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SPATIAL DISTRIBUTION OF *TRICHODERMA* SPP. IN EMBU AND TAITA REGIONS, KENYA

[DISTIBUCION ESPACIAL DE *TRICHODERMA* SPP. EN LAS REGIONES DE EMBU Y TAITA, KENIA]

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SUMMARY

The distribution of *Trichoderma* species in soils of Embu and Taita benchmark sites in Kenya with relation to land use practices was investigated. The study areas were chosen because of their significant land use intensification and for being biodiversity hot spots. Soil washing and dilution plate techniques were used to recover *Trichoderma* spp from the soil samples collected from different land use types. The fungal isolates were identified and assigned to nine species from Embu soils and eleven species from the Taita soils. Greater populations were observed in Embu than in Taita. Geographic differences between the regions explained the differences in ecological niches of the two regions that result to different soil assemblages and plant types in the regions and therefore variation in available substrate for the fungus. Land use at each site affected the distribution, richness and abundance of *Trichoderma* with napier grass having the highest abundance in both Embu and Taita while coffee had the lowest richness and abundance. *Trichoderma* favoured plants with shallow and widely spreading rooting systems, compared to the deeply rooted perennial coffee and tea trees. This underpins the importance of plant type and in effect land use system in the abundance of *Trichoderma*. The unevenness in the distribution of *Trichoderma* within the LUTs suggested that other factors influenced the distribution of *Trichoderma* apart from the LUTs. Unevenness was greater in Embu than in Taita. The differences could mostly be attributed to soil management practices employed by different farmers while managing their land and crops. *Trichoderma harziunum* was the most frequently isolated species and the most abundant in both Embu and Taita. Presence of *Trichoderma* species in some land use types and absence in others, provided a clue on the most preferred habitats, plants and/or crops. Considering the beneficial aspects of *Trichoderma* such as being antagonistic to the pathogenic fungi, crops or plants such as napier grass that induce high abundance and richness of *Trichoderma* can be used in crop rotations or in combinations with other crops to maintain high levels of the fungus in the soil.

Key words: Land use, *Trichoderma* distribution; Africa.

INTRODUCTION

Microorganisms in soil are critical to the maintenance of soil function in both natural and managed agricultural soils because of their involvement in key processes such as soil structure formation, decomposition of organic matter, cycling of carbon, nitrogen, phosphorus and sulphur. Microorganisms like *Trichoderma* also play key roles in suppressing soil borne plant diseases and promoting plant growth (Garbeva *et al*, 2004).

Trichoderma species are cosmopolitan fungi in soils decaying wood and vegetable matter. Their dominance in soil may be attributed to their diverse metabolic capability and aggressive competitive nature (Lewis and Papavizas, 1991; Haran *et al.*, 1996a; Haran *et al.*, 1996b; Eland, 2000). They colonize roots, attack, parasitize and gain nutrition from other fungi, thus enhancing root growth. *Trichoderma* species have developed rhizosphere competence through evolving numerous mechanisms for both attack of other fungi and for enhancing plant and root growth. These properties include mycoparasitism, antibiosis, competition for nutrients or space, tolerance to stress through enhanced root and plant development, solubilization and sequestration of organic nutrients, induced resistance, inactivation of the pathogens enzymes and these properties have been demonstrated by several scientists including Chet (1987, 1993); Hjeljord and Tronsmo, 1998; Altomore *et al*., 1999; Eland and Kapat, 1999; Howell *et al*., 2000; Yedidia *et al*., 1999. These diverse activities of *Trichoderma* render them a beneficial component of soil ecosystem. They play a key role in suppressing soil borne plant

diseases and promoting plant growth (Garbeva *et al*, 2004). This study was conducted to determine the influence of land use systems on the occurrence and distribution of *Trichoderma* species in Embu and Taita districts, Kenya. The two sites were chosen because of their land use intensity gradients and classification as biodiversity hot spots with several plant and animal species either endangered or vulnerable to extinction (Miller *et al.,*1993, Muya *et al.,* 2006, Newmark, 1998, Wass, 2000). Diversity, abundance and spatial distribution of *Trichoderma* species over the different LUTs in Embu and Taita were compared. Morphological and cultural characters were used to identify *Trichoderma* isolates using taxonomic keys compiled by Samuels *et al.*, (2004).

MATERIAL AND METHODS

Description of study site

In Embu site three windows were identified for study; Window 1 and 2 are 0.5km apart and both are 20km away from Window 3. Sixty sampling points which were 200m apart were marked in a grid-mesh construction using GPS markings in each of the windows. The points fell within eight land-uses namely; Tea (*Camellia sinensis*) Coffee (*Coffea arabica*), Maize/bean intercrop (*Zea mays*), Fallow (mainly *Digitaria abyssinica, Pennisetum cladestinum*), Napier (*Pennisetum purpureum*), Planted forests with Meru oak (*Vitex keniensis*), Planted forests with mixed eucalyptus (*Eucalyptus saligna, E. globulus*), and Indigenous forests.

The Taita site was divided into two windows; Window 1 covered annual and perennial cropping on bench-terraced uplands and agro forestry on steep hills while Window 2 occupies natural and planted forest, mixed cropping and horticulture on the bottomlands. The two windows are 0.5km apart. The sixty sampling points fell within seven land use systems. Coffee, Napier, Maize, Horticulture, Fallow, Indigenous forest and Planted forests.

Soil sample collection

Sampling points were marked in circles measuring 3m and 6m radii where 4 and 8 soil samples were augured at 0-10cm and 10-20cm depths respectively. The soil samples were mixed thoroughly into a single composite sample for *Trichoderma* species isolation. The soil was collected from the sixty points in Embu and sixty points in Taita and transported in paper bags to the laboratory where they were kept at 5^{o}C.

Isolation of *Trichoderma* spp.

The 120 soil samples were processed using the soil dilution plate (Johnson *et al*., 1959) and soil washing methods (Gams *et al*., 1987; Bills & Polishook, 1994). $^{1}/_{10}$, $^{1}/_{100}$, $^{1}/_{1000}$ dilutions of the samples were prepared. Before the setting of the organic matter and soil particles, 1 ml of the dilutions was applied to prepared plates of malt extract (MEA) and cornmeal agar (CMD) with 2% dextrose) both with 50mg/L streptomycin and 10mg/L cyclosporine.

For isolation using the soil washing technique, 10g of soil was sieved in a set of 4.0 mm, 1.0 and 0.5 mm sieve. This was done by suspending 10g of the soil in 2L tap water and pouring through the nest of the sieves. The procedure was then repeated with 2L of sterile water. After this treatment, the contents of the first mesh which were bigger in size were surface sterilized by transferring the contents into a sterile Petri dish with sterile water containing streptomycin. Organic particles floating on the surface of the water and the washed soil particles were picked up with a loop and forceps and transferred onto plates of MEA and CMD (Cornmeal agar with 2% dextrose) both with streptomycin 50mg /L and cyclosporine 10mg/L. Two replicates per media were used. The small pieces of debris retained on the other two sieves could not be surface sterilized because they were too small and porous. The debris was damp-dried on sterile paper towels and then dried over silica gel for 24 hours before plating on the isolation media. The plates were incubated at 25^{o}C for two weeks (Gams *et al*., 1987).

The colonies were counted and identified using the soil dilution plate method. The identified colonies were transferred to Petri dishes containing PDA (potato dextrose agar) and incubated at 15, 25, 30 and 35^{o}C for further identification to species level. Colonies developed from the isolates using the soil washing technique were also identified.

Identification of *Trichoderma* species

Genus identification of green fungus was undertaken using the method of Domsch *et al*., (1980). *Trichoderma* isolates were identified at species level following the taxonomic key of the genus *Trichoderma* by Samuels *et al*, (2004). Colony characters, growth rates in culture and morphological characters were used in identification. Microscopic examination was carried out by mounting the culture in lactophenol cotton blue but for size measurements KOH and water was used as the mounting fluid. A small amount of material was placed in a drop of 3% KOH on a slide and then replaced with water.

Statistical Analysis

Analysis of the distribution of *Trichoderma* across land use systems in Embu and Taita regions was done using R version 2.1.1. Ordination plots were used to compare species compositions with LUTs (Kindt and Coe, 2005). Variations in the data were examined by performing a multi-level Analysis of Variance (ANOVA) using Genstat 9th edition.

RESULTS

A total of 299 *Trichoderma* isolates were obtained from soil samples collected from Embu benchmark site (Plate 1). The number of isolates obtained method were 271 and 28 from the soil dilution plate and soil washing technique respectively. Identification of the isolates resulted into 9 species of *Trichoderma* (Tables 1a). Of the nine species seven were isolated with the soil dilution plate technique and two from both methods (*T. harzianum* and *T. citrinoviride*). From the soil samples collected from Taita benchmark site, 309 isolates were recovered (273 from soil dilution plates and 36 from the soil washing technique, Table 1b) and they were assigned to 11 species (10 species from soil plates and 1 species *T. harzianum* from soil washing technique). The most frequently isolated species from both Embu and Taita sites was *T. harzianum* (Table 1). This was also the most abundant species in the two regions. *T. harzianum* was observed across all the LUTs. *T. aggressivum* was isolated in Embu while *T. reesei*, *T. asperellum* and *T. polysporum* were observed only in Taita.

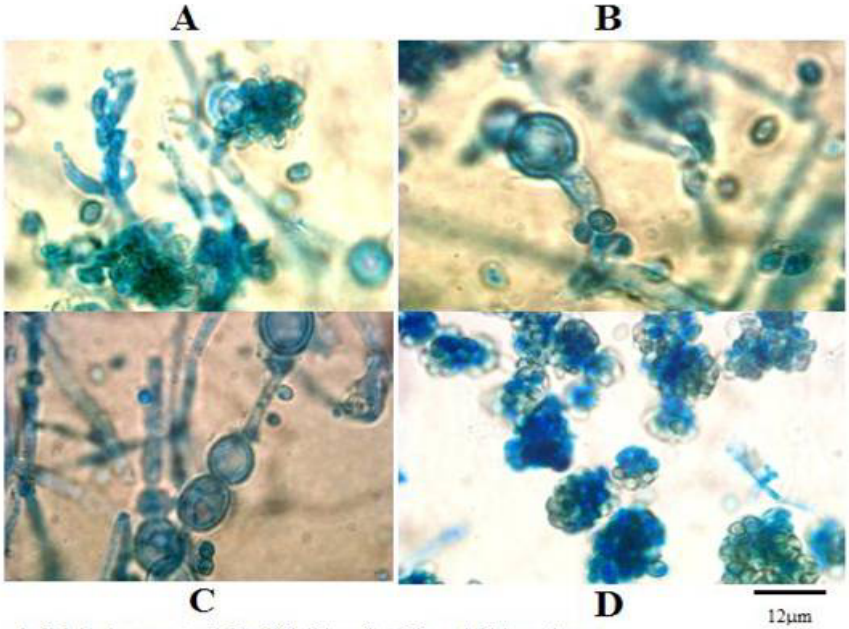
Table 1. Frequency of isolation of *Trichoderma* species from the different land use types.

Land use type	TC	TS	TH	TK	TAG	TAV	TV	TAS	TST	Total
Embu region										
Coffee	8	4	8	4	0	0	8	0	0	32
Fallow/Pasture	0	0	16	0	0	4	0	7	6	33
Maize	0	4	16	0	0	0	8	0	4	32
Napier	35	0	28	0	0	0	4	0	0	67
Indigenous forest	0	0	36	0	4	4	16	0	0	60
Planted forest	0	0	36	0	0	0	4	0	0	40
Tea	4	0	20	0	0	0	0	11	0	35
Total frequency of isolation	47	8	160	4	4	8	40	18	10	299

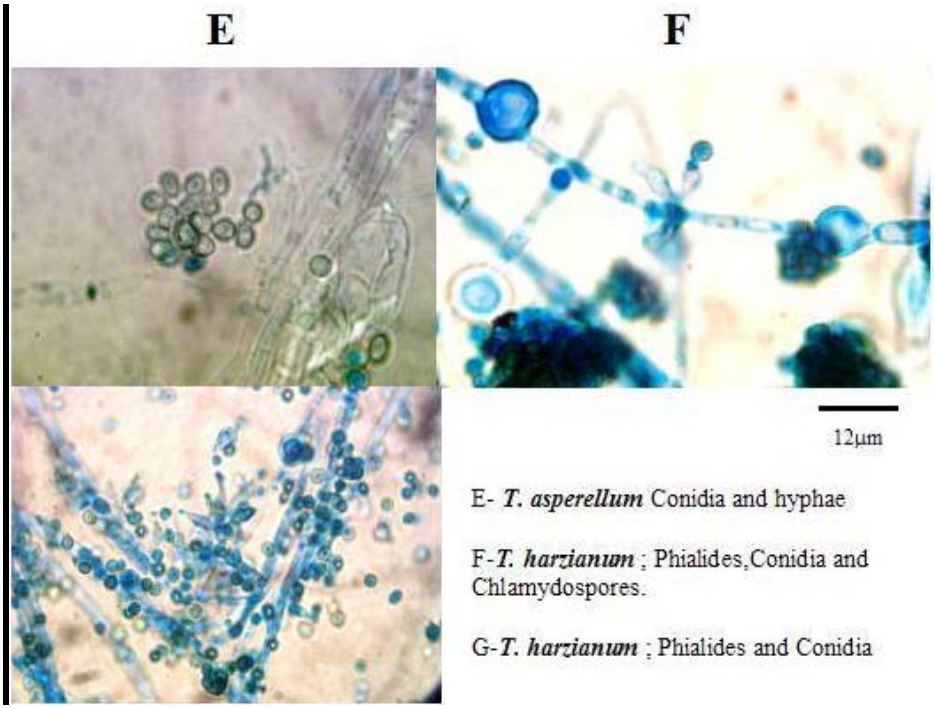
Land use type	TR	TS	TH	TA	TP	TK	TAV	TV	TAS	TST	Total
Taita region											
Coffee	2	0	4	0	0	7	0	0	0	0	13
Fallow	4	0	25	0	0	3	0	18	0	2	60
Horticulture	0	2	21	0	3	10	5	7	3	0	51
Indigenous forest	0	0	37	6	0	8	10	6	2	0	77
Maize	0	5	5	2	0	2	0	4	0	2	25
Napier	0	0	16	2	2	4	0	5	0	0	41
Planted forest	0	0	26	0	0	0	0	16	0	0	42
Total frequency of isolation	6	7	134	10	5	34	15	56	5	4	309

Key to fungal species

TC = *Trichoderma citrinoviride,* TK = *T. koningii,* TR = *T. reesei* TAV = *T. atroviride*
TS = *T. surrotunda* TV = *T. viride* TH = *T. harzianum* TST = *T. stromaticum*
TA = *T. aggressivum* TAS = *T. asperellum* TP = *T. polysporum*



A-***Trichoderma atroviride***; Phialides, Conidia and Chlamydospores.
B- ***T. atroviride***; Conidia and Chlamydospores.
C- ***T. atroviride*** ; Conidia and Chlamydospores
D- ***T. asperellum***; Conidia whorls



E- ***T. asperellum*** Conidia and hyphae

F-***T. harzianum*** ; Phialides,Conidia and Chlamydospores.

G-***T. harzianum*** ; Phialides and Conidia

Plate 1 : *Trichoderma* isolates showing conidia, phialides and chlamydospores

Distribution of *Trichoderma* was compared within LUTs. Napier LUT at the benchmark sites where soils under napier grass recorded the highest richness, abundance and diversity, Tables 2, 3 and 4. Despite horticulture, indigenous forests and maize LUTs having a higher total richness of seven species compared with napier grass with six species the latter recorded the highest mean abundance and diversity in both Embu and Taita. This implied that a sample of soil collected from napier grass soils would have more *Trichoderma* propagules than those from Indigenous forest. Planted forest and coffee LUTs are the least diverse. The effect of land use on the distribution of *Trichoderma* was significant both in Embu and Taita at α=0.05 (Embu :p=0.04937; Taita: p=0.005364), Table 5. The ANOVA also gives important information on the deviance explained. For Embu data, the model only explained 13.954 of the total or null deviance of 163.583 (8.53%) while for Taita the deviance explained is 19.37 of the total or null deviance of 134.890 (14.36%). This showed that there were other factors that influenced the occurrence of *Trichoderma* other than just the LUTs.

Table 2. Richness of *Trichoderma* across land use types in Embu and Taita benchmark sites.

Land use type	Mean	Total	Jackknife Estimate
Embu			
Coffee	0.444	5	6.888889
Fallow/Pasture	0.562	4	4.937500
Maize	0.500	4	5.875000
Indigenous forest	0.938	4	5.875000
Napier	1.214	3	3.928571
Tea	0.450	3	3.950000
Planted forest	0.556	2	2.944444
Taita			
Horticulture	1.786	7	7.000000
Indigenous forest	1.192	7	7.961538
Maize	1.000	7	8.857143
Fallow	0.906	6	7.937500
Napier	2.125	6	7.750000
Coffee	0.500	3	4.800000
Planted forest	0.800	2	2.000000

Table 3. Abundance of *Trichoderma* across land use types in Embu and Taita benchmark sites.

Land use type	Mean	Total	Jackknife Estimate
Embu			
Napier	9975.000	139650	269325.00
Indigenous forest	7389.375	118230	229070.63
Planted forest	3662.778	65930	128197.22
Maize	3831.250	61300	118768.75
Fallow/Pasture	1871.875	29950	58028.13
Coffee	1301.667	23430	45558.33
Tea	1164.000	23280	45396.00
Taita			
Indi. forest	2706.538	70370	138033.46
Planted forest	2240.000	44800	87360.00
Napier	5211.250	41690	78168.75
Horticulture	2883.571	40370	77856.43
Fallow	1135.312	36330	71524.69
Maize	1338.571	18740	36141.43
Coffee	972.000	9720	18468.00

Table 4. Diversity (Shannon) of *Trichoderma* across land use types in Embu and Taita benchmark sites.

Land use type	Mean	Total	Jackknife Estimate
Embu			
Tea	0.023	1.091	1.5612413
Coffee	0.020	1.047	1.4721721
Maize	0.060	0.914	1.1307564
Fallow/Pasture	0.063	0.844	0.9639275
Napier	0.192	0.722	0.7673178
Indigenous forest	0.099	0.532	0.5813863
Planted forest	0.011	0.079	0.1024284
Taita			
Horticulture	0.386	1.703	2.0190577
Maize	0.118	1.604	2.2119018
Indigenous forest	0.083	1.547	1.7866016
Napier	0.445	1.512	1.7973558
Fallow	0.128	1.342	1.5184411
Coffee	0.066	0.788	1.0804125
Planted forest	0.000	0.685	0.7333187

Table 5. Influence of land use types on the distribution of *Trichoderma* : Analysis of deviance table.

		Degrees of Freedom (df)	Deviance(Dev)	Residual df	Residual Dev	F-value
Embu Region	Land use types	6	13.954	111	149.6	0.049
Taita Region	Land use types	6	19.373	117	115.5	0.005

The correlations between the LUTs and species composition aws 0.9441, $p<0.01$ in Embu and 0.8243, $p<0.01$ in Taita indicating larger correlations in Embu than in Taita. The cluster analysis grouped LUTs in Embu into two major categories based on similarity in species composition, Fig 1. Tea, coffee and napier LUTs are more similar to each other as shown by the clustering tree in Fig. 1a and are less diverse as displayed by the ordination graph Fig 1b. Most of the species were recovered from the forests, maize and fallow cluster. Tea farms did not attract fungal diversity. In Taita the clustering pattern is quite different from Embu, with coffee being ecologically distant from napier and maize far from fallow (Figure 2a and 2b). The forests are clustered together in both sites and together with fallow and maize are on the same side of the ordination graphs. Most of the species were recovered from the forests, napier grass, horticulture and maize LUTs with coffee farms attracting the least.

No consistent associative relationships between individual species and their occurrence in different LUTs were observed. *T. harzianum, T. surrotunda and T. stromaticum* were more predominant in soils under forests, maize and fallow LUTs. A PCA analysis of the data found that Factors 1 and 2 explained 84% of the variance in Embu and 65% in Taita indicating strong representation of the described relationship.

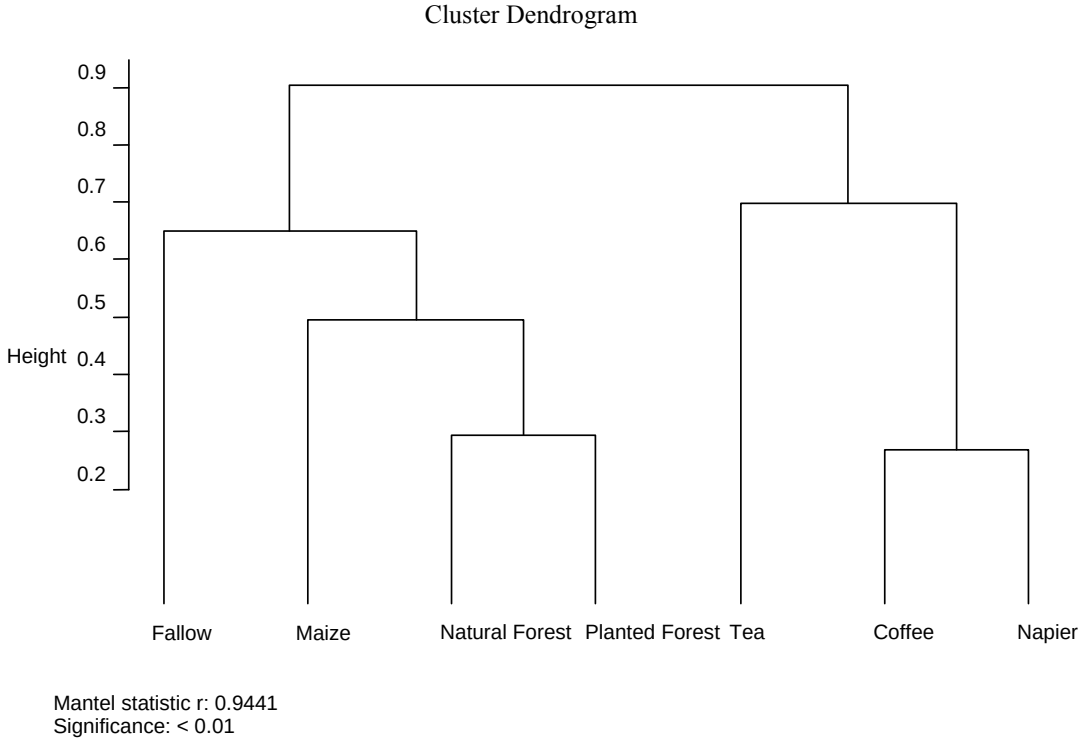
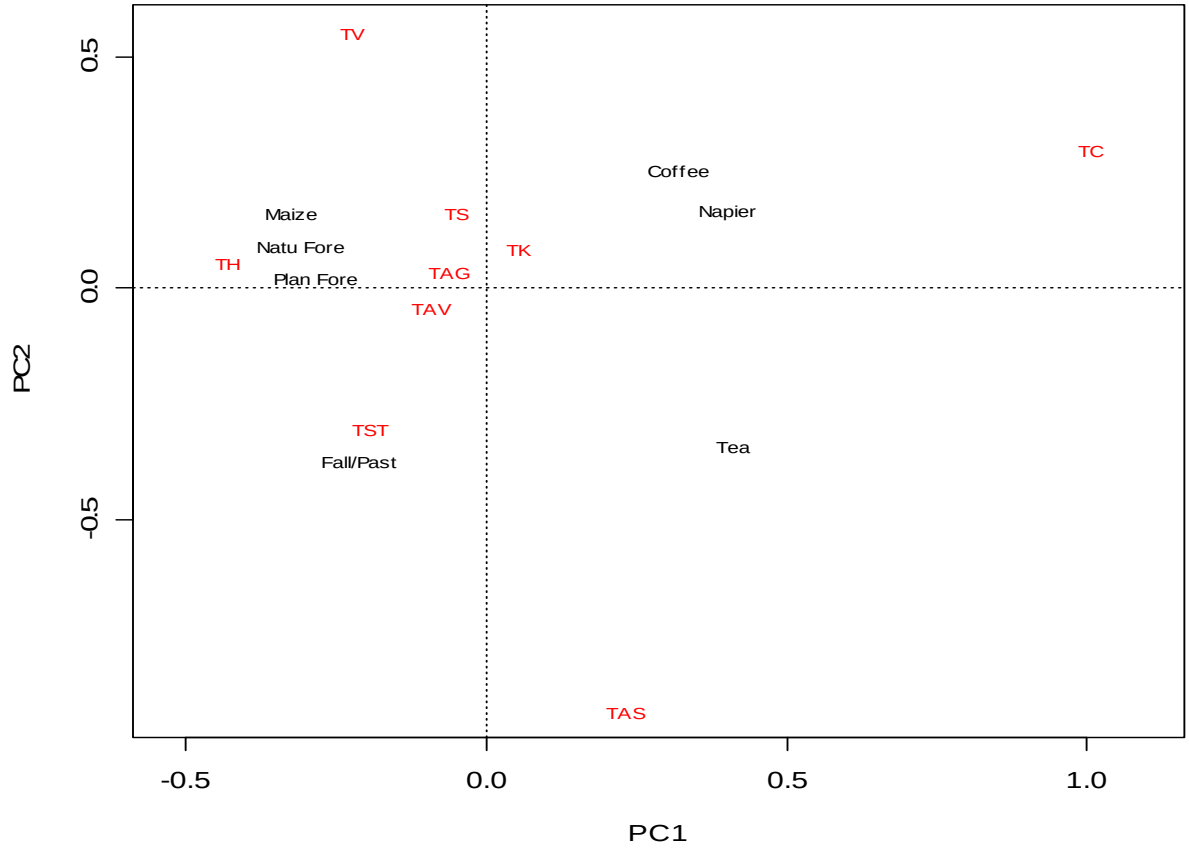


Figure 1a. Land use types clustering at the Embu benchmark site



Eigen values and their contribution to the variance

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Lambda	0.1711	0.08596	0.02694	0.01388	0.005235	0.00159
Accounted variance	0.5616	0.84365	0.93205	0.97760	0.994782	1.00000

Key to fungal species

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Figure 1b. Influence of LUT on *Trichoderma* spp. occurrence in Embu: Ordination graph

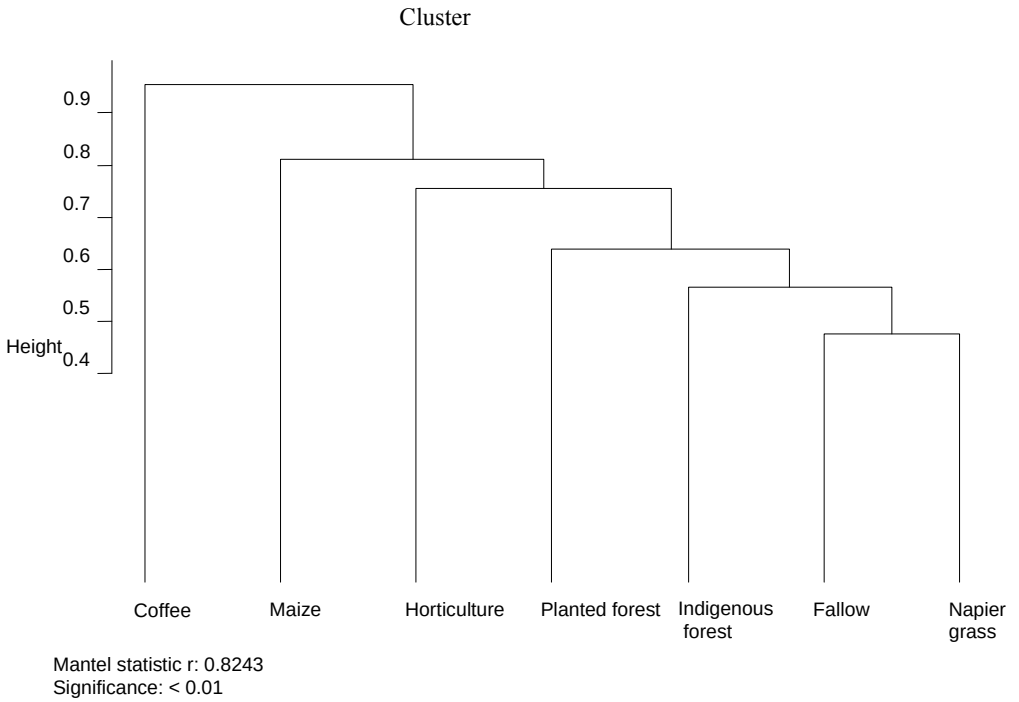


Figure 2a. Land use types clustering at the Taita benchmark site.

Planted forests in Taita were the most even in the distribution of *Trichoderma* while maize LUT was the most uneven. Although coffee had the least abundance of *Trichoderma*, in Taita, it had more even distribution of the fungus than fallow, napier, horticulture, indigenous forest and maize LUTs. The same results were evident in Embu where tea had the least abundance of the fungus and yet had the highest evenness, Figure 3 and 3b.

DISCUSSION

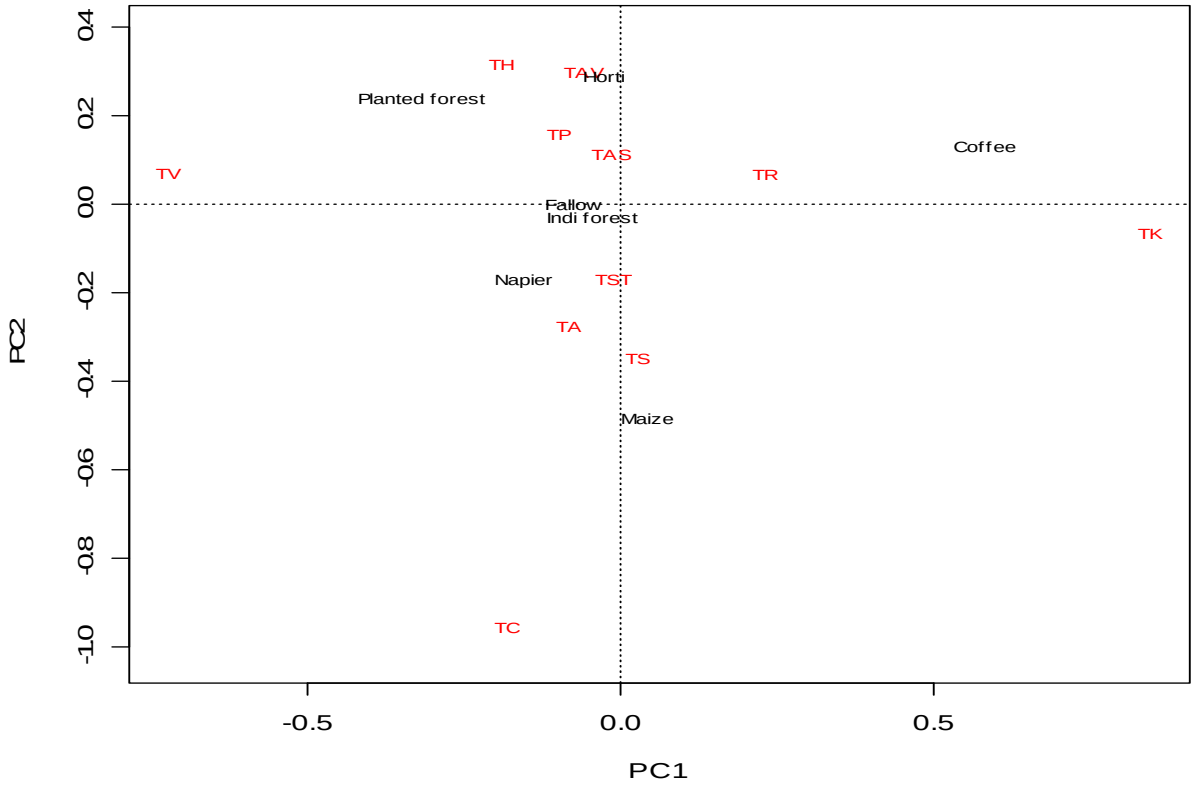
The soil dilution technique proved to be more useful in isolating *Trichoderma* sp. from the soil compared with the soil washing technique. *Trichoderma* is a fast growing fungus with proclivity for conidiation. The results of the abundance counts confirm that the propagules are so numerous in the soil making the simple dilution plating method adequate for a survey like this study. The soil washing technique aims at sieving out the spores of the many fungi in the soil and to end up with actively growing mycelium. As indicated by the poor isolation obtained from the soil washing technique, most of the *Trichoderma* propagules were spores and these were washed out in this technique. Hence for *Trichoderma* isolation, the dilution plating technique remains adequate. These findings agree with those of Klein and Eveleigh (1998) who reported that CFUs obtained through direct plating reflected the numbers of conidia lying dormant in the soil, rather than active mycelial mass. They further indicated that as *Trichoderma* spores are numerous, even washing of the samples may remove only an insignificant proportion of the conidiophores, 99% removal could still result in the majority of CFU colonies arising from conidia rather than mycelium.

Trichoderma is distributed in soils of Embu and Taita confirming that they are ubiquitous soil dwellers, though regional difference in richness, species occurrence and abundance were observed. Taita soils were more diverse compared with Embu soils and the differences could be due to geographical locations and soil type (Muya *et al*, 2005, Garbeva *et al.,* 2004). Influence of geographical location on distribution of *Trichoderma* was also reported by Turner *et al.,* (1997) through the use of RAPD-based screening. Even with a limited number of cultures available to him, Turner *et al.,* (1997) showed that some species of the fungus overlapped through much of their geographic ranges while others were recovered in only specific regions. The variations in distribution of *Trichoderma* could also be attributed to the isolation methods used and LUTs. For example the fallow in Taita may be different from a fallow in Embu either in terms of the number of years it has been left fallow or whether it is a re-current fallow.

Although LUTs were a major factor in determining the distribution, diversity and abundance of the fungus

other factors such as plant type, mixed cropping and rooting system also influenced these trends significantly. Horticulture and mixed cropping systems constituted varied ecological niches for the existence of the fungus in terms of litter types for decomposition and necrotrophisms with the host plant soil pathogens. Strains of *T harzianum* and *T. viride* are known to be strongly rhizosphere competent and proliferate best when there are abundant healthy roots (Hjeljord and Tronsmo, 1998; Yedidia, 1999) as they are also attracted by root exudates. This may explain why the fungus was abundant in soils under napier grass in both sites Further higher probability of *Trichoderma* occurrence in Taita may have been due to litter types and quality. Ibekwe *et al.*, (2002) and Grayston *et al.*, (1998) also reported that plant type occurring played a major role in determining the occurrence of fungi and fungal species.

Unevenness in distribution of the fungus within the LUTs suggested yet another factor influencing the distribution of *Trichoderma* apart from the LUTs and geographical locations. Unevenness was greater in Embu than in Taita. Damping was also confirmed by the ANOVA test with the deviance giving evidence that apart from the LUTs, there are other factors that influence the occurrence of *Trichoderma*. These differences can be attributed to variations in individual farmer soil management practices that would be expected with different land owners. Several researchers have reported that soil management practices such as crop rotation, tillage, fertilizer use, composts, manure, or pesticide applications and irrigation greatly affect soil microbial parameters (Roper and Gupta, 1995; Toresani *et al.*, 1998).



Eigen values and their contribution to the variance

	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6
Lambda	0.1091	0.09735	0.05784	0.03092	0.01375	0.008479
Accounted variance	0.3437	0.65038	0.83258	0.92998	0.97329	1.000000

Key to fungal species

TC = *Trichoderma citrinoviride*, TK = *T. koningii*, TR = *T. reesei* TAV = *T. atroviride*
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Figure 2b: Influence of land use type on *Trichoderma* species occurrence in Taita: Ordination graph.

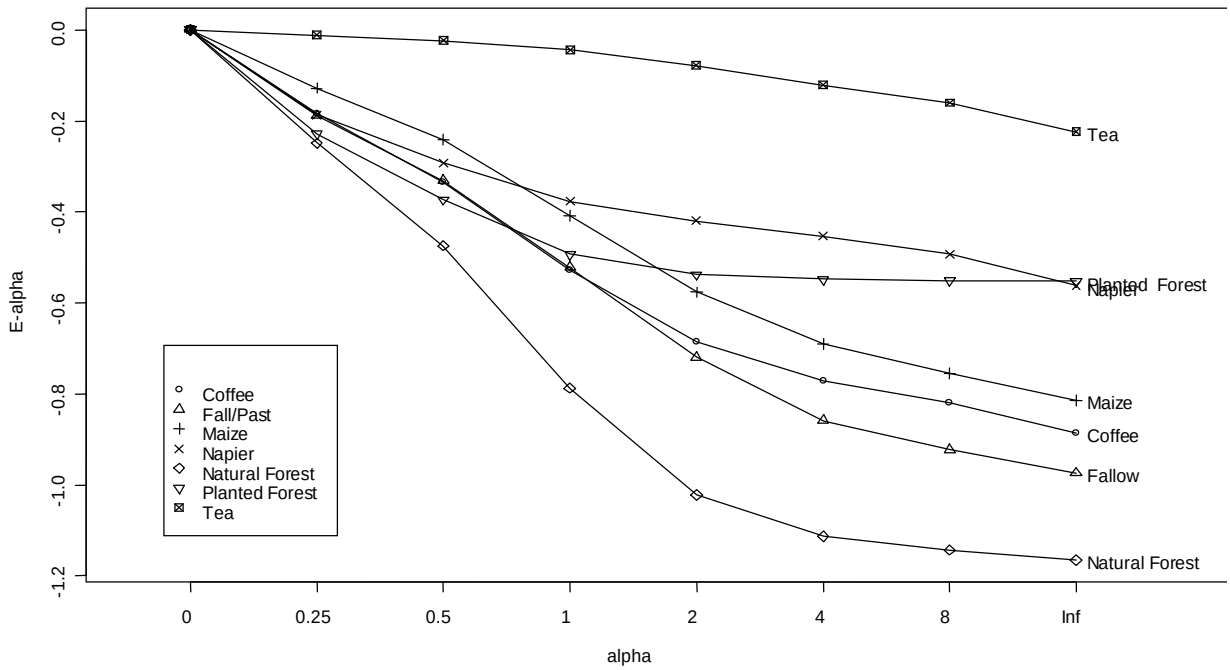

Figure 3a. Rényi evenness profiles in Embu benchmark site

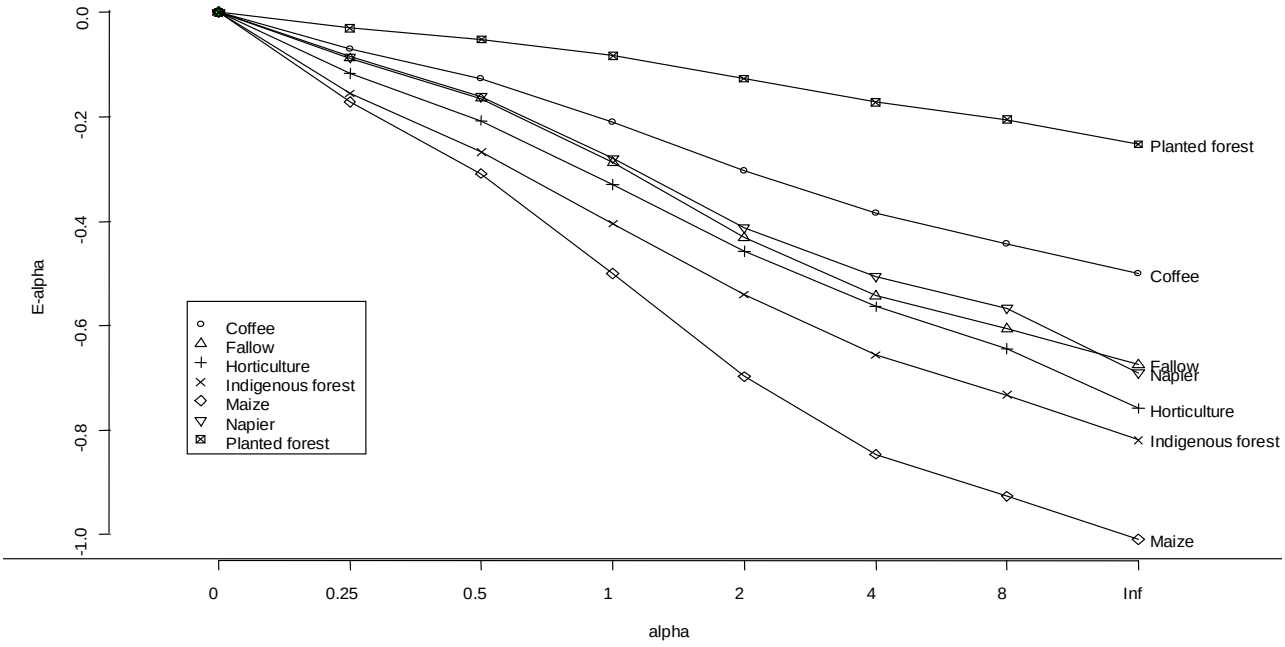

Figure 3b: Rényi evenness profiles in Taita benchmark site

CONCLUSION

Soil type, LUTs and soil management practice were some of the factors that influenced the distribution and abundance of *Trichoderma* sp. while soil type influence species occurrence, LUTs and management practice mostly determine species abundance and evenness.

The abundance of *Trichoderma* species in some LUTs, provided a clues on the most preferred habitats including plants and/or crops. Considering the beneficial aspects of *Trichoderma* such as being antagonistic to the pathogenic fungi and enhancing growth vigour in plants such as napier grass that attracted high abundance and richness of *Trichoderma* could be used in crop rotations or intercrops ensuring high levels of the fungus in the soils. Colonization of the younger roots by the fungus would have a dual effect of increasing efficient nutrient uptake by the plant while controlling soil borne plant pathogens.

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