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Diversity of the Bacterial Community of Three Soils Revealed by Illumina-Miseq Sequencing of 16S rRNA Gene in the South of Brazzaville, Congo

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Abstract The study was conducted on three soils (MFILOU, SNR and ORSTOM) at the south of Brazzaville in Congo. The aim of the work was to study the composition and diversity of the soil microbial community. Microbial diversity was assessed using the Miseq-Sequencing. The results showed that microbial diversity was represented by 1289 OTUs assigned to the Bacteria (1282 OTUs), Fungi (2 OTUs), Viridiplantae (3 OTUs) and metazoa (2OTUs) domain with 97% of similarity. The 1282 bacterial OTUs were affiliated with 12 phyla, 31 classes, 59 orders, 122 families, 288 genera et 521 species for all sites. The Proteobacteria (45.59%-29.92%), Firmicutes (27.27%-7.35%), Acidobacteria (16.74%-10.15%), Actinobacteria (12.35%-5.07%) and Nitrospirae are the most abundant common phyla for the three sites. The most dominant common classes were Alphaproteobacteria (38.87%-24.77%), Bacilli (27.20%-6.95%), Acidobacteria (16.47%-7.59%), Actinobacteria (12.35%-5.07%) and Nitrospira (9.81%-1.78%). The most abundant common orders for the 3 sites are represented by Rhizobiales (33.96%-20.38%), Bacillales (27.20%-6.95%), Acidobacteriales (16.49%-7.61%), Actinomycetales (10.20%-3.6%) and Nitrospirales (9.81%-1.78%). Bacillaceae (25.37%-5.60%), Acidobacteriaceae (16.49%-7.61%), Bradyrhizobiaceae (10.81%-4.61%), Nitrospiraceae (9.81%-1.78%) and Chitinophagaceae (4.24%-1.59%) were the best distributed common families in the microbial community of the three sites. Bacillus (25.27%-5.56%), yhe most abundant and common genera were Rhodoplanes (15.48%-5.30%), Bradyrhizobium (10.74%-4.39%), Nitrospira (9.81%-1.78%) and Acidobacterium (8.49%-6.51%). At species level, *Rhodoplanes spp.* (15.26%-5.10%), *Bradyrhizobium spp.* (10.59%-4.35%) and *Acidobacterium spp.* (8.49%-6.51%) were the most common and abundant in the three soils.

Keywords: *soil microbial community, microbial diversity, Illumina-Miseq*

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1. Introduction

Microbial communities of soil play a key role in ecosystems. Bacteria, fungi and archaea are closely related to nutrient and energy cycling, which support the structure and functional stability of ecosystem [1,2]. Kuczynski et al [3] have reported that composition and diversity of soil microbial communities are influenced by different soil characteristics and vegetation types under land-use change (soil carbon, nutrient depletion and reduced water holding capacity). Soil microbes play important roles in plant growth and health. The higher abundance of beneficial microbes (Bacillus, Agromyces, Micromonospora, Pseudonocardia, Acremonium, Lysobacter, Mesorhizobium, Microvirga, Bradyrhizobium, Acremonium, Chaetomium) are positively related to the higher soil quality, including better plant growth, lower disease incidence, and higher nutrients contents, soil enzyme activities and soil pH. Several authors found that soil texture represents one of the most important factors influencing the structure of microbial communities as well as, pH, cation exchange capacity, and organic matter content. Soil texture can affect microbial community structure directly by providing a suitable habitat for specific microorganisms which in turn making a maximum degradation process [4]. In other hand some works have found that soil water content is a main driver of soil microbial community structure rather than soil pH [5,6]. Zhang et al., [7]

showed that different vegetation types had different effects on soil bacterial community structure. Wang et al., [8] claimed that diversity and catabolic activity of the bacterial community are greatly impacted by the composition of different vegetation types. Several studies found that the microbial community composition and alpha-diversity varied significantly over time, whereas the change in beta-diversity was relatively small [9,10]. Among soil microbes, bacteria play a pivotal role in crop production, supplying nutrients to crops, stimulating plant growth, controlling the activity of plant pathogens, and improving soil structure [11]. Bacteria are integral components of soil, where their community structure and diversity have been found to be linked to many soil environmental characteristics, such as the physical and chemical properties of the soil [12,13].

In the Congo, apart from the studies by Lebonguy et al [14], no study on the composition and diversity of soil microorganisms in different ecosystems is available. These kinds of studies are need for improving the assessment of the status of microbial community and diversity of different ecosystems of Congo. Thus, the present study was carried out to improve the knowledge of the diversity and composition of the bacterial community in the rhizospheric soil of different types of legumes. The aim of the study was to know how the characteristics of these soils impact the diversity and composition of the bacterial community under the legumes. For this study, we used Illumina-Miseq sequencing of PCR amplified 16S rRNA genes from total DNA to assess bacterial diversity, community structure, and the relative abundance of bacterial taxa within three sites from the south of Brazzaville in Congo.

2. Material and Methods

2.1. Study Site and Soil Sampling

The study was conduted in south of Brazzaville in three sites : MFILOU (4°15'50,7600" S, 15°14'6,1512" E), SNR (4°16'41,4944" S, 15°15'5,7341" E) and ORSTOM (4°16'42,1439" S, 15°14'24,6538" E). The climate of Brazzaville is characterized by a rainy season from October to May and a dry and cool season from June to September. The average annual temperature is 26 ° C in the rainy season and 20 ° C in the dry season. The average annual rainfall is 1200mm. The soil is sandy loam. The vegetation of MFILOU and SNR was characterised by legumes and non-legumes while at ORSTOM, the main species were *Panicum maximum, Indigofera hirsuta, Mimosa pudica and Milletia laurentii.*

The rhizospheric soil samples were collected at five locations in each site. The soil samples were taken in the 0-20 cm horizon using a 5 cm diameter auger. The five soil samples of each site were put together to form one composite soil. The soil was then transported to the laboratory in iceboxes. At the laboratory, the stones, various debris and roots were removed from the soil by sieving with a sieve of 2mm mesh. Each sample was homogenized then 10 g of soils were put in the sterilized tubes and stored at -80°C for DNA extraction. The remaining soils were used for an analysis of the physicochemical properties of the soil.

2.2. Soil Physicochemical Properties

The soil pH was measured with a « OHAUS STARTER 3000 » pH meter using soil suspension with deionized distilled water (1:25 w/v). Total organic carbon content of soil samples was determined using Walkley-Black method [15]. Total nitrogen was determined using the Kjeldahl method described by Bremner and Mulvaney (1982) [16]. The ammoniacal nitrogen is extracted over 10g of soil after stirring for 30 minutes in 50ml of a 1N KCL solution on Shaker. After decantation and filtration on filter paper, the assay was carried out on a 10 ml aliquot of supernatant supplemented with 1 mL of Nessler's reagent [16]. The phosphorus was determined using the Olsen method [17]. Total iron was determined using DEB method [18]. The magnesium was extracted on 20g of soil with 150 mL of ammonium acetate at pH7. The mixture is stirred for 1 hour then filtered through a filter paper without ash. Then, the soil extract was used to determine spectrophotometrically the magnesium.

2.3. DNA Extraction, illumina-MiSeq Sequencing and Bioinformatic Analyses

DNA extraction, Miseq sequencing and bioinformatic analyses were performed by Mr DNA laboratories in USA.

Genomic DNA was extracted from 0.5 g of soil samples (dry weight) using the PowerSoil kit (MOBIO Laboratories, Carlsbad, CA, USA). The concentration of the extracted DNA was determined using a Nanodrop 2000C Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The DNA extract from the soil samples was kept at 80°C until it was used.

The V4 variable region of the bacterial 16S rRNA gene were amplified with primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGTWTCTAAT-3'). The following program was used to conduct the PCR reaction: 3 min of denaturation at 94°C, 30-35 cycles of 30 s at 94°C for amplification, 40 s at 53°C for annealing, 1min at 72°C for elongation, and a final extension at 72°C for 5 min.

After amplification, PCR products are checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Multiple samples are pooled together (e.g., 100 samples) in equal proportions based on their molecular weight and DNA concentrations. Pooled samples are purified using calibrated Ampure XP beads. Then the pooled and purified PCR product is used to prepare Illumina DNA library. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq following the manufacturer's guidelines. Sequence data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, sequences were joined, depleted of barcodes then sequences <150bp removed, sequences with ambiguous base calls removed. Sequences were denoised, OTUs generated and chimeras removed. Operational taxonomic units (OTUs) were defined by clustering at 3% divergence (97% similarity). Final OTUs were

taxonomically classified using BLASTn against a curated database derived from RDPII and NCBI (www.ncbi.nlm.nih.gov, http://rdp.cme.msu.edu).

2.4. Statistical Analysis

One-way analyses of variance (ANOVA) and relative abundance of phyla and classes were carried out using Excel 2013. For alpha diversity, all analyses were based on the OTU clusters with a cutoff of 3% dissimilarity. Chao1, Shannon and Simpson indexes, equitability J' were calculated using PAST software (v.3.26) to estimate the richness and diversity of the bacterial community of each site. Rarefaction curves with the average number of observed OTUs were generated using PAST software (v.3.01) to compare relative levels of bacterial OTU diversity across the three different sites. The dendrogram of site similarity index was plotted from the Bray-Curtis association matrix. The principal Component Analysis (PCA) and Redundancy analysis (RDA) were performed by CANOCO software (v 4.56) to assess the beta diversity. At the end, Venn diagrams were constructed to visualize shared and unique genera between sites.

3. Results

3.1. Soil Physicochemical Properties

Table 1 shows the results of physicochemical properties. In the three sites sand was the main content of the soil. While Clay was the lower soil component of these three sites. The pH of soil varied from 6.89% in the soil of ORSTOM to 7,21% in the soil of MFILOU. The highest carbon contents (16.2‰), total nitrogen (1.7‰), phosphorus (0.4‰), iron (0.37%), NH_4^+ (0.8‰) and magnesium (1.0‰) were observed in the soil of ORSTOM site. These chemical properties showed the lowest content in the two other soils (MFILOU and SNR).

Table 1. Soil characteristics

Sites	Clay	Slit	Sand	pH	C	N	P	Fe	NH_4^+	Mg
	%				‰					
MFILOU	5.50	21.91	67.52	7.21	10.5	1.0	0.3	2.5	0.02	0.5
SNR	4	15.33	78.74	5.01	14.2	1.2	0.2	3.3	0.02	0.7
ORSTOM	5.77	19.54	73.19	6.89	16.2	1.7	0.4	3.7	0.08	1.0

3.2. Bacterial Community Composition

The sequences obtained from 16S rARN sequencing of the soils were grouped with 97% of similarity sequence, in 1289 valid OTUs. Among these OTUs, 99.45% (1282 OTUs) have been assigned to the domain of Bacteria and 0.55% was attributed to Fungi, Metazoa and Viridiplantae. The bacterial OTUs was assigned to 12 phyla, 31 classes, 59 orders, 122 famillies, 288 genera et 521 species for all sites. ANOVA applied to phyla, classes, orders, families, genera and species does not show any significant difference between soils of the three prospected sites (p>0,05). From the 1282 bacterial OTUs, 960 OTUs were affiliated to MFILOU (with 10463 individuals), 825 OTUs to SNR (with 8062 individuals) and 855 OTUs to ORSTOM (with 11133 individuals). The dendrogram of similarity (Figure 1) between soils shows two clusters: the first was formed by ORSTOM, SNR and MFILOU were combined in the second cluster. The value of Bray-Curtis similarity between SNR and MFILOU was 0.59. The rarefaction curves (Figure 2) plotted with Shannon index and number of individuals showed that MFILOU has the greatest diversity followed by SNR and ORSTOM. On the other hand ORSTOM had the highest number of individuals than MFILOU and SNR.

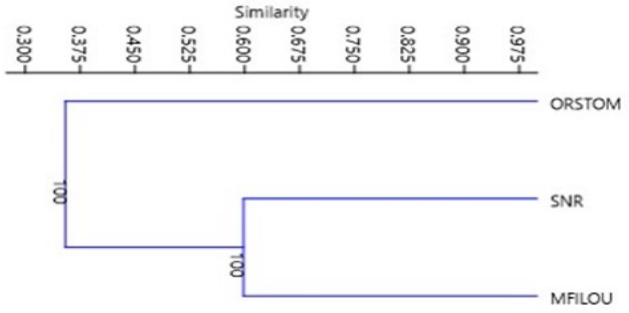

Figure 1. Dendrogram UPMGA of the three soils (Bray-Curtis Similarity and OTUs)

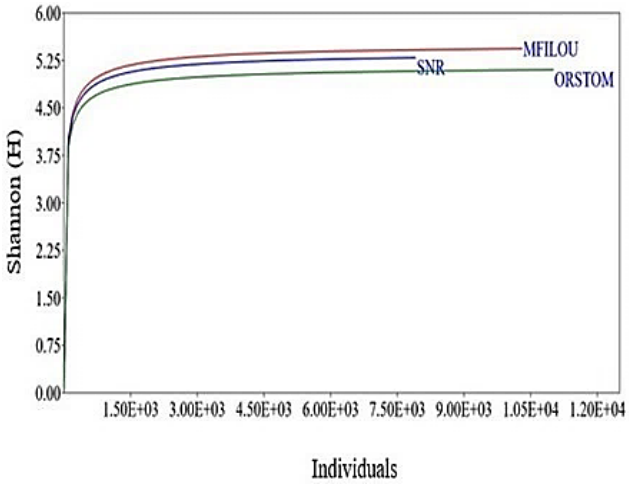

Figure 2. Rarefaction curve plotted against Shannon index and individuals

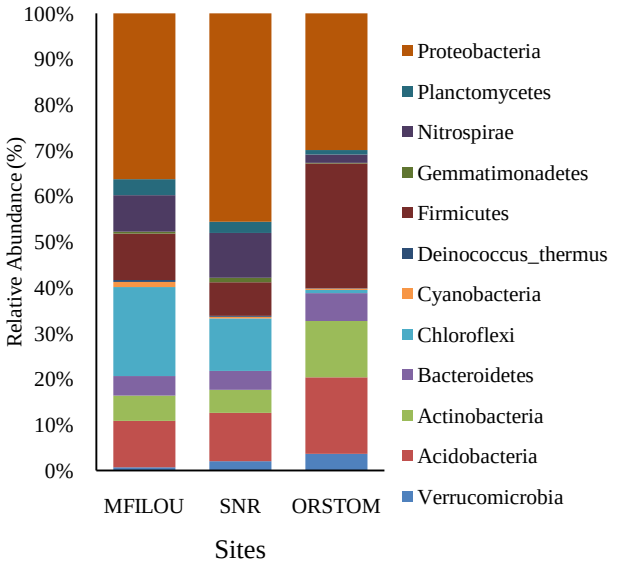

Figure 3. Relative abundance of the dominant phyla in the three sites

3.3. Relative Abundance of Phyla

Among the 12 phyla founded in the present study, only 7 for ORSTOM have a relative abundance ≥1% while this number was 9 for MFILOU and SNR; Proteobacteria is the most dominant phylum in all sites (Figure 3).

At MFILOU, the most representative phyla are: Proteobacteria (36.25%), followed by Chloroflexi (19.44%), Acidobacteria (10.15%), Firmicutes (10.13%), Nitrospirae (7.93%), Actinobacteria (5.55%), Bacteroidetes (4.26%), Planctomycetes (3.54%) and Cyanobacteria (1.12%). At SNR, the most dominant phyla are: Proteobacteria (45.59%), Chloroflexi (11.51%), Acidobacteria (10.58%), Nitrospirae (9.81%), Firmicutes (7.35%), Actinobacteria (5.07%), Bacteroidetes (4.10%), Planctomycetes (2.46%) and Verrucomicrobia (2%). At ORSTOM, Proteobacteria (29.92%) is followed by Firmicutes (27.27%.), Acidobacteria (16.74%), Actinobacteria (12.35%), Bacteroidetes (6.06%), Verrucomicrobia (3.62%) and Nitrospirae (1.78%). Chao1 indicated that phyla richness were the same in the 3 sites (Table 2). According to Shannon and Simpson indexes the phyla diversity of MFILOU (H'=1.89; 1-D= 0.79) was higher than in SNR (H'= 1.79; 1-D= 0.74) and ORSTOM (H'=1.74; 1-D= 0.78). The phyla equitability (J') showed that at MFILOU (J'=0.76) phyla were more equitably distributed than in SNR (J'=0.72) and ORSTOM (J'=0.70).

Table 2. Alpha diversity indexes and richness at phylum level

Alpha diversity index	MFILOU	SNR	ORSTOM
S (number of phylum)	12	12	12
N (number of individuals)	10463	8062	11133
Simpson index (1-D)	0.7975	0.7472	0.7873
Shannon index (H')	1.899	1.793	1.749
Equitability (e^H/S)	0.5565	0.5004	0.4792
Equitability (J')	0.7641	0.7214	0.7039
Chao-1	12	12	12

PCA plotted with phyla (Figure 4) showed that Nitrospirae, Proteobacteria and Gemmatimonadetes are abundant at SNR while Cyanobacteria, Deinococcus_thermus, Planctomycetes and Chloroflexi are abundant at MFILOU. On the other hand, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria and Verrucomicrobia are abundant at ORSTOM. The two first axis of PCA explained 100% of variations observed between soils at phylum level. Axis 1 explained87.3% of variation while axis 2 explained 12.7% of variation. By taking into account axis1, Nitrospirae, Proteobacteria and Gemmatimonadetes are negatively correlated to SNR while Cyanobacteria, Deinococcus_thermus, Planctomycetes and Chloroflexi are negatively correlated to MFILOU. On the other hand, Firmicutes, Bacteroidetes, Actinobacteria, Acidobacteria and Verrucomicrobia are positively correlated to ORSTOM. By taking into account axis 2, Nitrospirae, Proteobacteria and Gemmatimonadetes are negatively correlated to SNR while Actinobacteria, Acidobacteria and Verrucomicrobia are negatively correlated to ORSTOM. Cyanobacteria, Deinococcus_thermus, Planctomycetes, Chloroflexi, Firmicutes and Bacteroidetes are positively correlated to MFILOU.

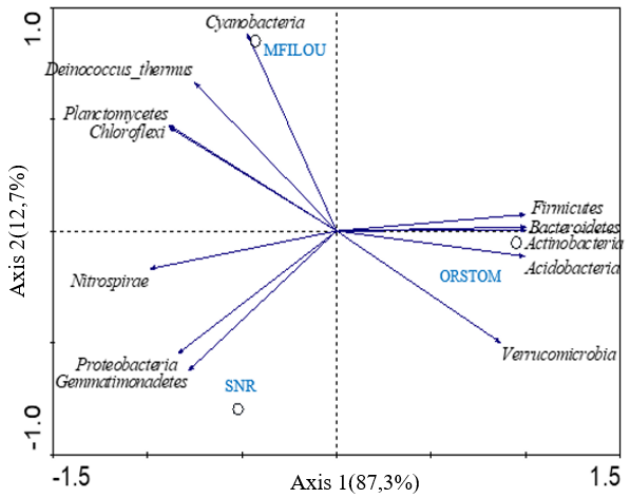

Figure 4. PCA plotted with the bacterial phyla

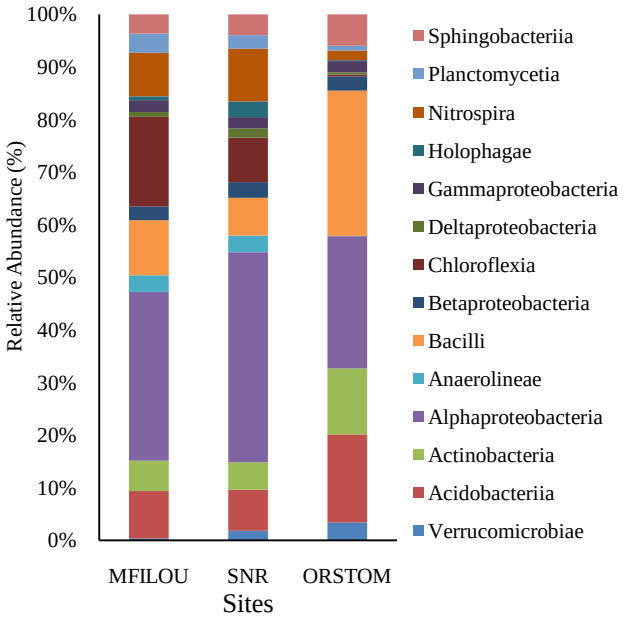

Figure 5. Relative abundances of the dominant classes in the three sites

3.4. Relative abundance of Classes

In this study 31 classes were obtained from the 3 sites. At MFILOU 11 classes were retrieved, 14 at SNR and 9 at ORSTOM with a relative abundance ≥1% (Figure 5). At MFILOU Alphaproteobacteria (30.73%) was the most dominant class followed by Chloroflexia (16.36%), Bacilli (10%), Acidobacteriia (8.63%), Nitrospira (7.93%), Actinobacteria (5.55%), Planctomycetia (3.49%), Sphingobacteriia (3.47%), Anaerolineae (3.03%), Betaproteobacteria (2.48%), Gammaproteobacteria (2.18%). While at SNR the most dominant class was Alphaproteobacteria (38.87%) followed by Nitrospira (9.81%), Chloroflexia (8.23%), Acidobacteriia (7.59%), Bacilli (6.95%), Actinobacteria (5.07%), Sphingobacteriia (3.87%), Anaerolineae (3.12%), Betaproteobacteria (2.91%), Holophagae (2.86%), Planctomycetia (2.45%), Gammaproteobacteria (2.1%), Verrucomicrobiae (1.77%) and Deltaproteobacteria (1.69%). However, at ORSTOM Bacilli (27.20%) was the most dominant class followed by

Alphaproteobacteria (24.77%), Acidobacteriia (16.47%), Actinobacteria (12.35%), Sphingobacteriia (5.86%), Verrucomicrobiae (3.33%), Betaproteobacteria (2.62%), Gammaproteobacteria (2.11%), Nitrospira (1.78%).

3.5. Relative Abundance of Orders

The 1282 OTUs were grouped into 59 taxonomic orders. Among these orders, 13, 15 and 12 were represented with a relative abondance ≥ 1% respectively at MFILOU, SNR and ORSTOM (Figure 6). At MFILOU, Rhizobiales (22.85%) is the most represented order followed by Chloroflexales (16.25%), Bacillales (10%), Acidobacteriales (8.65%), Nitrospirales (7.93%), Rhodospirillales (4.11%), Actinomycetales (4.10%), Planctomycetales (3.49%), Sphingobacteriales (3.47%), Sphingomonadales (3.45%), Anaerolineales (3.03%), Burkholderiales (1.96%) and Xanthomonadales (1.51%). At SNR, Rhizobiales (33.96%) is the most represented order followed by Nitrospirales (9.81 %), Chloroflexales (8.22%), Acidobacteriales (7.61%), Bacillales (6.95%), Sphingobacteriales (3.87%), Rhodospirillales (3.7%), Actinomycetales (3.6%), Anaerolineales (3.12%), Holophagales (2.86%), Planctomycetales (2.45%), Burkholderiales (2.40%), Verrucomicrobiales (1.77%), Xanthomonadales (1.63%) and Sphingomonadales (1%). At ORSTOM Bacillales (27.20%) is the most represented order followed by Rhizobiales (20.38%), Acidobacteriales (16.49%), Actinomycetales (10.20%), Sphingobacteriales (5.86%), Verrucomicrobiales (3.33%), Sphingomonadales (2.85%), Burkholderiales (2.49%), Xanthomonadales (2.03%), Solirubrobacterales (1.96%), Nitrospirales (1.78%) and Rhodospirillales (1.15%).

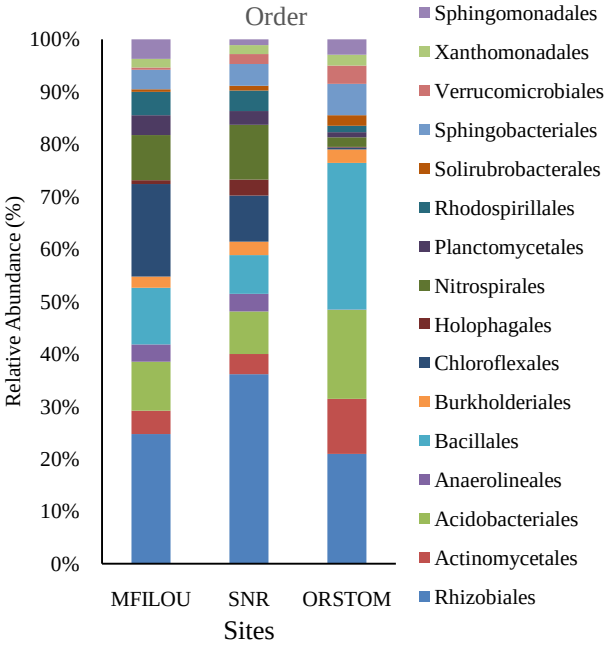

Figure 6. Relative abundance of the dominant bacterial order in the three sites

3.6. Relative abundance of Families

A total of 122 bacterial families were obtained in the tested soils. However, only 15, 17 and 16 families respectively at MFILOU, SNR and ORSTOM have a relative abundance ≥1% (Figure 7). At MFILOU, Chloroflexaceae (16.09%) is the most represented family followed by Hyphomicrobiaceae (10.56%), Acidobacteriaceae (8.65%), Bacillaceae (8.35%), Nitrospiraceae (7.93%), Bradyrhizobiaceae (4.61%), Rhodospirillaceae (4.05%), Sphingomonadaceae (3.35%), Anaerolineaceae (3.03%), Methylobacteriaceae (2.69%), Rhodobiaceae (2.21%), Sphingobacteriaceae (1.86%), Chitinophagaceae (1.59%), Sinobacteraceae (1.36%) and Rhizobiaceae (1.20%). At SNR, Hyphomicrobiaceae (19.75%) is the most represented family followed by Nitrospiraceae (9.81%), Bradyrhizobiaceae (8.12%), Chloroflexaceae (8.11%), Acidobacteriaceae (7.61%), Bacillaceae (5.60%), Rhodospirillaceae (3.58%), Anaerolineaceae (3.12%), Holophagaceae (2.86%), Sphingobacteriaceae (2.05%), Chitinophagaceae (1.81%), Rhodobiaceae (1.68%), Rhizobiaceae (1.66%), Verrucomicrobiaceae (1.6%), Sinobacteraceae (1.42%), Burkholderiaceae (1.17%) and Sphingomonadaceae (1%). At ORSTOM the most represented family is Bacillaceae (25.37%) followed by Acidobacteriaceae (16.49%), Bradyrhizobiaceae (10.81%), Hyphomicrobiaceae (6.40%), Chitinophagaceae (4.23%), Pseudonocardiaceae (4.20%), Verrucomicrobiaceae (3.26%), Sphingomonadaceae (2.78%), Nitrospiraceae (1.78%), Sphingobacteriaceae (1.63%), Streptomycetaceae (1.4%), Paenibacillaceae (1.30%), Sinobacteraceae (1.29%), Burkholderiaceae (1.16%), Conexibacteraceae (1.14%) and Methylobacteriaceae (1.10%).

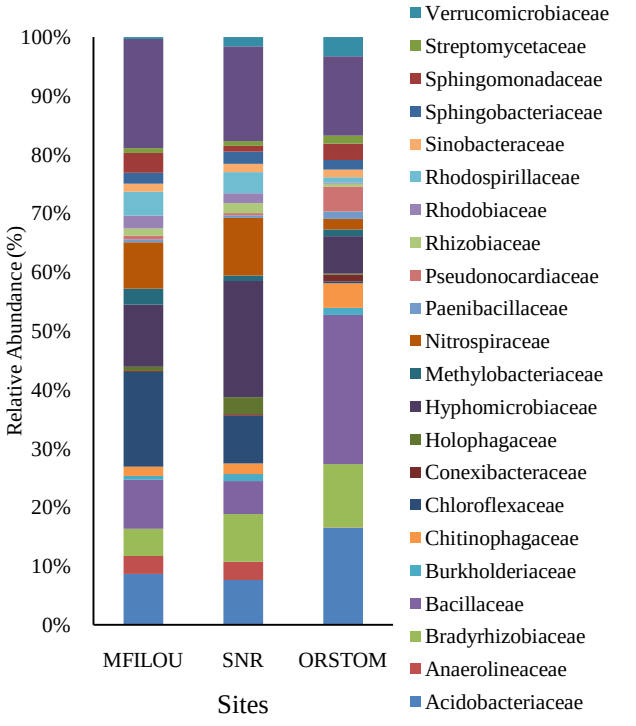

Figure 7. Relative abundance of the main family in the three sites

3.7. Relative Abundance of Genera

In this study 288 taxonomic genera have been founded and the most representative of them (with a relative abundance ≥1%) are presented in Figure 8. The most representative genera are Acidobacterium (8.49%)

followed by Bacillus (8.19%), Nitrospira (7.93%), Rhodoplanes (6.54%), Bradyrhizobium (4.39%), Skermanella (2.85%), Microvirga (2.63%), Sphingomonas (2.37%), Rhodobium (2.21%), Hyphomicrobium (2.10%), Pirellula (2.03%), Sphingobacterium (1.84%), Bellilinea (1.77%), Longilinea (1.26%), Steroidobacter (1.19%) and Pedomicrobium (1.01%) for MFILOU. While at SNR, Rhodoplanes (15.48%) is followed by Nitrospira (9.81%), Bradyrhizobium (7.93%), Acidobacterium (6.51%), Bacillus (5.56%), Holophaga (2.51%), Hyphomicrobium (2.30%), Skermanella (2.25%), Bellilinea (2.18%), Sphingobacterium (2.04%), Rhodobium (1.68%), Prosthecobacter (1.48%), Pirellula (1.42%), Steroidobacter (1.40%) and Pedomicrobium (1.04%). On the other hand, at ORSTOM Bacillus (25.27%) is followed by Bradyrhizobium (10.74%), Acidobacterium (7.10%), Rhodoplanes (5.30%), Terriglobus (4.39%), Pseudonocardia (3.58%), Flavisolibacter (3.36%), Prosthecobacter (3.17%), Candidatus koribacter (3.16%), Nitrospira (1.78%), Sphingobacterium (1.61%), Sphingomonas (1.56%), Steroidobacter (1.29%), Streptomyces (1.23%), Edaphobacter (1.22%), Conexibacter (1.14%) et Oxalophagus (1.01%).

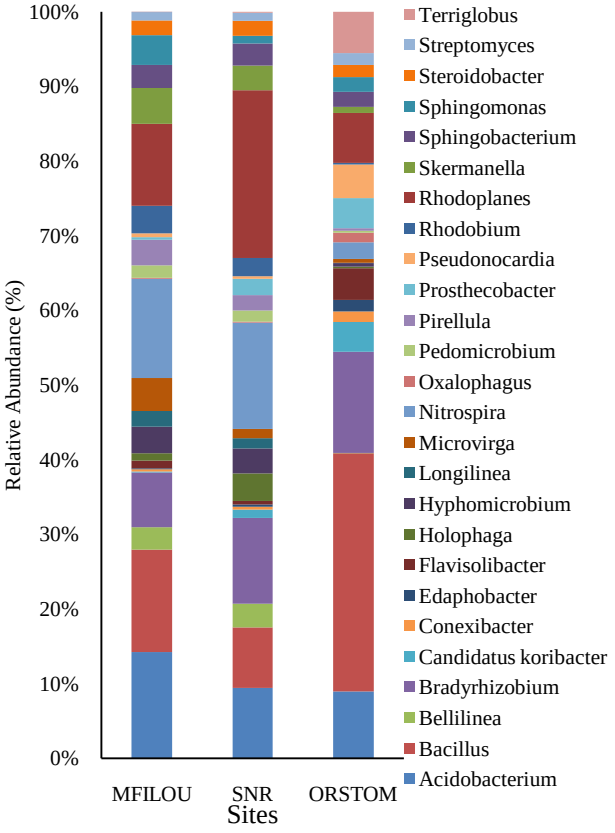

Figure 8. Relative abundances of the dominant genus in the three sites

3.8. Relative abundance of species

The bacterial OTU are distributed in 521 species and the most abundant are reported in Figure 9. The 5 most dominant species are *Chloroflexus spp*. (16.09%), *Acidobacterium spp*. (8.49%), *Nitrospira spp* (7.93%), *Rhodoplanes spp*. (6.42%) and *Bradyrhizobium spp*. (4.35%) for MFILOU; *Rhodoplanes spp*. (15.26%), *Nitrospira spp*. (9.81%), *Chloroflexus spp*. (8.11%), *Bradyrhizobium spp*. (7.87%) and *Acidobacterium spp*. (6.51%) for SNR; *Bacillus sp*. (11.17%), *Bradyrhizobium spp*. (10.59%), A*cidobacterium spp*. (7.07%), *Rhodoplanes spp*. (5.10%) and *Bacillus spp*. (4.86%) for ORSTOM.

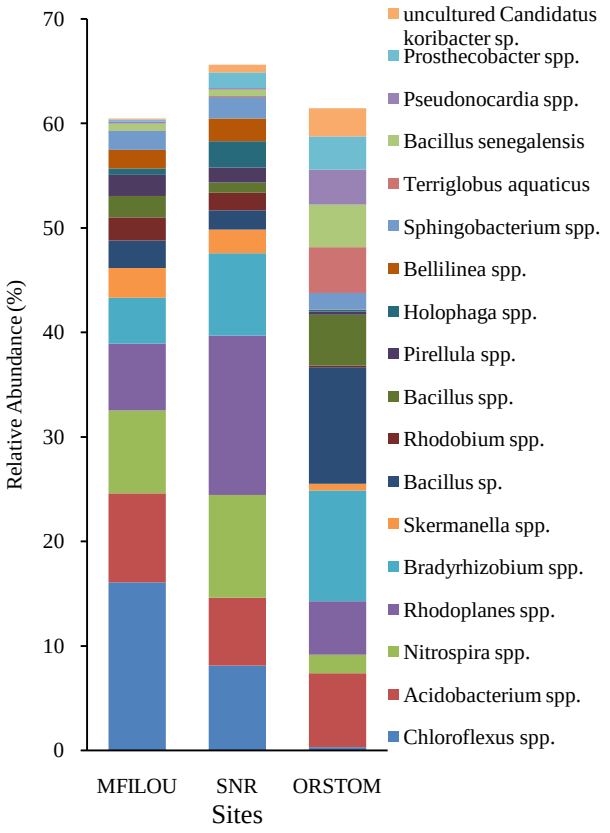

Figure 9. Relative abundance of the 18 most abundant species of the 3 sites

3.9. Alpha and Beta Diversity

Diversity index and richness were also calculated at genera level (Table 3). Chao-1 indicated that the genera richness were highest at MFILOU (280) than SNR (270.4) and ORSTOM (240.8). According to Shannon and Simpson indexes the diversity at MFILOU (H'=3.66, 1-D=0.942) and SNR (H'=3.61, 1-D=0.941) were similar and higher than ORSTOM (H'=3.37, 1-D=0.90). The genera equitability (J') was similar at MFILOU (0.672) and SNR (0.673). Venn diagram (Figure 10) showed that the three soils shared 152 genera. MFILOU and ORSTOM shared 24 genera while SNR and MFILOU 35 genera. On the other hand SNR and ORSTOM shared 17 genera. The number of genera specific to MFILOU, SNR and ORSTOM was respectively 23, 11 and 24.

Table 3. Alpha Diversity index and richness at genera level

Alpha diversity index	MFILOU	SNR	ORSTOM
S (nombre de genres)	234	215	217
N (nombre d'individus)	10463	8062	11133
Simpson index (1-D)	0,9421	0,9413	0,908
Shannon index (H')	3,667	3,618	3,37
Equitability (e^H/S)	0,1672	0,1732	0,134
Equitability (J')	0,6722	0,6736	0,6265
Chao-1	281	270,4	240,8

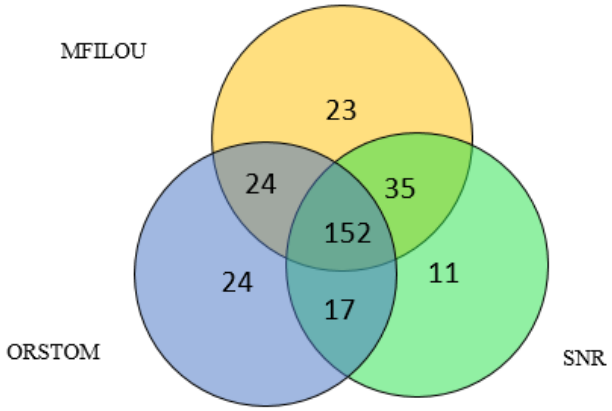

Figure 10. Venn Diagram of genus between the three sites

PCA was plotted with the 10 most abundant genera for all soils (Figure 11). Bacillus and Bradyrhizobium are abundant at ORSTOM while Acidobacterium Sphingomonas, Nitrospira, Hyphomicrobium, Rhodobium, Skermanella, and Microvirga are abundant at MFILOU; Rhodoplanes is abundant at SNR. Axis 1 and axis 2 explained 100% of variations observed between soil at genera level. Axis 1 explained 92.9% of variations while axis 2 explained 7.1% of variations. By taking in account axis1, Bacillus and Bradyrhizobium are positively correlated to ORSTOM. Acidobacerium and Sphingomonas are positively correlated to MFILOU while Microvirga Nitrospira, Hyphomicrobium, Rhodobium and Skermanella are negatively correlated to MFILOU. On the other hand, Rhodoplanes are negatively correlated to SNR. By taking in account axis 2, Bradyrhizobium is negatively correlated to ORSTOM while Bacillus is positively correlated to ORSTOM. Sphingomonas, Acidobacterium, Microvirga, Nitrospira, Hyphomicrobium, Rhodobium and Skermanella are positively correlated to MFILOU. Rhodoplanes is negatively correlated to SNR.

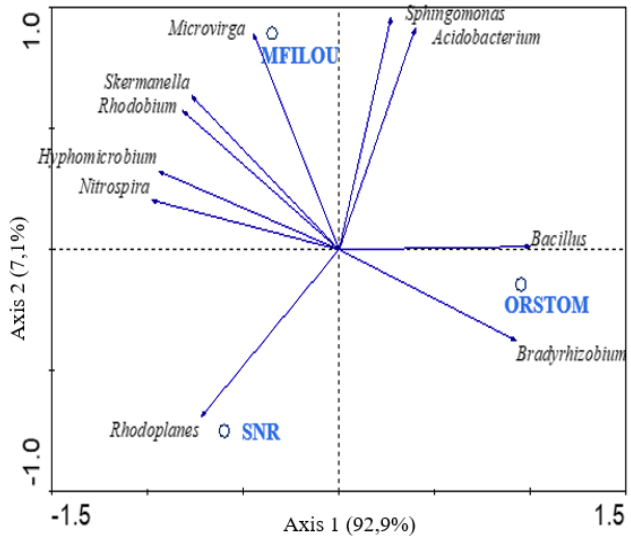

Figure 11. PCA plotted with the 10 most abundant genera of bacterial community

In this study, RDA was used to explore relationships between environmental factors and soil microbial community structure. RDA was plotted with the 10 most abundant genera and the physicochemical characteristics of soil. According to the RDA analysis the 12 environmental parameters (Table 3) accounted for 100% of the variation in the observed bacterial community structure. Axis 1 explained 92.9% of this variation and axis 2 explained the other 7.1% (Figure 12). We found that sand and kind of nitrogen components, and carbon were the driver of bacterial communities respectively at SNR for Rhodoplanes and ORSTOM for Bradyrhizobium and Bacilli while clay, pH and silt impacted the distribution of bacteria at MFILOU for Acidobacterium, Sphingomonas, Microvirga, Rhodobium, Skermanella, Ntrospira.

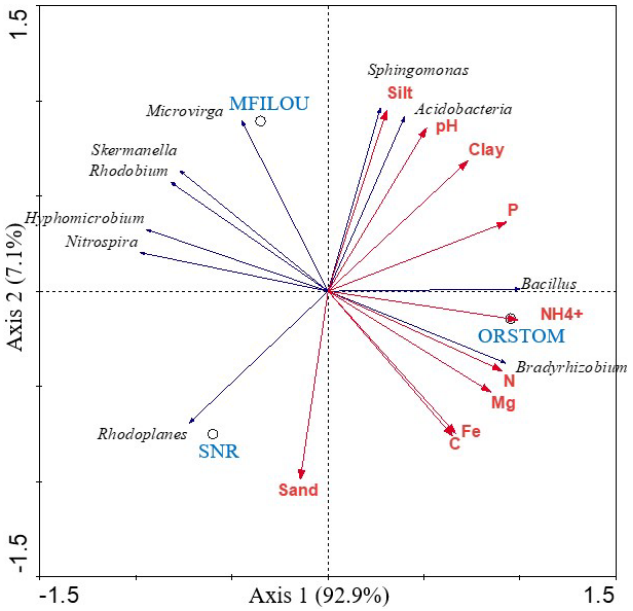

Figure 12. RDA plotted with the 10 most abundant genera and physiochemical properties of soils

4. Discussion

Soil is an extremely rich environment which harbors a multitude of microorganisms, among which bacteria are the most abundant in term of biomass and taxonomic diversity. Bacteria are the most plentiful and diverse group of microorganisms in the soil, and by number of species found per gram of soil [19]. These assertions agreed with our result in the fact that 99,45% of OTU found were attributed to bacteria. For the three sites prospected, at phylum level, all the bacteria were related to Proteobacteria, Chloroflexi, Actinobacteria, Acidobacteria, Planctomycetes, Nitrospira, Verrucomicrobia, Bacteroidetes, Gemmatimonadetes, Firmicutes, Deinococcus_thermus, Cyanobacteria. We found that Proteobacteria was the most dominant phylum in all sites (45,59% - 29,92%). Alami et al. [20] found in their study on the rhizospheric soil of *Coptis chinensis* that Proteobacteria, Actinibacteria, Chloroflexi, Acidobacteria, Bacteroidetes, Gemmatimonadetes were the dominant bacterial phyla. Among these phyla, Proteobacteria was the most dominant phylum as in our study. This kind of result was also found by Sun et al [21] in a tropical forest soil in China, where they found that Proteobacteria, Planctomycetes, Nitrospirae, Verrucomicrobia, Bacteroidetes, Chloroflexi, Acidobacteria and Actinobacteria constituted the major phyla. This assertion was assumed by several authors in their works, Wang et al [8] on the soil covered by halophytic vegetation in China, Zang et al

[22] in soil of mixed bamboo and broad-leaved forest in China, Wang et al [23] in a Mineral Sandy loam Soil with a lower pH in the Desert of Maine (USA), Goma-Tchimbakala and Lebonguy [14] in Sandy-Loam Soil Polluted by hydrocarbons in Congo. The present study and all the previous cited above have shown that the bacterial community of soils at phylum level is similar although belonging to different environments and having different characteristics.

Several studies found that the microbial community composition and alpha-diversity varied significantly over time, whereas the change in beta-diversity was relatively small [9] and [10]. In this study, alpha diversity represented by richness (S observed and Chao1) and diversity index (Shannon and Simpson indexes, equitability) declined from the cultivated soils of MFILOU and SNR to the soil of ORSTOM that was exempt of culture. This result is in accordance with several other studies in which it was demonstrated that continuous cultivation resulted in decrease of bacterial diversity [20]. According to Alami et al. [20] and Kennedy et al. [24], microbial richness and diversity have a crucial role in soil quality, health and ecosystem sustainability. These authors claimed that the reduction of soil microbial richness and diversity could contribute to alter plant performance and insufficient resistance against disease and pest in the continuous cultures. Hamarashid et al [25] have shown that bacterial population in loamy sand was higher than in sandy loam soil. In the present study, the result showed that in ORSTOM and MFILOU in which soils were sandy loam bacterial number was higher than in loamy sand soil of SNR. The dendrogram (UPMGA) has shown two clusters: the first formed by ORSTOM and the second by SNR and MFILOU. This result can be explained by the fact that SNR and MFILOU are agricultural soil therefore subject to several practices such as green manure amendment for soil enrichment while ORSTOM is a forest and was not subjected to any agricultural practices. Many previous studies showed that soil microbial communities structure and diversity are affected by many factors, including plant species, soil types, organic breeding, and agricultural management (e.g., fertilizer application, periodic outflow, and crop rotation) [19,26,27,28]. Venn diagram and the rarefaction curves plotted against Shannon index have shown that bacterial community of SNR and MFILOU is more diverse than of ORSTOM but bacterial community of ORSTOM is greater in term of number of bacteria than SNR and MFILOU. The great diversity and low number of bacteria of MFILOU and SNR can be explained by agricultural practices. The choice of cultivation method is essential to increase crop yield and suppress disease, and it may cause changes in microbial communities as different plant species secrete different types of root exudates, which can alter the structure of the soil bacterial community [29]. On the other hand, Chen et al [30] have shown that the microbial biomass in the soil with legumes is higher than soil with grass. At ORSTOM around soil sampling point (within a radius of 1m) the vegetation were composed by *Indigofera hirsuta* which is a spontaneous leguminous plant while at SNR and MFILOU vegetation were mixt comprising legumes and non-legume plant.

In this study, RDA showed that soil pH, clay and silt have an influence on distribution of Acidobabacterium, Sphingomonas, Microvirga, Rhodobium, Ntrospirae that found to be most related with soil of MFILOU while in soil of ORSTOM the main components that impacted bacteria distribution of Bradyrhizobium and Bacillus were phosphorus, total nitrogen, ammonium, and magnesium. Soil pH has been demonstrated to be one of the main factors determining soil microbial composition and diversity and has also been found to be correlate with soil bacterial phylogenic diversity, with peak diversity near neutral pH [12]. Some other studies showed that soil pH was closely correlated with bacterial diversity and soil microbial communities [31,32,33]. At SNR, bacterial distribution of Rhodoplanes was greatly related with the sand content. The findings of our study were in accordance with Lauber et al. [12] and Fierer et al [13] they claimed that bacteria are integral components of soil, where their community structure and diversity have been found to be linked to many soil environmental characteristics, such as the physical and chemical properties of the soil.

5. Conclusion

In conclusion, in this study we found that Alphaproteobacteria, Bacilli, Acidobacteria, Actinobacteria and Nitrospira were the main phyla in the three sites. Our finding revealed that bacterial richness and diversity were relatively high in agricultural soil (MFILOU AND SNR) than in soil under ORSTOM. We found that sand and kind of nitrogen components, and carbon were the driver of bacterial communities respectively at SNR and ORSTOM while clay, pH and silt impacted the distribution of bacteria at MFILOU.

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Conflict of Interest

The authors declare no conflict of interest in this work.

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