

# Diversity of Arbuscular Mycorrhizal Fungi Associated with Maize in the Eastern Part of Uganda <sup>†</sup>

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<sup>†</sup> Presented at the 2nd International Electronic Conference on Diversity (IECD 2022)—New Insights into the Biodiversity of Plants, Animals and Microbes, 1–15 March 2022; Available online: <https://iecd2022.sciforum.net/>.

**Abstract:** Improving maize yield is an utmost important objective for food security in Uganda. In the evaluation of soil microorganisms to crop production, it is important to assess the composition and diversity of Arbuscular Mycorrhizal Fungi (AMF) species at different agroecosystems. AMF play an important role in improving crop growth and yield. We present a study of the morphological diversity of native AMF species associated with the rhizosphere of maize in two locations in eastern Uganda (Amuria and Serere districts). The effects of soil chemical properties on this diversity were also assessed. AMF diversity was assessed by morphological identification of the spores extracted from soils samples by the wet sieving method. Spores abundance, species richness, and diversity were determined. A total of 19 AMF morphotypes were distributed in 7 genera (*Gigaspora*, *Scutellospora*, *Glomus*, *Acaulospora*, *Archaospora*, *Entrophosporaa*, and *Paraglomus*) were observed. *Glomus* species were abundant in all sites. Spores densities were higher in Amuria than in Serere. Soil pH, CEC, and phosphorus content influenced AMF distribution. Finding the species in various agroecological environments indicates that they are adapted to the environments. Maize grown in eastern Uganda is associated with a diversity of AMF that could be selected as a bio-fertilizer to improve crop production.

**Keywords:** Arbuscular mycorrhizal fungi; Morphotypes; species richness

Academic Editor: Ipek Kurtboke

Published: 14 March 2022

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## 1. Introduction

Maize (*Zea mays L.*), a tropical cereal crop, is one of the most economic and nutritional cereals grown in the African continent. It is used as a staple food and as livestock feed but industry production (biofuel) (Ekpa et al., 2019). In Sub Sahara Africa (SSA), particularly in the eastern region, maize is the main source of carbohydrates and protein which contribute to populations' food security (Mumo et al., 2021). Its dry grains contained a high level of carbohydrate (73%), protein (9.1–14%), and oligo-elements such as iron and zinc (Ochieng et al., 2021; Wu and Messing, 2014). In addition, another important beneficial attribute of this cereal is it is particularly adapted to regions of limited rainfall (under 600

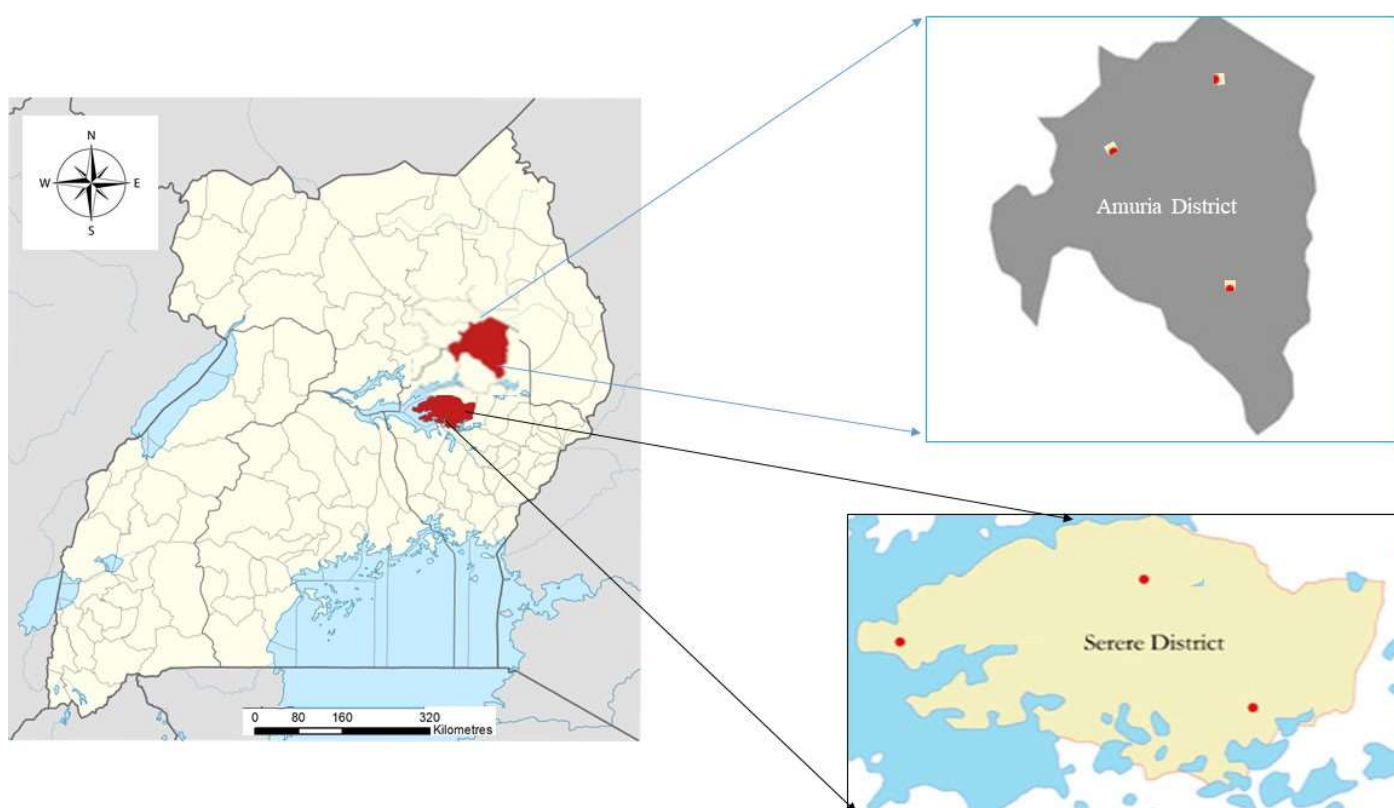
mm per year) and sub-humid to semi-arid (1000–1500 mm per year). In Uganda, it is the most important cereal crop, it accounts for 16% of caloric intake and per capita, maize consumption ranges from 28 to 125 kg per annum (Sharon et al., 2020). Maize is planted in all agro-ecological zones in Uganda, but more intensely in the eastern region (Mbale, Jinja, Iganga, and Teso sub-region). More than 90% of Uganda's maize is produced by smallholder farmers, of which about 60% of the annual production is consumed on the farm and its management is generally done with low or zero input technology due to the fairly capacity of farmers to buy chemical fertilizers (UBOS, 2016). It has been reported that 2.9 million tons of maize grain were produced in 2018 but this level of production is largely below the national demand (FAO, 2020). Constraints limiting maize production in Uganda include pests and diseases (Oloo, 2021) as well as low soil fertility and erratic rainfall pattern (Okoboi et al., 2012). The challenge of the National Agricultural Research Organization (NARO) in Uganda is the improvement of maize production using new affordable and sustainable technologies contributing to maintaining biodiversity and preserving the environment. It has been proven that the soil microbiota has an important role in the development of sustainable agriculture (Yadav, 2020.). Among the key microbial communities developing in the rhizosphere are arbuscular mycorrhizal fungi (AMF). This ancient group of fungi is obligate symbionts forming associations with a large majority of plant species (about 72% of terrestrial plant species) (Brundrett and Tedersoo, 2018). Several studies have been reported on their roles in mineral nutrition and water supply of plants as well as effects on improving plant resistance to abiotic and biotic stresses (Wu, 2017; Dowarah et al., 2021). Thus, AMF is used in agriculture to increase crop yields while reducing the applications of chemical fertilizer inputs (Liu et al., 2016). Numerous studies in Senegal, and Kenya have shown substantial yield increases through long-term mycorrhizal inoculation (Leye et al., 2015, Nyaga et al., 2015). Selection of efficient AMF and production of biofertilizer in quality and quantity are critical issues for the application of AMF technology in agriculture production in Africa. The use of indigenous mycorrhiza from soil has many benefits in restoration schemes, such as their high adaptation to local conditions and low ecological risk. However, little is known about native AMF associated with annual crops in fields in Uganda. The first studies on mycorrhizal symbiosis in Uganda were conducted on bananas in 2001 by Msiska (Msiska, 2007). One recent study has helped to identify the diversity of AMF in Soroti (Uganda) based on spore morphology (Sebuliba et al., 2010). Spores are one of the most important convenient characteristic features, they can help research in the identification of mycorrhizae rapidly and faster than sequence techniques. Many studies revealed that based on spore morphology is necessary when working on AMF. About 150 AMF species have been described employing morphological features of spores. Though several studies have been carried out on the use of AMF in crop yield, studies on the morphological diversity of AMF are almost missing in Uganda. To our knowledge, no information is available on the morphological diversity of AMF associated with maize crops grown in East African fields. Hence, the present work was conducted to find out the morpho-types of Arbuscular Mycorrhizal Fungi associated with maize (*Zea mays*) in eastern Uganda. This study is probably one of the first to assess the diversity of AMF associated with the rhizosphere of maize in East Africa. The results obtained will provide background information on the indigenous AMF associated with maize and a necessary step towards forecasting the integration of AMF biofertilizer in improving grain yields.

## 2. Materials and Methods

### 2.1. Study Area

This study arose from the need to achieve a long-term objective: to improve maize production in Uganda by using the diversity of native Arbuscular Mycorrhizal Fungi associated with maize rhizosphere. The study areas were Amuria and Serere districts as depicted in Figure 1. The Serere district is located in Eastern Uganda, in the Teso sub-

region, and covers approximately 1965 km<sup>2</sup> (Agwot, 2018), with an elevation about 1095 m above sea level (Nsabagwa et al., 2021). The Amuria district is also situated in the Teso sub-region, and covers a surface of 2588 km<sup>2</sup>, with an elevation of 1096 m above sea level. The area experiences a humid and hot climate, receiving bimodal rainfall with an annual average of 1350 mm, between March to May. There are decreasing light showers between June and August and heavier rains again between September and November. The dry season begins in December and lasts in February. The climate of the sub-region is modified by the large swamp wetland area that surrounds it. The minimum temperature is 18 °C and the maximum temperature is 31.3 °C. However, extremes usually occur in February, when the temperature can exceed 35°C. Teso-land slopes from East to West and lies at a lower altitude than Karamoja highlands and Sebei uplands sub-regions of Uganda, thereby receiving water discharges (Egeru et al., 2012). The economic activities in the sub-region are based on subsistence agriculture and livestock rearing. Farmers grow a diversity of crops, especially legumes and cereals including millet, sorghum, rice, and maize.



**Figure 1.** Study area and soil sampling sites.

## 2.2. Soils Sampling and Analysis

Soils were sampled from maize fields, at the end of the rainy season in December 2021. Cores were collected from 20 random points and the soils were pooled to obtain representative bulk samples. Samples were collected between 2–30 cm depth by removing the top 2 cm of soil and excavating maize rhizosphere soil. The fields selected were active experimental, conventionally managed fields in maize cultivation for multiple seasons. The soils were then placed in a zip-lock freezer bag and kept at 4 °C until their utilization. Soil samples from the fields were analyzed in Makerere University lab (Uganda) according to the procedure described by Okalebo et al. (2002). The soil samples were air-dried and used for the determination of physical and chemical properties including soil texture, pH, organic carbon (C), total nitrogen (N), total phosphorus (P), available phosphorus (P), and the CEC.

### 2.3. Extraction, Enumeration, and Morphological Identification of AMF Spores

Maize rhizosphere soils collected were wet sieved and sucrose centrifuged to extract AMF spores, following the method described by Gerdemann and Nicholson (1963). In summary, 100 g of rhizosphere soil were suspended in 1000 mL tap water, stirred for 1 min and the solutions were sieved sequentially through 400, 200, 100, and 50  $\mu\text{m}$  sieves under flowing tap water to separate the spores according to their size. The soil fraction in each sieve was collected into the beaker. Then, spore suspensions were transferred to 50 mL centrifugations tubes and centrifuged with a water sucrose solution (20% and 60% *w/v*) for 5 min at 4000 rpm (Oehl et al., 2003). The supernatant was decanted into a 32  $\mu\text{m}$  sieve, washed, and transferred to Petri dishes for quantification under a binocular and grouped according to their morphological characteristics (spore size, color, and hyphal attachment). The spore density was expressed as the total number of spores per 100 g of soil (Sasvári et al., 2012). Some spores morphotypes were mounted on slides in polyvinyl-lactic acid-glycerine (PVLG) and a mixture of PVLG with Melzer's reagent (1:1; *v/v*) (Brundrett et al., 1996) to observe wall structures and other specific attributes using a compound microscope at 400 $\times$  magnification (Motic, MIPLUS20). Then, the spores were identified based on descriptions and identification criteria presented in the International Culture Collection of (Vesicular)- Arbuscular Mycorrhizal Fungi website (<http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html>) and on the descriptions in the literature. Morphotypes were classified to the genus level and, when possible, to the species level according to the current valid taxonomy (Redecker et al., 2013).

### 2.4. AMF Roots Colonization Measurement

Capillary root segments were cleaned in 10% KOH solution for 20 min at 90  $^{\circ}\text{C}$  in a bain-marie, then rinsed with water several times using mesh and forceps, and acidified with 1% HCl solution for 30 min. Finally, the root pieces were stained with 0.05% trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol) for 10 min at 90  $^{\circ}\text{C}$  in a bain-marie. The stained root segments were placed in a 50% (*v/v*) glycerol solution for destaining. The destained root samples were then placed on slides and observed under a compound microscope. Root segments were randomly chosen from the destained root samples. Ten slides were prepared by mounting 10 root fragments on each; therefore, a total of 100 root fragments were prepared per treatment. Mycorrhizal colonization was estimated according to the method proposed by Trouvelot et al. (1986).

### 2.5. Statistical Analysis

The significance of differences between treatments concerning spore abundance, species numbers, and AMF diversity (Shannon-Weaver index) was tested using Fisher's least significant difference at  $p < 0.05$  after one-way ANOVA. Statistical analyses for spore abundance, species numbers, and AMF diversity (Shannon-Weaver index) were performed with Minitab software version 17. The data were subjected to an analysis of variance (ANOVA) test. The Fisher least significant difference (LSD) was used to separate the means at the 5% level of significance ( $p < 0.05$ ). Linear regression clarified the influence of soil chemical parameters on communities of AMF. Principal Components Analysis (PCA) was performed using XIStat excel extension 2020 to determine the relationship between soil chemical properties and spore's abundance. The Principal Component Analysis (PCA) analyzed species composition about soil chemical properties using XIStat.

The diversity was calculated from the Shannon-Weiner diversity index (H) from the formula

$$H = - \sum \left( \frac{n_i}{N} \right) \ln \left( \frac{n_i}{N} \right)$$

where  $n_i$  = the spore abundance for an individual species (S) and N = the total spore abundance of the population of all the species in a sample.

A low H value generally suggests a site with few species and a few dominant species, while a high H value suggests considerably more species. The more species we have, the more diverse the area.

### 3. Results

#### 3.1. Soils Chemical and Physical Characterization

The characteristics of the soils from the study sites are given in Table 1. The soil pH from Amuria was neutral ( $6.0 < \text{pH} < 7.5$ ) while the soils from Serere were strongly acidic ( $\text{pH} < 5.5$ ). The P differed also in content with significant variation between sites as the levels of P contents are 9.52 mg/kg and 4.62 mg/kg for Amuria and Serere, respectively. The Organic matter is moderate in Amuria (2.09%) and Serere (1.952%). The CEC is low (8.6 cmol/kg) in the Sandy Loam soil from Amuria and moderate (19.9 cmol/kg) in the Clay Loam from Serere. Total Nitrogen (TN) differed significantly between sites, it was 0.047% and 0.093 % for Amuria and Serere, respectively.

**Table 1.** Physical and chemical properties of soils in the study area.

Site	pH	%TN	%O.M	P (mg/kg)	K (mg/kg)	CEC	Soil Texture *
Amuria	6.15 a	0.047 a	2.09 a	9.52 a	108.3 a	8.6 a	Sandy Loam
Serere	5.0 b	0.093 b	1.952 a	4.62 b	73.7 b	19.9 b	Clay Loam

\* according to USDA classification. TN—total nitrogen content; O.M—organic matter content. Data within a row followed by the same letter are not statistically different ( $p > 0.05$ ) by Fisher's least significant difference test.

#### 3.2. Intensity of Maize Mycorrhization

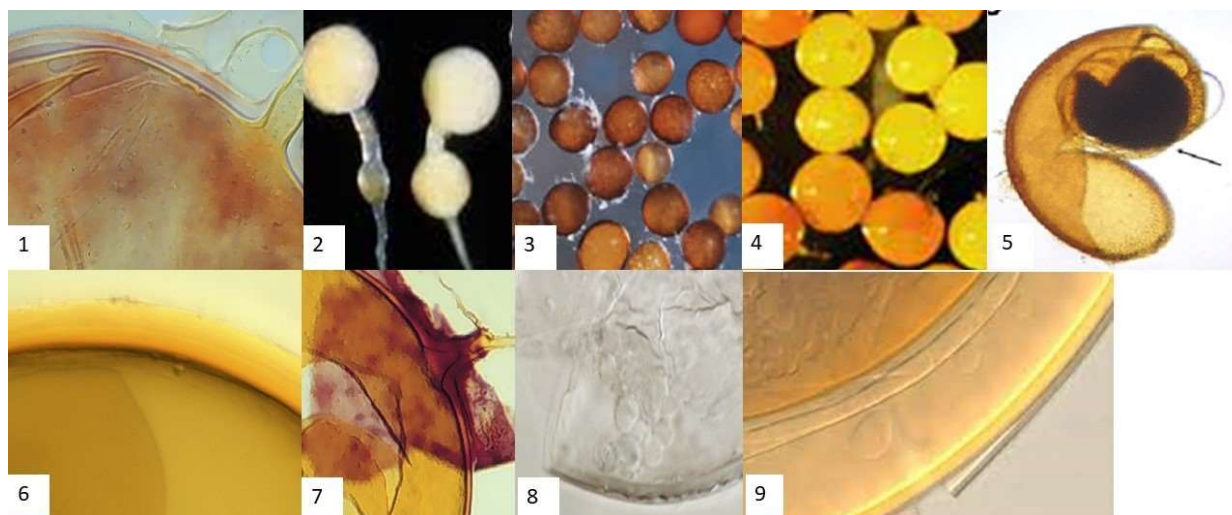
The microscopic observation of maize root samples showed the presence of hyphal, vesicular, and arbuscular features of AMF and the presence of spores within the roots. Statistical analysis of the frequency and the intensity of mycorrhization showed that there is no significant effect of the site on roots mycorrhizal colonization. We noticed that the roots isolated from Amuria fields showed almost a similar value for both mycorrhization intensity (56%) and compared to the Serere field roots that presented an intensity of (55%) (Table 3).

#### 3.3. Diversity and Abundance of AMF Spores

Seven genera of AMF were associated with maize in both sites. The genera were *Gigaspora*, *Scutellospora*, *Glomus*, *Acaulospora*, *Archaeospora*, *Entrophospora*, and *Paraglomus* described by Morton and Benny (1990). In total, 19 AMF morphotypes were recovered. Eight (8) species could be unequivocally assigned to known species of the Glomales, namely three species of the genus *Glomus*, two of the genus *Acaulospora*, one of the genus *Gigaspora*, one of the genus *Scutellospora*, and one of the genus *Entrophospora*. Eleven spore types could not be distinguished at the species level because they lacked enough distinct features. Figure 1 presents some AMF spores observed in the study area. As shown in Table, all of the 19 AMF species found in the present study were present in the samples from Amuria. Only 17 species were observed in the samples from Serere. Regarding relative spore abundance, most of the *Glomus* species showed no significant differences between Amuria and Serere. *Glomus* and *Acaulospora* spores were relatively more numerous than other AMF spore types in Amuria fields, whereas *Glomus* and *Entrophospora* spores were relatively more abundant than other AMF spore types in Serere fields. Interestingly, spores of *Paraglomus* were never found in Serere. Spores of the genera *Archaeospora*, *Gigaspora* and *Scutellospora* were found to be ubiquitous in all the sites and they were: *Archaeospora schenckii*, *Archaeospora* sp., *Gigaspora gigantean*, *Gigaspora* sp., *Scutellospora*, *Pelliculida*, *Scutellospora* sp.

The number of AMF spores per gram soil and the number of AMF species found per site (Table 3). The highest value was observed in Amuria, with 5.1 spores  $\text{g}^{-1}$  of soil ( $p <$

0.05) compared to Serere (3.7 spores  $g^{-1}$  of soil) (Figure 2). Likewise, the species numbers were higher in Amuria compared to Serere. The AMF species diversity as expressed by the  $H'$  did not have much difference between sites with higher diversity obtained in the soils from Amuria ( $H' = 1.84$ ) compared to Serere ( $H' = 1.81$ ) Table 3. The Shannon diversity index ( $H'$ ) showed similar values (3.35–3.38) for all soil microbial communities, and these differences between soils were not statistically significant (Table



**Figure 2.** AMF Spores isolated from the rhizosphere of maize in eastern Uganda. 1—*Glomus intraradices*. 2—*Entrophospora colombiana*. 3—*Acaulospora foveate*. 4—*Gigaspora gigantean*. 5—*Acaulospora spinose*. 6—*Glomus geosporum*. 7—*Glomus mossae*. 8—*Archaeospora schenckii*. 9—*Scutellospora pellucida*.

**Table 2.** Relative spore abundance (%) (mean of three field replicates) from each of AMF species distinguished in the soil samples from the different sites. Data within a row followed.

Genus	Morpho-Species	Amuria	Serere
<i>Acaulospora</i>	<i>Acaulospora spinose</i>	6.9	10.4
	<i>Acaulospora foveata</i>	10.2	6.9
	<i>Acaulospora</i> sp.	12.6	7.3
<i>Archaeospora</i>	<i>Archaeospora schenckii</i>	5.0	4.4
	<i>Archaeospora</i> sp.	13.1	3.1
<i>Entrophospora</i>	<i>Entrophospora colombiana</i>	6.6	10.7
	<i>Entrophospora</i> sp.1	15.3	6.8
	<i>Entrophospora</i> sp.2	5.7	11.8
<i>Gigaspora</i>	<i>Gigaspora gigantean</i>	8.6	9.0
	<i>Gigaspora</i> sp.	12.3	4.6
<i>Glomus</i>	<i>Glomus mossae</i>	7.3	7.8
	<i>Glomus geosporum</i>	6.7	5.1
	<i>Glomus intraradices</i>	10.9	8.2
	<i>Glomus</i> sp.1	6.3	4.4
	<i>Glomus</i> sp.2	5.6	4.7
<i>Scutellospora</i>	<i>Scutellospora. Pellucida</i>	13.3	8.2
	<i>Scutellospora</i> sp.	6.0	7.3
<i>Paraglomus</i>	<i>Paraglomus</i> sp.1	5.1	0.0
	<i>Paraglomus</i> sp.2	5.3	0.0
The total number of spores identified		310	226

Data within a row followed by the same letter are not statistically different ( $p > 0.05$ ). LSD Fisher's least significant difference at the 5% level.

**Table 3.** AMF spore abundance, species richness, and intensity of mycorrhization found in the field samples of the different sites.

	Amuria	Serere	LSD *
Number of AMF spores per gram soil (average of three field replicates)	5.1 a	3.7 b	2.34
Total Number of AMF species found at the field sites (sum of three field replicates)	19	17	-
$H'$	1.84 a	1.81 a	0.16
Intensity of mycorrhization	56%	55%	

The AMF diversity is expressed by the Shannon-Weaver diversity index ( $H'$ ) calculated from the no. of spores identified. Data within a row followed by the same letter are not statistically different ( $p > 0.05$ ).

### 3.4. Relationship between Soil Chemical Properties and Species Diversity

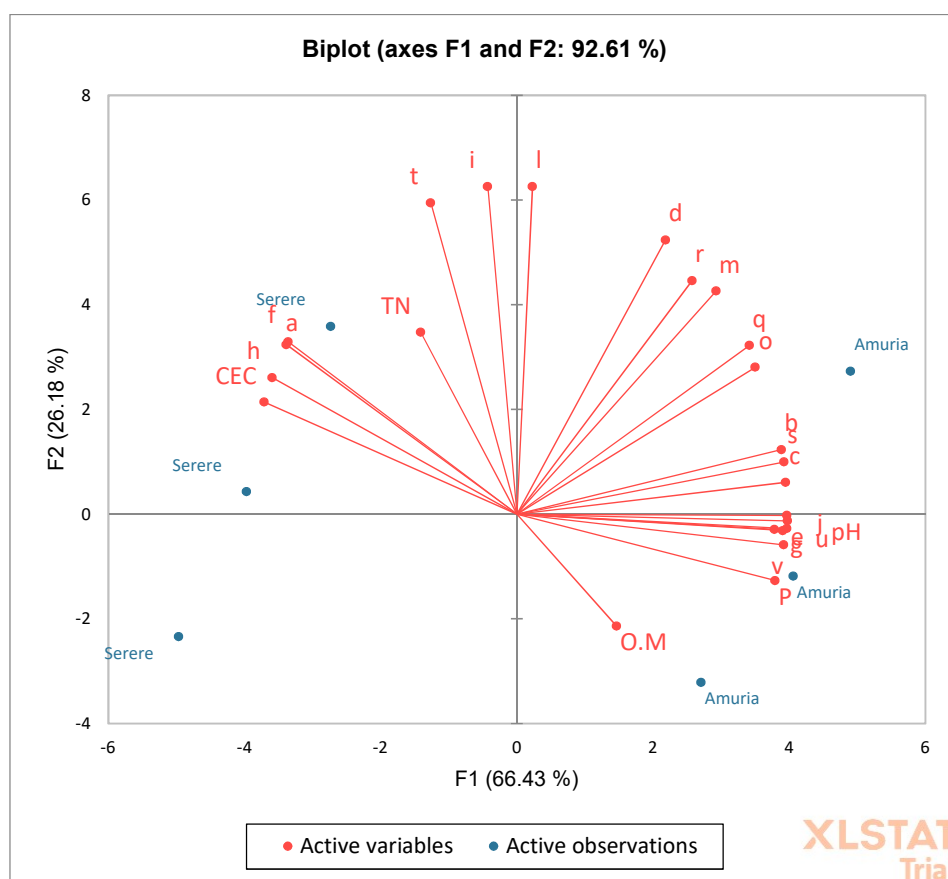
A strong positive and statistically significant correlation was observed between the soil chemical parameters (pH and P) and the species (b, c, e, g, j, m, o, q, s, u, and v) (Table 4). The same species were negatively correlated with the CEC. Chemical parameters (such as O.M and TN) were not correlated with the species abundance.

**Table 4.** Pearson's correlation coefficients between soil chemical properties and AMF community.

	Morphotypes																		
	a	b	c	d	e	f	g	h	I	j	l	m	o	q	r	s	t	u	v
pH	-0.806	0.911	0.952	0.494	0.953	-0.829	0.959	-0.888	-0.119	0.938	0.018	0.673	0.865	0.783	0.564	0.935	-0.383	0.909	0.917
TN	0.511	-0.198	-0.299	0.188	-0.357	0.546	-0.397	0.518	0.511	-0.314	0.463	0.117	-0.136	-0.006	0.105	-0.268	0.679	-0.300	-0.345
O.M	-0.500	0.262	0.298	-0.045	0.361	-0.403	0.357	-0.387	-0.392	0.357	-0.325	0.041	0.066	0.145	0.223	0.309	-0.350	0.345	0.388
P	-0.917	0.886	0.933	0.346	0.958	-0.896	0.951	-0.938	-0.302	0.953	-0.171	0.578	0.741	0.711	0.518	0.911	-0.485	0.929	0.947
CEC	0.981	-0.852	-0.889	-0.229	-0.940	0.967	-0.944	0.982	0.447	-0.938	0.283	-0.457	-0.654	-0.635	-0.368	-0.867	0.600	-0.948	-0.964

The values indicated in red are a positive correlation, and those in blue are a negative correlation. Abbreviation: pH—potential hydrogen; TN—total nitrogen; O.M—organic matter; P—phosphorus; CEC—cation exchange capacity; a—*Acaulospora spinose*; b—*Acaulospora foveata*; c—*Acaulospora* sp.; d—*Archaeospora schenckii*; e—*Archaeospora* sp.; f—*Entrophospora colombiana*; g—*Entrophospora* sp.1; h—*Entrophospora* sp.2; i—*Gigaspora gigantean*; j—*Gigaspora* sp.; l—*Glomus mossae*; m—*Glomus geosporum*; o—*Glomus intraradices*; q—*Glomus* sp.1; r—*Glomus* sp.2; s—*Scutellospora pellucida*; t—*Scutellospora* sp.; u—*Paraglomus* sp.1; v—*Paraglomus* sp.2.

To understand the correlation between the AMF community structure and soil chemical parameters, principal component analysis (PCA) was undertaken (Figure 3). A large number of species occurred in soils with lower CEC, sufficient available P, and tended to prefer neutral soils. The distribution of the species was mostly influenced by the pH, CEC, and P. The abundance of the majority of spores was positively correlated with P and pH and negatively correlated with CEC. This implied that the higher the pH and available P and the lower the CEC, the higher the species abundance and vice versa.



**Figure 3.** Principal component analysis of the species occurrence related to soil chemical properties.

## 4. Discussion

### 4.1. Intensity of Maize Mycorrhization

The Intensities of mycorrhization of maize roots observed in this study were high (about 55%) and show no significant differences from both sites. These results confirm those obtained by Houngnandan et al. (2009) and Tawaraya (2003) who observed a mycorrhization rate between 50% to 70%. This can be explained by the fact that maize roots are less abundant, stocky, and devoid of absorbent hairs, therefore particularly dependent on AMF (Garbaye, 2013). The root system of maize is characterized by the presence of adventitious roots which absorb nutrients only from the superficial soil layer. Thus, AMF helps root development and improves water and nutrients uptake from the soil.

### 4.2. Diversity and Abundance of AMF Spores

Taking into account all the AMF species identified directly in the field samples nineteenth (19) species were divided into seven genera: *Gigaspora*, *Scutellospora*, *Glomus*, *Acaulospora*, *Archaospora*, *Entrophospora*, and *Paraglomus* were observed under maize cultivation in all sites. This specific richness is higher than that obtained for the sorghum crops in Uganda by Sebuliba et al. (2010) and that observed by Fall et al. (2021) in pearl millet in Senegal, as well as by Bossou et al. (2019) in maize crop in Benin. On the other hand, this specific richness is lower than that obtained from the maize field (39 species) by Malembaka et al. (2021) in South Kivu (DR Congo). This difference could be due to the period of collection of soil samples and previous crops which are important parameters to consider in the evaluation of the density of spores in the soil. The seven genera of AMF have been described by Johnson et al. (2013); Mbogne et al. (2015); Pereira et al., (2016), and Crossay et al., (2018). *Acaulospora*, *Entrophospora*, and *Glomus* genera constituted the dominant genera in this area. This finding is in line with the results of Songachan and Kayang (2012) in



India. These authors demonstrated that *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, *Paraglomus*, *Archaeospora*, and *Funnelformis* are the most dominant genera associated with maize crops. The results reported in this study indicate dominance of spores of the *Glomus* genus. *Glomus* appeared to be the most dominant in croplands (Fall et al., 2021) and they are the principal maize colonizers (Borriello et al., 2012). The predominance of species of the genus *Glomus* in the study area suggests a better adaptation of this genus to the environmental conditions and a wide range of ecological niches (Blaszkowski et al., 2011). In addition, Hijri et al. (2006) and Alguacil et al. (2008) that maize monoculture also reduces AMF diversity. Maize monoculture is practiced in the fields where the soils are sampled. The result revealed that the area is rich in spores of *Archaeospora* and *Paraglomus* genera. This result is in opposition to those of Sasvári et al. (2011), who observed that *Archaeospora* and *Paraglomus* genera are not colonizers of maize grown in monoculture. The results about the diversity index indicate that the AMF community is very diverse regardless of the site. In this study, the high diversity of AMF species found associated with maize might mean that the mycorrhizal fungi host specificity is low in maize crops. Host specificity of AMF is a longtime debate among researchers. Many authors argued that AMF has no host-plant specificity (Sanders, 2002), but several studies demonstrated the preference of some AMF genera to some plant species (Jacquemyn et al., 2011). Thus, the maize crop has a relatively high mycorrhizal dependency (Hao et al., 2021). This mycorrhizal dependency is characterized by the type of crop, the soil properties, and the effect of the cropping system (Chiffot et al., 2009). The diversity index varying between 1.81 and 1.84 in the area might have resulted from the variability in soils properties and management practices observed in the study area where most of the fields are managed in a conventional setting with medium nutrients inputs. This is in line with the findings of Vieira et al. (2018) who observed that a high diversity of AMF species result from management practices that affect the nutrient status in the soil.

The number of AMF morphotypes identified was high which means that eastern Uganda has a high diversity of AMF species. Some AMF species could not be identified in the field samples; the morphological features of the spores were not distinct enough and, thus, these spores could only be grouped into a species group (for example *Aucolospora* sp.). Using bimolecular tools, this area could reveal more AMF diversity. Therefore, to ensure the species composition, molecular methods of analysis should be used to confirm the identification of these species.

The results from this study reveal that spore density varies between 3.7 to 5.1 spores per g of soil for all sites. This density is significantly lower than that obtained by Bossou et al. (2019) (about 1340 spores per g of soil) under maize in Benin but higher than that observed in DR Congo (0.7 to 1.5 spores per g of soil) by Malembaka et al. (2021). This difference could be due to the period of collection of soil samples and previous crops which are important parameters to consider in the evaluation of the density of spores in the soil. According to Bohrer et al. (2003) and Rosendahl, (2008), the number of spores is higher in the soil after long-term stress conditions. The number of undiscovered morphotypes maybe even high affirming that many species are yet to be discovered. Spores abundance was moderate in the field soils. Similar results of moderate abundance have been reported in the Willamette Valley of western Oregon (USA) in a field evaluation of AMF fungal diversity by Cheeke et al. 2013 by recording 15.42 and 16.05 spores g<sup>-1</sup>. Low spore abundance has been discovered in South Kivu (0.6 to 1.5 spores g<sup>-1</sup>) (Malembaka et al., 2021).

#### 4.3. Relationship between Soil Chemical Properties and Species Diversity

In this study, differences in the composition of AMF communities associated with maize were recorded and characterized. The difference in the composition of AMF diversity indicates the soil properties were the main factors of AMF communities associated with maize. Indeed, previous studies have demonstrated the influence of soil properties on the composition of AMF communities (Jansa et al., 2014; Xiang et al., 2016). The results

from this study show the existence of a strong relationship between some chemical parameters and the total species diversity and density in the study area. These results are consistent with those of Bossou et al. (2019). There is a positive and negative correlation between available P, pH, and CEC and spore density and diversity depending on the genus. The low number of morphotypes recovered in Serere compared to Amuria could be explained by the chemical properties of the Sandy loam soils which were neutral with a high level of available P. According to Zhu et al. (2020), AMF proliferation depends on pH, with a preference to slightly acidic conditions. This is in contradiction with the results of this study which reveals that AMF is more abundant in the neutral than the acidic soil. The genera *Acaulospora* and *Glomus* show a positive correlation with soil pH. This finding was consistent with the observation of Séry et al. (2016). In addition, AMF plays a major role in P mobilization for the host plant. Depending on the pH of the soil, this element is strongly adsorbed by iron, aluminum, or calcium in forms that are not available for plants (Hinsinger, 2001). Phosphorus is a limiting factor for the abundance of AMF spores, whether at very high or very low concentrations (Amijee et al., 1989; Lagrange et al., 2013). Mycorrhizal associations play a significant role in the mineralization of organic phosphorus and mobilize nutrients for the benefit of the host plant (Bi et al., 2019). In addition, the diversity and abundance of AMF species concerning soil chemical properties varied among species as some species are associated with some specific soil conditions. For instance, in this study, AMF species of the genus *Entrophospora* prefer acidic soil, low P content, and high soil CEC while the genus *Paraglomus* are only found in neutral soils with a high level of P content. Many species were found to be a generalist in all the sites, especially the species from *Archaeospora*, *Gigaspora*, *Scutellospora*, and *Glomus* genera which do not dependent on soil chemical properties. This study reveals that maize can associate with various ranges of AMF, thus a mycorrhizal-based fertilizer produced from the associated species could have higher chances of thriving in the region.

## 5. Conclusions

Our study is the first report on Arbuscular Mycorrhizal Fungi (AMF) diversity associated with maize in Uganda. The overall objective of this study was to highlight the diversity of AMF associated with maize in the eastern region of Uganda. Little is currently known about the AMF composition in the roots of other cereal or other plants in semi-arid regions of Uganda. Results showed a higher spore density and the dominance of the *Glomus* genus in the rhizosphere soil of maize. Nineteenth species divided into seven genera have been identified with an abundance of the *Glomus* genus. However, further research is needed to identify and explore more diversity of AMF species associated with this cereal. The molecular identification of the species is recommended for accurate identification. There is a strong relationship between the chemical parameters and the total spore density in the area as well as with the diversity. The soil pH and P contributed to the distribution of AMF species. Some of the species from the genera *Gigaspora*, *Acaulospora*, *Glomus*, *Archaeospora*, *Gigaspora*, and *Scutellospora* were found in all sites. These genera can be recommended for further research aiming at improving the agricultural benefits of indigenous AMF to enhanced root functions and subsequent productivity in farming systems in the region.

**Author Contributions:** A.F.F. contributed to the inception of the paper, research, and writing. G.N. contributed to the inception and reviews of the paper. J.S. contributed to reviews. H.F.-M. contributed to the inception of the work. A.B. contributed to the data analysis. I.B. and P.M.N. contributed to the write-up and review. All authors have read and agreed to the published version of the manuscript.

**Funding:** Regional Academic Exchange for Enhanced Skills in Fragile Ecosystem Management in Africa (REFORM), grant number: 2017–2861.

**Acknowledgments:** The authors are thankful to Keryose, Katende, Jude Ssebuwufu, and J.B. of the college of agriculture sciences and biotechnology department, Makerere University for providing laboratory facilities.

**Conflicts of Interest:** The authors declare that they have no conflict of interest.

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