Retraction Notice

Title of retracted article: Effect of Long-Term Inorganic Fertilization on Diversity and Abundance of Bacterial and Archaeal Communities at Tillage in Irrigated Rice Field

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Journal: Advances in Bioscience and Biotechnology (ABB)
Year: 2023
Volume: 14
Number: 1
Pages (from - to): 18-33
DOI (to PDF): https://doi.org/10.4236/abb.2023.141002
Paper ID at SCIRP: 122499
Article page: https://www.scirp.org/journal/paperinformation.aspx?paperid=122499
Retraction date: 2023-02-13

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☐ Some of the authors:
X Editor with hints from Journal owner (publisher)
Institution:
Reader:
Other:
Date initiative is launched: 2023-02-13

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Advances in Bioscience and Biotechnology, 2023, 14, 18-33
https://www.scirp.org/journal/abb
ISSN Online: 2156-8502
ISSN Print: 2156-8456

Effect of Long-Term Inorganic Fertilization on Diversity and Abundance of Bacterial and Archaeal Communities at Tillage in Irrigated Rice Field

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How to cite this paper: Dondjou, D.T., Fankem, H., Diedhiou, A.G., Mofini, M.-T., Mbodj, D., Pignoly, S., Manneh, B., Laplaze, L., Kane, A. and Taffouo, V.D. (2023) Effect of Long-Term Inorganic Fertilization on Diversity and Abundance of Bacterial and Archaeal Communities at Tillage in Irrigated Rice Field. *Advances in Bioscience and Biotechnology*, **14**, 18-33.
https://doi.org/10.4236/abb.2023.141002

Received: January 3, 2023
Accepted: January 12, 2023
Published: January 18, 2023

Abstract

Farmers largely rely on fertilizer application to maintain soil productivity and increase grain crop yield. However, the application of inorganic fertilizers can decrease soil quality and productivity. Therefore, soil microbes play important roles in energy flow and nutrient cycling, such as organic matter decomposition, biogeochemical cyclings of nitrogen, carbon, and other elements. In the present study, we collected soil samples from three different inorganic fertilization treatments in the Ndiaye site at tillage where irrigated rice was cultivated previously, over a period of 27-year (1991-2017) with different inorganic fertilization treatments in Saint Louis, region of Senegal whose two plots have been subjected to inorganic fertilization and one none. Soil bacterial and archaeal communities were analyzed by targeting the bacterial and archaeal 16S rRNA genes. Shannon diversity index and Simpson diversity index revealed the alpha-diversity of bacterial and archaeal com-

munities with more response of archaeal community to long-term different inorganic fertilization compared with the bacterial community. For bacteria, *Proteobacteria* was the preponderant phylum which was rather subdivided into classes of *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria*, followed by *Chloroflexi*, *Firmicutes*, *Actinobacteria* and *Acidobacteria*. For archaea, *Methanomicrobia* was the preponderant class followed by *Methanobacteria*, *Thaumarchaeota* and *Crenarchaeota*. For examining the effects of soil properties on bacterial and archaeal communities, we employed canonical analysis of principal coordinates, for both communities, we observed a spatial distribution according to each fertilization treatment, spatial distribution affected by the parameters of soil. This study provides more insights into the response of soil microbiome to long-term different inorganic fertilization treatments which will be helpful in managing soil microbes for sustainable agricultural productivity.

Keywords

Inorganic Fertilization, Soil, Microbiome, Tillage, Next-Generation Sequencing, 16S rRNA Gene, V4 Region, Senegal

1. Introduction

The global demand for crops is expected to rise by at least 60% by 2050 [1]. Rice is a major staple crop grown in many countries across the World with a total production of more than 510 million tons annually [2] feeding over 50% of the global population [3] [4] and is grown mainly in flooded paddy fields [5]. Rice consumption among urban dwellers has steadily grown, with a *per capita* consumption that has doubled [6]. To meet the rising demand from a growing world population, it has been estimated that rice production should increase by 40% by the end of 2030 [7]. In particular, increasing production is a major food sovereignty issue for Sub-Saharan African countries that consume a lot of rice and are currently largely dependent on importation to meet the demand [8].

Farmers largely rely on fertilizer application to maintain soil productivity and increase grain crop yield [9]. However, long-term application of inorganic fertilizers can decrease soil quality and productivity [9] [10]. It also has led to a series of environmental issues such as nitrate leaching or greenhouse gas emission [11] [12] [13]. Soil microbes play important roles in energy flow and nutrient cycling, such as organic matter decomposition, biogeochemical cyclings of nitrogen, carbon, and other elements [14] [15] [16]. They can also mitigate soil pollution and regulate greenhouse gas emissions [16]. In addition, soil microbial communities can supply some essential nutrients for crop growth [9] and their biomasses, diversities, and activities are sensitive indicators of soil quality, productivity, and sustainability in terrestrial ecosystems [11] [17] [18]. The growth, activity, and functional diversity of soil microbial communities can be affected by various factors, including climatic, vegetation, soil type, land use strategy, and

fertilization practices [16] [19].

To the best of our knowledge, the effect of long-term inorganic fertilization on diversity and abundance of bacterial and archaeal communities at tillage in irrigated rice fields has not been yet explored in Sub-Saharan Africa, particularly in Senegal. In the present study, we collected soil samples from three different inorganic fertilization treatments in Ndiaye site at tillage where irrigated rice was cultivated previously, over a period of 27-year (1991-2017) with different inorganic fertilization treatments in Saint Louis, region of Senegal whose two plots have been subjected to inorganic fertilization and one none. Soil bacterial and archaeal communities were analyzed with the Illumina MiSeq sequencing by targeting the bacterial and archaeal 16S rRNA genes. We hypothesized that the microbial communities in irrigated rice field soils will be only influenced by different inorganic fertilization treatments. Thus, the objective of our study was to examine the responses of bacterial and archaeal taxa in the soils to long-term inorganic fertilization treatments.

2. Materials and Methods

2.1. Site Description

Field experiments were conducted at the Africa Rice Sahel research station in Ndiaye (16˚11'N, 16˚15'W) located close to the coast (about 40 km inland) in the Senegal River Delta (SRV; Senegal, West Africa) during the hot and dry season 2017. The local climate is a typical Sahelian climate with a long dry period from October to June and a short-wet season from July to September [20]. The highest average temperatures are recorded in April-May and the lowers in December-January. At our experimental site, the soil is an orthothionic Gleysol, containing 40% - 54% clay, (smectite and kaolinite) with average permeability of 2.8 mm·d^{-1} [21]. Soil salinity is high due to the occurrence of marine salt deposits in the sub-soil [22]. During the hot dry season, maximal temperature varied from 25.5˚C to 41.8˚C and averaged 32.9˚C while minimal temperature varied from 13.2 to 25.2˚C and averaged 19.32˚C. Mean daily temperature was 26.1˚C during the HDS. Relative Humidity max and Relative Humidity min varied from 49% to 100% and 7.5% to 71%, and averaged 86.2% and 35.6%, respectively. Seasonal average relative humidity was 60.9%. The seasonal average precipitation is 7.5 mm. Daily income shortwave solar radiation varied from 9.8 to 26.3 MJ·m^{-2} and averaged 22.4 MJ·m^{-2}. Wind speed varied from 1.8 to 4.3 m·s^{-1} averaging 2.9 m·s^{-1}.

We worked on plots of 25 m^2 (5 × 5 m) separated by small dikes (30 cm high) and maintained in irrigated conditions, which received three different inorganic fertilization treatments in randomized blocks design from 1991-2017. UF: unfertilized plots (control); N: fertilized plots with only urea fertilization (120 kg·ha^{-1} urea) and NPK: fertilized plots with recommended fertilization (120 kg·ha^{-1} urea $(CONH_2)_2$, 26 kg·ha^{-1} superphosphate (P_2O_5), 50 kg·ha^{-1} potassium chloride (KCl).

2.2. Soil Sampling

The soil sampling was non-destructive and was performed following the diagonal sampling method: it consists of taking soil at a depth of 0 - 20 cm. From each plot, soil cores were collected and pooled into a single composite sample. Soil samples were placed in plastic bags in ice and transported to the laboratory where they were stored at 4˚C for 24 h before processing. Each composite soil sample was then divided into two subsamples of which one was used for soil chemical analysis and the other for microbial DNA-metabarcoding.

2.3. Soil Chemical Analysis

Soil properties were determined as we previously described in [23]. Briefly, soil pH was determined with a soil-to-water ratio of 1:2.5. Soil nitrate (NO_3^-) and ammonium (NH_4^+) were extracted with 2 M KCl and were quantified by Bran + Luebbe GmbH Auto Analyzer III. Total carbon (C) and total nitrogen (N) contents were quantified using Elemental Analyzer (Flash EA 1112 series, Thermo Finnigan, France). Soil available phosphorus (AP) was extracted using sodium bicarbonate and then measured by the molybdenum-blue method. The phosphorus (P) concentration was determined after dry mineralization by inductively coupled plasma atomic emission spectrometry (ICP-AES). Electrical conductivity (EC) and salinity were measured with a digital conductivity meter.

2.4. Soil DNA Extraction

DNA was extracted from samples of 250 mg using the Fast DNA Spin Kit for Soil (MP Biomedicals, Fountain Parkway, Solon, OH, USA), according to the manufacturer's instructions. DNA concentration and purity were determined using a NanoDrop ND-2000 UV-VIS spectrophotometer (Nano Drop Technologies, Wilmington) and DNA samples were stored at −20˚C before sequencing.

2.5. PCR Amplification and Sequencing

Amplification and sequencing of bacterial and archaeal DNA were performed at MR DNA (www.mrdnalab.com, Shallo water, TX, USA) by targeting the V4 variable region of the 16S rRNA gene. Bacterial DNA was amplified by using the universal primers 515F/806R [24], while archaeal DNA was amplified with the primers 349F/806R [25]. After amplification, the quality and relative concentration of the amplicons were checked by migration on 2% agarose gel. Multiple replicates were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled DNA samples were purified using calibrated Ampure XP beads. Then the pooled and purified amplicons were used to prepare DNA libraries following Illumina Truseq DNA library preparation protocol. Sequencing was performed on a MiSeq Illumina platform (2 × 300) following the manufacturer's guidelines.

2.6. Sequence Analysis

Sequence data were processed using MR DNA analysis pipeline

(https://www.mrdnalab.com/, Shallowater, TX, USA) as we previously described in [23]. In summary, raw Illumina MiSeq paired-end reads were assembled, and sequences were demultiplexed and formatted for processing using a Python script (http://drive5.com/usearch/manual/uparse_pipeline.html). Sequences were then separately quality-filtered and clustered into operational taxonomic units (OTUs) using UPARSE algorithm [26]. Briefly, sequences were quality-filtered allowing a maximum e-value of 0.5. Reads were then trimmed to 240-bp length, dereplicated and sorted by abundance, and singletons were removed prior to OTU determination at 97% sequence similarity threshold. Then, chimeric sequences were screened and removed using UCHIME [27] against the Gold database [28]. Finally, bacterial and archaeal OTU tables were generated by mapping back the reads from the entire dataset to the representative prokaryotic databases. The taxonomic affiliation of each OTU was obtained using BLASTn against a curated database derived from Green Genes [29], RDPII (http://rdp.cme.msu.edu), and NCBI (https://www.ncbi.nlm.nih.gov/).

2.7. Data Analyses

All statistical analyses were conducted in R v3.5.1 [30]. All levels of significance were defined at $P < 0.05$. Rarefaction analysis was first performed to normalize the filtered OTU with the package *rtk* in the phyloseq package [31]. Thus, the OTU tables were rarefied from 36181sequences to 15721 sequences for bacteria and from 51939 sequences to 25357 sequences for archaea. Alpha diversity was then estimated by the Shannon and Simpson indices of diversity at each rarefaction level for bacteria and archaea. Shapiro test was done and normality test was checked at p-value < 0.05. One-way analysis of variance (ANOVA; fertilization) was performed. Mean values were compared using Tukey's Honest Significant Differences test at the 5% significance level ($P < 0.05$). In order to assess the beta-diversity, all ordination analyses were performed using the R package *phyloseq* [31]. CAP was performed on Bray-Curtis dissimilarity with the *phyloseq* package. Permutational analysis of variance (PERMANOVA) was performed using the functions Adonis from the *vegan* package [32] in R software with 1000 permutations based on Bray-Curtis dissimilarity [33].

3. Results

3.1. Effect of Long-Term Inorganic Fertilization on Diversity of Bacterial and Archaeal Communities at Tillage

Shannon diversity index and Simpson diversity index were calculated to assess the effect of long-term fertilization treatments on the diversity of microbial communities.

The results showed that for the bacterial community, there was no significant difference between different fertilization treatments not only for Shannon diversity index but also for Simpson diversity index (Table 1). For archaeal community, there was no significant difference between different fertilization treatments for

Table 1. Results of statistical tests of alpha diversity of bacterial community in different long-term fertilization treatments.

Fertilization	Shannon	Simpson
UF	8.471 ± 0.2**a**	0.9995 ± 0.0001**a**
N	8.585 ± 0.062**a**	0.9996 ± 0.00003**a**
NPK	8.533 ± 0.1**a**	0.9995 ± 0.00008**a**

Means in the same column followed by the same letter are not significantly different ($p < 0.05$) according to Tukey's Honest Significant Differences (HSD). UF: unfertilized plots (control); N: plots with only urea fertilization (120 kg N ha^{-1}) and NPK: plots with recommended fertilization (120 kg N ha^{-1}, 26 kg P_2O_5 ha^{-1}, 50 kg KCl ha^{-1}).

Shannon diversity index, on the contrary, there was a significant difference between different fertilization treatments for Simpson diversity index (Table 2).

For examining the effects of soil properties on bacterial and archaeal communities, we employed canonical analysis of principal coordinates also called constrained analysis of principal coordinates (CAP). Thus, constrained analysis of principal coordinates (CAP) was performed to find associations between community composition and soil properties based on OTU level. The CAP profile indicated that the first Axis 1 (CAP1) and second Axis (CAP2) explained 18.4% and 15.6% of the total variations, respectively for the bacterial community (Figure 1) whilst 29.4% and 18.3% were explained respectively for archaeal community (Figure 2). Furthermore, salinity, soil nitrate (NO_3^-) and ammonium (NH_4^+) were the factors that affected more the composition of both microbial communities in soil (Table 3 and Table 4).

3.2. Effect of Long-Term Inorganic Fertilization on Relative Abundance of Bacterial and Archaeal Communities at Tillage

In unfertilized plots and fertilized plots, the relative abundances were assessed for both communities at tillage in the irrigated rice field.

For bacterial communities, *Proteobacteria* was the preponderant phylum which represented 35% - 39% of the total reads which are rather subdivided into classes of *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria* followed by *Chloroflexi* (21% - 23%), *Firmicutes* (11% - 12%), *Actinobacteria* (5% - 6%) and *Acidobacteria* (4% - 5%) in long-term different inorganic fertilization treatments (Figure 3). These all phyla accounted for an average of 80% of the total bacterial sequences in an irrigated rice field at tillage. Furthermore, we noticed the shifts of relative abundances of bacterial members according to each fertilization.

For archaeal communities, *Methanomicrobia* was the preponderant class which represented 33% - 46% of total reads followed by *Methanobacteria* (12% - 19%), *Thaumarchaeota* (12% - 16%) and *Crenarchaeota* (11% - 12%) in long-term different inorganic fertilization treatments (Figure 4). These all classes accounted

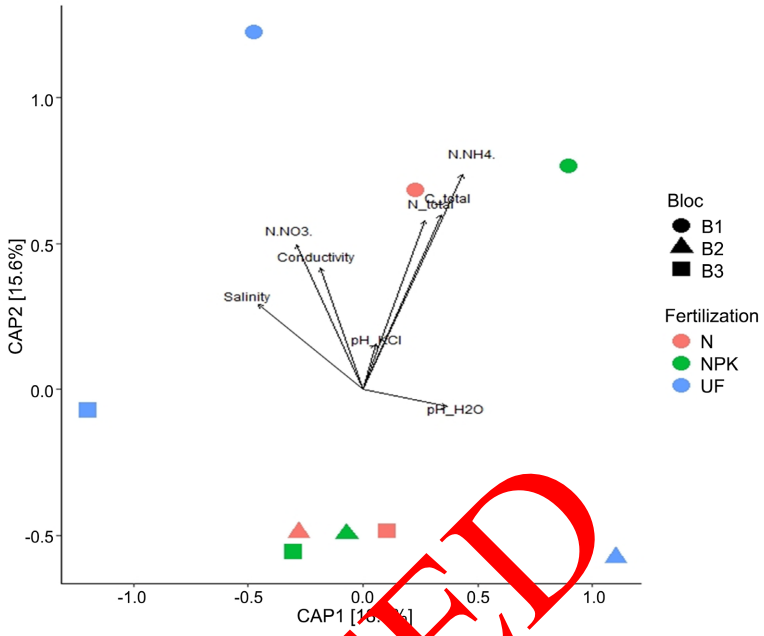

Figure 1. Effect of soil properties on bacterial community dissimilarity with permutational analysis of variance (PERMANOVA) based on OTU level. Beta-diversity of bacterial community. Separate CAP ordinations using Bray-Curtis distance were performed for bacterial community. From B1 to B3: different blocks. UF: unfertilized plots (control); N: plots with only urea fertilization (120 kg N ha^{-1}) and NPK: plots with recommended fertilization (120 kg N ha^{-1}, 26 kg P_2O_5 ha^{-1}, 50 kg KCl ha^{-1}).

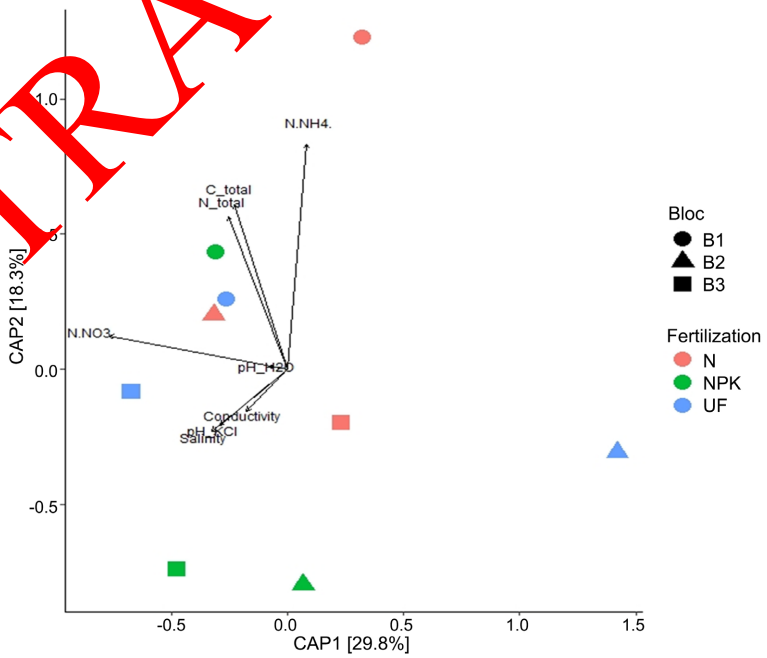

Figure 2. Effect of soil properties on archaeal community dissimilarity with permutational analysis of variance (PERMANOVA) based on OTU level. Beta-diversity of archaeal community. Separate CAP ordinations using Bray-Curtis distance were performed for archaeal community. From B1 to B3: different blocks. UF: unfertilized plots (control); N: plots with only urea fertilization (120 kg N ha^{-1}) and NPK: plots with recommended fertilization (120 kg N ha^{-1}, 26 kg P_2O_5 ha^{-1}, 50 kg KCl ha^{-1}).

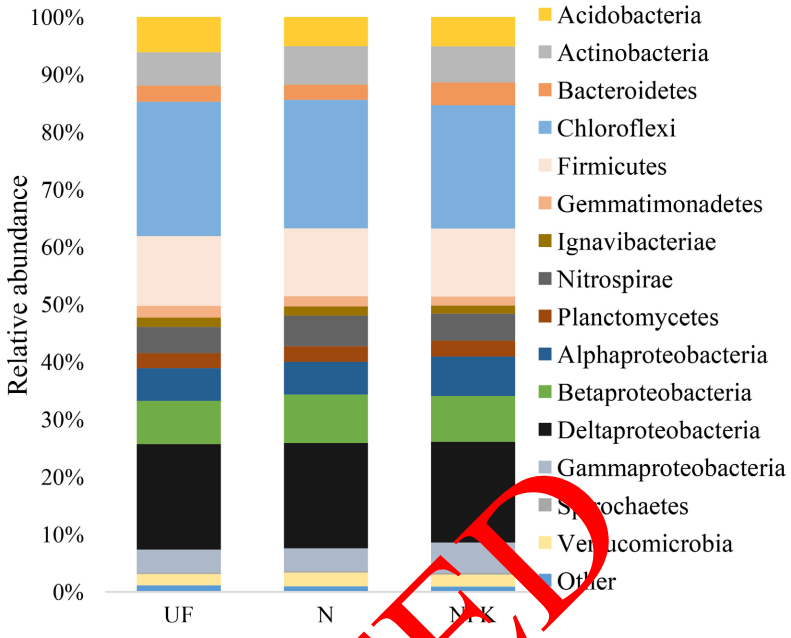

Figure 3. Relative abundance of bacterial community in different long-term fertilization treatments. UF: unfertilized plots (control); N: plots with only urea fertilization (120 kg N ha^{-1}) and NPK: plots with recommended fertilization (120 kg N ha^{-1}, 26 kg P_2O_5 ha^{-1}, 50 kg KCl ha^{-1}).

Table 2. Results of statistical tests of alpha diversity of archaeal community in different long-term fertilization treatments.

Fertilization	Shannon	Simpson
UF	5.754 ± 0.216**a**	0.9841 ± 0.0012**a**
N	6.026 ± 0.099**a**	0.9923 ± 0.0006**b**
NPK	5.792 ± 0.071**a**	0.987 ± 0.0024**ab**

Means in the same column followed by the same letter are not significantly different ($p < 0.05$) according to Tukey's Honest Significant Differences (HSD). UF: unfertilized plots (control); N: plots with only urea fertilization (120 kg N ha^{-1}) and NPK: plots with recommended fertilization (120 kg N ha^{-1}, 26 kg P_2O_5 ha^{-1}, 50 kg KCl ha^{-1}).

Table 3. Soil properties affecting the bacterial composition in different long-term fertilization treatments.

Soil properties	CAP1	CAP2
pH_H_2O	0.3652	−0.0589
pH_KCl	0.0548	0.1554
Conductivity	−0.1852	0.4157
Salinity	−0.4559	0.2914
N_NO_3^-	−0.2917	0.4947
N_NH_4^+	0.4332	0.7351
N_total	0.2712	0.5785
C_total	0.3399	0.5983

Table 4. Soil properties affecting the archaeal composition in different long-term fertilization treatments.

Soil properties	CAP1	CAP2
pH_H_2O	−0.08314	0.01069
pH_KCl	−0.29364	−0.20586
Conductivity	−0.17851	−0.15513
Salinity	−0.32825	−0.22868
N_NO_3^-	−0.76862	0.12496
N_NH_4^+	0.08264	0.83184
N_total	−0.25733	0.56486
C_total	−0.22930	0.60733

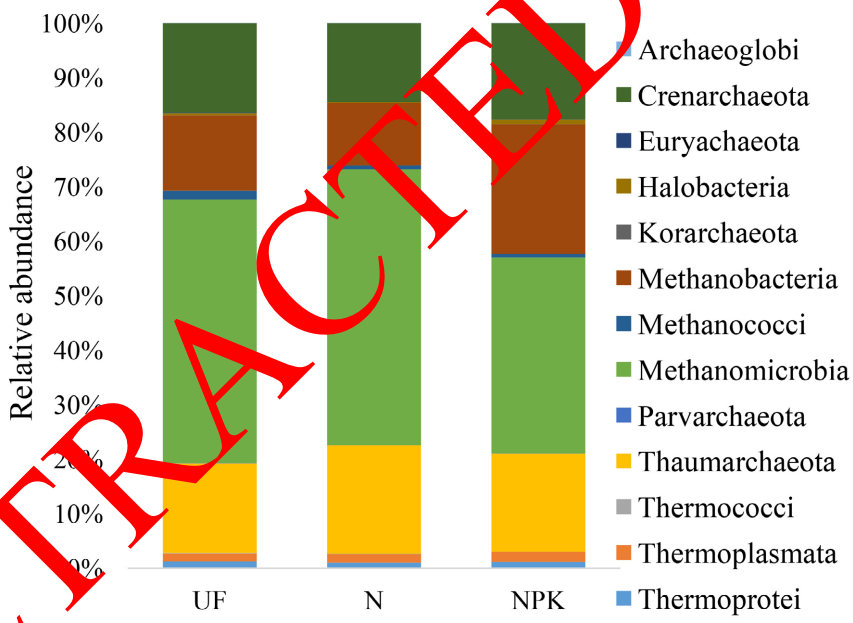

Figure 4. Relative abundance of archaeal community in different long-term fertilization treatments. UF: unfertilized plots (control); N: plots with only urea fertilization (120 kg N ha^{-1}) and NPK: plots with recommended fertilization (120 kg N ha^{-1}, 26 kg P_2O_5 ha^{-1}, 50 kg KCl ha^{-1}).

for an average of 80% of total archaeal sequences in an irrigated rice field at tillage. Moreover, we noticed the shifts of relative abundances of archaeal members according to each fertilization.

4. Discussion

4.1. Relations between Bacterial and Archaeal Diversity and Soil Chemical Properties

In this study, next-generation sequencing of the bacterial and archaeal 16S rRNA gene was used to explore the changes in bacterial and archaeal communities [34] [35] according to long-term different inorganic fertilization treatments. Micro-

biomes vary in abundance, composition and diversity according to many factors such as soil properties and fertilization practices [36]. The use of standard alpha/beta diversity metrics and composition can successfully reveal the changes of soil bacterial communities [9]. However, long-term different inorganic fertilization treatments rarely altered the bacterial community alpha diversity indexes, and similar findings were observed in paddy soil [10]. In contrast, some studies demonstrated that inorganic fertilizer application decreased the bacterial community diversity [9] [37]. Moreover, previous studies reported that nutrient additions did not strongly alter bacterial diversity but strongly affect archaeal diversity [38]. Thus, soil archaeal communities are sensitive to nutrient additions.

4.2. Relative Abundance of Bacterial and Archaeal Communities

The relative abundance revealed that for bacteria, *Proteobacteria* was the preponderant bacterial phylum which rather subdivided into classes (*Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria*), followed by *Chloroflexi*, *Firmicutes*, *Actinobacteria* and *Acidobacteria* which all accounted for an average of 80% of the total bacterial sequences in the irrigated rice field. [9] demonstrated that phyla *Proteobacteria*, *Acidobacteria*, *Chloroflexi* and *Actinobacteria* accounted for 74.3% on average of the total bacterial reads in paddy soils. [10] showed that the phyla *Proteobacteria*, *Chloroflexi*, *Acidobacteria* and *Nitrospirae* accounted for more than 70% of the total bacterial reads in paddy soils. Furthermore, most bacterial species found in the paddy soils belonged to the phyla *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Acidobacteria* and *Chloroflexi*, composing up to 87% of the total [39]. These results indicated that the bacterial dominant phyla remained relatively stable in the same use pattern soils [1]. *Proteobacteria* is the most abundant phylum from rice soil [9] [10] [40] [41]. *Proteobacteria* comprises several species that are considered to be fast-growing copiotrophs and thus proliferate in nutrient-rich soils [42]. They are also known to be rice-straw degraders [43]. *Acidobacteria* was described as an oligotrophic bacterium with slow growth and K-selected life strategy, and the abundance of *Acidobacteria* in soil was often higher under the lower nutrient-rich soils [44]. The ratio between *Proteobacteria* and *Acidobacteria* is thought to be an indicator of the nutritional status of the soil ecosystem [45] [46]. *Chloroflexi* are facultative anaerobic and have a recognized role as heterotrophic oligotrophs in soils, having the ability to survive on recalcitrant plant polymers [47] [48]. In the agroecosystems, *Actinobacteria* contribute to nutrient cycling and the degradation of organic compounds, including pesticides and herbicides [49] [50] [51] [52]. For archaea, the relative abundance revealed that, the preponderant class was *Methanomicrobia* followed by *Methanobacteria*, *Thaumarchaeota* and *Crenarchaeota* which all accounted for an average of 80% of the total archaeal sequences in the irrigated rice field. *Methanomicrobia* and *Methanobacteria* classes belong to *Euryarchaeota* phylum [53] which is the more abundant phylum present in wetland soils [54] [55] [56]. Besides, *Methanomicrobia* and

Methanobacteria are more abundant in wetland soils amended with inorganic fertilization [39] [53]. Therefore, soil archaeal communities are sensitive to nitrogen additions [38].

5. Conclusion

In this present study, to conclude, the results after long-term different inorganic fertilization treatments showed that Shannon diversity index and Simpson diversity index revealed the alpha-diversity of bacterial and archaeal communities with more response of archaeal community to long-term different inorganic fertilization compared with the bacterial community. For bacteria, *Proteobacteria* was the preponderant phylum which was rather subdivided into classes of *Alphaproteobacteria*, *Betaproteobacteria*, *Deltaproteobacteria* and *Gammaproteobacteria*, followed by *Chloroflexi*, *Firmicutes*, *Actinobacteria* and *Acidobacteria*. For archaea, *Methanomicrobia* was the preponderant class followed by *Methanobacteria*, *Thaumarchaeota* and *Crenarchaeota*. This study provides more insights into the response of soil microbiome to long-term different inorganic fertilization treatments, which will be helpful in managing soil microbes for sustainable agricultural productivity. However, further research is still needed to study the microbial communities which respond better to long-term different inorganic fertilization treatments.

Author Contributions

Conceptualization: Henri Fankem, Victor Désiré Taffouo, Donald Tchouomo Dondjou, Abdala Gamby Diedhiou, Aboubacry Kane, Laurent Laplaze, and Baboucarr Manneh; methodology: Donald Tchouomo Dondjou, Henri Fankem, Abdala Gamby Diedhiou, Baboucarr Manneh, Sarah Pignoly, and Daouda Mbodj; data analysis, Donald Tchouomo Dondjou, Marie-Thérèse Mofini, and Abdala Gamby Diedhiou; writing-original draft preparation: Donald Tchouomo Dondjou; writing-review and editing: Henri Fankem, Victor Désiré Taffouo, Abdala Gamby Diedhiou, Laurent Laplaze, Aboubacry Kane; supervision: Henri Fankem, and Victor Désiré Taffouo; funding acquisition: Henri Fankem, and Victor Désiré Taffouo. All authors read and agreed to the published version of the manuscript.

Acknowledgements

This work was supported by the Deutscher Akademischer Austauschdienst, Ph.D. grant to Donald Tchouomo Dondjou and Marie-Thérèse Mofini through the Centre d'Etude Régional pour l'Adaptation à la Sécheresse, Institut Sénégalais de Recherches Agricoles.

Conflicts of Interest

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

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