



RESEARCH PAPER

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Community Knowledge, Land Use Practices, and Fungal Microbial Volume in Soil from Protected and Non-Protected Areas of Itigi District, Tanzania

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Key words: Soil fungal, Protected forest, Non-protected land, Land management

<http://dx.doi.org/10.12692/ijb/25.4.181-192>

Article published on October 07, 2024

Abstract

Soil fungal microorganisms are vital for soil health and ecosystem balance, are highly threatened by human. Unsustainable land use practices such as improper land tillage and crop residue management, excessive use of industrial inorganic fertilizer and pesticide, overgrazing and deforestation lead soil degradation and disruption of fungal microbial activities. This study explores a case of Itigi District in Tanzania to determine the relationship between community knowledge, land use practices, and fungal microbial volume in soils from farmland and protected areas. Soil fungal isolation were done using Potato Dextrose Agar with streptomycin sulfate. A structured questionnaire collected information from 150 participants to assess knowledge. Data analysis was performed using SPSS. Results show that gender (female), higher education level, increase in income level and practical knowledge positively influences the adoption of practices that enhance soil fungal microbial activities. It also shows that protected forest soils have higher fungal volume compared to farmlands. This study underscores the need for awareness campaigns on sustainable soil management practices and the promotion of the use of organic manure to maintain soil fertility and ecosystem balance. Females showed a statistically significant difference in the use of organic manure ($P=0.0364$). Moreover, comparison of CFU/mL mean 4.175×10^6 and 1.308×10^7 from non-protected and protected areas respectively revealed that protected areas consistently exhibited higher fungal microbial growth, probably attributed by minimal human disturbance and richer organic matter. The study concludes that sustainable soil fungal management is essential for soil health and ecosystem services, with protected areas demonstrating superior fungal volume. Raising awareness about responsible land use practices is crucial for maintaining these vital microbial communities.

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Introduction

Soil fungal represent a diverse array of microscopic fungi that inhabit the earth's soil, playing crucial role in maintaining soil health and ecosystem balance (Srivastava *et al.*, 2023; Chen *et al.*, 2022). Approximately 144,000 fungal species have been identified globally (Satyanarayana *et al.*, 2019; Wijavawardene *et al.*, 2020), where about 4843 fungal species are found in African countries specifically in the Western Africa (Piepenbring *et al.*, 2020). Soil fungal communities play a crucial roles in land management, significantly influencing soil hearth, fertility, and ecosystem stability (Frąc *et al.*, 2018; Zhang *et al.*, 2022). Soil fungi are involved in the decomposition of organic materials and transform compounds, as a such playing a key role in soil formation (Chen *et al.*, 2022; Dhiman *et al.*, 2022).

Higher soil fungal microbes are linked to increased soil fertility, reduced erosion, and enhanced soil aggregation dynamics (Ren *et al.*, 2022). The fungi interact directly with plants but also play a crucial role in improving soil structure (Khaliq *et al.*, 2023), therefore, facilitate a variety of sustainability programs in agriculture, ecosystem conservation, and restoration particularly relevant in the context of soil rehabilitation and restoration of depleted natural resources (Kuyper & Suz, 2023; Chabay, 2018).

Fungi, as key players in soil ecosystems, provide multiple benefits that support both environmental sustainability and agricultural productivity. Saprotrophic fungi, for example, are responsible for the decomposition of organic matter, releasing essential nutrients back into the soil and contributing to nutrient cycling (Sterkenburg *et al.*, 2018). This process not only maintains soil fertility but also improves plant health by enriching the soil with nutrients. In parallel, biocontrol fungi suppress soil-borne plant pathogens, thereby reducing the incidence of plant diseases and enhancing agricultural productivity (Sharma *et al.*, 2014). By reducing the reliance on chemical pesticides, biocontrol fungi promote more sustainable farming practices, ensuring long-term crop resilience (Mehla, 2023). Despite the essential roles of soil fungi, their

populations and volume are increasingly at risk due to human activities such as unsustainable farming practices, inappropriate use of agricultural inputs, excessive tillage, overgrazing and deforestation (Zelleke *et al.*, 2019; Jinger *et al.*, 2023). These activities disrupt the fragile balance of soil ecosystems, leading to a decline in fungal microbial populations and volume, and the overall soil health (Daunoras *et al.*, 2024). This study explored Itigi district in the Singida region, Tanzania to; 1) investigate the impact of land use practices on soil fungal, microbial population and volume, 2) assess the population by counting Fungal Colony Forming Units (CFU) from soil samples collected in farmlands and protected forest areas to unveil the impact of human activity on soil fungal communities.

Materials and methods

Study site

The study was conducted in Mitundu and Mgandu wards located in Itigi district, in the Singida region, in central Tanzania. Itigi district lies between latitudes 5.70°S and 6.00°S and longitudes 34.50°E and 35.00°E (Kiondo *et al.*, 2019). The study area is bordered by Manyoni district to the east and the Ikungi district to the north. In the south, it shares a boundary with the Chunya district of the Mbeya region, and to the west, it shares boundaries with the Uyui and Sikonge districts of the Tabora region (Isinika *et al.*, 2021).

The district has an average temperature of 21.6°C and receives an average annual rainfall of 632 millimeters, with an elevation of 1,306 meters above sea level (Nyaombo, 2021). The predominantly soils texture classes are sand clay loam and sandy loam, which support favorable conditions for crop cultivation (Nyaombo, 2021). The vegetation is characterized by savanna woodlands and semi-arid glass land, with scattered trees, such as *miombo woodlands*, Umbrella Thorn Acacia (*Acacia tortilis*), Red Acacia (*Acacia seyal*), African myrrh (*Commiphora Africana*), and dense shrubs. The ground layer is covered with grasses like Bermuda grass (*Cynodondactylon*) and Red oat grass (*Themedatriandra*), Indian goosegrass

(*Eleusine indica*) (Makero,2017).The main ethnic groups in the study area are the Nyaturu, Kimbu, Sukuma, and Nyamwezi. The population of the Mgandu ward is 26,887, with 4,436 households, while

Mitundu ward has a population of 32,370 and 6,044 households (Frac *et al.*, 2018) (URT, 2022). These two wards were selected because many residents engage in agro pastoralism activities.

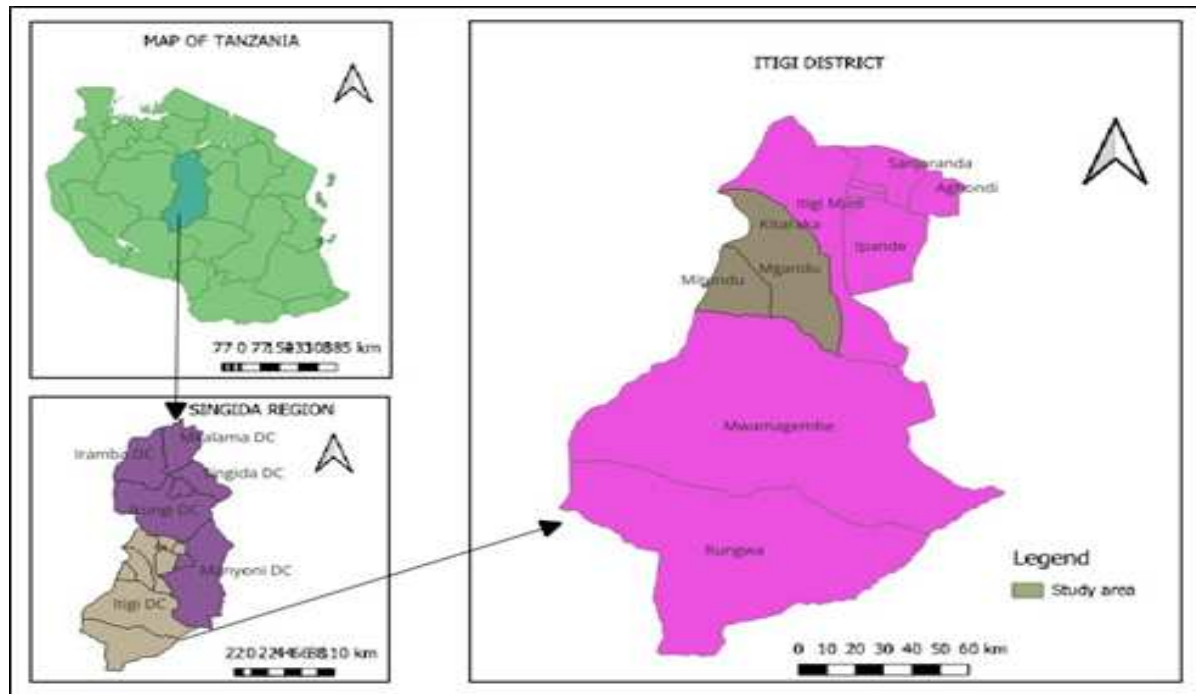


Fig. 1. Showing the study area.

Insert figure 1 here

Data collection and analysis

Structured questionnaire interviews were conducted to gather data on demographic characteristics from 150 households across two wards, aiming to assess knowledge on land use practices involving soil, which serves as a habitat for microbes, including fungi. The KoboToolbox platform (version 2023.2.4) was used as the data collection tool. From a total of 10,480 households, 140 farmers and 10 government employees were randomly selected, resulting in a sampling rate of 1.43% of the total population (Angelsen *et al.*, 2015). The household were those whose heads were 20 years and above. Priority was given to respondents engaged in agro-pastoralism, as their activities directly influence soil fungi and related ecological processes. Additionally, participants were required to have resided in the area for at least 1 to 10 years, ensuring they had sufficient experience and familiarity with the local environment and agricultural practices. Soil samples were collected

from both protected forest areas (PA) and non-protected areas (NPA), following the method established by Maes *et al.* (2020). In both areas, a total of 40 soil samples were taken from five points per quadrat 2m x 2m, mixed into a composite sample, and quartered to obtain about 0.5 kg of soil, (Naveen and Madhukar, 2022) with slight modifications such as collection of soil sample whereby 20cm depth was used instead of 15cm. Global Position System (GPS) Coordinates of the sampling sites were recorded using Garmin eTrex 10 devices with accuracy between 3 to 7 meters. The collected samples were packed in labeled zip-lock bags, with labels ranging from W1NPAS1 to W1NPAS10 and W2NPAS1 to W2NPAS10 for non-protected areas in Ward 1 and 2, and from W1PAS1 to W1PAS10 and W2PAS1 to W2PAS10 for protected areas in both wards. These samples were stored in a cool box and transported to the Nelson Mandela African Institute of Science and Technology (NM-AIST) Laboratory, where they were refrigerated at 4°C for slowing down the metabolic activities of fungi

and other microorganism until further isolation processes was conducted. After refrigeration, the samples were air-dried at room temperature for 24 hours in preparation for fungal isolation. The isolation was then carried out using the dilution plate count technique. A 1g of the soil sample from a 0.5kg composite soil was suspended in 9 ml of autoclaved distilled water and serially diluted from 10^1 to 10^7 using micropipettes. A 0.1 ml of solution from 10^4 , 10^5 , and 10^6 dilutions was plated onto a Potato Dextrose Agar (PDA) petri-dish with streptomycin sulfate ($100\mu\text{g mL}^{-1}$). Plates were incubated at 25°C for 3-7 days. After 5 days plate with colonies counts between 30 and 300 from 10^4 dilution were selected for viable fungal count (Gomez *et al.*, 2014) and calculated as colony-forming units (CFU) per mL using the equation 1 below.

$$\text{CFU/mL} = (\text{Number of colonies} \times \text{dilution factor}) \div \text{Volume plated (mL)}$$

Respondent's demographic characteristics were analyzed using multinomial logistic regression model to examine the influence of demographic factors on the land use practices and management. The model

assessed variables such as gender, age, education level, and occupation with the reference group being one of the demographic characteristics in each category.

The analysis provided estimates, standard errors, z value and p-value to identify significant predictors for the land practices such as choice of fertilizer, land preparation methods, and causes of soil degradation. The collected demographic data were subsequently imported into Statistical Package for the Social Sciences (SPSS). Also descriptive statistics was employed to determine the influence of gender on farmland preparation methods and the impact of education on causes of soil degradation in Mitundu and Mgandu wards.

Result and discussion

Influence of demographic characteristics on nutrient fertilizers use

The study employs logistic regression to examine the impact of demographic factors on the use of organic manure and inorganic fertilizer, yielding several key insights.

Table 1. Regression Analysis of Demographic Factors (Age, Gender, and Occupation) on nutrient fertilizer Use.

Variable	Estimate	Std. Error	z value	Pr (> z)
(Intercept)	0.9328	0.876	1.065	0.287
<i>20-30 reference group</i>				
31-40	0.507	0.53062	0.096	0.9239
41>	0.55070	0.48502	1.135	0.2562
<i>Female reference group</i>				
Female	-0.81484	0.3893	-2.093	0.0364 *
<i>Business reference group</i>				
Farmer	0.9209	0.6851	-1.344	0.025
Government employees	-4.381	1.423	-0.605	0.1789
<i>Non formal educational level reference group</i>				
Primary level	0.67520	0.47887	1.410	0.7572
Secondary level	0.50136	0.60908	0.823	0.4104
Tertiary level	0.5560	1.79833	0.309	0.7572
<i>Inorganic manure</i>				
(Intercept)	1.0511	0.7542	1.394	0.163
<i>20-30 reference group</i>				
31-40	-0.1664	0.5091	-0.327	0.744
41>	-0.5983	0.4553	-1.314	0.189
<i>Female reference group</i>				
Male	0.65	0.130	-3.580	0.65
<i>Business reference group</i>				
Farmer	-0.7164	0.5272	-1.359	0.374
Government employees	-0.250	0.040	-1.770	0.281
<i>Non formal educational reference group</i>				
Primary level	16.2424	0.4708	-0.675	0.093
Secondary level	-0.1640	0.5913	-0.277	0.781
Tertiary level	0.2424	0.0543	0.009	0.893

The common nutrients fertilizers resources that are used in the study sites are nitrogen, phosphorus and potassium (NPK), Calcium Ammonium Nitrate (CAN) and urea (Inorganic nutrient fertilizer sources) and animal manure (organic nutrient fertilizer source).

Notably, age 31-40 and those 41 and above years did not significantly influence the use of animal manure with high p-values (0.9239 and 0.2562), indicating no statistical significance (Table 1).

Table 2. Influence of gender on farmland preparation in soil fungal microbes' volume in Itigi and Mgandu wards in the Singida region, Tanzania.

Category	Variables	Coefficients	SE	t Start	p-value
(Tractor)	Intercept	0.24	0.05	4.6	8.09e-06
	Male	0.06	0.07	-2.36	0.08
	Female	-0.17	0.04	1.75	0.02
Oxen Plough	Intercept	0.33	0.064	5.122	9.113e-07
	Male	0.473	0.088	5.401	2.530e-07
	Female	-0.25	0.044	-5.733	5.187e-08
Hand hoe	Intercept	0.84	0.06	13.84	1.15e-28
	Male	-0.15	0.08	-1.75	0.08
	Female	0.04	0.04	1.08	0.28

This finding aligns with studies by Babasola *et al.*(2018) in Nigeria and Muluneh *et al.*(2022) in Ethiopia, which documented that the average age of the farmers who are using organic fertilizer resources in managing soil fertility is around 37 years. Given the findings of this study, it is suggested that age alone did not appear to be a significant determinant factor that may influence the use of organic manure. Gender appeared to play a significant role in the use of organic manure in the study sites, with females being less likely to use it compared to males with statistically significant estimate of -0.81484 and a p-value of

0.0364. In the studied wards, men are typically the farm owners and primary decision-makers in agricultural activities, which contributes to this disparity. According to Mensah *et al.* (2018), male-headed households are more likely to adopt organic manure due to factors such as household size and marital status. These findings highlight the importance of considering gender dynamics when promoting sustainable agricultural practices as the decision-making power and socio-economic roles of men and women significantly influence the adoption of organic manure usage.

Table 3. Influence of education level on sources of soil degradation.

Categories	Variables	Coefficients	SE	t start	p-value
Conversion to farmland	Intercept	0.06	0.04	1.68	0.09
	None formal	-0.03	0.14	-0.18	0.86
	Primary level	0.18	0.06	2.64	0.008
	Secondary level	0.04	0.04	1.07	0.28
	Tertiary level	-0.019	0.04	-0.46	0.65
Overgrazing	Intercept	0.620	0.053	11.73	6.51e-23
	None formal	0.093	0.188	0.492	0.623
	Primary level	-0.043	0.091	-0.479	0.633
	Secondary level	-0.054	0.053	-1.018	0.010
	Tertiary level	-0.031	0.054	-0.566	0.572
Deforestation	Intercept	0.617	0.052	11.727	6.512e-23
	None formal	0.092	0.188	0.482	0.622
	Primary level	-0.043	0.090	-0.479	0.633
	Secondary level	-0.054	0.053	-1.018	0.310
	Tertiary level	-0.031	0.054	-0.566	0.572

The use of inorganic fertilizer is less affected by age as shown by the insignificant estimates for the 31-40 years as well as 41 and above age groups, with estimate values of -0.1664 and -0.5983 and corresponding p-values of 0.744 and 0.189, respectively, as supported by Alyokhin *et al.*(2020) and Rahman *et al.* (2018). Occupation shows statistically significant differences in the use of

organic manure. Farmers have a p-value of 0.025, indicating a strong positive association with organic practices. In contrast, government employees, with a p-value of 0.1789, do not demonstrate a meaningful influence on organic manure use. These findings highlight that farmers are more likely to engage in sustainable agricultural practices compared to government employees (Table 1).

Table 4. Influence of age on sources of soil degradation.

Categories	Variables	Coefficients	SE	t Start	p-value
	Intercept	0.051	0.046	1.110	0.268
conversion to farmland	20-30	-0.145	0.124	-1.169	0.244
	31-40	0.091	0.056	1.617	0.110
	41<	0.089	0.024	3.667	0.0003
Overgrazing	Intercept	0.657	0.0582	11.281	9.247
	20-30	-0.034	0.157	-0.218	0.837
	31-40	0.086	0.071	1.219	0.224
	41<	-0.063	0.030	-2.036	0.043
Deforestation	Intercept	0.478	0.059	8.082	1.950e-13
	20-30	0.175	0.159	1.085	0.275
	31-40	0.007	0.072	0.109	0.813
	41<	0.015	0.031	0.478	0.633

These findings are consistent with earlier studies e.g., by Jekayinfa *et al.* (2005), Ferahtia, (2021), and Wasil *et al.* (2023) which emphasize that financial status, along with knowledge and practical experience, are the more influential factors in the adoption of organic fertilizer. However, in some under developing countries, the lack of knowledge on appropriate use of agricultural inputs by smallholder farmers further increases the negative effect on soil quality by reducing the availability of essential soil microbial fungi (Sanginga and Woome, 2009).

Insert table 1 here

Influence of gender related farmland preparation on soil fungal microbial volume

The analysis of land tillage using tractor, oxen plough, and hand hoe provides important insights into the impact of gender on soil fungi volume. The results reveal that females have a significant negative association with tractor use, with a coefficient of -0.17 ($p = 0.02$), indicating they are significantly less likely to use tractors when preparing crop fields compared to the male (Table 2). In contrast, tractor use in land tillage for males significantly differ from the female,

as shown by a coefficient of 0.06 and a p-value of 0.08. These findings align with studies by Jekayinfa *et al.*, (2005) and Alyokhin *et al.*, (2020), which demonstrated that the use of tractors land tillage can disrupt soil structure, leading to compaction and mechanical damage that adversely affect the growth, volume, and ecological functions of essential soil fungi. This disruption ultimately compromises soil health and long-term agricultural productivity. In contrast, for oxen plough, the intercept is highly significant, reflecting a strong baseline effect. Males have a significant positive association with a coefficient of 0.473 ($p = 2.530e-07$), indicating that they use oxen ploughs more frequently than the females. Conversely, females show a significant negative coefficient of -0.25 ($p = 5.187e-08$), suggesting that they use oxen ploughs less frequently compared to the males. Regarding hand hoe, the females also showed highly significant usage. Males exhibit a non-significant decrease in hand hoe usage with a coefficient of -0.15 and a p-value of 0.08, while females show no significant difference in hand hoe usage compared to the baseline with coefficient = 0.04, $p = 0.28$.

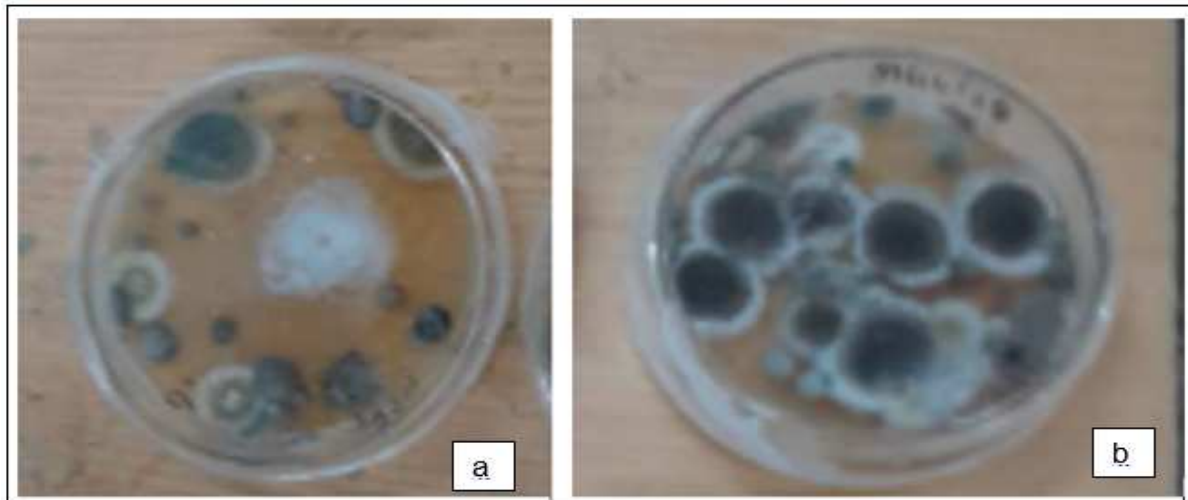


Fig. 2. Fungal colon cultures from (a) non-protected soils sample (b) Protected soils samples.

This indicates that gender does not significantly influence the use of hand hoes. Overall, while significant of gender differences are observed in the use of tractors and oxen ploughs, hand hoe usage does not exhibit significant gender-based variations. In this study, hand hoe and draft animal tillage are more common than tractor tillage. Previous studies such as by Nyamwange *et al.*, (2018) and Martinsen *et al.*, (2017) described that the hand hoe tillage can disrupts the hyphal networks of soil fungi in the topsoil, potentially affecting fungal volume. Guan *et al.* (2022) demonstrated that tillage methods, including no tillage and deep tillage, impacted fungal communities and their co-occurrence networks following residue removal and retention over two years. Their findings indicated that different tillage practices influenced the complexity of microbial co-occurrence networks in distinct ways: while deep tillage increased the complexity of fungal networks, it simplified fungal networks (García De León *et al.*, 2018).

Insert table 2 here

Influence of Education level on the sources of soil degradation

The results of this study indicates that education levels has influence on land conversion to farmland, with primary education significantly increasing the likelihood of such conversions. Individuals with primary education are more inclined to convert land into farmland compared to those with no formal

education, as indicated by a coefficient of 0.18 and a p-value of 0.008 (Table 3). However, other education levels, such as secondary and tertiary education, do not significantly impact farmland conversion, as their p-values are well above the conventional 0.05 threshold. Similarly, the intercept for farmland conversion is not statistically significant ($p = 0.09$). Studies by Majule *et al.*, (2012) and (Kanjanga *et al.*, 2022) suggested that education alone does not fully account for the land use change.

In contrast, secondary and tertiary education levels play a crucial role in mitigating overgrazing, with a significant negative association (coefficient = -0.054, p-value = 0.010). This suggests that individuals with secondary and tertiary education are less likely to contribute to overgrazing due to the understanding of sustainable land management and its environmental benefits, along with greater access to resources and technologies that support responsible grazing practices (García, *at al.*, 2018).

The intercept for overgrazing is highly significant ($p = 6.51e-23$), indicating a strong baseline effect, but other education levels, including primary and tertiary, do not show significant influence on overgrazing. The observation that education has no significant effect on deforestation suggests that factors beyond education probably are more influential in driving environmental changes. While education is often assumed to foster greater environmental awareness

and responsible practices (Van De Wetering *et al.*, 2022), the lack of a statistically significant association indicates that other socio-economic factors may play a more critical role. For instance, practices such as

farmland expansion, charcoal production, and urbanization can heavily impact forested areas regardless of the population's education level (Basnyat, 2009).

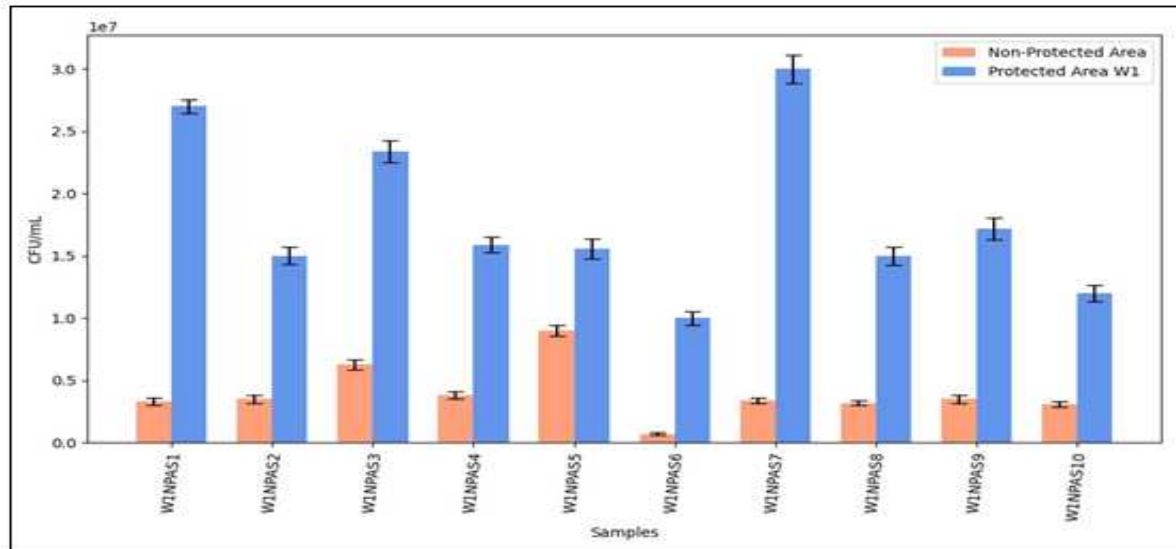


Fig. 3. Colon forming units from non-protected and protected areas sample soil from Ward I.

Note: 1. W1NPS1 to W1NPS10 =Ward 1 non-protected area sample 1 to 10

2. W2NPS1 to W2NPS10=Ward2 non-protected area sample 1 to 10.

The results of this study further indicated that age plays a role in land conversion and overgrazing (Table 4), particularly among individuals aged 41 and above. This age group is significantly more likely to convert forest land to farmland, with a positive association (coefficient = 0.089, $p = 0.0003$), while younger age groups (20-30 and 31-40) do not show significant

associations. For overgrazing, older individuals (41 and above) are less likely to contribute, as indicated by a significant negative coefficient (-0.063, $p = 0.043$). However, similar to education, age does not significantly affect deforestation rates, as none of the age groups examined show significant associations with deforestation.

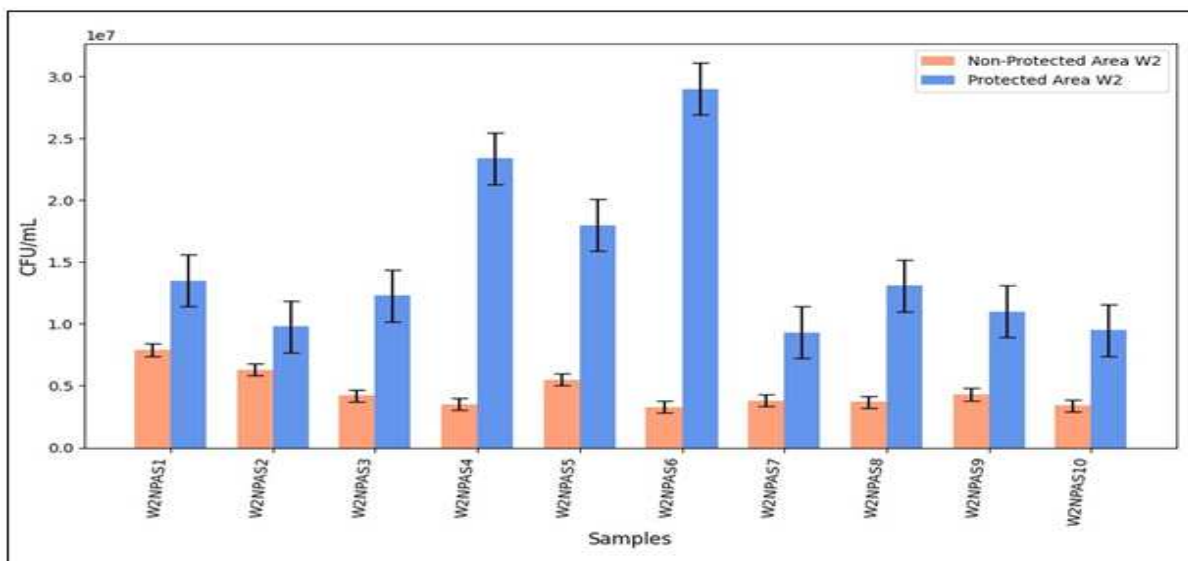


Fig. 4. Colon forming units from non-protected and protected areas sample soil from Ward I.

These findings align with Kodiwo *et al.*, 2015, who found that individuals with elementary and post-secondary education experienced higher soil erosion in Nyakach Sub-county, Kenya. In contrast, farmers had lower erosion depths, while civil servant households faced greater erosion. This emphasizes the need to consider socio-economic factors in addressing soil erosion and promoting sustainable land management.

Soil fungal microbial colony forming in protected verses non protected areas

Soil analysis from the non-protected area (NPA) revealed a range of colony-forming units (CFU) per milliliter. In the WINPA zone, CFU/mL values ranged from 380,000 to 9,000,000, while in WIPA, the

values spanned from 10,000,000 to 30,000,000. The mean CFU/mL for WINPA was 1.811×10^7 (Fig. 3). For W2NPA, CFU/mL values ranged between 3,300,000 and 7,900,000, with a mean of 4.59×10^6 . In contrast, W2PA showed values between 9,800,000 and 30,000,000, with a mean of 1.489×10^7 (Fig. 4). The overall mean CFU/mL for NPA samples was 4.175×10^6 , whereas the protected area (PA) samples had a higher mean of 1.308×10^7 (Fig. 5). These averages suggest a notable difference in soil fungal microbial density between PA and NPA, indicating a significantly lower presence of fungal microorganisms in NPA soils compared to PA. This disparity reflects the greater microbial activity and volume in protected areas, likely due to better soil conservation and ecological balance.

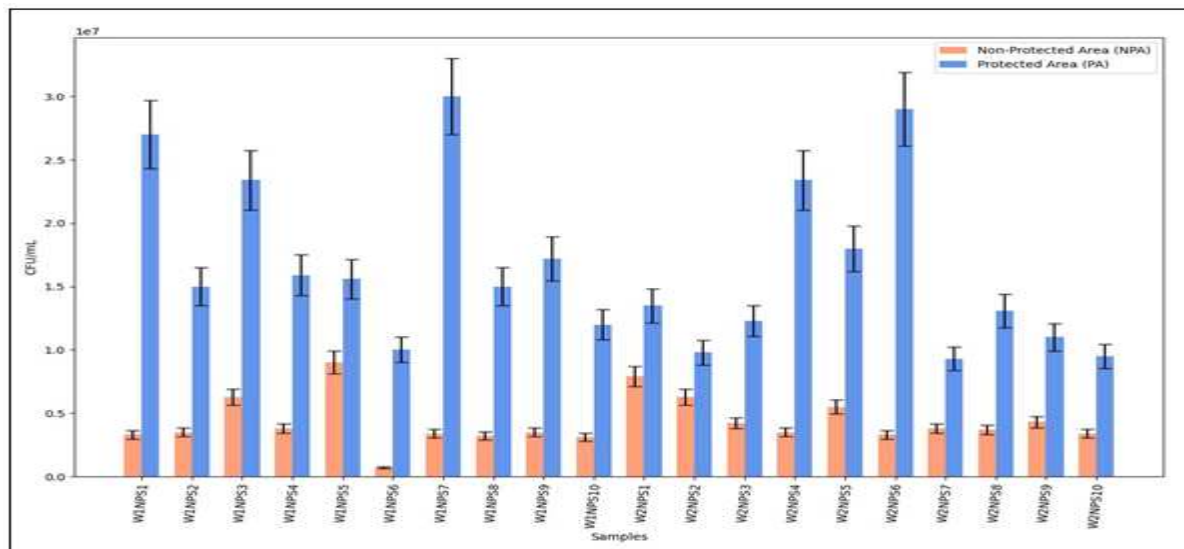


Fig. 5. Showing the combined bar charts of CFU/mL for the protected and Non-Protected areas in both wards.

Note: 1. W1PAS1 to W1PAS10=Ward 1 protected area sample 1 to 10

2. W2PAS1 to W2PAS10=Ward 2 protected area sample 1 to 10.

The results further indicated that soil samples collected from PA consistently had more fungal colonies than those from NPA (Fig. 2). The observed large number of fungal microbial colonies in PA soils could be explained by minimal human disturbance *viz.* frequent land tillage, overgrazing, and inappropriate use of inorganic fertilizers and pesticide that can negatively impact fungal microbial communities. Likewise, the green bars which represent soil samples collected from PA display significantly higher CFU values than the blue bars for

NPA (Fig. 2). In samples like W1PAS7 and W2PAS6, the difference is particularly notable, with PA having more than double of CFU/mL of NPA (Fig. 5), suggesting that protective regulations create a more favorable environment for fungal microbial proliferation by reducing environmental stress. Therefore, the findings of this study further highlight the importance of considering anthropogenic influences when assessing fungal microbial health (Kodiwo *et al.*, 2015) and ecosystem dynamics (Adomako and Ampadu, 2015). Nevertheless, a clear

trend emerges showing that soil samples collected from PA consistently outperformed those from NPA in nearly all study sites (Fig.5). This observation aligns with findings by Liu *et al.* (2020), Dukpa *et al.* (2020) and Guan *et al.* (2022), which indicate that inappropriate agricultural practices can diminish microbial volume and density by disrupting soil structure and lowering organic matter both vital for sustaining fungal communities (Martinsen *et al.*, 2017). This reinforces the notion that better land use practices play a crucial role in enhancing fungal microbial growth and overall soil health.

Conclusion

This study highlights the significant association between community knowledge and land use practices on soil fungal microbial volume. It shows that unsustainable land-use practices, such as overgrazing, deforestation, and excessive use of industrial fertilizers, contribute to soil degradation and disrupt fungal microbial activities. The study reveals that gender significantly influences the adoption of organic manure, with women less likely to use it compared to men, underscoring the importance of addressing gender disparities in agricultural decision-making.

Further, it shows that higher education level increasing income and practical knowledge have positively influenced use. Additionally, the study found that protected forest soils have higher fungal volume compared to farmlands, emphasizing the need to minimize human disturbance to preserve soil health. This study underscores the need for awareness campaigns sustainable soil management practices and promoting the use of organic manure to maintain soil fertility and ecosystem balance.

Acknowledgments

We would like to acknowledge the invaluable contributions of our research assistants and ward leaders. Their dedication and support were instrumental in the successful execution of this study. Their local knowledge and guidance were crucial in facilitating data collection and engaging with the

community. Thank you for your commitment and hard work. With gratitude hearts, we also express our sincere thanks to the Sisters of Charity of St. Vincent de Paul's Congregation and The Catholic Scholarship Program and Agroecology Hub in Tanzania (AEHT) project for sponsoring this research.

Funding

This research was funded by the Sisters of Charity of St. Vincent de Paul, the Catholic Scholarship and Agroecology Hub in Tanzania (AEHT) project.

Declarations ethical approval

All participants were fully informed about the nature, purpose, and potential risks and benefits of the study before agreeing to participate. Clear and comprehensive information sheets were provided, and written or verbal informed consent was obtained from all participants.

Competing interests

There are no competing interests.

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