Fungal Population in Farming Soils in a Community in Rivers State

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Abstract
Fungal population were studied from different farming soils in Rukpokwu community, in Rivers State. The control soil was collected from a non- farming soil. Soil samples were collected from different location using a hand auger at dept of 0-15cm and labeled A-F. Mycological and physicochemical analysis were done using standard procedures. The mean total fungal count ranged between 5.21×10^3 to 2.57×10^4 CFU/g. The lowest fungal counts were found in sample F (control), while the highest fungal counts were found in sample A. The predominant fungal isolates identified were Mucor sp, Microsporum sp, Candida sp, Penicillium sp, Aspergillus niger, Aspergillus flavus, Rhizopus sp, Rhodotorula sp, Geotrichum sp, Scopulariopsis sp, and Saccharomyces sp. Candida sp occurred more followed by A. niger while Rodiotorula sp, Rhizopus sp, Saccharomyces sp and Geotrichum sp shared similar frequency and occurred the least. The study showed that fungal populations in the various farms were higher than those in the control. The fungal isolates from the various soil samples differed only little. The nutrient composition of farm soil revealed that the soil samples contain nutrients necessary for fungus and crop growth. The results suggest that plants residue can play a pivotal role in fungal population in soil.

Keywords: farming soil, fungal population

Introduction

Fungal are an important aspect of the ecosystem and may be found in a variety of biodiversity locations, including soil, plants, animals, and water, with soil serving as the primary driver of the fungi kingdom. In the agricultural sector, fungal species play an important role. Fungus is thought to be an enormously diverse and little understood category of organisms, with an estimated 5 million fungi worldwide, of which 74,000–120,000 have been identified. These fungal species have been discovered in a variety of settings, including mines, deep oceans, glaciers, deserts, hot springs, and coastal regions (saline zones) (Tiwari et al. 2021, Yadav 2021).

Sustainable agriculture is an environmentally friendly strategy to developing techniques with minimal negative environmental consequences or activities. There is no doubt that activities like excessive soil tilling, deforestation, bush burning, and indiscriminate use of agrochemicals contribute to environmental degradation. In a word, sustainable agriculture aims to use soil-friendly practices and techniques that utilize nonrenewable resources sparingly (Figueiredo et al. 2017).

The diversity and composition of the plant community have a profound influence on fungal populations, which in turn affect plant growth through mutualism, pathogenicity, and their effect on nutrient availability and cycling (Wardle 2002; Wagg et al., 2014; Hannula et al. 2017). Fungi also aid in nitrogen fixation, hormone generation, biological control of root diseases, and drought resistance (Jayne & Quigley 2014; Baum et al. 2015, El-Komy et al. 2015). They're also vital for soil organic matter stability and residue breakdown. Fungi have a role in the breakdown of organic matter and the delivery of nutrients to plants. Their involvement in plant defense against pathogenic microbes as biological agents, which has an impact on soil health (Frac et al. 2018), is critical. All agricultural systems require good soil management, and reducing soil deterioration is a top goal for long-term output. Only by taking soil fungal biodiversity into consideration can this impact be realized. The study's major goal was to examine the fungal population and diversity in farming soils.

Materials and Methods
Description of Study Area

The area of study were farms in Rukpokwu, Obio/Akpor Local Government Area of Rivers State. Plants such as cassava, vegetables and plantain were mostly seen in the study area. The coordinates of the study areas are presented in Table 1. Sample locations were labeled A-F.

Table 1: **GPS coordinates of the farms under study**

SAMPLE LOCATIONS	GPS CORDINATES
A	$4^o54'26''$ N $7^o0'17''$ E
B	$4^o54'18''$ N $7^o0'17''$ E
C	$4^o54'30''$ N $7^o0'17''$ E
D	$4^o54'28''$ N $7^o0'13''$ E
E	$4^o54'29''$ N $7^o0'14''$ E
F (control)	$4^o54'28''$ N $7^o0'13''$ E

Collection of Sample
Soil samples were collected from three different points (1m apart) in a farm into clean bags using hand auger at a dept of 0-15cm to form composite a sample. Samples A-E were collected from five different farms in the above locations. While the control, sample F was collected from non-farming soil. The samples were put into a cooler containing ice packs and were transported to the Microbiology Laboratory, Rivers State University for analysis.

Sample Preparation
The ten-fold serial dilution as described by Amadi *et al.* (2014) was adopted. In this method, 1g of the soil was weighed and transferred aseptically into test tubes containing sterile 9ml diluent (normal saline)

which gave an initial dilution of 1:10ml. Subsequent dilutions were carried out by transferring 1ml from the initial dilution to another test tubes containing 9ml sterile diluent which gave rise to 1:100 dilution. This was repeated until a dilution of $1:10^{-6}$ was reached.

Enumeration and Isolation of Heterotrophic Fungi

Aliquot (0.1ml) of 10^{-2} and 10^{-3} dilutions were transferred on prepared Sabouraud Dextrose agar (SDA) plates which have been fortified with tetracycline antibiotics for the inhibition of bacterial growth. The plates were later spread evenly using sterile bent glass rod. Inoculation was done in duplicates and after inoculation, plates were incubated at room temperature (22-25 °C) for 4 days. Enumeration of fungal counts was carried out after incubation, while distinct fungal colonies were morphologically characterized and sub-cultured on fresh SDA plates for further identification.

Identification of Fungal Isolates

Isolates were identified using their morphological features such as colony color, shape, texture and size of colony followed by microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue and reference made to fungal identification manual (Sarah *et al.* 2016).

Physicochemical Parameters

Determination of Temperature

The temperature was determined in the site of collection. temperature for each sample was determined using a mercury-in-glass thermometer by adopting APHA standard methods (APHA 2012). About 10g of the soil sample was emptied into 100ml beaker containing 50ml distilled water. The thermometer was immersed into the samples such that the mercury bulb was well covered by the samples. The final readings were considered the actual reading and were taken after allowed to stabilize.

Determination of pH

The pH of soil sample was determined by APHA Standard Methods (APHA 2012). The meter was switched on and allowed for some time. It was then calibrated with buffer solutions of high pH range between 8 and 9 as well as a lower pH range between 1 and 6 by dipping the electrode into the buffer solutions. 10g of soil was weighed into 100ml beaker; 50ml of distilled water was then added to allow immersion of the electrode, mixing was carried out by stirring frequently for few minutes. Then beaker was allowed to stand for 15 minutes. The electrode was immersed into the sample. The pH values for each sample were recorded accordingly.

Determination of Total Nitrogen

Reagents: Analytical reagent grade AR chemical are highly recommended, selenium powder, lithium sulphate, hydrogen peroxide, 30% and sulphuric acid concentration.

Digestion Mixture: About 0.42g selenium powder 14g lithium sulphate was added to 350ml, 30% H_2O_2 and was properly homogenized, after which 420ml concentration of H_2SO_4 was gently added while cooling in an ice both. This was stored at 20^oC in stable for 4 weeks.

Procedure: total of 1.0g of oven dried soil sample was weighed into a labelled dry and clean digestion tube. Then 15 ml digestion mixture was added to each tube. This was digested at at 360^oC for 2 hours. The solution should now be colourless and remaining sand while if solution in still coloured, heat for a further 1 hour, allow to cool. Add about 25ml distilled water and mix well until no soil dissolved. Allow cooling, make up to 50ml with distilled water and mix well. Allow settling so that a clear solution can be taken from the top of the tube for analysis.

Determination of Total Organic Carbon (TOC)

About 0.2g of soil sample was measured into a 500ml conical flask. 10ml of 0.5M $K_2Cr_2O_7$ was added and swirled gently. 20ml of concentration H_2SO_4 was added rapidly and directly into suspension but with care to avoid splashing. Immediately swirl gently until the reagents are mixed for 1 minute. Flask was allowed to stand for 30 minutes. 200ml of distilled water and 10ml concentration H_3PO_4 was added cautiously to avoid splashing and mixture was cooled, 3 drops of Ferroin Indicator Solution was added. Solution was titrated to a deep green end-point with 0.25M Ferroin Ammonium Sulphate (FAS) solution (APHA 2012).

Determination of Phosphate (PO_4^{3-})

The phosphate levels for the samples were determined using an ultraviolet (UV) spectrophotometer. 25ml of 2.5% Acetic acid was added to 1g of soil sample and shaken for 30minutes. The suspension was filtered through a filter paper. 10ml of the extract was transferred into 50ml volumetric flask. Extract was diluted with distilled water until the flask is about 2/3 full. 2ml of Ammonium Molybdate reagent was added and mixed with extract. 2ml of stannous chloride was also added and mixed; the solution was diluted to 50ml mark with distilled water. The flask was allowed to stand for 30minutes, and the absorbance was measured at wavelength of 690nm (APHA 2012).

Statistical Analysis

The mean and standard deviation of fungal counts was calculated using statistical package SPSS (version 23). One-way ANOVA was carried out to check for significant differences while the Tukey Pairwise Comparisons at 95% Confidence interval was used in separating the means.

RESULTS

Fungal Population

The result for the fungal populations in farming soil is presented in Table 2. The mean total heterotrophic fungal counts of samples A, B, C, D, E and F are 2.57×10^4, 1.08×10^4, 7.83×10^3, 1.46×10^4, 7.56×10^3 and 5.21×0^3 cfu/g. Results showed that soil sample A had the highest fungal counts while soil sample F had the least fungal counts.

Table 2: Total Heterotrophic Fungal Counts in the Farms

Sample Location	Total Heterotrophic Fungal Counts
A	2.57×10^4
B	1.08×10^4
C	7.83×10^3
D	1.46×10^4
E	7.56×10^3
F	5.21×0^3

Characterization of Fungal Isolates

Results of the fungal isolates from the various farming soil is presented in Table 3. Results showed that all seventy-six fungal isolates belonged to *Mucor* sp, *Microsporum* sp, *Candida* sp, *Penicillium* sp, *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus* sp, *Rhodotorula* sp, *Geotrichum* sp and *Saccharomyces* sp.

The frequency occurrence of the fungal isolates is presented in Fig 1, while the percentage abundance is presented in Fig 2. The frequency occurrence of *Aspergillus niger, Aspergillus flavus, Microsporium* sp, *Candida* sp*, Scopulariopsis sp, Mucor* sp, *Pencillium* sp, *Rodiotorula* sp*, Rhizopus* sp, *Saccharomyces* sp and *Geotrichum* sp were 22.37, 19.74, 9.21, 31.58, 5.26, 2.63, 3.95, 1.32, 1.32, 1.32 and 1.32 %, respectively.

Results showing the distribution of the fungal isolates across the farms is presented in Table 4. Results showed that fungal isolates varied across the respective farms. *Candida* sp were the most occurring fungal isolates followed by *A. niger* while *Rodiotorula* sp*, Rhizopus* sp, *Saccharomyces* sp and *Geotrichum* sp which shared similar frequency were the least occurring fungal   isolates.

Table 3: Macroscopy and Microscopy of Fungal Isolates

Isolates	Macroscopy	Microscopy	Probable Identity
a.	Fluffy white cottony, white reverse	Aseptate hyphae bearing round sporangiospores	*Mucor* sp
b.	waxy, glabrous, convoluted thallus with a cream to buff-coloured surface and no reverse pigment	Spindle shaped macroconidia with verrucose thickwalled. Presence of irregular branching hyphae, prominent cross walls	*Microsporum* sp
c.	Cream small round raised colony	Spherical budding cells	*Candida* sp
d.	Green powdery surface surrounded by white lawn, brown reverse	Septate hyphae with septate conidiophores bearing conidia	*Penicillium* sp
e.	Black spores surrounded by white lawn-like growth	Aeseptate conidiophores bearing conidia	*Aspergillus niger*
f.	Light green lawn surrounded by white lawn-like growth	Septate hyphae with aseptate conidiophore bearing conidia	*Aspergillus flavus*
g.	Fluffy white to grey spores, brown reverse	Aseptate banana hyphae	*Rhizopus* sp
h.	Pink-red wrinkled colonies	Ovoid, elongate budding cells	*Rhodotorula* sp
i.	Flat white dry sued-like colonies with no reverse pigment	Cylindrical arthroconidia	*Geotrichum* sp
j.	White small creamy-shiny colony	Large oval cells, presence of budding cells	*Saccharomyces* sp

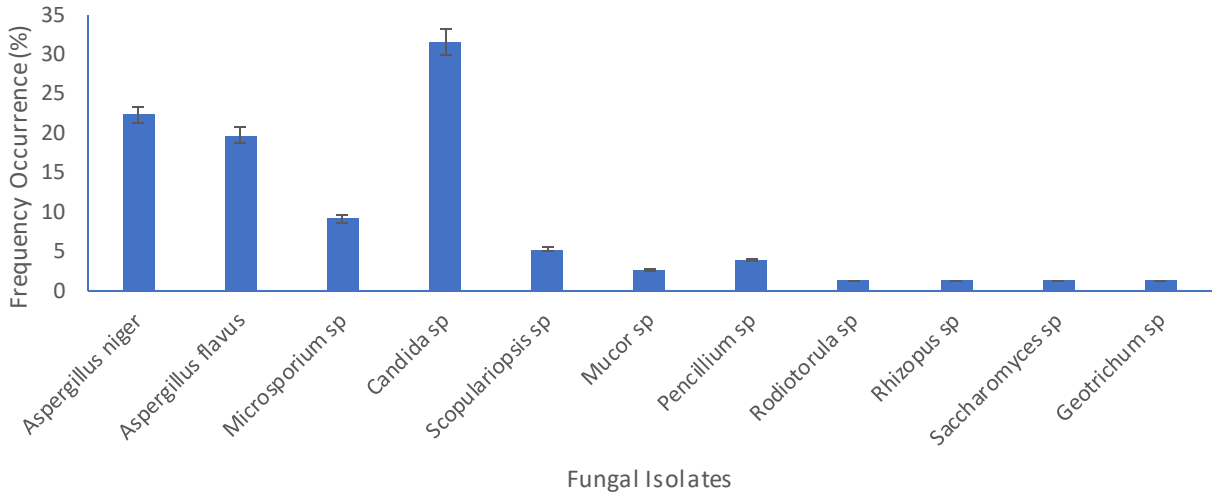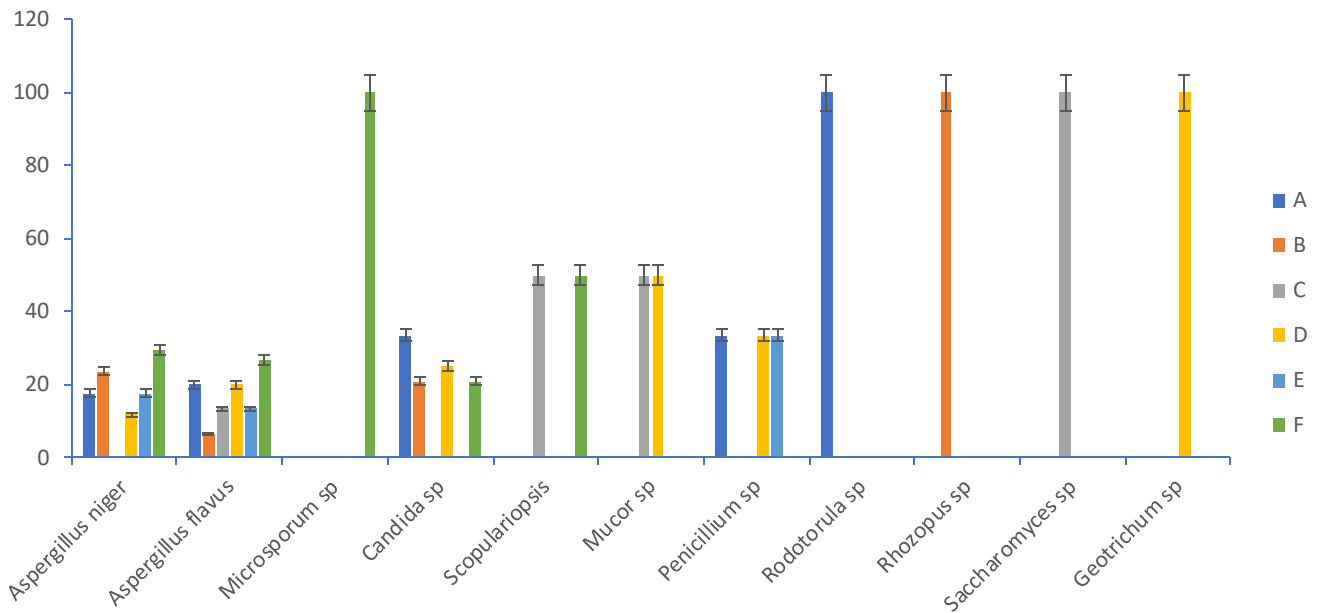| k.	Buff to brown like growth	chains of single-celled conidia *Scopulariopsis* sp produced in basipetal succession

Fig 1: Frequency occurrence of fungal Isolates

Fig 2: Percentage Abundance of Fungal Isolates in the Farms

Table 4: Distribution of Fungal Isolates Across the respective farms

Isolates	A	B	C	D	E	F
Aspergillus flavus	+	+	+	+	+	+
Aspergillus niger	+	+	_	+	+	+
Rhizopus sp	_	+	-	-	-	-
Mucor sp	-	-	+	+	_	_
Penicillium sp	+	_	_	+	+	_
Candida sp	+	+	_	+	_	+
Saccharomyces sp	_	_	+	_	_	_
Geotrichum sp	_	_	_	+	_	_
Microsporum sp	_	_	_	_	_	+
Scopulariopsis sp	_	_	+	_	_	_
Rhodotorula sp	+	_	_	_	_	_

Keys: +: present; -: not present

Physicochemical Parameters of Soil Samples

Results of the physicochemical parameters of the soil samples are presented in Table 5. Results showed that the pH, temperature, total nitrogen, phosphate, total organic carbon and potassium ranged from 7.70 - 8.32, 27.8 - 31.3°C, 0.010 - 0.112 mg/kg, 3.96 - 5.21 mg/kg, 0.44 - 1.65 mg/kg and 4.971 - 6.012 mg/kg.

Table 5. Physicochemical Parameters of Agricultural Soil from Various Farms

Sample location	pH	Temperature (°C)	Total Nitrogen (Mg/kg)	Phosphate (Mg/kg)	TOC (%)	Potassium (Mg/kg)
A	8.32	28.4	0.021	3.96	1.04	4.984
B	8.21	31.3	0.013	4.53	1.61	5.003
C	8.10	29.0	0.010	5.01	1.65	5.012
D	7.90	30.2	0.112	4.22	1.64	4.971
E	7.70	27.8	0.017	5.21	1.52	5.008
F	7.91	30.6	0.10	3.42	0.44	6.012

DISCUSSION

The total fungal population recorded in this study showed that soil from sample A had the highest fungal counts which was followed by sample D. The control recorded the least fungal count. Despite the disparity in the total heterotrophic fungal counts across the soil samples, fungal counts across the soil samples, there was no significant differences in the fungal counts across the sampling points. The plant residues drop to the soil and the death and decay of organisms may have provided the necessary organic carbon necessary for the high fungal population in the farming soils as compared to the control that recorded the Lowest fungal population which could be attributed to lack of vegetation. The result supports the findings that diversity and composition of plant community influence the fungal population, which in turn will affects plant growth through mutualism pathogenicity and their effect on nutrient availability and cycle (Wardle 2002, Wagg et al. 2014, Hannula et al. 2017).

A total of seventy-six fungal isolates were recorded. They belong to the genus namely; *Mucor*, *Microsporum*, *Candida*, *Penicillium*, *Aspergillus*, *Rhizopus*, *Rhodotorula*, *Geotrichium*, *Saccharomyces* and *Scopulariopsis*. These are fungal know to be habitat in soil and plant materials. The percentage abundance of the isolates was determined. *Aspergillus niger* was isolated in all the samples apart from sample C. The highest percentage abundance of *Aspergillus niger* was recorded in sample F, while the least was recorded in sample C. *Aspergillus flavus* was isolated in all the samples. The highest percentage abundance was in sample F while the least was recorded in sample B. *Microsporum* was only isolated in sample F. *Candida* sp was isolated in all the samples apart from soil sample C and E. Percentage abundance of *Candida* sp was highest in sample A while the least was in samples B and F. *Scopulariopsis* was isolated from only samples C and F which had the same percentage abundance. Mucor was isolated from only sample C and D, with sample percentage of abundance. *Penicillium* was only isolated in samples A, D and E, they all had similar percentage abundance. *Rodotorula*, *Rhizopus*, *Saccharomyces*, and *Geotrichum* were isolated from samples A, B, C and D, respectively. The frequency occurrence of the fungal isolates showed that *Candida* recorded the highest frequency occurrence followed by *Aspergillus niger*, *Aspergillus flavus*, *Microsporium*, *Scopulariopsis*, *Penicillium* and *Mucor*. *Rodiotorula*, *Rhizopus*, *Saccharomyces* and *Geotrichum* recorded similar percentage of occurrence and were observed to be the least occurring fungal isolates. The fungal diversity in the soil samples could be attributed to different environmental factors and soil nutrients, which confirms the findings of Nielsen et al. (2015) that fertile soil harbours an abundant active, diverse and adaptive microbial community. Also, according to Ellouze et al. (2013) soil fungal diversity could be influenced by plants. It was observed that the farms had different plants. In the present study, more fungal diversity was found in soil sample D than other soil sample, reason could be litter decomposition is very rich. Litter decomposition and soil fertility, the rate of decomposition of organic matter influences the rate of nutrients (Das et al. 2007). The least fungal diversity was observed in sample E, this may be because of very little amount of litter decomposition that was found in the location.

The pH of the various soil samples is generally alkaline. Fungal isolates are known to thrive in diverse environmental conditions and the form of asexual reproduction which they undergo is one of the major attributes for their inhabitations of different environment. Although, they thrive better in acidic environments but growth in other environmental pH have been reported.

According to Smith & Read (2008), soil fungi can grow in a wide range of soil pH but their population is more under acidic conditions because of severe competition with bacteria at neutral pH. Thus, the fungal population earlier reported could have been influenced by competition of other minerals since the soil samples were entirely alkaline and favours the growth of bacteria which thrive well in alkaline soils (Prescott et al. 2011). The pH of the soil and other nutrients could have been influenced by the different forms of chemicals or organic substances added in the soil. This agreed with Parham et al. (2002) who reported a significant increase in soil pH due to the addition of cattle manure while the addition of chemical fertilizer resulted in slightly lower pH values.

Like every physicochemical parameter, the temperature, total nitrogen, phosphate, total organic carbon and potassium concentrations in the soil samples of the different farms varied. The highest temperature was observed in farm B followed by Sample F (control) and farm D while farm E had the least temperature. The fluctuation in the temperature of the soil could be attributed to the amount of heat exchange as well as other factors like shade which limits the soil from receiving direct radiant energy from the sun. according to Elias et al. (2004) the temperature of the soil is determined by heat flow in the soil and heat exchanges between the soil and the atmosphere while Onwuka & Mang (2018) opined that Solar radiation is the primary source of soil temperature. It was also reported that the function of the soil's internal energy (Geiger et al. 2003). The principal sources of fluctuations in soil temperature are heat transmission in the soil and latent heat exchanges at the surface (Nwankwo & Ogugurue 2012, Zhao et al. 2007). The temperature of soils is a very important parameters as it controls many activities in the soil including nutrient availability and enzymatic activities of the microorganisms and other biotic life forms existing in the soil. Soil temperature, biological activities such as seed germination, seedling emergence, plant root development, and nutrient availability are all affected by the quantity of radiation absorbed by the soil (Probert 2000). Also, the rate of organic matter breakdown and mineralization of various organic components in the soil is said to be affected by soil temperature (Onwuka & Mang 2018). Furthermore, most soil microorganisms require temperatures ranging from 10°C to 35.6°C to function well (Onwuka & Mang 2018) and Low soil temperatures reduce microbial activity and at temperatures near the freezing point, most activities halt (Allison 2005). Thus, the soil temperature in the current study is within the required temperature for microbial activities and plant growth.

The nutrients availability of the soil which include nitrogen, potassium and phosphate were also investigated and findings showed that these nutrients fluctuated across the farms with no particular farm maintaining a high trend all through. For instance, farm D had the highest nitrogen content followed by Sample F (control) soil while farm B had the least nitrogen content. Also, farm E had the highest phosphate concentration followed by farms C and B while the control had the least phosphate concentration. While in terms of the potassium content, the highest concentration was observed in the control sample followed by farms E, C and D. These continuous fluctuations observed in the present study could be attributed to the different microbial activities underneath the soil as well as other abiotic and environmental factors including fertilizer applications carried out in the respective farms. Farm yard manure is a potentially important source of nitrogen, phosphorus and potassium (Swer et al. 2011). Thus, the variation observed in these nutrients across the farms in the present study could be directly or

indirectly influenced by the amount and type of manure applied in each respective farms as well as the microbiological activities. This is in agreement with the findings of Plaza et al. (2004). More so, microbial metabolic activities have been reported to play vital roles in the cycling of nutrients in the soil and ensuring that the nutrients are in a form that plants can use (Onwuka & Mang 2018). The organic carbon in soil is a crucial determinant for soil quality and productivity. There is a close relationship between soil enzyme activity and soil organic carbon, mainly affected by horizons and other factors (Deb et al. 2015). The maintenance of soil organic carbon is the main factor favouring soil microbial biomass and enzyme activity (Zhao et al. 2018). The results of the organic carbon in the farms showed that the control soil had the least total organic carbon while higher organic carbon was detected in the agricultural soil samples. The organic carbon in the current study is higher than the 0.08 reported by Nontobeko et al. (2021). The low values of total organic carbon recorded in the control soil could be attributed to reduced root biomass in the sampling site, which may decrease the labile (carbon) C input to mineral soil in the form of root excretions. This agreed with Nontobeko et al. (2021) who also made similar observations.

CONCLUSION

This study revealed that there was disparity in the population of fungal counts across the soil samples from the respective soil sample. Fungi isolated from the study were known to be habitat in soil and plant material. Fungal diversity was observed in the samples however, fungal diversity did not vary significantly among the areas. Fungi population plays a major role in the accessing soil fertility. The fungi isolated were able to thrive in alkaline pH, also the soil sample recorded values of some micronutrients which may have also supported the fungal growth.

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