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SOIL MICROBIAL LOADS IN THREE DIFFERENT LAND USE IN MICHAEL OKPARA UNIVERSITY OF AGRICULTURE RESEARCH AND TEACHING FARM, UMUDIKE, ABIA STATE

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ABSTRACT

The distribution and abundance of soil microbes were studied under three different land uses (arable land, forest land and pasture land) in Michael Okpara University of Agriculture, Research and Teaching Farms, Umudike, Abia State to observe the impact of land use on fungi and bacteria populations. Soil samples were collected at 3 sampling points from each land use type at 0 – 20 cm depth. From the data collected, the populations of *Bacillus* and *Aspergillus* species showed some level of significance at 5%. The results of some soil properties studied revealed that the soil chemical properties and microbial distribution varied with land use systems. The total viable count of *Bacillus* population under forest land ($18.00\times10^3\pm8.72$ CFU/g) was significantly ($p<0.05$) higher than pasture land ($3.00\times10^3\pm1.00$ CFU/g) and arable land ($8.67\times10^3\pm3.79$ CFU/g).Also, forest land was observed to have the highest total viable count of bacteria population of $1.52\times10^5\pm0.84$ CFU/g). Values of *Aspergillus* population was highest in arable land ($1.33\times10^3\pm0.58$ CFU/g) but was statistically similar ($p>0.05$) to forest land and pasture land. Population of fungi was significantly the same in the different land use types. There was significant difference ($p<0.05$) in soil pH, with arable land giving the highest mean value of 5.4±0.17. Organic carbon content was highest in pasture (3.33±0.17) and forest land uses (3.10±0.79) and significantly ($p<0.05$) different from that of arable land (2.10±0.22). In this study, land use affected microbial population and also influenced organic matter content.

Keywords: *Aspergillus* species, *Bacillus* spp., Arable, Pasture, Forest Lands

INTRODUCTION

Soil microorganisms are essential for ecosystem functioning and key drivers to nutrient cycling in soils (Val-Moraes *et al*., 2013; Nwokeh *et al.*, 2022). They are one of the factors that contribute to soil formation. The roles soil microorganisms play are basically that of sustainability of soil for crop production and ecosystem stability (ecostability). The functions of soil microbes facilitate nutrient cycling. Willger *et al*. (2009) reported that the fungus *Aspergillus fumigates* has the ability to recycle carbon and nitrogen within the environment. The beneficial ones, bacteria for instance, are involved in the detoxification of harmful substances in soil, and also facilitate breakdown of organic compounds (Haines-Young and Potschin, 2013). Nutrient cycling is dependent on the presence and population of microbes.

Different microbial entities carryout specific functions in soil nutrient cycling. Some agronomic practices, such as tillage, may increase crop yield, but at the same time have negative effects on microbial populations. Soil particles influence fungi diversity and degradation and regulate their distribution (Grundmann, 2004). Bacteria and fungi in soil enhance the sustainability, and decrease chance for soil to degrade (Aktar *et al*., 2009).

Land use system involves the modification and rearrangement of the soil system which may affect the activities of microbes and eventually lead to soil degradation if not properly controlled (Braimoh and Vlek, 2004). Biological activities and other soil physical and chemical properties are affected by change in land use system (Viollete *et al.*, 2009).With intensive land management, often leading to reduced soil organic carbon (SOC) storage, microbial activities have been negatively affected (Sanderman *et al*., 2017). That is to say continuous land use that exposes soil resource to harsh environmental conditions causes a sharp decline in the soil fertility.

The challenge with the understanding of the functions of soil microorganisms is to evaluate the factors that are likely to regulate their activities. The major objective of this work was to highlight the bacteria and fungi populations as affected by different land use systems, based on the distribution of *Bacillus* spp. and *Aspergillus* spp. in the area studied.

MATERIALS AND METHODS

Study Area

The study was carried out in Michael Okpara University of Agriculture Research and Teaching Farm, Umudike, Research and Teaching Farm, Ikwuano Local Government Area of Abia State, Nigeria. The area lies within latitude 5º27´N and longitude 7º35´E (Chukwu, 2012). The area has a characteristic bimodal rainfall, which starts in April and ends in October, with its peak in July and September (Nigeria Meteorological Agency, 2015). The vegetation of the area studied is Rainforest and the soil is derived from Coastal plain sand parent material. The average annual rainfall ranges between 1,800 and 2,500 mm / year, with the peak between July and August (Nuga and Akinbola, 2011).

Soil Sample Collection

Within each land use system, three sampling points were marked randomly. Soil samples were collected at 20 foot walk away from each other at a depth of 0 – 20 cm making a total of9 soil samples, 3 from each land use. The soil samples collected were carefully put into sterilized bottles and then taken for laboratory analyses.

Soil pH was determined in a 1:2.5 soil-water ratio (Thomas, 1996). Organic carbon was determined, following the procedure of Walkley and Black wet oxidation method as modified by Nelson and Sommers (1996).

Isolation of Soil Microbes

One gram of each soil sample was weighed on a mettler sensitive scale. A plastic rack was arranged with sterile test tubes having 9ml of sterile distilled water. Serial dilution method (Fawole and Oso, 1988) was used to isolate bacteria and fungi from the soil.

A pour plate method (Fawole and Oso, 1988) was used in plating all the soil samples. 1ml from dilution 10^{-3} (the dilution that was not too turbid and not too plain) was dropped into separate sterile Petri dishes with the aid of different sterile pipettes. A molten nutrient agar and Potato Dextrose agar were poured into these plates (10ml). The plates were rotated clockwise for easy mix-up of the sample and the media. Nutrient Agar (NA) was used to culture bacteria species while Potato Dextrose Agar (PDA) was used for the growth of Fungi species. They plates were allowed to solidify and later duplicated.

All the Potato Dextrose Agar plates (Fungi) were transferred into an incubator at 25ºC for 3 days while the Nutrient agar plates (Bacteria) were transferred into another incubator at 37ºC for 18 – 24 hours. Plates were observed daily for emergence of mycelia (Fungi) or colony (bacteria) growth.

With the aid of flamed wire-loop, discrete colonies from the bacteria plates were isolated after incubation and then sub-cultured to obtain a purified bacteria colony. Also pure fungi isolate was obtained with the aid of flamed surgical knife which was used to transfer the mycelia from Potato Dextrose agar plates onto a newly prepared PDA plates. All plates were incubated again appropriately. Purified colonies (culture) were transferred into slants and stored in a refrigerator at 4ºC for identification.

Identification of Purified Fungi Cultures

Macroscopic examination was done by physical characteristics of the mycelia like structure and colour of the mycelia. The morphology structures viewed included septate or on – septate mycelia, presence of sporangiophores, uniting bodies and special organs like Rhizoids. Each morphology structure of each isolates were matched with a Mycology Atlas (Barnett and Hunter, 1987).

Colony counting

After the incubation period, plates were observed and colonies were counted. The colony count from the counting meter was recorded. A mean of the count was obtained and multiplied with the appropriate diluting factor. The mean count was calculated as:

$$\text{Mean} = \frac{Total\ Viable\ Count(Colonies)}{Number\ of\ plates}$$

The estimation of viable number of bacteria and fungi (Total Viable Count) in each sample was expressed in Colony Forming Unit (CFU/g).

The Analysis of variance (ANOVA) in complete randomized design (CRD) was calculated using R Statistical Package. Significantly different means were separated using least significant difference at 5% level of probability ($p<0.05$). Correlation analysis was used to determine the relationship between the selected soil properties and soil microbes.

RESULTS AND DISCUSSION

The total viable count of bacteria and fungi in the soils studied are shown in Table 1. The highest mean total viable count of *Bacillus* spp ($18.00\times10^3\pm8.72$ CFU/g) was significantly higher ($p<0.05$) under the forest land (FL). While the pasture land (PL) recorded the lowest ($3.00\times10^3\pm1.00$ CFU/g). Forest habitat provides a viable ecosystem for soil microbes to reproduce and thrive well. Their sources of energy are stable. The high total viable count of *Bacillus* spp. under forest land could be attributed to the relatively dense structure of plants and a greater accumulation of litter and fine roots of forest (Wright *et al.,* 2005 and Sharma *et al.,* 2004). Organic matter content has been reported to influence the distribution of bacteria (Moscatelli *et al.,* 2007 and Yang *et al.,* 2010). In arable land, the removal of vegetative cover and top soil might have contributed to the low total viable count observed in *Bacillus* count (Sharma *et al.,* 2004).

With respect to the bacteria population, forest land (FL) also recorded the highest total viable count of bacteria ($1.52\times10^5\pm0.84$ CFU/g), and was significantly different from value obtained from the arable land (AL) which had the lowest colony forming unit of $0.51\times10^5\pm0.11$CFU/g. Bacteria population increases with increase in the inputs of organic carbon and nitrogen to soil (Fernandes *et al.,* 2002; Cerri *et al.,* 2003) thereby increasing the bacterial population. On the other hand, pressure mounted on soils by different land uses raises a challenge for soil microbial stability. Many microbes are exposed to unfavourable conditions which eventually cause a decline in their populations, functions and activities, which invariable affect soil productivity and sustainability. To resuscitate biological activities, agricultural soils must be left relatively undisturbed; and the use of organic manure is encouraged.

The highest total viable count of *Aspergillus* spp ($1.33\times10^3\pm0.58$ CFU/g) was observed under arable land (AL) whereas the pasture land (PL) had the lowest mean total viable count of $0.67\times10^3\pm1.15$ CFU/g with significant different at $p<0.05$. The higher distribution of *Aspergillus* under arable land than other land uses was contrary to the observations of Li *et al*. (2004). However, the higher population of *Aspsergillus* may be attributed to the clay content of the soil (McCulley and Burke, 2004). Soils with high clay content may lead to more stabilization of soil organic carbon and higher population of *Aspergillus* (Schimel *et al.,* 1994).

With reference to the population of fungi, forest land had the highest total viable count of $2.03\times10^5\pm1.10$CFU/g, whereas arable land (AL) recorded the lowest ($1.02\times10^5\pm0.93$CFU/g).The higher total viable count of fungi under forest land could be attributed to the presence of undisturbed and abundant organic matter on the forest ground (Karam *et al*., 2011).

Table 1: **Total viable count of microorganisms of soils studied**

Land use	**Total viable microbial count**			
	***Bacillus* spp.**	**Bacteria**	***Aspergillus* spp.**	**Fungi**
	→ ($\times10^3$CFU/g) ←		→ ($\times10^5$CFU/g) ←	
AL	8.67	0.51	1.33	0.17
FL	18.00	1.52	1.00	2.03
PL	3.00	1.33	0.67	1.57
$LSD_{0.05}$	11.02	1.00	1.88	1.67

AL = Arable Land, PL = Pasture Land, FL= Forest Land, CFU = Colony Forming Unit

A correlation analysis conducted showed that total viable count of bacteria had a positive significant correlation ($p<0.05$) with mean total viable count of fungi ($r = 0.706^*$), but a negative significant correlation ($p<0.05$) with soil pH ($r = -0.798^*$). There was a positive relationship ($p<0.01$) between total viable count of bacteria and organic carbon content ($r = 0.897^{**}$).

Total viable count of fungi population correlated significantly ($p<0.05$) but negatively with soil pH ($r = -0.743^*$). Fungi population also correlated significantly ($p<0.05$) and positively with organic carbon content ($r = 0.706^*$). Also a strong negative correlation ($r = -0.847^{**}$) was observed between soil pH and organic carbon content ($p<0.01$) (See Figures 1 – 6).

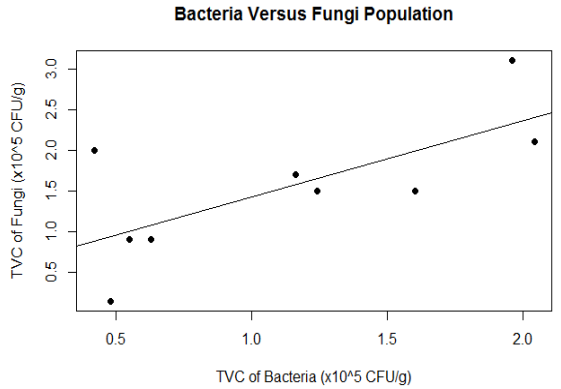

Figure 1: Positive significant correlation between bacteria and fungi populations in soil

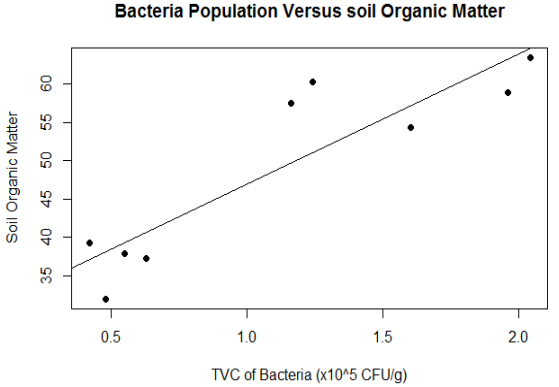

Figure 2: Positive significant correlation between bacteria population and soil organic matter

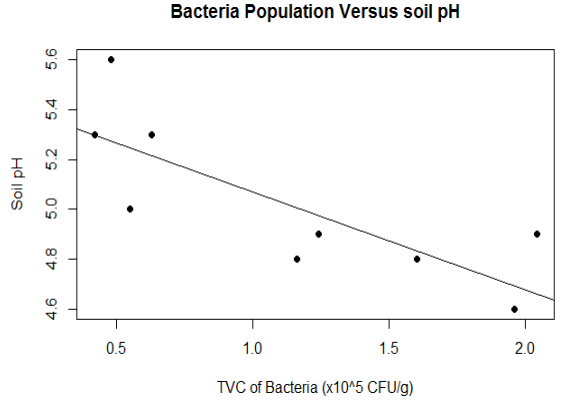

Figure 3: Negative significant correlation between bacteria population and soil pH

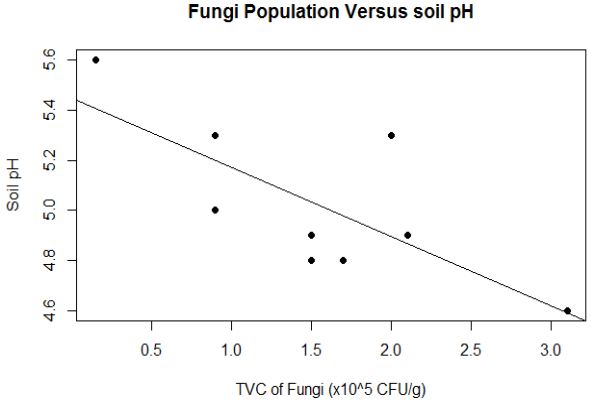

Figure 4: Negative significant correlation between fungi population and soil pH

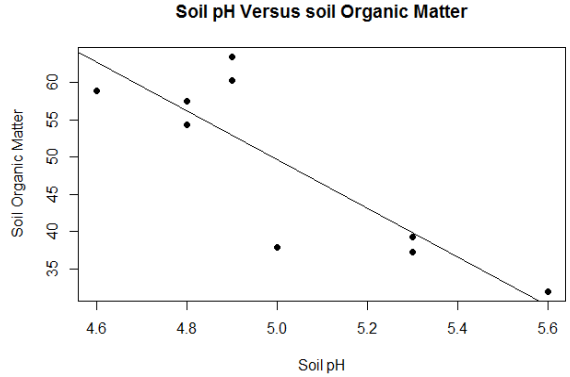

Figure 5: Negative significant correlation between soil organic matter and soil pH

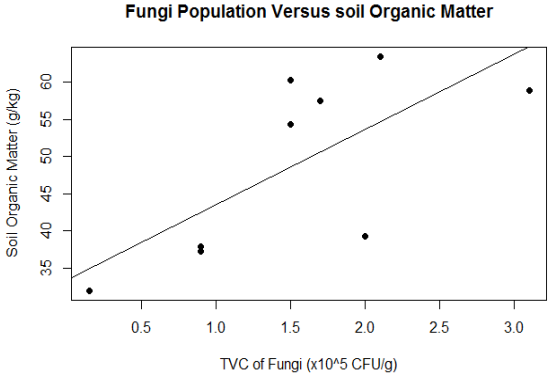

Figure 6: Positive significant correlation between fungi population and soil organic matter

Soil Organic matter

The organic matter and pH of the soils studied are shown in Table 2. Results obtained from this study revealed that forest land (FL) had the highest soil organic matter content with a mean value of 57.30 g/kg. While the arable land (AL) had the lowest (36.20 g/kg) organic matter contents. Land use significantly ($p<0.05$) influenced organic matter content. This findings also agreed with what was documented by Bizuhoraho *et al.* (2018). They reported that forest land in Rwanda had the highest organic matter content, while the lowest value was observed in cultivated land. The quality and quantity of vegetation of land use types causes variations in soil organic matter contents. Leaves, stems and barks from trees, flowers and fruits raise organic matter contents of forest land (Alemayehu and Sheleme, 2013; Bizuhoraho *et al.*,

2018). Forest lands provide a variety of litters than arable or pasture land.

Another factor that might result to high organic matter content in forest land may be due to low temperature and low decomposition rate of litters. Even though soil microorganisms enhance the decomposition of litters, environmental conditions such as temperature might reduce microbial activities, hence reduce decomposition rate. The mechanism involved is in enzyme activities; slight increase in temperature can facilitate enzymatic reactions which eventually increase rate of decomposition. So, in forest land, because of dense vegetation and much soil cover, rate of decomposition reduce, leaving high soil organic matter in place.

It is obvious that soil disturbance, tillage systems interrupt the stability of land and results to low organic matter and reduced microbial activities. This is usually evident in arable or cultivated lands (Malo *et al*., 2005).Organic matter serves as an ideal medium for fungal growth (Banning *et al*., 2008). This condition occurs because the forest is in equilibrium state and has an abundant plant dead material which is the main source of organic matter in the soils (Kourtev *et al*., 2002 and Bird *et al*., 2011).

Soil pH

With regards to pH, arable land (AL) had the highest pH value of 5.40, while forest land and pasture land had a lower pH of 4.83. The value of pH obtained in the arable land (5.40) is similar to the result obtained by Bizuhoraho *et al*. (2018) where the highest soil pH was found to be 5.3 in a cultivated land in Rwanda. It is likely, that inherent soil acidity on cultivated land is due to weathering and frequent use of inorganic fertilizers.

Oftentimes, soil acidity of a particular land can be induced by several anthropogenic activities. Soil pH, to a large extent influences the distribution and population of microbial species, especially those of fungi and bacteria origins (Siles and Margesin, 2016).

Table 2: Effect of land use on soil organic matter and soil pH

Land use	Soil properties (0 – 20 cm)	
	Organic Matter (g/kg)	Soil pH
AL	36.20	5.40
FL	57.30	4.83
PL	53.40	4.83
$LSD_{0.05}$	16.60	0.32

AL = Arable Land, FL = Forest Land, PL = Pasture Land,

CONCLUSION

The population of bacterial and fungal species in the different land uses is an indication of the effect of land use on microbial load and organic matter played an important role in the determination of the distribution and activity of microorganisms.

The continuous use of land for cultivation, provide a downward curve in microbial activities. Land used for agricultural purposes are put under pressure, and if not properly managed, substantially reduced microbial loads that contribute to soil health. It becomes important to develop bio-conservative strategies that will sustain frequently cultivated soils. Such strategies may include addition of organic materials, to enhance biomass build-up on arable lands. Forest soils exhibited higher microbial dominance and also enhances organic matter contents than the soils under arable land and pasture land.

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