

## Research Article

# Arbuscular mycorrhizal status of *Cordia africana* and *Millettia ferruginea* trees in traditional agroforestry land use systems of Sidama Regional State, southern Ethiopia

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### Article Info

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### Abstract

Arbuscular mycorrhizal fungi (AMF) enhance plant growth and productivity through nutrient acquisition, organic matter decomposition, improved soil health, increased resilience and stress tolerance. A higher percentage of root colonisation by AMF indicates a stronger symbiotic relationship and potentially greater benefits to the plant. A high abundance of AMF spores reflects their overall potential to colonise plant roots. The aim of this study was to determine the number of spores and root colonisation potential of AMF in the soil beneath *Cordia* and *Millettia* trees grown in enset and maize fields, and to estimate the extent of AMF colonisation of *Millettia* and maize seedling roots grown on these field soils. Six tree-crop combinations, making 36 plots, and two open maize plots in the traditional agroforestry systems of Sidama. At the field level, the study was conducted under the canopy of *Cordia africana* and *Millettia ferruginea* trees in different plots. To assess the relationship, root colonisation and spore counts of field soils sampled from different *Cordia* and *Millettia* trees in enset and maize plots, maize and *Millettia* plants were grown in the nursery. The tree-crop combinations induced higher spore counts and higher colonisation levels than in the open maize field. Significantly, lower numbers of spores were observed in soils under *Cordia* and *Millettia* trees grown in maize plots than under trees grown in enset coffee and enset plots. The order of colonised roots was: tree enset coffee < tree enset < tree maize for *Cordia* trees and tree enset < tree enset coffee < tree maize for *Millettia* trees. At the nursery level, a significantly higher level of root colonisation was observed for maize plants grown on soil from under tree-enset-coffee and enset plots than for those grown on soil from tree-maize and open maize plots. The percentage of AM colonised maize roots was significantly positively correlated with spore counts for field soils. Both maize and *Millettia* plants with high root colonisation achieved higher fresh weight. The presence of spore counts and root colonisation in the plants studied suggests a contribution of native AMF in improving plant growth and productivity.

**Keywords:** Number of spores, Open-maize plot, Root colonizations, Tree-enset, Tree-maize

## 1 Introduction

Mycorrhizal association can contribute to agricultural sustainability through improved mineral uptake, especially of low mobile ions (e.g. Sieverding, 1991), and biological N fixation (Ibijbijen et al., 1996). Mycorrhizal association can also improve nutrient cycling and soil structure quality (Hamel, 1996), plant community diversity and plant tolerance to biotic and abiotic stresses (Hamel 1996;

Smith and Read, 1997). Studies of some agroecosystems (Sieverding, 1991) and natural forests in the tropics (Janos, 1996) suggest that the presence of mycorrhizal associations can maintain plant diversity. Mycorrhizal association is also helpful in explaining the relative abundance of plant species in a given habitat (Reader, 1998). In tropical agroecosystems, AMF is the most common mycorrhizal symbiosis (Sieverding, 1991). In managed ecosystems, however, these contributions may be influenced by management practices. The most important management practices influencing AM associa-

tions are the intensity of agricultural, high and low input practices.

High-input farming practices in this context refer to the use of artificial fertilisers, intensive tillage, weed control with herbicides, chemical control of insects and diseases, and monoculture with high-yielding crop varieties, while low-input farming practices refer to mixed cropping, low fertiliser application, mainly of organic origin, hand weeding and no pesticide application (Sieverding, 1991). The effects of high-input farming practices include (i) reduction in the diversity and effectiveness of native mycorrhizal populations (Sieverding, 1991; Schreiner and Bethlenfalvai, 1995); (ii) high rates of P and N fertiliser application, which both reduce the effectiveness and alter the population structure of mycorrhizal fungi (Sieverding, 1991; Scullion et al., 1998); (iii) the use of pesticides, which can adversely affect mycorrhizal associations and communities (Perrin and Plenchette, 1993); (iv) intensive tillage, which reduces the amount of mycelium and adversely affects AM spore density in agricultural soils (Schreiner and Bethlenfalvai, 1995; Kabir et al., 1997; Miller et al., 1995; Gavito and Miller, 1998; Menendez and Scervino, 2001); (v) continuous monocropping, even in the case of perennial crops, which can lead to significant changes in the composition of native mycorrhizal fungal species (Sieverding, 1991). The effects of high-input agricultural practices include: (i) reduction in the diversity and efficacy of native mycorrhizal populations (Schreiner and Bethlenfalvai, 1995); (ii) high rates of P and N fertilization, which both reduce the efficacy and alter the population structure of mycorrhizal fungi (Scullion et al., 1998); (iii) the use of pesticides, which can adversely affect mycorrhizal associations and communities (Perrin and Plenchette, 1993); (v) continuous monocropping, even in the case of perennial crops, which can lead to significant changes in the composition of native mycorrhizal fungal species (Sieverding, 1991). The effects of high-input agricultural practices include: (i) reduction in the diversity and efficacy of native mycorrhizal populations (Schreiner and Bethlenfalvai, 1995); (ii) high rates of P and N fertilization, which both reduce the efficacy and alter the population structure of mycorrhizal fungi (Scullion et al., 1998); (iii) the use of pesticides, which can adversely affect mycorrhizal associations and communities (Perrin and Plenchette, 1993); (iv) intensive tillage, which reduces the amount of mycelium and adversely affects AM spore density in agricultural soils (Schreiner and Bethlenfalvai, 1995; Kabir et al., 1997; Miller et al., 1995; Gavito and Miller, 1998; Menendez and Scervino, 2001); (v) continuous monocropping, even in the case of perennial crops, which can lead to significant changes in the composition of native mycorrhizal fungal species (Sieverding, 1991). As certain crops have a preferential selection for certain AM fungal species and strains, cropping sequence may also influence species composition (Sieverding, 1991).

Low-input systems often involve reduced tillage, increased crop diversity, maintenance of vegetation cover, reduced chemical inputs, and also tend to establish more undisturbed ecosystems in which the mycorrhizal symbiosis can provide some of the ecological functions mentioned above (Sieverding, 1991; Kabir et al., 1997; Hamel, 1996). Such systems also influence the composition of AM fungal communities by increasing diversity and altering the relative abundance of fungal species (Douds et al., 1993) and increasing sporulation (Kurle and Pflieger, 1994) by having a higher infectivity of AMF spores than the high input systems (Sieverding, 1991; Hamel,

1996; Scullion et al., 1998). However, the selection and management of non-host plants can delay or inhibit the infective potential of propagules (Thompson, 1987; Sieverding, 1991). If at least one of the plant components in an intercropping or agroforestry system is mycorrhizal or potentially mycorrhizal, adequate inoculum levels are likely to be maintained (Sieverding, 1991; Michel-Rosales and Valdes, 1996).

The use of AM for the above benefits is not well advanced because inoculum cannot be readily produced in artificial (axenic) culture. The need for large quantities of inoculum may limit artificial inoculation of agricultural crops with AMF in the field (Sieverding, 1991). Therefore, production on roots and their environment in the field is of great importance for AMF. In the case of agroforestry, standing farm trees or tree inoculation at nursery level could help to overcome the need for large amounts of inoculum. However, in order to properly manage the mycorrhizal symbiosis on the farm, it is necessary to know the current status of the agroecosystem at farm, field and plot level.

In the traditional agroforestry land use systems of the Sidama, it is not known how farmers' practices in selecting and managing crop and/or tree components affect native mycorrhizal associations. In these systems, 87 tree species are reported (Zebene Asfaw 2003), of which *Cordia africana* and *Millettia ferruginea* are the second and fourth most abundant tree species, respectively. However, studies on the AM fungal hosting capacity of these two tree species in these traditional agroforestry systems are lacking. Research aimed at understanding the status of mycorrhizal fungi in a Sidama traditional agroforestry system in general and around these two tree species is valuable in determining appropriate management strategies as a background against which inoculation techniques will be developed. For this, it is necessary to understand the host capacity of these agricultural trees and the compatibility of the symbionts.

Some AMF have a wide host range. Therefore, the roots of standing crop trees may harbor inocula that are also of value to crops intercropped with them. Therefore, it was hypothesised that *Cordia africana* and *Millettia ferruginea* trees in the traditional agroforestry land use system of Sidama could promote greater reservoirs of AM inoculum for agricultural crops, with particular reference to maize and *Millettia /Ensete* plants grown on the underlying soils. This study was designed to (i) determine the number of spores of AM fungi in the soil under *Cordia* and *Millettia* trees grown on enset and maize fields, including without trees in maize monoculture; and estimate the extent of colonisation of *Cordia* and *Millettia* trees and maize roots by AMF(ii) assess the potential of field soils from the differently managed plots, under *Cordia* and *Millettia* trees, to cause AMF colonisation of maize and *Millettia*.

## 2 Materials and Methods

### 2.1 Description of the study area

This study was carried out in the Awassa Zuria District of the Sidama Zone in southern Ethiopia. The present report is a part of other work carried out at three sites namely Entaye (Enta), Haranfama (Hara) and Murancho Kutela (Figure 1). For this particular study, two sites, namely Entaye and Murancho Kutela, were randomly selected to collect soil and root samples for both nursery experiments and routine laboratory activities at the Wondo Genet College of Forestry.

### 2.2 Sampling plots within the fields

Sidama farms have about 10 different types of fields (Zebene Asfaw, 2003). For this study, we selected two predominant field types, namely enset and maize fields. A plot was defined as a relatively homogeneous area within a field, characterised by a single species or species mix and common management (Huxley, 1983). Soil samples for AMF spore counts ( $n=36$ ) and root extractions ( $n=72$ ) Table 2. For nursery level growth media, soil was collected from these plots. In the sample plots, the main weeds were first identified by the farmers themselves, then the botanical name was given based on the corresponding local names (Kelecha, 1980) or in the description by Edward et al. (1995).

**Tree and enset-coffee:-** These plots were characterised by multi-storey structure in which *Cordia* or *Millettia* are the upper storey. The age of the sample trees ranges from 24-32 and 17-25 year for *Cordia* and *Millettia*, respectively. Under both tree species at mid storey, a mixture of enset and coffee crop has been grown for the last five years. The ground cover, lower storey, includes Ethiopian kale, local cabbage "Tunaye", and pepper. The major weeds were *Snowdenia polystachya* "merge", *Galinsoga parviflora* "Butissa", *Commelina benghalensis* "Laluntie" *Digitaria abyssinica* "Hiele", *Cyperus spp.* "Gicha", *Agrostis semiverticillata* "serdo". No external input was used. Hand hoeing was carried out twice a year. The topsoil properties are indicated in Table 1.

**Tree and enset:-** These plots are also located in enset fields but without coffee. The main under growth was Ethiopian Kale, "Tuneye" and pepper. At these plots enset has been grown for 5 and 7 years beneath *Cordia* and *Millettia* plots, respectively. No external input is applied. Hand hoeing is carried out twice a year. The recorded weeds were *Amaranthus spp.*, *Snowdenia polystachya*, *Cyperus spp.*, and *Galinsoga parviflora*. Soil properties are indicated in Table 1.

### 2.3 Spore and root sampling

Topsoil samples (0-20 cm) for spore counts and estimation of mycorrhizal colonisation and soil for the nursery experiment were collected at the beginning of the main rainy season. The physicochem-

ical properties of all soil samples are shown in Table 1. To identify the specific plant roots in a composite soil sample is very difficult, but efforts were made to directly collect fine roots of standing plants to learn about specific characteristics, mainly their colour. Based on this fine root colour, we learnt that the maize root colour is white, while that of *Millettia* is cream and that of *Cordia* is very light brown. Using this common approach, we therefore made educated guesses based on these known root characteristics of these plants. For *Cordia* and *Millettia* grown in the top layer of enset mixed with coffee, soil samples were taken under the canopy at three distances in four different directions. Soils from each direction were pooled to obtain a composite sample. Finally, 400 g of the sample was taken separately for estimation of arbuscular mycorrhizal spores and root colonisation. Similarly, soil under *Cordia* and *Millettia* grown on enset coffee and scattered on enset and maize plots was also sampled.

For the nursery experiment, four lines, E-W and S-N, were established in both the enset and maize plots from the trunk to the canopy edge or open field. On each line from the trunk, three sample points were marked at 0.75 m, mid-canopy and canopy edge, and a fourth sample point was marked along the line in the open field. Soil samples of approximately 3 kg were then taken from a randomly selected line. During sampling, every effort was made to avoid disturbing the soil, such as repeatedly mixing by hand, as was done with the spore count samples. This precaution was taken because the process of repeated mixing can lead to loss of the hyphal network. Three replicates of soil samples from each plot were used to start the growth of maize and millet seedlings.

### 2.4 AMF spore estimation

As one of the objectives of the present study was to estimate the number of AMF spores in the soil on different plot types, it was decided to keep the samples separate (Table 2). Spores were isolated from 50 g subsamples of soil. The methods described by Brundrett et al. (1996) were used for spore extraction. The spore suspension was stored in a refrigerator at  $-4^{\circ}\text{C}$  for preservation, as suggested by Dalpe (1993), until counting. Spores were counted by placing them in an 8 cm diameter nematode counting dish. Some spores were densely grouped in sporocarps and it was difficult to count the number of spores per sporocarp, so in these cases the sporocarp was referred to as one spore. A dissecting microscope was used to count the spores.

### 2.5 Root colonization by AM fungi

#### 2.5.1 Root sub-sampling and cleaning

Each 400 g soil sample was immersed in a bucket of water and gently swirled to mix and remove most of the soil. To obtain a cleaner sample of root fragments, the soil with roots was washed vigorously and poured off, the liquid passed through 350-500  $\mu\text{m}$  sieves and the roots washed free of soil. The suspended root fragments were

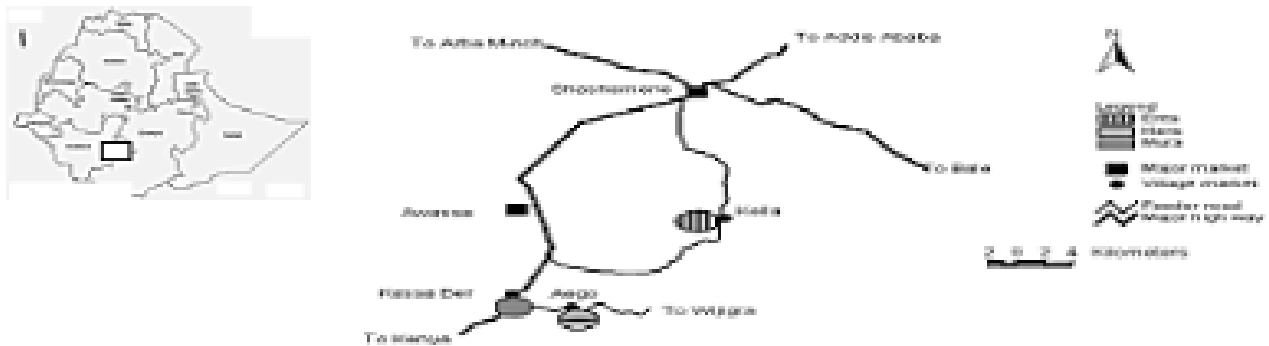


Figure 1: Map of the study sites showing the nearest markets and road network in Sidama, south Ethiopia.

Table 1: Top soil characteristics from which spore density, tree root colonisation and on which seedling of maize and *Millettia* was raised

Plot Type	Sand %	Silt %	Clay %	pH <sup>1</sup>	Na	K	Ca	Mg	CEC	T:N <sup>2</sup>	O:C <sup>3</sup>	P <sup>4</sup>
<b>Tree -enset-coffee</b>												
<i>Cordia</i>	36	34	30	6.7	0.7	2.7	15.4	3.1	28.5	0.4	3.1	12.6
<i>Millettia</i>	36	34	30	6.3	0.9	1.7	16.7	3.0	29.9	0.4	3.1	9.5
<b>Tree and enset</b>												
<i>Cordia</i>	36	36	28	7.0	0.6	2.0	17.7	3.0	27.7	0.3	3.4	15.4
<i>Millettia</i>	36	35	29	6.6	0.8	1.4	14.0	2.1	27.3	0.4	2.7	9.0
<b>Cordia-maize<sup>5</sup></b>												
CT	33	35	32	6.5	0.5	2.2	18.1	3.2	29.0	0.3	3.5	9.4
MC	35	34	31	6.5	0.5	1.9	17.1	3.2	27.4	0.3	3.4	8.4
CE	35	33	32	6.4	0.4	1.9	16.7	2.9	26.4	0.3	3.1	7.4
OF	36	33	31	6.2	0.4	1.6	16.0	2.8	25.9	0.3	2.8	5.7
<b>Millettia- maize</b>												
CT	39	31	30	6.2	0.7	1.0	12.4	2.1	29.1	0.3	2.7	6.6
MC	39	35	26	6.1	0.8	1.2	12.6	2.2	29.0	0.3	2.9	6.4
CE	40	34	26	6.0	0.8	1.0	12.0	2.0	28.8	0.3	2.5	4.9
OF	38	34	28	6.0	0.7	1.3	10.7	1.92	26.5	0.2	1.3	4.6

<sup>1</sup> pH in H2O 1:2.5; <sup>2</sup> T:N total nitrogen in %; <sup>3</sup> O:C organic carbon; <sup>4</sup> Available phosphorus ppm; <sup>5</sup> For maize-tree plots at laterally increasing distance from the tree trunks: CT= 0.75 m, MC= mid canopy, CE= canopy edge OF= open-maize field

rapidly decanted onto the 50-100 µm sieve and the decanting was repeated until all the root fragments were on the 50 µm sieve. The fine 50 µm sieve was placed in a pan of shallow water so that the floating roots and any debris could be picked up. Roots were sorted into two categories, living <math>\leq 1</math> and 1-2 mm, representing fine roots. Depending on the type of sample, up to 0.5-1.5 g of the <math>\leq 1</math> mm categories were taken and samples were stored in 50% ethanol until clarified and stained (Brundrett et al., 1996).

### 2.5.2 Root clearing and staining

The duration of autoclave sterilisation can vary depending on the type and density of the plant root. Autoclave sterilisation at 121°C is usually the same temperature for most materials. To standardise sterilisation we used a range of times and replicated three times. Based on this, the better time was found to be 20 minutes for *Millettia* and 25 minutes for *Cordia*. Young roots of maize and *Millettia* plants grown in the nursery were autoclaved for 5 minutes.

From the fresh field soil samples, the roots were gently washed with tap water to remove adhering soil particles. The roots were then cut into short segments (typically 1-2 cm) using a sharp scalpel. The roots were boiled in 10% KOH solution for 15-20 min at 90-0°C in a boiling water bath, rinsed several times with water and acidified with 1% HCl solution. Root samples were stained by adapting the techniques developed by Phillips and Hayman (1970) as modified by Koske and Gemma (1989). Roots were stained by boiling in 0.05% trypan blue at 90 0°C for 10 min, followed by destaining in acidic glycerol at room temperature. The stained root segments were mounted on slides in acidic glycerol, the coverslips placed over them and gently tapped with the blunt end of a pen. The stained roots were poured onto 50 cm sieves and destaining solution. The stained root segments were evenly distributed in a petri dish marked with grid lines (the method of intersecting grid lines on the bottom to form approximately 1.3 cm squares and root colonisation of individual species was estimated as described by Brundrett et al. (1996)). Cross-checks were made at a higher magnification of x200 (McGonigle et al., 1990). In most cases, *Cordia* roots were crushed when examined to confirm the presence of arbuscules, vesicles and/or In

Table 2: Number of soil samples collected from different plots for estimation of number of AM spores (n=36) and root colonisation n=72).

Tree Plot type	No. of samples species	Spore	growth maize	substrate <i>Millettia</i>
Tree and enset-coffee	<i>Cordia</i>	3	3	3
	<i>Millettia</i>	3	3	3
Tree and enset	<i>Cordia</i>	3	3	3
	<i>Millettia</i>	3	3	3
Tree and maize	<i>Cordia</i>	12	12	12
	<i>Millettia</i>	12	12	12

general, the intersections of the grid with the root were examined for the presence of AMF structures. The presence of colonisation in a root segment was recorded if hyphae (only), vesicles or arbuscules were found. Approximately 100-150 sections were examined for each sample. Total root colonisation was calculated as % colonisation = total number of positive segments/total number of segments examined x 100.

## 2.6 Nursery experiment

The aim was to assess the potential of field-collected soil to induce root colonisation of the two important plants in the study area. Thirty-six soil samples were used as growth medium in the nursery (Table 2). Maize and millet were used as host plants. Both species started germination after 5-8 days. A local variety of maize was purchased from the local market and sown. For *Millettia*, a locally collected seed source was used after four months of storage. Four seeds of each species were sown on 7 July 2000 in soil contained in a perforated plastic pot of approximately 3000 ml, with three replicates of each soil type and host seedling species. Plants were thinned to one per pot 15 days after sowing. All plants were harvested 45 days after sowing. Feeder roots were cut from the main root system, chopped into 1 cm lengths and mixed to represent fine roots  $\pm$  2 mm. Then, 1.5 g of fine roots  $\pm$  1 mm were collected for clearing and staining as described above. The percentage of root colonisation was calculated and the total fresh weight of the plant was determined.

## 2.7 Data analysis

To assess the relationships between spore counts in field soils and AM colonisation percentage n of maize and *Millettia* plants grown on these soils from the same sample plots, spore counts per sample were log-transformed. Data from colonised roots were also arcsine transformed to conform to the normal distribution (Sokal and Rohlf, 1981). Pearson's correlation was used to assess the relationships between the number of spores in field soils and the degree of root colonisation in the nursery experiment. The transformed values were also used for the ANOVA calculation. One-way ANOVA with STATISTICA (1999) was used to test for differences between the number of spores and the degree of root colonisation in each sampling plot and the data at the nursery level. Where significant F values were obtained ( $P < 0.05$ ), the plot means were separated by

the Duncan test. Results for spore counts and percent root colonisation are presented as back-transformed data.

## 3 Results

### 3.1 Number of AM spores and root colonization in the field

#### 3.1.1 Spore number

Spore counts varied from 3 to 31 and 1 to 24 g<sup>-1</sup> dry soil under *Cordia* and *Millettia* canopy, respectively, at enset-coffee, enset and open maize fields (Tables 3) at the different and grown at increasing distances from tree trunks of seated trees (Table 4). Soil samples beneath *Cordia* and *Millettia* had about the same number of spores. There were significant differences ( $n = 24$ ,  $F = 3.807$ ,  $p < 0.004$ ) in number of spores between soil samples from varying tree-crop combination plots. Significantly, ( $p < 0.05$ ) lower numbers of spore were observed in soils under *Cordia* and *Millettia* trees grown on maize plots than under tree grown in enset-coffee and enset plots (Table 3). Numbers of spores were variable among soil samples collected at increasing distances from tree trunks (Table 4). However, the number of spores under the canopy did not significantly ( $p < 0.05$ ) vary with increasing distances from tree trunks (Table 4). In the maize field outside the canopy the number of spores was about half the number under the canopy.

#### 3.1.2 Percentage of AMF root colonization

Tables 3 shows mean spore count g<sup>-1</sup> dry soil from under *Cordia* and *Millettia* canopy, enset-coffee, enset and open maize fields at the different sites. Spore counts varied from 3 to 31 and 1 to 24 g<sup>-1</sup> dry soil under *Cordia* and *Millettia* canopy, respectively. Soil samples beneath *Cordia* and *Millettia* had about the same number of spores. There were significance differences ( $n = 36$ ,  $F = 3.807$ ,  $p < 0.004$ ) in number of spores between soil samples from varying tree-crop combination plots. Significantly, ( $P < 0.05$ ) lower numbers of spore were observed in soils under *Cordia* and *Millettia* trees grown on maize plots than under tree grow in enset-coffee and enset plots (Table 3).

Numbers of spores were variable among soil samples collected at increasing distances from tree trunks (Table 4). However, the number of spores under the canopy did not significantly ( $p < 0.05$ ) vary with increasing distances from tree trunks. In the maize field outside the canopy the number of spores was about half the number under the canopy (Table 4).

For *Cordia* and *Millettia* trees scattered on maize fields, there was a non-significant tendency for lower proportion of colonized roots with increasing distances from the tree trunks (Table 4). At all distances from tree trunk, higher root colonization was also noticed for *Millettia* than for *Cordia* trees.

## 3.2 Nursery grown plants

### 3.2.1 Levels of AM colonization

Table 5 shows the mean level of root colonization while the overall value ranged from 4 to 66% for maize and 3 to 37% for *Millettia*. Colonization of roots by AMF was relatively more intensive in maize than in *Millettia* seedlings. There were significant differences ( $n = 24$ ,  $F = 2.671$ ,  $P < 0.030$ ) in levels of root colonization between maize plants grown on soil samples from different tree crop combinations, with the lowest colonization in soil from maize fields. Although not significant, root colonization of nursery-grown *Millettia* plants tended to be higher than values on soils from under *Cordia* trees at enset-coffee plots, followed by soil from under *Millettia* trees at enset plots. The lowest root colonization was noted in soils from maize fields. The percentages of AMF colonized roots of maize plants grown on soil from under *Cordia* trees at enset-coffee plots were 25% greater than those grown on soil from under *Cordia* tree canopy at maize plot. For both maize and *Millettia* plants grown on soils collected at laterally increasing distances from tree trunks, no consistent pattern of colonization was observed (Table 6). The relationships between estimates of AMF colonization of maize roots and *Millettia* seedlings grown in the nursery and the number of spores in the soil samples used as growth media was determined. The percentage of AM colonized roots of maize was significantly positively correlated with the number of spore counts from field soils (Table 7).

The same letter(s) following mean values indicate no significant ( $P < 0.05$ ) difference between tree species.

### 3.2.2 Fresh weight of plant

Soils used as growth media in the nursery, from different plots beneath *Cordia* and *Millettia* trees, had no significant effects on the fresh weight of either maize or *Millettia* plants 45 days after sowing. In all cases, maize plants attained higher fresh weight than *Millettia* (Fig. 2a, 2b, 3a, and 3b). Fresh weights of plants grown in the soil collected from under *Cordia* and *Millettia* trees grown in enset fields (Fig. 2a and 3a) were greater than from maize fields. Although not

statistically significant ( $P < 0.05$ ), a decrease in fresh weight was noticed for both maize (Fig. 2b) and *Millettia* (Fig. 3b) plants grown on soils from plots at laterally increasing distances from *Cordia* and *Millettia* tree trunks.

## 4 Discussion

Studies on the effect of *Cordia* and *Millettia* trees on the number of AM fungi spores in field soils are lacking. It is therefore difficult to compare the results of this study with those of others, as most of the studies on spore numbers and colonization levels have been carried out on other trees, crops, and/or land-use systems under different ecological conditions. Spore counts and/or root colonization levels are commonly used to study mycorrhizal associations (Dalpe, 1993; Brundrett et al., 1996). However, the number of spores and the percentage of mycorrhizal colonization of root systems are not always good measures of the potential effectiveness of mycorrhization (Reich and Barnard, 1984; Merryweather and Fitter, 1998). Despite these limitations, both the number of spores and the percentage of infection in roots in the soil are used as a means of assessing mycorrhizal association.

### 4.0.1 Number of spores

Depending upon the seasons, AM fungi spore formation could be different at a given site (Allen et al., 1998). Since the sampling period of this work was 6 weeks after the onset of the main rainy season, the high number of spores could be related to seasonality effect, as suggested by other workers for other species (Brundrett et al., 1996; Janos, 1996).

The number of AM fungi spores ranged 1-31 per gram of dry soil, which is comparable with some of the previous findings from various land use types (Table 8). The number of spores was approximately the same under *Cordia* and *Millettia* canopies (maximum 31 and 25 g<sup>-1</sup> soil, respectively). The occurrences of spores reflect many factors: such as the ability of native AM fungi to produce spores, the successful development of AM fungi and host tree/plants, or suitability of the environmental conditions. Soil physical, chemical, and biological conditions are well-known to affect spore germination and mycelial dispersion of AM fungal species (Sieverding, 1991). Soil beneath *Cordia* and *Millettia* at maize fields had a significantly lower number of spores than in enset fields. Similarly, soils beneath trees at enset-coffee and at enset plots in enset fields contained four to five as many spores as in open-maize plots on maize fields.

The increased number of spores beneath *Cordia* and *Millettia* trees at enset-coffee and enset plots could probably be explained by four major factors or by their combined effects: (1) less soil disturbance/tillage (Gavito and Miller, 1998; Menendez and Scervino, 2001), (2) higher number of plant species (Schreiner and Bethlenfalvay, 1995), (3) more active biological conditions (Sieverding, 1991), and (4) higher cover/vegetation as compared with plots in

Table 3: Number of spores g [-1] soil dry weight and percent length of colonised roots of *Cordia africana* and *Millettia ferruginea* grown at different sites. Mean ± std of three replications.

Plot type	species	Spore g [-1] soil	Colonised root length in%
Tree-enset- coffee	<i>Cordia</i>	20a ± 11.0	44.49 ±11.0
	<i>Millettia</i>	15a ± 6.7	41.47±17.0
Tree-enset	<i>Cordia</i>	14a± 8.8	38.01±8.4
	<i>Millettia</i>	17a± 6.5	48.38±11.4
Tree-maize	<i>Cordia</i>	8bc± 4.8	30.31±12.2
	<i>Millettia</i>	6bc± 4.1	39.76±14.0

The same letter(s) following mean values indicate no significant (P > 0.05) difference between tree species

Table 4: Mean number of spore g[-1] dry weight soil and percent length of colonised roots of *Cordia africana* and *Millettia ferruginea* grown at increasing distances from tree trunks of scatted trees in maize fields. Mean ± std of three replications

Distances from	Spore g[-1]soil		Colonised root length %	
	<i>Cordia</i>	<i>Millettia</i>	<i>Cordia</i>	<i>Millettia</i>
CT at 0.75 m	9±3.4	7±5.2	31.54±17.1	44.06±14.3
Mid Canopy	6±6.6	6±4.2	30.30±10.3	39.58±17.4
Canopy edge	8±5.5	7±4.8	29.09±9.5	35.65±10.9
open-maize field	4±3.9	5±2.6	-	-

open-maize fields (Sieverding, 1991; Janos, 1996). Although those factors have not been critically investigated in this study, based on farmers’ knowledge on frequency of tillage, one could speculate that tillage could influence mycorrhizal inoculum/spore. Farmers practice relatively higher frequency of tillage for maize than enset plots. Available reports for different species and land use types indicated a lower AM fungi spore density in disturbed soils than in less disturbed (Miller et al., 1995; Gavito and Miller, 1998; Menendez and Scervino, 2001) or undisturbed soils (Lovera and Cuenca, 1996). There are also several reports of low AM fungi spore number in disturbed maize fields (Jasper et al., 1991; Douds et al., 1993; McGonigle and Miller, 1996; Kabir et al., 1998; Boddington and Dodd, 2000). Variation in spore number could also be related to the differences between soil properties of studied plots.

Regarding biological conditions, the host genotype, vegetation cover, and microbial activities could influence AM symbiosis (Janos, 1996; Smith and Read, 1997). The difference in number of spores between the *Cordia* and *Millettia* trees could be explained by the hosting ability for AM fungi of individual tree species. As having dominantly perennial plant species and high vegetation cover, the enset-coffee and enset plots have some characters of forest-dominated ecosystems. The numbers of spores recovered from tree-enset combination plots are higher than those reported from some tropical natural forests (Table 8). However, in the tropical moist forests, the number of spores tends to be lower (Janos, 1980; Sieverding, 1991; Fischer et al., 1994) than in polyculture agricultural systems. In these systems, like the Sidama traditional agroforestry land use, the spore numbers are much greater than in intensive or high input systems (Sieverding, 1991). In general, the effect of vegetation and microorganisms on the amount of spore/inoculum is site-specific (Sieverding, 1991; Fischer et al., 1994).

The number of spores did not significantly vary with increasing distances from tree trunks (Table 4), which was observed only on maize fields with a generally low number of spores. At the closest

distances from tree trunks, the number of spores was only slightly higher than further away from the crown cover. This is in agreement with Musoko et al. (1994) who reported that distance from *Terminalia superba* trees trunk has no influence on the number of spores, although more spores were observed close to the trunks. The open field samples were taken well outside the area less influenced by tree roots and the farmers are growing maize under the tree canopy with almost the same intensity of weeding and tillage practice as outside the canopy. The lower spore number outside the crown canopy in the maize field sample may thus indicate that the high spore number under the canopy is influenced by the root system of trees.

#### 4.0.2 Root colonization in the field

The degree of AM fungi colonization of *Cordia* and *Millettia* trees was variable, with fairly high standard deviation. This could be explained by differences in rapid root growth between trees and field plots during the wet period when the sampling was carried out. The seasonal influence on colonization of grass roots and common forbs and graminoids was reported by Allen (2001) and Michelsen et al. (1993), respectively. In this investigation, levels of root colonization were relatively high for both *Cordia* (25 to 57%) and *Millettia* (33 to 62%) reflecting their mycotropic nature. This is comparable with 27 to 57% root colonization for lime grown in three agroecosystems reported by Michel-Rosales and Valdes (1996). Difference in AM colonization in the soil beneath *Cordia* and *Millettia* trees grown at enset-coffee and enset, and at maize plots examined in this study, probably resulted from interactions between tillage practices and species composition/cover. The relatively higher plant cover and richness at enset plots may explain differences in mycorrhizal infection between soil samples collected from various plots. At tree enset-coffee and tree enset plots, the infection of new roots probably occurred directly from the roots in a manner more rapid and efficient way than from germinating spores. Moreover, as being a perennially dominated and more diverse system, the tree enset-coffee and

Table 5: Mean proportion of colonised roots of maize and *Millettia* seedlings grown in nursery soils taken from under the canopy of *Cordia* and *Millettia* trees grown in different fields. Mean  $\pm$  std of three replications.

Plot type	Colonised root of maize seedling (%)	Colonised root of <i>Millettia</i> seedling (%)		
Tree-enset-coffee	51.9a $\pm$ 13.6	32.4a $\pm$ 8.4	27.4 $\pm$ 10.2	19.5 $\pm$ 10.6
Tree-enset	42.9ab $\pm$ 11.4	48.2ab $\pm$ 14.3	21.7 $\pm$ 8.1	26.0 $\pm$ 8.4
Tree-maize	24.9b $\pm$ 13.2	26.4b $\pm$ 9.8	15.2 $\pm$ 8.2	12.4 $\pm$ 7.1

Table 6: Mean proportion of colonised roots of maize and *Millettia* seedlings grown in nursery soils taken at increasing distances from the trunk under *Cordia* and *Millettia* trees grown on maize fields. Mean  $\pm$  std of three replications.

Distances from trunk	Colonised roots of maize seedling (%)	Colonised roots of <i>Millettia</i> seedling (%)		
CT (0.75 m)	31.1 $\pm$ 16.9	25.2 $\pm$ 12.9	12.5 $\pm$ 9.5	15.4 $\pm$ 9.5
Mid Canopy	25.6 $\pm$ 6.72	26.8 $\pm$ 10.5	11.1 $\pm$ 3.1	11.2 $\pm$ 8.8
Canopy edge	18.1 $\pm$ 15.45	27.1 $\pm$ 10.3	15.9 $\pm$ 10.7	10.5 $\pm$ 3.8
Open-maize field	24.0 $\pm$ 13.42	23.1 $\pm$ 14.9	19.8 $\pm$ 13.0	11.4 $\pm$ 6.1

tree enset combination plots are probably suitable to host various AM fungi species. It is possible that due to low frequency of tillage practices for these tree-enset coffee and tree enset plots, plant roots suffer less damage and the hyphae network is less disturbed. This is in agreement with Michel-Rosales and Valdes (1996) who reported a higher percentage (57%) of root colonization for lime trees grown in gardens than those grown (27%) in plantations. Boddington and Dodd (2000) reported a greater length of extra-radical mycelium of AM fungi in soil under agroforestry systems than in a monoculture. McGonigle et al. (1999) also reported that reduced tillage increased the mycorrhizal association. However, different species of AM fungi that colonize host roots can respond to soil disturbance in different ways (Merryweather and Fitter, 1998). Although not quantitatively and qualitatively assessed in this study, it is possible that tree species or maize might selectively favor only certain AM fungal species, which may initiate relatively low root infection or are less compatible at this stage of development.

Slight spatial differences were detected in levels of mycorrhizal colonization. The lack of significant differences among plots at increasing lateral distances from tree trunks could be attributed to the fact that roots might be extended at least up to the edge of the crown with uniform distributions of AM fungal infection.

#### 4.0.3 Root colonization in nursery

At nursery level, relatively more intensive root colonization was observed for maize plants than for *Millettia* plants. The literature does not appear to contain details of mycorrhizal dependency of *Millettia*. The high colonization of maize could be due to its high mycorrhizal dependency, as it is well known to be a facultative host for AM fungi (Sieverding, 1991). However, soil under *Cordia* and *Millettia* trees at enset-coffee and at enset plots resulted in significantly higher levels of maize root colonization than in soil from beneath tree-maize and open-maize plots.

The difference in number of spores that occurred as a result of differences in tree-crop plots and open-maize were reflected in the levels of root colonization of maize and *Millettia* grown in the nursery.

Significantly positive correlation of spore numbers in field soil with the level of colonization on maize roots (Table 7) indicates that the original set of AM fungi might play a role in the functional relationship between the roots and the fungi, as well as to support hyphae for nutrient uptake. These results agree with the conclusion that level of colonization was positively correlated with the number of AM fungi spores (Simpson and Daft, 1990; Frank and Morton, 1994; Frioni et al., 1999; Oliveira and Sanders, 1999). On the other hand, it is in disagreement with the lack of relationships between spore number and root colonization as suggested by Merryweather and Fitter (1998).

The relatively poor correlation of the spore number in the soil samples before planting and level of root colonization in *Millettia* plants could partially be explained by the low number of suitable spore of AM fungi species, lack of compatibility of AM fungi or most probably a too small *Millettia* plant not capable of acting as an effective host at this stage of development. The low proportion in the extent of AM colonization of maize and *Millettia* roots with increasing distances from the tree trunk was not significantly related to the number of spores in field soil.

#### 4.0.4 Plant size

Both maize and *Millettia* plants with a high level of root colonization attained higher fresh weight when grown in soil under *Cordia* at enset-coffee, and *Millettia* at enset plots. The differences in fresh weight might also be related to the differences in the level of soil fertility, since soil fertility indicators under tree-enset combinations were relatively higher than in all other soil samples (Table 1). Apart from these, there was no readily apparent explanation of the difference in fresh weight between plants grown on soils from different plots.

Regarding AM association, we believe that this is the first report to deal with *Cordia* and *Millettia* trees in the Sidama traditional agroforestry land use systems. It could contribute the base line data necessary for future in-depth studies, on the relationship to environmental components of this complex system. For better understanding, of



Table 7: Pearson correlation between log-transformed spore density and arcsin-transformed proportion of AM colonization of maize and *Milletia* seedlings grown in soils from under *Cordia* and *Milletia* canopy of different fields and in soils from open maize fields.

Species	r	p	n
Maize	0.340	0.043	36
<i>Milletia</i>	0.131	0.447	36

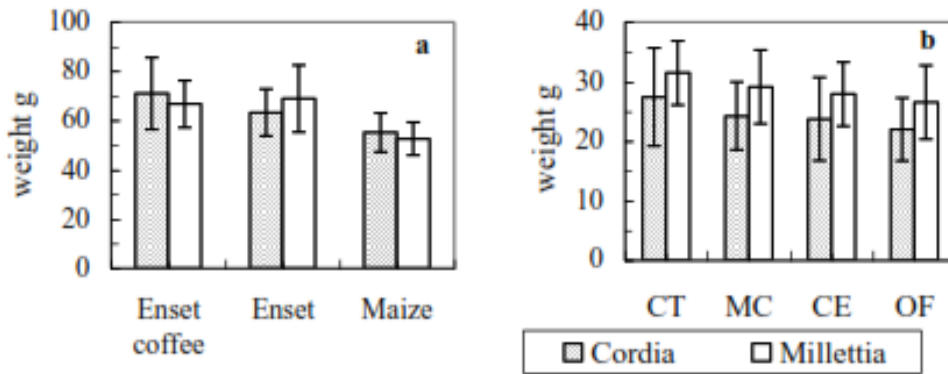


Figure 2: Mean and standard error fresh weight of maize plants grown on soils from beneath *Cordia* and *Milletia* trees (a) grown at enset and maize fields and (b) at laterally increasing distances from tree trunks at maize fields. CT = distance at 0.75 m, MC = at mid-canopy, CE = at canopy edge, and OF = at open maize field.

the factors that influence AM colonization, continuous sampling is needed, during both dry season and rainy seasons. Furthermore, detailed investigations are needed to understand the reasons for occurrences and species diversity of AM fungi as well as the predominant species.

## 5 Conclusions

In conclusion, tree-enset-coffee and tree-enset combinations can induce a higher number of spores and level of colonization than tree-maize plots or the open maize field. Soil under *Cordia* and *Milletia* in maize fields had a significantly lower number of spores than in enset fields. The number of spores did not vary significantly with

increasing distance from the tree trunk. Although not statistically significant, differences were observed in the degree of root colonization for both *Cordia* and *Milletia* trees grown in different plots. In general, the proportions of colonized roots were in the following order: tree enset coffee > tree enset > tree enset maize for *Cordia* trees and tree enset > tree enset coffee > tree enset maize for *Milletia* trees.

Soil under *Cordia* and *Milletia* trees in enset coffee and enset plots resulted in significantly higher levels of maize root colonization than soil under tree maize and open maize plots. The high number of spores in the field soil had a positive, statistically significant effect on the colonization of maize and *Milletia* plants in the nursery. Both maize and *Milletia* plants with high root colonization achieved higher fresh weight when grown in soil under *Cordia* in enset coffee and *Milletia* in enset plots.

Table 8: Spore density g-1 soil of AMF recorded at different places under three major land uses.

Land use	Country	Site/species/forest type	Spore no. g-1 dry soil	References
Tropical natural forest	Argentina	Semi-humid deciduous	5-23	Fontenla et al., 1998
Tropical natural forest	Mexico	Deciduous moist forest	2-28	Allen et al., 1998
Tropical natural forest	Costa Rica	Moist forest	120	Johnson and Wedin, 1997
Tropical natural forest	Cameroon	Deciduous moist forest	2-5	Musoko et al., 1994
Tropical natural forest	China	Deciduous moist forest	6-19	Zhao et al., 2001
Woodland	UK	Oak	1-5	Merryweather and Fitter, 1998
Woodland	UK	Sycamores	1-50	Merryweather and Fitter, 1998
Agriculture	Colombia	Cassava	7-28	Sieverding, 1991
Agriculture	USA	Orchard	5-36	Reich and Barnard, 1984
Agroforestry	Senegal	Plantation	1-8	Ingleby et al., 1997
Agroforestry	Indonesia	Tree-maize	4-28	Boddington and Dodd, 2000
Agroforestry	Mexico	Citrus/dry tropics	1-4	Michel-Rosales and Valdes, 1996

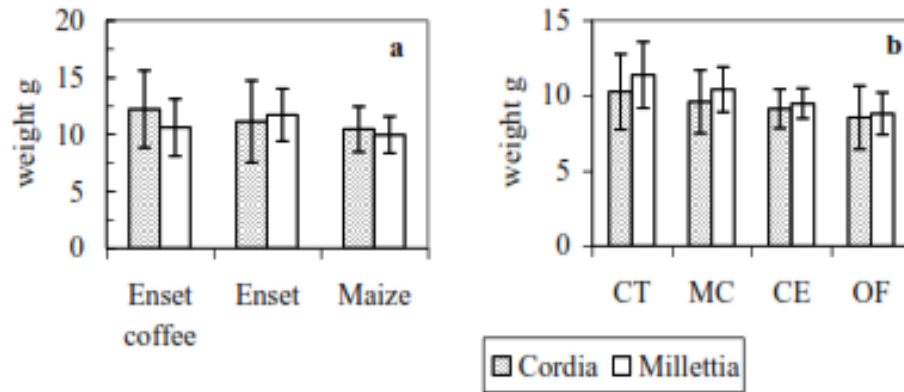


Figure 3: Mean and standard error fresh weight of *Millettia* plants grown on soils from beneath *Cordia* and *Millettia* trees (a) grown at enset and maize fields and (b) at laterally increasing distances from tree trunks at maize fields. CT = distance at 0.75 m, MC = at mid-canopy, CE = at canopy edge, and OF = at open maize field.

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