



Comparative Study of Soil Bacteria from the Rhizosphere of Two Selected Tree Species (*Anogeissus leiocarpa* and *Pterocarpus erinaceus*) in Shere Hills, Plateau State, Nigeria

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ABSTRACT: The comparative study of soil bacteria from the rhizosphere was carried out using samples obtained from the soil (rhizospheres) of *Anogeissus leiocarpa* and *Pterocarpus erinaceus* using purposive sampling method. The soil samples were collected at a depth of 0-30cm and 30-60cm around the rhizosphere of the studied tree species and away from the rhizosphere used as a control. The method by Ibitoye (2008) was used to test for physico-chemical properties and method by Cheesborough (2006) was used for stocking, culturing, incubating and gram staining. Result for the mean physical properties shows, *P. erinaceus* had highest mean values for clay and silt which wasn't significant ($p=0.067$ and 0.381 respectively). *A. leiocarpa* had the second highest clay and silt. Sand and bulk density was highest for the control which was highly significant ($p=0.000$ and $p=0.044$ respectively). For mean chemical properties among plant species, pH, EC, OC, OM, N, Na, Ca and CEC were highly significant ($p<0.05$), while p, K, Mg and EA were not significant. *Pterocarpus erinaceus* had the highest mean for all the chemical elements assessed with ten (10) bacteria species identified. *Anogeissus leiocarpa* had the second highest biochemical elements present with six (6) species of bacteria identified, *Bacillus subtilis* was most common. It is therefore necessary that these tree species are allowed in their natural habitat to preserve and conserve these microbes in the ecosystem.

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According to a general view, the rhizosphere includes plant roots and the surrounding soil. The rhizosphere inhabiting microorganisms compete for water, nutrients and space and sometimes improve their competitiveness by developing an intimate association with plant (Hartmann *et al.*, 2009). These microorganisms play important roles in the growth and ecological fitness of their host. An understanding of the basic principles of rhizosphere microbial ecology, including the function and diversity of microorganisms that reside there, is necessary before soil microbial technology can be applied in the rhizosphere. Trees represent a multitude of habitats; such as the phyllosphere or rhizosphere, but they also substantially affect the remaining parts of the ecosystem. This is mainly because, as the dominant primary producers, they supply the bulk of the carbon that enters the ecosystem, and while some of this carbon is in the form of simple organic molecules, a significant fraction, such as the complex biomass of wood, litter, or roots, is composed of recalcitrant biopolymers (Defreitas *et al.*, 2015). Trees also largely contribute to the spatial

heterogeneity of forest ecosystems by multiple means, including the penetration of soils by various guilds of roots, generation of patches of litter and ground vegetation, and changes of the morphology of the terrain during uprooting or the production of deadwood. Phytopathogenic organisms, bacteria as well as fungi, have coevolved with plants and show a high degree of host specificity (Raaijmakers *et al.*, 2009). Forest microorganisms contribute greatly to the carbon balance in their ecosystems. They play an important role as decomposers, symbiots, or pathogens influencing the carbon turn over and retention and the availability of other nutrients (Baldrian, 2017). Microbial communities are vital in mediating the biogeochemical cycles and understanding of their role in ecosystem processes is essential for the prediction of the forest response-the future environmental conditions (Graham *et al.*, 2016). Microbial contribution to carbon and nitrogen cycling in forest soils is important, and may depend on tree species (Keenan *et al.*, 2015). Because of the fact that a high proportion of root-associated bacteria and fungi possess

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an antagonistic potential against other microorganisms, rhizosphere microorganisms are an important bio resource for bioactive substances, i.e. antibiotics, biosurfactants, enzymes and osmo-protective substances. In this respect, an enormous untapped pool of biological resources might be harbored in hot spots of plant diversity, such as tropical rainforests (Gabriele *et al.*, 2009). More comparative, biogeographic studies are needed to determine the nutrients and the microbial communities within the rhizosphere of different plant populations. Soil is one of the last great scientific frontiers (Lal, 2004) and the rhizosphere is the most active portion of that frontier in which biogeochemical processes influence a host of landscape and global scale processes. A better understanding of these processes is critical for maintaining the health of the planet and feeding the organisms that live on it (Morrissey *et al.*, 2004). Clearly, there are also disadvantageous relationships arising from rapidly proliferating and competitive soil microbes and plant pathogenicity and there is a fascinating story behind a plant's successful or unsuccessful attempts to counteract these threats. Understanding these microbe-catalyzed processes is critical in maintaining the role of forests in the future. Forest is subjected to multiple modes of disturbances, amongst which are insects' outbreak, fires, other activities etc. They are also significantly threatened by a combination of anthropogenic factors ranging from climate change to environmental pollution, population encroachment and inappropriate management practices (Gauthier *et al.*, 2015), which together may easily shift the balance of carbon cycling processes. The presence and abundance of specific plant species can influence soil microbial community composition and function (Edwards and Zak, 2010; Eisenhauer *et al.*, 2010). Knowledge of microbial diversity and function in soils is limited because of the taxonomic and methodological limitations associated with studying these organisms (Kirk *et al.*, 2004). Most soil microorganisms are still unknown (Crecchio *et al.*, 2004). *Anogeissus leiocarpa* and *Pterocarpus erinaceus* have low germination potential and are highly disturbed in the study site, hence the need to understand the organisms found within the rhizosphere that could play a great role in the growth and ecological health of the threatened study plant species.

The study is aimed at determining the soil bacteria from the rhizosphere of *Anogeissus leiocarpa* and *Pterocarpus erinaceus*.

MATERIALS AND METHODS

Study site: This research was carried out in Shere hills, North Central Nigeria, on the plateau composed of the guinea savannah zone of Nigeria. The dominant habitat type in this ecosystem is the rocky outcrop. There are also fragments of riparian forest that have

been exploited by the surrounding communities for fuel wood. The area still holds some of the best natural vegetation of the Jos plateau (Hulme, 2007) and also a habitat to endemic and endangered birds and plants.

Soil sample collection: Purposive sample was used to locate the studied plant species in the study site. Selected plants species were marked with Global positioning system (GPS) and ribbons at three points. The soil samples were collected around the rhizosphere of the two studied plant species at 0-30 cm and 0-60cm depth, soil samples were collected from adjacent points away from any plant species (control) at each point making a total of twenty four samples. The distance from the tree to 50cm falls within the rhizosphere, while soil collected at distance >50cm is a control (non-rhizosphere). Samples were collected during the raining season (June – September, 2020).

Physicochemical properties: The procedure followed to test for physico-chemical properties (silt, sand, clay, pH, organic matter etc.) was essentially the same as described by Bibitoye (2008). The bulk density was determined using the core sampler. It consists of cylinders fitted that collect soil sample. The sampler was pressed vertical into the soil surface to fill the sampler but not so far as to compress the soil, it was carefully removed, from the ground, the two cylinders was separated, then the soil was retained undisturbed in the inner cylinder, it was trimmed; weigh calculated. The particle density of a soil sample was calculated from two measured quantities, mass and volume (Ibitoye, 2008). Pycnometer (Specific gravity bottle) was employed.

Stocking, Culturing and Incubating: Suspension for serial dilution was prepared using; 1g of soil sample weighed was poured into 10ml of deionized (distilled) water. The suspension was well swirled to make the stock evenly distributed. The serial dilution was done by taking the stock containing 10ml of deionized water. 1ml of suspension using syringe from the stock (10^0), was transferred to B = 10^1 which contains 9ml of deionized water; from B = 10^1 , 1ml was taken and transferred to C = 10^2 which also contains 9ml of deionized water, also 1ml of suspension was taken and transferred to D = 10^3 . Two suspension bottles were selected 10^1 and 10^3 . Culturing was done using the suspensions and media prepared. 1ml of suspension was taken from B = 10^1 using syringe and poured at the middle of the sterilized petri dish, then some media was also poured, mixed evenly and labeled appropriately. Incubation process was done by first leaving the cultured media to solidify for minutes after which all the dishes were incubated for 24 hours.

Gram Staining: This procedure was based on the techniques/manuals by Monica Cheesbrough (2006), used by Microbiology laboratory, University of Jos. Ethanol was used to clean the slides, table and area so as to sterilize the environment, each slide was passed over the spirit lamp so as to burn off foreign microorganisms. A drop of water was dropped on slide, wire loop was used to pick the samples and was smeared on the wet area on the slide. The crystal violet was dropped on it and was left for half a minute (30 seconds). After 30 seconds it was washed off with deionized (distilled) water, and ethanol properly; Lugol's iodine was dropped on the slide for 1 minute after which ethanol and water was used to wash off the chemical; then Safarane was dropped on it for another half a minute (30 seconds), it was also washed off using ethanol and water and was allowed to dry, these processes were repeated for each slide. After drying, it was observed under the light microscope for presence of bacteria. Subsequently, identification of unknown bacteria was conducted. The procedure was done by first preparing suspension using nutrient Broth Agar in which 1.3g of nutrient broth agar was added to 110ml of distilled water it was swirled well and was distributed into final containers (universal bottles) It was autoclaved at 121°C for 15 minutes. It was allowed to cool so as not to kill the bacteria when hot, wire loop was used to pick from colony of each cultured petri dishes and was inoculated, into the nutrient broth suspension then was incubated for several hours; until ready for use. Bacteria were identified using Frankland *et al.* (1995), in the microbiology laboratory of the University of Jos, Plateau state.

Statistical Analysis: The data obtained were subjected to analysis of variance (ANOVA) to determine their significance difference at 5%.

RESULTS AND DISCUSSION

Table 1 below, shows the result for mean chemical properties among plant species. pH, EC, OC, OM, N, Na, Ca and CEC were highly significant ($p < 0.05$), while p, K, Mg and EA were not significant. *Pterocarpus erinaceus* had the highest mean for all the chemical elements assessed except for EA which was highest for the samples collected away from *P. erinaceus* rhizosphere (control) but it wasn't significant with the values obtained to tree species. *Annoyessus leiocarpus* had the second highest mean for all the chemical parameters assessed.

Table 2 below shows result for the mean physical properties among plant species. *P. erinaceus* had highest mean values for clay and silt which wasn't

significant ($p = 0.067$ and 0.381) respectively. *A. leiocarpus* had the second highest clay and silt. Sand and bulk density was highest for the control which was highly significant ($p = 0.000$ and $p = 0.044$ respectively).

Table 3 below showed that all *A. leiocarpus* samples at different depths tested positive to gram staining and a total of six (6) species of bacteria were identified with *Streptococcus* observed only at point 1 (30-60cm) at a lower concentration. *Bacillus subtilis* was most common, it was found at all points. Table 4 below shows that all samples collected from rhizosphere of *Pterocarpus erinaceus* tested positive to gram staining, with ten (10) bacteria species found. *Bacillus subtilis* and were common at two points, but there was variation in the species of bacteria found at the three points where samples were collected. *Bacillus subtilis* was observed at two points (0-30cm) at low concentrations while *Bacillus coagulans* was observed at the two points at a high concentration.

The result of this work showed that there is nutrient elements found within the rhizosphere of the plant species; and that they also contribute to its development and growth. The rhizosphere, that is, the narrow zone surrounding and influenced by plant roots, is a hot spot for numerous organisms and is considered as one of the most complex ecosystems on Earth (Hinsinger *et al.*, 2009; Raaijmakers *et al.*, 2009). Most members of the rhizosphere microbiome are part of a complex food web that utilizes the large amount of nutrients released by the plant. Given that these rhizodeposits are a major driving force in the regulation of microbial diversity and activity on plant roots, Cook *et al.* (1995) postulated that plants may modulate the rhizosphere microbiome to their benefit by selectively stimulating microorganisms with traits that are beneficial to plant growth and health. According to Hinsinger (2001) it was stated that, plant growth-promoting rhizobacteria may positively influence plant phosphorus (P) availability by enhancing mobilization. The presence of pH level, Electrical conductivity, Organic matter, Nitrogen, Sodium, Calcium, Cation exchange capacity and Organic carbon in the soil can was higher in *Pterocarpus erinaceus* followed by *Annoyessus leiocarpus* with high level of significance. After the pH, the presence of organic matter is the most important indicator of the soil quality. Organic matter indeed covers the major reserves of nutritive substances for plants (Rattan *et al.*, 2005).

Table 1: Mean chemical properties of different soil samples around plant species

Soil samples	CHEMICAL PROPERTIES											
	pH	EC	OC	OM	N	P	K	Na	Ca	Mg	EA	CEC
<i>P. erinaceus</i> (control)	2.182 ^b	0.008 ^a	0.481 ^c	0.810 ^b	0.025 ^b	0.000 ^a	0.026 ^b	0.003 ^b	0.343 ^d	0.143 ^a	0.550 ^a	1.070 ^c
<i>Pterocarpus erinaceus</i>	2.227 ^a	0.009 ^a	1.164 ^a	2.013 ^a	0.057 ^a	0.001 ^a	0.032 ^a	0.004 ^a	0.493 ^a	0.163 ^a	0.534 ^a	1.274 ^a
<i>Anogeissus leiocarpa</i>	2.210 ^a	0.009 ^a	0.640 ^b	0.102 ^d	0.033 ^b	0.000 ^a	0.031 ^{ab}	0.0003 ^c	0.423 ^b	0.160 ^a	0.527 ^a	1.192 ^b
<i>A. leiocarpa</i> (control)	2.190 ^b	0.007 ^b	0.417 ^d	0.743 ^c	0.022 ^b	0.000 ^a	0.029 ^{ab}	0.003 ^{ab}	0.348 ^c	0.146 ^a	0.522 ^a	1.088 ^c
S.E	0.006	0.000	0.005	0.003	0.004	0.000	0.002	0.000	0.001	0.009	0.009	0.006
p-value	0.003*	0.003*	0.000*	0.000*	0.002*	0.233^{ns}	0.067^{ns}	0.000*	0.000*	0.347^{ns}	0.203^{ns}	0.000*

Means on the same column with the same superscript do not differ significantly from each other ($P = 0.05$). Where: * = Significant; ns = Non-significant; SE = Standard error; EC = Electrical conductivity; OC = Organic carbon; OM = Organic matter; N = Nitrogen; P = Phosphorous; K = Potassium; EA = Exchange acidity; CEC = Cation exchange capacity; Na = Sodium; Ca = Calcium; Mg = Magnesium.

Table 2: Mean physical properties of different soil samples around plant species

Soil samples	PHYSICAL PROPERTIES				
	Clay	Silt	Sand	Bulk Density	Particle Density
<i>P. erinaceus</i> (control)	3.480 ^b	4.713 ^a	25.187 ^a	0.524 ^a	0.816 ^a
<i>Pterocarpus erinaceus</i>	6.812 ^a	6.000 ^a	20.520 ^c	0.484 ^b	0.821 ^a
<i>Anogeissus leiocarpa</i>	4.480 ^{ab}	5.333 ^a	21.854 ^b	0.483 ^b	0.821 ^a
<i>A. leiocarpa</i> (control)	3.480 ^b	4.677 ^a	25.188 ^a	0.522 ^a	0.817 ^a
p-value	0.067^{ns}	0.381^{ns}	0.000*	0.044*	0.947^{ns}

Means on the same column with the same superscript do not differ significantly from each other ($P = 0.05$). Where: * = Significant; ns = Non-significant; SE = Standard error

Table 3: Result of gram staining processes carried out on the colonies of *Anogeissusleiocarpa*

Depth	Colony color	Conc.	Observation color	Shape	Gram staining	organism bacteria
Al ₃ 30-60cm	Red	10 ³	Purple	Rod like	Gram positive	<i>Mitushi nitrobacterium</i>
Al ₃ 30-60cm	Red	10 ¹	Purple	Rod like	Gram positive	<i>Bascillus cereus</i>
Al ₃ 0-30cm	Red	10 ¹	Purple	Rod like	Gram positive	<i>Bascillus megaterium</i>
Al ₃ 0-30cm	White	10 ³	Purple	Rod like	Gram positive	<i>Bascillus subtilis</i>
Al ₁ 30-60cm	White	10 ¹	Purple	Cocci chains	Gram positive	<i>Streptococcus spp.</i>
Al ₁ 30-60cm	Red	10 ³	Purple	Rod Like	Gram positive	<i>Bascillus subtilis</i>
Al ₁ 0-30cm	White	10 ¹	Purple	Rod Like cluster	Gram positive	<i>Bascillus subtilis</i>
Al ₁ 0-30cm	White	10 ³	Purple	Rod Like cocci	Gram positive	<i>Bascillus megaterium</i>
Al ₂ 0-30cm	Red	10 ¹	Purple	Rod like chains	Gram positive	<i>Bascillus cereus</i>
Al ₂ 0-30cm	White	10 ³	Purple	Rod like chains	Gram positive	<i>Bascillus cereus</i>
Al ₂ 30-60cm	White	10 ³	Purple	chains like cluster	Gram positive	<i>Bascillus subtilis</i>
Al ₂ 30-60cm	Red	10 ¹	Purple	Rod likes chains	Gram positive	<i>Bascillus cereus</i>

Al₁ – *Anogeissus leiocarpa* point 1; Al₂ – *Anogeissus leiocarpa* point 2; Al₃ – *Anogeissus leiocarpa* point 3.

Table 4: Result of grain staining processes carried out on the colonies of *Pterocarpuserinaceus*

Depth	Colony color	Conc.	Observation color	Shape	Gram staining	organism bacteria
Ptn ₃ 30-60cm	Red	10 ³	Purple	Cluster black Rod like	Gram positive	<i>Bascillus coagulans</i>
Ptn ₃ 30-60cm	Yellow	10 ¹	Purple	Cluster	Gram positive	<i>Mitushi nitrobacterium</i>
Ptn ₃ 0-30cm	Yellow	10 ¹	Purple	Cluster	Gram positive	<i>Bascillus subtilis</i>
Ptn ₃ 0-30cm	Red	10 ³	Purple	Cluster cocci	Gram positive	<i>Streptococcus</i>
Ptn ₁ 30-60cm	Red	10 ¹	Purple	Thread like spiral cocci	Gram positive	<i>Micrococcus luteus</i>
Ptn ₁ 30-60cm	Yellow	10 ³	Purple	Cluster sphere	Gram positive	<i>Azotobacter</i>
Ptn ₁ 0-30cm	Red	10 ¹	Purple	Cluster with black dot	Gram positive	<i>Bascillus subtilis</i>
Ptn ₁ 0-30cm	Yellow	10 ³	Purple	Cluster with black dot	Gram positive	<i>Bascilluscoagulans</i>
Ptn ₂ 0-30cm	Red	10 ¹	Purple	Cluster thread like	Gram positive	<i>Nitrobacterium</i>
Ptn ₂ 0-30cm	Yellow	10 ³	Purple	Rod like cluster	Gram positive	<i>Enterobacter cloacae</i>
Ptn ₂ 30-60cm	Red	10 ³	Purple	Cluster spiral	Gram positive	<i>Clostridium</i>
Ptn ₂ 30-60cm	Yellow	10 ¹	Purple	Cluster colorless bubble	Gram positive	<i>Rhizobium</i>

Ptn₁ – *Pterocarpus erinaceus* point; Ptn₂ – *Pterocarpus erinaceus* point 2; Ptn₃ – *Pterocarpus erinaceus* point 3

Pterocarpus erinaceus belongs to the family leguminosae, it is a nitrogen fixing plant, which may contributed to adding other elements in the soil as observed from the result and may inversely have contributed to the presence of more microorganisms (bacteria) in the soil as compared to *Anoigessus leiocarpus*, according to David (2013) these changes in pH can influence the availability of other plant essential micronutrients (e.g., Zn, Ca, Mg). Plant community traits have a significant influence on soil physico-chemical properties, substrate supply to the soil microbes through plants (Martin *et al.*, 2016). Therefore, this means that nitrogen fixing and nutrient mineralization process carried out by soil microbes are crucial for plant nutrition in natural ecosystems, because these reaction is to metabolize recalcitrant forms of nitrogen (N), phosphorus (P) and sulphur (S) to liberate these elements for plant nutrition (Van der Heijden *et al.*, 2008). Species of bacteria found in samples collected from *Pterocarpus erinaceus* was higher than *A. leiocarpus* and these confirm to other studies that shows a number of free-living rhizosphere bacteria and fungi enhancing plant growth directly. Rhizosphere bacteria play vital roles in plant nutrition, growth promotion, and disease interactions. Several studies have indicated that bacteria are the most numerous inhabitants of the rhizosphere, although they account for only a small portion of the total biomass due to their small size (Bashan and de-Bashan, 2005). Studies revealed that plant community traits have a significant influence on soil physicochemical properties, substrate supply to the soil microbes through plants (Martin *et al.*, 2016) and in turn soil microbial diversity and activity (Barbiet *et al.*, 2016). This confirms to our study where the ontrol had less chemical elements as well as poor physical properties with high sand content, bulk density and low clay, silt and particle density. The growth of soil microbes is usually carbon limited, so the high amounts of sugars, amino acids, and organic acids that plants deposit into the rhizosphere represents a valuable nutrition source (Bais *et al.*, 2004) in natural soil the vast majority of N, P and S atoms are organically bound, while in the atmosphere the vast majority of N is contained in the N₂ molecule. Due to the different metabolic capacities of plants and microbes, these nutrient sources are minimally bio available to plant, but can be metabolized by various soil microbes. This means that nitrogen fixing and nutrient mineralization processes carried out by soil microbes are crucial for plant nutrition in natural ecosystems, because these reactions metabolize recalcitrant forms of N, P, and S to liberate these elements for plant nutrient (Van der Heijden *et al.*, 2008). The soil microbial community is diverse, heterogeneous and difficult to characterize (Singh *et*

al., 2004). Plant-microbial relationships are often plant species-specific (Berg and Smalla, 2009). For example, legume species are often associated with particular strains or species of rhizobia bacteria for fixing nitrogen in their root nodules. Plants exude chemicals from their roots that can foster beneficial microbes in the rhizosphere (Ehrenfield *et al.*, 2005; Bais *et al.*, 2004; Compant *et al.*, 2010). For example, *Arabidopsis thaliana* exudes malic acid in the presence of a beneficial bacterium, *Bascillus subtilis*, protecting the roots from the pathogen (Rudrappa *et al.*, 2008). Therefore, studies also revealed on the microbiome of different plants species and accessions revealed strong variations, leading to the hypothesis that exudates are crucial in shaping plant-microbe interactions (Hartmann *et al.*, 2008).

Conclusion: Rhizosphere is a unique ecological zone of the soil that is heavily loaded with nutrients. Since the bacteria are the most abundant organisms that reside in the rhizosphere and influence the plant growth by direct or indirect mechanisms, they must therefore be exploited to develop eco-friendly and safe replacement for chemical based fertilizers. It is paramount that both plant species which are critically threatened with low germination/growth rates should be protected and conserved to avoid global extinction and losing the organisms found around them.

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