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ORIGINAL ARTICLE

Arbuscular mycorrhizal fungi with different soil fertility amendment practices in agricultural landscapes of Kenyan highlands

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Abstract Several interrelated and site-specific agronomic factors ranging from agroecological conditions to systems management practices have been shown to variably affect arbuscular mycorrhizal fungi (AMF) diversity in the soil. Also, there have been various attempts in the past to evaluate the potential of AMF field inoculation but a majority focussed on the use of exotic strains, disregarding the potential of the existing naturally occurring strains. In an attempt to address these problems, our study aimed to develop 'best-bet practice' based on soil fertility amendment practice (SFAP) that encourages occurrence and diversity of AMF in the soil. Control treatment (no application) was compared with three (3) SFAP used singly or in combination with AMF or two other soil nutrients enhancing organisms (*Bacillus* and *Trichoderma*) which included the following: (1) Mavuno (macro- and micronutrients and secondary nutrients) fertilizer, (2) calcium ammonium nitrate (CAN) plus triple super phosphate (TSP) and (3) cattle manure. Maize (*Zea mays* L.) and common bean (*Phaseoli vulgaris* L.) were planted at on-station and on-farm plots for two consecutive cropping seasons with the experiment replicated in two benchmark sites of Embu and Taita-Taveta Districts. Embu site recorded a lower soil pH and also very low phosphorus levels compared to Taita site. The number of AMF spores per kg of soil was very low, ranging from 30 to 100, at Embu in the first season and application of SFAP resulted in no significant difference. However, in the second season, use of *Trichoderma* + CAN plus TSP was shown to significantly stimulate AMF species in the soil, with a 250 % increase in species density compared to use of *Bacillus* + Manure. At Taita, after the first cropping season, significant change in spore density was only recorded from AMF applied singly with a 66.1 %

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increase in spore density compared to Control treatment. In comparison, after the second cropping season, use of AMF applied singly, AMF + CAN plus TSP and AMF + Manure increased spore density by 135.4, 109.6 and 100 % respectively compared to Control treatment. Use of AMF applied singly increased species density in the soil by 100 and 81.1 % compared to CAN plus TSP and *Trichoderma* treatments respectively after first season at Taita site: while after the second cropping season, application of AMF + CAN plus TSP, AMF + Manure and AMF + Mavuno increased AMF species density in the soil by 60.3, 51.5 and 55.9 % respectively compared to Control treatment. These findings provide evidence that it is possible to increase the number of AMF spores in the soil through inoculation with native species and also possibly stimulate dormant species through other SFAP treatments.

Keywords Arbuscular mycorrhizae fungi · Soil fertility amendment practices · Native species · Spore density · Species density · AMF inoculation

Introduction

Poor soil fertility and nutrient depletion continue to represent huge obstacles to securing needed harvests in Africa (Vanlauwe and Giller 2006; Sanchez 2002). This has led to recommendation of integrated soil fertility management (ISFM) methods which advocates for improvements in efficiency regarding use of mineral fertilizer through its combination with organic resources while producing longer-term beneficial environmental impacts (Sanginga and Woomer 2009).

Consequently, mycorrhizal association has received attention as part of an increasingly popular paradigm that considers an active and diverse soil biological community as essential for increasing the sustainability of agricultural systems (Cardoso and Kuyper 2006). Arbuscular mycorrhizal fungi (AMF) are attributed to deliver various essential agroecosystem services, such as nutrient uptake, soil aggregation, and carbon sequestration (Gianinazzi et al. 2010).

AMF form symbiotic association with over 80 % of all crop plants in agriculture, the most important phylum being Glomeromycota (Schussler et al. 2001). However, the occurrence of AMF is low in many tropical soils and landscapes (Jefwa et al. 2009) and many factors have been shown to contribute this. In general, conventional intensive agricultural practices have a negative impact on the AMF association and thus agricultural soils are AMF impoverished, particularly in the number of species (Helgason et al. 1998; Menéndez et al. 2001). These agricultural production systems also lead to low diversity of AMF compared to a natural ecosystem (Jefwa et al. 2009) and tend to propagate *Glomus* species due to low diversity of hosts, which is severe in case of monoculture (Oehl et al. 2003). However, it does not necessarily follow that the resultant mycorrhizal symbiosis is less effective in taking up nutrients and promoting plant growth, than with the case of more species. In order to benefit from mycorrhizal associations emphasis has to be on agricultural practices that promote the occurrence and functioning of AMF (Cardoso and Kuyper 2006). Therefore, a better understanding of how these microorganisms are affected by various soil fertility amendment practices is an important step towards ISFM.

Numerous studies have reported the positive effect of AMF inoculation on crop production (Douds et al. 2007; Nyaga et al. 2014) and this has been in an effort to bolster and optimize the indigenous AMF populations. However, a majority of past field inoculation attempts have focussed on the use of exotic strains, disregarding the potential of the existing naturally occurring strains (Izaguirre-Mayoral et al. 2000; Njeru et al. 2014). This could be cited as a possible reason for failure in field inoculation attempts. Native species have been regarded as more adapted to the soil environment than introduced strains whereby they may out-compete the added AMF (Izaguirre-Mayoral et al. 2000; Klironomos 2003). Gainful use of AMF in a low-input farming context requires selection of a suitable combination of plant host, fungal partner and agricultural management practices (Sawers et al. 2008). Consequently, field inoculations under prevailing environmental conditions and under different soil fertility amendment practices become necessary to determine the success or failure of possible use of AMF inoculum.

The current study hypothesized that: (1) use of organic Soil Fertility Amendment Practices (SFAP) increase AMF spore and species density in the soil, (2) use of native AMF species as an inoculum increase spores density in the soil, (3) the number of AMF

spores and species density in the soil reduce with sampling depth as number of possible host roots are expected to decrease with depth, and iv) AMF spores and species density increase with increase in cropping season as a result of a build-up.

The specific objectives of this study were to: (1) evaluate the effect of ex-situ use of organic and inorganic soil fertility amendments practices (SFAP) on AMF spore and species density in the soil in comparison to use of native species as inoculum, (2) assess the influence of sampling depth on AMF spore and species density in the soil, and (3) investigate the contribution of cropping seasons on AMF spore and species density in the soil.

Materials and methods

Study site

The study was conducted in Mount Kenya region of Embu District and Taita Hills of Taita Taveta District. Biophysical measurements, sampling and data collection were done in study areas labelled "windows" and each window being a frame established on a grid, having a spatial extent of 6 km (Moreira et al. 2008).

Embu District is in Central Kenya (lat 03°30′S; long 37°30′E, and altitude 1480 m above sea level) while Taita Hills (lat 3°25′; long 38°20′, and altitude of 2228 metres above sea level) are situated in the Taita-Taveta District in South-Eastern Kenya. Embu District receives a total annual rainfall of between 1200 and 1500 mm in two rainy seasons, 'long rains' (March–June) and 'short rains' (mid-October–December). Mean monthly temperature ranges between 14 °C and 19.5 °C. Taita district receives an average annual rainfall of 1500 mm in the highlands and 250 mm in the lowlands and the mean monthly temperature ranges between 17.4 and 34.5 °C.

The communities in both study areas are mainly smallholder subsistence farmers and land use is dominated by intensive agriculture. The soils in Taita Taveta benchmark site are classified as Haploic Acrisols and Eutric Cambisols while those from Embu are Rhodic Nitisols and Humic Nitisols (Muya et al. 2009). The soils at Embu are characterized by the presence of a lower soil pH and phosphorus levels compared to Taita soils (Table 1).

Experimental design

An on-station experiment was established with a total of 90 plots with sixteen (16) treatments replicated five (5) times and separated by 1 m wide strips under randomized block design. Similar treatments were applied at both Embu and Taita except for *Bacillus* treatment which was not established at Taita where a total of twelve (12) were established translating to 60 plots. The rest of treatments comprised three (3) bio-inoculant treatments (AMF, *Trichoderma* and *Bacillus*) and three (3) fertilizer practices (cattle manure, mavuno and TSP plus CAN), two crops (beans and maize), and an untreated control. Plots measuring 3 m × 3 m divided by 1 m pathway were demarcated.

Inoculation with bio-inoculants and application rate for different SFAP

The source of mycorrhizae inoculant used was derived from native species from the two respective experimental sites which have a history of maize and bean intercropping during on-season and associated weed species during off-season. Each site received AMF inoculums that had been cultured one and a half years in sorghum then transferred to leek (*Allium porrum* L.) 4 weeks before planting to generate infective mycelia and infected root fragments that are more infective. The cultures were initiated and maintained at the National Museums of Kenya and inoculum produced as by Munro et al. (1999). The inoculum consisting of native species was applied by coating seeds at planting with thick slurry (20 g ml^{-1}) in a crude state comprising of spores, mycelia and infected root fragment. AMF spore abundance in trap culture soils and infective propagules was calculated by MPN per 20 g air dried soil. The mean spore abundance in soils from the trap culture was 64.3 spores per 250 g dry soil.

The *B. subtilis* (isolate K194) used in this study was supplied by the Microbial Resource Centre (MIRCEN), University of Nairobi and was only applied at Embu experimental site. For *Trichoderma* inoculation, seed was coated with *Trichoderma* at rate of 2 g of *Trichoderma* kg^{-1} of seeds using gum arabic as a sticker.

Cattle manure was applied at a rate of 40–60 tons $plot^{-1}$ while Mavuno [macronutrients:

Table 1 Analysis of variance showing the mean values of soil pH and soil nutrients between the two experimental sites at two soil depths

	Embu		Taita	
	Depth 1 (0–10 cm) (n = 15)	Depth 2 (10–20 cm) (n = 15)	Depth 1 (0–10 cm) (n = 16)	Depth 2 (10–20 cm) (n = 13)
pH (H_20)	4.3 ± 0.1[a]	4.3 ± 0.1[a]	5.0 ± 0.1[b]	4.8 ± 0.2[b]
Nitrogen (g kg^{-1})	3.1 ± 0.1[a]	2.9 ± 0.1[a]	1.7 ± 0.2[b]	1.7 ± 0.2[b]
Organic C (g kg^{-1})	23.6 ± 0.7[a]	23.0 ± 0.6[a]	20.8 ± 2.8[a]	20.6 ± 2.9[a]
Phosphorus (mg kg^{-1})	13.6 ± 1.3[a]	10.7 ± 0.8[b]	40.5 ± 11.1[c]	43.4 ± 15.0[c]
Potassium (g kg^{-1})	5.2 ± 0.7[a]	3.9 ± 0.6[a]	4.6 ± 0.6[a]	4.4 ± 1.0[a]
Calcium (g kg^{-1})	30.4 ± 2.5[a]	45.3 ± 12.1[a]	20.4 ± 2.6[b]	20.1 ± 3.2[b]
Magnesium (g kg^{-1})	11.3 ± 3.4[a]	7.7 ± 1.5[a]	18. 4 ± 2.8[b]	18.2 ± 5.0[b]
Manganese (g kg^{-1})	11.9 ± 1.2[a]	10.4 ± 1.3[a]	4.3 ± 0.7[b]	4.2 ± 0.8[b]
Cooper (mg kg^{-1})	5.8 ± 0.7[a]	6.2 ± 0.7[a]	2.7 ± 0.5[b]	2.7 ± 0.6[b]
Iron (mg kg^{-1})	36.7 ± 5.0[a]	37.3 ± 3.2[a]	52.4 ± 5.0[b]	65.1 ± 9.3[b]
Zinc (mg kg^{-1})	10.0 ± 0.9[a]	9.4 ± 0.9[a]	6.6 ± 0.7[b]	6.3 ± 0.6[b]
Sodium (g kg^{-1})	2.2 ± 0.1[a]	2.3 ± 0.2[a]	1.6 ± 0.3[b]	1.5 ± 0.2[b]

[a,b,c] Values within rows with the same letter do not differ significantly at $p < 0.05$; n = sample number

nitrogen (N) 10 %, phosphorus (P_2O_5) 26 %, potassium (K_2O) 10 %, sulphur (SO_4) 4 %, calcium (CaO) 10 %, magnesium (MgO) 4 % and micronutrients: zinc, copper, molybdenum, boron and manganese] was applied at a rate of 40 kg ha^{-1}. Triple superphosphate (TSP) + calcium ammonium nitrate (CAN) was applied at the rate of TSP = 200 kg ha^{-1} and CAN = 150–200 kg ha^{-1}.

Maize and bean establishment

At Embu benchmark site Hybrid 513 (maize) and Mwitemania (GLP 92) beans were planted being the common varieties used by local communities at the beginning of the project. At Taita benchmark site, Hybrid 513 and Mwezi moja (GLP-1004), maize and bean varieties were planted respectively. Each plot (3 m × 3 m in dimensions) had four rows of maize on 90 cm spacing with three rows of beans equally spaced between the maize rows. Wet planting was done at the depth of 2.5–4 cm with 2 seeds per hole (seed rate; 20–25 kg ha^{-1}). Thinning was carried out and single plant per hole was retained: a total of 40 maize and 30 bean plants per plot were allowed to grow to maturity. Crops were established consecutively in the short (August–January) and long rains (February–July) in year 2008–2009.

Soil sampling and analysis

For AMF spores isolation, soil samples were collected using a soil corer 5 cm diameter and 5 cm deep. From each plot, 5-sub samples (four samples near the square plot corners and one at the centre of the plot) were taken and mixed to form a composite sample from each plot whereby collection was done in two depths (0–10 and 10–20 cm). To determine change in AMF population, two sequential soils sampling timing were done; initial sampling after administration of SFAP and the first maize and bean intercrop harvest (season 1/January 2009) and last, after the second maize and bean intercrop harvest (season 2/July 2009).

For soil chemical analysis, samples collected at the start of the experiment were subjected to laboratory analysis. The following soil properties were analysed: available nutrients (P, K, Na, Ca and Mg) using the Mehlich double Acid Method (Anderson and Ingram 1993), total organic carbon using Calorimetric method (Nelson and Somers 1982), total nitrogen using Kjeldahl method (Page et al. 1982), and soil pH—water in 1:1 (w/v) soil—water suspension with pH meter.

AMF spore processing and identification

Spores were extracted from 250 g soil sample in portions of 50 g per extraction by water and sucrose

centrifugation method modified by using a 270 and 45 μm mesh sieves and 60 % w/v of sucrose (Jenkins 1964). Spores were examined with a 40× stereomicroscope, aided by both reflected light and white background (specifically used for colour determination) and a transmitted light (to aid morphological character recognition). Only fresh spores were counted in small petri dishes. Fresh spores were recognized by the appearance of the oily contents as either a diffuse milky white substance or as multiple oil globules, or one of a few large oil droplets within the spore, and the absence of parasitism. The spores were distinguished into morphotypes with colour of spore, spore size, attachments on spore and surface appearance of spore used as the diagnostic features and the number of spores counted for each morphotype. The Edinburgh Botanic Gardens colour chart for fungi was used to determine spore colour. Voucher specimens were prepared for each AMF morphotype and further described under a compound microscope with spore germination characteristics, spore wall characteristics, type of spore wall, size and number of layers and reaction to Melzer's reagent used as diagnostic features (Morton 1988). The spores were matched with species described by International Culture Collection of VA Mycorrhizal Fungi (INVAM) West Virginia University Website in some instances for taxonomic references and also using updated classification of AMF by Redecker et al. (2013). Effort was made to identify spores up to species level but where this proved impossible they were identified up to unnamed morphospecies. Voucher specimens are kept at the National Museum of Kenya collection, Nairobi.

Statistical analysis

Normality test (Shapiro–Wilk) on the raw data showed that the samples did not follow a normal distribution. Therefore a non-parametric test, Kruskall–Wallis test, was performed for more than two independent samples while a Mann–Whitney (U) test was carried out for two independent samples using SPSS version 22 (IBM Corp. Released 2013). Significance levels of $P \leq 0.05$ were used, unless stated otherwise.

Results and discussion

Contribution of sampling depth and cropping season on AMF spores and species

Sampling depth and cropping season were shown to have an influence on the number of AMF spores and species detected. Analysis of changes in spore and species densities with sampling depth showed significant differences at Embu whereby depth 2 recorded 20.7 % higher spore density and 12.4 % more species compared to depth 1 (Table 2). At Embu, higher AMF spores and species densities were recorded from depth 2 while at Taita, depth 1 recorded higher AMF spores and species. These contrasting results on AMF spores' occurrence between depths is assumed to be explained by soil P which was the only soil nutrient found to be significantly different between sampling depths within the same site (Table 1). Therefore, the study did not support the hypothesis that AMF spores and species count reduce with sampling depth as number of host roots reduce with depth but was shown to be related to soil P availability.

At both sites, higher soil P levels in the soil resulted in less AMF spores and species count. The effects of AMF are manifested only in conditions of optimum P levels, hence where soil conditions have extremely low P level AMF will not be effective (Picone 2002). In extremely high P, AMF is equally ineffective and could also be detrimental with carbon reallocated to the fungi from the plant a factor that might account for decline in grain yield in the crop in cases where its function in P uptake is no longer required. Negative effects of high phosphorus levels on AMF colonisation was previously reported from a study by Jefwa et al. (2009) at Embu, though not from similar plots to the current study. Under optimum P conditions, the carbon relocation is offset by increased uptake of phosphorus by the crop as a result of mycorrhizal contribution (Grigera et al. 2006).

The study had also hypothesized that the number of AMF spores and species count increase/build up with increase in number of cropping season. Analysis of changes in AMF communities in reference to cropping seasons supported this hypothesis and showed a significant difference in spore abundance between first and second seasons for the combined sites (Table 2). In total, a 30.3 % increase in spore density

Table 2 Relative AMF spore and species abundance in the spoil at Embu and Taita experimental sites discriminated by sampling depth and cropping seasons

Site	Variables	Spores count (kg^{-1})	Species count (50 g^{-1})
Taita	Depth 1 (0–10 cm)	744.6[a]	7.5[a]
	Depth 2 (10–20 cm)	649.8[a]	7.2[a]
Embu	Depth 1 (0–10 cm)	129.8[a]	2.3[a]
	Depth 2 (10–20 cm)	156.8[b]	2.6[b]
Combined sites	Depth 1 (0–10 cm)	270.2[a]	3.51[a]
	Depth 2 (10–20 cm)	271.2[a]	3.68[a]
Taita	Season 1 (January 2009)	551.2[a]	6.96[a]
	Season 2 (July 2009)	843.4[b]	7.77[b]
Embu	Season 1 (January 2009)	160.4[a]	2.53[a]
	Season 2 (July 2009)	119.0[b]	2.39[a]
Combined sites	Season 1 (January 2009)	239.4[a]	3.43[a]
	Season 2 (July 2009)	312.0[b]	3.82[b]

[a,b] Values within columns with the same letter are not significantly different at the 5 % level. This applies only for values under same variable and same site

was recorded in the second season compared to the first. Analysis at experimental site level recorded a significant difference in both spore and species densities from Taita site whereby a 53.1 and 11.6 % increase in spore and species densities respectively was recorded in second season compared to the first. At Embu site, a significant difference was only recorded on spore density whereby a 25.9 % reduction in number of spores was recorded from Embu in season 2 compared to season 1 after cultivation of maize and beans. As shown in Table 3, the number of AMF spores per kg of soil at Embu site was very low; ranging from 30 to 100 in the first season and this explains the failure of the cropping season to record AMF spore or diversity change despite maize being a mycotrophic crop. The rate of infection of AM fungi in plants is strongly influenced by amount of spore propagules (Lekberg and Koide 2005; Mohammad et al. 2004). Low numbers of propagules in field soils may result in low level of colonization (Smith and Read 1997).

Influence of SFAP on occurrence and diversity of AMF

Ex-situ use of organic and inorganic SFAP was shown to have an influence on AMF communities in the soil. At Embu significant difference was only recorded for species density in the second cropping season whereas at Taita site a significant difference in spore and species density was recorded with application of different SFAP for both seasons (Table 3). At Embu, use of different SFAP in the first season showed no significant difference compared to Control treatment. As earlier explained, the number of AMF spores per kg of soil was very low; ranging from 30 to 100 in the first season and this explains the failure of the different SFAP to record changes. A significant difference in species density in the second season was only recorded from *Trichoderma* + CAN plus TSP treatment compared to *Bacillus* + Manure. *Trichoderma* + CAN plus TSP treatment recorded highest number of AMF species in the second season but this value was not significantly different from Control treatment. Use of *Trichoderma* + CAN plus TSP was shown to significantly stimulate AMF species in the soil compared to use of *Bacillus* + Manure with a 250 % increase in species count. Previous study by Johansson et al. (2004), also reported that some bacterial strains promote germination of AMF spores and can increase the rate and extent of root colonisation by AMF.

The highest spore density was recorded from AMF applied singly and lowest from CAN plus TSP treatment in both seasons at Taita site. After the first cropping season at Taita, significant change in spore density was only recorded from AMF applied singly with a 66.1 % increase in spore density compared to Control treatment. Other SFAP which involve AMF inoculation such as, AMF + CAN plus TSP and AMF + Manure treatments also recorded higher spore density compared to Control treatment while all other treatments recorded lower density but the changes were not significantly different. At the same site after the second cropping season, significant increase in spore density compared to Control treatment was recorded from AMF applied singly,

Table 3 Relative abundance of AMF spore (kg^{-1}) and species (50 g^{-1}) of soil under different soil fertility amendment practices as discriminated by experimental sites and cropping seasons

Treatment	Embu				Taita			
	Spore density		Species density		Spore density		Species density	
	Season 1 (January 2009)	Season 2 (July 2009)	Season 1 (January 2009)	Season 2 (July 2009)	Season 1 (January 2009)	Season 2 (July 2009)	Season 1 (January 2009)	Season 2 (July 2009)
AMF	41.8 ± 9.5(11)	85.0 ± 33.3(8)	1.5 ± 0.3(11)	2.0 ± 0.4(8)^{a,b}	950 ± 100.3(10)^{a}	1676 ± 157.8(10)^{a}	9.6 ± 0.8(10)^{a}	9.7 ± 0.5(10)^{a,b}
AMF + CAN plus TSP	78.5 ± 20.6(13)	147.5 ± 41.0(8)	1.8 ± 0.4(13)	2.4 ± 0.4(8)^{a,b}	770 ± 118.0(10)^{a,b}	1492 ± 119.8(10)^{a,b}	7.7 ± 0.8(10)^{a,b}	10.9 ± 0.3(10)^{a}
AMF + Manure	92.9 ± 24.4(14)	80.0 ± 16.1(10)	1.9 ± 0.3(14)	1.7 ± 0.2(10)^{a,b}	640 ± 56.3(10)^{a,b,c}	1090 ± 111.0(10)^{b,c}	8.0 ± 0.5(10)^{a,b}	10.6 ± 0.5(10)^{a}
AMF + Mavuno	100.0 ± 24.3(7)	80.9 ± 31.4(8)	1.7 ± 0.4(7)	1.4 ± 0.3(8)^{a,b}	544 ± 67.6(10)^{b,c,d}	1424 ± 107.7(10)^{a,b}	7.2 ± 0.6(10)^{a,b}	10.3 ± 0.6(10)^{a}
Bacillus	53.3 ± 15.6(12)	47.5 ± 17.7(8)	1.2 ± 0.3(12)	1.3 ± 0.4(8)^{a,b}	–	–	–	–
Bacillus + CAN plus TSP	95.0 ± 25.4(12)	40.0 ± 6.7(10)	2.3 ± 0.4(12)	1.4 ± 0.3(10)^{a,b}	–	–	–	–
Bacillus + Manure	63.6 ± 14.5(11)	30.0 ± 12.3(6)	1.6 ± 0.3(11)	0.8 ± 0.3(6)^{a}	–	–	–	–
Bacillus + Mavuno	62.9 ± 25.6(7)	37.5 ± 16.7(8)	1.3 ± 0.3(7)	1.1 ± 0.48(8)^{a,b}	–	–	–	–
CAN plus TSP	91.7 ± 26.7(12)	65.0 ± 34.0(8)	1.9 ± 0.3(12)	1.8 ± 0.3(8)^{a,b}	294 ± 40.3(10)^{d}	310 ± 37.6(10)^{d}	4.8 ± 0.4(10)^{b}	4.8 ± 0.4(10)^{c}
Control	92.0 ± 38.8(15)	82.9 ± 30.4(7)	1.7 ± 0.3(15)	1.6 ± 0.3(7)^{a,b}	572 ± 78.7(10)^{b,c,d}	712 ± 93.8(10)^{c,d}	6.6 ± 0.4(10)^{a,b}	6.8 ± 0.4(10)^{b,c}
Manure	30.0 ± 10.9(10)	92.0 ± 21.5(5)	1.1 ± 0.3(10)	2.4 ± 0.2(5)^{a,b}	482 ± 74.7(10)^{b,c,d}	532 ± 63.9(10)^{d}	7.2 ± 1.5(10)^{a,b}	6.6 ± 1.0(10)^{c}
Mavuno	76.9 ± 17.7(13)	54.0 ± 14.6(10)	2.3 ± 0.5(13)	1.5 ± 0.3(10)^{a,b}	434 ± 54.0(10)^{b,c,d}	486 ± 53.3(10)^{d}	6.4 ± 0.8(10)^{a,b}	6.8 ± 0.6(10)^{b,c}
Trichoderma	76.4 ± 21.9(11)	72.0 ± 19.8(10)	1.8 ± 0.4(11)	1.8 ± 0.3(10)^{a,b}	392 ± 51.7(10)^{c,d}	392 ± 51.7(10)^{d}	5.3 ± 0.8(10)^{b}	5.3 ± 0.8(10)^{c}
Trichoderma + CAN plus TSP	65.5 ± 16.2(11)	94.0 ± 18.9(10)	1.5 ± 0.4(11)	2.8 ± 0.4(10)^{b}	–	–	–	–
Trichoderma + Manure	46.3 ± 8.9(16)	82.2 ± 34.7(9)	1.4 ± 0.2(16)	1.4 ± 0.4(9)^{a,b}	534 ± 63.9(10)^{b,c,d}	656 ± 76.4(10)^{c,d}	7.0 ± 1.0(10)^{a,b}	7.0 ± 0.9(10)^{b,c}
Trichoderma + Mavuno	47.7 ± 15.9(13)	37.1 ± 14.7(7)	1.6 ± 0.4(13)	1.4 ± 0.5(7)^{a,b}	450 ± 61.7(10)^{b,c,d}	508 ± 86.0(10)^{d}	6.8 ± 0.8(10)^{a,b}	6.7 ± 0.8(10)^{b,c}
P value (Kruskal–Wallis Test)	0.477	0.147	0.697	0.048*	<0.0001***	<0.0001***	0.008**	<0.0001***

Columns with hyphen (–) represents missing results. Values in parenthesis indicate the sample size

Significant differences within columns are indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. Where significant difference was seen within a column Kruskall–Wallis test was followed by pairwise Mann–Whitney Test

^{a,b,c,d} Values within a column carrying the same letter are not significantly different at $p < 0.05$ (Mann–Whitney Test)

AMF + CAN plus TSP and AMF + Mavuno treatments. Use of AMF applied singly increased spore density by 135.4 % compared to Control treatment while use of AMF + CAN plus TSP and AMF + Manure too increased by 109.6 and 100 % respectively. The study therefore confirms that use of native AMF species has potential to constitute an environmentally friendly method of soil fertility amendment over time to improve the AMF reservoir in the soil in order to accrue the benefits associated with the fungal-crop association. Application of inorganic fertilizer (such as TSP plus CAN and Mavuno) and also organic amendments such as manure in combination with AMF inoculum was also shown to be a better practice than use of individual fertilizers or manure alone. The current study demonstrates that formation and function of AMF is generally affected negatively by higher soil fertility and corroborates other studies such as Grant et al. (2005), Kahiluoto et al. (2001), Mäder et al. (2000) and Oehl et al. (2004).

Even though a significant difference in species density was recorded with application of different SFAP after first season at Taita site; this was as a result of significant difference in AMF applied singly treatment compared to CAN plus TSP and *Trichoderma* and not to Control treatment. Use of AMF applied singly increased species density in the soil by 100 and 81.1 % compared to CAN plus TSP and *Trichoderma* treatments respectively.

After the second cropping season at Taita, significant increase in species density was recorded with application of AMF + CAN plus TSP, AMF + Manure and AMF + Mavuno compared to Control treatment. Application of AMF + CAN plus TSP, AMF + Manure and AMF + Mavuno increased AMF species density in the soil by 60.3, 51.5 and 55.9 % respectively compared to Control treatment. The three treatments also recorded significant increase in species density in soil when compared to Manure, Mavuno, *Trichoderma*, *Trichoderma* + CAN plus TSP, *Trichoderma* + Manure and *Trichoderma* + Mavuno. Use of AMF applied singly significantly increased species density in the soil compared to CAN plus TSP, Manure and *Trichoderma* treatments.

We therefore conclude that continuous use of soluble fertilizers negatively impact on total populations of AMF and may stimulate some species while reducing others and this observation is similar to other previous studies by Howeler et al (1987), Liu et al (2000) and Treseder and Allen (2002).

The study hypothesized that use of organic SFAP increases AMF spore abundance and diversity in the soil. At Embu experimental site, application of manure led to a significant increase in AMF spores and species densities in the soil while application of *Trichoderma* + CAN plus TSP increased species density significantly in the soil (Table 4). The observation is supported by previous studies that have shown that farmyard manure does not seem to suppress AMF and may even stimulate them (Joner 2000; Alloush and Clark 2001). Also, low-input systems such as organic farming have been shown to be more favourable to AMF diversity and mycorrhizal root colonization (Gosling et al. 2006). Positive effects of organic amendments on AMF are attributed to low P contents in organic inputs that are released slowly over time (Ryan et al. 1994). Organic inputs are also associated with increased levels of soil organic matter, improved soil structure, water retention capacity and microbial activity that stimulate AMF growth (Ryan et al. 1994).

At Taita, the number of AMF spores was lowest from CAN plus TSP treatment for both seasons. The study showed that use of inorganic fertilizer applied singly (without use of AMF inoculum) had negative effect of AMF abundance in the soil. Varying results have previously been reported on the effect of organic and inorganic fertilizers use on AMF activity. For example, use of excess phosphorus fertilizer may lead to reduced AM colonisation of roots and AMF spore density in soil (Kahiluoto et al. 2001).Use of other readily soluble fertilizers, particularly, N fertilizers has also been reported to have a negative impact on AM colonization and diversity in some cases (Gosling et al. 2010; Karunasinghe et al. 2009; Linderman and Davis 2004; Treseder and Allen 2002) but not in others (Jumpponen et al. 2005; Nyaga et al. 2014). Use of AMF inoculum in combination with inorganic fertilizer such as CAN plus TSP was reported to increase maize yield (Nyaga et al. 2014). Organic resources can increase soil organic matter and enhance soil microbial communities, but they often provide insufficient nutrients to build up longer-term soil fertility and sustain crop yields (Palm et al. 2001).

Use of native AMF species as an inoculum was hypothesized by current study to increase the number of AMF spores in the soil. The hypothesis was supported whereby; a significant increase in spore

Table 4 Differences in AMF spore and species densities recorded between the two cropping seasons (August–January and February–July 2009)

Treatment	Embu		Taita	
	Spore density	Species density	Spore density	Species density
AMF	ns	ns	***	ns
AMF + CAN plus TSP	ns	ns	***	**
AMF + Manure	ns	ns	**	***
AMF + Mavuno	ns	ns	***	**
Bacillus	ns	ns	–	–
Bacillus + CAN plus TSP	ns	ns	–	–
Bacillus + Manure	ns	ns	–	–
Bacillus + Mavuno	ns	ns	–	–
CAN plus TSP	ns	ns	ns	ns
Control	ns	ns	ns	ns
Manure	*	*	ns	ns
Mavuno	ns	ns	ns	ns
Trichoderma	ns	ns	ns	ns
Trichoderma + CAN plus TSP	ns	*	–	–
Trichoderma + Manure	ns	ns	ns	ns
Trichoderma + Mavuno	ns	ns	ns	ns

Columns with hyphen (–) represents missing results

ns not significant

Significant differences within columns are indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (Mann–Whitney Test)

density after AMF inoculation was recorded from Taita site (Table 4).

At the same site, application of AMF in combination with Manure, Mavuno and CAN plus TSP recorded significant increases in spore and species densities in the soil after the two cropping seasons. The current study utilised native AMF species for each experimental site which eliminates the problem of competition with native AMF. Use of native AMF may not be the best way for diversifying the AMF community in the soil but in the current study it is shown to be an easy way of ensuring a farmer is able to optimize the benefits that accrue with AMF symbiosis. This study showed that it is possible to increase the number of AMF spores in the soil through inoculation and also possibly stimulate dormant species through other SFAP treatments. As observed by Vanlauwe et al. (2010), the current study also proposes combined use of organic amendments and soluble fertilizers (ISFM) for long-term build-up and maintenance of soil fertility and soil organic matter and diverse microbial communities.

Use of native AMF has potential application, (a) among resource constrained farmers especially in sub-Saharan Africa as a cheap and environmental friendly technology, (b) at conventional land where high rates of minerals fertilizers have been used leading to impoverished AMF community, and (c) to improve plant growth and survival rate of AMF colonized plants.

Changes in occurrence and diversity of AMF between the two cropping seasons discriminated by SFAP

A total of 15 morphotypes were isolated and described from both Taita and Embu sites, a majority being Gigasporaceae (9), followed by Acaulosporaceae (4) and Glomaceae (2). There were variations in spore proportions of individual species after the first and second cropping seasons. The morphotypes with remarkable decrease with all forms of farm use include *Scutellospora* sp a, *Acaulospora* sp1, *Scutellospora* sp b, *Scutellospora nigra* and *Scutellospora* sp

Table 5 Species rank abundance after the first and second cropping seasons

Morphotypes	Proportion (%) Season 1 (January 2009)	Proportion (%) Season 2 (July 2009)
Scutellospora sp a	30.5	30.2
Acaulospora sp1	15.9	6.0
Scutellospora pellucida	2.8	3.5
Scutellospora sp c	0.0	0.2
Scutellospora nigra	10.1	10.2
Scutellospora sp e	11.8	10.2
Glomus sp1	0.0	0.0
Scutellospora sp b	30.2	25.3
Glomus sporocarpic	0.3	0.2
Scutellospora sp d	0.0	0.0
Acaulospora sp2	0.0	0.0
Acaulospora denticulata	0.0	0.0
Scutellospora sp f	0.1	0.1
Acaulospora sp3	0.6	1.8
Gigaspora sp1	0.0	0.0

e. Others recorded an increase in species count with application of different soil amendment practices over a period of two cropping seasons include *Acaulospora* sp3, *Scutellospora* sp c, and *Scutellospora pellucida* (Table 5).

The highest proportion after the first cropping season was that of *Scutellospora* sp a (30.5 %) followed by *Acaulospora* sp1 with a proportion of 15.9 % each. After the second cropping season, *Scutellospora* sp a had highest proportion as well with a value of 30.2 followed by *Scutellospora* sp b with a value of 11.3 %.

Conclusion

Soil fertility amendment practices had an effect on AMF spore density and diversity whereby use of native AMF inoculum when applied singly or in combination of other organic or inorganic SFAP was best in improving AMF communities in the soil. Thus, the study showed that it is possible to increase the number of AMF spores in the soil through inoculation and also possibly stimulate dormant species through other SFAP treatments. Utilisation of native AMF species can constitute an environmentally friendly method of soil fertility amendment over time.

Analysis of spore abundance and species density with seasons showed an increase at Taita and a reduction at Embu. Effect of sampling depth on AMF spore and diversity also showed contrasting results with experimental site and was shown to be related to differences in availability of soil P. These two important observations put emphasis on future studies that evaluation of AMF occurrence, diversity and inoculation potential in the field should be devised to take into account the prevailing soil nutrients status and initial AMF abundance in the soil and choice should be made whether to monitor AMF at sporulation or vegetative stage.

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