

## ABUNDANCE, DIVERSITY AND DEPTH DISTRIBUTION OF BACTERIAL AND FUNGAL POPULATION IN UNIVERSITY OF UYO ARBORETUM

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### ABSTRACT

The extent of the diversity of microorganisms in soil are seen to be critical to the maintenance of soil health and quality, as a wide range of microorganisms are involved in important soil functions. The objectives of this study were to investigate the abundance, diversity and depth distribution of bacterial population at University of Uyo Arboretum, and was conducted from 2022 to 2023. The soils were collected at a depth of 0-10cm, 10-20cm and 20-30cm; total of nine (9) samples were collected, samples were divided into two (2) parts, one for soil routine analysis while the other for soil microbial analysis. Aseptic techniques were deployed to handle samples meant for microbial studies. Soil analysis results showed that the percentage sand decreases with depth, while silt and clay increases. The pH was slightly acidic at all sample depths. The electrical conductivity (EC), soil organic carbon (SOC), soil organic matter (SOM), total nitrogen (TN), available phosphorus (AVP), potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) also vary with depth. seventeen (17) microbial species, ten (10) bacteria species (*Bacillus cereus*, *Bacillus subtilis*, *Serratia sp*, *Pseudomonas aeruginosa*, *Micrococcus sp*, *Clostridium sp*, *Dissulfurivibriospp*, *Staphylococcus albus*, *Staphylococcus aureus*, and *Proteus mirabilis*) and seven (7) fungi, *Aspergillus fumigatus*, *Aspergillus niger*, *Candida albicans*, *Rhizopus stolonife*, *Clasdosparum spp*, *Penicillum expansum* and *Vertiocillium spp*) were found in the 9 soil samples investigated. *Bacillus cereus* was found to be more prevalent in the upper layers of soil (0-10 cm and 10-20 cm) while *Bacillus subtilis* was found in higher abundance in the deeper layers (10-20 cm and 20-30 cm), *Serratia sp.* and *Pseudomonas aeruginosa* were identified but were swarming, meaning they were moving rapidly on the surface of the agar plate, *Micrococcus sp.* was found in all three soil layers, with the highest abundance in the topmost layer (0-10 cm), *Clostridium sp.* was found to be more prevalent in the upper two layers of soil (0-10 cm and 10-20 cm) and less so in the deepest layer (20-30 cm), *Staphylococcus albus* was

found only in the topmost layer (0-10 cm) while *Staphylococcus aureus* was found in the upper and lower layers (0-10 cm and 20-30 cm), *Proteus mirabilis* was detected but swarming too. Shannon Weiner's index exhibited that the bacterial and fungal community in the soil diversified and even, with a higher number of species at the surface soil layer. However, there was a decrease in both diversity and evenness with increasing soil depth, indicating a shift in the fungal community composition at deeper soil depths. The microbial population decreases with increase in depth in terms of aerobic organisms but the anaerobes increased with increase in depth.

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**Keywords:** *Abundance, Diversity, Bacterial, fungal, Arboretum*

## **INTRODUCTION**

Soils are considered one of the most diverse microbial habitats because of their extensive physical, chemical and biological heterogeneity (Seuradje *et al.*, 2017; Neufeld *et al.*, 2017). Soil is a vibrant habitat for huge diversity of life-forms. It shelters many animals from invertebrates such as worms and insects to mammals like rabbits, rodents and badgers. It is also habitat of microorganisms. All these forms of life interrelate with each other and with the soil to create continually changing conditions. This allows changes in soil fertility and the soil productivity. Microorganisms are very diverse and include all the bacteria, archaea and almost all the protozoa. They also include some fungi, algae, and certain animals such as rotifers. Microorganisms live in every part of the biosphere, including soil, hot springs, "7 miles deep" in the ocean, "40 miles high" in the atmosphere and inside rocks far inside within the Earth's crust. The mass of prokaryote, bacteria and archaea may be as much as 0.8 trillion tons of carbon (Eilers *et al.*, 2012) (Fierer *et al.*, 2012). Recent studies indicate that airborne microorganisms play role in precipitation and weather (Hardy *et al.*, 1990). Microorganisms are also exploited in biotechnology, both in traditional food and beverage preparation, and in modern technologies based on genetic engineering (Bourgaize *et al.*, 2004).

Soil microorganisms are vital for the continuing cycling of nutrient and for driving above ground ecosystems. It is important to study microbial diversity not only for the basic scientific research, but also to understand the link between diversity and community structure and functions. Soil bacterial and fungi plays pivotal roles in various biogeochemical cycle (Molin *et al* 1997).

Soil microorganisms also influence above-ground ecosystems by contributing to plant nutrition (George *et al.*, 1995) Plant health soil structure (Dodd *et al.*, 2000) and soil fertility. However, activity and species composition of microbes are generally influenced by many factors including physicochemical properties of soil, temperature and vegetation. The dynamics of soil microorganisms have important implication for the response of subsurface soil ecosystem to perturbations. Environmental conditions, such as temperature and moisture tend to influence soil physical and chemical characteristics in shallow to deeper regions due to additions, losses, transfer and transformation of matter and energy that occur during the formation and development of soil. (Quesada *et al.*, 2010). Understanding the diversity and dominance of indigenous microbial population represent one challenge of modern soil ecology. This study focuses on the abundance, diversity and depth distribution of bacterial population in University of Uyo Arboretum.

## **MATERIALS AND METHODS**

### **EXPERIMENTAL SITE**

The study was conducted in the arboretum belonging to Department of Forestry and Environmental Management, University of Uyo, Akwa Ibom State. The Arboretum lies between latitudes 4°35' and 5°35'N and longitudes 7°35' and 8°25'E. It was allocated to the Department of Forestry and Environmental Management by the Faculty of Agriculture of University of Uyo farm committee in 1994 for arboretum and Departmental Nursery, it covers an area of 1.08ha. The area was organized into compartment and assigned to cover different forestry operations: Gmelina woodlot, bush fallow, Cassia species woodlot, agroforestry. In 1995 planting season, Gmelina and cassia species woodlots were established along with all the trees marking the nursery boundary. Tree species planted in the Arboretum include: *Lovoawhghheineana*, *Mammeafricana*, *Pterocarpus species*, *Mimusopsdjave*, *Entandrophragma species* and *Naucleadiderrichii*. The area marked out for bush fallow was planted up in 1996 with *Anthonatha macrophylla* and *Dactyladeniabaterii* and was allowed to grow undisturbed into forest. About hundred metres from the Department of Forestry and Environmental Management of the University of Uyo to the entrance of the Aboretum is planted with two rows of Teak (*Tectonagrandis*) along the walkway, with none of the trees being below

20metres in height. This created a natural canopy which shaded the non vegetated walkway.

## **SAMPLE COLLECTION AND ANALYSIS**

At the study area, three different sampling points were selected where an auger was drilled into the soil at depths of 0-10cm,10-20cm, 20-30cm respectively. Total of nine (9) sample were collected. Aseptic technique was deployed to handle sample to the laboratory for immediate analysis. The samples were split into two (2) parts, one for soil routine analysis and the other for microbial analysis.

## **LABORATORY ANALYSIS**

### **Routine Soil Analysis**

The soil samples for routine soil analysis were air-dried, pulverized and sieved (through a 2mm diameter sieve), bagged, relabeled and stored. While samples for microbial study were stored at 4°C in a refrigerator.

These soil samples were used for the determination of the following soil properties: Particle size distribution was determined using the hydrometer method as described by Bouyoucos (1951) using Calgon as the dispersing agent. Soil reaction (pH) was determined using the glass electrode pH meter in water suspension at 1:2.5 soil-to-water ratio (Udo *et al.*, 2009).

Electrical conductivity was determined using the conductivity bridge (Rhoades, 1982). Organic carbon was determined by the dichromate wet oxidation method of Walkley and Black as modified by Nelson and Sommers (1982). Exchangeable Acidity (EA) was extracted using 1M KCL and determined by titration method. Exchangeable Bases (EB) were extracted with neutral ammonium acetate (NH<sub>4</sub>OAC) buffered at 7.0

Exchangeable Magnesium and Calcium were determined using ETDA complexometric titration while Exchangeable Potassium and Sodium was determined using flame photometer (Jackson, 1982). Available phosphorus was determined by the Bray P-I method (Bray and Kurtz, 1945) and its concentration in the extract was determined by the blue colour method of Murphy and Riley (1962). Effective Cation Exchange Capacity (ECEC) was determined by summation of exchangeable cations and exchange acidity. Percentage Base Saturation will be computed.

## **MICROBIAL ANALYSIS**

### **Serial dilution**

Ten-fold serial dilutions of the samples were made as described by Collins and Lyne (1976). **Inoculation and incubation**

One millilitre of appropriate ten-fold serial dilutions of the soil samples were inoculated onto Nutrient agar, sabouraud Dextrose agar plates in triplicate using pour plate method (Collins and Lyne 1976) and spread plates inoculated plates were incubated at 28±2°C for the enumeration of total heterotrophic bacteria and fungi visible discrete colonies in incubated plates were counted and expressed as colony forming units per gram (cfu/g) of soil sample.

**Maintenance of pure culture**

Discrete colonies were purified by repeated sub-culture unto appropriate agar media. Pure cultures were preserved on Nutrient agar slant and stored in the refrigerator (4±2°C and at ambient temperature 28±2°C) for further test.

**Characterization and Identification of microbial isolates**

Pure cultures of microbial isolates were identified based on cultural parameters, microscopic technique and biochemical tests, including carbohydrate utilization. Identification of bacterial isolates were accomplished by comparing the characteristics of the culture with that of known taxa as in (Holt *et al.*, 1994). Characterization and identification of fungal isolates was carried out as in (Domeshch *et al.*, 1980)

**DATA ANALYSIS**

Shannon Index were used to evaluate diversity, abundance, and coverage of the microbial community, respectively, and formulas are listed as follows:

$$\text{Shannon Index (H)} = - \sum_{i=1}^s p_i \ln p_i \tag{1}$$

The Shannon index is an information statistic index, which means it assumes all species are represented in a sample and that they are randomly sampled.

In the Shannon index, p is the proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N), ln is the natural log, Σ is the sum of the calculations, and s is the number of species.

$$\text{Simpson Index (D)} = \frac{1}{\sum_{i=1}^s p_i^2} \tag{2}$$

In the Simpson index,  $p$  is the proportion ( $n/N$ ) of individuals of one particular species found ( $n$ ) divided by the total number of individuals found ( $N$ ),  $\Sigma$  is the sum of the calculations, and  $s$  is the number of species.

**Margalef's index (d)** is a measure of species richness in a community and is calculated using the following formula:

$$D = \frac{(S - 1)}{\ln N} \quad (3)$$

Where:

$S$  = number of species

$N$  = total number of individuals in the community

**Menhinick's index (D)** is a diversity index used to measure the species richness of a community while taking into account the size of the community. The formula for Menhinick's index

$$D_{men} = \frac{S}{\sqrt{N}} \quad (4)$$

Where  $D$  is Menhinick's index,  $S$  is the number of species in the community, and  $N$  is the total number of individuals in the community.

This index is based on Simpson's diversity index,  $D$  and is defined as:

$$E = \frac{1/D}{S} \quad (5)$$

Where  $D$  is Simpson's diversity index and  $S$  is the number of species.

### Evenness index (E)

If  $H$  is the observed Shannon-Wiener index, the maximum value this could take is  $\log(S)$ , where  $S$  is the total number of species in the habitat.

Therefore the index is:  $J = H/\log(S)$ .

### Simpson Dominance index

$$C = \sum_{i=1}^{s_{obs}} p_i^2$$

(6)

Where  $p_i$  is the number of the  $i$ th species (i.e  $p_i = n_i/N$ )

Where  $n_i$  = number of individuals of the  $i$ th species,  $N$  = total number of individuals for all the species.

## RESULT AND DISCUSSION

The physicochemical parameters of the soil samples collected from the University of Uyo Arboretum showed variations with depth. The sand content decreased with depth while the silt and clay contents increased. This is typical of most soils where the sand fraction is found to be more prevalent in the upper layers, while the finer fractions (silt and clay) are more predominant in the subsoil layers. The pH of the soil samples is strongly acidic (pH 4.5 - 4.7), which is a common characteristic of soils in the humid tropics. The soil organic matter (SOM) content increased with depth, which could be due to the accumulation of organic matter in the lower soil layers as a result of microbial activities and the decomposition of plant residues. The total nitrogen (TN) content was generally low, which could be attributed to the acidic nature of the soil, which can limit microbial activity and nitrogen mineralization. The available phosphorus (AV.P) content was also low, which could be due to the strong adsorption of phosphorus by the soil colloids, making it less available to plants. The potassium (K), calcium (Ca), magnesium (Mg), and sodium (Na) contents of the soil samples were generally low. These micronutrients are essential for plant growth, and their low concentrations in the soil could limit plant growth and productivity. The electrical conductivity (EC) values of the soil samples were also low, indicating a low concentration of soluble salts in the soil. This could be due to leaching of soluble salts from the upper soil layers to the lower layers.

**Table 1** Soil Physicochemical characteristics with depth of experimental site

Physicochemical parameters	DEPTH		
	0-10cm	10-20cm	20-30cm
Sand (%)	82.48	63.2	65.4
Silt (%)	5.44	10.00	6.00
Clay (%)	12.08	26.8	28.6
pH	4.7	4.50	4.60
EC (cmol/kg)	0.11	0.08	0.10
SOC (g/kg <sup>1</sup> )	2.85	2.75	3.63
SOM (g/kg <sup>1</sup> )	4.93	4.70	6.27
TN (g/kg <sup>1</sup> )	0.12	0.12	0.09
AV.P (mg/kg)	52.91	31.66	41.66
K (mg/kg)	0.17	0.05	0.06
Ca (mg/kg)	4.00	5.80	9.20
Mg (mg/kg)	11.09	5.46	2.67
Na (mg/kg)	0.17	0.15	0.24
EA (cmol/kg)	5.28	1.92	3.36

**Data are average of three (3) replicates**

The distribution of bacteria in soil is known to be influence by several factors such as soil type, pH, moisture temperature and nutrient availability

**Table 1** showed *Bacillus cereus* and *Bacillus subtilis* as two common soil bacteria that are known to have a wide distribution in different soil types. They are Gram-positive, spore-forming bacteria that can survive in harsh environmental conditions. *Bacillus cereus* was found to be more prevalent in the surface soil (0-10 cm and 10-20 cm) while *Bacillus subtilis* was found in higher abundance in the deeper layer (10-20 cm and 20-30 cm). These results are consistent with previous studies that have reported the distribution of *Bacillus spp.* in soil (Ceci *et al.*, 2016; Fierer *et al.*, 2012). *Serratia sp.* and *Pseudomonas aeruginosa* were identified as swarming, meaning they were moving rapidly on the surface of the agar plate. *Serratia spp.* are known to be motile, Gram-negative bacteria that are commonly found in soil and water environments. They are facultative anaerobes and can utilize a wide range of carbon sources. *Pseudomonas aeruginosa*, on the other hand, is an obligate aerobe that is commonly found in water and soil environments. The swarming behavior of *P. aeruginosa* has also been found to be influenced by various environmental factors, such as pH, temperature, and nutrient availability (Bever *et al.*, 2012). These findings are in consistent with previous studies



that have been reported Type IV pili and twitching motility (Mattick *et al.*, 2002)

*Micrococcus sp.* was found in all three soil layers, with the highest abundance in the topmost layer (0-10 cm). *Micrococcus spp.* are Gram-positive, aerobic bacteria that are commonly found in soil and water environments. They are known to have a wide range of metabolic capabilities and can utilize a variety of carbon sources. *Clostridium sp.* was found to be more prevalent in the upper two layers of soil (0-10 cm and 10-20 cm) and less so in the deepest layer (20-30 cm). *Clostridium spp.* are aerobic, Gram-positive bacteria that are commonly found in soil environments. They are known to play an important role in the decomposition of organic matter in soil. *Staphylococcus albus* was found only in the topmost layer (0-10 cm) while *Staphylococcus aureus* was found in the upper and lower layers (0-10 cm and 20-30 cm). Both *Staphylococcus albus* and *Staphylococcus aureus* are Gram-positive bacteria that are commonly found in soil and water environments. They are known to have the ability to form biofilms and can persist in the environment for long periods of time. *Proteus mirabilis* and *Dissulfurivibrio sp.* were detected but swarming. *Proteus mirabilis* is a Gram-negative, facultative anaerobe that is commonly found in soil and water environments. *Dissulfurivibrio spp.* are anaerobic, sulfate-reducing bacteria that are commonly found in soil and sediment environments.

**Table 2 Soil Microbial population with depth at the experimental site**

BACTERIA	/ % Abundance		
	DEPTHS		
	0-10cm (%)	10-20cm (%)	20-30cm (%)
<i>Bacillus cereus</i>	49 (20.76)	64 (25.30)	40 (20.62)
<i>Bacillus substilis</i>	56 (23.72)	103 (40.71)	20 (10.31)
<i>Serratia sp</i>	(swarming)		
<i>Pseudomonas aeruginosa</i>	(swarming)		
<i>Micrococcus sp</i>	64 (27.12)	34 (13.44)	70 (36.08)
<i>Clostridium sp</i>	46 (19.49)	52 (20.55)	41 (21.13)
<i>Dissulfurivibrio sp</i>	(Swarming)		
<i>Staphylococcus albus</i>	14 (5.93)	0 (0)	0(0)
<i>Staphylococcus aureus</i>	7 (2.97)	0 (0)	23 (11.86)
<i>Proteus mirabilis</i>	(swarming)		
<b>TOTAL</b>	<b>236 (100)</b>	<b>253 (100)</b>	<b>194 (100)</b>

Number in bracket are percentage occurrence

The results in Table 2 showed that the composition and abundance of fungal communities vary with depth in the soil. Some fungal species were

more abundant in the upper soil layers (0-10cm), while others are more abundant in the deeper layers (10-20cm and 20-30cm). For instance, *Candida albicans* is the most abundant fungal species overall, with the highest abundance at 10-20cm and 20-30cm depths. *Aspergillus niger* and *Aspergillus fumigatus* are also present, but in lower abundance. The presence of different fungal species in soil is important for the maintenance of soil health and the cycling of nutrients. For example, some fungal species, such as *Aspergillus* and *Penicillium*, are known to be involved in the decomposition of organic matter and the cycling of nutrients in soil. Other fungal species, such as *Candida albicans*, can have both beneficial and detrimental effects on plants and soil health, depending on the context. The abundance and diversity of soil fungi are influenced by a range of environmental factors, including soil pH, moisture, temperature, and nutrient availability. Further studies on the soil properties at different depths in the University of Uyo Arboretum could provide insight into the environmental factors influencing the fungal communities observed.

**Table 3: Abundance of Fungi population at the various depth in University of Uyo Arboretum**

FUNGI	Depths		
	0-10cm (%)	10-20cm (%)	20-30cm (%)
<i>Aspergillus fumigatus</i>	2 (10.57)	2 (6.89)	0 (0)
<i>Aspergillus niger</i>	5 (26.32)	0 (0)	0 (0)
<i>Candida albicans</i>	7 (36.84)	24 (82.76)	15 (88.24)
<i>Rhizopus stolonife</i>	1 (5.26)	1(3.45)	0 (0)
<i>Clasdosparum sp</i>	1 (5.26)	1 (3.45)	2 (11.76)
<i>Penicillium expansum</i>	2 (10.57)	0 (0)	0 (0)
<i>Vertiocillium sp</i>	1 (5.26)	1 (3.45)	0 (0)
<b>TOTAL</b>	<b>19 (100)</b>	<b>29 (100)</b>	<b>17(100)</b>

**Table 3** shows several diversity indices for bacterial communities at different depths in the soil: 0-10cm, 10-20cm, and 20-30cm. These indices provide different measures of the number and relative abundance of bacterial species in each depth. Here's an interpretation of each index: Species Richness Index (d): This index measures the number of different bacterial species present in the soil. The higher the value, the greater the species richness. In this case, the highest species richness is found at 0-10cm depth, with a value of 0.915. Shannon-Wiener Index (H): This index takes into account both the number of species and their relative abundance. The higher the value, the greater the diversity. In this case, the highest diversity is found at 0-10cm depth, with a value of 1.61.

Shannon's Index (H'): This is another version of the Shannon-Wiener Index that gives a measure of the evenness of species distribution in the community. The higher the value, the more evenly distributed the species. In this case, the highest evenness is found at 0-10cm depth, with a value of 1.424.

Evenness Index (Ei): This index also measures the evenness of species distribution, but in a slightly different way. The higher the value, the more evenly distributed the species. In this case, the highest evenness is found at 10-20cm depth, with a value of 0.925. Dominance Index (C): This index measures the degree of dominance of the most abundant species in the community. The lower the value, the lower the dominance. In this case, the lowest dominance is found at 0-10cm depth, with a value of 0.290. Simpson's Index (D): This index measures the probability that two individuals randomly selected from the community belong to the same species. The lower the value, the greater the diversity. In this case, the greatest diversity is found at 0-10cm depth, with a value of 0.784. Simpson's Index (Di): This is another version of Simpson's Index that gives a measure of the dominance of the most abundant species in the community. The higher the value, the greater the dominance. In this case, the highest dominance is found at 0-10cm depth, with a value of 1.408.

Overall, these indices suggest that the bacterial communities in the top 10 cm of soil are the most diverse and even, with the lowest dominance and the highest species richness, diversity, and evenness. The diversity and evenness decrease with depth, while the dominance increases. This is due to changes in environmental factors such as nutrient availability, temperature, moisture, and oxygen availability, which may influence the growth and survival of different bacterial species.

**Table 4: Summary of Result for Bacteria Diversity at the Experimental Site**

PARAMETERS	DEPTH		
	0-10cm	10-20cm	20-30cm
Species Richness Index (d)	0.915	0.542	0.759
Shannon-Wiener Index (H)	1.612	1.31	1.51
Shannon's Index (H')	1.424	0.7302	0.781
Evenness Index (E)	0.835	0.925	0.904
Dominance Index (C)	0.215	0.290	0.242
Simpson's Index (D)	0.784	0.710	0.757
Simpson's Index (D')	1.275	1.408	1.321

**Table 4** explains the summary of results for fungi shows the values of various diversity indices for three different soil depths, namely 0-10cm, 10-20cm, and 20-30cm. The species richness index (d) indicates the number of different fungal species present in the soil samples. The highest species richness index was observed at the surface soil layer (0-10cm), with a value of 2.037, whereas the lowest was observed at 20-30cm soil depth, with a value of 0.353. The Shannon-Wiener index (H) measures the diversity and evenness of fungal species present in the soil. The highest Shannon-Wiener index was observed at 0-10cm soil depth, with a value of 1.66, indicating high diversity and evenness of fungal species present in the soil. The Shannon's index (HI) and evenness index (Ei) also show similar trends, with higher values at the surface soil layer and decreasing values with increasing soil depth. The dominance index (C) and Simpson's index (D) measures the dominance and diversity of fungal species, respectively. The dominance index (C) shows a decreasing trend with increasing soil depth, indicating a decrease in the dominance of particular fungal species. The Simpson's index (D) shows the opposite trend, with higher values at deeper soil depths, indicating a decrease in the diversity of fungal species.

**Table 5: Summary of Result for Fungi**

PARAMETERS	DEPTH		
	0-10cm	10-20cm	20-30cm
Species Richness Index (d)	2.037	1.188	0.353
Shannon-Wiener Index (H)	1.66	0.69	0.36
Shannon's Index (H')	1.658	0.685	0.355
Evenness Index (E)	0.852	0.428	0.522
Dominance Index (C)	0.816	0.340	0.269
Simpson's Index (D)	0.235	0.306	0.207
Simpson's Index (D')	4.255	3.267	4.830

## CONCLUSION

The distribution of bacteria in soil is influenced by several factors and can vary based on the specific soil type and environmental conditions.

It appears that *Candida albicans* is the most abundant fungi species, particularly in the deeper soil layers (20-30cm). *Aspergillus niger* and *Aspergillus fumigatus* are also present, but to a lesser extent, while the other species have relatively low abundance. Moreover, if this soil is being used for agricultural purposes, it may be beneficial to monitor and manage the microbial populations to prevent the growth of harmful

species that could affect crop yields. Such management practices may include crop rotation, soil amendments, and the use of fungicides, among others. Bacterial communities in the top 10 cm of soil are the most diverse and even, with the lowest dominance and the highest species richness, diversity, and evenness. The diversity and evenness decrease with depth. This is due to changes in environmental factors such as nutrient availability, temperature, moisture, and oxygen availability, which may influence the growth and survival of different bacterial species. The results presented in this research is consistent with previous studies that have reported the distribution of soil bacteria (Ceci *et al.*, 2016; Fierer *et al.*, 2012).

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