



## Studies on Fertility Indicators of Forest Soil under Different Tree Canopy Shades in a Tropical Low-Land Rain Forest of Otuoke Bayelsa State, Nigeria.

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### Abstract

The study was carried out to investigate whether tree canopy shades have relationships with soil fertility indicators. Purposeful random- within- block sampling method was used. A homogenous forest stand with same site quality was purposefully demarcated into three sample areas of 1 hectare each in the order of limited canopy cover, and total canopy cover. An open field within the same terrain was also demarcated. A 10m×10m sub-samples were demarcated within each sample area and three sample plots were randomly selected from each sample area for investigation. Twenty trees (10/sample plot) within the nearest neighbour under limited and total canopy covers were randomly picked. Twenty leaves were collected from each sample tree for leaf area index measurement. Soil samples were collected from 0-30cm depth under each canopy shade and taken to the laboratory for soil and microbial load analyses. Chi- square test shows that microbial load was significantly ( $P < 0.05$ ) higher under close canopy than open field. Microbial population was  $501 \times 10^5$  CFU under close canopy,  $415 \times 10^5$  CFU under limited canopy and  $201 \times 10^5$  CFU in open field. Results showed that closed canopy has the highest microbial load with mean value of  $167 \times 10^5$ , followed by limited canopy  $138 \times 10^5$ . Open canopy had the lowest microbial load  $67 \times 10^5$ . The coefficient of determination ( $R^2$ ) was 0.15 for closed canopy, and  $R^2$  was 0.21 for limited canopy shade. In all, there were weak positive correlations between tree canopy shades and soil microbial load.

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### Introduction

Tree canopy have obvious impacts that bring about changes in belowground soil conditions and the consequences of these changes are less understood. It is a known fact among subsistent farmers that soil under tree canopy seems to be more fertile than those away from tree shade even within the same parcel of land or cropping area. On the other hand, some rural farmers avoid tree shades while planting and many times embark on clear felling before planting, believing that tree shades impedes productivity. However, Theodorou (1984) reported that tree canopy can influence the microbial population of soil under it and Graystone *et al.* (2001) and Garbeva *et al.* (2006) have also posited that tree canopy can influence the composition of underlying soil microbial communities. Unanaonwi and Ake (2016) stated that soil microbial load is an indicator of soil fertility, measured by crop yield and forest productions. That is to say a fertile soil harbours

more microorganisms than an infertile or less fertile soil. Tree canopy with its biodiversity is a crucial part of the earth systems, offering essential services to both the ecosystem and human, it also affect soil biota as it plays a significant role in determining the soil physicochemical properties. Canopy-shade support the functions of soil which includes decomposition, nutrients cycling, soil respiration, invasion resistance and ecosystem services essential to mankind and crops (Huo *et al.*, 2014). The change in soil physicochemical properties depends on the litter quality and quantity and the canopy architecture (Sharma and Anderson, 2013). Studies revealed that soils were sandier and slightly acidic under canopies of medium and large tree compared to small trees, which have slightly alkaline soil (Volkenhube *et al.*, 2004). Soils in the interspaces have significantly higher silt and clay content than beneath trees (Zemmrch *et al.*, 2010). Thus, distribution of general

soil fertility, organic matter, nitrogen, phosphorus and potassium, as well as microbial activities becomes spatially and vertically concentrated under the tree canopy. Soil under canopies was found to have significantly higher levels of organic matter, calcium, magnesium and  $p^H$  than those in open grassland (Mlambo, 2005). Macdonald and Fenniak, 2007). Canopy-shade also contributes immensely to the stock of soil organic carbon which is essential for good soil structure and nutrient availability that support soil biota and aboveground productivity. The types of tree canopy present in an area have a great impact on the quality of the soil of that area as the microbial biomass and soil are strongly influenced by each other ( Isichei and Moughalu, 1981). The characteristics of a soil are undergoing continual changes and the rates of these changes are highly dependent on the type and density of tree canopy (Kim *et al.*, 1995). Tree canopy and soil may act as significant sinks or sources of atmospheric carbon dioxide, depending on land use, forest management and environmental conditions. The most important aspect of tree canopy in terms of its influence on nutrient cycling is its role as the source of leaf litter.

## Materials and Methods

2.1 Demarcation of Sampling Sites -The site was purposefully demarcated into three sample areas of 1 hectare each in the order of limited canopy cover, total canopy cover and open field. 10m×10m sub-

## Data Collection and Estimation of Canopy Cover

Twenty trees (10/sample plot) within the nearest neighbor under limited and total canopy cover were randomly picked. Mean distances of trees within the nearest neighbors were recorded. Twenty Leaves were collected from each sample tree for leaf area index measurement. Leaf area index (LAI) is a dimensionless quantity that characterizes plant canopies. It is defined as the one-sided green area per unit ground surface area ( $LAI = \text{leaf area}/\text{ground area } M^2/M^2$ ) in broadleaf canopies (Chen *et al.*, 1997). The area of the collected leaves was measured by a leaf area meter. The measured leaf areas were then divided by the ground surface area and were recorded for the different canopies. LAI was estimated with a direct optical method (Pierce and Running, 1988). LAI was determined directly by taking a statistically significant sample from a plant canopy, measuring

## Soil study for microbial load

Three soil samples per plot were collected at 0-30cm using soil auger. All soil samples per canopy cover were homogenized by hand and 1kg of each was

Characteristics of the canopy determine the amount and composition of leaf litters produced, which largely determine the amount of nutrients to be recycled, the composition of the soil microbial and faunal communities and the resulting availability of nutrients in mature forests. Canopy litter (foliage, reproduce tissue and fine woody debris) accounted for 66 to 86% of the mass, 63 to 90% of nitrogen, and 49 to 92% of the phosphorus returned annually in aboveground litter (Wason *et al.*, 2003). However, several mechanisms may account for the increased fertility under trees, nutrients are returned to the soil through deposition of litter, root decay and exudation, as well as leaching of tree nutrients in rain fall. Biological processes appear to be particularly important with tree sites being characterized by higher macrofaunal and microbial activity, as well as higher mineralization rates, lower bulk density and better water infiltration than treeless location. Although studies have demonstrated that tree population and canopy shade can influence soil fertility status (Haicher *et al.*, 2008) such a study is not yet documented within the region.

samples were demarcated within each sample area and three sample plots were randomly selected from each sample area for investigation.

the leaf per sample plot and dividing it by the plot land surface area (Brenda, 2003). The equation given by Blanco and Watson (1947) was used to calculate leaf area, from leaf length and width measurements.  $LA = L * W * A$

Where LA= leaf Area, L=leaf length, W=leaf maximum width, A= (constant) 0.75.

The total leaf area of the leaves in a given canopy is as the ratio of total leaf area to the total land area available to the tree (Bianco and Folegetti, 2003). LAI was estimated simply by measuring the width and length of the leaf that represent the Mean leaf area of the plant in each canopy cover ( Lovett and Lindberg, 1993). Ground surface area was calculated using the equation  $L \times W$ , where  $LAI = \text{Leaf Area}/\text{Ground Surface}$ .

dried at 37 °c for 24 hours and taken to the laboratory for microbial and biochemical test analyses. Mac-Conkey agar, Blood agar, Nutrient agar and cystine Lactose Electrolyte Deficient agar

(CLED) were used for the cultivation of soil bacteria. The media were then autoclaved at 121<sup>0</sup>c for 15 minutes after which it was then dispensed into sterile plastic petri dishes. The freshly served soil was carefully mixed and pulverized with spatula on the larger piece of paper. One gram of soil was transferred immediately to the conical flask containing 150ml of normal saline. The soil was stirred for 15 minutes. The soil suspension was then serially diluted with 1ml of the soil suspension added to 9ml test tube of normal saline. Dilution ratios included: 10<sup>0</sup>, 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>, 10<sup>-7</sup>, 10<sup>-8</sup>, 10<sup>-9</sup>. For plate count experiment, 200ul aliquots from 10<sup>-5</sup> dilution was transferred to petri dishes and semi-solid

media were poured in the Petri dishes containing diluents and was spread evenly. This was followed by incubation at 37<sup>0</sup>c for 24 hours. Identification and characterization of bacteria isolates was carried out. The Preliminary characterization of the isolates involved the examination of colony morphology and culture features such as color, pigmentation, elevation, shape, size and growth form. Gram staining and other biochemical tests were carried on isolates. The effect of three different soils was investigated by determining the number of colonies visible on plates after 24 to 72 hours. The viable counts after 72 hours of incubation were recorded.

**Statistical Analysis:** The data generated was analyzed statistically using Chi-square test and Correlation analysis. The microbial isolates and

counts of the sites were analyzed with Test of Relationship using the Chi-Square Test.

**Results and Discussion**

The result shows that closed canopy has the highest microbial load with mean value of 167 x 10<sup>-5</sup>, followed by Limited canopy with 138 x 10<sup>-5</sup>. The open canopy had the lowest microbial load of 67x10<sup>-5</sup>. Enumeration of bacteria viable cell counts against three different sites of soil i.e. closed canopy, limited canopy closure and open field showed that the viable

cell counts were significantly different between the three areas. The research has revealed that tree canopy shade has significant effect on the bacterial and fungal load of the soil under it with weak correlations.

Table 1. Soil Microbial Load under Closed canopy, Limited canopy and open field (Tree canopy shades)

Replicates	Closed	Limited	Open Field
Replicate 1	227×10 <sup>5</sup>	194×10 <sup>5</sup>	83×10 <sup>5</sup>
Replicate 2	145 ×10 <sup>5</sup>	169 ×10 <sup>5</sup>	108 ×10 <sup>5</sup>
Replicate 3	129×10 <sup>5</sup>	52 ×10 <sup>5</sup>	10 ×10 <sup>5</sup>
"	167 ×10 <sup>5</sup>	138 ×10 <sup>5</sup>	67 ×10 <sup>5</sup>
Total	501×10 <sup>5</sup>	415 ×10 <sup>5</sup>	201×10 <sup>5</sup>

Table 2. Chi-square test of effects of canopy shades on soil microbial and bacteria load (Tree canopy shade)

Replicates	Closed	Limited	Open field	Total
1 O	227	194	83	504
1 E	226.1	187.3	90.7	504
2 O	145	169	108	442
2 E	189.3	156.8	75.9	442.0
3 O	129	52	10	191
3 E	85.7	71.0	34.4	191.0

O: Observed

E: Expected

X<sup>2</sup> = 70.011 df = 4 P- value = 0.001\* no of cases = 1117

Table 3. Correlation between soil microbial load and Leaf Area Index under closed canopy

		Microbes	LAI
Closed canopy MICROBE N	Pearson Correlation	1	0.383
	Sig. (2-tailed)		0.750
	N	3	3
Closed canopy LAI	Pearson Correlation	0.383	1
	Sig. (2-tailed)	0.750	
	N	3	3

The correlation coefficient ( $r$ ) of 0.383 shows that the relationship between microbial load and leaf area index under closed canopy is not strong although positive. That is, the correlation coefficient is close to zero hence the correlation is poor. The coefficient of

determination ( $r^2$ ) is 0.15. This implies that 15% of the variation in soil microbial load has been accounted for by the variation in canopy shade (LAI) under closed canopy

Table 4. Correlation between soil microbial load and limited canopy shade (LAI)

		Limited microbes	Limited canopy LAI
Limited canopy microbes	Pearson Correlation	3	0.455
	Sig. (2-tailed)		0.699
	N	3	3
Limited canopy LAI	Pearson Correlation	0.455	1
	Sig. (2-tailed)	0.699	

The correlation coefficient ( $r$ ) of 0.455 shows that the relationship between microbial load and leaf area index under limited canopy is not strong. That is, the correlation coefficient is close to zero hence the correlation is poor. The coefficient of determination ( $r^2$ ) is 0.21, implying that 21% of variation in soil microbial load under limited canopy has been accounted for by the variation in closed canopy shade (LAI). The p-value 0.699 also indicates that the null hypothesis cannot be rejected. These results (Tables 1, 2, 3, and 4) clearly demonstrate the importance of tree canopy cover and its impact on soil microorganisms. Table 1 shows soil fungal and bacteria load under three different canopy closures. A wide range of different media have been used to estimate the size of microbial load of soil and to isolate representatives of its community. However, it has been known for a long time that the number of bacteria that are able to form colonies on microbiological media is generally only a small part of the total number of bacteria in the soil. The counts were higher for closed canopy compared to those obtained from limited canopy closure and the

open field. Canopy characteristics affect the amount and composition of leaf litter produced, which largely determines the amount of nutrients to be recycled and resulting nutrient availability. There is a greater amount of some nutrients beneath the canopies than in open field which may be attributed to greater organic matter inputs from leaf fall than it occurs in open field (Bossi *et al.*, 2005). Although effects of tree canopy on soil nutrient availability were thought to be brought about largely through differences in the decomposition rate of their foliar litter, recent studies indicate that the effect of tree canopy can be better predicted from the mass and nutrient content of litter produced, hence total nutrient return, from litter decay rate. In table 2, the greater canopy complexity in closed canopy creates similar heterogeneity in nutritional characteristics of the belowground microbial load

The essence of using linear correlation to further conduct analysis on leaf area index of the three sites (closed canopy, limited canopy and open field) and their microbial load respectively is to measure the degree of association as well as the direction of

association of Leaf Area Index (canopy shade) and corresponding soil microbial load. Table 3 shows a positive but weak correlation between closed canopy and soil microbial load. This implies that variation in closed canopy shade will bring about 15% variations in soil microbial load. Although weak, the result still indicated that tree canopy shade correlates with soil microbial load.

The remaining 85% unexplained could be attributed to topography, soil drainage and perhaps tree age which were not object of the study. The correlation between microbial load and leaf area index under limited canopy, table 4, is also positive and weak, with a coefficient of determination ( $r^2$ ) of 0.21, implying that only 21% of the variation in soil microbial load is accounted for by the variation in tree canopy shade.

### Conclusion

Soil microbial population is a function of soil fertility and soil fertility is a factor of great concern in agricultural and forest productions. Leaving more trees on the farm lands would be an option for boosting soil fertility. As the land in Federal University Otuoke succeeds from forest into field due to developmental activities, the capacity of the underlying soil to store microorganism has clearly been altered. The succeeding open fields need to be afforested in order to maintain and if possible further improve its part of standing bio-mass.

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