001.

standards) [33, 34]. This richness in mineral nitrogen in the soil could partly explain the variation in yield observed in *G. max* depending on the treatments applied and growing seasons. This clay-sand-loam soil was rich in clay. According to [35], the fertility scale for available phosphorus, the soil at the cultivation site is very poor in available phosphorus. The results showed a high cation exchange capacity (CEC). Previous report [36] mentioned that soil phosphorus deficiency and the impact of acidity on soil cation exchange capacity (CEC) are characteristic of tropical soils. Of the four exchangeable bases identified, the percentage of calcium (Ca2+) was the highest. This mineral, which is involved in important physiological processes for plants, such as photosynthesis, fruiting, cell permeability, and ionic balance [37], also affects soil structure, nutrient availability, and soil pH, sometimes directly influencing the establishment of symbiosis [38, 39].

Mycorrhizal status assessments of plant roots showed mycorrhization index values that varied depending on the treatments applied, with high frequency and intensity values in the presence of *B. japonicum* + *T. asperellum* and *B. japonicum* in *S. bicolor* and *B. japonicum* + *T. asperellum* in *G. max*. These different treatments would have an impact on the activation of soil microbiome activities, such as the activity of arbuscular mycorrhizal fungi. The presence of mycorrhizal structures in the roots allowed for the assessment of symbiosis. This variation in

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mycorrhizal status parameters observed would be influenced not only by root turnover in plants but also by the soil properties. Root colonization by AMF can be influenced by spore density and the species of AMF present in the soil. The variability in response observed could be due to the fact that the efficiency of the symbiosis depends not only on the AMF but also on the compatibility between the plant and the fungus [40]. It is known that plant-AMF symbiosis is costly in terms of carbohydrates. This carbon cost would influence the quality of symbiosis and the diversity of AMF associated with the plant.

Analysis of the diversity of native arbuscular mycorrhizal fungi in the rhizosphere revealed a low species richness among the communities present in *S. bicolor* and *G. max*. This low species richness of AMF is attributed not only to the impact of the different types of fertilizers applied, but also to the natural ecological dominance of *S. bicolor* and *G. max* in our cultivated plots, a specific interaction in response to the hormonal effect emitted by the host plants (*S. bicolor* and *G. max*), or even the nature of the soil at the cultivation site (including environmental pressures). Furthermore, previous report [41] indicated that spore abundance depends on the physicochemical properties of the soil. The variation in spore density could also be attributed to the presence of diverse plant cover, which would contribute to the creation of a vast network of hyphae and interconnections with the roots of host plants, improving not only biomass but also soil microbial activity [42]. However, the abundance of *S. bicolor* and *G. max* plants would limit the creation of that network in this work. Shannon indices close to 0 highlight very low biological diversity and, consequently, a poverty of arbuscular mycorrhizal fungi strains in the soil influenced by the dominant plant cover, such as S. bicolor and G. max which are used as hosts and the characteristics of the soil. These indices were lower in the rhizosphere of *G. max* plants compared to *S. bicolor* plants. It is certainly true that the introduction of *T. asperellum* strains would limit the action of arbuscular mycorrhizal fungi by competing for soil nutrients. However, the presence of the few AMF observed here, despite their low diversity, might be the result of improved root health through the stimulation of plant immunity and increased nutrient availability by *T. asperellum* introduced into *S. bicolor*. Conversely, in *G. max*, by colonizing plant roots, the latter, through the secretion of antifungal enzymes, would limit the penetration of hyphae released by AMF into the roots, consequently influencing diversity. Therefore, the complex interaction between *T. asperellum* and AMF diversity observed in this study could depend on the plant type (*S. bicolor* and *G. max*), AMF present in the soil, and soil conditions of the cultivation site.

Similarly, based on morphological characteristics and the INVAM identification key [26], seven genera were identified (*Acaulospora* sp., *Claroideoglomus* sp., *Entrophospora* sp., *Funneliformis* sp., *Gigaspora* sp., *Glomus* sp., and *Scutellospora* sp.), belonging to four families: Acaulosporaceae, Claroideoglomeraceae, Gigasporaceae, and Glomeraceae. Before sowing, the genera *Acaulospora* sp., *Glomus* sp., *Scutellospora* sp., and *Claroideoglomus* sp. were recorded in the soil at the cultivation site. In *S. bicolor*, during the first growing season, the genus *Glomus* sp. was present in the rhizosphere of the plants for all applied treatments. Under crop rotation and non-rotation conditions in the second growing season, in addition to the presence of AMF of the genus *Glomus*, it should be noted that the genus *Acaulospora* sp. was also present in all treatments except the control and S. bicolor plants treated with *B. japonicum* (RH). However, in general, *Glomus* sp. appeared to be the most frequently recorded treatment for both plants under all growing conditions. The presence of only *Glomus* sp. spores in the rhizosphere of *G. max* plants fertilized with *Trichoderma asperellum* indicates their co-involvement in good soil biological activity through a positive interaction between the two beneficial fungi. It is certainly true that the study [43] highlighted that Cameroonian soils are richer in AMF of the genus *Glomus* sp., but the report [44] showed that during co-inoculation with *Trichoderma asperellum* + *Glomus* sp., Trichoderma strain would promote sporulation and root colonization by the *Glomus* sp. strains, probably due to its ability to modify the rhizosphere and stimulate root health.

The growth parameters assessed to evaluate the effect of the applied fertilizers were significant, with maxima observed in the presence of the *Trichoderma* *asperellum* + *Bradyrhizobium japonicum* treatment. Furthermore, these very high growth parameters during the first growing season decreased during the second season, regardless of whether crop rotation was in place or not. This observation could be explained by the depletion of nutrients in the soil, resulting in low microbial activity for the degradation of organic matter. Additionally, the soil at the site was poor in organic matter, which has already impacted its physical and mineral fertility. The poor organic matter content of the soil at the cultivation site prevented crop rotation, and the various types of biofertilizers applied did not promote soil stability. Maffia [45] also emphasizes that crop rotation contributes to maintaining the balance and structure of soil nutrients. Furthermore, the yield obtained was not significant in the first growing season, or the second season under crop rotation conditions, despite some significant maxima observed in *S. bicolor* and *G. max* plants with the *Bradyrhizobium japonicum* and *Trichoderma asperellum* treatments, respectively. These treatments increased the yields by 45% and 29% under non-rotation conditions. Non-rotation appears to have favored these treatments, facilitating nitrogen fixation necessary for growth in *S. bicolor*, while *Trichoderma asperellum* is known for its ability to absorb essential minerals through root exudate. The lack of correlation between yield and mycorrhization indices indicates that *S. bicolor* and *G. max* plants are not entirely dependent on the symbiosis established with native arbuscular mycorrhizal fungi (AMF) species in the rhizosphere. This suggests that while the applied biofertilizers are effective in establishing symbiosis, the nature of the soil used may limit their effectiveness. The presence of organic matter may have contributed to increased soil microbial activity and, consequently, increased yields. Our results contradict with Zani [46], which showed that crop rotation improved carbon storage in the soil.

5. Conclusions

This study highlighted that crop rotation and applied treatments influence the diversity of arbuscular mycorrhizal fungi present in the soil. No crop rotation favored the presence of a greater number of AMF genera compared to crop rotation. However, despite the applied treatments, the genus *Glomus* sp. appeared to be the most abundant for all treatments applied under all growing conditions. The rhizosphere of *G. max* plants, fertilized with *T. asperellum* under all growing conditions, was rich only in AMF of the genus *Glomus* sp. The results of the assessment of the impact of applied fertilizers on growth and yield highlighted the limited capacity of crop rotation between *S. bicolor* and *G. max*, despite the presence of indigenous AMF alone, to maintain the biological quality of soils in the absence of external nutrient inputs when soils are poor in organic matter.

Disclaimer (artificial intelligence)

Authors hereby state that no generative AI tools such as Large Language Models (ChatGPT, Copilot, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript

Authors' contributions

Conceptualization, M.D.M., M.H.D, D.A.C.; data curation, D.A.C., M.T.L., N.B.A.M.; formal analysis, D.A.C.; investigation, D.A.C., M.D.M., N.B.A.M.; A.S.B., N.O., N.I., M.I.L., K.C.L., M.D.R.T.D., W.R.C.; methodology, M.D.M., T.A.M.K., N.B.A.M.; project administration, D.A.C.; resources, M.T.L., N.B.A.M., A.S.B., N.O., N.I., M.I.L., K.C.L., M.D.R.T.D., W.R.C.; software, M.D.M., T.A.M.K., W.R.C., D.A.C.; supervision, M.H.D., T.A.M.K.; validation, M.D.M., M.T.L., D.A.C.; visualization, M.T.L., A.S.B., N.O., N.I., M.I.L., K.C.L., M.D.R.T.D., W.R.C., D.A.C.; writing – original draft, M.D.M., M.H.D., N.N., D.A.C.; writing – review & editing, M.D.M., M.H.D., N.N., D.A.C.

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Availability of data and materials

This manuscript includes all data generated or analyzed during this study. Other necessary data about this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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