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ARTICLE

Assessment of Fungi Species Associated with a Multicultural Orchard and Cultivated Land in Bingham University Landscape, Karu Nasarawa State, Nigeria

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ABSTRACT

Assessment of fungal species associated with a multicultural orchard and cultivated land in Bingham University landscape was carried out, with the ultimate aim of identifying the fungi species present in soil under different agricultural practices. A total of 30 soil samples were collected and the composite from each land use pattern was analyzed in the laboratory using standard methods. Soil type, percentage Soil Moisture (SM), percentage Organic Carbon (OC) and percentage Organic Matter (OM) were measured using standard methods; fungi species were isolated and identified on the basis of mycelia and spore characteristics, after staining with lactophenol-in-cotton blue. The results showed that, four types of soil exist in the sites including sandy, clayey, silt and loamy. SM ranges between 3.6% -5.7%, and OC in the sandy soil was the highest 1.01% in the orange plantation (Op5) followed closely by loamy soil on cultivated site C with 0.97% OM and 0.56% OC and least in clay soil with 0.72% OM and 0.42% OC. The results of colony forming unit per gram (cfu/g)in relation to land use type, Cultivated Site C (CC1-CC5) had the highest (262 cfu/g) and mango plantation (Mp1-Mp5) had the least with 156 cfu/g. Pictorial representation of isolated fungal species are indicative of suspected presence of *Aspergillus Spp, Mucor Spp, Cladosporium Spp, Fusarium Spp, Aspergillus Spp* etc. This qualitative study concluded that fungal species population in the soil depends on the management practice in place and the moisture content of the soil.

1. Introduction

Some fungi species are among soil organisms that control ecosystem functioning through decomposition and nutrient cycling and may serve as indicators of land-use change and ecosystem health [1-3]. The indigenous microbial populations in soil are of fundamental importance for ecosystem functioning in both natural and managed agricultural soils because of their involvement in such

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key processes such as soil structure formation, organic matter decomposition, nutrient cycling and toxic removal [1,4]. Soil is a most precious natural resource that contains the most diverse assemblages of living organisms and soil organic matter is important in relation to soil fertility, sustainable agricultural systems, and crop productivity [5].

Fungi constitute an important part of the soil ecosystem, playing a central role in the biotic and abiotic interactions in the environment, participating in the decomposition of organic matter and the recycling of soil nutrients to make them available to plants [6]. Therefore, communities of soil fungi are involved in soil fertility [7] and contribute to the alleviation of soil degradation [8].

Soil fungi are an immensely diverse group of organisms, and their diversity is affected by the local environmental conditions [9], including the chemical and physical soil characteristics, which determine to a great extent the composition of fungal communities [10].

Soil degradation is triggered by human activities (anthropogenic), which influence the biodiversity of soil [11], and it impacts fungal diversity because soil characteristics influence the presence, distribution, and abundance of fungal species, and the soil characteristics depend on the soil degradation level. Every soil particle has a different micro-spatial composition of fungal species, which is influenced by different micro-habitats in the soil [12].

Every species of fungi requires specific conditions for development, reproduction, and propagation, including different ranges of temperature, moisture, carbon reservoirs, seasons, soil depth, or chemical factors [13]. Soil compaction decreases soil fertility through decreasing storage and supply of water and nutrients, which entails a reduction in the activities and diversity of fungal communities [14].

Also, soil moisture is assumed to be very important for microorganisms, because water availability is fundamental for different processes. The soil pH has a strong influence on species richness of soil fungi, diversity, and community structure [15]. The composition and proportion of the soil components have appreciable effects on nutrient concentrations and soil texture, thereby influencing the community of soil fungi [16]. Different land use pattern was therefore assessed in order to understand the effects of different agricultural practices on fungal species.

In view of these significant roles played by fungi in the soil, this study was therefore designed to assess the fungi species associated with the multicultural orchard and an adjacent agricultural land in Bingham University landscape.

2. Materials and Methods

The study was carried out in Bingham University karu, Nigeria, located in Auta Balefi, Karu, Nasarawa state. It has a tropical climate with two distinct seasons; rainy and dry (harmattan) seasons. The University is geographically located at latitude 8°50ºN and longitude 7°52ºE. (Garmin Etrex GPS)

The soil samples were collected along transect lines running through the study sites at interval of 10 meters apart and 10 meters along the transect to avoid overlap, and samples were taken 1 meter to the North, South, East and West of the point (compass direction). Five soil samples each at auger depth of 2 m were collected within

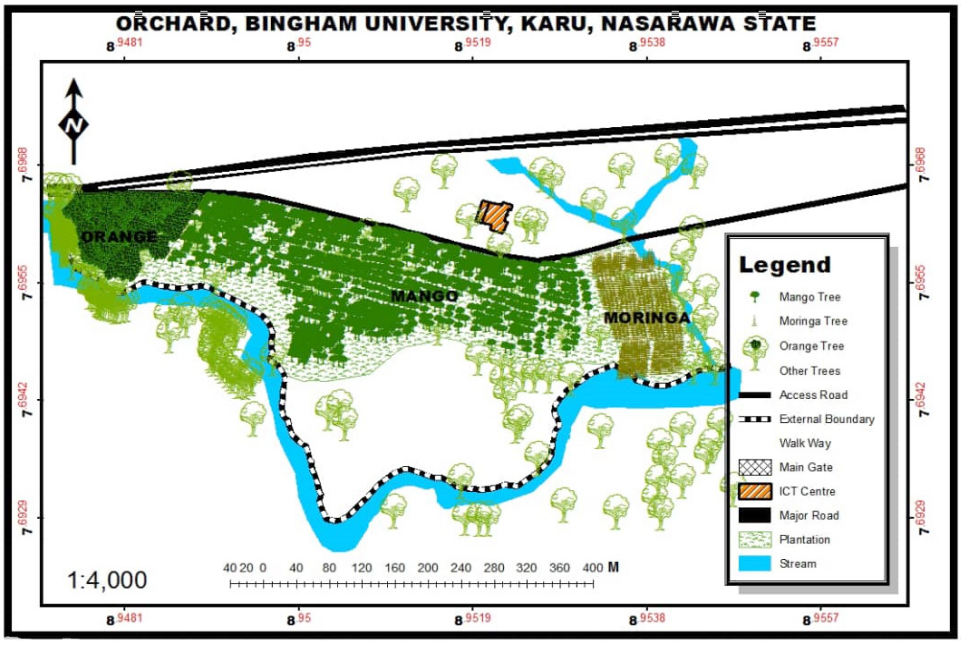


Figure 1. Map of the Study area showing the Multicultural Orchard (*Moringa oliefera*, *Mangifera* spp and *Citrus* Spp)

the Citrus Spp (Op1-Op5), *Mangifera* Spp (Mp1-Mp5) and *Moringa oliefera* (Zp1-Zp5) stands, and from the cultivated land A, B and C, which were denoted as CA1-CA5, CB1-CB5 and CC1-CC5 respectively. At each point, composite soil samples were obtained after homogenising the soil samples collected from each of the quarters at a sampling point. The composite samples were then taken the laboratory and analysed.

After the soil samples were collected, the samples were dried at room temperature and sieved with a 2 mm mesh sieve, the unwanted materials such as stones, granules, plant parts, and leaves were discarded from samples. The air dried and sieved soil samples were used for the estimation of soil organic carbon.

There are various methods for estimating OM in soil [17]. Loss of weight can be used as a direct measure of the OM contained in the soil. Percentage organic matter (%OM) was calculated by using the formula below;

$\%OM = W_1\text{-}W_2 / W_2 \times 100$

Where,

W_1 is the initial weight

W_2 is weight after the soil samples were dried

Percentage organic carbon (%OC) is calculated by; %OM X 0.58

Soil moisture was estimated by subtracting the weight of the dry soil from the weight of the moist soil, and then dividing by the weight of the dry soil [17].

The soil samples were cultured on potato dextrose agar (PDA) medium because it contains Potato infusion which provides a nutrient base for the rich growth of most fungi and Dextrose serves as a growth stimulant [18]. The incorporation of tartaric acid (TA) in the medium lowers the pH to 3.5, thereby inhibiting bacterial growth. . The potato dextrose agar was weighed and mixed with the appropriate mL of distilled water and was shaken thoroughly. The medium was autoclaved for 15 min. Autoclaved medium was poured in sterile petri dish (15 mL/plate) and allowed to solidify [18].

Soil samples from various locations were taken and serial dilutions were made [18]. For this, 10 g sample was taken and added in tube containing distilled water and mixed thoroughly. This represented 10-1 dilution. Under aseptic conditions, 10^{-2} to 10^{-9} dilutions of samples were prepared [18].

For fungal population analysis, serial dilution method [18,19] was carried out, followed by using potato dextrose agar medium for the culturing. The inoculated plates were incubated at 25 °C. Colony forming units (CFU) were estimated by counting the number of colonies after 48 hours. Fungi were identified according to their macroscopic and microscopic features.

The isolation of fungi from soil was carried out in order to identify each fungus. The serial dilution method was used to dilute the soil sample as described by [18-20] with the purpose of minimizing the fungi in the soil in each dilution. Each sample was diluted five times and labelled as 10^{-1} until 10^{-9}. 10g of soil will be added in 10 mL of sterile distilled water and was thoroughly shaken to mix the solution. The solution was diluted to a series of prepared vials containing 9 mL of sterile distilled water. 1 mL of the soil-distilled water solution was then transferred to the first vial by using a pipette. Subsequently, another 1 mL of the solution from the first vials was transferred to the second vial and the steps continued until the last vial. 0.1 mL of the solution in each vial was pipetted into the prepared PDA plate and incubated at room temperature for 48 hours [18]. The fungi grown were sub cultured on new potato dextrose agar plates and incubated for 48 hours. The sub cultured fungi on the agar were stained with a lactophenol cotton blue solution on glass slides and were viewed under the microscope to further be able to identify them [18].

The identification of the isolated fungi was done by its staining procedure. A fungal colony was first grown on the potato agar medium and its morphology was studied by using inoculating needles to place the colony taken on a glass slide and staining with lactophenol-in- Cotton Blue [20]. The stained and air-dried slides were further examined under microscope at 40 X magnification. The fungi were identified on the basis of mycelia and spore characteristics.

3. Results

3.1 Soil Parameters and Fungal Colonies Isolated from the Soil Samples

As shown in Table 1 below, four soil types (Clayey, Sandy, silt and loamy) were identified. In the *Citrus* Spp plantation (Op1-Op5), soil type include sandy and silt with percentage Organic Matter (OM) content between 0.74% - 0.87%, however, percentage Organic Carbon (OC) was in the range of 0.43% - 0.51%. The Percentage Moisture Content (MC) and number of fungal colonies isolated were between 4.0% -5.5% and 23.0 cfu/g - 37 cfu/g respectively.

Similarly, in the *Mangifera indica* plantation (Mp1-Mp5), sandy and loamy soil samples were found with percentage Organic Matter (OM) content between 1.01% - 0.71%. The percentage Organic Carbon (OC) was in the range of 0.41% - 0.58%, while the Moisture Content (MC) and number of fungal colonies isolated were between 3.8% -5.7% and 23.0 cfu/g - 37 cfu/g respectively.

Again, in the *Moringa oliefera* plantation (Zp1-Zp5), loamy soil samples were found with percentage Organic Matter (OM) content between 0.79% - 0.91%, percentage

Organic Carbon (OC) was in the range of 0.46% - 0.53%. Percentage Moisture Content (MC) and number of fungal colonies isolated were between 4.3% -5.4% and 35.0 cfu/g - 40 cfu/g respectively.

In the cultivated site one (CA1-CA5), clay soil samples were found with percentage Organic Matter (OM) content between 0.72% - 0.91%, percentage Organic Carbon (OC) was in the range of 0.42% - 0.53%. Percentage Moisture Content (MC) and number of fungal colonies isolated were between 3.6% -5.2% and 42.0 cfu/g - 55 cfu/g respectively.

Similarly, in cultivated site two (CB1-CB5), sandy, silt and clay were found with percentage Organic Matter (OM) content between 0.79% - 1.0%, percentage Organic Carbon (OC) was in the range of 0.46% - 0.58%. Percentage Moisture Content (MC) and number of fungal colonies isolated were between 4.3% -5.7% and 33.0 cfu/g - 46 cfu/g respectively.

In the cultivated site three (CC1-CC5), sandy and loamy soil were found with percentage Organic Matter (OM) content between 0.79% - 0.97%, percentage Organic Carbon (OC) was in the range of 0.46% - 0.56%. Percentage Moisture Content (MC) and Number of fungal colonies isolated were between 4.2% -5.3% and 49.0 cfu/g - 55 cfu/g respectively.

Table 1. Percentage Organic Matter, Organic Carbon, soil Moisture Content and No of fungal colonies formed (cfu/g)

Site	Soil type	% Organic Matter	% Organic Carbon	% Soil Moisture Content	No of fungal colonies formed (cfu/g)
Op1	Sandy	0.74	0.43	4.0	23
Op2	Sandy	0.84	0.49	4.6	25
Op3	Silt	0.81	0.47	4.4	31
Op4	Silt	0.87	0.51	4.7	37
Op5	Sandy	1.01	0.59	5.5	32
Mp1	Loamy	1.00	0.58	5.7	37
Mp2	Loamy	0.71	0.41	3.8	35
Mp3	Sandy	1.01	0.59	5.5	36
Mp4	Sandy	0.72	0.42	3.6	25
Mp5	Sandy	0.86	0.50	4.7	23
Zp1	Loamy	0.91	0.53	5.4	40
Zp2	Loamy	0.83	0.48	4.3	48
Zp3	Loamy	0.81	0.47	4.4	35
Zp4	Loamy	0.88	0.51	5.2	39
Zp5	Loamy	0.79	0.46	4.8	37
CA1	Clay	0.90	0.52	5.1	45
CA2	Clay	0.91	0.53	5.2	42
CA3	Clay	0.84	0.49	4.2	44
CA4	Clay	0.72	0.42	3.6	51
CA5	Clay	0.84	0.49	4.5	55
CB1	Sandy	0.95	0.55	5.4	40
CB2	Sandy	1.00	0.58	5.7	37
CB3	Silt	0.83	0.48	4.3	46
CB4	Clay	0.79	0.46	4.8	33
CB5	Clay	0.90	0.52	4.6	42
CC1	Sandy	0.90	0.52	5.1	50
CC2	Loamy	0.97	0.56	5.3	52
CC3	Loamy	0.84	0.49	4.2	56
CC4	Sandy	0.79	0.46	4.8	49
CC5	Sandy	0.88	0.51	5.2	55

Key: **Op1 -Op5** = Soil samples collected under the Orange (*Citrus* Spp) Plantation
Mp1-Mp5 = Soil samples collected under the Mango (*Mangifera* spp) Plantation
Zp1-Zp5 = Soil samples collected under the Zogale (*Moringa Oliefera*) Plantation
CA1-CA5 = Soil samples collected from cultivated **site one(A)**
CB1-CB5 = Soil samples collected from cultivated **site two (B)**
CC1-CC5 = Soil samples collected from cultivated **site three (C)**

3.2 Number of Fungal Colonies

Colonies formed from every plot of land were counted and the results were represented in Table 2 below. Op4 had the highest number of colonies from all samples collected from the orange orchard. From samples collected from the mango orchard, Mp1 had the highest number of colonies. Zp3 had the highest number of colonies formed from samples that were collected from the *Moringa oliefera* (Zogale orchard). CA5, CB3, CC3 had the highest numbers of colonies formed from the cultivated land A, B and C respectively. As shown in Table 2 above, Op4 had the highest number of colonies from all samples collected from the orange orchard. From samples collected from the mango orchard, Mp1 had the highest number of colonies. Zp3 had the highest number of colonies formed from samples that were collected from the *Moringa oliefera* (Zogale orchard). CA5, CB3, CC3 had the highest numbers of colonies formed from the cultivated land A, B and C respectively.

Cumulatively, in terms of fungi colonies forming units, cultivated land C ranked highest (262 cfu/g), followed by cultivated land A (237 cfu/g) and Moringa plantation with (199 cfu/g). The least was recorded on Mango plantation (156 cfu/g), while cultivated land C and orange had (198 cfu/g) and (158 cfu/g) respectively.

3.3 Estimation of Soil Moisture

The soil samples were used to estimate their moisture contents. The samples were weighed and calculated and

Table 2. Number of colonies formed at every point of collection at the Mango, Orange and Moringa, and Cultivated Land A, B and C

Land use Type	Plot	No of colonies counted(cfu/g)	Total (cfu/g)
Orange	Op1	**33**	**158**
	Op2	25	
	Op3	31	
	Op4	37	
	Op5	32	
Mango	Mp1	37	156
	Mp2	35	
	Mp3	36	
	Mp4	25	
	Mp5	23	
Moringa	Zp1	40	199
	Zp2	48	
	Zp3	35	
	Zp4	39	
	Zp5	37	
Cultivated Land A	CA1	45	237
	CA2	42	
	CA3	44	
	CA4	51	
	CA5	55	
Cultivated Land B	CB1	40	198
	CB2	37	
	CB3	46	
	CB4	33	
	CB5	42	
Cultivated Land C	CC1	50	262
	CC2	52	
	CC3	56	
	CC4	49	
	CC5	55	

the results were recorded as shown in Table 3 below. Op5 had the highest amount of moisture content from all samples collected from the orange orchard. From samples collected from the mango orchard, Mp1 had the highest amount of moisture content. Zp3 had the highest number of colonies formed from samples that were collected from the Zogale orchard. CA2, CB2, CC3 had the highest numbers of colonies formed from the cultivated land A, B and C respectively. The results above show the calculated amount of moisture content in all the soil samples. As shown in Table 3 above, Op5 had the highest amount of moisture content from all samples collected from the orange orchard. From samples collected from the mango orchard, Mp1 had the highest amount of moisture content. Zp3 had the highest number of colonies formed from samples that were collected from the Zogale orchard. CA2, CB2, CC3 had the highest numbers of colonies formed from the cultivated land A, B and C respectively.

Cumulatively, in terms of percentage SM content, cultivated land B ranked highest (24.8%), followed by cultivated land C (24.6%) and Orange plantation with (24.3%). The least was recorded on cultivated land A (22.6%), while cultivated Mango and Moringa plantation had (23.3%) and (24.1%) respectively.

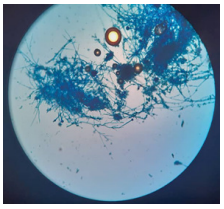
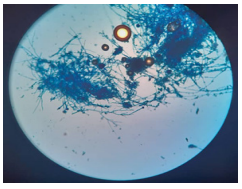
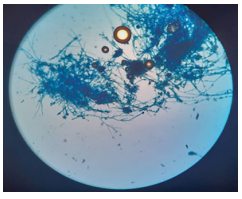
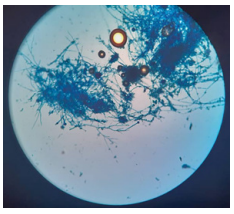
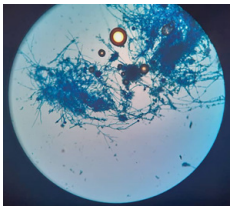
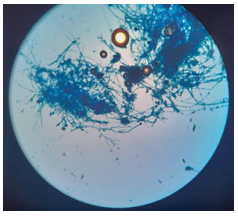
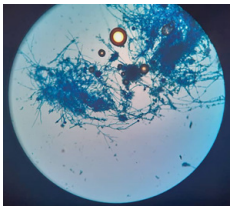
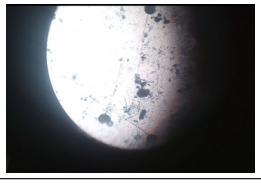
3.4 Suspected Fungal Species Isolated

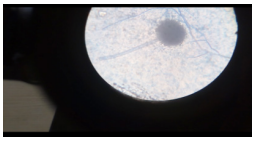
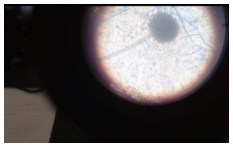
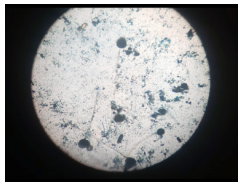
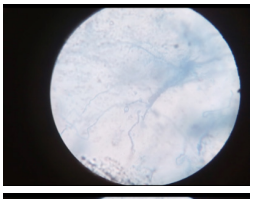
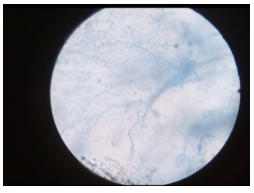
The fungi grown were stained on a slide using the lactophenol-in-cotton blue stain and were viewed under the microscope. The suspected fungal species were identified based on their morphological characteristics as recorded in Table 4 below.

Table 3. Percentage Soil Moisture Content

Land use Type	Plot	% Soil moisture	% Total
Orange	Op1	4.0	24.3
	Op2	4.4	
	Op3	4.7	
	Op4	5.5	
	Op5	5.7	
Mango	Mp1	5.7	23.3
	Mp2	3.8	
	Mp3	5.5	
	Mp4	3.6	
	Mp5	4.7	
Moringa	Zp1	5.4	24.1
	Zp2	4.3	
	Zp3	4.4	
	Zp4	5.2	
	Zp5	4.8	
Cultivated Land A	CA1	5.1	22.6
	CA2	5.2	
	CA3	4.2	
	CA4	3.6	
	CA5	4.5	
Cultivated Land B	CB1	5.4	24.8
	CB2	5.7	
	CB3	4.3	
	CB4	4.8	
	CB5	4.6	
Cultivated Land C	CC1	5.1	24.6
	CC2	5.3	
	CC3	4.2	
	CC4	4.8	
	CC5	5.2	

Table 4. suspected fungi species and their morphological characteristics and pictorial representation.

Suspected Fungi species	Macroscopic features	Microscopic features	Pictorial representation
Aspergillus oryzae	Colour of colony formed was brown.	Have conidial heads.	
Aspergillus wentii	Colour of colony formed was yellowish brown.	Have filaments, consists of colourless conidiaphores and spores.	
Aspergillus flavus	Colour of colony formed was yellowish green.	Have conidial heads, thick walled and vesicle bearing.	
Aspergillus niger	Colony formed was pale yellow.	Have filaments, consists of colourless conidiaphores and spores.	
Aspergillus versicolor	Colour of colony formed was whitish.	Have filaments with conidial heads and not so thick walls.	
Aspergillus clavatus	Colony formed was dark green in colour.	Have conidia produced from conidiophore which are in a columnar form.	
Aspergillus fumigates	Colony formed was dark green in colour.	Conidial heads on short columnar, have conical shaped terminal vesicles.	
Mucor racemosus	Colour of colony formed was grey.	Branched sporangiophores, needle like spores.	

Suspected Fungi species	Macroscopic features	Microscopic features	Pictorial representation
Mucor hiemalis	Colony formed was gray in colour.	Grows branched that are held by colume-shaped columnar.	
Mucor mucedo	Colour of colony formed was dark gray.	Broad hyphae and have spores.	
Mucor circinelloides	Colour of colony formed was dark gray.	Have branched sporangiophore with spherical shaped sporangium.	
Cladosporium cladosporioides	Colour of colony formed was dull greenish black.	Have one-celled conidia that is cylindrically shaped.	
Cladosporium herbarum	Colonies formed were pigmented and blue-black in colour.	Have one-celled conidia that is cylindrically shaped.	
Fusarium moniliforme	Colony formed was dark blue.	Have hyphae that are septate with simple conidiophores.	
Fusarium oxysporum	Colony formed was dark blue.	Have a thin wall. Conidiophores are short and simple.	
Fusarium semitectum	Colony formed was dark blue.	Have septate hyphae, conidiophores are simple.	

4. Discussion

Nature of soil can only determine the quantity and the quality of microbial activities, but cannot totally prevent them from interactions that could lead to ecosystem processes. Fungi are especially needed for such ecosystem processes like decomposition, water retention and environmental health. Soil fungi are an immensely diverse group of organisms, and their diversity is affected by the local environmental conditions [9], In all of the soil samples collected, parameter assessed varied and that had

reflected in the fungal colony counts/g of soil sample and also, on the percentage SM, OM and OC. For instance, cultivated Land A is characterized with sandy soil and some clay, hence it has more OM content and that is probably why in every plot of land, there more fungal colony counts in those plots. Loamy, clay and silt soils also exhibit high levels of microbial activity which shows that organic matter is pretty high in those soil types as well and it is good for fungi to thrive [10]. This outcome is probably due to fungi preference to slightly acidic conditions, low disturbance soils, perennial plants, internal nutrient sources directly from the plant, and highly stable forms of organic residues with high carbon to nitrogen (C:N) values and slower recycling time as stated by [21], and this also is the likely reason for the different level of microbial activities (fungal presence) with results in the percentage OM across the different soil types.

Fungi colonies forming units in cultivated land C ranked highest (262 cfu/g), followed by cultivated land A (237 cfu/g) and Moringa plantation with (199 cfu/g), and this could probably be due to high level of organic matter and carbon content and also the soil moisture content [10]. It can also be attributed to the soil type being loamy which provides for slightly acidic which permit for fungal activity [10]. The least was recorded on Mango plantation (156 cfu/g), while cultivated land C and orange had (198 cfu/g) and (158 cfu/g). This might be that land in the mango plantation is compacted and less input of carbon which consequently could not allow for ecological processes like decomposition of OM [9].

Soil Moisture (SM) is very important for fungal growth and it influences the amount of OM in soil. Cultivated land B ranked highest (24.8%), followed by cultivated land C (24.6%) and Orange plantation with (24.3%). The least was recorded on cultivated land A (22.6%), while cultivated Mango and Moringa plantation had (23.3%) and (24.1%). This outcome is unexpected, because cultivated land that are exposed have more SM than the soil under canopy in some cases. This might be as a result of the soil formation, type and the moisture holding capacity. The moisture level differs in the different plantation and this obviously affects the fungal activities which in turn affects the organic matter and carbon contents of the soil. This possibly affects the likely population of fungal species in the multicultural orchard. This study outcome agrees with reports by [22], it however differs from study report by [23] that reported a rather higher *fusarium* species as compared to *Aspergillus* species. And based on morphological characteristics, suspected fungal Species were *Aspergillus* spp, *Mucor* spp, *Cladosporium* spp, *fusarium* spp, and management system.

5. Conclusions

Fungal populations are strongly influenced by the diversity and composition of the plant community and in return affect plant growth through mutualism, pathogenicity and their effect on nutrient availability and cycling. They also play an important role in stabilization of soil organic matter and decomposition of residues. This study has been able to assess soil organic matter flux and fungal species diversity associated with the multicultural orchard and fringing forest in Bingham University landscape. This has been achieved by identifying varying quantities of organic matter and carbon content of soil samples, soil moisture content, number of fungi colonies and suspected fungal species collected from the areas under study. This is a preliminary and qualitative study, therefore further study is needed to properly identify the fungi species to generic level.

Authors' Contributions

Ihuma J.O. designed the study, handles the statistical analysis and is the principal investigator, Agida I.O. and Nashima T.N. handles literature search and sample collection and assist in the laboratory processes of the study alongside the principal investigator.

Conflict of Interest

The author collectively declares that there is no conflict of interest.

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