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Reducing mineral fertilizer use for sustainable agriculture: The influence of seed coating with arbuscular mycorrhizal fungal spores and *Leifsonia* bacteria on maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) production

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Arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) have been proposed as biofertilizers for sustainable agriculture. Nevertheless, many of the delivery approaches for these beneficial microorganisms have the disadvantage of limited applicability. The study aim was to evaluate the fertilizing potential of seed coating with microbial fertilizers on growth and yield parameters of maize and sorghum. Field trials were conducted using a seed coating technique where AMF spores (*Rhizophagus fasciculatus* and *Rhizophagus aggregatus*) and PGPR (*Leifsonia* sp.) were applied around the seeds of maize (*Early-Thai*) and sorghum (*Nganda*). Experiments included the following treatments: Coated seed, coated seed in combination with mineral fertilizer (NPK, 15-15-15) at 75 kg/ha, uncoated seed without fertilizer and NPK fertilizer applied at 150 kg/ha. Data were collected on root AMF colonization, plant growth, yield parameters and soil AMF spore richness and density. Research in this area suggests that there is little likelihood of a significant response from seed coating, despite high root AMF colonization rates. However, plants treated with microbial fertilizer in combination with 50% NPK fertilizer achieved significantly higher final heights and yields for both maize and sorghum. Plant production achieved reached that of NPK fertilizer at 150 kg/ha, suggesting that in our case of poor soils, the application of microbial fertilizer alone is not sufficient to achieve significant improvements in plant production. We also found that neither microbial fertilizer, nor mineral treatments had any effect on spore morphotype richness. However, seed coating further increased the spore density.

Key words: *Zea mays*, *Sorghum bicolor*, seed coating, arbuscular mycorrhizal fungal, plant growth promoting rhizobacteria, fertilizers, growth and yield, field trial.

INTRODUCTION

Maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) are among the staple food crops grown by most smallholder farmers in Sub-Sahara Africa. In the southern and eastern parts of Senegal, maize and sorghum are

the staple food for a large part of the population and were among the crops promoted in a special food production package to ensure food security in Senegal. After a long period of decline, maize and sorghum yields in Senegal have increased in recent years (ANSD, 2022). However, the factors that determine these increases, that is, soil fertility, continue to deteriorate, with a reduction in fallow land and low levels of fertilizer use (Sene et al., 2010), affecting crop production. In addition, smallholder farmers cannot afford mineral fertilizers because they are expensive.

Microbial inoculants offer low-cost alternatives to expensive mineral fertilizers and provide a means of maintaining or improving soil fertility (Hart et al., 2015; Garg et al., 2018; Begum et al., 2019; Aguégué et al., 2023). Many lines of scientific evidence demonstrate not only improved crop yield and resistance of mycorrhizal and/or nodulating plants to environmental stress, but also improvements in many food quality attributes, such as increased levels of desirable antioxidants, vitamins and minerals (Sene et al., 2010; Calvo et al., 2014; Fortin et al., 2015; Hart et al., 2015; Garg et al., 2018; Rocha et al., 2019). These soil microorganisms are now being promoted as smart fertilizers for a new green revolution in the 21st century (Sene et al., 2010; Fortin et al., 2015; Lesueur et al., 2016; Mohanty and Swain, 2018; Rocha et al., 2019).

Arbuscular mycorrhizal fungi (AMF) and plant growth promoting rhizobacteria (PGPR) are among these important soil-dwelling microorganisms and can have a strong influence on plant growth and productivity. They form mutualistic associations with over 80% of all vascular plants, affecting plant fitness and competitive interactions (Aguégué et al., 2023). They are well known for assisting host plants with phosphorus uptake (Smith and Read, 2008; Lu et al., 2023), but can also provide other benefits such as protection from pathogens (Cardoso and Kuyper, 2006; Sharma et al., 2023), assistance with the uptake of other nutrients such as nitrogen and copper, and improved water relations (Smith and Read, 2008; Sene et al., 2010; Lu et al., 2023; Sharma et al., 2023). AMF hyphae also play a role in the formation and structural stability of soil aggregates (Zhang et al., 2023) and contribute to the composition of plant community structures (Chen et al., 2023). On the other hand, plant growth promoting rhizobacteria (PGPR), isolated as free-living soil bacteria from the use rhizosphere of plants, can help reduce mineral fertilizer and increase plant growth and yield (Swarnika et al., 2022; Bhanse et al., 2022; Dhawi, 2023).

Several bacterial groups such as members of the genera *Azospirillum*, *Bacillus*, *Burkholderia*, *Klebsiella*, *Leifsonia* or *Pseudomonas* have been identified as PGPR for cereals through N_2 fixation, phosphate solubilization, phytohormone production or biological control of pathogens (Oleńska et al., 2020; Swarnika et al., 2022; Bhanse et al., 2022; Dhawi, 2023). In return, AMF and PGPR receive photosynthetic products from the host plant (Smith and Read, 2008; Swarnika et al., 2022; Lu et al., 2023).

Microbial inoculants have been variously supplied as a homogenized substrate form (such as peat, compost, vermiculite, perlite, sand, expanded clay) containing spores, mycorrhizal root fragments and hyphal segments (Dalpé and Monreal, 2004; Lesueur et al., 2016) and applied as a layer under the seeds. A liquid formulation has also been described, but contained only AMF spores (Barr, 2008). However, many of these approaches have the disadvantage of limited applicability (Lesueur et al., 2016; Rocha et al., 2019; Itelima et al., 2018). Relatively few have been scaled up for commercial use (Lesueur et al., 2016).

In fact, the application of AMF results in high costs per plant and is therefore not economically feasible in intensive agricultural fields (Oliveira et al., 2016; Rocha et al., 2018). In addition, these forms of inoculants easily contaminated and require special treatment before application in open fields (Rocha et al., 2018). Seed coating, a technique in which microbial fertilizers are applied around the seed, has the potential to allow the use of smaller amounts of inoculants (Oliveira et al., 2016), resulting in reduced costs and increased efficiency. Seed coating has been used as a biological tool to improve agricultural sustainability (Rouphael et al., 2017;

Rocha et al., 2019; Piri et al., 2019), but this technology is still in its infancy in West African countries. Based on the above considerations, field experiments were conducted in Ngéoul-Saloum during the 2016/2017 and 2017/2018 seasons to evaluate the fertilization potential of seed coating with a combination of AMF (*Rhizophagus fasciculatus* and *Rhizophagus aggregatus*) and PGPR (*Leifsonia* sp.) on the growth and yield parameters of maize and sorghum.

We also investigated the potential of seed coating to improve soil AMF spore richness and density. Specifically, we expected that seed coating with microbial fertilizers could improve the productivity of maize and sorghum.

This can lead to a significant reduction in mineral fertilizer use and improve soil AMF biodiversity.

MATERIALS AND METHODS

Experimental site description

The experiments were carried out from July to October during two

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consecutive growing seasons (2016/17 and 2017/18) in the open field at Ngueoul Saloum (14°25' N, 16°35' W), a traditional farming village in the central part of the Senegalese peanut (*Arachis hypogaea* L.) production zone. The experiments were carried out on farmer's fields. The fields were in fallow and covered with mixed pasture grasses and *Guerra senegalesis* Lam. shrub. At the beginning of the study, these fallow fields were ploughed. The maize plot has a pH of 5.93 and a sandy loam texture and contains 0.20 g/kg total N, 2.34 g/kg total C, 52.18 mg/kg total P, 1.45 mg/kg available P. The mineral composition of the sorghum plot has a sandy loam texture and has a pH of 6.00 and contains 0.17 g/kg total N, 2.21 g/kg total C, 50.13 mg/kg total P, 1.57 mg/kg available P. The climate of the area is typical of the semi-arid lands of West Africa with an average temperature of 25 to 35°C and an annual rainfall of 500 to 800 mm, mostly during a single, short rainy season of 3-4 months.

Plant materials

Seeds of selected cultivars (Nganda for *S. bicolor* and Early-Thaï for *Z. mays*) were provided by the Senegalese Institute for Agricultural Research (ISRA-Bambey). The vegetative cycles were 80 days for Early-Thaï and 90 days for Nganda. Their optimum yields were 2 to 3 tonnes per hectare for the former and 2.8 to 5 tonnes per hectare for the latter, respectively. All seeds were surface sterilized with 5% sodium hypochlorite (NaOCl) for 5 min, 70% ethanol for 3 min and thoroughly rinsed with sterile distilled water. The seeds were then dried in a sterile environment prior to coating.

Fugal materials and AMF inoculum production

The arbuscular mycorrhizal fungal (AMF) isolates *Rhizophagus fasciculatus* and *Rhizophagus aggregatus* were used for this study. The AMF strains were propagated on *Z. mays* for 12 weeks under greenhouse conditions on sterilized substrate (soil and sand 1:1 v/v). Spores were collected by wet sieving and decanting method (Gerdmann and Nicolson, 1963). They were suspended in sterile physiological water (8770 ppm NaCl, 270 ppm KH_2PO_4, 710 ppm Na_2PO_4) for seed coating.

Bacterial material and PGPR inoculum production

The bacterial strain ORS3454, identified as *Leifsonia* sp. (99% of 16S rDNA sequence similarity to *Leifsonia shenshuensis* (Diouf D. personal communication), was used for its plant growth promoting properties. This strain was collected from pond water containing wild rice (*Oryza barthii* A. Chev.) plants at Ndiaffate (Kaolack, Senegal (Diouf D. personal communication). It was grown in YEM medium (Vincent, 1970) for 48 h at 28°C with rotary shaking at 150 rpm. Liquid cultures were centrifuged at 8000 rpm for 10 min. Bacterial pellets were washed 3 times (8000 rpm, 10 min) and suspended in sterile physiological water (8770 ppm NaCl, 270 ppm KH_2PO_4, 710 ppm Na_2PO_4) for seed coating.

Seed pre-inoculation

Seeds were coated with the microbial consortium based on *R. fasciculatus* and *R. aggregatus* spores and ORS3454 bacterial cells. The spores and bacterial cells were first mixed in a small volume (20 ml) of sterile physiological water and then impregnated in a sterile peat substrate (pH 6.8) for a solid microbial fertilizer (Sene et al., 2021) at a rate of 5 x 10^8 cells and nearly 350 spores per 1 g to ensure that there were no less than 10^6-10^8 cells and 30-60 spores respectively on the surface of sorghum and maize seeds. A solution of gum arabic (10% w/v) was prepared prior to seed coating. This solution was used as an adhesive agent to glue the coating products to the seed. Peat substrate containing the microbial consortium was then gradually added and thoroughly stirred to ensure that each seed was coated with an even layer of coating materials. The seeds were coated just prior to sowing. The coated seeds were dried and stored in a ventilated and sterile environment (Sene et al., 2021).

Field trials and sampling

Plots of 62.37 m^2 (9.90 m x 6.30 m) were established with four replicates per treatment and a total of 4 experimental units in a complete randomized block design. The field experiments were laid out with two main treatments (seeds coated with the microbial consortium or uncoated seeds). An uncoated seed treatment and a full mineral fertilizer containing nitrogen, phosphorus and potassium (NPK, 15, 15, 15 for 150 kg ha^{-1}) were added as a negative and positive controls and compared with a coated seed treatment combined with reduced mineral fertilizer (NPK, 15, 15, 15 for 75 kg ha^{-1}) by 50%. Before setting up the experiments, soil samples were collected from each block and the most probable number (MPN) of soils was determined as described by Sene et al. (2012). Seeds (3 seeds for the maize and 10 seeds for the sorghum) were planted by hand in 5 cm deep furrows, with 90 cm spacing within and between rows. The plants were dismantled on the 8th day after emergence to a maximum of 2 plants per row. Weeds were removed by hand throughout the study, so that the cultivated plants were the only host for the AM fungi in the field plots. The field was rainfall watered and no fungicides or insecticides were used in the trials.

Plant growth and yield measurements

Plant height (average of 5 randomly selected main tillers) was measured on 10 plants per treatment at two times corresponding to three phenological stages of the crop: flowering (45 days after sowing), grain filling (60 days after sowing) and at grain maturity (90 days after sowing). At harvest, yield was assessed by the length of ten selected spikelets per treatment, the weight (g) of 1000 randomly selected seeds from each plot, the fresh weight of ten randomly selected plant stems taken in the field immediately after harvest from each plot, the number of rows and weight of grain on each spikelet and the crop yield (kg/ha) of each treatment.

Soil sampling

For the investigation of the mycorrhizal soil infectivity and AMF community diversity, bulk soil and root zone soil samples were collected from the plots (before sowing), at flowering and at harvest. Each soil sample was a composite of ten cores (6 cm diameter × 15 cm depth) taken from each of the field replications. Fresh soil samples were sieved <2 mm and homogenized in the laboratory to produce composite samples. Soil samples were then stored in a refrigerator prior to AMF analysis.

Field root sampling and study of mycorrhizal colonization

The fine living roots of five randomly selected plants per treatment were randomly collected at 60 days after sowing. These root samples were taken to the laboratory and root subsamples were drawn to examine the level of AMF colonization. Lateral roots, which are more likely to form mycorrhizae, were collected, cleared

in KOH (10% (w/v)) at 80 °C for 30 min and stained with trypan blue (0.05% (w/v) in 0.8% acetic acid solution) at 80°C for 35 min (adapted from Phillips and Hayman, 1970). Roots were cut into 1-2 cm pieces and placed on slides for microscopic observation (x250). A total of 100 root pieces were taken randomly from each sample. Mycorrhizal colonization was quantified according to the method of McGonigle et al. (1990).

Spore density and identification of AMF

One hundred grams of soil subsample was taken from each soil sample collected for spore isolation by wet sieving and decanting (Gerdmann and Nicolson, 1963) and flotation using the sucrose gradient method (Brundrett et al., 1996).

Mycorrhizal spores were examined under a dissecting microscope and spores of each morphotype were mounted in polyvinyl-lactoglycerol and 1:1 PVLG/Melzer's reagent for identification (Morton and Benny, 1990). Criteria for morphological characterization of spores were mainly based on spore size and color, wall structure and hyphal attachment (Morton and Benny, 1990). The morphological characteristics and their subcellular structures were observed under high-power light microscopy at 100 × and 400 × magnification. Identification was made to genus and, where possible, species level, following the species descriptions of the International Culture Collection of Arbuscular and Vesicular Arbuscular Fungi (http://invam.caf.wvu.edu).

Statistical analysis

All data were tested for normality and homogeneity using the Shapiro-Wilk test. Plant growth and yield data were statistically analyzed by one-way analysis of variance (ANOVA) using the R software v3.4.4 (R Core Team 2018) and expressed as mean value ± standard error. The significance of the data was assessed by Fisher's probable least-squares difference test with a significance cut-off at $P < 0.05$, based on the comparison of all treatments. Spore count and mycorrhizal colonization data for each treatment and plot were square-root transformed and subjected to a two-way ANOVA followed by the Tukey's multiple means tests to analyze how the response variable varied between treatments. Post-hoc analysis of means was performed using the Tukey HSD test. Means and standard errors are presented throughout and $P < 0.05$ is considered significant.

RESULTS

Mycorrhizal root colonization

Z. mays and *S. bicolor* plants were naturally colonized by autochthonous AMF as shown for the control plant roots (root colonization intensity of 28.2 ± 3.2 and 22.5 ± 2.8 for maize and 24.1 ± 3.8 and 22.8 ± 2.3 for sorghum at 60 days after sowing for the first and second growing season, respectively) (Table 1). However, in maize, the frequency (96.7 ± 4.9 and 93.3 ± 3.4 for the first and second year, respectively) and intensity (42.6 ± 3.9 and 32.4 ± 2.6 for the first and second year, respectively) of AMF colonization were significantly higher in plants from coated seeds than in the control plants (84.3 ± 4.6 and 84.0 ± 3.8 for the first and second year, respectively) and in plants treated with NPK fertilizer alone. In addition, root AMF colonization was significantly higher in plants from the coated seeds combined with NPK fertilizer at 75 kg/ha (36.8 ± 3.7 and 27.3 ± 2.5b for the first and second year, respectively) than in the control plants and in plants treated with NPK fertilizer at 150 kg/ha (27.6 ± 3.6 and 18.1 ± 2.1 for the first and second year, respectively). In sorghum, however, root colonization was higher (36.7 ± 3.7 and 27.9 ± 2.5 for the first and second year, respectively) when NPK was applied at 75 kg/ha in combination with seed coating. In addition, no significant difference was found between controls and plants of the coated seed treatment or when NPK was applied at 150 kg/ha (Table 1).

Plant growth and grain parameters

The effect of seed treatments on plant growth was assessed at 45, 60 and 90 days after sowing (DAS). In maize, plant height was significantly higher in the NPK fertilizer treatments at early stages (45 and 60 DAS) of plant growth compared to the controls. However, no significant difference was found between coated seed and control plants. In addition, the coated seed treatment combined with NPK fertilizer at 75 kg/ha significantly increased plant height and there was no significant difference compared to plants treated with NPK at 150 kg/ha. Similarly, these treatments also increased plant height at 90 DAS, but the difference between treatments was not significant for either the first or second growing season (Table 2).

Grain parameters [number of grains per ear (NGE), weight of grains per ear (WGE), weight of 1000 grains selected from each treatment (WGM) and total grain weight - TGWM (kg)] were higher in the NPK fertilizers treatments. However, the values were significant only for the NGE and TGWM parameters. Grain parameters were similar between NPK fertilizer at 150 kg/ha and coated seed treatment combined with NPK at 75 kg/ha. No significant difference was found between the coated seed treatment and the control plants (Table 2).

For *Sorghum bicolor*, plant height was significantly higher in plants from the coated seed treatments and in the NPK fertilizer treatments compared to the controls at the early stage (45 DAS) of plant growth. However, at 60 and 90 DAS, the differences were only significant with the NPK fertilizer application at 150 kg/ha and the coated seed combined with NPK at 75 kg/ha. As for the growth and up to 90 DAS, grain parameters were on average three and two times higher for plants treated with NPK fertilizer at 150 kg/ha and coated seed combined with NPK fertilizer at 75 kg/ha. The differences found between these treatments were significant for all grain parameters except WGS (Table 3).

Composition of AMF in the study plots and effects of treatments on AMF spore abundance

A total of 9 AMF morphotypes were identified in

Table 1. Root AMF colonization of maize and sorghum plants grown under field conditions during two consecutive growing seasons.

Treatment	Maize				Sorghum			
	2016-17/60 DAS		2017-18/60 DAS		2016-17/60 DAS		2017-18/60 DAS	
	Frequency	Intensity	Frequency	Intensity	Frequency	Intensity	Frequency	Intensity
T0	84.3 ± 4.6^{b}	28.2 ± 3.2^{ab}	84.0 ± 3.8^{ab}	22.5 ± 2.8^{ab}	80.7 ± 4.4^{a}	24.1 ± 3.8^{a}	84.7 ± 3.5^{a}	22.8 ± 2.3^{a}
T1	96.7 ± 4.9^{c}	42.6 ± 3.9^{c}	93.3 ± 3.4^{c}	32.4 ± 2.6^{c}	84.7 ± 4.7^{a}	29.4 ± 3.9^{ab}	87.3 ± 4.6^{a}	25.4 ± 2.7^{ab}
T2	72.5 ± 3.6^{a}	27.6 ± 3.6^{a}	78.7 ± 4.0^{a}	18.1 ± 2.1^{a}	88.5 ± 4.0^{a}	31.6 ± 3.6^{ab}	87.2 ± 4.5^{a}	26.5 ± 2.6^{ab}
T3	89.2 ± 5.6^{bc}	36.8 ± 3.7^{bc}	87.5 ± 3.3^{bc}	27.3 ± 2.5^{bc}	89.3 ± 4.9^{a}	36.7 ± 3.7^{b}	88.0 ± 4.0^{a}	27.9 ± 2.5^{b}

T0 = control, T1 = seed coated with AMF spores and PGPR, T2 = mineral fertilizer NPK (15-15-15) at 150 kg/ha, T3 = seed coated with AMF spores and PGPR + mineral fertilizer NPK (15-15-15) at 75 kg/ha, days after sowing (DAS). Within columns and for each crop species, values (mean ± standard error) followed by the same superscript letters are not significantly different (Fischer's protected LSD $P < 0.05$).

Table 2. Growth parameters and yield of maize plants grown under field conditions during two consecutive growing seasons.

Treatment	Plant growth and yield parameters of maize								
	Plant height (cm)				Plant yield				
	45 DAS	60 DAS	90 DAS		NGE	WGE (g)	WGM (g)	TGWM (kg)	
			2016-17	2017-18				2016-17	2017-18
T0	34.3 ± 3.7^{a}	177.1 ± 8.0^{a}	240.5± 15.0^{a}	233.4 ± 13.0^{a}	470.7 ± 21.8^{a}	102.9 ± 11.7^{a}	165.2 ± 13.6^{a}	6.49 ± 1.4^{a}	5.86 ± 1.1^{a}
T1	40.1 ± 4.1^{ab}	179.6 ± 9.3^{ab}	244.0 ± 14.26^{a}	240.8 ± 12.26^{a}	492.3 ± 22.5^{ab}	110.7 ± 14.5^{a}	178.7 ± 17.8^{a}	8.73 ± 1.7^{a}	7.68 ± 1.5^{a}
T2	45.8 ± 4.4^{b}	196.5 ± 8.5^{b}	254.7 ± 17.6^{a}	251.3 ± 15.6^{a}	535.9 ± 26.5^{b}	123.3 ± 14.3^{a}	197.0 ± 20.1^{a}	15.97 ± 2.6^{b}	14.89 ± 2.1^{b}
T3	42.1 ± 3.3^{b}	190.7 ± 8.2^{ab}	251.5 ± 14.26^{a}	247.9 ± 12.26^{a}	525.0 ± 24.9^{b}	117.7 ± 12.7^{a}	196.4 ± 20.7^{a}	13.93 ± 1.9^{b}	12.78 ± 2.0^{b}

T0 = control, T1 = seed coated with AMF spores and PGPR, T2 = mineral fertilizer NPK (15-15-15) at 150 kg/ha, T3 = seed coated with AMF spores and PGPR + mineral fertilizer NPK (15-15-15) at 75 kg/ha. Number of maize grains per ear (NGE), weight of maize grains per pocket (WGE), weight of 1000 maize grains selected from each treatment (WGM), total grain weight of maize - TGWM (kg), days after sowing (DAS). Within columns, values (mean ± standard error) followed by the same superscript letters are not significantly different (Fischer's protected LSD $P < 0.05$).

the two fields (Figure 1). They are divided into 7 genera (*Racocetra*, *Scutellospora*, *Dentiscutata*, *Gigaspora*, *Sclerocystis*, *Acaulospora* and *Glomus*), which are classified in 3 families (Glomeraceae, Acaulosporaceae and Gigasporaceae). The Gigasporaceae family is the most represented with 4 genera (*Gigaspora*, *Scutellospora*, *Racocetra* and *Dentiscutata*). This is followed by the family Glomeraceae with 2 genera (*Glomus* and *Sclerocystis*) and the family Acaulosporaceae. The genus *Glomus* is the most represented with 3 morphotypes, that is, 33.33% of the described species (*Glomus* sp1, *Glomus* sp2, *Glomus* sp3), followed by the genera *Racocetra*, *Gigaspora*, *Scutellospora*, *Acaulospora*, *Sclerocystis* and *Dentiscutata* with 1 species each, that is, 11.11% of the described species. Soil samples taken before sowing showed that the maize and sorghum plots did not differ significantly in terms of mycorrhizal inoculum potential (data not shown) and the density of the identified AMF species in the soils (Figure 2).

Compared to the results obtained before sowing, the density of AMF morphotypes in the maize plot at harvest was increased (Figure 3). All the AMF spore morphotypes identified before sowing were found in the soils sampled from

Table 3. Growth parameters and yield of sorghum plants grown under field conditions during two consecutive growing seasons.

Treatments	Plant growth and yield parameters of sorghum								
	Plant height (cm)		Plant yield						
	45 DAS	60 DAS	90 DAS		NGE	WG (g)	WGS (g)	TGWS (Kg)	
			2016-17	2017-18				2016-17	2017-18
T0	26.4 ± 2.30^{a}	44.9 ± 9.2^{a}	114.7 ± 13.7^{a}	110.1 ± 12.0^{a}	1421.1 ± 122.9^{a}	23.2 ± 5.1^{a}	14.9 ± 1.42^{a}	4.17 ± 1.07^{a}	3.38 ± 0.93^{a}
T1	33.4 ± 3.14^{b}	62.0 ± 8.8^{ab}	134.2 ± 15.3^{ab}	126.4 ± 14.8^{ab}	1612.1 ± 116.3^{a}	27.7 ± 5.2^{a}	15.3 ± 1.79^{a}	5.53 ± 1.44^{a}	4.25 ± 1.0^{a}
T2	35.9 ± 3.77^{b}	87.0 ± 7.7^{c}	171.4 ± 17.6^{c}	165.3 ± 15.1^{c}	2910.8 ± 146.1^{b}	56.0 ± 6.1^{b}	17.5 ± 2.28^{a}	9.07 ± 1.16^{b}	8.11 ± 1.5^{b}
T3	34.6 ± 3.23^{b}	72.6 ± 9.4^{bc}	162.8 ± 15.2^{bc}	147.2 ± 13.6^{bc}	2663.7 ± 136.9^{b}	50.9 ± 5.5^{b}	16.3 ± 1.43^{a}	7.87 ± 1.32^{b}	6.96 ± 1.42^{b}

T0 = control, T1 = seed coated with AMF spores and PGPR, T2 = mineral fertilizer NPK (15-15-15) at 150 kg/ha, T3 = seed coated with AMF spores and PGPR + mineral fertilizer NPK (15-15-15) at 75 kg/ha. Number of sorghum grains per ear (NGE), weight of sorghum grains per ear (WG), weight of 1000 sorghum grains selected from each treatment (WGS), total grain weight of sorghum - TGWS (k g), days after sowing (DAS). Within columns, values (mean ± standard error) followed by the same superscript letters are not significantly different (Fischer's protected LSD $P < 0.05$).

coated seed treatments, either alone or in combination with NPK fertilizer at 75 kg/ha. Interestingly, the results showed the appearance of a new *Sclerocystis* sp morphotype in the coated seed treatment and when combined with NPK at 75 kg/ha. In addition, the density of *Glomus* spore morphotypes was higher in the coated seed treatments compared to the uncoated and unfertilized control and the NPK fertilizer at 150 kg/ha. The density of AMF spores was lower for all morphotypes in the NPK fertilizer treatment at 150 kg/ha, except for the genera *Gigaspora*, *Acaulospora* and *Dentiscutata* for which the negative control treatment had the lowest spore density (Figure 3).

The density of AMF spore morphotypes in the sorghum plot was also increased compared to the pre-sowing samples (Figure 4). Except for the genera *Gigaspora*, *Dentiscutata* and *Acaulospora* which showed their highest spore density with the NPK fertilizer at 150 kg/ha, the other AMF genera were more abundant in the coated seed treatments. As for the maize plot, the morphotypes belonging to the genus *Glomus* were the dominant spores. The AMF spore density of *Glomus* sp2 was on average four and three times higher in the coated treatments than in the uncoated and in the fertilized treatments. The genus *Sclerocystis* was not found in the sorghum plot (Figure 4).

DISCUSSION

Root colonization is a prerequisite for the functioning of the AMF symbiosis and for the plant to reap the benefits of the plant-fungus association (Calvo et al., 2014; Fortin et al., 2015). Therefore, the primary goal of AMF application is to achieve root colonization (Smith and Read, 2008; Fortin et al., 2015). Here, we used PGPR bacteria to enhance root colonization of AMF to get improved plant growth and yield. Although the results of our previous study (Sene et al., 2021) highlighted that the technology of coating maize and sorghum seeds with AMF spores and PGPR cells using a 10% gum arabic solution was successful, it was necessary to confirm whether the use of coated seeds with beneficial microorganisms could also improve crop performance throughout the growing cycle under field conditions. The results of this study report the effects of the coated seeds co-inoculated with AMF spores and PGPR bacteria, alone or in combination with mineral fertilizers (NPK, 15-15-15) at 75 kg/ha, on AMF root colonization, growth and yield of maize and sorghum plants. The results showed that application of microbial fertilizers via seed coating was improved soil AMF spore density, which is effective in improving soil fertility and could reduce the dependence on mineral fertilizer. In maize, improved root AMF colonization rates were observed with the seed coating treatments up to 60 DAS. This agrees with the data of Rocha et al. (2018) and Paravar et al. (2023). In their study, these authors showed that PGPR can promote the germination of AMF spores to increase the rate and extent of root plant colonization by AMF.
Several other studies have focused on seed coating with AMF and have also shown increased root AMF colonization with such application of

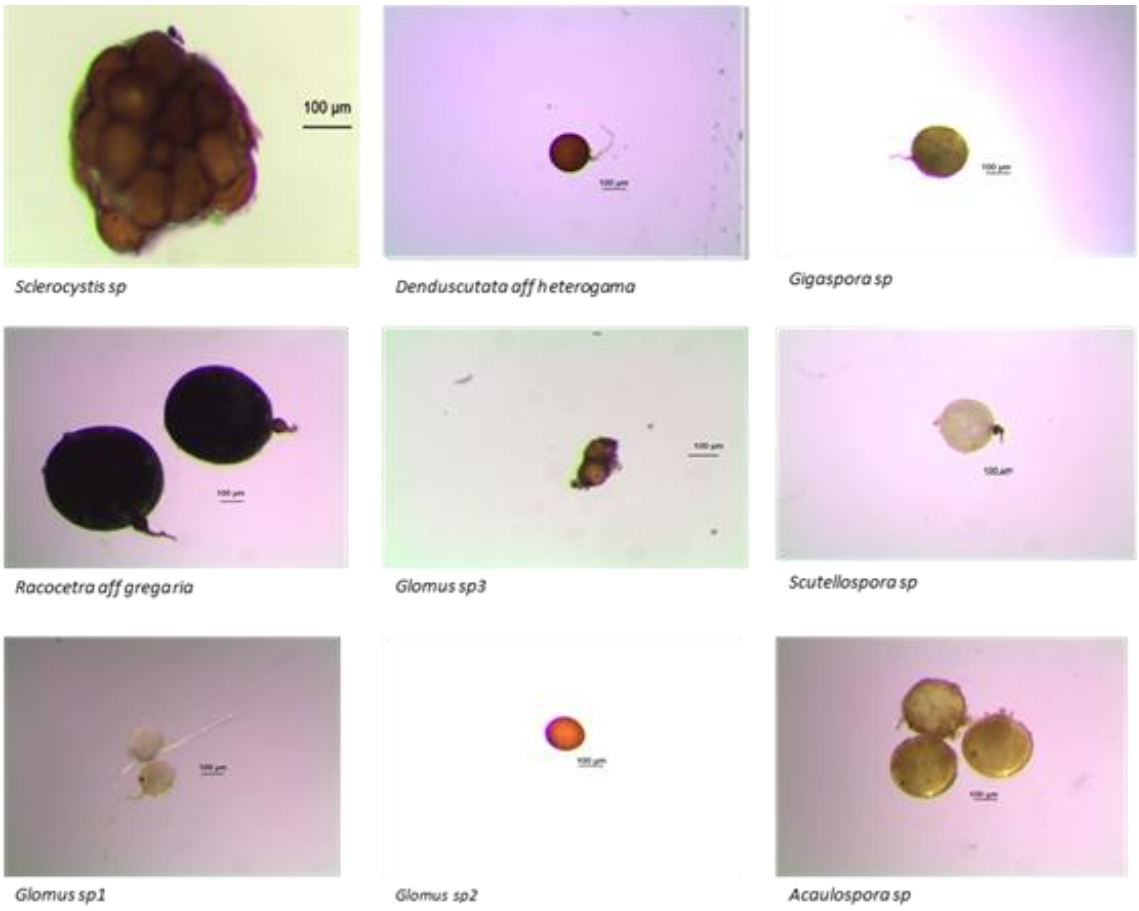

Figure 1. Morphotypes of AMF spores identified in soils sampled before sowing and after harvest.

mycorrhizal fertilizer (Ortas, 2012; Rocha et al., 2019; Begum et al., 2019).

However, we have demonstrated that the application of NPK fertilizer at the standard dose of 150 kg/ha for cereal crops in Senegalese soils significantly reduces root colonization by AMF in maize. Increasing soil P has been shown to decrease AMF formation in some species, probably through a direct effect on the development of extra-root hyphae or indirectly through the P content of the host plant (Rocha et al., 2018).

For the sorghum plants, the increase in root colonization by AMF was only found in the coated seed treatment at the early growth stage (45 DAS) of the plants (data not shown). Therefore, no significant increase was observed in sorghum plants from the coated seed treatment alone at 60 DAS. However, the sorghum seed coated with AMF spores and PGPR cells, in combination with the mineral fertilizer at 75 kg/ha or the mineral fertilizer at 150 kg/ha, significantly increased root colonization by AMF up to 60 DAS. This behavior between maize and sorghum crops confirms the reports of several other researchers (Ortas, 2012; Rozier et al., 2017; Accinelli et al., 2018; Kandhasamy et al., 2020) and suggests that the sensitivity of the plant-AMF-PGPR symbioses to mineral fertilizers varies with crop genotype. As maize and sorghum seeds have different sizes and shapes, and could differ in the amount of adherent AMF propagules and PGPR cells, this could also affect root colonization rates between these crop species.

Various research studies conducted on AMF over the last two decades have highlighted its myriad benefits on crop productivity, soil and plant health. Therefore, it is widely believed that AMF could be considered as a substitute for mineral fertilizers in the near future, as mycorrhizal application can effectively reduce the quantitative use of mineral fertilizer inputs (Ortas, 2012; Begum et al., 2019). Despite high AMF root colonization rates in the seed-coated treatment, maize and sorghum plant growth did not improve significantly until 90 DAS and the increase in yield parameters is not significant either. This is not in line with our initial hypothesis and the result did not reinforce the data from previous experiments combining AMF with PGPR fertilizers via seed coating (Lally et al., 2017; Rozier et al., 2017; Accinelli et al., 2018; Rocha et al., 2019) and reporting

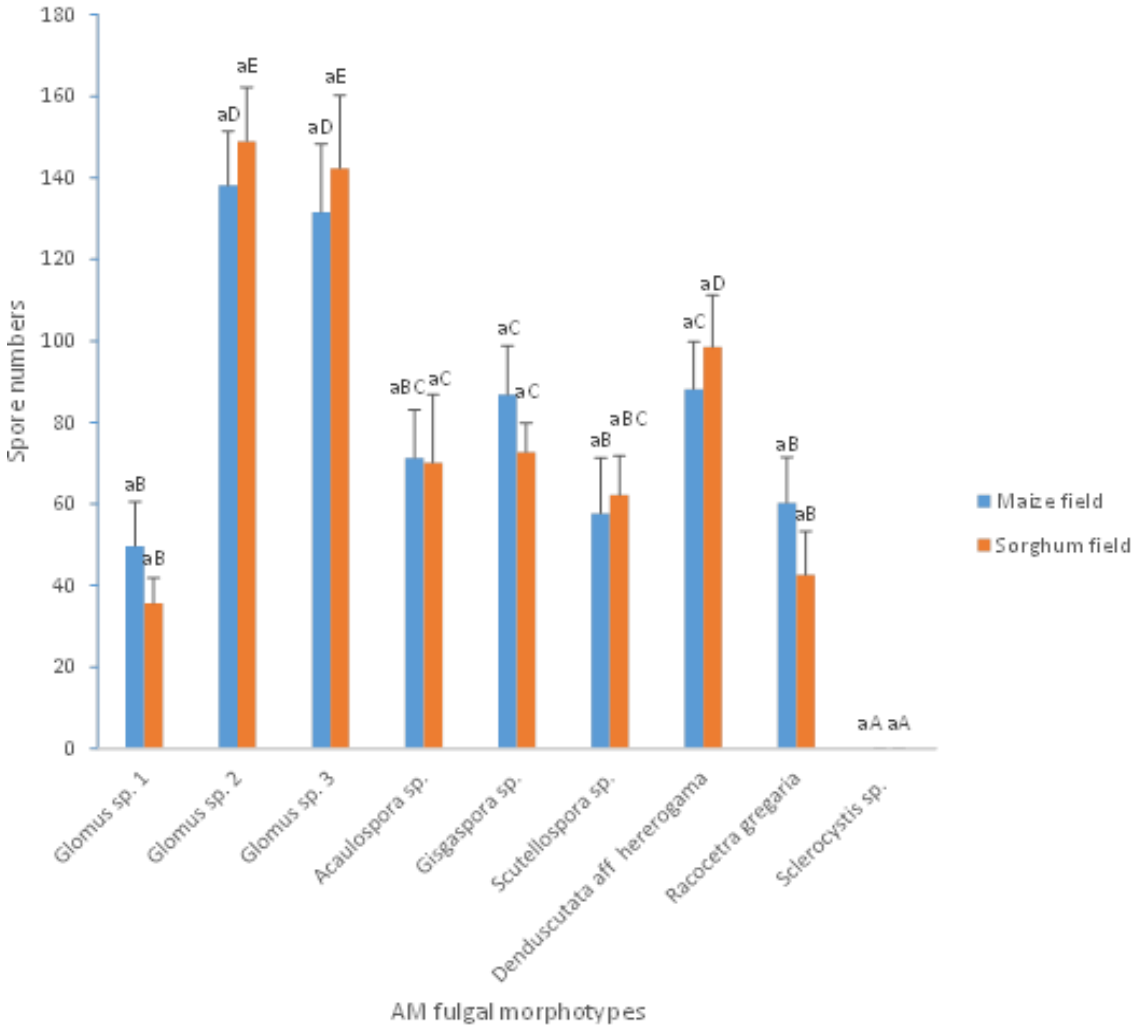

Figure 2. Abundance of AMF spores in soils sampled in the maize and sorghum fields before sowing. T0 = control, T1 = seed coated with AMF spores and PGPR, T2 = mineral fertilizer NPK (15-15-15) at 150 kg/ha, T3 = seed coated with AMF spores and PGPR + mineral fertilizer NPK (15-15-15) at 75 kg/ha. Lowercase letters compare the spore abundance between the maize and sorghum plots for each identified AMF species. Values with a common letter are not different at $p < 0.05$ according to the Tukey post-hoc tests. Capital letters compare the spore abundance of all AMF species found in the same maize or sorghum plot. Bars topped by the same letter indicate no significant differences at $p < 0.05$ according to the Tukey post-hoc tests.

improved plant growth or yield. Similarly, other studies (Cassán et al., 2020; Vafadar et al., 2014) showed that plant growth parameters such as grain number, grain weight, plant height, and biomass production increased synergistically with the addition of AMF and PGPR. In wheat, rice, and black gram, the combined application of PGPR and AMF increased plant yield by up to 41% compared to non-inoculated plants (Paravar et al., 2023). The authors can therefore hypothesis that soil nutrient deficiencies in our case study may limit the symbiotic effectiveness of inoculated strains with plants. The use of microbial inoculants has previously been shown to reduce the accumulation of nutrients such as N, P and K in agricultural soils (Adesemoye et al., 2008; Rocha et al., 2018). In this case, the benefits of microbial seed coatings on plant growth may be short term, depending on the availability of nutrients in the soil. In fact, not all published research shows positive effects of microbial seed coating inoculation on plant performance. For example, Diniz et al. (2009) coated sweet pepper seeds with a mixture of AMF and other beneficial PGPR and observed a negative effect on germination rate and plant height. Another explanation for this response may be related to the competitive ability of the inoculated AMF and PGPR species at the study site compared to the indigenous microorganisms. Inoculated AMF were previously tracked in the field using 454-pyrosequencing of the partial rRNA 18S gene, and these authors showed

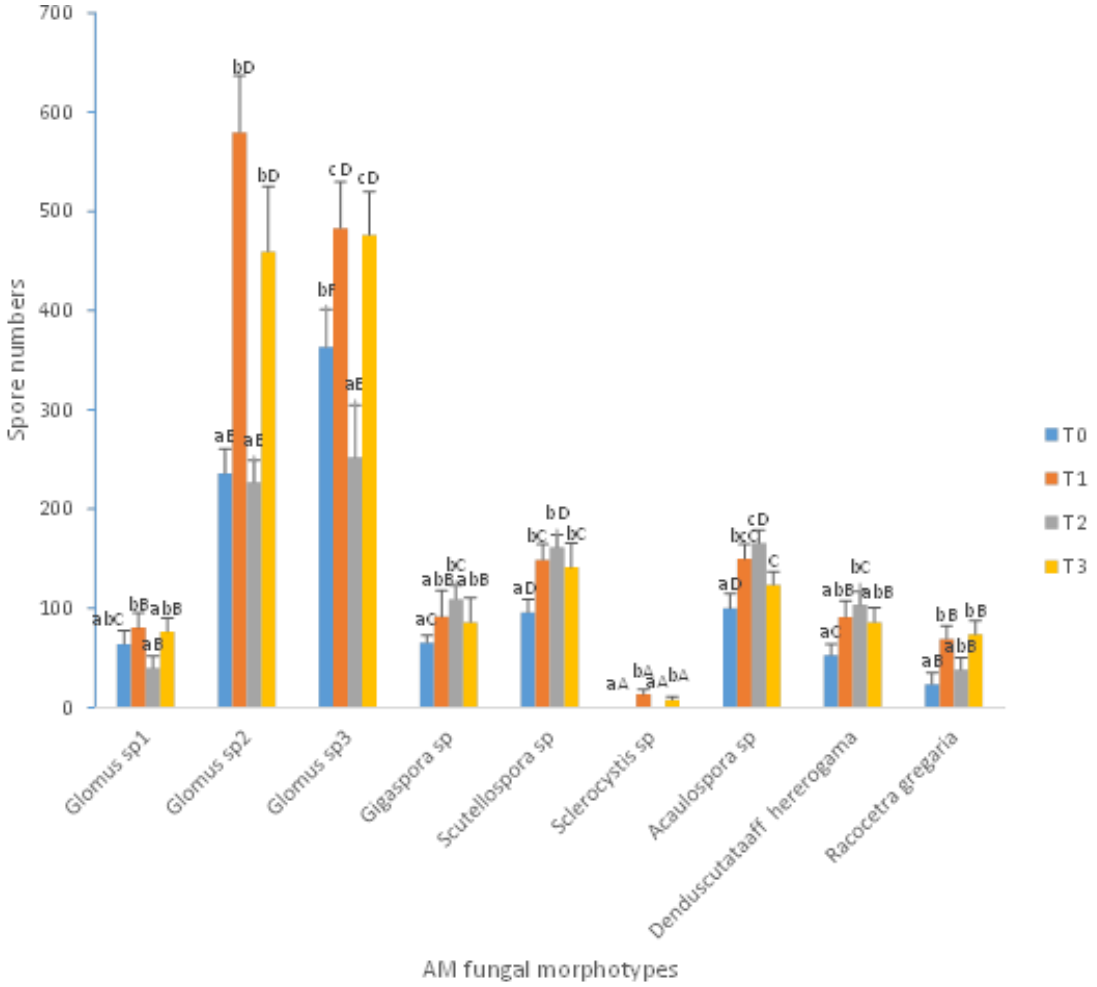

Figure 3. Abundance of AMF spores in treatments of the maize plots at plant harvest in the second growing season. T0 = control, T1 = seed coated with AMF spores and PGPR, T2 = mineral fertilizer NPK (15-15-15) at 150 kg/ha, T3 = seed coated with AMF spores and PGPR + mineral fertilizer NPK (15-15-15) at 75 kg/ha. Lowercase letters compare treatment effects on the spore abundance of each identified AMF species. Values with a common letter are not different at $p < 0.05$ according to the Tukey post-hoc tests. Capital letters compare the spore abundance of all AMF species found in the same treatment. Bars topped by the same letter indicate no significant differences at $p < 0.05$ according to the Tukey post-hoc tests.

that AMF taxa failed to colonize maize roots and lacked soil persistence (Berruti et al., 2017).

Maize and sorghum have a high demand for N and P, and their soil concentrations can affect AMF performance (Rocha et al., 2018; Ostmeyer et al., 2022). In our case of poor soils, the results suggest that the application of microbial fertilizer alone is not sufficient to achieve significant improvements in plant growth and yield. This is supported by the fact that plants treated with microbial fertilizer and combined with half dose of NPK fertilizer, achieved significantly higher final heights and yields for both maize and sorghum, whereas the unfertilized plants only achieved low heights and yields. Plant growth and yield of the coated treatment combined with 50% dose NPK fertilizer reached that of NPK fertilizer at the standard rate of 150 kg/ha. This could be due to the positive response of AMF colonization, which can increase nutrient and water uptake in the plant body, resulting in increased plant height and yield (Begum et al., 2019). Indeed, before the symbiosis takes place, this reduced dose of NPK may be sufficient to act as a "starter effect" for plant growth in the early stages of development. Previous research suggests that strategies combining both reduced rates of agricultural fertilizer and biofertilizer can be beneficial for plant development and nutrient uptake (Shaharoona et al., 2008; Adesemoye et al., 2010; Duarah et al., 2011; Mäder et al., 2011; Begum et al., 2019). These data and the increases in plant growth and yield that we achieved with the coated microbial fertilizer, while reducing the rates of agrochemical inputs, are relevant to sustainable and environmentally friendly agricultural practices and when

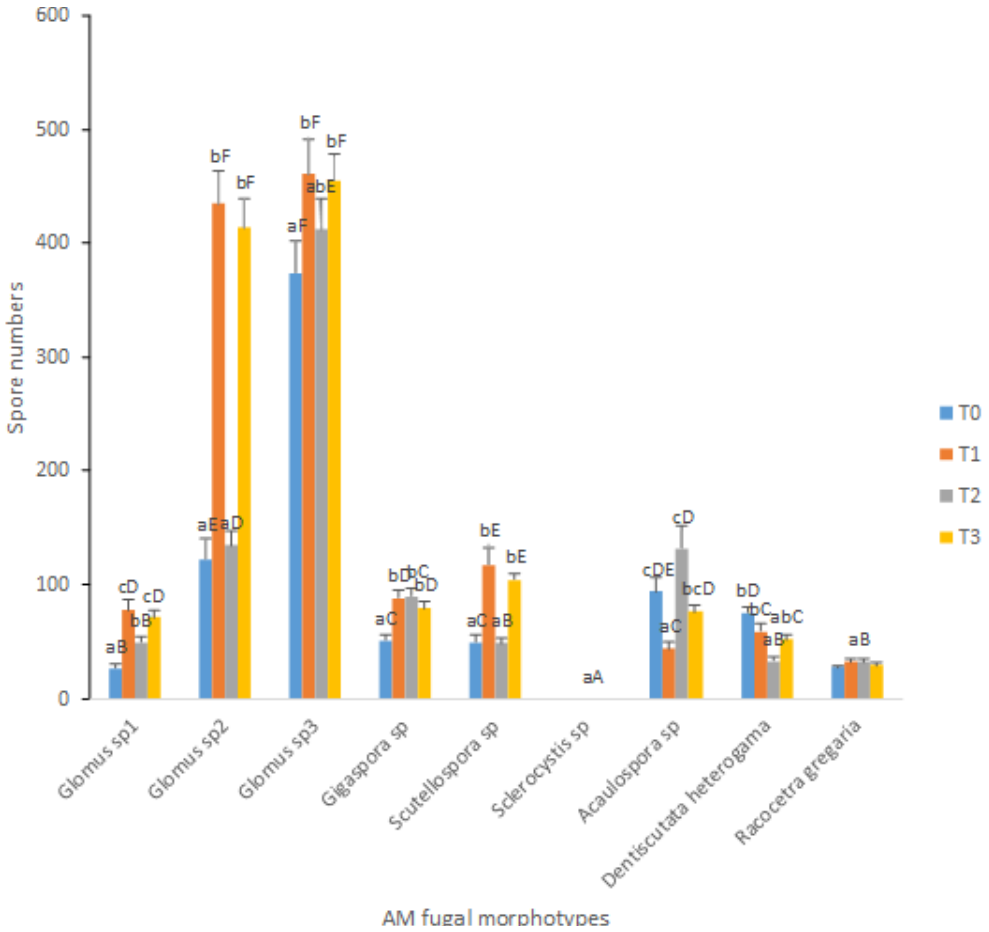

Figure 4. Abundance of AMF spores in treatments of the sorghum plots at plant harvest in the second growing season. T0 = control, T1 = seed coated with AMF spores and PGPR, T2 = mineral fertilizer NPK (15-15-15) at 150 kg/ha, T3 = seed coated with AMF spores and PGPR + mineral fertilizer NPK (15-15-15) at 75 kg/ha. Lowercase letters compare treatment effects on the spore abundance of each identified AMF species. Values with a common letter are not different at $p < 0.05$ according to the Tukey post-hoc tests. Capital letters compare the spore abundance of all AMF species found in the same treatment. Bars topped by the same letter indicate no significant differences at $p < 0.05$ according to the Tukey post-hoc tests.

considering the potential use of healthier maize and sorghum as livestock feed or industrial feedstock, as is a focus of the new green revolution (Fortin et al., 2015; Mohanty and Swain, 2018).

The effect of seed coating with beneficial AMF/PGPR and mineral fertilizers on AMF spore morphotype composition and density was also assessed in the current study. The context was not to determine total mycorrhizal diversity per se, but rather to comparatively explore the response of AMF spores between the treatments, an application considered entirely valid (Sene et al., 2012). The results showed that neither seed coating with beneficial microorganisms nor mineral fertilization had a significant effect on AMF spore richness over two growing seasons. Only the *Sclerocystis* sp. occurred in the coated treatment of the maize plot at harvest and was not found in the other treatments. This result confirms those of (Koffi et al., 2021), who showed an increase in spore density after cultivation and the appearance of new morphotypes that were not present in the soils before sowing. However, we found different responses of AMF morphotypes to treatments. The density of the small-spored morphotypes (*Glomus*) was particularly higher in the coated seed treatments, which are effective for soil life and fertility. Factors such as a more developed and colonized root system with AMF/PGPR inoculation could partially explain this increase. In contrast, most of the species with larger spores (*Gigaspora*, *Dentiscutata* and *Acaulospora*) showed their highest spore density with the mineral fertilizer, which is not consistent with reports by Egerton-Warburton and Allen (2000). These authors reported that increasing azote and/or phosphorus inputs were associated with the displacement of the larger-spored species due to a failure to sporulate.

Conclusion

Driven by the need for sustainable and environmentally friendly agricultural practices and safer and healthier food, the demand for microbial inoculants is increasing. Our hypothesis was that seed coating with microbial fertilizers would contribute to improving crop performance in maize and sorghum by reducing the use of mineral fertilizers. The results of this study showed that seed coating of maize and sorghum with arbuscular mycorrhizal fungi (AMF)/plant growth promoting rhizobacteria (PGPR) fertilizers increased root AMF colonization in maize plants, but not in sorghum, where the seed coating in combination with mineral fertilizer at a standard dose reduced by 50% increased root AMF colonization. In addition, the coating had no significant effect on plant growth or yield in either maize or sorghum.

However, the authors achieved significantly higher final height and yield in plants treated with the microbial fertilizer in combination with mineral fertilizer at a standard dose reduced by 50%.

The results of the current study also showed, that seed coating or mineral fertilizer, applied individually or together, had no significant effect on AMF spore richness.

However, they also found that the coated seed treatments increased the density of AMF spore morphotypes, which is effective for soil fertility. The results obtained showed that the application of AMF/ PGPR fertilizers could be adapted to reduced mineral fertilizer doses. This work is the first study to report on cereal seed coating as a tool for delivering beneficial microorganisms to agricultural crops in West Africa, and the data show that the seed coating can make a significant contribution to reducing the overuse of mineral fertilizers on poor soils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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