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Bacterial Communitie's Diversity of Rhizosphere's Soils of Two Legumes, *Cajanus cajan* and *Milletia laurentii*, Revealed by Illumina Miseq Sequencing of 16S rRNA Gene

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Abstract Microbial organic fertilizers have been shown to boost plant productivity. These microorganisms of interest are more numerous in the soil around the roots or rhizosphere. Objective of this study was to assess bacterial communities' diversity of in the rhizosphere of two legumes, *Milletia laurentii* and *Cajanus cajan*, growing on the same soil. First of all, the levels Mg, N, Fe, C total, P, NH_4^+ and particle size were determined by spectrophotometry, Kjeldahl method, Olsen method, Walkey-Black method, Nessler reagent, DEB method and Robinson pipette method, respectively. Next, bacterial diversity was determined by Sequencing Illumina Miseq of 16S rRNA gene. Results showed that contents of carbon, total nitrogen, ammoniacal nitrogen, phosphorus, iron and magnesium were slightly elevated in *Milletia* rhizosphere compared to *Cajanus*. According to the USDA's textural triangle, both soils have a sandy loam soil texture. In terms of diversity, all OTUs (1434) were divided into 30 phyla, 50 classes, 158 families and 314 genera for the 2 soils. Proteobacteria (58.62% - 48.71%), Acidobacteria (27.29% - 9.46%), Firmicutes (8.26% - 7.21%) and Bacteroidetes (13.70% - 2.53%) were most dominant phyla in both rhizospheres (*Cajanus* - *Milletia*). The most dominant classes were Alphaproteobacteria (51.44% - 38.90%), Acidobacteriia (26.57% - 8.67%), Bacilli (8.19% - 7.18%), Sphingobacteria (9.83% - 2.50%) and Gammaproteobacteria (4.27% - 3.39%). At the family level, Hyphomicrobiaceae (35.05%-24.22%), Bradyrhizobiaceae (17.32%-11.70%) and Bacillaceae (18.98%-6.49%) were most abundant. Finally, *Acidobacterium* (26.55%-4.58%), *Rhodoplanes* (21.63%-7.50%), *Bradyrhizobium* (17.27%-1.96%) and *Bacillus* (6.43%-6.29%) were the most abundant genera. Thus, bacterial diversity of the rhizosphere of these two legumes encourages their use for the isolation of bacteria with biofertilizing potential.

Keywords: *Illumina - Miseq, rhizosphere, bacterial diversity*

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1. Introduction

The need to increase agricultural production to meet the needs of a growing world population has led to the use of chemical and/or organic fertilizers. Unfortunately, the misuse and uncontrolled use of the latter is the cause of environmental pollution and public health problems [1]. Indeed, antimicrobials (antifungal, antibacterial and antiparasitic) are introduced into the soil with the use of manure from animals treated with drugs to prevent diseases and improve their growth. This has the consequence of distributing multidrug-resistant microorganisms in the environment that adapt to new environment contaminated by horizontal gene transfer with other bacteria [2,3]. In addition, manure of animal origin may contain heavy metals such as lead and cadmium but also pathogenic microorganisms [4]. [5] highlighted that agricultural and industrial development has led to an increase of Cd concentration in agricultural soils. However, toxicity of this metal leads to inhibition of carbon fixation, decreases the chlorophyll content and consequently photosynthetic activity of plants [6] In addition, the misuse of nitrogen fertilizers is at the origin of eutrophication of water bodies which leads to a decrease in the amount of dissolved oxygen with the risk of death of aquatic organisms [7].

Therefore it's interesting to opt for low-polluting and environmentally compatible soil amendment methods [1,8]. [9] state that microbial agents offer an attractive and feasible option for developing biological tools to replace or supplement chemicals. This idea has been supported by [10] who state that the exploitation of microorganisms as biofertilizers is considered an alternative to chemical fertilizers. This is because microbial fertilizers increase nutrient availability, solubilize phosphates, fix atmospheric nitrogen, produce phytohormones that improve plant growth, and protect the plant from pathogens [11]. In the other hand [12] point out in this sense that there is a need to explore the different mutualistic interactions between plant roots and microbiome of the rhizosphere. According to [9], the exploration of microorganisms that reside in the vicinity of the plant will make it possible to achieve this goal by moving to wards microorganisms in the rhizosphere. Similarly, [13] evaluated the effect of co-inoculation of *Rhizobium* and mycorrhizae on the agronomic performance of cowpea. Results of study of these authors showed that co-inoculation of crops increased pod yields. It's clear that beneficial application of the rhizosphere microbiome as a biofertilizer in agricultural practices has become an innovative and environmentally friendly technology to improve soil fertility and plant growth [14,15].

Thus, knowledge of the diversity of microbial communities colonizing the rhizosphere can be a first step in the screening of microorganisms that have beneficial effects on sustainable plant production. In Congo few studies have focused on microorganisms in the rhizosphere. Only [16] studied the diversity and structure of microbial communities in three soils south of Brazzaville. The results of these authors showed that microbial communities differ from one soil to another. But for the same soil, are the microbial communities of the rhizosphere specific to a given plant ? This study aims to look for microorganisms in the rhizosphere that can be used as biofertilizers. The objective is to assess the diversity and structure of bacterial communities in the rhizosphere of two legumes, *Milletia laurentii* and *Cajanus cajan*, growing on the same soil.

2. Material and Methods

2.1. Soil Survey and Sampling Site

The study was conducted in the Scientific City of Brazzaville: (4°16'42, 1439"S, 15°14'24, 6538" E). According to [17], the climate of Brazzaville called "transitional equatorial" is of the Low-Congolese type. This type of climate prevails over the South-West of the Congo and experiences moderate rainfall whose monthly distribution shows a very marked dry season of four to five months (May-September), framed by two periods of rain of which that of February to May is the most abundant. Relative humidity is always high around 75% [18]. Annual rainfall averages are of the order of 1200 to 1500 mm. Average temperatures hover around 25°C. However, there are monthly averages that sometimes reach 27°C in the rainy season and 19°C in the dry season [18].

For soil sampling, an equilateral triangle of 1 m side was drawn from the tree (*Cajanus Cajan* and *Milletia Laurentii*). Then the soils were taken from the horizon 0-10 cm, using an auger, at the vertices on each side and in middle of triangle. The four soil samples 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. In the laboratory, the soils were kept at 4°C until they were used. Before soil use, the stones and roots were removed. One of the batches of the two samples was used for the analysis of diversity of bacterial communities and the other for physicochemical characterization and cultivable strains' study.

2.2. Physicochemical Characterization of Soils

Total carbon of soil samples was determined by the Walkey-Black method [19]. Total nitrogen was determined using the Kjeldahl method described by [20]. Ammoniacal nitrogen was determined using Nessler's reagent [20]. Phosphorus was determined by Olsen's method [21]. Finally, total iron was determined by the DEB method [22]. Magnesium was determined by spectrophotometry. Particle size was determined by Robinson's pipette method [23].

2.3. Study of Bacterial Communities

2.3.1. DNA Extraction

DNA extraction, Illumina-Hiseq sequencing and bioinformatics analyses were carried out at Mr DNA laboratories (USA). Genomic DNA was extracted from 0.5g 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.

2.3.2. PCR Amplification and Illumina-Miseq Sequencing

The V4 region of 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 40s, 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).

2.4. Bioinformatics Analysis and Statistical Processing

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 (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu). The analysis of sequence data and calculation of the relative abundances of all taxa were carried out using Excel 2013 software. Then, the rarefaction curves and alpha diversity indices namely Chao1, Shannon, Simpson and equitability J' were plotted and calculated using the PAST software. Finally, bacterial community' diversity of two soils was determined by principal component analysis (PCA) to compare relative levels of genus and phylum diversity using Graph Pad software.

3. Results

3.1. Soil Characteristics

Table 1 shows physicochemical properties of two soils. The soil around *Cajanus* is composed of 75.17% sand, 18.33% silt and 6.5% clay. According to USDA' textural triangle, this soil was a sandy loam. For soil around *Milletia*, particle size analysis gave the following percentages: 67.69% for sand, 7.77% for clay and 24.54% for silt. The USDA textural triangle gives the same texture as the previous soil. The contents of carbon, total nitrogen, ammoniacal nitrogen, phosphorus, iron and magnesium were slightly high in the soil sample around *Milletia*. However, the equality of variances test (F-Test) between the two soils shows that the difference in the values obtained for all parameters is not significant ($P > 0.05$).

3.2. Composition of the Bacterial Community

3.2.1. Rarefaction Curve

The rarefaction curve obtained with the *Cajanus* soil sample shows that the maximum diversity (Shannon index) is reached from 1201 OTUs. When the number of OTUs is increased, the Shannon index no longer increases and the curve shows a plateau. Thus the sampling effort is reached. For the *Milletia* soil sample, maximum diversity was reached with 2010 OTUs. The increase in the sampling effort, i.e. number of sequences, does not lead to an increase in the Shannon index (diversity) illustrated by a plateau.

3.2.2. Alpha Diversity Analysis

The Illumina sequencing resulted in 45.412 and 32.781 raw sequences for *Cajanus* and *Milletia* soil, respectively. After bioinformatics treatment the number of sequences decreased by 15.316 and 15.359 respectively for the *Cajanus* and *Milletia* soil. These sequences were grouped into 471 OTUs for *Cajanus* soil and 963 OTUs for *Milletia* soil. Then OTUs were classified into phylum, class, order, family and genus in the 2 soils. For Sol *Cajanus*, eight (8) phyla were the most representative with a relative abundance > 1%. These are: Proteobacteria (48.71%), Acidobacteria (27.29%), Firmicutes (8.26%), Chloroflexi (5.83%), Actinobacteria (3.24%), Bacteroidetes (2.53%), Nitrospirae (1.41%) and Gemmatimonadetes (1.12%). While for Sol *Milletia*, seven (7) phyla were the most representative among which: Proteobacteria (58.62%), Bacteroidetes (13.70%), Acidobacteria (9.46%), Firmicutes (7.21%), Actinobacteria (5.05%), Nitrospirae (2.42%) and Planctomycetes (1.22%) (Figure 2).

Table 1. Physicochemical characteristics of soils

Soil type	Clay	Silt	Sand	C	N	NH4+	P	Fe	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

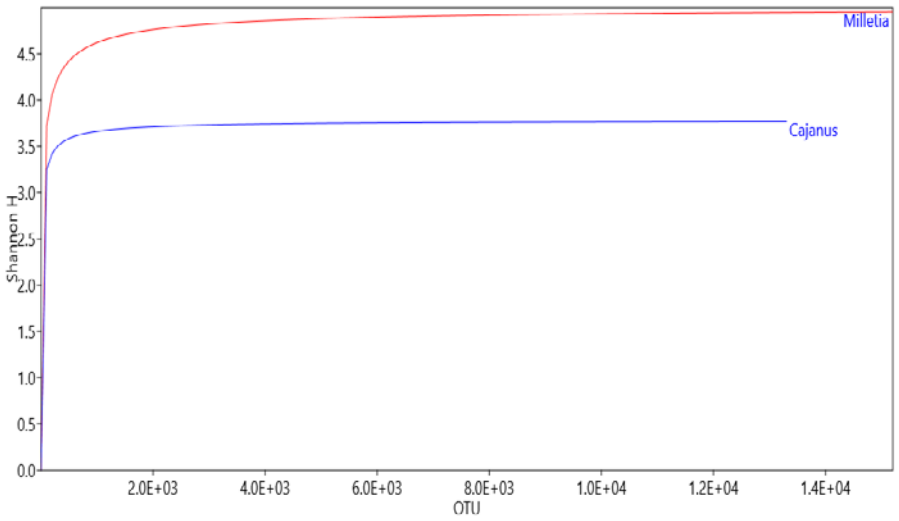

Figure 1. Rarefaction curve plotted as a function of the Shannon index and sequences

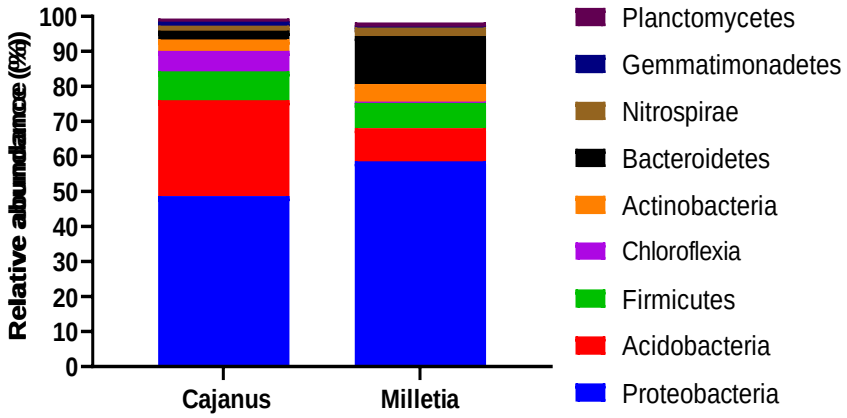

Figure 2. Relative abundance of the dominant phyla from both soils

Table 2. Alpha diversity of phyla

Alpha diversity	Cajanus Soil	Milletia Soil
S (Number of phylum)	9	9
N (Number of individuals)	95	95
Simpson index (1-D)	0.6062	0.6715
Shannon index (H')	1.437	1.33
Equitability (e^H/S)	0.4199	0.4677
Equitability (J')	0.6051	0.6541
Chao-1	9	9

The Chao 1 estimator shows that both soils have the specific richness of 9. However, the diversity was slightly higher in *Cajanus* soil (H'= 1.43; 1-D= 0.67) than in *Milletia* soil (H'= 1.33; 1-D= 0.60). With regard to equitability in both soils, sequences were unevenly distributed in the phyla. This shows a low equiabability.

The Venn diagram (Figure 3) shows that 20 OTUs are common to both soil samples. However, the number of OTUs specific to Soil *Cajanus* and *Milletia* was 1 and 4, respectively.

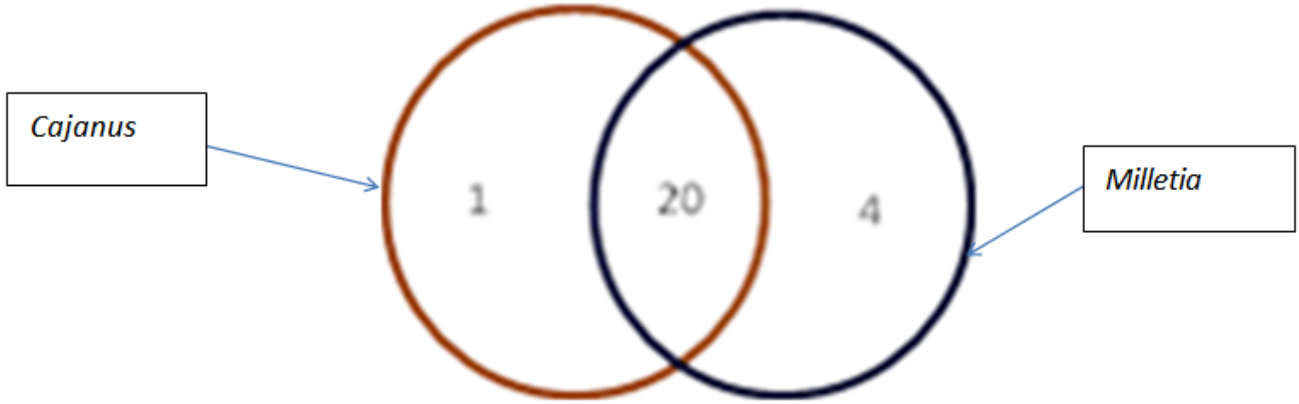

Figure 3. Venn diagram of the dominant genera of the two soils

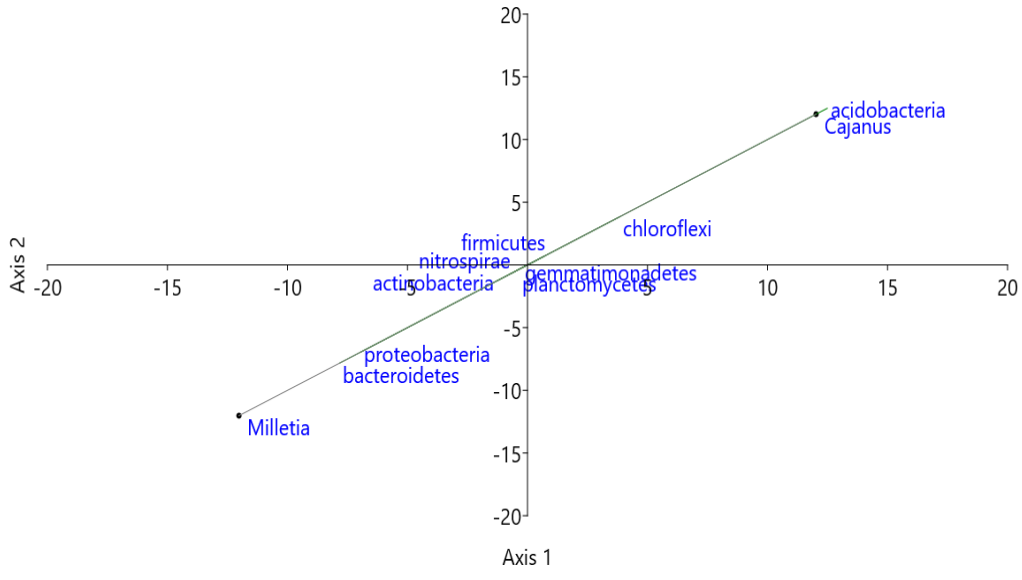

Figure 4. Principal Component Analysis (PCA) of the dominant phyla of the two soils

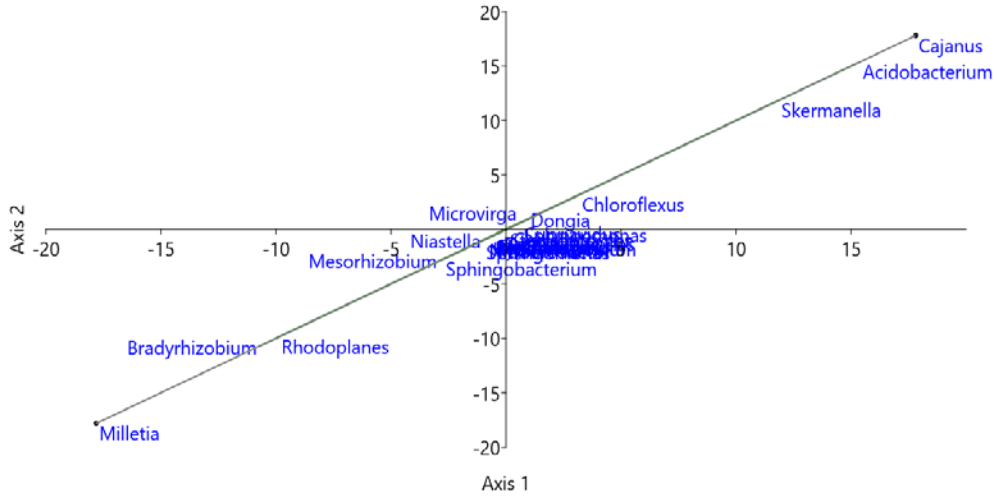

Figure 5. Principal Component Analysis (PCA) of the dominant genera of the two soils

Principal Component Analysis (PCA) was performed with the 9 most abundant phyla for both soils (Figure 4). The results show that Chloroflexi, Firmicutes and Acidobacteria are abundant in *Cajanus* soil while Gemmatimonadetes, Nitrospirae, Planctomycetes, Actinobacteria, Bacteroidetes and Proteobacteria abundant in *Milletia* soil. Taking into account axis 1, Gemmatimonadetes, Planctomycetes, Nitrospirae, Actinobacteria are negatively correlated and Proteobacteria is positively correlated. On the other hand, on axis 2, Chloroflexi, Firmicutes and Acidobacteria are positively correlated while Bacteroidetes is negatively correlated.

At the gender level, the PCA results showed that axes 1 and 2 explain 100% of the total variation (Figure 5). Axis 1 represents 61.52% and axis 2 38.48% of the observed variation. The PCA also shows that the genera Rhodoplanes, Bradyrhizobium, Sphingobacterium, Bacillus and Mesorhizobium are positively correlated with axis 1, but Acidobacterium, Skermanella, Chloroflexus are negatively correlated.

3.2.3. Relative Abundance of Classes

For *Cajanus* soil, 11 classes were the most representative with a relative abundance > 1%. These are: Alphaproteobacteria (38.90%), Acidobacteriia (26.57%), Bacilli (8.19%), Chloroflexia (5.13%), Gammaproteobacteria (4.27%), Betaproteobacteria (3.94%), Actinobacteria (3.24%), Sphingobacteriia (2.50%), Deltaproteobacteria (1.58%), Nitrospira (1.41%) and Gemmatimonadetes (1.12%). While the classes of Alphaproteobacteria (51.44%), Sphingobacteria (9.83%), Acidobacteriia (8.67%), Bacilli (7.18%), Actinobacteria (5.05%), Gammaproteobacteria (3.39%), Gemmatimonadetes (3.39%), Betaproteobacteria (3.28%), Nitrospira (2.42%) and Planctomycetia (1.17%) were the most representative in *Milletia* soil (Figure 6).

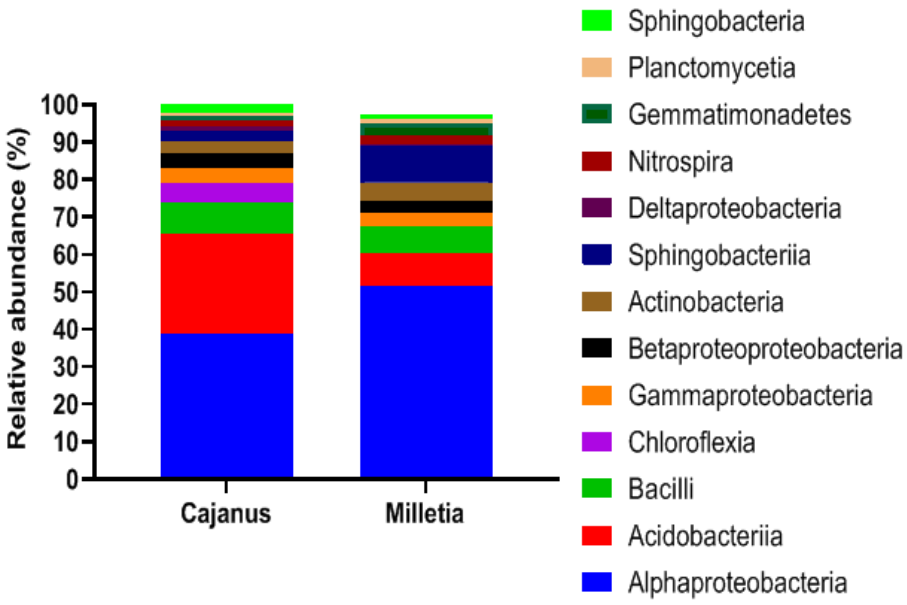

Figure 6. Relative abundance of the dominant classes of the two soils

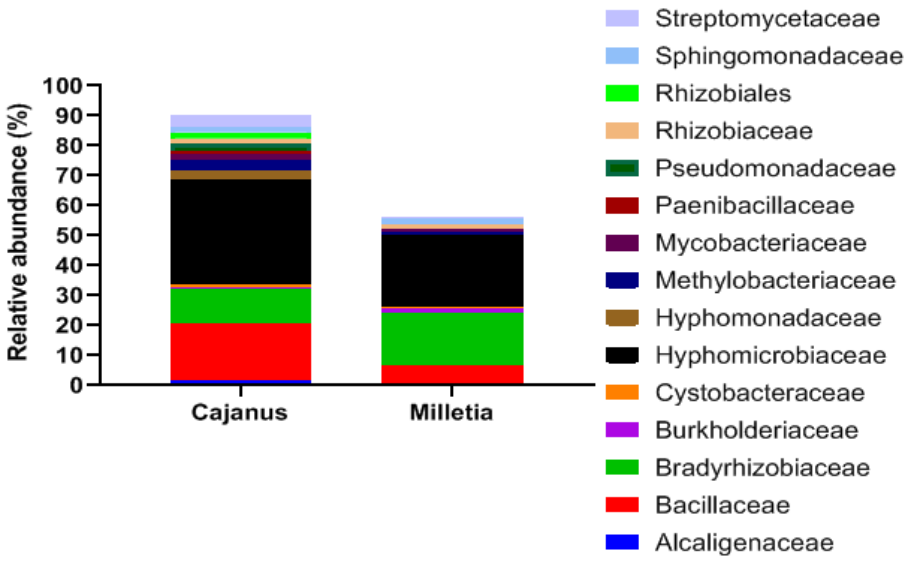

Figure 7. Relative abundance of dominant families of the two soils

3.2.4. Relative Abundance of Families

For *Cajanus* soil, 17 families were the most dominant with an abundance > 1%. These are: Hyphomicrobiaceae (35.05%), Bacillaceae (18.98%), Bradyrhizobiaceae (11.70%), Streptomycetaceae (3.66%), Methylobacteriaceae (3.44%), Hyphomonadaceae (3.00%), Pseudomonadaceae (2.40%), unclassified family Rhizobiales (2.07%), Sphingomonadaceae (1.91%), Mycobacteriaceae (1.75%), Rhizobiaceae (1.58%), Peanibacillaceae (1.58%) and Cystobacteriaceae (1.03%). While for *Milletia* soil, 14 families were the most representative among which: 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%), Sphingomonadaceae (1.97%), Burkholderiaceae (1.72%), Planctomycetaceae (1.17%), Mycobacteriaceae (1.08%) and Rhizobiaceae (1.08%) (Figure 7).

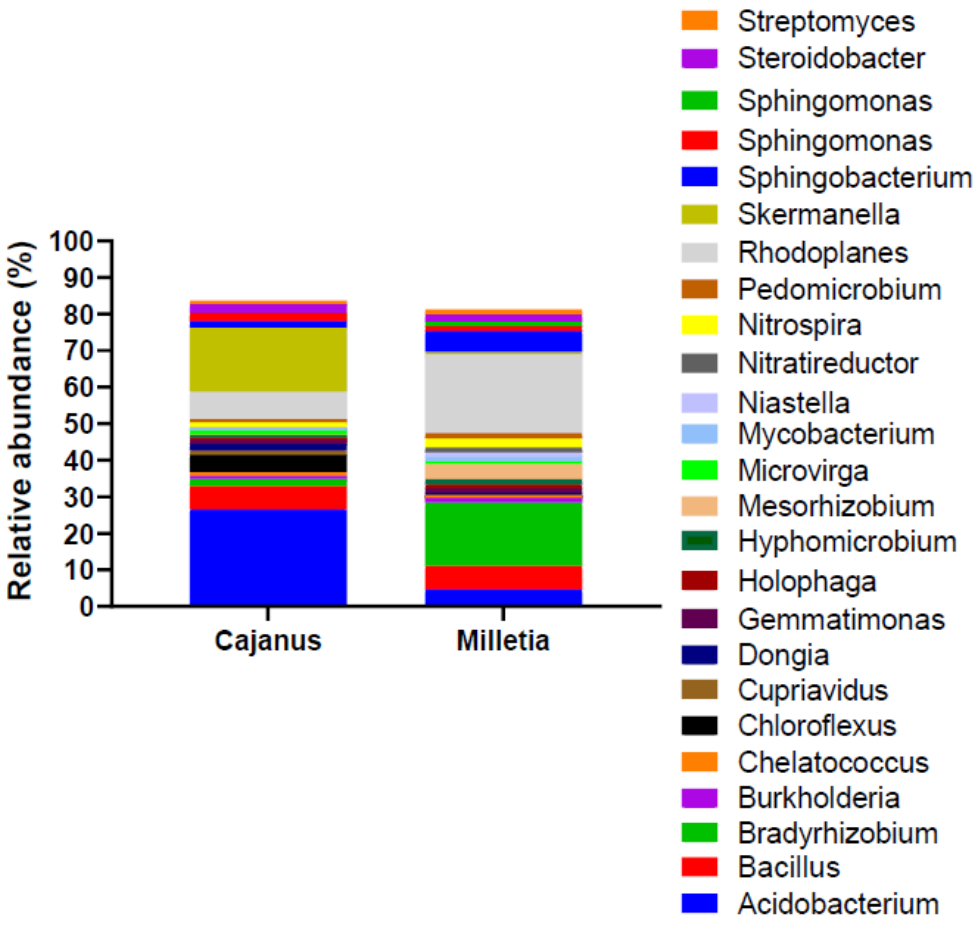

Figure 8. Relative abundance of dominant genera of the two soils

3.2.5. Relative Abundance of Genera

Sixteen genera were the most representative in the *Cajanus* soil, with a relative abundance > 1%. These genera are: 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%). While, in *Milletia* soil, the most representative genera (19) 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 8).

4. Discussion

The rhizosphere of legumes can contain bacteria that can stimulate plant growth and protect them from bio-aggressors. The latter can be used as biological fertilizers or biostimulant microorganisms [24,25]. Knowledge of this microflora can thus allow their isolation and selection. The objective of this study was to study the composition and diversity of bacterial communities in the rhizosphere of *Milletia laurentii* and *Cajanus cajan*, two legumes growing on the soil of the scientific city of Brazzaville, by Sequencing Illumina-Miseq of the 16S rRNA gene. Initially, particle size and physicochemical analyses were carried out. The results showed that soil samples under *Cajanus cajan* and *Milletia laurentii* have a sandy soil texture according to the USDA textural triangle. Although in the study by [16], soil texture was not presented, nevertheless, the clay, silt and sand contents found in our study were very similar to the results of these authors. For the mineral elements C, P, N, NH4+, Mg and Fe, the levels were slightly elevated in the Milletia soil. However, the F-test showed that the difference is not significant. This may be due to the fact that the two plants are not very far apart from each other. In addition, the relatively low content of mineral elements in the two soil samples can be explained by the fact that that Brazzaville capital of the Republic of Congo is located in the tropical zone where the rains are abundant. The latter leach the soils by depleting them of mineral elements. Carbon and nitrogen are two of the most important elements that affect soil productivity and environmental quality [26]. According to [27] it is accepted that the higher the C/N ratio of a product, the slower it degrades in the soil and the more stable humus it provides.

Regarding the diversity of bacterial communities, illumina sequencing resulted in 15316 and 15359 sequences respectively for *Cajanus* and *Milletia* soils. In both soils, analysis of the rarefaction curve showed that the sample effort was achieved illustrated by a plateau. The sequences obtained were grouped into 471 OTUs for *Cajanus* soil and 963 OTUs for *Milletia* soil with a similarity of 97%. Then, OTUs were classified at different taxonomic levels.

Proteobacteria was the most abundant phylum in both soil samples. These results are consistent with previous studies that have shown that Proteobacteria is the phylum the most representative in soils [16,28,29]. The predominance of this phylum is probably due to their metabolic capacity. Indeed, the bacteria belonging to this phylum intervene in biogeochemical cycles [30]. Nevertheless, other phyla have also been identified namely Acidobacteria (27.29%), Firmicutes (8.26%), Chloroflexi (5.83%), Actinobacteria (3.24%), Bacteriodetes (2.53%), Nitrospirae (1.41%) and Gemmatimonadetes (1.12%) for Cajanus soil and Bacteroidetes (13.70%), Acidobacteria (9.46%), Firmicutes (7.21%), Actinobacteria (5.05%), Nitrospirae (2.42%) and Planctomycetes for *Milletia* soil. The microbial community is therefore different from one soil to another. These phyla intervene in various ways in the soil. Indeed, Firmicutes produce metabolites necessary for the biocontrol and growth of plants. As for Acidobacteria, it was pointed out by [31] that bacteria belonging to this phylum are capable of to use nitrite as a source of nitrogen, to adapt to variations in macroelements and nutrients to soil acidity and the production of exopolysaccharides. Moreover, this diversity can be justified by the fact that many previous studies have shown that structure and diversity soil microbial communities are affected by many factors including plant species, soil types, biological selection and farm management [16,32,33,34]. Thus, *Milletia* and *Cajanus* being two different legumes, the leaves that fall around the trunk to form humus and the exudates released at the roots may not have the same composition. This probably influences the composition of the bacterial community. The study of the diversity α at the phylum level showed that the two rhizosphere soil samples have a specific richness of 9 (Chao 1 estimator). However, the diversity was slightly higher in *Cajanus* soil (H' = 1.43; 1-D = 0.67) than in *Milletia* soil (H' = 1.33; 1-D = 0.60). With regard to equitability in both soils, the sequences were unevenly distributed in the phyla. This shows low equitability. According to [35] and [36], bacterial richness and diversity play a crucial role in soil quality and ecosystem sustainability. These authors claimed that reducing soil richness and bacterial diversity could contribute to altering plant performance and insufficient resistance to diseases and pests in continuous crops. At the class level, Alphaproteobacteria were more dominant in both soils (*Milletia* and *Cajanus*). These results are consistent with those found by [37]. However, the abundance of this class was higher in the *Milletia* soil (51.44%) than in the soil of the *Cajanus* rhizosphere (38.90%). The predominance of this class in the soil *Milletia* is probably due to the difference in composition of the leaves and exudates released by the latter since these two legumes grow on the same soil and enjoy the same climate of Brazzaville. [38] claim that Alphaproteobacteria is widespread in soil but is also dominant in nodules, stems and leaves. A field investigation by these authors had indicated that the aerial parts of the plants (leaves) harbor bacterial communities complex and highly variable and that only a small number of bacterial taxa belonging mainly to Alphaproteobacteria

is plant-specific. Other classes have also been identified, these are: Acidobacteriia (26.57%), Bacilli (8.19%), Chloroflexia (5.13%), Gammaproteobacteria (4.27%), Betaproteobacteria (3.94%), Actinobacteria (3.24%), Sphingobacteriia (2.50%), Deltaproteobacteria (1.58%), Nitrospira (1.41%) and Gemmatimonadetes (1.12%) for *Cajanus* soil, and Sphingobacteria (9.83%), Acidobacteriia (8.67%), Bacilli (7.18%), Actinobacteria (5.05%), Gammaproteobacteria (3.39%), Gemmatimonadetes (3.39%), Betaproteobacteria (3.28%), Nitrospira (2.42%) and Planctomycetia (1.17%) for *Milletia* soil. These classes have also been identified in previous studies [16,30].

The Hyphomicrobiaceae family was the most dominant in both soil samples with a relative abundance of 35.05% and 24.22% respectively for the *Cajanus* and *Milletia* rhizospheres. These results are different from those found by [16]. In the study of these authors, it is the family bacillaceae that was dominant in the soil of the Scientific City of Brazzaville, with a relative abundance of 25.37%. While Hyphomicrobiaceae accounted for only 6.40%. These differences may be related to the sampling point that is not the same and the sampling period. Indeed, the collection of ORSTOM soil, in the study of [16], was carried out at the geographical coordinate point 4°16'42.1439" S and 15°14'24.6538" E. While in our study the soil was taken around the point of latitude -4.27825 and longitude 15.24118. Although the points are located in the same site, the environment, in terms of vegetation, is not the same. However, Hyphomicrobiaceae were dominant in SNR soil in the study by [16]. An other authors [39] founded in their study more Hyphomicrobiaceae in the raw soil than in the rhizosphere. Other families have been identified such as: Bacillaceae (18.98%), Bradyrhizobiaceae (11.70%), Streptomycetaceae (3.66%), Methylobacteriaceae (3.44%), Hyphomonadaceae (3.00%), Pseudomonadaceae (2.40%), unclassified family Rhizobiales (2.07%), Sphingomonadaceae (1.91%), Mycobacteriaceae (1.75%), Rhizobiaceae (1.58%), Peanibacillaceae (1.58%) and Cystobacteriaceae (1.03%). While for *Milletia* soil, the most representative families were Bradyrhizobiaceae (17.32%), Bacillaceae (6.49%), Sphingobacteriaceae (5.80%), Chitinophagaceae (4.02%), Flavobacteriaceae (3.70%), Nitrospiraceae (2.42%), Xanthobacteriaceae (2.30), Sinobacteriaceae (2.11%), Sphingomonadaceae (1.97%), Burkholderiaceaes (1.72%), Planctomycetaceae (1.17%), Mycobacteriaceae (1.08%) and Rhizobiaceae (1.08%). These families have also been identified in previous studies. For example, [40] identified, among others, in the rhizosphere of Paeonia jishanensis Sphingomonadaceae, Bradyrhizobiaceae, Chitinophagaceae, Planctomycetaceae, Pseudomonadaceae and Flavobacteriaceae. [38] showed that at the lower taxonomic ranks within Alphaproteobacteria, sequences belonging to members of Methylobacteriaceae and Sphingomonadaceae are more abundant in stems than in soil and nodules. In addition, Methylobacteriaceae and Sphingomonadaceae have been found as endophytes in a number of plants [38]. Bacteria in these families can benefit from plant life through their ability to use methanol (as a carbon source) released by the metabolism of the pectin that makes up the plant cell wall [38].

The Venn diagram made from the dominant genera showed that the two soil samples of the rhizosphere of *Milletia* and *Cajanus* have 20 common genera confirming the proximity of sampling points. Nevertheless, four genera have been specific to the *Milletia* rhizosphere and one genus specific to the *Cajanus* rhizosphere. This difference can be justified by the composition of the leaves and exudates secreted at the roots. In terms of genera, *Acidobacterium* was the most dominant genus in the *Cajanus* rhizosphere with a relative abundance of (26.55%) while in the *Milletia* rhizosphere, the genus *Rhodoplanes* was the most dominant with a relative abundance of 21.63%. These results are different from those found by [16] at ground level in the scientific city of Brazzaville (e.g. ORSTOM). Indeed, these authors found *Bacillus* as the dominant genus with a relative abundance of 25.27%. However, *Acidobacterium* (8.49%) and *Rhodoplanes* (15.48%) were more abundant in the rhizosphere of MFILOU and SNR respectively. According to [41], different plant species secrete different types of root exudates, this can change the structure of microbial communities at the rhizosphere. The following genera have also been identified with abundances > 1%. These are: *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%). While, in the *Milletia* soil, the most representative were *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%). These genera have been identified in previous studies [30; 40; 16; 42; 43]. Bacteria belonging to its different taxonomic genera play an important role in plant growth. Indeed, bacteria of the genus Bradyrhizobium enrich the medium with nitrogen which can also promote the transfer of nitrogen in the medium to non-legume plants, through root-root contact, mycorrhizal networks, root exudates and following the decomposition of nitrogen-enriched residues [42]. With regard to the genus *Bacillus*, [44] have shown that they form a group of bacteria with very diverse enzymatic activities (proteolytic, amylolytic, pectinolytic, cellulolytic, lipasic) and produce metabolites such as bacteriocins and other antimicrobial molecules. Also, they possess the ability to withstand harsh environmental conditions due to their stability and natural rigidity. [42] point out that *Bacillus* secrete several metabolites not only to improve plant growth but also to inhibit the microbial growth of pathogens in the soil by degrading cell walls. As for bacteria belonging to the genus Mesorhizobium, [45] point out that they are symbiotic bacteria of legumes, nitrogen fixers, belonging to the group of rhizobacteria. The latter promote plant growth by solubilizing phosphate which leads to an increase in crop productivity. [46] showed that Rhizobium/Bradyrhizobium co-inoculation

increases root weight and shoots, plant vigor, nitrogen fixation and grain yield of various legumes. Thus, Rhizobium, Gram-negative bacteria living in the soil, promote plant growth by fixing atmospheric nitrogen, in symbiotic association with legumes and also improve soil fertility [47]. With regard to Sphingobacterium, [48] also indicated that they are producers of siderophores that provide iron to plants that is present in the soil as insoluble ferric oxide by making it unavailable to plant pathogens. As for [49], bacteria of the genus Streptomyces, also identified in our study, produce abundant metabolites that play various roles in agriculture such as plant growth and resistance to plant pathogens. It was indicated in the study conducted by [50] that Streptomyces can produce metabolites including cellulase and natamycin under the conditions of sound-based solid-state fermentation. These bacteria belong to the group of rhizobacteria that promote plant growth. It has been reported by [44], that these bacteria can alleviate abiotic stress in plants and can help resist cold stress by inducing the production of antioxidants and the secretion of phytohormones in plants. Thus, several of the identified genera may have an interest in agriculture and/or biotechnology.

5. Conclusion

The diversity and structure of bacterial communities in the rhizosphere of two legumes *Cajanus cajan* and *Milletia laurentii* growing on the same soil were determined using the Illumina Miseq technique targeting the 16S rRNA gene. The results showed that the *Cajanus* rhizosphere is slightly more diverse than the *Milletia* rhizosphere. Nevertheless, the rhizosphere of these two soils contains phyla that contain bacteria such as *Bradyrhizobium, Bacillus, Sphingobacterium, Mesorhizobium, Rhizobium, Streptomyces* capable of producing phytohormones, antimicrobial molecules to eliminate plant pathogens, mineralize organic matter and even fix atmospheric nitrogen. Thus, these soils can be used as a source for the isolation of microbial biofertilizers.

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Conflict of Interest

The authors do not declare any conflict of interest in this work.

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