



Co-occurrence and Diversity of Soil *Trichoderma* and *Fusarium* species from Different Land Use Intensities in Machakos County, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Authors PMW, SAO and JWK designed and supervised the study. Author PKM carried out the study, managed the literature searches and wrote the first draft of the manuscript. Author JMM carried out data analyses. All authors read and approved the final manuscript.

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ABSTRACT

Soil fungi are important components of the soil biota and their diversity is a good indicator of soil health. Soil fungi respond differently to land use practices and to their relative populations. The co-occurrence and diversity of *Trichoderma* and *Fusarium* species against various land use types (LUTs) was investigated. The genus *Trichoderma* contains many important species with potential for biocontrol of soil-borne plant pathogens as well as high saprophytic capacity while the genus *Fusarium* has many species that are highly pathogenic to plants and with potential for mycotoxin production. This research adopted a cross-sectional study design. Soil samples were collected from 3 land-use types (LUTs) in Kabaa irrigation scheme in Machakos County, Kenya, that is, intensive land-uses under irrigation and rain-fed agriculture and undisturbed lands. From the top soil layer, 100 soil samples were collected. The samples were processed and 369 *Trichoderma*

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and 1,546 *Fusarium* isolates recovered. *Fusarium* had a higher abundance than *Trichoderma* in the highly disturbed lands. In the undisturbed lands, *Trichoderma* had a higher abundance than *Fusarium*. There was a clear negative correlation between *Trichoderma* and *Fusarium* occurrence and diversity. The study further revealed that disturbance had a positive effect on *Fusarium* but a negative one on *Trichoderma*.

Keywords: Biocontrol; biodiversity; *Fusarium*; land-use type; soil health; *Trichoderma*.

1. INTRODUCTION

Soil is a highly complex ecosystem, made up of many components with diverse functionality [1,2]. It provides microhabitats for soil microflora [3]. Soil microflora has a major role on soil functioning [4]. Soil microbiota regulates soil fertility as they are involved in biochemical transformations and mineralization activities [5]. They determine the status of soil health as some infect plants while others diminish populations of pathogenic species [6]. This has an overall effect on plant growth. Different land use systems have greater influence on the activity of soil microflora [3]. Clearance and subsequent cultivation of land for crop production has a great effect on soil microflora diversity [4]. Some farming practices like increased intensity in land-use may cause an imbalance in soil microflora affecting biological properties of soil.

Soil fungi play an important role in the soil ecosystem. The fungi form an important component of soil microflora [7]. Apart from providing mankind with very useful pharmaceutical products, such as antibiotics and other valuable substances, including organic acids, enzymes, pigments and secondary metabolites used in the food industry and fermentation, some play a pivotal role in mineralization and soil health. Many of them are biological control agents for plant pathogens and insect pests [7]. On the other hand, some of them are very harmful causing food spoilage and diseases to plants, animals and humans with significant economic losses and produce mycotoxins in certain products [7].

Soil fungal populations are affected by various factors. The amounts of soil organic and inorganic matter have a direct effect on the fungal populations. Soil tillage and application of agrochemicals in the soil affects populations of fungi in the soil [4]. The types and numbers of fungi in the soil depend on factors such as the amount and type of nutrients, soil organic matter, soil moisture level, degree of aeration, pH, plant

types, soil temperature etc [8,9]. The factors can be influenced by intensity of land-use.

In recent years, Kenya has experienced food shortages arising from declining farm productivity owing to unreliable weather, prevalence of pests and diseases, low fertility levels and high input costs in the face of a rising population [10]. Increased population coupled with falling food production makes the food security situation in the country precarious and vulnerable. This has led to drastic land-use changes to adapt to the current situation. As agricultural intensification is becoming a real phenomenon, it is believed to have a major impact on biodiversity and functionality [11]. As the system gets more intensive, biodiversity decreases and its ecological function and sustainability may be affected.

Although numerous studies have shown that changes in land use also have a significant effect on soil microbial communities [12,13], there is a poor understanding of how land-use (and the associated changes in plant and soil properties) may affect the abundance of specific taxonomic fungal groups. According to Okoth [14], there is a clear need to study the biodiversity of soil microorganism in Kenya and evaluate the effect of external factors including agricultural intensification on soil biodiversity and ecosystem function at various temporal and spatial scales. Data on effects of agricultural intensification on soil microbial diversity in Kenya is scanty. Furthermore, soil *Trichoderma* and *Fusarium* levels can serve as key indicator organisms on the status of soil health [15]. These fungi are relevant ecologically, are cosmopolitan in nature, abundant and easily enumerated and identified [16]. Their populations change in the soil in response to land-use changes can easily be monitored. Their correlation in the soil can therefore serve as a pointer to the status of soil health as the genus *Trichoderma* has many species with biocontrol potential for crop pathogens while genus *Fusarium* has many species that cause serious diseases of crops [17].

Trichoderma has a cosmopolitan distribution and is associated with soil, plant roots and soil debris. It has been isolated from different climatic ecosystems [18]. About 150 species of *Trichoderma* have so far been described but the actual number is estimated to be more than 200 species [19]. The genus *Fusarium* has many species that are highly pathogenic to plants worldwide [20]. These species are widely distributed in both cultivated and uncultivated soils in both tropical and temperate areas [20]. Some produce mycotoxins in food and others are pathogenic to man and other animals [20]. The species *Fusarium* have ability to rapidly change its host and morphology.

Relatively few studies have tried to compare quantitatively the fungal diversity among different habitats in Kenya based on land use types [10,21,22,23]. Comparative studies about the effects of land intensification on co-occurrence and diversity of soil *Trichoderma* and *Fusarium* are scarce. There is need to assess these fungi due to the prominent role they play in soil ecosystems. The genus *Trichoderma* has many species that have biocontrol potential for plant diseases. On the other hand, the genus *Fusarium* has many species that are highly pathogenic to crops. The relative abundance of these two genera may therefore serve as an indicator of status of soil health. This study therefore sought to determine the co-occurrence and diversity of *Trichoderma* and *Fusarium* in different land use types in Kenya.

2. MATERIALS AND METHODS

2.1 Study Area

This research was conducted in Kabaa irrigation scheme. This area is located in Machakos County in Kenya. The area is semi-arid with moderately high temperatures. The study area was divided into two regions; Kauti and Mwala. The areas have diverse land-use systems which vary from intensive land use to undisturbed areas under grasses and thorn bushes. This allowed comparison of results. Kauti scheme, which is more recent in cultivation, has alfisols and acrisols soils and is found in the Upper Midland zone 4. Mwala scheme, which has a longer history of cultivation, has cambisol soils and is found in Lower Midland zone 4. Crops grown in these schemes are mainly vegetable crops and fruits.

2.2 Soil Sampling

Samples of soil were obtained from irrigated and rain-fed farmlands under intense land use as well as from undisturbed lands. Soil sampling was replicated in the two schemes. Twelve soil subsamples were collected at each sampling point (Fig. 1), from the top 20 cm and thoroughly mixed to form a composite sample from which a 100 g soil ample was obtained for analyses.

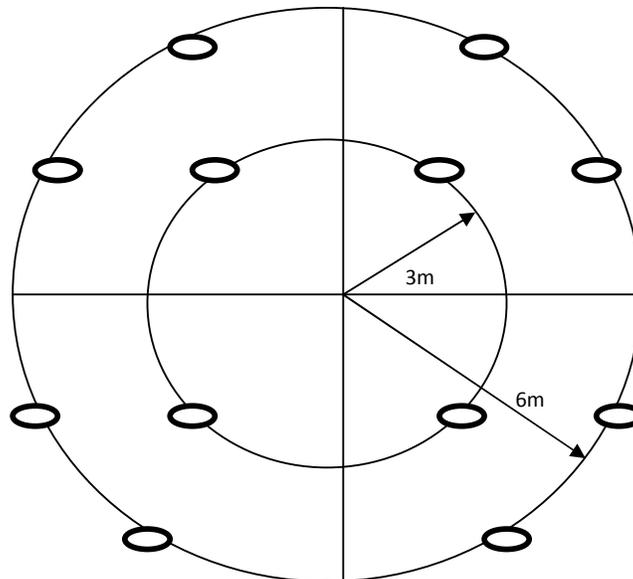


Fig. 1. The twelve soil sub-sampling points in each sampling point

A sterilized auger was used to obtain samples from each point. The auger was sterilised between points. The soil from each point was mixed before obtaining a subsample of 100 g. Samples were placed in containers separately and placed in an ice cooled box and moved to University of Nairobi, Chiromo campus mycology laboratory and stored at 5°C. During analyses, 10 g of soil from each sample were air-dried at 27±1°C for a period of 5 days, and sieved with 0.5 mm sieve. This was done in order to obtain fine particles that were processed for quantification of *Trichoderma* and *Fusarium*.

2.3 Isolation of *Trichoderma* and *Fusarium* species from the Soil

2.3.1 *Trichoderma* isolation and identification

A selective medium for *Trichoderma* was used to isolate this fungus from the soil according to McLean et al. [24]. The soil was diluted with distilled water up to the third dilution. Three replicate plates each were done for the second and third dilutions. This was followed by incubation at 20°C for a period of ten days as described by Klein and Eveleigh [25]. Sub-culturing of *Trichoderma* isolates was done on ½ strength PDA amended with chloramphenicol in order to obtain pure cultures. The plates were incubated at 20°C for 7 days in the dark and then another 7 days at four temperature ranges i.e. 15, 25, 30 and 35°C under 24 h blue light in order to encourage sporulation [24]. Taxonomic keys of Samuels et al. [26] and [27] were used to identify the respective *Trichoderma* species from the isolates. Identification was based on colony characteristics, growth rates and morphological features.

2.3.2 *Fusarium* Isolation and identification

Fusarium isolates were obtained from the soil samples using serial dilution plating technique. This method is given by Burgess et al. [28] and uses medium selective for *Fusarium*. Three replicates each for the 2nd and 3rd dilutions were prepared. The inoculated plates were incubated at 25°C day / 20°C night temperatures and 65% relative humidity. Cool white fluorescent lights were provided during growth with a 12 h photoperiod. This was continued for a period of ten days. Colonies growing on the selective media were transferred to Spezieller Nährstoffarmer Agar (SNA) media. This was done in order to obtain distinct colonies [29].

Colonies were then transferred on to 2% Tap Water Agar (TWA) media in order to obtain single spore cultures. Germinating spores on TWA were transferred to SNA, Carnation-Leaf-Agar (CLA) and Potato-Dextrose-Agar (PDA) media and incubated for 10 to 20 days at 25 °C under fluorescent lamps with a 12 h photoperiod. *Fusarium* isolates were identified up to species level using cultural and morphological characters [28,30,29,31,32].

2.4 Data Analyses

Data obtained were standardized before analyses. This was due to the differences in numbers of samples obtained in the diverse land use categories. Data on abundance and diversity of the target fungal species relative to land use type were analysed by GenStat computer package. Due to wide range on abundance of the fungi, data were first log transformed for normality before analyses. The effect of land use on co- occurrence of the fungi was evaluated using ANOVA. Shannon diversity (H') indices were obtained for both fungi across the land use types. For the analyses, significance was determined at $p < 0.05$. Tukey test was used to separate the means that were significantly different at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Abundance, Richness and Diversity of *Trichoderma* species Across the Various Land Uses

A total of 369 *Trichoderma* isolates were obtained from the entire area under study. These were identified into eleven *Trichoderma* species; *T. virens*, *T. spirale*, *T. atroviride*, *T. viride*, *T. harzianum*, *T. crassum*, *T. koningii*, *T. tomentosum*, *T. asperellum*, *T. brevicompactum* and *T. hamatum*. *Trichoderma harzianum* was significantly ($p < 0.01$) the most prevalent species, followed by *T. koningii* and *T. viride*, respectively. *Trichoderma hamatum* was least frequent. Increase in intensity of land use lowered the abundance of *Trichoderma* (Fig. 2). Undisturbed lands had the highest numbers of *Trichoderma*. This study revealed that rainfed farmlands had a higher *Trichoderma* than irrigated farms. History of disturbance also had a bearing on the occurrence of *Trichoderma*. The older areas in terms of cultivation had lower *Trichoderma* levels (Fig. 2).

Trichoderma richness between different LUTs was not significant ($p = 0.203$). The study revealed that undisturbed lands had a higher *Trichoderma* richness than disturbed lands for

the two regions (Fig. 3). Diversity of *Trichoderma* species was negatively related to intensity of land use, with undisturbed lands having a higher *Trichoderma* species diversity (Fig. 4).

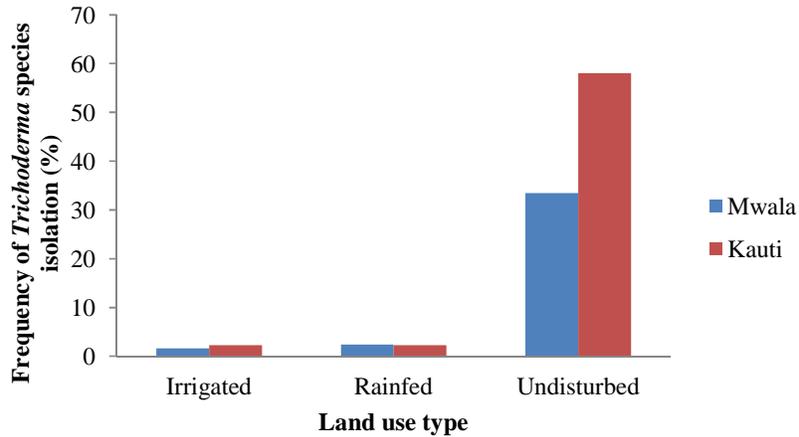


Fig. 2. Frequency of isolation of *Trichoderma* in different land-use types in the two regions

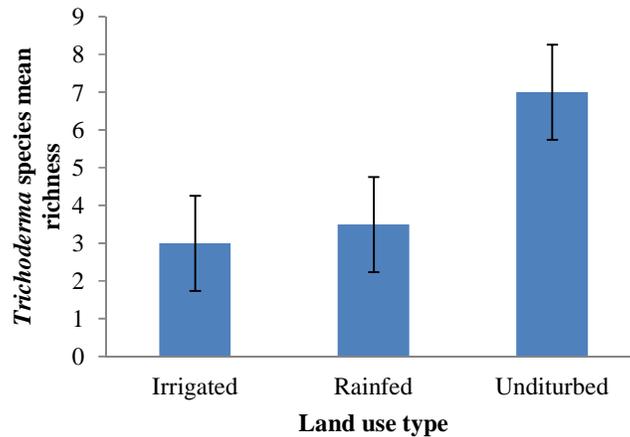


Fig. 3. *Trichoderma* mean richness with standard error bars for different land use types

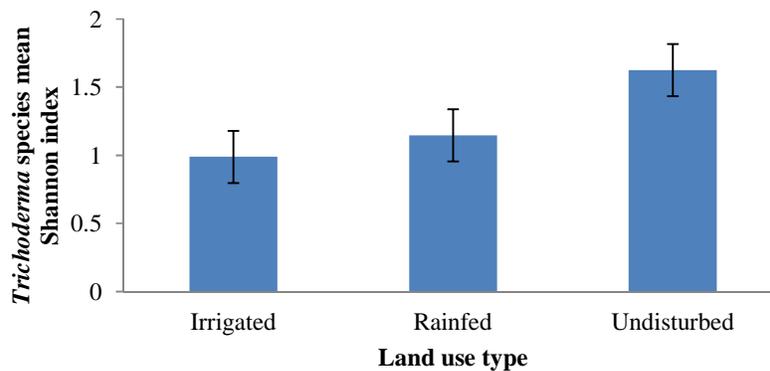


Fig. 4. *Trichoderma* species diversity with standard error bars for different land use types

3.2 Abundance, Richness and Diversity of *Fusarium* species Across the Land Use Types

In this study 1,546 *Fusarium* isolates were recovered. This resulted into 12 *Fusarium* species, namely; *F. solani*, *F. oxysporum*, *F. acuminatum*, *F. compactum*, *F. proliferatum*, *F. nygamai*, *F. beomiforme*, *F. equiseti*, *F. verticillioides*, *F. chlamyosporum*, *F. semitectum* and *F. merismoides*. *Fusarium oxysporum* was the most prevalent species while *F. merismoides* was the least abundant species. There was significant difference ($p = 0.047$) in abundance of *Fusarium* species. A positive correlation between land use intensity and history of cultivation and

Fusarium abundance was evident (Fig. 5). Least occurrence was observed in the undisturbed lands. The study further revealed that irrigated farmlands had a higher prevalence of *Fusarium* than rainfed lands.

There was no significant difference in *Fusarium* species across the LUTs ($p = 0.825$). However, intensity in land use had a positive influence on richness with intensively cultivated lands having a higher *Fusarium* species richness (Fig. 6). There was no significant difference in *Fusarium* species diversity among the LUTs ($P = 0.063$). Rainfed land-use type had the highest diversity of *Fusarium* while irrigated lands had the least (Fig. 7).

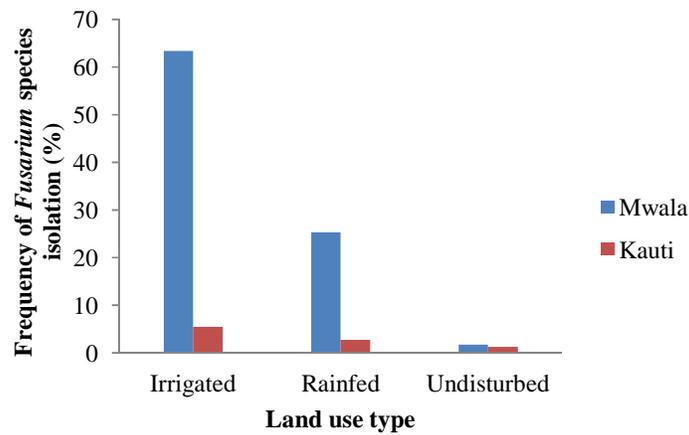


Fig. 5. Frequency of isolation of *Fusarium* isolates from the three land-use types in the two regions

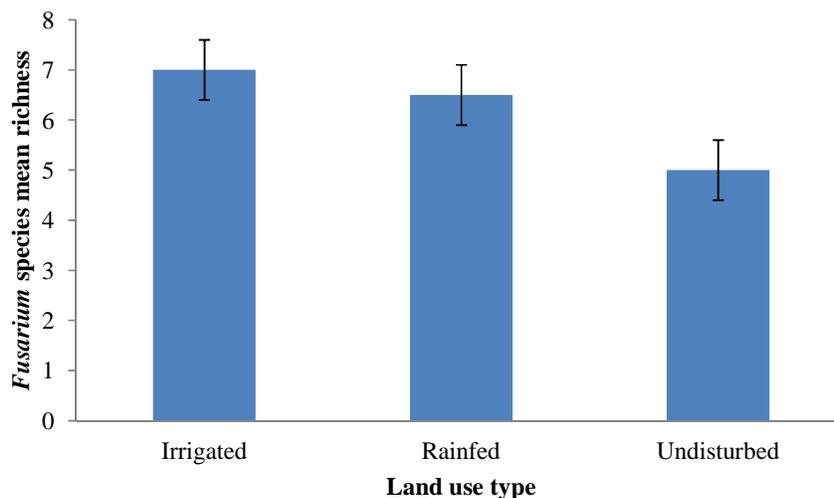


Fig. 6. *Fusarium* mean richness with standard error bars in different land use types

3.3 Relationship between *Trichoderma* and *Fusarium* across the Land Use Types

The results of this study further revealed that there is a negative correlation between *Trichoderma* and *Fusarium* abundance (Fig. 8). In land areas with high abundance of *Trichoderma*, low numbers of *Fusarium* isolates were realized. The abundance of *Fusarium* was high in the intensely cultivated lands where *Trichoderma* occurrence was low. Undisturbed lands had higher *Trichoderma* levels whereas the

Fusarium abundance was low (Fig. 8). A negative relationship between *Trichoderma* and *Fusarium* richness across the land use types was also observed (Fig. 9). Intensively cultivated lands had a higher *Fusarium* richness but lower *Trichoderma* richness (Fig. 9). However, the trend was reversed in undisturbed lands where there was a higher *Trichoderma* richness than that of *Fusarium*. Further, a negative relationship between *Trichoderma* and *Fusarium* diversity was also revealed. All the land use under study had a lower *Trichoderma* diversity than that of *Fusarium*.

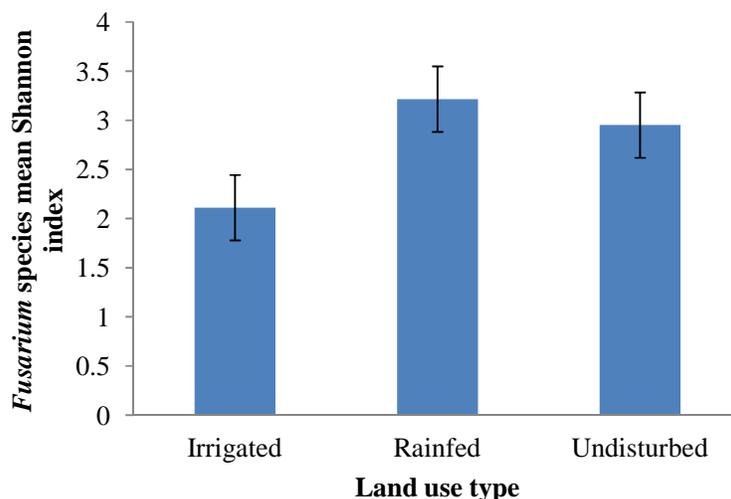


Fig. 7. *Fusarium* species diversity with standard error bars for different land use types

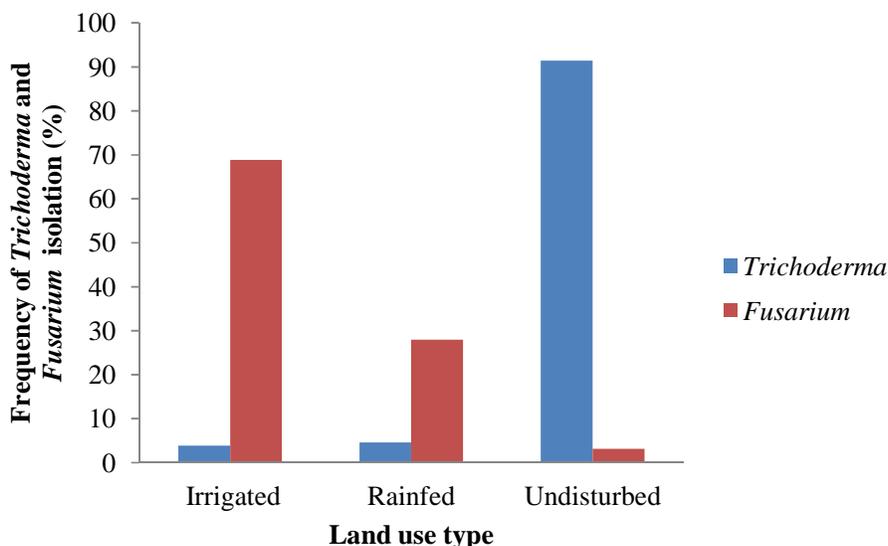


Fig. 8. Frequency of isolation of *Trichoderma* and *Fusarium* for different land use types

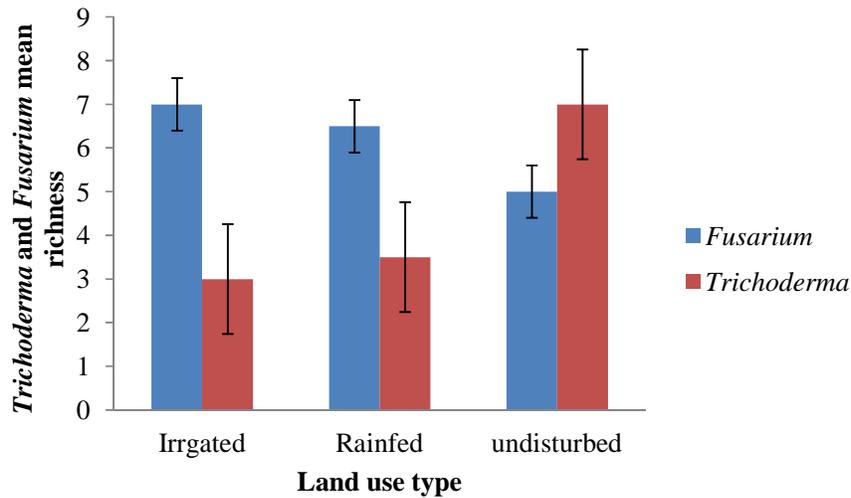


Fig. 9. *Trichoderma* and *Fusarium* mean richness with standard error bars for different land use types

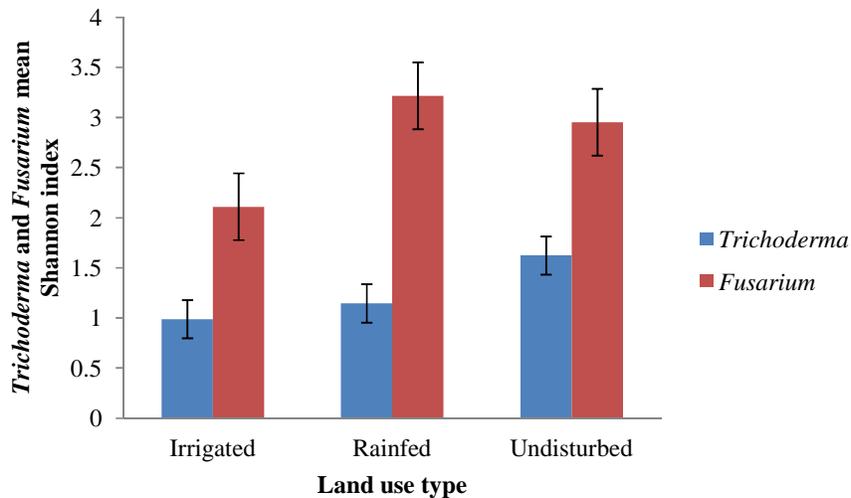


Fig. 10. *Trichoderma* and *Fusarium* mean Shannon index with standard error bars for different land use types

This study has revealed that both *Trichoderma* and *Fusarium* genera are widely distributed in the study area. Members of these two genera are ubiquitous occurring in different ecosystems [33]. Many members of these genera are abundant in the soil due to their diverse adaptations. The difference in the occurrence of these fungi with land use intensities is evident. These fungi are important members of soil micro flora, participating in many biologically important processes. According to Hoyos-Carvajal and Bissett [34], where *Trichoderma* is found is

determined by substrate availability, prevailing climatic conditions and other ecological interactions. Members of this genus are able to survive in different areas due to high reproductive ability, ability to compete effectively and distinct metabolism [35]. Results of this study revealed that *Trichoderma harzianum* was the most abundant and diverse species. This fungus is a powerful competitor multiplies fast in soils rich in organic matter. Organic materials exuded by plant roots also increases numbers of this fungus. These factors may explain why this

fungus is very abundant in soils under study. In a study by Okoth et al. [21] in two ecologically diverse regions in Kenya, *T. harzianum* was the most abundant species. Other studies have confirmed high levels of this fungus in various parts of the world [36,37].

There was significant difference in abundance of *Trichoderma* with respect to LUTs ($p < 0.01$). The prevalence of this fungus varied negatively with the intensity of land use and the history of cultivation. This was replicated in the two regions. Intensively cultivated lands had a lower *Trichoderma* occurrence (Fig. 2). This was evident from the two regions under study. Similarly, lands with longer history of cultivation had a lower abundance of this fungus (Fig. 2). The less disturbed lands are naturally balanced to support a higher *Trichoderma* diversity. Undisturbed lands could be marked by a high diversity of plants, which could account for higher *Trichoderma* diversity. Agricultural activities may negatively impact on *Trichoderma* in the soil. Growth of similar annual crops continuously may reduce the variety of organic material in the soil. Harvesting of plant material every season may reduce organic matter in the soil. This may account for low abundance and diversity of *Trichoderma* in the soil of cultivated lands. Okoth et al. [21] also reported similar results where a higher occurrence of *Trichoderma* was obtained from the less disturbed soils. A study by Bourguignon [38] indicated that diverse crops and cropping regimes affected *Trichoderma* populations. A study by Celar, [39] confirmed that onions may decrease *Trichoderma* levels in the soil.

Many factors could also explain for the low levels of *Trichoderma* in the intensively cultivated soils. Irrigation may have a negative impact on *Trichoderma*. A study by Eastern and Butler, [40] demonstrated that excess soil water level reduces spore germination and growth of *Trichoderma*. The rise in pH also inhibits *Trichoderma* population. Rise in pH could be attributed to the application of fertilizers in the soil by the farmers. Tillage affects soil physical and chemical environment. Janusauskaite et al. [41] demonstrated decrease in organic carbon and nitrogen with increased tillage. This could account for lower *Trichoderma* levels in the cultivated lands. Hence diverse land use practices have major effects on the microclimates where soil microflora live and multiply [42]. A study by Lehman et al. [43], revealed that there are many factors that change

and modify ecology of fungi. The prevalence of *Trichoderma* species in this study seems to be more directly determined by the environment and anthropogenic activities.

This study further revealed that *Fusarium* species are abundant in the soils under study. These results are similar to those of Silvestro et al. [44]. In their study, they noted that the genus *Fusarium* is a cosmopolitan with most species being ubiquitous in soil [45]. However, differences in how species are spread in the study area were evident. The difference in composition and abundance of soil *Fusarium* could be attributed to differences in type of plants grown, soil characteristics, as well as land management practices [46].

Fusarium oxysporum was the most abundant species in this study. These results are in agreement with of a previous study in Kenya [22]. This fungus has serious crop pathogens [45] and its high prevalence is a cause for concern as it may be indicative of the status of health of soils under study. Increase in intensity of land use increased *Fusarium* abundance. Undisturbed farmlands had the least *Fusarium* abundance. The results were replicated in the two regions. The results are in agreement with those of Maina et al. [22] and Marasas et al. [47]. Manipulation of the soil during tillage could assist in the dispersal of propagules [48]. Tillage modifies the soil conditions by enhancing aeration and hence fungal activities. Irrigation water is able to percolate through the spaces aiding in dispersal [49]. Application of inorganic fertilizers by the farmers may have positively impact on abundance of soil fusaria [22]. *Fusarium* levels were low in undisturbed soils probably due to suppression antagonistic microorganisms.

Similarly, disturbed lands had a higher *Fusarium* richness for the two regions. These findings are similar to those reported by Nesci et al. [50]. This could be explained by increased ability of soil to sustain a higher level of fusarial species. Diversity in *Fusarium* species between the areas under study did not differ significantly. Rainfed farmlands had a higher diversity probably due to a higher diversity of plant species, soil physical-chemical parameters and farming practices [28,51].

The results of this study further revealed that there is a negative correlation between *Trichoderma* and *Fusarium* abundance (Fig. 8).

Farmlands with high abundance of *Trichoderma* had low numbers of *Fusarium* isolates. Undisturbed lands had higher *Trichoderma* levels while the *Fusarium* abundance was low. The intensely cultivated lands had a higher abundance of *Fusarium* while *Trichoderma* occurrence was low. A negative relationship between *Trichoderma* and *Fusarium* richness across the land use types was also observed (Fig. 9). Intensively cultivated lands had a higher *Fusarium* richness but lower *Trichoderma* richness. However, the trend was reversed in undisturbed lands where there was a higher *Trichoderma* richness than that of *Fusarium*. Further, a negative relationship between *Trichoderma* and *Fusarium* diversity was also revealed. All the land uses under study had a lower *Trichoderma* diversity than that of *Fusarium*. *Trichoderma* is known to have biocontrol activity against *Fusarium* species and this may explain the low *Fusarium* abundance, richness and diversity in areas with high *Trichoderma* abundance. *Trichoderma* spp. can be antagonists against other fungal species, especially phytopathogenic species [52].

Agricultural intensification, the main driver of land use change, is characterized by management practices such as frequent cultivation, use of agrochemicals, mono and mixed cropping, annual cropping with limited incorporation of perennial crops and organic materials [11,53]. These land-use trends influence fungal abundance, richness and functioning. Crop management may cause different gradients of disturbance depending on the crop and soil fertility level [54]. Evidence of the consequences of agricultural intensification on soil fungal community is still limited. The present study has shown that as land use intensifies, there is likelihood of change in fungal community structure. There may be loss of useful fungal genera like *Trichoderma* due to declining organic matter while the phytopathogenic genera like *Fusarium* may increase due to prevalence of susceptible hosts and enhanced dispersal.

Therefore, soil fungal community possess the ability to give an integrated measure of soil health, an aspect that cannot be obtained with physical, chemical measure and/or analyses of diversity of higher organisms. Soil mycoflora respond to changes in land use more rapidly and may therefore function as excellent indicators of changes in soil health [21]. Hence enhanced land-use intensification may portend a deleterious effect on soil health as it leads to

lower abundance and diversity of *Trichoderma* while increasing population of potentially pathogenic *Fusarium* species.

4. CONCLUSION

This study has revealed a negative correlation between *Trichoderma* and *Fusarium*. With increased land use intensification, the levels of *Trichoderma* decreases while those of *Fusarium* increases. This trend is further confirmed with increase in period of disturbance. It is clear that as land use becomes more intense, there is change in fungal soil balance. The population of the potentially pathogenic *Fusarium* species increases while that of *Trichoderma* species declines. This trend does not auger well for soil's capacity to maintain its health. *Trichoderma* contains many species with biocontrol potential for many phytopathogens.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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