

## Full Length Research Paper

# Occurrence and abundance of arbuscular mycorrhizal fungi (AMF) in agroforestry systems of Rubavu and Bugesera Districts in Rwanda

Jean de Dieu Habiyaemye<sup>1,2\*</sup>, Catherine Muthuri<sup>3</sup>, Vivienne Matiru<sup>4</sup>, John Nyaga<sup>3,4</sup>, Athanase Mukuralinda<sup>5</sup>, Vicky Ruganzu<sup>6</sup>, Motohito Yoneda<sup>6</sup> and Fergus Sinclair<sup>3</sup>

<sup>1</sup>Pan African University Institute for Basic Sciences, Technology and Innovation, Nairobi, Kenya.

<sup>2</sup>University of Rwanda-College of Education, Rwanda.

<sup>3</sup>World Agroforestry Center, Nairobi, Kenya.

<sup>4</sup>Jomo Kenyatta University of Agriculture and Technology, Kenya.

<sup>5</sup>World Agroforestry Center, Rwanda.

<sup>6</sup>Rwanda Agriculture Board, Rwanda.

Received 4 August, 2014; Accepted 12 December, 2014

Arbuscular mycorrhizal fungi (AMF) help to facilitate mobilization of nutrients from soil to plant. The study was carried out in humid Rubavu and semi-arid Bugesera districts in Rwanda. We hypothesized that the presence of tree species in farming systems enhances mycorrhizal fungal density. The occurrence and abundance of AMF in the soil around main agroforestry tree species in these regions was studied. Tree species in Rubavu included *Alnus acuminata*, *Markhamia lutea*, *Grevillea robusta* and *Eucalyptus* sp. and in Bugesera *Acacia polyacantha*, *Senna spectabilis*, *Grevillea robusta* and *Eucalyptus* sp. AMF spores were isolated from soil samples collected under and outside the trees canopies. Results show significant differences in spore density between species. The density of AMF spores was highest under *A. acuminata* and *A. polyacantha* and lowest in *Eucalyptus* sp. and *G. robusta* in Rubavu and Bugesera, respectively. Generally, the mean spore abundance (spores/g of soil) was significantly higher in Bugesera (3.1-6.6) than Rubavu (1.6-4.4). Spores abundance was also affected by distance from the tree trunk and tree size. The present work is the first attempt to study the AMF communities associated with tree species in agroforestry systems in Rwanda. We propose further studies relating mycorrhizal diversity in the agroforestry systems to performance and yields of crops.

**Key words:** Arbuscular mycorrhizal fungi, spore abundance, agroforestry system.

## INTRODUCTION

Crop productivity is declining in Rwanda mainly due to declining soil fertility and other challenges like high

population density, water scarcity, land degradation, land fragmentation and deforestation notwithstanding. Most

\*Corresponding author. E-mail: [jdhabiyaemye@gmail.com](mailto:jdhabiyaemye@gmail.com) or [jdhabiyaemye@yahoo.fr](mailto:jdhabiyaemye@yahoo.fr).

options to improve productivity involve the use of expensive inputs that inherently increase risks that farmers are often unwilling or unable to bear. The government of Rwanda is interested in eco-efficient means to raise land productivity that farmers can afford to adopt like scaling up the adoption of farm trees. This study targets the semi-arid and humid districts in Rwanda, sites of an ongoing Australian funded project investigating the effects of trees on water, nutrients and crops performance and hence food security. The study focused on investigating the occurrence and abundance of arbuscular mycorrhizal fungi (AMF) in the rhizosphere of the key agroforestry tree species found in these two contrasting districts.

AMF are root-inhabiting soil fungi which form obligate symbiotic associations with over 80% of terrestrial plant families (Smith and Read, 2008; Van Hoeyk et al., 2001; Harley and Smith, 1983). They are ubiquitous in almost all plant communities in both natural and managed ecosystems, but the number has decreased due to tillage, fertilization, removal of topsoil, erosion, fumigation and over-fertilization (Raja and Tang, 2005). They are widespread in tropical soils and associated with a wide variety of plant species, including most commercial crops (Sieverding, 1991) and trees (Atayese et al., 1993; Adjoud-Sadadou and Halli-Hargas, 2000). These very important organisms form an interface between soils and plant roots (Ingleby, 2007; Power and Mills, 1995) increasing the absorptive surfaces of the roots (Manjunath and Habte, 1988). Extra-radical hyphae of the AMF extend beyond the root and act as extensions of the root system in acquiring nutrients from the soil (Rhodes and Gerdemann, 1975). AMF can therefore absorb mineral nutrients from soil through their extended intricate hyphal network and deliver them to their host plants in exchange for carbohydrates (Oehl et al., 2003). AMF can also enhance tolerance of abiotic stresses such as drought and metal toxicity (Meharg and Cairney, 2000).

AMF are not host-specific (Ingleby, 2007). Because of this, the same fungi can associate with tree and crop species and therefore have the potential to enhance both tree and crop growth in agroforestry systems. In this situation, the tree species can act as a 'reservoir' of AMF fungi, from which roots of germinating crop seedlings can quickly form mycorrhizal associations (Ingleby, 2007).

Arbuscular mycorrhizal associations are characterized by structures called arbuscules and vesicles which are produced inside the host plant root cells together with asexual spores which they produce in the soil (Ingleby, 2007). Though hyphal networks, dead root fragments and other organic material occupied by fungal structures are important, AMF spores have traditionally been considered to be the most important propagules of AMF (Chandrasekara et al., 2005; Brundrett and Abbott, 1994). Therefore, analysis of spore populations in soils is currently the most used method to assess the species density and diversity of AM fungal communities (Chandrasekara et al., 2005). However, the interpretation

of these results remains conditional as isolates of AMF vary greatly in spore production; some isolates produce spores copiously, while others rarely or never sporulate (Chandrasekara et al., 2005).

All the soils harbor AMF spores despite the different structural and chemical differences of the cropping fields (Don-Rodrigue et al., 2013). However, the major factors affecting their diversity, abundance and distribution in agro-ecosystems are soil pH, availability of phosphorous (P), nitrogen (N), organic matter and water. These factors could also affect the crop production in different agro-ecosystems (Porrás-Soriano et al., 2009).

The relationship between plants and AMF species abundance and diversity is not completely understood for most natural ecosystems (Bainard et al., 2011). Even though species richness of mycorrhizal fungal communities has been correlated with the species richness of plant communities in temperate grasslands and tropical agro-ecosystems (Nancy and David, 1997; Eom et al., 2000), agricultural soils have a low density and diversity of AMF as compared to natural ecosystems. When a soil is put to agricultural use, it undergoes a series of physical changes, like tillage and fertilizer use, which can negatively affect microorganism population (Bellgard, 1994).

Declining of soil fertility and crop production are challenges to food security in Rwanda. Past studies in Rwanda have concentrated on water and nutrient cycling and nothing has been done on the role of AMF in productivity systems. Trees are being incorporated in the agricultural lands to provide ecosystem services and products for example, firewood, fruits and furniture. Therefore, there is a need to document the AMF around trees in these systems as a preparatory phase to understand their contribution in these systems as they provide several free ecosystem services. Research has shown that there is greater soil biota in agroforestry systems than in agriculture systems (without trees) with greater biodiversity generally reported near the trees but the effect varies with tree species (Barrios et al., 2010). In our study, the agroforestry systems of Rubavu and Bugesera districts were taken to represent the humid and semi-arid agroecological zones of Rwanda, respectively.

## MATERIALS AND METHODS

### Site description

Bugesera district is located in eastern province of Rwanda. Its altitude varies between 1300 and 1667 m with soft slopes. Its relief mainly constituted of a succession of low plateau, dry valleys and swamps. The annual precipitation ranges between 700-900 mm with the mean atmospheric temperature between 21 and 29°C. Soils in the region are sandy-loam of moderate fertility (JICA, 2006; MINITERE, 2003). Dominant crops and trees observed are relatively homogenous across Bugesera district - crops: banana, maize, beans and cassava; and trees: *Acacia*, *Senna spectabilis*, *Grevillea robusta*, *Eucalyptus* (Kiptot et al., 2013; CRA, 2005; <http://www.bugesera.gov.rw/>).

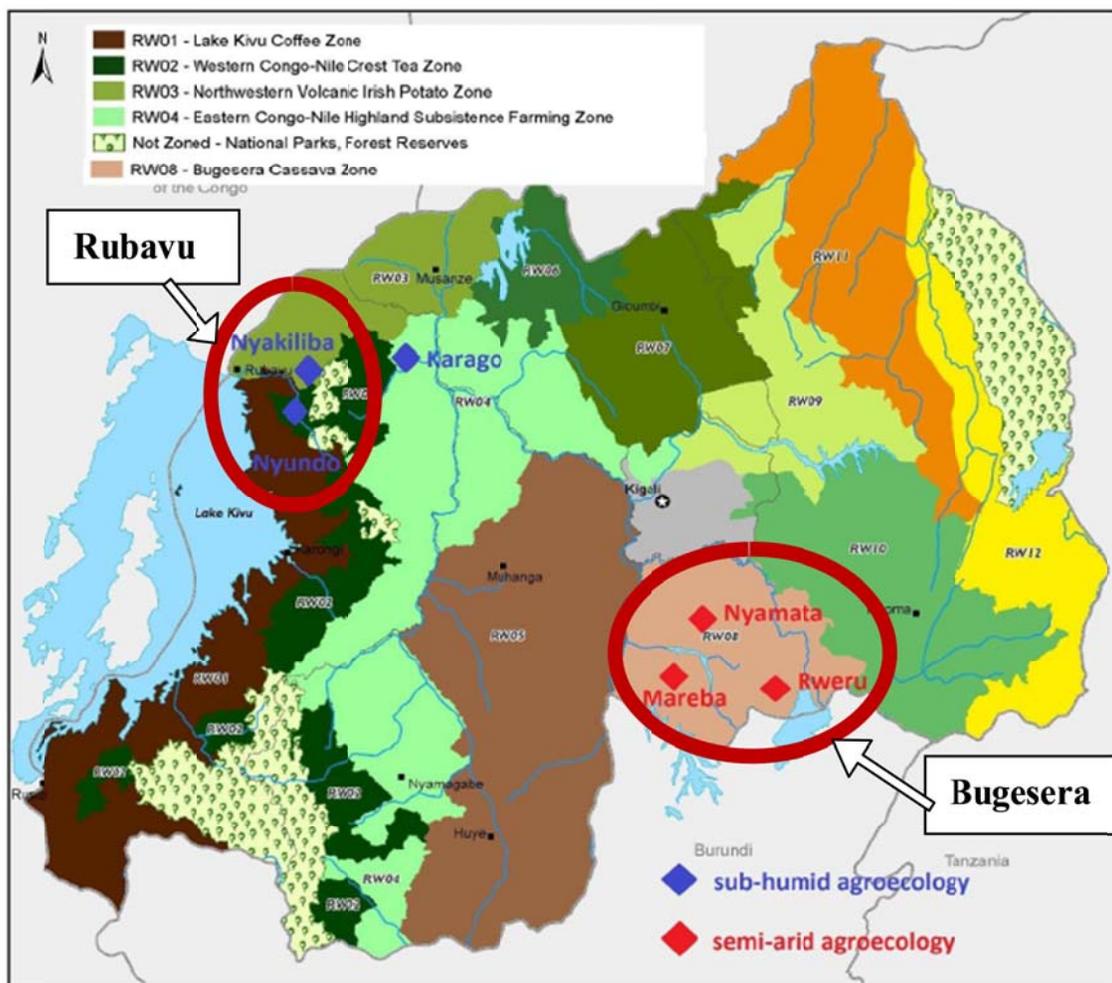


Figure 1. Agroecological Map of Rwanda with selected sites (Districts/sectors).

Rubavu is one of the western province districts of Rwanda. The region is characterized by an elevation ranging between 2000 and 3000 m and higher slopes (the mean slope is 35%). Temperatures are generally cool with an average of 10°C. Its mean annual rainfall is 1800 mm (Nyandwi and Mukashema, 2011). Major crops in the region include maize, climbing beans, irish potatoes, wheat and vegetables such as carrots and cabbages along with tea plantations on valley bottoms. The dominant trees observed are *Alnus acuminata* along the contours, *Markhamia lutea* on farm, *Eucalyptus* woodlots, *Grevillea robusta*, bamboo, avocado and some indigenous trees such as ficus (Kiptot et al., 2013).

### Sampling

The rhizosphere soil was sampled from Nyundo and Rweru sectors of Rubavu and Bugesera districts, respectively (Figure 1). These two sites represent areas where there are complimentary ongoing project activities on tree-crop interactions with a wide range of participatory trials by the farmers.

Soil samples were collected using a soil auger to a depth of 0-10 cm. This was done around each tree of the four most common tree species found in the agroforestry systems: *Alnus acuminata*, *Markhamia lutea*, *Grevillea robusta* and *Eucalyptus* sp. (in Rubavu)

and *Acacia polyacantha*, *Senna spectabilis*, *Grevillea robusta* and *Eucalyptus* sp. (in Bugesera).

Three replications for each tree species were sampled and this is on the same day. Soil samples were taken at three different positions from the tree trunk, that is, 1) 0.5 m from the tree trunk, 2) the edge of the tree canopy and, 3) 3 m from the edge of the tree canopy. At every position, the soil was sampled in the east and west of the tree and samples from the same position were pooled. A total of 72 soil samples were collected (Table 1) and stored in sealed plastic bags kept at 4°C until spores were extracted, counted and analyzed.

Additional information about sampled tree replicates, sizes of the trees, geographic coordinates of the sites and some soil chemical characteristics are provided in Tables 2 and 3.

### Extraction of AMF spores

Soil samples were air-dried before extraction and counting of AMF fungal spores. AMF spores extraction was done using the method adapted from Ingleby (2007). A 50 g sample of air-dried soil was mixed with water to obtain a 1 L suspension, and the suspension was strongly agitated to disperse the soil aggregates and release AMF spores. The liquid was then poured onto a nest of two sieves:

**Table 1.** Summary of soil samples collected from the field

Agroforestry system	Number of tree species	Number of replicates per species	Sampled sites around a tree	Total soil samples
Rubavu	4	3	3	4X3X3=36
Bugesera	4	3	3	4X3X3=36
Grand total				72

**Table 2.** Geographical coordinates and critical chemical characteristics of rhizospheres of the sampled trees (Rubavu agroforestry system).

Tree species	Sampled tree replicate	Geographic coordinates of the sites			Important chemical characteristics of the site*	
		Latitude S	Longitude E	Elevation (m)	Mean pH	Mean P (ppm)
<i>Alnus acuminata</i>	1	01°44'33"	029°20'54"	2025	4.9	10.32
	2	01°44'28"	029°20'59"	1998	4.9	10.32
	3	01°44'27"	029°21'01"	2006	4.9	10.32
<i>Markhamia lutea</i>	1	01°44'50"	029°21'00"	2114	4.9	10.32
	2	01°44'04"	029°21'14"	1967	4.9	10.32
	3	01°44'01"	029°21'16"	1959	4.9	10.32
<i>Grevillea robusta</i>	1	01°44'28"	029°20'57"	2007	4.9	10.32
	2	01°44'51"	029°21'01"	2126	5.1	10.07
	3	01°44'50"	029°21'00"	2119	5.8	19.73
<i>Eucalyptus</i> sp.	1	01°44'50"	029°21'00"	2116	5.8	19.73
	2	01°44'43"	029°20'47"	2089	5.1	10.07
	3	01°44'52"	029°21'01"	2144	5.1	10.07

\*Chemical characteristics of the sampled sites provided by Rwanda Agriculture Board (RAB), 2014.

**Table 3.** Geographical coordinates and critical chemical characteristics of rhizospheres of the sampled trees (Bugesera agroforestry system).

Tree species	Sampled tree replicate	Geographic coordinates of the sites			Important chemical characteristics of the site*	
		Latitude S	Longitude E	Elevation (m)	Mean pH	Mean P (ppm)
<i>Senna spectabilis</i>	Tree No 1	02°17'30"	030°15'23"	1336	5	24.98
	Tree No 2	02°17'39"	030°15'14"	1352	5	24.98
	Tree No 3	02°17'40"	030°15'13"	1349	5.1	8.8
<i>Acacia polyacantha</i>	Tree No 1	02°17'28"	030°15'05"	1329	6.1	58.4
	Tree No 2	02°17'30"	030°15'04"	1324	5.1	8.8
	Tree No 3	02°17'28"	030°15'02"	1329	6.1	58.4
<i>Grevillea robusta</i>	Tree No 1	02°17'32"	030°15'22"	1342	5	24.98
	Tree No 2	02°17'33"	030°15'22"	1339	5	24.98
	Tree No 3	02°17'28"	030°15'24"	1334	5	24.98
<i>Eucalyptus</i> sp.	Tree No 1	02°17'38"	030°15'14"	1353	5	24.98
	Tree No 2	02°17'38"	030°15'14"	1344	5.1	8.8
	Tree No 3	02°17'37"	030°15'12"	1342	5	24.98

\*Chemical characteristics of the sampled sites provided by Rwanda Agriculture Board (RAB), 2014.

**Table 4.** Abundance of AMF spores around four most common tree species in the agroforestry system of Rubavu district.

Tree species	Sampled tree replicate	Tree size		AMF spores abundance (Number of spores/g of soil)		
		Height (m)	Diameter at breast height (m)	0.5m from tree	End of tree crown	3m from tree crown
<i>Alnus acuminata</i>	1	15.5	0.22	6.6	5.8	4.2
	2	14.0	0.20	3.5	5.1	3
	3	11.5	0.18	3.1	2	1.6
	Mean and SE*			4.4(±1.9)	4.3(±2.0)	2.9(±1.3)
<i>Markhamia lutea</i>	1	10.5	0.14	1.1	2.2	1.7
	2	13	0.17	1.8	2.3	1.6
	3	14.5	0.18	2.9	2.8	2.5
	Mean and SE*			1.9(±0.9)	2.4(±0.3)	1.9(±0.5)
<i>Grevillea robusta</i>	1	22	0.27	1.7	2.8	3
	2	21.5	0.26	1.6	3	2.8
	3	19.5	0.24	2.5	1.5	1.8
	Mean and SE*			2.0(±0.5)	2.4(±0.8)	2.5(±0.6)
<i>Eucalyptus</i> sp.	1	17.5	0.25	1.8	2.1	1
	2	19	0.27	1.3	3	2.2
	3	16.8	0.22	2	2.2	1.6
	Mean and SE*			1.7(±0.3)	2.4(±0.5)	1.6(±0.6)

\*Mean values are the mean of n; SE = Standard Error.

200  $\mu$  size on top to allow passage of spores but retain large soil and organic matter particles, and 45  $\mu$  on the bottom to retain AMF spores yet allow passage of the finest soil particles. The collected residue in the smallest sieve was washed and transferred into 50 ml centrifuge tubes and centrifuged with water for 5 min at 1,800 rpm. The supernatant was then discarded and the pellet re-suspended in 48% (w/v) sucrose and centrifuged again for 1 min at 1,800 rpm. The supernatant (with spores) was poured onto 45  $\mu$  sieve and rinsed with water to remove the sugar. The remaining debris on the sieve were transferred to a Petri dish for initial observation and collection of AMF spores under dissecting microscope with 40x magnification. Spore abundance was expressed as the number of AMF spores per gram of soil.

### Statistical analysis

The analysis of variance (ANOVA) was used to assess the data. Comparison among tree species and between the two agroforestry systems was carried out at  $p=0.05$  significant level.

## RESULTS

The distribution of AMF on the basis of spore density showed difference among the experimental tree species. Spore abundance in the rhizosphere of different tree species from the agroforestry systems of Rubavu district is shown in Table 4. The mean spore count varied from 1.7 to 4.4 spores  $g^{-1}$  soil at 0.5 m from tree trunk, 2.4 to 4.3 spores  $g^{-1}$  soil at the end of tree canopy and 1.6 to 2.9 spores  $g^{-1}$  soil at 3 m from tree canopy. *Alnus acuminata* had the highest AMF spores across all the

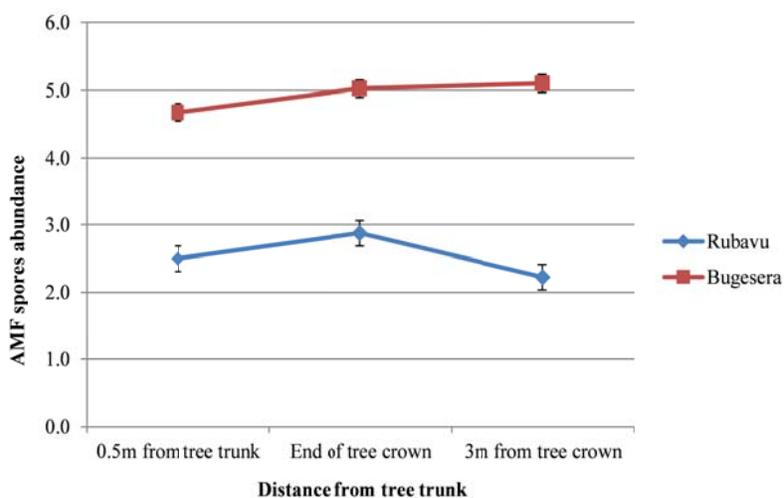
three positions from the tree trunk, that is, 4.4 spores  $g^{-1}$  soil at 0.5 m from tree trunk, 4.3 spores  $g^{-1}$  soil at the edge of the canopy and 2.9 spores  $g^{-1}$  soil at 3 m from the canopy. The tree species showed a significantly greater density of AMF spores than *Markhamia lutea* ( $p=0.001$ ), *Grevillea robusta* ( $p=0.02$ ) and *Eucalyptus* sp. ( $p=0.01$ ) which among themselves do not show any significant difference. Within trees of the same species, there was generally a positive correlation between AMF spores density and size of the sampled trees. Around individual tree species, AMF spores abundance varied with the distance from the tree trunk, but not consistently. *A. acuminata* showed a decrease of AMF spore density with the distance from the tree, *M. lutea* and *Eucalyptus* sp. showed higher density of AMF spores at the end of the tree canopy and *G. robusta* gave AMF spore density increasing with the distance from the tree.

Table 5 shows the abundance of AMF spores around four most common tree species in the agroforestry system of Bugesera. The mean spore count varied from 3.1 to 6.6 spores  $g^{-1}$  soil. *Acacia polyacantha* and *Senna spectabilis*, with no significant difference between the spores count associated with their rhizospheres, showed the highest AMF spores with an average of 6.1 and 6.0 spores  $g^{-1}$  soil, respectively. *Senna spectabilis* showed a greater density of AMF spores than *G. robusta* and *Eucalyptus* sp. ( $p=0.0002$  and  $0.030$ , respectively). *A. polyacantha* with no significant difference with *G. robusta*, showed a greater density of AMF spores than *Eucalyptus*

**Table 5.** Abundance of AMF spores around four most common tree species in the agroforestry system of Bugesera.

Tree species	Sampled tree replicate	Tree size		AMF spores abundance (Number of spores/g of soil)		
		Height (m)	Diameter at breast height (m)	0.5m from tree	End of tree crown	3m from tree crown
<i>Senna spectabilis</i>	1	7.5	0.14	9.9	8.2	7.5
	2	6.0	0.13	6.4	5	3.6
	3	5.5	0.11	3.4	4.4	6.1
	Mean and SE*			6.5(±3.2)	5.8(±2.0)	5.7(±1.9)
<i>Acacia polyacantha</i>	1	7.0	0.17	2.9	5	5.4
	2	7.6	0.19	4.8	4.9	5.9
	3	8.2	0.22	8.7	9.2	8.5
	Mean and SE*			5.4(±2.9)	6.3(±2.4)	6.6(±1.6)
<i>Grevillea robusta</i>	1	12.0	0.17	3.2	3.4	4.9
	2	11.5	0.16	3.4	2.6	1.8
	3	10.5	0.14	3.6	3.4	3
	Mean and SE*			3.4(±0.2)	3.1(±0.4)	3.2(±1.5)
<i>Eucalyptus</i> sp.	1	11.0	0.14	2.9	4.1	4.6
	2	13.5	0.21	2	5.8	6.5
	3	11.5	0.16	5.3	4.9	4.3
	Mean and SE*			3.4(±1.7)	4.9(±0.8)	5.1(±1.2)

\*Mean values are the mean of n; SE = Standard Error.



**Figure 2.** Variation of AMF spore abundance (number of spores/g of soil) with position from tree trunk in Rubavu and Bugesera agroforestry systems.

sp. ( $p=0.022$ ). Within each tree species, AMF spore density was positively correlated with tree size. Around individual tree species, lack of constancy in variation of AMF spore density was also noticed. Around *Senna spectabilis* and *G. robusta*, a decrease of AMF spores density with the distance from tree trunk was generally noticed while the density increased as moving away from the tree around *Acacia polyacantha* and *Eucalyptus* sp.

The difference in AMF spore density between the two agroforestry systems was also noticed (Figure 2). The

spore count varied from 3.1 to 6.6 spores per gram of soil in Bugesera and 1.6 to 4.4 spores per gram of soil in Rubavu. The mean AMF spore density in Bugesera agroforestry system was significantly higher with  $p=0.036$  than in Rubavu.

## DISCUSSION

Various studies were previously conducted to determine AMF abundance in different rhizospheres. Abundance of

775 to 1240 spores  $100\text{ g}^{-1}$  soil were found in *A. albida* Del. in Senegal (Diop et al., 1994); 500 to 1500 spores  $100\text{ g}^{-1}$  soil in *A. farnesiana* and *A. planifrons* in moderately fertile alkaline soils in India (Udaiyan et al., 1996); 110 to 2600 spores  $100\text{ g}^{-1}$  soil in tropical forest and pasture (Picone, 2000) and 5 to 6400 spores  $100\text{ g}^{-1}$  soil in a valley savanna of the dry tropics (Tao et al., 2004). By contrast, low spore densities of 11 to 32 spores  $100\text{ g}^{-1}$  soil were detected in dry deciduous woodlands of Northern Ethiopia associated with different *Acacia* species (Birhane et al., 2010). Low AMF spore numbers were also recorded in a survey of *Acacia* tree species (49 to 67 spores  $100\text{ g}^{-1}$  soil) in India (Lakshman et al., 2001) and in *Acacia* and *Prosopis* tree species (8 to 51 spores  $100\text{ g}^{-1}$  soil) in Senegal (Ingleby et al., 1997).

In comparison with the above findings, the abundance of AMF spores got in the agroforestry systems of Rubavu and Bugesera agroforestry systems is generally moderate, that is, 1.6 to 4.4 spores/g of soil and 3.1 to 6.6 spores/g of soil in Rubavu and Bugesera, respectively.

The moderate level of AMF spores density found in the current study agrees with research findings of Picone (2000) who found a total of 110-770 AMF spores  $100\text{ g}^{-1}$  in forest and 830-2600 spores  $100\text{ g}^{-1}$  in pasture. Conversion from natural habitats to agricultural lands has been identified as one of the leading causes for loss of biodiversity worldwide. As shown by previous researchers, some modern agricultural practices such as continuous monoculture, non host crop in rotation and tillage can impact on the AMF association, both directly, by damaging or killing AMF and indirectly, by creating conditions unfavorable to AMF. These practices especially tillage can also cause soil erosion, reduce soil fertility and disrupt biodiversity in general including the previous crops. In cultivated lands, therefore, AMF population, species composition and diversity are often decreased as compared to natural ecosystems (Helagson et al., 1998; Mathimaran et al., 2007).

The abundance of AMF is also influenced by many factors including soil P and pH. However, in this study, the influence of P was not recorded. In their study, Zerihun et al. (2013) showed a significant negative correlation between AMF spore density and available P. These findings were similar to some reports from India and Northern Europe (Udaiyan et al., 1996; Kahiluoto et al., 2001). In contrast to this, Muleta et al. (2007) observed a positive relationship between spore number and available P in soil samples from natural coffee forest in Ethiopia. They suggested that available P level in their study sites was not high enough to inhibit mycorrhizal development. Similarly to this view of Muleta et al. (2007), the available P in our sampled sites may not be at a level of inhibiting AMF spores development.

Another important soil factor on AMF development is pH and this is positively correlated with AMF abundance. Low soil pH negatively affects AMF species richness (Don-Rodrigue et al., 2013). Therefore, the low pH in our

sampled sites (Mean pH = 4.9-5.8 in Rubavu and 5.0-6.1 in Bugesera) may also be one of the causes of the moderate abundance of AMF spores in the regions, that is, 1.6 to 4.4 spores/g of soil and 3.1 to 6.6 spores/g of soil in Rubavu and Bugesera, respectively.

Results obtained in this study are supported by various findings from previous studies. Various researches showed that rhizosphere in close proximity to trees has a greater spore densities relative to the soil beyond the tree canopies (Mutabaruka et al., 2002; Pande and Tarafdar, 2004; Prasad and Mertia, 2005). In addition, studies in agroforestry coffee (*Coffea arabica* L.) systems observed higher spore densities in the rhizosphere of coffee plants under shade trees as compared to monocultural coffee systems (Muleta et al., 2007; Muleta et al., 2008). The explanation was given by Tadesse and Fassil (2013) that greater numbers of spores under the tree canopies in agroforestry systems may be due to greater amount of roots at this specific site. In contrast, other studies have shown no effect or in some cases a negative effect of trees on AMF (Boddington and Dodd, 2000; Leal et al., 2009). Spores of AMF may also occur in clumped distributions in the field, not correlated with root distribution (Douds and Millner, 1999).

The difference in spore density between the two agroforestry systems could be explained partly by a certain number of reasons. Most of Bugesera parts are made of valleys and a succession of low plateaux. This relief implies soft and middle slopes and smooth flow of rainwater that do not much transport away AMF spores, and then resulting in the accumulation of AMF spores in the soil around the host plants. This is not the case at Rubavu district as the region is characterized by a higher elevation and higher slopes causing strong flows of rainwater and erosion transporting away both soil and AMF spores. This could explain partly the higher density of AMF spores at Bugesera zone than Rubavu. This is in agreement with Chandrasekara et al. (2005) who concluded that there is a decreasing trend in spore density and spore diversity with increasing elevation. The researchers concluded that higher density of spores at lower elevations could be explained by the accumulation of spores, which are coming down with rainwater.

Another possible explanation for the higher density of spores at Bugesera zone than Rubavu may be the fact that during the period the soil samples were collected, Bugesera zone was experiencing a dry period and most of possible host crops were out of season. The AMF may have remained as spores without germinating. This was not the case at Rubavu as it was the period of much rain, and the soil samples were collected in agricultural lands with growing maize where very many AMF spores might have germinated to colonize the maize roots. This is supported by the view of Janos (1980) who pointed out that the variation in spore population could be attributed to many factors in a given site. The researcher argues that AMF spores always need live root contacts for

germination since they are obligate fungi; they may persist as spores in the absence of suitable hosts.

Environmental differences are also important factors in determining spore production by AMF. It is known that high temperature can increase AMF sporulation (Guadarrama and Álvarez-Sánchez, 1999). These characteristics could also be used to explain the reason for the higher abundance of AMF spores in Bugesera characterized by a climate quite hot with the annual temperatures averaging between 21 and 29°C (<http://www.bugesera.gov.rw/>) as compared to Rubavu with an average temperature of 10°C (Nyandwi and Mukeshimana, 2011).

In conclusion, this work represented one of the first attempts to study the native AMF communities associated with some common tree species of agroforestry system in Rwanda. We recommend that the study be extended by characterization of the fungi as well as analyzing their effect on some common crops cultivated in the country with the aim of selecting and developing well performing and adapted inoculum to enhance tree growth and crop production.

### Conflict of interest

The authors did not declare any conflict of interest.

### ACKNOWLEDGEMENTS

The authors are grateful to Rwanda Agriculture Board (RAB) for providing research facilities. This work was carried out under the aid of Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI) and the project “Trees for Food Security” sponsored by Australian Centre for International Agricultural Research (ACIAR) and working within World Agroforestry Center (ICRAF).

### REFERENCES

- Adjoud-Sadadou D, Halli-Hargas R (2000). Occurrence of arbuscular mycorrhizae on aged Eucalyptus. *Mycorrhiza* 9:287-290.
- Atayese MO, Awotoye OO, Osonubi O, Mulongo K (1993). Comparison of the influence of hedgerow woody legumes and cassava at the top and base of a hillslope in alley cropping system. *Biol. Fertil. Soils* 16:198-204.
- Bainard LD, Klironomos JN, Gordon AM (2011). Arbuscular mycorrhizal fungi in tree-based intercropping systems: a review of their abundance and diversity. *Pedobiologia* 54:57-61.
- Bellgard SE (1994). The bi-functional nature of the mycelial network associated with plants colonized by vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza News* 6:1-15
- Birhane E, Kuyper TW, Sterck FJ, Bongers F (2010). Arbuscular mycorrhizal associations in *Boswellia papyrifera* (frankincense-tree) dominated dry deciduous woodlands of Northern Ethiopia. *For. Ecol. Manage.* 260:2160-2169
- Boddington CL, Dodd JC (2000). The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. I. Field studies in an Indonesian ultisol. *Plant Soil* 218:137-144.
- Brundrett MC, Abbott LK (1994). Mycorrhizal fungus propagulus in the Jarrah forest I. Seasonal study of inoculum levels. *New Phytol.* 127: 539-546.
- Chandrasekara CMCP, Madawala Weerasinghe HMSP, Gunatilleke IAUN, Seneviratne G (2005). Spatial distribution of arbuscular mycorrhizas along an elevation and adaphic gradient in the forest dynamics plot at Sinharaja, Sri Lanka. *Cey. J. Sci. (Bio. Sci.)* 34:47-67.
- CRA (2005). Assessing the Impact of Decentralisation on Service Delivery in Rwanda. Final Report produced for the World Bank and MINALOC. Kigali, Rwanda.
- Diop TA, Gueye M, Dreyfus BL, Plenchette C, Strullu DG (1994). Indigenous arbuscular mycorrhizal fungi associated with *Acacia albida* Del. in different areas of Senegal. *Appl. Environ. Microbiol.* 60:3433-3436.
- Don-Rodríguez R BV, Nandjui J, Sery JMD, Fotso B, Ainoa JA, Kouadio MSA, Seydou Coulibaly S, Niamke S, Zeze A (2013). Abundance and diversity of Arbuscular mycorrhizal fungal (AMF) communities associated with cassava (*Manihot esculenta* Crantz) rhizosphere in Abengourou, East Côte d'Ivoire. *J. Ecol. Nat. Environ.* 5(11):360-370.
- Douds DDJ, Millner PD (1999). Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agric. Ecosyst. Environ.* 74:77-93
- Eom AH, Hartnett DC, Wilson GWT (2000). Host plant species effects on arbuscular mycorrhizal fungal communities in tallgrass prairie. *Oecologia* 122:435-444
- Guadarrama P, Álvarez-Sánchez FJ (1999). Abundance of arbuscular mycorrhizal fungi spores in different environments in a tropical rain forest, Veracruz, Mexico. *Mycorrhiza* 8:267-270.
- Harley JL, Smith SE (1983). *Mycorrhizal symbiosis*. Academic Press, London.
- Helagson T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998). Ploughing up the wood-wide web? *Nature* 394:431.
- Ingleby K (2007). *Mycorrhizal training manual: Assessment of mycorrhizal diversity in soils and roots, and nursery inoculation to improve the survival and growth of seedlings*. Centre for Ecological & Hydrology. Midlothian.
- Ingleby K, Diagne O, Deans JD, Lindley DK, Neyra M, Ducousso M (1997). Distribution of roots, arbuscular mycorrhizal colonisation and spores around fast-growing tree species in Senegal. *For. Ecol. Manage.* 90:19-27
- Janos DP (1980). Mycorrhizae influence Tropical succession. *Tropical succession (Suppl.)* 12:(2)56-64.
- JICA (Japan International Cooperation Agency) (2006). Sustainable rural and agricultural development in Bugesera district, Eastern province of Rwanda. Progress report one. Ministry of Agriculture and Animal Resources of Rwanda. Kigali, Rwanda.
- Kahiluoto H, Ketoja E, Vestberg M, Saarela I (2001). Promotion of AM utilization through reduced P fertilization 2. Field studies. *Plant Soil* 231:65-79.
- Kiptot E, Kinuthia R, Mutaganda A (2013). ACIAR 'Trees for Food Security' project. The extension system in Rwanda: a focus on Bugesera, Rubavu and Nyabihu districts. Online copy available on [http://worldagroforestry.org/sites/default/files/Extension%20in%20Rwanda\\_Final%20report.pdf](http://worldagroforestry.org/sites/default/files/Extension%20in%20Rwanda_Final%20report.pdf)
- Lakshman HC, Rajanna L, Inchal RF, Mulla FI, Srinivasulu Y (2001). Survey of VA-mycorrhizae in agroforestry and its implications on forest trees. *Trop. Ecol.* 42(2):283-286.
- Leal PL, Sturmer SL, Siqueira JO (2009). Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from soils under different land use systems in the Amazon, Brazil. *Braz. J. Microbiol.* 40:111-121.
- Manjunath A, Habte M (1988). Development of vesicular arbuscular mycorrhizal infection and the uptake of immobile nutrients in *Leucaena leucocephala*. *Plant Soil* 106:97-103.
- Mathimaran N, Ruh R, Jama B, Verchot L, Frossard E, Jansa J (2007). Impact of agricultural management on arbuscular mycorrhizal fungal communities in Kenyan ferralsols. *Agric. Ecosyst. Environ.* 119: 22-32.
- Meharg AA, Cairney JWG (2000). Co-evolution of mycorrhizal symbionts and their hosts to metal contaminated environments. *Adv. Ecol. Res.* 30:69-112.
- MINITERE (The Ministry of Lands, Environment, Forestry, Water and

- Mines) (2003). Environment Policy. Kigali, Rwanda.
- Muleta D, Assefa F, Nemomissa S, Granhall U (2007). Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia. *For. Ecol. Manage.* 241:145-154.
- Muleta D, Assefa F, Nemomissa S, Granhall U (2008). Distribution of arbuscular mycorrhizal fungi spores in soils of smallholder agroforestry and monocultural coffee systems in southwestern Ethiopia. *Biol. Fertil. Soils* 44:653-659.
- Mutabaruka R, Mutabaruka C, Fernandez I (2002). Research note: diversity of arbuscular mycorrhizal fungi associated to tree species in semiarid areas of Machakos, Kenya. *Arid Land Res. Manage.* 16: 385-390.
- Nancy CJ, David AW (1997). Soil Carbon, nutrients, and mycorrhizae during conversion of dry tropical forest to grassland. *Ecol. Appl.* 7 (1): 171-182.
- Nyandwi E, Mukashema A (2011). Excessive deforestation of Gishwati Mountainous forest & biodiversity changes. Participatory Geographic Information Systems (P-GIS) for natural resource management and food security in Africa. Available on [http://www.crdi.ca/Documents/ICT4D\\_article\\_forests\\_nyandwi\\_EN.pdf](http://www.crdi.ca/Documents/ICT4D_article_forests_nyandwi_EN.pdf)
- Oehll F, Sieverding E, Ineichen K, Mäder P, Boller T, Wilmken W (2003). Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. *Appl. Environ. Microbiol.* 69 (5) 2816-2824.
- Pande M, Tarafdar JC (2004). Arbuscular mycorrhizal fungal diversity in neem-based agroforestry systems in Rajasthan. *Appl. Soil Ecol.* 26: 233-241.
- Picone C (2000). Diversity and abundance of arbuscular-mycorrhizal fungus spores in tropical forest and pasture. *Biotropica* 32:734-750.
- Porrás-Soriano A, Sorano-Marintín ML, Porrás-Piedra A, Azcón P (2009). Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *J. Plant Physiol.* 166:1350-1359.
- Power ME, Mills LS (1995). The keystone cops meet in Hilo. *Tree* 10:182-184.
- Prasad R, Mertia RS (2005). Dehydrogenase activity and VAM fungi in tree-rhizosphere of agroforestry systems in Indian arid zone. *Agroforest. Forum* 63:219-223.
- Raja P, Tang CK (2005). Commercial potential of arbuscular mycorrhiza fungi application in the agriculture, soil rehabilitation and conservation sectors. *FNCA Biofertilizer Newsletter* 5:6-10.
- Rhodes LH, Gerdemann JW (1975). Phosphate uptake zones of mycorrhizal and non mycorrhizal onions. *New Phytol.* 75:555-561.
- Sieverding E (1991). Vesicular-arbuscular mycorrhiza management in tropical agro systems. German Technical cooperation (GTZ). Eschborn.
- Smith SE, Read DJ (2008). *Mycorrhizal Symbiosis*, 2nd edn. London, UK: Academic Press.
- Tadesse C S, Fassil AT (2013). Arbuscular mycorrhizal fungi associated with shade trees and *Coffea Arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia. *Afrika Focus* 26 (2):111-131
- Tao L, Jianping L, Zhiwei Z (2004). Arbuscular mycorrhizas in a valley-type savanna in southwest China. *Mycorrhiza* 14:323-327.
- Udaiyan K, Karthikeyan A, Muthukumar T (1996). Influence of edaphic and climatic factors on dynamics of root colonization and spore density of vesicular-arbuscular mycorrhizal fungi in *Acacia farnesiana* Willd. and *A. planifrons* W.et. A. *Trees* 11:65-71.
- Van Hoewyk D, Wigand C, Groffman PM (2001). Endomycorrhizal colonization of *Dasiphora floribunda* a native plant species of calcareous wetlands in Eastern New York State, USA. *Wetlands* 21:431-436
- Zerihun B, Mauritz V, Fassil A (2013). Diversity and abundance of arbuscular mycorrhizal fungi associated with acacia trees from different land use systems in Ethiopia. *Glob. J. Microbiol. Res.* 1(1):23-35.