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Impact of pH, EC, OC and TN on Microbial Population Across Different Soil Depth of Balangir, Odisha

Samapika Dash, Arupa Nanda, Namrata Chouhan, Monty Kujur*

School of Biotechnology, Gangadhar Meher University, Amruta Vihar, Odisha, India, PIN- 768001
*Corresponding Author: mkujur@gmuniversity.ac.in

ABSTRACT: The present study shows the variation of soil microbial population across different depth in agricultural soil (AS), Pesticide treated soil (PTS) and forest soil (FS) of Patanagarh, Balangir district, Odisha. Soil samples were collected from four depth intervals (0, 15, 30 and 60 cm) to analyse the vertical changes in soil characteristics and fertility status. Microorganisms play a vital role in improving growth and performance of different crops. Microbial population was estimated from different soil types and presented in CFU/g of soil. Total bacterial population was increased with increased rate from top layer to 30cm depth which was decreasing with increasing depth due to alteration in physicochemical properties. The reduction in microbial population in PTS relative to AS may be attributed to the adsorption of microorganisms onto agrochemicals, which failed to safeguard themselves from pesticides. In forest soil, bacterial population was higher as compare to other because of high organic matter ($r= 0.785$) and available nutrients ($r= 0.838$; $p<0.01$) in soil with favourable PH ($r= 0.865$). Fungal population also followed same pattern as bacteria and relative proportion of fungi increases from top layer to 15cm and found to be highest in 30cm depth soil because of increase nutrients concentration (OC; $r=0.696$;TN; $r=0.729$;$P<0.01$) ,PH($r= 0.771$) and EC($r= 0.729$; $p<0.01$). Fungi are predominantly located in topsoil (up to 10 cm in depth) and are infrequently encountered at 60 cm depth. Additionally, the lowest ACM count in 60cm and highest count was found in 30cm depth, can be explained by the fact that actinomycetes are relatively regulated by physicochemical attributes and available nutrients.

INTRODUCTION

One of the most important natural resources for terrestrial life is soil, which serves as the basis for biogeochemical cycles, agriculture, and ecosystems. It functions as a dynamic repository of nutrients, water, microbes, organic matter, and minerals that together support plant growth and have an impact on environmental processes. Soil characteristics vary significantly throughout depths due to its heterogeneous and stratified nature, which ultimately shapes soil functioning, ecosystem production, and land management outcomes. In contemporary agricultural and environmental research, it has become more important to understand the state of soil at various depths, including its physicochemical, biological, and structural properties (Brady & Weil, 2016). The intricate relationships between climate, parent material, vegetation, land use, and human activity, all of which have an impact on soil fertility and health, are shown by soil depth profiles.

One key attribute that changes with depth is the amount of soil organic matter (SOM). Soil organic matter (SOM) is fundamental to nutrient cycling, soil aggregation, moisture retention, and carbon sequestration. Plant litter adds organic residues to the surface layers, leading to higher soil organic matter content in surface layers than in subsurface layers (Cotrufo *et al.,* 2019). Leaching, root activity and mineral weathering also generally cause soil pH to change with depth. Changes of pH

with soil depth might influence the availability of nutrients and microbial activity and, thus, the biogeochemical processes in the soil profile (Mengel and Kirkby, 2012). Distribution of macro and micro nutrients (N, P, K, Ca, Mg and trace minerals) is an important component of a complete soil evaluation. The nutrient pools in surface layers are mostly from organic inputs, fertilisers, and biological activity, while nutrients in subsurface layers are derived from weathering of parent material or translocation mechanisms (Havlin *et al.,* 2020).

Depth influences biological parameters such as microbial biomass, enzyme activity, earthworm populations, and root density to a great extent. The highest microbial diversity is found in the top soil layers, which contain the highest carbon inputs, nutritional availability and oxygen levels (Fierer, 2017). Some specialised bacteria grow in deeper anoxic or low energy environments, although microbial activity and diversity drop with depth. The deep dispersion of biological components influences nitrogen fixation, decomposition, nutrient mineralisation and soil ecosystem health. Evaluation of biological condition at various depths offers more profound understanding of soil health than chemical and physical characteristics. Environmental and ecological studies require stratified soil data for understanding processes like groundwater recharge, carbon sequestration, erosion and pollutant movement. Vertical soil contaminants such as pesticides, heavy metals, and nitrates affect human health and the ecology (Alloway, 2012).

Microbial community topologies in soils regulate nutrient cycles (Harris and Birch, 1989) and accelerate metabolic activities that promote soil genesis (Monson *et al.,* 2006). The pattern of land use and availability of minerals in the soil measure the existence of microbial communities by supplying nutrients and habitats. The activity and interaction of microorganisms influence the physical, chemical and biological aspects of the soil (Tangjang *et al.,* 2015). Microbial diversity may influence soil processes (Cavigelli and Robertson, 2000; Balser *et al.,* 2002). If deep and surface microbial populations are similar then microbial processes should also be similar. In deeper layers of the soil there is the possibility of specific microbial communities which differ from those on the surface (Fritze et al., 2000; Blume *et al.,* 2002). From the foregoing facts, the present study focuses on the determination of microbial population from different depths of soil profiles to monitor the variation in microbial population in different layers of soil.

MATERIALS AND METHODS

Study site- Soil samples were collected from Balangir, Patanagarh of Western Odisha. To maintain diversity in microbial population three types of soil viz. agriculture soil, pesticide treated soil and neighbouring forest soil areas were selected. The agricultural soil used for the study was gathered from the land of local farmers where vegetables such as brinjal (*Solanum melongena*), tomato (*Solanum lycopersicum*), cauliflower (*Brassica oleracea*), pumpkin (*Cucurbita pepo*), and rice were grown. Flowers like blue pea (*Clitoria ternatea*), marigold (*Calendula officinalis*), and Datura (*Datura stramonium*) were also cultivated along with vegetables. Fertility induction of soil vegetable pills, vermicompost, and green manure (1:1:1; 1 part green manure; 1 part vegetable pills; 1 part cattle dung) was put in the field. Pesticide-treated soil was subjected to streptomycin. Composition: Streptomycin Sulphate (90% v/v; Tetracycline hydrochloride 10% v/v) pesticide thrice at every 15-day interval at local farmer land cultivated with cauliflower, Solanaceous vegetables in rabi season, followed by rice as a kharif crop. The forest soil chosen for the present study was of the red soil type and bordered by plants like *Bambusa vulgaris, Diospyros melanoxylon, Butea monosperma, Tectona grandis,* etc.

Sampling- Sampling was done by employing the general soil sampling protocol of Dash and Kujur (2024); earlier given by Parkinson et al. (1971). For sampling, each site is divided into 5 blocks, and five soil samples must be collected randomly from 0 cm, 15 cm, 30 cm, and 60 cm soil depth by spading pits of (15 x 15 x 15) cm^3 size in each block (without considering small rocks, pebbles, or fragments) and referred to as sub-samples, which are then mixed thoroughly to form the composite sample. The samples were each labelled separately and aseptically packed in polypropylene vials to prevent mixing and delivered to the laboratory for testing. Samples were air-dried, homogenized, sieved (2 mm mesh size), and stored at 4°C for further characterization.

Physicochemical characterization and Microbial enumeration from soil samples

Soil pH was tested to evaluate the acidity and basicity (ISSS 2002). The soil electrical conductivity (ISSS 2002) was used to determine soluble salts. Soil organic carbon was determined by the Walkley and Black titration method. (Mishra, 1968). Soil profiles were sampled at different depths (ISSS 2002) for determination of total nitrogen (TN), available phosphorus (AP), and available potassium (AK).

Soil bacteria were counted by the serial dilution method by following methods of Dash and Kujur (2024). Colonies formed were quantified and represented as colony-forming units (CFU/g soil). Besides, enumeration of total heterotrophic aerobic bacterial population (HAB) need to be count using nutrient agar (Gray, 1990). Actinomycetes population (ACT) from mine spoil was enumerated from different soil profiles using starch-casein agar [10g soluble starch; 0.3g casein; 0.2g KNO_3; 2g NaCl; 2g K_2PO_4; 0.5g $MgSO_4.7H_2O$; 0.02g $CaCO_3$; 0.07g $FeSO_4$ per liter; 1.5% agar, pH 7.2] (Hunter-Cevera and Eveleigh, 1990). In order to prevent bacterial and fungal contamination 40 µl/ml streptomycin and griseofulvin in 50 µl/ml was treated (Alharbi *et al.*, 2012). Fungal population (FUN) was enumerates using Rose Bengal agar [5g papaic digest of soyabean meal; 10g dextrose; 1g K_2PO_4; 0.5g $MgSO_4$; 0.05g Rose Bengal per liter; 1.5% agar; pH-7.2] supplemented with streptomycin (50µl/ml) to inhibit bacterial contaminants (Alef and Nannipieri, 1995).

RESULTS

Physicochemical analysis from different soil

In agricultural soil, the top layer had a neutral pH (7.21±0.01). Soil pH increased at 15 cm depth (7.42±0.001), indicating mild alkalinity, and further increased upto 30 cm depth (7.51±0.005). pH measured 6.42±0.003 in the 60 cm layer of soil, indicating a somewhat acidic character. Pesticide-treated soil had pH values of 6.813±0.005 at the top layer, 7.086±0.065 at 15 cm depth, and 7.33±0.05 at 30 cm deep. Pesticide-treated soil with 60 cm depth had a low pH, indicating a slightly acidic soil. pH levels in forest soil were somewhat alkaline (7.43±0.026) in the top layer, 7.52±0.011 in 15cm depth, and 7.59±0.001 in 30 cm depth. A 60cm profile of forest soil revealed somewhat acidic soil (6.221± 0.012), as shown in Table 1.

Soil sample electrical conductivity was determined and shown in Table 1. Electrical conductivity in agricultural soil was calculated as 0.276±0.011 mS/cm in the top layer, 0.306±0.005 in 15 cm, and 0.323±0.005 in 30 cm depth. Electrical conductivity increased from top layer to 30 cm depth of soil, then reduced to 60 cm (0.213±005 mS/cm). Electrical conductivity in pesticide-treated soil was 0.273±0.005 mS/cm in the top layer, increasing to 0.296±0.005 mS/cm at 15cm depth, and maximum at 30cm depth (0.286±0.005 mS/cm). The lowest electrical conductivity (0.186±0.005 mS/cm) was observed in soil depth of 60cm. Electrical conductivity in forest soil increased from top layer (0.306±0.005 mS/cm) to 15cm (0.333±0.015 mS/cm) and peaked at 30cm (0.351±0.026 mS/cm). Slightly poor electrical conductivity was observed at 60 cm soil depth (0.193±0.015 mS/cm).

In agricultural soil, accumulation of organic carbon was found to be 1.838± 0.053 % in top layer of soil which increases to 2.078±0.055 % in 15cm depth of soil and found to be greatest in 30cm (2.873±0.046%) depth of soil. In 60cm depth of soil, (1.119±0.011%) of organic carbon was accumulated. In pesticide treated soil, low organic carbon was accumulated in 60cm soil profile i.e 0.915±0.013 %. From top layer of soil (1.661± 0.039 %), organic carbon concentration was increases towards 15 cm (1.826±0.006%) depth and highest concentration was found in 30cm (1.916±0.001%) depth in pesticide treated soil samples. In case of forest soil, organic carbon accumulation increases from top layer (2.255± 0.034%) to 15cm (2.325± 0.005%) and highest concentration was shown by 30cm depth of soil (2.431±0.052%) which was further decreases with increasing depth upto 60cm (1.116±0.006%).

Table 1 showed the total nitrogen percentages in agricultural, pesticide, and forest soil depths. The soil has a greater content of total nitrogen (3.866± 0.04%) at 30 cm depth. The soil nitrogen concentration increased from top layer (3.186±0.013%) to 15cm (3.903±0.006%) and peaked at 30cm (3.866±0.04%). Nitrogen concentration was lower in pesticide-treated soil at 60cm (1.108±0.006%) depth, but higher at 30cm (3.033±0.058%). Nitrogen levels in soil were 2.243±0.483% in the top layer and 2.933±0.045% at the 15cm depth. Forest soil had higher total nitrogen concentration at 30cm deep (3.969±0.036%) and lower nitrogen concentration at 60cm depth (1.201±0.002%). In the top layer of forest soil, 3.360±0.156 % nitrogen was detected, while 3.874±0.513% was found in 15cm depth.

Table 1 Concentration of physicochemical variables along different soil depth (0-60cm) in three distinct soil profiles (Values are Mean± stdev)

		PH	EC	OC	TN
AS	0cm	7.21±0.01	0.276±0.011	1.838±0.053	3.166±0.043

	15cm	7.42±0.001	0.306±0.005	2.078±0.055	3.903±0.066
	30cm	7.51±0.005	0.323±0.005	2.873±0.046	3.866±0.04
	60cm	6.42±0.003	0.213±0.005	1.119±0.011	1.143±0.048
PS	0cm	6.813±0.005	0.273±0.005	1.661±0.039	2.243±0.483
	15cm	7.086±0.065	0.296±0.005	1.826±0.006	2.933±0.045
	30cm	7.33±0.05	0.286±0.005	1.916±0.001	3.033±0.058
	60cm	6.153±0.055	0.186±0.005	0.951±0.013	1.108±0.006
FS	0cm	7.43±0.026	0.306±0.005	2.255±0.034	3.360±0.156
	15cm	7.52±0.011	0.333±0.015	2.325±0.005	3.874±0.0513
	30cm	7.59±0.001	0.351±0.026	2.431±0.052	3.969±0.636
	60cm	6.221±0.012	0.193±0.015	1.116±0.0064	1.201±0.002

*Values are Mean± Stdev

Microbial enumeration from different soil depth

In the current study, enumeration of microbial communities and their relative availability was expressed in terms of colony forming unit (CFU/g soil) (Table 2). The variations occur in terms of their relative abundance of microbiota among different depth of soil and were shown in terms of $\log_{10}$ transformed of CFU/g soil.

Table- 2 Relative distribution and abundance of different microbial populationfrom (0-60cm) cm soil depth among seven different soil profiles.

Sites	Total bacteria	Fungi	Actinomycetes
AS (0 cm)	21.9×10^{-4}	23.9×10^{-4}	26.3×10^{-4}
AS (15cm)	24.6×10^{-5}	17.91×10^{-5}	31.5×10^{-5}
AS (30cm)	17.6×10^{-6}	11.19×10^{-6}	33.5×10^{-6}
AS (60cm)	11.9×10^{-3}	8.2×10^{-3}	16.1×10^{-3}
PS (0cm)	18.5×10^{-4}	11.9×10^{-4}	22.21×10^{-4}
PS (15cm)	13.9×10^{-5}	8.5×10^{-5}	28.31×10^{-5}
PS (30cm)	8×10^{-6}	7.16×10^{-6}	29.3×10^{-6}
PS (60cm)	7.3×10^{-3}	5.3×10^{-3}	14.5×10^{-3}
FS(0cm)	30.9×10^{-4}	21.8×10^{-4}	29.1×10^{-4}
FS (15cm)	33.9×10^{-5}	23.7×10^{-5}	32.4×10^{-5}
FS (30cm)	34.1×10^{-6}	24.9×10^{-6}	33.2×10^{-6}
FS (60cm)	19.5×10^{-3}	11.9×10^{-3}	11.9×10^{-3}

The current study specified the increasing pattern of total bacteria count from top layer (21.9×10^{-4}) to 15 cm depth (24.2×10^{-5}) and was highest in 30cm depth (17.6×10^{-6}) in case of agricultural soil. Lowest population was in 60 cm depth of soil represented as 11.9×10^{-3} CFU/g of soil. The result shows increasing pattern in the population of bacteria with increasing

depth of soil upto 30cm in agricultural soil. Fungal population was highest in 30 cm depth of soil i.e 11.19× 10^{-6} CFU/g of soil. Fungal population in top layer of agricultural soil was found to be 23.9× 10^{-4} which was increases in 15 cm depth of soil (17.91× 10^{-5}) increases further upto 30 cm depth of soil (11.19 ×10^{-6} CFU/g of soil). In 60 cm depth of soil (8.2× 10^{-3} CFU/g of soil) lowest fungal population was found. Actinomycetes population was shown increasing pattern from top layer to 30 cm depth of soil. Actinomycetes population was found to be 26.3×10^{-4} CFU/g of soil in top layer of soil which was slightly increases upto 31.5× 10^{-5} CFU/g of soil. Highest population was found in 30cm of soil (33.91× 10^{-5}) and lowest was found in 60 cm soil (16.1×10^{-3} CFU/g of soil).

Variation in relative abundance of microbial population in different soil depth across pesticide treated soil was calculated and presented in Table 2. The relative proportion of total bacteria count was shown increasing pattern from top layer to 30 cm depth of soil. The lowest count was found in 60 cm depth of soil (7.3× 10^{-3} CFU/g of soil) as compare to top soil (18.5× 10^{-4} CFU/g of soil). The bacterial count was 18.5×10^{-4} in 0 cm of soil followed by 13.9× 10^{-5} in 15 cm depth and 8× 10^{-6} CFU/g of soil was found in 30 cm depth of soil. In pesticide treated soil, highest fungal count was found in 30 cm depth of soil (7.16× 10^{-6} CFU/g of soil) whereas 60 cm depth soil contains lowest count (5.3× 10^{-3} CFU/g of soil). The relative abundance of fungi was present as 11.9×10^{-4} CFU/g of soil in top layer of soil, 8.5× 10^{-5} CFU/g of soil in 15cm of depth , 7.16×10^{-6} CFU/g of soil in 30cm depth and further 60cm depth of soil contains 5.3× 10^{-3} CFU/g of soil contains fungi. Actinmycetes population was found to be higher in 30cm soil depth (29.3× 10^{-6} CFU/g of soil) as compare to top layer (22.21×10^{-4} CFU /g of soil) followed by 15 cm depth of soil (28.31× 10^{-5} CFU/g of soil). Lowest count of actinomycetes was found in 60cm depth of soil (14.5×10^{-3} CFU/g of soil) in forest soil.

In case of forest soil, relatively high proportion of bacteria was found in 30 cm depth of soil 34.1× 10^{-6} CFU/g of soil whereas top layer contains 30.9× 10^{-4} CFU/g of soil followed by 33.9×10^{-5} CFU/g of soil in 15 cm depth of soil. Lowest count of bacteria was found in 60 cm depth of soil (19.5× 10^{-3} CFU/g of soil). In fungal population lower proportion of fungal count was found in 60 cm depth of soil (11.9× 10^{-3} CFU/g of soil) as compare to other depths. In top layer of soil 21.8×10^{-4} CFU/g of soil was found followed by 23.7× 10^{-5} CFU/g of soil in 15 cm depth and 24.9× 10^{-6} CFU/g of soil in 60 cm of soil. In case of actinomycetes, the count was found to be higher in 30 cm depth (33.2× 10^{-6} CFU/g of soil) as compare to top layer (29.1× 10^{-4} CFU/g of soil) and 15cm depth of soil (32.4 × 10^{-5} CFU/g of soil. Lowest count was found in 60 cm depth of soil (11.9× 10^{-3} CFU/g of soil) and presented in Table 2.

DISCUSSION

A vital part of the soil ecosystem, the microbiome affects processes like soil fertility and formation, plant development and stress tolerance, nutrient turnover, and carbon storage. Nonetheless, our comprehension of the soil microbiome predominantly stems from research focused on surface strata. As a result, deeper soil layers constitute a hitherto undervalued component of the soil microbiome. These deeper layers are known as "subsoils" and are described as soil that is located below the surface layer. Thus, current study is focused on the determination of variation in microbial population and how they are effected by physicochemical properties of agricultural soil, pesticide treated soil and forest soil.

Table 3 Analysis of Variance ($p<0.05$) between different variables of physicochemical and microbial populations across different sites

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
BAC	Between Groups	4.158	8	.520	376.682	.000
	Within Groups	.025	18	.001		
	Total	4.182	26			
FUN	Between Groups	1.796	8	.225	161.947	.000
	Within Groups	.025	18	.001		

	Total	1.821	26			
ACT	Between Groups	2.341	8	.293	38.275	.000
	Within Groups	.138	18	.008		
	Total	2.479	26			
PH	Between Groups	12.653	8	1.582	94.022	.000
	Within Groups	.303	18	.017		
	Total	12.956	26			
EC	Between Groups	.610	8	.076	119.082	.000
	Within Groups	.012	18	.001		
	Total	.622	26			
OC	Between Groups	5.173	8	.647	344.738	.000
	Within Groups	.034	18	.002		
	Total	5.207	26			
TN	Between Groups	206098.52	8	25762.316	11.350.26	.000
	Within Groups	44.805	18	2.489		
	Total	206143.33	26			

*Significance at 0.05 level

The soil reaction, measured by pH, was seen to be in the acidic range to mild alkaline range of 6.42-7.51 in agricultural soil, slightly acidic to neutral of 6.15-7.33 in pesticide treated soil and slightly acidic to mildly alkaline of 6.22-7.52 in forest soil of different depth (Significant at $p<0.05$ level (Analysis of Varince Table 3). Slight acidification of soil in case of pesticide treated soil was seen (Jha and Singh, 1991) because of mineral deposition (Sahani and Behera, 2001).In top layer of all three types of soil, pH was ranged within neutral range because of availability of organic matter and available nutrients in soil. The organic matter accumulation was increases from top layer (neutral) to 30 cm (alkaline) depth in soil. Thus, soil organic matter stabilizes soil P^H showed positive correlation ($r= 0.823$; $p<0.01$) which is crucial for regulating nutrient input (TN: $r= 0.976$; $p<0.01$; Table 4) and availability for plant absorption (Campbell *et al.,* 1996).

Electrical conductivity quantifies the movement of ions in reaction to electric current. The measurement of electrical conductivity (EC) alongside pH offers comprehensive insights into the chemical composition of soil (Smith and Doran, 1997). The electrical conductivity of a soil solution increased with rising ion concentration. Numerous reports elucidate the role of electrical conductivity (EC) in regulating key physicochemical properties of soil (NagaRaju *et al.,* 2016; Kekane *et al.,* 2015; Kumar *et al.,* 2012; Kirkham *et al.,* 2017). In current study, agricultural soil shows electrical conductivity within a range of 0.213-0.323 mS/cm, 0.186-0.273 mS/cm in pesticide treated soil and 0.193-0.306 mS/cm.EC of pesticide treated soil was less than that of agricultural and forest soil because in pesticide treated soil, pH was slightly acidic, act as an important soil quality indicator and accumulates less organic matter than agricultural and forest soil. In case of all types of soil, EC was increasing in soil with increasing depth of soil upto 30 cm, but after that EC become decreased might be due to unfavourable soil conditions.

Dead and decomposing plant and animal leftovers, as well as dead plant tissue including roots and leaves that are broken down by bacteria and fungus, are major sources of organic carbon (OC).The organic carbon content exhibited an increasing trend from the surface layer to a depth of 30 cm, with significant variation across distinct profiles reported at the $p<0.05$ level in the analysis of variance research (Table 3). At a depth of 60 cm, the organic matter content was diminished due to soil acidification, resulting in reduced nutrient availability for plant growth. Soil organic carbon increases from the upper layer,

promoting macroaggregation formation that reduces compaction risk and enhances soil water retention capacity. Furthermore, elevated SOC levels buffer soil pH (r= 0.823; p<0.01) and improve soil electrical conductivity (r= 0.859; p<0.01) (Canqui *et al.,* 2013). The acidic pH causes 60 cm deep soil to be susceptible to cation leaching, resulting in reduced availability of organic matter for plant nourishment (Goswami 2009).The AS (1.119-2.873%) exhibited a greater concentration of organic carbon than PS (0.915-1.826%), attributable to the heightened application of pesticides, which has resulted in soil contamination with hazardous chemicals, so modifying microbial populations, enzyme activities, and soil processes (Perucci and Scorponi, 1994; Jaymadhuri *et al.,* 2005). Additionally, it is determined that elevated enzymatic activity correlates with increased organic matter content (Tu, 1995). The increase in organic carbon significantly enhances the concentration of total nitrogen (TN: r= 0.818) from the surface layer to a depth of 30 cm, with variation noted at a significance level of p<0.01. The total nitrogen content increased from 0.003% in the topsoil to 0.214% at a depth of 30 cm, likely due to the successful establishment of leguminous herb species such as Cassia and Acacia, which promote symbiotic associations and indicate a favourable nitrogen potential of the soil (Visser and Parkinson, 1983; Dutta and Agrawal, 2002).Analysis of Variance indicates significant variation among distinct soil profiles at the p<0.05 level.Total nitrogen content was less in 60cm depth requires development of plausible management strategies to restore the physicochemical and biological properties of soil (Table 4).

Table 4 Pearson's coefficient of correlation physicochemical variables and microbial population across three distinct soil profiles (0cm, 15cm, 30cm, 60cm soil depth)

	BAC	FUN	ACT	PH	EC	OC	AN
BAC	1						
FUN	.948**	1					
ACT	.984**	.954**	1				
PH	.865**	.771**	.880**	1			
EC	.836**	.756**	.867**	.958**	1		
OC	.785**	.696**	.806**	.823**	.859**	1	
AN	.838**	.729**	.861**	.976**	.944**	.818**	1

**Significance at p<0.01 level

Microbial population was estimated from different soil types and presented in CFU/g of soil. Although the majority of these studies concentrate on topsoil communities, little is known about how subterranean communities evolve with continuous soil age and what factors are crucial in forming these communities. Subsoils exhibit significant variations in environmental conditions relative to topsoils; for instance, the concentrations of carbon and nutrients diminish sharply with increasing soil depth (Hansel et al., 2008; Turner et al., 2016; Stone et al., 2015). Consequently, subsoils include unique microbial communities that are adapted to energy and substrate-limited circumstances (Blume *et al.,* 2002; Fierer *et al.,* 2003; Hansel *et al.,* 2008; Hartmann *et al.,* 2009). Total bacterial population was increased with increased rate from top layer to 30cm depth which was decreasing with increasing depth due to alteration in physicochemical properties (Naylor *et al.,* 2022). The reduction in microbial population in PTS relative to AS may be attributed to the adsorption of microorganisms onto agrochemicals, which failed to safeguard themselves from pesticides (Perucci and Scarponi, 1994; Jayamadhuri and Rangaswamy, 2005). In forest soil, bacterial population was higher as compare to other because of high organic matter (r= 0.785) and available nutrients (r= 0.838; p<0.01) in soil with favourable P^H (r= 0.865).

Fungal population also followed same pattern as bacteria and relative proportion of fungi increases from top layer to 15cm and found to be highest in 30cm depth soil because of increase nutrients concentration (OC; r=0.696;TN; r=0.729;P<0.01), P^H(r= 0.771) and EC(r= 0.729; p<0.01). Fungi play a crucial role in maintaining soil structure. The fundamental unit of fertile soil is the soil aggregate, which is cohesively maintained by microbial polymers, while fungal hyphae consolidate the

micro-aggregates into macro-aggregates (Kibunja *et al.,* 2010). Fungi are predominantly located in topsoil (up to 10 cm in depth) and are infrequently encountered at 60 cm depth. The majority of soil fungus are opportunistic (zymogenous). Numerous environmental parameters, including pH, moisture content, soil organic carbon content, sufficient aeration, and a relatively high concentration of usable substrates, affect the relative distribution of fungal populations (Kennedy *et al.,* 2008). A comparatively elevated fungal population in FS relative to others was noted , potentially attributable to the conducive moisture levels, ample organic matter, and humus accumulation in FS, which may have facilitated the colonization of soil microbes in the subsequent period (Arunachalam *et al.,* 1997).

Actinomycetes are gram-positive, aerobic bacteria that make up the majority of microbial populations found in soil (Kuster, 1968). Their abundance and prevalence in specific soil are significantly affected by various factors, including soil temperature, pH, organic carbon content, aeration, and moisture content (Arifuzzaman *et al.,* 2010), which may explain the elevated actinomycetes (ACT) count in as compare to other soil. Additionally, the lowest ACT count in 60cm and highest count was found in 30cm depth, can be explained by the fact that actinomycetes are relatively regulated by physicochemical attributes and available nutrients.(Naylor *et al.,* 2022).

Table 5 Partia Eta Square value of all variables in three distinct soil profiles from Multi Variate Analysis of variance (MANOVA; $p<0.05$)

	0cm	15cm	30cm	60cm
BAC	0.943*	0.992*	0.996*	0.923*
FUN	0.782*	0.956*	0.998*	0.992*
ACT	0.536*	0.729*	0.899*	0.128*
P^H	0.977*	0.984*	0.997*	0.936*
EC	0.559*	0.699*	0.767*	0.493*
OC	0.941*	0.951*	0.999*	0.593*
AN	0.951*	0.978*	0.988*	0.797*

*Significant at 0.05 level

Multivariate analysis of variance (MANOVA) was used to elucidate efficacy of variables across the study region concerning varying depths. Wilk's Lambda score demonstrated statistical significance of microbial populations at the $p<0.05$ level across all variables in the top layer of agricultural, pesticide-treated, and forest soils. Furthermore, the partial Eta squared values for Wilk's Lambda, which are 92.3%, 94.9%, and 99.9%, indicate a significant contribution to variance in the top layer, 15cm and 30 cm layer of soil, demonstrating successful microbial establishment due to sufficient resource availability compared to other layers, thereby facilitating effective microbial colonization to sustain ecological balance within soil profiles. In comparison to fungi and actinomycetes, bacteria are dominating in their contribution to soil fertility and its accessory characteristics.The fungal population accounted for 78.2%, followed by 95.6% and 99.85%, indicating an increasing trend in fungal population with depth, which enhances water infiltration and expands the root surface area for nutrient translocation to plants. The effective influence of pH (0cm: 97.75%; 15cm: 98.4%; and 30cm: 99.95%) considerably affects differences in microbial populations. Total nitrogen (95.1% - 98.8%) significantly contributes to enhancing the nutrient content for microbial populations at various soil depths. The variance in microbial populations across three distinct soil profiles is illustrated in Table 5.

CONCLUSION

The present work concentrated on a comprehensive analysis of three separate soil profiles to clarify the differences in physicochemical attributes and microbial populations. The pH, electrical conductivity, organic carbon, and total nitrogen exhibited an increasing trend from the surface layer to a depth of 30 cm due to the enhancement of pH from slightly acidic to alkaline conditions. The population of total bacteria, fungus, and actinomycetes increased from the surface layer to a depth of

30 cm due to enhanced soil physicochemical properties. Their population diminished to a depth of 60 cm due to adverse soil conditions. In PTS, the microbial population was diminished compared to AS and FS due to exposure to agrochemicals in the field, which may elevate heavy metal concentrations in the soil. Forest soil exhibited great fertility due to optimized soil factors at various depths. Overall, our results demonstrate the dominance of the bacterial population in extremely fruitful regions, namely at the 15 cm and 30 cm layers. Further research is necessary to further understanding of the processes and variations in patterns related to long-term soil development and the specific role of microbes in cycling of nutrients.

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