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Arbuscular mycorrhizal fungi associated with shade trees and *Coffea arabica* L. in a coffee-based agroforestry system in Bonga, Southwestern Ethiopia

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In a first step to understand the interactions between *Coffea arabica* L. trees and mycorrhizae in Ethiopia, an investigation of the current mycorrhizal colonization status of roots was undertaken. We sampled 14 shade tree species occurring in coffee populations in Bonga forest, Ethiopia. *Milletia ferruginea*, *Schefflera abyssinica*, *Croton macrostachyus*, *Ficus vasta*, *F. sur*, *Albizia gummifera*, *Olea capensis*, *Cordia africana*, *Ehretia abyssinica*, *Pouteria adolfi-friederici*, *Pavetta oliveriana*, *Prunus africana*, *Phoenix reclinata* and *Polyscias fulva*. Coffee trees sampled under each shade tree were all shown to be colonized by arbuscular mycorrhizal fungi (AM fungi). Four genera and 9 different species of AM fungi were found in the soils. *Glomus* (*Sp1*, *Sp2*, & *Sp3* & *Sp4*), *Scutellospora* (*Sp1* & *Sp2*) and *Gigaspora* (*Sp1* & *Sp2*) were found under all 14 shade tree species, whereas *Acaulospora* (*Sp1*) occurred only in slightly acidic soils, within a pH range of 4.93-5.75. Generally, roots of the coffee trees were colonized by arbuscules to a greater degree than those of their shade trees, the arbuscular colonization percentage (AC%) of the former being higher than the latter (significant difference at 0.05 level). Though differences were not statistically significant, the overall hyphal colonization percentage (HC%) and mycorrhizal hyphal colonization percentage (MHC%) were shown to be slightly higher under coffee trees than under their shade trees. However, the differences were statistically significant at 0.05 level in the case of HC% values of coffee trees under *Pouteria adolf-friederici* and MHC% under *Cordia africana*. Spore density and all types of proportional root colonization parameters (HC%, MHC%, AC% and vesicular colonization percentage, VC%) for both coffee and shade trees were negatively and significantly correlated with organic soil carbon, total N, available P, EC and Zn. Correlation between arbuscular colonization for coffee (AC%) and organic carbon was not significantly positive at a 0.05 level. Incidence of specific spore morphotypes was also correlated with physical and chemical soil properties. Results indicate that AM fungi could potentially be important in aforestation and help to promote coffee production activities in Ethiopia providing an alternative to expensive chemical fertilizer use, and would offer management methods that take advantage of natural systems dynamics that could potentially preserve and enhance coffee production.

Key words: aforestation; agroforestry, coffee groves, ecology, integrated production systems, rhizosphere, sustainable agriculture, symbiosis

1. Introduction

Forests are important gene reservoirs and natural regeneration areas for many economically important plants around the world (Wolf, 1999; Daba, 2002). *Coffea arabica* L., or arabica coffee, is a non-alcoholic stimulant beverage crop and one of the most valued crops in world trade. It has for many years remained second in value only to oil as a source of foreign exchange in numerous developing countries (Tefestewolde, 1995; ICO, 2007). Ethiopia is believed to be the country of origin of arabica coffee (Paulose and Demel, 2000). It represents over 70% of the world's coffee production (ICO, 2009). In 2002, it contributed to more than 60% of Ethiopia's foreign exchange earnings, over 5% of GDP, 12% of agricultural output, and 10% of government revenues (CSA, 2002). According to somewhat older data, it also employs circa 25% of domestic labour force (EIAR, 1996). About 55% of the country's production is exported while the balance is consumed locally (Mesfin, 1991). In Ethiopia, coffee grows in natural coffee groves and managed agroforestry systems, totaling about 500,000 hectares (Aga *et al.*, 2003).

More than 60% of coffee plants grow under shade trees in evergreen forest areas situated in southwestern Ethiopia (Paulose and Zebene, 1994). Shade tree genera include *Albizia*, *Acacia*, *Bersama*, *Cordia*, *Croton*, *Dracaena*, *Entada*, *Erythrina*, *Ficus*, *Leucaena*, *Millettia*, and *Syzygium* (FAO, 1968; Demel and Tigeneh, 1991). Like many crops, coffee and shade trees associate symbiotically with arbuscular mycorrhizal fungi (AM fungi) (Sieverding, 1991; Cardoso, 2003; Muleta *et al.*, 2007).

Mycorrhizae are thought to influence plant community composition and plant productivity (Van der Heijden *et al.*, 1998). Moreover, success of any reforestation intervention is likely to depend on the co-establishment of diverse AM fungi together with seedlings in the nursery (Sieverding, 1991; Francis and Read, 1994). Benefits from mycorrhizae are greatest and most obvious under low input subsistence agriculture systems in developing countries in the tropics (Sieverding, 1991).

Propagules of mycorrhizae in coffee soils enhance coffee plant growth, increase P and Zn uptake of young coffee seedlings in nursery conditions and improve their establishment after transplantation (Lopes *et al.*, 1985; Rivera *et al.*, 2003; Vaast and Zasoski, 1992). They also impart tolerance against a number of plant parasitic nematodes (Vaast *et al.*, 1998). These positive effects of mycorrhizal fungi could have significant importance for low input agriculture (Douds *et al.*, 2000).

A number of recent studies have described the relationship of mycorrhizal symbiosis with *Acacia polyacantha* obtained from a dry savannah woodland ecosystem (Yonase, 2005), and *Erythrina brucei* from a highland woodland ecosystem (Shasho, 2002). Mycorrhizae (esp. *Glomus* spp.) were also shown to colonize tamarind roots in different ecological zones in Senegal, which is a country with an essentially dry climate. Inoculation of young seedlings helped to generate increased drought tolerance and can thus be considered an active tool in tamarind management (Bourou *et al.*, 2010 & 2011). Mycorrhizal colonization rates of a number of tree species in an afromontane forest were also estimated (Tesfaye *et al.*, 2003a). On top of that, the number of coffee shade tree species and density of AM fungi spores in Bonga natural coffee forest soils of southwestern Ethiopia

(in the same forest as the present study but at different locations) were also investigated (Muleta *et al.*, 2007). The latter authors identified the dominant coffee shade tree species, evaluated their densities, and quantified and characterized AM fungi populations particularly within the rhizosphere of coffee plants. In Brazil, Cardoso *et al.* (2003) indicated that greater numbers of spores in the deeper soil layers of agroforestry and coffee stands may be due to greater amounts of roots at those depths. Additionally, the same authors explained that greater mycorrhizal incidence at deeper soil layers in the agroforestry system may change the dynamics of phosphorus cycling in soil, making this nutrient more available to plants. In Ethiopia there is almost no information available on the interrelationship between diversity of AM fungi, their density, root colonization rate (RCR) and the nutrient dynamics of coffee and their shade trees.

The present study was therefore initiated with the following objectives, to (a) quantify and identify spores of AM fungi occurring under the different shade tree species, and (b) investigate the relationship between AM fungi, root colonization, and physical and chemical soil parameters in a number of natural coffee forest sites in Bonga, Ethiopia, where some natural forest relics are found.

Figure 1: A sketch of shade tree and understory coffee plants

Materials and methods

Study site

This study was carried out in Bonga coffee forest situated 15 km from Bonga town, Ethiopia (Fig. 2).

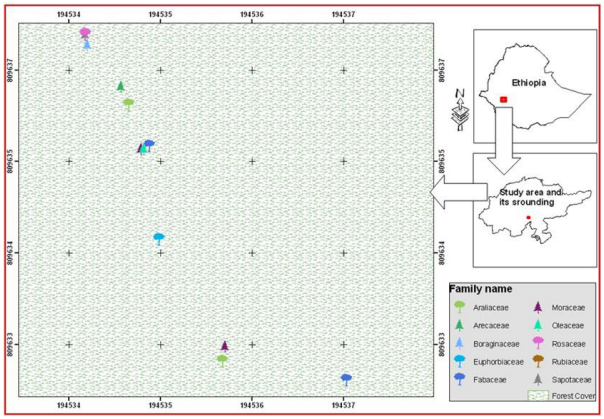

Figure 2: GPS coordinates of sample shade tree species in the study area (coordinates of shade tree species in the figure were taken from table 1). Source: Adopted from Ethiopian Map Agency, digitized and modified using GIS technology

The forest was identified and demarcated by the government of Ethiopia as a coffee forest and forest genetic reservoir for conservation (MOA, 1998). The site is one of the national coffee forest areas defined in Southern Nations and Nationalities People's Regional State (SNNPRS) of the country. Its altitude ranges 1750-2000 masl. Average annual rainfall amounts to over 1700 mm. Mean annual temperature ranges between 18-20°C, with mean daily temperature minima of 8.7°C and corresponding mean maxima of 29.9°C (Taye and Burkhard, 2011).

Sampling roots and AM fungi

Point locations of 14 dominant shade tree species were determined using Garmin 12 Handheld GPS Receiver (Table 1). Root samples of the shade trees and their associated coffee trees (3 coffee plants under each shade tree) were collected by excavating soil using a handheld hoe starting from the shade tree's trunk base and working out towards the fine roots within a 3 m radius. Soil samples taken at 50 cm depth were washed over 2 (to restrain bigger roots) and 0.5 mm sieves. Per sample, twenty fine roots were randomly collected from the 0.5 mm sieve and brought into the laboratory, carefully washed with tap water, cut into 1 cm pieces and placed in 50% alcohol until processed (Frioni *et al.*, 1999). The area under each tree canopy was partitioned into six sectors of 600 each. A total of 600 g of triplicates sample (each replica having 200 g composite soil) was collected at 50 cm depth from under the canopy of each tree and brought to the laboratory for spore extraction.

Scientific name	Family name	Location
Milletia ferruginea	Fabaceae	07019'.376''N 036014'.947''E
Schefflera abyssinica	Araliaceae	07019'.383''N 036014'.899''E
Croton macrostachyus	Euphorbiaceae	07019'.430''N 036014'.874''E
Ficus vasta	Moraceae	07019'.389''N 036014'.900''E
Ficus sur	Moraceae	07019'.465''N 036014'.867''E
Albizia gummifera	Fabaceae	07019'.466''N 036014'.870''E
Olea capensis	Oleaceae	07019'.465''N 036014'.868''E
Cordia africana	Boraginaceae	07019'.505''N 036014'.846''E
Ehretia abyssinica	Boraginaceae	07019'.509''N 036014'.845''E
Pouteria adolfi-friederici	Sapotaceae	07019'.509''N 036014'.845''E
Pavetta oliveriana	Rubiaceae	07019'.509''N 036014'.845''E
Prunus africana	Rosaceae	07019'.509''N 036014'.845''E
Phoenix reclinata	Arecaceae	07019'.489''N 036014'.859''E
Polyscias fulva	Araliaceae	07019'.482''N 036014'.862''E

Table 1: Coffee shade tree species and their respective geographical locations in Bonga coffee forest studied for AMF colonization

AM fungi spores were separated from the soil by the wet-sieving/gradient centrifugation technique (Brundrett *et al.*, 1996). Spores were counted from 100 g soil aliquots using an AJ05 model Turret Dissecting Microscope with a magnification of 40x. Spores were grouped into genera of different species according to a number of morphological characteristics such as: spore size, shape, colour, wall structure, hyphal attachment (simple, swollen or bulbous) and Melzer's solution reaction (INVAM, 2004; Merryweather, 2004). Permanent slides were prepared for each different spore morphotype with polyvinyl-alcohol and polyvinyl-alcohol plus Melzer's solution (Merryweather, 2004). The diameter of

spores was measured using a cc12 model camera mounted on an Olympus Bx 51 microscope which was connected to the AnalySIS ® Soft Imaging Systems GmbH version 3.2 software program.

Roots were cleared in 10% KOH (Kormanik and McGraw, 1982; Brundrett *et al.*, 1994). Dark-pigmented roots were further bleached with 10% H_2O_2 and acidified with 1% HCl. Cleared roots were stained in trypan blue (0.05% in 14:1:1 lactic acid: glycerol: water). Proportional colonization (colonization of roots by AM fungi) was estimated using the magnified intersection method. A hair line graticule inserted into an eyepiece acted as the line of intersection with each root at x 200 magnification under the compound microscope (McGonigle *et al.*, 1990). Percentage of root length colonization (%RLC) was calculated from 100 or more intersections for each root sample (around 10 fine root pieces per slide). At each intersection, there were six possible mutually exclusive outcomes. The line might intersect at points p, q, r, s, t and u where, "p" represents intersection at no-fungal structures, "q" arbuscules, "r" mycorrhizal vesicles, "s" arbuscules and mycorrhizal vesicles at a time, "t" mycorrhizal hyphae but no arbuscules or mycorrhizal vesicles, and "u" hyphae not seen to be connected to arbuscules or mycorrhizal vesicles.

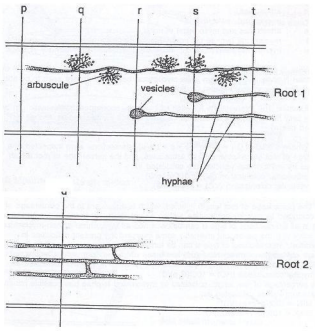

Figure 3: The eyepiece hairline can make 6 types of intersections (p, q, r, s, t, and u)
Source: Adopted from Practical Methods in Mycorrhizal Research. (1994). Ed. M. Brundrett, L. Melville and L. Peterson

Soil sampling

A total of 1 kg of composite soil was taken from each sector at 50 cm depth within 3 m diameter under each shade tree rhizosphere for physical and chemical soil analyses. Soil samples were analyzed for pH, organic matter (OM), available P (AvP), total N (TN), Mg, K, Ca, Na, Zn, cation exchange capacity (CEC) and exchangeable cations (EC). Soil pH was potentiometrically (1:1 sample) measured by using distilled water (Van Reeuwijk, 1993). OM was determined by the wet combustion procedure of Walkley and Black (Van Ranst *et al.*, 1999). TN was determined by the wet or oxidation procedure of the Kjeldahl method (Bremner and Mulvaney, 1982). AvP content of the soil was determined by Olsen method (Van Reeuwijk, 1993). CEC and EC of soils were determined by 1M ammonium acetate (pH 7) method as presented in the percolation tube procedure (Van Reeuwijk,

1993). Effective CEC was calculated as the sum of exchangeable cations extracted by ammonium acetate buffered at pH 7 plus 1M KCl extractable Al. Available Zn was determined by the diethylenetriaminepentaacetic acid (DTPA) method (Tan, 1996).

Data analysis

Data were statistically analyzed using the SPSS V.11.0 program (SPSS Inc., Chicago, IL., USA). Analysis of variance (ANOVA) was applied to spore numbers and colonization percentages. Means were compared by Duncan's Multiple Range Test at $P \leq 0.05$.

Results

AM spores

Four genera and 9 species of AM fungi were identified from the different rhizosphere soils and are presented in table 2 where characteristics exhibited by the different spore types are also indicated. Type 1 (*Glomus* spp.) with four different species, type 2 (*Gigaspora* spp.) with two different species, and type 3 (*Scutellospora* spp.) with two different species were found under all tree species. Spores of type 4 (*Acaulospora* spp.), which contains only one species, were found under 64% of tree species and at lower densities than the first three types (genera) (Tables 2 and 3).

Type/Genus	Colour	Shape	Diameter (µm)	Subtending hyphae	Hyphal attachment	Species
I (*Glomus*)	light yellow (honey) to brown (black)	spherical	150-175	+	simple	Sp1, Sp2, Sp3, Sp4
II (*Gigaspora*)	white to gray	globose	350-600	+	Bulbous /Swollen	Sp1, Sp2
III (*Scutellospora*)	brown	globose to ellipsoidal	100-150	+	Bulbous /Swollen	Sp1, Sp2
IV (*Acaulospora*)	brown to black	round to oblong	> 400	–	–	Sp1

Table 2: Characteristics of Arbuscular Mycorrhizal (AM) fungi spores (based on 15 samples from each type) found in this study. (Where: + = presence of subtending hyphae / - = absence of subtending hyphae (sessile) / Sp = species)

Spore densities recorded in the present study are high, which compares well to other studies. For example, low counts of AMF spores ranging from 4 to 67 spores per 100 g of dry soil were reported by Muleta *et al.* (2003) for coffee groves. Similarly, in soils from a dry savannah wood land ecosystem under *Acacia polyacantha*, Yonase (2005) reported a mean of 57.9 spores per 100 g of dry soil. Spore densities showed considerable variations across tree species (Table 3). The highest spore density was found under *F. vasta*, *C. macrostachyus* and *S. abyssinica*, the lowest occurred under *E. abyssinica*, *P. africana* and *P. fulva*. Spore density also varied significantly among different members of the same family of shade trees: *S. abyssinica* and *P. fulva* in Araliaceae, and *F. vasta* and *F. sur* in Moraceae (Table 3).

Species	Total	Type 1	Type 2	Type 3	Type 4
Milletia ferruginea	670 ± 17^{f}	521 ± 14^{h}	86 ± 5^{f}	56 ± 3^{bc}	7 ± 2^{c}
Schefflera abyssinica	997 ± 11^{c}	807 ± 12^{c}	129 ± 4^{c}	61 ± 4^{bc}	–
Croton macrostachyus	1098 ± 31^{b}	892 ± 15^{b}	149 ± 8^{b}	57 ± 2^{bc}	–
Ficus vasta	1313 ± 36^{a}	1089 ± 17^{a}	171 ± 7^{a}	$53 \pm {}^{2c}$	–
Albizia gummifera	805 ± 17^{e}	631 ± 5^{f}	106 ± 2^{d}	63 ± 2^{ab}	5 ± 2^{c}
Olea capensis	910 ± 15^{d}	728 ± 15^{d}	123 ± 4^{c}	59 ± 2^{bc}	–
Cordia africana	760 ± 17^{e}	576 ± 10^{g}	$101 \pm 8d^{e}$	68 ± 3^{a}	15 ± 2^{ab}
Ehretia abyssinica	578 ± 11^{h}	396 ± 4^{j}	125 ± 2^{c}	51 ± 2^{c}	6 ± 2^{c}
Pouteria adolfi-friederici	787 ± 13^{e}	623 ± 8^{f}	93 ± 3^{ef}	60 ± 2^{b}	11 ± 2^{bc}
Pavetta oliveriana	827 ± 22^{e}	654 ± 4^{e}	102 ± 2^{d}	54 ± 3^{c}	17 ± 2^{a}
Ficus sur	753 ± 8^{e}	592 ± 9^{g}	103 ± 3^{d}	49 ± 3^{c}	9 ± 2^{bc}
Prunus africana	635 ± 9^{g}	483 ± 9^{i}	96 ± 3^{e}	50 ± 3^{c}	6 ± 1^{c}
Phoenix reclinata	674 ± 19^{f}	563 ± 17^{g}	73 ± 3^{g}	38 ± 2^{d}	–
Polyscias fulva	638 ± 14^{g}	487 ± 6^{i}	81 ± 3^{f}	$58 \pm 2b^{c}$	12 ± 2^{b}

Table 3: Spore density per spore type in the rhizosphere of shade trees (spores per 100 g dry soil, n=6)
Means followed by the same letter in the same column are not significantly different at 0.05 level, ± s.d.

Root colonization

All shade tree species were shown to be colonized by mycorrhizae (Table 4). Arbuscules and vesicles were observed in all tree species. General hyphal colonization percentage (HC%) varied between members belonging to the same family. While in Fabaceae, colonization of *M. ferruginia* and *A. gummifera* was statistically similar, it differed in Araliaceae, *S. abyssinica* (76%) and *P. fulva* (56%), Moraceae, *F. vasta* (81%) and *F. sur* (65%) and Boraginaceae, *C. africana* (67%) and *E. abyssinica* (52 %) ($P<0.05$) (Table 4).

Species	HC (%)	MHC (%)	AC (%)	VC (%)
Milletia ferruginea	69 ± 3^{bc}	51 ± 3^{bc}	5 ± 0.9^{de}	11 ± 2^{bc}
Schefflera abyssinica	76 ± 7^{ab}	57 ± 2^{ab}	6 ± 0.6^{cd}	12 ± 3^{bc}
Croton macrostachyus	81 ± 5^{a}	60 ± 4^{ab}	4 ± 0.5^{e}	14 ± 0.6^{b}
Ficus vasta	81 ± 3^{a}	62 ± 4^{a}	3 ± 0.5^{ef}	16 ± 0.6^{a}
Albizia gummifera	71 ± 3^{b}	51 ± 4^{bc}	6 ± 0.6^{cd}	12 ± 0.6^{bc}
Olea capensis	73 ± 5^{b}	55 ± 3^{ab}	5 ± 0.3^{de}	12 ± 0.6^{bc}
Cordia africana	67 ± 3^{bc}	47 ± 2^{bc}	7 ± 0.3^{b}	10 ± 0.1^{bc}
Ehretia abyssinica	52 ± 3^{de}	39 ± 2^{c}	6 ± 0.5^{c}	6 ± 0.3^{d}
Pouteria adolfi-friederici	69 ± 3^{bc}	52 ± 3^{b}	5 ± 0.1^{d}	11 ± 0.6^{bc}
Pavetta oliveriana	72 ± 4^{b}	53 ± 6^{bc}	6 ± 0.5^{c}	12 ± 0.3^{bc}
Ficus sur	65 ± 4^{bc}	45 ± 4^{c}	9 ± 0.5^{a}	8 ± 0.3^{d}
Prunus africana	54 ± 3^{d}	40 ± 3^{c}	3 ± 0.4^{f}	7 ± 0.2^{d}
Phoenix reclinata	61 ± 2^{c}	43 ± 3^{c}	8 ± 0.5^{ab}	8 ± 0.6^{d}
Polyscias fulva	56 ± 4^{cd}	42 ± 5^{c}	5 ± 0.7^{de}	8 ± 0.6^{d}

Table 4: Root colonization percentages of coffee grove shade trees, Bonga, Ethiopia
(Where: HC, hyphal colonization = 100[(G-p)/G]; MHC, mycorrhizal hyphal colonization = 100[(q+r+s+t)/G]; AC, arbuscular colonization = 100(q+s/G); VC, vesicular colonization = 100(r+s/G).Where: G = (p+q+r+s+t+u) intersections inspected, p: no fungal structures, q: arbuscules, r: mycorrhizal vesicles, s: arbuscules and mycorrhizal vesicles, t: mycorrhizal hyphae but no arbuscules or mycorrhizal vesicles and u: hyphae not seen to be connected to arbuscules or mycorrhizal vesicles).

Percentages of coffee roots colonized by total hyphae (HC%) were generally higher than for the respective associated shade tree species. Mycorrhizal hyphal colonization (MHC%) of coffee trees varied: under the canopy of *F. vasta*, *F. sur*, *C. macrostachyus*, *S. abyssinica*, *O. capensis*, *P. adolfi-friederici*, *A. gummifera*, *P. oliveriana*, *M. ferruginea* and *C. africana* we evidenced the highest colonization percentages. Coffee trees under *P. reclinata* and *P. fulva* showed significantly lower colonization percentages. At the other end of the spectrum, coffee trees under *E. abyssinica* and *P. africana* exhibited the lowest colonization percentages (Table 5). Arbuscular colonization (AC%) is found to be greater in coffee trees than in their respective shade trees in almost all cases (Tables 4 and 6). However, in most cases vesicular colonization (VC %) is larger in shade tree species than in the coffee trees that grow underneath.

Shade trees over coffee	HC (%)	MHC (%)	AC (%)	VC (%)
Milletia ferruginea	76 ± 4^{b}	56 ± 4^{a}	13 ± 2^{bc}	5 ± 2.0^{def}
Schefflera abyssinica	80 ± 4^{ab}	60 ± 4^{a}	15 ± 2^{ab}	4 ± 0.3^{f}
Croton macrostachyus	83 ± 5^{ab}	61 ± 7^{a}	16 ± 2^{ab}	2 ± 0.6^{h}
Ficus vasta	87 ± 5^{a}	64 ± 9^{a}	18 ± 2^{ab}	4 ± 0.3^{f}
Albizia gummifera	78 ± 5^{ab}	57 ± 7^{ab}	19 ± 2^{a}	2 ± 0.5^{h}
Olea capensis	80 ± 4^{ab}	57 ± 3^{a}	18 ± 3^{ab}	3 ± 0.3^{g}
Cordia africana	73 ± 3^{bc}	55 ± 5^{ab}	12 ± 2^{bc}	7 ± 0.3^{cd}
Ehretia abyssinica	58 ± 5^{c}	39 ± 1^{c}	8 ± 2^{c}	9 ± 0.4^{b}
Pouteria adolfi-friederici	78 ± 4^{ab}	57 ± 5^{a}	14 ± 2^{b}	6 ± 0.6^{e}
Pavetta oliveriana	79 ± 6^{ab}	56 ± 4^{a}	14 ± 2^{b}	7 ± 0.3^{d}
Ficus sur	71 ± 3^{bc}	57 ± 9^{ab}	10 ± 2^{bc}	8 ± 0.4^{c}
Prunus africana	59 ± 6^{c}	36 ± 7^{c}	9 ± 2^{c}	7 ± 0.3^{d}
Phoenix reclinata	67 ± 4^{c}	47 ± 3^{b}	9 ± 2^{c}	10 ± 0.5^{a}
Polyscias fulva	60 ± 4^{c}	42 ± 4^{bc}	8 ± 1^{c}	8 ± 1.0^{bcd}

Table 5: Root colonization percentages of coffee plants under shade trees in Bonga, Ethiopia (Where: HC, hyphal colonization = 100[(G-p)/G]; MHC, mycorrhizal hyphal colonization = 100[(q+r+s+t)/G]; AC, arbuscular colonization = 100(q+s/G); VC, vesicular colonization = 100(r+s/G).Where: G = (p+q+r+s+t+u) intersections inspected, p: no fungal structures, q: arbuscules, r: mycorrhizal vesicles, s: arbuscules and mycorrhizal vesicles, t: mycorrhizal hyphae but no arbuscules or mycorrhizal vesicles and u: hyphae not seen to be connected to arbuscules or mycorrhizal vesicles).

Species	pH	OM	TN	P	EC	CEC	Na	K	Ca	Mg	Zn
Milletia ferruginea	5.10	2.366	0.261	3.60	0.049	27.70	0.21	0.07	6.41	2.93	1.298
Schefflera abyssinica	4.90	2.111	0.231	1.33	0.025	29.20	0.19	0.10	3.63	2.34	0.511
Croton macrostachyus	4.70	1.890	0.176	1.05	0.027	22.25	0.15	0.12	2.64	1.91	0.265
Ficus vasta	4.83	2.274	0.206	0.91	0.021	26.15	0.14	0.12	3.22	2.05	0.133
Albizia gummifera	4.93	2.119	0.233	1.25	0.029	28.25	0.15	0.25	5.23	2.25	0.374
Olea capensis	4.93	2.604	0.277	1.17	0.046	32.10	0.14	0.50	5.98	3.42	0.644
Cordia africana	5.75	2.333	0.270	2.65	0.064	31.15	0.12	0.22	9.33	3.52	2.542
Ehretia abyssinica	5.50	3.048	0.337	4.00	0.061	30.05	0.14	0.11	9.68	2.55	2.838
Pouteria adolfi-friederici	5.10	2.525	0.306	1.40	0.059	29.65	0.18	0.08	6.50	3.37	1.591
Pavetta oliveriana	5.20	2.707	0.348	1.31	0.082	30.05	0.15	0.06	7.42	2.78	1.963
Ficus sur	5.25	2.289	0.291	1.82	0.051	29.45	0.11	0.33	6.69	3.13	0.857
Prunus africana	5.38	2.654	0.291	3.40	0.051	29.45	0.55	0.14	7.22	2.19	2.159
Phoenix reclinata	4.45	2.814	0.312	2.60	0.076	26.80	0.10	0.21	5.35	2.33	1.062
Polyscias fulva	5.63	2.449	0.304	3.05	0.06	32.25	0.13	0.71	7.52	2.96	1.853

Table 6: Physico-chemical characteristics of rhizosphere soil under each shade tree species in Bonga, Ethiopia
Units used: pH (H_2O, 1:2.5); EC in ds/m; Na, K, Ca, Mg and CEC in Cmol(+)/kg; TN and OM in %; AvP and Zn in ppm

Soil characteristics and mycorrhization (Tables 6 and 7)

Spore densities, HC, MHC and AC were inversely correlated with OM, total N, available P, EC and Zn (Table 7.a). Only HC % was negatively correlated with pH (r = 0.540), whereas VC was not significantly correlated with any of the soil characteristics tested. A similar correlation trend was observed between coffee trees and soil characteristics, except for AC versus OM (Table 7.b).

	Soil parameters							
	pH (H2O)	OM	TN	AvP	EC	CEC	Zn	
(a) For shade trees (correlations between tables 4 and 6)								
HC	-0.540*	-0.647*	-0.704**	-0.825**	-0.627*	-0.472ns	-0.736**	
MHC	-0.528ns	-0.691**	-0.741**	-0.823**	-0.642*	-0.495ns	-0.74**	
AC	-0.503ns	-0.645*	-0.712**	-0.791**	-0.621*	-0.510ns	-0.703**	
VC	-0.021ns	0.062ns	0.292ns	0.37ns	0.357ns	0.176ns	0.038ns	
SD	-0.509ns	-0.599*	-0.742**	-0.795**	-0.688**	-0.514ns	-0.727**	
(b) For coffee trees under each shade tree (correlation between tables 5 and 6)								
HC	-0.555*	-0.696**	-0.708**	-0.853**	-0.592*	-0.486ns	-0.76**	
MHC	-0.54*	-0.647*	-0.704**	-0.823**	-0.627**	-0.472ns	-0.738**	
AC	-0.503ns	0.645ns	-0.712**	-0.791**	-0.621*	-0.510ns	-0.703**	
VC	-0.021ns	0.062ns	0.292ns	0.037ns	0.357ns	0.176ns	0.038ns	

*Table 7: Correlation between soil parameters and AM fungi of shade trees and the respective coffee trees underneath (Where: **- Correlation is significant at the 0.01 level; * - correlation is significant at the 0.05 level. ns, non significant at 0.05 level; SD, Spore density; HC, hyphal colonization = 100[(G-p)/G]; MHC, mycorrhizal hyphal colonization = 100[(q+r+s+t)/G]; AC, arbuscular colonization = 100(q+s/G); VC, vesicular colonization = 100(r+s/G).Where: G = (p+q+r+s+t+u) intersections inspected. (p, q, r, s, t & u are same as in the above tables)).*

Discussion

Four genera totaling 9 species of mycorrhizae were recovered from soils sampled from Bonga natural coffee forest, Ethiopia. *Glomus* was the dominant genus in all soils under each tree species both in terms of species diversity and spore density (Tables 2 and 3). This is in line with findings not only from dry afromontane forests of Ethiopia (Tesfaye *et al.*, 2003b) and Bonga natural coffee forest (Muleta *et al.*, 2007), but also from the tropical rain forest of Xishuangbanna, Yunnan, China (Zhao *et al.*, 2001), tropical rain forest in Mexico (Guadarrama and Alvarez-Sanchez 1999), and arid and semi-arid lands of north Jordan (Mohammad *et al.*, 2003).

This dominance of *Glomus* could be attributed to several factors. In the present study, it is observed that the acidic nature of the rhizosphere soil may have favoured this genus (Table 6). As we indicated earlier, *Glomus* species (with the exception of Glomus mosseae) have been found to be distinctly acid-tolerant (Mosse, 1972; 1973).

The least-occurring spore type, *Acaulospora*, was absent under *S. abyssinica*, *C. macrostachyus*, *F. vasta*, *O. capensis* and *P. reclinata* and when present was only evidenced at low spore densities (Table 3). The reasons for the low occurrence of this spore type are unclear. Low figures may be influenced by host types as a number of studies indicate that host-plant preferences exist (Halgason *et al.*, 2002; Vandenkoornhuyse *et al.*, 2003; Johnson *et al.*, 2003). This could either be due to differences in root anatomy or exudates from plant roots (Gamalero *et al.* 2004; Norman *et al.*, 1996). Cardoso *et al.* (2003) also indicated that spore production or colonization could be influenced by the length of plant roots.

However, the number of species (especially *Glomus*) isolated as spores from any forest may be lower than that of pot cultures (Brundrett *et al.*, 1999). The number of species recovered in this study might be underestimated due to the fact that some species may not produce their spores in forest soil.

F. vasta, *C. macrostachyus* and *S. abyssinica* harboured relatively greater number of spores than other hosts. Fewest spores were encountered under *E. abyssinica*, *P. africana* and *P. fulva*. Spore numbers were negatively associated with available P, total N, and Zn in soil, and variably associated with organic matter, EC and CEC. Because mycorrhizae help to increase uptake of minerals in plants, the levels of P and Zn in the plant may regulate root colonization and spore formation (Smith and Read, 1997). It has been shown that high levels of phosphorus in soil and plant are able to inhibit mycorrhiza formation (Douds and Schenck, 1990) and influence the diversity of AM fungi in field soils (Cuenca and Menses, 1996).

Generally, a larger number of mycorrhizal spores were extracted under each tree in this study than in other studies. In this work, the lowest average number of AM fungi spores extracted (578/100 g) was larger than those presented by Yonas (2005) who reported 57.9 spores per 100 g dry soil under *Acacia polyacantha* in a dry savannah woodland ecosystem. Similarly, Shasho (2002) reported more than 300 spores per 100 g soil beneath *Erythrina brucei* from a highland woodland ecosystem. These differences in spore density may be due to variations in environment, host trees and edaphic factors between study places. The higher spore number in this coffee forest may be due to the low level

of soil disturbance where almost no tillage is practiced in the forest. Similar results were obtained in Brazil by Cardoso *et al.* (2003).

The pattern of root colonization was also found to vary among shade trees (even between members of the same family). The variations in some species with relation to the degree of colonization and presence/absence of AM fungi in the same or different collecting places indicate that environmental factors influence the presence or absence of mycorrhizae and their colonization level (Alexander, 1989). Similarly, St. John (1980) indicated such a variation of root colonization to occur at genus and family levels.

A direct relationship was found between colonization percentage and spore density in the soil in each tree species. Jasper *et al.* (1993) and Frank and Morton (1994) similarly observed sporulation to be positively correlated with mycorrhizal colonization. Therefore, this may indicate that most of the spores in this study are colonizing ones.

Generally, the coffee tree roots were more heavily colonized by arbuscules (AC%) than their respective shade trees (significant difference at the 0.05 level). General hyphal colonization (HC%) and mycorrhizal hyphal colonization (MHC%) were also seen to be slightly higher in coffee trees than in their respective shade trees. However, these differences were only statistically significant in *Pouteria adolf-friederici* (in case of HC%) and *Cordia africana* (in case of MHC%).

The reasons for the relatively higher coffee root colonization are unclear. However, the implications may be important. Greater colonization in coffee may indicate lower relative available P, Zn and N for coffee than for its companions.

It is not clear why AC% seems to be higher in coffee roots than in tree roots, whereas VC% is lower in coffee than in shade trees. However, this may be because coffee tree roots are more colonized by non-vesicle forming mycorrhizal species, or it might be explained by the fact that coffee trees need to have nutrients in the short term rather than to store them in vesicles (nutrient storage sites). This would support the idea that transfer of assimilates from one plant to another is facilitated more through arbuscules than by vesicles (Grime *et al.*, 1987).

Spore density and proportional colonization (for both coffee and shade trees) were negatively correlated with organic carbon, total N, available P, EC and Zn (Tables 7.a and 7.b). However, the correlation between arbuscular colonization for coffee (AC%) and organic carbon was positive but not significant at 0.05 level (Table 7.b). Abiotic factors had minimal influence on mycorrhizal colonization variation (Bohrer *et al.*, 2004).

The present result showed that correlations were very significant between edaphic factors and mycorrhizal colonization except for vesicular colonization (VC%) (Tables 7.a and 7.b). This strongly supports the idea that both climatic and edaphic factors could influence the mycorrhizal colonization (Staddon *et al.*, 2003). AM fungi could enhance plant uptake of P and other nutrients, especially in nutrient-deficient environments (Smith and Read, 1997). Similarly, Cardoso *et al.* (2003) postulated that greater mycorrhizal activity in the deeper soil layers may be important in making more P available to the plant and thus increase the efficiency of nutrient recycling processes in agroforestry systems.

This may again be related to root architecture. Nutrient availability had a much stronger effect on root architecture than arbuscular mycorrhiza (Cruz *et al.*, 2004). Mycorrhizae increase the uptake capacity of the active root zone. However, it is well-known that high soil P suppresses mycorrhizal activity mainly through its effect on the P concentration in plants (Bowen 1987; Menge *et al.*, 1978).

The importance of mycorrhizae to coffee has been reported by several investigators indicating that coffee plants will be heavily mycorrhized under natural conditions. Benefits include enhanced growth and increased P and Zn uptake by young coffee seedlings in nursery conditions (Lopes *et al.*, 1985; Siqueira *et al.*, 1998); enhanced tolerance to nematodes (Vaast *et al.*, 1998) and increased survival of coffee plants after field transplanting and in agroforestry systems (Vaast and Zasoski, 1992). Agroforestry systems can increase soil nutrient availability and accelerate P cycling because the deeper tree roots retrieve nutrients from lower soil horizons (Young, 1997), enhance the chemical and physical quality of soils and increase soil microbial activity (Cooper *et al.*, 1996).

Conclusions

A diverse population of mycorrhizae was observed as spores in a coffee forest in Bonga, Ethiopia. Presence of spores was associated with high levels of mycorrhizae on the roots of both the forest species and coffee plants growing under these forest species. Mycorrhizal colonization in coffee was higher than in the companion forest species, indicating that mycorrhizae may be more helpful for coffee than for the companions. Colonization of both coffee and shade trees was correlated with several edaphic characters of the soil in which they were growing. These observations indicate that management of coffee groves should take into account the impact of various practices on the mycorrhizae, and try to maintain high levels of colonization. The specific management practices needed to maintain mycorrhizae in this system are unclear, but absence of soil disturbance and proper management of litter fall (OM) may be important, and are worth further investigation.

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