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Diversity and distribution of arbuscular mycorrhizal fungi in maize (*Zea mays*) cropping fields in South Kivu, Democratic Republic of Congo

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Arbuscular Mycorrhizal Fungi (AMF) occur naturally in agroecosystems and interact symbiotically with crops, facilitating nutrition. This study aimed at assessing the occurrence, abundance and diversity of AMF communities in the maize cropping system and their relation to soils properties in two agroecological zones in South Kivu, eastern DR Congo. Soil samples were collected from eight sites, with 4 sites in highland at 1400 to 2000 m altitude above sea level (in Katana, Kavumu, Mulamba and Mugogo) and four in the lowland at <1000 m (in Luberizi, Bwegera, Luvungi and Kamaniola). Spores were extracted from the field soils, morphologically identified and counted. AMF spores occurrence, abundances, species richness, and diversity were determined. A total of 38 AMF morphotypes distributed in 11 genera were obtained with the majority being from Gigasporaceae, Acaulosporaceae, and Glomeraceae families. This is the first report on the occurrence of these species in the eastern of DR Congo. *Acaulospora excavata*, *Acaulospora bireticulata*, *Densitiscutata erythropha*, *Funneliformis mosseae* and *Scutellospora pellucida* were ubiquitous in all the agroecologies. Spores densities were higher in the highland with the highest recorded in Mulamba. Soil pH and phosphorus content influenced AMF distribution. The many different ubiquitous species indicate adaptation to a wide range of physicochemical environments and could reduce the cost of AMF inoculants production for the region. Maize agroecosystems are rich in AMF diversity and selection of appropriate fungal species from the Gigasporaceae, Acaulosporaceae and Glomeraceae as biofertilizer could contribute in improving crops production.

Key words: Arbuscular mycorrhizal fungi, diversity, occurrence, agroecological zones, maize, South Kivu.

INTRODUCTION

Arbuscular Mycorrhizae Fungi (AMF) are a group of soil fungi from the phylum Glomeromycota, forming symbiotic associations with almost 80% of terrestrial plants (de

Oliveira Júnior et al., 2017; Solaiman et al., 2014). They are considered as the most important group of soil microorganisms and essential for ecosystem functioning

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because they play a fundamental role in soil fertility and biodiversity. AMF are ubiquitous in every ecosystem and through the mycorrhizae, they enhance plants nutrients and water absorption (Quoreshi, 2008; Liu et al., 2015). In low Phosphorus (P) soils, up to 80% of P up taken by crops is absorbed through mycorrhizae association. Other nutrients uptake like N, Zn, and Cu is also facilitated by the mycorrhizae (Smith and Smith, 2011; Solaiman et al., 2014). The AMF have been widely recognized to induce resistance to drought (Augé, 2001), biotic stress and to contribute to soil aggregate stability and soil erosion prevention (Nwaga et al., 2003; Liu et al., 2015; Eulenstein et al., 2016).

Soil fungi span across all agro ecologies and different fungi species can colonize one root at the same time. Their functional diversity, distribution and respective activities in the soil can influence the productivity of agroecosystems (Eulenstein et al., 2016; Ndonga, 2018; Habiyaemye et al., 2018; Sendek et al., 2019) and they can be affected by edaphic and climatic conditions and soil management (Oehl et al., 2003).

Maize is the second most produced crop in the world and in the sub Saharan Africa (SSA) (Santpoort, 2020). It is largely grown in DRC and in South Kivu province, a prone region for its production, where it is produced in all its agroecological zones which have different climatic and edaphic conditions (Badibanga, 2013; MinAgri, 2018). Maize is highly mycorrhizal and can associate with a diversity of AMF. Its production and drought resistance are enhanced by good management of AMF that colonize its roots (Bona et al., 2016) and by inoculation of maize with mycorrhizal biofertilizers (Nwaga et al., 2003; Borriello et al., 2012; Cozzolino et al., 2013; Eulenstein et al., 2016; Sendek et al., 2019).

Application of AMF in agriculture or/and good management of indigenous AMF in fields is beneficial for soil fertility, degraded soils restoration, soil reclamation, crops production and crops resistance to environmental stresses (Solaiman et al., 2014; Crespo, 2015; Teixeira et al., 2017). Therefore, in the agroecological outlook, mycorrhizal symbiosis could be a very interesting options to enhance soil fertility and improve plant growth, but their natural communities should be understood firstly as native species have been regarded as more adapted to soils environments than introduced strains (Tchabi et al., 2010; Alexandra, 2017; Habiyaemye et al., 2018).

AMF communities in the Southern part of DRC were found to have high diversity in soils of different farming systems including *Allium cepa* and *Phaseolus vulgaris* (Alexandra, 2017). However, there are few reports on the AMF associations in the maize cropping fields in South Kivu. Little is also known on the effects of edapho climatic conditions on the community structure of AMF in maize cropping fields. The objectives of this work were therefore to determine the occurrence, diversity and the distribution of AMF communities in rhizosphere of maize crop in two different agro ecological zones namely the highland and

lowland in South Kivu province, eastern DR Congo.

MATERIALS AND METHODS

Study area

This study was conducted in the South Kivu province in the eastern part of DRC, in two agroecological zones considered promising for cereal crops production, especially maize, rice, mil and sorghum (IPAPEL, 2011; Muhindo et al., 2017), namely lowland (<1000 m above sea level, asl) and highland (1400-2000 m asl). The climate in the highland is the high altitude tropical climate that falls within AW3 type of Köppen-Geiger classification (Kottek et al., 2006) type with the average annual rainfall of 1411 mm and average daily temperature of 16.45°C, while in the lowland the climate is semi-arid falling in the type Aw4 with the average rainfall of 978 mm and average daily temperature of 23.95°C (Muhindo et al., 2017; Kottek et al., 2006). In each agroecological zone, 4 sites were considered and they were respectively Luberizi, Bwegera, Luvungi and Kamaniola in the Lowland, and Katana, Kavumu, Mulamba and Mugogo in the Highland (Figure 1). Two main climatic seasons occur in the area; the long rainy season (September to May) and the short dry season (June to August).

Soils sampling and analysis

Soil samples were collected from maize fields during the dry season, at the end of August 2018, when the roots activities are declined and fungal sporulation increased (Brundrett et al., 1996; Jefwa et al., 2006).

Sampling was done using a stratified random sampling plan (Dalpé and Hamel, 2008). In each site, 5 field were selected on a transect separated with around 1 km from each other and presenting apparent homogeneity. In each field, three sub samples were randomly collected in the rhizosphere at the depth of 0 to 20 cm using a soil auger and mixed thereafter to make a composite sample of around 1.5 kg subsamples. In total, 20 samples were collected per AEZ and thus 40 samples for both AEZs. The fields selected were fields where maize was grown or grew the previous season, sole or in association with other crops but in this last case, samples were collected right beside the maize roots. Samples were air dried for analysis. Soil pH was determined at a ratio of 1:2.5 in water using a pH meter (Metrohm 632pH-meter). The available P was extracted following the Bray1 P method as described in Jones Jr (2001). Organic carbon was determined following the method of Nelson and Sommers (1975). The CEC was determined following the neutral ammonium acetate (1 N NH₄C₂H₃O₂, pH 7.0) extraction method and the soil texture was determined using the hydrometer or Boyoucoucous method (Okalebo et al., 2002).

Spores extraction and morphotypes identification

Dried soil samples were taken to the Mycology laboratory, Botany Department, East Africa Herbarium in Nairobi for spores extraction and species identification. The wet sieving and sucrose gradient methods were used to extract spores from 50 g from each soil sample (Brundrett et al., 1996; Ingleby, 2007). Three to four healthy spores, observed in a dissecting microscope, were mounted on microscope slides for description of the morphotypes, mounted with Polyvinyl Alcohol-Lacto-Glycerol (PVLG) and stained with Melzer's reagent (Brundrett et al., 1996). Spores were gently cracked open to allow detection of spore substructure features that were observed under a compound microscope at a magnification of 100, 400 and 1000X. Morphotypes identification was based on

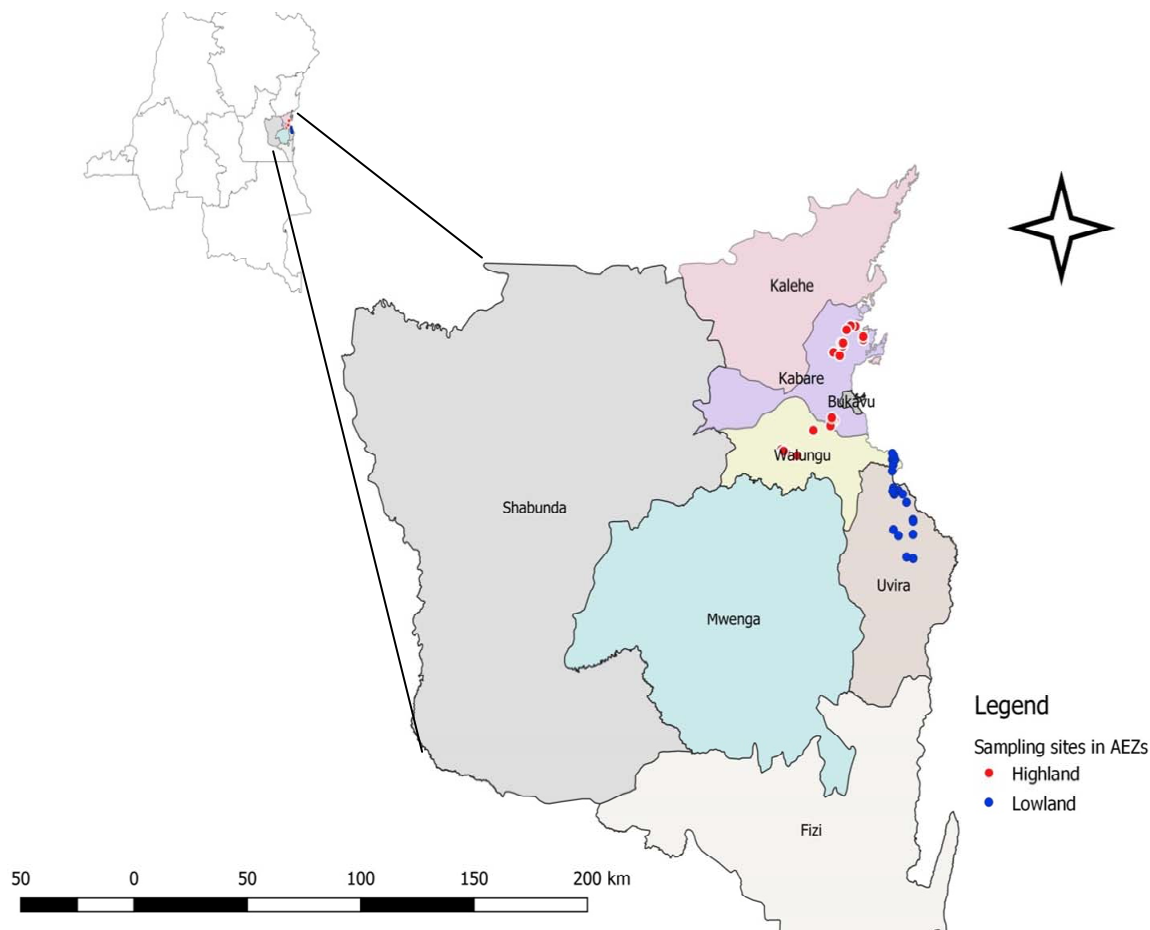


Figure 1. Study sites in South Kivu.

descriptions and identification criteria presented in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi (INVAM) collection websites (<http://invam.wvu.edu/> and <http://www.zor.zut.edu.pl/>) (INVAM, 2019) and on the descriptions in the literature (Oehl et al., 2011; Walker et al., 2018). Species classification was done according to Redecker et al. (2013).

Spore density was expressed as the number of AMF spores per gram of soil (Sasvári et al., 2012). The total number of spores per sample was determined by counting all the spores recovered in each sample. The species richness was expressed as the number of species recovered in each site and the occurrence frequency was the number of samples in which a given species was isolated over the total number of samples \times 100%.

Statistical analysis

The diversity was derived from the Shannon-Weiner diversity index. Shannon index is a measure of community diversity which takes into account both species richness and evenness (Weaver and Shannon, 1963) and was computed according to the formula: $Sh (H') = P(X_i/X_0) \log(X_i/X_0)$, where X_i = the spore abundance for an individual species and X_0 = the total spore abundance of the population of all the glomale species. The analysis of variance was done to compare spores densities and the Fisher's least significant difference (LSD) used to separate the means at 0.05 p-value.

Hierarchical cluster Analysis (HCA) based on Ward's minimum variance and Euclidian distance was applied to determine the relationship between field sites based on soils properties. Species composition in relation to environmental variables and elevations was analyzed by the Principal Component Analysis (PCA) using SPSS software.

RESULTS

Soils chemical and physical characterization

The pH varied between 4.6 and 7.44. The lowland soils were neutral while the highland and lowland were neutral to acidic in general. The P content differed also in content with significant variation between sites as the levels varied from low ($<17 \text{ mg kg}^{-1}$), medium ($17 - 34 \text{ mg kg}^{-1}$) to high ($>34 \text{ mg kg}^{-1}$) P contents. The Organic C is mostly moderate (1-3%) to high ($>3\%$) and is spread almost uniformly in all the AEZs. The CEC vary from low ($<12 \text{ cmol kg}^{-1}$) to moderate ($12 - 25 \text{ cmol kg}^{-1}$) with the lowest values present in the sandy soils in Luvungi and Bwegera and in Burhale and Luhihi (Table 1). These classification were done according to Okalebo et al. (2002), Jones Jr

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

Site	pH		P Bray 1 (mg/kg)		Organic C(%)		CEC		Texture*
	Average	Range	Average	Range	Average	Range	Average	Range	
Katana	5.89	5.0-7.46	41.85	7.92-76.3	3.11	2.7-3.8	13.32	9.1-18.6	Clay, Clay loam, Silt clay
Kavumu	6.28	6.09-6.50	68.19	40.4-72.1	4.25	2.71-6	14.22	9.8-18	Sandy clay loam-Silty clay
Mugogo	5.45	5.24-5.64	46.68	6.27-30.7	2.98	1.82-5.97	10.78	7.4-21	Clay, Clay loam, Silt clay
Mulamba	5.35	4.6-6	15.3	3.7-21.2	2.88	2.15-4.37	7.84	4.2-10.6	Clay, clay loam
Bwegera	6.63	6.2-7.44	23.34	11.5-55	2.69	1.45-6.51	7.36	1.5-14.4	Clay, Sandy loam, Loam
Kamaniola	6.92	6.59-7.33	81.6	45.1-108.7	3.58	2.05-5.5	25.04	19.8-33	Clay, Sandy clay loam
Luberizi	6.72	6.19-7.12	20.6	11.7-34.1	3.42	2.5-5.1	13.6	11-16.0	Clay, Sandy clay, Sandy loam
Luvungi	6.53	7.41-7.13	40.05	27.1-59.6	2.34	1.21-3.55	6.58	4.1-13	Sandy clay, Sandy loam

*The textural classes were determined using the average values of sand, silt and clay percentages in each site (USDA Classification).

(2001) and Hazelton and Murphy (2016).

Species composition

In total, 38 AMF morphotypes were recovered, belonging to 11 different genera. Twenty eight species could be unequivocally identified. Five morphotypes were not distinguishable but had similarities with some species and they were thus affiliated to the species that they have more overlapping morphological features with (e.g. *Acaulospora excavata* aff.). Five other morphotypes were not described up to the species level since they lacked enough distinct features, could not be named at the species level (e.g. *Glomus* and *Scutellospora* species). One species was not identified; it seemed to have not been described yet and might presumably be found for the first time (species u, Figure 3). The majority of species obtained belonged to Gigasporaceae (17 morphotypes) with 6 species in the Gigaspora, 5 morphotypes in the Scutellospora, 4 species in the Dentiscutata and 2

morphotypes on the *Racocetra* genera. The Acaulosporaceae (13 morphotypes) came in the second position with all the morphotypes in the genus *Acaulospora* followed by the Glomeraceae (5 morphotypes) with 2 morphotypes of *Glomus*, 2 species of Funneliformis and 1 species of Rhizophagus. The Clariodeoglomeraceae, Diversisporaceae and Pacisporaceae appeared with one species each in the *Clariodeoglomus*, *Globifera* and *Pacispora* genera, respectively. The Figure 2 presents some AMF spores observed in the study area.

Abundance and occurrence in the field soils

The occurrence frequency and abundance of spores in each site as observed in field soils are presented in Table 2. Some species of AMF were generalists, since they were found to be ubiquitous in all the AEZs and they were: *A. excavata*, *Acaulospora rhemii*, *Acaulospora* species, *Dentiscutata erythropha*, *Funneliformis coronatum*, *Funneliformis mosseae*, *Gigaspora*

gigantea, *Glomus* species, *Scutellospora pellucida*, *Scutellospora castanea* and *Scutellospora* species. The other species with a high occurrence percentage were *Acaulospora bireticulata*, *F. mossae*, *Gigaspora margarita*, *Gigaspora rosea*, *Glomus* spp., *Racocetra castanea* and *Racocetra* species, with the *S. pellucida* occurring in at least 15% of the field sampled. Species densities, richness and occurrence frequency

Table 2 presents the AMF morphotypes occurrence, specific spore densities as observed in the field soils.

Species Shannon diversity index

The Shannon-Weiner diversity index (H'), species richness in each site and spore densities as observed in field soils are presented in the Table 3. Based on the assumptions made for the species diversity and the need to emphasize on species richness, the H' diversity index was used. Species diversity differed within AEZs with higher diversity obtained in the highland with soils from

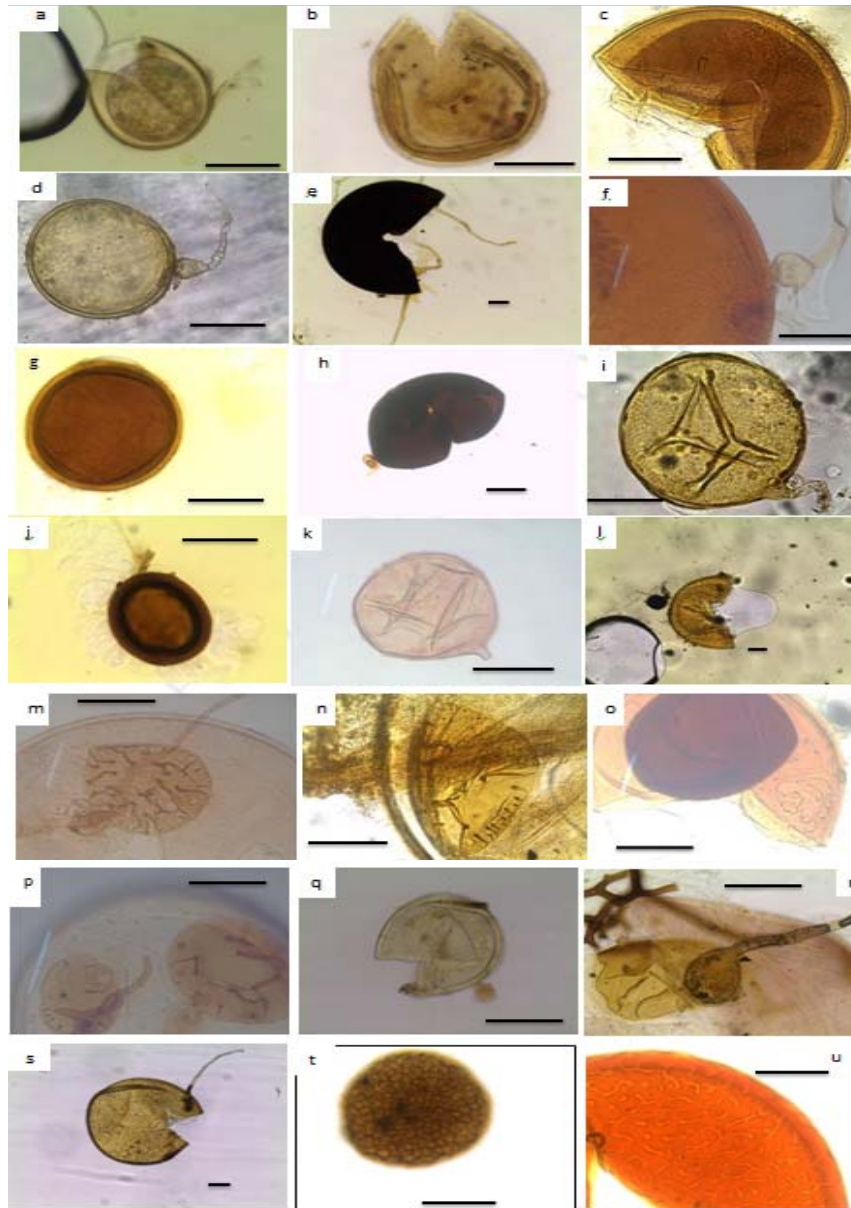


Figure 2. AMF Spores isolated from rhizosphere of maize croplands in South Kivu. a) *Acaulospora spinosissima*, b) *Acaulospora reducta*, c) *Acaulospora spinosa*, d) *Gigaspora margarita*, e) *Gigaspora albida*, f) *Gigaspora gigantea*, g) *Glomus ambisporum*, h) *Dentiscutata nigra*, i) *Funneliformis mossae*, j) *Glomus ambisporum*, k) *Funneliformis mossae*, l) *Pacispora robiginia*, m) *S. castanea*, n) *D. erythropha*, o) *Scutellospora calospora*. Each scale bar represents 100 μ m

Mugogo (H' of 2.58); Katana (H' of 2.44) and Mulamba (H' of 2.26) recording the highest diversity index.

Spore densities

In the soils, spores densities varied significantly with the highland recording higher values than the lowland in general. The highest value was observed in Mulamba,

with 1.5 spores g^{-1} ($p < 0.05$) and the rest of sites showing no statistical significant difference (Figure 3).

Sampling effort and morphotypes recovery

It was observed that in overall that the more samples collected, the more new morphotypes were recovered in all the AEZs and the curves were still rising. In the

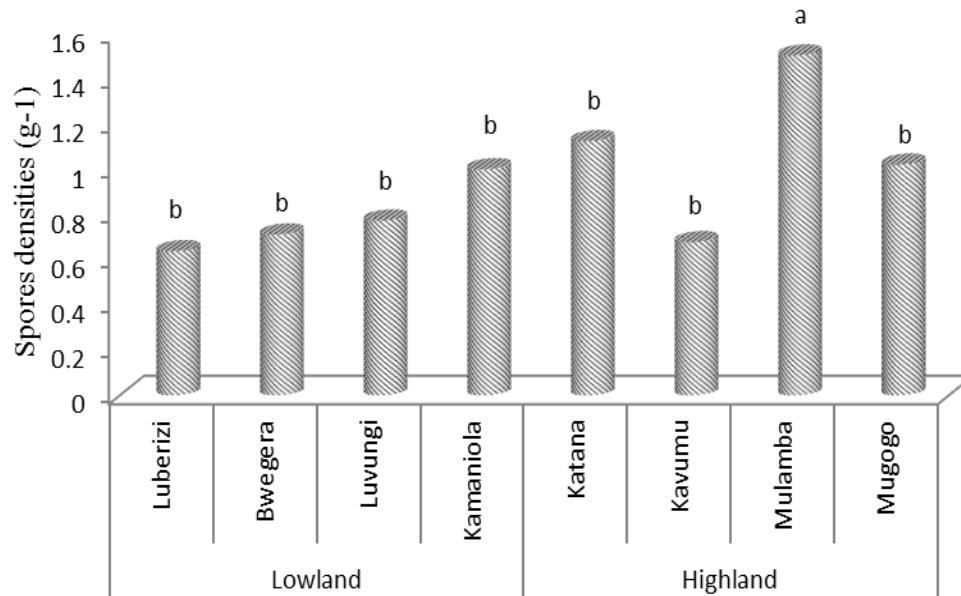


Figure 2. Spore densities in the field soils.

Lowland out of 20 samples, 24 morphotypes were recovered while in the highland out of 20 samples 28 morphotypes were recovered (Figure 4).

Similarities between fields

The different fields were clustered based on the chemical properties and the dendrogram (Figure 5) presents the hierarchical cluster obtained. Based on their chemical properties, two major groups of fields were observed but in general in each AEZ the fields have very diverse properties and fields from all the AEZs were almost spread homogeneously. In the first major group 12 fields from lowland were grouped with 13 fields from highland and in the second group of fields from lowland had similarities with 6 fields from highland. Similarity of field sites from different agroecologies suggested that in all the AEZs the properties were variables in the maize cropping systems.

Soil properties influence on species composition, and relationship between fields

A large number of species occurred in soils with lower organic carbon, lower CEC, lower available P specifically, and tended to prefer acidic soils (Figure 6). The distribution of the most dominant families, namely the Gigasporaceae, Acaulosporaceae and Glomeraceae was mostly influenced by the pH and P. The occurrence of the majority of spores was negatively correlated with P and pH. This implied that the higher the pH and available P,

the lower the species occurrence and vice versa.

DISCUSSION

Soils chemical and physical characterization

Soils were found to be acidic to neutral in the study area. The acidity in Mulamba and Mugogo soils results from the intense weathering and lixiviation due to high precipitations occurring in the region, with mostly hilly lands, where alteration and nutrients removal processes have led to nutrients losses in the soils as confirmed by Crawford et al. (2008). In general in South Kivu, the most dominant soils in croplands are acidic soils (Heri-Kazi, 2011; Muhindo et al., 2017; Bagula et al., 2014; Kulimushi et al., 2018), especially Ferralsols, Nitisols and Ultisols (Ngongo et al., 2009; Bashagaluke, 2014).

In the SSA, most of agricultural soils are acidic and have been found to be mostly impoverished in P content and organic matter (Nziguheba et al., 2016).

The level of P in the soils varied from low to high and this could be linked to the high fixation, low content in the parental materials and low organic or mineral P input in agroecosystems. The low organic residue incorporation, continuous tillage and conventional agriculture account for the decline in the chemical status of these soils (Ngongo et al., 2009; Lambert et al., 1979).

In the lowland the soil texture varied from clay to Sandy loam while in the highland it varied between clay and silt clay. The intensive weathering might also have been the cause of the fine textures in the hilly highland fields while in the lowland, in addition to a moderate weathering, the

Table 2. Species occurrence frequencies and spores abundance.

Species	Lowland				Highland				Occurrence frequency (%)
	Luberizi	Bwegera	Luvungi	Kamaniola	Katana	Kavumu	Mulamba	Mugogo	
<i>A. bireticulata</i>	-	-	-	-	-	10	-	22	5
<i>A. brasiliensis aff.</i>	-	-	-	-	9	-	-	-	3
<i>A. capsicula</i>	28	-	10	-	-	-	-	-	5
<i>A. excavata</i>	-	-	24	-	-	-	-	-	3
<i>A. excavata aff.</i>	-	-	20	-	8	-	-	-	5
<i>A. foveata</i>	-	13	-	-	-	-	-	-	3
<i>A. reducta</i>	-	-	-	2 (50)	-	-	10	-	8
<i>A. rhemii</i>	-	-	-	27	12	-	-	15	8
<i>A. scrobiculata</i>	-	-	-	-	18	-	-	-	3
<i>A. spinosa</i>	-	-	-	-	-	-	-	20	3
<i>A. spinosissima</i>	-	-	-	20	-	-	-	-	3
<i>A. tuberculata</i>	-	-	-	5	-	-	-	-	3
<i>Acaulospora spp.</i>	-	5	-	-	8	3	23	14	13
<i>Clariodeoglomus spp.</i>	-	-	-	2 (20)	-	-	-	-	5
<i>D. heterogama</i>	15	-	7	-	-	-	-	-	5
<i>D. nigra</i>	-	-	-	-	4	-	2 (30)	10	10
<i>D. reticulata</i>	-	-	-	-	-	-	-	4	3
<i>D. erythropha</i>	-	-	-	14	22	-	2 (99)	16	13
<i>D. globifera</i>	-	-	-	-	-	-	-	13	3
<i>F. coronatum</i>	-	-	-	5	-	-	13	-	5
<i>F. mossae</i>	-	-	-	20	-	2 (30)	-	4	10
<i>G. albida</i>	-	-	-	-	-	-	8	-	3
<i>G. gigantea</i>	-	32	-	-	20	-	-	-	5
<i>G. margarita</i>	-	-	-	-	2 (22)	-	21	-	8
<i>G. rosea</i>	-	-	-	-	-	-	13	-	3
<i>Gigaspora spp.</i>	-	-	-	-	-	6	-	10	5
<i>G. decipiens</i>	-	10	-	-	-	-	-	-	3
<i>G. ambisporum</i>	-	-	-	-	-	1	-	-	3
<i>Glomus spp.</i>	-	-	-	5	-	-	2 (16)	18	10
<i>P. robiginia</i>	-	-	-	-	22	-	-	-	3
<i>Racocetra sp. aff.</i>	-	-	-	-	-	-	18	8	5
<i>R. intraradices</i>	-	-	-	-	-	-	2 (42)	-	5
<i>S. calospora aff.</i>	-	-	-	-	12	-	-	-	3
<i>S. pellucida</i>	10	2 (23)	5	-	30	-	-	15	15
<i>S. Castanea</i>	9	16	-	-	29	-	15	-	10

Table 3. Contd.

<i>S. scutata</i>	-	-	-	-	-	50	-	-	3
<i>Scutellospora</i> spp.	-	-	-	2 (31)	-	-	15	10	10
Unidentified species	-	-	-	-	-	-	-	3	3
Total number spores	62	99	66	197	216	100	323	182	-

*The number 2 before the brackets means the specific species was recovered twice in two fields in that specific site. In the brackets is the number of spores counted of the specific species.

Table 3. Shannon H' index and species richness.

AEZ	Site	Shannon H'	Species richness
Lowland	Luberizi	1.276	4
	Bwegera	1.647	6
	Luvungi	1.449	5
	Kamaniola	1.803	10
Highland	Katana	2.44	13
	Kavumu	1.258	6
	Mulamba	2.266	13
	Mugogo	2.588	15

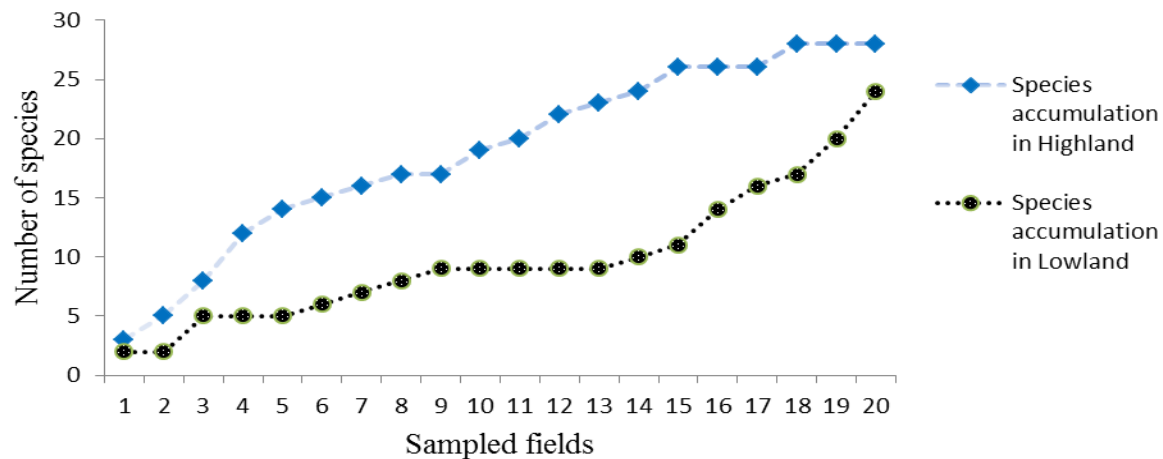


Figure 3. Sampling effort and morphotypes recovery.

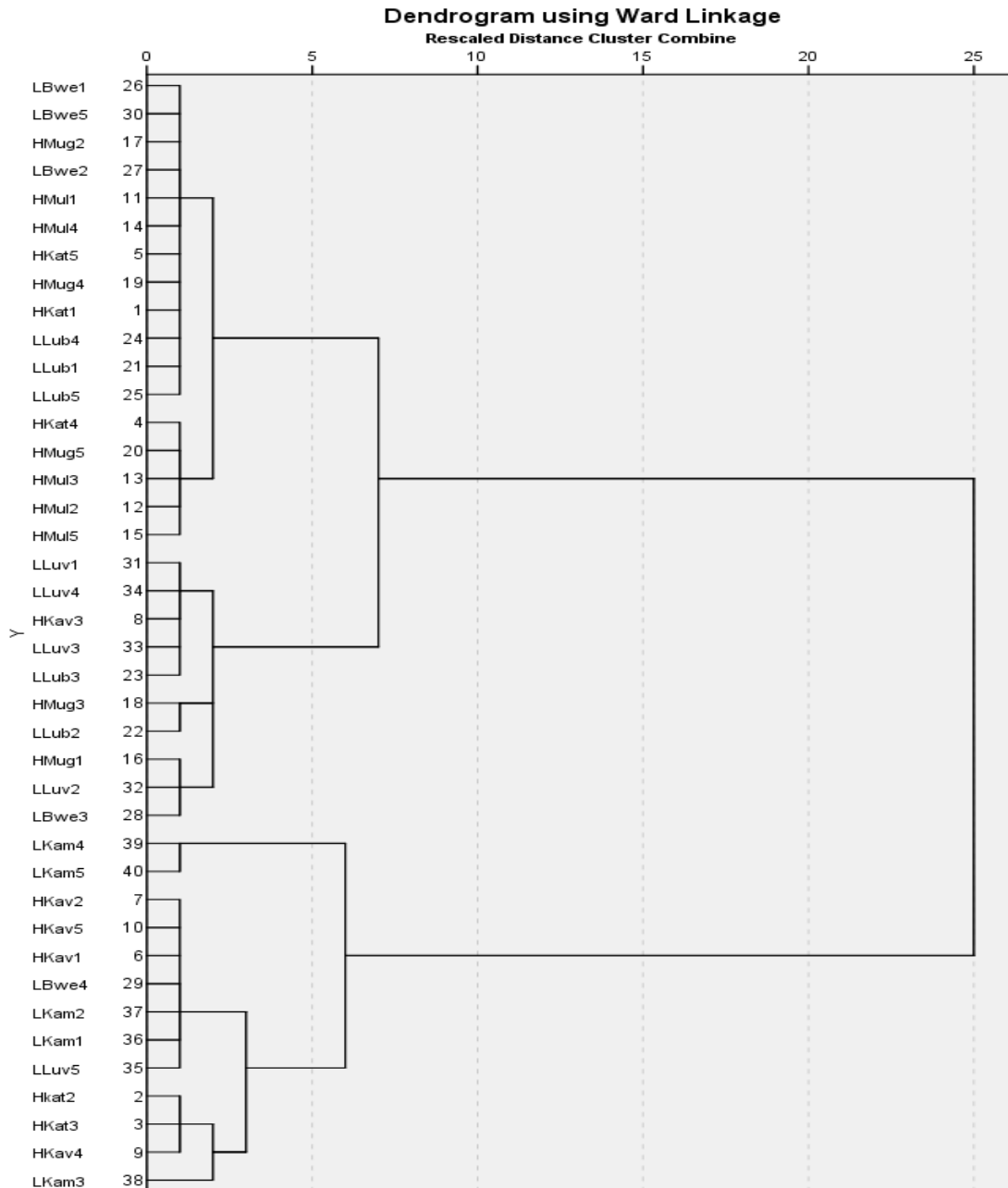


Figure 4. Dendrogram of the Hierarchical Cluster analysis of fields based on chemical properties. H=Highland, L= Lowland, Kat=Katana, Kav=Kavumu, Mul=Mulamba, Mug=Mugogo, Lub=Luberizi, Bwe=Bwegeera, Luv=Luvungi, Kam=Kamaniola. 1 to 5 are the field numbers in each site.

nature of the bedrock may explain the various textures observed.

Species composition

The number of AMF morphotypes identified was high and means that South Kivu harbors a high diversity of AMF species. Most of these AMF have been described

elsewhere (Oehl et al., 2006; Krüger et al., 2011; Oehl et al., 2014; Pereira et al., 2016; Crossay et al., 2018). AM fungal diversity has never been extensively studied in the region and in the world the number of described species is continuously increasing (Redecker et al., 2013).

Gigasporaceae, Acaulosporaceae and Glomeraceae constituted the dominant families in this area. The dominance of species from 2 to 3 genera in the AMF communities was found to be the trend in Brazilian

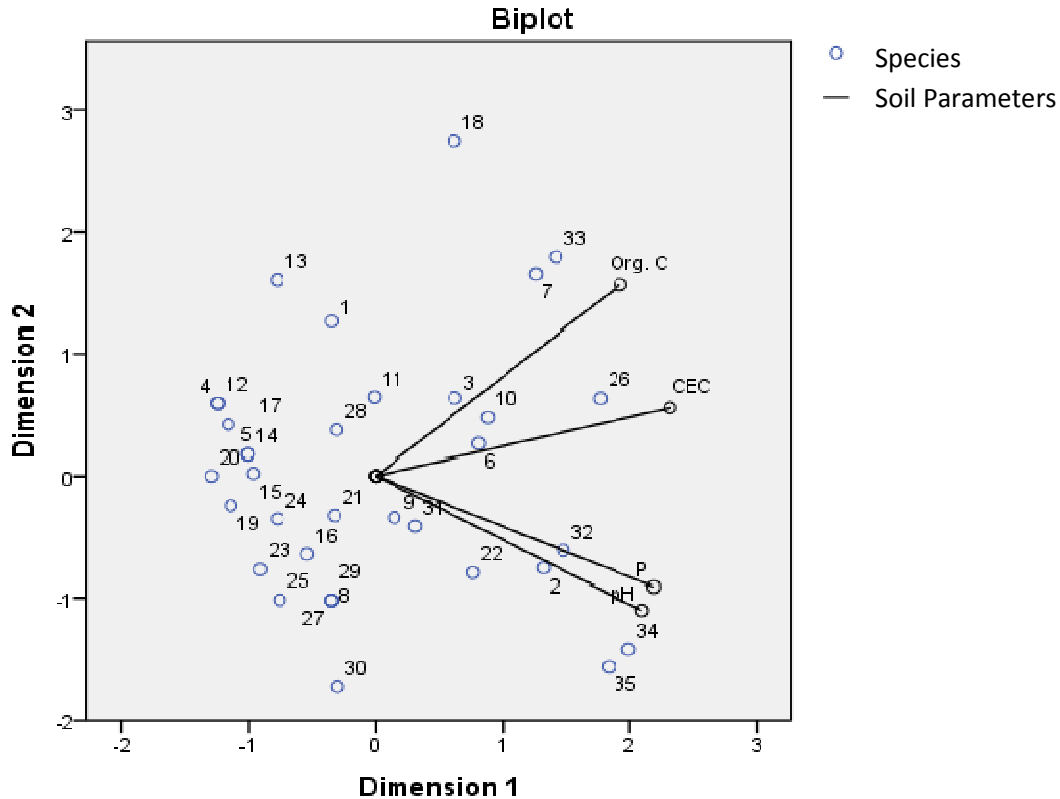


Figure 5. Principal component analysis of the species occurrence related to soil chemical properties. 1. *A. bireticulata*, 2. *A. brasiliensis* aff., 3. *A. capsicula*, 4. *A. excavata*, 5. *A. excavata* aff., 6. *A. foveata*, 7. *A. reducta*, 8. *A. rhemii*, 9. *A. scrobiculata*, 10. *A. spinosa*, 11. *A. spinosissima*, 12. *A. tuberculata*, 13. *Acaulospora* spp., 14. *Clariodeoglomus* spp., 15. *D. heterogama*, 16. *D. nigra*, 17. *D. reticulata*, 18. *D. erythropha*, 19. *D. globifera*, 20. *F. coronatum*, 21. *F. mossae*, 22. *G. albida*, 23. *G. gigantea*, 24. *G. margarita*, 25. *G. rosea*, 26. *Gigaspora* sp., 27. *G. decipiens*, 28. *G. ambisporum*, 29. *Glomus* spp., 30. *P. robiginia*, 31. *Racocetra* spp. aff., 32. *R. intraradices*, 33. *S. calospora* aff. 34. *S. pellucida*, 35. *S. castanea*, 36. *S. scutata*, 37. *Scutellospora* spp., 38. Unidentified species.

ecosystems (Teixeira et al., 2017; Pereira et al., 2016), which might be similar to the ecosystems in DRC.

In the Katanga province in DRC, 65 different spore morphotypes were recovered from the field soil (Alexandra, 2017). Likewise, Stürmer and Siquiera (2011) retrieved 43 species from Amazonian ecosystem in western Brazil. With currently only 338 AMF species described worldwide (<http://www.amf-phylogeny.com/>, as of January 2021), the AMF morphotypes described in this work represented 11% of all species; showing that this area is a hotspot of AMF diversity. For an ensured species composition, molecular methods of analysis should be used to confirm the identification of these species.

Diversity and occurrence

AMF description, occurrence and diversity can be accurately based on spores morphology since spores

serve as a mean of preservation of fungal strains in prolonged drought conditions (Rosendahl, 2008). This important number of diverse of AMF species observed associated with maize might mean that the mycorrhizal fungi's specificity is low in the cereals. The diversity index varying between 1.27 and 2.58 in the area might have resulted from the variability in vegetation types, soils properties and management practices observed in the study area where most of the fields are managed in a smallholding setting with subsistence agriculture and low nutrients inputs.

The high diversity of AMF species could result from the heterogeneity among the habitats evaluated, management practices and the cropping system (Oehl et al., 2003; Borriello et al., 2012; Belay et al., 2013; Gomes et al., 2015; Vieira et al., 2018). This finding agrees with Alexandra et al. (2017) who observed that the community diversity and colonisation ability were influenced by local management practices that affect the nutrient status in the soil, and found that spores abundance and root

colonisation were determined by the site factor rather than the crop or the mineral P fertilization. The dominance of *Gigaspora*, *Acaulospora*, *Scutellospora* and *Glomus* genera is fairly consistent with the findings of Gomes et al. (2015) who found the genera *Glomus*, *Dentiscutata*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Funneliformis* to be the most dominant in the rhizosphere of maize crops.

These morphotypes have been shown resilient to climatic and edaphic conditions (Teixeira et al., 2017; Alexandra, 2017; Belay et al., 2013). *Glomus* did not appear to be the most dominant in field soils probably because the classification has significantly changed and many species formerly classified in the *Glomus* genus, have been moved to other genera (Redecker et al., 2013); or because a large number of *Glomus* spp. are sensitive to low pH and Aluminium toxicity, but still they are the principal maize colonizers (Borriello et al., 2012; Sasvári et al., 2011). The fungistatic effect of acidic soils has been reported to hinder *F. mossae* from forming mycorrhizae with maize (Siqueira et al., 1984).

In an overall perspective, the more samples collected the more new morphotypes were recovered and this could be explained by the difference in the edapho-climatic conditions of the area studied. The low number of morphotypes recovered in the lowland comparing to the highland could be explained by the chemical properties of the soils from the lowland which were mostly neutral with higher level of available P.

The number of undiscovered morphotypes may be even high seeing this trend and the global trend where in 2003 only 150 were described (Oehl et al., 2003) while in 2019, they were more than 315 morphotypes recognized as AMF species (http://www.amf-phylogeny.com/amphylo_species.html) and the number keep on increasing with Oehl et al. (2011) affirming that many species are yet to be discovered.

Spore densities

Spores densities were very low in the field soils. Similar results of low densities have been reported in India in a survey of AMF fungal diversity by Lakshman et al. (2001) by recording 0.49 to 0.67 spores g^{-1} , but also in Senegal in a survey of AMF in cropping systems by Ingleby et al. (1997) by recording 0.081 to 0.51 spores g^{-1} . AMF proliferation depends on pH, with a preference to slightly acidic conditions, spatial and temporal variation as different AMF have different growth length and germination sparkling conditions, age of the host plants, soils disturbance, and differential sporulation ability of AMF taxa and fungal species (Jansa et al., 2014; Walker et al., 2018).

These specifications related to climate, soils conditions and intrinsic species characteristics could explain the variation in AMF spores densities among the studied

sites. The sporal formation in mycorrhizal fungi is very variable and is driven by many factors such as species specific nature (as some species have a periodic sporulation, others cannot form spores at all), host plant, temperature and soils conditions among others (Rosendahl, 2008; Smith and Smith, 2011; Gomes et al., 2015).

Soil properties influence on species composition, and relationship between fields

This study's findings agreed with this conclusion of others researchers who came to a common agreement that soil acidity is one of the most important factor defining microbial communities by acting as an environmental filter (Belay et al., 2013; Alexandra, 2017; Teixeira et al., 2017). AMF occurrence showed a negative correlation with soil pH. *Gigaspora*, *Acaulospora*, *Scutellospora*, *Dentiscutata* and *Glomus* were the most frequent AMF genera and this finding was consistent with the observation of Songachan and Kayang (2012) who found *Acaulospora* and *Glomus* were the most frequently encountered in croplands in India, and Séry et al. (2016) who found *Acaulospora* to be the most frequent in acidic to neutral soils in Ivory Coast. The distribution and densities of AMF species in relation to soil chemical properties varied among species as some species were only associated to some specific soil conditions. It has been recognized that acidic to neutral soils are harbor a good number of AMF species, like the *Acaulospora* genus which was found to be associated in acidic soils of pH below 6.5 in Brazil (Gomes et al., 2015; Teixeira et al., 2017). As obligate symbionts, AMF have to associate to roots in order to survive and in neutral soils, the plants prefer not to associate with AMF but to absorb nutrients directly from the solution, and this is why naturally the mycorrhizal fungi thrive mostly in acidic conditions (Smith and Smith, 2011; Solaiman et al., 2014). The generalist species might have a less specificity level and may be able to symbiotically associate with other cereal crops found in the region but also they may have plant protective effect against soils contaminated with pesticides, heavy metals, etc., and soils acidity (Teixeira et al., 2017).

Other significant changes in AMF communities composition were found to be driven by the changes in the soil available P, CEC and SOM; with the distribution of most of the species negatively correlated with these parameters. Fields were found to be evenly clustered and many species were found to be ubiquitous in all the AEZs, especially the species from *Gigaspora*, *Glomus* and *Acaulospora*. This could be explained by the fact that maize was found to be grown in the lowland and highland in the region, grown sole or associated with beans, cassava, sweet potato, sorghum, etc. Other studies have reported that the geographical locations, land use and

management are critical factors influencing the distribution of AMF taxa in the agroecosystems (Oehl et al., 2003; Nandjui et al., 2013; Jansa et al., 2014; Habiyaemye et al., 2018). Within the limits of this study area and target crop, there was a high occurrence frequency of some species, indicating that they have a wide geographic range. This confirmed the results obtained by Oehl et al. (2003) and Borriello et al. (2012) and this meant that maize can associate with a broad range of mycorrhizal fungi and a mycorrhizal inoculum produced from species from *Gigaspora*, *Glomus* and *Acaulospora* could have higher chances of thriving in the region.

Conclusion

The findings of this study revealed that up to 38 AMF morphotypes were recovered in maize cropping fields in South Kivu, meaning that there is a high diversity in AMF in the maize agroecosystems in the study area. The molecular identification of the species is recommended for their accurate identification. The soil pH and P contributed to the distribution of AMF species. Some of the species from the families Gigasporaceae, Acaulosporaceae and Glomeraceae were ubiquitous. These families are recommended for further research aiming at improving the management of indigenous glomale fungi and their agricultural benefits, but also a selection of an appropriate inoculum to improve root functions and subsequent productivity in farming systems in the region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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