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Vescular Arbuscular Mycorrhiza Diversity and Morphotypes, from Different Land Use of the Serengeti National Park, Tanzania

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Abstract

Vesicular Arbuscular Mycorrhiza (VAM) are members of the kingdom fungi that constitute an important part of the savannah ecosystem of Serengeti National Park (SNP). Three different land use types; indigenous woodland, natural grasslands with different degree of protection based on whether they are found outside or inside the park, and cropland were explored for the presence of VAM fungi. A cross relationship of the land use effect to the VAM morphotypes, diversity and abundance as well as soil chemical properties were evaluated. Roots of 80 plant species belonging to 20 genera were examined. Morphological characterization of the VAM morphotypes and diversity were observed using the light microscope after root staining with Trypan blue in 0.05% w/v lactoglycerol preceded by fixation, tissue clearing, rinsing, bleaching and acidification.

The results show that the dominant groups of VAM identified belong to *Scutellospora*, *Glomus*, *Acaulospora*, and *Gigaspora* genera. Species in the genus *Scutellospora* were dominant followed by species in *Glomus* and *Acaulospora* while the least were from *Gigaspora*. This difference in species dominance may be due to differences in soil parameters such as soil pH which ranged from 5.59-7.49 in different land use types. With respect to the morphotypes, the examined VAM fungi in SNP were found to exhibit two main morphotypes; the Arum and Paris type. Generally the Arum morphotype was dominant comprising of (57%)

followed by the Paris that constituted 40% while the undifferentiated morphotype constituted 3%. Noticeably, the Paris-type was more appropriate for slow growing plants dominating in the grassland with scarce and less vegetation while the Arum were dominant in the woodland constituting the fast growing vegetation.

This shows that Arum-type are very important to the fast growing forest and may be useful in reforestation compared to the Paris morphotype.

Keywords: Serengeti, Land use types, Paris-type, Arum-type, VAM fungi

1. Introduction

Vesicular arbuscular mycorrhizas (VAM) are extremely successful fungi that form mutualistic symbioses with about two thirds of all plant species (Smith & Read 1997). VAM fungi are of great ecological importance since arbuscular mycorrhizae are the most widespread plant symbiosis that often improves plant productivity (Fedderman *et al*., 2010). The main advantage of mycorrhizae to the host plants are increased efficiency of mycorrhizal roots versus non mycorrhizal roots caused by the active uptake and transport of nutrients especially immobile minerals like P, Zn and Cu (Phiri *et al*., 2003; Jamal *et al*., 2002). This work is achieved by the interconnected networks of external hyphae which act as an additional catchment and absorbing surface in the soil (Sharma, 2004).

Gallaud (1905) described two morpho types of the VAM fungi as the Arum type and Paris type. The Arum-type form extensive intercellular hyphae in air spaces between cortical cells and invaginate the plasma membrane of the cells as short side branches to form arbuscules while the Paris type, the colonization spreads from cell to cell in the cortex thus develop intracellular hyphal coils that frequently have intercalary arbuscules. Most species of plants including grasses, herbs, and tropical trees form Vesicular-arbuscular mycorrhiza (Gerdemann, 1965, 1968). While the VAM fungi are benefited with carbon substrates from plants and shelter in return to the host plant they provide enormous benefits to their host. Among them include improving plant nutrition, (Van der Heijden *et al*., 1998), help to control pests and fungal pathogens (Pozo *et al*., 1996, 2010), increase uptake and transport of soil nutrients (Cox *et al*., 1975; Abbot & Robson, 1984; Newman & Reddel, 1987) enhancing water movement, Auge, 2001), promote positive diversity (Laura *et al*., 2010) as well as reducing pathogenic infections to their host (Dehne, 1982; Fitter, 1986).

In developed countries VAM fungi have been substantially studied (Cox *et al*., 1975; Abbot & Robson, 1984; Newman & Reddel, 1987, Laura *et al*., 2010) due to its importance in forest conservation. Although SNP is hot spot of biodiversity, the studies on the fungi and specifically the VAM fungi has been completely neglected, yet successful conservation efforts in any ecosystems may require understanding of fungi communities in terms of ecology and distribution. Apart from Tibuhwa *et al*. (2011) and Tibuhwa (2011, 2012) who mainly examined the macro-fungi, no any studied on fungi that have been done in the SNP. This study therefore, examined the VAM fungi and establishes the effect of the land types to the VAM morphotypes, diversity and abundance in the SNP.

2. Materials and Methods

2.1 Site Description

The study was conducted in the Serengeti National Park Tanzania (Figure 1). Serengeti ecosystem is made up of protected land (Serengeti National Park, The protected area in the east is surrounded by semi -arid rangelands characterized by less rainfall (c.800 mm per year) while the west side is wet with high rainfall (c.1200 mm per year) comprising small-scale subsistence agriculture. The annual distribution of the rainfall across the study area is characterized by two rainy seasons and two dry seasons. The short rains are from October to December and long rains occur between March and May. The main dry period is from June to September with lesser dry spell in January and February. The land use system in this region

comprises of natural woodland and grassland inside and outside the park. Natural woodland and grassland inside the park are characterized by minimal human disturbances while woodland and grassland outside the park are characterized by heavy human disturbances associated with overgrazing by the cattle and wild animals and other human related activities such as charcoal burning, cutting of trees for timber, fire wood as well as harvesting of non-timber products. Agricultural systems outside the protected core of SNP comprise of small-scale subsistence farming of cassava, millet, maize and beans.

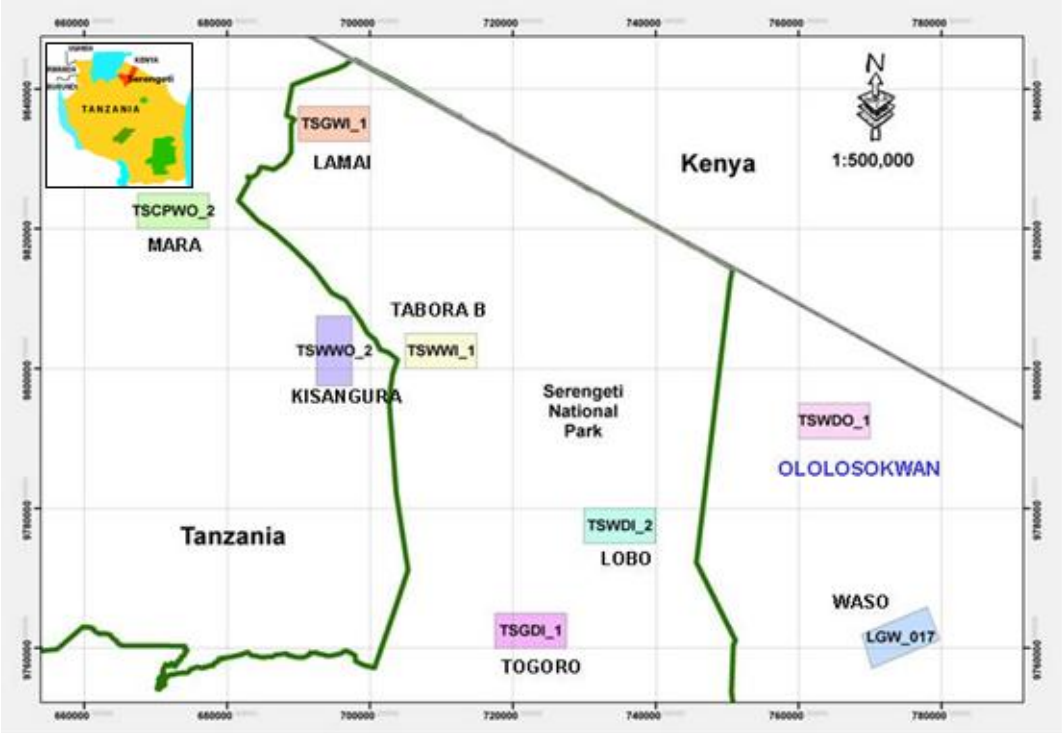


Figure 1. Map of Serengeti National Park showing studied sites.

2.2 Experimental Design

A one-year survey was conducted during the short rain of September-November 2009 and long rains of March-June 2010. The study was conducted in pre-established study land use/habitat categories containing uniform land use differing according to major land use types viz: (i) indigenous woodland (ii) natural grasslands and (iii) Cropland with mixed subsistence cropping (Figure 2). For each land use, plots of 10 x 5 km were selected inside the park (protected) and outside the park (unprotected) and one crop land from both dry and wet region of the park. The plots included (i) Kibeyo woodland outside the park (TSWWO), (ii) Tabora B woodland inside the park (TSWWI), (iii) Gibaso grassland outside the park (TSCPWO), (iv) Lamai grassland inside the park (TSGWI) and (v) Nyansurura crop land (TSCPWO) in the wet region of SNP. In the dry side the study plots included (i) Ololosokwan woodlands outside the park (TSWDO), (ii) Lobo woodland inside the park (TSWDI), (iii) Togoro Plains grassland outside the park (TSGDI), (iv) Wasso grassland inside the park (TSGDO) and (v) Mdito crop land (TSCPDO). In each sampling plot (10 x 5 km) in each land use types, four transects each measuring 1 x 0.05 km were laid out, 1 km away from

each other and 0.5 km away from the road. Roots of trees with VAM-fungi were collected twice during dry season September-November, 2009 and wet season April-June 2010 in each transects.



a b c

Figure 2. Some land use types (a) Woodland, (b) Grass land (c) Crop land.

2.3 Roots with VAM-Fungi Sampling and Staining

For the purposes of avoiding the possibility of collecting roots from another species, only roots attached to main root of the plants were collected and used for mycorrhizal assessment. Roots were stained with Trypan blue in 0.05% w/v lactoglycerol preceded by fixation, tissue clearing, rinsing, bleaching and acidification according to (Kormanik & Mc Graw 1982). A concentration of 0.05% w/v in lactoglycerol was used to stain cleared roots. Prolonged clearing times with KOH were used to remove more phenolic pigments from roots. Post-clearing bleaching with alkaline hydrogen peroxide (0.5% NH4OH and 0.5% H2O2 v/v in water) was used to removes phenolic compounds left in cleared roots.

2.4 Microscopic Observation and Identification

Morphological characterization of VAM fungi from stained and mounted sections of the roots were directly observed on a light compound digital microscope following (Kormanik & McGraw 1982) protocol. The VAM fungi were identified based on their spores, vessicles colour and filament shapes, and arbuscules structures using available identification guide by Kormanik & McGraw 1982 (at http://www.ableweb.org/volumes/vol-9/7-charvat.pdf). The photos of the observed demarcating features were taken direct on the digital microscope (MOTIC IMAGES PLUS 2, Japan). The morphotypes were determined based on Hyphal growth types according to Armstrong & Peterson (2002) while the arrangements spores, hyphae, vesicles, arbuscules and other additional characters were applied to place the studied specimen to their respective genera according to Gerdemann & Trappe (1974), Walker & Sanders (1986), Morton & Benny (1990), Schenck & Pérez (1990).

2.5 Mycorrhiza Morpho-type Assessment

A stained root sample was gently placed on the observation slide covered with a cover glass and tapped gently to squash and it. About 10 cm length of root in each plant individual was examined. To establish the morphological type of the VAM, the morphology of AM colonization and the presence of Arbuscular-mycorrhiza structures such as arbuscules, vesicles and coils were assessed. Observation was done on a light microscope Olympus (OM BX 50, JAPAN) with Normasiki interference contrast optics. In order to avoid the confusion

of VAM with other fungi such as endophytic fungi which are symptom less, and occurring very commonly in the roots of plants (Addy *et al*., 2005) only hyphae that formed the arbuscules or those with coordinated development in young roots were considered.

2.6 Soil Sample Collection

Soil sample were collected from each transect at a depth of 0, and 10 cm using soil auger. Collecting 3 different samples and mixing them made composite sample. The soil samples were then kept in the polythelyne bags, properly sealed to prevent moisture loss and contaminations before transported to the Botany Department of the University of Dar es Salaam for Laboratory analysis. The associated chemical properties including soil reaction (pH), soil texture, Total soil Nitrogen, Available soil Phosphorus, Total carbon, Al^{3+}, Na and K were established.

2.6.1 *Soil Sample Analysis*

Soil pH was measured electrometrically using a Metrohm E510 pH meter. This was done according to Kormanik & McGraw (1982) using 1:1 soil water mixture, which was allowed to equilibrate for 30 minutes. Then the pH or stirred suspension was read from the pH meter and recorded as pH in water (pH). The soil organic matter was determined using the Walkley-Black potassium dichromate method while available phosphorus determined using spectrophotometer method by ascorbic acid method as described by Olsen & Sommers (1982). The total soil Nitrogen was determined by using a semi-microKjeldah digestion according to Allen (1989). Soil texture was determined by pipette method as described by Gee & Bauder (1986).

3. Results and Discussion

3.1 VAM Diversity

In this study, VAM fungi species belonging to four genera (*Acaulospora, Glomus, Gigaspora* and *Scutellospora*) were observed across different land use system in the SNP. Throughout the studied area species in the genus *Scutellospora* were dominant followed by species in *Glomus* while the least was from *Gigaspora*. This result is partly similar to that of Mathimaran *et al*. (2007) and Muchane *et al*. (2012), who also both reported on the dominance of *Acaulospora* and *Scutellospora* species in Western Kenya and Maasai Mara respectively, however, in this study the dominant species in the SNP were found to belong to *Scutellospora* and *Glomus* genera. This difference in species dominance may be due to differences in soil parametes such as soil pH which is well known in affecting the VAM distribution (Gai & Liu, 2003). For example, according to Muchane *et al*. (2012), the soil pH in the Masai Mara ranges from 6-6.42. However, the pH of all land use types in this study ranged from 5.59-7.49 (Figure 3 a&c). This difference in soil pH might probably alter the dominance of the VAM fungi in the studied area, although the two areas are in close proximity with many similar climatic conditions (Tibuhwa *et al*. 2011).

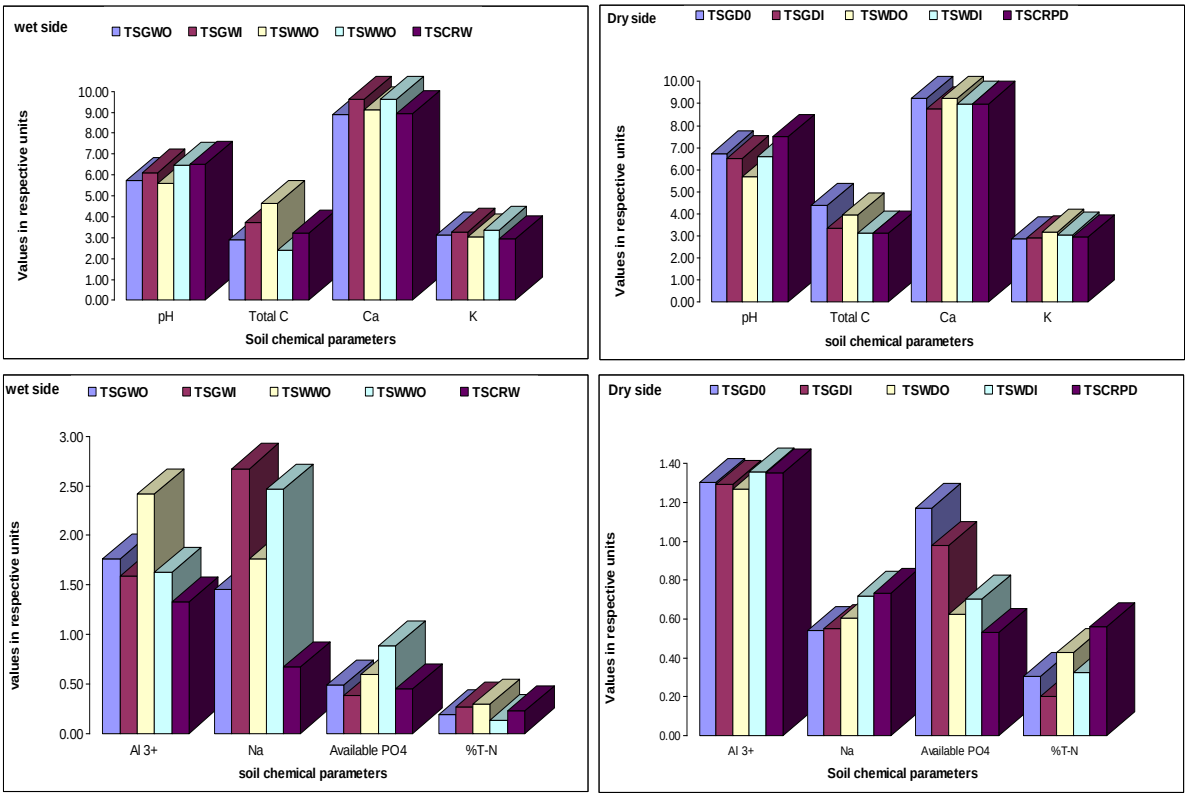


Figure 3. Variation of soil parameters with respect to different land use types in the SNP

The roots systems of the plants were used in this study; it thus, included all the spores that might not be sporulated and species that may not have associated with the trap cultures. It was however, interesting to note that species of the genus *Gigaspora* were the least in the studied area. This finding concurs with other various studies which reported on low density of *Gigaspora* species (Schalamuk *et al*., 2006; Jefwa *et al*., 2009, Muchane *et al*. 2012) in their studied areas. Our study thus concurs with opinion of low level of occurrence of the species in this genus in many ecosystems compared to other species.

3.2 VAM Morphotypes

The results of this study show the dominance of Arum morphotype in the SNP (Figure 4&5). This dominant colonization by Arum type might be attributed by the type of plants found in the SNP. It is well known that the host plant exerts a control over the intercellular hyphal proliferation and arbuscle formation. The host plant undergoes major modifications in its cells such as vacuoles shrinkage, cytoskeleton reorganization, and other cellular organelles proliferation to accommodate the arbuscules (Pozo *et al.* 1996). These host plant anatomical characters strongly influences VAM morphotype hence their identity (Brundrett & Kendrick, 1990, Yamato 2004). The dominance of Arum type results in this study, suggest that arbuscules, coils, entry points and vesicles to the majority of SNP plants is suitable to longitudinal growing by the linear hyphae between the plant cells. According to Ocampo *et al*. (1980), the correlation between entry points-vesicles and entry points-arbuscules suggests VAM of Arum type to form high root colonization structures, while the entry points-coils relationship suggests low root colonization by Paris type. Nevertheless, our results show that intermediate VAM morphotype colonization exists where by the fungus colonized root,

growing intracellularly from cell to cell as in Paris type, but the arbuscules was formed terminally as in Arum type (Table 1).

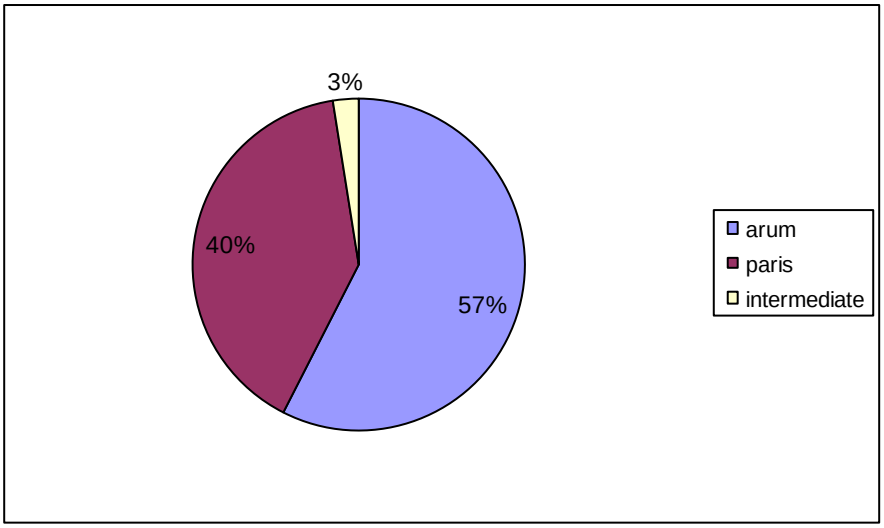
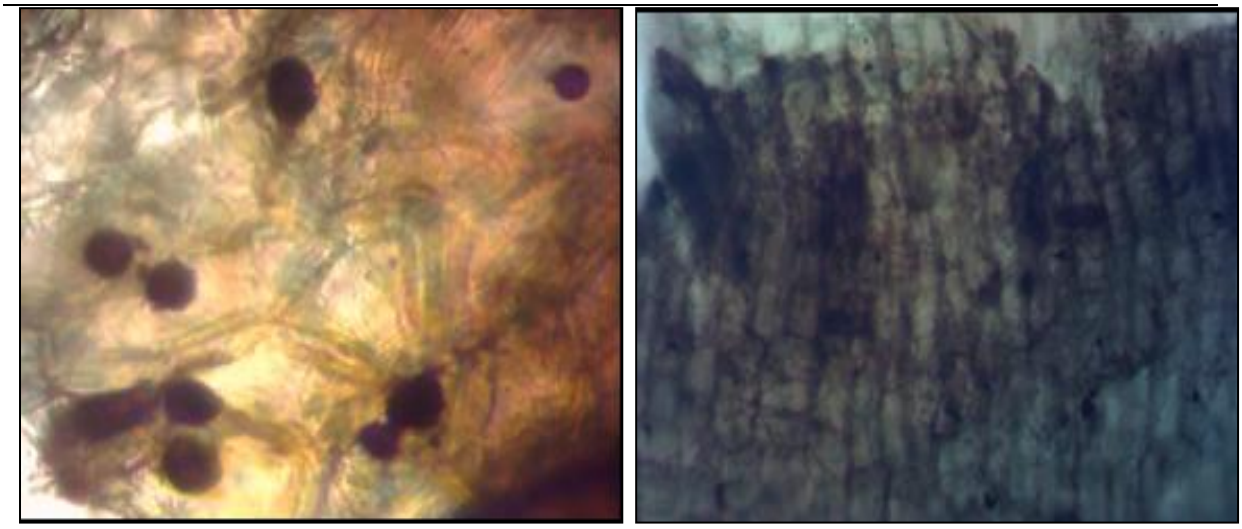


Figure 4. Distribution of the VAM fungi morphotypes showing the overall dominance of Arum type.

This result concur with that of (Muthukumar & Parkash, 2009) who also noted the co-occurrence of both types in plant families of Arecaceae, Poaceae, Euphorbiaceae, Fabaceae, Rubiaceae, Solanaceae and Lamiaceae which has also been recently reported by Smith & Read (1997). Recent study has however revealed many exceptions to the main morphological types including the existence of paired arbuscules in some roots of *Linum* species (Dirk *et al*., 2003). Our study concur with the opinion that more studies need to be done on different genera and families in order to ascertain the mechanism involved in forming a particular VAM morphotypes. In this study it was also revealed that the land use type affect the distribution of VAM morphotypes. Wood land was found dominated with Arum unlike the grassland which was dominated by Paris type (Figure 6). Yamato (2004) in his study also noted the influence of ecology and environmental factors to on the VAM morphology the result which concurs with our observation. Generally the functional differences between the two VAM morphotypes have not yet been elucidated although Brundrett & Kendrick (1990) suggested that probably the slower colonization of AM fungi in the Paris type might be advantageous to maintain the energy supply to the fungi at a manageable level for plants growing slowly in a relatively dark environment; This study in addition presume that the Paris type spend a lot of energy for intracellular hyphal growth compared to Arum type with intercellular hyphae growing in air spaces between cortical cells.



a b

Figure 5. Photo showing different VAM morphotypes (a) Paris-type (b) Arum-type

Although the physiological and functional disparity between Arum type and Paris type is still not clear but it has been reported that the development of Arum-type is faster than that of Paris type (Brundrett &Kendrick, 1990; Cavagnaro *et al*., 2001). This study thus suggests that Arum-type is very important to the fast growing forest and may be useful in reforestation compared to the Paris morphotypes.

The soil chemical analysis revealed that there is enormous variation in the soil chemical properties such as pH, total N, available P, and C, between different land use sampling sites while other parameters of Ca, Na, K, and $Al3^{+}$ in meq/100g vary slightly with land use types (Figure 3 b&d). For example in the dry side of SNP, the lowest soil pH (5.67) was observed from TSWDO samples which are considered to be acidic, whereas the highest pH was observed in TSCRP (7.49) which is considered to be alkaline (Figure 3 a&c). The highest available phosphate was observed in TSGDI (1.17 meq/100g), whereas other samples possessed comparatively low soil 'P' up to 0.53 meq/100g found in TSCRPD.

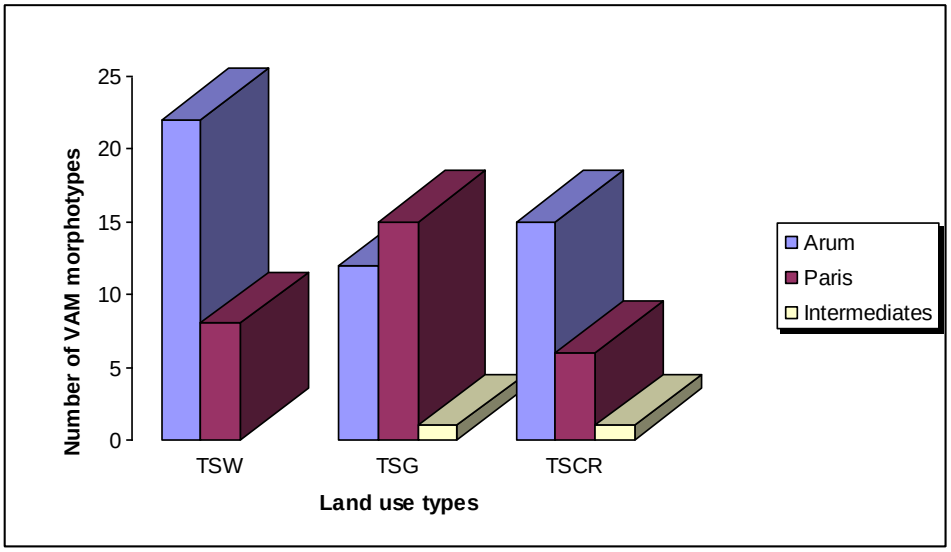


Figure 6. Distribution of VAM morphotypes according to land use types

3.3 The Influence of Soil Properties, Climatic Conditions on VAM Fungi Diversity and Distribution

The total Nitrogen was found high (0.56) in the cropland compared to other land use types in the dry side, while the least value was from the TSGDI. The high nitrogen content found in the cropland might be attributed by high organic matter brought in by mixed crops especially the legumes which in nature are nitrogen self-sufficient (Heichel *et al*., 1991). The low nitrogen content in the grassland inside the park might be caused by absolutely less vegetation cover, which do not even attract wild animal which would have added up the nitrogen content through their wastes (see Figure 2b).

Table 1. Studied mycorrhizal tree roots and the observed VAM morphotypes

Side	Plot	Family	Life form	Species	Morphotype
Wet side	**TSWWI**	Leguminoseae	Climber	*Pseudovigna sp.*	Arum
'	'	Phaseoleae	Grass -like	*Virgina unguiculata*	Arum
'	'	Fabaceae	Shrub	*Crotalaria labunoides*	Arum
'	'	Leguminosae	Herbaceous	*Tephrosia villosa*	Arum
'	'	Vitaceae	Tree	*Cyphostema ademocaule*	Arum
'	'	Caesalpinaceae	Herbaceous	*Cassia mimosoides*	Arum
'	**TSWWO**	Fabaceae	Tree	*Acacia nilotica*	Arum
'	'	Caesalpinaceae	Herbaceous	*Cassia occidentalis*	Arum
'	'	Papilionoideae	Shrub	*Ormocarpum kirkii*	Arum
'	'	Fabaceae	Tree	*Acacia seyal var. seyal*	Arum
'	'	Leguminosae	Herbaceous	*Zornia setosa*	Arum
'	'	Fabaceae	Tree	*Acacia seyalvarfestula*	Arum
'	'	Fabaceae	Tree	*Acacia robusta*	Arum
'	'	Caesalpinaceae	Herbaceous	*Cassia elata*	Arum
'	**TSWCR**	Leguminosae	Herbaceous	*Tephrosia punila*	Arum
'	'	Leguminosae	Herbaceous	*Zornia bracteata*	Arum
'	'	Papilionaceae	Herbaceous	*Crotolaria lanceolata*	Paris
'	'	Fabaceae	Tree	*Acacia nilotica*	Arum
'	'	Mimosoideae	Tree	*Albizia amara*	INT
'	'	Leguminoseae	Shrub	*Indigofera cuniata*	Paris
'	'	Leguminosae	Herbaceous	*Rynchosia minima*	Paris
'	'	Fabaceae	Shrub	*Alycircapus glumecense*	Paris
'	'	Leguminosae	Herbaceous	*Desmodium barbatus*	Arum
'	'	Caesalpinaceae	Herbaceous	*Cassia mimosoidea*	Arum
'	'	Fabaceae	Herbaceous	*Tylossema sp.*	Arum
'	'	Papilionaceae	Herbaceous	*Crotolaria spinosa*	Arum
'	**TSGWI**	Papilionoideae	Shrub	*Ormocarpus kirkii*	Paris
'	'	Caesalpinaceae	Herbaceous	*cassia mimosoides*	Arum
'	'	Leguminoseae	Shrub	*Indigofera volkensii*	Paris
'	'	Papilionoideae	Climber	*Dolichos oliver*	Paris
'	'	Mimosaceae	Shrub	*Dichrostachys cinerea*	Arum

'	'	Fabaceae	Tree	*Acacia drepanolobium*	Arum
'	**TSGWO**	Papilionoideae	Shrub	*Ormocarpus kirkii*	Paris
'	'	Fabaceae	Tree	*Acacia polyacantha*	Paris
'	'	Fabaceae	Tree	*Acacia seyal var. seyal*	Arum
'	'	Papilionoideae	Shrub	*Stylosanthus frruticosa*	Arum
'	'	Papilionaceae	Herb	*Crotolaria burker*	Arum
'	'	Mimosaceae	Shrub	*Dichrostachys cinerea*	Paris
'	'	Papilionaceae	Herbaceous	*Crotolaria burkensii*	Arum
'	'	Leguminosae	Herbaceous	*Desmodium barbatus*	Paris
Dry side	'				
'	**TSWDO**	Fabaceae	Tree	*Acacia nilotica*	Paris
'	'	Fabaceae	Tree	*Acacia drepanolobium*	Paris
'	'	Leguminoseae	Shrub	*Indigofera volkensii*	Paris
'	'	Papilionoideae,	Shrub	*Ormocarpus kirkii*	Paris
'	'	Fabaceae	Tree	*Acacia brevispica*	Paris
'	'	Leguminosae	Herbaceous	*Rhynchosia minima*	Arum
'	'	Caesalpinaceae	Herbaceous	*Cassia sp.*	Paris
'	'	Leguminosae	Climber	*Macrotyloma axillare*	Arum
'	'	Papilionaceae	Herbaceous	*Crotolaria good formis*	Paris
'	**TSGDI**	Fabaceae	Tree	*Acacia drepanolobium*	Arum
'	'	Papilionoideae,	Shrub	*Ormocarpia kirkii*	Arum
'	'	Papilionaceae	Herbaceous	*Crotolaria labunoides*	Arum
'	'	Mimosaceae	Shrub	*Dichrostachys cinerea*	Paris
'	'	Fabaceae	Tree	*Acacia robusta*	Arum
'	'	Fabaceae	Shrub	*Crotolaria spinosa*	Paris
'	'	Leguminoseae	Shrub	*Indigofera volkensii*	Paris
'	'	Caesalpinaceae	Herbaceous	*Cassia mimosoidea*	Arum
'	'	Labiatae	Herbaceous	*kotschyia sp.*	Arum
'	**TSWDI**	Fabaceae	Tree	*Acacia drepanolobium*	Paris
'	'	Papilionaceae	Herb	*Crotolaria lanceolata*	Paris
'	'	Mimosaceae	Shrub	*Dichrostachys cinerea*	Paris
'	'	Papilionaceae	Herbaceous	*Crotolaria spinosa*	Arum
'	'	Papilionoideae,	Shrub	*Ormocarpus kirkii*	Paris
'	'	Leguminoseae	Shrub	*Indigofera volkensii*	Arum
'	'	Leguminosae	Herbaceous	*Rhynchosia virta*	Paris
'	'	Labiatae	Herbaceous	*kotschyia sp.*	Paris
'	'	Fabaceae	Tree	*Acacia robusta*	Paris
'	**TSGDO**	Papilionaceae	Herbaceous	*Indigofera cuniata*	Arum
'	'	Leguminoseae	Shrub	*Indigofera volkensii*	Arum
'	'	Leguminosae	Herbaceous	*Rhynchosia minima*	Paris
'	'	Papilionaceae	Herbaceous	*Crotolaria spinosa*	INT
'	'	Fabaceae	Tree	*Acacia drepanolobium*	Arum
'	**TSDCR**	Papilionoideae	Climber	*Dolichos oliver*	Arum

'	'	Leguminoseae	Herbaceous	*Phaseolus vulgaris*	Arum
'	'	Leguminoseae	Shrub	*Indigofera arrecta*	Arum
'	'	Leguminosae	Herbaceous	*Tephrosia villosa*	Arum
'	'	Leguminosae	Herbaceous	*Rhyichosia minima*	Paris
'	'	Leguminoseae	Shrub	*Indigofera vokensii*	Paris
'	'	Fabaceae	Tree	*Acacia fortilis*	Arum
'	'	Caesalpinaceae	Herbaceous	*Cassia mimosoides*	Arum

The wetness and dryness of the SNP soil was found to influence the occurrence, diversity and distribution of VAM fungi population. The areas such as Kibeyo, Tabora B, Lamai, Nyansurula, Gibaso are quite wet, while the areas like Mdito, Lobo, Ololosokwani, Togoro and Wasso are in the dry side (Figure 1). The dry side is characterized by irregular and scarce rainfall as well as long dry and hot dry enchantment while the wet region is characterized by regular rainfall and relatively cooler dry seasons. These climatic conditions cause the drier side to have low soil organic matter content and low nutrients and water availability, which would consequently result to poor soils, accompanied with low productivity, limited plant growth and diversity as well as less microbial populations (Alguacil *et al*., 2009). It is also well known that high plant species richness and diversity is associated with high mycorrhiza community (Johnson *et al*., 1991; Sieverding, 1990). This study result shows low levels of carbon and nitrogen in the drier side than in the wetter side (Figure 3 a&c). These disparities would therefore be associated with less species composition and richness of the VAM fungi observed in this study from the dry side.

4. Conclusion

VAM fungi belonging to three genera were found in the SNP dominated by the Arum morphotype. The Paris-type was more appropriate for slow growing plants dominating in the grassland with scarce and less vegetation while the Arum were dominant in the woodland constituting the fast growing vegetation. This shows that Arum-type are very important to the fast growing forest and may be useful in reforestation compared to the Paris morphotype. This is the first study on the VAM of SNP which used morphological characterization to identify the taxa to the genus level. The study thus recommends more studies using molecular to ascertain the taxa to the species level.

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Glossary

SNP: Serengeti National Park.

VAM: Vesicular Arbuscular Mycorrhiza.

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