Effects of Manure and Chemical Fertilizer on Bacterial Community Structure and Soil Enzyme Activities in North China

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Abstract: The application of organic fertilizer affects soil microbes and enzyme activities. In this study, we explored the effects of various long-term different fertilization treatments (manure, M; chemical fertilizer, NP; manure + chemical fertilizer, MNP; and no fertilizer, CK) on bacterial community structure and soil sucrase, urease, and alkaline phosphatase activities in Shaping, Hequ, China. High-throughput sequencing was used to amplify the third to the fourth hypervariable region of the 16S ribosomal RNA for analysis of the bacterial community structure. Enzyme activities were determined by colorimetry. Soil treated with MNP had the highest bacterial Abundance-based Coverage Estimator index and enzyme activities. The principal coordinates analysis results showed significant differences among the various fertilization treatments ($p < 0.001$). Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, and Chloroflexi were consistently dominant in all soil samples. The redundancy analysis and Monte Carlo permutation tests showed that the soil bacterial communities were significantly correlated with alkali-hydrolyzable nitrogen, organic matter, urease, and alkaline phosphatase. Our results reveal the fundamentally different effects that organic and inorganic fertilizers have on soil bacterial communities and their functions.

Keywords: bacterial community; long-term fertilization; soil enzyme activities; high-throughput sequencing

1. Introduction

Soil microorganisms and enzyme activities are important indicators in the characterization of soil fertility and these play a vital role in soil material transformation and energy flow [1,2]. Soil bacteria are one of the largest functional microbial subgroups in soil, participating in soil respiration, nutrient transformation, organic matter decomposition, and other processes [3,4]. Soil enzymes secreted by microorganisms play critical roles in nutrient cycling (such as nitrogen fixation, phosphorus adsorption and desorption, potassium release, etc.), as well as soil structure maintenance and crop production [5,6]. Organic matter in soil serves as a substrate for a diverse set of soil microorganisms, promoting enzymatic activity [2]. Previous studies have shown that soil microorganisms and enzyme activities are highly sensitive to changes in soil properties, including pH, the degree of organic matter, moisture content, the presence of mineral substances, etc. Furthermore, soil microorganisms are also regarded as vital factors driving the production and turnover of extracellular enzymes and their activities in soil [5,7]. Studies showed that the physical and chemical properties of soil could be largely influenced by fertilization management practices [8,9].

Fertilization is an important measure in agricultural production, which can improve soil nutrients and increase crop yield. Recently, a large number of chemical fertilizers

have been imported into farmland to ensure food supply, and the result was an imbalance in soil nutrients, as well as a series of environmental problems [8,10]. Manure, however, could partly prevent many environmental problems caused by chemical fertilizers and also improve crop quality. It might be due to the fact that numerous beneficial microorganisms and organic materials enter soil through manure application, leading to changes in the soil environment that include differences in enzyme activity. Thus, it is necessary to investigate the soil's bacterial community structure and soil enzymatic activity. One of the main challenges in microbiological research is to improve the identification of bacteria. Traditional methods have various defects. For instance, the plating method can only reflect culturable microorganisms that account for only about 1% of the total population [11,12]. Furthermore, neither single-strand conformation polymorphism (SSCP) nor terminal restriction fragment length polymorphism (T-RFLP) are suitable for bacterial analysis because they only capture the dominant community members as selected by their PCR primers [13]. High-throughput sequencing technology, however, has facilitated bacterial research, allowing for huge amounts of information to be obtained, and for the bacterial diversity in the environment to be fully reflected [14]. In this study, we explored the effects of the long-term application of manure and chemical fertilizer on bacterial community structure with high-throughput sequencing technology, and we studied the soil's enzymatic activities by colorimetry in North China. The objectives of this study were as follows: (1) to elucidate the influences of different fertilization on bacterial community structure and soil enzyme activities, (2) and to search for the most suitable fertilization method.

2. Materials and Methods

2.1. Field Description and Experimental Design

The fertilization experiment was established in a maize field in Shaping, Hequ, China (39°12′18″ N, 111°15′41″ E). The altitude is 1089 m, with a frost-free period of about 140 days. The average temperature is 8.8 °C with an effective accumulative temperature of 3000–3360 °C per year. The experimental site has a light loam loess, classified as Hapli-Ustic Cambosols in the World Reference Base for Soil Resources (WRB) [15,16]. Analysis of soil samples taken from the experimental area showed that the basic physical and chemical properties of the surface 0–20 cm of soil before planting were as follows: organic matter (OM), 5.64 g/kg; total nitrogen (TN), 0.45 g/kg; total phosphorus (TP), 1.23 g/kg; alkali-hydrolyzable nitrogen (AN), 34.90 mg/kg; available phosphorus (AP), 2.69 mg/kg; cation exchange capacity (CEC), 6.97 cmol/kg; pH, 8.2.

The long-term fertilization trial began in 1988, and it lasted for 31 years. From 1988 to 2008, millet (*Panicum miliaceum* L.) and potatoes (*Solanum tuberosum* L.) were rotated; from 2009 to 2013, maize (*Zea mays* L.) was continuously cropped; from 2014 to 2017, potatoes and millet were rotated; and since 2018, potatoes and maize have been periodically rotated. The samples were obtained on 25 July 2019, when the crop was maize. The four treatments were arranged in a randomized block design with three replications, with each plot being 24 m². The four treatments were (1) M—manure, 22,500 kg/ha; (2) NP—N: 120 kg/ha, P_2O_5: 75 kg/ha; (3) MNP—manure, 22,500 kg/ha, N: 120 kg/ha, P_2O_5: 75 kg/ha; and (4) CK—control treatment without fertilizer. Nitrogen was applied in the form of urea, while phosphorus was applied as superphosphate. The manure was made up of local cow dung with an average N content of 3.64 g/kg, P_2O_5 content of 2.46 g/kg, and K_2O content of 7.87 g/kg. The fertilization details are shown in Table 1. All of the fertilizers were applied as basal fertilizers before planting.

2.2. Soil Sampling and Analysis of Soil Properties

Soil were sampled from five points in each plot and mixed into one sample. There were 12 composite samples in total. After removing visible stone and debris, samples were collected in sterile plastic bags in an ice-box and transported to the laboratory. One part was used for analyzing physicochemical properties and enzyme activities after sieved through

a 2 mm screen, and the other part was stored in $-80°C$ refrigerator for DNA extraction and sequencing.

Table 1. Experimental treatments and fertilization.

Treatments	Chemical Fertilizer (kg/hm^2)			Manure (kg/hm^2)			Total Nutrient (kg/hm^2)		
	N	P$_2$O$_5$	K$_2$O	N	P$_2$O$_5$	K$_2$O	N	P$_2$O$_5$	K$_2$O
Manure (M)	0	0	0	80	55	180	80	55	180
Chemical fertilizer (NP)	120	75	0	0	0	0	120	75	0
Manure + Chemical fertilizer (MNP)	120	75	0	80	55	180	200	130	180
No fertilizer (CK)	0	0	0	0	0	0	0	0	0

2.3. Soil Physicochemical Analytical Procedures

Soil TN, TP, and total potassium (TK) were analyzed using a Vario Max element analyzer (Elementar Vario PYRO cube and Isoprime100, Hanau, Germany). Soil pH was determined at a soil/water ratio of 1:2.5 with a pH meter. OM was determined by $K_2Cr_2O_7$ oxidation method. Soil AN was determined by the alkaline hydrolysis diffusion method. AP was determined by $NaHCO_3$-extracted method. Available potassium (AK) was analyzed by CH_3COONH_4-extracted method. The details can be found from the reference [17].

2.4. Soil Enzyme Activities' Determination

In this study, 3, 5-dinitrosalicylic acid colorimetry was used for sucrase (SUC) activity, and the weight of glucose (mg) in 1 g of soil after incubation for 24 h was used to represent SUC activity. The urease (URE) activity was indicated by sodium phenol-sodium hypochlorite colorimetric method, and the weight of NH_3-N (mg) in 1 g soil after incubation for 24 h was used to represent the URE activity. The alkaline phosphatase (ALP) activity was represented by the phenyl disodium phosphate method. Detailed procedures for the three enzymes can be found from the reference [18].

2.5. Soil DNA Extraction and High-Throughput Sequencing

Total DNA of each sample was extracted form 0.5 g soil using the Fast DNA SPIN Isolation Kit (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's instructions. The bacterial third to the fourth hypervariable region of the 16S ribosomal RNA (16S rRNA) was amplified using primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Specific barcodes of 7-bp were incorporated into the primers for multiplex sequencing. The first round PCR reactions were performed in a total volume of 50 μL mixture containing 10 μL of 5×Q5 Reaction Buffer, 10 μL of 5 × Q5 High-Fidelity GC Enhancer, 1 μL of dNTPs, 1 μL of each primer (10 μM), 0.2 μL of Q5 High-Fidelity DNA Polymerase (Transgen, Beijing, China), 2 μL of temple DNA and 24.8 μL of ddH$_2$O. PCR process were as follows: 5 min of initial denaturation at 95 °C, followed by 15 cycles of denaturation at 95 °C for 1 min, annealing at 50 °C for 1 min and extension at 72 °C for 1 min, and a final extension of 7 min at 72 °C.

PCR products from the first PCR step were purified through VAHTS^{TM} DNA Clean Beads (Vazyme, Nanjing, China). A second round PCR was then performed in a 40 μL reaction system which contained 20 μL 2 × Phusion High-Fidelity Master Mix (New England Biolabs, Boston, MA, USA), 8 μL ddH$_2$O, 10 μM of each primer and 10 μL PCR products from the first step. PCR process was as follows: an initial denaturation at 98 °C for 30 s, followed by 10 cycles of denaturation at 98 °C for 10 s, annealing at 65 °C for 30 s and extension 72 °C for 30 s, and a final extension at 72 °C for 5 min. Finally, all PCR products were quantified by Quant-iT™ dsDNA HS Reagent (Thermo Fisher, Waltham, MA, USA) and pooled together. High-throughput sequencing analysis of bacterial 16S rRNA genes

was performed with the purified and pooled sample using the Illumina Hiseq 2500 platform (2 × 250 paired ends) at Biomarker Technologies Corporation (Beijing, China).

2.6. Bioinformatics Analysis

The Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) pipeline was employed to process the sequencing data [19]. Briefly, raw sequencing reads with exact matches to the barcodes were assigned to respective samples and identified as valid sequences. The low-quality sequences were removed. Paired-end reads were assembled using FLASH (version 1.2.7) [20]. After chimeras' detection with UCHIME (version 4.2), the remaining high-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold by UCLUST [21]. A representative sequence was selected from each OTU using default parameters. OTU taxonomic classification was conducted by BLAST searching the representative sequences set against the Silva Database (version 132) [22]. An OTU table was further generated to record the taxonomy of the OTUs and the abundance of each OTU in each sample. OTUs containing less than 0.001% of total sequences across all samples were discarded. To minimize sequencing depth differences across samples, an averaged, rounded, and rarefied OTU table was generated by averaging 100 evenly resampled OTU subsets under the 90% minimum sequencing depth for further analysis.

Sequence data analyses were mainly performed using QIIME and R (version 3.1.2, vegan package) [23,24]. The Abundance-based Coverage Estimator (ACE) and Shannon index were calculated based on the OTU table using QIIME. β-diversity analysis was performed to investigate the bacterial communities' structural variation across samples using principal coordinate analysis (PCoA), which was based on the OTU level compositional profiles [25]. The significance of bacteria structure differentiation among groups was assessed by permutational multivariate analysis of variance (PERMANOVA) [26] with the distance legend of 0.01. The taxonomic compositions and abundances were visualized using MEGAN [27]. A Venn diagram was generated to visualize the shared and unique OTUs among groups based on the occurrence of OTUs across groups regardless of their relative abundances [28]. Redundancy analysis (RDA) and Monte Carlo permutation tests were carried out using Canoco for Windows 4.5 (Cabiy Information Techonogy Co., LTD., Shanghai, China). The species compositional similarity was analyzed by unweighted pair-group method with arithmetic mean (UPGMA) with R software.

2.7. Statistical Analysis

Statistical analyses were carried out using a one-way analysis of variance (ANOVA) procedure in the SPSS software (IBM SPSS Statistics version 22) to check the normal distribution and homoscedasticity, and to detect differences in soil parameters, soil enzyme activities, the bacterial relative abundance, α-diversity indices, etc. Significant differences between data were determined with the least significant difference (LSD) test at the $p = 0.05$ level.

3. Results

3.1. Soil Physicochemical Characteristics

As shown in Table 2, long-term fertilization significantly promoted soil TP, AN and AP ($p < 0.05$) compared to the control. The TN level was the highest in treatment MNP, followed by treatment M, and then treatment NP, while M was not significantly different from MNP. The TP value was the highest in treatment MNP, followed by treatment NP and treatment M. Again, however, there was no significant difference between NP and MNP. Soil pH (H_2O) values decreased with the application of fertilizers, although no significant differences were observed. F- and p-values of the factors can be found in Table S1.

Table 2. Soil physicochemical properties under different treatments.

Treatments	pH	AN (mg/kg)	AP (mg/kg)	AK (mg/kg)	OM (g/kg)	TN (g/kg)	TP (g/kg)	TK (g/kg)
M	8.40 ± 0.23 a	63.09 ± 3.06 ab	22.53 ± 1.73 a	244.78 ± 16.67 a	11.76 ± 2.26 a	0.81 ± 0.10 a	0.80 ± 0.03 b	12.79 ± 0.65 a
NP	8.51 ± 0.13a	59.15 ± 7.35 b	14.40 ± 5.52 b	71.27 ± 4.25 c	7.82 ± 0.57 b	0.74 ± 0.14 ab	0.84 ± 0.04 ab	12.58 ± 0.34 a
MNP	8.40 ± 0.18 a	74.55 ± 4.55 a	26.90 ± 2.80 a	223.90 ± 3.04 b	12.27 ± 1.14 a	0.85 ± 0.12 a	0.85 ± 0.12 a	12.36 ± 0.74 a
CK	8.59 ± 0.10 a	37.98 ± 1.23 c	5.10 ± 0.70 c	60.01 ± 8.30 c	6.52 ± 1.48 b	0.56 ± 0.06 b	0.66 ± 0.03 c	12.78 ± 0.17 a

Note: M: manure; NP: NP chemical fertilizer; MNP: manure + NP chemical fertilizer; CK: no fertilizer. AN: alkali-hydrolyzable Nitrogen; AP: available phosphorus; AK: available potassium; OM: organic matter; TN: total nitrogen; TP: total phosphorus; TK: total potassium; Different letters in the same column indicate significant differences (ANOVA followed by LSD post hoc test, $n = 3$, $p < 0.05$, average value, SD standard deviation).

3.2. Bacterial α-Diversity

After the removal of chimeras, a total number of 748,247 sequences were obtained in this study, which are available through the NCBI Sequence Read Archive (Accession: PRJNA706481). The OTU numbers in the samples did not significantly increase with the number of sequences sampled and the curve gradually flattened out (Supplementary Figure S1), indicating that the sample size was large enough and that the library could satisfactorily characterize the bacterial communities. The ACE and Shannon indices under different treatments were estimated. As shown in Table 3, the ACE was the highest in treatment MNP with a combination of manure and chemical fertilizer, followed by treatments M, NP, and CK, respectively. The Shannon index in the M treatment was significantly higher than in the CK treatment. However, no significant differences were observed between the other treatments.

Table 3. α-diversity indices of the bacteria under different treatments.

Treatments	ACE	Shannon
M	1828.12 ± 6.06 b	6.57 ± 0.01 a
NP	1769.66 ± 7.89 c	6.53 ± 0.02 ab
MNP	1867.82 ± 8.81 a	6.55 ± 0.05 ab
CK	1665.51 ± 5.54 d	6.48 ± 0.02 b

Note: M: manure; NP: NP chemical fertilizer; MNP: manure + NP chemical fertilizer; CK: no fertilizer. Different letters in the same column indicate significant differences (ANOVA followed by LSD post hoc test, $n = 3$, $p < 0.05$, average value, SD standard deviation).

3.3. Soil Bacterial Community Composition

As shown in Figure 1, 1701 OTUs were shared by different treatments, accounting for 23.4% of the total OTU of all the samples. Numbers of specific OTUs of treatments M, NP, MNP, and CK were 1, 0, 25, and 0, respectively. The combined application of manure and chemical fertilizer notably increased the number of unique OTUs in treatment MNP compared with the other treatments.
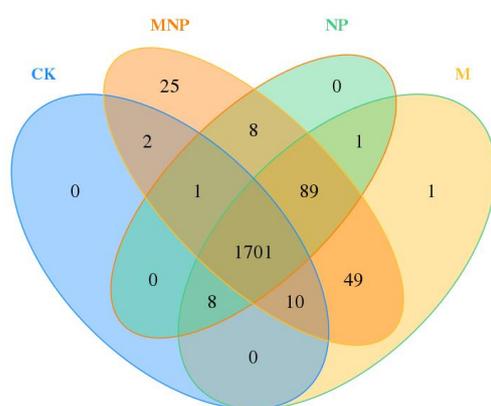
Figure 1. Venn Diagram showing unique and overlapped OTUs between different fertilization treatments. M: manure; NP: NP chemical fertilizer; MNP: manure + NP chemical fertilizer; CK: no fertilizer.

3.4. Effect of Different Treatments on Soil Bacterial Communities

PCoA was used to compare the similarity of soil bacterial communities among different treatments at the OTU level. As shown in Figure 2, the plot identified two principal component factors related to the percentage abundance of groups, explaining 63.91% (PC1) and 7.99% (PC3) of the total variation. PCoA showed that the four treatments could be separated clearly. The three repeats of each treatment were clustered together, showing good repeatability. Samples of treatment NP were parted from the other treatments by the first axis, while samples of treatments NP and CK were separated from samples of treatments M and MNP by the second axis. Samples of treatments M and MNP tended to cluster together, which could also be seen in the clustering tree (Supplementary Figure S2). In Figure 3, PERMANOVA showed significant differences in the bacterial community structures among treatments M, NP, MNP, and CK at the OTU level ($p < 0.001$).

3.5. Taxonomic Composition Analysis at the Phylum and Genus Level

The taxonomic distributions of bacterial communities were evaluated at different levels of classification. As shown in Figure 4, Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, and Chloroflexi were the most abundant phyla, accounting for approximately 90% of the bacteria detected in all 12 soil samples. Compared with treatment CK, the percentage of Proteobacteria increased by 2.49% in treatment M, 0.08% in treatment NP, and 1.94% in treatment MNP. The percentage of Gemmatimonadetes increased in treatments M, NP, and MNP by 0.29%, 1.58%, and 0.45%, respectively. However, compared with CK, the relative abundance of Chloroflexi decreased in treatments M, NP, and MNP by 0.97%, 0.53%, and 1.11%, respectively. The bacterial difference significance analysis can be found in Table S2.
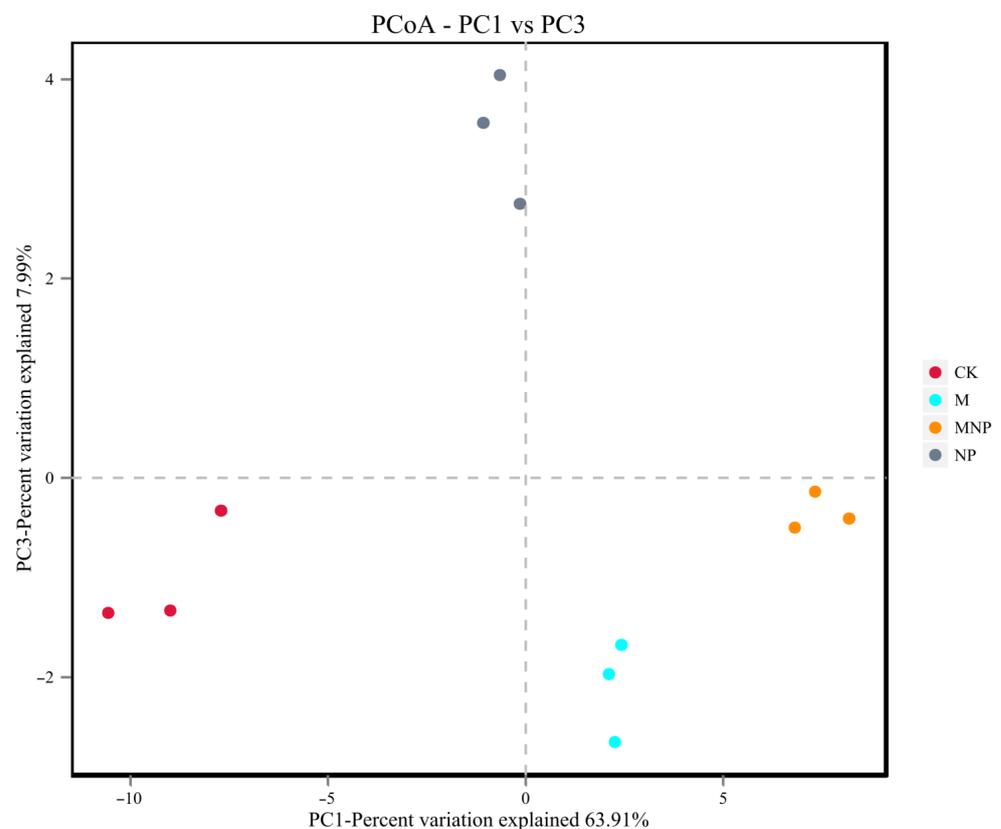

Figure 2. PCoA of the bacterial community compositions in soil under different treatments based on OTUs. M: manure; NP: chemical fertilizer; MNP: manure + chemical fertilizer; CK: no fertilizer.
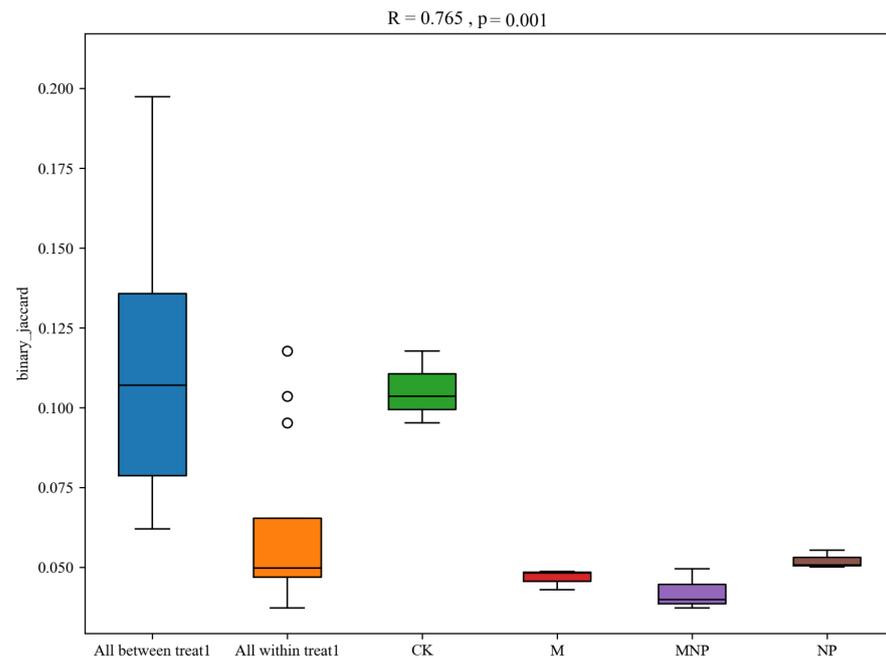
Figure 3. PERMANOVA analysis under different treatments. M: manure; NP: chemical fertilizer; MNP: manure + chemical fertilizer; CK: no fertilizer.

As shown in Figure 5, at genus level, the top ten genera accounted for about 32% of the total genera and belonged to four phyla: Gemmatimonadetes, Acidobacteria, Proteobacteria, and Actinobacteria. Compared with CK, the percentage of *MND1* decreased by 1.42% in treatment M, 1.56% in treatment NP, and 2.26% in treatment MNP, respectively. The relative abundance of *RB41* decreased by 0.23%, 0.86%, and 0.85% in treatments M, NP, and MNP, respectively. On the contrast, the percentage of *Arthrobacter* increased by 0.03% in treatment M, 0.88% in treatment NP, and 0.29% in treatment MNP, respectively. The relative abundance of *Steroidobacter* increased by 0.60%, 0.21%, and 0.74% in treatments M, NP, and MNP, respectively. The bacterial difference significance analysis can be found in Table S3.
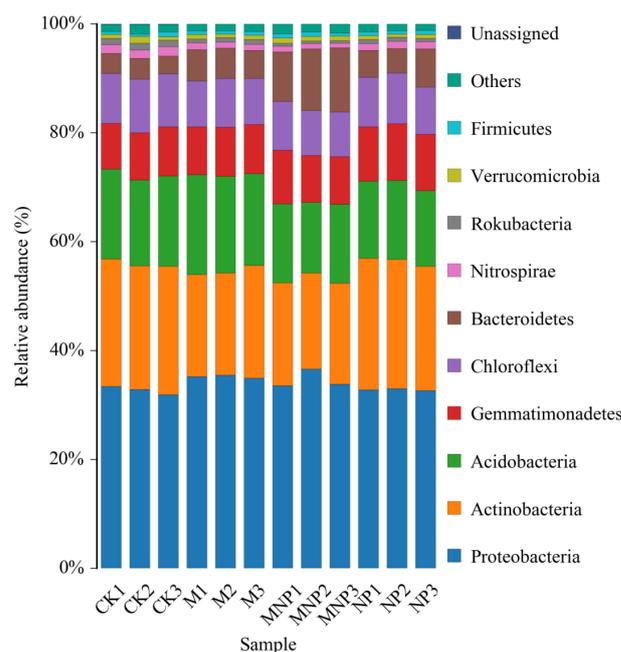

Figure 4. Taxonomic composition and abundance distribution of bacteria at the phylum level under different treatments. M: manure; NP: chemical fertilizer; MNP: manure + chemical fertilizer; CK: no fertilizer.
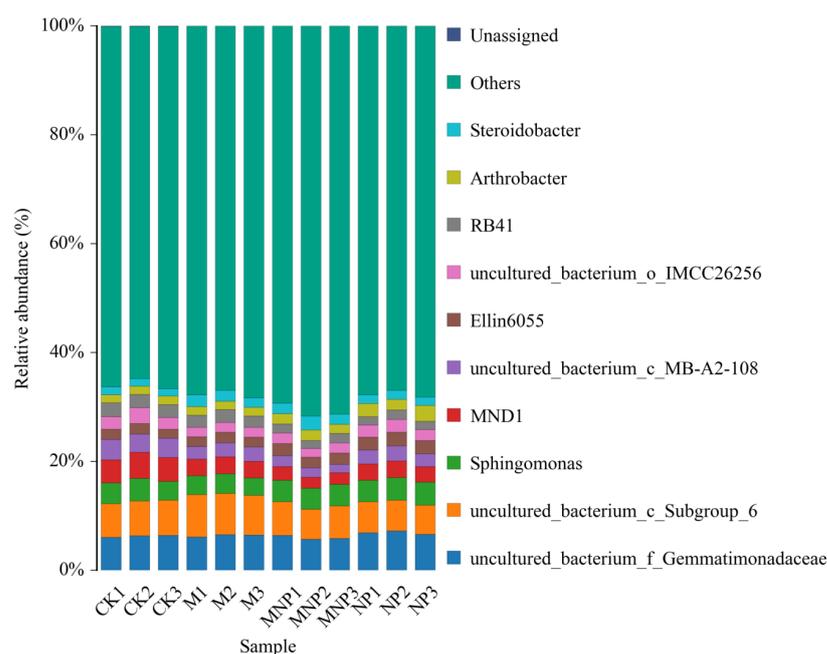
Figure 5. Taxonomic composition and abundance distribution of bacteria at the genus level under different treatments. M: manure; NP: chemical fertilizer; MNP: manure + chemical fertilizer; CK: no fertilizer.

3.6. Effect of Different Treatments on Soil Enzyme Activities

Fertilization significantly increased soil enzyme activities compared with CK (Table 4). Three enzyme activities were the highest in MNP treatment, in which the nutrients were likewise the most abundant, followed by the M and NP treatments. There was no significant difference in the activities of the three soil enzymes between treatments M and NP, but they were slightly higher in the M treatment than in the NP treatment. Compared with CK, SUC activity increased in treatments M, NP, and MNP by 124.1%, 80.9%, and 145.6%, respectively. The increasing range of URE and ALP were 63.9–109.7% and 4.11–67.9%.

Table 4. Effects of different treatments on soil enzyme activities.

Treatments	SUC Activity (mg·Glucose·g^{-1}·24 h^{-1})	URE Activity (mg·NH$_3$-N·$^{-1}$·24 h^{-1})	ALP Activity (mg Phenol·g^{-1}·24 h^{-1})
M	19.55 ± 3.227 ab	1.983 ± 0.021 b	1.820 ± 0.080 b
NP	15.78 ± 1.520 b	1.900 ± 0.290 b	1.404 ± 0.153 bc
MNP	21.42 ± 0.708 a	2.537 ± 0.218 a	2.264 ± 0.278 a
CK	8.723 ± 0.083 c	1.210 ± 0.128 c	1.348 ± 0.016 c

Note: M: manure; NP: NP chemical fertilizer; MNP: manure + NP chemical fertilizer; CK: no fertilizer. Different letters in the same column indicate significant differences (ANOVA followed by LSD post hoc test, $n = 3$, $p < 0.05$, average value, SD standard deviation).

3.7. Correlations

We performed RDA to understand the correlations between phylum-level bacterial community composition and soil properties and that of enzyme activities. These eight environmental variables together explained 82.9% of the bacterial community variances. In addition, the first axis of the RDA accounted for a higher variation of 62.9%, whereas the second axis accounted for a lower of 20.0% (Figure 6a). The bacterial community composition had significant correlation with AN (F = 10.892, $p = 0.002$) and OM (F = 10.847, $p = 0.002$).
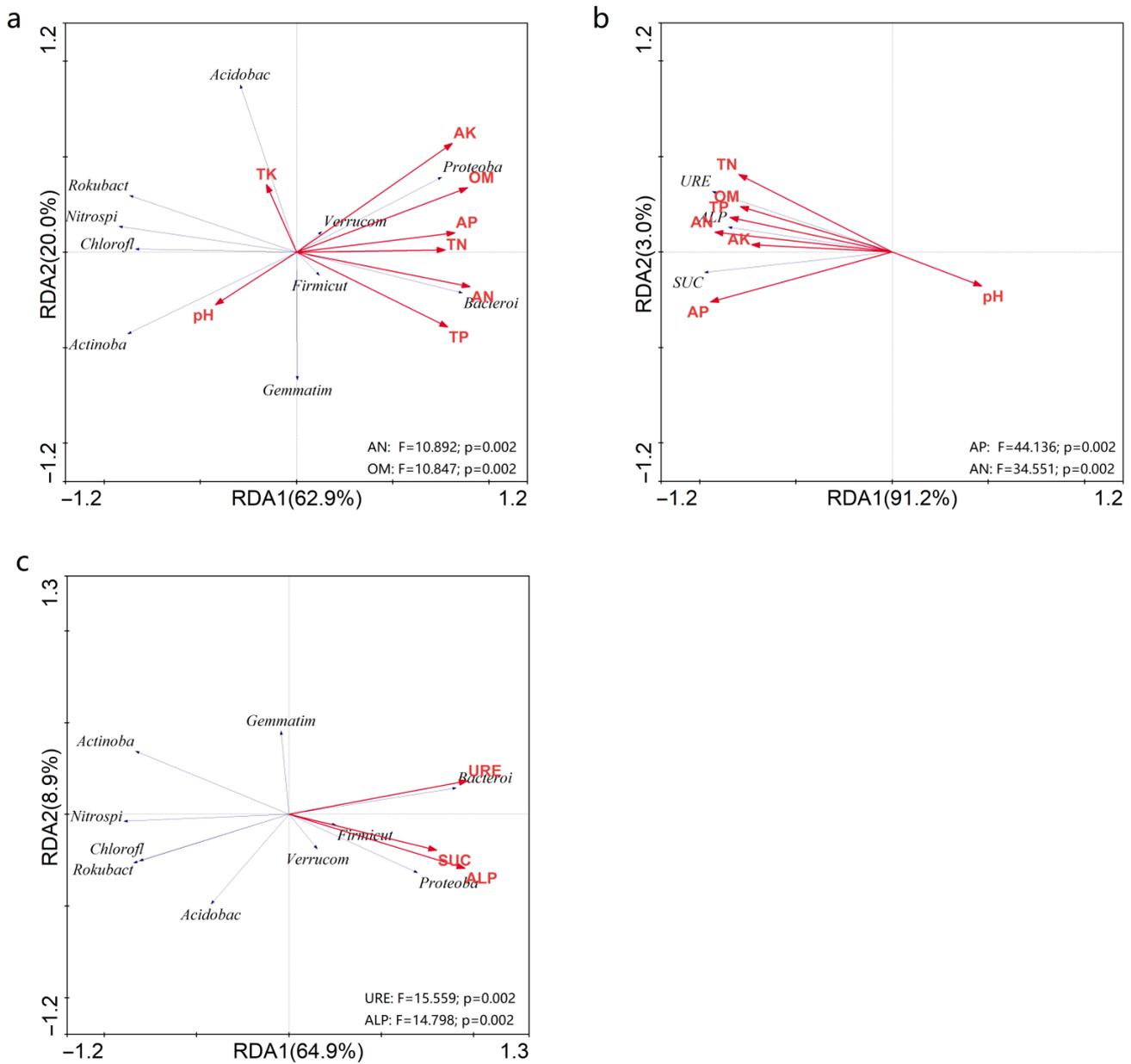
Figure 6. RDA depicting the relationship between the soil properties, the phylum-level soil bacteria and soil enzyme activities. (**a**) The relationship between soil bacterial community composition and properties. (**b**) The relationship between soil enzyme activities and properties. (**c**) The relationship between soil bacterial community composition and enzyme activities.

Figure 6b illustrated the relationship between enzyme activities and soil characteristics. The first and second axes explained 91.2% and 3.0% of the total change of the soil enzyme activities. The enzyme activities showed significant correlation with AP (F = 44.136, p = 0.002) and AN (F = 34.551, p = 0.002).

RDA between phylum-level bacterial community composition and soil enzyme activities was illustrated in Figure 6c and soil enzyme activities were taken as explanatory variables. The first and second axes explained 64.9% and 8.9% of the total changes of the bacterial community. The bacterial community composition showed significant correlation with URE (F = 15.559, p = 0.002) and ALP (F = 14.798, p = 0.002).

4. Discussion

4.1. Effects of Treatments on the Soil Physicochemical Properties

In the present study, no fertilizer had been applied in treatment CK for 31 years, leading to the lowest chemical properties. Long-term fertilization improved soil nutrients including AN, AP, AK, OM, TN, and TP. In this study, fertilization lowered soil pH, which was consistent with the findings of Wang, who reported a decline in soil pH with the application of fertilizer [29]. pH was regarded as one of the main factors related to fertilization management. A large number of studies have shown that soil pH decreased with excessive application of chemical N fertilizer, resulting in soil acidification [30,31]. In this study, no significant differences were observed in these treatments, which might be due to the fact that the calcareous soils containing higher carbonates to buffer soil pH [32].

4.2. Effects of Treatments on the Bacterial Diversity and Community Composition

Alpha diversity analysis showed significant difference ($p < 0.05$) of ACE index under different fertilization measures, and these were consistent with the previous results [8]. The combination of manure and chemical fertilizer significantly increased the bacterial ACE index compared with the other fertilization measures. The main reason was that organic materials themselves contain a variety of nutrient elements, which could increase the exogenous carbon, promote the formation of aggregate structure, and provide good conditions for the growth and reproduction of bacteria [33].

PCoA and PERMANOVA showed significant differences ($p < 0.001$) in bacterial community structure under different fertilization treatments, consistent with the results of Liang, who documented a clear separation of bacteria under different fertilization modes [34]. Fertilization directly affected the soil's bacterial community structure by changing nutrients in the soil and by further impacting the biological activity of bacteria in soil. Additionally, the change of soil pH caused by fertilization had an indirect effect on the bacterial community structure. This change might be due to the fact that the optimal pH range for bacterial growth and reproduction was narrow [35]. The application of manure could cause significant differences on bacterial community structures compared with chemical fertilizer [36].

The availability of soil nutrients was usually linked with the transformation from oligotrophic microorganisms to copiotrophic microorganisms. For example, a high nutrient availability in soil promoted the growth and reproduction of copiotrophic microorganisms, while in nutrient-restricted soil environments, the number of oligotrophic microorganisms that grow slowly would increase [37,38]. Proteobacteria, Actinobacteria, Acidobacteria, Gemmatimonadetes, and Chloroflexi were the most abundant phyla, accounting for approximately 90% of the total bacteria, consistent with the previous results reported in different systems [39,40]. Among these phyla, Proteobacteria is considered to be a kind of copiotrophic microorganism widely existing in soil and whose abundance is relatively higher in treatments with manure. Most of the taxa within Acidobacteria are thought to be slow growing, oligotrophic microorganisms [37]. In the present study, the relative abundance of Acidobacteria was higher in CK without fertilizer than in NP and MNP by 2.16% and 2.34%, respectively, which indicated that Acidobacteria was more suitable to nutrient-restricted soil environments [38]. Gemmatimonadetes is dominant in soils with low water content [41]. The NP treatment in this study seemed to be more suitable for its survival. Chloroflexi is considered an oligotrophic bacteria in the soil and prefers to decompose some refractory organic compounds [37]. In the present study, the relative abundance of Chloroflexi decreased in treatments with fertilizer compared with CK. However, the abundance was relatively higher in treatments M and MNP, compared with treatment NP.

Most of dominant genera are widely distributed in the environment. For example, *Sphingomonas* has been found in both aqueous (both fresh- and seawater) and terrestrial habitats, plant root systems, and others. *MND1* can be detected more frequently in soil, aquatic and subsurface ecosystems [40]. *Arthrobacter* is commonly found in soils, the aerial surfaces of plants, and wastewater sediments [41]. Some of the genera have the ability

to use organic matter to grow and reproduce. For example, *Sphingomonas* distributes widely in the environment due to its ability to use a wide range of organic compounds and to grow and survive in low-nutrient conditions. *Arthrobacter* can degrade unusual and polymeric compounds and plays an important role in biodegrading agrochemicals and pollutants. In the present study, the relative abundance of these genera tended to be higher in treatments with manure than the others, which was consistent with these properties. In the previous study, *Steroidobacter* was reported to be a complex organic compound degrading bacteria [42], whose abundance was the highest in MNP treatment, followed by M treatment, and then by NP treatment in this study. On the contrary, the abundance of *RB41* was the highest in CK treatment, consistent with Yan who observed the highest relative abundance of *RB41* in abandoned farmland compared to other treatments with fertilizer [43].

4.3. Effect of Different Treatments on Soil Enzyme Activities

Enzymatic activity in soil is largely related to the microorganisms present, to available nutrients and to root exudates, as well [44,45]. Compared with CK, fertilization significantly increased the activities of SUC, URE, and ALP ($p < 0.05$). With the increase of nutrients input, soil enzyme activities gradually increased. Enzyme activities were the highest in treatment MNP. The results indicated that soil enzyme activity was directly affected by the amount of fertilizer applicated. Previous studies have shown that manure application was of paramount importance to improve soil enzyme activities, which significantly increased enzyme activities more than that of chemical fertilizer application [46].

4.4. Relationship between Soil Bacteria Community Composition and Environmental Factors and Soil Enzyme Activities

SUC, URE, and ALP were mainly involved in soil's carbon, nitrogen, and phosphorus cycles, which were not only limited by soil nutrients, but also affected by other soil properties. In this study, AP and AN were two significant factors related to the changes of enzyme activities, which were mainly caused by fertilization regimes. Part of the soil enzymes are derived from the decomposed materials of bacterial cell exudates and residues, and in turn, soil bacterial diversity is greatly affected by soil enzyme activities [47]. Moreover, the composition of the bacterial community was influenced by its environment, including soil properties. Furthermore, through the RDA analysis, we found that AN, OM, URE, and ALP were main factors attributed to bacterial community structure in this region. This might be due to OM-provided substrates and energy for bacterial reproduction and metabolism [2]. Alkali-hydrolyzable nitrogen and carbon were coupled to participate in many bacterial metabolic processes. Previous studies also reported that microbial community composition had a significant correlation with URE [47].

In the present study, MNP treatment had the best effect on the improvement of bacterial diversity and enzyme activity, not only because of the maximum input of nitrogen, phosphorus, and potassium, but also because of the key advantages of combining of manure and chemical fertilizer application. Admittedly, manure contains a variety of nutrient elements, which can increase the input of exogenous carbon, promote the formation of aggregate structures, reduce soil bulk density, enhance the activity of microorganisms, promote the growth of crop roots and above-ground parts, and even increase the activity of soil enzymes [48–50]. However, a single application of manure alone is not conducive to the growth of crops, possibly because the slow and delayed release of nutrients in organic fertilizer cannot meet the needs of crops for nutrients in time [51]. Chemical fertilizers can provide quick nutrients, but studies have shown that a single application of chemical fertilizer can easily lead to soil degradation and is not suitable for the growth and reproduction of microorganisms. By contrast, manure has a good buffering ability to protect crops from adverse fluctuations and enhance the anti-interference ability of the soil [52,53]. Thus, manure and chemical fertilizer cooperation can foster strengths and circumvent weaknesses of either technique, and this can provide a more balanced supply of nutrients, just as it can also promote the growth of crops more effectively.

5. Conclusions

Various degrees of difference in bacterial community structures, soil enzyme activities and soil properties were observed with manure, chemical fertilizers, or both. As a whole, the combination of manure and chemical fertilizer showed great advantages, which could be a promising method in agricultural production. The proportion of the combination, however, has yet to be explored for different crops in the future.

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