Scholars Bulletin *(Biology)*

An Official Publication of "Scholars Middle East Publishers", Dubai, United Arab Emirates
Website: http://scholarsbulletin.com/

ISSN 2412-9771 (Print)
ISSN 2412-897X (Online)

Effects of Tree Canopy Shade on Soil Bacterial and Fungal Load

Unanaonwi Okpo Esio*[1], Okezeke, Roy Nebolisa[2]

[1]Senior Lecturer, Department of Biology Federal University Otuoke, Nigeria
[2]Department of Microbiology, Federal University Otuoke , Nigeria

***Corresponding author**
Unanaonwi Okpo Esio

Article History
Received: 21.12.2017
Accepted: 27.12.2017
Published: 30.12.2017

DOI:
10.21276/sb.2017.3.12.21

Abstract: Effects of tree canopy shades on Below Ground Microbial Load (BGML) were investigated within Federal University Otuoke. A homogenous forest stand was purposefully demarcated into three sample areas of 1 hectare each in order of limited canopy, 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 area for investigation. Twenty trees (10/sample plot) under limited and total canopy cover were randomly picked. Twenty Leaves were randomly collected from each sample tree for leaf area index measurement. Soil samples were collected from 0-30cm under each canopy shade and taken to the laboratory for microbial load analyses. Chi-square test and correlation analyses were used in data analyses. Results showed that closed canopy has the highest microbial load with mean value of 167 x 10^{-5}, followed by Limited canopy (138 x 10^{-5}). Open canopy had the lowest microbial load of 67x10^{5}. Chi- square test shows that microbial load was significantly ($P<0.05$) higher under close canopy than open field. Microbial population was 501 x 10^{-5} CFU under close canopy, 415 x 10^{-5} CFU under limited canopy, *and* 201 x 10^{-5} CFU in open field. The coefficient of determination (R^2) was 0.15 for closed canopy, $R^2 = 0.21$ for limited canopy. There were weak positive correlations between canopy shades and soil microbial load. The practice of clearing woody trees to improve grassland for livestock production is not recommended. More trees on agricultural lands could improve soil health by attracting below ground populations.

Keywords: Tree canopy shade, Leave area index, Below- ground population, Homogenous forest, Silviculture, forest health, soil fertility, Agriculture and forest productions

INTRODUCTION

Tree canopy are formed from the spread of branches with the leaves, and the more of the branches removed, the more open the crown, which in turn have implications on soil environment. Understanding ecological linkage between the tree canopy and belowground biota is an important challenge for our knowledge on the maintenance and stability of ecosystem processes [1]. Soil microorganism, play fundamental roles in biogeochemical cycling and are major drivers of terrestrial ecosystem diversity and productivity, and hence, the relationship between soil microbial community and tree canopy attributes has greater attention than ever before, nevertheless, direct comparisons of plant-microbial community diversity remain limited due to the knowledge gap between microbiology and general ecology [2].

Tree canopy have obvious impacts that bring about changes in belowground soil microbial communities and the consequences of these changes are less understood. It is 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 tree felling before planting, believing that tree shades impedes productivity. However, [3], reported that tree canopy can influence the microbial population of soil under it and [4, 5] have also posited that tree canopy can influence the composition of underlying soil microbial communities [6]. 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. Although numerous studies have demonstrated that below-ground biodiversity can be influenced by tree canopy-shade [7], such a study is almost virtually absent in the Nigerian tropical lands. The objective of this research is to ascertain whether tree canopy shade has any effect on below- ground fungal and bacteria load, so as to make recommendations that could be useful for land use practice, for agriculture and forest productions.

LITERATURE REVIEW

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. An estimated 50-90 percent of life above and below ground in the Tropical Forest revolves around the tree [8]. Primary tropical rainforest is vertically divided into at least five layer; the over story, the canopy, the understory, the shrub layer and the forest floor, [9]. Each layer has its own unique plant and animal species interacting with the ecosystem around them. The over storey refers to the crowns of emergent trees when soar 20-100 feet above the rest of the canopy. The canopy is the dense ceiling of closely spaced trees and their branches, while the understory is the term for more widely spaced, smaller tree species and juvenile individuals that form a broken layer below the canopy. The shrub layer is characterized by shrubby species and juvenile trees that grow only 5-20 feet off the faorest floor [10]. Canopy-shade support the functions of soil which includes decomposition, nutrients cycling, soil respiration, invasion resistance and ecosystem services essential to mankind and crops [11]. 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 productivit, [12].

The change in soil physicochemical properties depends on the litter quality and quantity and the canopy architecture, [9]. 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 [12]. Soil in the interspaces have significantly higher silt and clay content than beneath trees, [13]. 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 [14]. And soil under canopies was found to have significantly higher levels of organic matter, calcium. Magnesium and p^H than those in open grassland [15].

A great deal of research has sought to answer the question of how land cover change impacts the storage of organic carbon. Soil is a mixture of organic and inorganic materials. The organic part consists of living thing and their remains while the inorganic part is made up of rocks and minerals. 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 [16]. 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 [17].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 the canopy in terms of its influence on nutrient cycling is its role as the source of leaf litter. Characteristics of the canopy determine the amount and composition of leaf litter 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 [18].

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 numerous studies on the effects of tree canopy on soil microorganisms are available, they rarely focus on microbial communities' belowground level [19-21]. Surveys on effects of pure tree species in a tropical region, as well as those focusing in vegetation gradients or chronosequences contributed to the current overall picture concerning tree influences on soil microbial communities [22, 23] has been acknowledged. Tree canopy provide the organic carbon required by the decomposer subsystem, and the decomposer subsystem in turn breaks down dead plant material, and indirectly regulates the plant growth and community composition by determining the supply of available soil nutrient [1]. Given the predominant functional associations between tree canopy and soil microbes, high tree canopy is usually proposed to be linked to high belowground diversity because of diverse litter types and root exudates [24].

Given the predominant functional associations between tree canopy and soil microbes, high tree canopy is usually proposed to be linked to high belowground diversity because of diverse litter types and root exudates [24]. Apart from diversity comparison, our understanding of the association of plant-microbial diversity is quite limited upon which this study is predicated for a Tropical Moist Rain Forest of Southern Nigeria.

MATERIAL AND METHODS

Place of investigation

The Federal University Otuoke (FUO), in Ogbia Local Government of Area (LGA) of Bayelsa state, Nigeria, is one of nine new federal universities established by the Federal Government of Nigeria In February 2011. The university is located in the Niger Delta region of Nigeria.

The campus occupies a 2,000,000 square meters (200 hectares) land donated by the indigenes of Otuoke Community in Bayelsa state. The site is geographical located at latitude $N04^0$,47' 32.7" and Longitude $E006^0$ 19' 31.4". The area lies in the low land rain forest belt of Nigeria. Traditional occupation of the people in the area is farming and fishing.

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

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=leaf area/ground area M^2/M^2) in broadleaf canopies [25]. 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 [26]. LAI was determined directly by taking a statistically significant sample from a plant canopy, measuring the leaf per sample plot and dividing it by the plot land surface area [27]. The equation given by [29] 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.

We calculated the total leaf area of the plants in a given canopy as the ratio of total leaf area to the total land area available to the plants [28].

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 [29]. Ground surface area was calculated using the equation L×W, where LAI = Leaf Area/ Ground Surface.

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 dried at 37^{0c} 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^{0c} 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:10^0,10^{-1},10^{-2},10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9}. For plate count experiment, 200ul aliquots from 10^{-5} 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^{0c} 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 enumeration of bacteria viable cell counts against three different sites of soil i.e. Close canopy Area C.C.A, Limited Canopy Area L.C.A and Open Field O.F, showed that the viable cell counts were significantly different between the three areas. 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.

Analytical Procedure

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.

Test of Relationship using the Chi-Square Test

Test statistic

$$\chi^2_{\alpha,(r-1)(c-1)} = \sum_{i=1}^{r}\sum_{j=1}^{c}\left(\frac{O_{ij} - E_{ij}}{E_{ij}}\right)^2$$

Where O = observed frequency

E = expected frequency

(r-1)(c-1) = degree of freedom

α = level of significance

RESULTS AND DISCUSSIONS

The research has revealed that tree canopy shade has significant effect on the bacteria and fungal load of the soil under it with weak correlations.

Table-1: Soil fungal and bacteria Load under Closed canopy, Limited canopy, and open field Tree canopy shades

Replicates	**Closed**	**Limited**	**Open Field**
Replicate 1	227×10^5	194×10^5	83×10^5
Replicate 2	145×10^5	169×10^5	108×10^5
Replicate 3	129×10^5	52×10^5	10×10^5
Mean	167×10^5	138×10^5	67×10^5
Total	501×10^5	415×10^5	201×10^5

The result shows that closed canopy has the highest microbial load with mean value of 167 x 10^{-5}, followed by Limited canopy with 138 x 10^{-5}. The open canopy had the lowest microbial load of $67x10^5$.

Table-2: Chi-square test of effects of canopy shades on fungal 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

$X^2 = 70.011$ df = 4 P- value = 0.001* no of cases = 1117

Table-3: Correlation between soil fungal and bacteria load, and Leaf Area Index under closed canopy

		MICROBES	LAI
Closed canopy MICROBE N	Pearson Correlation	1	0.383
	Sig. (2-tailed)		0.750
		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 that15% 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 fungal and bacteria load, and limited canopy shade (LAI)

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

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 [30]. 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 [31]. 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. To this end, allowing more trees on the farm lands would be an option for boosting soil health for productivity. 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.

REFERENCES

1. Wardle, D. A., Bonner, K. I., & Nicholson, K. S. (1997). Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. *Oikos*, 247-258.
2. Finzi, A. C., Van Breemen, N., & Canham, C. D. (1998). Canopy tree–soil interactions within temperate forests: species effects on soil carbon and nitrogen. *Ecological applications*, *8*(2), 440-446.
3. Theodorou, N. J., & Tzafestas, S. G. (1984). A canonical state-space model for three-dimensional systems. *International journal of systems science*, *15*(12), 1353-1379.
4. Grayston, S. J., Griffith, G. S., Mawdsley, J. L., Campbell, C. D., & Bardgett, R. D. (2001). Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. *Soil Biology and Biochemistry*, *33*(4), 533-551.
5. Garbeva, P., Postma, J., Van Veen, J. A., & Van Elsas, J. D. (2006). Effect of above-ground plant species on soil microbial community structure and its impact on suppression of Rhizoctonia solani AG3. *Environmental Microbiology*, *8*(2), 233-246.
6. Unanaonwi, O. E., & Ake, B. I. (2016). Evaluation of Soil Health under Different Forest Stands in a Tropical Moist Rain Forest of Otuoke,Southern Nigeria. *I nt. J. Curr. Res. Aca. Rev 5* (8), 67-74.
7. Morin, X., Fahse, L., Scherer-Lorenzen, M., & Bugmann, H. (2011). Tree species richness promotes productivity in temperate forests through strong complementarity between species. *Ecology Letters*, *14*(12), 1211-1219.
8. Berendse, D., Binkley, O. C., Campoe, B., gaPaltl, M., & Forrester, D. I. (2013). Light absorption and use efficiency in forests: why patterns differ for trees and stands for. *Ecol. Manage*, 288, Academic Press, New York, PP.2, 5-13.
9. Brevik, J., Chol, C. G., Lorimer., Vanderwerker, W. G., Cole, G. L., & Martin, B. (2015). A crown model for spatially explicit forest and models. *Ecol. Manage*, *107*(1), 6,19-46.
10. Dassot, M., Constant, T., & Fournier, M. (2011). The use of terrestrial LiDAR technology in forest science: application fields, benefits and challenges. *Annals of Forest Science*, *68*(5), 959-974.
11. Grams, T. E., & Andersen, C. P. (2007). Competition for resources in trees: physiological versus morphological plasticity. *Progress in botany*, 356-381.
12. Enquist, B. J., West, G. B., & Brown, J. H. (2009). Extensions and evaluations of a general quantitative theory of forest structure and dynamics. *Proceedings of the National Academy of Sciences*, *106*(17), 7046-7051.
13. Olsthoorn, A. F. M., Bartelink, H. H., Gardiner, J. J., Pretzsch, H., Hekhuis, H. J., & Franc, A. (1999). Management of mixed-species forest: silviculture and economics.
14. Mlamb, H., Pretzsch, A., Metle, T. (2005). Linkiy leaf biomass allometry to the tree-level leaf biomass allometry. Trees, Volume 22, Academic Press New York, PP.611-622.
15. Macdolnald, A., & Fenniak, P. (2007). Responses of crown architecture in Betula pendula to competition are dependent on the species of neighboring trees. *Trees*, Volume 24, university press U.S.A, PP. 411-424.
16. Isichei, & Muoghalu C. A. (1981). In-forest assessment of timber stiffness in Noway spruce (piceaabies (L)Kurst.) Eur. J. wood prod., Volume 71, University Press London. PP.429-435.
17. Kim, C., Sharik, T. L., & Jurgensen, M. F. (1995). Canopy cover effects on soil nitrogen mineralization in northern red oak (Quercus rubra) stands in northern Lower Michigan. *Forest Ecology and Management*, *76*(1), 21-28.
18. Wason, B., Sariano, A., & Sala. O. E. (2003). Emergence and survival of *bromussetifolius* in difference micro sites of apatogonian arid steppe. *Isr.J. Brot*, *35*, 91-100.
19. Maestre, F. T., Bautista, S., Cortina, J., & Bellot, J. (2001). Potential for using facilitation by grasses to establish shrubs on a semiarid degraded steppe. *Ecological Applications*, *11*(6), 1641-1655.
20. Thomis, M. T., Franco, A. S., & Nobel, P. S. (2012). Interaction between seed-lings of Agave desert and the nurse plant Hilaria rigida. *Ecology 69*, 1731-1740.
21. Urbanova A., Keity, M.J., & Kellman, M. (1979). Soil enrichment by neotropical savanna trees. *J. Ecol*, *67*, 565-577.
22. Uroz, A., and Paine, S.T. (2016). Disturbance, Patch formation and community structure. Proc. *Nat Acad. Sci*, *71*, 2744-2747'
23. Tiedemann, A. R., & Klemmedson, J. O. (1977). Effect of mesquite trees on vegetation and soils in the desert grassland. *Journal of Range Management*, 361-367.
24. Zeng. A., Vetagg, O. R., & Start-Hill. (2016). Micro-site effects of trees and shrubs in dry savannas. *J. Vej. Sci. 3*, 337-344.
25. Weltzin, J. F., & Coughenour, M. B. (1990). Savanna tree influence on understory vegetation and soil nutrients in northwestern Kenya. *Journal of vegetation science*, *1*(3), 325-334.
26. Chen, J. M., Rich, P. M., Gower, S. T., Norman, J. M., & Plummer, S. (1997). Leaf area index of boreal forests: Theory, techniques,and measurements. *Journal of Geophysical Research: Atmospheres*, *102*(D24), 29429-29443.

27. Nüsslein, K., & Tiedje, J. M. (1999). Soil bacterial community shift correlated with change from forest to pasture vegetation in a tropical soil. *Applied and environmental microbiology*, *65*(8), 3622-3626.
28. Bréda, N. J. (2003). Ground-based measurements of leaf area index: a review of methods, instruments and current controversies. *Journal of experimental botany*, *54*(392), 2403-2417.
29. Watson, D.J, (1947). Comparative physiological studies on the growth of Field crops. Variation in net assimilation rate and leaf area between years. *Annals of Botan*y. 11:41-76.
30. Blanco, F. F., & Folegatti, M. V. (2003). A new method for estimating the leaf area index of cucumber and tomato plants. *Horticultura Brasileira*, *21*(4), 666-669.
31. Lovett, G. M., & Lindberg, S. E. (1993). Atmospheric deposition and canopy interactions of nitrogen in forests. *Canadian Journal of Forest Research*, *23*(8), 1603-1616.