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ASSESSMENT OF SOIL MICROBIAL DIVERSITY OF DIFFERENT RUBBER PLANTATION AGES

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ABSTRACT

A study was carried out to investigate composition and diversity of the soil microbial communities in rubber plantations of different ages. Soil samples were collected from five different plantations aged 10, 13, 18, 23, 28 years old (latitudes 6º9′27.08, 6º9′27.78, 6º9′20.59, 6º9′28.21, 6º9′27.60 (E) and longitude 5º35′49.61, 5º36′38.13, 5º35′27.95, 5º36′11.37, 5º37′12.50 (N) respectively) at Rubber Research Institute of Nigeria, Iyanomo, Benin City. The laboratory experiment was carried out at the Faculty of Agriculture main laboratory and the International Institute for Tropical Agriculture (IITA) Bioscience Centre. The experiment was laid out in Completely Randomized Design (CRD) with three replications. The results showed changes in soil microbial diversity and composition over the different ages of rubber tree plantations. The ages of the rubber tree plantations had no significant effect on the microbial populations as it varied irrespective of the ages. The soil microbial diversity tend to increase with increasing rubber tree plantation ages and were highest in 28 year-old whereas 13 and 18 year-old had the lowest values of diversity. Microbial activity increased with increasing rubber ages with the highest being at 28 year old (73.2 µg/g Soil). The diversity of the soil microbes increased with the increase in diversity of the vegetation. Soil microbial diversity and composition in the different stages were closely related with soil properties. Soils with lower pH values of 4.0 and 4.1 had less diversed microbial communities than others.

Keywords: Microbial diversity, *Hevea brasiliensis*, Soils, Rubber plantation

INTRODUCTION

Hevea brasiliensis is native to the tropical rainforest of the Amazon basin. It is the major economic source of latex due to its singular ability to renew its bark thus permitting a sustained latex harvest (Gomez, 1982). The tree is a member of the Spurge family called *Euphorbiaceae* which can produce natural latex (Purseglove, 1987). The tree is highly valued for its latex content, which is very significant in world's industrialization. *Hevea brasiliensis* leads to significant changes in land use, environment and ecological processes, including soil condition, biodiversity and carbon sequestration (Kiriya and Sukanya, 2019). Previous researches had predicted that soil fertility in rubber plantations decreases with the age of the trees. However, types of vegetation, fertilizer application and intercropping with suitable crops can help to maintain soil fertility.

The extraction of latex and weed control from rubber plantations are key factors in the decrease in soil nutrients and change in diversity of microorganisms. Nielsen and Winding; (2002); reported that microbial diversity is a very crucial component for soil health maintenance. Minerals form a considerably large component of the rubber (latex), which is removed via current tapping system, and thus rubber trees will absorb nutrients from soil to meet growth and production needs. A healthy soil will guarantee normal growth of rubber trees as; soil properties influence the growth and productivity of *Hevea brasiliensis.* Soil quality which refers to the three soil properties including physical, chemical and biological properties, exerts a considerable influence on plant growth development, nutrients availability and reduces soil environmental stress effect on plant (Elliot et al., 1996). Healthy soil plays a major role as habitat for various forms of living things ranging from microflora, microfauna, mesofauna, macrofauna to megafauna. The activities of these group of microbes help in maintaining a healthy, fertile and productive soil by breaking down organic wastes into bioavailable nutrients which aid plant germination and growth (Bardgette *et al*., 1999). In this study, the vegetation, cultural practices carried out in the plantations and soil properties greatly influenced the diversity and composition of microbes of different rubber ages. The objectives of the study were to evaluate the variations in soil microbial diversity and fertility in different aged rubber plantations in Iyanomo, Benin City, Edo state, Nigeria.

MATERIALS AND METHODS

Soil sampling: This study was conducted at Rubber Research Institute of Nigeria, Iyanomo, Benin City, Crop Science laboratory, Faculty of Agriculture University of Benin, Benin City and the International Institute for Tropical Agriculture (IITA) Bioscience Centre Ibadan. Soil samples were collected from five different rubber tree plantations aged 10, 13, 18, 23 and 28 years within the institute at 0 -15 cm depth. Three composite soil samples were obtained from each rubber tree plantation and bulked into one. As a result, a total of fifteen soil composite samples were collected from the five plantations. The topography, soil types and rubber tapping method of these plots were basically consistent, conventional cultivation and management measures were used for the rubber tree plantations. The cultivars of rubber trees in the plantations were mixed. The soils of the study area was classified as Alagba series (Orimoloye and Akinbola, 2013).

Soil Sample Preparation and Analysis

Physical and chemical properties determination: The soil samples were air-dried, ground and passed through a 2mm sieve and used for the determination of the physical and chemical properties of the soil using standard laboratory procedures.

Microbial population determination (dilution method using selective media): Isolation of microorganisms from the soil samples were carried out using Serial dilution technique and pour plate method. The nutrient agar plates were incubated at 28 ^{o}C for 24hrs, the potato dextrose agar plates were incubated at 28 ^{o}C for 72hours and the starch casein agar plates were incubated at 28 ^{o}C for 5 days. The number of colonies was counted and identification of microorganisms was carried out using morphological and molecular techniques.

Statistical analysis: The data collected were subjected to analysis of variance (ANOVA) using statistic Analytical System (SAS 9.0). Test of significant difference in means was statistically compared using Least Significance Difference at 5% level of probability

RESULTS AND DISCUSSION

Latex extraction and weed control in rubber plantations are key factors in the decrease in soil nutrients and changes in diversity of microorganisms. The result of the physical and chemical properties of the soils is presented in Table 2. The pH of the soils of the plantations were acidic and low in some nutrients. The acidic nature of the soil had a negative effect on the microbial population and diversity most especially bacteria because they are more sensitive to acidic environment than fungi. The overall nutrient status of the soils was low maybe due to tapping activities which tend to lower the nutrients in soil as the rubber plantation ages. The amount of soil nutrient was lowest in the high yield period where tapping rate is high (13 and 18 year old rubber tree plantations). These results were consistent with previous research results (Wang *et al*., 1999). Minerals form a considerably large component of the latex which is removed via the current tapping system and thus rubber trees will absorb nutrients from the soil to meet growth and production needs (Zhou et al., 2017). In this study, soil properties (especially soil pH) greatly influenced the diversity and composition of microbes in the plantations.

Table 1: Site parameters and characteristics of the five rubber tree plantations

Rubber plantation (Age in years)	Longitude (N)	Latitude (E)	Vegetation	Cultural practices
28	6º9′27.08″	5º35′49.61″	Highly Dense	Weeding, Intercropped with cocoa
23	6º9′27.78″	5º36′38.13″	Dense	Weeding
18	6º9′20.59″	5º35′27.95″	Sparsely dense	Weeding, Herbicide
13	6º9′28.21″	5º36′11.37″	Sparsely dense	Herbicide trial
10	6º9′27.60″	5º37′12.50″	Dense	Intercropped with pineapple

Source [Google map, Rubber Research Institute of Nigeria Iyanomo, Benin City, Edo state.]

Soil microbial population and composition: The result in Table 3 reveals the microbial populations in the rubber tree plantations. Microbial population was highest in the 28 year old rubber tree plantation with colony count of 11.3 x 10^{6}cfu/g and 2.7 x 10^{6}cfu/g for bacteria and fungi respectively. Colony count was lower in the plantations with lower pH values. The microbial communities varied between the different aged rubber tree plantations (Table 4 and 5). Variation of soil microorganisms is related to the succession of vegetation (Table 1) at different stages of growth of rubber trees. The predominant bacteria genera were *Bacillus, Enterobacter, Salmonella and Klebsiellia* Sp (Table 4). The dominant fungi genus was *Aspergillus* belonging to the phyla *Ascomycota. Aspergilli sp were* present in all the rubber tree plantations (Table 5). Other fungi genera isolated in the plantations include; *Fusarium*, *Penicillium*, *Mucor* and *Cladosporum*. As shown in this research, the microbial composition of the soils of the rubber plantation was consistent with results of previous findings (Akan et al., 2017). Some of the microorganisms isolated from the soils of the plantations are quite beneficial microbes. The abundance of these microbes in soils positively influences soil quality, including plant growth, lower disease incidence, higher nutrient contents, soil enzyme activities and soil pH (Wang, et al., 2017). *Bacillus* sp which was the dominant bacteria in the rubber plantations is generally effective for suppressing some bacteria diseases (Wang et al., 2018).

Table 2: Physical and chemical properties of the different aged rubber plantation soils Iyanomo Benin City

	Age of rubber plantation (years)				
Soil Properties	10	13	18	23	28
Sand	843	814	820	822	824
Silt	8.40	5.50	5.64	9.40	9.90
Clay	138.8	180.2	180.0	161.1	166.1
Ph	4.5b	4.0a	4.1a	4.6b	5.0c
Total N(gkg^{-1})	2.34	2.30	2.25	2.50	2.50
Organic carbon (gkg^{-1})	25.0b	20.1c	18.5d	25.4b	29.7a
Available P (mgkg^{-1})	14.6	12.1	13.3	15.5	18.6
Potassium (cmolkg^{-1})	0.42	0.26	0.34	0.40	0.59
Ca (cmolkg^{-1})	2.02	1.95	1.81	1.95	2.0
Mg (cmolkg^{-1})	0.80	0.75	0.81	1.40	0.91
Na (cmolkg^{-1})	0.26	0.20	0.21	0.24	0.29
Soil bulk density(g cm^{-3})	1.14ab	1.08b	1.10b	1.13ab	1.13ab

Free living nitrogen fixing bacteria or associative nitrogen fixers belonging to the species *Enterobacter* and *Klebsiellia* were also isolated in soils of the study are as they were attached to the roots of the trees and efficiently colonized the root surfaces. *Ascomycetes* fungi, the dominant fungi phyla observed in this study are important decomposers in carbon cycle, which can secrete digestive enzymes to break down organic substances (e.g. cellulose, lignocellulose, lignin in plant litters) into smaller molecules. It was observed in this study that beneficial microbes were present in all the plantations though at varying frequencies. This means that rubber tree plantation soils (28, 23 and the 10 year old rubber tree plantations) with higher abundance of these beneficial microbes are healthier than the others.

Table 3: Soil microbial population in the different aged rubber soils in Iyanomo

	Colony count (x10^{6}cfu/g)	
Rubber plantation (Year)	Bacteria	Fungi
10	6.0c	1.3b
13	8.3b	2.3a
18	3.0d	1.7ab
23	3.7d	1.0b
27	11.3a	2.7a
LSD	1.41	1.05

Means followed by the same letter(s) are not significantly different at 5% level of probability

Table 4: Microbial isolates from Soil Samples of the different aged rubber plantations

Rubber plantation (Age in years)	Accession number	Bacteria isolates	Strain
10	MN606201	*Stapyloccocus epidermidis*	SAMC-RUSOL(10)1
	MN606203	*Halomonas campaniensis*	SAMC-RUSOL(10)3
	MN606202	*Enterobacter tabaci*	SAMC-RUSOL(10)2
	MN606204	*Streptomyce spp*	SAMC-RUSOL(10)4
	MN606205	*Staphylococcus aureus*	SAMC-RUSOL(10)5
	MN606206	*Bacillus cereus*	SAMC-RUSOL(10)6
	MN606208	*Salmonella spp*	SAMC-RUSOL(10)8
	MN606207	*Klebsiellia pneumonia*	SAMC-RUSOL(10)7
13	MN606195	*Bacillus subtilis*	SAMC-RUSOL(13)1
	MN606196	*Bacillus sphaericus*	SAMC-RUSOL(13)2
	MN606197	*Enterobacter tabaci*	SAMC-RUSOL(13)3
	MN606198	*Klebsiella pneumonia*	SAMC-RUSOL(13)4
	MN606199	*Staphylococcus aureus*	SAMC-RUSOL(13)5
	MN606200	*Escherichia coli*	SAMC-RUSOL(13)6
18			
	MN606189	*Enterobacter tabaci*	SAMC-RUSOL(18)1
	MN606190	*Bacillus mycoides*	SAMC-RUSOL(18)2
	MN606191	*Staphylococcus aureus*	SAMC-RUSOL(18)3
	MN606192	*Bacillus cereus*	SAMC-RUSOL(18)4
	MN606193	*Escherichia coli*	SAMC-RUSOL(18)5
	MN606194	*Salmonella bongori*	SAMC-RUSOL(18)6
23	MN606182	*Bacillus sphaericus*	SAMC-RUSOL(23)1
	MN606183	*Klebsiella pneumoniae*	SAMC-RUSOL(23)2
	MN606184	*Salmonella enterica*	SAMC-RUSOL(23)3
	MN606185	*Bacillus cereus*	SAMC-RUSOL(23)4
	MN606186	*Citrobacter freundii*	SAMC-RUSOL(23)5
	MN606187	*Staphylococus epidermidis*	SAMC-RUSOL(23)6
	MN606188	*Escherichia coli*	SAMC-RUSOL(23)7
28	MN606172	*Proteus mirabilis*	SAMC-RUSOL (28)1
	MN606173	*Streptomyces gramineus*	SAMC-RUSOL(28)2
	MN605174	*Klebsiellia pneumoniae*	SAMC-RUSOL(28)3
	MN606175	*Halomonas campaniensis*	SAMC-RUSOL(28)4
	MN606176	*Salmonella bongori*	SAMC-RUSOL(28)5
	MN606177	*Bacillus mycoides*	SAMC-RUSOL(28)6
	MN606178	*Bacillus subtilis*	SAMC-RUSOL(28)7
	MN606179	*Staphylococcus aureus*	SAMC-RUSOL(28)8
	MN606180	*Klebsiellia peumoniae*	SAMC-RUSOL(28)9
	MN606181	*Citrobacter freundii*	SAMC-RUSOL(28)10

Table 5: Microbial isolates from Soil Samples of the different aged rubber plantations

Rubber plantation (Age in years)	Accession number	Fungi isolate	Strain/strain number
10	MN606283	*Fusarium Oxysporum*	SAMF-RUSOL(10)2
	MN606284	*Aspergillus niger*	SAMF-RUSOL(10)3
	MN606285	*Aspergillus flavus*	SAMF-RUSOL(10)4
	MN606282	*Penicillium chrysogenum*	SAMF-RUSOL(10)1
13	MN606281	*Aspergillus niger*	SAMF-RUSOL(13)1
	MN606282	*Aspergillus flavus*	SAMF-RUSOL(13)2
18	MN606277	*Aspergillus niger*	SAMF-RUSOL(18)1
	MN606278	*Aspergillus flavus*	SAMF-RUSOL(18)2
	MN606279	*Aspergillus flavus*	SAMF-RUSOL(18)3
23	MN606273	*Aspergillus niger*	SAMF-RUSOL(23)1
	MN606274	*Fusarium oxysporum*	SAMF-RUSOL(23)2
	MN606275	*Aspergillus flavus*	SAMF-RUSOL(23)3
	MN606276	*Mucor minutus*	SAMF-RUSOL(23)4
28	MN606267	*Penicillium chrysogenum*	SAMF-RUSOL(28)1
	MN606272	*Aspergillus flavus*	SAMF-RUSOL(28)6
	MN606268	*Aspergillus niger*	SAMF-RUSOL(28)2
	MN606269	*Fusarium oxysporium*	SAMF-RUSOL(28)3
	MN606270	*Mucor minutus*	SAMF-RUSOL(28)4
	MN606271	*Cladosporium spp*	SAMF-RUSOL(28)5

Soil microbial activity: The result in table 6 reveals that there was significant ($p<0.05$) in the microbial activities of the soils of the rubber plantations. Soil microbial activities (a parameter that expresses the overall microbiological activity of the soil) in the soils show that there was higher activity in the 28 year old rubber tree plantation (73.2) than other plantations. Microbial activities increased with increasing rubber ages following the pattern of vegetation in the plantations. This suggests that the responsiveness of microbial activity to the age of the rubber tree plantations was influenced by vegetation and other factors. The microbial activities in the 13 and 18 years old rubber tree plantations had lower values which was a suggestive of low microbial diversity and composition. The soils of the rubber plantations with higher microbial activities were more diverse and also had a higher microbial biomass.

Table 6: Soil microbial activities in the different aged rubber tree plantations

Rubber plantation (Age in Years)	Soil Microbial Activities (µg/gSoil)
10	16.93c
13	14.06c
18	17.46c
23	43.18b
28	73.20a
LSD	5.3

Means followed by the same letter(s) are not significantly different at 5% level of probability

Soil microbial diversity: The leaf coverage provided by rubber trees and their root system regulates the microclimate, allowing a range of secondary plants to flourish and providing protection of soil against dehydration and the erosive effect of rain (Jarin, 1997). The result of microbial diversity is presented in Table 7 which shows that there was a significant difference in the number of genera of isolated microorganisms in the soils of the different aged rubber plantation. The 28 year old rubber tree plantation recorded higher levels of diversity of microbial population and had a greater microbial load in the soil than the other plantations; even though its effect was not significantly different from the 10 year old rubber tree plantations. The diversity of microbial populations was lower in the 13 and 18 years rubber tree plantations then increased at the older ages (23 and 28 years). This was consistent with previous research results (Zhou et al., 2017). The decrease in diversity with an increase in the ages of rubber trees is suggestive that other factors other than the rubber ages had influenced this trend. The diversity of the microbial population was lowest in the high yield periods (13 and 18 year) which happened to be the peak of tapping activities. This could be as a result of the high nutrient intake by the rubber plant to meet growth and production needs. The extraction of latex and weed control from rubber plantations are key factors in the decrease in soil nutrients and change in diversity of microorganisms (Kiriya and Sukanya, 2019). The vegetation on these plantations (13 and 18 years old) was sparse due to the herbicide application carried out on the soil (Table 1). This suggests that the variation of soil microbial diversity in different stages of rubber tree plantations is not directly influenced by the ages of the rubber tree plantations. Other related factors such as soil properties, vegetation and cultural practices carried out in the plantations are the main drivers of soil microbial diversity. The change in soil microbial community may not be directly affected by vegetation but it can drive soil microbial community changes through indirect mechanisms such as altering pH, Litter chemistry, Root density and carbon secretions, (Prescott and Grayston, 2013). Diversity and composition of soil microbial community in this study were closely related to soil properties and vegetation in the rubber tree plantations.

Soil microbial diversity was related to the succession of vegetation in different stages of the rubber tree plantations. Previous researchers found that plant diversity, composition, and production during succession affect the composition and diversity of soil microbial communities due to the bi-directional exchanges between above and below ground communities (Bardgett and shine, 1999). With an increase in age of rubber tree, the vegetation (Table 1) in 10, 23, and 28 year old evolved into a complex vegetation communities. The diversity of the soil microbes increased with increased diversity of vegetation. However, the microbial diversity decreased in the 13 and 18 years old rubber tree plantation which most likely was due to tapping activities and the cultural practices (weeding and herbicide application) carried out which led to a decreased level of vegetation. This also proves the importance of vegetation as another important factor affecting soil microbial community. This was consistent with previous research carried out by Zhou et al., (2017). The decrease in microbial diversity in the 13 and 18 year rubber tree plantation may also be related to the decrease in soil pH values (acidic condition). Soils with near – neutral pH values have higher diversity than the acidic ones. Soil pH is an important factor affecting the Soil microbial community (Fierer and Jackson, 2006). Soil acidity is linked to the decrease of available carbon for soil microbes thereby acting as an environmental filter for selecting specific microbial groups and regulating soil microbial community composition (Wang et al., 2017). It was observed in this study that soils of the rubber plantations with higher pH values (Table 1) had higher diversity of microbes. Microbial diversity is a very crucial component for soil health maintenance. Furthermore the microbial community could be an indicator of soil health and quality. A healthy soil will guarantee normal growth of rubber trees.

Table 7: Microbial diversity in soils of the different aged rubber plantation

	No of genera isolated	
Rubber plantation (Age in years)	Bacteria	Fungi
10	7.0ab	3.0b
13	5.0c	1.0c
18	5.0c	1.0c
23	6.0bc	3.0b
27	8.0a	5.0a
LSD	1.17	0.71

Means followed by the same letter(s) are not significantly different at 5% level of probability

CONCLUSION

This study discovered some variations in soil microbial diversity and composition over the different ages of rubber tree plantations. The variations in soil microbial diversity and composition in the different aged rubber tree plantations were closely related to soil properties as well as the management practices carried out in the plantations. The diversity of the soil microbes increased with the increased diversity of vegetation in the plantations. There was an increase in the microbial activity in the soils with increasing age of the rubber tree plantations which could be as a result of the prevailing vegetation on the plantation and other factors. Microbial diversity was highest in the older ages of rubber plantations (23 and 28 years). The ages of the rubber tree plantations had no significant effect on the microbial populations as it varied irrespective of the rubber tree ages. Soil properties greatly influenced plant health and soil microbial community as soils with a lower pH had a less diverse microbial community. The healthier soils had higher abundance of beneficial microbes. Microbial diversity studies should be carried out at intervals in rubber tree plantations as it will help the researcher to uncover the critical period of nutrient loss and overall plant health of the rubber trees. Rubber tree growth and latex yield can be optimized by minimizing human disturbances of plantation vegetation which can lead to changes in or suppression of the soil microbial community.

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