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INFLUENCE OF SOIL CHEMICAL AND PHYSICAL PROPERTIES ON *TRICHODERMA* SPP OCCURRENCE IN TAITA REGION

[INFLUENCIA DEL LAS PROPIEDADES QUIMICAS Y FISICAS SOBRELA OCURRENCIA DE TRICHODERMA SPP EN LA REGION DE TAITA, KENIA]

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SUMMARY

Agricultural intensification has been cited as one of the causes of loss of biodiversity. Taita Taveta, a coastal highland region is such like area which has experienced land conversion from forest cover into farmlands with high soil intensification. Soil samples were collected across seven land use types and analysed for *Trichoderma* occurrence. The fungus was recovered from the soil using the dilution plate and soil washing technique. The remaining soil samples were used to analyse for pH, acidity, organic carbon (C) total nitrogen (N), organic matter (OM), calcium (Ca) phosphorus (P) sodium (Na), potassium (K), magnesium (Mg), Manganese (Mn), Iron (Fe), and Zinc (Zn), and copper (Cu). Soil texture, bulk density, porosity and available water capacity was also determined.

Land use type (LUT), plant cover, and soil physical and chemical properties influenced *Trichoderma* occurrence. The frequency of isolation of *Trichoderma* spp. was highest in indigenous forests followed by fallow and horticulture LUTs. Maize and Coffee LUTs recorded the least. The most frequently occurring species was *T. harziunum* which was isolated from all land use types LUTs. Carbon, N, organic matter and Fe were high in soils collected from forests thus suggesting they influenced fungal diversity. The forests had clay loam soils with higher porosity and water retention capacity compared with the cultivated LUTs and fallow which were characterized with clay soils and high bulk density, pH, Na, P, K, Ca, Mg, Cu showing the effect of land conversion on soil properties and *Trichoderma* occurrence. The influence of plant type and land management is also seen in the cultivated LUTs with the same soil properties but with different diversity and abundance of *Trichoderma*. The diversity of soil factors observed in the maize plots explained the influence of land management on soil physical and chemical characteristics which in turn determined the fungal distribution. Soil depth (0-20cm) did not influence soil factors though fungal diversity, abundance and evenness varied with depth suggesting the influence of substrate availability. Occurrence of *Trichoderma* spp. and distribution in soil is determined by a number of interacting biotic and abiotic factors.

Keywords: Soil physical and chemical properties; *Trichoderma* spp.; land use

INTRODUCTION

Trichoderma is a genus of fungi that is present in all soils, where they are the most prevalent culturable fungi. They are saprophytes with high antagonistic activities against soil-borne fungal pathogens. Many species in this genus can be characterized as opportunistic avirulent plant symbionts. These abilities make them quite competitive in the soil rhizosphere and thus the fungus is available commercially for control of soil born pathogens (Butt *et al*., 2001). As more strains are being suggested for bio-control preparations, their effectiveness requires an indepth understanding of their soil and root ecology.

Land conversion and intensification alter the biotic interactions and patterns of soil resource availability. For example addition of fertilizers in soils alters their chemical and physical properties which in turn affect the environment of soil inhabitants (Kaiser, *et al*., 1992). The physical properties of soil that are improved by amendments include soil structure, porosity, and water-holding capacity. Poor soil physical characteristics directly constrain root growth and in turn may have direct effect on microbial rhizosphere inhabitants. Several species of *Trichoderma* have been found to have antagonistic and root colonizing behavior (Subhendu and Sitansu, 2007). They are also the main decomposer of cellulose in acid soils where bacteria and actinomycetes become inactive. In other soils *Aspergillus* and *Fusarium* are known to participate more in cellulose decomposition

(Saria *et al.,* 2005). Understanding the effect of soil factors on *Trichoderma* spp may provide information on abiotic soil factor that influence on the bio-control activity of the fungus. Understanding bio-control mechanisms and their regulation in situ could be applied in selection of strains to be applied at different soil environments.

The objective of this study was to assess the effect of soil properties in different land use systems on the distribution of *Trichoderma* spp.

MATERIALS AND METHODS

Study site description

The study was conducted in Taita Taveta which is a coastal highland with the highest point which is mountain indigenous and planted forests at 1952 metres above the mean sea level. Agroforestry and shrubs are found on the footslopes, hills and uplands while annuals and perennials dominate the bench-terraced uplands. Mixed cropping and horticulture dominate the bottomlands. The major soil types are Plinthic Lixisols, Plinthic Acrisols, Dystric Cambisols and Chromic Luvisols.The variation in soil characteristics has been reported to vary with land use types associated with land use intensification gradients. The steep land use intensity gradients from forest (LUI<0.05) to horticultural and maize-based systems (LUI>40%) corresponded with the observed soil quality trends, where the highest level of soil organic carbon recorded was 7.6% in the forest and the lowest value of 1.6% in intensively cultivated maize-based and horticultural systems. Similarly the highest level of nitrogen and phosphorous were recorded in natural grassland and forest with values ranging from 0.47 to 8.7%, while the lowest was observed in the most intensively cultivated horticultural and maize-based systems with values ranging from 0.18 to 0.47% (Muya *et al*., 2009)

Soil sample collection

Sixty points which were 200m apart were marked in a grid-mesh construction using GPS markings. The points fell within seven land use systems (LUTs): Coffee farming (*Coffea arabica*), Maize based farming (*Zea mays*), Horticulture, Fallow plots (mainly *Indigofera arrecta* and *Ocimum gratissimum*), Napier farms (*Pennisetum purpureum*), Planted forests of *Pinus* and *Cupressus*, and Indigenous forests (mainly *Craibia zimmermannii* and *Cola greenwayi.*
Circles of 3m and 6m radius were marked round each sampling point where four and eight soil samples were taken at 0-10cm and 10-20cm depths using soil auger respectively. The samples were mixed thoroughly to make a composite sample which was used for *Trichoderma* isolation The soil were from the sixty points were placed in paper bags then transported to the laboratory where they were stored at 2- 5^{o}C.

Soil chemical characteristics

The remaining soil samples were used for pH was determined in 1:1 (w/v) soil – water suspension with pH meter. Total nitrogen was determined by the Kjeldahl method (Page *et al.,* 1982). Available nutrients (P, K, Na, Ca and Mg) were determined using Mehlich method (Anderson and Ingram, 1993).Total organic carbon was determined by oxidation, (Nelson and Sommer, 1982).

Isolation of *Trichoderma* spp.

Trichoderma spp. were isolated from the 120 soil samples using the soil dilution plate (Johnson *et al*., 1959) and soil washing methods (Gams *et al*., 1987; Bills & Polishook, 1994). Soil dilutions were prepared and one milliliter of each of the dilutions applied to plates containing malt extract (MEA) and cornmeal agar (CMD) with 2% dextrose) both with streptomycin 50mg/L and cyclosporin 10mg/L antibiotics.

The soil washing technique was used for isolation of *Trichoderma* and , 10g of soil was sieved in a nest of 4.0 mm, 1.0 and 0.5 mm sieve. This was done by suspending 10g of the soil in two litres 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 large organic particles 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 antibiotics. 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 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 carried out 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 characteristics, growth rates in culture and morphological characters were used for 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.

Soil chemical characteristics

The remaining soil samples were used for pH was determined in 1:1 (w/v) soil – water suspension with pH meter. Total nitrogen was determined by the Kjeldahl method (Page *et al.,* 1982). Available nutrients (P, K, Na, Ca and Mg) were determined using Mehlich method (Anderson and Ingram, 1993).Total organic carbon was determined by oxidation, (Nelson and Sommer, 1982).

Statistical Analysis

Distribution of *Trichoderma* across land use systems in Embu and Taita regions was compared using R analysis version 2.1.1, (Kindt and Coe, 2005) and analysis of variance (ANOVA). Ordinations were done to relate species compositions in with LUTs and soil physical and chemical characteristics. Shannon diversity index was used to compare the diversity and abundance of *Trichoderma* with depth. Renyi profiles compared the evenness of the fungus with depth.

RESULTS AND DISCUSSION

Soil Characteristics and Land Use Types

Acidity, pH, C, N, Fe, and OM were significantly different with land use but not with depth (Table 1). The readings of these soil characteristics were highest in the forests and this could have been the reason for the high diversity and abundance of this fungus in this LUT. Mineral nutrition is essential for growth, sporulation and stimulation of fungal secondary metabolism (Griffin, 1994). High total N availability increased sporulation, production of antifungal anthroquinone pigments, hyphal growth rate (Fargasova, 1992), and antagonistic activity of *Trichoderma* spp. against wood rot fungus *Serpula lacrymans* (Score and Palfreyman, 1994). Soil nitrate levels were positively correlated with cellulose production (Widden and Breil, 1988) and may favor competitiveness of the bio-control agent with the pathogen. Magnesium increased growth of *T. viride* (Shukla and Mishra, 1970), and copper enhanced conidiogenesis and biomass nitrogen in other hyphomecetes (Ismail *et al*., 1991; King *et al*., 1982). However, there seemed to be other factors that favored the occurrence of the fungus in fallow and horticulture LUTs that recorded higher fungal isolation than the planted forest. These other factors could be the variability of substrate types for the fungus that would be found in these LUTs.

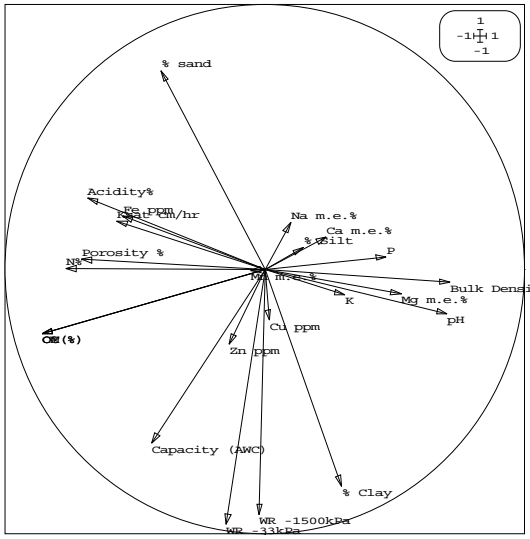
The ordinations of soil properties with LUTs showed that cultivated land were quite similar in their soil chemical and physical characteristics and correlated positively with Na, P, K, Ca, Mg, Cu and pH. The forest soils correlated with organic matter, nitrogen, acidity, iron and porosity though they produced larger ellipses indicating high variability within them (Fig 1a, b). The eigen values of the first and second axes constrained to the soil characteristics was 0.4375. The distribution of the inertia indicated that axes 1 and 2 accounted for 43.75% of the variations. The sum of all canonical eigen values revealed that the variables that significantly contributed to the description of the variations in the soil characteristics with LUTs explained 65.3% of the total variation observed. The factor grouping forest soils on one side and those from cultivated fields on another could be associated with fertility management practices with soil inputs in the cultivated fields influencing the soil properties. The second factor could be parent rock resulting into the wide variation observed within the forest soils. This grouping also shows the negative effects of land intensification on the occurrence of *Trichoderma* which was greater in the indigenous forest.

Soil texture ranged from sandy loam in indigenous forest through sandy clay loam in fallow, horticulture and napier LUTs to clay in maize LUT (Fig 1c). Soil porosity and bulk density varied significantly with LUT with the forest soils recording highest porosity and lowest bulk density (Table 2). The run off from the forests at the hill tops could have resulted to the clay type of soil found in the cultivated fields which are on the slopes and valley bottoms. The soil texture varied extensively but correlated with *Trichoderma* distribution wit the forests (with clay loam soils) favoring fungal occurrence rather than clay soils found in cultivated fields. The proportions of soil textural components such as clay has a major influence on water flow, oxygen availability, and the matrix for fungal growth and dispersal (Schippers *et al*., 1987). Clay minerals have been reported to reduce respiration of *T. viride* (Stotzky and Rem, 1967).

Table 1: Soil chemical characteristics as influenced by land use intensification

LUT	Mean values													
	pH	Exchange Acidity	%C	%N	OM %	Ca cmol/kg	P ppm	Na cmol/kg	K cmol/kg	Mg cmol/kg	Mn cmol/kg	Fe ppm	Zn ppm	Cu ppm
Coffee	4.789	0.390	1.777	0.203	3.06	2.06	14.4	0.188	0.245	2.98	0.340	41.1	3.77	0.675
Horticulture	4.776	0.330	1.569	0.195	2.70	2.18	53.4	0.356	0.311	2.66	0.807	52.3	3.42	1.075
Maize	4.587	0.307	1.683	0.203	2.89	2.57	12.5	0.201	0.383	2.19	0.695	31.1	4.50	1.903
Napier	4.934	0.340	1.891	0.284	3.25	3.40	58.2	0.245	0.758	3.71	0.527	44.1	6.16	1.760
Fallow	4.408	0.781	1.681	0.247	2.89	3.50	42.1	0.329	0.409	2.38	0.398	48.2	1.88	0.802
Planted Forest	3.832	1.858	2.480	0.388	4.27	2.80	8.2	0.280	0.249	1.44	0.597	93.5	4.01	1.099
Indigenous Forest	3.724	1.192	2.554	0.424	4.39	2.72	27.2	0.268	0.232	1.71	0.612	81.8	3.40	1.550
P values	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.064	0.127	0.359	0.017	0.019	0.021	<0.001*	0.003*	0.005*

*Significant at p = 0.005



Num. Eigenval. R.Iner. R.Sum |Num. Eigenval. R.Iner. R.Sum |
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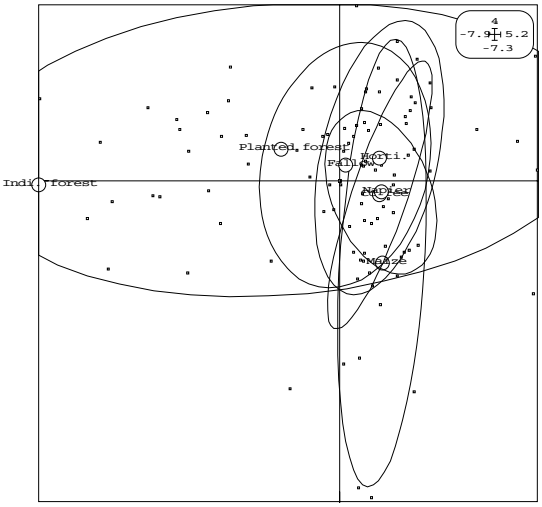
03 +2.8621E+00 +0.1193 +0.5568 |04 +2.3101E+00 +0.0963 +0.6530 |

Figure 1a: Correlation circle of the soil characterization



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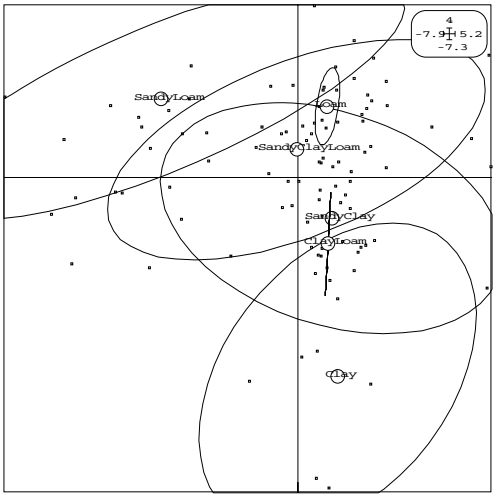

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Figure1b: Ellipses for the land use types in relation to soil properties correlation circle
Figure.1c: Ellipses for the land use types in relation to soil texture correlation circle

Table 2: Variation of soil physical properties with land use and depth: Mean values and Analysis of variance

Land Use Type (LUT)	Mean values					
	Clay	Silt	Sand	Available water capacity	Porosity	Bulk density
Coffee	30.5	17.00	52.5	0.2068	54.80	1.197
Fallow	29.2	14.72	56.1	0.2008	56.84	1.143
Horticulture	26.6	17.60	55.8	0.2004	54.00	1.217
Indigenous Forest	28.3	11.25	60.4	0.2110	58.58	1.095
Maize	40.6	13.29	46.1	0.2091	55.50	1.174
Napier	30.8	10.50	58.8	0.2001	55.62	1.174
Planted Forest	28.2	9.38	62.4	0.2069	70.69	0.775
P - value with LUT	0.018	0.076	0.002*	0.369	<0.001*	<0.001*
P - value with depth	0.856	0.986	0.772	0.138	0.005*	0.005*

*Significant at p=0.005

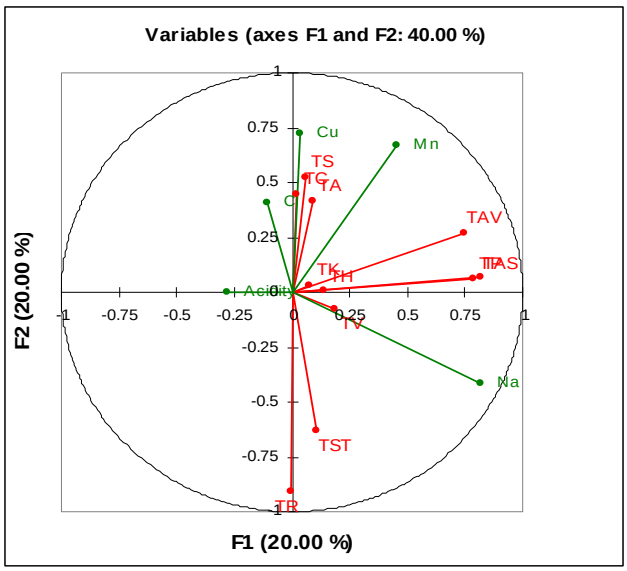

Figure 2: Correlation of *Trichoderma* spp. with soil chemical nutrients

KEY

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*		

Trichoderma spp correlated negatively with acidity (Fig 2). Soils in planted forests were more acid with the least fungal occurrence. Of the soil variables that significantly varied with land use (C, Cu, Fe, N, Zn) C, Cu, N were high in the indigenous forests correlating positively with fungal occurrence. Fe correlated positively with C and was high in the soils of the forests. However the most common species, *T. harziunum*, showed very low loading with the nutrients. *T. Surrotunda, T. citrinoviride* and *T. aggressivum* were attracted to Cu and C while *T. atroviride, T. asperellum, T. polysporum, T. harziunum, T. viride* and *T. koningii* were attracted to Mn and Na. However these nutrients also correlated positively or negatively with each other (Table 3).

The soil samples resulted into 309 isolates whose identification resulted into 11 species, with the highest total frequency of isolation (TFS) of 77 from indigenous forest followed by fallow and horticulture LUTs with a values of 60 and 51 respectively (Fig 3). Maize (TSF=25) and Coffee (TSF=13) LUTs recorded the least. Planted forest and napier LUTs had total frequency of isolation of 42 and 41.The most frequently occurring species was *T. harziunum* which was isolated from all land use types LUTs.

Top soil recorded a higher abundance, diversity and evenness compared to the soil beneath. Fungal richness was not influenced by depth examined (0-20cm) (Table 4, Fig 4). Substrate availability in the top soil is the reason for this pattern of distribution. Studies on colonization and succession of fungi during decomposition has shown that *Trichoderma* are late colonizers of fresh litter and normally found underneath the litter on top soil, Takashi, 2005; Takashi, *et al*., 2006. Here they probably degrade non-lignified holocellulose. The cellulolytic activities of the fungus explain its occurrence mostly on the top soil. The unevenness in distribution of the fungus in the lower depth can also be attributed to availability of substrate, the fungus preferring root rhizosphere. *Trichoderma* spp have been reported to flourish in plant root rhizosphere than most soil fungi (Tsahouridou and Thanassoulopoulos, 2002). Younger roots have been recorded to produce more of these exudates than older once (Garland, 1996, Harman and Kubicek Vol.2 1998, Picard *et al.*, 2000). Plant roots growing in soils are a major source of carbon and energy to microorganisms in the form of root exudates, cells detached from old parts of the roots or the roots itself after plant death. The most common species was *T. harziunum*.

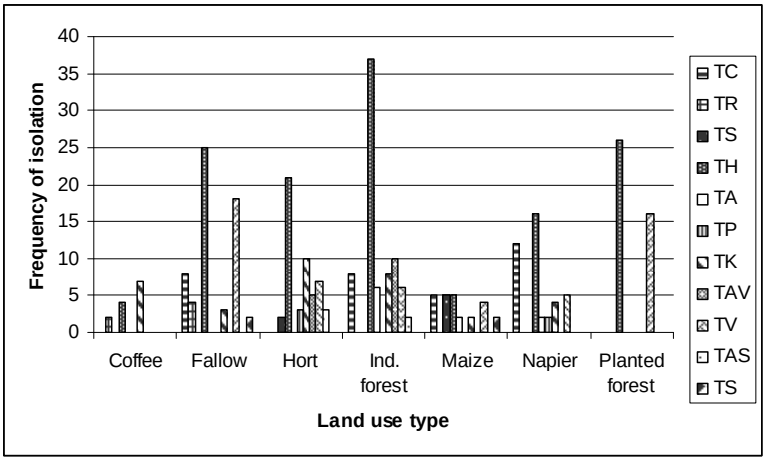

Figure 3: Frequency of isolation of *Trichoderma* species from different land use types

KEY

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*		

Table 3: Pearsons correlation coefficients matrix among soil properties

Variables	Acidity	N	C	P	K	Ca	Mg	Mn	Cu	Fe	Zn	Na
pH	-0.749	-0.352	-0.292	0.494	0.401	0.290	0.623	-0.056	-0.094	-0.439	0.236	0.098
P values	< 0.0001*	< 0.0001*	0.001*	< 0.0001*	< 0.0001*	0.001*	< 0.0001*	0.540	0.298	< 0.0001*	0.008*	0.279
Acidity		0.263	0.139	-0.362	-0.332	-0.184	-0.529	0.021	0.046	0.478	-0.225	-0.026
P values		0.003*	0.124	< 0.0001*	0.000*	0.041*	< 0.0001*	0.816	0.614	< 0.0001*	0.012*	0.771
Nitrogen			0.718	-0.150	-0.030	-0.016	-0.123	0.089	-0.008	0.265	0.446	0.019
P values			< 0.0001*	0.096	0.741	0.861	0.174	0.327	0.929	0.003*	< 0.0001*	0.834
Carbon				-0.211	-0.025	0.021	-0.137	-0.037	-0.080	0.237	0.360	0.015
P values				0.018*	0.787	0.813	0.128	0.680	0.375	0.008*	< 0.0001*	0.865
Phosphorus					0.086	0.411	0.543	-0.168	-0.268	-0.154	-0.032	0.528
P values					0.342	< 0.0001*	< 0.0001*	0.062	0.003*	0.088	0.724	< 0.0001*
Potassium						0.329	0.226	0.044	-0.024	-0.113	0.254	-0.059
P values						0.000*	0.012*	0.628	0.793	0.212	0.004*	0.514
Calcium							0.481	-0.211	-0.173	0.081	-0.061	0.488
P values							< 0.0001*	0.019*	0.055	0.369	0.498	< 0.0001*
Magnesium								-0.077	-0.091	-0.133	0.225	0.252
P values								0.398	0.317	0.141	0.012*	0.005*
Manganese									0.110	0.066	0.434	0.094
P values									0.222	0.464	< 0.0001*	0.297
Copper										-0.050	0.033	-0.283
P values										0.584	0.713	0.001*
Iron											-0.104	0.091
P values											0.249	0.313
Zinc												-0.122
P values												0.176

Positive and negative correlation coefficient with probability values beneath them. *=Significant p-values – p<0.05.

Table 4: Effect of soil depth on *Trichoderma* richness, abundance and diversity

	Mean	Total	Chao	Bootstrap	Jackknife
Richness					
0 – 10cm	1.274	11	11.020	11.981	11.98387
10 – 20cm	0.935	11	13.222	12.769	14.93548
Abundance					
0 – 10cm	2801.774	173710			344618.2
10 – 20cm	1424.355	88310			175195.6
Shannon					
0 – 10cm	0.189	1.753			1.875305
10 – 20cm	0.094	1.593			1.734360

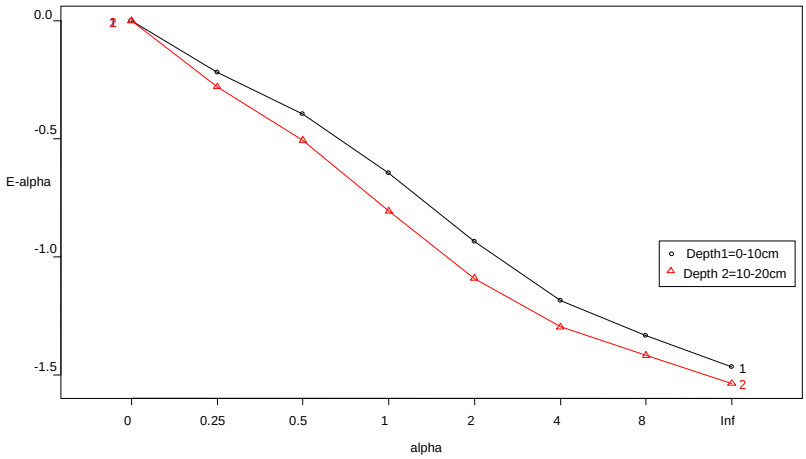

Figure 4: Renyi Profile curves for evenness of *Trichoderma*

CONCLUSION

Many factors interact to determine the occurrence of *Trichoderma* spp. Some of these factors are substrate availability which is determined by plant type; parent rock which determines soil type and land intensification which influences land management and in turn soil physical and chemical characteristics. The mode of exertion of the function of these three major drivers (land intensification, soil type and plant/crop type) is a complex interaction between for example the fungus and soil, plants, and other soil microorganisms. In some situations it is the soil that is the key factor determining fungal occurrence and diversity while in others it is the plant type.

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