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Rhizosphere Soil Microbial Characteristics and Enzyme Activities under *Cajanus cajan* and *Milletia laurentii* Grown in Scientific Center of Brazzaville, Republic of the Congo

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Abstract

This study was conducted at the scientific center of Brazzaville. The objective was to assess the microbial characteristics and enzymes activities in the rhizosphere soil of *Cajanus cajan* and *Milletia lauurentii*. These plants have great importance in food and forestry. Microbial diversity management in the rhizosphere is the key for sustainable crop production or forest durability. DNA metagenomic sequencing was used to analyze the whole bacterial diversity, the microbial biomass was determinate by the fumigation-extraction method and the enzymes by the p-nitrophenol-β-D-glucoside for β-glucosidase, the p-nitrophenyl-N-acetyl-β-D-glucosaminide for β-Glucosaminidase. Dehydrogenase and acid phosphatase were quantified using 2,3,5-tryphenyl tetrazolium chloride and p-nitophenylphosphate respectively. The results show that, in *Cajanus cajan* culturable bacteria genera were mainly Acidobacterium, Skermanella, Rhodoplanes, Bacillus, Chloroflexus, Steroidobacter, Sphingomonas and Bradyrhizobium while in *Milletia laurentii*: Rhodoplanes, Bradyrhizobium, Bacillus, Sphingobacterium, Acidobacterium, Mesorhizobium, Nitrospira were the principal genera. In the two rhizosphere soils investigated, the uncultured bacteria exhibited relatively higher abundance, often for the same genera, than culturable bacteria. Metagenomic studies have revealed more bacterial diversity in each compared to when culturable bacteria were taken into account alone. The MBC and MBN were higher in the rhizosphere of

Milletia than in rhizosphere of Cajanus. The same trend was observed with the enzyme activities. PCA of culturable and NMDS of unculturable soil bacteria genera shows that factors mainly involved in the carbon cycle such as MBC, members of the microbial community *i.e.* Acidobacterium, Skermanella, Chloroflexus, sand, C, β-glucosaminidase and dehydrogenase, were strongly correlated with *Cajanus cajan*. On the other hand, the MBN, Mesorhizobium, Bradyrhizobium, Burkholderia, Nitrospira, Nitratireductor, N, NH_4, β-glucosidase and acid phosphatase involved in the N cycling, silt and clay were predominantly founded in the rhizosphere soil of *Milletia laurentii*. This study showed that metagenomic sequencing could improve the assessment of the microbial diversity structure of the rhizosphere.

Keywords

Metagenomic, Microbial Diversity, Uncultured Bacteria, Microbial Biomass, Enzyme

1. Introduction

Legumes rich in protein (21.7%), vitamins, and essential amino acids (lysine, phenylalanine, valine, leucine and isoleucine) contribute significantly to food security in many countries around the world [1]. Seeds of these plants are also rich in fatty acids such as linoleic acid and palmitic acid [2]. Legumes have a high potential to improve soil fertility through fixation of atmospheric nitrogen [3]. For restoring soil fertility, legumes can be used as green manure. *Cajanus cajan* is part of this category of plant. *Milletia laurentii* is a forest legume that has a high commercial value due to the technological quality of its wood. *Cajanus cajan* and *Milletia laurentii* have the ability to fix atmospheric nitrogen in association with certain bacteria present in their rhizosphere [4]-[6]. The soil influenced by plant roots, called rhizosphere, is the zone in which several interactions take place between roots and soil microbial [7] [8]. In this area, the microorganisms interact with each other and with other organisms. Some microorganisms are beneficial and promote plant growth and productivity while others cause disease [8] [9]. The microbial community structure that is involved in rhizosphere zone has a distinct composition and activity characteristic compared to bulk soil. Indeed, the roots of a plant release a wide variety of chemical compounds which make it possible to select the microorganisms in the rhizosphere. In turn, plant-associated microorganisms, through different mechanisms, influence plant health and growth [10]. Many studies reveal differences in rhizodeposition among different grain legumes, which is very likely to influence their respective structure and function in the rhizosphere [11].

This study aims to assess structure of the bacterial community, their biomass and enzyme activities in the rhizosphere soils of two legumes: *Cajanus cajan* and *Milletia laurentii*. These plants have great importance in food and forestry. The

main objectives of this study were (i) to characterize the bacterial community in the rhizosphere of the two legumes, (ii) to compare their bacterial profiles, and (iii) to assess effects of soil characteristics on microbial biomass, enzymes activities and on the bacterial community.

2. Material and Methods

2.1. Description of Site

The study was conducted in the Scientific City of Brazzaville (4˚16'42, 1439"S, 15˚14'24, 6538"E). The climate of Brazzaville is characterized by two seasons. The rainfall occurs between September and May whereas the dry season takes place between June and August. Annual rainfall averages are 1200 to 1500 mm. Relative humidity is always high around 75%. Temperatures are often around 25˚C [6].

2.2. Soil Sampling

Soil sampling was taken at 1m from the tree (*Cajanus cajan* and *Milletia laurentii*) in the 0 - 10 cm horizon, using an auger. Four soil subsamples were taken near each tree (three tree per specie). The subsamples of each tree were mixed to form a composite sample. Each composite sample was separated in two and packaged in sterile glass jars and transported to the laboratory using a cooler. At the laboratory, the stones and roots were removed and the soils were kept at 4˚C until the use. One part of soil samples was used for the analysis of the bacterial communities and the other part was used for physicochemical characterization and isolate cultivable bacteria.

2.3. Soil Analyses

Soil texture was assessed using the pipette method, pH was measured with pHmeter. Total carbon of soil was determined by the Walkley-Black method [12]. determined The Kjeldahl method [13] was used to assess total nitrogen. Ammoniacal nitrogen was determined using Nessler's reagent [13]. Phosphorus was determined by Olsen's method [14]. The DEB method [15] was used to determine total iron, the Magnesium was determined by spectrophotometry.

2.4. Soil Physicochemical Characteristics

The soil physicochemical characteristics used in **Table 1** was determined by [6].

Table 1. Characteristics of rhizospheric soils of Cajanus and Milletia.

Sol types	Clay	Slit	Sand	C	N	P	Fe	NH_4^+	Mg
	%			‰					
Cajanus	6.5	18.33	75.17	14.2	1.2	0.02	0.2	3.3	0.02
Milletia	7.77	24.54	67.69	16.2	1.7	0.08	0.4	3.7	0.08

2.5. Bacterial Community Structure

DNA extraction, Illumina-Hiseq sequencing and bioinformatics analyses were

carried out at Mr DNA laboratories (USA). Genomic DNA was extracted from 0.5 g of dry soil sample using the PowerSoil kit (MOBIO Laboratory, Carlsbad, CA, USA) following the manufacturer's instructions. The concentration of the extracted DNA was estimated using the Nanodrop 2000C spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Finally, DNA extracts from soil samples were stored at 80˚C until use. The bacterial 16S rRNA gene was amplified with primers 515F (5'-GTGCCAGCMGCCG CGGTAA-3') and 806R (5'-GGACTACHVGGTWTCTA AT-3'). The PCR reaction was conducted as follows: a denaturation was carried out at 94˚C for 3 minutes, 30 - 35 denaturation cycles at 94˚C for 30 s for amplification, hybridization at 53˚C for 40 s, then elongation at 72˚C for 1 minute and a final extension at 72˚C for 5 minutes. After amplification, PCR products were visualized by electrophoresis on 2% agarose gel. Then, the two samples were grouped and purified together in equal proportions based on their DNA concentrations. The samples were purified using the ampure XP calibrated ball method. Then, pooled and purified PCR products are used to prepare the Illumina DNA bank. Sequencing was performed at MR DNA (www.mrdnalab.com, Shalowater, TX, USA). Sequences were assembled and the barcodes eliminated. Then 150 bp sequences and chimeras were removed. The OTUs were defined by grouping the sequences at 3% divergence. The final OTUs were taxonomically classified using the BLAST program against the organized database derived from RDPII and NCBI (https://www.ncbi.nlm.nih.gov/, https://rdp.cme.msu.edu).

2.6. Microbial Biomass

Soils were rehydrated at 50% of field capacity. They were then pre-incubated for 7 days at 30˚C under aerobic conditions to allow microbial activity to stabilize [16]. Twenty grams of moist soil were extracted with 50 mL of 0.5 M K_2SO_4 after stirring for 30 min. Another 20 g of moist soil was put into 100mL Erlenmeyer flasks. This soil was incubated under vacuum in a desiccator in the presence of 25mL of Chloroform without ethanol for 24 hours. The experiment was carried out in triplicate. After the incubation, chloroform was removed by ventilation then the soil was extracted with K_2SO_4 as described above. Organic carbon in the soil extracts was measured by the HACH TM COD method after heating at 150˚C for 2 hours [17]. The dosage was carried out by colorimetry with the DR890. Extractable nitrogen was measured by the spectrophotometric method after mineralization of N to NH_4^+ using indophenol blue [18]. Microbial Biomass carbon (MBC) and Microbial Biomass nitrogen (MBN) were calculated as differences of C and N concentrations between fumigated and non-fumigated extractions. Factors of extraction efficiency of 0.45 and 0.54 were used for MBC and MBN respectively [19]: MBC = Ec/KE_C with KE_C = 0.45 and Ec = (Organic C extracted from fumigated soil) − (Organic C extracted from non-fumigated soil). The microbial carbon biomass is expressed as µg C/g soil; MBN = E_N/KE_N with KE_N = 0.45 and E_N = (organic N extracted from fumigated soils) − (organic N extracted from non-fumigated soils). The microbial nitrogen biomass is expressed as µg N/g soil.

2.7. Enzymes Activities

2.7.1. Determination of β—Glucosidase and β-Glucosaminidase

β-Glucosidase was measured following the method proposed by [20]. Briefly, 1 g of soil was mixed with 4 mL of modified universal buffer (pH 6), 1 mL of a 0.025M toluene solution and 1mL of a p-nitrophenol-β-D-glucoside solution. The mixture is then incubated at 37˚C for 1 hour. The released p-nitrophenol is quantified using a UV-visible spectrophotometer at 410 nm. β-Glucosaminidase was quantified according to the method described by [21]. Briefly, to 1 g of soil are added: 4 mL of 0.1M acetate buffer (pH 5.5) and 1mL of a 10mM p-nitrophenyl-N-acetyl-β-D-Glucosaminide solution. The mixture is incubated at 37˚C for 1 hour. Then, 1mL of a 0.5 M $CaCl_2$ solution and 4 mL of 0.5 M NaOH are added to stop the reaction. The mixture is stirred then filtered on Watman No. 2 filter paper. The intensity of the filtrate coloring is measured using a spectrophotometer at 405 nm.

2.7.2. Determination of Acid Phosphatase and Dehydrogenase

Acid phosphatase was determined by adding to 1 g of dry soil: 4mL of modified universal buffer (pH 6.5), 1mL of a 0.025M toluene solution and 1mL of p-nitophenyl phosphate. The mixture is incubated at 37˚C for 1 hour. The released p-nitrophenol (PNP) was quantified using a spectrophotometer at 410 nm [22] [23].

Dehydrogenase activity was determined according to the method of [24]. This method is based on the estimation of the concentration of 2,3,5-triphenyl formazan (TPF) released by dehydrogenases when the soil is incubated with a 2,3,5-tryphenyl tetrazolium chloride (TTC) buffer solution at 30˚C for 24 hours. In short, in a beaker we mix 20 g of soil with 0.2 g of calcium carbonate and 4mL of reagent. The optical density of the formed TPF was determined at 485 nm using U-V spectrophotometer.

2.8. Statistical Analysis

To assess family and genus level of culturable bacteria distribution, a heatmap was done with the relative abundance of each family or genus. Alpha diversity indices: Shannon index, Simpson index, Evenness, Equitability and Chao-1 was calculated using the Past software 4.03. To compare the properties under the rhizospheres of *Cajanus cajan* and *Milletia laurentii* on a multivariate scale, principal components analysis (PCA) was performed with Past software 4.03. The non metric multidimensional scaling (NMDS) plot, cosine method, was used to short a graphical ordination (two synthetic axes) of the impact of soil characteristics, microbial biomass and enzymes activities on distribution of uncultured bacteria under the rhizospheres.

3. Results

3.1. Composition of the Microbial Community

From Illumina sequencing, 45,412 and 32,781 raw sequences were obtained respectively for the rhizosphere soil of *Cajanus cajan* and *Milletia laurentii*. These

sequences mainly belong to culturable and uncultured soil bacteria. After statistical processing, 1434 OTUs (Operational Taxonomic Unit) were obtained from the valid sequences. In this work, only families and genera of culturable soil bacteria as well as the genera of uncultured soil bacteria were taken into account.

3.2. Relative Abundance of Culturable Soil Bacteria Families

Figure 1 shows that the nine families most representative culturable soil bacteria under *Cajanus cajan* were: Hyphomicrobiaceae (35.05%), Bacillaceae (18.98%), Bradyrhizobiaceae (11.70%), Streptomycetaceae (3.66%), Rhizobiaceae (3.65%), Methylobacteriaceae (3.44%), Hyphomonadaceae (3.00%), Pseudomonadaceae (2.40%), Sphingomonadaceae (1.91%). The most dominant genera of culturable soil bacteria under *Milletia laurentii* were: Hyphomicrobiaceae (24.22%), Bradyrhizobiaceae (17.32%), Bacillaceae (6.49%), Sphingobacteriaceae (5.80%), Chitinophagaceae (4.02%), Flavobacteriaceae (3.70%), Nitrospiraceae (2.42%), Xanthobacteriaceae (2.30), Sinobacteriaceae (2.11%).

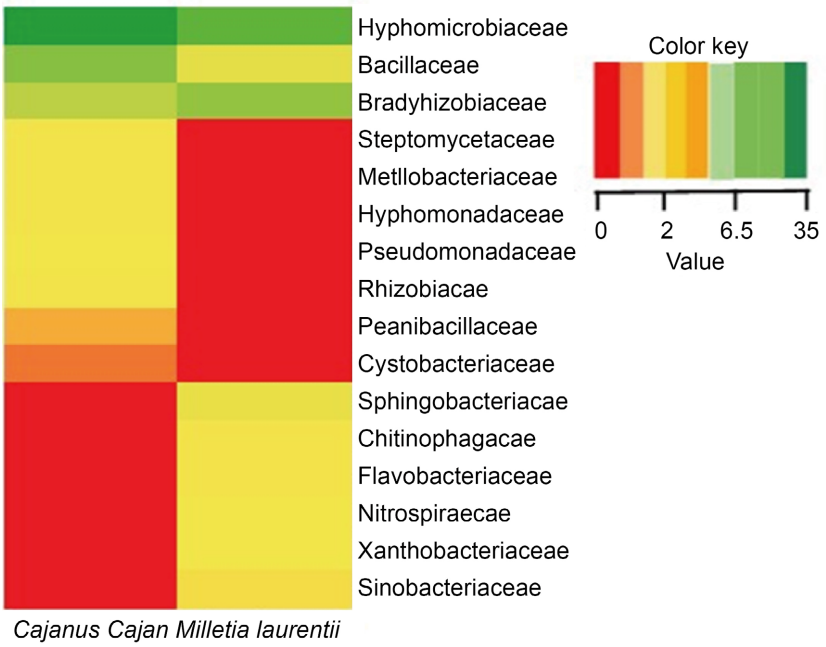

Figure 1. Family-level of bacterial distribution from Cajanus and Milletia rhizosphere samples. Row represents the relative abundance of each family and column sample of different rhizosphere. The relative abundance for each bacterial family was indicated by color key.

3.3. Relative Abundance of Culturable Soil Bacteria Genera

Figure 2 shows that the most dominants genera under the rhizosphere soil of *Cajanus cajan* were: Acidobacterium (26.55%), Skermanella (17.52%), Rhodoplanes (7.50%), Bacillus (6.29%), Chloroflexus (4.74%), Steroidobacter (2.34%), Sphingomonas (2.32%), Bradyrhizobium (1.96%), Dongia (1.80), Sphingobacterim (1.74%), Nitrospira (1.37%), Microvirga (1.30%), Cupriavidus (1.12%), Gemmatimonas (1.12%), Chelatococcus (1.03%) and Streptomyces (1,02%). In the

rhizosphere soil of *Milletia laurentii* the principal dominants genera were : Rhodoplanes (21.63%), Bradyrhizobium (17.27%), Bacillus (6.43%), Sphingobacterium (5.68%), Acidobacterium (4.58%), Mesorhizobium (4.06%), Nitrospira (2.42%), Steroidobacter (2.05%), Hyphomicrobium (1.53%), Pedomicrobium (1.53%), Streptomyces (1.45%), Burkholderia (1.45%), Nitratireductor (1.33%), Niastella (1.33%), Sphingomonas (1.22%), Gemmatimonas (1.22%), Mycobacterium (1.08%) and Holophaga (1.08%).

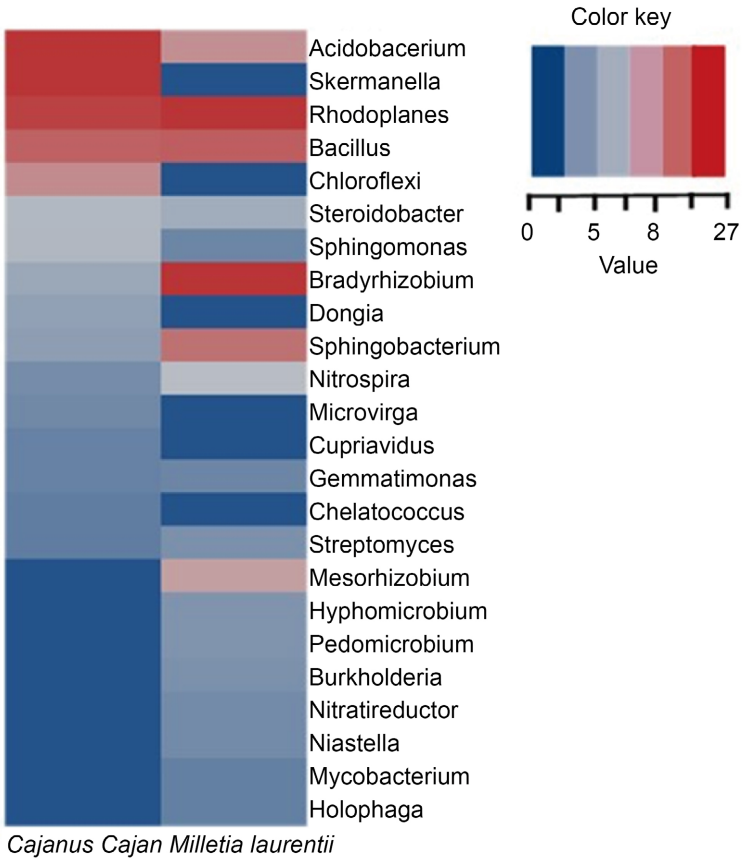

Figure 2. Genus-level of bacterial distribution from *Cajanus cajan* and *Milletia laurentii* rhizosphere samples. Row represents the relative abundance of each genus and column sample of different rhizosphere. The relative abundance for each bacterial genus was indicated by color key.

3.4. Relative Abundance of Uncultured Soil Bacteria Genera

Table 2 shows that the nine predominants of uncultured soil bacteria genera (relative abundance ≥1%) in the rhizosphere soil of *Cajanus cajan*, were mainly: Acidobacterium (34.02%), Skermanella (15.83%), Nitrosovibrio (8.29%), Chloroflexus (7.99%), Sinorhizobium (4.79%), Polyangium (4.31%), Blastopirellula (2.98%), Opitutus (2.47%), Sphingobacterium (2.45). The relative abundance of other genera was between 1.01 and 1.74%. Nine genera (Blastopirellula, Candidatus lariskella, Chitinophaga, Ktedonobacter, Nitrosovibrio, Opitutus) were exclusively founded among genera of uncultured bacteria in the rhizosphere soil. On

Table 2. Relative abundance (%) of Uncultured soil bacteria genera.

Genera	*Cajanus cajan*	*Milletia laurentii*
Acidobacterium	**34.02**	**4.55**
Bacillus	1.28	**3.17**
Blastopirellula	0.03	**3.39**
Bradyrhizobium	0.21	**8.73**
Candidatus lariskella	0.53	2.12
Chitinophaga	1.42	1.07
Chloroflexus	**7.99**	0.67
Dongia	1.62	0.68
Edaphobacter	0.02	1.47
Flavisolibacter	0.14	2.25
Flavobacterium	0.07	1.95
Gemmata	0.13	**3.03**
Gemmatimonas	1.01	0.08
Ktedonobacter	0.02	1.46
Niastella	0.23	1.66
Nitrosovibrio	**8.29**	0.8
Nitrospira	1.25	1.02
Opitutus	2.47	0.55
Pedosphaera	0.01	1.47
Polyangium	**4.31**	0.17
Prosthecobacter	0.01	1.34
Pseudolabrys	0.01	1.24
Rhizobium	0.13	1.61
Rhodoplanes	1.02	**12.59**
Sinorhizobium	**4.79**	0.09
Skermanella	**15.83**	1.02
Sphingobacterium	2.45	1
Spirulina	1.16	0.08
Steroidobacter	1.12	0.08

the other hand, in the rhizosphere soil of *Milletia laurentii* (Table 2), uncultured soil bacteria were predominant in the genera Rhodoplanes (12.59%), Brayrhizobium (8.73%), Chloroflexus (7.99%), Acidobacterium (4.55%), Blastopirellula (3.39%), Gemmata (3.03%), Flavisolibacter (2.25%), Candidatus (2.12%), Flavobacterium (1.95%) while the other genera of uncultured bacteria had relative abundances between 1.02 and 1.93%. Table 2 also reveals that relative abundance of uncultured soil bacteria genus Acidobacterium, Skermanella, Chloroflexus,

Nitrosovibrio and Sinorhizobia were higher under rhizosphere of *Cajanus cajan* than under *Milletia laurentii*. However, the results indicated that a high uncultured soil bacteria genera of Rhodoplanes, bacillus, Blastopirellula, bradyrhizobium, Gemmata were most observed under rhizosphere soil of *Milletia laurentii* than under *Cajanus cajan*. Distribution of the other uncultured soil bacteria genera was linked with the stand.

Table 3(a) shows that under rhizosphere soil of *Cajanus cajan* all uncultured soil bacteria genera had high relative abundance than culturable soil bacteria outside of genus Skemanella. The results indicated that six culturable soil bacteria (Sphingomonas, bradyrhizobium, Microvirga, Cupriavidus, Chelatococcus and streptomyces) were not retrieved among the uncultured soil bacteria genera. On the other hand, several uncultured soil bacteria were not in the list of genera of culturable soil bacteria for example: Nitrosovibrio, Sinorhizobium and Polyangium. Ten genera of culturable soil bacteria were not found among the uncultured soil bacteria genera. This was the case for Sphingomonas, Bradyrhizobium (Table 3(b)). On the other hand, thirteen uncultured soil bacterial genera have not been listed among the culturable soil bacteria, for example: Blastopirellula, flavobacterium, rhizobium, Skermanella.

Table 3. Comparison of relative abundance of culturable and uncultured soil bacteria genera in each rhizosphere. (a) Rhizosphere soil of *Cajanus cajan*); (b) Rhizosphere soil of *Milletia laurentii.*

(a)			
Culturable soil Bacteria Genera	%	Uncultured Soil Bacteria Genera	%
Acidobacterium	**26.55**	**Acidobacterium**	**34.02**
Skermanella	**17.52**	**Skermanella**	**15.83**
Rhodoplanes	**7.5**	**Nitrosovibrio**	**8.29**
Bacillus	**6.29**	**Chloroflexus**	**7.99**
Chloroflexus	**4.74**	**Sinorhizobium**	**4.79**
Steroidobacter	**2.34**	**Polyangium**	**4.31**
Sphingomonas	**2.32**	**Blastopirellula**	**2.98**
Bradyrhizobium	**1.96**	**Opitutus**	**2.47**
Dongia	**1.8**	**Sphingobacterium**	**2.45**
Sphingobacterium	1.74	Candidatus lariskella	1.93
Nitrospira	1.37	Dongia	1.62
Microvirga	1.3	Chitinophaga	1.42
Cupriavidus	1.12	Bacillus	1.28
Gemmatimonas	1.12	Nitrospira	1.25
Chelatococcus	1.03	Ktedonobacter	1.20
Streptomyces	1.02	Spirulina	1.16
		Steroidobacter	1.12
		Rhodoplanes	1.02
		Gemmatimonas	1.01

Continued

(b)			
Culturable Soil Bacteria Genera	%	Uncultured Soil Bacteria Genera	%
Rhodoplanes	**21.63**	**Rhodoplanes**	**12.59**
Bradyrhizobium	**17.27**	**Bradyrhizobium**	**8.73**
Bacillus	**6.43**	**Acidobacterium**	**4.55**
Sphingobacterium	**5.68**	**Blastopirellula**	**3.39**
Acidobacterium	**4.58**	**Bacillus**	**3.17**
Mesorhizobium	**4.06**	**Gemmata**	**3.03**
Nitrospira	**2.42**	**Flavisolibacter**	**2.25**
Steroidobacter	**2.05**	**Candidatus lariskella**	**2.12**
Hyphomicrobium	**1.53**	**Flavobacterium**	**1.95**
Pedomicrobium	1.53	Niastella	1.66
Burkholderia	1.45	Rhizobium	1.61
Streptomyces	1.45	Pedosphaera	1.47
Niastella	1.33	Edaphobacter	1.47
Nitratireductor	1.33	Ktedonobacter	1.46
Gemmatimonas	1.22	Prosthecobacter	1.34
Sphingomonas	1.22	Pseudolabrys	1.24
Holophaga	1.08	Chitinophaga	1.07
Mycobacterium	1.08	Nitrospira	1.02
		Skermanella	1.02

3.5. Alpha Diversity of Uncultured Soil Bacteria Genera

Table 4 shows that alpha diversity was different under the two rhizosphere soils. Specific richness evaluated by chao-1 had the same value in the two rhizospheres (chao-1 = 44). The others indices exhibited weak differences between: (H = 1.84; 1-D = 0.76), (H = 1.76; 1-D = 0.75) under Cajanus and Milletia respectively. The same trend was observed for evenness and equitability: in Cajanus ($e^{H/S}$ = 0.14; J = 0.49) and in Milletia ($e^{H/S}$ = 0.13; J = 0.47). However, the number of individuals was higher in Milletia than in Cajanus.

Table 4. Diversity index of uncultured soil bacteria genera.

Diversity index	*Cajanus cajan*	*Milletia laurentii*
Taxa_S	44	44
Individuals	1556	1806
Simpson1-D	0.76	0.75
Shannon_H	1.84	1.76
Evenness_$e^{H/S}$	0.14	0.13
Equitability_J	0.49	0.47
Chao-1	44	44

3.6. Microbial Biomass and Enzyme Activities

Soil of rhizosphere associated with Milletia has high levels MBC (688 mgC·kg^{-1} soil) and MBN (186.83 mgN·Kg^{-1} soil) compared to the rhizosphere of Cajanus (MBC 621 mgC·Kg^{-1} soil and MBN 138.57 mgN·Kg^{-1} soil) (Figure 3). Enzyme activities into the rhizosphere was different with enzyme (Table V). High β-Glucosidase activity (163 mg·pN·kg^{-1}·soil·h^{-1}) was observed under Milletia compared in the soil of rhizosphere of Cajanus (121 mg·pN·kg^{-1}·soil·h^{-1}). However high β-Glucosaminidase activity (64 mg·pN·Kg^{-1}·soil·h^{-1}) was obtained under Cajanus compared to Milletia (57.50 mg·pN·kg^{-1}·soil·h^{-1}). Table 5 shows that Acid phosphatase activity was lower in soil under Cajanus than in Milletia, whereas dehydrogenase activity was higher under Milletia than under Cajanus (1.03 µg TTC·g^{-1}·soil·h^{-1}).

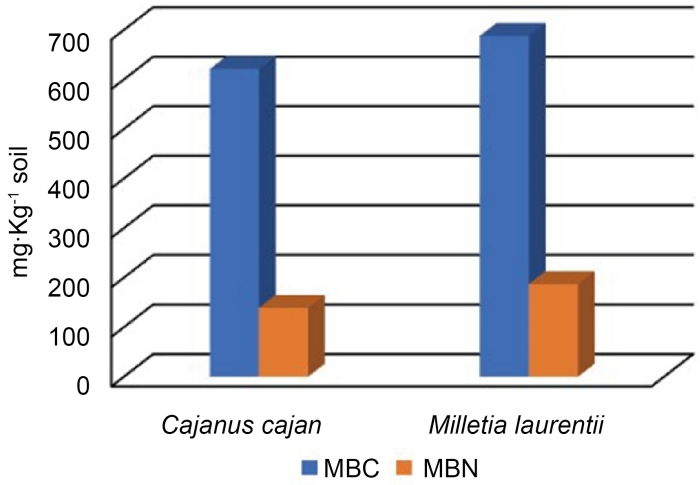

Figure 3. Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN).

Table 5. Enzyme activities under the rhizosphere soils.

Rhizosphere	Enzyme			
	mg·pN·kg^{-1}·soil·h^{-1}			µg TTC·g^{-1}·soil·h^{-1}
	β-Glucosidase	β-Glucosaminidase	Acid Phosphatase	Dehydrogenase
Cajanus	121 ± 1.8	64 ± 3.3	410 ± 43	1.03 ± 0.05
Milletia	163 ± 3.6	57.50 ± 3.1	544 ± 35	1.62 ± 0.33

3.7. Soil Microbial Biomass and Enzyme Activities Linkage to Plant Rhizospheres

Figure 4 shows that MBC and β-Glucosaminidase and dehydrogenase enzymes were more founded in soil of rhizosphere of *Cajanus cajan*, whereas MBN β-Glucosidase and acid phosphatase enzymes have a favorite distribution in soil rhizosphere of *Milletia laurentii*.

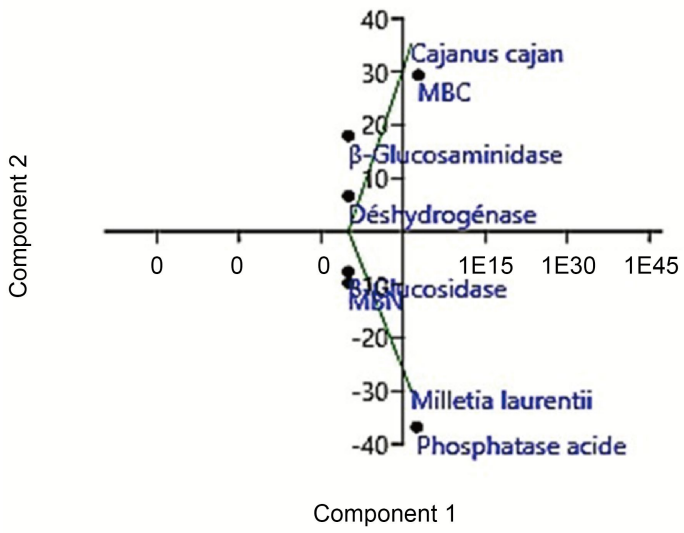

Figure 4. Distribution of microbial biomass and enzyme activities under *Cajanus cajan* and *Milletia laurentii.*

3.8. Effects of Soil Physicochemical, Microbial Biomass and Enzymes on Culturable Soil Bacteria Genera

Considering the component 1, PCA (**Figure 5**) shows that MBC, the genera (Acidobacterium, Skermanella, Chloroflexi), enzyme (*β*-glucosaminidase) and the sand were positively correlated with the rhizosphere of *Cajanus cajan*. MBN, the genera (Mesorhizobium, Bacillus Bradyrhizobium, Rhodoplanes, Burkholderia, Nitratireductor, Hyphomicrobium, Sphingobacterium, Nitrospira), enzymes (*β*-glucosidase, acid phosphatase), slit, clay, and NH_4 were mainly related with the rhizosphere of *Milletia laurentii*. Taking into account the component 2, we observed that: Acidobacterium, MBC, enzyme *β*-glucosaminidase and the sand were positively correlated with *Cajanus cajan* whereas Skermanella, Chloroflexi, Mesorhizobium, Rhodoplanes, Bradyrhizobium and NH_4 were negatively correlated

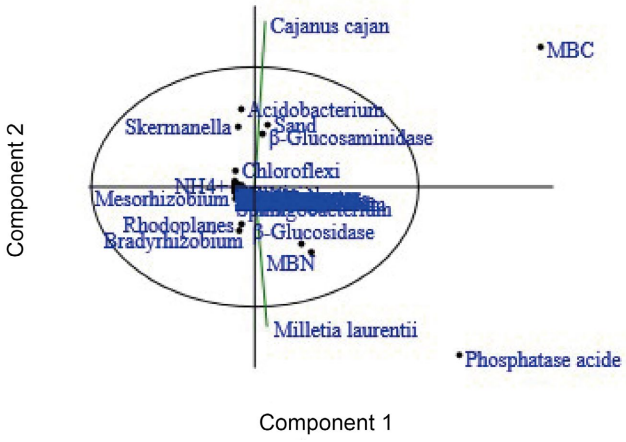

Figure 5. Effects of soil physicochemical parameters, microbial biomass and enzymes activities on the abundantly of culturable soil bacteria genera in the two rhizospheres.

with the rhizosphere of this plant. On the other hand, Skermanella, Cloroflexi, Mesorhizobium, Rhodoplanes, Bradyrhizobium and NH_4 had a negative relationship with Milletia, in the same time positive correlation take place between this plant and MBN, Nitrospira, Bacillus, Burkholderia, Nitratireductor, Hyphomicrobium, Sphingobacterium, the enzymes acid phosphatase and β-glucosidase.

3.9. Effects of Soil Physicochemical, Microbial Biomass and Enzymes Activities on Uncultured Soil Bacteria Genera

The NMDS was used to check the links of uncultured soil bacteria genera with the soil physicochemical, microbial biomass and enzymes. The NMDS (based on both cosine similarity) for genus assemblage, relative abundance was used without transformation. The NMDS graphic shows two groups (Figure 6). The first group included genera: Nitrospira, Chitinophage, Opitutus, Steroidobacter, Nitrosovibrio, Spirulina, Acidobacterium, Gemmatomonas, Sphigobacterium, Dongia, Skermanella, Cloroflexus. The group of these uncultured bacteria were linked with rhizosphere of *Cajanus cajan*. Under this plant the mainly soil properties were sand, clay, C, NH_4, MBC and β-glucosaminidase. The second group was related to rhizosphere of *Milletia laurentii*. Its composition was: Ktedonobacter, Bacillus, Candidatus, Nistella, Rhizobium, Rhodplanes, Flavisolibacter, Bradyrhizobium, Flavobacterium, Edaphobacter. Outside the nature of the plant, in this rhizosphere, nine soil properties (Slit, P, Mg, N, Fe, MBN, Acid Phosphatase, Dehydrogenase) could impact the distribution of the genera.

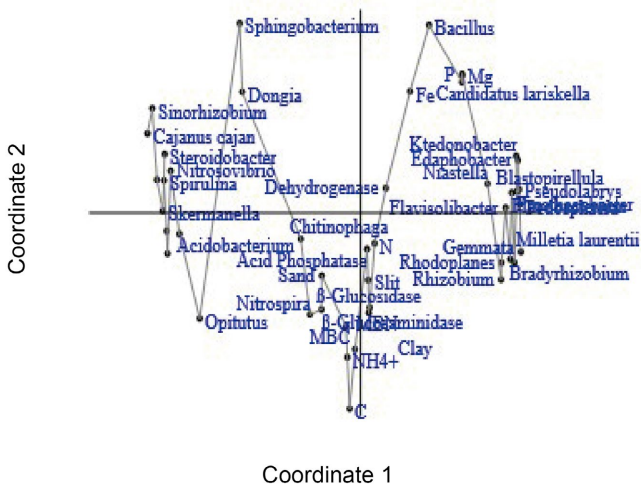

Figure 6. The NMDS (based on both cosine similarity) for genus assemblage, relative abundance was used without transformation.

4. Discussion

The results obtained in the present study revealed several differences in characteristics of soil microbial community, microbial biomass and enzyme activities. However, the major genera of the culturable bacterial community we found in our work were widely reported in several studies [5] [25] [26] For example, these genera

were composed under rhizosphere soil of *Cajanus cajan* and *Milletia laurentii* by: Acidobacterium, Skermanella, Rhodoplanes, Bacillus, Chloroflexi, Steroidobacter, Sphingomonas, Bradyrhizobium, Dongia, Burkholderia, Sphingobacterium, Nitrospira, Microvirga, Cupriavidus, Gemmatimonas, Chelatococcus, Streptomyces, Mesorhizobium, Hyphomicrobium, Pedomicrobium, Nitratireductor, Niastella, Sphingomonas, Mycobacterium and Holophaga.

As it was observed by other authors, that uncultured soil bacteria were relatively more abundant than culturable bacteria into the soil and particularly under the rhizosphere [27]. It was the case in this work that explores the uncultured soil bacteria under the rhizosphere of *Cajanus cajan* and *Milletia lauretii*. For example, under the rhizosphere of Cajanus: the total relative abundance of Acidobacteria genus (60%) was composed by 26% of culturable soil bacteria and 34% of uncultured soil bacteria respectively. The same trend was observed with Chlorflexus that was composed by a high level of uncultured bacteria (7.99%). The top common genera found under the two plants were Bacillus, Optitus, steroidobacter Gemmata, Candidatus. These soil bacteria widely found by others in several studies [28]. Differences of diversity between *Cajanus cajan* and *Milletia laurentii* probably were due to difference into the physicochemical composition of the rhizosphere soil under the plants. This finding is in agreement with the results obtained by [25] [29] in their studies.

PCA of culturable and NMDS of unculturable soil bacteria genera shows that factors mainly involved in the carbon cycle such as MBC, members of the microbial community (i.e. Acidobacterium, Skermanella, Chloroflexus), soil properties (sand, total carbon), enzymes β-glucosaminidase and dehydrogenase, were correlated with the rhizosphere soil of *Cajanus cajan*. Several authors [30]-[32] claimed that Acidobacteria are active members of soil bacterial communities and thus they are widely distributed in different soils. The members of Acidobacteria play a key role in the degradation of soil organic matter and thus impacting carbon turnover dynamics [33]-[35]. On the other hand, MBN, Mesorhizobium, Bradyrhizobium, Burkholderia, Nitrospira, Nitratireductor, Rhodoplanes, total nitrogen, NH_4, enzymes β-glucosidase and acid phosphatase involved in the nitrogen cycle, silt and clay were predominantly founded in the rhizosphere soil of *Milletia laurentii*. This study revealed high abundances of major bacterial genera in the soil. Our study was in agreement with the findings of [34] [35]. This work shows that the difference of distribution among the major uncultured soil bacteria genera depending of rhizospheric soil characteristics, root exudates and plant species [24] [36]. In the soil under Milletia, the major uncultured soil bacteria community was composed by: Actinobacteria, Bradyrhizobium, Rhodoplanes, Blastopirellula, Bacillus, Gemmata, Flavisolibactter, Candidatus, Flavobaterium, Rhizobium, and Nitrospira. This finding is in agreement with the study of [37]. They found that in the genera recorded the dominant bacteria were belonged to the uncultured bacteria. In other studies, an important role is attributed to uncultured soil bacteria in C and N dynamics, in decomposition of organic matter or N fixation and as they are

considered either producers polysaccharide-degrading enzymes [33] [37] [38]. Previous studies have been emphasized that Flavobacterium are recognized for their role in soil metabolic functioning and it is possible that they could provide a strong contribution to the mineralization of primary-production organic matter. The uncultured soil bacteria alpha diversity indices were slightly higher under *Cajanus cajan* than under *Milletia laurentii*. These findings are consistent with the differences observed in the composition of the microbial community structure. These results could significate that the uncultured bacteria had a weak number of genera in the rhizosphere soil of Milletia. Such results were recorded by [28] [39] in their studies.

In this work the rhizosphere soils exhibited differences in the distribution of the group of enzymes activities. These differences could express differences among biogeochemical functioning of cycling of carbon, nitrogen and phosphorus. Several authors claimed that a strong ecological linkage was observed between carbon and nitrogen cycling with high enzyme activities specially with β-glucosaminidase and β-glucosidase respectively [25] [40]. Our results showed that β-glucosaminidase was correlated with rhizosphere soil of *Cajanus cajan* whereas β-glucosidase was more under rhizosphere of *Milletia laurentii*. These results suggest that the transformation and turnover of organic matter is predominant under cajanus while in the rhizosphere of Milletia the mineralization of nitrogen is potentially more important. Acid phosphatase activity has a great importance in the functioning of the phosphorus cycling and it high level turnover under the rhizosphere soil of *Milletia laurentii* as demonstrated in our results. Acid phosphatase catalyzes the release phosphate how it was demonstrated in other studies [41] [42]. Dehydrogenase activity plays an important role in the carbon cycling by catalyzing the oxidation of organic matter and thus contributing to its decomposition. All those enzyme activities occurred in complex environment where microbial biomass and physicochemical properties of soil, the nature of plants and their rhizodepositions play a major role. Indeed, plant root release many kinds of compounds with different chemistry characteristics and concentration depending on the plant species and environmental conditions. According to [43] rhizodeposition include quorum-sensing molecules, molecules such as carbohydrates, phenolics, organic acids, amino acids, and proteins and polysaccharides. These exudates could have an attractive influence on symbiotic and mutualistic microorganisms to develop around the roots and have a protective or inhibitory effect on disease-causing pathogens [44]. Other root exudates could be use as nutrients in the metabolism of the microorganisms evolving in the rhizosphere [45]. In this case, these exudates improve microorganism growth. We founded that MBC was correlated with rhizosphere of *Cajanus cajan* whereas MBN was correlated with rhizosphere soil of *Milletia laurentii*. Establishment of symbiotic relationships improve plant growth and productivity. The finding enhances the understanding how root exudation improves plant health and sustainability of agricultural stand.

5. Conclusion

The current study investigated the microbial structure and the enzymes in the rhizosphere of the grain legume *Cajanus cajan* and the forest legume *Milletia laurentii*. It was observed that contribution from culturable and uncultured soil bacteria varied by environment induced in the rhizosphere by the plant functioning. Despite the abundance of bacterial species in soil, more of them were attributed to uncultured bacteria. These species play a key role in the C and N cycling, release phosphate and other nutrients through their exoenzymes. The enzymes activities occurred in a complex environment in which microbial composition, the exudates of the rhizosphere, and soil characteristics play an important role for their distribution and functioning. These mechanisms enhance the growth and productivity of the plants.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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