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Long-Term Chemical Fertilization Drove Beneficial Bacteria for Rice Soil to Move from Bulk Soil to the Rhizosphere

Jian Xiao [1,2,3,†], Jianglin Zhang [2,3,†], Yajie Gao [2,3], Yanhong Lu [2,3], Xue Xie [2,3,4], Changyu Fang [2,3,5], Yulin Liao [1,2,3,*] and Jun Nie [1,2,3,4,*]

1 Longping Branch, College of Biology, Hunan University, Changsha 410125, China; xiaojian1997@hnu.edu.cn
2 Hunan Soil and Fertilizer Research Institute, Changsha 410125, China; zhangjianglin@hunaas.cn (J.Z.); gyj06107048@163.com (Y.G.); luyanhong6376432@163.com (Y.L.); xiexue@hunau.edu.cn (X.X.); 18684663642@163.com (C.F.)
3 Scientific Observing and Experimental Station of Arable Land Conservation (Hunan), Ministry of Agriculture, Changsha 410125, China
4 College of Resource, Hunan Agricultural University, Changsha 410128, China
5 College of Agronomy, Hunan Agricultural University, Changsha 410128, China
* Correspondence: yulliao2006@126.com (Y.L.); neijun197@163.com (J.N.)
† These authors contributed equally to this work.

Abstract: Overuse of chemical fertilizer (CF) causes damage to soil and the environment. To reveal the process of the response of crop rhizospheric and bulk soil fertility and the bacterial community to long-term CF conditions, CF application and nonfertilization (CK, control) treatments were used in a long-term (12-year) fertilization experiment. Long-term CF application significantly increased the soil organic matter, total nitrogen, and available phosphorus contents ($p < 0.05$), increased the available nitrogen (AN) and potassium (AK) contents to varying degrees, and decreased the soil pH in both rice rhizospheric soil and bulk soil. In addition, the bacterial Shannon and Ace indices in rice rhizospheric soil under the CF treatment were all higher than those under the control (CK) treatment, and the bulk soil bacteria showed the opposite trend. The LEfSe results showed that *unidentified_Gammaproteobacteria* and *Geobacter* (genera) were significantly enriched in the rhizospheric and bulk soil of rice under the CK treatment, respectively. Gemmatimonadetes (phylum) and Nitrospirae (phylum) + *Thiobacillus* (genus) were significantly enriched in the rice rhizospheric and bulk soil under the CF treatment. Only AK and AN had strong positive correlations with soil bacteria. Long-term CF application accelerated the migration of soil bacteria from the bulk soil to the rhizosphere, thus improving soil fertility and nutrient cycling.

Keywords: paddy soil; fertilization; rhizosphere; bulk soil; bacterial community

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1. Introduction

Rice (*Oryza sativa* L.) plays a crucial role in agricultural fields worldwide as one of the main food crops [1]. China has the second largest cultivation area and is the largest producer in the world [2]. Yuan et al. [3] and Zhao et al. [4] suggested that the distribution of grain production will change with increasing population and the improvement of people's living standards in the future. However, the increase in crop yield is overdependent on the use of chemical fertilizers (CFs) in the process of food production in China [5]. To date, in conventional agricultural fertilizer management, agricultural producers manage fields with high CF inputs to maintain soil productivity and increase crop yields [2,6]. The amounts of nutrients required by crops are often far less than the amount of CF (i.e., the amount of conventional CF input) [6,7]. Potential harms, such as soil acidification [8], declines in soil organic matter (SOM) and soil fertility [9], and dramatic reductions in soil biodiversity [10], can be caused by excess nutrients in the soil.

Soil microbes play an important role in maintaining soil fertility and health by decomposing litter and recycling nutrients [11]. Wu et al. [10] found that soil microbial community

structures can be affected by changing soil properties through tilling, irrigating, fertilizing, etc. For example, fertilization directly changes soil nutrients and then affects the structure and diversity of soil microbial communities [12].

Previous studies have reported that microbial populations associated with the nitrogen cycle are positively or negatively affected by long-term chemical or mineral fertilizer application [13]. Thus, Wu et al. [10] reported that soil quality, soil fertility, and the soil microbial community can be negatively affected by repeatedly overusing CFs. Sun et al. [14] reported that soil pH was significantly reduced, soil bacterial diversity was decreased, and soil bacterial community composition was significantly changed after long-term CF application. However, Geisseler and Scow [15] reported that in comparison with a nonfertilized treatment, fertilizer application increased soil microbial biomass by 15.1%.

In the rhizosphere, the organic compounds secreted by plants enrich diversified microbial communities to provide beneficial nutrients for plants [16–18]. Edwards et al. [19] exhaustively characterized the root-associated microbiome of rice to support a multistep model for the assembly of a root microbiome from soil. Previous studies reported that the compositions of soil microbial communities in rhizospheric soil were significantly different from those in bulk soil [20,21]. In the rhizosphere of plants, their roots coordinate development and interact with rhizospheric microorganisms [22]. The structure, composition, and functioning of plant-associated rhizospheric microbiota can be shaped by free-living soil microorganisms. Beneficial effects, including nitrogen fixation [23], organic phosphate mineralization [24], production of plant growth regulators or phytohormones [25,26], production of siderophores [27], stress tolerance [28], and biological control [29,30], are facilitated by the rhizospheric microbiome. There are differences in the microbial metabolism and ecological processes between rhizospheric soil and bulk soil [18] because bulk soil is far away from plants' living areas and is not affected by plants.

Fertilizers that are applied to soil have an aftereffect and leave lasting residuals on crops. Compared with short-term tests, long-term positioning tests can be used to objectively characterize the effects of different management measures on the physical, chemical, and biological properties of soil. Many studies have reported the effects of soil quality, soil fertility, the soil microbial community, and crop growth indicators on yield with long-term CF application. However, few studies [31,32] have investigated the effects of the bacterial community structure in rhizospheric and bulk soils after long-term CF application. For this reason, in the present study, the effects of long-term (12-year) CF application on bacterial community structures in rhizospheric and bulk soils of rice were investigated through a 12-year field experiment. The main objective of the study was to provide a theoretical basis for revealing the migration mechanism of rice soil microorganisms under long-term chemical fertilizer application.

2. Materials and Methods

2.1. Location and Experimental Design with Rice

The continuous outdoor experiment began in 2008 in Sanxianhu Village (GPS coordinates 29°13′03″ N, 112°28′53″ E), Nan County, Yiyang City, Hunan Province, China, which is located in a humid subtropical monsoon zone. The altitude, annual average temperature, and precipitation were approximately 28.8 m, 16.6 °C, and 1237.7 mm, respectively. At the experimental location, the soil was a typical sandy purple clay soil developed from Dongting Lake sediment with 7.8% sand, 72.6% silt, and 19.6% clay [33]. Before transplanting rice, the soil chemical properties of the initial plow-layer soil (0–20 cm) were as follows: soil pH, 8.18 ± 0.12 (n = 3); soil organic matter (SOM), 31.85 ± 0.48 g kg^{-1} (n = 3); total nitrogen (TN), 3.04 ± 0.06 g kg^{-1} (n = 3); alkaline hydrolyzable nitrogen (AN), 169.9 ± 2.51 mg kg^{-1} (n = 3); available P (AP), 15.4 ± 0.65 mg kg^{-1} (n = 3); available K (AK), 81.9 ± 1.30 mg kg^{-1} (n = 3).

The conventional fertilizers used were urea (46% N, Xinlianxin, Xinxiang, China), calcium superphosphate (12% P_2O_5, Shuoling, Huaihua, China), and potassium chloride (60% K_2O, Lingkelongzi, Changsha, China). The treatment and control were as follows:

(i) Nonfertilization was used as the control (CK) and (ii) the recommended conventional chemical fertilizer application for local paddy fields, as used by local farmers, was used as the treatment (CF, 150 kg ha^{-1} urea, 75 kg ha^{-1} P_2O_5, and 120 kg ha^{-1} K_2O) [34]. The area of each test plot was 20 m^2 (4 m × 5 m), with three replicates; they were randomly arranged, and the transplanting spacing of the rice was 0.20 m × 0.20 m. Around each plot, polyethylene films were draped over ridges (0.3 m wide and 0.15 m above the ground) to prevent the transfer of water and nutrients between plots. Early rice (Yuanzao No. 1 (Hunan Academy of Agricultural Sciences, China) was planted from 2008 to 2010, and Xiangzaoxian No. 25 (Hunan Academy of Agricultural Sciences, China) was planted after 2010) was planted in middle or late April and harvested in early July. Since 2008, Huanghuazhan (Hunan Academy of Agricultural Sciences, China) has been planted as late rice in mid- or late July and harvested in early November. We ensured that other identical field management practices (irrigation, weeding, etc., other than fertilization) were consistent between the CF treatment and the control.

2.2. Soil Sampling

In mid-July 2019, bulk and rhizospheric soil samples were collected from the 6 plots. Debris (grass roots, litter, stones, etc.) and surface soil (0–1 cm) were removed before soil sampling. Five rice plants with the same growth potential were selected from each plot. The unvegetated soil cores (5 cm in diameter) adjacent to the rice plants (i.e., bulk soil) were sampled at a depth of 0–20 cm. The loosely adhered soil was shaken off by violently shaking the rice roots, and the soil attached to the roots was called rhizospheric soil. The roots were forcibly shaken with sterile forceps to separate the roots from the soil, and then five soil samples from each plot were mixed together to generate a composite sample. Finally, all of the soil separated from the roots was sieved by using a 2 mm mesh sieve and put into a sterile zip-lock bag [35,36].

2.3. Analysis of Soil Chemical Properties and Soil Bacterial Diversities

A pH meter (FE20K, Mettler Toledo, Switzerland) was used to measure the soil pH; the soil organic matter (SOM) and total nitrogen (TN) contents were determined with the wet digestion (120 °C, 2 h) and Kjeldahl methods, respectively [37]; the soil available nitrogen (AN), phosphorus (AP), and potassium (AK) contents were determined by using microdiffusion after the alkaline hydrolysis method, the Olsen method, and flame photometry, respectively [37].

The extraction, PCR amplification, and sequencing of the total genomic DNA of the soil samples were completed according to previous protocols [36]. The primer pair 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) was used to amplify the soil bacterial 16S rRNA gene in the V4 hypervariable region [38,39]. A library was constructed by using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA), and Qubit and Q-PCR were used to quantify the constructed library. Finally, NovaSeq6000 was used for sequencing, and 250 bp paired-end reads were generated. The raw reads of the soil bacteria were deposited in the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA955403).

The UPARSE software (Version 7.0.1001, http://drive5.com/uparse/, accessed on 2 April 2023) was used to perform the sequence analysis. Sequences with ≥97% similarity were assigned to the same operational taxonomic units (OTUs). A representative sequence for each OTU was screened for further annotation. The SILVA database (Release 132, http://www.arb-silva.de/, accessed on 2 April 2023) was used to annotate the taxonomic information. All alpha diversity indices were calculated with QIIME (Version 1.7.0) and displayed with the R software (Version 3.3.1). Whether the sequencing findings accurately reflected the microorganisms in the sample was shown by the index of coverage [39]. The higher the diversity index (Shannon index, http://www.mothur.org/wiki/Shannon, accessed on 2 April 2023) and richness indices (Chao1 index, http://www.mothur.org/wiki/Chao, accessed on 2 April 2023, and Ace index, http://www.mothur.org/wiki/

Ace, accessed on 2 April 2023) of a community, the higher the diversity and richness of that community [40]. Graphics of the bacterial community compositions and Venn diagrams were generated with the R (Version 3.3.1) software. The "vegan" package was used, and graphs for a heatmap analysis of the bacterial communities were created by using the R language (Version 3.3.1) tool. The "WGCNA" package, "stat" packages, and "ggplot2" package were used for weighted UniFrace Principal Coordinate Analysis (PCoA) in the R software (Version 3.3.1). Unweighted Pair–Group Method with Arithmetic Means (UPGMA) Clustering was performed as a type of hierarchical clustering method to interpret the distance matrix by using the average linkage and was conducted with the QIIME software (Version 1.7.0). The MUSCLE software (Version 3.8.31) was used to conduct multiple sequence alignment and construct phylogenetic relationships. LEfSe (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload, accessed on 2 April 2023) was used to perform linear discriminant analysis (LDA) on samples according to different grouping conditions based on the taxonomic composition to identify clusters that had a significant differential ($p < 0.05$, LDA ≥ 3.0) impact on sample delineation. FAPROTAX [38] was employed to predict the bacterial functions involved in processes of the biochemical cycle as an artificially constructed functional classification database. The "pheatmap" package was run with the R (Version 3.3.1) software to conduct a heatmap analysis of the Spearman correlation between the soil chemical properties and soil bacteria. The data processing and microbial diversity analysis were the same as those in previous studies [39,40].

2.4. Statistical Analyses

The results are shown as the mean ± standard deviation (SD) values calculated in Microsoft Excel 2019. One-way analysis of variance (ANOVA), a two-tailed Duncan test, and multiple comparisons ($p < 0.05$) were used to evaluate significant differences. IBM SPSS Statistics (Version 26.0, IMB SPSS Inc., Chicago, IL, USA) and Origin 2021 (OriginLab Corporation, Northampton, MA, USA) were used to analyze and plot the experimental data, respectively.

3. Results

3.1. Soil Chemical Properties

In comparison with the CK treatment, the soil pH in the CF treatment was similar in both the rhizospheric and bulk soil, and there were no significant differences ($p > 0.05$). In contrast, the opposite trends were shown in the AN and AK contents. Furthermore, the SOM, TN, and AP concentrations in both the rhizospheric and bulk soil were significantly increased by the CF treatment ($p < 0.05$) (Table 1).

Table 1. Soil chemical properties in rice fields after long-term chemical fertilization.

Treatment	pH	SOM (g kg^{-1})	TN (g kg^{-1})	AN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
RCK	7.89 ± 0.06 a	29.67 ± 0.60 bc	2.73 ± 0.15 bc	167.23 ± 7.68 ab	13.90 ± 0.41 b	78.49 ± 3.33 ab
RCF	7.77 ± 0.12 a	32.50 ± 0.61 a	3.13 ± 0.19 a	176.97 ± 9.64 a	18.97 ± 0.82 a	82.73 ± 11.64 a
BCK	7.75 ± 0.09 a	28.37 ± 0.85 c	2.65 ± 0.24 c	144.87 ± 4.47 b	13.65 ± 0.19 b	67.97 ± 1.12 b
BCF	7.73 ± 0.08 a	31.23 ± 2.57 ab	3.03 ± 0.05 ab	155.63 ± 20.72 ab	18.91 ± 0.76 a	71.54 ± 1.06 ab

All data values indicate the mean ± SD ($n = 3$). Different lowercase letters in the same column indicate significant differences ($p < 0.05$). Prefix R, rhizospheric soil; Prefix B, bulk soil. The same applies below in all tables and figures.

3.2. Soil Bacterial Alpha and Beta Diversities

The soil bacterial OTU numbers rose as a function of the number of samples according to a boxplot of species accumulation (Figure 1a). With the saturation of the OTU numbers, the curve became asymptotically stable, and fewer new OTUs were added to each soil sample, showing sufficient sequencing depth to adequately characterize the rice soil bacterial compositions (Figure 1b) with a good coverage of >99.4%, and the data were reliable (Figure 1c). Moreover, the results showed that the rhizospheric and bulk soil bacterial

Shannon (describing bacterial diversity) and Ace (describing bacterial richness) indices in the paddy field were not significantly changed by the CF treatment compared with the CK treatment (Figure 1d,e). However, we found, quite interestingly, that the bacterial Shannon and Ace indices in the rice rhizospheric soil under the CF treatment were all higher than those under the CK treatment, and the bulk soil bacteria showed the opposite trend (Figure 1d,e). The results of the PCoA showed that the soil bacterial compositions in each group were clustered separately, and they were quite similar (Figure 1f). All of the above results suggest that the bacterial diversity and richness in the rice rhizospheric soil were increased, and the bacterial diversity and richness in the rice bulk soil were decreased by the long-term application of CF.

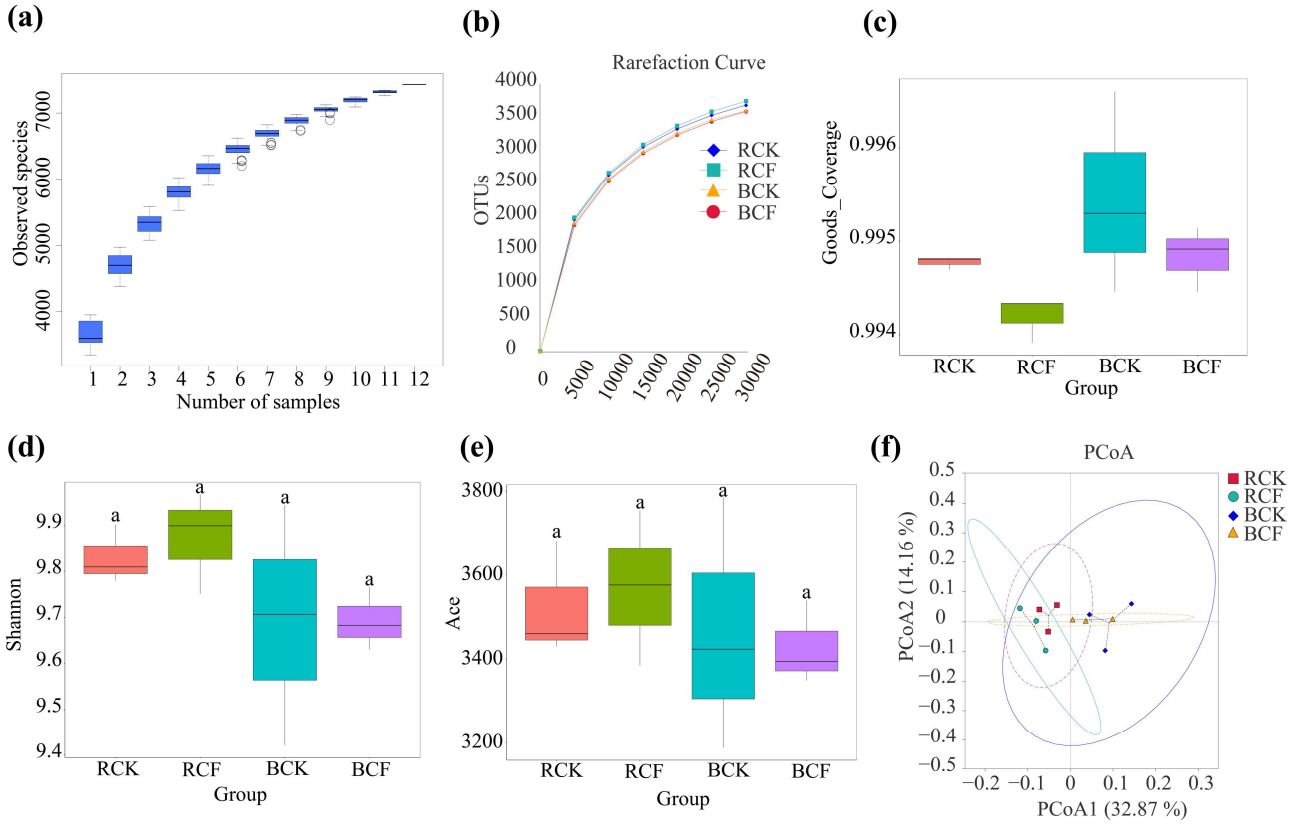

Figure 1. Soil bacterial alpha and beta diversities at the OTU level after long-term chemical fertilization. (**a**) Species accumulation boxplot for soil bacteria. (**b**) The rarefaction curve for soil bacteria. (**c**) Good coverage of soil bacteria. (**d**) Shannon index for soil bacteria. (**e**) Ace index for soil bacteria. (**f**) Soil bacterial PCoA. Different color blocks indicate different groups. Different lowercase letters indicate significant differences ($p < 0.05$).

3.3. UPGMA Cluster Analysis and Species Phylogenetic Tree

The results of UPGMA showed that the cluster of the RCF and RCK treatments was a branch and that of the BCK and BCF treatments was another branch, indicating that there were differences in the bacterial community compositions, relative abundance, and phylogenetic relationships between the rhizospheric and bulk soil of rice under long-term lack of fertilization (CK) and long-term fertilization (CF) (Figure 2a). In both rice rhizospheric soil and bulk soil, there was little difference in the soil bacterial community compositions, relative abundance, and phylogenetic relationships between the CK and CF treatments (Figure 2a).

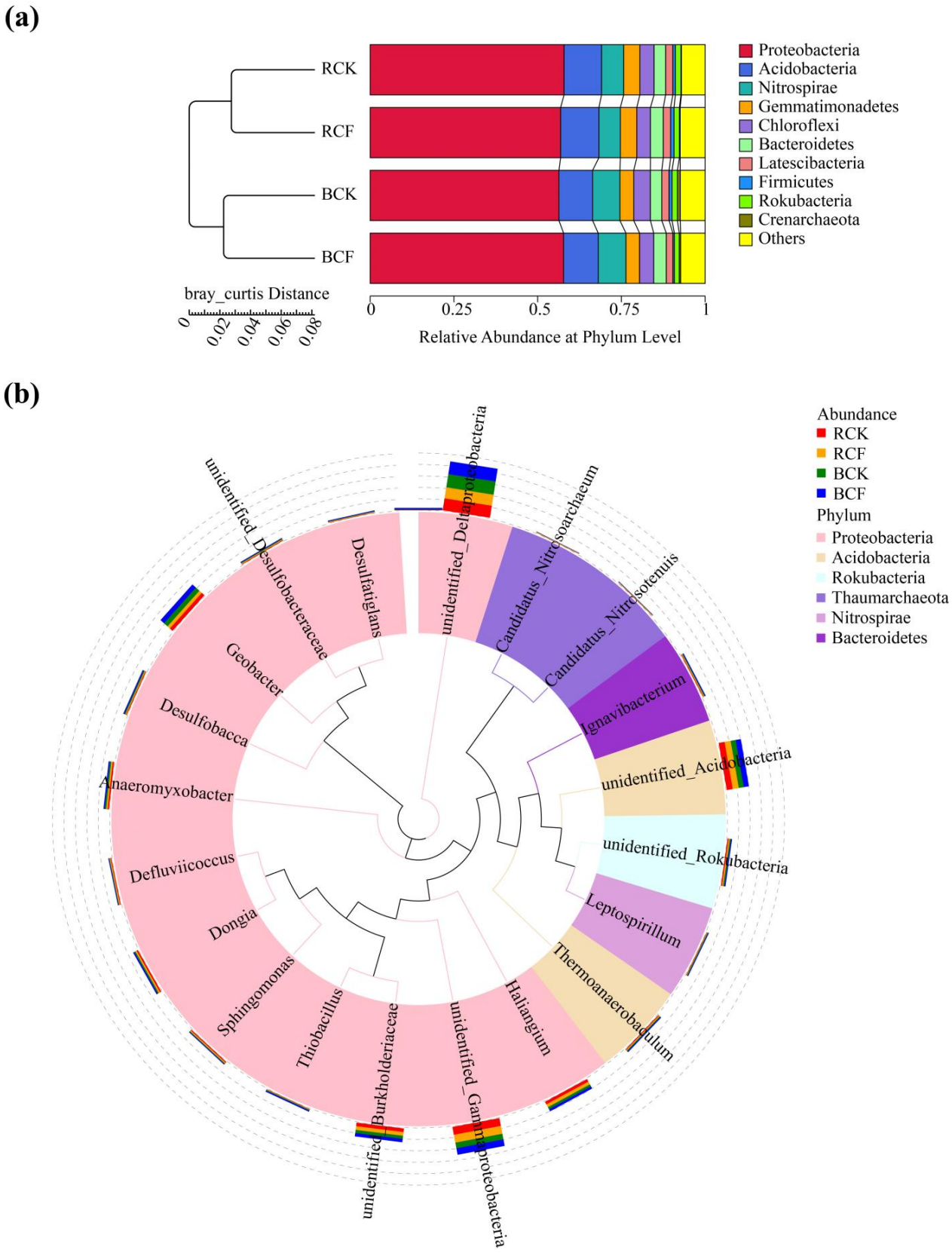

Figure 2. Bray–Curtis UPGMA clustering tree (**a**) and species evolutionary tree (**b**). The colors of the branches and fan-shaped sections represent their corresponding phyla, and the stacked columns on the outside of the fan ring represent the abundance distribution information of different species in the genus.

The representative sequences of the top 20 most abundant soil bacterial genera were obtained by using a multisequence comparison to further study the species' phylogenetic relationships at the genus level (Figure 2b). The top 20 bacterial genera in the soil in terms of relative abundance mainly belonged to Proteobacteria, Acidobacteria, Rokubacteria, Thaumarchaeota, Nitrospirae, and Bacteroidetes. The soil bacteria in the rhizospheric soil and bulk soil samples were mainly distributed in the following six genera: *unidentified_Deltaproteobacteria*, *unidentified_Gammaproteobacteria*, *unidentified_Acidobacteria*, *unidentified_Burkholderiaceae*, *Geobacter*, and *Haliangium*. *Unidentified_Deltaproteobacteria* and *unidentified_Gammaproteobacteria*, which were relatively abundant, both belonged to the branch of Proteobacteria (Figure 2b).

3.4. Soil Bacterial Composition

In the rhizospheric soil of rice, the common dominant (i.e., relative abundance percentages > 1.00%) bacterial phyla were Proteobacteria, Acidobacteria, Nitrospirae, Gemmatimonadetes, Chloroflexi, Bacteroidetes, Latescibacteria, and Rokubacteria in both the CK (averages of 57.88%, 11.22%, 6.59%, 4.84%, 4.24%, 3.45%, 2.16%, and 1.67%, respectively) and the CF (averages of 56.86%, 11.43%, 6.41%, 4.90%, 4.09%, 3.81%, 2.18%, and 1.62%, respectively) treatments (Figure 3a). In addition, Firmicutes was a unique dominant bacterial phylum in the CF treatment. In the bulk soil of rice, the dominant bacterial phyla were Proteobacteria, Acidobacteria, Nitrospirae, Chloroflexi, Gemmatimonadetes, Bacteroidetes, Latescibacteria, and Rokubacteria in both the CK (averages of 56.35%, 10.04%, 8.15%, 4.96%, 4.14%, 3.41%, 2.17%, and 1.60%, respectively) and the CF (averages of 57.78%, 10.32%, 8.27%, 4.22%, 4.06%, 3.74%, 1.98%, and 1.46%, respectively) treatments (Figure 3a).

At the genus level, Haliangium, Defluviicoccus, unidentified_Acidobacteria, Sphingomonas, Dongia, unidentified_Gammaproteobacteria, and unidentified_Burkholderiaceae were enriched in the rhizospheric soil of rice. Geobacter, Desulfobacca, Sulfurifustis, unidentified_Deltaproteobacteria, and Leptospirillum were enriched in the bulk soil of rice. Candidatus_Nitrosotenuis and Candidatus_Nitrosoarchaeum were enriched in the rhizospheric soil of rice under the CF treatment. Thiobacillus, Ignavibacterium, and Thermoanaerobaculum were enriched in the bulk soil of rice under the CF treatment (Figure 3b).

The numbers of soil bacteria at the OTU level in the RCK, RCF, BCK, and BCF treatments were 3719, 3775, 3781, and 3739 OTUs, respectively. Furthermore, the numbers of unique soil bacteria in the RCK, RCF, BCK, and BCF treatments were 338, 394, 400, and 358 OTUs, respectively (Figure 3c).

3.5. Soil Bacterial Communities with Statistically Significant Differences

The effect size (LEfSe) in linear discriminant analysis (LDA) was calculated to identify high-dimensional biomarkers (from phylum to species) with significantly different soil bacterial abundances in the rhizospheric and bulk soils of rice between the CK and CF treatments (Figure 4). Cladograms were used to show different groups (Figure 4a), and LDA scores of three or greater were confirmed with the LEfSe tool (Figure 4b). In total, 18 abundant soil bacterial clades exhibited significant differences ($p < 0.05$, LDA ≥ 3.0) (Figure 4b). The LEfSe results showed that *unidentified_Gammaproteobacteria* (genus) was significantly enriched in the rhizospheric soil of rice under the CK treatment, *Geobacter* (genus) was significantly enriched in the bulk soil of rice under the CK treatment, Gemmatimonadetes (phylum) was significantly enriched in the rhizospheric soil of rice under the CF treatment, and Nitrospirae (phylum) and *Thiobacillus* (genus) were significantly enriched in the bulk soil of rice under the CF treatment.

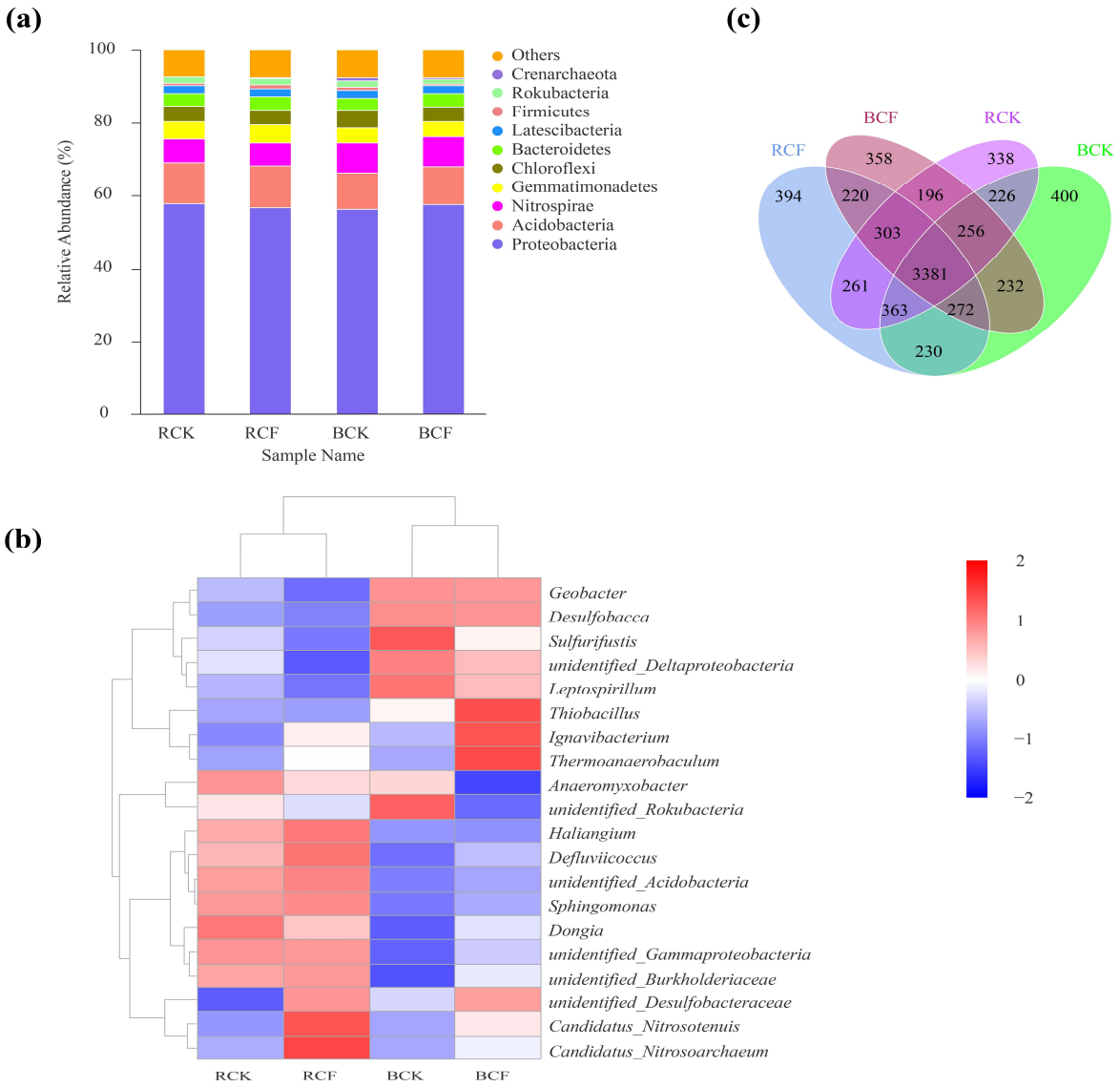

Figure 3. Soil bacterial composition of rice after long-term chemical fertilization. (**a**) Relative abundance of the top ten soil bacterial phyla. (**b**) Heatmap of the top 20 most abundant soil bacterial genera based on relative abundances. (**c**) Venn analyses of soil bacteria at the OTU level.

3.6. Functional Predictions of the Soil Bacterial Communities with FAPROTAX

The proportions of soil bacterial abundances related to human_pathogens_pneumonia, aerobic_chemoheterotrophy, animal_parasites_or_symbionts, human_pathogens_all, chemoheterotrophy, and predatory_or_exoparasitic functions were higher in the rice rhizospheric soil than in the rice bulk soil. In addition, the guilds related to iron respiration and nitrification of soil bacteria were enriched in the bulk soil compared to the rhizospheric soil. Furthermore, in comparison with the CK treatment, the guilds related to respiration_of_sulfur_compounds and sulfate_respiration in soil bacteria were enriched in the rhizospheric and bulk soil of rice under the CF treatment (Figure 5).

3.7. Soil Chemical Factors Structuring Soil Bacterial Communities after Long-Term Fertilization

Spearman correlation heatmaps were used to show the relationships between the top 10 soil bacterial phyla (Figure 6a) and genera (Figure 6b) and the soil chemical factors. First, Acidobacteria and Gemmatimonadetes showed a significant positive correlation with the AN ($r = 0.79$ and $r = 0.66$) and AK ($r = 0.62$ and $r = 0.74$) contents, respectively. Second, Nitrospirae and Crenarchaeota showed significant negative correlations with the

AN (r = −0.68 and r = −0.71) and AK (r = −0.72 and r = −0.66) contents, respectively. Furthermore, Chloroflexi showed a significant negative correlation with the SOM (r = −0.67), TN (r = −0.67), and AN (r = −0.61) contents. Finally, Latescibacteria showed a significant positive correlation with the AN (r = 0.62) content (Figure 6a). Moreover, only the AN and AK contents were correlated with the top 10 bacterial genera. The AK content was significantly positively correlated with *Haliangium* (r = 0.75), *unidentified_Acidobacteria* (r = 0.60), and *unidentified_Gammaproteobacteria* (r = 0.78) and significantly negatively correlated with *Desulfobacca* (r = −0.80) and *Thiobacillus* (r = −0.81). In addition to the same results as those for the AK content in the Spearman correlation heatmap, AP content was positively correlated with *unidentified_Burkholderiaceae* (r = 0.62) (Figure 6b).

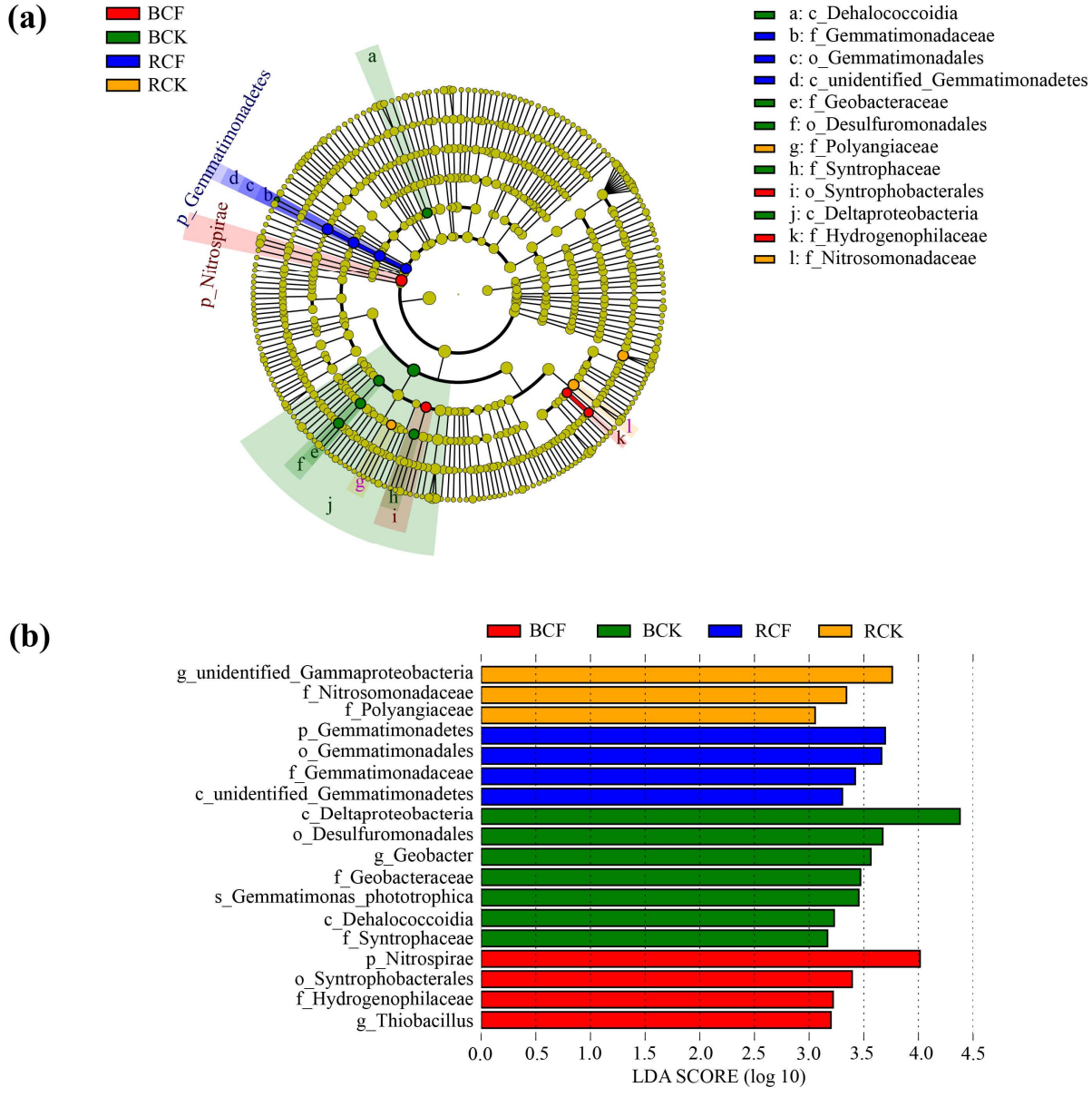

Figure 4. Soil bacterial cladograms (**a**) and biomarkers with LDA scores ≥ 3.0 (**b**) after long-term chemical fertilization. Different levels are indicated by different prefixes (p, phylum; c, class; o, order; f, family; g, genus; s, species).

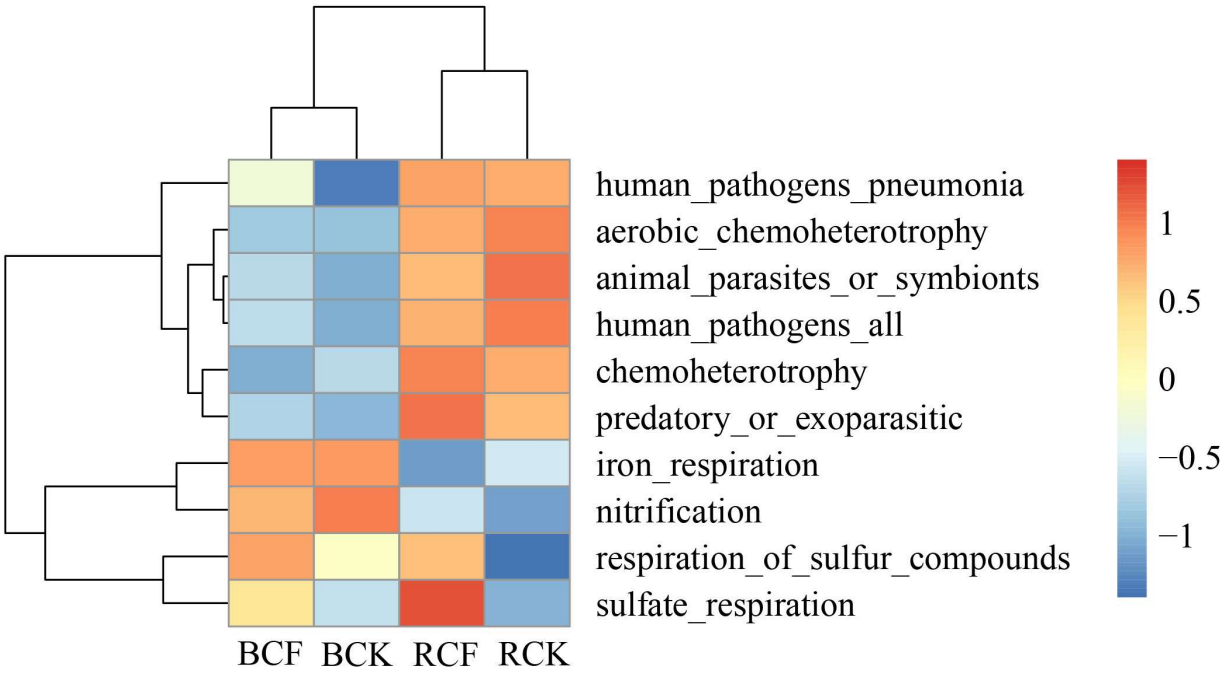

Figure 5. Heatmap of functional predictions of the soil bacterial communities. The X and Y axes are the treatment groups and soil bacterial functional predictions, respectively, and each lattice represents a Spearman correlation coefficient between a treatment group and a bacterial functional prediction. Red represents a positive correlation, whereas blue represents a negative correlation.

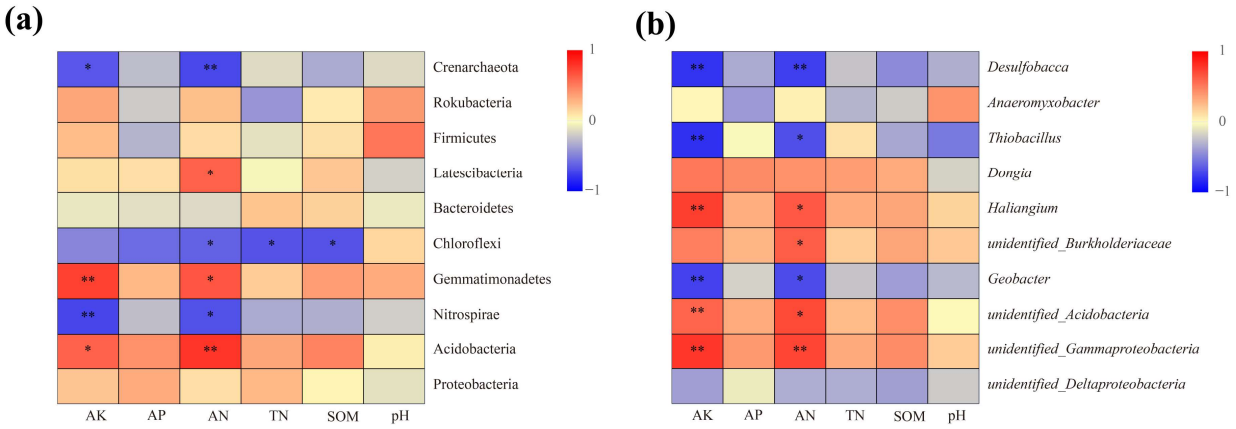

Figure 6. Correlation heatmap of soil physicochemical properties and the top 10 soil bacterial phyla (**a**) and genera (**b**). The X and Y axes are the soil physicochemical factors (pH, soil pH value; SOM, soil organic matter; TN, soil total nitrogen; AN, soil alkaline hydrolysable nitrogen; AP, soil available phosphorus; AK, soil available potassium) and soil bacterial phyla or genera, respectively, and each lattice represents a Spearman correlation coefficient between a soil physicochemical factor and a bacterial phylum or genus. Red represents a positive correlation, whereas blue represents a negative correlation (* $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$).

4. Discussion

4.1. Effects on Soil Chemical Properties after Long-Term Chemical Fertilization

In the present study, the results showed that some soil chemical properties were significantly changed by the long-term application of chemical fertilizers, which was similar to the findings of previous studies [10,41–43]. Zhong et al. [44] and Dan et al. [43] reported that the SOC and TN contents (as organic substrates) were significantly increased by the long-term application of synthetic chemical fertilizers, which was consistent with our results in both the rhizospheric and bulk soils (Table 1). Pinggera et al. [45] found that

regulating the C/N ratio by changing the N input was beneficial for the decomposition of organic matter by microorganisms, the release of organic nutrients, and the increase in SOM content, which may explain the increased SOM in the rhizospheric soil and bulk soil with the CF treatment in this study. Jin et al. [6] also verified this explanation by changing the N input content. On the other hand, we found that the SOM content in the rhizospheric soil was higher than that in the bulk soil, which was similar to the findings of a previous study [46]. This result was interpreted to mean that root exudates promoted soil organic carbon decomposition [46,47]. Previous studies found that the soil pH was significantly reduced after long-term fertilization in calcareous soil [48], Hapli-Udic Cambisol [49], and acidic soil [50]. In our study, long-term fertilization also reduced soil pH (in both the rhizospheric and bulk soils) in the typical sandy purple clay soil, and there were no significant differences, which may have been caused by differences in soil types and N inputs. Wu et al. [10] also reported that the soil pH was not significantly changed by the application of chemical fertilizers.

4.2. Effects on Soil Bacterial Diversity after Long-Term Chemical Fertilization

Previous studies reported that conventional fertilization reduced the soil bacterial richness in the plow layer (0–20 cm depth) of wheat [51] and maize [44]. Tang et al. [52] also discovered that CF alone reduced the soil microbial richness and Shannon indices in the plow layer of rice. In this study, the bacterial Shannon (used to measure diversity) and Ace (used to measure richness) indices in rice bulk soil with the CF treatment were all lower—but not significantly—than those with the CK treatment (Figure 1d,e); thus, these results were consistent with those of the aforementioned studies. On the other hand, we also found that the bacterial diversity and richness in the rhizospheric soil were higher than those in the bulk soil. Tang et al. [46] also reported the same results, explaining that root exudates enriched microorganisms and accelerated microbial activities and that extracellular enzyme activities were higher in the rhizosphere [53]; the soil quality and soil fertility indices were different [54].

4.3. Effects on Soil Bacterial Communities after Long-Term Chemical Fertilization

The ecological consistency of microbial communities can be revealed by elucidating the soil microbial taxa at the phylum level [6,55]. In our study, Proteobacteria was the most dominant phylum, and the relative abundances of Acidobacteria and Bacteroidetes increased in both the rhizospheric and bulk soils after long-term (12-year) application of chemical fertilizers according to high-throughput sequencing, which was consistent with the findings of a previous study by Wang et al. [42]. The possible reason is that Acidobacteria are acidophiles, and the long-term fertilization reduced the pH of the rhizospheric soil and bulk soil, which was not conducive to growth. Tang et al. [46] found that Acidobacteria were the main C source and thrived in both rhizospheric and bulk soils. Hungria et al. [56] also suggested that the enrichment of Acidobacteria in the plant rhizosphere may be due to their ability to utilize root exudates. In this study, we also found that Acidobacteria were the dominant bacteria in the rice rhizospheric and bulk soils. However, the abundance of Acidobacteria in the rhizospheric soil was higher than that in the bulk soil of rice, regardless of whether the CF treatment or the CK treatment was used. Wu et al. [57], Bulgarelli et al. [58], and Tang et al. [46] all suggested that the enhanced Acidobacteria in roots could be generally adapted to the plant rhizosphere as saprophytic bacteria.

Bacteroidetes are copiotrophes that rapidly proliferate in eutrophic environments [59] and mineralized organic matter [60], and they are mainly distributed in the rhizosphere [61]. In this study, the proportions of the abundance of Bacteroidetes between different treatments were in the following order (from large to small): RCF > RCK > BCF > BCK, which was consistent with the findings of previous studies [61].

Long-term fertilization results in the enrichment of specific microorganisms that can use these nutrients efficiently [42]. According to the results of the LEfSe analyses, 18 abundant soil bacterial taxa in the two treatments were distinctly different. *Geobacter* is an

obligate anaerobic N-fixing bacterium that is commonly observed in flooded conditions or anoxic environments in deep soils and plays an important role in nitrate reduction and iron restoration [62–64]. This explained why *Geobacter* responded to the nitrogen requirements of rice growth by reducing nitrite in the nutrient-poor environment. Nitrospirae can oxygenate nitrite into nitrate under aerobic conditions and play an important role in the nitrogen cycle [65]. Nitrate production leads to pH reduction in the soil. *Thiobacillus* is a beneficial soil bacterium that plays an important role in the soil sulfur cycle. Sulfuric acid is produced after the oxidation of sulfur by *Thiobacillus*, and the soil pH is reduced [66]. The significant enrichment of Nitrospirae and *Thiobacillus* led to nitrate and sulfate production in the rice bulk soil with the CF treatment, which might have caused the pH of the bulk soil to decrease under the CF treatment [66].

5. Conclusions

Overall, in this study, we demonstrated that long-term CF had different effects on rhizospheric and bulk soil fertility, soil bacterial diversity, and the structure and function of soil bacterial communities. After long-term fertilization, rhizospheric soil fertility indices (such as pH, SOM, TN, AN, AP, AK) and the soil bacterial diversity and richness were higher than those in the bulk soil. Based on our study, long-term fertilization can improve the fertility of rhizospheric and bulk soils, increase the rhizospheric soil bacterial diversity and richness, and decrease the bulk soil bacterial diversity and richness. Only AK and AN had significant correlations with soil bacteria. These results provide useful insights for studying nutrient accumulation, turnover, and microbial migration in rhizospheric and bulk soils under long-term fertilization strategies.

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