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Effects of Different Fertilizers on Rhizosphere Bacterial Communities of Winter Wheat in the North China Plain

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Received: 5 November 2019; Accepted: 7 January 2020; Published: 9 January 2020

Abstract: The application of bioorganic fertilizer affects rhizosphere microbes and further improves soil fertility in farmlands. However, the effects of different fertilizers on rhizosphere bacterial community diversity and structure of winter wheat remains unclear. In this study, we explored the effects of different fertilization treatments (no fertilizer added, CK; nitrogen fertilizer, NF; bioorganic fertilizer, BOF) on the rhizosphere bacterial community of winter wheat in the North China Plain. Rhizosphere soil treated with BOF had a higher Shannon index than that of CK and NF. The relative abundance of the *Proteobacteria* treated with BOF was significantly higher than that of NF, while the *Acidobacteria* and *Planctomycetes* were significantly lower. The redundancy analysis (RDA) and Mantel test showed that soil bacterial communities were significantly correlated with pH, nitrate, available phosphorus (AP), and available potassium (AK). Our findings indicated that BOF increased bacterial diversity and the relative abundance of copiotrophic bacteria in rhizosphere soil, while NF reduced bacterial diversity and increased the relative abundance of oligotrophic bacteria. The increase in copiotrophic bacteria in the rhizosphere of winter wheat could indicate an increase in soil nutrient availability, which might have positive implications for soil fertility and crop production.

Keywords: fertilization treatment; bacterial communities; rhizosphere; North China plain; illumina high-throughput sequencing

1. Introduction

Microorganisms play an important role in maintaining soil fertility and ecosystem function [1,2]. The rhizosphere is the area in the soil that is immediately adjacent to and affected by the roots of the plant [3]. The roots of the plant secrete a large amount of carbon-containing organic material into the soil, which is the carbon source, nitrogen source, and energy required for the growth and reproduction of soil microorganisms. A large number of microbes accumulate around the roots of plants [4], resulting in a distinction between the soil nutrient environment and soil microbial community structure in the non-rhizosphere [5]. The rhizosphere becomes the region with the strongest interaction between plant roots, soil, and microorganisms [6]. Rhizosphere microbes become an important medium between plant roots and soil, playing a significant role in the soil material cycle and energy flow [7,8].

The application of fertilizer is an important management measure in agricultural production, which has the effect of promoting crop growth and increasing yield [9,10], and it also affects soil microbes [11,12]. Currently, the extensive use of chemical fertilizers leads to a decrease in soil fertility and a series of environmental problems [13–15], while bioorganic fertilizer not only increases soil fertility through the input of beneficial microorganisms and organic materials [16,17] but also prevents many of the environmental problems caused by chemical fertilizers. Studies have shown that different fertilization treatments have a significant impact on soil microbial biomass and community structure [18–21], but rhizosphere microbes and bulk microbes are not been differentiated in these studies. Thus, it is necessary to investigate the microbial community in rhizosphere soil [22].

In this study, we conducted a pot experiment using winter wheat to investigate the effects of different fertilizer treatments on bacterial community diversity and structure in rhizosphere soil, and to determine which soil properties are important factors affecting bacterial communities. Understanding the response of rhizosphere microorganisms to different fertilization treatments will assist with providing a theoretical basis for scientific, rational, and effective fertilization strategies, which in turn will improve soil fertility and productivity and reduce the waste of fertilizer resources.

2. Materials and Methods

2.1. Site Description

The experimental field is located at the Yucheng Comprehensive Experiment Station of Chinese Academy of Sciences (36°50′ N, 116°34′ E). The station is located in Yucheng City, Shandong Province, and is located in the North China Plain of the alluvial plain in the lower reaches of the Yellow River, at an average altitude of 20 m. The climate is warm-temperate and sub-humid monsoon climate with an average annual precipitation of 593 mm, an average temperature of 13.1 °C, and a frost-free period of 220 days. The soil is calcaric fluvisols according to the FAO-UNESCO system. Soil texture is silt loam with 12% sand, 66% silt, and 22% clay. The crop planting system is a twice-yearly rotation of winter wheat and summer maize.

2.2. Experimental Design and Soil Sampling

Three treatments were established in the experiment: (1) control, with no fertilizer added, CK; (2) nitrogen fertilizer (urea; 46% N) added, NF; and (3) bioorganic fertilizer (1.78% N; produced by ETS, Biological Technology Development Co., Ltd., Tianjin, China) added, BOF. Three replicates (pots) were conducted for each treatment. All fertilizer treatments received the same total amount of N addition at a rate of 200 kg N ha^{-1}, which reflected the total nitrogen input in a growing season of farmland crops in the North China Plain. The NF treatment received 3.07 g urea per pot, and the BOF treatment received 79.38 g bioorganic fertilizer per pot. Fifty percent of the nitrogen fertilizer and 100% of the bioorganic fertilizer were applied as the base fertilizers in the different treatments, and the remaining nitrogen fertilizer was applied during the returning green stage. Soil samples were collected from the top 20 cm of a field, and after air-drying, were passed through a 2 mm sieve for later use. The size of pots used in the experiment was 30 cm in diameter and 23 cm in height. A total of 20 kg of soil was packed into each pot. The soil water content was adjusted to 65% of the field water holding capacity. In each pot, 46 wheat seeds were sown in two rows. In order to ensure that the environmental conditions were similar to those in the field, the soil-filled pots were buried in the field soil.

The experiment began in October 2016 and samples were collected in May 2017. When sampling, the root system of the whole potted plants were removed from the pots, and the roots were tapped to loosen the soil. The soil attached to the surface of the roots was collected using a sterile brush, and known as the rhizosphere soil sample (R). The soil collected from the rootless area next to the plant root system was called the bulk soil sample (B). The collected soil samples were passed through a 2 mm sieve to remove plant debris and other impurities from the roots. Some soil samples were air-dried for

analyzing soil properties; some were stored at a −20 °C for measuring soil microbial biomass; others were stored at −80 °C for determining soil bacterial communities.

2.3. Measurement of Soil Properties and Soil Microbial Biomass

The soil pH value was determined by potentiometry. Soil water content was determined by the drying method. Soil organic matter (SOM) was determined by the external heating method with potassium dichromate ($K_2Cr_2O_7$). Soil total nitrogen (TN) was determined by the semi-micro Kjeldahl digestion method. Ammonium nitrogen in the soil was determined by the indophenol blue colorimetric method. Nitrate was determined by dual-wavelength ultraviolet spectrophotometry. Available phosphorus (AP) was determined by the molybdenum anti-colorimetric method. Available potassium (AK) was determined by ammonium acetate extraction-flame spectrophotometry. Microbial biomass carbon and nitrogen (MBC; MBN) were determined by the chloroform fumigation method [23,24].

2.4. DNA Extraction and Amplicon Sequencing

Soil total DNA was extracted using the PowerSoil DNA Extraction Kit (MoBio Laboratories Inc., Carlsbad, CA, USA). The V4 region gene fragment was amplified using primer 515F (GTGCCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT). The modified primers contain different Barcodes to distinguish between different samples. The polymerase chain reaction (PCR) volume was 50 μL, which included ddH_2O 23 μL, Premix Taq DNA polymerase 25 μL, DNA template amount 1 μL (5–10 ng), forward primer 0.5 μL (20 ng μL^{-1}), and forward primer 0.5 μL (20 ng μL^{-1}). The PCR amplification reaction conditions were: 95 °C, 5 min; 30× (94 °C, 45 s; 56 °C, 45 s; 72 °C, 45 s); 72 °C, 10 min. Each sample was amplified three times. After PCR amplification, three tubes of the same sample were combined, purified using the GeneJET kit (Thermo Scientific Inc., Waltham, MA, USA), and the PCR product concentration was determined using NanoDrop 2000 (Thermo Scientific Inc., Waltham, MA, USA). PCR products were detected by electrophoresis with 2% agarose gel. The PCR products were mixed at the same volume according to the concentration of the PCR products. After thoroughly mixing, 2% agarose gel electrophoresis was used to detect the PCR products. The gel recovery kit (Qiagen, Hilden, Germany) was used to recover the products for the target band. The library was constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA). The constructed library was quantified by Qubit and Q-PCR. After the library was qualified, it was sequenced using the Illumina HiSeq2500 platform (Novogene Bioinformatics Technology Co., Ltd., Beijing, China).

2.5. Biological Information Analysis and Data Processing

The sample data were resolved according to the Barcode sequence and the PCR amplification primer sequence, and each sample was spliced using FLASH (version 1.2.7) after truncating the Barcode and primer sequences [25]. Data processing was performed using Qiime (version 1.9.1) [26]. All valid sequences of all samples were clustered using Uparse (version 7.0.1001) [27], and the sequences were clustered into operational taxonomic units (OTUs) by default with 97% identity. Species annotation of OTUs representative sequences was determined by species annotation analysis using the Mothur method and SILVA SSU rRNA database [28,29]. Fast multi-sequence alignments were performed using MUSCLE (version 3.8.31) to obtain phylogenetic relationships for all OTUs representative sequences [30]. Finally, the data of each sample was homogenized, according to the minimum amount of data in the sample. All sequences obtained in this study had been registered in the NCBI's Sequence Read Archive database (accession numbers: SRR10362754–SRR10362771).

2.6. Statistical Analysis

The Observed species, Chao1, Shannon, Simpson, and abundance-based coverage estimator (ACE) indices were calculated using Qiime software (version 1.9.1). Soil properties, microbial biomass, and soil bacterial alpha diversity index of different fertilization treatments were compared by one-way ANOVA

using SPSS software (version 20.0). Pearson correlation analysis of soil bacterial alpha diversity index and soil properties was calculated using SPSS software. Ade4 data package and ggplot2 data package of R software (version 2.15.3) were used for the principal component analysis (PCA). The Mothur software (version 1.30.1) was used for analysis of molecular variance (AMOVA). Species composition analysis of significant differences between groups was performed by R software for the T- test. Vegan data package of R software was used to conduct a redundancy analysis (RDA) and Mantel test of bacterial community structure.

3. Results

3.1. Effects of Different Fertilizers on Soil Properties and Microbial Biomass

Compared to the control, the pH of the rhizosphere and bulk soil increased significantly in BOF, while NF did not change (Table 1). There was no significant effect on SOM and TN in the rhizosphere and bulk soil in BOF, while soil TN increased in NF. Both the BOF and NF treatments had no effect on soil ammonium. The concentration of nitrate in the rhizosphere and bulk soil was the lowest in BOF and was significantly lower in rhizosphere soil than that in CK and NF. The concentration of AP and AK increased in the rhizosphere soil in BOF. The AP concentration increased by 66.14% in comparison to both CK and NF treatment, and AK increased by 12.50% and 27.35%, respectively. The nitrate in the rhizosphere soil of each treatment was lower than that in the bulk soil, which may be related to the large amount of nitrate absorption in the rhizosphere of the winter wheat roots. The other soil properties were higher in the rhizosphere than in the bulk soil (except for AP in the NF treatment).

MBC was the highest in the rhizosphere and non-rhizosphere in NF, followed by BOF, both of which were higher than CK. MBN in the rhizosphere and bulk soil was lower in NF and BOF than that in CK. Rhizosphere MBC and MBN content were higher than those in non-rhizosphere soil.

Table 1. Effects of different fertilizer treatments on soil properties and microbial biomass (mean ± SD).

Item	CKR	CKB	NFR	NFB	BOFR	BOFB
pH	8.32 ± 0.02 b	8.22 ± 0.03 c	8.30 ± 0.06 b	8.21 ± 0.03 c	8.40 ± 0.05 a	8.31 ± 0.06 b
SOM (g kg^{-1})	16.74 ± 0.75 a	16.43 ± 0.65 ab	15.86 ± 0.97 ab	15.14 ± 1.71 ab	16.64 ± 0.23 ab	14.84 ± 0.77 b
TN (g kg^{-1})	0.94 ± 0.06 ab	0.86 ± 0.02 b	1.02 ± 0.02 a	0.99 ± 0.06 a	0.98 ± 0.08 ab	0.97 ± 0.10 ab
Ammonium (mg kg^{-1})	6.10 ± 0.12 a	5.89 ± 0.25 a	6.28 ± 0.45 a	5.99 ± 0.40 a	6.24 ± 0.14 a	6.11 ± 0.16 a
Nitrate (mg kg^{-1})	22.87 ± 4.62 ab	23.24 ± 1.44 ab	22.16 ± 2.89 ab	24.17 ± 5.97 a	12.65 ± 3.29 c	16.49 ± 1.32 bc
AP (mg kg^{-1})	10.16 ± 0.49 b	10.08 ± 0.37 b	10.16 ± 0.91 b	10.48 ± 1.61 b	16.88 ± 0.21 a	12.88 ± 2.19 b
AK (mg kg^{-1})	116.00 ± 2.90 b	108.27 ± 1.67 c	102.47 ± 4.42 cd	98.60 ± 5.02 d	130.50 ± 2.90 a	115.03 ± 1.67 b
MBC (mg kg^{-1})	423.27 ± 51.69 ab	334.59 ± 36.63 c	474.41 ± 23.76 a	432.71 ± 26.99 ab	432.47 ± 25.70 ab	394.42 ± 25.27 b
MBN (mg kg^{-1})	42.74 ± 5.03 a	36.37 ± 3.25 abc	40.06 ± 1.47 ab	35.59 ± 3.83 bc	39.16 ± 1.14 ab	32.16 ± 3.763 c

Note: Different lowercase letters mean indicate significant differences among different treatments at $p < 0.05$. SOM, soil organic matter; TN, total nitrogen; AP, available phosphorus; AK, available potassium; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; CKR, rhizosphere of no fertilizer; CKB, bulk of no fertilizer; NFR, rhizosphere of nitrogen fertilizer; NFB, bulk of nitrogen fertilizer; BOFR, rhizosphere of bioorganic fertilizer; BOFB, bulk of bioorganic fertilizer.

3.2. Effects of Different Fertilizers on Soil Bacteria Diversity Index

High-throughput sequencing results showed 739,971 effective sequences obtained from all samples, and 3562–3853 OTUs obtained after clustering. Compared with CK and NF, the Shannon diversity index for rhizosphere soil treated with BOF was the highest, which increased by 0.79% and 1.60%, respectively, while the Shannon diversity index for rhizosphere soil treated with NF was the lowest (Table 2). The Simpson, Chao1, and ACE indices did not differ between treatments. In addition, there was no significant difference in the bacterial diversity index between rhizosphere and non-rhizosphere soil in each treatment. The Observed Species Index was the significant positive correlation between with soil pH and AK and was significantly negatively correlated with nitrate. The Shannon index was significantly positively correlated with soil pH, AP, and AK and significantly negatively correlated with nitrate. Both the Chao1 and ACE indices were significantly positively correlated with pH (Table 3).

Table 2. Effects of different fertilizer treatments on soil bacterial alpha diversity index (mean ± SD).

Treatment	Observed Species	Shannon	Simpson	Chao1	ACE
CKR	3853 ± 47 a	10.09 ± 0.07 ab	0.998 ± 0.001 a	5141.34 ± 245.51 a	5270.75 ± 185.09 a
CKB	3652 ± 191 a	10.05 ± 0.03 ab	0.998 ± 0.001 a	4696.15 ± 650.13 a	4712.37 ± 605.82 a
NFR	3692 ± 94 a	10.01 ± 0.07 b	0.997 ± 0.001 a	4838.85 ± 65.13 a	4941.59 ± 10.71 a
NFB	3562 ± 238 a	10.06 ± 0.06 ab	0.998 ± 0.001 a	4302.26 ± 614.79 a	4399.41 ± 588.79 a
BOFR	3853 ± 130 a	10.17 ± 0.09 a	0.998 ± 0.001 a	5032.43 ± 335.56 a	5084.55 ± 337.89 a
BOFB	3838 ± 244 a	10.16 ± 0.10 a	0.998 ± 0.000 a	4803.29 ± 599.45 a	4934.92 ± 602.96 a

Note: Different lowercase letters mean indicate significant differences among different treatments at $p < 0.05$. ACE, abundance-based coverage estimator; CKR, rhizosphere of no fertilizer; CKB, bulk of no fertilizer; NFR, rhizosphere of nitrogen fertilizer; NFB, bulk of nitrogen fertilizer; BOFR, rhizosphere of bioorganic fertilizer; BOFB, bulk of bioorganic fertilizer.

Table 3. Pearson correlation coefficients between the alpha diversity index of the soil bacterial community and soil properties.

Item	Observed Species	Shannon	Simpson	Chao1	ACE
pH	0.756 **	0.619 **	0.172	0.652 **	0.687 **
SOM	−0.062	−0.121	−0.259	0.101	0.075
TN	0.004	0.152	0.010	−0.027	0.018
Ammonium	0.094	0.220	−0.029	−0.023	0.034
Nitrate	−0.560 *	−0.730 **	−0.463	−0.418	−0.410
AP	0.354	0.616 **	0.180	0.239	0.218
AK	0.544 *	0.601 **	0.169	0.440	0.426

Note: * $p < 0.05$; ** $p < 0.01$. ACE, abundance-based coverage estimator; SOM, soil organic matter; TN, total nitrogen; AP, available phosphorus; AK, available potassium.

3.3. Effects of Different Fertilizers on Soil Bacterial Community Structure

Sequencing results showed a total of 43 phyla, including *Proteobacteria* (29.67%–34.15%), *Acidobacteria* (14.24%–19.08%), *Actinobacteria* (11.01%–12.78%), *Bacteroidetes* (7.41%–9.10%) and *Thaumarchaeota* (3.90%–5.30%) as the main dominant groups (Figure 1a). At the order level, relatively high abundance groups were *Xanthomonadales* (4.52%–5.49%), *Sphingomonadales* (3.17%–4.75%), and *Sphingobacteriales* (3.73%–4.94%), *Rhodospirillales* (3.63%–4.88%) (Figure 1b).

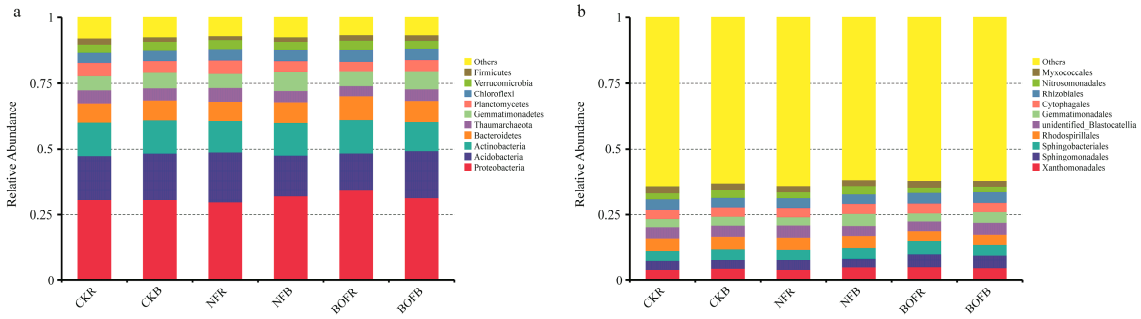

Figure 1. Bacterial community composition at the (**a**) phylum level and (**b**) order level in different fertilization treatments. CKR, rhizosphere of no fertilizer; CKB, bulk of no fertilizer; NFR, rhizosphere of nitrogen fertilizer; NFB, bulk of nitrogen fertilizer; BOFR, rhizosphere of bioorganic fertilizer; BOFB, bulk of bioorganic fertilizer.

The PCA showed that soil bacterial structure differed under the different treatments. While the CK and NF treatments clustered together (and thus similar), the BOF treatment was clearly separated and therefore different from the other two treatments (Figure 2). There were some differences between the rhizosphere and bulk soil. In addition, the results of the AMOVA indicated that there was a significant difference in bacterial community structure between the different fertilization treatments ($p < 0.001$).

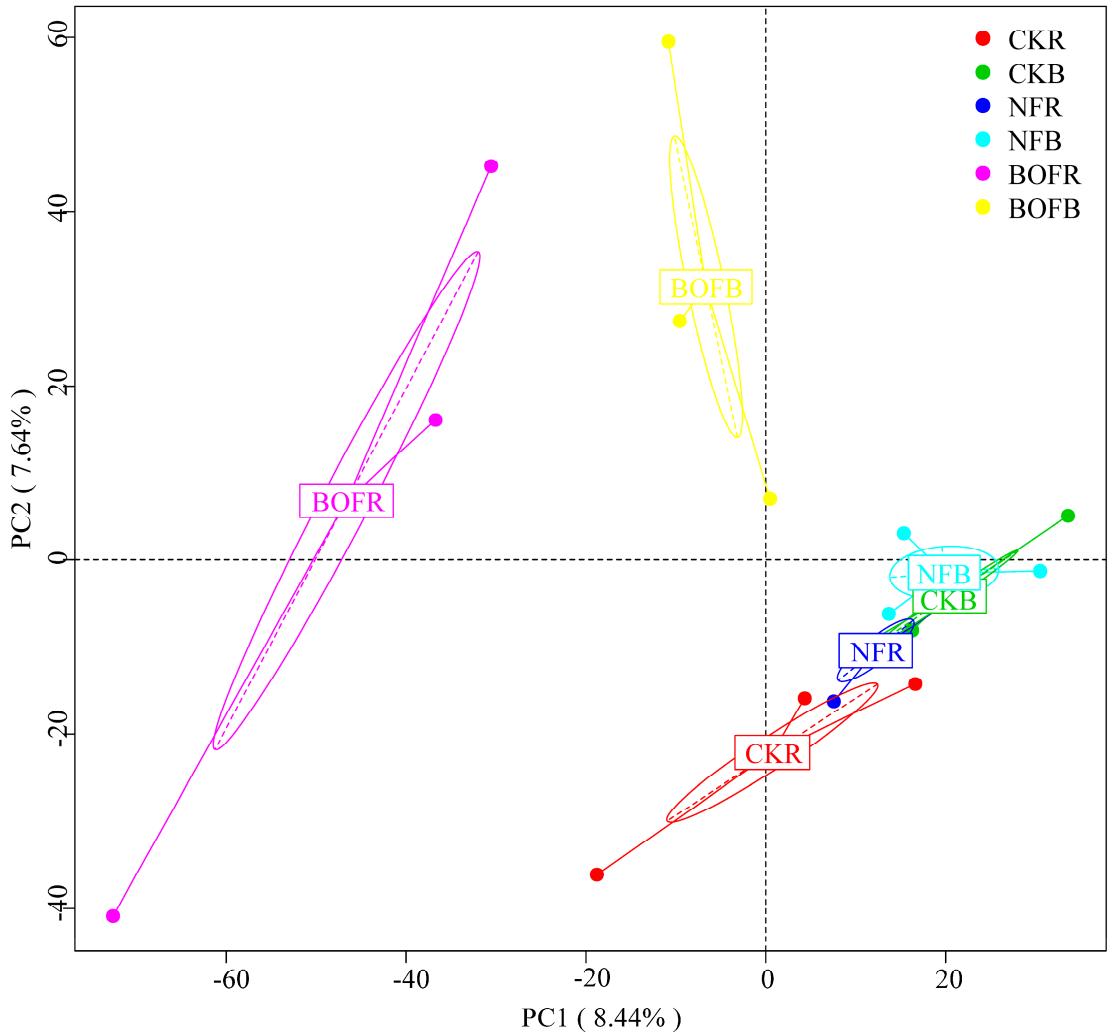

Figure 2. Principal components analysis of bacterial community structure in different fertilization treatments. CKR, rhizosphere of no fertilizer; CKB, bulk of no fertilizer; NFR, rhizosphere of nitrogen fertilizer; NFB, bulk of nitrogen fertilizer; BOFR, rhizosphere of bioorganic fertilizer; BOFB, bulk of bioorganic fertilizer.

Based on the analysis of soil bacterial community structure, we analyzed the effects of the different treatments on soil bacterial species. As shown in Figure 3a,b, we compared the bacterial species composition in rhizosphere soil treated with NF and BOF at the phylum and order level. The relative abundance of *Proteobacteria* in the BOF treatment was significantly higher than that in NF, while the relative abundance of *Acidobacteria* and *Planctomycetes* was significantly lower than NF. For the order level, the relative abundance of *Sphingomonadales* and *Sphingobacteriales* in the BOF treatment was significantly higher than that in the NF treatment.

3.4. Correlation between Bacterial Community and Soil Properties

The changes in soil bacterial community structure were related to soil properties. We used the envfit function to screen out four soil properties that best reflect the changes in soil bacterial community structure, and conducted RDA on the screened soil properties and bacterial community structure. The results showed that the soil bacterial community was significantly correlated with soil pH ($p < 0.01$), nitrate ($p < 0.01$), AP ($p < 0.01$), and AK ($p < 0.05$) (Figure 4). Similarly, the Mantel test showed that soil pH ($p < 0.05$), nitrate ($p < 0.01$), AP ($p < 0.01$), and AK ($p < 0.01$) were important factors affecting soil bacterial community.

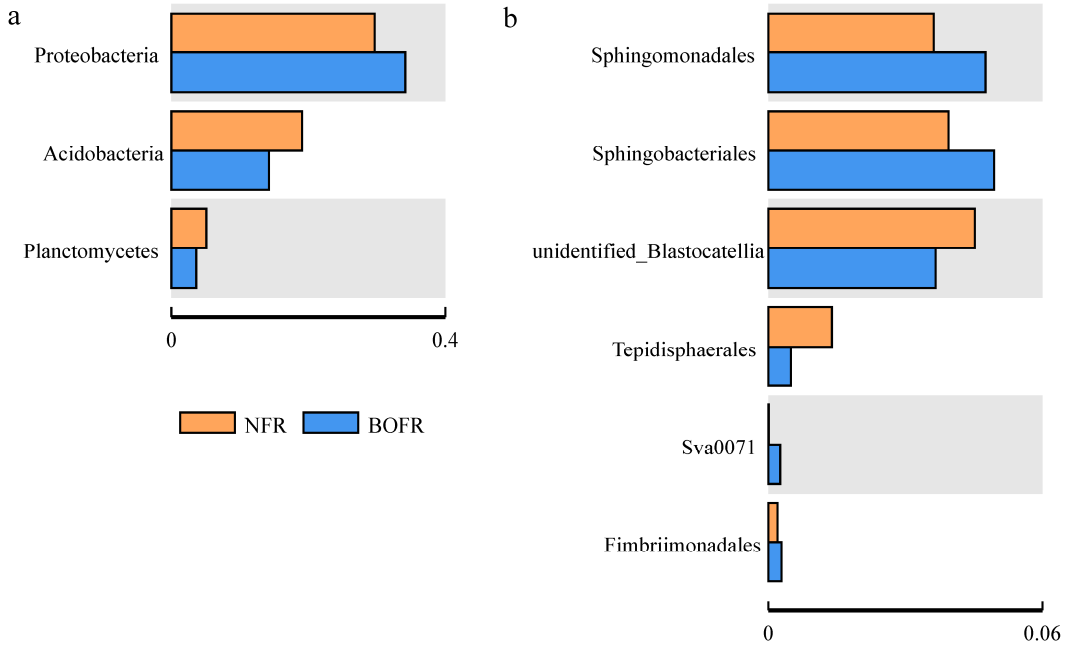

Figure 3. T-test analysis of bacterial community structure at the (**a**) phylum level and (**b**) order level between NFR and BOFR. NFR, rhizosphere of nitrogen fertilizer; BOFR, rhizosphere of bioorganic fertilizer.

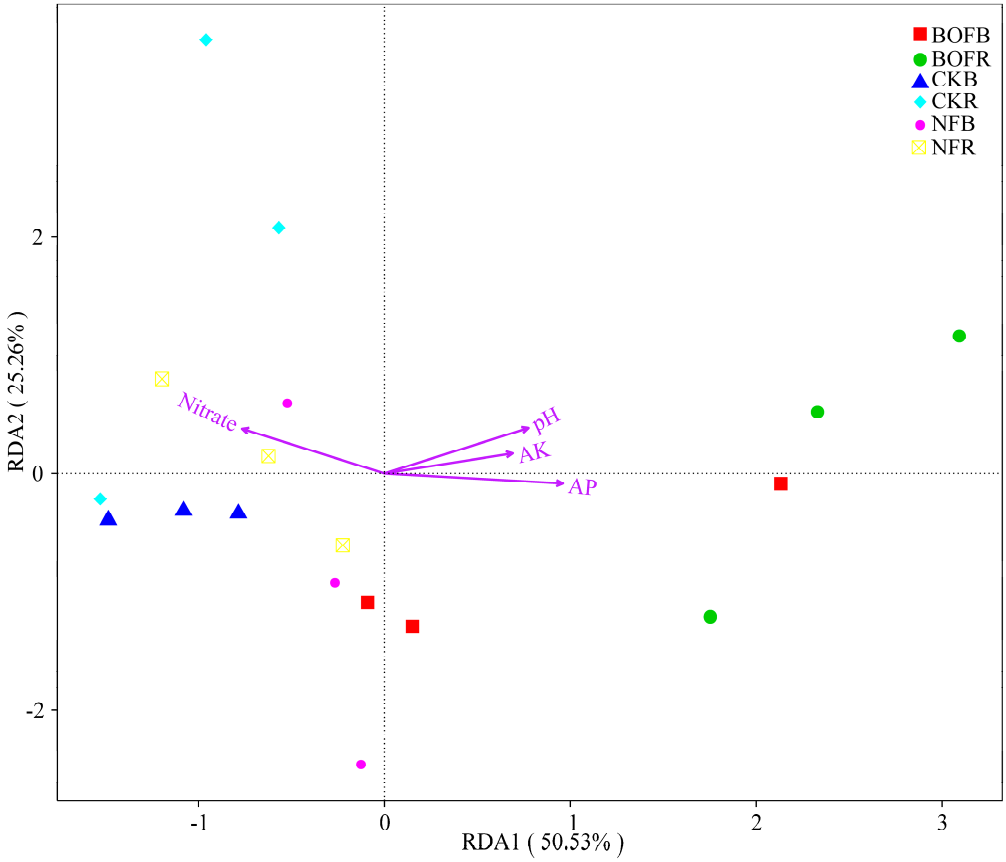

Figure 4. Redundancy analysis of bacterial community structure and soil properties. AP, available phosphorus; AK, available potassium; CKR, rhizosphere of no fertilizer; CKB, bulk of no fertilizer; NFR, rhizosphere of nitrogen fertilizer; NFB, bulk of nitrogen fertilizer; BOFR, rhizosphere of bioorganic fertilizer; BOFB, bulk of bioorganic fertilizer.

4. Discussion

The diversity index is an important indicator for evaluating the diversity of bacterial communities. The higher the diversity index the higher the richness and diversity. Studies have shown that different fertilization treatments have different effects on soil bacterial community diversity and that chemical fertilizers lead to reduced community diversity [31–33], while organic fertilizers could increase bacterial community diversity [11,34,35]. Similar results were obtained in the present study. The Shannon index was the highest for the BOF treatment, regardless of whether the soil was from the rhizosphere or non-rhizosphere (Table 2). In the rhizosphere, the Shannon index for the BOF treatment was higher than that of CK, while the NF treatment was lower than CK, indicating that bacterial diversity in the rhizosphere increased under BOF, but decreased under NF. This may be due to two reasons: Firstly, the bioorganic fertilizer itself contains microorganisms [14,36], while the nitrogen fertilizer does not. These microorganisms enter the soil with the application of BOF, which increases the bacterial diversity in the soil. Secondly, the bioorganic fertilizer contains a large amount of organic matter that can be absorbed and utilized by soil microorganisms, thereby promoting the growth and development of bacteria [37,38]. These substances are not present in nitrogen fertilizer. In addition, the soil alpha diversity index was significantly correlated with soil pH, nitrate, AP, and AK, indicating that soil pH, nitrate, AP, and AK were important factors affecting the diversity of bacterial communities.

Bacteria is an important part of soil microbes. They participate in soil nutrient cycling and play a significant role in maintaining the stability of the entire soil ecosystem [39]. Studies have shown that the majority of the dominant soil bacteria are similar, which includes about 10 bacterial groups [40,41]. Ai et al. [42] showed that the relative abundance of *Proteobacteria* in the rhizosphere soil of wheat was the highest. Wang et al. [22] found that *Proteobacteria*, *Chloroflexi*, *Acidobacteria*, and *Actinobacteria* were the dominant bacterial groups in the wheat-rice rotation system. The results of the present study showed that the dominant bacterial phyla and orders in the rhizosphere and bulk soil were similar (Figure 1a,b), but the relative abundances were significantly different (Figure 3a,b). The dominant phyla in the rhizosphere and non-rhizosphere of each treatment were *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Thaumarchaeota*. The dominant orders were *Xanthomonadales*, *Sphingomonadales*, *Sphingobacteriales*, and *Rhodospirillales*.

Previous studies have shown that the dominant groups of bacteria in farmland soils are similar, but their relative abundance is affected by soil type or texture and crop varieties [43–45]. In the present study, different fertilization treatments changed the soil properties of the rhizosphere (Table 1) and therefore affected the relative abundance of the dominant bacterial groups. Our results showed that the relative abundance of rhizosphere *Proteobacteria* treated with bioorganic fertilizer was significantly higher than that treated with nitrogen fertilizer while *Acidobacteria* was significantly lower (Figure 3a). Studies have shown that *Proteobacteria* are copiotrophic bacteria, and *Acidobacteria* are oligotrophic bacteria [46,47]. Thus, our findings indicated that the BOF treatment increased the relative abundance of copiotrophic bacteria while the NF treatment increased the relative abundance of oligotrophic bacteria. Because bioorganic fertilizer contains exogenous microorganisms and organic matter, nutrient availability in the rhizosphere increases, providing a suitable environment for the growth of copiotrophic bacteria, while nitrogen fertilizer does not contain exogenous microorganisms and organic matter, and this is coupled with long-term nitrogen fertilization in this region. A large amount of the application of nitrogen fertilizer results in low soil nutrient availability, which is beneficial to the growth of oligotrophic bacteria. Additionally, we determined the bacterial communities of the bioorganic fertilizer used in this study. The results showed that *Proteobacteria* was the dominant phylum. This might be another reason why the relative abundance of *Proteobacteria* in the BOF treatment was higher than that in the NF treatment.

The relative abundance of *Sphingomonadales* and *Sphingobacteriales* in the rhizosphere of the BOF treatment was significantly higher than that of the NF treatment (Figure 3b). *Sphingomonadales* and *Sphingobacteriales* belong to *Proteobacteria* and *Bacteroidetes*, respectively. Both *Proteobacteria* and *Bacteroidetes* are copiotrophic bacteria. This further illustrated that the bioorganic fertilizer increased

the abundance of copiotrophic bacteria. Eichorst and Kuske [48] showed that *Sphingobacteriales* are copiotrophic microbes that live in a highly carbon-efficient environment that has the ability to break down cellulose and chitin while also degrading nitrogen-containing substances. Lopes et al. [49] found that *Sphingomonadales* are involved in the degradation of cellulose and lignin. These two microorganisms may have promoted the decomposition of soil organic matter in the rhizosphere environment of the BOF treatment, which would increase nutrient availability for crop absorption and utilization. We considered that this might be the reason why the bioorganic fertilizer improved nutrient availability in rhizosphere soil and increased winter wheat yield.

Different fertilizer applications changes the physical and chemical properties of the soil, which in turn affects the soil bacterial community structure [50,51]. Previous studies have found that pH, nitrate, and AP were important factors in soil affecting microbial community structure [52–54]. Consistent with the previous studies, the present study found that the bacterial community structure in soil under different fertilization treatments was significantly correlated with soil pH, nitrate, AP, and AK (Figure 4). This may explain the changes in bacterial community structure under different fertilization treatments. The concentrations of soil nitrate, AP, and AK represent soil nutrient availability, which affects the soil bacterial community structure. Our findings showed that the bioorganic fertilizer improved nutrient availability in rhizosphere soil, which promoted the growth of copiotrophic bacteria, while nitrogen fertilizer reduced nutrient availability, which was more conducive to the growth of oligotrophic bacteria.

Different fertilization treatments affected bacterial community diversity and structure in rhizosphere soil. This study only analyzed the overall changes in the bacterial community in rhizosphere soil and did not study microbes with special functions. These microorganisms are involved in important soil metabolic pathways. Therefore, whether the differences in the functional microbial diversity and structural caused by different fertilization treatments lead to soil fertility and ecosystem function changes need to be investigated.

5. Conclusions

The BOF treatment increased the bacterial diversity in rhizosphere soil of winter wheat in the North China Plain, while the NF treatment reduced bacterial diversity. Bioorganic fertilizers increased the relative abundance of copiotrophic bacteria in the rhizosphere, while nitrogen fertilizer increased the relative abundance of oligotrophic bacteria. Soil pH, nitrate, AP, and AK were important factors affecting the bacterial communities of rhizosphere soil.

Author Contributions: R.L. and Z.O. designed the experiments; R.L., R.H., J.L., Y.L., S.H., and H.G. performed the research and analyzed the data; R.L. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Science and Technology Service Network Initiative (STS) of the Chinese Academy of Sciences (KFJ-STS-ZDTP-049), the Strategic Priority Research Program of the Chinese Academy of Sciences (XDA23050103) and the National Key Research and Development Program of China (2016YFD0300808).

Acknowledgments: We thank the staff of Yucheng Comprehensive Experiment Station of the Chinese Academy of Sciences for field and lab assistance. We also thank the editors and anonymous reviewers for their constructive comments in the improvement of this manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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