agronomy

MDPI

Article

Effects of Continuous Return of Bt Corn Straw on Soil Nutrients, Enzyme Activities, and Microbial Communities

Chenning Zhang [1,2,3,†], Xiao Lv [1,2,3,†], Xiaomin Liang [1,2,3], Peng Peng [1,2,3] and Yuanjiao Feng [1,2,3,*]

[1] Key Laboratory of Agro-Environment in the Tropics, Ministry of Agriculture and Rural Affairs, South China Agricultural University, Guangzhou 510642, China; zhangcn@stu.scau.edu.cn (C.Z.); lvxiao678678@sina.com (X.L.); xmliang@stu.scau.edu.cn (X.L.); peng102910@sina.com (P.P.)
[2] Guangdong Engineering Research Center for Modern Eco-Agriculture and Circular Agriculture, Guangzhou 510642, China
[3] Department of Ecology, College of Natural Resources and Environment, South China Agricultural University, Guangzhou 510642, China
* Correspondence: yjfeng@scau.edu.cn
† These authors contributed equally to this work.

Abstract: The impact of *Bacillus thuringiensis* (Bt) corn straw returning on the soil ecosystem has attracted significant attention. In this study, taking the homologous conventional corn 5422 as a control, we explored the effects of Bt corn (5422Bt1 and 5422CBCL) straw return after five consecutive cycles on soil nutrients, enzyme activities, and microbial communities. The results showed that in the 5422Bt1 treatment, the levels of available phosphorus (AP), total nitrogen (TN), and sucrose enzyme (SUC) activities significantly increased. In the 5422CBCL treatment, organic matter (OM), alkaline nitrogen (AN), and AP contents, as well as SUC and acid phosphatase (ACP) activities, significantly decreased, while available potassium (AK) and TN contents significantly increased. Through Illumina high-throughput sequencing, it was found that the OTU abundance of soil fungi and bacteria changed after straw returning, and there were no significant differences in alpha diversity (α-diversity) among the three treatments. Redundancy analysis (RDA) indicated that soil nutrients and enzyme activities also affect the soil microbial communities. In summary, Bt corn straw returning affects soil nutrients, enzyme activities, and the structure of microbial communities. Overall, this study revealed the impact of continuous Bt corn straw returning on the soil ecosystem, providing a theoretical basis for subsequent studies.

Keywords: straw return; soil features; soil enzyme; soil microbial community

Citation: Zhang, C.; Lv, X.; Liang, X.; Peng, P.; Feng, Y. Effects of Continuous Return of Bt Corn Straw on Soil Nutrients, Enzyme Activities, and Microbial Communities. *Agronomy* **2024**, *14*, 2737. https://doi.org/10.3390/agronomy14112737

Academic Editor: Witold Grzebisz

Received: 29 October 2024
Revised: 17 November 2024
Accepted: 18 November 2024
Published: 20 November 2024

1. Introduction

The year 2023 marks the 28th anniversary of the successful global commercialization of genetically modified (GM) crops. As of 2023, the global planting area of GM crops has reached 206.3 million hectares, 121 times that of 1996, accounting for about 13% of the world's 1.5 billion hectares of arable land. From 1996 to 2023, the cumulative global planting area of GM crops exceeded 40 billion hectares [1]. GM crops, with traits such as insect resistance, cold tolerance, and virus resistance, bring significant benefits to agriculture, the environment, and consumers [2,3]. So far, 32 different non-food and food-transgenic crops have been developed worldwide, including soybeans, corn, cotton, and canola [4]. The insect resistance trait in GM crops originates from the expression of insecticidal crystal proteins produced by a class of Gram-positive bacteria called Bacillus thuringiensis (Bt) [5]. Bt crops can accumulate Bt proteins in various tissues and organs, such as leaves, stems, roots, and seeds. Studies have shown that Bt proteins released by Bt crops can enter the soil ecosystem through root exudates and straw return, directly or indirectly causing changes in the physical and chemical properties of soil and impacting soil nutrients, enzyme activities, and soil microbial communities [6,7].

Currently, numerous studies are examining the soil nutrients, enzyme activity, and microbial communities of Bt crops throughout the planting and decomposition processes [8–10]. Returning GM corn straw to the soil is a primary pathway for Bt proteins to enter the soil, altering soil physicochemical properties (SPP) and microbial communities. Zeng et al. [11] showed that the Bt protein from Bt corn straw, after being returned to the soil, does not accumulate in the soil and has no significant impact on soil nutrient contents. Wang et al. [12] showed that returning Bt corn straw to the soil significantly reduced the soil AP and AK contents. Yan et al. [13] showed that returning the Bt corn variety DK647BTY straw to the soil significantly increased the soil OM content. Soil enzymes are involved in a wide range of essential metabolic activities in the soil, reflecting the intensity and dynamics of various biochemical processes. They are the key factors in determining nutrient cycling and organic matter decomposition in the soil and play an important role in assessing microbial functions in soil ecosystems [14,15]. Some studies have shown that the decomposition of Bt corn straw has no significant effect on the activities of soil phosphatase, dehydrogenase, protease, and aromatic sulfate esterase [16]. However, other research has found that the activity of certain soil enzymes can change during the decomposition process of Bt corn straw. During the decomposition of Bt corn 34B24 straw, soil protease and acid phosphatase activities showed no significant difference compared to those occurring during the decomposition of the conventional corn variety 34B23 straw. However, at certain sampling times, the activities of soil dehydrogenase and sucrase were significantly higher than those occurring during the decomposition of 34B23 straw. In contrast, the activity of soil URE varied, being either higher or lower than that of the 34B23 straw treatment [12]. Under field conditions, the return of Bt corn straw increased the soil SUC, URE, and protease activities [13]. Shu et al. [17] found that Bt corn straw significantly improved soil nutrient and AN content. Wang et al. [18] found that the addition of Bt rice straw significantly increased the number of fungi, while the quantities of bacteria, actinomycetes, and denitrifying bacteria significantly decreased. Mulder et al. [19] discovered that the Bt protein released from Bt corn straw added to the soil temporarily promoted soil microbial populations in the short term. Research findings indicate that the effects of returning Bt crops to the soil on soil nutrients, enzyme activities, and microbial communities vary. This variation arises from differences in Bt crop varieties, methods and timing of straw return, experimental environments and conditions, as well as inherent soil characteristics. Therefore, it is essential to analyze the results in the context of specific conditions during experiments.

Soil microorganisms play an important role in the biological activity of soil. These microorganisms participate in nutrient cycling and organic matter transformation and alter the soil environment through various biochemical and physiological mechanisms, making them indispensable members of the soil ecosystem [20]. The major studies are more focused on the effects of the short-term straw return of Bt crops on soil ecosystems. However, there are few reports on the impact of the continuous straw return of Bt crops on soil microbial communities. In this study, we hypothesized that the straw returning of the two Bt corn varieties 5422Bt1 and 5422CBCL would have unique impacts on the soil ecosystem. Therefore, this study uses the straw from conventional corn 5422, Bt corn 5422Bt1, and Bt corn 5422CBCL as materials, employing high-throughput sequencing technology to conduct an in-depth investigation into the diversity, structure, and composition of soil fungal and bacterial communities after five consecutive returns of Bt corn straw to the soil. Additionally, this study will examine the interplay between microbial communities, soil nutrients, and enzyme activities, aiming to provide a theoretical framework for understanding the ecological impacts of continuous Bt corn straw return.

2. Materials and Methods

2.1. Overview of Experimental Site and Corn Varieties

The soil used in this experiment was collected from the surface layer (0–20 cm) of a conventional corn plot of the South China Agricultural University teaching experimental farm. The soil is red clay, which was air-dried, passed through a 1 mm sieve, and

thoroughly mixed to serve as the experimental soil for this study. Before the experiment, the basic SPP were as follows: pH 6.0, OM 20.89 ± 0.49 g/kg, TN 1.01 ± 0.03 g/kg, TP 0.80 ± 0.06 g/kg, TK 20.71 ± 0.95 g/kg, AN 137.20 ± 10.35 mg/kg, AP 95.35 ± 2.91 mg/kg, and AK 183.81 ± 7.48 mg/kg. The tested varieties were Bt corn 5422Bt1 (Bt11) and Bt corn 5422CBCL (Mon810), from Beck's Hybrids, USA. This study used their common homologous conventional corn variety 5422 as a control.

2.2. Experimental Design and Sampling

The two Bt corn varieties (5422Bt1 and 5422CBCL) and their homologous conventional corn variety 5422 were planted in a greenhouse. At maturity, the straw was collected (divided into leaves and stems), freeze-dried, crushed, and screened. The leaves and stems were then thoroughly mixed in a 1:1 ratio and stored in a −40 °C freezer before returning the straw to the field. The equipment was cleaned and disinfected before the straw was freeze-dried, crushed, and sieved. A total of 6.0 g of each type of corn straw was added to a plastic cup containing 100 g of soil (height 10 cm, diameter 8 cm). After mixing the straw with the soil, 53 mL of distilled water was added to maintain a moisture content of 50%. Each sample was portioned into 36 plastic cups, which are all kept in the laboratory corridor, without temperature control, light protection, or rain exposure. The samples were placed in positions with similar light exposure. The temperature of the area was recorded. The positions of the plastic cups were exchanged at regular intervals to avoid differences caused by light exposure time and intensity. Water was replenished every three days by the weighing method to maintain a moisture content of 50%. After the first straw return, 6.0 g of the corresponding variety's straw was re-added every 120 days, continuing for a total of five returns.

After adding the straw, samples were taken at 30, 60, and 120 days, and for each sampling, four plastic cups were selected as four replicates for each treatment. The mixtures in the plastic cups were thoroughly mixed on a sterile workbench and then divided into portions, which were then stored in a −4 °C freezer, to determine soil enzyme activity and nutrient content. The soil enzyme activities (URE, SUC, and ACP) and soil available nutrients (OM, AN, AP, and AK) were measured in samples taken at 30, 60, and 120 days after each straw addition, while total soil nutrients (TN, TP, and TK) were measured in samples taken 120 days after each straw addition. After five consecutive straw additions, samples were collected and placed into sterilized cryotubes and subsequently stored in a −80 °C freezer to determine soil microorganisms.

2.3. Soil Nutrient and Enzyme Activity Analysis

Soil OM was determined using the potassium dichromate volumetric method; soil AN, AP, and AK were determined by alkali hydrolysis diffusion, sodium bicarbonate, and ammonium acetate-flame photometry, respectively. Soil TN, TP, and TK were determined using the potassium dichromate–sulfuric acid digestion method, the sulfuric acid–perchloric acid digestion method, and the NaOH fusion–flame photometry method, respectively [21]. Soil enzyme (URE, SUC, and ACP) activities were determined using the methods of Guan Songyin [22] and Zhou Likai [23].

2.4. Illumina HiSeq Sequencing

The DNA of all soil samples was extracted using the Fast DNA Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA), and DNA quality was checked using 1% agarose gel electrophoresis. The resulting DNA samples were stored at −80 °C prior to PCR amplification. PCR amplification of the bacterial V3–V4 variable region was performed using the specific primers (338F 5′-ACTCCTACGGGAGCAG-3′) and (806R 5′-GGACTACHVGGGTWTCTAAT-3′), as well as the fungal ITS primers F primer ITS-1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and R primer ITS-2 (5′-GCTGCGTTCTTCATCGATGC-3′). Each sample underwent three replicates. The PCR products of the same sample were mixed and subjected to electrophoresis using 2% agarose gel for detection. The PCR products were recovered from the gel using the AxyPrep DNA Gel Recovery Kit (Axygen, Union City, CA, USA) and eluted with

Tris-HCl. After mixing the PCR products of the same sample, they were electrophoresed on a 2% agarose gel. Based on the preliminary electrophoresis results, the PCR products were quantified using the QuantiFluor™-ST blue fluorescent quantification system (Promega, Madison, WI, USA). Then, the products were mixed in the appropriate proportions according to the sequencing requirements for each sample, and the mixture proceeded to the next step of library construction. Adapter ligation was performed, followed by magnetic bead selection to remove adapter dimers. PCR amplification enriched the library template, and sodium hydroxide was used for denaturation, resulting in single-stranded DNA fragments.

After the sequencing library passed quality control, it was sequenced on the Illumina MiSeq PE300 platform by Anoroad Gene Technology (Beijing) Co., Ltd., Beijing, China. The process is as follows: one end of the DNA fragment is complementary to the primer base and is fixed on the chip; the other end randomly complements another nearby primer, which is also fixed in place, forming a "bridge." PCR amplification generates DNA clusters, and the DNA amplicons are linearized into single strands. Modified DNA polymerase and dNTPs, labeled with four types of fluorescent markers, are added, allowing for the synthesis of one nucleotide per cycle. The surface of the reaction plate is scanned with a laser to read the type of nucleotide incorporated during the first round of reactions for each template sequence. The "fluorescent groups" and "terminator groups" are chemically cleaved to restore the 3′ sticky end, enabling the incorporation of the second nucleotide. The fluorescent signals collected in each round are analyzed to determine the sequence of the template DNA fragments. The raw data are stored in the NCBI SRA (Sequence Read Archive, http://www.ncbi.nlm.nih.gov/Traces/sra, accessed on 12 April 2023) database. The sequences were then quality-controlled and filtered using Trimmomatic and FLASH (V 1.2.7) software. The sequence direction was corrected, based on the box sequence at the sequence ends, and valid data were obtained by identifying and distinguishing the samples according to the barcode tag sequences. Usearch (version 7.1, http://drive5.com/uparse/, accessed on 12 April 2023) software was used to cluster non-redundant sequences (excluding singletons) into OTUs at 97% similarity, removing chimeras during the clustering process to obtain representative sequences of OTUs. Sequences with greater than 97% similarity to the OTU representative sequences were selected to generate the table.

2.5. Network Construction and Analysis

To standardize the OTU data obtained from high-throughput sequencing, upload the data to the Molecular Ecological Network Analyses Pipeline (MENA) website and construct a Pearson correlation matrix. Based on random matrix theory (RMT), set an appropriate threshold to build a microbial molecular ecological network to study changes in the soil microbial community following the return of Bt corn stalks to the soil. Import the network file, node attribute file, and edge attribute file into Cytoscape 3.8.0 to visualize the microbial molecular ecological network and obtain the complete network structure. In this network, nodes represent species within the community, and connections between nodes indicate interactions between species. The degree represents the number of nodes directly connected to a given node, path length represents the shortest distance between two nodes, clustering coefficient reflects the connectivity among a node and its neighboring nodes, and modularity characterizes the modular features within the molecular ecological network.

2.6. Data Analysis

Microsoft Office Excel was used to sort out the data, and one-way ANOVA was conducted using SPSS 25.0 software to compare the SPP and enzyme activities among different treatments. RDA and principal component analysis (PCA) were conducted to identify the correlation among SPP, soil enzyme activities, and soil microbial communities. RDA and PCA analyses of soil microorganisms were performed using the "vegan" package in R language (V 4.3.2) software, while the Pearson correlation analysis was conducted using the "psych" package. Graphs were generated using Origin 2018 and the ggplot2, heatmap, and plspm packages in R (V 4.3.2).

3. Results

3.1. Effects of Continuous Return of Bt Corn Straw on Soil Nutrients and Enzyme Activities

The contents of soil OM, AN, AP, AK, TN, TP, and TK in the three treatments increased with the extension of straw return time. This may be due to the increased frequency of straw return, resulting in variations in soil nutrients due to differences in straw nutrient content. Differences in soil nutrients between the three treatments were observed at individual times.

The results of the two-way analysis of variance are shown in Table 1. It can be seen from the table that the time of straw returning to the field has an extremely significant impact on soil nutrients. The type of straw has an extremely significant impact on OM, AN, AP, AK, and TN ($p < 0.01$). The above results demonstrate that the continuous return of Bt corn straw to the soil has had a significant impact on the content of soil nutrients. We analyzed the significance among the three treatments at the same timepoints after straw was returned to the soil in order to clarify the impact of Bt corn straw returning to the field on soil nutrients. As shown in Table S1, compared to the control, the soil OM content in the 5422Bt1 straw treatment significantly increased at 420 days, 480 days, and 540 days after return, while the TN content significantly increased at 480 days and 600 days after return, and significantly decreased or showed no significant change at other timepoints. AN content significantly decreased at 30 days and 360 days after return, and AP significantly reduced at 300 days and 360 days after return. AK content significantly decreased at 30 days and 150 days after return, while at other timepoints, it significantly increased or showed no significant difference. TP and TK contents showed no significant changes during the straw return process. During the straw return process of the 5422CBCL straw treatment, the soil OM content significantly increased at 30 days, 60 days, 150 days, and 270 days after return; the AN content significantly increased at 180 days and 300 days after return; the AP content significantly increased at 150 days after return; and the TN content significantly increased at 480 days and 600 days after return. At other timepoints, the contents significantly decreased or showed no significant difference. The AK content significantly decreased at 30 days after return, while at different timepoints, it significantly increased or showed no significant difference. After five consecutive straw returns, the soil nutrients were measured for each treatment. The results showed that the soil AP and TN content in the 5422Bt1 straw treatment significantly increased, while the soil OM, AN, AK, TP, and TK contents showed no significant difference compared to those of conventional corn 5422. In the 5422CBCL straw treatment, the soil OM, AN, and AP contents decreased significantly, while the AK and TN contents increased. The TP and TK contents in the soil showed no significant difference compared to those of conventional corn 5422.

Table 1. The results of variance analysis on the impact of different continuous straw returning treatments on soil nutrients.

Treatments	OM		AN		AP		AK		TN		TP		TK	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Time	105.539	<0.001 ***	128.673	<0.001 ***	428.818	<0.001 ***	1823.782	<0.001 ***	126.578	<0.001 ***	349.837	<0.001 ***	24.253	<0.001 ***
Straw treatment	57.317	<0.001 ***	47.501	<0.001 ***	119.422	<0.001 ***	38.734	<0.001 ***	19.900	0.002 **	1.799	0.244	3.342	0.106
Time × Straw treatment	12.938	<0.001 ***	8.556	<0.001 ***	13.531	<0.001 ***	4.216	<0.001 ***	8.080	<0.001 ***	1.553	0.192	0.953	0.494

Abbreviations: straw treatment—5422, 5422Bt1, 5422CBCL treatment; OM—organic matter; AN—available nitrogen; AP—available phosphorus; AK—available potassium; TN—total nitrogen; TP—total phosphorus; TK—total potassium; ** $p < 0.01$; *** $p < 0.001$.

The results of the two-way analysis of variance are shown in Table 2. It can be seen from the table that both the time of straw returning to the field and the type of straw have a significant impact on the activities of soil URE, SUC, and ACP ($p < 0.01$). The above results demonstrate that the continuous return of Bt corn straw to the soil has had a significant impact on the content of soil enzyme activities. The impact of Bt corn straw on

soil enzyme activities after being returned to the soil at the same timepoints were analyzed through one-way analysis of variance. As shown in Table S2, compared to the control, the URE activity in the 5422Bt1 straw return process significantly decreased at 390 days; the SUC activity significantly decreased at 60 days, 150 days, 480 days, 510 days, and 540 days; and the ACP activity significantly reduced at 150 days and 180 days, while at other timepoints, the enzyme activities significantly increased or showed no significant difference. In the 5422CBCL straw return process, the URE activity significantly decreased at 30 days and 390 days. The SUC activity significantly decreased at 30 days, 60 days, 150 days, 390 days, 480 days, 510 days, and 600 days after return, while the ACP activity significantly decreased at 180 days and 600 days after return. At other timepoints, enzyme activities significantly increased or showed no significant difference. Overall, after five consecutive straw returns, compared to that for the 5422 straw treatment, the SUC activity in the soil of the 5422Bt1 straw treatment significantly increased. At the same time, there was no significant difference in URE and ACP activities. In the 5422CBCL straw treatment, the URE activity significantly increased compared to that of the 5422 straw treatment, whereas the SUC and ACP activities significantly decreased.

Table 2. The results of variance analysis on the impact of different continuous straw returning treatments on soil enzyme activities.

Treatments	URE		SUC		ACP	
	F	***p***	**F**	***p***	**F**	***p***
Time	76.332	<0.001 ***	47.989	<0.001 ***	171.517	<0.001 ***
Straw treatment	18.412	0.001 **	7.208	0.014 *	72.553	<0.001 ***
Time × Straw treatment	16.301	<0.001 ***	12.293	<0.001 ***	4.246	<0.001 ***

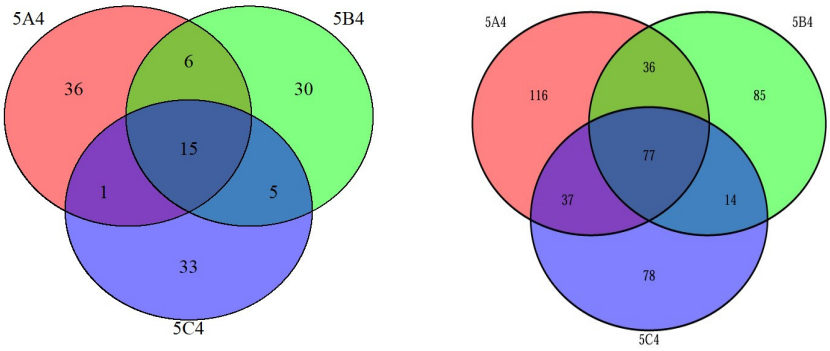
Abbreviations: straw treatment—5422, 5422Bt1, 5422CBCL treatment; URE—urease; SUC—sucrase; ACP—acid phosphatase; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2. Alpha Diversity (α- Diversity) of Soil Microorganisms After Continuous Return of Bt Corn Straw

Soil samples collected after five consecutive returns of straw were sequenced using the Illumina MiSeq PE300 device by Anoroad Gene Technology (Beijing) Co., Ltd., Beijing, China. The dilution curves for soil fungi and bacteria showed that the OTU dilution curve for each sample flattened as the number of reads increased, indicating that the sequencing data volume had reached an analyzable level. Clustering was performed at a 97% similarity level for the high-throughput sequences.

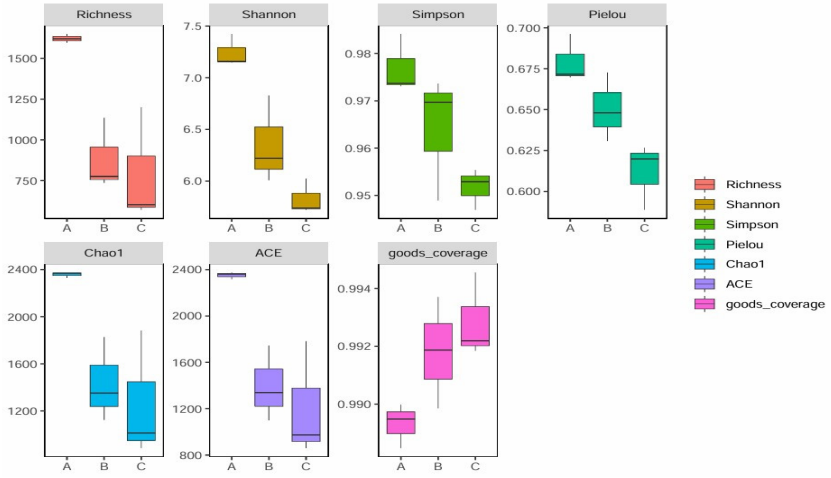
As shown in Figure 1, a total of 126 fungal OTUs and 443 bacterial OTUs were detected, with the number of soil fungi and bacteria ranked from highest to lowest, as follows: 5422Bt1, 5422CBCL, and 5422. Across all soil samples, there were 15 shared fungal OTUs, with 33 unique OTUs in 5422, 36 unique OTUs in 5422Bt1, and 30 unique OTUs in 5422CBCL. Additionally, 5422Bt1 and 5422 shared 16 OTUs, while 5422CBCL and 5422 shared 20 OTUs. In terms of bacterial OTUs, there were 77 shared OTUs across all treatments, with 78 unique OTUs in 5422, 116 unique OTUs in 5422Bt1, and 85 unique OTUs in 5422CBCL. Furthermore, 5422Bt1 and 5422 shared 114 OTUs, while 5422CBCL and 5422 shared 91 OTUs.

α-diversity reflects the diversity and richness of soil microbial communities. In this study, the richness, Shannon, Simpson, Chao1, and ACE indices were used to evaluate the abundance, evenness, and diversity of the fungal and bacterial communities. As shown in Figure 2, the alpha diversity indices indicated that the soil microbial diversity tended to increase in the 5422Bt1 straw treatment (Figure 2A) and the 5422CBCL straw treatment (Figure 2B) compared to that of the homologous conventional corn 5422 straw treatment, although the differences were not significant. Therefore, we infer that compared to the homologous conventional corn 5422, the continuous return of Bt corn straw 5422Bt1 and 5422CBCL has no significant impact on the diversity and richness of the soil fungal and bacterial communities.

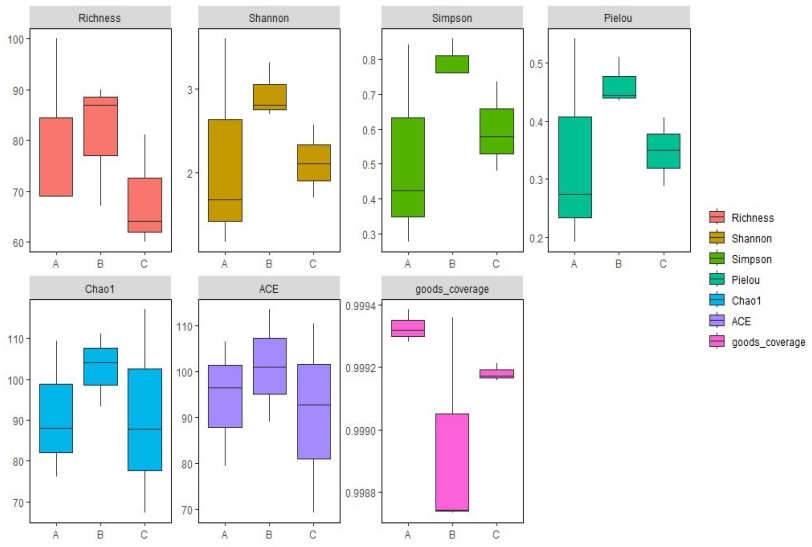


(**A**) (**B**)

Figure 1. Shared and unique OTU counts of fungal (**A**) and bacterial (**B**) communities under different straw treatments. Note: 5A4 represents the 5422Bt1 straw treatment; 5B4 represents the 5422CBCL straw treatment; 5C4 represents the 5422 straw treatment.



(**A**)



(**B**)

Figure 2. Effects of different straw treatments on the alpha diversity of fungal (**A**) and bacterial (**B**) communities. Note: 5A4 represents the 5422Bt1 straw treatment; 5B4 represents the 5422CBCL straw treatment; 5C4 represents the 5422 straw treatment.

Through sequencing analysis, a total of four phyla of fungi were detected in the soil, with Ascomycota (accounting for 84.22%) as the dominant phyla. This phylum comprised 88.78% in the 5422Bt1 straw treatment, 75.48% in the 5422CBCL straw treatment, and 90.98% in the 5422 straw treatment. Compared to the control, the 5422CBCL straw treatment increased the relative abundance of Basidiomycota. Compared to the control, the 5422CBCL straw treatment increased the relative abundance of Basidiomycota (Figure 3A).

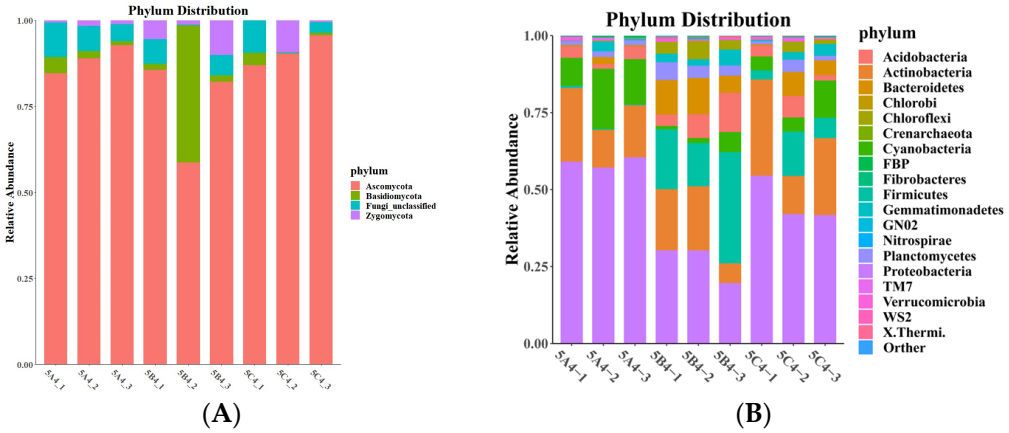


Figure 3. Dominant phyla of soil fungi (**A**) and bacterial (**B**) communities under different straw treatments. Note: 5A4 represents the 5422Bt1 straw treatment; 5B4 represents the 5422CBCL straw treatment; 5C4 represents the 5422 straw treatment.

The dominant bacterial communities at the phylum level across all samples primarily consist of Proteobacteria, Actinobacteria, and Firmicutes. The soil bacterial communities from the 5422Bt1 and 5422 straw treatments are similar, mainly composed of Proteobacteria and Actinobacteria. In addition, compared to the 5422 straw treatment, the bacterial community in the 5422CBCL straw treatment is primarily composed of Proteobacteria (26.74%), Actinobacteria (15.68%), and Firmicutes (23.21%). The 5422CBCL straw treatment significantly reduced the relative abundance of Proteobacteria and Actinobacteria in the soil, while significantly increasing the content of Firmicutes (Figure 3B).

3.3. Beta Diversity (β- Diversity) of Soil Microorganisms After Continuous Return of Bt Corn Straw

A principal component analysis (PCA) was performed on the distance matrix between samples to create a PCA plot. From the perspective of the fungal community, PC1 and PC2 explained 74.41% and 13.11% of the variation in the fungal community composition, respectively, together accounting for 87.52%. There were significant differences in the soil fungal community composition among the 5422Bt1, 5422CBCL, and the 5422 straw treatments (Figure 4A).

From the perspective of the bacterial community, PC1 and PC2 explained 65.53% and 25.57% of the variation in bacterial community composition, respectively, together accounting for 91.10%. The similarity between the 5422Bt1 and 5422 straw treatments was high, while there was a significant difference in the soil bacterial community between the 5422CBCL and 5422 straw treatments (Figure 4B).

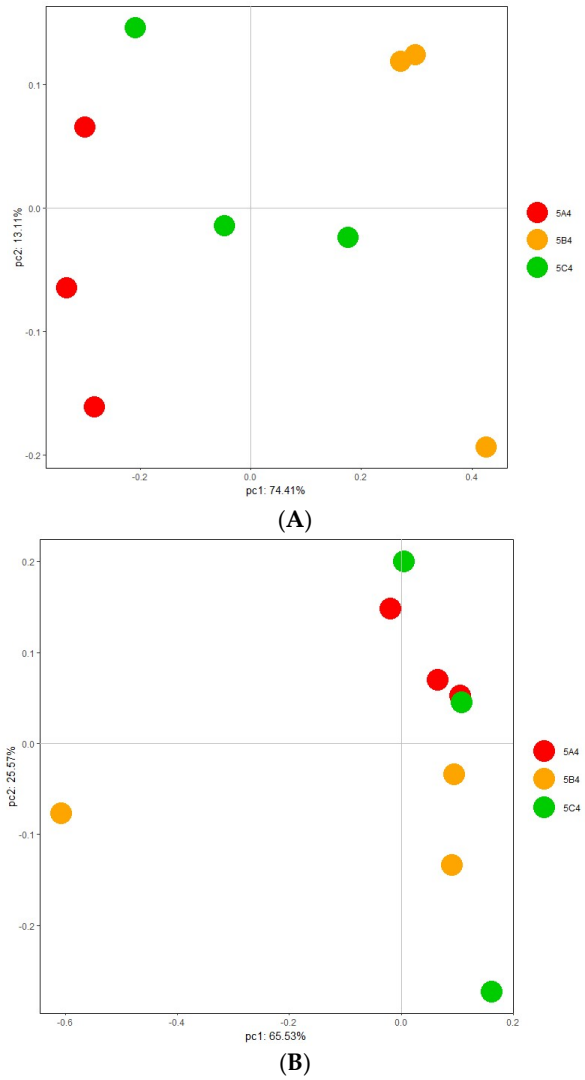


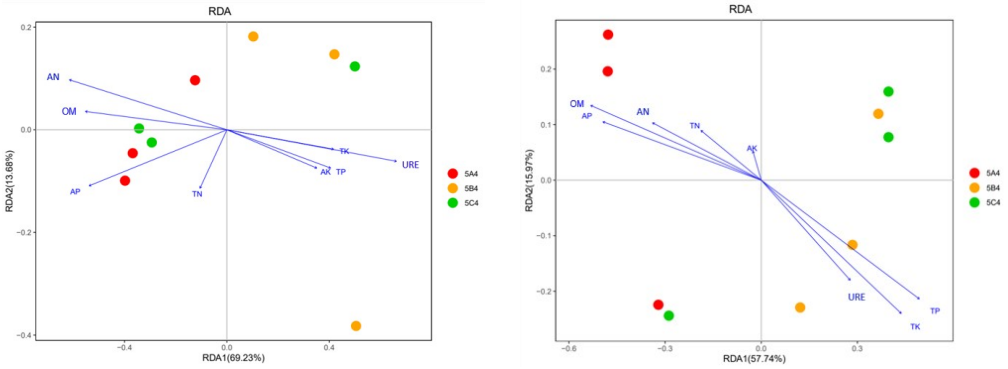
Figure 4. PCA analysis of differences in soil fungal (**A**) and bacterial (**B**) community structures under different straw treatments. Note: 5A4 represents the 5422Bt1 straw treatment; 5B4 represents the 5422CBCL straw treatment; 5C4 represents the 5422 straw treatment.

3.4. Effects of Environmental Factors on Soil Microbial Communities

RDA (redundancy analysis) of the screened environmental factors was conducted for the fungal and bacterial communities in the soil at the end of the continuous straw return. The results showed that RDA1 and RDA2 explained 69.23% and 13.68% of the variation in the fungal community, respectively. The fungal community was significantly influenced by URE, AP, and AN, with URE having the greatest impact, followed by AN content, while AK content had the least influence (Figure 5A). RDA1 and RDA2 explained 57.74% and 15.97% of the variation in the bacterial community, respectively. The bacterial community was significantly influenced by TP, TK, and OM, with TK having the greatest impact, followed by TP, while AK had the least influence (Figure 5B).

To further explore the correlation between soil fungi and bacteria (at the genus level) with environmental factors, a correlation heatmap was utilized for analysis. The heatmap was used to analyze the impact of environmental factors on the top 10 species of the fungal community. The results of the correlation between fungi at the genus level and environmental factors indicate that OM, AN, AP content, and SUC are significantly positively correlated with unclassified Sordariales. At the same time, URE and ACP are significantly negatively correlated with it. Additionally, AP content and SUC are significantly negatively correlated with unidentified fungi. URE is significantly positively correlated with unclassified Sor-

dariomycetes. AN content is significantly negatively correlated with Trichosporon. URE is significantly negatively correlated with unclassified fungi. AP is significantly negatively correlated with unclassified Chaetomiaceae, while ACP is significantly positively correlated with it. TK is significantly negatively correlated with other fungi (Figure 6A).



(**A**) (**B**)

Figure 5. RDA of soil fungal (**A**) and bacterial (**B**) communities in regards to the environmental factors. Note: 5A4 represents the 5422Bt1 straw treatment; 5B4 represents the 5422CBCL straw treatment; 5C4 represents the 5422 straw treatment.

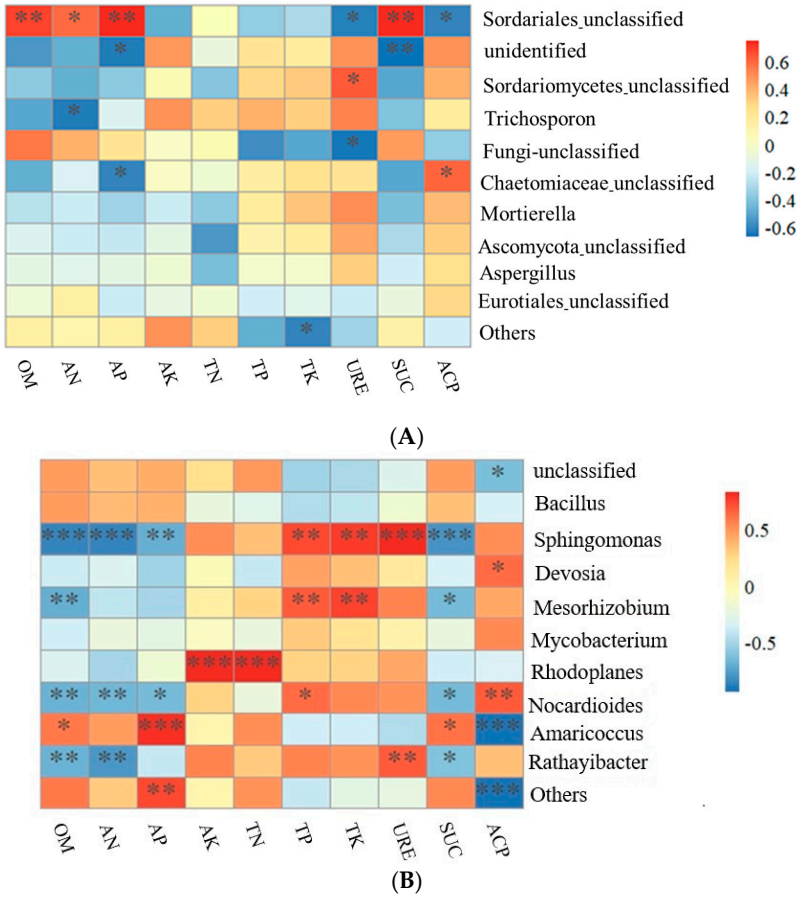


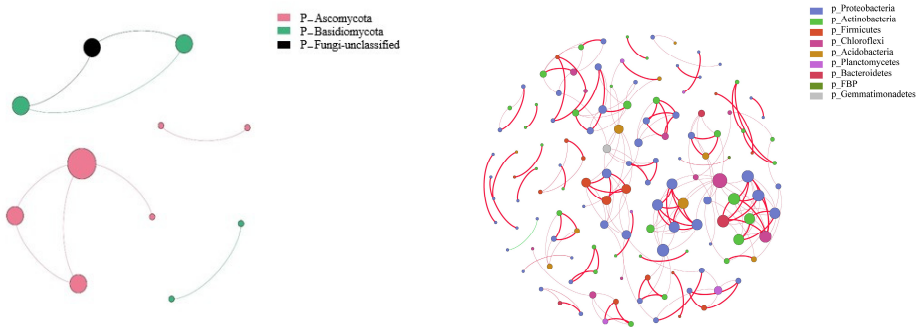
Figure 6. Analysis of correlation of soil fungal (**A**) and bacterial (**B**) communities with environmental factors. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

A correlation heatmap was used to analyze the impact of environmental factors on the top 10 species of the bacterial community. The results of the correlation between bacteria at the genus level and environmental factors indicate that ACP is significantly negatively

correlated with unclassified bacterial and significantly positively correlated with Devosia. OM, AN, AP, and SUC are significantly negatively correlated with Sphingomonas and Nocardioides, while TP, TK, and URE are significantly positively correlated with Sphingomonas. OM and SUC are significantly negatively correlated with Mesorhizobium, while TP and TK are significantly positively correlated with it. AK and TN are significantly positively correlated with Rhodoplanes. TP and ACP are significantly positively correlated with Nocardioides. OM, AP, and SUC are significantly positively correlated with Amaricoccus, while ACP is significantly negatively correlated with it. OM, AN, and SUC are significantly negatively correlated with Rathayibacter, while URE is significantly positively correlated with it. AP is significantly positively correlated with other bacteria, whereas ACP is significantly negatively correlated with them (Figure 6B).

3.5. Effects of Continuous Return of Bt Corn Straw on Soil Microbial Networks

A co-occurrence network diagram based on phylum level was constructed to explore the co-occurrence characteristics of fungi and bacteria. In the fungal co-occurrence network at the phylum level, Ascomycota and Basidiomycota account for a large proportion, with Ascomycota showing the highest relative abundance (Figure 7A). The bacterial community network diagram shows that at the phylum level, Proteobacteria, Actinobacteria, and Firmicutes account for a large proportion, with Proteobacteria having the highest relative abundance (Figure 7B).



(**A**) (**B**)

Figure 7. Phylum-level correlation network of soil fungal (**A**) and bacterial (**B**) communities.

The soil fungal community network contains 11 nodes and 9 edges. The soil bacterial community network contains 1354 nodes and 206 edges, indicating many nodes and connections, which suggests strong interactions among species and complex interspecies relationships. The predominance of positive correlations in the network indicates that the relationships among bacterial species are largely cooperative or symbiotic. Comparing the properties of fungal and bacterial networks, the bacterial network has a greater number of nodes and connections, with more complex interconnections. Additionally, the longer average degree, modularity, and average diameter of bacteria indicated that the bacterial community structure was stable, the efficiency of material and information transfer was high, and it could effectively cope with various changes in the soil environment (Table 3).

Table 3. The influence of different straw treatments on soil nutrients.

Sample	Node	Edges	Average Degree	Clustering Coefficient	Modularity	Average Diameter
ITS	11	9	1.63	0.889	0.667	2
16S	1354	206	3.052	0.822	0.878	6

Notes: ITS refers to the internal transcribed spacer region sequence of fungi and can be used for fungal community analysis; 16S refers to the 16S rRNA gene sequence of bacteria and can be used for bacterial community analysis.

4. Discussion

4.1. Effects of Continuous Return of Bt Corn Straw on Soil Nutrients

Soil is a crucial site for material cycling and energy transformation processes in ecosystems, and the cultivation of Bt crops and the return of straw to the soil may have an impact on soil nutrient content [11,16,24,25]. Through a straw decomposition experiment, we analyzed the effects of long-term continuous return of Bt corn straw on soil nutrient content. The experiment found that soil nutrient content in the three treatments increased with the continuous return of corn straw, consistent with previous research findings [11,26]. After five consecutive straw return treatments, compared to the straw treatment of the homologous conventional corn variety 5422, the soils treated with 5422Bt1 straw showed no significant differences in OM, AN, AK, TP, and TK, while the contents of AP and TN significantly increased. This may be because the 5422Bt1 straw has a higher TN and TP content than those found in conventional corn, which influenced the soil nutrient cycling during straw decomposition. At the same time, the soil nutrient content in the 5422CBCL treatment exhibited complex changes, with no significant differences in TP and TK content, while OM, AN, and AP contents significantly decreased, and AK and TN contents significantly increased (Table S1). This result is generally consistent with the study by Wang et al. [12]. The changes in soil nutrients after the return of the two Bt corn varieties showed obvious complexity, and this difference may be caused by the variations between varieties. Therefore, during the Bt corn straw return process, soil nutrient management should be optimized based on the specific characteristics of the varieties.

4.2. Effects of Continuous Return of Bt Corn Straw on Soil Enzyme Activity

Soil enzyme activity is of significant importance in soil ecosystems, as it originates from crop root exudates and is stimulated by soil microorganisms, playing a key role in OM decomposition and nutrient cycling [27]. Numerous studies have shown that soil enzyme activity exhibits complex changes after the return of Bt crops, and these complex changes are likely influenced by various factors such as soil type and Bt protein concentration [28]. In the related experiments conducted in this study, we compared the changes in soil enzyme activity under different treatments. The experiment found that, compared to the straw treatment of the conventional variety 5422, after five consecutive straw return treatments, the soil SUC activity in the 5422Bt1 straw treatment significantly increased, while the URE activity and ACP activity were not significantly affected (Table S2). From the perspective of soil fertility and nutrient cycling, the significant increase in sucrase activity suggests that the decomposition and conversion efficiency of OM in the soil may have improved, which to some extent, reflects that the continuous return of 5422Bt1 straw helps enhance soil fertility and can play a positive role in promoting soil carbon cycling. Meanwhile, after five consecutive straw return treatments, the soil enzyme activity under the 5422CBCL straw treatment also showed significant changes. URE activity in the soil significantly increased. As one of the key enzymes involved in nitrogen mineralization, URE can convert urea into inorganic nitrogen, which helps improve the efficiency of nitrogen cycling in the soil [29]. However, at the same time, the 5422CBCL straw treatment significantly reduced the activity of SUC and ACP. Based on the analysis of the existing research, this is likely because the Bt protein in the 5422CBCL straw inhibited the activity of enzymes involved in phosphorus cycling and organic carbon OM decomposition [29]. The two Bt corn straw treatments showed both the promotion and inhibition of SUC, URE, and ACP activity at certain sampling times, indicating that the return of 5422Bt1 and 5422CBCL straw to the soil has a complex effect on soil enzyme activity. After straw return, there was either no significant change or a declining trend in soil enzyme activity. On one hand, this may be due to differences in straw varieties and the products of straw decomposition, while on the other hand, it may be due to the soil lacking proper water and air exchange, which hinders microbial growth, ultimately leading to changes in soil enzyme activity. Therefore, in future studies on Bt corn straw return, we should consider the differences in soil enzyme activity caused by different types of straw and the substances contained within them.

4.3. Effects of Continuous Return of Bt Corn Straw on Soil Microbial Communities

In the complex network of soil ecosystems, soil microorganisms play an important role in the straw decomposition process [30]. Straw return, as an effective method to improve soil fertility and maintain crop productivity, has a significant impact on soil microbial communities, which deserves in-depth investigation. This impact is even more complex and meaningful in the context of genetically modified crop straw return [31–33]. This study conducted a systematic analysis of the changes in soil microbial communities after the return of 5422Bt1 and 5422CBCL straw. After five consecutive straw return treatments, compared to the control, we found that both treatments had a certain promoting effect on the diversity of soil fungal and bacterial communities, although the effect was not significant (Figure 2). This is consistent with previous studies [34,35]. Further analysis of the microbial community composition at the phylum level revealed that the two Bt corn treatments and the control had similar phylum-level compositions, with Ascomycota and Basidiomycota being the dominant fungal phyla (Figure 3A). During the initial stages of straw return, Ascomycota degrades the easily decomposable parts of the straw, while Basidiomycota mainly degrades the more difficult-to-decompose organic matter in the later stages of straw decomposition [36]. However, there were no significant differences in the fungal community composition among the three treatments. This is similar to the findings of Liu et al. [37]. However, the relative abundance of Basidiomycota in the 5422CBCL straw treatment increased, which may be because this treatment accelerated the succession of the soil fungal community, promoting the degradation of more difficult-to-decompose OM. The study results showed that the dominant bacterial phyla in the three treatments were Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes (Figure 3B), and Proteobacteria is the most abundant microbial phyla in soil, which is consistent with the results for the dominant bacterial communities in the soil after corn straw return [38,39]. In the soils of the three straw return treatments, the relative abundance of Proteobacteria was the highest. Proteobacteria is the most abundant microbial phylum in the soil, and it can live under both facultative or obligate anaerobic conditions, preferring dry soil environments [38,40]. Compared to the control, the 5422CBCL straw treatment caused significant changes in the bacterial community structure, with a significant decrease in the relative abundance of Proteobacteria and Actinobacteria and a significant increase in the relative abundance of Firmicutes. This may be due to changes in soil nutrient and enzyme activity levels induced by the 5422CBCL treatment, which in turn altered the soil bacterial community structure [41–43].

In soil ecosystems, the interactions between soil microbial communities and the surrounding environment are crucial, profoundly affecting the functioning and stability of the entire system. Soil fertility is widely recognized as a key factor shaping microbial community diversity [44,45]. The results of the RDA analysis indicate that URE is an important factor influencing fungal community changes, while TK content is an important factor affecting bacterial community changes (Figure 5). Meanwhile, further correlation heatmap analysis revealed that the fungal community was significantly correlated with OM, AK, and SUC ($p < 0.01$) (Figure 6A), while the bacterial community showed a highly significant correlation with OM, AN, AP, AK, TN, URE, SUC, and ACP ($p < 0.001$) (Figure 6B). We speculate that the dynamic changes in soil microbial communities may be the result of the synergistic effects of various soil physicochemical properties. Previous studies have provided key insights for further exploring the mechanisms of soil microbial community changes observed in this study. The changes in URE activity may reshape the fungal community composition by affecting the nitrogen supply balance, which might be related to the specific nitrogen utilization preferences of the fungi [45]. The changes in TK content likely influence the bacterial community, which may be closely related to the bacteria's potassium uptake and metabolism pathways. Potassium plays a key role in maintaining bacterial cell membrane potential stability and osmotic pressure balance. Previous studies have found that soil URE and phosphatase play crucial roles in the N and P cycles. The former converts organic nitrogen into inorganic nitrogen to provide a biological nitrogen source [46], while

the latter hydrolyzes organic phosphorus into inorganic phosphorus to assist in plant phosphorus absorption [47]. Meanwhile, soil physical and chemical properties such as pH and available potassium have also been found to be closely related to the construction of fungal or bacterial community structures [48]. Therefore, physical and chemical properties such as AK, OM, and ACP are the main driving factors for the changes in the soil microbial community structure [41,47,48].

Analyzing the structural characteristics of ecological networks can detect the complex relationships between species and the stability of ecological network structure; the more connections there are, the stronger the microbial interactions will be [49,50]. The results of this study indicate that returning corn straw to the soil increased the complexity of the bacterial network while reducing the complexity of the fungal network, suggesting that bacteria play a dominant role in the process of straw return (Figure 7, Table 3). This is consistent with the findings of previous studies. Xu et al. [51] found that long-term straw incorporation can increase the degree of interaction in the soil bacterial network

5. Conclusions

The five continuous returns of Bt corn straw to the soil exerted an impact on soil nutrients and enzyme activities. The 5422Bt1 straw treatment increased some soil nutrients and enzyme activities, while the 5422CBCL straw treatment caused some soil nutrients and enzyme activities to increase and decrease in different situations. The dominant phyla of the soil fungal and bacterial communities under the three treatments were Ascomycota, Basidiomycota, Proteobacteria, Actinobacteria, and Firmicutes. The fungal community was relatively stable, with no significant differences in its α-diversity and relative abundance. The 5422CBCL straw treatment significantly changed the composition of the bacterial community and affected the relative abundance of specific bacterial phyla. There were differences in the fungal community composition between the 5422Bt1 and 5422CBCL treatments and the 5422 treatment, and there were differences in the bacterial community composition between the 5422CBCL treatment and the 5422 treatment. The community structure composition was related to a variety of soil indicators. The soil microorganisms showed complex correlations with environmental factors at the genus level. This experiment provided crucial evidence and clues for further research on the influence of the continuous return of Bt corn straw on the soil microbial community and its relationship with ecological functions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112737/s1, Table S1. The influence of different straw treatments on soil nutrients; Table S2. The influence of different straw treatments on soil enzyme activities.

Author Contributions: Conceptualization, Y.F.; investigation, Y.F. and X.L (Xiao Lv).; data curation, C.Z., P.P. and X.L. (Xiaomin Liang); methodology, Y.F. and X.L. (Xiao Lv); software, X.L. (Xiao Lv) and P.P.; formal analysis, C.Z., P.P. and X.L. (Xiaomin Liang); writing—original draft, C.Z. and X.L. (Xiaomin Liang); writing—review and editing, Y.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the National Natural Science Foundation of China (31370543, 41101279).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We thank Cindy Nakatsu (Purdue University, West Lafayette, IN) for providing us with corn seeds.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Agbio, I. Global GM Crop Area Review. Available online: https://gm.agbioinvestor.com (accessed on 28 February 2024).
2. Brookes, G. Genetically Modified (GM) Crop Use 1996–2020: Environmental Impacts Associated with Pesticide Use Change. *GM Crop. Food* **2022**, *13*, 262–289. [CrossRef] [PubMed]
3. Sharkey, S.M.; Williams, B.J.; Parker, K.M. Herbicide Drift from Genetically Engineered Herbicide-Tolerant Crops. *Environ. Sci. Technol.* **2021**, *55*, 15559–15568. [CrossRef]
4. Kanaujia, A.; Gupta, S. Tracing scientific and technological development in genetically modified crops. *Transgenic Res.* **2024**, *18*, 1–22. [CrossRef]
5. Campos, S.O.; Santana, I.V.; Silva, C.; Santos-Amaya, O.F.; Guedesa, R.N.C.; Pereira, E.J.G. *Bt*-induced hormesis in *Bt*-resistant insects: Theoretical possibility or factual concern? *Ecotoxicol. Environ. Saf.* **2019**, *183*, 109577. [CrossRef] [PubMed]
6. Wyk, D.A.B.V.; Adeleke, R.; Rhode, O.H.J.; Bezuidenhout, C.C.; Mienie, C. Ecological guild and enzyme activities of rhizosphere soil microbial communities associated with *Bt*-maize cultivation under field conditions in North West Province of South Africa. *J. Basic Microbiol.* **2017**, *57*, 781–792. [PubMed]
7. Liu, Y.B.; Li, J.S.; Luo, Z.L.; Wang, H.R.; Liu, F. The fate of fusion Cry1Ab/1Ac proteins from *Bt*-transgenic rice in soil and water. *Ecotoxicol. Environ. Saf.* **2016**, *124*, 455–459. [CrossRef]
8. Guan, Z.J.; Wei, W.; Huo, Y.L.; Stewart, C.N.; Tang, Z.X. Dynamics of Cry1Ac protein and soil enzyme activity in the rhizosphere of transgenic *Bt* oilseed rape. *Plant Soil Environ.* **2024**, *70*, 580–589. [CrossRef]
9. Fazal, A.; Yang, M.K.; Han, H.W.; Lu, G.H.; Hao, C.Y.; Lai, X.H.; Song, Y.H.; Ma, H.K.; Yin, T.M.; Qi, J.L.; et al. Impact of dual *Bt*-transgenic maize (2A7) on soil microbial communities and enzyme activities: A comparative study with control variety Z58. *Environ. Res.* **2024**, *263*, 120006. [CrossRef]
10. Yang, S.K.; Lin, X.; Xu, X.H.; Sun, H.W.; Li, F.; Hao, C.F.; Lu, X.B. Effects of Insect-Resistant Maize 2A-7 Expressing mCry1Ab and mCry2Ab on the Soil Ecosystem. *Plants* **2022**, *11*, 2218. [CrossRef]
11. Zeng, P.; Feng, Y.J.; Zhang, W.C.; Zhang, Y.F.; Dong, W.C.; Wang, J.W. Changes of Bt protein in soil after planting Bt corn and straw returning and their effects on soil nutrients. *Chin. J. Appl. Ecol.* **2014**, *25*, 1997–2003.
12. Wang, J.W.; Feng, Y.J.; Luo, S.M. Effects of Bt corn straw decomposition on soil enzyme activity and soil fertility. *Chin. J. Appl. Ecol.* **2005**, *16*, 524–528.
13. Yan, S.L.; Zhao, L.; Sun, H.W.; Tian, X.Y.; Li, F.; Lu, X.B. Effects of transgenic Bt corn on soil enzyme activities under field conditions. *Acta Ecol. Sin.* **2011**, *31*, 4244–4250.
14. Zhang, J.; Jin, K.; Luo, Y.; Du, L.; Tian, R.; Wang, S.; Shen, Y.; Zhang, J.; Li, N.; Shao, W.; et al. Responses of Soil Enzyme Activity to Long-Term Nitrogen Enrichment and Water Addition in a Typical Steppe. *Agronomy* **2023**, *13*, 1920. [CrossRef]
15. Guan, P.T.; Yang, J.J.; Yang, Y.R.; Wang, W.; Zhang, P.; Wu, D.H. Land conversion from cropland to grassland alleviates climate warming effects on nutrient limitation: Evidence from soil enzymatic activity and stoichiometry. *Glob. Ecol. Conserv.* **2020**, *24*, e013828. [CrossRef]
16. Stotzky, G. Persistence and biological activity in soil of insecticidal proteins from *Bacillus thuringiensis* and of bacterial DNA bound on clays and humic acids. *J. Environ. Qual.* **2000**, *29*, 691–705. [CrossRef]
17. Shu, Y.H.; Zhang, Y.T.; Zeng, H.L.; Zhang, Y.H.; Wang, J.W. Effects of Cry1Ab Bt maize straw return on bacterial community of earthworm *Eisenia fetida*. *Chemosphere* **2017**, *173*, 1–13. [CrossRef] [PubMed]
18. Wang, H.X.; Chen, X.; Tang, J.J.; Ye, Q.F.; Wang, Z.H. Effects of transgenic Bt rice straw degradation on soil microbial culturable groups. *Acta Ecol. Sinica.* **2002**, *24*, 89–94.
19. Mulder, C.; Wouterse, M.; Rutgers, M.; Posthuma, L. Transgenic maize containing the Cry1Ab protein ephemerally enhances soil microbial communities. *AMBIO* **2007**, *36*, 359–361. [CrossRef]
20. Philippot, L.; Chenu, C.; Kappler, A.; Rillig, M.C.; Fierer, N. The interplay between microbial communities and soil properties. *Nat. Rev. Microbiol.* **2024**, *22*, 226–239. [CrossRef]
21. Bao, S.D. *Soil and Agricultural Chemistry Analysis*, 3rd ed.; China Agriculture Press: Beijing, China, 2000; pp. 25–114.
22. Guan, S.Y. *Soil Enzymes and Their Research Methods*, 1st ed.; China Agriculture Press: Beijing, China, 1986; pp. 340–344.
23. Zhou, L.K.; Zhang, Z.M. Methods for the determination of soil enzyme activities. *Chin. J. Soil Sci.* 1980; 37–38+49. [CrossRef]
24. Song, Y.Y.; Liu, J.W.; Li, L.K.; Liu, M.Q.; Chen, X.Y.; Chen, F.J. Evaluating the effects of transgenic *Bt* rice cultivation on soil stability. *Environ. Sci. Pollut. Res.* **2020**, *27*, 17412–17419. [CrossRef]
25. Lu, H.H.; Wu, W.X.; Chen, Y.X.; Zhang, X.J.; Devare, M.; Thies, J.E. Decomposition of *Bt* transgenic rice residues and response of soil microbial community in rapeseed–rice cropping system. *Plant Soil* **2010**, *336*, 279–290. [CrossRef]
26. Li, J.F.; Shu, Y.H.; Wang, F.; Wang, J.W. Effects of Cry1Ab-expressing Bt rice straw return on juvenile and adult *Eisenia fetida*. *Ecotoxicol. Environ. Saf.* **2019**, *169*, 881–893. [CrossRef] [PubMed]
27. Luo, L.; Meng, H.; Gu, J.D. Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *J. Environ. Manag.* **2017**, *197*, 539–549. [CrossRef]
28. Tang, H.M.; Xiao, X.P.; Tang, W.G.; Lin, Y.C.; Wang, K.; Yang, G.L. Effects of winter cover crops residue returning on soil enzyme activities and soil microbial community in double-cropping rice fields. *PLoS ONE* **2014**, *9*, e100443.
29. Li, Z.L.; Cui, J.; Mi, Z.R.; Tian, D.S.; Wang, J.S.; Ma, Z.L.; Wang, B.X.; Chen, H.Y.H.; Niu, S.L. Responses of soil enzymatic activities to transgenic *Bacillus thuringiensis* (Bt) crops-A global meta-analysis. *Sci. Total Environ.* **2019**, *615*, 1830–1838. [CrossRef]

30. Cong, P.; Wang, J.; Li, Y.Y.; Liu, N.; Dong, J.X.; Pang, H.C.; Zhang, L.; Gao, Z.J. Changes in soil organic carbon and microbial community under varying straw incorporation strategies. *Soil Tillage Res.* **2020**, *204*, 104735. [CrossRef]
31. Chen, Z.M.; Wang, H.Y.; Liu, X.W.; Zhao, X.L.; Lu, D.J.; Zhou, J.M.; Li, C.Z. Changes in soil microbial community and organic carbon fractions under short-term straw return in a rice–wheat cropping system. *Soil Tillage Res.* **2017**, *165*, 121–127. [CrossRef]
32. Su, Y.; Lv, J.L.; Yu, M.; Ma, Z.H.; Xi, H.; Kou, C.L.; He, Z.C.; Shen, A.L. Long-term decomposed straw return positively affects the soil microbial community. *J. Appl. Microbiol.* **2019**, *128*, 138–150. [CrossRef]
33. Oh, S.D.; Jang, Y.J.; Park, S.Y.; Lee, K.J.; Lee, S.K.; Lee, S.K.; Yun, D.W.; Suh, S.J.; Chae, J.C. Assessment of the effect on soil microbial communities of genetically modified soybean and a hybrid from crossing with wild soybean. *Plant Biotechnol. Rep.* **2021**, *15*, 855–862. [CrossRef]
34. Li, Z.L.; Bu, N.S.; Chen, X.P.; Cui, J.; Xiao, M.Q.; Song, Z.P.; Nie, M.; Fang, C.M. Soil incubation studies with Cry1Ac protein indicate no adverse effect of *Bt* crops on soil microbial communities. *Ecotoxicol. Environ. Saf.* **2018**, *152*, 33–41.
35. Zhou, X.L.; Liang, J.G.; Luan, Y.; Song, X.Y.; Zhang, Z.G. Characterisation of *Bt* maize IE09S034 in decomposition and response of soil bacterial communities. *Plant Soil Environ.* **2021**, *67*, 286–298. [CrossRef]
36. Francioli, D.; Schulz, E.; Lentendu, G.; Wubet, T.; Buscot, F.; Reitz, T. Organic amendments: Microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Front. Microbiol.* **2016**, *7*, 1446. [CrossRef] [PubMed]
37. Liu, X.S.; Zeng, X.; Zhu, Y.H.; Wang, W.; Huang, S.Q.; Qiao, X.X.; Wang, Z.H.; Di, H.; Qu, J.J. Degradation of betaine aldehyde dehydrogenase transgenic maize BZ-136 straw and its effects on soil nutrients and fungal community. *Front. Microbiol.* **2023**, *14*, 1180310. [CrossRef] [PubMed]
38. Eilers, K.G.; Lauber, C.L.; Knight, R.; Fierer, N. Shifts in bacterial community structure associated with inputs of low molecular weight carbon compounds to soil. *Soil Biol. Biochem.* **2010**, *42*, 896–903. [CrossRef]
39. Xia, X.Y.; Zhang, P.P.; He, L.L.; Gao, X.X.; Li, W.J.; Zhou, Y.Y.; Li, Z.X.; Li, H.; Yang, L. Effects of tillage managements and maize straw returning on soil microbiome using 16S rDNA sequencing. *J. Integr. Plant Biol.* **2019**, *61*, 765–777. [CrossRef]
40. Zhang, T.; Xu, F.; Huai, B.D.; Yang, X.; Sui, W.Z. Effects of land use change on soil bacterial community diversity in wetlands along the lower reaches of Huajiang River. *Environ. Sci.* **2020**, *41*, 4273–4283.
41. Sun, H.; Wang, Q.X.; Liu, N.; Li, L.; Zhang, C.G.; Liu, Z.B.; Zhang, Y.Y. Effects of different leaf litters on the physicochemical properties and bacterial communities in *Panax ginseng*-growing soil. *Appl. Soil Ecol.* **2017**, *111*, 17–24. [CrossRef]
42. Qiu, W.J.; Luan, L.; Zheng, J.; Hu, K.J.; Xu, Q.S.; Sun, B.; Jiang, Y.J. Effects of straw returning methods on rhizosphere nitrogen-fixing bacteria community and peanut yield. *J. Plant Nutr. Fert.* **2021**, *27*, 2063–2072.
43. He, Y.L.; Cui, H.Z.; Wang, B.B.; Ma, K. Effects of continuous maize straw returning on soil microbial community composition. *J. Agric. Sci.* **2022**, *43*, 27–35.
44. Yan, F.; Zhao, X.; Shao, L.J.; Wang, X.Y.; Liang, Y.B.; Chen, Y.H. Effects of different vegetation restoration types on soil microbial community structure in the restoration area of quarry in northern Hebei Province. *Environ. Sci.* **2024**, 1–16. [CrossRef]
45. Chen, F.; Yu, G.; Sun, Y.B.; Zhang, H.L.; Tian, X.; Xia, B. Characteristics of soil microbial community structure and its environmental driving factors in farmland around mercury mining area. *Environ. Sci.* **2022**, *43*, 4342–4352.
46. Li, C.Y.; Wang, Y.W.; Wang, R.; Li, L.L.; Liu, C.L. Effects of straw returning methods on physical and chemical properties and microbial community of paddy soil in Northeast China. *Acta Microbiol. Sin.* **2022**, *62*, 4811–4824.
47. Gu, S.Y.; Guo, X.J.; Cai, Y.T.; Zhang, Z.H.; Wu, S.; Li, X.; Zhang, H.H.; Yang, W. Residue management alters microbial diversity and activity without affecting their community composition in black soil, Northeast China. *PeerJ* **2018**, *6*, 5754. [CrossRef] [PubMed]
48. Sun, M.Q.; Liu, H.R.; Liu, X.D.; Wang, H.J.; Wang, N.; Gao, Y.; Luan, T.H.; Wang, L.C.; Chen, B.Y. The Impact of Straw Returning on the Structure of Soil Bacterial Community in Maize Farmland in Semi-arid Areas. *J. Northeast Norm. Univ. (Nat. Sci. Ed.).* **2021**, *53*, 123–129.
49. Montoya, J.M.; Pimm, S.L.; Sole, R.V. Ecological networks and their fragility. *Nature* **2006**, *442*, 259–264. [CrossRef]
50. Xiao, X.; Laing, Y.T.; Zhou, S.; Zhuang, S.Y.; Sun, B. Fungal community reveals less dispersal limitation and potentially more connected network than that of bacteria in bamboo forest soils. *Mol. Ecol.* **2018**, *27*, 550–563. [CrossRef]
51. Xu, Z.Y.; Sun, R.H.; He, T.Y.; Sun, Y.Z.; Wu, M.C.; Xue, Y.H.; Meng, F.Q.; Wang, J. Disentangling the impact of straw incorporation on soil microbial communities: Enhanced network complexity and ecological stochasticity. *Sci. Total Environ.* **2023**, *863*, 160918. [CrossRef]