academicJournals

Vol. 10(29), pp. 1111-1120, 7 August, 2016
DOI: 10.5897/AJMR2016.7933
Article Number: 2722B0A59835
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Mycorrhiazal association in relation to soil properties and plant species density in Sidama of Southern Ethiopia

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Received 25 January 2016; Accepted 5 May, 2016

In Sidama, agroforestry represents land-use systems with deliberate management of multipurpose trees and shrubs that grow in intimate association with annual and perennial agricultural crops and/or livestock. The interaction of microbiota with the trees, shrubs and crops make the system fertile, productive and sustainable. One of the beneficial microbiota which has symbiotic association with most of the plants in agroforestry is arbuscular mycorrhizal fungi (AMF). In November and December of 2012, root and rhizosphere soil samples of 21 plant species from nine peasant associations (PAs) (villages within districts where 300 to 500 families live) were collected from the agroforestry practices in Sidama of Southern Ethiopia for the determination of diversity of AMF based on selected soil parameters and plant species density. Findings on the diversity of AMF based on soil properties showed that at moderate to low P and N concentrations the rate of root colonization and spore density was high in comparison with the rhizosphere soils with the highest P and N concentration. The highest percentage of total colonization was recorded for shade trees *Millettia ferruginea* (84%) *and Erythrina brucei* (80%) followed by intercropped perennial crops *Ensete ventricosum* (86%), *Catha edulis* (85%) and *Coffea arabica* (80%) and the lowest percentage colonization was recorded for *Rhamnus prinoides* (53%) and *Colocasia esculenta* (52%). Though found in almost all homegarden agroforestry practices and with broad coverage in Sidama agroforestry, some crops and vegetables such *Brassica integrifolia* and *Cucurbita pepo*, grown intercropped were found to be non-mycorrrhizal as none of the AMF structures were recorded. The highest number of AM spore population was recorded in rhizosphere soils of *Croton macrostachyus* (1066±19.33) and *Catha edulis* (1054±53.12) and the lowest spore density was recorded for *Dioscorea alata* (100.00±2.89) spore per 100 g of dry soil. The percentage fungal colonization in any individual plant species and spore population in the rhizosphere soils of that species did not correlate to each other and percentage root colonization and spore density of all plants in the agroforestry of Sidama were found significantly different at P<0.05 level.

Key words: Arbuscular mycorrhizal fungi (AMF), dependency, rhizosphere, parameters, colonization, density.

INTRODUCTION

Forests are cleared for agriculture and settlement in different parts of Ethiopia. Agroforestry is an age old traditional practice which gradually replaced forests. It is a sustainable management system for land that

combines agricultural crops, trees, forest plants and/or animals simultaneously or sequentially, and applies management practices that are compatible with the cultural patterns of the local population (Raintree, 1997). It is practiced on Homegarden (Millat-e-Mustafa, 1997; Zebene, 2003), cropland (Roy, 1997), forestlands (Emiru et al., 2010; Wang et al., 2004). Although the role of agroforestry in conserving biological diversity is being appreciated in many parts, the sustainability of these practices is a major concern in Ethiopia.

Low soil fertility, acidity and deficiency in major nutrients such as phosphorus and nitrogen are some of the problems related to successful agroforestry systems. The conversion of forest to different agricultural systems causes loss of plant species that expose the soil to water and wind erosion. Besides, the removal of plant species from forests and agroforests may be accounted for the loss of biodiversity, not only of plants, but also the microbiota important for the soil health as a whole. Therefore, maintenance of floristic diversity in agroforestry is number one crucial condition for sustainable productivity of the agroforests. One of such situations is planting of mycorrhizal seedlings that can be a good measure to increase the yield of the plants for the sustainable management for land that will increase the overall production.

Arbuscular mycorrhizal fungi are widespread and symbiotic copious members of the soil biota, a generally acknowledged key factor in agricultural ecosystem functioning and sustainability (Verbruggen and Kiers, 2010). These symbiotically associated fungi play an important role to the plants in agroforestry practices by enhancing establishment and growth, increasing nutrient and water uptake, maintaining diversity by accelerating the ability of host plants to compete for resources, contributing to efficient recycling of nutrients and thus to long term stability and stabilization of the soil (Smith and Read, 1997; Jasper, 1992). AMF are widespread in tropical soils and associate with a wide range of plant species, including most commercial crops (Sieverding, 1991) and trees (da Silva Sousa et al., 2013). They are keystone organisms and form an interface between soils and plant roots, and are sensitive to changes in soil physicochemical properties and plant conditions (Power and Mills, 1995).

Under the insufficient concentration of nutrients in the root zone of plants, exploring further in the soil with its extended extra radical hyphae, AMF supply phosphorus, macro and micro soil nutrients to roots which has symbiosis with AMF. In these symbiotic associations both partners benefit from each other under certain conditions (Demir, 2008; Rhodes, 1980; Bolan et al., 1987; Li et al., 1991). Fungi take some organic matter and carbohydrates from the plant. In return, AMF supply nutrients such as phosphorus, nitrogen (N), calcium (Ca), copper (Cu), manganese (Mn), sulphur (S) and zinc (Zn) (Sieverding, 1991; Ortas, 2002). But when the soil is rich in necessary nutrients, plants may not waste more energy for the association with AMF and go for the cheapest source of energy, and individual plant species and plant communities in natural and farming systems affect the distribution and diversity of AMF species (Dalpe et al., 2000; Jefwa et al., 2006).

Therefore, according to the previous investigators, AMF spore density, diversity and rates of root colonization are dependent on soil physicochemical properties (Mahmud et al., 1999) and plant species density and richness (Allsopp and Stock, 1992). Though there are some research reports (Zebene, 2003) on mycorrhizal association of agroforestry trees, information is scarce on spore density and diversity of AMF based on soil parameters and type of plant species density and richness in Sidama agroforestry. Therefore, the objective of the present investigation is to study diversity of AMF based on selected soil parameters and plant species density in selected sites of Sidama agroforestry practices.

MATERIALS AND METHODS

Sampling site

This study was conducted in Sidama Administrative region of Southern Ethiopia during dry season in November and December of 2012. It lies between 06°45´33" and 06^{0}54´713" N and 038°27´432" and 038^{0}31´788"E and 1740 to 2135 m above the sea level. The study area is characterized by a moist to sub-humid warm sub-tropical climate with annual rainfall of 1000 to 1800 mm and mean annual temperature of 15 to 20°C. The main agricultural system in the region is the Tree-enset-coffee based home-garden agroforestry systems commonly practiced in Southern Ethiopia. The study was undertaken in 36 home gardens located in 9 peasant association (PAs, smallest Ethiopian administrative unit, where 300 to 500 families live) distributed over two woredas or district. Each district has 40 to 60 PAs where these agroforestry systems are practiced. In this study, we selected only 9 PAs (Table 2) because these PAs are located at closest proximity to the original forest relics from where the agroforestry practices were emerged. From each PAs, 4 home gardens were selected randomly and soil and root samples were collected from 23 commonly occurring plant species in selected agroforestry practice (Table 1).

Soil sampling and analysis

Rhizosphere soils from under the canopy of the 23 plant species

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Table 1. Plant species present in Sidama agroforestry systems the time of sampling.

Latin names	Family	Amharic	Sidamigna
Ensete ventricosum (Welw.) Cheesman)	*Musaceae*	Enset	Wesse
Catha edulis (vahl.)Forssk.ex Endl	*Celastraceae*	Khat	Khat
Millettia ferruginea Hochst	*Fabaceae*	Birbira	Hengedicho
Erythrina brucei Schweinf	*Fabaceae*	Korch	Wellako
Cordia africana Lam.	*Boraginaceae*	Wanza	Wadicho
Croton macrostachyus (HochstExDel.)	*Euphorbaceae*	Bisana	Mesincho
Prunus africana (Hook.f.)Kalkm.	*Rosaceae*	Tikur Inchet	Garbicho
Vernonia amygdalina Del.	*Asteraceae*	Grawa	Hecho
Persea americana Mill.	*Lauraceae*	Avokado	Abukato
Mengifera indica L.	*Anacardiaceae*	Mango	Mango
Saccharum officinarum L.	*Poaceae*	Shenkorageda	Shenkora
Ricinus communis L.	*Euphorbiaceae*	Castor	Qenboo
Coffea arabica L.	*Rubiaceae*	Coffee	Buna
Zea mays L.	*Poaceae*	Bokolo	Bedela
Phaseolus vulgaris L.	*Fabaceae*	Common bean	Wahe
Ipomoea batatas (L.) Lam	*Convolvulaceae*	Sikuar dinich	Metatessa
Solanum tuberosum L.	*Solanaceae*	Potato	Dinich
Cucurbita pepo L.	*Cucurbitaceae*	Pumpkin	Baqula
Brassica integrifolia (West) O.E. schulz	*Brassicaceae*	Kale	Shana
Cappisicum annuum L.	*Solanaceae*	*Chilly*	*Mitmita*
Rhamnus prinoides L' herit	*Rhamnaceae*	Gesho	Taddo
Dioscorea alata L.	*Dioscoraceae*	Boyna	Bohe
Colocasia esculenta (L.)Schoot.	*Araceae*	Godere	Qolchoma

Table 2. Geographical location and altitudinal ranges of the Peasant Associations (PA's)

Study site(PAs)	Latitude	Longitude	Altitude
Ferro I	06°46'30"N	038°29'00"E	1795-1896
Ferro II	060°49'602"N	038°29'135"E	1860-1940
Halekena	06°45'333"N	038°27'432"E	1740-1825
Taramesa	06°086'055"N	038°45'605"E	1745-1868
Tellamo	06°083'629"N	038°48'489"E	1825-1906
Gonowa goda	06°084'609"N	038°50'916"E	1855-1990
Abela Lida	06°54'993"N	038°29'317"E	1945-1960
Galakohireye	06°54'864"N	038°30'315"E	2006-2011
Arossa	06°54'713"N	038°31'788"E	2111-2135

which included trees, crops and vegetable species were collected during dry season (November-December) in year 2012 from each sampling site (PAs). Soil sample collection was carried out at four 10m X 10m quadrats from each PAs. The soil was sampled to a depth of 0 to 15 cm in 828 sampling points using a soil auger. A composite soil sample of 500 g was collected from each plant species. In total (23x9x4) samples were collected for analysis from all the 9 PAs (sampling sites), that is, 23x4 samples from each sampling site. The soil samples were air-dried at room temperature for two weeks and preserved at 4°C for analysis of soil physicochemical properties, AMF spore abundance and AMF diversity. About 0.5 mg of fine root samples from each plant were also collected, washed with tap water, preserved in 50% ethanol, and stored at 4°C for percentage root colonization analysis.

Soil analysis were undertaken at the Southern Nations, Nationalities and Peoples Region (SNNPR) Soil Laboratory and Debrezeite Agricultural Research Center following standard procedures and methods: Soil textural fractions were analyzed following the hydrometric method after removing organic matter using H_2O_2 and dispersing the soils with sodium hexameta-phosphate (Black et al., 1965). Soil pH was determined by potentiometric methods using 1:2.5 soil: water ratio. Soil organic

carbon (SOC) was determined by the Walkley-Black oxidation method (Schnitzer, 1982). Total nitrogen (TN) was determined using the Kjeldahl distillation method (Bremner, 1982), and available phosphorous (AP) was determined using Olsen's extraction method (UV/visible Spectrometer, Lambda EZ 201) (Olsen et al., 1965). Available potassium (Av. K) was determined by Sodium Acetate method (Jones, 2001).

Root colonization

The root pieces collected and preserved were stained according to Phillip and Hayman (1970) with some modifications. The root pieces were cleared in 10% KOH solution for 15 to 20 min at 90°C in a boiling water bath. Then, they were rinsed in water several times and acidified with 1% HCl solution. Pigmented roots were bleached in H_2O_2 for about 20 min and again washed with water. Finally the root segments were stained for 10 min in 0.05% trypan blue at 90°C for about 10 min and subsequently distained at room temperature in acidic glycerol.

The stained root segments were mounted in acidic glycerol on slides. The roots were then observed under the compound microscope (200x objective magnification). The presence of colonization in a root segment was recorded if hyphae (only), vesicle or arbuscule were found. About 100 to 150 intersections were examined for each sample. Total root colonization was calculated using the following formula: % Colonization = Total number of positive segments/Total number of segments studied × 100.

Spore population

From each soil sample 100 g soil was mixed in a 2 L capacity beaker containing 1.5 L of water. The soil in water was agitated by stirring vigorously by hand and left to settle down for about five minutes. The suspension was then sequentially (480, 100, 50 and 38 µm) sieved following the wet sieving and decanting method (Gerdemann and Nicolson, 1963). The last pellet (38 µm) was suspended in 60% sucrose solution and thoroughly mixed and centrifuged at 2000 rpm for 1 min and the spores were rinsed carefully with tap water and transferred into plastic petri-dishes.

The AMF spores and sporocarps of each sample were counted under 4x stereomicroscope. The spore densities were expressed as the numbers of spores and sporocarps per 100 g^{-1} of dry soil. Representative Morphospecies were mounted on slides with polyvinyl-lactic acid-glycerol (PVLG) or PVLG mixed with Melzer's reagent (1:1 v/v). Taxonomic differentiation was made based on the descriptions of the International Culture Collection of Vesicular/Arbuscular Mycorrhizal Fungi (http://invam.caf.wvu.edu; 2005), and following descriptions by Schenck and Pěrez (1990).

Statistical analysis

Data on spore abundance and root colonization was log(x) and arcsine (the inverse sine of the square root of the proportion) transformed using PAST3 (ver. 1.0.0.0) and SPSS software package (version 20.0) respectively, prior to analysis to meet assumptions of ANOVA such as normality and homogeneity of variance.

Significance of differences in AM fungal spore abundance and percentage of root colonization between the samples was tested using Duncan's Multiple Range Test at $p < 0.05$ after one-way analysis of variance (ANOVA) with the SPSS software package (version 20.0) (Fisher et al., 1970). Pearson's correlation analysis was used to study the relationship between AMF and soil parameters.

RESULTS

Soil physicochemical properties under different plant species

The physicochemical properties of soil samples of each plant are shown in Table 3. The soil in the study area was slightly acidic with mean pH of 5.87; mean organic carbon, total nitrogen, available phosphorus and potassium being 2.73, 0.30, 12.39 and 1.10 respectively. When individual plant species were considered, all plant species studied significantly affected the rhizosphere soil chemical properties (Table 3). They showed variation in soil pH (H_2O) from 5.0 (acidic) - 7.0 (neutral), whereas soil nitrogen content was in between 0.19 (medium) - 0.36 % (high).

Similarly, phosphorus (Olson) content varied from the lowest 6.75 mg P/kg (*Solanum tuberosum*) to the highest 18.25 mg P/kg (*Persea americana*). Potassium was highest in the rhizosphere soil of *Ipomoea batatas* (2.18cmol (+)/kg) and the lowest (0.55 cmol (+)/Kg) was recorded from *Prunus africana* and *Ricinus communis.* Organic carbon (OC) content of soil sample fall in between 2.04 (high) to 3.45 (very high).

AMF root colonization and spore density

The AMF root colonization on the different agroforestry plant species is presented in Table 4. The highest percentage root length colonization (RLC) was recorded in *Ensete ventricosum* (86%) and the lowest in *Rhamnus prinoides* (53%) and *Colosica esculenta* (52%) respectively (Table 5). All plants were infected with AMF arbuscules and vesicles except *Brassica integrifolia* and *Cucurbita pepo* species (data not shown) (Table 4).

In case of vesicle formation, the highest colonization was found in *Ensete ventricosum* and *Coffea arabica* each 20.33% followed by *Zea mays* 18.67% and *Catha edulis* (17%) and the lowest in *Persea americana* (9.67%), *Mangifera indica* (9.33) and *Discorea alata* (9%). Considering arbuscule formation, the highest percentage was observed in *Ricinus communis* (18%), *Persea americana* (18%) followed by *Ensete ventricosum* (15%) and *Solanum tuberosum* (15.33%). The lowest arbuscular colonization was recorded for *Colocasia esculenta* (5%). In most of the records arbuscular and vesicular colonization are not significantly different at $P<0.05$ level.

The highest number of spore population/100 g dry soil was recorded in the rhizosphere soil of *Croton*

Table 3. Rhizosphere soil chemical properties of plants.

Plant species	pH(H_2O)	OC%	TN%	AP(Olson) (mg/kg)	Kcmol (+)/kg
Ensete ventricosum (Welw.) Cheesman)	6.49±.12^{b}	2.41±.23^{a}	0.26 ±0.02^{c}	10.20±1.3^{e}	0.61±0.33^{a}
Catha edulis (vahl.)Forssk.ex Endl	5.02±0.00^{a}	2.04±0.02^{a}	0.23±0.00 ^{b}	7.12±0.01^{b}	0.88±0.01^{a}
Millettia ferruginea Hochst	6.530.03^{b}	2.46±0.01^{a}	0.36 ±0.01^{d}	9.13±0.01^{d}	1.01±0.00^{b}
Erythrina bruce Schweinf	6.11±0.01^{b}	2.48±0.03^{a}	0.31 ±0.00^{d}	9.10±0.05^{d}	1.16±0.01^{b}
Cordia africana Lam.	7.12±0.01^{c}	3.45±0.03^{a}	0.36 ±0.01^{d}	15.33±0.02^{g}	1.22±0.01^{b}
Croton macrostachyus (HochstExDel)	6.50±0.06^{b}	2.50±0.06^{a}	0.33 ±0.02^{d}	8.15±0.01^{c}	0.98±0.01^{ab}
Prunus africana (Hook.f.)Kalkm.	5.71±0.32^{a}	2.64±0.01^{ab}	0.29±0.00 ^{d}	16.12±0.01^{h}	0.55±0.01^{a}
Vernonia amygdalina Del.	5.60±0.08^{a}	3.15±0.00^{c}	0.34±0.01^{d}	17.07±0.06i	1.19±0.05^{b}
Persea americana Mill.	6.40±0.12^{b}	2.63±0.00^{ab}	0.29 ±0.01^{d}	18.25±0.14^{j}	0.88±0.01^{a}
Mengifera indica L.	5.20±0.23^{a}	2.74±0.01^{ab}	0.36±0.02 ^{d}	16.50±0.06^{h}	0.75±0.02^{a}
Saccharum officinarum L.	5.35±0.02^{a}	2.31±0.00^{a}	0.29±0.01^{d}	9.23±0.03^{c}	1.35±0.0^{b}
Ricinus communis L.	5.38±0.03^{a}	2.63±0.04^{ab}	0.29 ±0.00^{d}	8.21±0.01^{c}	0.55±0.06^{a}
Coffea arabica L.	6.50±0.06^{b}	3.21±0.01^{c}	0.32 ±0.01^{d}	7.68±0.01^{b}	1.21±0.02^{b}
Zea mays L.	5.63±0.04^{a}	2.51±0.29^{a}	0.30 ±0.01^{d}	8.93±0.04^{c}	0.71±0.02^{a}
Phaseolus vulgaris L.	5.51±0.29^{a}	3.14±0.02^{c}	0.33 ±0.01^{d}	7.68±0.28^{b}	1.14±0.01^{b}
Ipomoea batatas (L.) Lam	5.14±0.09^{a}	2.53±0.00^{a}	0.30 ±0.01^{d}	7.35±0.03^{b}	2.18±0.01^{c}
Solanum tuberosum L.	5.33±0.09^{a}	2.71±0.02^{ab}	0.35 ±0.00^{d}	6.75±0.03^{a}	1.15±0.01^{b}
Cappsicum annuum L.	6.05±0.48^{b}	2.47±0.02^{a}	0.30 ±0.01^{d}	14.52±0.25^{f}	0.95±0.03^{ab}
Rhamnus prinoides L' herit	5.67±0.22^{a}	2.65±0.00^{ab}	0.34 ±0.01^{d}	16.01±1.16^{h}	1.20±0.02^{b}
Dioscorea alata L.	5.93±0.08^{ab}	2.37±0.07^{a}	0.19 ±0.01^{a}	17.09±0.03i	1.34±0.01^{b}
Colocasia esculenta (L.)Schoot.	5.23±0.09^{a}	2.78±0.01^{ab}	0.27 ±0.01^{c}	16.60±0.12^{h}	2.22±0.01^{c}
Mean Total	5.87±0.00^{ab}	2.73±0.05^{ab}	0.30 ±0.01^{d}	12.39±0.54^{ef}	1.10±0.05^{a}

OC, Organic carbon; TN, Total nitrogen; AP, Available phosphors, K, Potassium. Similar letters in columns show not significant difference between plant species at p<0.05.

*macrostachyus (*1066) followed by *Catha edulis* (1054) and *Coffea arabica (*995) and the lowest density was recorded in *Dioscorea alata* (100 spores/100 g soil) (Table 4). From the data (Table 4), it is also observed that the percentage root colonization and spore population do not correlate to each other. Current result in this study reveals that under highest P and N concentrations spore density decreased in soils from rhizospheres of some plant species, although the opposite is true for the other species. For soils from the rhizospheres of *P. americana* (P, 19.25; N 0.29), *Vernonia amygdalina* (P, 17.07; N 0.34), *M. indica* (P, 16.50; N, 0.36), and *D. alata* (P, 17.09; N, 0.19) was recorded, 550 and 80, 600 and 78, 580 and 80 and 100 and 66 spores per 100 g soil and percentage root colonization respectively.

On the other hand for agroforestry plant species with low to medium P concentration such as *Catha edulis* (P, 7.12; N, 0.23) *Croton macrostachyus* (P, 8.15; N, 0.33, *Coffea arabica* (p,7.68; N, 0.32) was recorded 1054 and 85, 1066 and 64 and 995 and 80 spores per 100 g soil and percentage root colonization respectively. This result indicates that different plant species respond to AMF differently under low, medium and high concentrations of nutrients in their rhizospheres.

Pearson's correlation coefficient (Table 4) showed that spore density was not significantly correlated with soil pH, OC, total nitrogen and potassium and significant negative correlation with available phosphorus (r = -0.346, p = 0.005) and electron conductivity (r = -0.441, p =0.00) at P<0.01 level (2-tailed). Spore density was also negatively correlated with total nitrogen, but not significantly different.

Pearson's correlation coefficient between root colonization and soil parameters showed that it is negatively correlated to available phosphorus (r = -0.324, p = 0.010) and potassium (r = -0.301, p = 0.017) and significantly different at P<0.01 and P<0.05 level (2-tailed) respectively. It was positive with pH and total nitrogen but not significantly different and was negative but significantly different between root colonization and organic carbon, and negative but not significantly different in between EC and root colonization.

AMF spore diversity

In general 8 different AMF genera were identified (Table

Table 4. Root colonization and spore density of AMF in agroforestry plant species.

Plant species	AMF structural colonization (%) and spore density			
	AC	VC	RLC	SD/100 g^{-1} dry soil
Ensete ventricosum (Welw.) Cheesman)	15.00±2.08^{ab}	20.33±0.33^{a}	85.73±3.73^{a}	630.00±2.90^{d}
Catha edulis (vahl.) Forssk.ex Endl	16.67±2.33^{a}	17.00±1.15^{b}	84.74±1.24^{a}	1054.00±53.12^{a}
Millettia ferruginea Hochst	11.67±0.88^{b}	16.67±1.76^{b}	83.80±2.22^{ab}	686.00±2.31^{d}
Erythrina brucei Schweinf	13.67±1.67^{abc}	14.67±1.33^{bc}	80.23±1.04^{b}	768.00±39.26^{cd}
Cordia africana Lam.	12.00±2.31^{c}	14.00±3.00^{bc}	72.33±1.57^{c}	880.00±49.07^{c}
Croton macrostachyus (HochstExDel.)	10.00±0.58^{c}	14.33±0.33^{c}	64.11±3.20^{d}	1066.00±19.63^{a}
Prunus africana (Hook.f.)Kalkm.	11.67±0.33^{b}	9.67±0.88^{e}	57.67±2.69^{d}	675.00±14.43^{d}
Vernonia amygdalina Del.	7.00±1.53^{c}	13.67±2.40^{cbc}	77.78±1.91^{b}	600.00±46.19^{d}
Persea americana Mill.	18.33±3.84^{a}	15.33±3.18^{bc}	80.32±2.47^{ab}	550.00±21.94^{e}
Mengifera indica L.	10.33±1.20^{c}	9.33±0.88^{de}	79.97±0.77^{b}	580.00±69.28^{d}
Saccharum officinarum L.	13.67±4.18^{abc}	16.00±4.04^{ab}	68.87±2.25^{b}	625.00±14.43^{d}
Ricinus communis L.	18.00±5.69^{ab}	14.00±4.1^{bcd}	74.25±4.31^{bc}	800.00±86.60^{c}
Coffea arabica L.	11.33±1.45^{c}	20.33±2.19^{a}	80.14±1.89^{b}	995.00±2.89^{b}
Zea mays L.	12.67±0.33^{b}	18.67±1.76^{ab}	80.59±1.82^{b}	700.00±46.19^{c}
Phaseolus vulgaris L.	11.33±1.67^{c}	15.00±0.58^{b}	73.27±1.10^{c}	495.00±83.72^{d}
Ipomoea batatas (L.) Lam	12.00±0.58^{b}	15.00±0.58^{b}	70.62±1.96^{c}	665.00±39.84^{d}
Solanum tuberosum L.	15.33±5.55^{ab}	12.00±2.52^{c}	73.04±1.63^{c}	520.00±11.55^{e}
Cappisicum annuum L.	12.33±1.45^{b}	10.00±3.00^{dcd}	72.67±1.76^{c}	632.00±27.71^{d}
Rhamnus prinoides L' herit	9.67±1.20^{c}	9.67±0.88^{de}	53.33±5.02^{d}	751.00±5.20^{c}
Dioscorea alata L.	11.33±1.45^{c}	9.00±1.53^{de}	60.52±4.67^{d}	100.00±2.89^{f}
Colocasia esculenta (L.)Schoot	5.00±1.0^{d}	14.33±7.17^{c}	51.50±7.17^{d}	660.00±92.38^{cd}

AC, Arbuscular colonization; VC, vesicular colonization; RLC, Total root length colonization; SD, Spore Density. Similar letters in columns show not significant difference between groups at p<0.05.

Table 5. Pearson's correlation coefficient in between AMF parameters and soil chemical properties.

Parameter	SD	RLC	pH	OC	T.N	Av.P	Ec	K
Root colonization	0.243^{ns}	1						
pH	0.101^{ns}	0.129^{ns}	1					
Organic carbon	0.120^{ns}	-0.010^{ns}	0.281*	1				
Total nitrogen	-0.002^{ns}	0.121^{ns}	0.006^{ns}	0.236^{ns}	1			
Available P	-0.346**	-0.324**	0.088^{ns}	0.227^{ns}	0.142^{ns}	1		
Electron conductivity(Ec)	-0.441**	-0.105^{ns}	0.085^{ns}	0.163^{ns}	0.384**	0.182^{ns}	1	
Potassium(K)	-0.130^{ns}	-0.301*	-0.198^{ns}	0.137^{ns}	0.043^{ns}	0.057^{ns}	0.138^{ns}	1

significant at the 0.05 level (2-tailed); ns, Not significantly different.

6): *Acaulospora, Claroidioglomus, Funneliformis, Glomus, Gigaspora, Rhizophugus, Septoglomus, and Scutellospora of which Glomus* and *Acaulospora* were the dominant genera found in all species. The highest percentage of *Glomus* was recorded in *C. arabica* (15.27%) and *E. ventricosum* (12.68%) the lowest being recorded in *Cappisicum annuum and C. esculenta* (0.29%). The highest percentage of *Acaulospora* was recorded in three species; *E. ventricosum, C. arabica, and Zea mays* with 14.93, 10.85 and 11.76% respectively. The highest percentage was recorded for *Claroideoglomus* (24.18%) *in Croton macrostachyus, Funneliformis* (33.9%) in *E. ventricosum, Gigaspora* (22.94%) in *Catha edulis, Rhizophugus* (28.67%) in *E. ventricosum, Septoglomus* (20%) in *Saccharum officinarum,* and *Scutellospora* (16.67%) in *E. ventricosum.* In this study, observed Plant species density (Figure 1) in the agroforestry does not induced consistent spore formation and percentage root colonization values in all plant species. *C. esculenta* with its relative abundance value of 0.1% (982) showed

Table 6. Percentage AMF diversity in the 21 plant species in Sidama agroforestry.

Plant species	Different Genera of AMF (%)								
	Ac.	***Clar.***	***Fun.***	***Glo.***	***Giga.***	***Rhi.***	***Sep.***	***Scut.***	***Sp.No***
Ensete ventricosum (Welw.) Cheesman)	14.93	14.38	33.9	12.68	17.65	28.67	13.33	16.67	8
Catha edulis (vahl.)Forssk.ex Endl	3.18	-	10.17	9.8	22.94	16	-	2.38	6
Millettia ferruginea Hochst	4.07	14.38	11.86	11.82	2.35	12.67	6.67	5.95	8
Erythrina brucei Schweinf	4.07	5.23	16.95	2.31	3.53	3.33	-	1.19	7
Cordia africana Lam.	3.62	5.23	5.08	0.86	3.53	0.67	-	2.38	7
Croton macrostachyus (HochstExDel)	9.95	24.18	6.78	9.51	1.76	7.33	13.33	8.33	8
Prunus africana (Hook.f.)Kalkm.	3.17	1.31	-	1.73	1.18	6	-	4.76	6
Vernonia amygdalina Del.	2.71	2.61	3.39	4.9	1.18	6	13.33	4.76	8
Persea americana Mill.	4.07	-	-	1.73	1.18	2.67	-	3.57	5
Mengifera indica L.	1.36	1.31	-	3.17	-	2	-	3.57	5
Saccharum officinarum L.	3.62	2.61	-	3.46	0.58	3.33	20	7.14	7
Ricinus communis L.	3.18	-	3.39	1.44	2.53	0.67	-	3.57	6
Coffea arabica L.	10.85	17.64	-	15.27	17.65	2	-	13.1	6
Zea mays L.	11.76	-	-	12.1	14.1	0.67	13.33	4.76	6
Phaseolus vulgaris L.	4.07	1.31	3.39	1.44	2.53	3.33	-	4.76	7
Ipomoea batatas (L.) Lam	2.26	3.92	-	2.59	2.94	2	-	3.57	6
Solanum tuberosum L.	1.8	-	3.39	1.73	1.76	-	13.33	5.95	6
Cappisicum annuum L.	3.18	1.31	-	0.29	-	-	-	-	3
Rhanmnus prinoides L'herit	2.26	1.96	1.69	0.58	-	-	-	-	4
Dioscorea alata L.	1.36	1.31	-	0.86	1.18	-	6.67	1.19	6
Colocasia esculenta (L.)School.	0.45	1.31	-	0.29	1.18	-	-	-	4
Mean	9.91	7.29	2.814	16.29	8.05	6.95	0.71	3.90	

Ac., Acaulospora; Claro., Claroidioglomus; Fun., Funneliformis; Glo., Glomus; Giga., Gigaspora; Rhi., Rhizophugus; Sep., Septoglomus; Scut., Scutellospora.

percentage colonization of 52%, spore density of 660 and for *D. alata* with its relative abundance value of 0.05% (511) was recorded root colonization of 61%, more than *C. esculenta* (Figure 1) and the least spore density of 100 spore/100 g soil.

On the other hand for species with the highest value of percentage abundance 0.22% (2107) in *E. ventricosum*, was recorded the highest percentage colonization (86%) and medium number of spore density (630 spores/100 g dry soil).

For *C. macrostachyus* with percentage abundance value of about 0.008% (75), was recorded 1066 spores/100 g soil and 64.11% root colonization.The next highest spore density (1054 spores/1001 g soil) and root colonization (85%) was recorded for perennial crop *Catha edulis* with its percentage abundance value of about 0.02% (188), followed by *C. arabica,* 995 spores 100-1 g soil and 80% colonization with its abundance value of 0.12% (1147) respectively. This indicates that in some plant species (e.g. *E. ventricosum*) density decreased the spore production but in some other plant species such as *C. arabica* plant species density has increased spore production.

DISCUSSION

The effects of plant species density and its associated soil chemical properties in Sidama agroforestry homegardens were studied to determine their influence on AMF diversity, spore density and root colonization. The arbuscular mycorrhizal fungi spore population and root colonization pattern varied in different plant species in Sidama homgarden agroforestry systems. The study on diversity of AMF in relation to soil parameters and plant species density did not show significant differences in AMF diversity indices (Shannon-Weaver diversity index, Simpson's Index of Diversity (data not shown)) due to plant species density and soil physical and chemical properties. However, there were differences in AMF spore density and root colonization between plant species. Shifts in AMF species composition with increasing or decreasing plant species density may be explained in part by differential responses of individual AMF species to individual host plant species and edaphic factors in the rhizospheres of each plant species.

According to Koide and Dickie (2002), three mechanisms have been proposed to account for plant

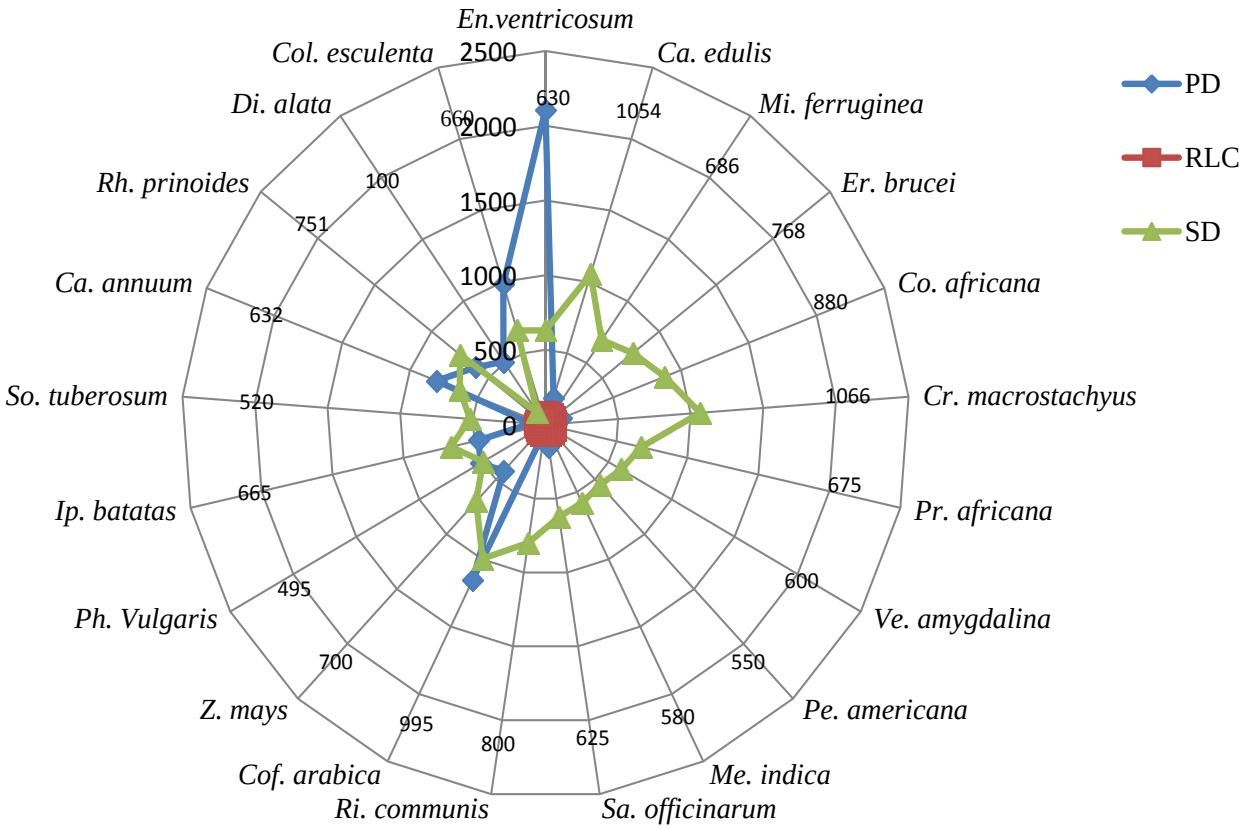

Figure 1. Effect of plant species density on root colonization and spore density. PD, plant density; RLC, total root length colonization; SD, Spore density, *En, Enset; Ca, Catha; Mi, Millettia; Er, Erythrina; Co, Cordia; Cr, Croton; Pr, prunus; Ve, Vernonia; Pe, Persea; Me, Mengifera; Sa, Saccharum; Ri, Ricinus; Cof, Coffee; Z, Zea; Ph, Phaseolus; Ip, Ipomoea; So, solanum; Ca, Cappisicum; Rh, Rhamnus; Di, Discorea; Col, Colocasia*

density-dependent regulation of mycorrhizal colonization. According to their report, higher plant together with higher root density causes, (a) an increase in overlap of phosphorus depletion zone, (b) a competition for light results in less carbohydrate available for mycorrhizal fungi and c) an increase in cost benefit ratio of mycorrhizal colonization. As plant density increase, competition for light increases and photosynthesis per plant declines and plants become limited more by carbon than by P reducing the root colonization. However, in some cases, increasing plant density has been associated with increasing AMF colonization (Eissenstat and Newman, 1990; Allsopp and Stock, 1992).

Most of the plants included in this study are multiple purpose trees and perennial crops with course root systems. The variation in the percentage of colonization in the roots and the AM population in the rhizosphere soils of different plants recorded in the present study, therefore might explain the generally held view that plants with coarse roots gain more AMF (Mahmud et al., 1999) compared to those with fine roots. These differences might be due to the presence of diverse type of AMF in the rhizosphere soils of individual plant species or might be a manifestation of greater host susceptibility to AMF (Mehrotra, 1998).

In this study 8 different AMF genera were identified: *Acaulospora, Claroidioglomus, Funneliformis, Glomus, Gigaspora, Rhizophugus, Septoglomus,* and *Scutellospora* of which *Glomus* and *Acaulospora* were the dominant genera found in all species. This is not surprising since it was previously shown that *Glomus* and *acaulospora* species are most abundant among the glomeromycotan genera in tropical areas (Gerdemann and Trappe, 1974; Blaszkowski, 1989; Talukdar and Germida, 1993; Zerihun Belay et al., 2014). *Glomus* species is widely distributed regardless of the type and intensity of disturbance in the different ecosystems, whereas *Acaulospora* is dominant in least disturbed agro ecosystems (Snoeck et al., 2010).

In our study, the total spore density, in general, did not correlate with mycorrhizal colonization, possibly because of the presence of a diverse population of AM fungal species or according to Biermann and Linderman (1983), it could be due to the fact that there are AMF species that

rely more on extensive formation of hyphal networks instead of survival through spore formation as primary infective propagules. The relationship between spore numbers and mycorrhizal colonization has been found positive by several workers (Giovannetti and Nicolson, 1983) but negative by others (Louis and Lim, 1987). Some workers have also found no relationship between mycorrhizal colonization and spore density of AMF (Diaz and Honrubia, 1994). The factors like edaphic or climatic condition; host fungus compatibility, root properties and soil microorganisms might influence the abundance of spore population and mycorrhizal associations with a particular tree species.

It is noticeable in the present study that the wide spread presence of *Glomus* and *Acaulospora* in the rhizospheres of selected plants, makes them favorable fungi for mass multiplication as well as seedling inoculation for their better establishment.

The present survey of AM fungi on Sidama agroforestry plant species reveals that AM fungi are common, occurring in 21 of the 23 plant species examined. However, when establishing new agroforestry practices the tree species must possess adequate amounts of mycorrhizal colonization at the planting stage in order to survive better and perform well in adverse agroforestry sites. More studies are needed to select the suitable indigenous AM fungal strains for the production of quality plant and expanding the awareness of the role of AMF in agroforestry systems to the small holder peasants for better organic and sustainable production should be initiated.

Conclusion

The investigation reveals that AM fungi are common, occurring in 21 out of 23 plant species examined. The study also demonstrated the influence of different plant densities on the diversity of arbuscular mycorrhizal fungi. For some plant species such as *E. ventricosum* was recorded up to 8 AMF species compared to the least (3 species) for *Cappisicum annum*. As to the dependency of AMF on soil parameters, plants exhibited variable patterns; some with high concentration of P showed lower spore density and root colonization, while others shows the opposite phenomena. *Glomus* and *Acaulospora were* dominant in this study. They could be used for mass multiplication as well as seedling inoculation for better establishment of major trees and perennial crops which can also serve as inoculums sources. Mycorrhization of agroforestry plants has gained considerable attention over the last few years because of their role as bio-fertilizers for improving host growth. More studies are needed to select the suitable indigenous AM fungal strains for the production of quality plant and expand the awareness of the role of mycorrhiza in agroforestry systems to the small holder farmers.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors would like to acknowledge Hawassa College of Teacher Education for financial and logistic supports; Department of Microbiology, Cellular and Molecular Biology, Addis Ababa University and College of Agriculture, Hawassa University for their support with chemicals and laboratory equipment. A great thanks goes to Haramaya University SGS and School of NRMES and the Soil Science program for offering me the opportunity to purse my study. Finally, they would like to thank Vestberg Mourith (PhD) and Zerihun Belay (PhD) for their unreserved comments on taxonomic positions of AMF spores.

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