Scientific Research Publishing

Advances in Microbiology, 2023, 13, 477-498
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

16S rRNA Gene-Based Metagenomic Analysis of Soil Bacterial Diversity in Brazzaville, Republic of the Congo

Irène Marie Cécile Mboukou Kimbatsa[1,2], Itsouhou Ngô[1,2*], Armel Ibala Zamba[1,3], Faly Armel Soloka Mabika[1,2], Thantique Moutali Lingouangou[1,2], Joseph Goma-Tchimbakala[2,3], Etienne Nguimbi[1,2,3]

[1]Laboratoire de Biologie Cellulaire et Moléculaire (BCM), Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, Republic of the Congo
[2]Unité de Recherche en Bioinformatique et Microbiologie Moléculaire, Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville, Republic of the Congo
[3]Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN), Brazzaville, Republic of the Congo
Email: irenemboukou@gmail.com, *itsoung21@gmail.com, armel.ibalazamba@umng.cg, armelmabika@gmail.com, thantiquemoutali40@gmail.com, goma.tchimbakala@gmail.com, etienne.ng1612@gmail.com

How to cite this paper: Kimbatsa, I.M.C.M., Ngô, I., Zamba, A.I., Mabika, F.A.S., Lingouangou, T.M., Goma-Tchimbakala, J. and Nguimbi, E. (2023) 16S rRNA Gene-Based Metagenomic Analysis of Soil Bacterial Diversity in Brazzaville, Republic of the Congo. *Advances in Microbiology*, **13**, 477-498.
https://doi.org/10.4236/aim.2023.139031

Received: August 3, 2023
Accepted: September 22, 2023
Published: September 25, 2023

Abstract

Soil contains a great diversity of microorganisms, among which are bacteria. This study aimed to explore bacterial diversity in soil samples in Brazzaville in the Republic of the Congo. Environmental DNA was extracted. The illumina MiSeq sequencing was held and the diversity indices have been computed. Illumina MiSeq sequencing revealed 21 Phyla, four of which were abundant: Proteobacteria, Acidobacteria, Actinobacteria and Bacteroidetes. Soil microbial communities in the studied samples were phylogenetically diverse but with a stable community structure. 17 classes are represented with relative abundances of Rihzobiales, Bacillales, Actinomycetales and Acidobacteriales. 40 families, the Alphaproteobacteria, the Bacilli and the 12 Actinobacteria. 83 orders among which the Rhizobiales are the most abundant followed by Bacillales and the least abundant followed by the Flavobacteriaceae. Of the 28 genera listed, the Bradyrhizobium is the most dominant in Mw3 and Mw4. 25 listed species, Bradyrhizobium, Bacillus, Actinoplanes, and Candidatu coribacter Acidobacterium are the most abundant species. The Shannon indices of Mw3 and Mw4 are equal, the H'max of Mw4 is greater than the H'max of Mw3. The Simpson index of Mw4 is equal to the Simpson index of Mw3, and the Pielou index (J) of Mw4 is less than the R of Mw3, but very close. This study opens interesting perspectives on the knowledge and exploitation of telluric bacteria in several areas of life.

Keywords

Metagenomic, Sequencing, 16S rRNA Gene, Soil, Bacteria

1. Introduction

Over the past 20 years, soil microbial ecology has undergone a real technological revolution in the way it characterizes communities of microorganisms thanks to the advent of molecular biology tools [1]. These recent advanced techniques are based on the direct extraction of genetic information (DNA) from the soil and the characterization of bacterial and fungal sequences from this nucleic acid matrix. These approaches have the advantage of overcoming the biases associated with the culture of soil-borne microorganisms, of which it is often estimated that only 0.1% to 1% of them can be cultivated on synthetic media. These recent advanced techniques, thus, offer new perspectives in terms of resolution and understanding of the distribution of diversity in the role of soil and bran in the biological functioning of concentrates.

In addition, these molecular tools are easy to standardize and their costs have been falling steadily over the past ten years. These advantages, therefore, make it possible to work at medium throughput, making it possible today to characterize microbial communities on large sets of samples (several hundred to several thousand) that integrate large spatial or temporal scales. In most European countries, there are soil monitoring networks that consist of a systematic determination of soil characteristics and their large-scale temporal and spatial variations [2]. However, most of these monitoring networks often only take into account the pedoclimatic parameters of the soils without any integration of biological parameters and even less of the diversity of native organisms. If we reframe the interest of these networks in a dynamic of large-scale characterization of soil biological diversity, they become very relevant study supports.

Soil microbial communities dominate the biogeochemical cycle, while playing a key role in natural ecosystems [3]. Soil microorganisms stimulate the transformation and recycling of organic matter residing in the soil and of elemental nutrients, such as C and N [4].

They are incredibly active and diverse, and play invaluable roles in maintaining soil structure, conserving soil fertility, soil formation and development, and system stability [5]. Soil microbes also play an essential role in plant growth and crop production [6].

Most bacteria are harmless to humans, some even being essential for the body to function properly. However, there are many pathogenic species that cause infectious diseases such as cholera, syphilis, anthrax, and tuberculosis [7] [8]. Humans very early on used the properties of bacteria to feed and heal themselves. Today, the fields of application are very varied. In the food industry, bacteria, such as Lactobacillus, Lactococcus or Streptococcus, combined with yeasts and

molds, are involved in the preparation of fermented foods, such as cheeses, yogurts, and beer [9]. Recently, many studies have been published on the influences of different land use patterns on soil microbiological communities, as well as on their metabolic activity and functional capacity [10] [11], including chemical and organic fertilizers and anthropogenic interventions [11] [12].

The main reservoir in terms of cell number and diversity is found at the soil level with around 108 - 109 bacteria per gram of soil. However, more research is needed to explain the effects on soil microorganisms of certain types of land use, such as secondary forests, plantations and agricultural land. In agriculture, certain bacteria can be used instead of pesticides in biological control to fight plant parasites [13] (e.g. *Bacillus thuringiensis*), and other bacteria will have a beneficial effect on the growth of plants, such as PGPRs [14].

In the medical field, bacterial isolates have been selected on the basis of their capacity to produce secondary metabolites that can be used as drugs and genetic engineering has made it possible to improve their performance or even to cause bacteria to produce compounds initially derived from other organisms (production of antibiotics, insulin, growth hormone, ...) [15] [16] [17]. However, the majority of these bacteria have not yet been identified or characterized because they remain recalcitrant to *in vitro* culture on culture media that may have been offered to them [18].

These limits imposed by *in vitro* culture have allowed the development of independent approaches. The advent of metagenomics in the 1990s restricted the study of bacteria to their DNA directly extracted from the environment [19]. These very numerous bacteria with very low numbers also constitute a reservoir of genetic information and in particular of genes that can be transferred between the different members of the bacterial community by horizontal transfer mechanisms that are a powerful engine of bacterial evolution, allowing in particular the very rapid adaptation of these microorganisms to biotic or abiotic changes in their environment [20].

In the Republic of the Congo, the land area is of great importance in the various localities, from an agricultural and cultural point of view. Bacteria will be like in other lands presenting a diversity, certainly correlating with many other factors.

In this work, the objective is to explore bacterial soil diversity, thus determining through the taxonomy of different phyla, classes, orders, families and operational taxonomic units, the metagenomic sequencing of 16S RNA gene has been used on the specific illumina. Two soil-sample composite has been concerned in this study, all were from Brazzaville in the Republic of the Congo. The only environmental parameter that was measured for this study was the pH.

2. Materials and Methods

2.1. GPS Coordinates of Stations, Climate Characteristics, Soil Sampling and Processing

The study was carried out in Brazzaville (situation de Brazzaville GPS). In Brazza-

ville, the climate is characterized by a rainy season from October to June and a dry and cool season from July to September. The average annual rainfall is 1250 mm and the average annual temperature is 28˚C. Two stations were used: MW3 (4˚16'14.4"S; 15˚15'43.1"E; altitude 315 m) and MW4 (4˚16'15.8"S; 15˚15'09.3"E; altitude 314 m).

All samples were collected from November 2016 to September 2017. Six soil samples were collected in clean, dry and sterile glass Falcone tubes with a sterile spatula. Three soil samples were collected by station. For each station, the three samples were mixed to have a composite soil, in the purpose of getting more bacterial diversity of each station. In the same stations, sample collection sites were separated by 100 m. All samples were transferred to the Laboratory of Cellular and Molecular Biology of the Faculty of Sciences and Techniques at University Marien Ngouabi, under sterile conditions for analysis. Table 1 shows the GPS coordinates of Stations in this study.

2.2. pH Measurement

The pH was determined with a model pH Tester type pH meter on a solution with a ratio of weight of soil to volume of sterile distilled water of 1/2.5. Table 2 shows the pH values of different sites.

2.3. Characterization of Soils Bacterial Diversity

2.3.1. DNA Extraction

DNA of soils microorganisms can be extracted using many procedures. In this study, soil microbial metagenomic DNA was isolated with a soil DNA kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. DNA extracts were stored at −20˚C for the next PCR amplification.

Table 1. GPS coordinates of stations.

Stations	Composites	Sites	GPS coordinates
1	MW3	1. Beach Brazzaville	04˚16'09.4"S 015˚17'51.0"E A: 272 m
		2. Bassin du Terminal du Congo	04˚15'29.0"S 015˚17'58.0"E A: 276 m
		3. Mamiwata	04˚16'39.9"S 015˚17'00.1"E A: 272 m
2	MW4	4. Station Puma	04˚16'14.4"S 015˚15'43.1"E A: 315 m
		5. Ministère des Affaire Étrangères	04˚15'58.1"S 015˚15'31.2"E A: 310 m
		6. Tennis Club	04˚16'15.8"S 015˚15'09.3"E A: 314 m

Table 2. Different pH values of the stations used for soil samples collection.

Composites	**MW3**			**MW4**		
Sites	**1**	**2**	**3**	**4**	**5**	**6**
pH	6.8	6.5	6.4	6.7	6.5	7

2.3.2. PCR Amplification of 16S rRNA Gene and Sequencing

16S rRNA gene amplification and high throughput sequencing were performed. The universal 16S rRNA gene primers were 515F (5'-GTGCCAGCMGCCGCGG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The barcode and adapter have been incorporated between the adapter and the front primers. The PCR was carried out in ABI GeneAmp 9700 (USA). The PCR mix contained 10 ng of DNA template plate, 2 μL of 2.5 mM dNTP, 0.8 μL of both primers, 0.4 μL of FastPfu polymerase, 4 μL of 5x FastPfu buffer and sterile bidistilled H2O in a total volume of 20 μL of PCR amplification. The PCR process consisted of an initial 5 min denaturation at 95˚C, followed by 27 cycles of denaturation at 95˚C for 30 s, and annealing at 55˚C for 30 s, with extension at 72˚C for 45 s. The same sample was mixed with the PCR product with detection by electrophoresis on 2% agar. Using the AxyPrepDNA gel extraction kit (Axygen Biosciences, Union City, CA, US), PCR products were cut, eluted with Tris-HCl and subjected to detection of 2% agarose electrophoresis at the same time. The QuantiFluor™-ST blue fluorescence quantitative system (Promega, US) for PCR products was used for detection.

The final DNA fragments completed with the primer base and were attached to a chip, for a laser. Chemical cutting of "fluorescent groups" and "end groups" was performed, and the viscosity at the end of 3' was restored. During this time, the second nucleotide was aggregated and the fluorescent signal results in each round were recalculated and collected, while the template DNA obtained has been illumina Miseqsequenced.

2.3.3. Bioinformatic Analyses of Sequences and Statistics

The overlap relationship was used to obtain the PE readings while performing quality control and filtering the quality of the sequence at the same time. OTU taxonomist analysis, cluster analysis and diversity index analysis were performed after distinguishing the samples. At the same time, we have carried out various analyzes of various indices. The detection of the sequencing depth was also performed based on an OTU clustering analysis. The analysis of the community structure in each classification level was conducted by the taxonomy information. Based on the above analysis, the study was carried out on a serial analysis of community structure, system development and visualization. According to the similarity levels, all the sequences were taken using the OTU division.

Meanwhile, using biological information from OTU, below 97% similarity level, statistical analysis was performed. All optimized sequences were mapped to the representative OTU sequence, and those sequences which have the level of similarity of greater than 97% were selected when generating the OTU form.

The analysis of the community structure in each classification level was conducted by the taxonomy information.

The frequency curve was obtained from the sequencing depth of the sample. A rarefaction analysis was conducted with a 97% OTU of similarity, using Mothur

and R language tools to create a graph.

The indices of bacterial diversity were as follows: Chao—the Chao1 estimator (http://www.mothur.org/wiki/Chao); Ace—the ACE estimator (http://www.mothur.org/wiki/Ace); Shannon—Shannon index (http://www.mothur.org/wiki/Shannon); Simpson—the Simpson index (http://www.mothur.org/wiki/Simpson); and the Sequencing Depth Coverage Index—Good's Coverage (http://www.mothur.org/wiki/Coverage).

After processing all reads, the statistical processing of the dataset comprised a total of 10,230 high quality sequences, following removal of chimeras, with an average of 1543 sequences obtained from each soil sample. After several levels of sequence processing, quality filtering and sequence number normalization were followed by 3% dissimilarity clustering. To determine the rarefaction curves, richness and diversity, 80,000 readings were randomly selected from each sample.

These analyzes were carried out exclusively on the basis of data collected in Brazzaville as part of the biodiversity study (structure of bacteria communities according to the variables of the sites chosen at random). Microsoft Excel was used for statistical analyzes of the data. The heat map representation of the relative abundance of bacterial OTUs among the samples was developed using Excel.

A Principal Component Analysis (PCA) was also performed based on composition profiles at the class level. We used CANOCO 4 software for Windows [21], and the PRIMER version 5 program [22].

PCA was used to study the relationship between different species and different sites. However, to study the distribution of the species of bacteria in the samples (Mw3 and Mw4), we used canonical analysis techniques [23].

3. Results

3.1. GPS Coordinates of Sites and pH Values

Table 1 and Table 2 show respectively the GPS coordinates of different sites of soils sample collection and the pH values of each site before the composite soil. The pH values are all comprise in between 6.5 and 7.00, around the neutral composition.

3.2. Composition of the Bacterial Community

A total of 10,230 OTUs were obtained for the two soils. Following the filtering 1543 valid OTUs were retained with 97.7% similarity. These OTUs are divided into 17 phyla including 8 phyla for MW3 soil and 9 phyla for MW4 soil, 10 classes including 5 classes for Mw3 and 5 classes for Mw4, 15 orders including 7 orders for Mw3 and 8 orders for Mw4, 33 families including 15 families for Mw3 and 17 families for Mw4, 28 genera including 14 genera for Mw3 and 14 genera for Mw4, and 28 species including 14 species for Mw3 and 14 species for Mw4.

3.2.1. Relative Abundance of Phyla

Figure 1 shows the relative abundances of the two soils (Mw3 and Mw4). The

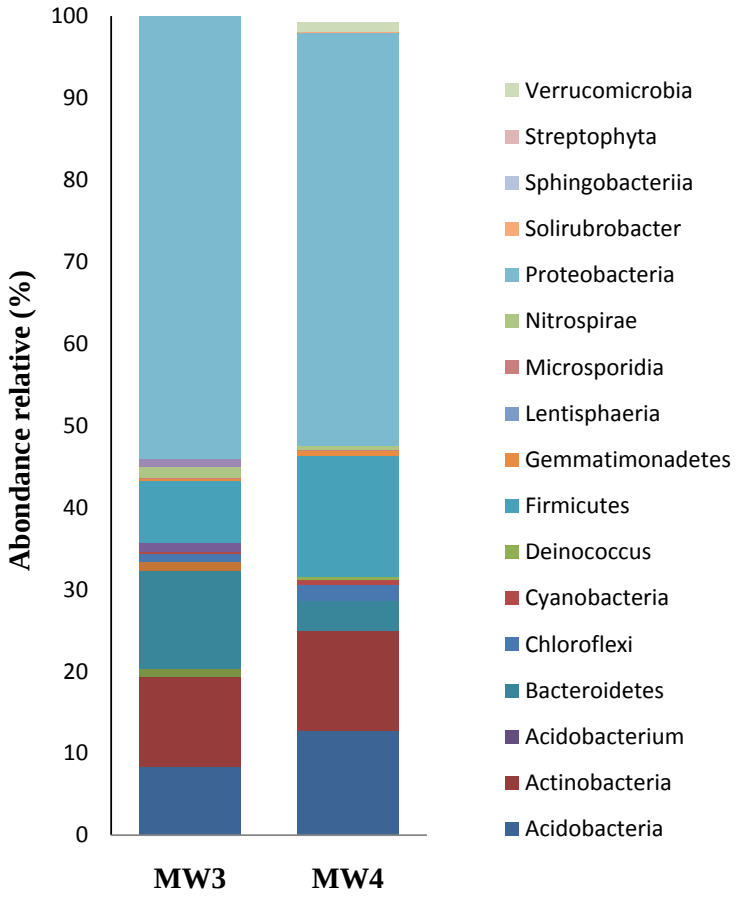

Figure 1. Taxonomy and relative abundances of the most representative phyla in soil samples (Mw3 and Mw4).

most abundant phyla are Protéobacteria with Mw3 55.33% and Mw4 50.51% followed by Firmicutes (Mw3 7.63% and Mw414.81%), Acidobacteria with Mw3 8.38% and Mw4 12.78% and Actinobacteria with Mw3 10.92% and Mw4 12.15% respectively. On the other hand, the least abundant are verrucomicrobia and solirubrobacteria with parent abundances of less than 1%.

3.2.2. Relative Class Abundance

Figure 2 shows the different classes of the two soils. The dominant classes are represented: Aphaproteobacteria with Mw3 45.50% and Mw4 39.72% respectively, followed by Actinobacteria 12.07% in soil Mw3 and 9.63% in Mw4, Acidobacteria with 10.97% in Mw3 soil sample and 8.71% in Mw4. Bacteriodetes (Mw3 12.95 and 3.67 Mw4). Verrucomicrobia are the least abundant class.

3.2.3. Relative Abundance of Orders

The relative abundance of orders is shown in **Figure 3**. Of the 15 orders listed, Rhizobiales, Bacillales, Actinomycetales, Acidobacteriales are the most abundant orders. The relative abundance of Rhizobials is 40.37% in the Mw3 sample while it is 34.97% in Mw4. Bacillales have a relative abundance of 8.44% in Mw3 and 11.35% in Mw4. The relative abundance of Actinomycetales is 9.44% in Mw3

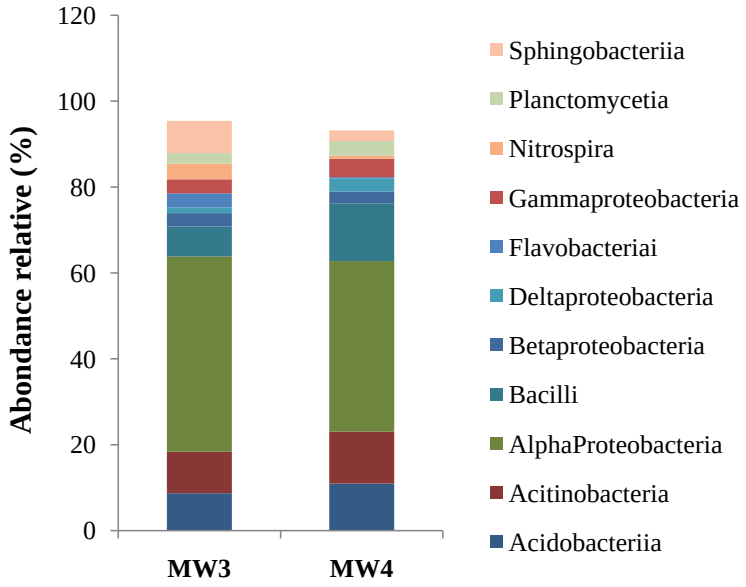

Figure 2. Taxonomy and relative abundances of the most representative classes in soil samples (Mw3 and Mw4).

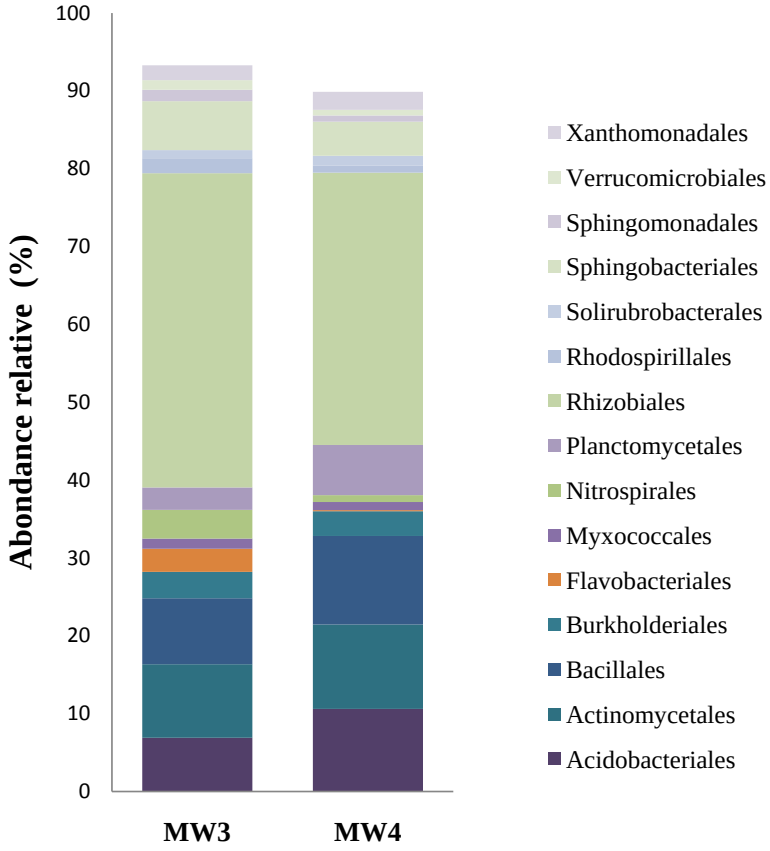

Figure 3. Taxonomy and relative abundances of the most representative orders in soil samples (Mw3 and Mw4).

and 10.84% in Mw4. On the other hand, the relative abundance of Acidobacteriales is 10.65% in Mw4 and 6.90 in Mw3. The Xanthomonadeles represent the I

order which has the lowest abundances in the two soils.

3.2.4. Relative Abundance of Families

Figure 4 presents 33 families with the highest abundances: Catenulisporaceae have a higher relative abundance in Mw3 (23.89%) than in Mw4 (16.53%), Hyphomicrobiaceae are more abundant in Mw3 12.40% that in Mw4 10.85%, the Bacillaceae in Mw3 4.8% and Mw4 10.01% while the abundance of Acidobacteriaceae is 7.48% Mw3 and 8.67% for Mw4. Vibrionaceae are least abundant in the two soils.

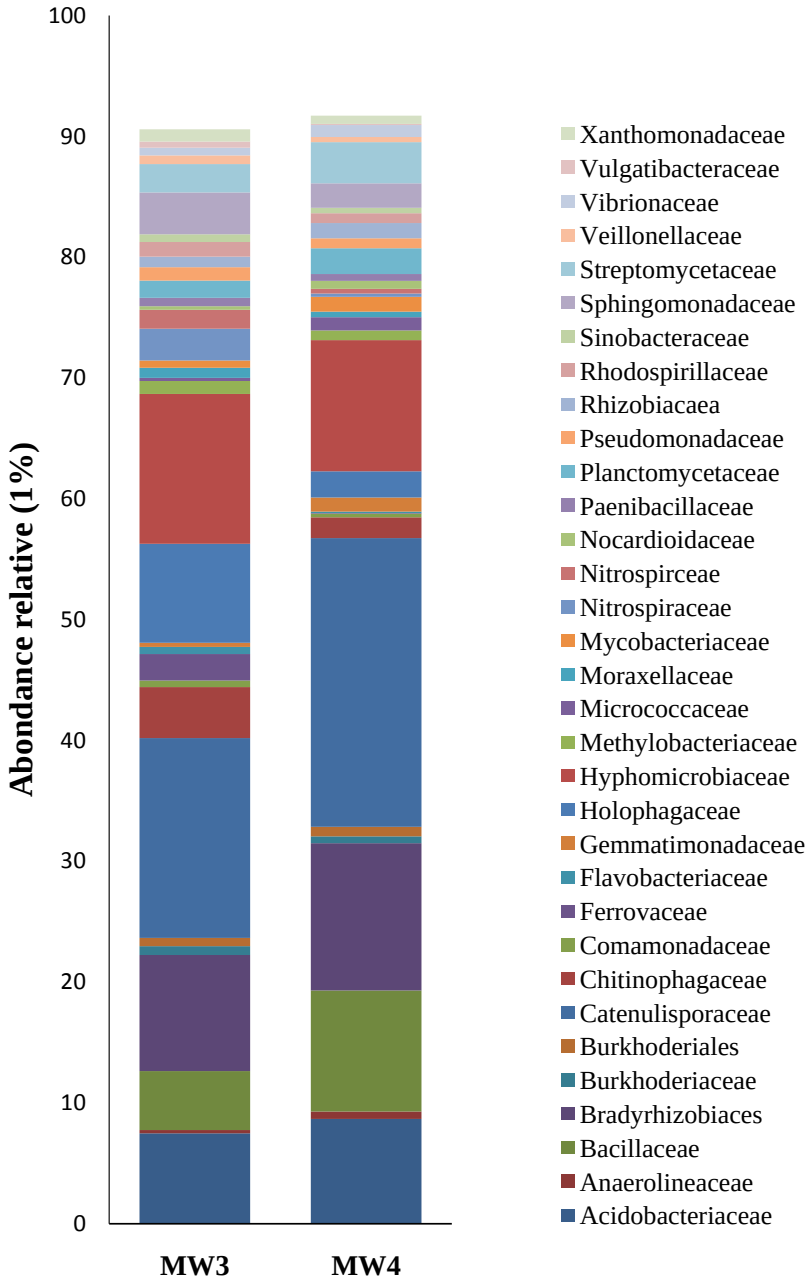

Figure 4. Taxonomy and relative abundances of the most representative families in soil samples (Mw3 and Mw4).

3.2.5. Relative Abundance of Genera

Figure 5 shows that among the 28 listed genera, Bradyrhizobium are the most dominant in Mw3 (30.21%) and Mw4 (28.93%) followed by Bacillus with respectively 16.19% in Mw3 and 23.88 in Mw4, Actinoplanes with a relative abundance of 19.91% in Mw3 and 12.89% in the soil sample of Mw4, and Acidobacterium with 11.83% in the soil of Mw3 and 11.83 in that of Mw4. The least abundant genera are Pedomicrobium and Brevibacterium.

3.2.6. Relative Abundance of Species

The relative abundance of species is presented in Table 3. Among the 25 species listed, Bradyrhizobium, Bacillus, Actinoplanes, Candidatu coribacter Acidobacterium are the most abundant species. The relative abundance of Bradyrhizobium is 30.21% in the Mw3 sample while it is 28.93% in Mw4. Bacilli have a relative abundance of 16.19% in Mw3 and 23.88% in Mw4. The relative abundance of Actinoolanes is 12.97% in Mw3 and 12.89% in Mw4. On the other hand, the relative abundance of Acidobacterium is 11.83% in Mw4 and 11.83 in Mw3. The least abundant species are the Pedomicrobium and Mycrovirga.

3.2.7. Analysis of Alpha Diversity

Analysis of Table 4 shows that the specific richness S and the number of individuals are higher in soil Mw4 than in soil Mw4. The Shannon and Simpson indices are similar in both soils of Mw3 and Mw4 while the H'max fairness index

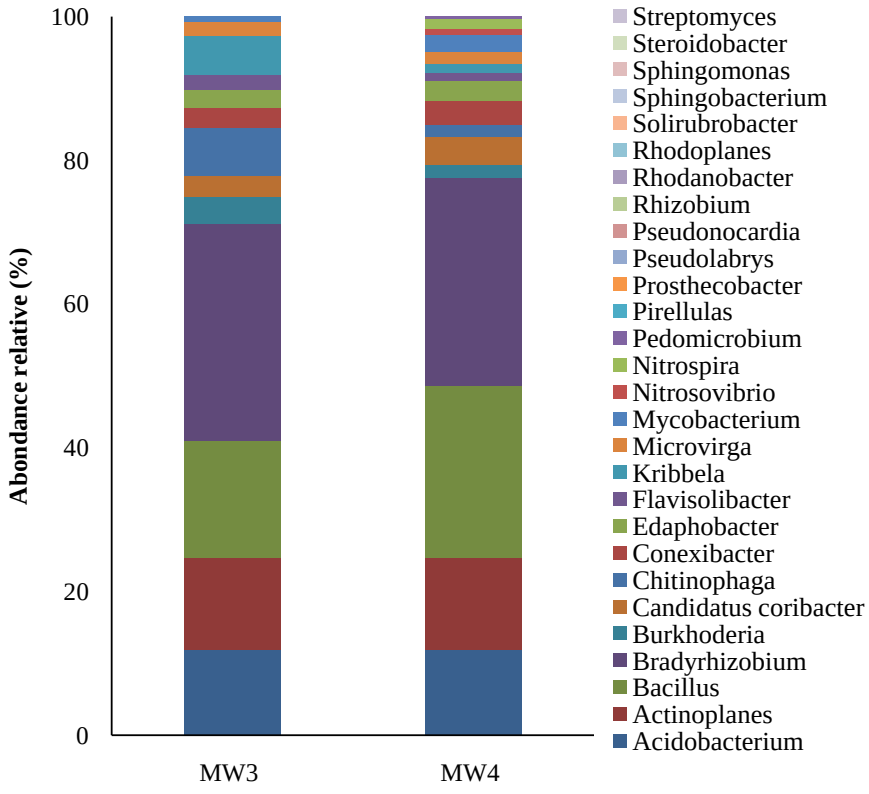

Figure 5. Taxonomy and relative abundances of the genera in soil samples (Mw3 and Mw4).

Table 3. Representation of bacterial genus in soil samples Mw3 and Mw4.

Genus	Values of the two soil (%)	
Acidobacterium	Mw3	Mw4
	11.83%	11.83%
Bradyrhizobium	30.21%	28.93%
Bacillus	16.19%	23.88%
Actinoplanes	12.97%	12.89%
Candidatus coribacter	3.06%	3.78%
Kribbela	5.46%	1.28%
Burkhoderia	3.67%	1.97%
Chitinophaga	6.69%	1.68%
Edaphobacter	2.44%	2.69%
Conexibacter	2.69%	3.43%
Mycobacterium	4.21%	1.64%
Pedomicrobium	1.38%	1.11%
Mycrovirga	1.21%	1.64%

Table 4. Values of the diversity indices in the two soils.

Stations	S	N	R/J	H'	H'max	Simpson
MW3	363	4066	0.77	4.56	5.89	0.97
MW4	440	7755	0.75	4.55	6.09	0.97

S: Number of species; N: Number of individuals; R/J: Fairness index or Pielou; H': Shannon index; H'max: Maximum.

is higher in the soil of Mw4 than in Mw4. On the other hand, the Pielou index (J) of Mw4 is lower than that of Mw3.

Rarefaction curve

Rarefaction methods consist of estimating the number of species for a certain number of individuals. Figure 6 shows the rarefaction curves of the two soils, at 200 OTU the two curves have almost 1000 sequences. From 350 OTU the number of sequences is, the Mw3 curve shows a plateau and the Mw4 curve continues to grow.

3.2.8. Beta Diversity Analysis

Principal Component Analysis (Figure 7) shows that the first two axes explain 100% of the variations in the frequency of bacteria in the Mw3 soil sequence classes. Axe 1 shows that 63.7% bacteria are predominantly grouped into the 0 - 500 class. These are: Alphaproteobacteria, Anaerolinea, Blastocatellia, Betaproteobacteria, Bacteroidetes, Chloroflexia, Clostridia, Cyanobacteria, Cytophagales, Cytophagia, Dehalococcoidia, Deinococci, Deltaproteobacteria, Flavobacteriai, Chloroflexia, Clostridia, Cyanobacteria, Cytophagales, Cytophagia, Dehalococcoidia,

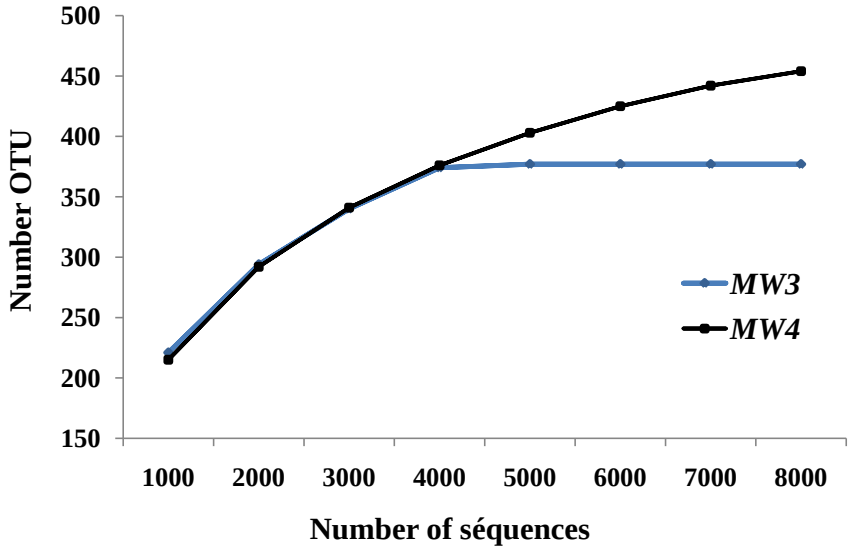

Figure 6. Rarefaction curves for the two soils (Mw3 and Mw4).

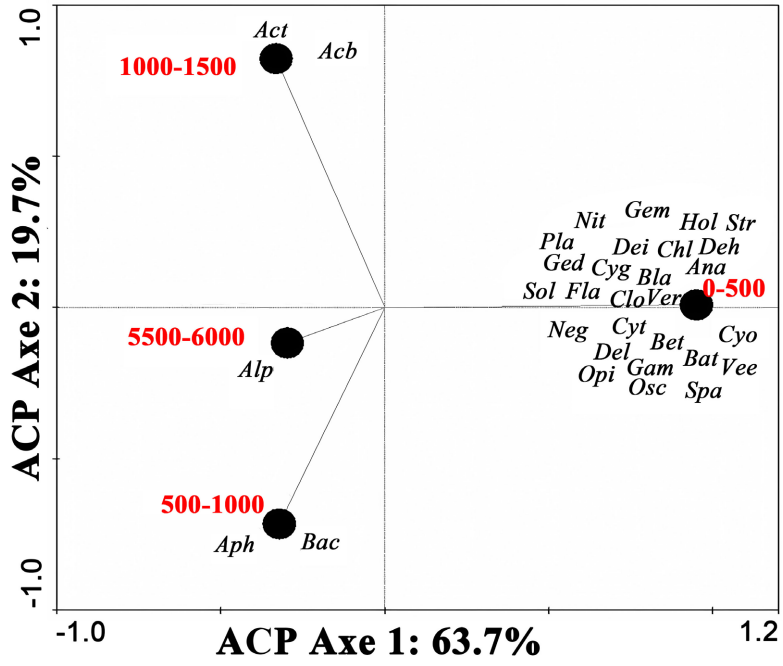

Figure 7. PCA of bacteria at the Mw3 site distribution taking into account the two axes (Axe 1 and Axe 2).

Deinococci, Deltaproteobacteria, Flavobacteriai, Gammaaeproteobacterobacteria, Holgaae, Opinospiondycitrata, Holgaae, Opinospiondycitrata, Holgaae, Opinospiondycitrata, Holautesitimonositratai, Holautesi Hol Planctomycetia, Spartobacteria, Streptophyta and Verrucomicrobiae. Axe 2 with a value of 19.7% shows that Alphaproteobacteria, are frequently found in the sequence class 5500 - 6000, Acidobacteria and Acitinobacteria are common in the range of 1000 - 1500 sequences while Bacilli and Gammaproteobacteria are abundant in the 500 - 1000 sequence class.

Component analysis for Mw4 soil

PCR (Figure 8) shows that the first two axes explain 100% of the variations in the frequency of bacteria in the Mw4 soil sequence classes. Axe 1 shows that 64.9% bacteria are predominantly grouped into the 0 - 500 class. These are: Alphaproteobacteria, Anaerolinea, Blastocatellia, Betaproteobacteria, Bacteroidetes, Chloroflexia, Clostridia, Cyanobacteria, Cytophagales, Cytophagia, Dehalococcoidia, Deinococci, Deltaproteobacteria, Flavobacteriai, Gemimaeimono, Neptycycinpha, Oscilleae, Nacomycidata, Hollyae, Nacidomycidata, Hollyae, Nacidomycitrata, Plane, Neptune, Neptune, Neptune, Plane, Neptune, Neptune, Neptune, Neptune, Neptune, Neptune Spartobacteria, Solibacter, Streptophyta, Verrucumicrobiaceae and Verrucomicrobiae. The axe with a value of 24.4% shows that Alphaproteobacteria, are frequently found in the sequence class 5500 - 6000, Gammaproteobacteria are common in the range of 500 - 1000 sequences while Bacilli, Acidobacteria and Acitinobacteria are abundant in the 1500 - 2000 sequence class.

4. Discussion

The aim of this study was to compare the composition and the diversity of the bacterial community of two soils sample. Sequencing revealed significant differences and similitudes in the microbial taxonomic composition of both MW3 and MW4 soils. The study showed that the bacterial communities of the two soils

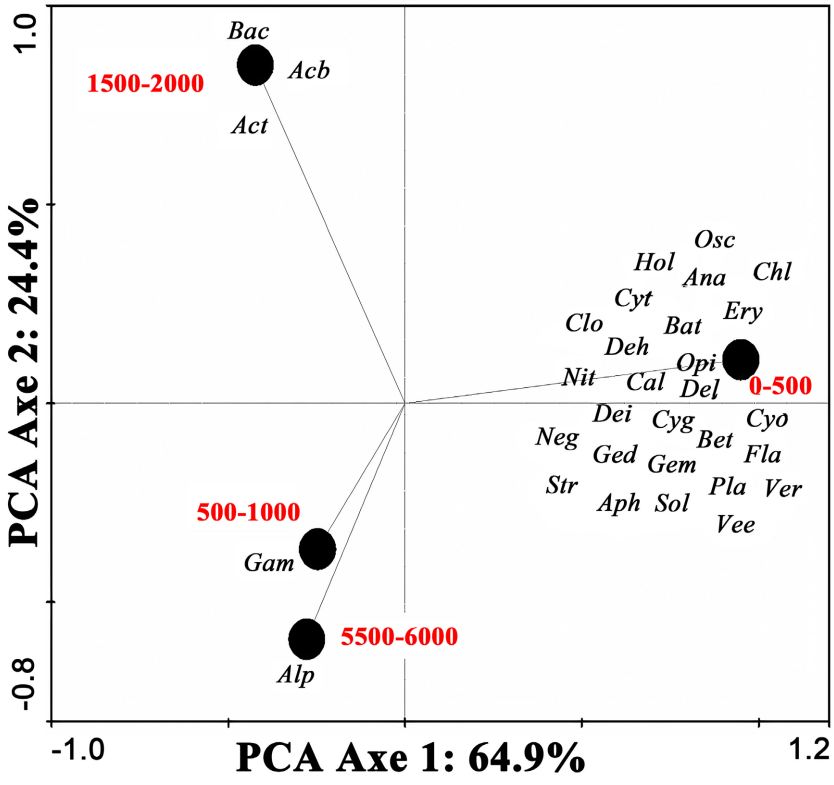

Figure 8. PCA of bacteria at the Mw4 site distribution taking into account the two axes (Axe 1 and Axe 2).

were dominated by five phyla: Proteobacteria, Acidobacteria, Actinobacteria, Firmicutes and Solirubrobacterer. These five phyla represented more than 90% of the sequences in each of the soils examined. Our results were nearly similar to those reported in a meta-analysis conducted by Janssen [6]. According to the same author, the soils were similar to each other because all soils were made up of the same five bacterial groups and where the rarer phyla never had an abundance of 10%. Many other studies have reported the same type of observation [24] [25].

General characteristics of soil bacterial communities Although we collected an average of 1501 sequences per soil, We still did not study the full extent of bacterial diversity in individual soils, so we can only conclude that typical soil harbors more of 1000 phylotypes (if we define phylo-types at the 97% sequence similarity level). This result is to be expected; a number of other studies have used modeling approaches [26] or more in-depth surveys than those described here [27] to demonstrate that soil bacterial communities harbor an enormous number of unique taxa. Since most bacterial soil taxa can be considered rare [28] it is not possible to document the full extent of bacterial diversity in a given soil, even if a Complete pyrosequencing is assigned to a single sol [29] [30]. Not only do individual soils harbor a large amount of phylogenetic diversity, but at our depths of investigation, soil communities shared a small percentage of their phylotypes, and most phylotypes were only found in one soil. Again, this is not surprising, as other studies have also shown a high degree of endemism at finer levels of taxonomic resolution [31]. However, it is important to recognize that the degree of overlap between soil pairs would likely increase if individual soils were studied more comprehensively.

The relative abundances of the dominant taxa correspond roughly to those reported in a meta-analysis by Janssen [6]. Although we focus here on the variability of bacterial communities across a range of soil habitats, it is important to recognize that soils are more similar to each other than to other microbial habitats [32] [33]. Because all soils were generally composed of the same five bacterial groups and hence, the rarer phyla never had an abundance of 10%.

These differences may be due to the strong influence of pH on the composition of Acidobacterial and Actinobacterial communities, the relative abundances of certain taxa within these groups being strongly influenced by changes in soil pH, with soils of distinct pH preventing minimal competition of taxa. Soil pH as a predictor of bacterial community structure although there is a high degree of variability in the composition of the bacterial community across the range of soils examined here, the overall composition of the bacterial community and (to a lesser extent) diversity was surprisingly predictable at this scale of the survey considering only a single parameter, soil pH. This influence of soil pH on overall community composition was evident even at a very coarse level of taxonomic resolution, where we saw the relative abundances of some bacterial phyla (e.g. we used lineage-specific analyzes to examine changes in composition and diversity within the community within individual phylum.

However, we found that pH was often significantly correlated with the structure of these phyla in the range of soils examined. Although soil pH was the best predictor of the composition and diversity of bacterial communities relative to other soil and site characteristics that were measured, much of the variability in bacterial community structure remains unexplained. As we have not measured other factors, our analysis concerning environment factor were limited. For example, salinity has been shown to be an important factor in the global distribution of bacteria, but it is not systematically measured in most soil studies [34].

Our results are similar to those [35]. The phyla Alphaproteobacteria, Actinobacteria, Planctomycetes, Firmicutes, Gammaproteobacteria, Acidobacteria, Betaproteobacteria and Deltaproteobacteria have been identified as the majority in other soil studies, or also in other environments such as marine sediments, oceans and mammalian digestive tracts. Their strong cosmopolitan character can be explained by their great dispersal capacity but also by their great ability to colonize different types of environments.

The most represented in the two soils are: Aphaproteobacteria in Mw3 45.50% and Mw4 39.72%,, Actinobacteria in Mw3 12.07% and Mw4 9.63% and Acidobacteriia in Mw3 10.97% and Mw4 8.71% are most abundant in the two soils and the least abundant in both soils are verrucomicrobia these results are similar to those [36] on the other hand our results are different from that of [37] which worked in freshwater sediments in comparison, a high abundance of Alphaproteobacteria and Betaproteobacteria appeared on the other hand in us we have the Deltaproteobacteria and the Gammaprotéobacteria.

Illumina results revealed differences in microbial composition between the two soils, our results suggest that Mw3 and Mw4 may play a major role in structure the composition of the bacterial community (Rhizobials from sample Mw3 have a relative abundance of 40.37% and soil Mw4 has an abundance of 34.97%, Bacillales have an abundance of 8.44% in Mw3 and of 11.35% in Mw4, Actinomycetales of Mw3 have an abundance of 9.44% and 10.84% in Mw4 and Acidobacteriales 10.65% in Mw4 and 6.90 in Mw3), our results are similar to Sanjiang who works in wetlands. In Nova Scotia soil (107.95%) was generally superior to that of DS (25.22%) and FS (28.01%) soils, which is consistent with the results of a wetland study plains in the Yellow River [8]. Many researchers have shown that proteobacteria are the dominant taxon in soils [38]. Nevertheless, the proportion of Proteobacteria in degraded soils was lower in two wetlands, while that of Firmicutes was higher. Our results differ. In particular, the comparison of communities by grouping sequences into phylotypes defined at the 97% similarity level has limitations in that these surveys will be far from exhaustive, and global models highlighted by comparing the overall phylogenetic structure can be more difficult to discern and quantify.

The Catenulisporaceae, Hyphomicrobiaceae, Bacillaceae and Acidobacteriaceae are the most abundant. The Catenulisporaceae in Mw4 23.89% and in Mw4 16.53%, Hyphomicrobiaceae in Mw3 12.40% and for Mw3 10.85%, Bacillaceae in

Mw3 4.8% and Mw4 10.01% and Acidobacteriaceae for Mw3 7.48% and for Mw4 8.67%. The vibrionaceae are respectively the least abundant by their percentage. Our results are close to the results of a study on the wetlands of the plains in the Yellow River [39].

Figure 5 presents 28 genera including Bradyrhizobium in Mw3 30.21% and Mw4 28.93%, Bacillus in Mw3 16.19% and Mw4 23.88, Actinoplanes in Mw3 19.91% and Mw4 12.89% and Acidobacterium in Mw3 11.83% and Mw4 11.83. The least abundant genera are pedomicrobium and brevibacterium our results are different from those [40], it has been confirmed that some genera of beta-proteobacteria inhabit extremely alkaline wetlands filled with historic steel slag. Epsilonproteobacteria are relatively abundant at oxico-anoxic interfaces such as intertidal wetlands.

Study by [41] found that soil pH was a key factor in determining microbial diversity and community composition. The soil pH in our study is 6.5 and the Simpson index was significantly positively correlated with soil pH. Published studies have also revealed that, especially in soils with a pH below 6.5, microbial diversity decreases with decreasing soil pH [42]. Other studies have indicated that the phylum Acidobacteria is widely distributed in various soil environments [43]. And that its abundance has been significantly correlated with soil pH [44]. However, our study did not confirm this, which could be due to the narrow drill of soil pH values found in this study.

Study by [45] found that soil pH was a key factor in determining microbial diversity and community composition. The soil pH in our study is 6.5 and the Simpson index was significantly positively correlated with soil pH. Published studies have also revealed that, especially in soils with a pH below 6.5, microbial diversity decreases with decreasing soil pH [46]. Other studies have indicated that the phylum Acidobacteria is widely distributed in various soil environments [47] and that its abundance has been significantly correlated with soil pH [48]. However, our study did not confirm this, which could be due to the narrow drill of soil pH values found in this study. [48] found that changes and distribution of bacterial communities were strongly correlated with soil carbon and nitrogen and other chemical properties. The results obtained in this study do not align well with our study because we did not take into account the physical parameters. We found that changes and distribution of bacterial communities were strongly correlated with soil carbon and nitrogen and other chemical properties. The results obtained in this study align well with a previous study, which found that several soil properties, including organic C and total N contents affect the composition of microbial communities in soils [49] [50] [51] [52]. Variations in microbial communities are a complete reflection of the impacts of these environmental factors. Variations in microbial communities are a complete reflection of the impacts of these environmental factors. Compared to forest and shrub lands, Shannon Simpson and Equitability and soil diversity indices were the most average, which is in agreement with previous studies [51]. This may be because

the soils are in a spaced protected forest and shrub area contains additional plant species and their developed root systems provide suitable habitat for soil microorganisms.

This may be because forest lands and shrubs contain additional plant species and their developed root systems provide suitable habitat for soil microorganisms. At the same time, their root secretions can provide resources for microorganisms, which is more conducive for the survival of various microorganisms. The bacterial diversity index showed that the two soils were not very disturbed by the degradation process, hence Shannon varies from 4.59 for Mw3 and 4.57 for Mw4, Simpson for Mw3 0.96 and 0.97 for Mw4 our results are close to those of [52], and similar to [53]. The study of the functional structure of bacterial populations through diversity indices (S, H and R) revealed that the habitat Mw3 is the least diversified than Mw4, the species seem to be fairly represented and that based on these results, there is apparently no major ecological problem at the sites.

A Principal Component Analysis (PCA) analysis was conducted to assess the relationships between the compositions of dominant bacterial phyla (or genera) and selected soil properties (soil pH, with the results summarized in **Figure 7** and **Figure 8**. The graphs PCA, based on dominant phyla and genera, were almost identical. The overall structures of the dominant phyla or genera under different land use types were significantly related to certain soil properties.

Phylum level, the eigenvalues of the first and second axes were respectively 63.7% and 19.7% Mw3 and 64.9% and 24.4%. The axes explained 83.4% and 89.3% of the total microbial variance.

Correlation analysis of dominant bacterial groups and soil environmental factors indicated that the relative abundance of Mw3. Axe 1 (63.7%), in its positive part, groups together bacteria whose abundances are between 0 - 500 (Alphaproteobacteria, Anaerolinea, Blastocatellia, Betaproteobacteria, Bacteroidetes, Chloroflexia, Clostridia, Cyanobacteria, Cytophagales, Cytophagia, Dehalococcoidia, Deinococci, deltaproteobacteria, Flavobacteriai, Gammaproteobacteria, Gemmatimonadales, Gemmatimondets, Holphagae, Negativicutes, Nitrospira, Opitutae, Oscillatoriophycideae, Planctomycetia, Spartobacteria, Streptophyta, Verrucomicrobiae) and its negative part, all batteries having abundances between 500 - 6000 (Alphaproteobacteria, Acidobacteria, Acitinobacteria, Bacilli, Gammaproteobacteria). Following Axe 2 (19.7%) two groups are defined, the first which is located in its positive part, includes the bacteria having abundances between 1000 - 1500 and the second on its negative part, includes the bacteria with abundances between 500 - 1000 (Gammaproteobacteria) and 5500 - 6000 (Alphaproteobacteria) was positively correlated with pH.

For Mw4 Axe 1 (64.9%), in its positive part, groups together bacteria whose abundances are between 0 - 500 (Alphaproteobacteria, Anaerolinea, Blastocatellia, Betaproteobacteria, Bacteroidetes, Caldilinea, Chloroflexia, Clostridia, Cyano-

bacterie, Cytophagales, Cytophagia, Dehalococcoidia, Deinococci, deltaproteobacteria, Flavobacteriai, Gammaproteobacteria, Gemmatimonadales, Gemmatimondets, Holphagae, Negativicutes, Nitrospira, Opitutae, Oscillatoriophycideae, Planctomycetia, Spartobacteria, Streptophyta, Verrucomicrobiaceae, Verrucomicrobiae) and its negative part, all bacteria having included abundances between 500 - 6000 (AlphaProteobacteria, Acidobacteria, Acitinobacteria, Bacilli, Gammaproteobacteria). Following Axe 2 (24.4%) two groups are defined, the first which is located in its positive part, includes the bacteria having abundances between 0 - 500 and those whose abundances are between 1500 - 2000 (Acidobacteria, Acitinobacteria and Bacilli) and the second which is located on its negative part, groups together the bacteria having abundances between 500 - 1000 (Gammaproteobacteria) and 5500 - 6000 (Alphaproteobacteria) were positively correlated with pH.

5. Conclusion

In summary, this study clearly illustrated the structures of bacterial communities and their variety in the Brazzaville sites, which revealed the influences of different land use types on bacterial diversity. These results greatly advance the elucidation of the effects and mechanisms of different soil types, which can alter the composition and diversity of bacterial communities in soils. The influence of the only measured environmental factor pH has been demonstrated, but was insufficient for other analyses.

Authors' Contributions

1) Itsouhou Ngô: has made the supervision of all of the editing of the manuscript.

2) Irène Marie Cécile Mboukou Kimbatsa: has been involved in all plans of practice practice, editing the manuscript and data analysis.

3) Armel Ibala Zamba: has conducted the practice and data analysis.

4) Faly Armel Soloka Mabika: has made the practice and data analysis.

5) Thantique Moutali Lingouagou: has made the practice and data analysis.

6) Joseph Goma-Tchimbakala: has been involved in data analysis.

7) Etienne Nguimbi: has edited the manuscript with the corresponding author.

Highlights

The diversity of bacteria on the soil has been holding with metagenomic 16SrRNA gene illumina MiSeq sequencing.

21 Phyla, 17 classes, 40 families, 83 orders, and 28 genera have been revealed.

The diversity indices have been used to understand the state of the bacterial composition of soil in Brazzaville.

This study opens interesting perspectives on the knowledge and exploitation of telluric bacteria in several areas of Brazzaville.

Conflicts of Interest

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

References

[1] Ranjard, L., Maron, P.A., Cuny, P., *et al.* (2017) La microbiologie moléculaire au service du diagnostic environnemental. *Étude et Gestion des Sols*, **24**, 9-31.

[2] Gardi, C., Montanarella, L., Arrouays, D., Bispo, A., Lemanceau, P., Mulder, C., Ranjard, L., Rombke, L., Rutger, M. and Menta, C. (2009) Soil Biodiversity Monitoring in Europe: Ongoing Activities and Challenges. *European Journal of Soil Science*, **60**, 807-819. https://doi.org/10.1111/j.1365-2389.2009.01177.x

[3] Barberán, A., *et al.* (2014) Why Are Some Microbes More Ubiquitous than Others? Predicting the Habitat Breadth of Soil Bacteria. *Ecology Letters*, **17**, 794-802. https://doi.org/10.1111/ele.12282

[4] Hendershot, J.N., Read, Q.D., Henning, J.A., Sanders, N.J. and Classen, A.T. (2017) Consistently Inconsistent Drivers of Microbial Diversity and Abundance at Macroecological Scales. *Ecology*, **98**, 1757-1763. https://doi.org/10.1002/ecy.1829

[5] Pester, M., Maixner, F., Berry, D., Rattei, T., Koch, H., Lücker, S., Nowka, B., Richter, A., Spieck, E. and Lebedeva, E. (2014) *Nxr*B Encoding the Beta Subunit of Nitrite Oxidoreductase as Functional and Phylogenetic Marker for Nitrite-Oxidizing *Nitrospira*. *Environmental Microbiology*, **16**, 3055-3071. https://doi.org/10.1111/1462-2920.12300

[6] Janssen, P.H. (2006) Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. *Applied and Environmental Microbiology*, **72**, 1719-1728. https://doi.org/10.1128/AEM.72.3.1719-1728.2006

[7] Lozupone, C.A. and Knight, R. (2007) Global Patterns in Bacterial Diversity. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 11436-11440. https://doi.org/10.1073/pnas.0611525104

[8] Lin, C., Li, X. and Jin, H. (2015) Soil Degradation Characteristics of Food-Plain Wetlands in Yellow River Source Zone. *Chinese Agricultural Science Bulletin*, **31**, 243-249.

[9] Khan, K.S., Mack, R., Castillo, X., Kaiser, M. and Joergensena, R.G. (2016) Microbial Biomass, Fungal and Bacterial Residues, and Their Relationships to the Soil Organic Matter C/N/P/S Ratios. *Geoderma*, **271**, 115-123. https://doi.org/10.1016/j.geoderma.2016.02.019

[10] Bastida, F., Zsolnay, A., Hernández, T. and García, C. (2008) Past, Present and Future of Soil Quality Indices: A Biological Perspective. *Geoderma*, **147**, 159-171. https://doi.org/10.1016/j.geoderma.2008.08.007

[11] Gyaneshwar, P., Kumar, G.N., Parekh, L.J. and Poole, P.S. (2002) Role of Soil Microorganisms in Improving P Nutrition of Plants. *Plant Soil*, **245**, 83-93. https://doi.org/10.1023/A:1020663916259

[12] Berg, G. (2009) Plant—Microbe Interactions Promoting Plant Growth and Health: Perspectives for Controlled Use of Microorganisms in Agriculture. *Applied Microbiology and Biotechnology*, **84**, 11-18. https://doi.org/10.1007/s00253-009-2092-7

[13] Xu, X., Stern, A., Liu, Z., Kan, B. and Zhu, J. (2010) Virulence Regulator AphB Enhances *toxR* Transcription in *Vibrio cholerae*. *BMC Microbiology*, **10**, Article No. 3. https://doi.org/10.1186/1471-2180-10-3

[14] Kumar, S. and Tuteja, U. (2009) Detection of Virulence-Associated Genes in Clinical Isolates of *Bacillus anthracis* by Multiplex PCR and DNA Probes. *Journal of Microbiology and Biotechnology*, **19**, 1475-1481. https://doi.org/10.4014/jmb.0902.101

[15] Hanniffy, S.B., Pelaez, C., Martinez-Bartolome, M.A., Requena, T. and Martinez-Cuesta, M.C. (2009) Key Enzymes Involved in Methionine Catabolism by Cheese Lactic Acid Bacteria. *International Journal of Food Microbiology*, **135**, 223-230. https://doi.org/10.1016/j.ijfoodmicro.2009.08.009

[16] Lucy, M., Reed, E. and Glick, B.R. (2004) Applications of Free Living Plant Growth-Promoting Rhizobacteria. *Antonie van Leeuwenhoek*, **86**, 1-25. https://doi.org/10.1023/B:ANTO.0000024903.10757.6e

[17] Lugtenberg, B. and Kamilova, F. (2009) Plant-Growth-Promoting Rhizobacteria. *Annual Review of Microbiology*, **63**, 541-556. https://doi.org/10.1146/annurev.micro.62.081307.162918

[18] Wong, S.L. (1995) Advances in the Use of *Bacillus subtilis* for the Expression and Secretion of Heterologous Proteins. *Current Opinion in Biotechnology*, **6**, 517-522. https://doi.org/10.1016/0958-1669(95)80085-9

[19] Zhou, Q.F., Li, M.Y. and Li, C.W. (2009) Cloning and Expression of a Novel Insulin-Releasing Peptide, Brevinin-2GU from *Escherichia coli*. *Journal of Bioscience and Bioengineering*, **107**, 460-463. https://doi.org/10.1016/j.jbiosc.2008.12.011

[20] Demain, A.L. (1999) Pharmaceutically Active Secondary Metabolites of Microorganisms. *Applied Microbiology and Biotechnology*, **52**, 455-463. https://doi.org/10.1007/s002530051546

[21] Rappe, M.S. and Giovannoni, S.J. (2003) The Uncultured Microbial Majority. *Annual Review of Microbiology*, **57**, 369-394. https://doi.org/10.1146/annurev.micro.57.030502.090759

[22] McHardy, A.C. and Rigoutsos, I. (2007) What's in the Mix: Phylogenetic Classification of Metagenome Sequence Samples. *Current Opinion in Microbiology*, **10**, 499-503. https://doi.org/10.1016/j.mib.2007.08.004

[23] Lawrence, J.G. (1999) Gene Transfer, Speciation, and the Evolution of Bacterial Genomes. *Current Opinion in Microbiology*, **2**, 519-523. https://doi.org/10.1016/S1369-5274(99)00010-7

[24] Ding, G.C., Piceno, Y.M., Heuer, H., Weinert, N., Dohrmann, A.B., Carrillo, A., Andersen, G.L., Castellanos, T., Tebbe, C.C. and Smalla, K. (2013) Changes of Soil Bacterial Diversity as a Consequence of Agricultural Land Use in a Semi-Arid Ecosystem. *PLOS ONE*, **8**, e59497. https://doi.org/10.1371/journal.pone.0059497

[25] Lagerlöf, J., Adolfsson, L., Börjesson, G., Ehlers, K., Vinyoles, G.P. and Sundh, I. (2014) Land-Use Intensification and Agroforestry in the Kenyan Highland: Impacts on Soil Microbial Community Composition and Functional Capacity. *Applied Soil Ecology*, **82**, 93-99. https://doi.org/10.1016/j.apsoil.2014.05.015

[26] Sun, R., Zhang, X.X., Guo, X., Wang, D. and Chu, H. (2015) Bacterial Diversity in Soils Subjected to Long-Term Chemical Fertilization Can Be More Stably Maintained with the Addition of Livestock Manure than Wheat Straw. *Soil Biology and Biochemistry*, **88**, 9-18. https://doi.org/10.1016/j.soilbio.2015.05.007

[27] Coolon, J.D., Jones, K.L., Todd, T.C., Blair, J.M. and Herman, M.A. (2013) Long-Term Nitrogen Amendment Alters the Diversity and Assemblage of Soil Bacterial Communities in Tallgrass Prairie. *PLOS ONE*, **8**, e67884. https://doi.org/10.1371/journal.pone.0067884

[28] He, Z.L., Xu, M.Y., *et al.* (2010) Metagenomic Analysis Reveals Amarked Divergence in the Structure of Belowground Microbial Communities at Elevated CO_2. *Ecology*

Letters, **13**, 564-575. https://doi.org/10.1111/j.1461-0248.2010.01453.x

[29] Yun, J.L., Zhang, H.X., Deng, Y.C. and Wang, Y.F. (2015) Aerobic Methanotroph Diversity in Sanjiang Wetland, Northeast China. *Microbial Ecology*, **69**, 567-576. https://doi.org/10.1007/s00248-014-0506-2

[30] Zhang, W., Chen, L., Zhang, R. and Lin, K.F. (2016) High Throughput Sequencing Analysis of the Joint Effects of BDE209-Pb on Soil Bacterial Community Structure. *Journal of Hazardous Materials*, **301**, 1-7. https://doi.org/10.1016/j.jhazmat.2015.08.037

[31] Zhang, J., Wang, P.C., Tian, H.M., Xiao, Q.Q. and Jiang, H.K. (2019) Pyrosequencing-Based Assessment of Soil Microbial Community Structure and Analysis of Soil Properties with Vegetable Planted at Different Years under Greenhouse Conditions. *Soil and Tillage Research*, **187**, 1-10. https://doi.org/10.1016/j.still.2018.11.008

[32] Acosta-Martínez, V., Dowd, S., Sun, Y. and Allen, V. (2008) Tag-Encoded Pyrosequencing Analysis of Bacterial Diversity in a Single Soil Type as Affected by Management and Land Use. *Soil Biology and Biochemistry*, **40**, 2762-2770. https://doi.org/10.1016/j.soilbio.2008.07.022

[33] Green, J.L., Bohannan, B.J.M. and Whitaker, R.J. (2008) Microbial Biogeography: From Taxonomy to Traits. *Science*, **320**, 1039-1043. https://doi.org/10.1126/science.1153475

[34] Hamady, M., Walker, J.J., Harris, J.K., Gold, N.J. and Knight, R. (2008) Error-Correcting Barcoded Primers for Pyrosequencing Hundreds of Samples in Multiplex. *Nature Methods*, **5**, 235-237. https://doi.org/10.1038/nmeth.1184

[35] Huse, S.M., Dethlefsen, L., Huber, J.A., Welch, D.M., Relman, D.A. and Sogin, M.L. (2008) Exploring Microbial Diversity and Taxonomy Using SSU rRNA Hypervariable Tag Sequencing. *PLOS Genetics*, **4**, e1000255. https://doi.org/10.1371/journal.pgen.1000255

[36] Borneman, J. and Triplett, E.W. (1997) Molecular Microbial Diversity in Soils from Eastern Amazonia: Evidence for Unusual Microorganisms and Microbial Population Shifts Associated with Deforestation. *Applied and Environmental Microbiology*, **63**, 2647-2653. https://doi.org/10.1128/aem.63.7.2647-2653.1997

[37] Cookson, W.R., Osman, M., Marschner, P., Abaye, D.A., Clark, I., Murphy, D.V., Stockdale, E.A. and Watson, C.A. (2007) Controls on Soil Nitrogen Cycling and Microbial Community Composition across Land Use and Incubation Temperature. *Soil Biology and Biochemistry*, **39**, 744-756. https://doi.org/10.1016/j.soilbio.2006.09.022

[38] He, Z.L., Piceno, Y., Deng, Y., *et al.* (2012) The Phylogenetic Composition and Structure of Soil Microbial Communities Shifts in Response to Elevated Carbon Dioxide. *The ISME Journal*, **6**, 259-272. https://doi.org/10.1038/ismej.2011.99

[39] Hartman, W.H., Richardson, C.J., Vilgalys, R. and Bruland, G.L. (2008) Environmental and Anthropogenic Controls over Bacterial Communities in Wetland Soils. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 17842-17847. https://doi.org/10.1073/pnas.0808254105

[40] Singh, R.P., Dhania, G., Sharma, A. and Jaiwal, P.K. (2007) Biotechnological Approaches to Improve Phytoremediation Efficiency for Environment Contaminants. In: Singh, S.N. and Tripathi, R.D., Eds., *Environmental Bioremediation Technologies*, Springer, Berlin, 223-258. https://doi.org/10.1007/978-3-540-34793-4_10

[41] Constancias, F., Saby, N.P.A., Terrat, S., Dequiedt, S., Horrigue, W., Nowak, V., Guillemin, J.P., Biju-Duval, L., Chemidlin Prévost-Bouré, N. and Ranjard, L. (2015) Contrasting Spatial Patterns and Ecological Attributes of Soil Bacterial Taxa across a

Landscape. *Microbiology Open*, **4**, 518-531. https://doi.org/10.1002/mbo3.256

[42] Nemergut, D.R., Cleveland, C.C., Wieder, W.R., Washenberger, C.L. and Townsend, A.R. (2010) Plot-Scale Manipulations of Organic Matter Inputs to Soils Correlate with Shifts in Microbial Community Composition in a Lowland Tropical Rain Forest. *Soil Biology and Biochemistry*, **42**, 2153-2160. https://doi.org/10.1016/j.soilbio.2010.08.011

[43] Nedashkovskaya, O.I. and Kim, S.B. (2015) Pontibacter. In: Sneath, P.H.A. and Bergey, D.H., Eds., *Bergey's Manual of Systematics of Archaea and Bacteria*, John Wiley & Sons, Hoboken, 1-4. https://doi.org/10.1002/9781118960608.gbm00272

[44] Baldwin, S.A., Khoshnoodi, M., Rezadehbashi, M., *et al.* (2015) The Microbial Community of a Passive Biochemical Reactor Treating Arsenic, Zinc, and Sulfate Rich Seepage. *Frontiers in Bioengineering and Biotechnology*, **3**, Article 27. https://doi.org/10.3389/fbioe.2015.00027

[45] Ji, Y., Liu, P. and Conrad, R. (2018) Response of Fermenting Bacterial and Methanogenic Archaeal Communities in Paddy Soil to Progressing Rice Straw Degradation. *Soil Biology and Biochemistry*, **124**, 70-80. https://doi.org/10.1016/j.soilbio.2018.05.029

[46] Wang, H., Sheng, Y., He, Y., Wu, J., Jiang, Y., Tam, N. and Zhou, H. (2012) Comparison of the Levels of Bacterial Diversity in Freshwater, Intertidal Wetland, and Marine Sediments by Using Millions of Illumina Tags. *Applied and Environmental Microbiology*, **78**, 8264-8271. https://doi.org/10.1128/AEM.01821-12

[47] Gans, J., Wolinsky, M. and Dunbar, J. (2005) Computational Improvements Reveal Great Bacterial Diversity and High Metal Toxicity in Soil. *Science*, **309**, 1387-1390. https://doi.org/10.1126/science.1112665

[48] Tringe, S.G., von Mering, C., Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., Podar, M., Short, J.M., Mathur, E.J., Detter, J.C., Bork, P., Hugenholtz, P. and Rubin, E.M. (2005) Comparative Metagenomics of Microbial Communities. *Science*, **308**, 554-557. https://doi.org/10.1126/science.1107851

[49] Elshahed, M.S., Youssef, N.H., Spain, A.M., Sheik, C., Najar, F.Z., Sukharnikov, L.O., Roe, B.A., Davis, J.P., Schloss, P.D., Bailey, V.L. and Krumholz, L.R. (2008) Novelty and Uniqueness Patterns of Rare Members of the Soil Biosphere. *Applied and Environmental Microbiology*, **74**, 5422-5428. https://doi.org/10.1128/AEM.00410-08

[50] Fulthorpe, R.R., Roesch, L.F.W., Riva, A. and Triplett, E.W. (2008) Distantly Sampled Soils Carry Few Species in Common. *The ISME Journal*, **2**, 901-910. https://doi.org/10.1038/ismej.2008.55

[51] Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G. and Triplett, E.W. (2007) Pyrosequencing Enumerates and Contrasts Soil Microbial Diversity. *The ISME Journal*, **1**, 283-290. https://doi.org/10.1038/ismej.2007.53

[52] Shen, C.C., Xiong, J.B., Zhang, H.Y., Feng, Y.Z., Lin, X.G., Li, X.Y., Liang, W.J. and Chu, H.Y. (2013) Soil pH Drives the Spatial Distribution of Bacterial Communities along Elevation on Changbai Mountain. *Soil Biology and Biochemistry*, **57**, 204-211. https://doi.org/10.1016/j.soilbio.2012.07.013

[53] Lauber, C.L., Hamady, M., Knight, R. and Fierer, N. (2009) Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. *Applied and Environmental Microbiology*, **75**, 5111-5120. https://doi.org/10.1128/AEM.00335-09