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Microbia Population and Diversity as Influenced by Soil pH and Organic Matter in Different Forest Ecosystems

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Abstract: Microbial populations were isolated and counted in agar-plated composite soil samples collected from stands of three different species and an adjacent natural forest in Akure forest reserve. The plantations were mature and unthinned stands of *Nauclea didderrichi, Gmelina arborea* and *Tectona grandis*. This was to assess the role of microbes in humus formation and soil fertility enhancement and to compare their population and species diversity in the monoculture stands and the natural forest. Soil pH and organic matter contents of the soil samples were also obtained, compared and correlated with microbial population. The results show that the soil samples consisted 33 species of bacteria and 23 species of fungi. The population of bacteria ranged between 26.14×10^6 and 360×10^6 MPN g^{-1} dried soil while that of fungi ranged between 2.50×10^6 and 23.34×10^6 MPN g^{-1} dried soil. Highest species diversity and population of the microbes were isolated in soil samples from the natural forest and the least from *Tectona grandis* stand. The correlation and regression results show that microbial diversity and abundance is highly influenced by soil pH and organic matter. There was no significant difference in organic matter and pH values of the samples from the different forest ecosystem ($p = 0.05$) but significant difference was discovered to exist in bacterial and fungal population ($p = 0.05$). The number and species diversity obtained for bacteria were more than that of fungi but there was close association in the abundance of the microbes obtained for all the soil samples.

Key words: Forest reserve, detoxification, soil fertility, nitrogen fixation, aboveground

INTRODUCTION

The many ecological, economical and environmental roles of the tropical rainforest ecosystem cannot be overemphasized. These roles include purification of air and water, regulation of water flow, detoxification and decomposition of wastes, generation and renewal of soil and soil fertility, carbon sequestration, biodiversity conservation, climate stabilization, moderation of temperature extremes, windbreaks, support for diverse culture and aesthetic beauty and landscape enrichment[1]. Other socioeconomic function is the supply of many products for rural livelihood. The products include timber, fruits, herbs, wildlife etc. People are now becoming aware of the dangers and cost of allowing the forest ecosystem to be degraded or lost[2]. Forest degradation and conversion could have impacts like global climate change, floods, landslides, loss of biodiversity etc. So, forest conservation should be the concern of the government at all levels, forest industries and private citizens.

The investigations of below ground biological interactions, microorganisms' population and diversity with the roles they play in forest ecosystem have not been adequately studied in Nigeria. Ecologists have just begun to study the contribution of biota to the dynamic interactions occurring among plant roots, animals and microbes in the forest ecosystem[3]. Dead plants and animals, animal droppings and leaves on forest floor are usually converted into fine organic matter (OM) when fed upon by small animals. These fine organic matters are further broken down into humus by microorganisms. Humus is a form of organic matter that cannot easily be decomposed further. Organic matter and humus have very high water and nutrient retention capacity and make them available to plant easily[4].

Humus contains the most essential nutrients (N, P and S) for plant growth and improves soil structure. Microorganisms are also responsible for the mineralization process in forest ecosystem. They act on the humus to release CO_2, water and nutrients, which could be absorbed directly by plants. The roles of microbes were summarized to include degradation of complex nutrient sources extracellularly, transportation of simple nutrients across cell membranes for metabolic processes and tolerating or deactivation of compounds that could inhibit fungal growth[5]. It has been reported that bacteria allow

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phosphorus, zinc, potassium and other minerals to be redeposited back into the nutritional bank[6]. The most important nutrient supply to the forest soil environment is derived from litter decomposition by actions of organism under conditions of high air temperature and soil moisture content[7]. These organisms mobilized the chemical elements in the litter and make them reabsorbable by plant roots. These microbes are able to perform these roles because of their ability to obtain nutrients through absorption. So, the study of microbial population and diversity as it affects humus formation and soil fertility is highly imperative.

The rate of humus formation and mineralization depend so much on microbial population and diversity in the ecosystem. Other factors are favourable weather condition (e.g. temperature and humidity), soil acidity, quality and quantity of litters and the physical and chemical environment. All these major factors are very adequate in tropical forest ecosystem. For instance, a study reported that some environmental factors affecting OM decomposition are usually higher in tropical than in temperate regions[8].

The objective of this study therefore was to evaluate and compare microbial populations and diversity, the main agents of humus formation and mineralization, obtained from different forest soil ecosystem. This will help to determine the interrelationships among organisms and the role of microbes in soil fertility maintenance. The study was limited to fungi and bacteria analysis because they present the highest values of biomass and respiratory metabolism and have greater participation in OM decomposition process[9]. The bacteria represent the major group responsible for 25-30% of the total soil microbial biomass[7]. The soil samples were collected from plantations of *Gmelina arborea, Nauclea didderrichii, Tectona grandis* and adjoining undisturbed natural forest (Permanent Sampling plot) in Akure forest reserve, SW Nigeria. *Gmelina aborea* is one of the fast growing exotic species in Nigeria and it has the largest plantation in the country. This is followed by *Tectona grandis,* another fast growing exotic species. *Nauclea didderrichii* is one of the few indigenous species that are grown in plantations in SW Nigeria.

MATERIALS AND METHODS

The study area: This study was carried out in Akure forest reserve located in Ifedore Local Government Area, Ondo State, Nigeria. This reserve was selected for the study because of the presence of a permanent sample plot demarcated in 1935 by Forestry Research Institute of Nigeria as strict nature reserve (representing an undisturbed natural forest ecosystem) and well-managed plantations of *Gmelina arborea, Nauclea didderrichii* and *Tectona grandis*. These constituted the different forest ecosystems where soil samples were collected. The plantations were established and managed by Ondo State Forestry and Wildlife Department.

Soil sample collection: Soil samples were collected at surface (surface soil 0-20 cm soil layer) using 3.5 mm diameter soil auger. Four points were taken from four sample plots of size 25×25 m randomly located in each of the forest types. The forest types are permanent sample plot representing a natural forest, teak stand established in 1991, Nauclea stand established in 1973 and Gmelina stand established in 1981. In all, sixteen-soil sub samples were collected, grouped and homogenized resulting in one composite soil sample. Three composite soil samples were retained, litters, roots and leaves were removed from the samples and they were taken to the laboratory for analysis.

Soil analysis: Soil biological properties assessment of the samples was limited to fungi and bacteria. The standard procedures for determining the total number of soil microbes were adopted for bacteria and fungi culturing[10]. Suspension of the soil samples was prepared with sterile water and a serial dilution of five factors was made for accurate counting. Then 1 mL of the appropriate dilution was carefully transferred to sterilized petri dishes containing sterile molten nutrient agar at about 37°C. This was mixed and allowed to solidify. It was then incubated for 24 h. The bacteria that grew into colonies were sub-cultured to obtain pure culture for easy identification. Identification was done according to Bergey's manual of determinative bacteriology.

For fungi culturing, serial dilution of the suspension was also transferred into petri dishes containing sterile, molten malt extract agar. This was kept in an incubator at 30°C for 5 days. Fungi that grew were sub-cultured to obtain pure culture for easy identification. Microscopic characterization was done for identification.

The soil pH was determined with the aid of glass electrode pH meter in soil solution of 0.01 mol L^{-1} calcium chloride while OM was determined after the soil sample furnace incineration at 55°C for 24 h[11]. All the soil analyses were done at the Department of Food and Industrial Microbiology of the Federal University of Technology, Akure, Nigeria.

Method of data analysis: Population values for bacteria and fungi (Most Probable Number-MPN) were logarithmically transformed-Ln (x + 1) where, x = MPN g^{-1}

dried soil x 10^6. All the data were subjected to one way analysis of variance (ANOVA), mean were separated, where significant differences occur by Fisher's protected Least Significant Difference (LSD)[12]. Correlation between microbial populations and soil pH and OM from each of the forest types was carried out to obtain the association between the variables. Regression analysis of the form $Y = b_0 + b_1X$ was also generated. This was used to assess the relationship between microbial population (Y) and soil pH and OM (X). The regression equation used for the bacterial and fungi population is of the form $LnY = b_0 + b_1LnX$ (where, Y is the dependable variables (bacterial population), X is the independent variable (fungi population), b_0 and b_1 are regression constants to be estimated and Ln is natural logarithm). All the equations were assessed to be able to determine their fitness for further use. The assessment criteria used are: (I) Correlation coefficient (r) which must be greater than 0.5, (ii) Coefficient of determination (R^2) which must be more that 50%, (iii) Regression Mean Square Error (RMSE) must be very small and (iv) F-ratio which must be significant ($p \leq 0.05$) for the equation to be of good fit[13]. All the statistical analyses were done with SPSS 10.0 computer software module on Pentium IV desktop computer.

RESULTS AND DISCUSSION

The diversity of bacteria and fungi encountered in the different forest soil environment with their relative abundance reveal that soils under forest cover contain different species of microorganism that are very important for humus formation. This is responsible for the usual fertile land under forest cover. The different species of bacteria and fungi identified in the forest soils are presented in Table 1 and 2 for bacteria and fungi, respectively.

The most frequently occurring species of bacteria encountered is the aerobic spore formers (*Bacillus* sp.) that were isolated in soil samples from all the forest types. This bacterium is able to survive adverse environmental conditions by producing extremely drought resistant endospores[14]. So it could be referred to as habitat generalist. This was followed by the members of the genus Rhizobium, which normally form symbiotic relationships with roots of leguminous plants. This colonization by rhizobia results in the formation of root nodules where atmospheric nitrogen that is then made available to plants is fixed. For fungi species, *Penicillum* sp. was prominent in all the soil samples (habitat generalist). This was followed by *Aspergillus* sp., which was encountered in three of the four sites.

It has been discovered that Cyanobacteria, Actinomycetes and other Rhizobacteria (Azotobacter) fix atmospheric nitrogen, thereby increasing soil fertility and cell materials[15]. Also, excretions from soil microorganisms affect water and air movement within the soil. A study reported that some bacteria could produce antibiotics that are very useful while some fungi function largely in the breakdown of complex organic molecules like lignin (a compound that is resistant to bacteria degradation)[16]. Bacteria are also very beneficial to trees by regulating inputs and outputs of nitrogen[17].

On the whole, a total of thirty-three species of bacteria and twenty-three species of fungi were isolated from all the soil samples. But the highest number of bacteria and fungi species was isolated from the natural forest. This was followed by soil samples from *Nauclea didderrichii* stand (10 species of bacteria and 8 species of fungi). The least number of the microbes was discovered in soil samples from *Tectona grandis* plantation. The difference in species and numbers of microbes found in the soil samples could be attributed to the variation in some factors such litter quality, soil

Table 1: Bacteria species diversity encountered in the different forest ecosystem

Natural forest	Nauclea	Gmelina	Teak
Serratia maccescens	*Clostridium sporogenes*	*Proteus vulgaris*	*Streptomyces* sp.
Bacillius cereus	*Rhizobium leguminosarium*	*Escherichia coli*	*Bacillus cereus*
Actinomyces sp.	*Bacillus polymyxa*	*Bacillus polymyxa*	*Staphylococcus aureus*
Escherichia coli	*Bacillius subtilis*	*Acinetobacter parapertusis*	*Proteus vulgaris*
Shigella dysenteriae	*Streptomyces* sp.	*Actinomyces* sp.	*Streptococcus faecalis*
Bacillus subtilis	*Kurthia* sp.	*Bacillus cereus*	*Bacillus cereus*
Bacillius megatarium	*Thermobacterium lactobacillus*	*Alcaligenes feacalis*	
Proteus vulgaris	*Bacillus meg terium*	*Acinetobacter Iwoffii*	
Coryneabacterium sp.	*Pseudomonas aeruginosa*	*Bacillus subtilis*	
Rhizobium leguminosarium	*Citrobacter freundii*		
Sarcina flora			
Alcaligenes feacalis			
Clostridium sporogenes			
Total 13	10	9	6

Table 2: Fungi species diversity encountered in the different forest ecosystem

Natural forest	Nauclea	Gmelina	Teak
Stachbotrys sp.	*Neurospora crassa*	*Gonatobotrys simplex*	*Boytrytis cinerea*
Gonatobotys simplex	*Penicillum* sp.	*Penicillum* sp.	*Fusarium* sp.
Rizopus sp.	*Aspergillus fumigatus*	*Mucor mucedo*	*Rhizopus* sp.
Wardomyces sp.	*Candian* sp.	*Varicosporium elodeae*	*Gonatobotrys simplex*
Aspergillus niger	*Streptomyces* sp.	*Aspergillus flavus*	*Penicillum* sp.
Boytrytis cinerea	*Aspergilus raperis*	*Penicillum* sp.	
Penicillum sp.	*Trichoderma vivide*	*Aspergillus niger*	
Fusarium sp.	*Wardomyces* sp.		
Choanephora cucurbitarum			
Varicosporium sp.			
Total 11	8	7	5

Table 3: ANOVA for soil parameter obtained from the different forest ecosystems

		Sum of squares	df	Mean square	F-calculated	sig.
pH	Forest types	1.580E-02	2	5.267E-03	1.368	0.320
	Error	3.080E-02	8	3.850E-03		
	Total	4.660E-02	11			
Organic matter	Forest types	33.039	3	11.013	2.754	0.112
	Error	31.995	8	3.999		
	Total	65.034	11			
Bacteria (LnX+1)	Forest types	10.306	3	3.435	150.133	0.000
MPN g^{-1} dried soil	Error	0.183	8	2.288E-02		
	Total	10.489	11			
Fungi (LnX+1)	Forest types	9.877	3	3.292	209.919	0.000
MPN g^{-1} dried soil	Error	0.125	8	1.568E-02		
	Total	10.002	11			

temperature, moisture content, pH and the nutrient status in the selected forest ecosystems. So changes affecting these species as a result of these factors may have profound effects on ecosystem functioning. Below and above ground communities are inextricably linked through complex interactions. So any disturbance or change in the environment that affects above ground vegetation will also affect soil biota[16]. The highest number and species diversity of the microbes in the natural forest could be attributed to the presence of different types of litter from the various plant species in the tropical natural forest ecosystem. This supported the survival of many species of micro and macro-organisms. So the processes of humus formation were usually rapid and occur throughout the year in this ecosystem. This is responsible for the availability of fertile land under natural forest and the subsequent increase in the rate of encroachment of this forest type by land hungers mainly for agricultural purposes.

In plantation ecosystem (monoculture), the number and diversity of the organisms were lesser than what was in the natural forest because of the few number of plant species available in the plantations. Only the microbes that could thrive with the few available plant species were present. Microbial diversity and abundance is less when there is few plant vegetation, frequent fire outbreak and coarse leaves that make decomposition very difficult. Litters with high content of more complex and slowly degradable phenolic compound and lignin often decompose very slowly[18]. The quality of plant residues depends on their relative contents of sugars, hemicelluloses, lignin and polyphenol that also determine the proportional content of nutrients. Based on this, plant residues were categorized into high quality and low quality residues[19]. The population, diversity and the proportional amount of nutrients to be released into the soil by the microbes during decomposition are direct function of litter quality.

Table 3 is the result of the one-way analysis of variance for assessing the presence of significant difference in relative abundance of the microbes (MPN), organic matter content and soil pH from the different forest soil. While there was no significant difference ($p \leq 0.05$) in soil pH and organic matter contents of the samples, a significant difference ($p \geq 0.05$) was discovered to exist in the number of bacterial and fungi isolated from the different forest soil environments. The LSD procedure for mean separation reveled that there was no significant difference between the population of bacteria from the stands of *Nauclea didderrichi* and *Gmelina arborea* and also in the fungi population from *Gmelina arborea* and *Tectona grandis* stands ($p \leq 0.05$). This shows that the population of microbes in these plantations does not differ significantly.

Highest number of the microbes (MPN) was obtained in samples from the natural forest and they significantly differ from what was obtained from

Table 4: The relative abundance of the microbes, organic matter content and soil pH from the different forest soil environments

Soil parameters	Natural forest	*Nauclea didderrichii*	*Gmelina arborea*	*Tectona grandis*
pH	6.19±0.3	6.26±0.05	6.20 ±70.2	6.15±0.02
Organic matter	12.77±1.42	12.68±0.78	14.37±1.41	9.77±0.86
Bacteria (MPN g^{-1} Dried soil)×10^6	360±12.77(a)	118.44±7.24(b)	138.67±19.73	26.14±1.63
Fungi (MPN g^{-1} Dried soil)×10^6	23.34±0.77 (a)	8.50±0.11(b)	2.89±0.40©	2.50±0.10©

Means follow with the same alphabets are not significantly different ($p \leq 0.05$)

Table 5: Regression equations with their assessment criteria for microbial population and soil properties in the different forest ecosystems

Natural forest	Nauclea stand	Gmelina stand	Teak stand
F = 0.67, pH-0.99, (R = 0.78,	F = 4.76-0.60, pH (R = 0.11,	F = 2.88-0.12, pH (R = 0.0.48,	F = 13.64-2.07, pH (R = 0.88,
R^2 = 61%, RMSE = 0.003,	R^2 = 1%, RMSE = 11.0	R^2 = 23%, RMSE = 0.19,	R^2 = 77%, RMSE = 0.002,
F-ratio = 11.23*)	F-ratio = 5.37*)	F-ratio = 4.21*)	F ratio = 75.22*)
F = 3.37-0.028OM (R = 0.74,	F = 2.26-0.08x (R = 0.89,	F = 1.93-0.2OM (R = 0.99,	F = 1.35-0.04OM (R = 0.98,
R^2 = 55%, RMSE = 0.007,	R^2 = 79%, RMSE = 0.02,	R^2 = 99%, RMSE = 0.004,	R^2 = 96%, RMSE = 0.001,
F-ratio = 25.64*)	F-ratio = 20.36*)	F-ratio = 121.14*)	F ratio = 105.14*)
B = 8.30-0.40, pH (R = 0.42,	B = 0.40, pH-21.85 (R = 0.74,	B = 0.99, pH-1.41 (R = 0.82,	B = 3.45, pH-17.95 (R = 0.93,
R^2 = 17%, RMSE = 3.70,	R^2 = 55%, RMSE = 0.06,	R^2 = 68%, RMSE = 0.01,	R^2 = 87%, RMSE = 0.003,
F-ratio = 8.13*)	F-ratio = 15.84*)	F-ratio = 9.35*)	F ratio = 89.45*)
B = 5.53 + 0.02OM (R = 0.96,	B = 3.46 + 0.10OM (R = 0.97,	B = 5.66-0.08OM (R = 0.87,	B = 2.56 + 0.07OM (R = 0.99,
R^2 = 93%, RMSE = 0.008,	R^2 = 94%, RMSE = 0.007,	R^2 = 75%, RMSE = 0.01,	R^2 = 99%, RMSE = 0.001,
F-ratio = 31.52*)	F-ratio = 41.45*)	F-ratio = 78.11*)	F ratio = 185.92*)
LnB = 8.96-0.99LnF (R = 0.90,	LnB = 8.96-0.99LnF (R = 0.75,	LnB = 14.24-4.43LnF (R = 0.90,	LnB = 4.67-1.55LnF (R = 0.99,
R^2 = 80%, RMSE = 0.04,	R^2 = 56%, RMSE = 0.06,	R^2 = 81%, RMSE = 0.005,	R^2 = 98%, RMSE = 0.001,
F-ratio = 51.61*)	F-ratio = 21.03*)	F-ratio = 52.14*)	F ratio = 116.32*)

F = Fungi, B = Bacterial, OM = Organic Matter

plantations (Table 4). This was as a result of plant species diversity in the natural forest ecosystem and the favourable environmental conditions for microbial activities. So, the conversion of the tropical lowland rainforest to plantations of fast growing species affected the microbial diversity and abundance of the study area. The risks and the changes in sites associated with plantation forestry practices had been reported[20] and the account of the impacts of plantation species on the tropical humid soils had also been given[21]. Apart from the negative impacts of plantation development on flora and fauna species diversity, the physical and chemical properties of the soil could also be adversely affected.

Also in this study, the population and species diversity of the bacteria were more than fungi in all the sites. This supports the claim that bacteria are by far the most abundant group of soil microbes in term of number[22].

The highest pH value (6.26±0.05) was obtained in soil samples from *Nauclea didderrichii* stand while the least (6.15±0.02) was obtained from *Tectona grandis* stand. The soil pH obtained (between 6.15 and 6.26) was discovered to be very favourable for microbial activities. For the organic matter content, the highest value (14.37±1.41) was present in the stand of *Gmelina arborea* due to biomass accumulation and abundant undergrowth on the forest floor. This is followed by Natural forest stand (12.77±1.42). The least amount of OM was in the stand of *Tectona grandis* 9.77±0.86 as a result of frequent fire outbreak in the stand. In the (Table 4) *Tectona grandis*, OM value was very low in the natural forest too because there is always rapid rate of biomass decomposition and humus formation in the ecosystem[20]. This is also responsible for the highest number and species diversity of bacteria and fungi obtained in the natural forest. This corroborates the findings of that the diversity and population of microorganisms depend on the rate of OM decomposition in the forest ecosystem[7].

A positive correlation was obtained between the microbes and soil pH and organic matter. High and positive correlation coefficient and coefficient of variation (R and R^2, respectively) values were obtained between bacteria and OM and also between fungi and OM for all the soil samples analyzed. The R-values ranged between 0.87 and 0.99 while the R^2 were between 74 and 99% for bacteria and OM. The r-values for fungi and OM ranged between 0.74 and 0.99 while the R^2 were between 55 and 99%. All the equations were significant ($p \leq 0.05$) with small standard errors. Further assessment shows that the equations have good fit and could be used to predict the population of the microbes in the study area and in other similar sites (Table 5).

The result of the double-log regression equations between bacteria (dependent variable) and fungi

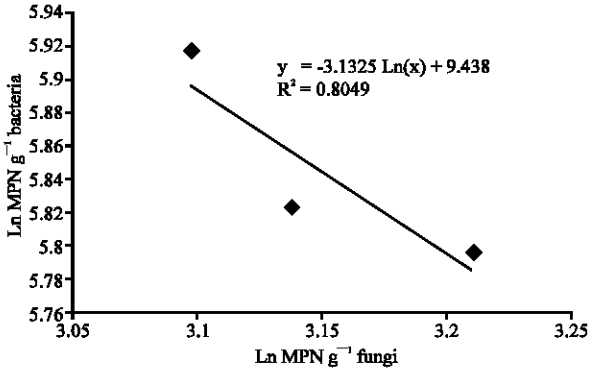

Fig. 1: Regression line plot for Ln MPN g^{-1} dried soil bacterial and fungi (Natural forest)

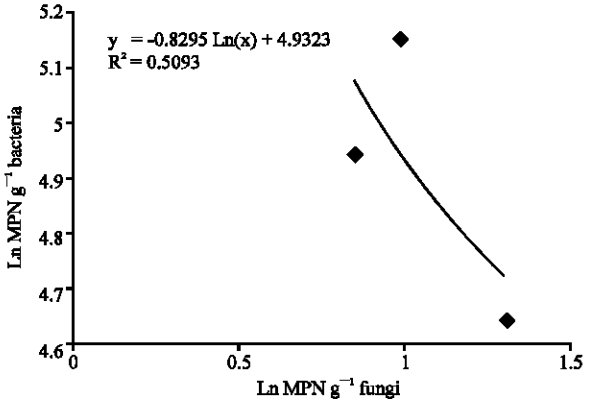

Fig. 2: Regression line plot for Ln MPN g^{-1} dried soil bacterial and fungi (Nauclea)

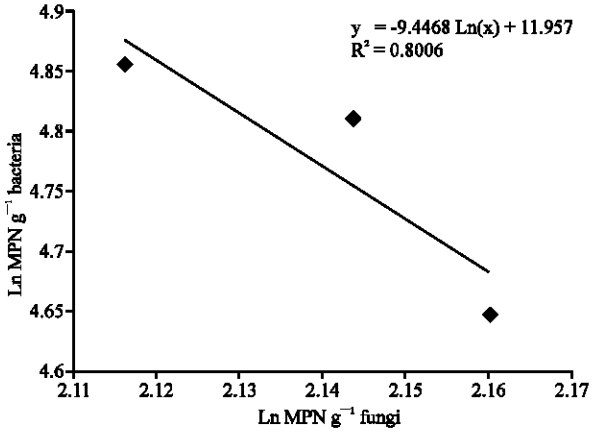

Fig. 3: Regression line plot for Ln MPN g^{-1} dried soil bacterial and fungi (Gmelina)

(independent variable) and the regression plots (Fig. 1-4) revealed that there was good relationship and positive correlation between the two microorganisms in all the four sites selected for this study. The correlation coefficient (positive and significant) ranged between 0.75 and 0.99 while the coefficient of determination ranged between

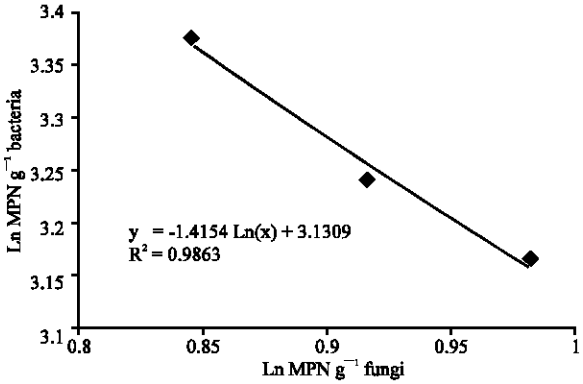

Fig. 4: Regression line plot for Ln MPN g^{-1} dried soil bacterial and fungi (Teak)

56 and 98%. All the equations were significant ($p<0.05$) with small regression mean square error. This shows that the relative abundance, species diversity and activities of the two microbes were well and equally favoured by the site conditions, pH and OM.

CONCLUSIONS AND RECOMMENDATIONS

The result of this study revealed that there is variation in the population and diversity of fungi and bacterial in soil samples from the different forest ecosystem. The pH values obtained from all the soil samples favour microbial activities as a result; organic matter decomposition and humus formation was enhanced. The highest number of microbes and species discovered to exist in the natural forest when compared with the plantations could be attributed to the quality of plant residues and the relative abundance and richness of plant and animal species in the ecosystem. Microbial diversity and abundance will also contribute to the fertility of soil under forest cover or fallow. So, degraded lands and abandon farmlands could be left under fallow for it to regain its vegetation and fertility. The equations with good fit developed in this study are recommended for further use in this kind of forest environment and similar ones. The conversion of the natural forest ecosystem to monoculture should be discouraged if flora and fauna diversity is to be conserved.

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