CHARACTERIZATION AND SCREENING OF NATIVE ARBUSCULAR MYCORRHIZAL FUNGI ISOLATES FROM MAIZE (*Zea mays* L.) AGRO ECOSYSTEMS IN SOUTH KIVU, DEMOCRATIC REPUBLIC OF CONGO

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University.

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I dedicate this work to my parents Sébastien Malembaka and Françoise Mwamini and my family for their care and encouragement during the pursuit of this degree.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

Absl	Above sea level
AEZ	Agro Ecological Zone
Al	Aluminium
AMF	Arbuscular Mycorrhizal Fungi
ATP	Adenosine Triphosphate
Ca	Calcium
CAN	Calcium Ammonium Nitrate
CEC	Cation Exchange Capacity
CV	Coefficient of Variation
DAP	DiAmmonium Phosphate
DRC	Democratic Republic of Congo
ECM	Ecto Mycorrhizae
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics Database
Fe	Iron
GPS	Global Positioning System
H	Hydrogen
HCA	Hierarchical Cluster Analysis
IITA	International Institute of Tropical Agriculture
K	Potassium
LAI	Leaf Area Index
LSD	Least Significant Difference
Mn	Manganese
N	Nitrogen
P	Phosphorus
PCA	Principal Component Analysis
pH	Hydrogen Potential
PVLG	PolyVignyl Lacto Glycerol
rpm	Revolutions per minute
SOM	Soil Organic Matter
SSA	Sub Saharan Africa
TSP	Triple Super Phosphate
USAID	United State Agency for International Development
WAT	Weeks After Transplantation
Zn	Zinc

ABSTRACT

Maize (*Zea mays*) is a staple food playing a crucial role in food security and income generation for many farmers but its productions is constrained by low soils fertility and poor crops husbandry in DRC. Phosphorus (P) is a critical macronutrient required for maize production and yet most deficient in the agricultural soils of South Kivu which are inherently low in P content coupled by high fixation capacity. Maize is a highly mycorrhizal plant but little is known about its mycorrhization in its cropping system in different agroecologies in South Kivu. The objectives of this study were to characterize the occurrence and diversity of native AMF and natural mycorrhizal infectivity potential of soils from maize cropping systems of South Kivu, DR Congo, and to screen and evaluate the influence of AMF isolates on maize growth, P and Zn nutrients uptake, and root colonization in dominant acidic soils of South Kivu namely Ferralsol and Nitisol, under controlled conditions. Soil samples were collected from maize rhizosphere in 12 villages spread across four territories representative of the 3 AEZs in South Kivu. A trap culture was initiated and AMF spores were extracted from field and trap culture soils and species identified using the morphotypes criteria. AMF diversity, frequency of occurrence and species richness were examined. Roots colonization was assessed after bio infectivity assay for soils mycorrhizal potential determination. Soil monospecies inoculums were produced and after screening, five native AMF strains named AMF1 (*Gigaspora gigantea*). AMF2 (*Gigaspora sp.*), AMF3 (*Gigaspora margarita*), AMF4 (*Rhizophagus intraradices*) and AMF5 (*Acaulospora. reducta*) were selected as probable good AMF biofertilizers. A greenhouse experiment was conducted using single specie inoculation and these inoculums, applied in a Ferralsol and a Nitisol, were compared to the inorganic P application, the commercial biofertilizer Rhizatech and the control. Plant height, chlorophyll content, shoot biomass, shoot P and Zn content, hyphal P contribution and root colonization were observed. Data were subjected to analysis of variance and means separated by the Fisher's least significant difference. A total of 45 strains of AMF distributed in 11 genera were obtained with the majority being from Gigasporaceae, Acaulosporaceae and Glomeraceae. *Acaulospopra excavata, Acaulospopra bireticulata, Dentiscutata erythropa, Funneliformis mosseae* and *Scutellospora pellucida* were ubiquitous in all the agroecologies. Species distribution was mostly influenced by the soil pH and exchangeable Al. At least 22% of the soils had a mycorrhizal infectivity potential as good as the Rhizatech. The soil AMF inoculum produced had low spore densities (0 - 2.85 spores g^{-1}) compared to the density of Rhizatech (4 spores g^{-1}). In the Ferralsol, there was no difference for the P content (p= 0.195),

but for the Zn content, AMF2 was the highest followed by AMF1. The roots were colonized equally (p = 0.252) in all the treatments. In the Nitisol, AMF4 equalized with the Pi application for the shoot biomass, followed by AMF1. The highest root colonization was observed in AMF4 (31%) and Rhizatech (28%). This is the first report on the study of native AMF species in South Kivu and there is probably a species that was found for the first time. The high number of ubiquitous species indicates adaptation to a wide range of physicochemical environments and could also reduce the cost of production of inoculants. Other methods of AMF inoculum production like the mass production using in-vitro techniques or the stimulation of naturally occurring AMF species in the fields should be tested. Furthermore, there is a need to screen for efficient AMF strains that could improve nutrients acquisition and maximize plants benefits from the symbiosis. Further studies assessing the performance of these selected efficient AMF *Gipaspora gigantea*, *Gigaspora sp.*, *Rhizophagus intraradices* and *Acaulospora reducta* with high spores densities are recommended.

CHAPTER ONE: INTRODUCTION

1.1. Background information

Maize (*Zea mays* L.) is the third staple food after cassava and plantain banana in the Democratic Republic of Congo (DRC) (Badibanga, 2013; Anonymous, 2018). The national production is low, with a mean yield of 0.77 tons ha^{-1} (FAOSTAT, 2018) and 1.13 tons ha^{-1} in the South Kivu province according to IPAPEL (2011) with a potential yield of 3.5 tons ha^{-1} (Badibanga, 2013) while the average yields in neighboring countries with the same agro ecological conditions are 1.57 tons ha^{-1} and 2.31 tons ha^{-1} in Rwanda and Uganda respectively (FAOSTAT, 2018). The maize produced locally is far from meeting the local demand, and the country is importing large quantities for both human and animal consumption (Ndege, 2011).

The poverty trap in which many farmers in Sub Saharan Africa (SSA) are caught, results from the soil degradation caused by declining soils fertility, nutrients mining, low inputs, poor and inappropriate management of agricultural resources beside the drought threat imposed by the climate change. Consequently, farmers experience low crops productivity and high yields gaps (Tittonell and Giller, 2013). This situation, among other factors, has led to the current food insecurity and malnutrition occurring in South Kivu (Mushagalusa *et al.*, 2017; USAID, 2017). With the current rise of world population, by 2050 the population in SSA is expected to increase by 2.5 fold and the demand for cereals will increase by approximately 3 fold, whereas current levels of cereal consumption depends substantially on imports (Van Ittersum *et al.*, 2016).

Crop production is mostly limited by low soils fertility constraints in the SSA and the climate change phenomenon (Van Ittersum *et al.*, 2016). Phosphorus (P) is a plant macronutrient playing an important role in plant's growth and production by intervening in essential metabolic processes like the energy transfer, biosynthesis of macromolecules and respiration (Fageria, 2016). Phosphorus is among the most deficient macronutrient in the agricultural soils in SSA due to their low P content but also the most unavailable due to its complexation by Calcium (Ca) in alkaline soils and with Aluminium (Al) and Iron (Fe) in acidic soils (Nziguheba *et al.*, 2016). Drought is an obvious threat and a limiting factor for crop production as high temperature and evaporation and low rainfall are currently being experienced. Much attention is being paid to adapt and mitigate its effects on crop production (Amerian *et al.*, 2001; Symanczik *et al.*, 2018).

Acidic soils are dominant in eastern DRC in particular (Jones *et al.*, 2013; Ngongo *et al.*, 2009), especially Ferralsols, Alfisols, Acrisols and Nitisols orders which are degraded soils often characterized by a low nutrient contents (Ngongo *et al.*, 2009; Batjes, 2011; FAO, 2015). Phosphorus deficiency in this region has been attributed to a combination of low native P and the prevalence of soils with high P-adsorption capacity (Batjes, 2011; Bationo *et al.* 2012), high nutrient depletion rates and low inputs (Smaling *et al.* 1997). With the highest P nutrient removal rate in the soils and lowest additions in agricultural soils, soil P is projected to be depleted by 2050 in the tropical regions (Balemi and Negisho, 2012) and the worse is that the easily accessible high-grade phosphate rock reserves are likely to be depleted by the end of this century (Schröder *et al.,* cited by Cozzolino *et al.*, 2013).

Presently, campaigns for promoting agricultural intensification are essentially based on the recommendation of the use of breed crops varieties, inorganic fertilizers and pesticides. Though providing some direct benefits, these measures can contribute unfortunately to soil degradation as they can speed up the acidification, leaching, underground water contamination, eutrophication processes and soil biodiversity loss if applied in excess (Brady and Weil, 2002; Solaiman *et al.*, 2014), and hence they are not sustainably productive (Quoreshi *et al.,* 2008).

There is a wide range of environmental friendly agricultural technologies and practices that have the potential to increase food production, adaptive capacity and resilience of agricultural production systems threatened by soil degradation and climate change effects (McCarthy *et al.*, 2011). Among these promising technologies, the biological means by using soil organisms as biofertilizers, biomanures and biopesticides. In the biofertilizers group, there are dinitrogen fixers, phosphate solubilizers and phosphorus mobilizers microorganisms that are used as inoculums (Mazid and Khan, 2014).

Arbuscular Mycorrhizal Fungi (AMF) is a group of soil microorganisms that form symbiotic associations with almost 80% of terrestrial plants. They play a crucial role in plant nutrition and protection against biotic and abiotic stresses. The phosphatase they produce increases P solubilization and plant uptake of P, N, and other less mobile soil nutrients such as Zn and Cu (Quoreshi *et al.*, 2008). They increase the solubilization of phosphates; up to 80% of P uptake by crops is absorbed through mycorrhizal associations with crops in soils with a low available P concentration in the soil solution (Solaiman *et al.*, 2014; Mazid and Khan, 2014).

AMF enhance acquisition of water by roots to the host plants (Parniske, cited by Symanczik *et al.*, 2018). Several studies have observed that some mycorrhizal plants are more tolerant to drought, maintain a high tissue hydration, high leaf water potential exchange and leaf area index (LAI) compared to non-mycorrhizal plants (Subramanian *et al.*, cited by Augé, 2001; Symanczik *et al.*, 2018; Fortin *et al.*, 2009). Maize roots AMF infection with *Glomus mosseae* and *Glomus intraradices* induced a higher leaf water potential in the mycorrhizal plants than in the no mycorrhizal ones (Augé, 2001).

Cereal crops, especially maize, have a high dependency and form symbiotic associations with soil fungi. There is a vast literature devoted to inoculation of maize with mycorrhizal biofertilizers that highlights how maize growth and production capacity are enhanced by AMF that colonizes its roots (Nwaga *et al.*, 2003; Cozzolino *et al.*, 2013; Crespo, 2015; Eulenstein *et al.*, 2016). Nwaga *et al.* (2003), found yield increase of 56 to 66% after mycorrhizae inoculation of maize on a Ferralsol in Cameroon while in India, mycorrhizae application of phosphate resulted in 10 to 20 percent increase in the yield of almost all the crops (Mazid and Khan, 2014).

1.2. Statement of the problem

Acidification, erosion and nutrient mining are the major soil degradation factors in Central Africa and they are being amplified by the climate change effects which bring in also drought (Vanlauwe *et al.*, 2015; Muhindo *et al.*, 2017). Because of the pressure on the land, continuous degradation is rampant in smallholder farming system which is characterized by low inputs of organic and inorganic fertilizers, inappropriate and poor soil fertility management (Tittonell and Giller, 2013; Vanlauwe *et al.*, 2015).

About 25 percent of tropical land area in SSA is covered by soils having high P adsorption capacity (Sanchez *et al.*, 1997). They are mostly acidic and the most dominant in South Kivu, especially the Ferralsols, Ultisols, Nitisols and Acrisols (Crawford *et al.*, 2008; Ngongo *et al.*, 2009). They have a low nutrient availability, especially P for which the plant-available levels are often below 10 mg kg^{-1} (Bray 1 method) (Kihara and Njoroge, 2013), while the optimum for maize is 15 to 20 mg kg^{-1} of soil (Brady and Weil, 2002; Jones *et al.*, 2013).

Different AMF species can colonize one root at the same time (Sylvia *et al.*, 1998) but the degree of colonization and mutual benefits varies between plants and the fungal species (Fortin *et al.*, 2009; de Oliveira Júnior *et al.*, 2017). AMF are the most important microorganism in agricultural ecosystems; they present many beneficial effects and have a

great ability of adaptation to different environments. The diversity, distribution and respective activities of AMF are wide and still to be actively studied and understood, especially in the context of tropical agriculture (Jefwa *et al.*, 2006). Little has been done towards the exploration of AMF strains in cropping systems in South Kivu and evaluation of their plant growth, yield promotion capacities and how they can cope with this low P concentration, solubilization rate and limited humidity conditions.

1.3. Justification

Agricultural brown revolution and the paradigm of ecological intensification which focuses on the yield potential, precision agriculture, good agronomic practices and soil quality restoration are required now than ever to meet the current and growing demand of food in Africa (Dobermann *et al.*, 2013; Van Ittersum *et al.*, 2016). Efforts are being made by researchers to find out the best fit agricultural technologies and management practices tailored to specific conditions of the complex smallholder farming systems in the country and the integrated soil fertility management principles implemented have yielded positive results (Vanlauwe *et al.*, 2017; Nziguheba *et al.*, 2016; Bationo *et al.* 2012).

The first attempt in the study of soils microbes in South Kivu was focused on rhizobia strains as dinitrogen fixers, associated with soybean for its growth and nodulation and gave out an effective local inoculant strain for soybean production (Ndusha, 2013). Use of mycorrhizal inoculants to crops is a common practice in agriculture (Brooks *et al.*, 2006) but despite AMF's immense benefits, limited research has been dedicated to the study of AMF in South Kivu's soils, their characterization, and crop influence on growth and their services for a sustainable agriculture. The use of effective mycorrhizae inoculant promoting plant growth could provide low cost approaches towards boosting maize yields in South Kivu province where chemical fertilizers are still scarce and expensive to be affordable by poor farmers (Crawford *et al*, 2008). Thus, this work takes the lead to study the AMF associated with maize grown in South Kivu, and it seeks to identify agricultural technologies tailored to specific local conditions from the diverse possibilities of technologies.

Efforts in identifying AMF microorganisms from soils, screening them and assessing their nutrients uptake and plant growth promotion capacities on specific crops in the regional agroecosystems settings should be done in order to support or not their agricultural use as biofertilizers (Dadd and Thomson, 1994; Massena *et al.*, 2011) in Africa (Jefwa *et al.*, 2006). Efficient native AMF strains have a high chance of thriving in their environment and have

proved to be effective in plant growth promotion, reason why for an appropriate use of this biofertilizers inoculation technology, selection of AMF strains adapted to the specific environment is required and needs a good knowledge on the occurrence and distribution of fungal species in their natural habitat along with their intrinsic mycorrhizal capacity.

1.4. Objectives

General objective

To contribute towards alternative soil fertility management through characterization and screening of indigenous mycorrhizal fungi isolates for production of a AMF inoculant for maize production in South Kivu, DR Congo.

Specific objectives

1) To characterize the occurrence and diversity of AMF and assess the natural mycorrhizal infectivity potential of soils from maize agroecosystems in South Kivu, DR Congo.

2) To screen and evaluate the influence of native AMF isolates on maize growth, P and Zn uptake under controlled conditions.

3) To evaluate the effect of AMF isolates on maize root colonization in acidic soils under controlled conditions.

1.5. Hypotheses

1) Agricultural soils from maize agroecosystems in South Kivu contain a high diversity of AMF species and most of the soils have a naturally high mycorrhizal potential to colonize maize.

2) The native AMF inoculum would enhance growth and improve P and Zn uptake

3) AMF inoculation with effective native AMF increases root colonization in acidic conditions.

CHAPTER TWO: LITERATURE REVIEW

2.1. Maize production and constraints in South Kivu

2.1.1. Maize production

Maize is the third staple food in DR Congo after cassava and plantain (Anonymous, 2018), playing a crucial role in sustaining food security and income generation for many smallholder farmers. It occupies about 67% of the land area allocated to cereals (IPAPEL, 2011) and grown in all the agroecologies of South Kivu (Appendix 7). The national production is low, with a mean yield of 0.77 tons ha^{-1} in 2016 (FAOSTAT, 2018) and 1.13 tons ha^{-1} in the South Kivu province according to IPAPEL (2011) while the average yields in neighbor countries with the same agro ecological conditions are 1.57 tons ha^{-1} (Rwanda) and 2.31 tons ha^{-1} (Uganda) (FAOSTAT, 2018). The yield gap is very high since the actual production in DR Congo is 0.77 tons ha^{-1} while the potential yield is 3.5 tons ha^{-1} (Badibanga, 2013). It means that the actual yield is only 22 % of the potential yield. This situation implies that there is a big potential for increasing the maize yield.

2.1.2. Constraints to maize production

Maize production has many constraints which prevent farmers from reaching the maximum yield. Low soil fertility, weeds, pests, diseases, drought, lack of access to quality seeds and poor and inappropriate agronomic practices are some factors limiting crop production. Soil fertility and inappropriate agricultural practices were identified among the major constraints in SSA (Sanchez *et al.*, 2003; Brady and Weil, 2002, Tittonell and Giller, 2013).

2.1.2.1. Soils acidity

Soils in the DR Congo are very variable but most of them are acidic (Ngongo *et al.*, 2009). The most predominant soils are, especially Ferralsols, Alfisols, Acrisols and Nitisols (Jones *et al.*, 2013; Ngongo *et al.*, 2009; Batjes, 2011; FAO, 2015). The worldwide extent of Ferralsols is estimated at some 750 million ha, mostly found in tropical Africa; particularly D.R. Congo, Central Africa Republic, Angola, Guinea and Madagascar (Sanchez *et al.*, 2003). Because of an intensive weathering under an aggressive climate, their chemical fertility is low since they are highly weathered with pH often below 5.5 and also more affected by erosion (Jones *et al.*, 2013; FAO, 2015). Because of the pressure on the land, luxiviation and nutrient mining, continuous acidification of soils is rampant (Tittonell and Giller, 2013; Vanlauwe *et al.*, 2015).

Generally, the tropical highly weathered soils are characterized by a low nutrients availability. In acidic soils, the base cations are more and more replaced by Al^{3+}, Mn^{2+} and H^+, reducing the supply of plant nutrients and making basic cations susceptible to leaching (Jones *et al.*, 2013). In the Ferralsols for example the dominant clay minerals are the 1:1 types such as kaolinite and oxyhydroxides of Fe and Al. The external surface for koalinite ranges between 5 and 30 m²/g, while other clays have surface areas ranging between 70 and 300 m²/g. The kaolinite and oxyhydroxide of Fe and Al have the capacity to retain negatively charged anions. This means that the soil becomes positively charged and binds anions, such as phosphate, nitrate and chloride (Brady and Weil, 2002). Because of the low Cation Exchange Capacity (CEC) of the occurring minerals, tropical soils mainly depend on Soil Organic Matter (SOM) to increase the nutrient status as its CEC ranges between 240 and 400 cmol (+) kg^{-1} while for 1:1 types of clays is below 15 cmol (+) kg^{-1}. In highly weathered soils the organic matter colloids, especially humus can even be responsible of up to 80 percent of the CEC (Bationo *et al.*, 2007; Brady and Weil, 2002).

2.1.2.2. Phosphorus low availability and high fixation

Phosphorus is a plant macronutrient, making up to 0.2% of the plant's dry weight. It is a key component in the complex nucleic acid structure (nucleotides and nucleic acids) and associated with complex energy storage and transformations Adenosine Triphosphate (ATP) and involved in the regulation of metabolic pathways and enzyme reactions (Smith and Smith, 2011). P is concentrated in the fast growing parts of plants, especially the root tips, young leaves and is found in large quantities in seeds and fruits. During the early development of the plant it benefits the root development and enhances the development of reproductive parts (Fageria, 2016).

After Nitrogen (N), P is the second most limiting element for crops production. In undisturbed tropical ecosystems, most of the P is replenished through nutrient cycling, and very little is lost. However, once the original vegetation is cleared (e.g. for agriculture), a substantial part of P is lost in eroded soil particles, water runoff and biomass removal. Within a few years most of the P is lost, and the remaining inorganic P is largely unavailable for plant uptake (Nziguheba *et al.*, 2016). Hence, acidic highly weathered soils in the warm humid tropics have very low capacity to supply P to plants (Batjes, 2011). The low P availability results from the extensive losses during long periods of intense weathering, the

low P capital, the dominant Al and Fe complexes and the nutrient mining under continuous cultivation (Ngongo *et al.*, 2009; Jones *et al.*, 2013).

The insoluble inorganic P originates from primary minerals such as apatites, hydroxyapatite and oxyapatite and is present in the soils at levels of 400 to 1200 mg kg^{-1}of soil, but up to 80% is present in forms quite unavailable for plants (Mahdi *et al.*, 2012). Soluble inorganic P appears as orthophosphate ions, usually $H_2PO_4^-$ and HPO_4^{2-} which can be taken up by plant roots. The quantity of available P is time specific and crop specific, depending on the amount of P that is being solubilized in the soil solution and the speed of uptake by the plants through root absorption during the crop life cycle. Because of the typically very low solubility of P minerals and the tendency of P equilibrium to favour the solid phase, the amount of this P ranges between 5 and 10 mg kg^{-1} in most tropical degraded soils (Bray 1 method) (Nziguheba *et al.*, 2016). Because the rates of diffusion are generally low (10^{-12} to 10^{-15} m^{2}s^{-1}) (Hocking, 2001) and inadequate to meet the uptake rates by plants, P uptake is reduced and a P depletion zone is created around the root surface. The rapid way of replenishing the available P in agriculture is with the use of mineral P fertilizers like the Triple Super Phosphate (TSP), DiAmmonium Phosphate (DAP), phosphate rocks (Nziguheba *et al.*, 2016) and its assimilation maximized through mycorrhizal associations (Smith and Smith, 2011).

The P application is quickly immobilized soon after application to the soil and becomes unavailable to plants again (Mahdi *et al.*, 2012). In acidic soils, fixation occurs mainly through the reaction of $H_2PO_4^-$ with the surfaces of insoluble oxides of Fe, Al and Mn. Additionally the kaolinite can also fix orthophosphates (Brady and Weil, 2002). In alkaline soils, the dominating process for P fixation is through fixation with Ca. Availability of P is thus highest with soil pH values between 5.5 and 7 as fixation is lowest within this range, while beyond and below that range, the P availability is limited (Mahdi *et al.*, 2012) (Figure 1).
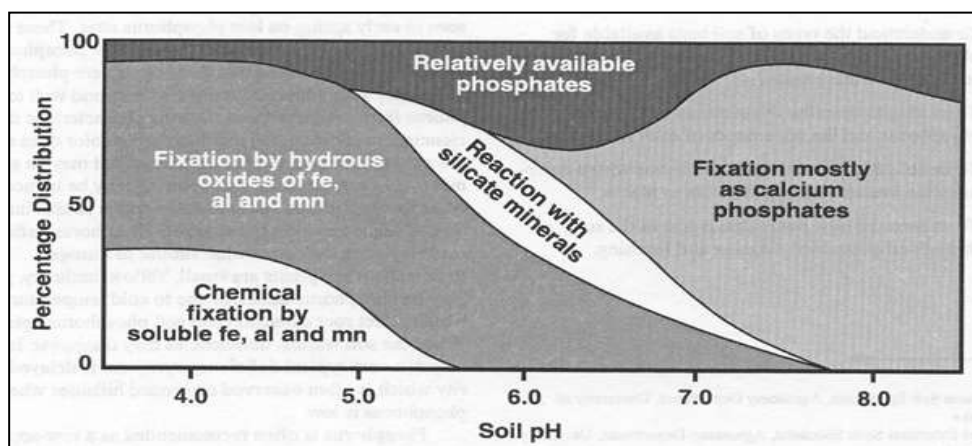
Figure 1: Soils pH and phosphorus availability (Source: Brady and Weil, 2002)

The insoluble organic P pool exists mainly in the forms of phosphate esters, nucleic acids and phospholipids. This form of P can be mineralized from and immobilized in the soil organic matter (Paul, 2014). Net mineralization generally occurs if the C:P ration of the organic matter is below 200:1 while immobilization occurs more likely if the C:P ratio is more than 300:1 (Brady and Weil, 2002; Mahdi *et al.*, 2012).

Zingore *et al.*, (2014) suggested that a rate of 30 kg ha^{-1} of P fertilizer should be added for a target yield of 4tons ha^{-1} in SSA after have filled the P sorption capacity of the soil; otherwise the target yield can be obtained by adding four times the fertilizer recommended (131 kg ha^{-1}) to compensate the amount to be fixed and lost (Brady and Weil, 2002). The P deficiency reduces the maize leaf area index (LAI), and therefore reduces the capacity and amount of absorption of the photosynthetically active radiation. It affects also negatively adventitious roots emergence. In case of deficient P, plants experience a poor development of the root system, leaves reddening, stunting growth, small ear size and a late maturity in general (Belfield and Brown, 2008).

2.1.2.3. Aluminium and manganese toxicity

Al is the most abundant metal in the soil, but its availability depends on soil pH and its toxicity depends on its concentration, chemical form, pH, growth conditions and plant species among others (Bojórquez-Quintal *et al.*, 2017). At neutral pH, the solid phase of Al in form of gibbsite ($Al(OH)_3$) dominates, but when the pH falls below 5, gibbsite solubilizes into the trivalent Al species Al^{3+}, as the equilibrium $Al^{3+} + 6H_2O \leftrightarrow Al(OH)_3$ shifts to the left (Brady and Weil, 2002). These ionic forms of Al species are extremely toxic for plants, as they inhibit root elongation, resulting in a stunted root system and greatly reduced uptake of water and nutrients (such as P). Additionally, increases in the Al^{3+} ion concentration is positively correlated with the increase of Mn^{2+} and both ions affect negatively the microbial community, which results in decreased mineralization and hence nutrient availability (Zheng, 2010).

2.1.2.4. Low soil organic matter content

The SOM influences on soil functions are tremendous and critical to maintain soil fertility as it provides high nutrient retention, high buffer capacity, improves physical and biological soil properties through the high water retention, good structural stability and microbial activities (McCauley *et al.*, 2009; Tittonell and Giller, 2013).

In most tropical soils, the SOM level is low. The main clay mineral, kaolinite, with a much smaller specific surface, leads to considerably fewer clay-humus complexes and thus to less long-term stabilization (Bationo *et al.*, 2007). However, tropic soils are rich in Fe and Al oxides that can retain organic matter as organometal complexes. Since this also leads to high P retention, it isn't always beneficial for crop production. As most of the factors mentioned above are influenced by land use and management practices, they also affect SOM and in the tropics the conventional agricultural practices affect negatively the SOM; hence the integrated soil fertility management practices implementation is being fostered (Bationo *et al.*, 2007; Tittonell and Giller, 2013).

2.1.2.5. Nitrogen, Potassium and Zinc constraints

Maize requires macronutrients (N, P, K) in high quantities but in different proportions. The micronutrients requirements depend on soil type and content in these specific elements, but it is better to apply them to maize crops to prevent deficiencies (Birch *et al.*, 2003).

Nitrogen content in the soil is generally low due to various reactions it undergoes quickly and which results to its lost through immobilization, volatilization, lixiviation or leaching. Nitrogen is uptaken by maize plant roots in form of nitrate (NO_3^-) or ammonium (NH_4^+), but it is mostly absorbed in the nitrate form because of the high mobility of this last in the soil solution and also the readily conversion of ammonium in nitrate in the soil by microorganisms (Zingore *et al.*, 2014). The rapid uptake of nitrogen starts in the middle of the vegetative growth with the maximum occurring at 6 to 8 weeks of growth, near silk. Therefore, the fractionation of N fertilizers is recommended to increase nutrients use efficiency and reduce losses. To supply nitrogen, it is better to apply the fertilizer in two to three splits and for a maize target yield of 4 tons ha^{-1}, the rate of 90, 30 and 60 kg ha^{-1}of N, P and K respectively are recommended in the tropical conditions generally but site specific recommendations are requested to be made after a thorough assessment of the right nutrient management and target yield (Zingore *et al.*, 2014).

Potassium (K) intervenes in a number of plant physiological processes and responses to stresses; not only in stomatal opening and closing, regulated by osmosis and ionic balance, but also in activating many enzymes and influencing photosynthesis, nutrient transport and assimilation (Birch *et al.*, 2003). In acidic soils, K can be deficient depending on other soil properties and its deficiency is mainly corrected by the inorganic fertilizers application in form of muriate of potash mostly (KCl). Potassium deficiency alters plant water relationships that reduces the leaf elongation rate and therefore results in reduction of LAI (Belfield and Brown, 2008).

Zinc (Zn) is a micronutrient mostly available in neutral to slightly acidic soils. According to Birch *et al.* (2003), maize grown in acidic soils and alkaline soils may present acute deficiency symptoms including leaves light streaking from the leaves edges towards the leaf center with the tip remaining green, and a general stunted growth, among others (Belfield and Brown, 2008).

2.2. Arbuscular Mycorrhizal Fungi

2.2.1. Description, characterization and occurrence

The term "mycorrhiza" means "fungus - root" and stands for the symbiotic relationship that exist between a group of fungi and the roots of higher plants. The "arbuscular" name comes from the branched tree-like hyphal structures termed "arbuscules" that they produce and which occurs within the root cortical cells. AMF are obligate symbiotic fungi associated with various plants in the clades of Pteridophytes, Gymnosperms and Angiosperms (Steinberg and Rillig, 2003). The taxonomy of AMF was based solely on the morphological and anatomical characteristics of spores, but recently, modern and accurate methods DNA based methods have helped in a more precise and clear phylogenetic analysis (Diop *et al.*, 2015; Redecker *et al.*, 2013). The mycorrhizae has three major components namely: the root, fungal structure and the extra radical hyphae. The root supplies carbon through sugar to the fungus via its structure in the cortical cells and the water and nutrients absorbed in the extraradical hyphae are supplied to the plants in turn (Brundrett *et al.*, 1996).

Among the many types of mycorrhizal associations existing, the arbuscular and vesicular are the most common. Arbuscules are the finely divided hyphae in the cortex and which invade cortical cells inter and intra cellularly. The vesicles are membrane bound organelles of varying shapes inside and outside the cortical cells. Vesicles and arbuscules and large spores constitute the diagnostic feature of the mycorrhizal associations (Figure 2) (Fortin *et al.*, 2009).
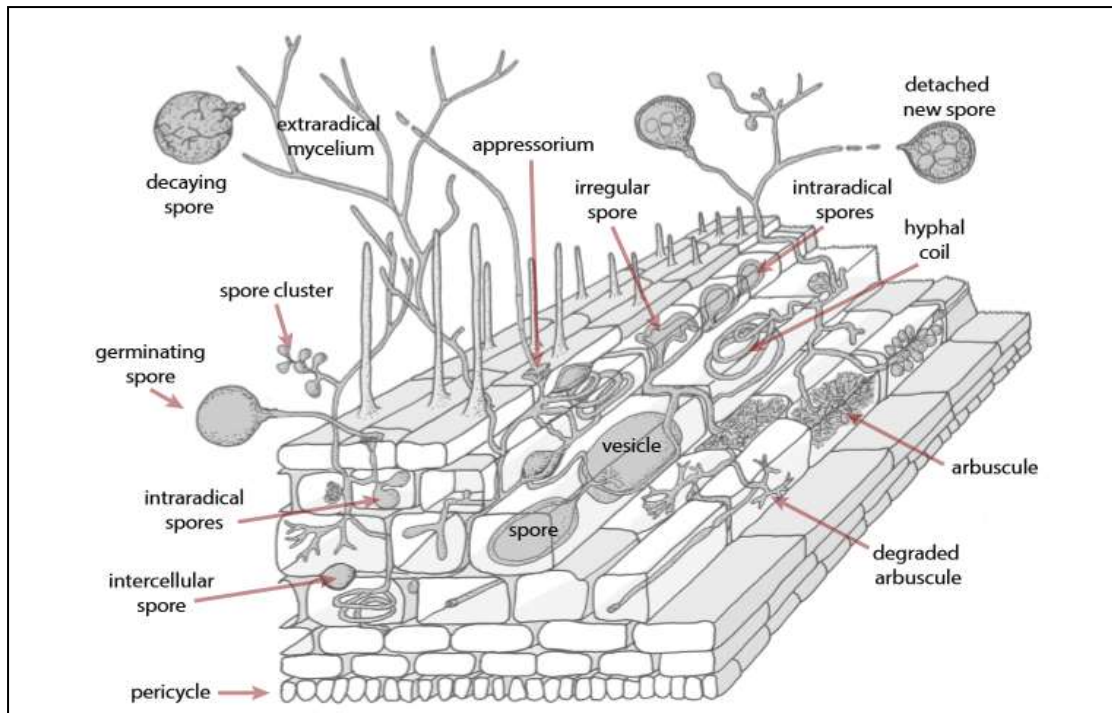
Figure 2: AMF structure (Source: Walker, 2013)

The term "arbuscular mycorrhizal fungi" (AMF) is preferentially used in the literature to represent the fungu-root association rather than "vesicular-arbuscular mycorrhizal" (VAM) fungi because of the absence of vesicles in two plant genera forming the mycorrhizae with the fungi, while the Ecto Mycorrhizal (ECM) Is formed with fungi from Basidiomycetes and associates with nearly 2% of higher plants only (Habte and Osorio, 2001).

AMF can reproduce either asexually or sexually. The spore dormancy can be over under favorable environmental conditions and AMF can germinate by undergoing series of structural and morphological changes. The changes are grouped into three major stages: asymbiotic, pre-symbiotic and symbiotic. The asymbiotic stage is the resting stage in which the extraradical hyphae produces naturally spores in soil after the association with the host is over (Fortin *et al.*, 2009; Bago and Becard, 2002). The spores thus produced can have, in this dormancy status, a lifespan of one or even two years depending on the kind of species or genera. In this spore or resting stage, the fungi is not host dependent and the energy reserves in form of lipid and trehalose is preserved to be used during the spore germination (Smith and Read, 2008). Spores are formed in case of lack of carbon metabolism due to a delay of contact with a host plant.

The pH, temperature, moisture, carbon dioxide and inorganic nutrients availability influence the relief of spore dormancy (Bago and Becard, 2002; Rosendahl, 2008). In this pre-symbiotic stage, spores germinate; produces branches towards the host and form the approssiora at the contact of the root. The appressiora are enlargement occuring on the epidermal cell walls of the host root. The third stage or the symbiotic stage is the physical contact and penetration and formation and development of the intra radicular hyphae, arbuscules and vesicles (Brundrett *et al.*, 1996; Bago and Becard, 2002).

2.2.2. Taxonomy and diversity

AMF belong to the Glomeromycota division. Glomeromycota (informally Glomeromycetes) is one of the five divisions or phylum in the fungi kingdom. It gathers 4 orders, 12 families and 34 genera. The genera Funneliformis, Septoglomus, Glomus and Rhizophagus are the more populated with approximately 230 described species. The fungi phylogenetic classification as summarized by Redecker *et al.* (2013), based on genetic analysis of Large Sub Unit sequence is presented in the Figure 3.
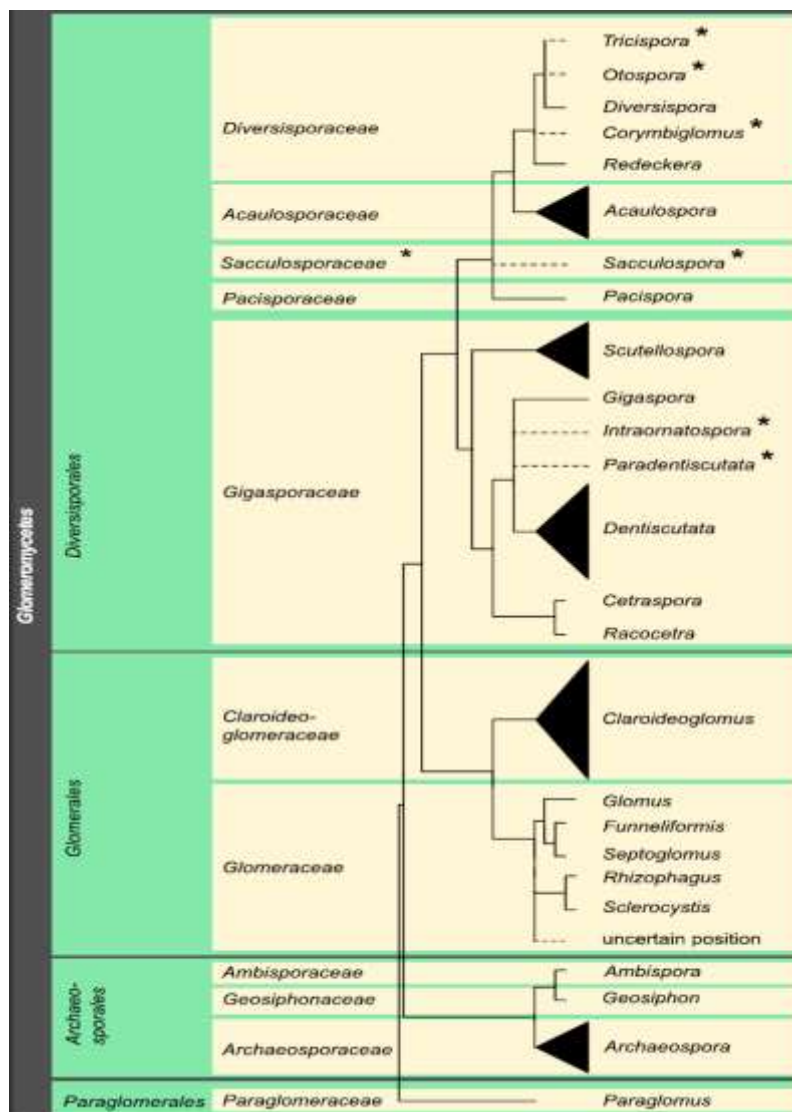
Figure 3: Phylogenetic classification of Glomalean fungi (Source: Redecker *et al.*, 2013)

2.2.3. Mechanism of mycorrhizal nutrients uptake

A root colonized by AMF has two options for nutrients uptake: the direct and the mycorrhizal pathways involving different cell types and transporters. The direct path is the by the root hair while the nutrient absorption via the extraradical hyphae is the indirect pathway (Smith and Smith, 2011).

Plant-microorganisms interaction is managed using biochemical signals triggering selectively the responses in symbiotic partners. Many plant compounds can influence the AM symbiosis among which the strigolactones and some plant flavonoids stimulate the fungi growth (Cruz *et al.*, cited by Solaiman *et al.*, 2014), etc. On the other side, the AMF produces symbiotic signaling compounds like the lipochitooligosaccharide and plants detect microbe-derived compounds and adjust their responses according to the specific microorganisms that is present (Solaiman *et al.*, 2014). The P is uptaken into the extraradical hyphae against an electrochemical potential gradient, by P transporters energized by H+-ATPases (Bucher, 2007) (Figure 4).

The molecular mechanisms driving the mycorhizae formation and function have not yet been fully elucidated but transcript profiling has revealed that a number of genes are up or dowm regulated in the symbiosis (Kistner *et al.*, 2005). Furthermore, the molecular mechanisms promoting Pi efflux from the intraradical hyphae to the roots cells are unknown so far (Smith and Smith, 2011).
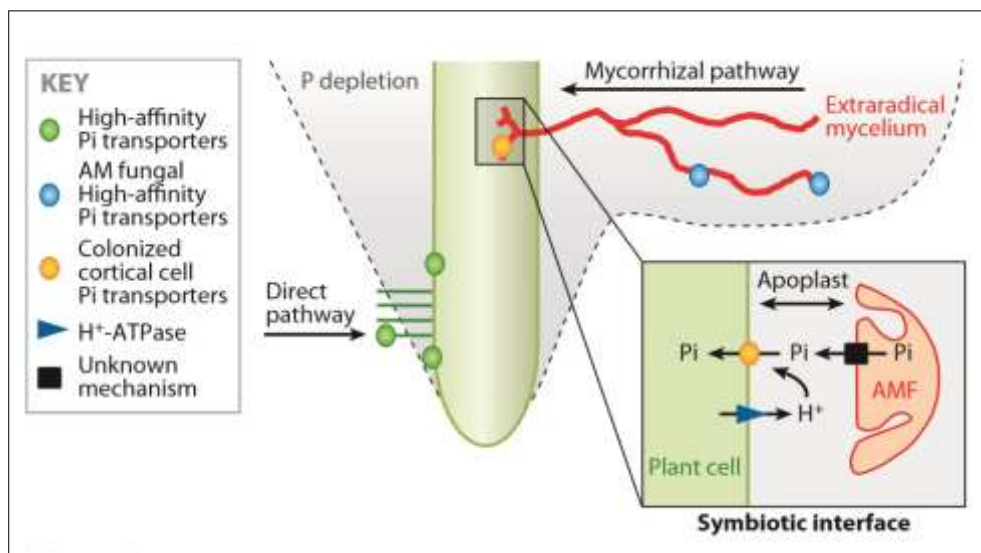
Figure 4: Representation of pathways of phosphorus uptake in a mycorrhizal root (Source: Smith and Smith, 2011)

2.2.4. Screening and inoculum production

For initiating an inoculation program, identification of the most suitable AMF species specific for a given plant species is necessary. Researchers have demonstrated that the necessity of selecting an effective to be used as biofertilzer emerges from the diversity observed in the performance of AMF species since different AMF species react differently in terms of water or nutrient uptake or plant growth promotion that they induce (Dodd and Thomson, 1994; Ortas *et al.*, 2011; Barenjee *et al.*, 2013; Gomes *et al.*, 2015).

Screening AMF consist in inoculating AMF on a specific crop for a specific target for selection of the most effective. Screening and selection of AMF and Ecto Mycorrhizal Fungi (ECM) for application in agriculture, forestry or horticulture, is a strategy allowing determining if inoculation is the appropriate management option instead of stimulation of the indigenous mycorrhizal communities in the agricultural soils (Dodd and Thomson, 1994). Wu *et al.* (2002), when screening the AMF in China, found that indigenous AMF species isolates of *Glomus manihotis* and *Glomus caledonium* from acidic soils in China were as effective as imported strains from Australia in plant growth improvement and recommended in revegetation efforts.

In order to benefit from AMF as a biofertilizer, AMF propoagules (inoculums) have to be inoculated into a target soil (Berruti *et al.*, 2016). There are different technologies of AMF production which differ in terms of substrates and costs. They can be substrate based, especially the crude inoculum and the field soil inoculum, or substrate free methods using in-vitro techniques through hydroponics or aeroponics (Habte and Osioro, 2001; Akhtar and Abdullah, 2014; van der Heijden *et al.*, 2015). The soil crude inoculum production is the more reliable and convenient method of producing AMF inoculum, and the more suitable in the research context (Berruti *et al.*, 2016; Habte and Osioro, 2001). In smallholder farming, the low cost technology of stimulating AMF in the soil through good agriculture practices appears to be more practicable (Alexander *et al.*, 2017). Currently, the in-vitro cultivation methods such as hydroponic system is being used widely for the mass production of AM fungi and mostly recommended for large scale use of AMF biofertlizer (Akhtar and Abdullah, 2014; van der Heijden *et al.*, 2015).

2.2.5. Infectivity potential

Agricultural soils contain naturally fungal propagules that abundance determines the inoculum reservoir of the soil and thus its colonization potential. This natural mycorrhizal

potential of soils is influenced by management practices and the cropping system (Jefwa *et al.*, 2008). The soil disturbance by the tillage and the soil erosion results often in the loss or significant reduction of mycorrhizal propagules occurring in the soil and leads subsequently to the reduction of the natural inoculum potential of the indigenous fungi for mycorrhizal formation. This situation can jeopardize the sustainability of agroecosystems since mycorrhizal symbioses are essential components of natural ecosystems, particularly in neutral to acidic soils like the dominant Ferralsols in the tropics where their essential role is sustaining the nutrients supply to crops (Solaiman *et al.*, 2014).

Lambert *et al.* (1979) pointed out that a high mycorrhizal infectivity potential implies a high competition between native and introduced strains. The mycorrhizal potential of a soil can be enhanced through agricultural practices favoring the proliferation of fungi in the soil like the minimum tillage, conservation practices, soil mulching, etc. (Alexander, 2017; Solaiman *et al.*, 2014; Dodd and Thomson, 1994).

Beside the spore abundance determination, the soil mycorrhizal potential assessment involves plants infectivity assay and observation of the roots colonization (Brundrett *et al.*, 1996; Dalpé and Hamel, 2008).

2.3. Benefits of Arbuscular Mycorrhizal Fungi on maize growth and soil properties

2.3.1. Host preference and maize mycorrhizal dependency

AMF are not host specific but they may have preferences for certain plants (de Oliveira Júnior, *et al.*, 2017). Different types of AMF strains can colonize a vast range of plants, both herbaceous or woody plants, and one root can be colonized by more than one strain at the same time. Sylvia and Chellemi (2001) define the specificity, infectivity and effectiveness as three parameters that determine root colonization. Specificity of fungi species refers to the ability of the fungus to colonize root cells of particular plant species, while infectivity is the amount of colonization or the proportion of colonized roots and effectiveness is the plant's response to colonization. AMF do not always cause plant growth increases (Fayé *et al.*, 2013). Notable cases of plant growth depression apparently caused by AMF in "non-host" species or in host species when phosphate availability in soil solution is high have been observed. The proliferation of indigenous AMF is influenced by soil properties such as soil pH, temperature, soil carbon and nitrogen. The level of benefit to plant growth thus varies depending on the fungal species community and host combination but also the prevailing environmental conditions (Lovelock *et al.*, 2003).

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Most crops species are colonized by AMF and maize is one of the good plants hosts that form good and effective symbiotic association with mycorrhizal fungi (Habte and Osioro, 2001; Cozzolino *et al.*, 2013). From the trials of different researchers (Nwaga *et al.*, 2003; Cozzolino *et al.*, 2013; Crespo, 2015), agricultural productivity of crops colonized by effective mycorrhizal fungi is improved thanks to the benefits of the symbiosis and artificial inoculation of maize by isolated local fungi strains. Their findings have proved a clear improvement in maize growth and yield thanks to the improvement of P, Zn and Cu uptake, particularly in acidic conditions.

2.3.2. Impact on maize growth

Approximately 80% of plants from all phyla of land plants are identified as hosts of fungi which colonize their roots by forming mycorrhizae (Solaiman *et al.*, 2014). AMF, both indigenous strains and exotic strains have has been proved to increase productivity in mycorrhizal maize (Zabinski *et al.*, 2014; Nwaga *et al.*, 2003; Cozzolino *et al.*, 2013; Crespo *et al.*, 2015; van der Heijden *et al.*, 2008). From the vast literature existing, the major benefits of AMF to host plants include nutrients uptake, drought resistance improvement and suppression of root pathogens.

AMF hyphal network in the soil allows access to a greater volume of soil and water therefore influence plant resistance to environmental stress conditions (Ahanger *et al.*, 2014). Subsequently mycorrhization increases root hydraulic conductivity and maintenance of cellular water pressure (Pereira *et al.*, 2016b). Mycorrhizal infection of maize with *Glomus mosseae* and *Glomus intraradices* resulted in maintenance of higher leaf water potential in mycorrhizal than in non mycorrhizal plants (Augé *et al.*, 2001; Amerian *et al.*, 2001).

When colonizing the roots, AMF act as biocontrol agents by enhancing plant tolerance and protection against pathogens (Azcon-Aguilar *et al.,* 2002). The ability of AMF to enhance plant vigor from the increased nutrient uptake enables plants to resist to the pathogen by root exudates production that inhibit pathogens development and increased competition for space of infection between the fungi and pathogen at the roots surface. Accumulation of phenols from AMF colonization has been reported to cause both localized and systemic resistance to some pathogens (Madiba, 2014).

Some root infecting pathogens fungi*,* or root rotting pathogens like *Phytophthora* and *Rhizoclonia* wilt when the roots are colonized by AMF. Mukasa-Mugerwa (2005) studied biocontrol potential of AMF inoculant on *Fusarium* using different maize cultivars and

showed an increased tolerance to the pathogen. Pozo *et al.,* (2002) used tomato plants and observed that *Glomus mosseae* reduced the infection of the pathogen *Phytophlhora parasitca* in tomato roots.

2.3.3. Impact on soils properties

AMF are tributed to enhance mainly the phosphorus solubilisation and uptake, restoration of degraded lands and soil aggregation. AMF are known to enhance the uptake of the macronutrient, mainly P and N to some extent, and micronutrients such as Cu, Zn, Fe, etc. from the soil (Gomes *et al.*, 2015; Quoreshi *et al.*, 2008).

The less availability of organic P and low solubility of rock phosphates makes it difficult for plants to readily utilize P from soils. Beside the mineral P, the other forms of P in the soils, either in acidic soils ($FeHPO_4$) or alkaline soils ($CaHPO_4$) are unavailable for plants absorption. AMF intervene to enhance nutrient uptake through (1) exposition of a larger surface area per unit volume of soil which makes the fungi much more efficient in the uptake of P than roots since the hyphae diameter is 2.5 to 5 times smaller than plant root diameter; (2) the spread of their extraradical hyphae into the soil in the surrounding allowing them to explore the soil micropores unaccessible to normal roots, and (3) hydrolyzing the unavailable P with the aid of secreted enzymes such as phosphatases (Smith and Smith, 2011).

Though bacteria are more effective in P solubilization, other fungi besides glomeromycota solubilize also the P in soils, especially *Penicillium* and *Aspergillus* which are the most powerful solubilizers among fungus (Whitelaw, 2000). Bacteria also help AMF to colonize better the plants and studies have shown that dual inoculation with AMF and bacteria like rhizobia results in high yields (Aguk, 2013; Kundu, 2012). The uptake of less mobile micronutrients (Zn, Cu, Mn,) by AMF in maize can be positively influenced by the soil P nutrition (Cardoso and Kuyper, 2006; Cavagnaro, 2014).

AMF is potentially able to be used in detoxification of heavy metal polluted environments and in phytoremediation. However in such processes, selection of AMF species with appropriate phytobionts properties is of great importance and need to be done carefully (Nwaga *et al.*, 2003; Entry *et al.,* 2002; Teixeira *et al.*, 2017).

AM fungi secrete the glomalin from the hyphae. It is a proteinic substance, glue-like, water soluble and heat stable that can contribute to the improvement of the structural stability in the soil by binding soil particles and forming well aerated macroagreates. The Glomalin is AMF

specific and it can't be produced from uncolonized plant roots and that's why AMF activity in the soil can be studied through its detection. (Lovelock *et al.,* 2004).

2.4. Research gap

As far as soils sustainable management and maize production improvement in the eastern DRC are concerned, studies in the context of mycorrhizal fungi exploration and screening for efficient AMF are still lacking. In the eastern DRC, efforts have been made in studying the indigenous microorganisms and their potential use in agriculture but they have so far limited to bacteria colonizing leguminous crops (Ndusha, 2013). Ndonda (2018) and Alexandra (2017) tried the manipulation of indigenous mycorrhizal fungi in the soils for cassava and vegetables production respectively in DRC and identified good agronomic practices inducing higher AMF densities, higher root colonisation and maximum nutrient uptake.

Maize growth and yield have been reported to be improved by efficient isolates of AMF and this is not yet proved with isolates from maize agrocosystems from DR Congo.

CHAPTER THREE: MATERIALS AND METHODS

3.1. Study area

South Kivu province is located in the eastern part of DR Congo and lies between $1^o34'05''$N, $4^o59'59''$S, $26^o47'54''$ W and $29^o15'07''$ E, with the altitude varying from as low as 600m in Uvira to more than 2200m above sea level (absl) in Kabare. The province falls in the tropical regions of the earth where up to 6 AEZs are distinguished based on different soil and climate characteristics prevailing. According to the Köppen-Geiger climate classification, different climate zones occur in the region and they include: the equatorial, warm semi-arid, humid subtropical, temperate subtropical and cool subtropical climate (Kottek *et al.*, 2006).

The study area was in 3 AEZs considered promising for cereal crops production, especially maize, rice, mil and sorghum (IPAPEL, 2011; Muhindo *et al.*, 2017). These AEZs are spread in 4 territories namely Kalehe, Kabare, Walungu and Uvira and in each territory 3 villages were selected for sampling. The 3 AEZs, namely the equatorial zone of high altitude (equatorial highland: >1800m absl: in Kalehe and Walungu), the tropical zone of low altitude (tropical lowland:<1000m, in Uvira) and the tropical zone of medium altitude (midland: 1400-1800m located in Kabare, Kalehe and Walungu territories), constituted the study area where sampling was done. The figure 5 indicates the sampling points in the province. Additional characteristics of the AEZs in South Kivu are provided in the Appendix 6.
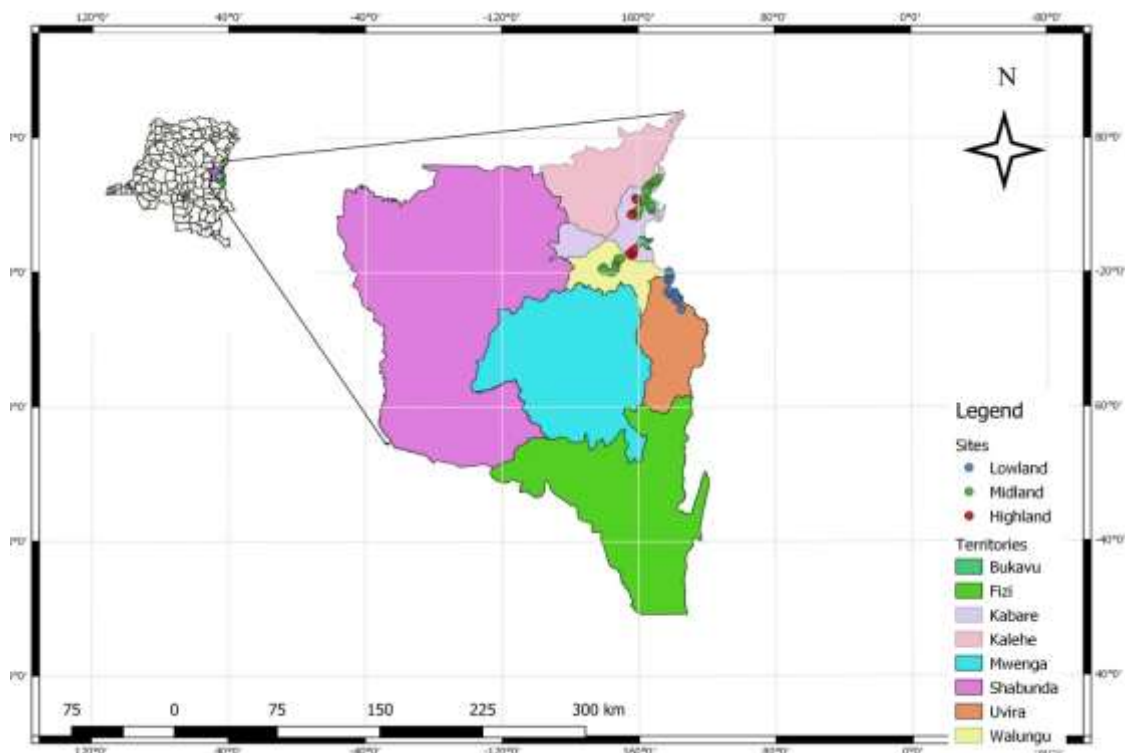
Figure 5: Study area in the South Kivu province, DR Congo

Two main climatic seasons occur in the area; the long rainy season (September to May) and the short dry season (June to August). The climatic data of the study area during the investigation year are presented in the Figure 6.
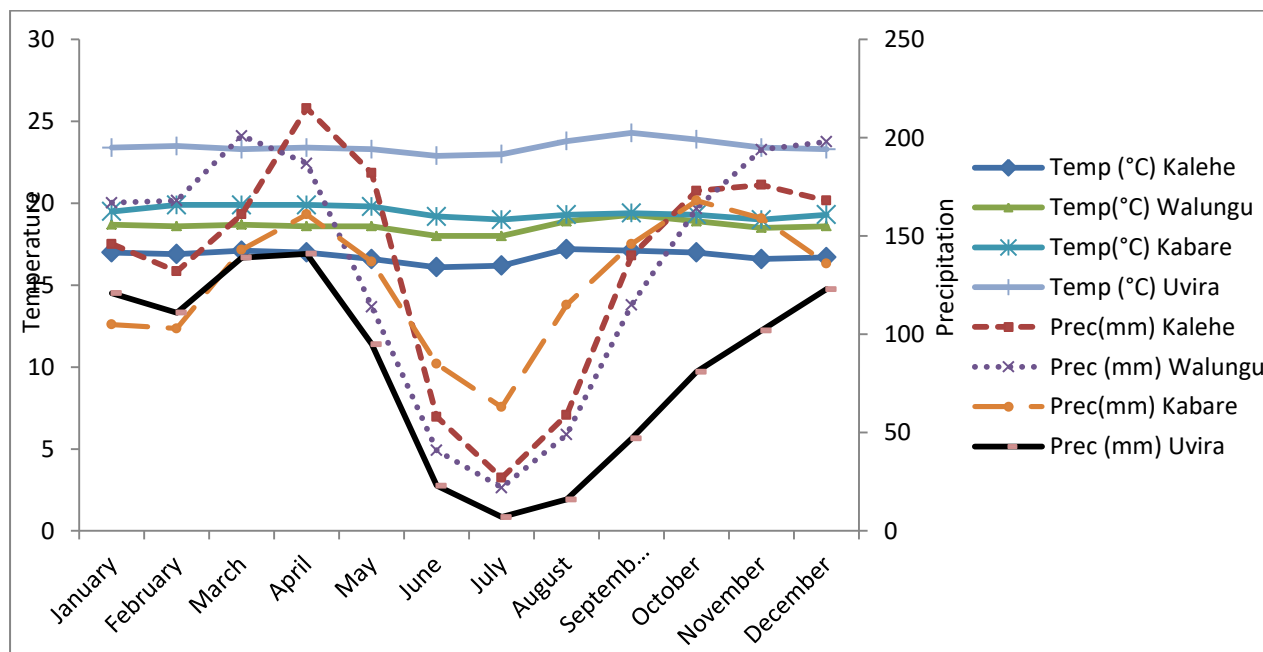
Figure 6: Climatic characteristics of the study area

Source: https//Climate-data.org (online): Accessed on 12th April 2019

3.2. Soil sampling

Soil samples were collected from cereals fields during the dry period in early September 2018 when the roots activities is declined and fungal spores are expected to be formed in numbers (Brundrett *et al.*, 1996; Jefwa *et al.*, 2006), in 3 AEZs represented in 4 different territories in South Kivu.

The soil sampling process was done following the method described by Dalpé and Hamel (2008). In each AEZ, 3 villages were randomly selected and in each village 4 to 6 samples, weighing 1.5 kg each, were randomly collected in the rhizosphere at the depth of 0 - 20 cm. The selected fields in each village were occupying different positions in the landscape; going from the summits to the toeslopes. The fields selected were fields where maize was grown or grew more than one cultural season, sole or in association with other crops, mostly leguminous, sorghum, cassava or banana plants. In total there were 60 samples. Geographic localization of sampling points was done using a GPS (Garmin). Additional information on agricultural practices and management of the sampling sites were collected and are presented in Appendix 1.

Immediately after sample collection, samples were sealed in well labeled bags to avoid mixing and air dried for a week in order to slowdown the growth of microorganisms (Ferguson and Woodhead, cited by Habte and Osorio, 2001). For further studies, dried samples were then exported to the laboratories of Soil Physics and Soil Chemistry at the Faculty of Agriculture, University of Nairobi and at the Mycology laboratory at the National Museums of Kenya in Nairobi. Each sample was then divided in four sub-samples for: physic-chemical analysis, AMF spores extraction, natural mycorrhizal potential assessment and trap culture initiation.

3.3. Soil physical and chemical analysis

Soil samples were homogenized and sieved at 2mm for analysis. The analysis done was for the determination of pH, exchangeable acidity, available P, CEC, total C and soil texture and they were conducted in the laboratory of soil chemistry and soil physics at the laboratories at the Agriculture faculty, University of Nairobi.

Soil pH was determined in water, pH H_2O at a ration 1:2.5 using a pH meter (Metrohm 632pH-meter). About 6 g of 2 mm sieved soil were mixed with 15 ml of water and shaken for 30 minutes after what samples were allowed to stand for 5-10 minutes before the immersion of electrodes for recording the readings.

The exchangeable Al was determined by the titration method using unbuffered, neutral salt, in this case KCl (McLean, 1965) as presented in Okalebo *et al.* (2002), to determine the degree of Al content in the soils when the pH was lower than 5.5. About 10 g of air-dry and 2 mm sieved soils were placed into plastic 50 ml capacity containers. About 25 ml of 1 M KCl was added and the contents were shaken for 30 minutes. After standing for 30 minutes, contents were filtered and leached with five successive aliquots of 25 ml of 1 M KCl. About 5 drops of phenolphthalein indicator were added in each filtrate then after titration with 0.1 M NaOH to the appearance of a permanent pink colour. To obtain the exchangeable acidity value, the following formula was applied:

Exchangeable acidity (cmol(+) kg^{-1}) = (ml NaOH sample - ml NaOH blank) $\times$ 10 [Equation 1]

The available P was extracted following the Bray P (Bray and Kurtz, 1945) method as described in Jones (2001). The extraction reagent was prepared by mixing 30 mL 1 N NH$_4$F with 50 mL 0.5 N HCl in a 1000 mL volumetric flask and dilute to volume with water. About 50 ml of extraction reagent were added to 5 g of 2 mm sieved air dried soil, shaken for 5 min and filtered directly using a Whatman paper during 10 minutes. The extract was retained for P concentration determination by the molybdenum blue method. About 3 ml aliquot of extract were pipetted in 50 ml volumetric flasks, mixed with 5ml of ascorbic and molybdate reagents, shaken and filled to the trait. After resting for 5 minutes, the absorbance reading was performed using a spectrophotometer (Spectronic 1001, Milton Roy company) at 882 μm wavelength. The P concentration was calculated using the following formula:

P(ppm)= Absorbance X $\frac{50ml}{5}$ X $\frac{50}{3}$ [Equation 2]

The CEC was determined following the neutral Ammonium acetate (1 N NH$_4$C$_2$H$_3$O$_2$, pH 7.0) extraction method (Anderson and Ingram, 1993). About 5 g of 2 mm sieved soil were set in a funnel with a filter paper, and then 50ml of NH$_4$OAC allowed to leach five times, making a total of 250 ml of NH$_4$OAC. The filtrate was discarded and the soil leached again two times with 50 ml of ethanol. The second filtrate is discarded and the soil was then leached finally with 25ml of 1 N KCl four times in 100 ml volumetric flasks and top up to the trait with the KCl solution. About 10ml of the filtrate was distilled for 2 minutes after adding 3 drops of mixed indicator, using the Kjeldhal distillator for the solution to turn green then titrated with 0.01 N H$_2$SO$_4$ and record the volumes tritrated. The CEC was determined using the following formula:

CEC (cmol+kg^{-1} soil) = $\frac{Volume\ titrated\ X\ 0.01\ N\ of\ the\ acid\ X 100ml\ of\ volume\ extracted\ X\ 100}{5g\ X\ 10ml\ of\ volume\ distilled}$ [Equation 3]

The organic carbon was determined following the method of Nelson and Sommers (1975). About 1g of 0.5 mm sieved soil was mixed in 500 ml volumetric flask with 10 ml of potassium dichromate and 20 ml of concentrated sulfuric acid then allowed to stand for 20 minutes. After complete oxidation from the heat from the solution and the external heating by digestion, the residual potassium dichromate (in oxidation) was titrated against ferrous ammonium sulphate. The difference between added and residual potassium dichromate, gave the measure of organic C content of soil, found using the formula:

TOC%= [$\frac{(Vblank - Vsample)x0.3x10/20.6}{weight}$]x $\frac{100}{77}$ [Equation 4]

The texture analysis was done to determine the particle size distribution of the soil samples using the hydrometer or Boyoucous method (Bouyoucos, 1962) as described in Okalebo *et al.* (2002). Sub samples of 2 mm sieved soil of 50 g each were used. Hydrogen peroxide was added to break the organic matter and calgon (sodium hexmetaphosphate) to disperse the particles. The prepared and dispersed samples were then transferred to 1 litre measuring cylinders and filled to the 1litre mark with deionised water. Stirring was done to ensure that all material at the bottom was suspended. A blank solution (without the soil sample) was also prepared and poured into a l litre cylinder. A hydrometer was immersed and allowed to float freely. Hygrometer and temperature readings were taken respectively 40 seconds after shaking after and 3h after standing. The particles sizes were calculated as follow:

Sand (%) = [(sample weight-first reading)/sample weight]x100 [Equation 5]

Clay (%) = [(second reading-blank)/sample weight)] X100 [Equation 6]

Silt (%) = 100 - Clay (%) - Sand (%) [Equation 7]

3.4. Characterization of Arbuscular Mycorrhizal strains

3.4.1. Isolation from field soils

The wet sieving and sucrose gradient methods were used to extract spores from 50 g from each soil sample (Brundrett *et al.,* 1996). The soil samples were suspended in water and then decanted through a series of 2 sieves (with 0.350 mm and 0.045 mm). The contents of the finest sieve were transferred separately with some water to 50 ml falcon tubes, shaken and centrifuged at 1750 revolutions per minute (rpm) for 5 minutes. After centrifugation, the

water supernatant was discarded and then the 50% sucrose solution (250 g of sugar dissolved in 500 ml of water) was added to the settled soil in the tubes to full it at almost 75%. The content was thoroughly shaken and the samples were centrifuged at 1750 rpm for 1 to 2 minutes (Ingleby, 2007). The supernatant was poured into 0.045 mm sieve then spores were washed thoroughly with running water then transferred into petri dishes for examination using dissection microscope.

Three to four healthy spores were mounted on microscope slides, for description of the morphotypes, and stained with Polyvinyl Alcohol-Lacto-Glycerol (PVLG) and Melzer's reagent (Brundrett *et al.*, 1996) which compositions are presented in the Appendix 3. Spores were gently cracked open to allow detection of spore substructure features under a compound microscope at a magnification of 100X, 400X and 1000X; and their morphological characteristics such as size, color, shape, and surface texture, surface ornamentation, wall layers, germination shield, germination orb, wrats, sub cellular structure, sporogenous cells, cicatrix and hyphae position were observed. The sizes were determined using a dissecting microscope equipped with an ocular micrometer. The descriptions are presented in the Appendix 2 but the full descriptions of species are on the INVAM website. The identification was based on descriptions and identification criteria described in the International Culture Collection of Arbuscular and Vesicular-Arbuscular Endomycorrhizal Fungi (INVAM) found on http://invam.wvu.edu/ and http://www.zor.zut.edu.pl/ collection websites) (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).

Spores abundance was calculated as the number of spores per gram of soil (Sasvári *et al.*, 2012; Estrada *et al.*, 2013). The total number of spores was determined by counting all the spores recovered under the dissecting microscope at 100 X magnification. The species richness was expressed as the number of species recovered in each site and the occurrence defined as the number of samples in which a specific species was recovered over the total number of samples per sites.

3.4.2. Trap culturing and spores isolation from trap soils

For an accurate spore abundance assessment, a trap culture using maize as a trap crop was set in a greenhouse at Kabete field station for 4 months. The growing medium was a mixed soil and sand at a 2:1 ratio (v/v), sterilized by autoclaving, then 4 kg of the medium was placed in 5L pots. Approximately 300g of soil samples were applied in a band at 3 to 4cm below the

surface (Brundrett *et al.*, 1996) (Figure 7) and the pots were watered up to field capacity 2 to 3 times a week without fertilizer. The soil used, collected from Kabete experimental field station, had a low content of available P (4.6mg kg^{-1} Olsen), which could allow a good development of mycorrhizas (Habte and Osioro, 2001). Pre-germinated maize seeds were sown and CAN fertilizers was applied as blank at 3 weeks after planting at a rate of 50 kg ha^{-1} to supply more N to plants.

Plants were allowed to grow up to 4 months and in the last 3 weeks, the watering frequency was reduced to once a week to favor the spores' formation. Soils were carefully recovered and 50g from each pot were used to identify abundant AMF morphotypes (Habte and Osioro, 2001; Brundrett *et al.*, 1996) following the procedure described in the section 3.4.1.
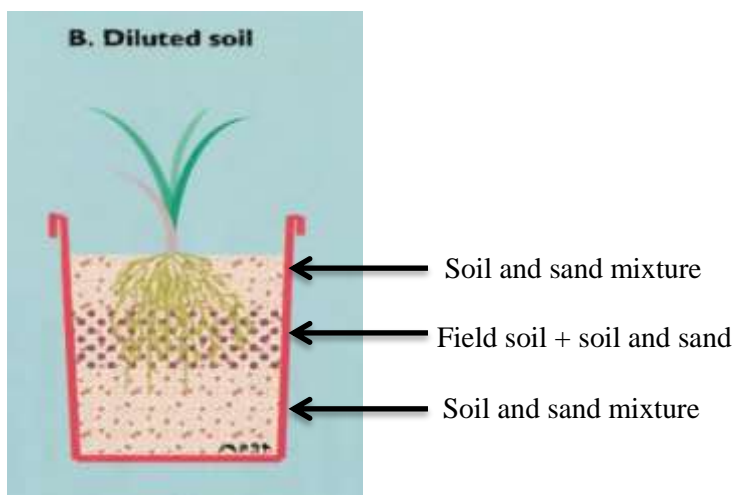
Figure 7: Diluted soil for trap culturing (Source: Brundrett *et al.*, 1996)

3.5. Determination of the infectiveness potential of indigenous mycorrhizae

An infectivity assay was conducted (Dalpé and Hamel, 2008). The maize seeds were grown in the soils under examination for 3 weeks, enough time for the infection to take place (Dalpé and Hamel, 2008) in small pots of 150 ml in a greenhouse. Maize seeds were sown directly in the pots and watered twice to thrice a week as needed with tap water. At the end of the experiment, the shoots were cut off, and roots recovered from the soils, preserved in labeled containers in 70% ethanol for clearing and staining following the procedure of roots staining (Brundrett *et al.*, 1996; Ingleby, 2007). The roots were cleared with 2.5% KOH clearing solution in water bath at 70°C for 1 hour. To remove phenolic substances, the bleaching solution was added after rinsing the roots in water 4 times and then left standing in the solution for 30 minutes. After that, roots were rinsed again with tap water; acidified with 1% HC1 and left for 1 hour. The HCl was decanted and separated from the roots without rinsing

them. The Trypan blue staining solution was added to the roots and left standing for at least 24 hours after which they were decanted and a storage solution was added to destain the roots (Ingleby, 2007). Twenty pieces of fine roots segments cut into pieces of almost 1cm long each were randomly picked and mounted on slides. They were then observed under a compound microscope to assess the intensity of infection by AMF used for observation.

3.6. Production of soil inoculums

AMF soil inoculums were produced following the methods described by Brundrett *et al.* (1996) and modified in Ingleby (2007) were followed. After trapping, 26 isolates were selected to produce inoculum. Single species (of at least 50 spores) of the selected isolates were used to grow soil "crude" inoculum in the greenhouse using a sterile substrate (soil and sand mixed at a proportion of 1:1 (w/w)), with sorghum as the trap plant. The spores of the selected isolates were introduced to the soils and mixed (Figure 8). (Habte and Osioro, 2001; Berutti *et al.*, 2016).

AMF Spores inoculation

Soil inoculated

Soil inoculum (roots + spores)
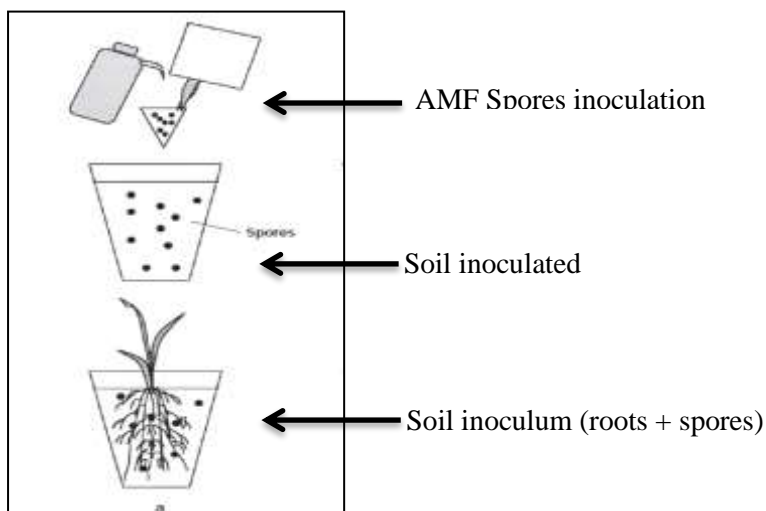
Figure 8: AMF monospecies inoculum production setup (Source: Habte and Osioro, 2001)

The soil used was collected from Kabete field station and responded to the criteria of slight acidity (its pH was 6.12) and low available P content (4.6 mg kg^{-1}). It was sterilized in an autoclave at 120°C for 30 minutes. Pots of 500ml capacity pots were filled with the mixed substrate and pierced at the bottom for drainage.

Sorghum was used for the inoculum production since it is a highly mycorrhizal, high roots density plant and more resistant than maize (Habte and Osioro, 2001). Three healthy pregerminated seeds were sown in each pot before applying approximately 1 cm of sterile

sand over to prevent cross contamination. The nutrient solution for mycorrhizal plants, adapted from Ingestad by Ingleby (2007) was used, mixed with irrigation water. Its composition is presented in the Appendix 4. Irrigation was done 2 to 3 times per week to field capacity.

To determine the inoculum potential, fine roots were collected and assessed for colonization while spores from 50 g of soil from each selected treatment was used to assess the density of viable spores. Roots colonization was determined using the Trypan blue method and the density of viable spores determined by the number of spores extracted (Brundrett *et al.,* 1996). Five strains were selected to be tested as biofertilizer based on the highest colonization percentage and spores density, as presented in the Appendix 5. They were named (as they represent) AMF1 (*Gigaspora gigantea*). AMF2 (*Gigaspora sp.*), AMF3 (*Gigaspora margarita*), AMF4 (*Rhizophagus intraradices*) and AMF5 (*Acaulospora. reducta*) respectively (Appendix 6).

3.7. Influence of indigenous Arbuscular Mycorrhizal Fungi on maize growth, P and Zn uptake and root colonization.

An experiment was established in the greenhouse at Kabete field station to evaluate the effects of the inoculum produced from infigenous AMF, the mineral P fertilization and the commercial fungal inoculant on plant biomass, nutrients uptake and root colonization.

3.7.1. Soils preparation

Two soils were used in the greenhouse. The first, locally called Kalongo, which is reddish, acidic and less productive, and classified as Ferralsols (FAO, 2015), was collected from the Walungu territory in South Kivu (Bagula *et al.*, 2014) and the second a Nitisol from Kabete field station (FAO, 2015; Jones *et al.*, 2013; Karuku *et al.*, 2012). These two soils are among the dominant agricultural soils in the origin area of the strains in South Kivu (Ngongo *et al.*, 2009; Bagula *et al.*, 2014; Bashagaluke, 2014). Soils were potted two weeks prior to planting and about 4 kg of soil placed in a 5 L plastic pot.

The two soils were subject to physical and chemical analyses as described in the section 3.3.

3.7.2. Experimental design and treatments

The experiment was carried put in a Randomized Complete Block Design (RCBD) with three replicates. In total 8 treatments comprising of the five strains of AMF selected as most promising AMF isolates namely AMF1, AMF2, AMF3, AMF4 and AMF5 (Table 3), , two controls: P and -P mineral fertilizer, and Rhizatech commercial AMF inoculant (obtained

from Dudutech Company Ltd, Kenya).With three replications for each treatment, the total number of treatmest was 48.

Table 1 presents the treatments, species compositions and forms of application and the locations coordinates of the fields where these strains were collected.

Table 1: Description of the treatments applied in the experiment

Treatment	Rate	Form of application	Specie composition
1. AMF1	100 g (per pot)	Soil inoculum	*G. gigantea*
2. AMF2	100 g (per pot)	Soil inoculum	*Gigaspora sp.*
3. AMF3	100 g (per pot)	Soil inoculum	*G. margarita*
4. AMF4	100 g (per pot)	Soil inoculum	*R. intraradices*
5. AMF5	100 g (per pot)	Soil inoculum	*A. reducta*
6. Rhizatech	50 g (per pot)	Soil inoculum	Mixture *(G. mosseae, G. intraradices, G. etunicatum, and G. aggregatum)*
7. Control-P			-
8. Control+P	45kg P_2O_5 ha^{-1}	DAP	

Figure 9 presents the experimental design used and the random arrangement of the pots in the greenhouse.

In Ferralsol								
Rep1	AMF4	Ctrl+P	AMF3	AMF2	Rhizatec	AMF1	Ctrl-P	AMF5
Rep2	AMF3	AMF2	AMF1	AMF5	Ctrl-P	AMF4	Ctrl+P	Rhizatec
Rep3	AMF1	Ctrl-P	Rhizatec	Ctrl+P	AMF4	AMF5	AMF3	AMF2
In Nitisol								
Rep1	Ctrl-P	AMF4	AMF2	AMF3	Rhizatec	AMF1	AMF5	Ctrl+P
Rep2	AMF2	AMF5	Ctrl-P	AMF3	Ctrl+P	Rhizatec	AMF4	AMF1
Rep3	Rhizatec	Ctrl+P	AMF1	AMF4	Ctrl-P	AMF3	AMF2	AMF5

Figure 9: Experimental design and treatments layout in the greenhouse

Basal application of N and K were applied at the rate of 100 kg N ha^{-1} and 60 kg K ha^{-1} in form of Urea and KCl (muriate potash). A split application of urea was done at sowing (50 kg ha^{-1}) and at 4 weeks after sowing (50 kg ha^{-1}).

The maize HD 02, a hybrid variety with a growth period of 3.5 months was used and inoculation was done at the sowing day. The inoculums, 100 g of soil containing at least 2 spores g^{-1} and sorghum chopped roots were mixed with the soil soil before transplantation. The seeds of maize were surface sterilized and pregerminated one week before transplanting

two per pot. The nutrient solution (Appendix 4) was applied to plants once a week and the other days, plants were watered with tap water. to the experiment was held for 14 weeks after which destructive sampling was done. Shoots were cut off using a knife and soils containing roots were taken to the laboratories for analyses. The greenhouse temperature was recorded using a thermometer and it was varying between 20 ^{o}C and 33°C.

3.7.3. Data collection

For the plant growth, plant height, chlorophyll content and shoot dry biomass were taken. The height was observed at 7 weeks and 11 weeks after transplantation (WAT) using a tape measure, and consisted of the height from the collar to the top of the youngest fully developed leaf. The chlorophyll content was recorded one at 11 weeks using a SPAD chlorophyll meter (SPAD 502 Plus) (Gekas *et al.*, 2013). Each observation was done in duplicate in each treatment.

For the nutrients uptake, dried shoot samples were ground and analyzed for total P and Zn. Phosphorus concentration in the shoot was determined after a wet digestion of the dried plant sample with a mixture of concentrated sulphuric acid (H_2SO_4), selenium powder and salicylic acid, and measured using a spectrophotometer (Spectronic 1001), while Zn level in the shoot was quantified using atomic absorption spectrophotometer (SBUCK 210 VGP) preceded by the digestion (Okalebo *et al.*, 2002).

Fine maize roots were sampled at the end of the experiment with two replicates per treatment. Roots were rinsed, cleared by boiling in 2.5% (w/v) KOH for 15 minutes in autoclave, bleached acidified and stained with Trypan blue in lactoglycerol following the method described in Ingleby (2007). Thirty pieces of 1cm roots fragments per treatment were mounted on slides and coverslip in PVLG, and then placed under a compound microscope to observe the fungal propagules. The colonization frequency was recorded as the number of root fragments infected with AMF propagules as presented in Brundrett *et al.* (1996).

The hyphal phosphorus contribution was calculated to evaluate the contribution of mycorrhizae in the total phosphorus uptaken (van der Heijden and Kuyper, cited by Gai *et al.*, 2006) using the formula:

$$\text{Hyphal P contribution(\%)} = [\frac{\text{P uptake of mycorrhizal plant } - \text{ P uptake of no mycorrhizal plant}}{\text{P uptake of mycorrhizal plant}}] \times 100$$

3.8. Data computation and statistical analysis

The ecological diversity index used was the Shannon-Weiner diversity index as it is a useful measurement of communities' diversity since it takes into account the species richness and evenness at the same time (Weaver and Shannon, 1963). The Shannon-Weiner index was computed according to the formula: $Sh\ (H) = P(Xi/X_0)\ \log(Xi/X_0)$ [Equation 8], where $Xi =$ spore abundance for an individual species and $X_0 =$ total spore abundance of the population of all the glomale species. The Hierarchical Cluster Analysis (HCA) based on Ward's minimum variance and Euclidian distance was applied to determine the natural cluster in the AMF communities using XLSTAT Software. Species composition in relation to environmental variables was analyzed by the Principal Component Analysis (PCA) using the Canoco 5.11 software (Šmilauer and Lepš, 2014). Spores abundance, plant biomass, chlorophyll accumulation and nutrient concentration were subjected to analysis of variance (ANOVA). Before the analysis, the normality test was performed to check the data normality.. Means were separated using the Fisher Least Significant Difference (LSD) test at 0.05% p-value. Roots colonization percentages were separated using t-test.

CHAPTER FOUR: RESULTS

4.1. Occurrence and diversity of AMF and natural mycorrhizal infectivity potential of soils from maize agroecosystems

4.1.1. Soils physical and chemical properties

Sandy soils were more dominants in the lowland, especially in Luvungi and Bwegera, and in the midland in Luzira. Soils from the highland and midland sites had more clay content. In general, soil texture was variable across the sites and varied from clayey to sandy loam. The chemical properties of the selected soil parameters indicate that soils in the region varied considerably (Table 2). The pH varied between 4.32 and 7.28. The lowland soils are neutral while the highland and midland 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 mg kg^{-1}), medium (17 - 34 mg kg^{-1}) to high (>34 mg kg^{-1}) P contents. The Organic C is mostly moderate (1-3%) to high (> 3%) and is spread almost uniformly in all the agroecologies. The CEC vary from low (<12 cmol kg^{-1}) to moderate (12 - 25 cmol kg^{-1}) with the lowest values present in the sandy soils in Luvungi and Bwegera and in Burhale and Luhihi. These classification were done according to Okalebo *et al.* (2002); Jones Jr (2001) and Hazelton and Murphy (2016). The Al could not be classified as high or low since plants differ in their sensitivity to it, but also AMF can have a beneficial or toxic effect on plants, depending on many other factors like its concentration, its chemical form, the growth condition and the plant species (Bojórquez-Quintal *et al.*, 2017) and plant species react differently to the levels of Al.

Table 2: Physical and chemical properties of soils in the study area

Parameter	Lowland			Midland						Highland		
	Bwegera	Kamanyola	Luvungi	Katana	Kavumu	Luhihi	Kasheke	Luzira	Mulamba	Munanira	Burhale	Mugogo
pH 1:2.5(H_2O)	6.50±0.09	6.98±0.3	6.67±0.4	6.27±0.6	5.80±0.7	5.47±0.7	4.92±0.6	5.58±0.2	5.17±0.4	5.4±0	5.03±1.4	5.39±1.2
P Bray1 (mg kg^{-1})	42.92±40.4	83.44±29.8	28±8.5	57.1±38.	56.92±13.5	13.91±13.2	27.06±14.3	15.23±5.6	17.04±15.3	80.95±0	7.62±1.19	33.25±25.3
Organic C (%)	1.88±0.3	3.54±1.3	3.20±1.6	3.3±0.4	5.06±1.2	2.77±0.1	2.84±0.6	2.97±0.4	2.10±0.8	3.34±0	2.12±0	2.38±1.8
CEC(cmol+kg^{-1})	4.93±2.4	22.55±7.2	7.3±3.4	14.76±3.5	15.46±2.9	9.62±1.3	10.21±3.2	11.81±3.4	8.10±2.08	17±0	8.8±0	12.38±6.0
Exch Al^{3+} (cmol+kg^{-1})	0±0	0±0	0±0	0±0	0.52±0.09	0.06±0.03	0.42±0.08	0±0	0.31±0.34	0±0	0.14±0	0.04±0.00
Soils texture classes*	Sandy loam	Clay	Sandy clay loam	Clay	Clay	Clay	Clay loam	Sandy clay loam	Clay	Clay	Clay	Clay

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

4.1.2. Species composition

In total, 45 AMF morphotypes wre recovered. They were found to belong to 11 different genera. Thirty two species could be unequivocally identified, while 4 strains were not distinguishable but had similarities. They were affiliated to the species that they have more overlapping morphological features with (e.g. *S. cerradensis* aff.). Seven strains were not described up to the species levelsince they lacked enough distinct features, could not be named at the species level (e.g. *Glomus sp*; *Gigaspora sp.*). One species was not identified; it seemed to have not been described yet and might presumably be found for the first time (species L, Figure 10).

The majority of species obtained belonged to Gigasporaceae (19 strains) with 6 strains in the Gigaspora Gedermann and Trappe, 5 strains in the Scutellospora Walker and Sanders and 4 species in each of the Dentiscutata Sieverd., F.A. Souza & F. Oehl and Racocetra Sieverd., F.A. Souza & F. Oehl genera. The Acaulosporaceae (15 strains) came in the second position with all the strains in the genus Acaulospora Gederman and Trappe, followed by the Glomeraceae (7 strains) with 3 strains of Glomus Tul. & C. Tul. emend C. Walker & A. Schüßler, 2 strains of Funneliformis C. Walker & A. Schüßler and 2 strains of Rhizophagus C. Walker & A. Schüßler. The Clariodeoglomeraceae appeared with one species in the Clariodeoglomus C. Walker & A. Schüßler, same as for Diversisporaceae with one species in the genus Globifera C. Walker & A. Schüßler and Pacisporaceae with one species in the genus Pacispora Sieverd. & F. Oehl.

4.1.3. Diversity and occurrence in the field and trap culture soils

The occurrence frequency, Shannon-Weiner diversity index, species richness in each site and spore densities are presented for both field soils and trap culture soils (Table 3 and 4). Based on the assumptions made for the species diversity and the need to emphasize on species richness, the Shannon-Weiner H diversity index was used. Species diversity differed within agroecologies (Table 3), with higher diversity obtained in soils from Kamaniola in the lowland (with diversity index H of 2.292); Mulamba (2.11), Katana (2.05) and Kasheke (2.04) in the midland and Burhale (2.2), Mugogo (2.43) and Munanira (2.23) in the highland.

Some species of AMF were generalists, since they were found to be ubiquitous in all the agroecologies and they are: *Acaulospopra excavata, Acaulospopra bireticulata, Dentiscutata erythropa, Funneliformis mosseae* and *Scutellospora pellucida*. Of these, *S. pellucida* was prominent in the midland followed by the lowland then less abundant in the highland. The

other species with a high occurrence percentage were *A. bireticulata, F. mossae, Gigaspora margarita, Gigaspora rosea, Glomus sp, R. castanea* and *Racocetra sp*, each of them occurring at least in 25% of the twelve field sites.

Five species were found exclusively in the highland, which include *A spinosa*, *Acaulospora sp2, Funneliformis mossae aff., Racocetra corraloidea* and the unidentified species. Three other species were exclusively found in the lowlands, namely *Acaulospora capsicula*, *Clariodeoglomus sp*, and *F. corronatum.*

Figure 10 presents representative images of plates of the AMF morphotypes recovered from the samples collected in the study area. The description is presented in Appendix 2.
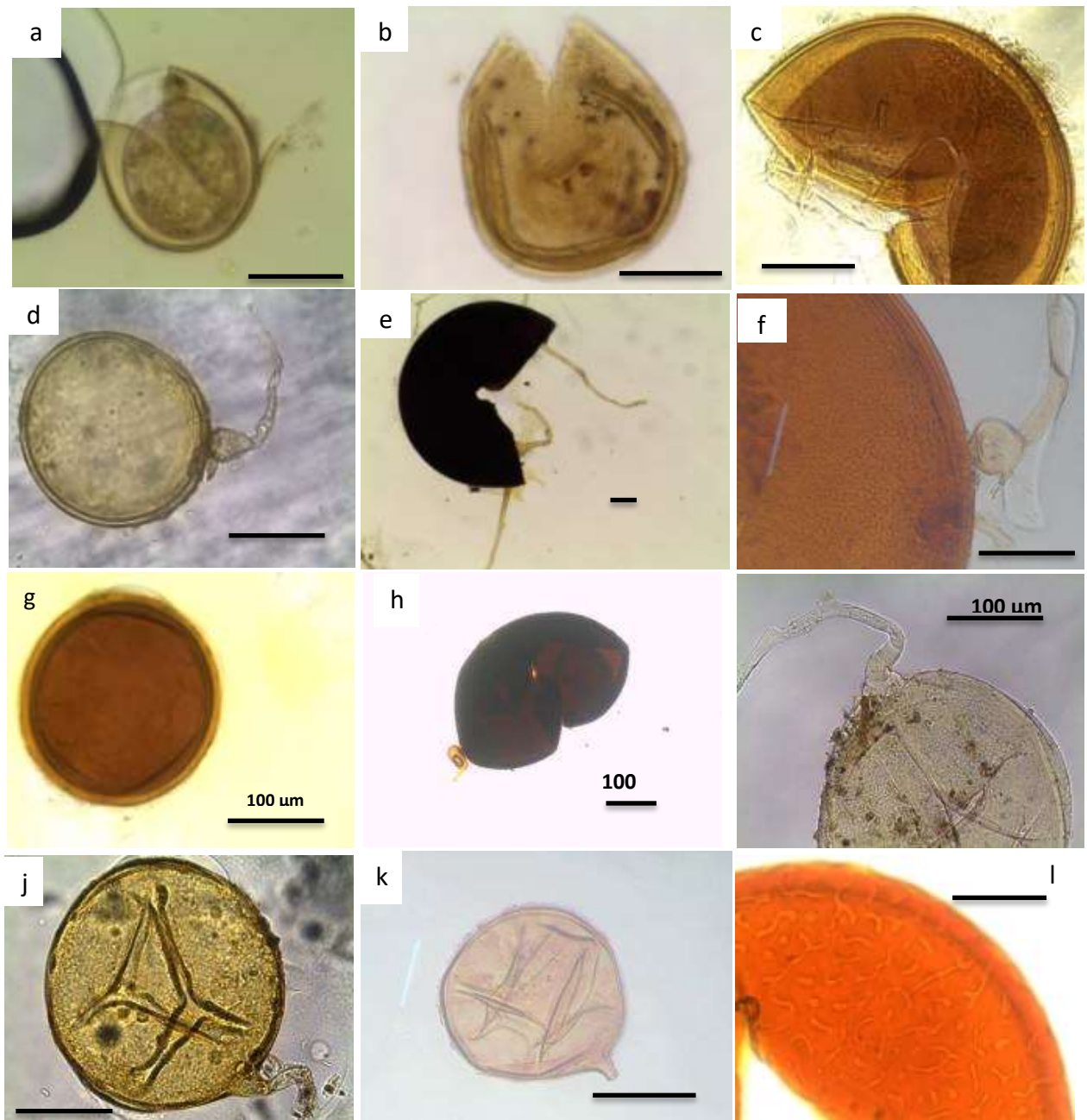
Figure 10: AMF Spores isolated from rhizosphere of maize croplands in South Kivu.

a) *Acaulospora spinosissima* ; b) *Acaulospora reducta*, c) *Acaulospora spinosa*; d) *Gigaspora margarita;* e) *Gigaspora albida*; f) *Gigaspora gigantean*; g) *Glomus ambisporum*; h) *Dentiscutata nigra*; i) *Funneliformis mossae* aff.; j) *Funneliformis mossae;* k) *Funneliformis mossae*, l) New, unidentified species

Each scale bar represents 100µm.
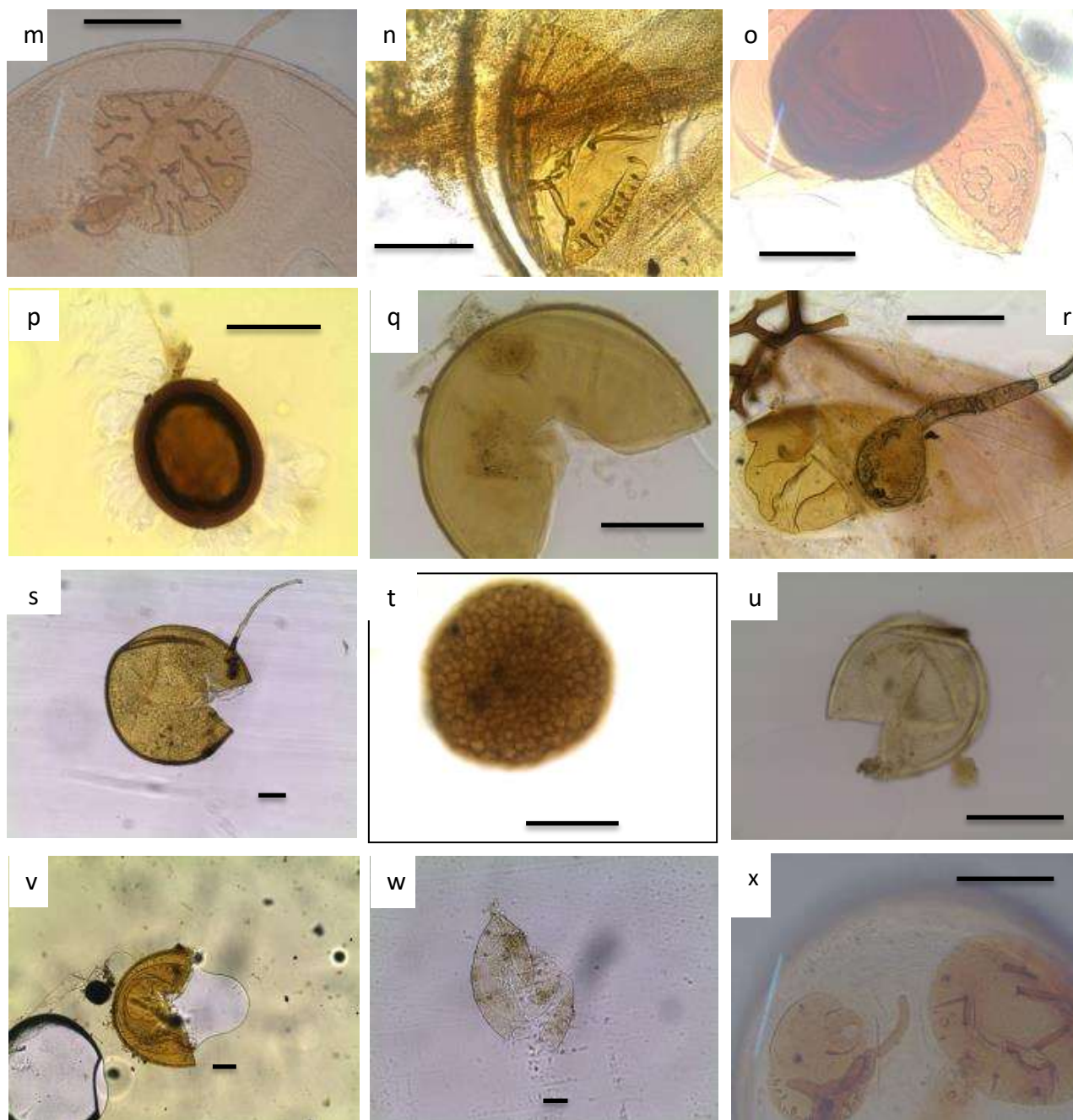
Figure 10 continued: m) *Scutellospora castanea* ; n) *Dentiscutata erythropa* ; o) *Scutellospora calospora*, p) *Glomus ambisporum* ; q) *Scutellospora sp.* ; r) *Racocetra pellucida ;* s) *Gigaspora rosea* ; t) *Acaulospora bireticulata* ; u) *Acaulospora reducta* ; v) *Pacispora robiginia* ; w) *Scutellospora pellucida* ; x) *Scutellospora scutata.*

Each scale bar represents 100µm.

Tables 3 and 4 present the AMF morphotypes occurrence, specific spore densities and their Shannon-Weiner H index as observed in the field soils and trap soils respectively.

Table 3 : Species occurrence frequencies, spores abundance, and Shannon-Weiner H diversity index in the field soils

Species*	Lowland			Midland						Highland			Occurrence frequency (%)
	Bwegera	Luvungi	Kamaniola	Luzira	Kasheke	Katana	Luhihi	Kavumu	Mulamba	Munanira	Burhale	Mugogo	
A. alpine				1(9)									8
A. bireticulata			1(29)					1(10)		1(10)	1(10)		33
A. capsicula		1(10)											8
A. denticulata								1(11)					8
A. excavata		1(20)	1(24)			2(12)				1(2)		1(17)	50
A. foveata							1(4)						8
A. reducta			2(54)	1(6)									25
A. rehmii			1(27)							2(18)	1(15)		33
A. scrobiculata						1(18)					1(7)		17
A. scrobiculata aff.					1(3)				1(23)				17
A. spinosa												1(20)	8
A. spinosissima			1(20)					1(3)					17
A. tuberculata					1(18)								8
Acaulospora sp.1					1(8)								8
Acaulospora sp.2								1(3)				1(14)	17
Clariodeoglomus sp.			1(20)										8
D. heterogama								1(4)			1(30)		17
D. nigra				1(19)			1(4)		2(24)		1(11)	1(10)	50
D. reticulate					1(1)				1(8)			1(1)	25
D. erythropa			1(14)				1(22)		1(72)			1(16)	33
D. globifera										1(4)	1(12)		17
F. coronatum			1(5)										8
F. mossae			1(20)					2(30)			1(10)	1(4)	42
G. albida								1(3)					8
G. gigantean	1(32)			1(3)	1(24)		1(20)						33
G. margarita							2(22)	1(21)		1(12)			33
G. rosea				2(20)						1(8)	1(13)		33
Gigaspora sp.				1(13)	1(16)	2(26)				1(12)	1(18)	1(10)	58
G. decipiens	1(10)												8
G. ambisporum							1(15)	1(10)				1(20)	25
Glomus sp.1			1(5)		1(14)	1(4)	1(12)		1(8)				42
Glomus sp.2						1(20)			1(8)			1(18)	25
P. robiginia						1(12)							8
R. castanea	1(16)					1(29)			2(28)	1(26)			42
R. coralloidea											1(8)		8
R. fulgida					1(24)						1(2)		17
Racocetra sp.		2(33)	1(9)						1(18)				33
R. fasciculatus aff.					1(24)								8
R. intraradices									2(26)				17
S. calospora aff.					1(8)								8
S. pellucida	2(23)	1(5)		1(4)	1(4)		1(30)			1(20)	1(2)	1(15)	75
S. scutata								1(50)					8
S. cerradensis aff.										1(20)			8
Scutellospora sp.			1(13)							1(10)		1(10)	25
Unidentified sp												1(8)	8
Total spores number	**81**	**68**	**240**	**74**	**128**	**137**	**129**	**122**	**239**	**142**	**130**	**171**	
Shannon H	**1.4**	**1.1**	**2.2**	**1.8**	**2.1**	**2.0**	**1.9**	**1.6**	**2.1**	**2.2**	**2.2**	**2.1**	
Species richness	**4**	**4**	**12**	**7**	**9**	**9**	**8**	**8**	**11**	**11**	**11**	**13**	

Table 4: Species occurrence frequencies, spores abundance, and Shannon-Weiner H diversity index in the trap culture soils

	AEZs												Occurrence frequency (%)
	Lowland					Midland				Highland			
Species*	Bwegera	Luvungi	Kamaniola	Luzira	Kasheke	Katana	Luhihi	Kavumu	Mulamba	Munanira	Burhale	Mugogo	
A. alpina				1(25)									8.3
A. bireticulata								1(28)		1(21)	1(20)		25.0
A. brasiliensis						1(10)							8.3
A. capsicula		1(19)											8.3
A. dilatata					1(11)								8.3
A. foveata aff.			1(50)										8.3
A. excavata			1(19)			1(10)						1(21)	25.0
A. foveata			1(15)										8.3
A. reducta			11(92)						1(7)				25.0
A. rehmii			1(48)				1(5)			11(78)	1(25)		33.3
A. scrobiculata						1(60)							8.3
A. scrobiculata aff.				1(12)					1(31)				16.7
A. spinosa											1(26)		8.3
A. tuberculata			1(9)		1(21)								16.7
Acaulospora sp.	1(7)	1(15)		1(8)		1(18)						1(40)	41.7
C. pellucida	11(40)	1(17)			11(118)		1(28)			11(41)	1(26)	1(78)	75.0
D. erythropa							1(25)		1(110)		1(51)		25.0
D. globifera											1(21)	1(12)	16.7
D. heterogama		1(5)						1(17)			1(20)		25.0
D. nigra							1(8)		11(65)	1(18)	1(28)		41.7
D. reticulate					1(16)				1(47)			1(5)	25.0
F. coronatum		1(19)							1(29)				16.7
F. mossae		1(34)						1(15)			1(59)	1(16)	33.3
G. albida									1(53)				8.3
G. ambisporum							1(18)						8.3
G. decipiens	1(25)				1(52)								16.7
G. gigantea	1(42)			1(28)		1(24)							25.0
G. gigantea aff.					1(83)								8.3
G. margarita							11(22)		1(94)	1(8)			33.3
G. rosea				1(28)						1(23)	1(15)		25.0
Gigaspora sp.			1(14)		1(13)	2(43)				1(50)	1(31)	1(28)	58.3
Glomus sp.						1(20)			2(24)			1(30)	33.3
P. robiginia			1(14)			1(43)							16.7
R. castanea	1(23)					1(7)			2(35)				25.0
R. Coralloidea											1(10)		8.3
R. fulgida					1(64)						1(45)		16.7
R. intraradices									2(101)				16.7
Racocetra sp. aff.									1(75)				8.3
Racocetra sp.		2(37)								1(15)			25.0
R. fasciculatus aff.					1(25)								8.3
S. calospora							1(17)						8.3
S. scutata								1(31)					8.3
Scutellospora sp			1(21)			1(18)					1(5)	1(15)	33.3
Total spores number	**137**	**93**	**321**	**103**	**415**	**229**	**147**	**91**	**671**	**254**	**346**	**281**	
Shannon H	**1.48**	**1.45**	**2.06**	**1.57**	**2.05**	**1.97**	**2.17**	**1.34**	**2.31**	**1.86**	**0.41**	**2.16**	
Specie richness	**5**	**5**	**10**	**5**	**11**	**9**	**8**	**4**	**12**	**8**	**12**	**11**	

*the number 1or 2 before the brackets means the specific species was present one and two times. In the brackets is the number of spores counted of the specific species.

4.1.4. Spore densities in field and trap culture soils

There was a clear increase in the spores density between the field soils and the trap plants (Figure 10) , by a factor of two in some soils like the soil from Kasheke (0.7 to 2.3 spores g^{-1}) ; Mulamba (0.9 to 2.7 spores g^{-1}) and Burhale (0.7 to 1.8 spores g^{-1}). However, after trapping, the density in Kavumu's soils decreased (0.4 to 0.3 spores g^{-1}). The spore densities in the field soils were low, with the means varying from 0.28 to 1.16 spores spores g^{-1} of soil. No statistical difference were observed between agroecologies in their spore densities in the field soils (p>0.05).

Significant differences were observed in the soils from the fields, across the sampling sites (p<0.001; CV=33.12%, LSD= 0.079), with the highest density on average recorded in Mulamba and the lowest in Luzira. After trapping soils from Mulamba, Kasheke and Burhale produced the highest spore densities with 2.68, 2.11 and 1.73 spores g^{-1} respectively.
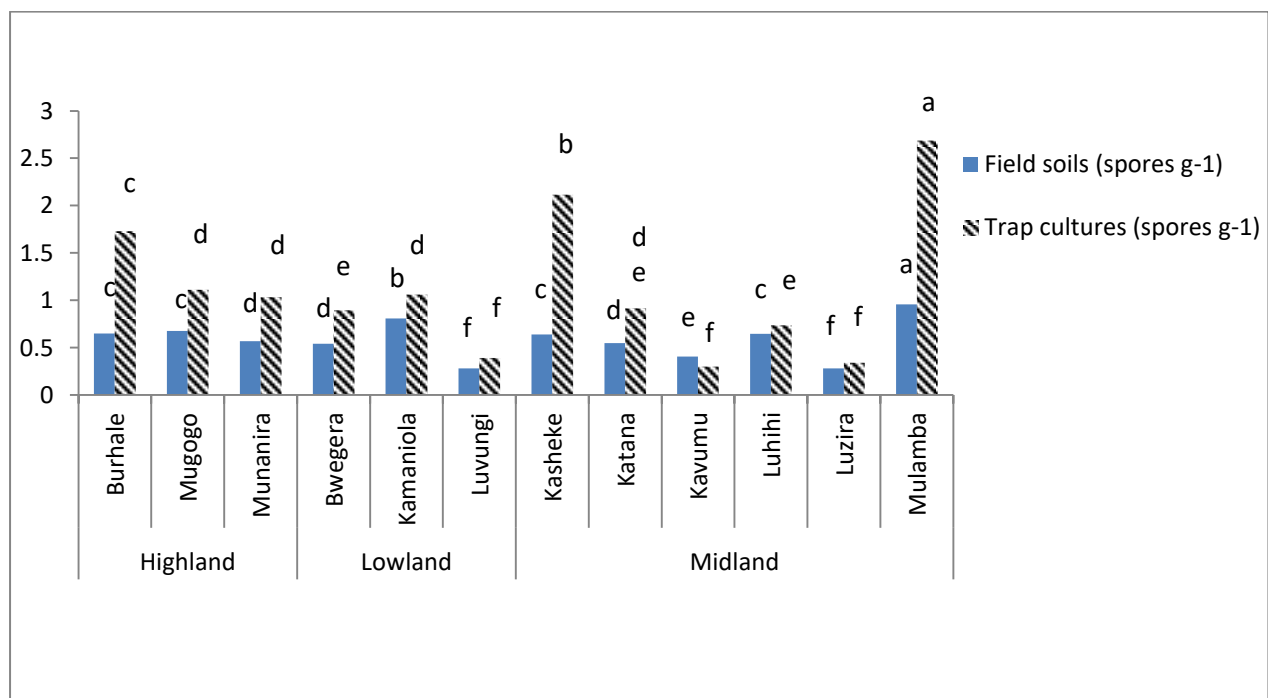
Figure 11: Spore densities in the field and trap culture soils

4.1.5. Sampling effort and morphotypes recovery

It was observed that in overall the more samples collected, the more new morphotypes were recovered in all the AEZs, as presented in the Figure 12. In each of the low and highland, 15 samples were collected and in the midland up to 30 were collected.

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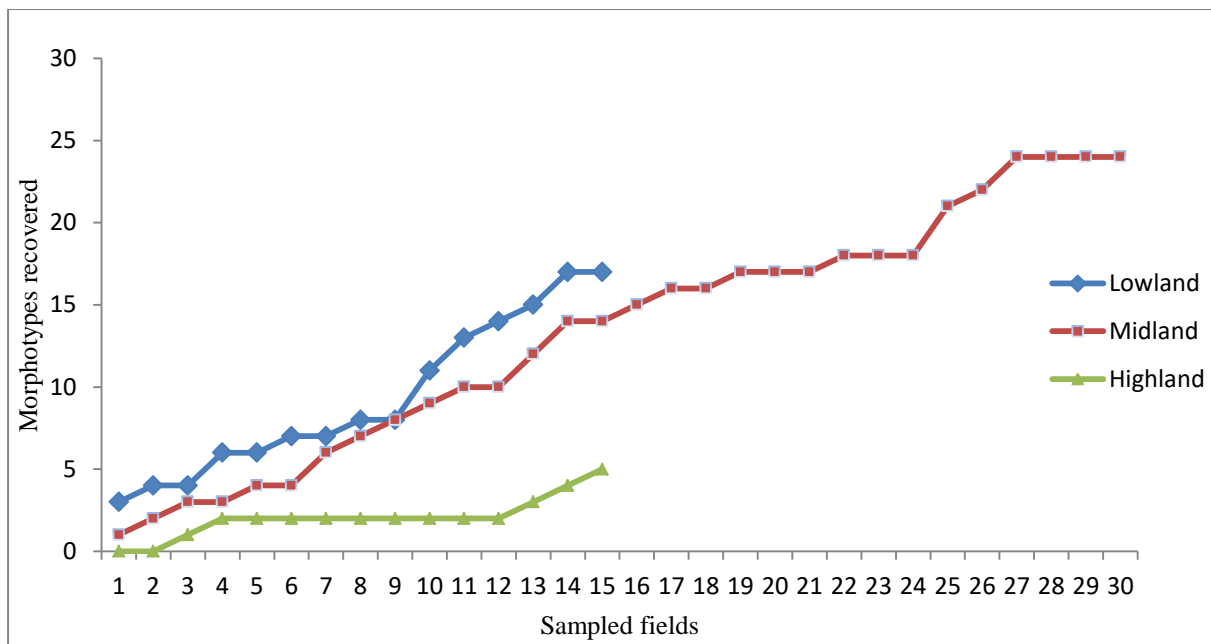
Figure 12: Sampling effort and morphotypes recovery

4.1.6. Species clustering

The dendrogram representing the similarities between the AMF morphotypes based on the chemical poperties of the field soils generated using the HCA is presented in the Figure 13. It is interesting to note that, though sampled in three different agroecologies and croppings systems, species from the Acaulospora and Glomus genera could be found in all the clusters. They are the most widespread in the region and more generalist, with the *Glomus sp.1* being present in the first cluster and last cluster. Species from the Gigaspora genus were also widespreadin the cluster but to a lesser extent.

Glomus sp.1
A. denticulata
G. albida aff.
D. nigra
Glomus sp.2
R. intraradices
R. castanea
D. reticulata
Acaulospora sp.2
R. coralloidea
D. nigra
D. erythropa
New species
R. fasciculatus aff.
A. tuberculata
S. pellucida
S. pellucida
Gigaspora sp.
D. nigra
D. erythropa
G. margarita
S. pellucida
G. margarita
G. gigantea
G. ambisporum
D. nigra
D. erythropa
G. rosea
D. heterogama
D. globifera
R. fulgida
S. pellucida
A. rhemii
D. reticulata
D. nigra
G. margarita
G. ambisporum
A. scrobiculata
Glomus sp.1
Gigaspora sp.
R. castanea
A. alpina
S. pellucida
Gigaspora sp.
D. erythropa
A. scrobiculata aff.
R. castanea
Racocetra sp.
G. rosea
G. gigantea
D. nigra
R. intraradices
Glomus sp.1
G. gigantea
D. reticulata
Gigaspora sp.
R. fulgida
A. scrobiculata aff.
Glomus sp.1
G. margarita
A. bireticulata
G. rosea
S. cerradensis aff.

Figure 13: Dendogram representing similarity between AMF morphotypes

The species recovered exclusively in the highland which are *A. spinosa, Acaulospora sp2., F. mossae aff., R. coraloidea* and the new species did not appear pertaining in the same clusters,

some for the *A. capsicula, Clariodeoglomus sp.,* and *F. coronatum* that were recovered exclusively in the soils from lowland.

4.1.7. Influence of soil properties on species occurrence

Projections presenting species occurrence as influenced by environmental soil factors on the factorial plan showed that Axes 1 and 2 accounted respectively for 50.14 and 25.07% of variability between sites (Figure 14). The organic carbon, CEC, P and pH were positively correlated among them but all were negatively correlated with the Al. The occurrence of the majority of spores is negatively correlated with Al and P, which show a very distinct distance with the axes and from other soil parameters. This implied that the higher the pH, the lower the species occurrence, the higher the exchangeable Al the lower the species occurrence and the higher the pH the lower the Al and vice versa. The distribution of the most dominant families, namely the Gigasporaceae, Acaulosporaceae and Glomeraceae, is mostly influenced by the Al and pH. A large number of species occurred in soils with lower organic carbon, lower CEC, lower available P specifically, and tended to prefer lower pH with lower available Al soils.
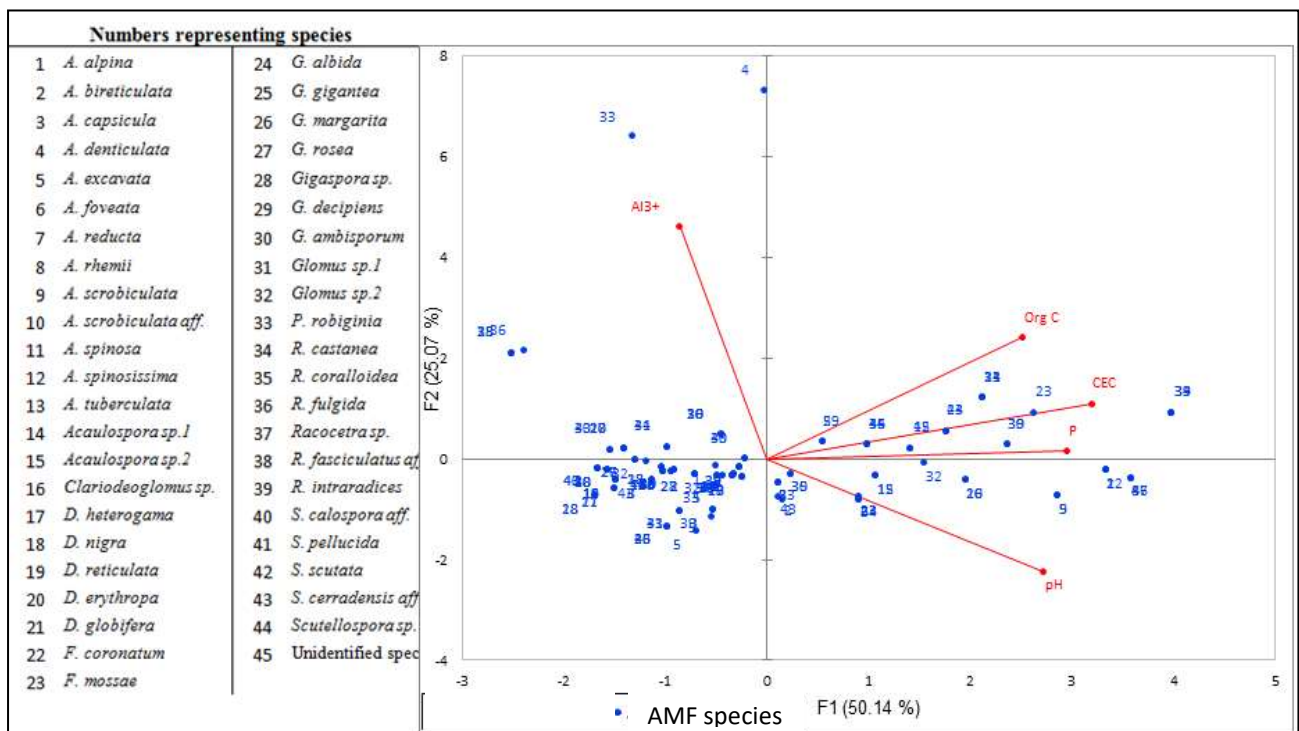
Figure 14: Principal Component Analysis of the species occurrence related to soil properties

4.1.8. Infectiveness potential of indigenous mycorrhizal fungi

The number of propagules (arbuscules, vesicles and hyphae) counted in the 20 cm maize root segments (Figure 15) and a significant difference between the mycorrhizal potential of the

different soils was observed (p<0.001; LSD= 38.8 and CV=62.7%). In total 21.66% of field soils under investigation were as infective as the Rhizatech biofertilizer used as control in the root colonization and these highly myzorrhizal soils were found to be in all the three AEZs. At least 3 soils from the highland, namely KMun1,KMun3 and WMug6; 7 from the midland, namely Kbkat2, Kbkav1, Kbkav7, Kbluh6, WMul2, WMul6 and Kkash4, and 3 in the lowland, namely Pkam7, Pkam5 and ULuv4 outperformed the Rhizatech, in terms of number of AMF propagules produced in the maize root.
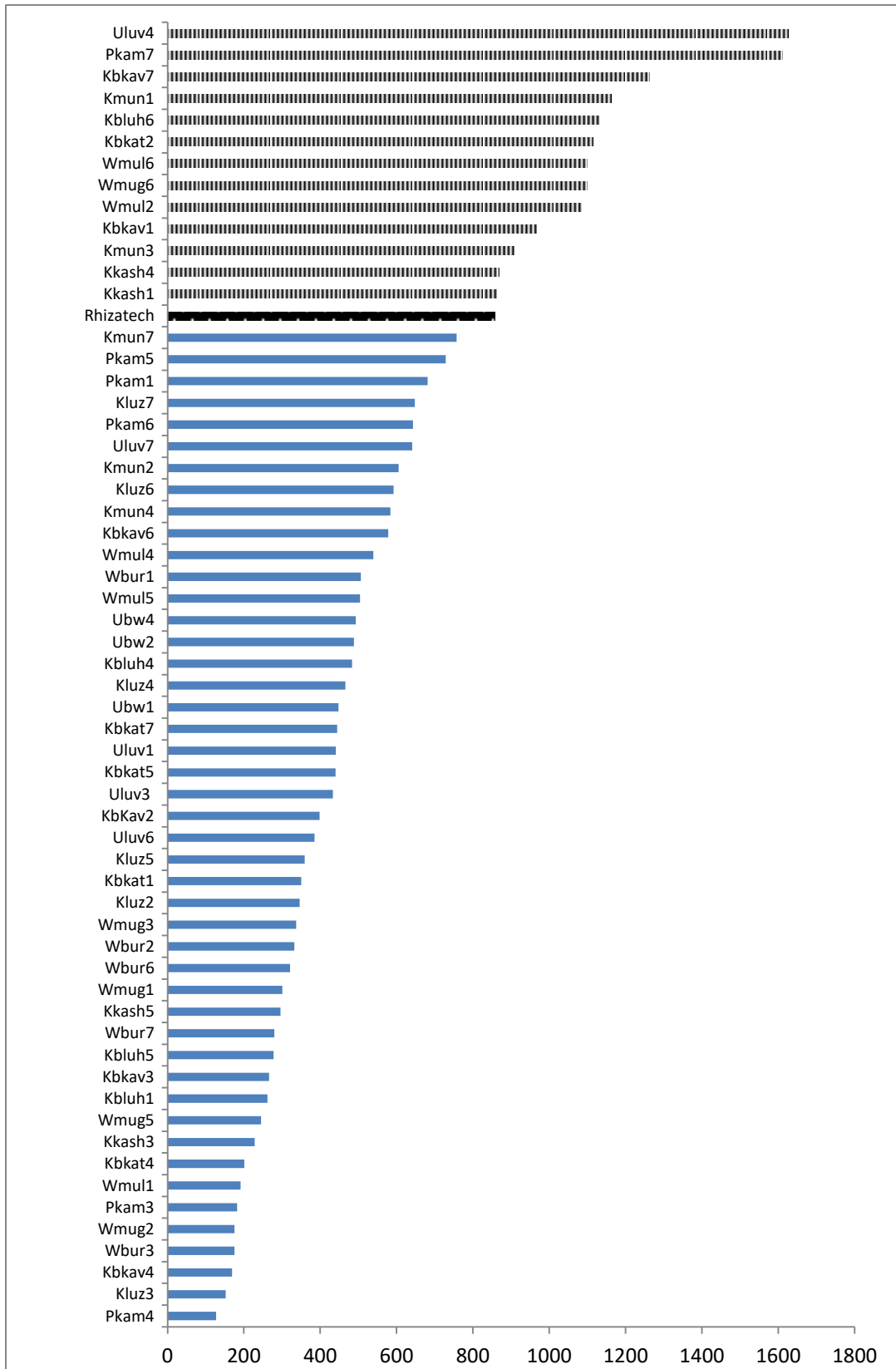
*The codes used to represent the AMF strains represents the territories and villages of origin and the number correspond to the number of the field sampled in the specific village:Kmun=Kalehe Munanira, Kluz=KaleheLuzira, Kkash=Kalehe Kasheke, Kbkat=Kabare Katana, KLuh=Kabare

Figure 15: Total number of propagules observed in maize roots

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Luhihi, WMul=Walungu Mulamba, WBur=Walungu Burhale, WMug=Walungu Mugogo, PLuv=Plaine Luvungi, Pkam = Plaine Kamaniola.

4.2. Influence of indigenous Arbuscular Mycorrhizal Fungi on maize growth, P and Zn uptake under controlled conditions

4.2.1. Physical and chemical properties of the experimental Ferralsol and Nitisol

The physical and chemical properties of the selected soil parameters of the Ferralsol and Nitisol used in the experiment are presented in the Table 5. Both soils were acidic with the Ferralsol being of moderate acidity and the Nitisol of low acidity. The available P was moderate with an average of 15.75 mg kg^{-1} in Ferralsol and high with average of 27.65 mg kg^{-1} in Nitisol, considering the moderate range of 13-27 mg kg^{-1} (Jones *et al.*, 2001), and the difference between these two soils was significant (p=0.024). The total nitrogen was low and medium, same as for the organic carbon in the Ferralsol and the Nitisol respectively, with no significant difference. The CEC varied between the two soils and was low (10.5 cmol kg^{-1}) in the Ferralsol than in the Nitisol (21.2 cmol kg^{-1}) with a significant difference between the two (p=0.043). There was a low density of AMF spores in both soils.

Table 5 : Physical and chemical properties of the experimental Ferralsol and Nitisol

Soil property	Units	Ferralsol (Mean±SD)	Rating	Nitisol (Mean±SD)	Rating
pH H$_2$O		5.77±0.12	Moderate acidity	6.3±0.07	Low acidity
Available P	mg kg^{-1}	15.75±1.9	Moderate	27.65±1.6	Moderate
Total N	%	0.12±0.01	Low	0.17±0.02	Medium
CEC	cmol kg^{-1}	10.5±2	Low	21.2±3.7	Moderate
Organic C	%	0.78±0.52	Low	1.89±0.14	Moderate
Sand	%	29±1		40±2	
Silt	%	26±3		22±1	
Clay	%	45±2		38±1	
Texture class		Clay		Sandy clay loam	
AMF population	Spores /g soil	1.23±0.15		0.87±007	

The analyses were done in duplicate and the ratings done according to Okalebo *et al.* (2002); Jones Jr (2001) and Hazelton and Murphy (2016).

4.2.2. Influence of indigenous Arbuscular Mycorrhizal Fungi on maize growth, P and Zn uptake

Table 6 presents the plants height, chlorophyll content and the shoot dry biomass in the Ferralsol. Significant differences were observed (p<0.001) for the height of plants with the Pi treatment having the best height at both 7 and 11 WAT (124 and 141 cm respectively). It was

followed by Rhizatech, AMF2, AMF4 and AMF5. The control-P gave the least height (68.8 cm at 11 WAT.

The chlorophyll content level varied among treatments. The P fertilization significantly increased the chlorophyll content at 7 WAT (p=0.009) and was equal with the treatment AMF2, AMF3 and AMF4. Rhizatech, AMF1 and Control-P recorded the lowest concentration of P at the 7WAT. At 11 WAT, some treatments increased significantly (p<0.001) their concentration and the highest cholorophyll content were recorded in AMF4 with the SPAD meter reading of 48.4. The, AMF1, AMF2, Rhizatech, AMF3, and Control +P, followed with 44.03, 43.9, 43.8, 41.8 and 41.1 values respeticively. The Control-P had the lowest Chlorophyll content.

Overall, the P application increased the dry biomass and recorded the highest biomass (39.2g; p<0.0001) over all the other treatments. It was followed by the treatment AMF2 (17.45g), which was also followed by AMF4, AMF5 and Rhizatech application which constituted the last group (Table 6).

Table 6: Maize height, chlorophyll content and shoot biomass at 7 and 11 WAT, in Ferralsol

Treatment	Height at 7 WAT (cm)	Height at 11 WAT (cm)	Chlorophyl(7 WAT)	Chlorophyl(11 WAT)	Shoot biomass (g)
AMF1	$84.66^b \pm 5.55$	$92c \pm 8.16$	$35.33^{bc} \pm 1.078$	$44.03^b \pm 0.85$	$7.316^d \pm 1.56$
AMF2	$91^b \pm 13.49$	$131.5^{ab} \pm 19.18$	$40.66^{ab} \pm 3.68$	$43.9^b \pm 2.45$	$17.45^b \pm 0.80$
AMF3	$67^c \pm 2.94$	$69^d \pm 4.54$	$42.33^{ab} \pm 2.29$	$41.83^b \pm 3.20$	$5.037^d \pm 0.37$
AMF4	$95.33^b \pm 7.40$	$121^b \pm 2.44$	$39.4^{ab} \pm 1.59$	$48.4^a \pm 1.88$	$12.57^c \pm 1.46$
AMF5	$91.33^b \pm 9.84$	$124^b \pm 14.69$	$36.06^{bc} \pm 4.16$	$35.3^c \pm 0.57$	$12.05^c \pm 1.18$
Control+P	$124.33^a \pm 4.78$	$141.5^a \pm 2.85$	$42.76^a \pm 2.08$	$41.13^b \pm 3.27$	$39.27^a \pm 0.96$
Control-P	$63.33^c \pm 2.35$	$68.83^d \pm 5.10$	$32.61^c \pm 1.94$	$34.6^c \pm 0.43$	$12.05^c \pm 2.85$
Rhizatech	$90^b \pm 8.16$	$134.33^{ab} \pm 6.54$	$38.16^b \pm 0.84$	$43.86^b \pm 0.61$	$11.58^c \pm 2.38$
Pvalue	**<0.001**	**<0.001**	**0.009**	**<0.001**	**<.0001**
LSD$_{0.05}$	**13.11**	**16.63**	**4.22**	**3.43**	**3**
CV%	**22.1**	**27.04**	**11.03**	**11.75**	**66**

Table 7 presents plants heights and chlorophyll content recorded at 7 and 11 WAT, and the shoot dry biomass at the end of the experiment in the Nitisol. The height varied significantly among treatments at 7 WAT. The Pi application increased significantly the height rapidly at 7 WAT (130.6cm) but at 11 WAt, the AMF4 and AMF5 had the best heights with 189 and 174.5 cm respectively (p<0.001). The chlorophyll content varied between treatments at 7 WAT (p=0.001) and at 11 weeks (p=0.047). At 7 WAT, the best results were obtained from

AMF1, AMF2, AMF3, AMF4, AMF5 and +Pi, and the last from Rhizatech and Control-Pi. At 11 weeks, the treatments AMF1, AMF2, AMF3, AMF4 still gave the highest chlorophyll content followed by Rhizatech, AMF5 and +Pi, and Control-P.

Treatments varied for the shoots dry biomass (p<.0001) (Table 7) and the highest biomass was from the control +P (47.7g) and AMF4 (44g). They were followed by AMF1 (31g), then by AMF3 (28.1g) and lastly AMF2, AMF5, Rhizatech and Control-P.

Table 7: Maize height, chlorophyll content and shoot biomass at 7 and 11 WAT, in Nitisol

Treatment	Height (7WAT) (cm)	Height (11 WAT, cm)	Chlorophyll (7 WAT)	Chlorophyll (11 WAT)	Shoot biomass (g)
AMF1	$107.33^{cd}\pm7.40$	$170^b\pm9.79$	$43.63^a\pm1.96$	$42.66^a\pm1.22$	$31.08^b\pm0.77$
AMF2	$91.33^{de}\pm5.43$	$159^{bc}\pm1.63$	$40.2^a\pm4.42$	$44.96^a\pm4.38$	$20.72^d\pm2.87$
AMF3	$98^{de}\pm5.88$	$160^{bc}\pm0.81$	$43.3^a\pm2.40$	$44.2^a\pm3.10$	$28.19^c\pm1.20$
AMF4	$117^b\pm5.71$	$189^a\pm13.88$	$42.3^a\pm2.69$	$44.4^a\pm2.12$	$44.06^a\pm1.92$
AMF5	$98.33^d\pm5.79$	$174.5^{ab}\pm11.02$	$40.46^a\pm0.75$	$40.43^b\pm2.36$	$17.47^{de}\pm0.67$
Control+P	$130.66^a\pm8.73$	$152^c\pm1.63$	$42.26^a\pm1.48$	$36.9^b\pm1.41$	$47.72^a\pm1.74$
Control-P	$93^{de}\pm1.41$	$126^d\pm11.22$	$32.06^b\pm2.28$	$40^b\pm1.63$	$17.47^{de}\pm2.22$
Rhizatech	$85.33^{de}\pm13.07$	$111.5^d\pm10.20$	$34.06^b\pm1.71$	$40.2^b\pm1.14$	$14.96^e\pm1.50$
P value	**0.002**	**<0.001**	**0.001**	**0.047**	**<.0001**
LSD	**12.62**	**15.39**	**4.17**	**4.12**	**5.55**
CV	**15.77**	**16.75**	**12.17**	**8.71**	**45.5**

Means followed by the same letter in a column were not significant at p=0.05; LSD = Least Significant Difference; CV = Coefficient of variation

The results obtained after quantification of P and Zn elements in the maize tissue and the hyphal P contribution calculated are presented in the Table 8.

In the Ferralsol, there was no significant difference between treatments (p=0.195) for the plant P content, but in the Nitisol, the difference was observed (p=0.015) and treatments were grouped into three groups, with the Pi fertilization giving the high P content along with AMF1, AMF2, AMF3, AMF5 and Rhizatech. AMF4 and Control were the second and last group respectively.

Plant Zn content differed in Ferralsol (0.007). AMF2, AMF1, AMF5 and Pi fertilization gave the highest contents, followed by AMF3 and Rhizatech. The control treatment resulted in the lowest Zn content. However, in the Nitisol, no significant difference was observed among treatments (p=0.414).

The Hyphal P contribution, being the difference between the P uptake in the mycorrhizal inoculant and the control, varied between 23.41 and 51.82 in the Ferralsol; with the Rhizatech and AMF3 having the highest hyphal P contribution. In the Nitisol, it varied between 28 .11 and 48.87.

Table 8: Plant P, hyphal P contribution and Zn concentrations

Treatments	Plant P (g/kg)		Hyphal P contribution (%)		Plant Zn (mg/kg)	
	Ferralsol	Nitisol	Ferralsol	Nitisol	Ferralsol	Nitisol
AMF1	1.12	1.70^{ab}	46.21	48.87	11.78^{ab}	10.56
AMF2	0.90	1.65^{ab}	33.33	47.16	13.70^{a}	8.51
AMF3	1.24	1.78^{a}	51.43	40.94	9^{bc}	6.39
AMF4	1.09	1.21^{b}	44.73	28.11	9.33^{b}	7.87
AMF5	0.78	1.66^{ab}	23.41	47.56	11.13^{ab}	11.15
Control+Pi	0.96	1.31^{ab}			10.71^{ab}	8.90
Control-Pi	0.60	0.87^{c}	0	0	5.84^{c}	8.13
Rhizatech	1.25	1.59^{ab}	51.82	45.16	6.83^{bc}	7.91
P-value	**0.195**	**0.015**			**0.007**	**0.414**
LSD$_{0.05}$	**-**	**0.4**			**3.18**	**-**
CV%	**30.2**	**20.8**			**27.5**	**24**

Means followed by the same letter in a column were not significant at p=0.05; LSD = Least Significant Difference; CV = Coefficient of variation

4.3. Influence of indigenous Arbuscular Mycorrhizal Fungi on root colonisation

The frequencies of colonization of maize roots are presented in the Ttable 9. The number of root fragments colonized were not significantly different between treatments in the Ferralsol (p=0.252) but they were significantly different in the Nitisol (p=0.005). AMF4, Rhizatech and AMF5 treatments colonized the most of roots, with 31.6%, 28.3% and 20% respectively. In the control, 11.6% of roots were colonized in the Ferralsol but none in the Nitisol.

Table 9: Root colonization frequency

Treatments	Ferralsol (%)	Nitisol (%)
AMF1	23.33	11.66[bc]
AMF2	18.33	10b[c]
AMF3	36.66	16.66[b]
AMF4	25	31.66[a]
AMF5	35	20[ab]
Control+Pi	11.66	0[c]
Control-Pi	6.66	13.33[b]
Rhizatech	45	28.33[a]
P-value	**0.252**	**0.005**
LSD$_{0.05}$	**-**	**12**
CV%	**45**	**54.2**

Means followed by the same letter in a column were not significant at p=0.05; LSD = Least Significant Difference; CV = Coefficient of variation

CHAPTER FIVE: DISCUSSION

5.1. Occurrence and diversity of AMF and natural mycorrhizal infectivity potential of soils from maize agroecosystems

5.1.1. Physical and chemical properties of soils from study sites

Acidic soils were found to be dominant in the study area. The soils from the midland (Kasheke, Luhihi and Mulamba) and highland (Burhale) were the most acidic, with pH < 5.5; while neutral soils predominate in the lowland Uvira (pH 6.5 - 6.9). This is the result of intense weathering and luxiviation due to high precipitations characteristic of the region. The acidity and the presence of Al in the mid and highland must have resulted from the natural conditions of soils formation in the highland and midland with mostly hilly lands, where alteration and removal processes have led to nutrients losses in the soils and this had earlier been confirmed by Crawford *et al.*(2008). These results agree with other researchers who have confirmed that 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 poor in P content, CEC and organic matter (Nziguheba *et al.*, 2016). The level of P in the soils varied from low to high; this could be associated with the diversity of the parental materials and of the agricultural organic or mineral inputs. The low organic residue incorporation, continuous tillage and conventional agriculture account for decline in the chemical status of the soils. This is consistent with Lambert *et al.*(1979) and Gomes *et al.* (2015) who found that the highland and midland could be more favorable for the proliferation of mycorrhization seeing the slight acidity conditions. From the clayey and sandy loam textures present, the area reveals a diversity of textures, meaning various soils physical properties like water retention, aeration and roots development (Brady and Weil, 2002). But since species distribution was not significantly different between AEZs, we can assume that the texture did not affect much the AMF distribution.

5.1.2. Species composition

The AMF morphotypes identified were many and various authors have described them also (Oehl *et al.*, 2006; Krüger *et al.*, 2011; Oehl *et al.*, 2014; Pereira *et al.*, 2016a ; Crossay *et al.*, 2018). This large number of species recovered could mean maize can associate with many

AMF species. It could not be very surprising since the sampling was done in different sites and agroecologies with very many variable edapho-climatic conditions. Furthermore, the diversity of fungal species has never been extensively studied in the region and in the world the number of described species is continuously increasing; it has passed from 150 species in 2003 (Oehl *et al.*, 2003) to 315 species in 2019. Therefore, some of the strains that were not identified might have not been described yet. Gigasporaceae, Acaulosporaceae and Glomeraceae constitute 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 ecosystems (Teixeira *et al.*, 2017; Pereira *et al.*, 2016a), which might be similar to the ecosystems in Congo. In Katanga, another province in DR Congo, 65 different spore morphotypes were isolated from the field soil (Alexander, 2017). Likewise, Tchabi *et al.*(2010) isolated 60 AMF species in the a tropical savanna in Benin, and Stürmer and Siquiera (2011) also retrieved 43 species from Amazonian ecosystem in western Brazil. This large number of AMF morphotypes discovered diversely is contrasting regarding the only 17 AMF species isolated in cropping systems in the tropical humid tropical highland in Kenya (Jefwa *et al.*, 2009) and in Malawi (Jefwa *et al.*, 2006). This confirms that continuous farming with high inputs reduces the AMF diversity. With currently only 315 AMF species described worldwide (http://www.amf-phylogeny.com/, as of October 2019), the AMF morphotypes described in this work represent 14.2% of all species; showing that this areas is a hotspot of AMF diversity.

5.1.3. Diversity and occurrence in the field and trap culture sols

The description, occurrence and diversity of AMF can be accurately based on spores morphology and spores since they are the propagules serving as a mean of preservation of fungal strains in prolonged drought conditions (Rosendahl, 2008). The high diversity of AMF associating with maize might mean that the mycorrhizal fungi's specificity is low in the cereals or it might have been brought by the less specificity of other crops associated with maize. Higher diversity indices were recorded mostly in the midland after trapping. The Shannon-Weiner varies between 1.5 and 3.5 generally and in ecological investigations it increases as the species richness and evenness increase (Weaver and Shannon, 1963). The index variability of 0.41 to 2.31 throughout the sites can be a result of a variability in vegetation types and management practices observed in the study area, and the low maize specificity. Also, the high diversity of AMF species can also be a result of different types of weeds, different types of plants associated to maize and mostly the heterogeneity among the

habitats evaluated (Oehl *et al.*, 2003; Borriello *et al.*, 2012; Belay *et al.*, 2013; Muketa *et al.*, 2008; Gomes *et al.*, 2015; Vieira *et al.*, 2018).

The findings of the Gigaspora, Acualospora, Scutellospora and Glomus genera as dominant morphotypes is fairly consistent with the findings of Gomes *et al.* (2015) in a similar study on the diversity of AMF in tropical agroecosystems in Brazil that presented the genera Glomus, Dentiscutata, Gigaspora, Scutellospora, Acaulospora and Funneliformis as the most dominat in the rhizosphere of maize crops and these morphotypes have been shown resilient to climatic and edaphic conditions (Texeira *et al.*, 2017; Alexander *et al.*, 2017; Belay *et al.*, 2013). The reason why in this study the Glomus didn't appear to be the most dominant in field soils is 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 species are sensitive to Al, but still they are the principal maize colonizers, especially in high agricultural inputs cropping systems (Borriello *et al.*, 2012; Sasvári *et al.*, 2011). The fungistatic effect of acidic soils has been reported to hinder *Funneliformis mossae* from forming mycorrhizae with maize (Siqueira *et al.*, 1984).

In this study, in the small holding farming system with low inputs, maize was mostly intercropped with beans, cassava, sweet potatos and the degree of interpcropping might have brought in the high species diversity. This finding agrees with Alexandra *et al.* (2017) who observed that the community diversity and colonisation ability are influenced by local management practices that primarily affect the nutrient status in the soil and found that the spore abundance and colonisation percentage was determined by the site factor, but not by the crop nor by the mineral P fertilization. Up to eleven strains were not identified at specie level since sometimes the morphological based characterization fails to distinctively identify some strains, depending on the developmental stage of the spore but identification of fungal species in a particular environment has been proved to be of invaluable importance for more accuracy about the species phylogeny and properties (Walker *et al.*, 2018; Redecker *et al.*, 2013; Smith and Smith, 2011).

5.1.4. Spore densities in field and trap soils

Spore densities were very low in the field soils; they varied between 0.27 to 0.95 spores g^{-1} of soil, while after trapping they increased significantly (p<0.001) and reached up to 2.85 spores g^{-1} of soil on average, but some soils could individually reach 4 spores g^{-1}. Similar results of low densities have been reported in India in a survey of AMF fungal diversity in

Acacia trees 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 Acacia and porsopis by Ingleby *et al.* (1997) by recording 0.081 to 0.51 spores g^{-1}. AMF spore densities were also recorded in a survey of Acacia tree species (0.49 to 0.67 spores g^{-1}) in India (Lakshman *et al.*, 2001) and in Acacia and Prosopis tree species (0.08 to 0.51 spores g^{-1} of soil) in Senegal (Ingleby *et al.*, 1997). 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 as for their propagation they need to obtain sucrose from the plant they associate with, soils disturbance, and differential sporulation ability of AMF taxa and fungal species (Walker *et al.*, 2018; Jansa *et al.*, 2009). These specifications related to climate, soils conditions and intrinsic species characteristics could explain the variation in AMF spore densities between the study sites.

The trap culture produce high densities with many samples giving densities of up to 4 spores g^{-1}, comparing to the field soils and which is the same as the densities of some commercial mycorrhizal inoculants (Mukhongo *et al.*, 2017). A more accurate AMF diversity and density is well detected after trapping the spores on a mycorrhizal hosting plant as during trap culturing, multiplication of infective propagules is boosted and non-infective strains eliminated (Habte and Osario, 2001; Séry *et al.*, 2016; Mukhongo *et al.*, 2016). In the trap culture, maize was actively growing for a period of four months and in the last month the reduced irrigation applied gradually might have helped abundant spores to be formed and this may explains the high densities in the trapped soils and this argument agrees with Jefwa *et al.* (2006).

The spore formation in mycorrhizal fungi is very variable and is driven by many factors such as species specific nature as some species cannot form spores at all, host plant, temperature and soils conditions among others (Rosendahl, 2008; Smith and Smith, 2011; Gomes *et al.*, 2015). Thus, the real contribution of AMF communities in agroecosystems functioning cannot be derived from survey of AMF based on spores only. Therefore, composition and distribution of glomalean fungi in other cropping systems would be much informative since it may help understand better the extent of these fungi distribution and may their utilization in improving root functions and subsequently the farming systems productivity.

5.1.5. Sampling effort and morphotypes recovery

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 agroecologies studied. The low rate of morphotypes recovered in the lowland comparing to the mid and highland could be explained by the chemical properties of the soils from the midland which were slightly acidic a higher level of available P. In total, 45 morphotypes were recovered from the maize cropping system alone but 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.

5.1.6. Species clustering influenced by cropping systems and management

Species were found to be evenly distributed in the area, especially the morphotypes from Gigaspora, Glomus and Acaulospora which appeared in all the clusters. This could be explained by the fact that maize was found to be grown in the lowland, midland and highland in the area; grown sole or associated with beans, cassava, sweet potato, sorghum, etc. and the fields were dominated by weeds of various types, mostly *Digitaria sp, Galinsoga sp, Bidens sp, Commelina sp*, etc. These cropping patterns of the area were observed almost all over the different agroecologies studied and might explain the great diversity in all the zones but with no difference between them. These findings are inagreement with other studies that 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).

The composition of indigenous AMF varies along soil and landscape gradients (Jansa *et al.*, 2014). Within the limits of this study area and target cropping system, there was a high occurrence frequency of some species, indicating that they have a wide geographic range. This confirms the results obtained by Oehl *et al.* (2003) and Borriello *et al.*, (2012) and means that maize can associate with a broad range of mycorrhizal fungi. In the study area, the cropping system is characterized by a smallholder farming system where there is no or low inputs of organic fertilizers, low mineral fertilizers inputs, continuous farming and characterized by low yields (Tittonell and Giller, 2013).

Agricultural management practices such as intercropping, tillage, crop rotation or organic management influence the AMF communities. Mathimaran *et al.* (2007) agreed that frequent and deep tillage, tillage on the hilly land, monocropping and intensive chemical fertilization usually bring about negative changes in the AMF communities composition and densities.

A high abundance of certain taxa has been found in intercropping than in mono cropping system (Alexander (2017). Moreover, generally cereals crops are known to be less colonized than grassland species or C4 grasses (Tang *et al.* 2016). The results obtained in this work are in accordance with this statement since maize was mostly intercropped with either beans, cassava, sweat potato etc. and a number of taxa as many as what other researchers have found in other cropping systems than the maize sole was observed (Tchabi *et al.*, 2010).

When evaluating altitude gradients in Brazil, Sylvia *et al.*, (Sylvia *et al.*, cited by Teixeira *et al.* (2017), reported that some 51 AMF species were present, and the most representative genera were Glomus and Acaulospora. The findings of this research correlate with this one indicated above since the sampling was done not only in different agroecosystems, but in each village selected the samples were taken in all the landscape position, from the summit to the bottom for getting the whole picture of the fungi in maize fields in the areas. Therefore, the results obtained in this work could have a broad implications as an alternative for improving sustainably soils fertility and restoration of degraded or polluted soils since this wide geographic range means that an effective inoculant can have higher chances of thriving when applied on maize in the region.

5.1.7. Influence of soil properties on species occurrence in the fields

In this study, AMF occurrence showed a negative correlation with soil pH. Gigaspora, Acaulospora, Scutellospora, Dentiscutata and Glomus were the most frequent AMF genera, which is in accordance with the study of Songachan and Kayang (2012) that found Acaulospora and Glomus to be most frequently encountered in croplands in India, and Séry *et al.* (2016) that found Acaulospora to be the most frequent in acidic to neutral soils in Ivory Coast. Jansa *et al.* (2014) confirmed that in agricultural lands, soils types and their geographical distribution are more important determinants of indigenous AMF communities. There are some species that are only associated to some soil conditions, like the Acaulospora genus which was found to be associated in acidic soils of pH below 6.5 in Brazil (Teixeira *et al.*, 2017; Gomes *et al.*, 2015).

The distribution and densities of AMF species to soil chemical properties varies among species. For example the genus Acaulospora has been often associated to lower pH values but in this study, it appeared to be spread evenly in acidic and neutral soils (Table 3); the same applies to Gigaspora, Dentiscutata, Racocetra and Scutellospora. It has been recognized that acidic to neutral soils are harbor a good number of AMF species. Naturally the mycorrhizal fungi are known to thrive mostly in acidic conditions. As obligate symbionts, the 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 (Smith and Smith, 2011; Solaiman *et al.*, 2014).

Each AEZ investigated proved to have a certain number of species uniquely found in the environment. However, some species of AMF were in all the environments, are thus generalists. It was the case of *A. excavata, D. erythropa* and *S. pellucida*. The generalist may even have a less specificity level and might be able to associate symbiotically with all the cereals found in the region but also they might have plant protective effect against soils contaminated with pesticides, heavy metals, etc. and soils acidity (Teixeira *et al.*, 2017).

There was no evidence of soil P content affecting much the AMF strains occurrence. This is in accordance with the findings of Teixeira *et al.* (2017) and Songachan and Kayang (2012) who observed that AMF distribution is not influenced by the P level in the soil. The available P level was very variable within agroecologies from low to high content throughout (Table 3) and the same trend was followed by the species diversity as they didn't show any difference between the three regions. Similar studies have found that the available P might influence or not the structure and diversity of AMF communities in the fields (Gomes *et al.*, 2015; Mathimaran *et al.* 2007, Nandjui *et al.*, 2013). Alexandra (2017) found that spores abundance and the colonization level were not affected by P fertilization but by sites factors and specific management practices in agricultural systems in Lubumbashi in DR Congo.

Generally, soils fungal communities are strongly affected by the soil chemistry that determines their occurrence and distribution in acidic soils (Gomes *et al.*, 2015). This study's findings agree 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 (Alexander, 2017; Belay *et al.*, 2013; Teixeira *et al.*, 2017). Among the soil chemical properties, the pH has been found to be the most important driver of AMF communities composition in agroecosystems, especially in the low agriculture inputs

conditions (Oehl *et al.*, 2003). 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. Contrarily, the exchangeable Al detected in the soils showed a negative correlation with the other selected soil properties and this is because as the pH rises the exchangeable soluble Al^{3+} changes into the insoluble form $Al(OH_3)$ and releasing the exchange sites on soils colloids for basic cations. The Acaulospora, Glomus and Racocetra are the genus that had shown a positive correlation with the Al. Some trends in this direction have been detected previously in other studies. For example, Racocetra, Glomus, Rhizophagus and Funneliformis have shown high distribution in acidic soils with a high Al toxicity and not limed soils (Gomes *et al.*, 2015).

In a similar study where the mycorrhizal communities in maize cropping systems in acidic soils in Brazil were evaluated, Gomes *et al.* (2015) observed a decrease in AMF diversity with the liming of acidic soils. The low AMF diversity in the less acidic soils with less exchangeable Al may be explained by the time the AMF needed to take to repopulate the soil that was just taken neutral conditions, or the lack of accommodation of the specific species to the neutral conditions since many AMF species prefer slightly acidic conditions (Ladygina and Hedlund, 2010).

5.1.8. Infectiveness potential of indigenous mycorrhizae

From the findings, 22% of the fields soils studied proved to be as infective as the commercial mycorrhizal biofertilizer inoculant Rhizatech in the capacity to colonize roots of maize. The high inherent mycorrhizal potential of these soils mean that they contain AMF propagules ready to infect crop roots and imply that their natural mycorrhizal content can be as effective as commercial biofertilizers application regarding plants nutrition (Requena *et al.*, 1996; Ndonda, 2018; Tchabi *et al.*, 2010; Wu *et al.*, 2002). However, this test should still need to be run in field conditions to confirm this result of the bioinfectivity assay (Dalpé and Hamel, 2008). For the other soils that were low in mycorrhizal potential, inoculation by a commercial AMF biofertilizer or implementation of agricultural management practices favoring the proliferation of mycorrhizae should be tested to improve the mycorrhizal potential of these soils and therefore their fertility (Dodd and Thomson, 1994).

Inoculation aims at improving the inoculum infectivity and effectivity potential of a soil with the ultimate target of enhance plant productivity or restoration of degraded lands. However, the mycorrhizal symbiosis is trapped in an environmental complexity during the interaction

with the plant and the relationship doesn't always result in a positive response on the roots functions and the plant in general (Fayé *et al.*, 2013). The need to inoculate can be determined after assessing the natural mycorrhizal potential of a soil. If not competitive with the indigenous fungal strains, an effective AMF inoculum cannot establish good mycorrhizae in a soil. (Requena *et al.*, 1996; Dodd and Thomson, 1994); therefore, evaluation of exotic strains in an agricultural soil is needed before concluding on a inoculation strategy under field conditions (Cozzolino *et al.*, 2013; Sylvia *et al.*, 1993; Sylvia and Chellemi, 2001). Furthermore, the AMF inoculation is a success and an important management practice only when the native mycorrhizal potential of a soil is not very adequate in bot quality and quantity (Smith and Smith, 2011).

5.2. Influence of indigenous Arbuscular Mycorrhizal Fungi on maize growth, P and Zn uptake under controlled conditions

5.2.1. Physical and chemical properties of the experimental Ferralsol and Nitisol

The soils used in this study, Ferralsol and Nitisol, were both acidic common to agroecosystems in tropical SSA, especially the eastern of DR Congo (FAO, 2015). The acidity level varied between the two soils; with the Ferralsol found to be more acidic (pH=5.8) than the Nitisol which was slightly acidic (pH=6.3). The Ferralsol had low nutrient content such as N, P and low CEC comparing to the Nitisol which had moderate nutrients contents for the same parameters. These results reflect the natural properties of these soils as described in FAO (2015) and what recognizes the Ferralsol as the more weathered soils, reddish, more acidic and with low nutrients contents and a high P sorption capacity comparing to the Nitisol which, though being also weathered acidic tropical soils, are more fertile and productive since they contain high proportions of weatherable minerals and their surface horizons can have high proportions of organic matter (FAO, 2015; Okalebo *et al.*, 2002). Also, the low nutrient content of the soils could be emanating from the conventional continuous cropping associated with nutrients mining and no incorporation of harvest organic residue or application of fertilizers in the fields. This continuous removal of organic residues via harvest leads to the shrinkage of organic matter that could improve soil fertility (Achieng *et al.*, 2010). It is obvious that in the Ferralsol and Nitisol, P is one of the most limiting nutrient and it implies often a low nutrients use efficiency when inorganic P is applied in acidic soils (Okalebo *et al.*, 2007; Achieng *et al.*, 2010). This could be the reason why the

hyphal P contribution was not significantly higher in the inoculated treatments than in the control.

The AMF densities and colonisation have been proved to be significantly influenced by the soils properties in which they are growing. AMF have been shown to be more abundant and effective in acidic soils, with the neutral soils tending to suppress the mycorrhizae association (Smith and Read, 2008; Solaiman *et al.*, 2014). This may be one of the reasons why the native AMF root colonization increased to equal the Rhizatech in the Ferralsol (Table 9). High concentration of available P reduces also the mycorrhizae formation (Lambert *et al.*, 1979; Smith and Smith, 2011); meaning that in the Nitisol with moderate rate of P (31mg kg^{-1}) and a slight acidity (pH 6.3) than the Ferralsol (pH 5.8), the colonisation could have not been very effective. This could be the explanation to the low density of spores obtained in the initial status of Nitisol, but also the low colonization of maize roots, comparing to the level in the Ferralsol.

The organic carbon and the CEC influence the supply of nutrients to plants; with the high level of organic matter increasing the bioavailability of nutrients and water, and high CEC providing more nutrient supply (Brady and Weil, 2002). The two acidic soils used had different amount of organic carbon and CEC, with the moderate values for Nitisol; meaning that the later had a high water retention capacity and high capacity of supplying P and Zn to maize plants. In a similar study, Mukhongo *et al.* (2017) found that with high total carbon content in Nitisol, there is a high water content; increased K nutrient uptake and roots colonisation compared to Ferralsol under fields' conditions in Uganda.

5.2.2. Influence of indigenous Arbuscular Mycorrhizal Fungi on maize growth, P and Zn uptake

The findings of this study revealed that different AMF showed functional diversity in terms of maize growth, Pi and Zn uptake. The P application improved significantly maize growth in both Ferralsol and Nitisol. Other studies have also found that the application of P in acidic soils increases its availability and its uptake by roots (Onwonga *et al.*, 2013; Templer *et al.*, 2017) through the direct pathway of P uptake (Figure 3). The high available P in soils tends to suppress the mycorrhizae formation (Lambert *et al.*, 1979) and this could explain why the P treatment ranked among the highest height and biomass in both Ferralsol and Nitisol.

At 7 WAT, the P application treatment (SPAD meter readings: 42) showed more greenness than all the other treatments but the strains AMF4 (48), AMF1 (44) and Rhizatech (43)

presented the highest concentration of chlorophyll in the leaves (p<0.001) in the Ferralsol at 11 WAT. In the Nitisol, AMF1 (44), AMF2 (44), AMF3 (44) and AMF4 (42) presented the highest chlorophyll content at 11 weeks (p=0.047). This can be explained by high availability of P for uptake in the P application treatments and towards the end of the season, more nutrients might have been mobilized and more resistance to drought might have been conferred to plants by these strains. The Chlorophyll Meter Readings have been positively correlated to maize growth and yield (Gekas *et al.*, 2013). The chlorophyll concentration in the leaves is a key indicator of the magnitude of physiological activities occurring, and is dictated by the water and nutrients availability (Gekas *et al.*, 2013).

By increasing the root surface area and serving as extra roots, the hyphal growth enhances nutrients uptake per unit area through the mycorrhizal pathway (Smith and Smith, 2011). The developmental stages, the external hyphae activities, hyphal transport rate and the ease of solute interchange at the interface between the arbuscule and the root cells variably dictates the mycorrhizal fungi species efficiencies in nutrients uptake (Bucher, 2007; Smith and Smith, 2011). Attention was paid on P and Zn content of the shoots as their availability and concentrations are reported to be increased by the AMF (Bucher, 2007; Crespo, 2015).

In the Ferralsol, the lack of significant difference in the P content could be acidity of the soil (pH=5.7) as the P applied might have been sequestered by Al and Fe sesquioxides. AMF1, AMF2, AMF3, AMF5 and Rhizatech were as effective as P fertilization in P content in Nitisol. The significant influence of biofertilizers on increasing P in Nitisol (p= 0.015) and Zn in Ferralsol (p=0.007) was attributed to the functioning of AMF and probably to the plant growth promoting bacteria (PGPB) that might had been present in the soil and are known to be abundant in the slightly acidic soils and intervene in improving root growth and development (Kavoo-Mwangi *et al.*, 2013) though not studied. They may have help in increased nutrient uptake. P concentration was higher in the Nitisol than in the Ferralsol, which reflects their initial soil available P (31 and 15 mg kg^{-1}, respectively). The Ferralsol was clay and the Nitisol (sandy clay loam), meaning that more water and nutrient could be supplied by the Ferralsol but since it had lower contents, the Nitisol might have supplied more nutrients to plants.

Mineral P applicant significant increase of P and Zn uptake and shoot biomass might have been a result of an increased P and Zn availability, as P uptake is positively correlated to Zn uptake. This is consistent with the similar findings obtained by Onwonga *et al.* (2013) and

Templer *et al.* (2017), when assessing the P concentration in maize after application of P in acidic soils.

The hyphal P contribution was low since it is believed that up to 85% of the P can be uptaken through hyphae but here it varied between 23.1% - 51.4% in Ferralsol and 28.1% - 48.7% in Nitisol; meaning that the AMF applied had not contributed greatly to the acquisition of P. This could be due to the low spore densities or the low diversity (monospecies inoculum) or the long time required by some AMF strains to establish the mycorrhizae association with the host and spread their extraradical hyphae in the roots. This is consistent with Tian *et al.* (2013) who proved that increasing the diversity of AM fungi in maize roots through co-inoculation leads to higher colonization, expression of Pi transporters enzymes, as well as mineral P uptake in maize shoots.

Mycorrhizae increased the uptake of Zn in maize and this result is in line with the findings of Lambert *et al.* (1979) that proved that mycorrhizal inoculation alone increases the concentration of P and Zn in the plant but when the P is added to soils, the mycorrhizal activity is suppressed by P fertilization and Zn concentration is reduced significantly. When working on phosphate transport in maize under AMF colonization, Tian *et al.* (2013) found that co-inoculation with different AMF species (*Glomus deserticola, Glomus intraradices, Glomus mosseae* and *Gigaspora gigantea*) resulted in the highest expression level of phosphate transporter gene *ZEAma:Pht1;6* as well as the highest P uptake; suggesting a high diversity of AM colonization may transfer more P to the intraradical hyphae within maize roots.

5.3. Influence of indigenous Arbuscular Mycorrhizal Fungi on root colonisation

Generally, root colonization was low in both soils and varied between 0 and 45%. In the Ferralsol, there was no significant difference but a high coefficient of variation meant high variations between treatments. This could be due to either the low performance of all the AMF strains in the Ferralsol since it has been proved that AMF perform very well in slightly acidic soils (Lambert *et al.*, 1979); or the low concentration of the propagules of the crude inoculum used. Low colonization levels were also observed by Aguk (2013), but in contrast, other authors found colonization levels varying between 41-73% (Ngakou *et al.,* 2006) and 48-68% (Džafić *et al.,* 2010) on maize. AMF have functional diversity in term of roots colonization and induction of nutrients uptake (Feddermann *et al.*, 2010; Smith and Smith, 2011; Tian *et al.*, 2013). The Gigasporaceae and Glomearaceae have different colonization

strategies (Feddermann *et al.* 2010). The lower degree of colonization found for AMF1 (*Gigaspora margarita*) and AMF3 (*Gigaspora gigantea*) reflected their low colonization ability of fungi belonging to Gigasporaceae; which agrees with the findings of Feddermann *et al.* (2010). The AMF2, AMF4 and AMF5 gave a relatively higher level of colonization of roots, statistically equal to the Rhizatech in the Nitisol. These findings confirm the results obtained by other researchers who found that the Glomeraceae had a high affinity to colonize maize (Tian *et al.*, 2013; Gomes *et al.*, 2015) but also the Acaulosporaceae had a high colonization of roots than the Gigasporaceae (Séry *et al.*, 2016). This is probably the reason why commercial inoculants are made from mixed species. However, Kouadio *et al.* (2017) found native AMF outcompete the exotic commercial AMF biofertilizers.

In general, a significant impact of AMF biofertilizers on growth, nutrients uptake and root colonization was not very much felt and this is possibly because of the short duration of the experiment and/or the low available P in the Ferralsol and its acidity. In the Nitisol the high P detected might have hindered the mycorrhizal formation to take place. According to Mukhongo *et al.* (2016), mycorrhizal treatments low responses in inducing annual crops growth can be attributed to a late start of the colonization process. The benefits from the colonization can come only after the specifivity, infectivity and effectiveness are met between the two symbionts (Sylvia and Chellemi (2001) and this depends on the developmental stage of the propagules (vesicles, arbuscles and hyphae) as some propagules like spores may take longer to germinate and infect roots, but also on the amount of carbon the fungus is obtaining from the plant. Furthermore, Kavoo-Mwangi *et al.* (2013) found that Rhizatech could failed to impact immediately the growth of banana plantlets in the tissue culture grown for about 5 months but came to induce a significant growth in the same planlets only after maintaining them in field up to 7 months. This could be the reason why in some treatments the Rhizatech resulted in the better growth, chlorophyll content and biomass. The same could have applied to the indigenous AMF strains whose effects were not observed. However, Aguk (2013) and Kundu (2012) reported positive effects of AMF inoculation on potatoes after only four months of cultivation.

The low level of root colonization in all the treatments among indigenous AMF strains could be due to the low spore densities in monospecies based inoculants; the low spore densities in both experimental soils (1.23 spores g^{-1} for Ferralsol and 0.87 spores g^{-1} for Nitisol) compared to the 7 spores g^{-1} used by Séry *et al.* (2016) or 50 propagules g^{-1}) applied by Kavoo-Mwangi *et al.* (2012). The failure of some strains to produce a high potential soil

inoculum might have resulted from the no suitability of the specific strain to the environmental conditions or a slow development of the strains. This finding confirmed the statement that AMF inocula can play a modest role in crops growth improvement, but are not always successful and in some cases the real positive benefits are not always obvious (Corkidi *et al.*, 2004; Fayé *et al.*, 2013).

Monospecies inoculum production is more demanding in term of labour and challenging seeing the often unpredictable outcome. The crude monospecies inoculum had low spore densities; the five selected native AMF's densities varied between 1.94 and 2.82 spores g^{-1}, and this implies that the changes of failing to produce high mycorrhizal crops and to induce important improvement in the growth of crops were high. From the results of Séry *et al.*, (2016), dual inoculation of yam with AMF of *A. colombiana* and *A. appendicula* contributed significantly to the growth and production comparing to the single inoculation. Besides that, many AMF inoculant manufactured products contain more than one specie; case of the Rhizatech inoculant which contains up to 4 species namely the *G. aggregatum, G. intraradices, G. mossae* and *G. etunicatum*, which are often generalist, for an easy adaptation of the product to a wide range of crops and environments.

Soil from the rhizosphere of a plant harbouring AMF is commonly used as an inoculum (Berruti *et al.*, 2016; Mukhongo *et al.*, 2016; Ndonda, 2018; Alexpander, 2017). This applies only after ensuring that the soil contains an important number of infective propagules such as colonized root fragments, spores and hyphae in abundance. This implies that the soil has to be evaluated and reliable information regarding these infective propagules abundance, diversity and infectivity has to be available. Other methods of inoculum production like the in-vitro cultivation methods or hydroponic systems can enhance the AMF biodiversity and establishment (van der Heijden *et al.*, 2015; Akhtar and Abdullah, 2014) and a AMF friendly management such as cover cropping, organic farming and conservation agriculture (Lehman *et al.*, 2012; Alexander, 2017) are worth to be tested since they have been proved to sustain the persistence of AMF community (Mukhongo *et al.*, 2016).

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

This study was conducted to characterize the native AMF, assess the mycorrhizal infectivity potential of soils from maize cropping systems in South Kivu, and to screen and assess the role the native AMF plays on maize (Z*ea mayz* L.) growth, P and Zn uptake and root colonization in acidic soils, under controlled conditions. Up to 45 AMF strains were obtained from field soils, meaning that there is a great diversity in AMF in the maize agroecosystems in the study area. The soil pH and exchangeable Al mostly influenced the distribution of AMF species. Some of the species from the families Gigasporaceae, Acaulosporaceae and Glomeraceae were ubiquitous. The natural mycorrhizal infectivity potential of soils is very much variable, and in overall, 22% of sampled fields proved to be as highly infective as the commercial AMF biofertilizer.

The monospecies soil crude inoculum production from native AMF species resulted in a low to moderate concentration of propagules (0-2.8 spores g^{-1}), compared to the concentrations in the commercial inoculum (4 spores g^{-1}). After producing the inoculum, five AMF strains out of 26 strains were selected based on their spore densities and roots colonization, which were morphologically identified as strains of *Gipaspora gigantea*, *Gigaspora sp., Gigaspora margarita*, *Rhizophagus intraradices* and *Acaulospora reducta..* In both Ferralsol and Nitisol, the AMF inoculum produced influenced the growth, the chlorophyll concentration, the P uptake and the root colonization differently; with some treatments equalizing the P application on the shoot biomass and P uptake and also equalizing with the Rhizatech in the root colonization.

From these findings, the following conclusions were drown:

1. The soils from maize cropping system in South Kivu have a high AMF diversity dominated by species from the Gigasporaceae, Acaulosporaceae and Glomeraceae families.

2. Almost a quarter of maize field soils have a mycorrhizal infectivity potential as high as the commercial AMF inoculant Rhizatech under controlled conditions.

3. Strains of *Gipaspora gigantea*, *Gigaspora sp*, *Gigaspora margarita, Rhizophagus intraradices* and *Acaulospora reducta* proved to be potential AMF biofertilizers for maize.

4. In the Ferralsol, inoculums made of strains of *Gipaspora gigantea, Gigaspora sp.* and *Rhizophagus intraradices* proved to be the best while in the Nitisol, *Rhizophagus intraradices, Gipaspora gigantea* and *Gigaspora sp.* based inoculums revealed to be the best inoculums.

6.2. Recommendations

1. Investigation of the composition and distribution patterns of glomale species in different farming systems in the region and use of molecular techniques for an accurate characterization and identification of the AMF isolates.

2. Stimulation of indigenous AMF communities in maize cropping fields in their natural environment through implementation of best fit site specific agricultural husbandry and conservation agriculture practices to promote mycorrhizal symbiosis in the fields.

3. Production of AMF soil inoculum with multiple species focusing on strains of *Gipaspora gigantea*, *Gigaspora sp*, *Gigaspora margarita, Rhizophagus intraradices* and *Acaulospora reducta* that guarantees better effectiveness of AMF biofertilizers for maize.

4. Further and extensive agroecologically based evaluation of commercial and effective AMF biofertilizers in the less infective soils.

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APPENDICES

Appendix 1: Field sites description

Territory	Site/Village	Code	AEZ zone	Toposequence	Crop Associated With	Cultural System	Weeds
Kalehe	Munanira	KMun1	Midland	plateau	maize, bean, cassava	association	bidens pilosa, comelina difusa,
Kalehe	Munanira	KMun2	Midland	summit	bean, plantain, maize		bidens pilosa, lantana camara
Kalehe	Munanira	KMun3	Midland	summit	bean, sorghum, sweet potato	association	lantana camara, bidens pilosa, comelina difusa
Kalehe	Munanira	KMun4	Midland	slight slope	pasture,	pasture	digitaria vestida
Kalehe	Munanira	KMun7	Midland	slight slope	roseau, cassava, plantain	association	digitaria, bidens pilosa, Crassocephalum montuosum
Kalehe	Luzira	KLuz2	Midland	toeslope	pasture	fallow, pasture	setaria barbata, digitaria
Kalehe	Luzira	KLuz3	Midland	toeslope	maize, taro, trispacum, papaye	assocation	galinsoga ciliata, Cynodon dactylon
Kalehe	Luzira	KLuz4	Midland	plateau	Sweet potato, cassava	association	digitaria, bidens sp, Conyza sumatrensis,
Kalehe	Luzira	KLuz5	Highland	steep slope	groundnut, maize	association	Bidens sp
Kalehe	Luzira	KLuz6	Midland	toeslope	maize et cassava	association	comelina difusa, bidens pilosa, Adenopus abyssinicus
Kalehe	Luzira	KLuz7	Midland	plateau	bean, maize	association	digitaria vestida, bidens
Kalehe	Kasheke	KKash1	Midland	slight slope	bean, maize, cassava	association	-
Kalehe	Kasheke	KKash3	Midland	steep slope	bean, maize	association	-
Kalehe	Kasheke	KKash4	Midland	plateau	bean, maize, cassava	association	comelina difusa, bidens pilosa, lantana camara
Kalehe	Kasheke	KKash5	Midland	toeslope	soja, taro, canne à sucre, tomate	association	digitaria vestida
Kabare	Katana	KbKat1	Midland	plateau	bean, cassava, soja, taro	association	bidens, lantana
Kabare	Katana	KbKat2	Midland	slight slope	bean, maize, groundnut	association	-
Kabare	Katana	KbKat4	Midland	plateau	bean, maize, groundnut	association	comelina, bidens, lantana
Kabare	Katana	KbKat5	Midland	plateau	maize, soja	association	Cymbopogon citratus
Kabare	Katana	KbKat7	Midland	plateau	fallow	fallow	comelina, bidens
Kabare	Luhihi	Kabluh1	Midland	toeslope	bean, maize, cassava, sunflower, sorghum	association	galinsoga ciliata, bidens pilosa, digitaria vestida

Kabare	Luhihi	Kabluh4	Midland	plateau	Sorghum+maize	association	comelina, bidens, digitaria
Kabare	Luhihi	Kabluh5	Midland	plateau	sorghum, plantain	association	comelina, bidens, lantana
Kabare	Luhihi	Kabluh6	Midland	slight slope	sorghum, cassava	association	comelina, bidens
Kabare	Kavumu	KabKav1	Highland	slight slope	bean, maize, cassava	association	galinsoga, bidens, digitaria
Kabare	Kavumu	kabKav2	Midland	plateau	bean, maize, cassava	association	galinsoga, comelina
Kabare	Kavumu	KabKav3	Midland	toeslope	maize,,bean, sorghum	association	comelina difusa, galinsoga
Kabare	Kavumu	KabKav4	Midland	plateau	maize,,bean, cassava	association	amaranthus, comelina difusa, galinsoga
Kabare	Kavumu	KabKav6	Highland	plateau	tomate, naiis, bean, igname, canne a sucre	association	galinsoga, bidens , lantana
Kabare	Kavumu	KabKav7	Midland	plateau	coffe, bean, maize, plantain	association	comelina, bidens, digitaria and lantana
Walungu	Mulamba	WMul1	Midland	slight slope	fallow, being ploughed	-	sida acuta, Digitaria abyssinica, Crassocephalum vitellinum
Walungu	Mulamba	WMul2	Midland	slight slope	bean, cassava, sweet potato	association	digitaria vestida, Cyathea manniana
Walungu	Mulamba	WMul4	Midland	slight slope	maize, bean, cassava	association	digitaria, galinsoga
Walungu	Mulamba	WMul5	Midland	summit	aubergine, cassava, roseau,	association	Digitaria sp
Walungu	Mulamba	WMul6	Midland	toeslope	maize, aubergine, taro	association	digitaria, "lwibaye"
Walungu	Burhale	WBur1	Midland	summit	bean, sweet potato, igname, cassava	association	digitaria vestida
Walungu	Burhale	WBur2	Midland	steep slope	cassava, bean, potato	association	digitaria vestida
Walungu	Burhale	WBur3	Midland	slight slope	bean, maize, cassava, potato	association	digitaria vestida
Walungu	Burhale	WBur6	Midland	slight slope	sorghum, sweet potato, cassava	association	digitaria vestida
Walungu	Burhale	WBur7	Midland	slight slope	maize, bean	association	-
Walungu	Mugogo	WMug1	Highland	steep slope	maize, cassava, petit pois, pomme de terre	association	digitaria vestida, Senna occidentalis
Walungu	Mugogo	WMug2	Highland	toeslope	maize, sweet potato	association	comelina, galinsoga ciliata, digitaria
Walungu	Mugogo	WMug3	Highland	slight slope	maize, groundnut, petit pois, pomme de terre	association	comelina, bidens, lantana, digitaria
Walungu	Mugogo	WMug5	Highland	steep slope	bean, cassava, sweet potato, onion	association	digitaria, nshenga
Walungu	Mugogo	WMug6	Highland	slight slope	maize, cassava, plantain	association	comelina, bidens, galinsoga, lantana, digitaria
Uvira	Bwegera	UBw1	Lowland	plaine	fallow en plein labour	fallow	-
Uvira	Bwegera	UBw2	Lowland	plaine	fallow labouré	fallow	Ploughed field
Uvira	Bwegera	UBw4	Lowland	plaine	Fallow, during d'intersaison	fallow	sida acuta, bidens pilosa, Euphorbia

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Uvira	Luvungi	ULuv1	Lowland	plaine	fallow ,during d'intersaison		-
Uvira	Luvungi	ULuv3	Lowland	plaine, toeslope	maize, cassava	association	Ploughed field
Uvira	Luvungi	ULuv4	Lowland	plaine	maize, palmeraie	association	-
Uvira	Luvungi	ULuv6	Lowland	plaine	maize, canne à sucre, bananeraie	association	"kakera", "mutija"
Uvira	Luvungi	ULuv7	Lowland	plaine	rice	monocuiture	comelina, digitaria vestida
Uvira	Kamanyola	PKam1	Lowland	plaine	maize	monoculture	comelina, digitaria vestida
Uvira	Kamanyola	PKam3	Lowland	plaine	fallow cultivée	fallow	-
Uvira	Kamanyola	PKam4	Lowland	plaine	fallow cultivée	fallow	-
Uvira	Kamaniola	PKam5	Lowland	plaine flat	fallow	Fallow	-
Uvira	Kamaniola	PKam6	Lowland	plaine flat	fallow, in interseason	association maize cassava	-
Uvira	Kamaniola	PKam7	Lowland	plaine flat	maize+cassava+plantain	association maize cassava	bidens pilosa

Appendix 2: AMF morphotypes description

Code	Color	Size*	Shape	Hyphae	Surface Texture	Surface ornamentation, wall layers, germination shield, germination orb, warts, sub cellular structure, sporogenous cells, cicatrix and hyphae position**	Genus	Species*
KMun1	Yellow cream	S, M	globose	yes	rough, shining	Affiliated to Acaulospora rehmii since the ornamentation is the main feature observed like a labyrinth but not yet very clearly developed; with a form of labyrinth and smoothly bend inside causing these depressions. Two walls layer group easily detected, they are continuous and the inner one is thicker. The spore didn't break, reason why in Melzer, it appear to have not picked the very well the reagent.	Acaulospora	A. rehmii aff.
	Ochraceous	S, M, L ++ small spores	globose and subglobose	yes	smooth, shining	Two wall layer groups. The inner wall separated slightly with the outer. The cell content formed	Racocetra	Racocetra sp.

		, unable to be picked				pustules. The subtending hyphae are typical for Diversisporaceae. It is more of a Racocetra than Scutellospora or Gigaspora. Ochraceous to yellow.		
	Suffron brown	M, L	globose	yes	rough, not shining	Laminated wall with a hyphae hidden behind. The hyphae plug is not visible, thus the species level determination was not possible. When crashed the inner content of the cells are poured out and form pustules.	Gigaspora	Gigaspora sp.
	Brick suffron	S,++	Gflobose	No	smooth	Young spore, not identified		
KMun2	White	L	Globose, subglobose, elliptical	yes	smooth, shining	young spore, not identified		
	Ochrc	S, M, L	globose	yes, some	smooth, not shining	young spore, not identified		
	Cinnamon	L	globose	yes	rough, not shining	ornamented surface like escavata. Walls not well seen, picked melzer. No sporogenous cell. It has stained purple to dark purple in Melzer's reagent	Acaulospora	A. escavata
KMun3	Yellow cream	M, L	Globose	yes	smooth, shining	Two layer groups. The layer group1 were the outer layer, hyaline and flexible. The layer group2 was the inner layer, which produces a red-brown reaction in Melzer's reagent and ornamentation typical for Elegans.	Acaulospora	A. bureticulata aff.
	Ochraceous	S, M, L	Globose and sub Globose	yes	smooth, shining	Spore has picked completely the melzer reagent and the germination shield slightly identifiable but has a typical hyphae insertion of scutellospora genus	Scutellospora	S. cerradensis aff.
	Cinnamon	M, L	Globose	yes	smooth, not shining	The spore is big, presence of warts and germ tubes, thick wall layers that doesn't stain. Typical hyphae insertion to the cell with the sporogenous cell	Gigaspora	G. rosea
	Ochrc	L	Globose	yes	smooth, not	Big spore, didn't stain in the PVLG. Three wall	Gigaspora	G. margarita

					shining	layer groups, sporogenous cell with a large plug not very clear, few warts observable		
KMun4	Cinnamon	S, M	subglobose, oblong	yes	rough, shining	Contaminated spores. Small size. Wall seems to be one group, very rigid and thick and continuous. When it breaks, the wall content reemain strong.	Glomus	Glomus sp.
	Ochraceous	S, M: very few spores	globose and subglobose	yes	rough, smooth, shining	All the walls stained in Melzer. Have thin wall layers, 2 groups.Has a germination shield not clearly seen in PVLG. Has an hyphae of scutellospora	Scutellospora	Scutellospora sp
KMun7	Yellow cream	S, M	globose	yes	rough, shining	clear surface ornamentation in labyrinth shape,	Acaulospora	A. rehmii
	Ochraceous	S, M, L	globose	yes	smooth, shining	germination shield	Scutellospora	S. pellucida
KLuz3	Cream ivory	M, L	globose and subglobose	no	rough, shining	no real clear feature that may help to detect even the genus	not identified	
	Brick	L	globose	yes	rough, not shining	no real clear feature that may help to detect even the genus	not identified	
	Ochraceous	M	globose	yes	rough, not shining	Features and Ornamentation uniform, typical of Acaulospora alpina, as described in Oehl *et al.* (2006)	Acaulospora	A. alpina
KLuz4	Sulfur yellow	M	globose	yes	rough, shining	germination shield and hyphae and a sporogenous cell typical for pellucida	Scutellospora	S. pellucida
	Ochrc	S, M	globose	yes	smooth, not shining	Uncle features, not identified, not identified, few detectable features		
	Ochraceous	S, M	globose and subglobose	no, some yes	rough, shining	Big spore, with two hyphae apparently, but the connection of the sporogenous cell and the spore is hidden. Stained completely in reddish brown in Melzer. Wall groups seem to be two, but are not visible but the inner one seems to have shrinked slightly	Gigaspora	Gigaspora sp.
	Straw	M, L	globose and	yes	rough,	Unclear features, could not stain properly, on the		

			subglobose		shining	same slide with the Ochrc spore. Not identified,		
KLuz5	Yellow	L	Globose and subGlobose	yes	smooth, shining	Big spore, yellow brown. Three laminated wall layers which are thick and stick to each other, don't crack completely, warts under development, sporogenous cell with a typical plug of G.rosea	Gigaspora	G. rosea
	Ochraceous	L	Globose and subGlobose	yes	smooth, shining	Big spore, the ochraceous is Greenish yellow. Three laminated wall layers, stained completely in Melzer, and cracked slightly. The warts are clearly seen, concentrated on the plug. The plug is connected till to the last inner wall layer.	Gigaspora	G. gigantea
	Cinnamon *	S	Globose , subglobose	yes	rough, not shining	Unclear features, spore not identified		
KLuz6	Yellow cream	M, L	globose	yes	smooth, shining	Big spore, with a characteristic color of gigaspore: pale cream yellow to pale yellow-brown. Of subglobose shape. Spore with laminated wall, with three layers (L1, L2, and L3), the first two adherent and L3 differentiating just a slightly. The spore has a hyphae, difficult to detect, with hyaline layers not very evident in pvlg. The occlusion seems to be a plug concolorous with the laminate layer of the spore wall.	Gigaspora	Gigaspora rosea aff.
	Ochraceous	S, M, L	globose and subglobose	yes	smooth, rough, shining	no clear features detected, not identified		
	Fuscous black	M	globose	sno	smooth, not shining	Two wall layers, Ornamentation and hyphae features, plug and sporogenous cell clearly typical for the nigra. Medium to large spores a bit red brown	Dentiscutata	D. nigra
KLuz7	Ochraceous	S, L	globose and subglobose	no, some yes	rough, shining	no clear features for identification, spore seem to be young		

	Buff	M	globose and subglobose	no	rough, not shining	The wall layers are not clearly detected but the distinctive features are the typical irregular numerous pits on the surface of the spore. No sporogenous saccule observed.	Acaulospora	A. reducta aff.
KKash1	Ochraceous1	S, M	globose and subglobose	No	Smooth, shining	The spore is hyaline/white. Of a subglobose shape, Three layers (L1, L2 and L3) that are adherent that are of almost equal thickness and continuous. The outer layer L1 is an outer permanent rigid hyaline layer, and tightly adherent to L2. Easily distinguished from L2 when spores are placed in Melzer's reagent, where L1 is nonreactive and L2 stains dark red-purple.	Cetraspora	C. pellucida
	Ochraceous2	S, M	Subglobose to elliptic	No	shining	young spore, with the wall layer seems to be ornamented, ornamentation typical for Acaulospora	Acaulospora	Acaulospora sp.
	Ochraceous	S, M	globose and subglobose	yes	smooth, shining	White spore, with a germination shield typical for fulgida, the hyphae seem to be many but the plug is unclear	Racocetra	R. fulgida
	Sulfur yellow	M	globose	no	rough	Yellow to white, Two wall layers, hyaline very well separated. Germinal orb visible. Unclear saccule plug on the cell due to some dusts, but also the ornamentation not very noticeable	Acaulospora	A. scrobiculata aff.
	Brick	S, M	globose	no, some yes	smooth, not shining	Big spore. Three thick and laminated wall layers and cracked slightly. The warts are clearly seen, concentrated on the plug. The plug is connected till to the last inner wall layer.	Gigaspora	G. gigantea aff.
KKash3	Pale ochraceous	L	globose	yes	smooth, slight shining	Two to three layer groups, L1, L2 and L3, sporogenous subtending hyphae. Germination shield hyaline to yellow brown, and clearly visible	Scutellospora	S. pellucida
	Cinnamon	S	globose	yes	Smooth, not shining	Two wall layers seemed to be laminated, saccule neck cicatrix, and a circular to ovoid cicatrix and a germination orb on the surface with specific	Acaulospora	A. tuberculata

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						ornamentation of tuberculata		
	Ochraceous	S, M	globose	yes	rough, some smooth, shining	Thick wall layers, stained in Melzer. Spore seemed to be still under development. Subtending and hyaline hyphae and a germination shield close to fasciculatus are seen	Rhizophagus	R. fasciculatus aff.
KKash4	Straw cream	M, L	globose and subglobose	yes	smooth, shining	Big spore, the ochraceous is Greenish yellow. Three laminated wall layers, stained completely in Melzer, and cracked slightly. The warts are clearly seen, concentrated on the plug. The plug is connected till to the last inner wall layer.	Gigaspora	G. gigantea
	Cinnamon	S	globose	yes	smooth, not shining	Big spore, laminated wall layer groups. Difficult to detect the specie since the hyphae is not visible	Gigaspora	Gigaspora sp.
	Ochraceous	S, M	globose and subglobose	yes	smooth, shining	no clear detectable features. Not identified		
	Cigar brown brick	L	globose	no	smooth, not shining	Ornamentation and germination shield as distinctive features for reticulata were visible	Dentiscutata	D. reticulata
KKash5	Brick	S, M, ++ small spores	globose	yes, some no	rough, smooth, not shining	With so many small spores, rick to brown, trapped in the many hyphaes. The hyphae plug typical of glomus	Glomus	Glomus sp.
	Cigar brown	S, M	globose	no	smooth, not shining	No hyphae seen, spore completely broken, wall with two layers thick and yellow brown. Circular to ovoid scar as cicatrix indicating the contact with a saccule neck. Spore seem to be dead	Acaulospora	A. dilatata
	White cream	M, L	globose and subglobose	yes	smooth, shining	Three laminated wall layers, typical for gigantea, and cracked slightly. The warts are clearly seen, concentrated on the plug. Plug is connected till to the last inner wall layer.	Gigaspora	G. gigantea
KbKat1	Ochraceous	S, L	globose and subglobose	yes	smooth, shining	Two spore wall groups adherent layers (L1 and L2), with the inner laminate layer thicker than the outer layer.The outer rigid layer, has a smooth surface. The inner layer is well visible in the melzer	Racocetra	R. castanea

						reagent. It a layer that is rigid. The germination shield not very distinct in the image, but it is visible to be circular to slightly oblong. Shield has margins with only shallow convolutions, with the surface smooth		
	Yellow cream	S	globose, irregular	yes	rough, smooth, shining	Big spore, thick and laminated wall, color of characeetistic of gigaspora. Has an hyphe but the plugbetwenn it and the spore is hidden	Gigaspora	Gigaspora sp. Aff.
	Brick	S, L	globose	yes	smooth, rough and not shining	Small spore, continuous, thick and strong walls groups. No much feature to differnciate to the specie level.	Glomus	Glomus sp
KbKat2	Ochraceous	S, M	globose and subglobose	no	smooth, not shining	Ormanemnation typical for scrobiculata, in Melzer the spore picked the color but in PVLG it remained whitish	Acaulospora	A. scrobiculata
	Yellow cream	S	globose	yes,	smooth	Spore with a rigid thick wall, small size, the hyphae is present but not easy to detect. Spore completely cracked but walls maintained they cohesion. Affiliated to glomus sp. With so many small spores, rick to brown, trapped in the many hyphaes. The hyphae plug typical of Glomus	Glomus	Glomus sp.
	Yellow cream 2	S, most of them are of small size, M	globose	yes	smooth, not shining	Shrinking inside wall, ornamented surface like the excavata but small spore. no hyphae detected. Pereira *et al*., 2015a.	Acaulospora	A. excavata aff.
KbKat4	Ochraceous	S, M	globose	yes	rough, smooth, shining	spore looking like the new specie of Acaulospora descibed by Oehl *et al* in 2014. 2 distinct wall layer groups, not adhering to one another, the inner wall, continuous and rigid, holding the cell content. Its surgace is rough	Pacispora	P. robiginia
	Pale cream	S, M	globose and subglobose	yes	smooth, shining	Spore with a rigid and continuous wall, the limation is not well seen. Big spore, with s hyphae	Gigaspora	Gigaspora sp.

						and sporogenous cell detected but the plug is unclear		
	Fuscous black	M	globose	no	smooth, not shining	no features, has not displayed inner features in the reagents	not identified	
KbKat5	Straw pale	S, M, L	sub globose to elliptical	yes	smooth, shining	Spore has a subglobose to ellipsoid shape, of small size. It has 2 layer groups. The outer layer is a rigid layer, smooth, pale yellow and so tightly adherent to the second layer. The second (inner) layer consisting of very fine adherent .this inner layer show some shrinkage inside.No germination shield detected, nor hyphae	Scutellospora	S. calospora aff.
	Cinnamon	S, L	Globose	yes	smooth, not shining	Spore ornamemented like an Acaulospora but the ornamentation is not lear enough to detect the features well	Acaulospora	Acaulospora sp.
	Cinnamon2					Weird and unclear walls, with inner content seeming to have formed pustules, like the brasiliensis species but we prefer to not go to the species level	Acaulospora	Acaulospora sp.
	Ochraceous	M	Globose	no	rough, not shining	No clear features observed	not identified	
KbKat7	Ochraceous	S, M,	globose	yes	rough, not shining	Ornamentation typical for excavata. Pereira *et al*., 2015a.	Acaulospora	A. excavata aff.
	Yellow cream	S	globose, oblong	yes	smooth, shining	Spore with a rigid thick wall, small size, the hyphae is present but not easy to detect. Spore completely cracked but walls maintained they cohesion. Affiliated to glomus sp. With so many small spores, rick to brown, trapped in the many hyphaes. The hyphae plug typical of glomus	Glomus	Glomus sp.
Kabluh1	Pale ochraceous	S, M, L	globose, irregular	yes, some	smooth, shining	Spore of small size, with yellowish, thick wall layer which seem to form one group, not laminated, no hyphae. It seems that the warts are starting to	Glomus	Glomus sp.

						form at the surface of the spore		
	Cinnamon	S	globose	yes	rough, not shining	Spore walls with lacunes	Acaulospora	A. foveata
Kabluh4	Pale straw ochraceous	S ++, M	globose	no, some yes	smooth, shining	No clear and distinctive features in the staining solutions. Not identified,		
	Ivory	M, L	globose to subglobose	no	smooth, shining	Big spore, with 4 wall layer groups distinct and continuous. The two first are hyaline and much separated from the two inner layers. The Inner layers stained completely in melzer. Spore with a cicatrix on the walls surface.	Scutellospora	S. calospora
	Brick	S	globose and subglobose	no, only 2	smooth, rough, not shining	Spore with a rigid thick wall layers. Hyphae within hyphae. Spore completely cracked but walls maintained they cohesion. brick to brown, trapped in the many hyphaes. Stained in Melzer	Glomus	G. ambisporum
	Yellow cream	M, L	Not detected	No	Not shining	Spore full of dust, and no much distinctive features observed. not identified		
	Cinnamon	M	Subglobose	no	not shining	Cinnamon to white, spores seems to be under development. Two wall layer clearly separated, no hyphae but with ornamentation started forming, more close to some Acaulospora	Acaulospora	Acaulospora sp.
Kabluh5	Fucous black	L, ++ small	globose	yes	rough, not shining	Large sporogenous cell head. Ornamentation and hyphae features, plug and sporogenous cell clearly typical for the nigra. Drak rebd brown in Melzer	Dentiscutata	D. nigra
	White cream	S, M, L	globose and subglobose	yes	smooth, not shining	Two to three layer groups, L1, L2 and L3, sporogenous subtending hyphae. Germination shield hyaline to yellow brown, and clearly visible	Racocetra	R. pellucida
	Ochraceous	S, M, L, ++ small ochraceous, brick	globose	yes	smooth, shininhg	Ormanementation of erythropa	Dentiscutata	D. erythropa

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	Ivory cream	S, M	globose	yes, some	rough, shining	Big spore, with laminated wall layers, did not crack. Warts not very visible but present. Large hyphae and plug with a sporogenous cell of margarita.	Gigaspora	G. margarita
Kabluh6	Ochraceous	S, M	Globose and subGlobose	yes	smooth, shining	wall laminated, the hyphae is present and at the plug of the hyphe to the spore, the wall bend slightly	Gigaspora	G. gigantea
	Ochraceous1	M	subglobose	no	rough	ornamented like acaulospora	Acaulospora	A. rehmii aff.
	Ochrc	S, L	globular	yes	smooth, not shining	Wall layer specific for margarita	Gigaspora	G. margarita
KabKav 1	Straw	M	globose	yes	smooth, not shining	no much features, young spore, not identified		
	Brick	L	subglobose	no	rough, not shining	Small spore, hard and thick wall layers continuous and adherent, didn't crack. Inner content no visible.	Glomus	G. ambisporum
KabKav 2	Yellow	S, M	Globose	yes	smooth, not shining	Funnel shaped hyphae at the insertion, clearly detected. Couldn't stain very well in Melzer	Funneliformis	F. mossae
KabKav 3	Cinnamon	S, M	globose and subglobose	no	rough, not shining	Outer wall layer ornamentation in form of granular excrescences, typical of denticulata.	Acaulospora	A. denticulata
	Ochraceous	S	globose and subglobose	yes	smooth, shining	young spore, completely crashed	not identified	
KabKav 4	Ochrc	L	globose	no, some yes	smooth, rough, not shining	The layer group1 werehyaline and flexible. The layer group2 was the inner layer, which produces a red-brown reaction in Melzer's reagent and ornamentation typical for Elegans.	Acaulospora	A. bireticulata (= A. elegans)
	Brick	S, M	globose	no	smooth, not shining	not identified, seemed to be young	Not identified	
KabKav 6	Yellow	M	Globose	no	smooth, shining	Spore young, but showing some ornamentation that will form like some species in Acaulospora.	Acaulospora	Acaulospora sp. Aff.
	White	L	subglobose	no	smooth, some shining	Two wall layer groups, separated largely. The inner wall layer picked Melzer solution and bears the specific ornamentation, slightly pitted and	Acaulospora	A. spinosissima

						uniform. The neck of the saccule clearly visible. Matching the features of spinosissima as described by Oehl *et al.* (2014)		
KabKav 7	Pale	M,L	Globose	yes	Smooth, shining	Pale to hyaline. Most of them big, with rigid many wall layers, laminated but lacked enough distinctive features to be classified in the Gigaspora	Gigaspora	Gigaspora sp.
	Brick	M, L	Globose	yes	rough,shining	Ornamentation typical for heretogama	Dentiscutata	D. hetergama
	Pale ochraceous	S, M, L	Globose and subglobose	yes, some	smooth, shining	Germination shield typical for S. scutata, seen without ambiguity, and hyphae connected to the germination shield	Scutellospora	S. scutata
	Straw	S	Globose	yes	smooth, not shining	Funnel shaped hyphae at the insertion, clearly detected. 2 layer groups in the spore and in the hyphae,	Funneliformis	F. mossae
WMul1	Ochraceous	S, M, L, ++ small brick, ochraceous	globose	yes	smooth, shining	Clear hyphae of margarita, with warts and distinctive wall layers	Gigaspora	G. margarita
	Fuscous black	M, L	globose	yes	smooth, not shining	Two wall layers, Ornamentation and hyphae features, plug and sporogenous cell clearly typical for the nigra. Stained to darkish color in Melzer	Dentiscutata	D. nigra
	Ochrc	M	Globose and subGlobose	yes	smooth, shining	Ornamentation and germination shield as distinctive features for reticulata were visible	Dentiscutata	D. reticulata
WMul2	White	L	Globose and subGlobose	yes	smooth, some shining	Germination shield typical for castanea	Racocetra	R. castanea
WMul4	Yellow cream	M, L	Globose and subGlobose	yes	smooth and shining	The hyphae and plug of the sporogenous cell is clearly of the nigra	Dentiscutata	D. nigra
	White	M, L	Globose, irregular	yes	smooth, not shining	White to pale cream, staining in melzer. Three wall layer groups, with the third laminae. Typical hyphae and subtending hyphae plug of rhizophagus, long and branched hyphae. No	Rhizophagus	R. intraradices

						ornamentation, no sporogenous cell,		
	Ochrc	L	Globose	no	rough, not shining	Big spore, stained to dark brown in Melzer, Multicaule, hyphae specific of albida	Gigaspora	G. albida
	Brick yellowish	S	Globose	no	smooth, not shining	Wall layer didn't crack, seen rigid and small spre mor close to glomus	Glomus	Glomus sp.
WMul5	White ivory	L	globose	yes	smooth, not shining	germination shield and hyphae typical for scutellospora castanea	Scutellospora	S. castanea
	Yellow cream	L	globose	yes	smooth, not shining	Two wall layers. Spores with so much dust stuck on the surface, no much distinctive features seen.	Not identified	
	Ochraceous	S, M, L, ++ small spores	globose and subglobose	yes	smooth, shining, not shining	Germination shield and hyphae are very cleary detected and distinct	Dentiscutata	D. erythropa
	Ochraceous1	S, M	Globose	No	Smooth, shining	The Outer wall consists of three layers, hyaline to subhyaline. The outer wall layer is laminated, dark yellow to light brown and has innumerous irregularly-shaped, often edged to sometimes dumbbell-shaped pit as described by Pereira *et al*., 2015a.	Acaulospora	A. reducta
	Brick	S, M	globose and subglobose	yes	smooth, not shining	no clear features, but ornamentation of acaulospora scrobiculata and a small typical germinal orb	Acaulospora	A. scrobiculata aff.
WMul6	Straw	S, M	globose	yes	shining and smooth	unable to identify due to lack of clear detective features, young spore	not identified	
	Ochraceous	S, M, L	globose and subglobose	yes	shining and smooth	Hyphae connections of intraradices	Rhizophagus	R. intraradices
	Brick	S, L	globose	yes, some	rough, not shining	Spore with a rigid thick wall, small size, the hyphae is present but not easy to detect. Affiliated to glomus sp. The hyphae plug typical of glomus. No much content detected	Glomus	Glomus sp.
	White	S, M	globose and subglobose	yes, some	smooth, shining	Funnel shaped hyphae, but specific for coronatum	Funneliformis	F. coronatum
WBur1	Yellow	M, L	globose and	yes	smooth,	laminated walls, warts , hyphae with sporogenous	Gigaspora	G. rosea

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	cream		subglobose		shining	cell		
	Cinnamon1	S, M	subglobose and elliptical	yes	smooth, rough, shining	Big spore, picked melzer, laminated wall layers, hyphae plug of dentiscutata and typical shape of erythropa	Dentiscutata	D. erythropa
	Cigar brown	L	globose	yes, some	smooth, not shining	Bi spore, with two wall layers, Ornamentation and hyphae features, plug and sporogenous cell clearly typical for the nigra.	Dentiscutata	D. nigra
	Ochraceous	S	globose	yes	smooth, shining	big spore, with inner content forming pustules, but all the other cell's features are not well seen to be cleary detected	not identified	
WBur2	Sulfur yellow	L	globose	yes	smooth, shining	White spore, with a germination shield typical for fulgida	Racocetra	R. fulgida
	Cinnamon	S, M, L	globose	yes	smooth, not shining	Three wall layer groups with the L2 very laminae, yellow brown, thick and plastic when pressure is applied, but the inner layer (L3) is elastic and clearly separated with the other ones Inner content for pastules. Germination shield hardy visible. Spore pasitited	Diversispora	D. globifera
	Ochraceous	S, M, L	globose and subglobose	yes	smooth, shining	Germination shield easily identified as of heterogama	Dentiscutata	D. heterogama
	White	M	Globose	Yes	Not shining	Typical wall layer of globifera. Three wall layer groups with the L2 very laminae, red brown, thick and plastic when pressure is applied it breaks but the inner layer groups is elastic.	Diversispora	D. globifera
WBur3	Cinnamon	S	globose	no	smooth, not shining	Spore with a rigid thick wall layers. Hyphae within hyphae. Spore completely cracked but walls maintained they cohesion. brick to brown, trapped in the many hyphaes. Stained in Melzer	Glomus	G. ambisporum
	Yellow cream	L	globose and subglobose	yes, some no	rough, shining	No clear feature but the spore looks more closed to Acaulospora since the wall seem to be slightly ornamented	not identified	

	Ochraceous	S, M	globose	no, some yes	smooth, not shining	Three wall layer groups which are hyaline, with the outer layer having define boundaries. Germinal walls not seen. It didn't pick the Melzer reagent, ornamented typically like the scrobiculata, no sporiferous saccule,	Acaulospora	A. scrobiculata
WBur6	White cream	M, L	globose	yes	smooth, shining	Two to three layer groups, L1,L2 and L3, sporogenous subtending hyphae. Germination shield hyaline to yellow brown, and clearly visible	Racocetra	R. pellucida
	Ochraceous	S, M, L	globose	yes	smooth, shining	The hyphae plug is not visible, thus the species level determination was not possible. Big spore, with two hyphae apparently, but the connection of the sporogenous cell and the spore is hidden. Stained completely in reddish brown in Melzer. Wall groups seem to be two, but are not visible.	Gigaspora	Gigaspora sp.
	Cinnamon	S, M	globose and subglobose	yes, some no	rough, not shining	no distinctive features, young spore	not identified	
WBur7	Cream white	S, M	globose	yes	smooth, shining	Funnel shaped hyphae at the insertion, hyphae with two hyaline layers.	Funneliformis	F. mossae
	Ochraceous	S, M	globose	yes	smooth, shining	Small spores, with three layers distinct (L1, L2, and L3). The L1 is continuous, hyaline and more thick than all the other layers. The L2 is adherne to L1; it is pale yellow to browm, a bit deep and in which the ornamentations seems to form a complex labyrinthian pattern typical of rehmii, when viewed from the spore surface, but it is not clearly viewed in these young spores here present. The L3 is much far from the 2 first layers and appreas as a distinct structure holding the cell content. The cicatrix or scar is a bit visible in one photo but not cleary visible. The inner layer stains slightly in Melzer,	Acaulospora	A. rehmii aff.

						maybe because spores are young.		
	Saffron	M	globose and subglobose	yes, some no	smooth, shining	Two layer groups. The layer group1 were the outer layer, hyaline and flexible. The layer group2 was the inner layer, which produces a red-brown reaction in Melzer's reagent and ornamentation typical for Elegans.	Acaulospora	A. bireticulata
WMug1	Sulfur yellow	S	globose	no	smooth, shining	Young spore, no distinct layers but affiliated to Acaulospora since the ornamentation presented look like Acaulospora escavata. Picked melzer slightly, no sporifefous saccule observed.	Acaulospora	A. escavata aff.
	Yellow cream	S, M	globose	yes	smooth, not shining	young spore, its wall is not yet well formed but is a Gigaspora	Gigaspora	Gigaspora sp.
	Purpish date	M	globose	no	rough, not shining	Yellowish in PVLG, Has 2 wall layer groups. Not laminated as Gigaspora. Hyphae with a plug unclear to be seen due to some dust, but most similar to the hyphae of scutellospora. Some warts can be seen on the surface.	Scutellospora	Scutellospora sp. Aff
WMug2	Ochraceous	M, L	globose	no	smooth, shining	four layer groups, two first are hyaline; two inner layers picked melzer. Big spores , no saccule observed but ornemantation typical for spinosa even in the external walls	Acaulospora	A. spinosa
	Brick	S, M	globose	no	rough, not shining	Spore with a rigid thick wall, small size, the hyphae is present but not easy to detect. With so many small spores, rick to brown, trapped in the many hyphaes. The hyphae plug typical of glomus	Glomus	Glomus sp.
	Cinnamon	S, M, L ++ small	globose and subgrlobose	yes	smooth, rough, not shining	no clear features, seem to be many different species, but some are broken, others infected, etc.	not identified	
	Cinnamon1	S, M	Globose	No	No	Typical wall layer of globifera. Three wall layer groups with the L2 very laminae, red brown, thick and plastic when pressure is applied it breaks.	Diversispora	D. globifera aff.

						Spores lacked enough other characteristics for specification		
	Saffron	M, L	globose	yes	smooth, rough, shining	germination shield of pellucida	Scutellospora	S. pellucida
	White ivory	L	globose and subglobose	yes	smooth, shining	Funnel shaped hyphae at the insertion, hyphae with two hyaline layers. Didn't stain in Melzer	Funneliformis	F. mossae
WMug3	Dark brick	L	Globose	no	rough, not shining	Ornamentation and germination shield as distinctive features for reticulata were visible	Dentiscutata	D. reticulata
	Ochraceous	S	Globose and subglobose	yes	smooth, shining	Young spore, no hyphae but with ornamentation started forming, more close to some Acaulospora	Acaulospora	Acaulospora sp
	Cinnamon	M, L	Globose	yes	smooth, not shining	Yellow to cinnamon in pvlg, picked melzer reagent. Has distinctive germination shield. Three wall layers. The L2 very thick. Hyphae	Racocetra	R. Coralloidea
WMug5	Brick	M	Globose	no	smooth, not shining	Typical wall ormanemnation, not yet encountered in all the literature consulted and in all the database of AMF species descriptions	New, unidentified species	-
	Cigar brown	M, L	Globose	yes, some no	smooth, not shining	Two wall layers, Ornamentation and hyphae features, plug and sporogenous cell clearly typical for the nigra. Medium to large spores a bit red brown	Dentiscutata	D. nigra
	Yellow cream	S, M, L	globose and subglobose	yes	smooth, shining	Germination shield detected without ambiguity	Dentiscutata	D. erythropa
UBw1	Brick	S, M, L	globose and subglobose	yes	smooth, not shining	Big spore, the ochraceous is a bit brick but the three thick laminated wall layers, stained in Melzer, and cracked slightly. The warts are clearly seen, concentrated on the plug. Plug is connected till to the last inner wall layer.	Gigaspora	G. gigantea
	White cream	L	globose	yes, some	smooth, shining	Germination shield and hyphse plug specific for castaneae	Scutellospora	S. castanea
	Ochraceous	L	globose	yes	rough, not	Two to three layer groups, L1,L2 and L3,	Cetraspora	C. pellucida

					shining	sporogenous subtending hyphae. Germination shield hyaline to yellow brown, and clearly visible		
UBw2	Yellow cream	S, M	globose and subGlobose	yes	smooth, shining	Two to three layer groups, L1,L2 and L3, sporogenous subtending hyphae. Germination shield hyaline to yellow brown, and clearly visible	Scutellospora	S. pellucida
	Ochraceous	M, L	globose and subGlobose	no, some yes	rough, not shining	Big spores in majority, stained in Melzer, three wall layer groups, with L2 very laminae and L3 the germinal layer is concolorous and adherent to the laminate layer. hyphae and sporogenous cell typical for decipiens, connected to the L3. Germ tube present but not clearly visible	Gigaspora	G. decipiens
ULuv3	White cream	S, M	Globose, subGlobose, oblong	yes	smooth, shining	Two wall layer groups. They smooth, The inner wall separated slightly with the outer. The hyphae plug not visible. It is more of a Racocetra than Scutellospora or Gigaspora. Ochraceous to yellow. Could broke wider. Spore lacking other distinctive features. Seem young	Racocetra	Racocetra sp.
	White	M	Subglobose	Yes	rough	immature spores of heterogama, but have already a distinct germination shield	Dentiscutata	D. heterogama
	Brick	S	Globose	no	smooth, not shining	Young spore, completely crashed	not identified	
	Ochraceous	S	Globose	yes	smooth, shining	Big spore, two wall layer, with a capsicule visible at the wall surface as a plug of the saccule that couldn't be seen	Acaulospora	A. capsicula
ULuv4	White	S, L	globose	yes	smooth, shining	completely white in PVLG, a young pellucida	Scutellospora	S. pellucida
	Yellow cream	M, L	globose and subglobose	yes	smooth, shining	completely crashed, inner cell content formed pustules and the cell walls and other features are not easy to detect	not identified	
	Yellow	M	globose and subglobose	yes	smooth	young spore, completely crashed but has some foreseen ornamentation of an Acaulospora	Acaulospora	Acaulospora sp.

ULuv6	Pale ochraceous	S, M	globose	yes	shining and smooth	Two wall layer groups. Spore completely broken. The visible ornamentation if of excavate as described by Pereira *et al*., 2015a., but no other feature detected to confirm.	Acaulospora	A. excavata aff.
	Ochraceous	S	globose	no	smooth, shining	Not identified due to lack of enough detectible features		
ULuv7	Brick	S, ++ spores of small size and other damaged already	globose	no, some yes	rough, not shining	Two wall layer groups. They smooth, The inner wall separated slightly with the outer. The hyphae plug not visible. It is more of a Racocetra than Scutellospora or Gigaspora. Ochraceous to yellow. Could broke wider. Spore lacking other distinctive features. Seem young	Racocetra	Racocetra sp.
PKam1	Brick	S, L	globose and subglobose	no	rough, not shining	No enough features detected for specification even in a genus. Young spore, not identified		
	Ochraceous	S, ++ spores of small size	globose	yes, some no	smooth, shining	Shrinking inside wall, ornamented surface like the excavata. No hyphae or saccule detected. Match with description of Pereira *et al*., 2015a.	Acaulospora	A. excavata
PKam3	White	L	subglobose	yes	smooth, shining	Thick two layer groups with very laminae and continuous. It picks melzer slightly, broke completely. No hyphae detected but its homogenous color in Melzer and the wall layers helped to classify it.	Clariodeoglomus	C. etunicatum
	Cinnamon	S	Globose	no	rough,not shining	White to yellowish brown. Funnel shaped hyphae plug. Two wall layers groups even in the hyphae germinal tube, but hyphae specific for coronatum at the plug and the septum	Funneliformis	F. coronatum
	Ochraceous	S, M, L , ++ small ochraceous	Globose and sub Globose	yes	smooth, shining	Mostly small to medium spores, funnel shaped hyphae at the insertion, hyphae with two hyaline layers and seemed to be branched. Didn't stain in Melzer	Funneliformis	F. mossae
PKam4	White cream	M	subglobose	no	smooth,	young spore with no much visible features	not identified	

						shining		
	Brick	S, M	Globose, elliptic, and subglobose	yes	rough, not shining	Most spores with an elongated morphology, with the hyphae as of Gigaspora. Germination shield and hyphae cleary detected and distinct as of the erythropa	Dentiscutata	D. erythropa aff.
	Ochraceous	S, M, L, ++ small ochrc	globose and subglobose	yes	smooth, shining	Many hyphae, two to three wall layer groups, thick and broke as glomus but seem to have not reacted in Melzer and very distinc layer than the ones of glomus	Clariodeoglomus	Clariodeoglomus sp.
PKam5	Ochric suffron	M	Globose	no	smooth, not shining	Spore with a rigid thick wall, small size, the hyphae is present but not easy to detect. Affiliated to glomus sp. With so many small spores, rick to brown, trapped in the many hyphaes. No hyphae	Glomus	Glomus sp.
	Ochraceous	S, M	Globose and subGlobose	Yes, some no	smooth, not shining	Ochraceous to yellow. Completely broken, one wall layer visible, hyphae plug close to racocetra but very unclear to be seen. Spore lacking other distinctive features. Seem young	Racocetra	Racocetra sp. Aff.
PKam6	Yellow cream	S, M	Globose and sub Globose	yes	smooth and shininh	Hyphae, big spore, no laminated wall, germination shield close the Scutellospora	Scutellospora	Scutellospora sp. Aff
	Ochraceous	M	subGlobose	no	smooth	Spore present a germination shield, very difficult to detect,	Scutellospora sp. Aff	
	Brick	M	Globose	no	smooth and rough	Young spore, no feature, not identified		
	Cinnamon	M	Globose	yes	smooth	The Outer wall consists of three layers, hyaline to subhyaline. The outer wall layer is laminated, dark yellow to light brown and has innumerous irregularly-shaped, often edged to sometimes dumbbell-shaped pit. No sporogenous saccule observed. Spore with a big cicatrix but not very distinct on the surface. Judged as A. Reducta from experts	Acaulospora	A. reducta

	Yellow	M and S	Globose and sub Globose	Yes, some no	smooth	Spores are looking so young, but the ornamentation under development are very similar to the one of rehmii	Acaulospora	A. rehmii aff.
PKam7	Brick	S	globose	yes,	smooth, not shining	The ornamentation seem to be of the bireticulata but the spore is full of dust for clear specification	Acaulospora	A. elegans aff.
	Cigar brown	L	globose	no, some yes	smooth, rough not shining	no feature, completely dark, not identified		
	Ochraceous	S, M, ++ small ochraceous and brick	globose and subglobose	yes	smooth, shining	The spore surface is dark yellow to light brown and has a lot of irregularly-shaped, large pits with small depressions. No sporogenous saccule observed. Spore with a big cicatrix but not very distinct on the surface	Acaulospora	A. reducta
	Ochraceous1	M	globose and subglobose	yes	smooth, shining	Three wall layers, saccule neck cicatrix, and a circular to ovoid cicatrix on the surface. Germination orb not clearly visible	Acaulospora	A. tuberculata
	Cream	S	globose	yes	smooth, shining	Two wall layer groups, separatd largely. The inner wall layer picked Melzer solution and bear the specific ornamentation, slightly pitted and uniform. The neck of the saccule clearly visible. Matching the features of spinosissima as described by Oehl *et al.* (2014)	Acaulospora	A. spinosissima

*Size estimation: S = 45 μm ∼ 150 μm, M = 150 μm ∼ 300 and L = ∼ 300 μm and above

**Species description was done with the lab technicians and the doctors who have acquired significant knowledge in this field

Source of description criteria (besides images, the description and identification were sustained by hand drowing and consultation of experts):

- http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html

- http://www.zor.zut.edu.pl/

- http://www.amf-phylogeny.com/

- Oehl *et al.*, (2011) ; Oehl *et al.* (2014); Pereira *et al.*, (2016a)

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Appendix 3: PVLG and Melzer reagents composition

PVLG		Melzer	
Chemical	Quantity	Chemical	Quantity
Polyvignyl	16.6g	Iodine	1.5g
Lactic acid	100ml	Chloral hydrate	100g
Glycerol	10ml	Potassium iodide	5g
Water	100ml	Water	100ml

Appendix 4: Nutrient solution for mycorrhizal plants-adapted from Ingestad by Ingleby (2007)

Stock solutions (amounts in g / liter)

Solution B	Solution C
NH_4NO_3 - 140.2	HNO_3 - 1.6
KNO_3 - 37.2	H_3BO_3 - 0.57
KH_2PO_4 - 41.3	$Fe_2(SO_4)_3$ - 2.5
K_2SO_4 - 14.0	$Ca(NO_3)_2$ - 14.3
	$Mg(NO_3)_2$ - 26.0
	$MnSO_4$ - 0.55
	$CuCl_2$ - 0.032
	$ZnSO_4$ - 0.036
	$NaMoO_4$ - 0.007

To make 10 litres of our standard feeding solution, we use 10.65 ml B and 6.25 ml C. This solution contains 10 ppm P, 62.5 ppm N and 34.5 ppm K.

Appendix 5: Mycorrhizal inoculum production

Sample Code	Spores Density	Roots Colonization frequency (%)	Geograhic localisation	
			Long E	Lat S
Kmun1	0.38	30.0	28° 54' 48.06"	2° 5' 43.08"
KMun7	0.92	36.66	28° 54' 22.46"	2° 5' 56.54"
Kluz5	1.2	23.33	28° 51' 59.18"	2° 7' 58.4"
Kkash1	2.12	46.66	28° 51' 17.75"	2° 9' 5.76"
Kkash3	0.96	40	28° 51' 18.43"	2° 9' 23.65"
Kkash5	2.32	40	28° 51' 22.54"	2° 10' 5.66"
Kbkat2	0.44	0	28° 50' 4.16"	2° 13'
Kbkat4	0.48	20	28° 48' 48.74"	2° 14' 1.18"
Kbluh4	0.98	20	28° 52' 54.19"	2° 17' 9.46"
Wmul1a	2.6	50	28° 34' 39.4"	2° 42' 3.06"
Wmul1b	1.12	16.66	28° 34' 39.4"	2° 42' 3.06"
Wmul5a	1.42	36.66	28° 33' 50.15"	2° 42' 0.86"
Wmul5b	1.6	26.66	28° 33' 50.15"	2° 42' 0.86"
Wmul6	2.82	43.33	28° 34' 5.56"	2° 41'
WBur1	1.2	26.66	28° 37' 16.68"	2° 42'
WBur2	1.12	13.33	28° 38' 45.38"	2° 41'
WBur7	0.68	13.33	28° 41' 9.28"	2° 37' 7.93"

WMug2	0.42	16.66	28° 45' 39.49"	2° 35' 14.5"
WMug3	0.06	0	28° 21' 15.3"	2° 36' 4.86"
WMug6	0.88	20	28° 46' 2.32"	2° 33'
Pluv3	0.12	3.33	29° 2' 29.44"	2° 51'
Pluv4	0.42	20	29° 0' 27.72"	2° 51'
Pluv7	0.64	10	29° 0' 16.27"	2° 50' 9.71"
Pkam2	0.34	10	28° 59' 59.17"	2° 46'
Pkam7a	0.76	40	29° 0' 1.08"	2° 42'
Pkam7b	1.94	40	29° 0' 1.08"	2° 42'
Rhizatech	4			

Five strains that have shown to produce a high spore density and high roots colonization were selected for effectiveness assessment. They are Kkash1, Kkash5, WMul1a, WMul6 and Pkam7b, and they were named (as they represent) AMF1 (*Gigaspora gigantea*). AMF2 (*Gigaspora sp.*), AMF3 (*Gigaspora margarita*), AMF4 (*Rhizophagus intraradices*) and AMF5 (*Acaulospora. reducta*) respectively.

Appendix 6: Agro ecological Zones of South Kivu

No		AEZs	Altitude (m)	Rainfall (mm)	T° mean (en °C)	KOP	Location	Agricultural vocation
1	Low altitude	Equatorial zone of low altitude	<1000	>1600	>29°C	Af	Shabunda, Kitutu, Kibemela, Ikose	Oil Palm, Coffea, rice, maize, Groundnuts, cassava, banana plantain
2		Tropical zone of low altitude	<1000	<1600	>24°C	Aw1-3	Kamaniola, Luberizi, Kiliba, Uvira, Baraka, Dine, Mboko, Lubarika	Sugar cane, coton, rice, sorhum, millet, maize, cassava, tArrowroot, yam
3	Mean altitude	Equatorial zone of medium altitude	1000 – 1400	>1300	20°C-23°C	A3	Kamituga, Mungombe, Bunyakiri, Nyambo, Isopo	rice, sorghum, millet, bean, banana plantain, cassava, Sweet potato, Oil palm
4		Tropical zone of medium altitude	1400 - 1800	>1300	17°C – 20°C	Cw	Mwenga, Walungu, Idjwi, Kalehe, Bukavu, Kabare, Cirunga, Kasika, Katana, Nyangezi	Thea, quinquina, coffea . banana plantain, sweet potato, bean maize, soja, groundnut, sorghum, banana
5	High altitude	Equatorial zone of high altitude	1800 and more	>1300	12°C - 17°C	Cw	Kabare, Mulume Munene, Kahuzi Biega, Kadjedje, Kalonge, Nyabibwe, Burhinyi, Ikoma, Izege, Luwinja, Kaziba	Tea, quinquina, tobacco, potato, peas, apple, wheat, Oat, maize, Pear tree
6		Tropical zone of medium and high altitude	1000 and higher	< 1600	< 23°C	Am	Fizi Itombwe, Minembwe, Miki, Katobo, Uvira, Sange	Rainfed and equatorial crops according to altitudes, dry season pastures

Source: IPAPEL (2011)