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Composition of coffee shade tree species and density of indigenous arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest, southwestern Ethiopia

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Abstract

The composition of coffee shade tree species and density of arbuscular mycorrhizal fungi (AMF) spores in Bonga natural coffee forest of southwestern Ethiopia were investigated. This study is the first report on AMF populations of Ethiopian natural coffee forests. The main purposes were to systematically identify the dominant coffee shade tree species, evaluate their densities and quantify and characterize populations of arbuscular mycorrhizal fungi particularly in the rhizosphere of coffee plants. Sample plots of 400 m^2 with coffee plants and dominant shade tree species were selected. Sampling of soil was carried out at a depth of 0–15 cm from the rooting areas of shaded and unshaded coffee plants for analysis of some soil parameters and quantification of AMF spores. Nineteen dominant shade tree species belonging to 14 plant families were identified in considered 10 quadrates. In terms of their stand dominance, *Millettia ferruginea* (Hochst.) Baker had the highest frequency of occurrence (22.3%) followed by *O. welwitschii* Friis & P.S. Green (15.5%). High density (503 stems/ha) and/or percentage (66%) of *Coffea arabica* L. were recorded. All soil samples yielded AMF spores and the counts ranged from 4 to 67 spores 100 g^{-1} of dry soil. Notably higher mean counts of AMF spores were found under leguminous shade trees compared to non-leguminous ones. AMF spore counts were significantly positively correlated with coffee counts and available soil P content. Five genera of AMF were identified based on spore morphology. *Glomus* dominated members of Glomeromycota. The other genera found were *Gigaspora*, *Acaulospora*, *Entrophospora* and *Scutellospora* in order of occurrence. The present investigation has documented species richness among dominant coffee shade tree species along with a fair distribution of relevant numbers and types (genera) of AMF to stimulate coffee growth. Thus, Bonga natural coffee forest seems to be an ideal focal forest for in situ coffee genetic resources conservation and promotion of organic coffee production.
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1. Introduction

Coffee (*Coffea arabica* L.) is a tropical crop grown in 75 countries with a total production close to 108 million t of beans (Bhattacharya and Bagyaraj, 2002). The arabica coffee plant, as the principal economic species, contributes 70% of the world's commercial coffee (Orozco-Castillo et al., 1994). It is a means of subsistence for a constantly fast growing population of the tropics as a complementary or even sole source of income (Bhattacharya and Bagyaraj, 2002).

Coffee is Ethiopia's major export crop, accounting for over 60% of total value of exports (FTFE, 2004). Studies reveal that Ethiopian coffees (arabica coffees) rank high in intrinsic quality of the bean (Bhattacharya and Bagyaraj, 2002). Coffee grows in many parts of the country though the commercially important coffee is from the southwestern, southern and eastern regions. Coffee is grown in natural forests and under agroforestry systems. It has been estimated that more than 60% of the coffee plantations in evergreen forest areas of southwestern Ethiopia are under naturally growing shade trees (Dubale and Mikru, 1994). Additionally, in eastern part of Ethiopia (Harerge) most farmers traditionally grow coffee as an important cash crop under shade trees (Teketay and Tegineh, 1991). The well-known and dominant shade trees reported from Ethiopia embody genera like *Albizia*, *Acacia*, *Bersama*, *Cordia*, *Croton*,

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Dracaena, *Entada*, *Erythrina*, *Ficus*, *Leucaena*, *Millettia*, and *Syzygium* (FAO, 1968; Teketay and Tegineh, 1991). The presence of mycorrhiza under some of these shade tree species has been shown in dry afromontane forests of Ethiopia (Wubet et al., 2003).

Recently, it has been reported that in a managed natural plantation forest at Yayu, southwestern Ethiopia, more than 40% of the important shade trees were legumes, i.e., species of *Albizia*, *Acacia*, and *Millettia* (Taye, 2001). These legume plants harbor important nitrogen-fixing microorganisms (rhizobia) to enrich the soil. The nodulation status of some of these legumes by root nodule bacteria of the genera *Rhizobium* and *Bradyrhizobium* has been reported (Granhall, 1987; Assefa and Kleiner, 1998). The significance of tree and herbaceous legumes in many agricultural production sectors is well addressed (Granhall, 1994; Snoeck et al., 2000) and nitrogen-fixing systems often offer economically attractive and ecologically sound means of reducing external inputs and improving soil fertility (Bohlool et al., 1992). More precisely, the addition of nitrogen to coffee systems by leguminous shade trees has been reported in many countries (Babbar and Zak, 1994).

The main general advantages derived from shade trees are well documented (Beer et al., 1998; Muschler, 2001). The fundamental consideration is the suppression of the major pest of coffee, couch grass (*Digitaria scalarum*). Other favorable considerations for shade encompass temperature regulation, cheaper production, reduction of hail damage and better growth under high altitude conditions (Baggio et al., 1997; Beer et al., 1998), maintenance of biodiversity (Perfecto et al., 1996), reduction of nutritional imbalances and die-back (Beer et al., 1998), and increment in alternative income sources (CEC, 1999). Additionally, Muschler (2001) in his investigation made on coffee attributes in Costa Rica clearly elaborated the main benefits obtained from shading: (1) higher weights of fresh fruits; (2) larger beans; (3) higher ratings for visual appearance of green and roasted beans; (4) higher ratings for acidity and body; and (5) absence of off-flavors.

Presence of mycorrhizal associations can maintain plant diversity in agroecosystems (Sieverding, 1991) and natural forests in the tropics (Husband et al., 2002). The positive effects of mycorrhizal fungi on plant nutrition, health, and soil stability have valuable agro-biotechnological importance for low-input agriculture in developing tropical countries with subsistence agriculture (Douds et al., 2000). Several studies (Habte and Bittenbender, 1999; Colozzi and Cardoso, 2000) have revealed the occurrence of AM fungal propagules in coffee soils. The importance of mycorrhiza to coffee has been repeatedly reported (Vaast et al., 1998). These benefits include enhanced growth and increased P and Zn uptake of young coffee seedlings in nursery conditions (Lopes et al., 1985), enhanced tolerance to nematodes (Vaast et al., 1998) and increased survival of coffee plants after field transplanting as reviewed by Vaast and Zasoski (1992).

To date, information regarding density and identification of dominant coffee shade tree species in Bonga natural coffee forest is extremely scanty. Moreover, arbuscular mycorrhizal fungi spore density is not evaluated in any of the natural coffee forests in the country and there is a strong need to generate relevant data on such important components of the ecosystem. Thus, this study was initiated with the following major objectives:

a. To systematically identify and determine the densities of dominant coffee shade tree species in Bonga natural coffee forest.
b. To evaluate the spore densities of mycorrhizal fungi in the rooting zone of coffee plants under different shade trees and to assign these spores to their respective fungal genera based on morphological parameters.
c. To relate the densities of coffee plants to the direct/indirect influence (shade, edaphic factors) of dominant shade trees as well as the abundance and distribution of AMF.

2. Materials and methods

2.1. The study site

Bonga natural coffee forest is situated 7 km away from Bonga town and 445 km south of the capital, Addis Ababa, Ethiopia. The study area is located at $07°19'$N and $036°14'$E. The altitudinal range in the study area is from 1750 to 2000 masl. The yearly average annual rainfall in southwestern part of Ethiopia is about 1600 mm. The mean annual temperatures of this region range from 15 to 25 °C with the mean daily temperature minima and maxima ranging from 9.7 to 16.3 and 20 to 30.4 °C, respectively. Currently, due attention has been given to this forest as a natural coffee forest and forest genetic resources conservation site (Alemu A., pers. comm., 2003).

2.2. Vegetation sampling and environmental parameters

A reconnaissance survey was made across the Bonga natural coffee forest during the main rainy season in June–July 2003. Homogenous stands were visually checked and selected on the basis of abundance and availability of coffee plants and their respective shade tree species. To assess the composition of different shade tree species, a total of 10 quadrates, measuring 20 m × 20 m were laid down at an interval of half a kilometer in between all the quadrates. In each plot, all dominant coffee shade trees were quantified and taxonomically identified. Coffee plants (*C. arabica* L.) were quantified in all sample plots. The height of each shade tree species was measured and recorded using clinometers. The identification and nomenclature of coffee shade tree species follow Hedberg and Edwards (1989), Edwards et al. (1995) and Hedberg et al. (2003).

Altitude was recorded using an ''Everest altimeter''. Light penetration was measured at four directions under the canopy of each considered shade tree species, 2–3 m away from stem base using a light meter (LI 250 Light meter, LI-COR, USA). The measurements were taken between 11:30 and 12:00 h on sunny days with clear sky in July 2003. Thus, recorded values were the mean of four measurements and the shade conditions

were ranked conveniently as follows. Full/complete shade was characterized by little light penetration (1–10%) and good shade by sufficient radiation (11–70%). For partial shade, the situation was ranked as semi-shade (71–85%).

2.3. Soil sampling

Within the 10 major plots, seventeen 10 m × 10 m (100 m^2) subplots were formed around different dominant coffee shade tree species. These and one 10 m × 10 m in an open area with unshaded coffee plants were used for soil sample collections. The following woody tree parameters were chosen as criteria for selecting shade trees of interest. Within each major plot (quadrate), dominant trees of different species with a diameter at breast height of 1.5 m and above, a height exceeding 10 m and a large canopy were included. Soil samples for AMF spore counts and soil chemical analyses were randomly collected at a depth of 0–15 cm from the rooting area of coffee plants of 5–10 adult/mature coffee stems which were growing under a shade tree canopy and in an open area (unshaded ones) using a soil auger of 5 cm diameter and mixed to obtain representative samples for each considered plot. The composite samples (triplicate for AMF spore counts and one for soil chemical analyses) were collected intentionally from the immediate vicinity of coffee roots so as to be particularly relevant for the coffee plant environment. In all cases, about 1 kg of soil was collected in polyethylene bags and brought to the laboratory within 2 days. Thereafter, the samples were stored at 4 °C until analysis. During sample collection, close observations were made regarding the understorey plants, degree of litter accumulation and general conditions of the coffee plants.

2.4. Soil chemical analysis

Some soil chemical parameters like total N, available P and soil organic carbon were measured at National Soil Laboratory, Ethiopian agricultural research organization (EARO), Addis Ababa, Ethiopia following standard procedures. Available P was extracted and P concentration was evaluated color-imetrically according to Olsen and Sommers (1982).

2.5. Arbuscular mycorrhizal fungi (AMF) spore extraction and enumeration

Spore isolation was carried out following techniques used by Brundrett et al. (1996). Spores were extracted from 50 g sub-samples of soil by wet sieving method in a nest of three soil sieves with different mesh sizes (i.e., 425, 106 and 45 μm) and sucrose density centrifugation. The centrifuged samples were observed under a stereomicroscope at 40× magnification for quantification and characterization. Additionally, a compound microscope was used to identify the quantified spores to their respective genera. The identification was based on spore size, color, surface ornamentation, wall structure as well as presence and absence of subtending hyphae with reference to the descriptions provided by INVAM (2004) and following descriptions given by Brundrett et al. (1996).

2.6. Statistical analysis

To measure diversity index of the dominant coffee shade tree species in Bonga natural coffee forest, the Shannon–Weaver index was employed. Pearson correlation analysis was used to explore the relationship among some soil parameters like available P, soil organic C and total N. ANOVA was used to test differences between the numbers of spores at each sampling plot using SPSS version 10 and Tukey's HSD post hoc test to test for mean separation ($p < 0.05$).

3. Results

3.1. Vegetation structure

The Bonga natural coffee forest was densely populated by different shade plant species and had a complex vegetation structure with seven height strata. The lower stratum (<2 m) contained many coffee seedlings and saplings. *Desmodium* and sometimes the seedlings of *Dracaena* species (data not shown) also occurred abundantly as major understorey plants. In sampled plots, whenever there was a dense population of *Desmodium* species around coffee plants, the aggressive understorey weeds were either totally absent or rarely encountered (data not shown). The second shrubby stratum consisted of coffee plants with a height of 3–6 m. The third (up to 10 m), fourth (10–15 m) and fifth (20–25 m) strata corresponded to large trees. These strata included mainly *Bersama abyssinica*, *Phoenix reclinata*, *Ehretia cymosa*, *Pittosporum viridiflorum*, *Croton macrostachyus*, *Ficus vasta*, *Cordia africana*, *Polyscias fulva*, *Millettia ferruginea*, *Prunus africana*, *Schefflera abyssinica*, and *Sapium ellipticum*. The top

Table 1
Dominant coffee shade tree species in Bonga natural coffee forest

| Scientific name and authority	Family name	Count[a]	%
Millettia ferruginea (Hochst.) Baker	Fabaceae	23	22.3
Olea welwitshii Friis & P.S. Green	Oleaceae	16	15.5
Schefflera abyssinica (Hochst.ex A.Rich.) Harms	Araliaceae	12	11.7
Phoenix reclinata Jacq.	Palmae	11	10.7
Prunus africana (Hook.f.) Kalkam	Rosaceae	10	9.7
Dracaena steudneri Schw.ex Engl.	Dracaenaceae	8	7.8
Croton macrostachyus Del.	Euphorbiaceae	4	3.9
Albizia gummifera (Gmel) C.A.Sm	Fabaceae	2	1.9
Cordia africana Lam.	Boraginaceae	2	1.9
Ficus vasta Forssk.	Moraceae	2	1.9
Pavetta oliveriana Hiern	Rubiaceae	2	1.9
Pouteria adolfi-friederici (Engl.) Baehni.	Sapotaceae	2	1.9
Sapium ellipticum (Krauss) Pax	Euphorbiaceae	2	1.9
Vepris dainellii (Pich.-Serm.) Kokwaro	Rutaceae	2	1.9
Bersama abyssinica Fresen.	Melianthaceae	1	1.0
Ehretia abyssinica R.Br.ex Fresen	Boraginaceae	1	1.0
Pittosporum viridiflorum Sims	Pittosporaceae	1	1.0
Polyscias fulva (Hiern) Harms	Araliaceae	1	1.0
Teclea nobilis Del.	Rutaceae	1	1.0
Total		103	100

[a] Total count in 10 quadrates.
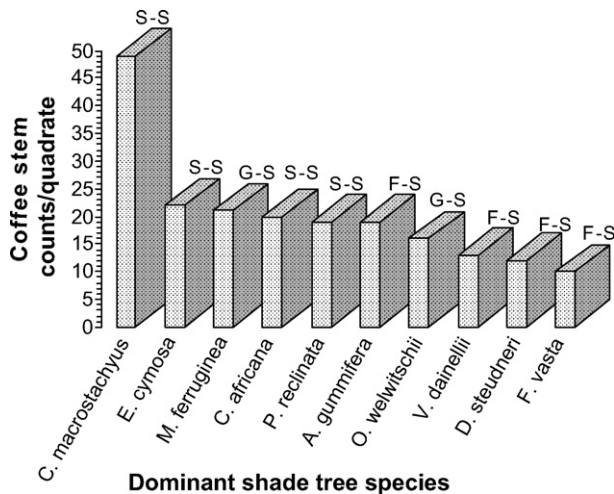
Fig. 1. The influence of the degree of shading on density of coffee plants in Bonga natural coffee forest. S-S: semi-shade, G-S: good shade and F-S: full shade.

Table 2
Species richness and Shannon–Weaver diversity index of dominant shade tree species in Bonga natural coffee forest

Quadrate #	Species richness[a]	H′
1	4	1.09
2	5	1.30
3	5	1.39
4	6	1.68
5	4	1.28
6	4	1.33
7	9	2.00
8	7	1.76
9	4	1.15
10	5	1.55

H′ = Shannon–Weaver diversity index.
[a] Species richness refers only to the number of dominant shade tree species in sampled plots based on the criteria set in Section 2.

strata (i.e., sixth and seventh layers), which respectively range from 25 to 30 and 30 to 35 m high were composed of emergent trees (*Albizia gummifera*, *Olea welwitschii* and *Pouteria adolfi-friederici*) representing the upper canopy of the coffee stand. The mean shade tree height in the study area was 19.7 m.

The density and percentage occurrence of each dominant coffee shade tree species encountered in all quadrates are presented in Table 1. The degrees of shading and coffee counts had an inverse relationship. Whenever the condition was either good shade or semi-shade, higher coffee counts were recorded as compared to the full shade situation except in quadrate 4 under the canopy of *A. gummifera* (Fig. 1). However, shade conditions (% light) and AMF spore counts were not related ($R^2 = 0.06$, $p = 0.34$). Coffee stem counts varied between 10 and 49 per quadrate corresponding to an average of 503 stems/

ha. The highest stem count was made under the canopy of *C. macrostachyus*.

A total of 103 individuals of shade trees, with a corresponding density of 258 individuals/ha, were encountered in all the plots (Table 1). There were 19 species belonging to 14 families. The Shannon–Weaver diversity index ranged from 1.09 to 2.00 (Table 2). The highest and lowest diversity index was recorded in quadrates 7 and 1, respectively. The coffee shade trees were dominated by *M. ferruginea* followed by *Olea welwitschii*, *S. abyssinica*, *P. reclinata*, *P. africana*, *Dracaena steudneri* in this order (Table 1). The common coffee forest shade tree species *Cordia*, *Croton* and *Albizia* were rarely encountered (<5% of shade tree species) at the study site.

3.2. Soil chemical analyses

Soil chemical analytic data are presented in Table 3. The relatively highest value of total nitrogen, organic carbon and

Table 3
Some chemical characteristics of the top 0–15 cm soil of the shaded coffee system used in the study from Bonga natural coffee forest

Quadrate #	Shade tree species/site of sampling	Organic C			Available P	
		Total N (%)	%	C/N ratio	mg kg^{-1}	P/N ratio
1	*M. ferruginea*	0.29	3.99	14	2.36	0.08
2	*E. cymosa*	0.31	5.11	16	3.82	0.12
3	*A. gummifera*	0.49	5.85	12	3.48	0.07
	O. welwitschii	0.69	5.99	9	3.84	0.06
4	*S. abyssinica*	0.60	4.49	7	1.10	0.02
5	*C. macrostachyus*	0.32	3.59	11	0.68	0.02
6	*F. vasta*	0.36	3.59	10	0.68	0.02
	P. adolfi-friderici	0.30	3.43	11	0.96	0.03
7	*P. reclinata*	0.25	2.20	9	1.20	0.05
8	*D. steudneri*	0.38	3.59	9	1.72	0.05
	P. africana	0.36	3.51	10	1.18	0.03
9	*V. dainellii*	0.47	5.19	11	4.12	0.09
10	*C. africana*	0.35	3.75	11	4.32	0.12
	Open area	0.32	3.03	9	1.64	0.05

The reported values were from composite samples (5–10 sub-samples).

Table 4
Mean spore densities of AMF in soil samples, shade conditions and *Desmodium* abundance in Bonga natural coffee forest

Quadrate #	Coffee shade tree species/site of sampling	Shade condition	*Desmodium* population	Spore counts (100 g^{-1} soil)[a]
1	*M. ferruginea*	Good shade (49.7%)	+	67.3 a ± 1.5
2	*E. cymosa*	Semi-shade (77.0%)	+++	54.3 ab ± 6.0
	M. ferruginea	Full shade (8.2%)	+	18.3 defg ± 15.6
3	*O. welwitschii*	Good shade (59.3%)	+	9.7 fg ± 3.2
	A. gummifera	Good shade (51.4%)	+	41.7 bc ± 7.6
4	*A. gummifera*	Full shade (5.6%)	n/a	35.7 bcd ± 9.3
	S. abyssinica	Full shade (0.6%)	n/a	8.7 fg ± 3.1
5	*C. macrostachyus*	Semi-shade (76.4%)	+	4.3 g ± 2.3
6	*F. vasta*	Full shade (1.4%)	++	8.3 fg ± 3.8
	P. adolfi-friderici	Good shade (43.7%)	+++	5.7 g ± 2.5
7	*P. reclinata*	Semi-shade (81.4%)	+	21.0 cdefg ± 6.6
	M. ferruginea	Semi-shade (76.0%)	+++	33.3 bcde ± 5.5
8	*D. steudneri*	Full shade (9.9%)	n/a	8.3 fg ± 4.0
	P. africana	Good shade (63.3%)	n/a	9.3 fg ± 5.5
9	*V. dainellii*	Full shade (8.9%)	+	27.7 cdef ± 2.5
	S. abyssinica	Full shade (3.5%)	+	4.3 g ± 1.5
10	*C. africana*	Semi-shade (79.9%)	+	31.3 cde ± 15.8
	Open area	No shade (99.9%)	n/a	13.3 efg ± 2.1

+++ = densely populated *Desmodium* (≥20 plants/m^2 near sampled coffee plant). ++ = moderately populated *Desmodium* (10–19 plants/m^2 near sampled coffee plant). + = very few *Desmodium* (1–9 plants/m^2 near sampled coffee plant). n/a = no *Desmodium* around sampled coffee plant.

[a] Mean values (of triplicates) followed by the same letter(s) indicate no significant difference ($p > 0.05$) at 95% confidence interval.

available P were detected in soil samples collected from the root zone of coffee plants under the canopy of *O. welwitschii* and *C. africana*, respectively, but the lowest organic carbon and total nitrogen was measured under *P. reclinata* (Table 3). Available P was lowest under *C. macrostachyus* and *F. vasta*. Organic C and total N were highly correlated ($R^2 = 0.55$; $p < 0.001$) and available P was related to C ($R^2 = 0.48$; $p < 0.01$) but not significantly to N.

3.3. Arbuscular mycorrhizal fungi spore counts

Arbuscular mycorrhizal fungi spores were recorded from all soil samples. The spore counts were found to be quite variable. Average spore densities ranged from 4 to 67 spores 100 g^{-1} of dry soil (Table 4). There were significant differences ($n = 18$, $F = 21.42$, $p < 0.001$; Table 4) in number of spores among soil samples. The highest arbuscular mycorrhizal fungi spore counts were recorded under the canopies of *M. ferruginea* in quadrate 1 and *E. cymosa* (quadrate 2). Under *A. gummifera* (quadrates 3 and 4), *C. africana* (quadrate 10), *V. dainellii* (quadrate 9) and *P. reclinata* (quadrate 7) counts were also greater than 20 spores 100 g^{-1} of dry soil (Table 4). Spore densities of AMF recorded in the open area (quadrate 10) was relatively low (<15 spores 100 g^{-1} dry soil) as compared to under most tree species (Table 4). Similarly, low densities (<10 spores 100 g^{-1} dry soil) of AMF spores were recorded from soil samples collected under the canopy of the coffee shade trees *C. macrostachyus* (quadrate 5), *S. abyssinica* (quadrates 4 and 9), and *P. adolfi-fredirici* (quadrate 6) (Table 4). The spore densities of coffee plant root zones were significantly ($p < 0.05$) higher under

leguminous shade trees than under non-leguminous ones (39 ± 16 and 16 ± 5, respectively). The understorey cover of the legume (*Desmodium* species) was not correlated with spore counts but rather with overall light conditions, i.e., cover under semi-shade was approximately double (++) that under good or full shade (+) (Table 4). AMF spore counts were positively related to available P ($n = 14$, $R^2 = 0.31$, $p = 0.04$; Fig. 2) and C/N ratio ($R^2 = 0.64$, $p = 0.001$) but not related to organic carbon ($R^2 = 0.1$, $p = 0.27$) or total nitrogen ($R^2 = 0.05$, $p = 0.45$).

The identified genera of arbuscular mycorrhizal fungal species are presented in Table 5. One or more genera were encountered in the root zones of coffee plants (shaded and unshaded). A total of five genera of AMF were recovered from
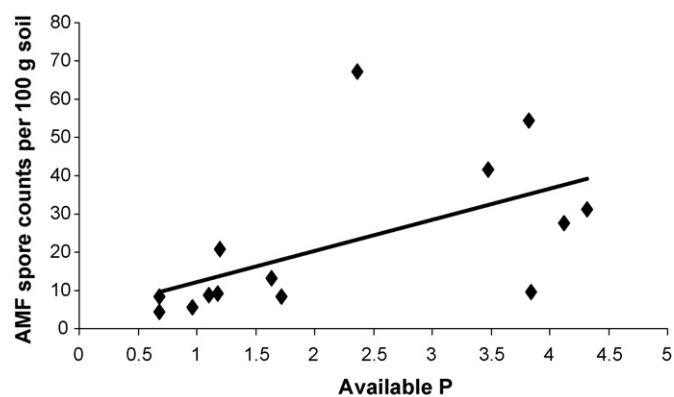

Fig. 2. Spore density of AMF and available P (mg kg^{-1}) in soil samples collected from Bonga natural coffee forest. For the linear regression shown, $R^2 = 0.32$, $p = 0.002$.

Table 5
Frequency distribution of arbuscular mycorrhizal fungal genera in soil samples and coffee counts (stems/quadrate) in Bonga natural coffee forest

Quadrate #	Identified AMF genera					Shade tree species/site	Coffee counts
	AC	EN	GL	GS	SC		
1	X		X	X	X	*M. ferruginea*	21
2	X	X	X	X	X	*E. cymosa*	22
	X	X	X			*M. ferruginea*	
3	X			X		*O. welwitschii*	16
	X		X	X		*A. gummifera*	
4	X			X		*A. gummifera*	19
	X	X				*S. abyssinica*	
5			X			*C. macrostachyus*	49
6	X	X		X	X	*F. vasta*	10
		X		X		*P. adolfi-friderici*	
7		X	X	X		*P. reclinata*	19
		X	X	X		*M. ferruginea*	
8			X	X		*D. steudneri*	12
	X		X	X		*P. africana*	
9	X		X	X		*V. dainellii*	13
			X	X		*S. abyssinica*	
10	X	X	X			*C. africana*	20
	X		X			Open area	

AC = *Acaulospora*, EN = *Entrophospora*, GL = *Glomus*, GS = *Gigaspora* and SC = *Scutellospora*.

Bonga natural coffee forest. The highest and least diversity was recorded under *E. cymosa* (five genera) and *C. macrostachyus* (one genus), respectively (Table 5). *Glomus* was constantly recovered (78% or 14 out of 18 soil samples; Table 5). Thus, the AM fungal floras of the soil samples were dominated by this genus followed by *Gigaspora* (72%; 13/18), *Acaulospora* (67%; 12/18) and *Entrophospora* (39%; 7/18) (Table 5). The occurrence of *Scutellospora* was quite low, i.e., it was recorded in only 3 of the 18 soil samples (17%). The number of coffee plants increased significantly ($R^2 = 0.83$, $p = 0.002$; Fig. 3) with increasing mean spore counts/quadrate (Table 4) considering those quadrates where at least duplicate soil samples were analyzed (quadrates 1 and 5 excluded). No such relation was found with distribution of AMF genera (Table 5).
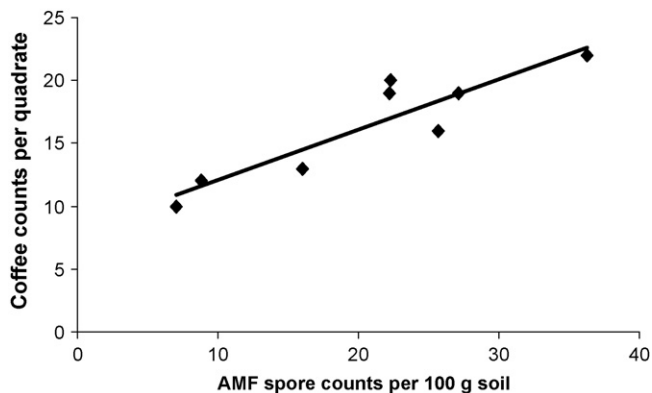

Fig. 3. Coffee and spore counts regression correlation analysis. For the linear regression shown, $R^2 = 0.83$, $p = 0.04$.

4. Discussion

4.1. Vegetation variables

The findings of the present study showed a forest system, composed of seven height strata. The universally reported shade tree species in Ethiopian natural coffee forests (FAO, 1968; Teketay and Tegineh, 1991) like *C. africana*, *C. macrostachyus*, *F. vasta*, *M. ferruginea* and *A. gummifera*, held the fourth, fifth and sixth strata. Dominant coffee shade tree species in Bonga natural coffee forest had a moderate number of total stems (overall mean 258 stems/ha). However, the corresponding value reported from Yayu natural coffee forest was almost 10-fold greater (Taye, 2001). This could be attributed to differences in counting of shade trees but more likely to differences in human activities in both forests. In the latter, disturbance was relatively low and the shade tree species were also more diverse. In contrast, selective cutting, overgrazing and wood collection are frequently found at present in Bonga natural coffee forest (Ersado, 2001; Stellmacher, 2005).

The Shannon–Weaver index revealed moderate levels of diversity among the sampled plots that ranged from 1.08 to 2.00 (Table 2). In general, the measured values seem low for an intact natural forest indicating that Bonga coffee forest has been disturbed (Ersado, 2001; Stellmacher, 2005). The main explanation for this observation is, however, the selective choice of study objects in terms of dominant shade tree species, i.e., not all vegetation in the sample plots was included. Nevertheless, the recorded values were not extremely different

from the figure (2.92) reported by Senbeta and Teketay (2003) for overall plant diversity index determined at one of the dry afromontane forests, Kiphee nature reserve in southern part of Ethiopia. Thus the overall picture is still that there is both heterogeneity and structural complexity of Bonga natural coffee forest in particular with respect to shade tree species.

During the reconnaissance survey we observed an extremely large number of coffee seedlings and saplings in the study area. Coffee stem count made under the canopy of *C. macrostachyus* (quadrate 5) was much higher than in the other quadrates. The main reason for encountering higher number of coffee stems at this particular sampling plot could be transplantation of coffee seedlings from full shade conditions within the forest by the owner of the coffee forest because the shade condition under *C. macrostachyus* was semi-shade. Coffee growers in the area prefer sites with about 50% light penetration to increase coffee growth and yield (Muleta et al. unpubl.).

The high density (66% of total shade trees, 503 stems/ha) of *C. arabica* recorded in the Bonga natural coffee forest is far greater than what has been reported elsewhere (Taye, 2001) and indicates the coffee forests potential for conservation of the wild coffee gene pool and for promotion of the globally renewed interest in production of organic coffee. Aga et al. (2003) have shown the presence of genetic variability among the Ethiopian forest coffee populations and recommended the suitability of southwestern forests for *in situ* conservation of wild coffee plants. The authors further noted that coffee improvement requires natural populations that are likely to be the source of new resistance genes to both biological and environmental stresses.

It appears that the degree of shading had effect on counts of coffee plants. In most cases the counts were lower under full shade than semi- and good shade conditions. The impact of the degree of shading on bean quality and yield has been reported (Faminow and Rodriguez, 2001). They found coffee under 30–50% shade produces less than unshaded coffee, but require less investment in labor and materials and (dubiously) produce higher quality coffee. Similarly, a review by Beer et al. (1998) revealed that the upper limit of acceptable shade for coffee (a C3 plant) is between 40 and 70%. In the present investigation, somewhat, better coffee counts were obtained under semi- and good shade (49.7–81.4%) than under full shade (Fig. 1). Photosynthetic rates of coffee are at a maximum at intermediate shade levels in many of the climatic conditions found in the tropics (see Beer et al., 1998). Additionally, in some cases, our results showed that relatively higher AMF spore counts (above 30 spores 100 g^{-1} dry soil) in coffee root zones were recorded under good and semi-shade conditions (Table 4; quadrates 1, 2, 3, 7 and 10).

In terms of shade tree species composition, Bonga natural coffee forest is by far more heterogeneous than what has been reported from southern (Teklay and Malmer, 2004), south-western (FAO, 1968) and eastern (Teketay and Tegineh, 1991) parts of the country. In the present study, *M. ferruginea*, *O. welwitschii*, *S. abyssinica*, *P. reclinata* and *P. africana* were encountered as dominant shade tree species with percentage occurrence of 22.3, 15.3, 11.7, 10.7 and 9.8%, respectively.

Except *M. ferruginea*, these trees are normally not reported as coffee shade tree species (FAO, 1968; Teketay and Tegineh, 1991). Nevertheless, our results mostly agree with what has been investigated at Yayu national forest priority area with regard to shade tree composition (Taye, 2001).

M. ferruginea, *A. gummifera*, *C. africana*, *C. macrostachyus* and *D. steudneri* comprised 40% of the total number of plant species. This value is far less than reported for other Ethiopian coffee forests (Teketay and Tegineh, 1991). At Bonga natural coffee forest, leguminous trees such as *M. ferruginea* and *A. gummifera* represent 24% of the dominant shade tree species. Investigations made at Yayu national forest priority area revealed higher percentage (40%) of leguminous trees (Taye, 2001).

The highest stem counts (57 stems/ha) of *M. ferruginea* along with another important legume tree, *A. gummifera* (5 stems/ha) and the frequent abundance of the understorey vegetation containing a *Desmodium* species at the study area, however, emphasize the important roles of leguminous plants. *Desmodium* was encountered in 13 of the 18 sampling plots with varying degrees of abundance (Table 4). We also observed frequent nodulation on roots of *Desmodium*. Presumably, such understorey leguminous plants may thus supply some fixed nitrogen to larger non-nitrogen-fixing nearby plants including coffee shrubs. Snoeck et al. (2000) for instance, demonstrated that nearly 30% of the nitrogen fixed by legumes like *Desmodium* and *Leucaena* was transferred to associated coffee trees. Apart from nitrogen fixation, *Desmodium* species play pivotal roles in suppression of parasitic nematodes (Herrera and Marban-Mendoza, 1999) and control of weeds (Bradshaw and Lanini, 1995) in coffee plantations. The visual observations in Bonga natural coffee forest also revealed that sampled plots with a dense population of *Desmodium* showed absence or poor growth of aggressive understorey weeds.

4.2. AMF populations and soil features

Under the canopy of *O. welwitschii* the values for both total organic C and total N were higher than under other shade trees (Table 3). During soil sampling we could observe an excessive accumulation of litter under the canopy of this particular plant. We have no evident explanation for this but should remark that in the same plot, *A. gummifera* was another dominant shade tree showing high amounts of AM spore counts, total soil carbon and nitrogen. Direct/indirect transfer of nutrients between these trees through joint mycorrhizal hyphae could be hypothesized because fixed nitrogen can be transferred to non-nitrogen-fixing plants via AM fungal hyphae (Mårtensson et al., 1998).

Available P was slightly higher under the canopy of *C. africana* than under the other shade tree species (Table 3). Teklay and Malmer (2004) have demonstrated the presence of high P particularly in leaves of *C. africana* during their decomposition study at Wondo Genet, Ethiopia and concluded that the higher the initial P in leaves, the higher the amount of P was released which could explain the relatively higher amount of P under this particular shade tree species.

The lowest organic C and total N (Table 3) were measured under the canopy of *P. reclinata*. Possible reasons could be thin litter accumulation and/or resistance to decomposition (fibrous leaves). This plant does not have a large canopy and does not regularly drop its leaves. As a result farmers avoid this shade tree plant in their forests/farms.

In general, our results on soil chemical parameters (Table 3) were more or less similar to studies made in Mexico in Inga coffee and rustic-shade coffee systems (Romero-Alvarado et al., 2002). Their findings of 5.2–5.3% organic matter, 0.2–0.3% total nitrogen, and P (2.4–4.8 mg kg^{-1}) are within our ranges (Table 3). The soil ratios of P/N (Table 3) from 0.02 to 0.12 demonstrate that the Bonga ecosystem is clearly P-limited, since Ågren (2004) has shown that corresponding N/P values (8–50) are limiting for plant growth. The limited soil P and low pH values (4.3–6.1; T. Chanie unpubl.) in the study area imply a high dependency of coffee plants on mycorrhizal symbioses (Habte and Bittenbender, 1999).

All soil samples yielded AMF spores but the counts showed considerable variations. Spore densities recorded in the present study seem low as compared to other studies. For example, higher counts of AMF spores ranging from 55 to 1908 spores 100 g^{-1} of dry soil, with an average of 476 has been reported from the tropical rain forest of Xishuangbanna, southwest China (Zhao et al., 2001). Other studies in the tropics have revealed total numbers of spores which ranged from 100 to 10,000 spores 100 g^{-1} dry soil (Picone, 2000 and references therein). In Brazil, however, in soils under agroforestry and monocultural coffee systems, Cardoso et al. (2003) recorded counts ranging from 60 to 150 spores 100^{-1} g dry soil, i.e., more or less comparable to our results. By and large, enumeration of low spore densities could be attributed to several factors:

(1) It has been reported that under high nutrient contents particularly P, mycorrhizal development is poor (Sieverding, 1991). In such a case colonization rate and number of spores increased with decreased P level. Values recorded for available P and AMF spore counts under some shade tree species in the present investigation had inverse relationship supporting the author's observations but overall, available P and AMF spore counts were positively correlated (Fig. 2). More importantly, the recorded values of soil available P in the current study were not high enough to cause suppression of mycorrhizal development since the soil P levels found are characteristic for poor soils (Romero-Alvarado et al., 2002 and references therein).

(2) The spore counts in our study may be truly characteristic for the coffee plants only and not so much for the nearby shade trees. A review by Osorio et al. (2002) showed that coffee grows well in soils with low P availability and usually does not respond to P fertilization, which is perhaps due to the presence of effective rather than numerous AMF spores in the root environment. Both *Glomus* and other AMF have been reported effective for coffee plants (Osorio et al., 2002). Apart from *Glomus*, other genera of AM fungi found in coffee root zone samples collected from the Bonga coffee forest in decreasing order of frequency embodied *Gigaspora*, *Acaulospora*, *Entrophospora* and *Scutellospora*. Colozzi and Cardoso (2000) noted that *Acaulospora* spp. occurred more often in coffee plant rhizospheres. Interestingly, in the present investigation, only *Acaulospora* and/or *Glomus* occurred in all coffee rhizospheres sampled. Bhattacharya and Bagyaraj (2002) demonstrated that, of eight isolates of AMF, *Gigaspora margarita* and *Acaulospora laevis* were the best AM symbionts for arabica coffee. The only indication of shade tree influence on AMF genera distribution in the coffee rhizospheres in our study was that *Acaulospora* occurred more frequently (80%) under leguminous than under other shade trees (58%). In the open area without direct influence of any shade trees only *Glomus* and *Acaulospora* were found in the coffee plant rhizosphere.

(3) Our sampling was made during the heavy rainy season, which may have suppressed the number of spores due to the excess moisture that could either pose decomposition of spores or initiate spore germination. It has been claimed that intra- and extrametrical mycelium increases during the rainy season, because spore germination is favored. This in turn increases mycorrhizal colonization and decreases spore abundance (Ragupathy and Mahadevan, 1993). Evidently, Guadarrama and Álvaarez-Sánchez (1999) have demonstrated that the highest numbers of species and spores were observed during the dry season, with a marked decrease during the rainy season. Furthermore, multiple lines of evidences reveal that AM fungi differ in their seasonality with regard to sporulation (Schultz et al., 1999) and in Bonga and Yayu natural coffee forests high spore densities were recorded during early dry season (Muleta et al., unpubl.).

(4) Earthworms which may feed on mycorrhizal fungi spores were frequently encountered during our soil sampling processes (data not shown). The negative influence of the soil fauna such as earthworms (Brown, 1995) and arthropods (Picone, 2000) on AMF populations has been reviewed. Secondly, Mangan and Adler (2000) have demonstrated the consumption of arbuscular mycorrhizal fungi by terrestrial and arboreal small mammals.

Altogether, considering the densities of AMF spores (4–67 spores 100 g^{-1} soil) encountered under the canopies of diverse shade tree species, the numbers of spores recorded in Bonga natural coffee forest could not be assumed low for initiation of colonization of coffee plants since the later investigations in Bonga and Yayu natural coffee forests have demonstrated the presence of fair levels of mycorrhizal colonization in coffee seedlings ranging from 3 to 34% of root length (Muleta et al. unpubl.).

The highest spore counts were observed under *M. ferruginea* and *E. cymosa* (near *M. ferruginea*). In addition, the counts under the canopy of *A. gummifera* (quadrates 3 and 4; Table 4) were neither low and overall values for counts under leguminous shade trees were higher than under non-leguminous shade trees implying some influences also from the nearby

shade trees. Similar observations have been reported elsewhere under canopies of legume plants (He et al., 2004) and Colozzi and Cardoso (2000) have demonstrated that legume intercropping cultivation increased spores concentration of AMF in the soil. Furthermore, mycorrhizal dependencies of leguminous plants such as *Acacia* and *Albizia* have been well demonstrated earlier (Osonubi et al., 1991; Habte and Musoko, 1994; Ghosh and Verma, 2006).

Under some shade tree species and in the open area, the densities of AMF spores were very low as compared to others (Table 4). A wide range of environmental factors can influence AMF spore production (Cardoso et al., 2003). Our data do not yet allow an evaluation of how such factors could be correlated to actual spore counts.

Five different genera of arbuscular mycorrhizal fungi were recovered from soil samples obtained from Bonga natural coffee forest. The results revealed the presence of a fair diversity among populations of AM fungi because several soil samples were composed of sometimes up to four or five different genera in varying proportions. The highest and least diversity was recorded under *E. cymosa* and *C. macrostachyus*, respectively (Table 5). The observation of less diversity of AMF genera under *C. macrostachyus* coincided with low spore counts.

The presence of *Glomus* in most soil samples is in line with reports from dry afromontane forests of Ethiopia (Wubet et al., 2004), tropical rain forest in Mexico (Guadarrama and Álvaarez-Sánchez, 1999), tropical forest and pasture (Picone, 2000), and the tropical rain forest of Xishuangbanna, China (Zhao et al., 2001). Interestingly, *Glomus* species were also the most frequently encountered fungi in the fecal samples collected from terrestrial and arboreal small mammals in a Panamian cloud forest with 87% frequency of occurrence in the samples (Mangan and Adler, 2000). Indeed, this has a positive consequence in dispersal of AMF in a given habitat/ecosystem. Ninety four percent of the sampled coffee plants which were under the influence of different shade tree species revealed association with two or more AMF genera (Table 5).

5. Conclusion

In natural coffee forests, the presence of different trees species which are used to shade the coffee plants may possibly also help to promote diverse populations of AMF because of higher biodiversity. The investigated forest presented diversity with respect to both shade tree species and indigenous AMF populations. Coffee rhizospheres under leguminous trees harbored higher numbers of AMF spores. AMF spore and coffee stem counts were strongly positively correlated. The Bonga natural coffee forest, therefore, is an ideal focal forest for *in situ* coffee genetic resources and biodiversity conservation as well as for the promotion of organically grown coffee crops in order to supply specialty coffee.

Acknowledgements

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