Scientific Research Publishing

Advances in Microbiology, 2024, 14, 513-531
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Integrated Effects of Phosphate Rock and Chemical Fertilizers on the Dynamics of Soil Bacterial in Acidic Rice Paddy Soils of Man (Ivory Coast)

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How to cite this paper: Bongoua-Devisme, A.J., Kouakou, S.A.A.E., Kouadio, K.-K.H. and Bahan, F.M.L. (2024) Integrated Effects of Phosphate Rock and Chemical Fertilizers on the Dynamics of Soil Bacterial in Acidic Rice Paddy Soils of Man (Ivory Coast). *Advances in Microbiology*, **14**, 513-531.
https://doi.org/10.4236/aim.2024.1410035

Received: May 27, 2024
Accepted: October 25, 2024
Published: October 28, 2024

Abstract

In agricultural soils, phosphorus is often limited, leading farmers to employ artificial supplementation through both inorganic and organic fertilization methods due to its restricted availability. Soil fertilization has the potential to augment both the abundance and diversity of bacterial communities. Our study aimed to assess the effects of phosphate amendments, derived from natural phosphate rock, and chemical fertilizers (TSP, NPK), on the density and diversity of bacterial communities within the study plots. We developed and applied eight phosphate amendments during the initial cultivation cycle. Soil samples were collected post 1st and 2nd cultivation cycles, and the quantification of both total and cultivable phosphate-solubilizing bacteria (PSB) was conducted. Additionally, we analyzed bacterial community structure, α-diversity (Shannon Diversity Index, Evenness Index, Chao1 Index). The combination of natural phosphate rock (PR) and chemical fertilizers (TSP, NPK) significantly increased ($p < 0.05$) both total cultivable aerobic bacteria (24 to 8500 $\times 10^7$ bacteria/g dry soil) and phosphate-solubilizing bacteria (0.01 to 6.8 $\times 10^7$ PSB/g dry soil) in comparison to unamended control soils. The diversity of bacterial phyla (Firmicutes, Actinobacteria, Proteobacteria, Halobacterota, Chloroflexia) observed under each treatment remained consistent regardless of the nature of the phosphate amendment applied. However, changes in the abundance of the bacterial phyla populations were observed as a function of the nature of the phosphate amendment or chemical fertilizer. It appears that the addition of excessive natural phosphate rock does not alter the number and the diversity of soil microorganisms population despite successive

cultivation cycles. However, the addition of excessive chemical fertilizer reduces soil microorganisms density and structure after the 2nd cultivation cycle.

Keywords

Phosphate Amendments, Phosphate Solubilizing Bacteria, P-Cycle Genes, Chemical Fertilizer

1. Introduction

Côte d'Ivoire is one of the countries in sub-Saharan Africa where rice is the primary food staple [1]. Despite its importance, national rice production falls short of meeting the growing demands of the population [2] [3]. To address this gap, farmers frequently resort to using chemical fertilizers.

Fertilization plays a crucial role in agriculture by enhancing soil fertility and boosting crop yields [4], essential for meeting the food demands of a growing global population. Fertilizers provide vital nutrients such as nitrogen, phosphorus, and potassium, which are essential for plant growth and development. However, the widespread and often intensive use of both organic and inorganic fertilizers poses significant long-term risks to soil health and quality [5]. The application of fertilizers, particularly chemical ones, can lead to various adverse effects on soil properties and indirectly impact its microbial ecosystem [6]. Continuous application of chemical fertilizers can cause soil acidification, negatively affecting soil structure and fertility [7]. Imbalanced use of chemical fertilizers can alter soil pH, increase pest attacks, and exacerbate acidification [5]. Consequently, acidification can lead to the loss of essential nutrients, decreased soil pH, and reduced availability of micronutrients, ultimately resulting in poorer crop performance and soil degradation [8].

Fertilizers can also alter the composition and functioning of microbial communities [9]. They affect soil microorganisms indirectly by changing soil properties or directly through the addition of nutrients [10] [11]. Research has shown that prolonged use of chemical fertilizers can significantly reduce soil bacterial diversity, primarily due to a decrease in soil pH levels [12] [13]. However, some studies have yielded contradictory results, indicating that fertilizer application can quickly supplement nutrient elements, greatly increasing the available nitrogen and phosphorus, enhancing microbial activity, and accelerating nutrient transformation [4] [9]. While some research suggests that organic fertilizer application can increase soil microbial diversity compared to chemical fertilizers [9] [14], other studies indicate the opposite [12] [13] [15]. According to Zhang *et al.* (2022) [4], while moderate fertilizer use may enhance microbial activity and diversity, excessive application can lead to a decline in beneficial microbes and an increase in pathogenic species. Additionally, the use of combined inorganic and organic fertilization in rice field soils across South China was found to change the abundance

of soil microbial communities [16].

Given these concerns, there is a growing emphasis on sustainable fertilizer practices. Integrated nutrient management, which combines organic and inorganic fertilizers, crop rotation, and the use of biofertilizers, is gaining traction as a strategy to mitigate the adverse effects of fertilizers on soil health. Research increasingly focuses on understanding the complex interactions between fertilizers and soil ecosystems to develop practices that maintain soil fertility while minimizing environmental impact.

In this context, our study aims to evaluate the specific effects of phosphate amendments derived from natural phosphate rock and chemical fertilizers on soil bacterial communities. By examining changes in bacterial density and diversity, we seek to understand how different fertilization strategies influence soil health and microbial ecology. This knowledge is essential for developing sustainable agricultural practices that preserve soil integrity and ensure long-term agricultural productivity.

2. Materials and Methods

Study site

Our research took place in western Côte d'Ivoire over the period 2020-2021, specifically on two experimental plots at the National Center of Agricultural Research (CNRA) station. These plots are located in both lowland (7˚21'12''N; 7˚36'19''W) and plateau (7˚ 20'57''N; W 7˚36'19'') areas.

Before initiating the experiments, soil samples were systematically collected at a depth of 0 - 20 cm from various locations within the plot to ensure comprehensive coverage and representativeness. These individual subsamples were then combined into a composite sample, which was subsequently sieved (2 mm) and divided into two parts. The first part underwent thorough physico-chemical analysis, while the second part was stored at −4˚C for microbiological analysis. The soil's characterization before experimentation is presented in Table 1 and Table 2.

Prior to rice cultivation, the initial characterization of soils indicated that lowland soils are more sandy and poorer in organic matter than upland soils (Table 1), thus not favoring adequate storage of nutrients (calcium, phosphorus, nitrogen, potassium, carbon) compared to those richer in clay [17].

Plant material and Fertilizer material

Two rice varieties, namely WITA 9 for lowlands and IDSA 10 for uplands, were carefully chosen from the National Center of Agricultural Research (CNRA) of Ivory Coast, on the basis of the two ecologies studied.

For the enhancement of soil fertility, two types of phosphorus fertilizers were employed in this study. The first is a natural phosphorus fertilizer, specifically Morocco phosphate rock (PR), boasting a P_2O_5 content of 30% with a solubility of 3% in water (Table 3). The second is a chemical phosphorus fertilizer, Triple Superphosphate (TSP), also containing 30% P_2O_5. Both fertilizers were generously supplied by the Office Cherifien of Phosphate (OCP).

Table 1. Physico-chemical characteristics of the soils at 0 - 20 cm depth before experimentation.

Parameters	Values	
	Upland	Lowland
Clay (%)	29	6
Silt (%)	16	13
Sand (%)	55	81
pH water	5.2	5.6
pH KCl	3.6	4.3
Assimilable P ($g{\cdot}kg^{-1}$ dry soil)	5	2.1
Organic C ($g{\cdot}kg^{-1}$ sol sec)	143	69
Total N ($g{\cdot}kg^{-1}$ sol sec)	13	7
Organic Matter ($g{\cdot}kg^{-1}$ sol sec)	246	120
C/N	11	9.8
K^+ ($g{\cdot}kg^{-1}$ sol sec)	0.96	0.22
Na^+ ($mmol^+{\cdot}kg^{-1}$)	0.08	0.03
Ca^{2+} ($mmol^+{\cdot}kg^{-1}$)	4.96	2.47
Mg^{2+} ($mmol^+{\cdot}kg^{-1}$)	2.4	0.78
CEC ($mmol^+{\cdot}kg^{-1}$)	8.5	3.4
S/T (%)	15.66	10.27

Table 2. Total Bacterial (BT) and phosphate solubilizing bacteria (PSB) count. 10^5/g dry soil.

Ecology	BT	PSB	Pr
Lowland	23,000[a]	5[b]	0.005**
Upland	833[a]	1[b]	0.0001***

In the same line, numbers with the same letters are not significantly different according to Student's t test at Pr < 5%). ***Probability very highly significant at $p < 0.05$; **Highly significant at $p < 0.05$.

Table 3. Chemical composition of Morocco phosphate rock (PR).

Chemical composition	P_2O_5	CO_2	SO_3	CaO	MgO	Fe_2O_3	Al_2O_3	F_2O	H_2O	SiO_2
Content (%)	30	6.44	1.29	49.54	1.16	0.20	0.4	2.21	2.03	6.64

To supplement the soil with additional nutrients, various chemical fertilizers were introduced. Notably, a nitrogen fertilizer in the form of Urea (46% N) and an NPK fertilizer (15/15/15) were incorporated into the study to ensure a recommended dose of NPK for rice.

Trial Design

The experiment was carried out on each plot, with an area of 1250 m^2, including 1000 m^2 of usable surface, following a randomized complete block design. Each block, treated as a replication, had an area of 250 m^2, with 200 m^2 of usable surface, subdivided into 8 microplots of 25 m^2 each, where each microplot represented a treatment.

The following eight treatments resulting to the combination of different proportions of Morocco phosphate rock (MPR) and Triple Superphosphate (TSP) were applied in the field before sowing upland seeds or transplanting nursery plants in lowland areas. These treatments included an absolute control (T0a: 0% MPR and 0% TSP without NPK); a recommended dose of fertilizer for rice control (T0: 0% MPR and 0% TSP + NPK); T1 (100% MPR and 0% TSP+ NPK); T2 (90% MPR and 10% TSP + NPK); T3 (80% MPR and 20% TSP + NPK); T4 (40% MPR and 60% TSP + NPK); T5 (20% MPR and 80% TSP + NPK); T6 (0% MPR and 100% TSP + NPK).

A randomized complete block design with eight treatments and five replications per treatment was implemented in the field, treating each block as a replicate. Phosphorus fertilizers (MPR-TSP) were applied at an overall dose of 90 kg $P_2O_5 \cdot ha^{-1}$ or 300 kg TSP and/or RP$\cdot ha^{-1}$ in the plots, except for the control treatments (T0 and T0a), which received fertilizer only at the beginning of the first cycle. A ternary fertilizer composed of NPK 15-15-15 was uniformly applied to each plot, except for the control treatment T0a, at a dose of 200 kg$\cdot ha^{-1}$ as a basal fertilizer (recommended for rice).

Additionally, 100 kg$\cdot ha^{-1}$ of 46% Urea was spread: 50 kg$\cdot ha^{-1}$ at the tillering stage and 50 kg$\cdot ha^{-1}$ at the bolting stage. At the end of the second cropping cycle, soil samples were collected by treatment and plot ecology (lowland/upland), and stored in a fridge at −20˚C before being used for microbiological analysis.

Enumeration of cultivable bacteria: Total bacteria (BT) and phosphate solubilizing bacteria (PSB)

A most probable number (MPN) technique was used to enumerate viable and cultivable bacterial populations including the total bacteria (TB) and phosphate-solubilizing bacteria (PSB).

After rice harvest, soil samples were collected from experimental plots in both upland and lowland ecologies. Soil dilutions were prepared by combining 10 g of soil with 90 mL of sterile 8 per mille (w/v) NaCl solution, resulting in serial dilutions from 10^{-1} to 10^{-6}. Enumeration of each soil sample was conducted in triplicate using 96-well microplate readers (Thermo Multiskan FC microplate photometer). Titration devices were employed, with 200 µL of culture medium per well, inoculated with 20 µL of soil suspension dilutions. Negative control wells were included to prevent cross-contamination. Total bacteria were enumerated in nutrient broth (NB) medium (DifcoTM, France) at 8 per mille (w/v). For phosphate-solubilizing bacteria (PSB), Pikovskaya's medium (PVK medium) was employed, containing 10 g de glucose; 0.01 g ammonium sulfate hydrate; 0.2 g potassium

chloride; 0.2 g sodium chloride; 0.1 g magnesium sulfate heptahydrate; 0.002 g manganese sulfate monohydrate; 0.002 g iron sulfate heptahydrate; 0.5 g yeast extract and 5 g Tricalcium phosphate; 15 g agar. Both culture media (TB/PSB) were autoclaved at 110˚C for 15 minutes. Microplates were kept in complete darkness at a temperature of 28˚C ± 2˚C for seven days before inoculation, and bacterial counts were determined using the method by Bongoua-Devisme *et al.* [18].

Quantification and analysis of microbial diversity in rice plot soils

The examination of bacterial communities in upland and lowland soils employed molecular biology techniques, specifically the extraction of total DNA from indigenous bacteria at the Ecology Laboratory of the Institute of Research and Development in Agroenvironment (IRDA) in Quebec, Canada (Quebec, QC Canada).

DNA extraction and amplification

DNA extraction from soil samples involved processing 0.5 g of soil using the FastDNA Spin kit for Soil (MP Biomedicals, Solon, OH, USA) at the Laboratory of Microbial Ecology of IRDA (Québec, QC Canada), with three replicates per sample. The extracted DNA was stored at −20˚C for subsequent amplification and sequencing.

Bacterial DNA amplification focused on the V4 regions of prokaryotic 16S rRNA, employing primer sequences specific to the V4 regions of the SSU rRNA gene (515F and 806R) following the methods outlined by Apprill *et al.* [19] and Parada *et al.* [20] (**Table 4**). The chosen approach involved a dual-index, two-step PCR designed for Illumina MiSeq high-throughput sequencing. Consequently, DNA was amplified with primers 515F/806RB and sequenced to evaluate amplification bias and sequencing error rate as described [21]. Paired-end sequencing (2 × 300 base pairs) occurred on Illumina MiSeq at the genomic analysis platform at the Institute of Research and Development in Agroenvironment (IRDA) in Quebec, Canada (Quebec, QC Canada).

Table 4. List of the primers that were used in this study/primers used in this study.

Name of primers	Séquences (5' → 3')	References
515F (modified)	GTGYCAGCMGCCGCGGTAA	Parada *et al.* (2016) [20]
926R	CCGYCAAATTYMTTTRAGTTT	Parada *et al.* (2016) [20]
806R (modified)	GGACTACNVGGGTWTTCTAAT	Apprill *et al.* (2015) [19]

Quantitative PCR (qPCR)

The qPCR system utilized primers eub338/eub518 [22] to detect total bacteria. Detection was conducted in duplicate on a CFX96 instrument (Biorad, Hercules, CA, USA) using SYBR green qPCR mix (Qiagen, Toronto, ON, Canada), following the procedure outlined in Tekeu *et al.* [21]. The detection system was designed within a detection range spanning 4 LOG. This method served to quantify the relative abundance of bacteria in the soil samples, expressed as the number of targeted sequences (Amplified Units) per gram of dry soil ($AU \cdot g^{-1}$

dry soil). Subsequently, these values underwent normalization, and a log transformation was applied.

Predictive functions of phosphate-solubilizing bacteria

Phosphate-solubilizing bacteria are characterized by several biochemical activities, including acid production and enzyme. These functions are determined from taxonomic data obtained by analyzing the diversity of prokaryotes. As part of this study, several genes involved in phosphate solubilization by bacteria or that could serve as biomarkers were identified and mentioned as method described by Wan *et al.* [23] and Wu *et al.* [24].

Bioinformatics and biostatistics processing

Sequence analysis and grouping into sequence taxonomic units was performed on IRDA's LEM bioinformatics platform and involved various processing strategies Qiime2 [25] and R (R Core Team project 2014), including quality validation steps, reference bases and indices for measuring microbial richness, and comparative measures of microbial diversity. These were grouped into OTUs based on 97% sequence similarity. Taxon assignment was performed using the SILVA version 138 reference database [26], which was also used for the analysis of bacterial diversity. For the analysis of predictive functions related to phosphate solubilization, the data obtained from the taxonomic analysis were processed using the Picrust2 approach [27] and 5 marker genes related to this functionality were filtered. Bacterial diversity was assessed by species richness (S), Shannon diversity index (Sh), evenness index (E), and Chao1 index (C). Species richness (S) refers to the total number of species in a soil sample. The Shannon Diversity Index (Sh) considers both species richness and species abundance. It integrates both the total number of species present and the relative abundance of species [28]. It provides a measure of the species composition of an ecosystem, taking into account their distribution and relative abundance. Values range from 0 (a single species) to 4.5 (very high diversity).

Statistical analysis

Bacterial abundance was estimated by enumeration using a spectrophotometer at OD = 620 nm, and the results were processed by a statistical program to determine the Most Probable Number (MPN) of bacteria per gram of dry soil [18]. An analysis of variance (ANOVA) was performed for all measured parameters. When a significant difference was found, the means were compared using the Newman-Keuls test to identify homogenous groups at the 5% probability threshold.

3. Results

Treatment Effect on Cultivable bacterial communities in soil

Overall, regardless of the ecological setting (upland/lowland), the application of natural phosphate rock (PR) and chemical fertilizer (NPK, TSP) significantly increased ($p < 0.05$) the total aerobic bacterial (TB) and phosphate-solubilizing bacterial (PSB) counts in soils compared to unamended control soils (**Figure 1** and **Figure 2**).

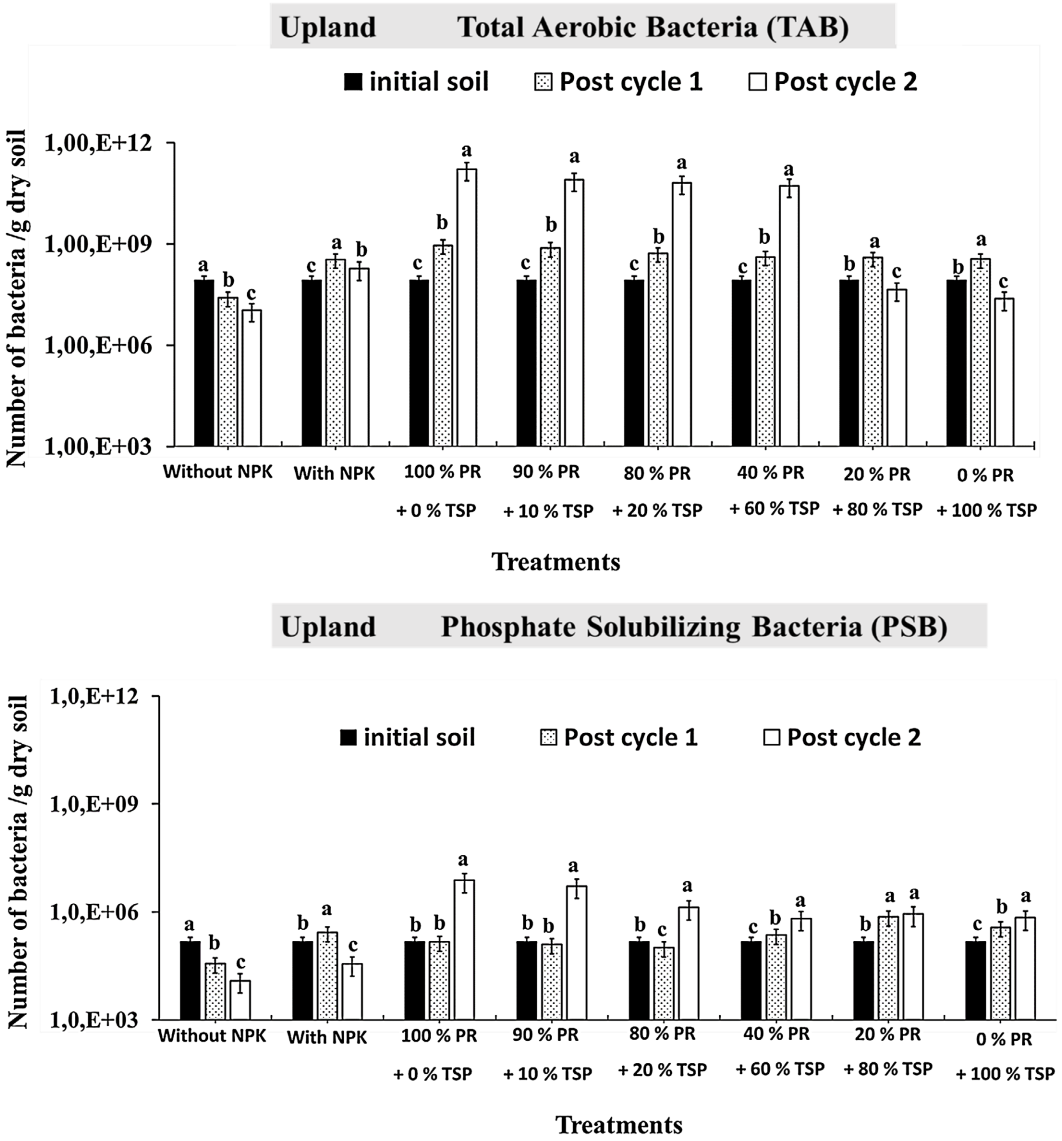

Figure 1. Number of cultivable bacteria/g dry soil under the different treatments in upland soils. TB (total aerobic bacteria), PSB (phosphate solubilizing bacteria). Histograms followed by the same letters (a, b) show significant differences according to Student's Test at Pr < 5%).

On upland soils, the total aerobic bacterial count ranged from 35.2 to 91 × 10^7 bacteria/g dry soil after post 1st cultivation cycle in presence of PR and/or TSP compared to controls (2.53 to 34.8 × 10^7 bacteria/g dry soil). Moreover, after 2nd cultivation cycle, the total aerobic bacterial count ranged from 2.4 to 16,700 × 10^7 bacteria/g dry soil in presence of PR and/or TSP compared to controls (1.1 to 18.5 × 10^7 bacteria/g dry soil). We noted during successive cultivation cycle, an increase of total aerobic bacterial number particularly when the phosphate amendment is rich in phosphate rock (Figure 1). For phosphate-solubilizing bacteria (PSB), the count ranged from 1.3 to 76 × 10^5 bacteria/g dry soil in amended soils compared to controls (0.1 to 2.6 × 10^5 bacteria/g dry soil) during successive

cultivation cycles. Regardless of cultivation cycle, we observed an increase of phosphate-solubilizing bacteria (PSB) number specially when the phosphate amendment is rich in phosphate rock (Figure 1).

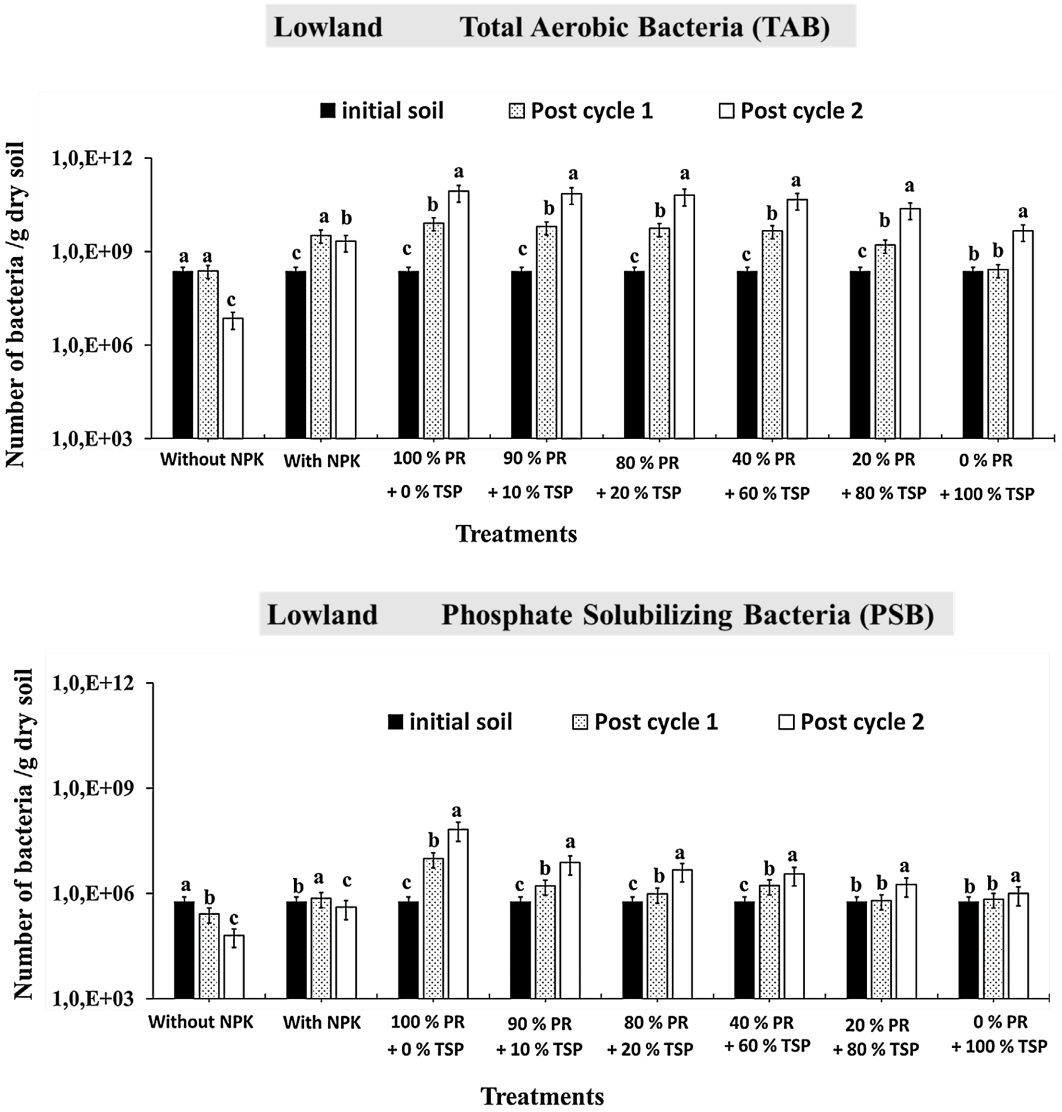

Figure 2. Number of cultivable bacteria/g dry soil under the different treatments in lowland soils after 1st nd 2nd cultivation cycles post. TB (total aerobic bacteria), PSB (phosphate solubilizing bacteria). Histograms followed by the same letters (a, b) show significant differences according to Student's Test at Pr < 5%).

In lowland soils, the total aerobic bacterial count varied from 27 to 8500 × 10^7 bacteria/g dry soil in amended soils compared to controls (0.7 to 340 × 10^7 bacteria/g dry soil), regardless of cultivation cycle (Figure 2). For phosphate-solubilizing bacteria (PSB), the count ranged from 69 to 6800 × 10^4 bacteria/g dry soil in amended soils compared to controls (0.6 to 7.2 × 10^5 bacteria/g dry soil).

Our results indicate a higher count of TAB and PSB bacteria when the phosphate amendment used is rich in Moroccan phosphate rock, *i.e.*, 40% to 100% PR (Figure 2). Comparing control T0 and T0a, our results show that the application

of mineral fertilizers (NPK) significantly increased the count of both TB and PSB bacteria (Figure 1 and Figure 2).

Treatment effect on bacterial α-diversity indexes

The total number of bacteria present in the lowland and upland plots varied from 58.5×10^7 to 156×10^7 UA/g dry soil and from 40.1×10^7 to 93.3×10^7 UA/g dry soil, respectively. (Table 5). Regardless of plot ecology (lowland or upland), our results indicate a significant increase in bacterial numbers (54.4×10^7 to 156×10^7 UA/g dry soil) when phosphate amendments (T1, T2, T3, T4, T5, T6) compared to the unamended control treatments (T0 and T0a), which ranged from 40.1×10^7 to 56×10^7 UA/g dry soil (Table 5).

Table 5. The bacterial *α*-diversity (Shannon, Chaos 1, Evenness, number of Operational Taxonomic Units (OTU), bacterial number Amplification Units (AU)/g dry soil.

Treatment	Upland					Lowland				
	Quantification et *α* diversity indexes					Quantification et *α* diversity indexes				
	Bacterial Number UA/g dry soil	Shannon	Chao1	Evenness	OTU number	Bacterial Number UA/g dry soil	Shannon	Chao1	Evenness	OTU number
T0a	$40.1 \times 10^{7\ bc}$	2.97^b	173.8^b	0.43^b	173^b	$51.5 \times 10^{7\ cd}$	2.48^b	171.5^c	0.45^b	218.5^c
T0	$47.9 \times 10^{7\ bc}$	2.92^b	182.6^b	0.44^b	173^b	$56 \times 10^{7\ cd}$	2.46^b	208.1^c	0.46^b	205.5^c
T1	$64.3 \times 10^{7\ ab}$	4.69^a	293.91^{ab}	0.83^a	293^a	$109 \times 10^{7\ b}$	5.47^a	423.91^{ab}	0.90^a	420^{ab}
T2	$66.3 \times 10^{7\ ab}$	4.80^a	318.15^a	0.83^a	317^a	$156 \times 10^{7\ a}$	5.64^a	519.86^a	0.90^a	515^a
T3	$93.3 \times 10^{7\ a}$	4.90^a	327.11^a	0.85^a	326^a	$125 \times 10^{7\ ab}$	5.50^a	435.33^{ab}	0.91^a	431^{ab}
T4	$73.6 \times 10^{7\ ab}$	4.96^a	322.10^a	0.86^a	320^a	$101 \times 10^{7\ b}$	5.47^a	431.55^{ab}	0.90^a	427^{ab}
T5	$58.7 \times 10^{7\ b}$	4.83^a	350.00^a	0.83^a	347^a	$72.1 \times 10^{7\ c}$	5.19^a	344.00^b	0.89^a	342^b
T6	$54.4 \times 10^{7\ b}$	4.83^a	343.10^a	0.83^a	341^a	$66.5 \times 10^{7\ c}$	5.35^a	381.15^b	0.90^a	379^b
MOY	62.32×10^7	4.36	288.85	0.74	286.25	92.14×10^7	4.7	364.425	0.78875	367.25
Pr	0.036	0.049	0.04	0.048	0.042	0.036	0.049	0.04	0.038	0.072

In the same column, data with the same letters (a, b) show significant differences according to Student's t test at Pr significant differences according to Student's t test at Pr < 5%).

The number of operational taxonomic units (OTUs) observed was significantly higher (293 to 515 OTUs) when phosphate amendments (T1, T2, T3, T4, T5, T6) were added, compared to the unamended control treatments (T0 and T0a), which ranged from 173 to 218.5 OTUs regardless of plot ecology (Table 5). Under the amended soils, the bacterial *α*-diversity indexes show that the Chaos index ranges from 293 to 520; the Shannon index is above 4 and the Evenness index tends to 1, regardless of plot ecology, compared to unamended soils where the Chaos index ranges from 171.5 to 208.1; the Shannon index is below 3 and the Evenness index tends to 0.5 (Table 5). This result reflects a strong diversity but with the same relative abundance of species in the bacterial population when phosphate

amendments were applied.

Treatment effect on the Relative Abundance of bacterial community composition

Five phyla (Firmicutes, Actinobacteria, Proteobacteria, Halobacterota, and Chloroflexia) were present regardless of the treatments applied and the ecology of the rice plots (upland/lowland). The Firmicutes phylum (35% to 53.25%) is the most dominant with 7 genera (Figure 3 and Figure 4), followed by the Halobacterota phylum (6.7% to 30%), Actinobacteria phylum (9% to 23.8%), and Proteobacteria phylum (5% to 21%) with two genera in each phylum (Figure 3 and Figure 4). While, the Chloroflexia phylum (4% to 9.58%) with two genera per phylum (Figure 3 and Figure 4) appears least abundant phylum in the both rice plots (Figure 3 and Figure 4). In the lowlands, the most representative genera are Bacillus sp. (9% to 17.5%) and Halobacterota Rice_cluster_I (6% to 15.5%), while in the uplands the most representative genera are Bacillus sp. (12% to 17.5%) and Clostridium sp. (17% to 19.5% in the lowlands) (Figure 4).

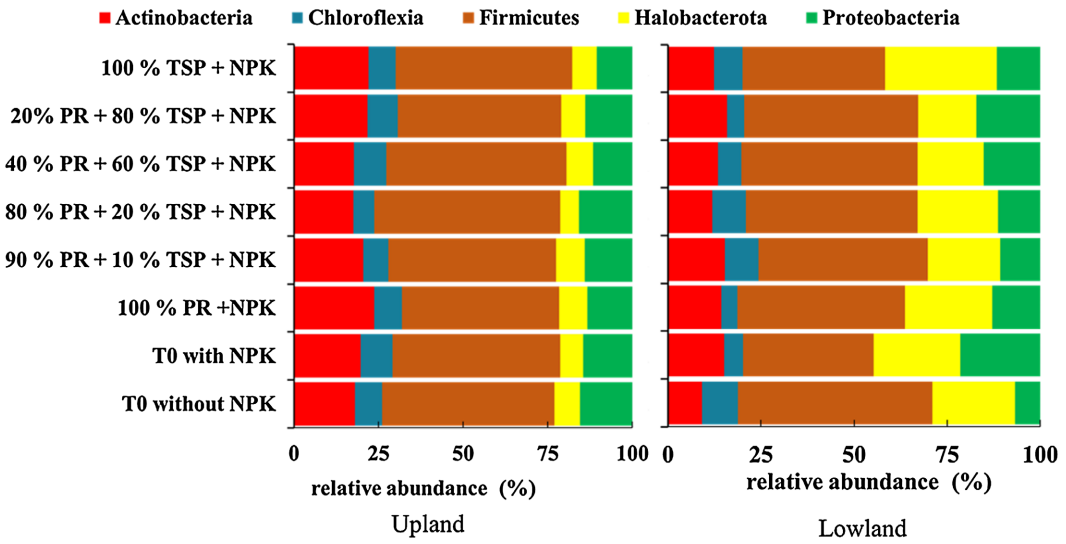

Figure 3. The relative abundance (%) of bacterial community composition among the primary prokaryotic groups detected in upland and lowland plots (groups greater than 1%) under different treatments.

The addition of NPK soluble fertilizer and/or phosphate amendment does not alter the soil indigenous bacterial community composition in the two rice plots studied, with the presence of five (5) phyla Firmicutes, Actinobacteria, Proteobacteria, Halobacterota, and Chloroflexia (Figure 3), regardless of the treatments applied and the ecological characteristics of the plots (upland/lowland).

However, a modification is observed in the abundance of the phyla population. Indeed, when comparing with the absolute control soil without soluble NPK fertilizer (T0 without NPK), it is noted that the addition of soluble NPK fertilizer decreases the relative abundance of the Chloroflexia phylum from 9% under T0 without NPK to 5% under T0 with NPK, and of the Firmicutes phylum from 52.3% under T0 without NPK to 35.1% under T0 with NPK (Figure 3). For the

Actinobacteria and Proteobacteria phyla, there is rather an increase in relative abundance, respectively, from 9% under T0 without NPK to 15.2% under T0 with NPK, and from 6% under T0 without NPK to 21% under T0 with NPK (Figure 4). Furthermore, it is noted that the addition of NPK does not alter the relative abundance of the Halobacterota phylum, where the relative abundance varies between 22% and 23% respectively under T0 without NPK and T0 with NPK (Figure 3).

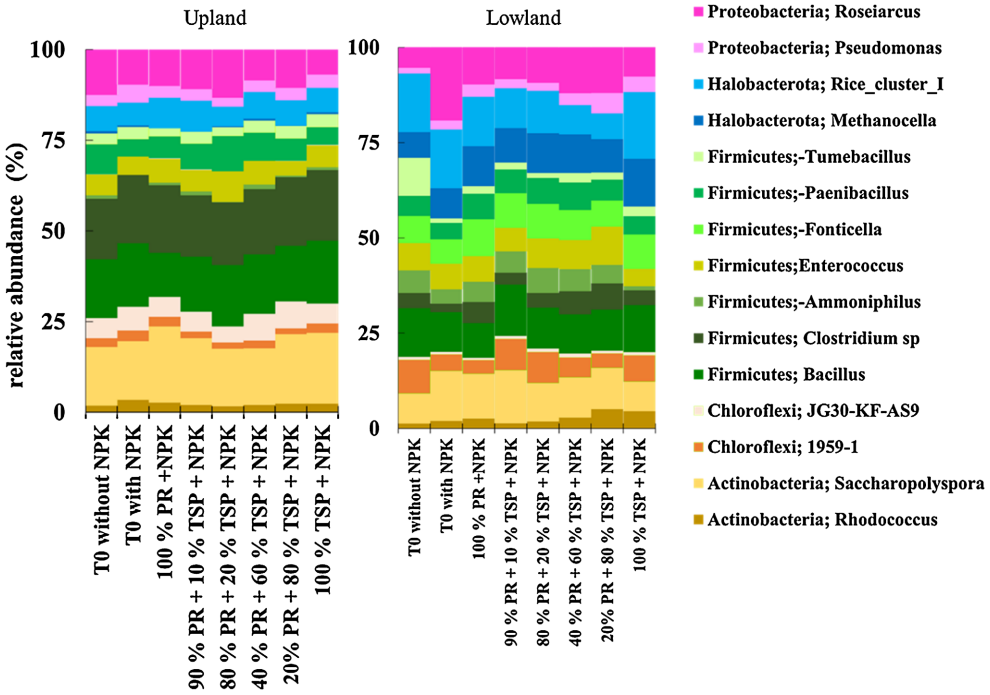

Figure 4. The relative abundance (%) of bacterial community composition among the primary prokaryotic genera detected in upland and lowland plots (genera greater than 1%) under different treatments.

Comparatively to the control treatment (T0 + NPK), our results show that when the phosphate amendment (AP) contains only phosphate rock (100% PR+NPK), the relative abundance of the five detected phyla on the upland is not significantly modified. However, in the lowland, the presence of phosphate rock (PR) alone in the AP decreases the relative abundance of the Proteobacteria phylum (from 21.5% to 12.97%), increases that of Firmicutes (from 35% to 45%), and does not affect Actinobacteria, Halobacterota, and Chloroflexia (Figure 3).

On the other hand, when the phosphate rock is associated with triple superphosphate (TSP), *i.e.*, under treatments T2, T3, T4, and T5, in the lowland, there is an increase in the relative abundance of the Chloroflexia phylum (from 5% to 9%) and Firmicutes (from 35% to 47%), and a decrease in the relative abundance of Halobacterota (from 23% to 15.5%) and Proteobacteria (from 21% to 10.7%), compared to the control treatment (T0+NPK). Moreover, under treatments T2, T3, T4, and T5, the relative abundance of the five detected phyla on the plateau (Firmicutes, Proteobacteria, Actinobacteria, Halobacterota, and Chloroflexia) is

not significantly modified, compared to the control treatment (T0 + NPK).

Furthermore, when the phosphate amendment contains only TSP, *i.e.*, under treatments T6, compared to the control treatment (T0 + NPK), the relative abundance of the five detected phyla on the upland is not significantly modified. However, in the lowland, the presence of TSP alone in the AP decreases the relative abundance of the Proteobacteria phylum (from 21.5% to 11.6%), increases that of Halobacterota (from 23% to 30%), and does not affect Actinobacteria, Firmicutes, and Chloroflexia (**Figure 3**).

4. Discussion

In soil, particularly in rice field soils, phosphorus is a limited nutrient and often exists in an insoluble form due to its reactivity with soil minerals [29]. Phosphorus exists in the soil in both organic and inorganic forms, and various microbes, including fungi, bacteria, and archaea, play crucial roles in extracting this nutrient.

Impact of Plot Topography on Bacterial Diversity and Abundance

The study conducted on rice field soils from both upland and lowland plots at the Man Research Station revealed the presence of a significantly higher number of bacteria, up to three times greater, in lowland soils compared to upland soils, regardless of the applied treatment ($p < 0.05$). Several factors may explain these variations in microbial biomass between these two ecologies, including the nature of organic compounds present in these plots rather than their quantity [30] [31], as well as the agricultural practices carried out in these two ecologies.

Despite the low organic content and limited mineralization in lowland soils, our findings suggest a better proliferation of bacteria in lowland soils than in upland soils. This outcome is likely attributed to divergent agricultural practices employed in these two ecological settings. In fact, in lowland areas, the cultivation technique is based on incorporating organic residues into the soil before transplanting, while on the upland, it involves burning and then direct seeding. According to Attard *et al.* [32] and Assémien *et al.* [33], burning reduces the density of soil bacterial communities and their various specific activities, justifying the higher number of total cultivable and phosphate-solubilizing bacteria observed under lowland plots than under upland plots. This result is in line with the works of [34] Khmelevtsova *et al.* [35] Szosboslay *et al.* and [31] Li *et al.* affirming that certain agricultural practices, especially plowing, affect the richness and diversity of the soil bacterial communities. Moreover, five phyla bacterial (Firmicutes, Actinobacteria, Proteobacteria, Halobacterota, Chloroflexi) were identified in plots of both ecologies, indicating stability in bacterial communities composition regardless of ecology.

However, a modification in the relative abundance of phyla is noted depending on the plot ecology, probably due to agricultural practices. This confirms the results of numerous previous studies reporting that agricultural practices have a strong impact on microbial communities [36] and also influence the abundance of the four denitrifying bacteria groups [33].

The taxonomic analysis of bacterial communities in the soils revealed that in lowland areas, the most representative genera are *Bacillus* sp. and Halobacterota Rice_cluster_I, while on the upland, the most representative genera are Bacillus sp. and *Clostridium* sp. (17% to 19.5%). This study suggests that depending on the topographical position of the plots, the composition of bacterial communities in soils not changes but modified the relative abundance of phyla, as proposed in the works of Vian [37].

Effect of phosphate amendments on soil bacterial diversity and abundance in the rice plots studied

The study on the effect of natural phosphate rock, and chemical fertilizers (TSP, NPK), on the density of bacterial communities and bacterial diversity in the studied plots revealed that the presence of the sole soluble chemical fertilizer (NPK), compared to the absolute control (soil potential), significantly influenced ($p < 0.05$) the number of cultivable bacteria (aerobic and phosphate-solubilizing) in all studied plots. However, the application of sole soluble NPK did not alter the composition of indigenous bacterial communities in the studied soils, with the presence of five phyla: Firmicutes, Actinobacteria, Proteobacteria, Halobacterota, and Chloroflexia, regardless of the applied treatments.

Conversely, the abundance of the phyla community showed variable modifications in response to NPK application. Specifically, compared to the unamended soil (absolute control without soluble NPK fertilizer - T0a), the application of soluble NPK fertilizer reduced the relative abundance of Chloroflexia by 4% and Firmicutes by 17.2%. However, it increased the relative abundance of Actinobacteria and Proteobacteria by 6.2% and 15%, respectively, with no effect on the relative abundance of Halobacterota. Thus, the application of soluble NPK fertilizer appears to impact the relative abundance but not the density and diversity of bacterial communities in the soils. Our results are in agreement with several previous studies that reported an increase in the abundance and activity of ammonium-oxidizing bacteria (AOB) in response to NPK fertilizer addition [33] [38].

The combination of natural phosphate rock from Morocco (PR) and chemical fertilizers (TSP, NPK) significantly increased ($p < 0.05$) the total cultivable aerobic bacteria (TB) and phosphate-solubilizing bacteria (PSB) in the soils, compared to unamended control soils. Furthermore, our findings revealed a higher abundance of total bacteria and phosphate-solubilizing bacteria (PSB) in soils amended with natural phosphate rock (PR), particularly when the phosphate amendment contained 40% to 100% PR. Interestingly, we observed that soil microbial communities, including total bacteria and PSB density, remained stable across successive cultivation cycles when PR-rich amendments from Morocco (containing 40% to 100% PR) were used.

This study underscores that prolonged use of chemical fertilizers can negatively impact soil bacterial density, consistent with previous research [39] [40]. However, combining natural phosphate rock with chemical fertilizers appears to mitigate this impact on soil microorganism density, as suggested by Iqbal *et al.* [41], who

found that combining manure with chemical fertilizer improved microbial biomass in a paddy field.

The quantification of the number of bacteria per amplification unit showed a significant increase of 28% in the number of bacteria UA/g dry soil and 40% to 57% in the number of Operational Taxonomic Units (OTUs) in amended soils (T1, T2, T3, T4, T5, T6), compared to unamended control treatments (T0 and T0a), regardless of the plot ecology.

The presence of phosphate amendments did not alter the composition of bacterial communities regardless of the applied treatments and plot ecology (upland/lowland). Moreover, microbial diversity indices (high Chao1, Shannon exceeding 4, and evenness approaching 1) reflect strong bacterial diversity with an identical species abundance in the population when phosphate amendments are applied. Our results also revealed that when phosphate rock is associated with triple superphosphate (TSP), *i.e.*, under treatments T2, T3, T4, and T5, the diversity of indigenous bacteria is not modified compared to the control treatment (T0 + NPK). However, an increase in the abundance of Chloroflexia by 4% and Firmicutes by 12%, as well as a decrease in Halobacterota by 7.5% and Proteobacteria by 10.3%, compared to the control treatment (T0 + NPK), was observed. This study demonstrates that the combination of phosphate rock and Triple Superphosphate in the medium also modifies bacterial density and diversity, as already demonstrated by Lori *et al.* [40].

Furthermore, the presence of TSP alone in the phosphate amendment did not cause a modification in bacterial density and diversity, but a decrease in the relative abundance of the Proteobacteria phylum by 10%, an increase in Halobacterota by 7%, and no effect on Actinobacteria, Firmicutes, and Chloroflexia. The presence of phosphate rock (100% PR + NPK) alone in the phosphate amendment did not affect the diversity of indigenous soil bacteria. However, a variable modification in the relative abundance of phyla was noted, with an 8.53% decrease for Proteobacteria, a 15% increase for Firmicutes, and no change in the abundance of Actinobacteria, Halobacterota, and Chloroflexia.

This study reveals that different phosphate amendments have varying influences on bacterial density, diversity, and phyla abundance. These results do not differ from those of Wang *et al.* [42], who observed changes in bacterial community composition after phosphorus addition. Taxonomic analysis of bacterial communities in the soils in response to phosphate amendment revealed variable modifications based on the nature of bacterial species. For example, the appearance of genera such as *Ammoniphilus* and *Fonticella*, and the consistent abundance of *Bacillus* sp. and *Clostridium* sp. after amendment, irrespective of the treatment applied. Thus, phosphate amendment affects the structure of bacterial communities, as indicated in the works of Lori *et al.* [40]. However, the nature of the phosphate amendment does not seem to affect the structure of soil bacterial communities. Furthermore, the presence of species, particularly Bacillus sp. and Pseudomonas sp., on these plots, known for their ability to solubilize phosphates

[43] [44].

5. Conclusion

The analysis of bacterial diversity in the acidic rice field soils revealed the presence of different bacterial groups, including species such as *Bacillus* sp. and *Pseudomonas* sp., known for their ability to solubilize and mineralize organic and inorganic phosphates. This study highlights variable changes in the density, structure, and abundance of bacterial communities following phosphate amendment. This variation is primarily attributed to the nature of the amendment. This study underscores that prolonged use of chemical fertilizers can negatively impact soil bacterial density. However, combining natural phosphate rock with chemical fertilizers appears to mitigate this impact on soil microorganism density.

Acknowledgements

We would like to sincerely thank the "Office Chérifien du Phosphate" (OCP-Africa) for the financial support and the National Center for Agronomic Research (NCAR) of Man and Gagnoa for the technical support provided to the realization of the ASORPRI research project. We also thank each farmer who lent his plot to carry out the field trials.

Conflicts of Interest

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

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