agronomy

MDPI

Article

Effects on Soil Bacterial Organisms in an Alfalfa Monocropping System after Corn Insertion and Nitrogen Fertilization

Zongyong Tong [1], Lixue Wang [2], Yu Wang [1], Xianglin Li [1] and Feng He [1,*]

[1] Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing 100094, China
[2] Institute of Animal Science and Veterinary Research, Tianjin Academy of Agricultural Sciences, Tianjin 300381, China
* Correspondence: hefeng@caas.cn

Abstract: Alfalfa (*Medicago sativa* L.) and corn (*Zea mays* L.) are common forage plants for feeding livestock, and their effects on soil bacterial organisms have been extensively studied. However, there is little comprehensive research on soil bacterial organisms and their function in a long-term alfalfa monocropping system after corn insertion and fertilization. The effects of alfalfa–corn rotation (AF: alfalfa monocropping, RA: alfalfa and corn rotation) and nitrogen fertilization (RA0 and RA15) were investigated in a field experiment. The results showed that fertilization significantly increased the aboveground biomass (AGB) and soil nitrate nitrogen, and corn insertion significantly decreased the nitrate nitrogen (NO_3^--N) ($p < 0.05$). In addition, the bacterial community structure among the treatments was significantly changed by the rotation and fertilization. The rotational system of RA significantly increased the soil bacterial diversity compared with AF ($p < 0.01$), and most of the soil bacterial communities were of the phyla Actinobacterota and Proteobacteria. The RA system had a lower relative abundance of Actinobacterota than the AF system. The bacterial function prediction found that the soil carbon and nitrogen cycle processes in RA were more active than those in AF. The RDA analysis revealed that NO_3^--N and pH were the main environmental factors affecting the bacterial community structure in the RA system.

Keywords: alfalfa; corn insertion; nitrogen fertilization; soil bacterial; microbial community

Citation: Tong, Z.; Wang, L.; Wang, Y.; Li, X.; He, F. Effects on Soil Bacterial Organisms in an Alfalfa Monocropping System after Corn Insertion and Nitrogen Fertilization. *Agronomy* **2023**, *13*, 253. https://doi.org/10.3390/agronomy13010253

Academic Editor: José David Flores-Félix

Received: 28 November 2022
Revised: 8 January 2023
Accepted: 12 January 2023
Published: 14 January 2023

1. Introduction

Alfalfa is one of the most important forages in the world; it is known as the "King of forage" and is widespread in large areas in China. Alfalfa is planted and mowed for hay to feed livestock, such as cows and sheep. Alfalfa, as a legume, could fix atmospheric nitrogen, which can be utilized by another crop through a particular rotation. The practice could enhance pre-crop effects and reduce chemical fertilizer application. Previous studies have focused on alfalfa and other crop rotational systems, such as soybean, wheat, oat, and corn [1–3]. Corn is extensively used in northern China as a rotational crop that has the advantage of large biomass and easy management. The crop rotation has many advantages over the continuous cropping of a single crop species, including improving the soil nutrients, crop biomass, and economic benefits [4,5].

Fertilization is an important and common agricultural practice to enrich soil nutrients. However, excessive use of nitrogen fertilizer usually leads to soil salinization and acidification, decreased plant growth and microbial diversity, and water eutrophication [6–8]. For the alfalfa–corn system, alfalfa can fix nitrogen from the air and provide nitrogen for succeeding crops. It is not clear whether the amount of fixed nitrogen could meet the needs of corn growth. Therefore, a long-term alfalfa–corn rotational system with proper nitrogen input may be a solution that can decrease the risk of chemical fertilizer abuse [9].

The cropping system and fertilization can affect the soil properties and soil microbial structure. Soil microbes are important components of agricultural ecosystems and play a

key role in nutrient cycling and plant growth [10,11]. In addition, plant-related microbial communities are essential to plant growth, development, and productivity [12,13]. Different types of plants alter microbial community structure and function in the soil through root exudates and crop residues [14,15]. The legume and Gramineae rotational system is a two-family cropping pattern that affects soil microbes via root symbionts. The effect of a long-term alfalfa monocropping system with the insertion of corn with different nitrogen fertilization rates on the composition of the soil microorganisms remains unclear.

In this study, the soil bacterial community and its functional groups were analyzed by high-throughput sequencing when a long-term alfalfa field was rotated with corn and treated with nitrogen fertilization. The aims of this experiment were mainly to (i) evaluate the effect of corn insertion with fertilization on the plant biomass and soil nutrients; (ii) elucidate the effect of N fertilization on the bacterial functional structure; and (iii) clarify the relationship between the soil microorganism community, the functional structure, and the soil nutrients in the alfalfa–corn rotation system.

2. Materials and Methods

2.1. Study Area

The experiment was conducted from 2016 to 2021 in Dagang county, south of Tianjin city, China. This zone has a field station with a latitude of 38°72′ N and a longitude of 117°21′ E and an altitude of 1450 m. The annual mean temperature was 12 °C, and the annual mean rainfall was 580–695 mm (which is primarily accumulated during a period of 6–8 months). The major soil of this experimental field has a brownish humus-rich surface horizon, which was classified as kastanozems; the organic matter content was 1.2% by the potassium dichromate method; the soil NO_3^--N was 7.13 mg·kg^{-1}; the available P was 5.39 mg·kg^{-1}; the available K was 128.5 mg·kg^{-1}, and the pH was 8.2.

2.2. Experimental Design and Treatments

This experiment consisted of two planting patterns: an alfalfa–corn rotational system (RA) and an alfalfa monocropping system (AF). The RA included two chemical fertilizer applications: RA0 (no urea) and RA15 (recommended urea). Three treatments were set in this experiment (Table 1). Each treatment had three replications, and the total was nine plots. Each plot shared an equal size of 24 m^2 (4 × 6 m). The chemical fertilizers were a nitrogen, phosphorus, and potassium (NPK) combined application. The fertilizers were applied to the soil by a strictly controlled and calibrated application; we mixed the fertilizers, weighed the amounts per 500 m^2, and spread them on the field using a fertilizer spreader.

Table 1. Fertilizer type and amount of different treatments.

Sample	Treatment	Fertilizer Type and Amount
RA0	No N fertilizer	P_2O_5 120 kg·hm^{-2}, K_2O 75 kg·hm^{-2}
RA15	N fertilizer	N 225 kg·hm^{-2}, P_2O_5 120 kg·hm^{-2}, K_2O 75 kg·hm^{-2}
AF0	No N fertilizer	P_2O_5 120 kg·hm^{-2}, K_2O 75 kg·hm^{-2}

The site had been planted with alfalfa since 2016; half of the field (RA0 and RA15) was planted with corn in June 2020 and June 2021 (with the alfalfa removed), and another half of the field retained perennial alfalfa.

2.3. Measurements

2.3.1. Aboveground Biomass (AGB)

The aboveground plants in 3 quadrats (1 × 1 m^2) of every treatment were cut down and dried in the oven at 65 °C for 72 h.

2.3.2. Soil Sampling

The soils were sampled to a 20 cm depth in October 2020 and 2021. After removing the plant residues and gravel, all the soil samples were filtered through a 2 mm filter and blended evenly. Then, each sample was divided into two groups: one group was used for the analysis of the soil nutrients, and the other was used for high-throughput sequencing. All the samples were sealed in sterile plastic tubes on ice and sent to the laboratory for further analysis.

2.3.3. Soil Nutrients

Soil pH, EC, nitrates (NO_3^--N), available phosphorus (AP), and available potassium (AK) were measured using the Palintest soil extraction and reagent kits SKW500 (Palintest Ltd., Kingsway, Team Valley, UK). Soil pH and EC were measured in aqueous solution (soil: water, 1:2 (*w/w*)) by a pH meter and conductivity meter. NO_3^--N and AP were measured by color comparison methods, while the AK test was measured by the turbidity method. Two milliliters of soil was added to the extraction solutions, which were 50 mL of 1 M calcium chloride, 0.5 M of sodium bicarbonate, and 0.1 M of magnesium acetate for NO_3^--N, AP, and AK, respectively.

The tubes were shaken for 1 min and filtered; then, the filtrate was added to 10 mL photometer tubes. Palintest reagent tablets (for NO_3^--N, AP, AK) and deionized water were added to the tube to a volume of 10 mL and left for 10 min. Inserting a blank tube made the photometer reset to zero; then, a sample tube was inserted, and the result was recorded.

2.3.4. High-Throughput Sequencing

The methods of DNA extraction, PCR, and sequencing are described in a previously published paper [16]. The microbial genomic DNA of the soil sample was extracted using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, CA, USA). The extracted DNA was checked by electrophoresis and a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, MA, USA). The V3-V4 region of the bacterial small-subunit (16S) rRNA gene was amplified with the primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR mixtures in 20 µL volumes contained 4 µL 5 × TransStart FastPfu buffer, 0.8 µL forward and reverse primer (5 µM), 0.4 µL TransStart FastPfu DNA Polymerase, and 10 ng template DNA. The PCR conditions were as follows: initial 95 °C for 3 min, 27 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and 72 °C for 10 min. The PCR products were confirmed by 2% agarose gel electrophoresis, purified by an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, CA, USA), and quantified by a Quantus™ Fluorometer (Promega, San Luis Obispo, CA, USA). The amplicon pools were high-throughput sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw reads data were deposited in the NCBI Sequence Read Archive (SRA) database with accession number PRJNA918782.

2.3.5. Sequencing Data Analysis

The raw reads were demultiplexed and merged by FLASH [16]. After removing chimeric sequences, the sequences with a 97% similarity cutoff were assigned to the same operational taxonomic units (OTUs) using UPARSE (http://drive5.com/uparse/, accessed on 1 August 2022). Representative sequences of each OTU were blasted with the 16S rRNA database (Silva SSU128) by the RDP Classifier (http://rdp.cme.msu.edu/, accessed on 1 August 2022) at a confidence threshold of 0.7.

All of the data were processed, and the graphs were plotted by Microsoft Excel 2010 (Microsoft, Seattle, WA, USA). The significance of the differences was analyzed using one-way ANOVA at the $p < 0.05$ level by SPSS Statistics 19.0 (IBM Corporation, Armonk, NY, USA). The differences in bacterial community structure among soil samples were analyzed by principal component analysis (PCA). The potential biomarkers in the bacterial community were detected by the linear discriminant analysis (LDA) effect size (LEfSe)

method with the Kruskal–Wallis test, and the LDA evaluated the effect size at the threshold score > 4.0. The FAPROTAX program was used to analyze the bacterial function according to the OTU abundance information. Redundancy analysis (RDA) was used to assess the relationship between the soil bacterial community structure and the environmental factors at the 95% confidence level. All of the above analyses were performed on the I-sanger platform (Majorbio Co., Ltd., Shanghai, China).

3. Results

3.1. Aboveground Biomass

The biomass of the RA0 treatments without N fertilization significantly decreased from 17.07 t·hm^{-2} in 2020 to 11.25 t·hm^{-2} in 2021, but the biomass of RA15 with N fertilization remained unchanged in the two years. The alfalfa yields (mowed four times per year) were consistent over the two years. In 2020, the biomasses of the three systems were not different, but in 2021 the AGB of RA15 was significantly larger than that of the others (Table 2).

Table 2. AGB in different systems.

Treatments	2020 (t·hm^{-2})	2021 (t·hm^{-2})
RA0	17.07 ± 1.96 Aa	11.25 ± 0.57 Bb
RA15	16.79 ± 1.18 Aa	16.44 ± 0.46 Aa
AF0	13.81 ± 0.86 Aa	12.97 ± 1.49 Ab

Note: Different capital letters within the same row indicate significant differences at the 0.05 level, and different lowercase letters within the same column indicate significant differences at the 0.05 level.

3.2. Soil Chemical Properties

Soil samples from the three treatments were collected to analyze the soil properties. The values of the soil properties did not differ in 2020 and 2021; the results in 2020 are presented (Table 3). The pH values and AP contents were not different in the three treatments, but the nitrate nitrogen (NO_3^--N) contents had significantly different values ($p < 0.05$). RA0 had the lowest value of 9.73 mg·kg^{-1}, and AF0 had the value of 22.05 mg·kg^{-1}, but RA15, with the N fertilizer addition, had the highest value of 49.03 mg·kg^{-1}. The AK content of AF0 had a lower value of 145.91 mg·kg^{-1} compared to the two corn insertion treatments (Table 3).

Table 3. Soil properties in different treatments.

Treatments	pH	NO_3^--N (mg·kg^{-1})	AP (mg·kg^{-1})	AK (mg·kg^{-1})
RA0	8.03 ± 0.05 a	9.73 ± 0.11 c	20.51 ± 5.13 a	220.49 ± 45.40 a
RA15	7.98 ± 0.15 a	49.03 ± 3.50 a	22.05 ± 3.83 a	197.80 ± 28.82 a
AF0	7.81 ± 0.05 a	22.05 ± 4.51 b	13.33 ± 1.68 b	145.91 ± 16.85 b

Note: Different lowercase letters within the same column indicate differences at the 0.05 level.

3.3. Soil Bacterial Diversity

The soil samples were analyzed for bacterial diversity based on high-throughput sequencing. The corn insertion significantly affected the Sobs index (Figure 1). RA15_20 and RA0_20, and RA15_21 and RA0_21 showed no significant change in bacterial diversity, which revealed that nitrogen fertilizer in the alfalfa–corn system had nearly no effect on the bacterial α-diversity. However, in 2020, the Sobs index of RA0 and RA15 with the corn insertion was significantly higher than that of AF0. For RA0 and RA15, the bacterial diversity significantly decreased in 2021 compared to 2020 ($p < 0.05$).

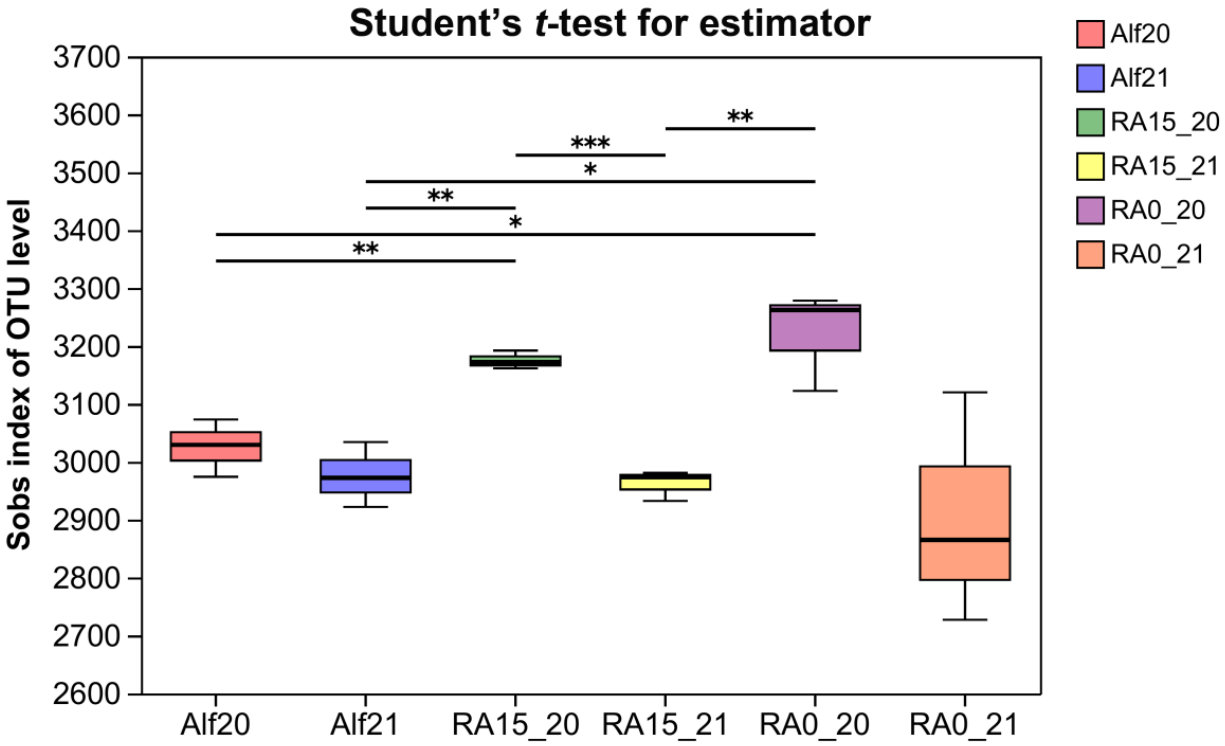

Figure 1. Soil bacterial α-diversity (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

3.4. Soil Bacterial Community Composition and Structure

The total OTUs of the soil samples were blasted with the 16S rRNA database, and most of them were Actinobacterota (27.0–36.4%), Proteobacteria (20.9–26.0%), Acidobacteriota (9.9–17.0%), and Chloroflexi (9.7–14.3%) at the phylum level (Figure 2A). The nitrogen fertilizer had a lower relative abundance of Acidobacteriota, but a higher relative abundance of Chloroflexi than no fertilizer in the RA system. Interestingly, the RA system had a lower relative abundance of Actinobacterota than the AF system, but in the second year, the relative abundance of Actinobacterota in the RA system significantly increased and was higher than that in the AF system.

The first two components explained 24.44% and 14.13% of the total variability in the principal component analysis (PCA) (Figure 2B). Three groups were well clustered (AF0_20 and AF0_21, RA0_20 and RA15_20, and RA0_21 and RA15_21), and the AF treatments were greatly separated from the RA rotation treatments regardless of the fertilization. Nitrogen fertilization had no effect on the components in the RA system in the same year; however, the bacterial community structure of the RA system did differ significantly between the two years.

To determine the classified bacterial taxa with significant abundance differences among the six treatments, LEfSe analysis was performed at LDA scores > 4. The results revealed that Thermoleophilia (class) and Galellales (order) had higher relative abundance in AF0_20; Tistrellales (order), Geminicoccaceae (family), Frankiales (order), and Geodermatophilaceae (family) were enriched in AF0_21; Nocardioidaceae (family), Propionibacteriales (order), and Nocardioides (genus) were enriched in RA0_20; Actinobacterota (phylum) and Actinobacteria (class) had higher relative abundance in RA0_21; and Chloroflexi (phylum), Anaerolineae (class), and SBR1031 (order) had higher relative abundance in RA15_20 (Figure 3A,B).

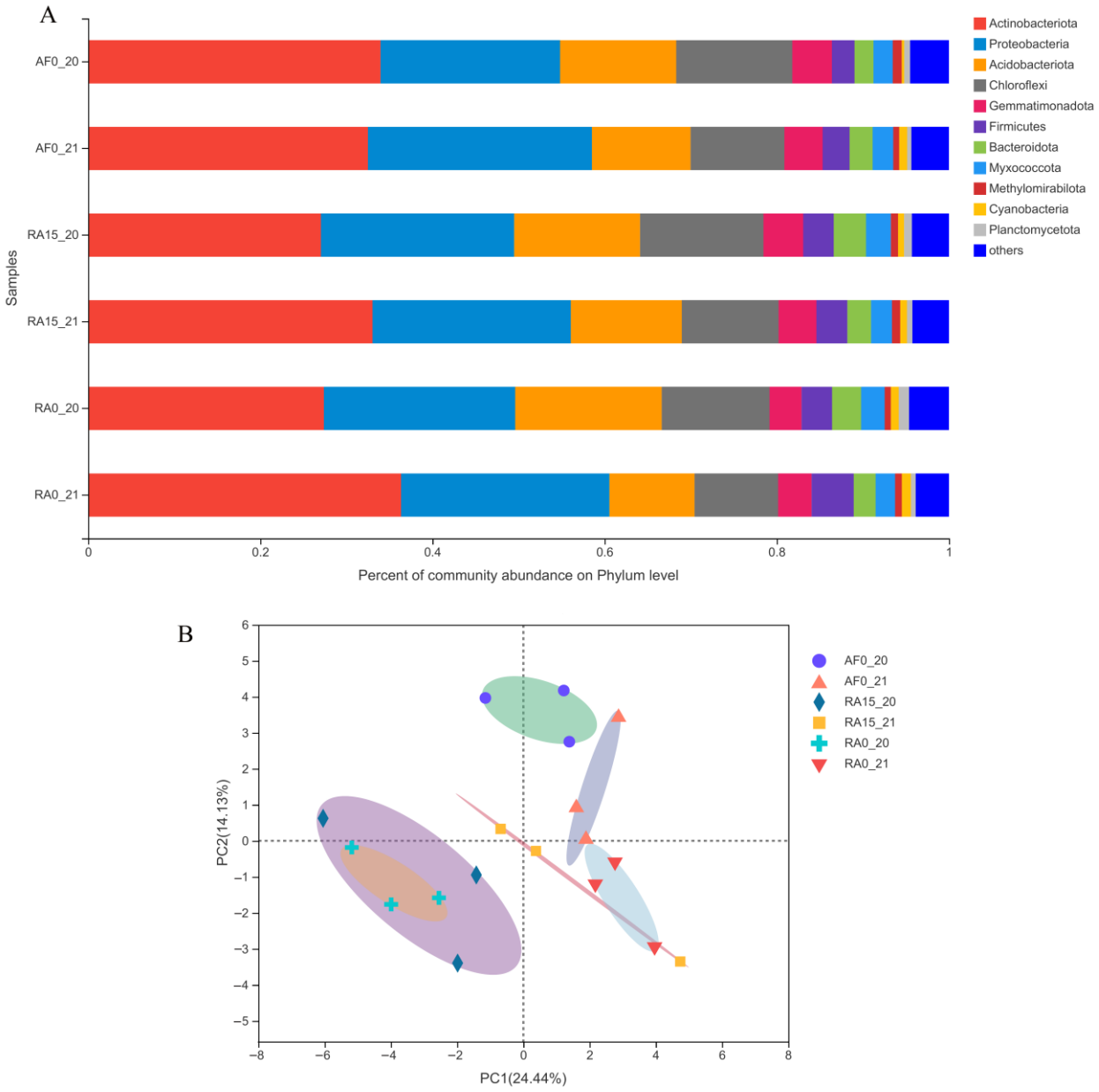

Figure 2. Dominant bacterial phyla (**A**) and principal component analysis (PCA) of the bacterial community (**B**).

3.5. Soil Bacterial Functional Prediction

The functions of the soil bacterial community were analyzed by the FAPROTAX program. A total of 55 functional categories related to the bacterial community were obtained. Most of the functional groups were assigned to chemoheterotrophy (35.05%), aerobic chemoheterotrophy (33.22%), nitrate reduction (4.25%), and aromatic compound degradation (3.52%) (Figure S1). The Kruskal–Wallis test was adopted among the treatments (Figure 4). The results revealed that the "aromatic compound degradation" group in the RA15 treatment was significantly higher than that in the AF0 and RA0 treatments ($p < 0.05$), and the "fermentation" groups in the RA treatments were significantly lower than those in the RA treatment ($p < 0.05$).

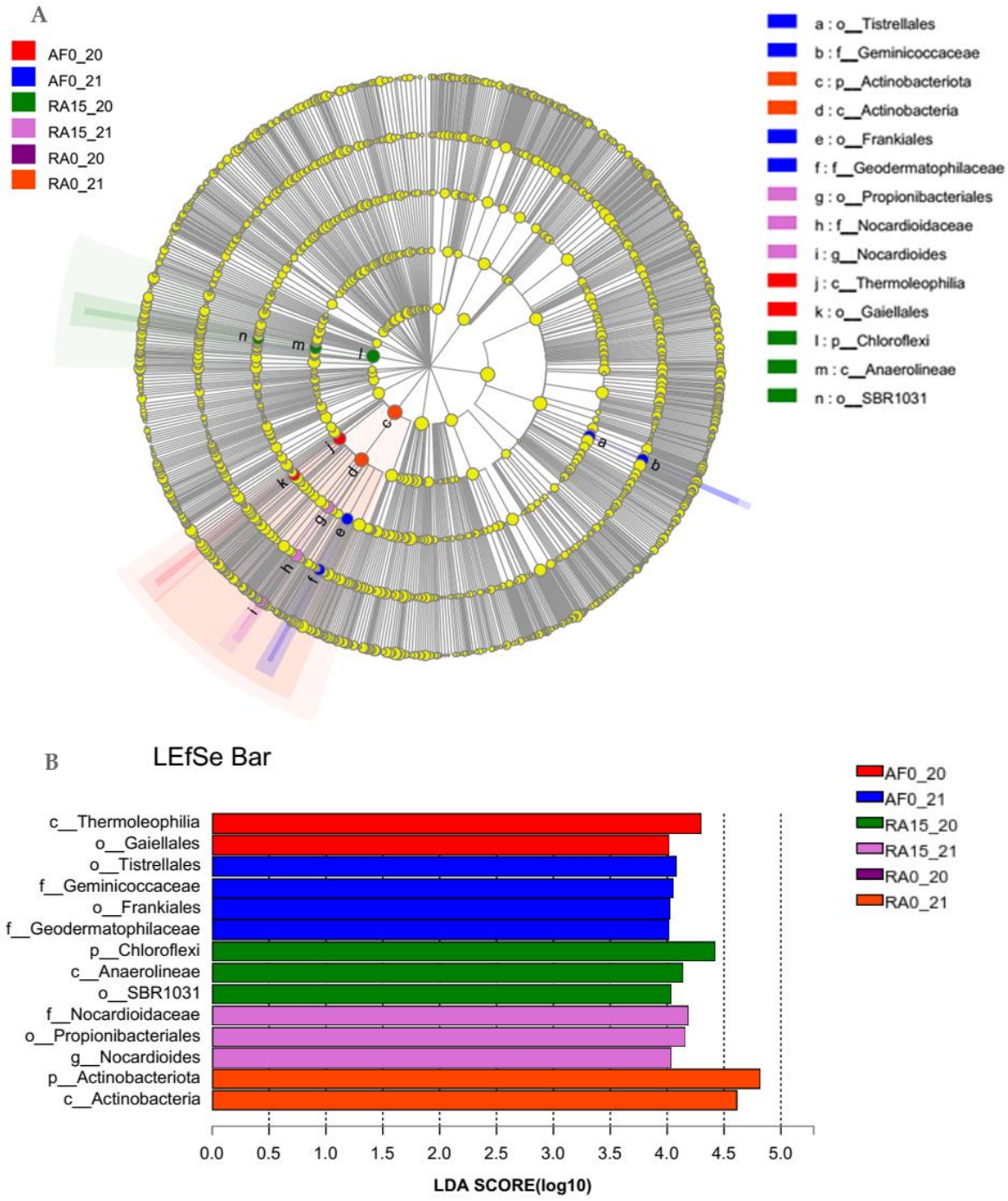

Figure 3. LEfSe cladogram (**A**) and bar (**B**) of different treatments.

3.6. Correlation between Bacterial Community and Soil Properties

RDA analysis was used to evaluate the correlation of the soil properties and the bacterial community structure. The first two axes (representing the bacterial community compositions) together accounted for 14.04% of the total community variation. NO_3^--N was positively correlated with the bacterial community in RA0 in 2020, and the AP was more correlated with the RA0 and AF0 treatments without N fertilization (Figure S2).

Spearman's test was performed to study the correlation of the environmental factors and the bacterial communities. The results showed that the AP was significantly positively correlated with Myxococcota, Chloroflexi, and Proteobacteria, and the AK was significantly positively correlated with Firmicutes, Myxococcota, and Proteobacteria (Figure 5).

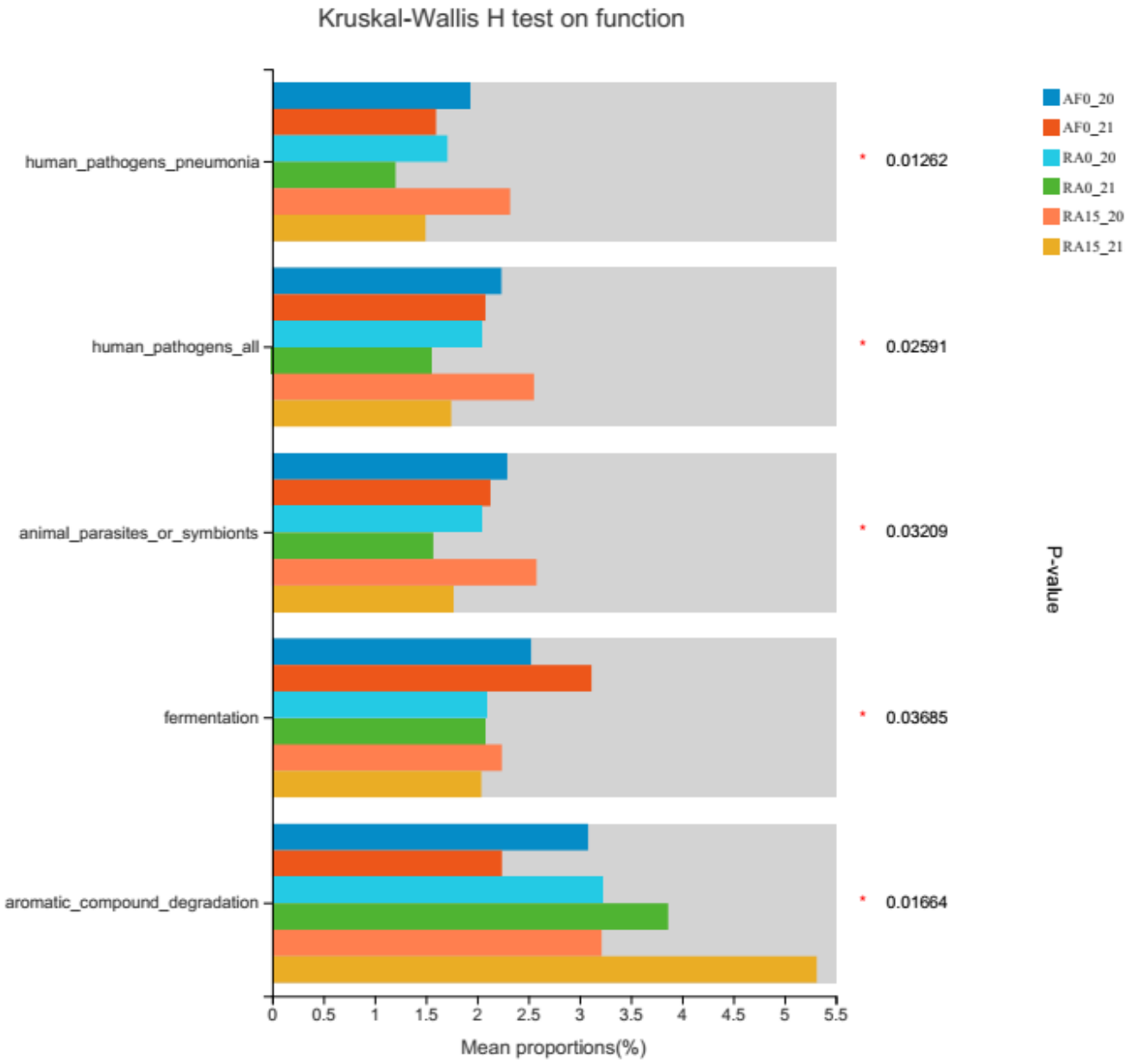

Figure 4. The Kruskal–Wallis test of the bacterial community.

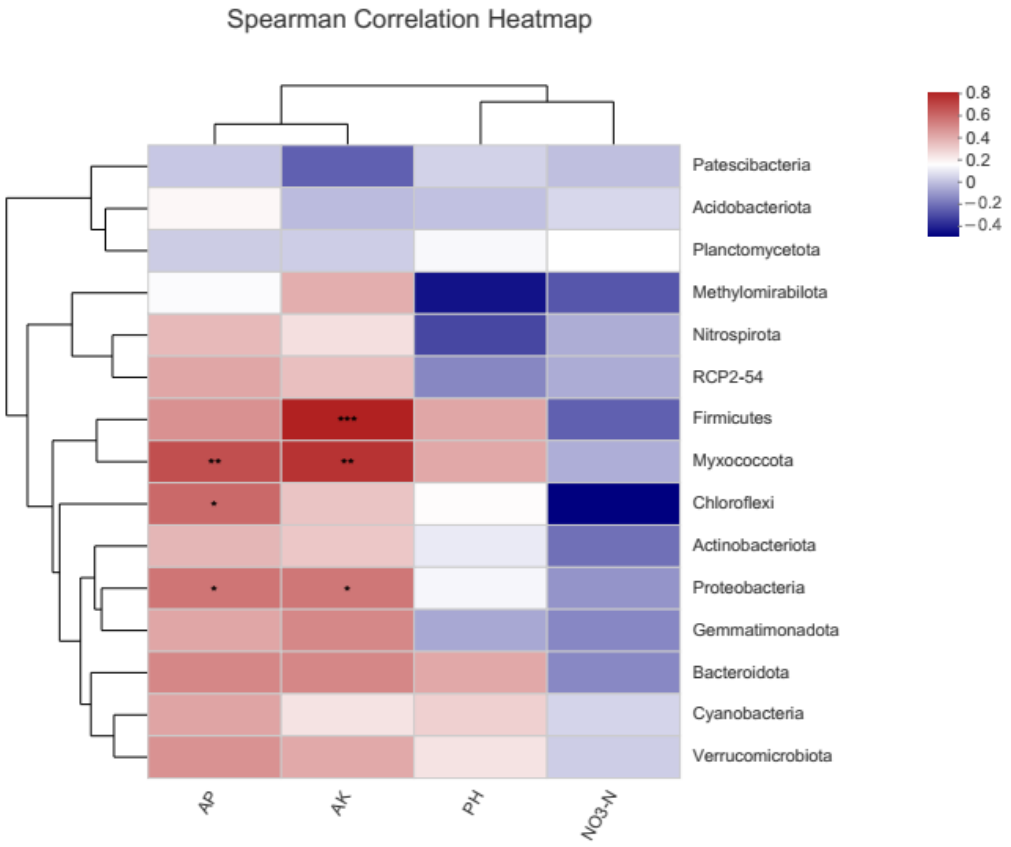

Figure 5. RDA of soil bacterial community structure associated with soil properties (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

4. Discussion

4.1. Effects of Corn Insertion and Fertilization on AGB and Soil Properties

In this study, the AGB of the three systems was not significantly different in 2020. In previous studies, the fields of alfalfa–corn systems generally increased compared with the monocropped alfalfa [17–19]. These studies collected the first-cut alfalfa and corn fields in a rotational system. However, in this research, only the corn biomass in the RA treatments and the biomass in the alfalfa cut four times in the AF treatment were collected; so the AGBs of RA15 with nitrogen fertilization and AF0 without fertilization were consistent over the two years. In 2020, the AGBs of RA0, RA15, and AF0 did not differ; however, in 2021, the AGB of RA0 significantly declined compared to those of RA15 and AF0. This result can be explained by the fact that alfalfa utilized nitrogen from the air by root azotification, offered nitrogen to the succeeding corn, and retained biomass in 2020, but in 2021, the fixed nitrogen by the alfalfa was largely used, and the corn needed the input of exogenous nitrogen sources to maintain the fields.

Corn insertion significantly decreased the NO_3^-−N content of the soil in RA0 compared to AF0 ($p < 0.05$), as corn has no root azotification and only absorbed nitrogen from the soil that was intrinsic or fixed by the alfalfa. When nitrogen fertilizer was applied in RA15, the nitrogen supplies significantly exceeded the demand for corn; so, the NO_3^--N content of the soil in RA15 significantly increased compared with that in RA0 and AF0. However, in 2020 the NO_3^--N content of RA0 was much less than that of RA15, and the AGB of the two treatments was not different. A possible reason is that we sampled the 0–20 cm soil layer to determine the soil properties. The nitrogen fixed by the alfalfa roots was in the deeper soil layer, or the corn, as Gramineae, absorbed most of the nitrogen and less nitrogen remained in the soil. In addition, RA15 significantly increased the AP and AK

contents in the soil (Table 3) because we applied P and K fertilizers in RA0 and RA15, and the supplied amounts exceeded the demand for corn growth.

4.2. Effects of Rotation and Fertilization on Bacterial Communities

In the three systems, fertilization in the alfalfa–corn system had no significant effect on bacterial diversity, but corn insertion increased the α-diversity, and the diversity of the alfalfa–corn system decreased gradually over time. In previous studies, the fertilization regime had a greater impact on soil microbe properties than crop rotation or the growth stage in the wheat–soybean rotational system [10], and NPK fertilization generally decreased the soil bacterial diversity [7]. However, NPK fertilization cannot significantly affect soil bacterial diversity in the soybean–corn system [20]. The results confirmed this conclusion, possibly because the promotional effect of the rotational system counteracted the reduction effect of the fertilization in a complex microbial network.

The PCA and LEfSe analysis revealed that corn insertion and nitrogen fertilization affected the bacterial community structure (Figures 2 and 3). Similarly to the published results, Actinobacterota and Proteobacteria usually accounted for most of the phyla of the bacterial community [21]. Nitrogen fertilization could increase the relative abundance of Proteobacteria and Actinobacteria but reduced the abundance of Acidobacteria [7,22]. Our results showed that nitrogen fertilization had no significant effect on the abundance of Proteobacteria and Actinobacteria, perhaps because of the corn insertion. The Actinobacteria abundance in the RA0 treatments in the second year significantly increased compared with that in the first year (Figure 3B). A possible reason is that the phylum Actinobacteria could decompose crop straw and complex polymers [23] when corn was harvested and straw was pulverized in the field as a metabolic substrate of microorganisms. Our results also confirmed the reduction effect of nitrogen fertilization on the abundance of Acidobacteria. However, the abundance of Chloroflexi increased with the N input in the RA15 system in our study (Figure 3B), possibly because Chloroflexi is a heterotrophic bacterium and prefers the organic matter released from the dead biomass of alfalfa residues and corn straw in rotational systems [24].

FAPROTAX analysis could predict and explain the function of different bacterial communities. The "aromatic compound degradation" group was significantly increased in the RA treatments compared with the AF treatment ($p < 0.05$) (Figure 4), and the main predictor was followed by the groups "chemoheterotrophy" and "aerobic chemoheterotrophy", which were the predominant groups in the bacterial communities (Figure S1). According to previous studies, these groups are involved in the soil carbon cycle process [25]. The rotational system offered more aboveground plants and residues that were returned to the soil [20], and this organic matter enriched the "aromatic compound degradation" group. It was also found that the "fermentation" group of AF0 was significantly higher than those of RA0 and RA15 (Figure 4), perhaps because the alfalfa was mowed four times per year, with more input of residues and organic matter into the soil, which improved the soil bacterial fermentation process.

RDA can be used to analyze the correlation of the environmental factors and the bacterial communities. Our results revealed that corn insertion and fertilization might alter the effects of the environmental factors on the bacterial community structure [10,26]. The NO_3^--N value positively correlated with the bacterial community in RA0 in 2020. The rotation treatments decreased the NO_3^--N content and further confirmed this conclusion. The AP was more correlated with the RA0 and AF0 treatments, perhaps because nitrogen fertilization enhanced the corn absorption of the AP from the soil. The phylum Proteobacteria is a copiotrophic bacteria which grows faster under nutrient-rich conditions [27], and our results confirmed that the Proteobacteria were significantly positively correlated with the AK and AP.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13010253/s1, Figure S1: the functional heatmap of bacterial community, Figure S2: RDA of soil bacterial community structure associated with soil properties.

Author Contributions: Conceptualization, X.L. and F.H.; methodology, F.H.; validation, X.L.; formal analysis, Y.W.; investigation, Z.T.; data curation, L.W.; writing—original draft preparation, Z.T.; writing—review and editing, F.H.; visualization, Z.T.; supervision, Y.W.; project administration, L.W.; funding acquisition, X.L. and F.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Inner Mongolia Science and Technology Project (2021GG0220 and 2021GG0225), China Agriculture Research System of MOF and MARA (CARS-34), and the Agricultural Science and Technology Innovation Program (ASTIP-IAS14).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting the findings of this study are included in the article.

Acknowledgments: We thank Zhao Ma, Hongyu Xu, and Bao Wei for their assistance with field data collection and Lihong Miao for materials and accounting.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Singer, J.W.; Moore, K.J. Living Mulch Nutritive Value in a Corn-Soybean-Forage Rotation. *Agron. J.* **2010**, *102*, 282–288. [CrossRef]
2. Jarecki, M.; Grant, B.; Smith, W.; Deen, B.; Drury, C.; VanderZaag, A.; Qian, B.D.; Yang, J.Y.; Wagner-Riddle, C. Long-Term Trends in Corn Yields and Soil Carbon under Diversified Crop Rotations. *J. Environ. Qual.* **2018**, *47*, 635–643. [CrossRef] [PubMed]
3. Coulter, J.; Delbridge, T.; King, R.; Allan, D.; Sheaffer, C. Productivity, economics, and soil quality in the Minnesota variable-input cropping systems trial. *Crop Manag.* **2013**, *12*, 1–11. [CrossRef]
4. Karpinski, I.; Ridder, R.; Rajmis, S.; Schwarz, J.; Klocke, B.; Kehlenbeck, H. Crop rotation versus monoculture: Economic analysis of long-term (18 years) field trials in rye Fruchtfolge versus monokultur: Betriebswirtschaftliche betrachtung eines dauerfeldversuches im roggenanbau uber 18 jahre. *J. Fur Kult.* **2020**, *72*, 298–310. [CrossRef]
5. Gollner, G.; Starz, W.; Friedel, J.K. Crop performance, biological N fixation and pre-crop effect of pea ideotypes in an organic farming system. *Nutr. Cycl. Agroecosystems* **2019**, *115*, 391–405. [CrossRef]
6. Savci, S. Investigation of Effect of Chemical Fertilizers on Environment. In Proceedings of the 3rd International Conference on Environmental Science and Development (ICESD), Hong Kong, China, 5–7 January 2012; pp. 287–292.
7. Dai, Z.M.; Su, W.Q.; Chen, H.H.; Barberan, A.; Zhao, H.C.; Yu, M.J.; Yu, L.; Brookes, P.C.; Schadt, C.W.; Chang, S.X.; et al. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. *Glob. Chang. Biol.* **2018**, *24*, 3452–3461. [CrossRef] [PubMed]
8. Liu, J.S.; Cui, Y.; Li, X.F.; Wilsey, B.J.; Isbell, F.; Wan, S.Q.; Wang, L.; Wang, D.L. Reversal of nitrogen-induced species diversity declines mediated by change in dominant grass and litter. *Oecologia* **2018**, *188*, 921–929. [CrossRef] [PubMed]
9. Macholdt, J.; Piepho, H.P.; Honermeier, B. Does fertilization impact production risk and yield stability across an entire crop rotation? Insights from a long-term experiment. *Field Crops Res.* **2019**, *238*, 82–92. [CrossRef]
10. Guo, Z.B.; Wan, S.X.; Hua, K.K.; Yin, Y.; Chu, H.Y.; Wang, D.Z.; Guo, X.S. Fertilization regime has a greater effect on soil microbial community structure than crop rotation and growth stage in an agroecosystem. *Appl. Soil Ecol.* **2020**, *149*, 103510. [CrossRef]
11. Naylor, D.; Sadler, N.; Bhattacharjee, A.; Graham, E.B.; Anderton, C.R.; McClure, R.; Lipton, M.; Hofmockel, K.S.; Jansson, J.K. Soil Microbiomes under Climate Change and Implications for Carbon Cycling. In *Annual Review of Environment and Resources*; Gadgil, A., Tomich, T.P., Eds.; Annual Reviews: San Mateo, CA, USA, 2020; Volume 45, pp. 29–59.
12. Wang, Y.; Liu, H.J.; Shen, Z.Z.; Miao, Y.; Wang, J.; Jiang, X.T.; Shen, Q.R.; Li, R. Richness and antagonistic effects co-affect plant growth promotion by synthetic microbial consortia. *Appl. Soil Ecol.* **2022**, *170*, 104300. [CrossRef]
13. Wu, Z.H.; Liu, Q.S.; Li, Z.Y.; Cheng, W.; Sun, J.M.; Guo, Z.H.; Li, Y.M.; Zhou, J.Q.; Meng, D.L.; Li, H.B.; et al. Environmental factors shaping the diversity of bacterial communities that promote rice production. *BMC Microbiol.* **2018**, *18*, 51. [CrossRef] [PubMed]
14. Kuzyakov, Y.; Hill, P.W.; Jones, D.L. Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature. *Plant Soil* **2007**, *290*, 293–305. [CrossRef]
15. Zheng, S.Q.; Zhang, J.M.; Chi, F.Q.; Zhou, B.K.; Wei, D.; Kuang, E.J.; Jiang, Y.; Mi, G.; Chen, Y.P. Response of the chemical structure of soil organic carbon to modes of maize straw return. *Sci. Rep.* **2021**, *11*, 6574. [CrossRef] [PubMed]

16. Shang, L.; Wan, L.; Zhou, X.; Li, S.; Li, X. Effects of organic fertilizer on soil nutrient status, enzyme activity, and bacterial community diversity in Leymus chinensis steppe in Inner Mongolia, China. *PLoS ONE* **2020**, *15*, e0240559. [CrossRef] [PubMed]
17. Yu, C.; Wang, Y.; Ma, L.; Su, L.; Gao, L.; Zhou, P.; An, Y. An annual rotation model of alfalfa and corn in Yangtze River area. *Pratacultural Sci.* **2022**, *39*, 996–1005.
18. Chen, J.S.; Zhu, R.F.; Zhang, Q.; Kong, X.L.; Sun, D.Q. Reduced-tillage management enhances soil properties and crop yields in a alfalfa-corn rotation: Case study of the Songnen Plain, China. *Sci. Rep.* **2019**, *9*. [CrossRef] [PubMed]
19. Chahal, I.; Hooker, D.C.; Deen, B.; Janovicek, K.; Van Eerd, L.L. Long-term effects of crop rotation, tillage, and fertilizer nitrogen on soil health indicators and crop productivity in a temperate climate. *Soil Tillage Res.* **2021**, *213*, 105121. [CrossRef]
20. Rao, D.; Meng, F.; Yan, X.; Zhang, M.; Yao, X.; Kim, K.S.; Zhao, J.; Qiu, Q.; Xie, F.; Zhang, W. Changes in Soil Microbial Activity, Bacterial Community Composition and Function in a Long-Term Continuous Soybean Cropping System after Corn Insertion and Fertilization. *Front. Microbiol.* **2021**, *12*, 638326. [CrossRef]
21. Fierer, N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol.* **2017**, *15*, 579–590. [CrossRef]
22. Ramirez, K.S.; Lauber, C.L.; Knight, R.; Bradford, M.A.; Fierer, N. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* **2010**, *91*, 3463–3470; discussion 3503–3414. [CrossRef]
23. Bhatti, A.A.; Haq, S.; Bhat, R.A. Actinomycetes benefaction role in soil and plant health. *Microb. Pathog.* **2017**, *111*, 458–467. [CrossRef]
24. Chen, R.; Yao, J.; Ailijiang, N.; Liu, R.; Fang, L.; Chen, Y. Abundance and diversity of nitrogen-removing microorganisms in the UASB-anammox reactor. *PLoS ONE* **2019**, *14*, e0215615. [CrossRef]
25. Liang, S.; Deng, J.; Jiang, Y.; Wu, S.; Zhou, Y.; Zhu, W. Functional Distribution of Bacterial Community under Different Land Use Patterns Based on FaProTax Function Prediction. *Pol. J. Environ. Stud.* **2020**, *29*, 1245–1261. [CrossRef] [PubMed]
26. Wu, B.; Luo, H.; Wang, X.; Liu, H.; Peng, H.; Sheng, M.; Xu, F.; Xu, H. Effects of environmental factors on soil bacterial community structure and diversity in different contaminated districts of Southwest China mine tailings. *Sci. Total Environ.* **2022**, *802*, 149899. [CrossRef] [PubMed]
27. Bastian, F.; Bouziri, L.; Nicolardot, B.; Ranjard, L. Impact of wheat straw decomposition on successional patterns of soil microbial community structure. *Soil Biol. Biochem.* **2009**, *41*, 262–275. [CrossRef]