agronomy

MDPI

Article

From Residue to Resource: A Physicochemical and Microbiological Analysis of Soil Microbial Communities through Film Mulch-Enhanced Rice Straw Return Strategies

Xinyue Wang [1], Jie Huang [1], Lili Yang [2], Yuanhuan Li [2], Bing Xia [1], Hailin Li [1] and Xiaohua Deng [1,*]

[1] College of Agriculture, Hunan Agricultural University, Changsha 410125, China; 1468909857@stu.hunau.edu.cn (X.W.); 2624555016@stu.hunau.edu.cn (J.H.); xiabing@hunau.edu.cn (B.X.); lihl@hunau.edu.cn (H.L.)

[2] College of Plant Protection, Hunan Biological and Electromechanical Polytechnic, Changsha 410125, China; yanglili@hnbemc.edu.cn (L.Y.); liyuanhuan@hnbemc.edu.cn (Y.L.)

* Correspondence: yzdxh@hunau.edu.cn; Tel.: +86-13974934919

Abstract: Promoting rice straw in situ return is an important strategy for improving soil quality. From 2018 to 2021, we investigated the effects of rice straw return with microbial agents and film covering technology on soil physical and chemical properties at different layer depths, as well as the soil microbial community structure, in Hunan, southern China. This study was designed to evaluate the effects of microbial agents (T1), film mulch covering (T2), and the application of microbial agents combined with film mulch (T3) on the soil physicochemical properties and microbial community after rice straw in situ return. The results show that, after three years of continuous treatment, T3 significantly increased the soil temperature by 17.76–22.97%, T2 significantly increased the water content by 34.27–46.23%, and T1 and T3 significantly increased the soil pH. The addition of microbial agents combined with film mulch resulted in a notable increase in both the number of OTUs and the Chao1 index of soil microorganisms. Additionally, the model of promoting rice straw in situ return (the application of a microbial agent combined with film mulch) was shown to promote the growth of beneficial soil microorganisms. RDA was used for the investigation, and the findings showed that soil microorganisms were significantly influenced by the TOC content, pH, and water content. These findings provide evidence of an effective method for accelerating the decomposition of late rice straw and guiding soil improvement in tobacco–rice rotation regions.

Keywords: rice straw; soil quality; microbial agents; sustainable agriculture; soil microbial structure

Citation: Wang, X.; Huang, J.; Yang, L.; Li, Y.; Xia, B.; Li, H.; Deng, X. From Residue to Resource: A Physicochemical and Microbiological Analysis of Soil Microbial Communities through Film Mulch-Enhanced Rice Straw Return Strategies. *Agronomy* **2024**, *14*, 1001. https://doi.org/10.3390/agronomy14051001

Academic Editor: Francesc Xavier Prenafeta Boldú

Received: 26 March 2024
Revised: 29 April 2024
Accepted: 8 May 2024
Published: 9 May 2024

1. Introduction

Soil quality plays an important role in agricultural production since it directly affects yields and product quality, which are vital for agriculture's sustainable development [1]. The protection and rational use of soil resources have become increasingly important in light of the growing population and limited agricultural area. In the tobacco–rice rotation areas of southern China, straw is returned to the field in situ through a rotary tiller before tobacco is transplanted [2]. In recent years, the return of late rice straw to the field as a resource utilization method has attracted much attention, as it can improve soil fertility as well as soil structure [3]. Straw contains abundant nitrogen, phosphorus, potassium, lignin, cellulose, starches, lipids and proteins, and trace elements, making it a valuable biomass resource [4]. The decomposition of straw can improve soil fertility by increasing the content of organic matter, available phosphorus, and available potassium [5]. As a result, it improves the structure and function of soil and regulates its moisture and nutrient content, gas and heat flows, and microbial community structure and function [6]. However, there are also a series of problems with returning straw directly to the field, such as slow decomposition, which affects the soil's physical and chemical properties and hinders crop

emergence [7]. Returning straw to the field can impact greenhouse gas emissions by contributing to methane production in rice fields [8]. Therefore, it is of great importance to optimize the strategy of returning straw to the field to minimize the emission of greenhouse gases and improve the utilization rate of straw resources and the yields in tobacco–rice rotation areas.

Microorganisms play a significant role in soil ecosystems, having major impacts on processes such as nutrient cycling, organic matter transformation, and fertility formation in soil [9,10]. The application of microbial agents provides a new method of soil improvement that is expected to accelerate straw decomposition, promote nutrient release, increase land productivity, reduce costs, simplify operations, and prevent secondary pollution [11,12]. Soil quality is determined by the structure and richness of soil microorganisms, which play a critical role in crop production [13,14]. Therefore, this new approach has received extensive attention in soil ecological research [15–17].

Soil physicochemical properties have a significant influence on the soil microbial community structure. There is a strong correlation between microbial community composition and soil properties, such as pH, total nitrogen, available nitrogen, available phosphorus, available potassium, and soil organic matter [18–20]. Different soil properties can affect specific microbial phyla or groups, such as Acidobacteria and Chloroflexi, which are positively correlated with pH, potassium, and TOC content [21]. Temperature also plays an important role in influencing the microbial community composition of soil [22]. Additionally, the application of microbial agents can significantly increase the abundance of bacteria and fungi in degraded grasslands [23].

It is important to consider the soil physicochemical properties and the structure of the microbial community when researching the impacts of farm management on crop growth. Plastic mulch film covering is commonly used to improve growing conditions by controlling evaporation and enhancing temperature regulation [24,25], thus providing a more suitable living environment for soil microorganisms. Covering corn straws with plastic film has been shown to promote straw decomposition [26]. Promoting straw return to the field has several benefits for farmland fertility and provides nutrients for crop growth [27]. In addition to enhancing dissolved organic carbon and microbial biomass carbon in soil, it enhances nitrogen fractions and microbial biomass nitrogen in the soil and reduces or avoids the need for straw burning, promoting a virtuous cycle of agricultural production. For soil fertility and environmental protection, it has a great deal of importance.

Though returning rice straw to fields has many advantages, issues such as delayed crop emergence and slow decomposition call for this practice to be optimized for sustainable agricultural production. This study investigates the effects of mulching and microbial agents on promoting straw return to the field and their impacts on soil physicochemical properties and microbial communities. Our approach integrates comprehensive field experiments and advanced analytical techniques to provide novel insights into the potential synergistic effects of these factors on enhancing soil fertility and sustainability in agricultural systems. By elucidating the mechanisms underlying the interactions between mulching, microbial communities, and soil health, our study contributes to the growing body of research aimed at developing more effective and sustainable agricultural practices. The findings presented herein not only advance our understanding of soil microbial ecology but also offer practical implications for optimizing straw management strategies in agricultural settings.

2. Materials and Methods

2.1. Site Description

The field experiment was conducted from 2018 to 2021 in Chaling County, Zhuzhou City, Hunan Province (26°46′59″ N, 113°45′0″ E). The experiment location is a typical tobacco–rice rotation area in southern China. This field has been implementing tobacco–rice rotation since 2010; rice straw is returned to the field every year, while tobacco straw is moved out of the field. There are 1744.7 sunshine hours, an average annual temperature

of 17.9 °C, and 1423 mm of precipitation in the region, which has a subtropical monsoon climate. The soil texture is loamy. The soil properties within the top 0–20 cm of soil were as follows: pH 5.8, organic matter 25.47 g/kg, total nitrogen (TN) 1.21 g/kg, total organic carbon (TOC) 14.25 g/kg, alkaline N 38.92 mg/kg, available P 52.16 mg/kg, and available K 212.13 mg/kg.

2.2. Materials

The powdered microbial agent was produced by Henan Wobao Biotechnology Co., Ltd., Hebi, China, and included *Bacillus*, filamentous fungi, and lactic acid bacteria, with 10 billion living bacteria per gram. The microbial agent underwent constant temperature fermentation (*Bacillus* 30 °C, filamentous fungi 28 °C, lactic acid bacteria 30 °C), used glucose as a source of nutrition, and involved the solid-state fermentation of filamentous fungus and continuous fermentation of *Bacillus* and *lactic acid bacteria*. The microbial agent was stored at a temperature of 20–25 °C in a dark and dry environment. The plastic mulch films used in this study were purchased from Qingzhou Yalong Plastic Industry Co., Ltd., Qingzhou, China. The plastic mulch films were black in color, 0.01 mm thick, and 1.2 m wide.

2.3. Experimental Design

Four treatments were established in the fixed-site (2018–2021) field trial: rice straw return (CK), rice straw return + microbial agent (T1), rice straw return + plastic mulch films (T2), and rice straw return + microbial agent + plastic mulch films (T3). For the four treatments, all of the rice straw was returned to the field and chopped into a size ranging between 5 and 10 cm using a rotary tiller in late December in 2018, 2019, and 2020. The dosage of the microbial agent was 30 kg/ha, broadcasted uniformly before soil tillage. The ridge was covered with plastic mulch films after soil ridging and removed 30 days after transplanting. Each treatment was repeated three times, with each plot area measuring 30 m^2.

After 3 years of continuous treatments, the soil samples were collected on 12 April 2021 (30 days after transplanting). From three plots, soil samples (0–20 cm) were collected for each treatment, and the loose soil was shaken from the dug-out tobacco plants. Then, these samples were mixed fully for one replication of the treatment and divided into two equal parts. In one part, the soil physical and chemical characteristics were determined naturally at room temperature, and in the other, soil microbial diversity was determined by storing the sample at a temperature of −80 °C.

2.4. Chemical and Physical Analyses

Using a ZD-2000 environmental temperature detector (Taizhou Zhengda Science and Education Equipment Factory, Taizhou, China), the soil temperature in different cultivation layers was measured with a temperature probe buried in the soil. The basic physical and chemical properties of the soil were evaluated according to the method described by Bao [28]. The soil water content was determined using the drying method. The soil pH was measured using the potentiometric method. The potassium dichromate method and the Kjeldahl method were used to determine the total organic carbon and total nitrogen contents.

2.5. Soil DNA Extraction and Gene Sequencing

A DNA extraction kit (E.Z.N.A.® Stool DNA Kit, Omega, Inc., Norcross, GA, USA) was used to isolate DNA from various samples. The 16S rRNA gene regions V3 and V4 were amplified using the primer sets 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). A slightly modified version of primers ITS1FI2 (5′-GTGARTCATCGAATCTTTG-3′) and ITS2 (5′-TCCTCCGCTTATTGATATGC-3′) was used to amplify the ITS2 region of the eukaryotic (fungi) small-subunit rRNA gene [29]. PCR amplification was performed under the following conditions: an initial

denaturation at 98 °C for 30 s; 32 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s, and extension at 72 °C for 45 s; and then final extension at 72 °C for 10 min. PCR products were confirmed via electrophoresis on 2% agarose gels. Qubit (Invitrogen, Carlsbad, CA, USA) was used to quantify the PCR products after purification with AM Pure XT beads (Beckman Coulter Genomics, Danvers, MA, USA). Sequencing of the amplicon pools and the assessment of library size and quantity were conducted using the Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and the Kapa Biosciences Library Quantification Kit for Illumina (Woburn, MA, USA). The libraries were sequenced on the NovaSeq PE250 platform.

2.6. Statistical Analysis

LC-Bio sequenced the samples using an Illumina NovaSeq platform according to the manufacturer's recommendations. By removing the barcode and primer sequence, paired-end readings were assigned to samples based on their unique barcodes. Paired-end reads were merged using Pear. According to Fqtrim (v0.94), raw reads were quality-filtered in accordance with specific filtering conditions. A chimeric sequence was filtered with Vsearch software (v2.3.4). DADA2 was used to de-replicate the feature table and feature sequence.

Using QIME2 software (2020.8), alpha diversity values were calculated for bacterial and fungal communities within the soil samples and evaluated with Chao1 and Shannon diversity indices. As a measure of genus complexity, the distance matrix of QIIME2 was used to perform beta diversity analysis between the samples [30]. The data were then subjected to principal component analysis (PCA) using Origin 8.1.

SPSS 19.0 was used for all statistical analyses, whereas one-way ANOVA and multiple comparative analysis (Duncan) were used for the significance level of the soil physicochemical properties and bacterial and fungal diversity. Origin 8.1 was used to analyze the bacterial and fungal OTUs in the different soil samples and perform a correlation heatmap analysis. The relationship between the soil physicochemical properties and the microbial community was examined with Canoco 4.5 using redundancy analysis (RDA).

3. Results

3.1. Effects of Different Treatments on Soil Physicochemical Properties

The soil temperature of T3 was significantly increased by 22.97% and 17.76%, respectively, compared with CK in the 0–10 cm and 20–30 cm soil tillage layers (Figure 1a). The soil temperature of T2 significantly increased by 15.05% compared with CK in the 0–10 cm soil tillage layer (Figure 1a). Moreover, T2 and T3 also significantly increased the water content by 46.23% and 34.26%, respectively, compared with CK in the 10–20 cm layer (Figure 1b). The results indicate that plastic mulch films can increase the soil temperature and water content.

The TOC content of T1 and T3 significantly increased compared with CK and T2 in the four different soil tillage layers (Figure 1c). The TOC content of T2 had no significant effect in the 0~10 cm soil tillage layer but notably increased in the 10–40 cm soil tillage layer compared with CK (Figure 1c). The TOC content of T3 in the four different soil tillage layers increased by 15.18%, 14.33%, 18.25%, and 21.56% compared with the CK, respectively (Figure 1c). The TN content of T1, T2, and T3 notably increased compared with CK in the four different soil tillage layers (Figure 1d). Moreover, the TN content of T3 was significantly higher than in the other treatments, with an increase of 49.12%, 44.92%, 40.76%, and 14.87% compared with CK, respectively (Figure 1d). After 3 years of continuous treatment, the soil TOC and TN contents were increased by the straw return with the application of plastic mulch films (Figure 1e). Microorganisms grow better in soil with an appropriate C/N ratio. The C/N ratio of T2 significantly increased in the 0–30 cm soil tillage layer compared with T1 (Figure 1e).

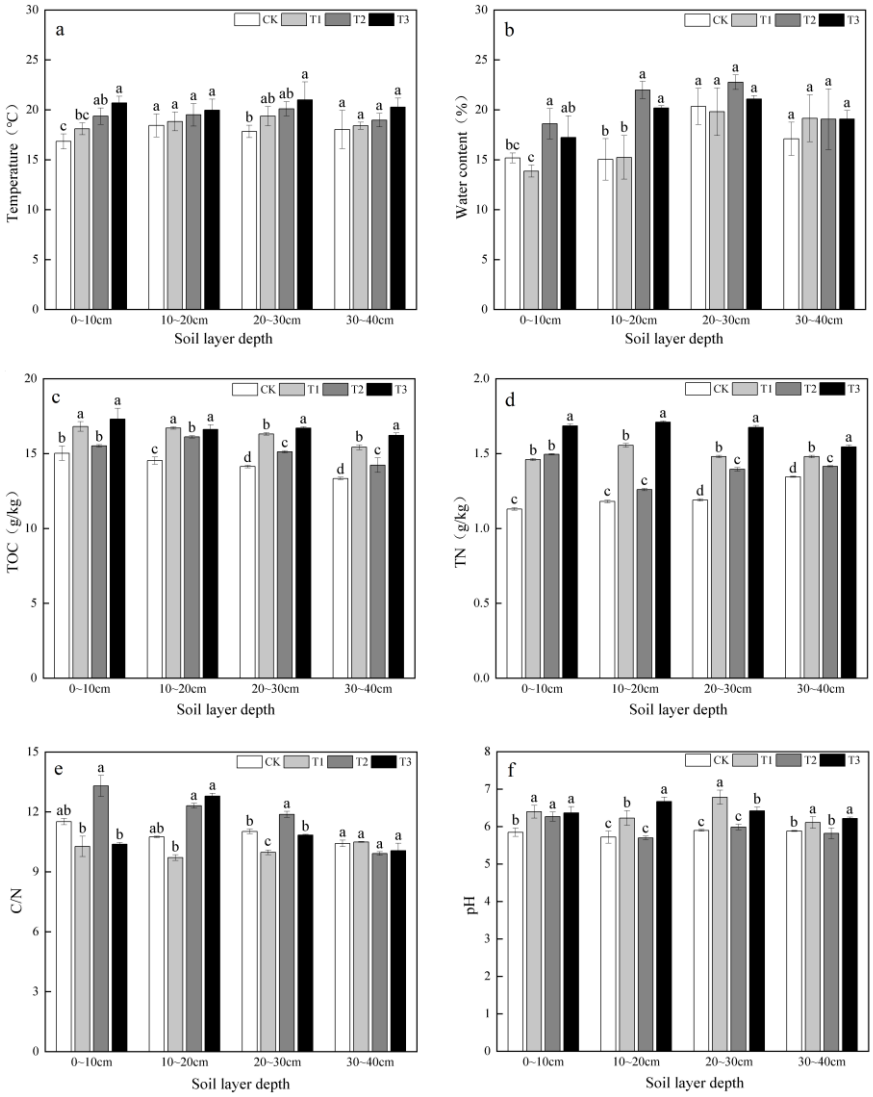

Figure 1. Effects of different treatments on soil temperature (**a**), water content (**b**), TOC (**c**), TN content (**d**), C/N ratio (**e**), and pH (**f**) under different soil layer depths. Different letters (a, b, c, d, ab and bc) indicate significant differences between different treatments according to one-way ANOVA (Duncan, $p < 0.05$), the same as below.

The soil pH values of T1, T2, and T3 significantly increased by 9.41%, 7.18%, and 8.83%, respectively, compared with CK in the 0–10 cm soil tillage layer (Figure 1f). There was no significant difference between T2 and CK in soil pH in the 10–40 cm soil tillage layer, but T1 and T3 did significantly increase the soil pH values in the 10–40 cm soil tillage layer (Figure 1f). The results show that the microbial agents significantly improved the soil acidification issues, while the plastic mulch films had no significant effect on soil pH, suggesting that the application of microbial agents could supply a suitable soil environment for plants.

3.2. Predominant Bacterial and Fungal Communities at the Phylum and Genus Levels of Different Treatments

Proteobacteria, *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, *Gemmatimonadetes*, and *Planctomycetes* were the main bacterial categories in the soil samples at the phylum level (Figure 2a). The relative abundance of *Proteobacteria* was 24.57–29.44%, which was the highest, followed by *Acidobacteria* and *Chloroflexi*, with relative abundances of 17.40–26.46% and 11.79–30.33%, respectively (Figure 2a). The T1, T2, and T3 treatments notably increased the relative abundance of *Acidobacteria*, which increased by 33.57%, 52.03%, and 36.88%, respectively,

but the relative abundance of *Chloroflexi* decreased by 61.14%, 58.66%, and 53.60%, respectively (Figure 2a). The relative abundance of *Actinobacteria* in all treatments was as follows: CK > T3 > T1 > T2. The highest relative abundance of soil fungal communities at the phylum level was *Ascomycota* (63.68–33.26%), followed by *Basidiomycota* (13.89–30.38%) and *Mortierellomycota* (9.09–16.77%) (Figure 2b). The relative abundance of *Ascomycota* was in the following order: CK > T3 > T1 > T2 (Figure 2b). The relative abundance of *Basidiomycota* was the highest in T2 (30.38%) and lowest in CK (13.89%), while the relative abundance of *Mortierellomycota* and *Zygomycota* was higher in T1 and T2 compared with CK and T3 (Figure 2b).

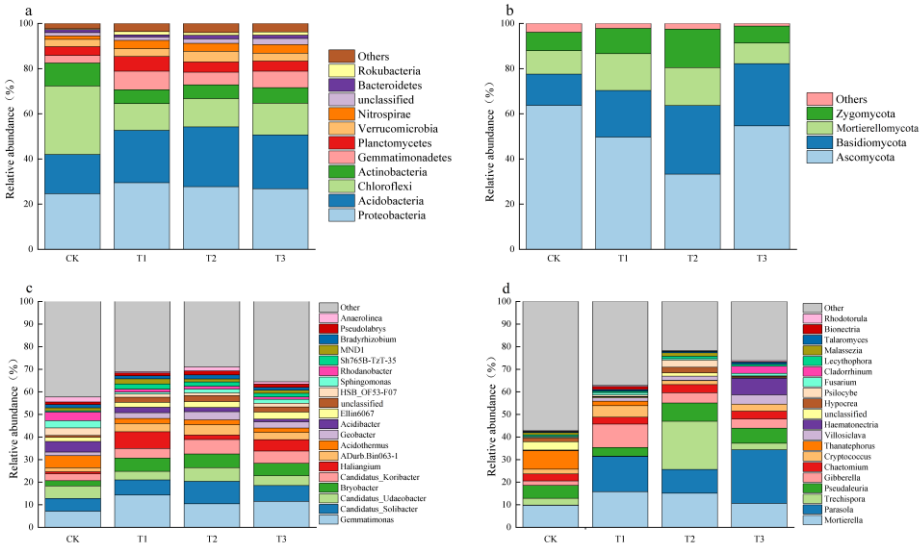

Figure 2. Composition and relative abundance of the bacterial communities at the phylum level (**a**), fungal communities at the phylum level (**b**), bacterial communities at the genus level (**c**), and fungal communities at the genus level (**d**).

The predominant bacterial communities in the different treatments at the genus level were *Gemmatimonas*, *Candidatus_Solibacter*, *Candidatus_Udaeobacter*, *Bryobacter*, *Candidatus_Koribacter*, and *Haliangium* (Figure 2c). Compared with the CK treatment, the relative abundance of the genera *Gemmatimonas*, *Candidatus_Solibacter*, *Bryobacter*, *Candidatus_Koribacter*, and *ADurb.Bin063-1* increased significantly in the treatment groups ranging from 45.88% to 100.53%, 18.19% to 75.93%, 134.44% to 158.95%, 38.49% to 110.36%, and 79.17% to 153.16%, respectively; however, the relative abundance of *Acidothermus*, *Acidibacter*, *HSB_OF53-F07*, *Sphingomonas*, and *Rhodanobacter* in the treatment groups significantly decreased by 58.09–64.24%, 48.30–75.60%, 51.16–65.47%, 45.52–74.15%, and 66.28–73.76%, respectively (Figure 2c). In addition, the predominant fungal genera were *Mortierella, Parasol, Trechispora, Pseudaleuria*, and *Gibberella* (Figure 2d). The relative abundance levels of *Parasola* in CK, T1, T2, and T3 were 0.07%, 10.92%, 7.36%, and 16.63%, respectively (Figure 2d). The relative abundance of *Gibberella* significantly increased by 106.91–426.20%, while the relative abundance of *Thanatephorus* decreased by 76.23–99.53% in the treatment groups (Figure 2d).

3.3. Richness and Diversity of Bacterial and Fungal Communities in Different Soil Treatment Samples

Soil bacterial diversity and richness are important indicators of soil quality and play a significant role in soil health [31,32]. In the results for all treatments, the numbers of clean tags and OTUs of soil bacteria were 62,728.33–67,734.33 and 3644.30–52,912.50, respectively (Table 1). In addition, the numbers of clean tags and OTUs of soil fungus were 81,289.33–78,691.33 and 370.70–498.50 (Table 1). The clean tags of bacteria in CK were significantly fewer than those in T3, while the number of clean tags of bacteria in T3 was the highest (Table 1). The number of OTUs, Chao1 index, and Shannon index of T3 were significantly higher than those of CK, while the number of OTUs, Chao1 index, and Shannon index of bacteria in T3 were the

highest among the different treatments (Table 1). However, there was no clear difference in the number of clean tags of soil fungus among the different treatments. Compared to the CK treatment, the number of OTUs of soil fungus noteworthily increased by 34.34% and 34.47% under the T1 and T3 treatments (Table 1). In addition, the Chao1 and Shannon index values of fungus in T1 and T3 were significantly higher than those in CK ($p < 0.05$) (Table 1). The specialization percentages of T1, T2, T3, and CK were 17.65%, 22.18%, 28.64%, and 16.49%, respectively, in the bacterial community (Figure 3). In the fungal community, the specialization percentages of T1 and T2 were 22.22% and 16.43%, respectively (Figure 3). The specialization percentage of T1 was higher, which indicated that the microbial agents had a greater effect on the soil microorganisms.

Table 1. Effects of composting on the richness and diversity of the bacterial and fungal communities in the soil.

	Treatment	Clean Tags	OTUS	Chao1	Shannon
Bacteria	CK	62,728.33 ± 206.00 b	2912.50 ± 20.50 b	2668.20 ± 58.90 b	9.80 ± 0.30 b
	T1	66,010.00 ± 267.59 ab	3040.30 ± 87.20 b	3073.10 ± 75.70 ab	10.40 ± 0.30 a
	T2	65,664.00 ± 115.84 ab	3472.30 ± 83.60 a	3520.70 ± 22.20 ab	10.50 ± 0.10 a
	T3	67,734.33 ± 108.62 a	3644.30 ± 28.10 a	3664.80 ± 44.10 a	10.60 ± 0.10 a
Fungi	CK	80,622.33 ± 45.46 a	370.70 ± 13.70 b	372.10 ± 14.20 b	5.20 ± 0.10 b
	T1	79,248.67 ± 167.19 a	498.00 ± 49.50 a	499.60 ± 50.30 a	5.80 ± 0.40 a
	T2	78,691.33 ± 65.55 a	399.00 ± 33.90 b	399.60 ± 34.80 b	5.80 ± 0.50 a
	T3	81,289.33 ± 108.23 a	498.50 ± 12.30 a	498.60 ± 12.50 a	6.10 ± 0.60 a

Different letters (a, b, and ab) indicate significant differences between different treatments according to one-way ANOVA (Duncan, $p < 0.05$).

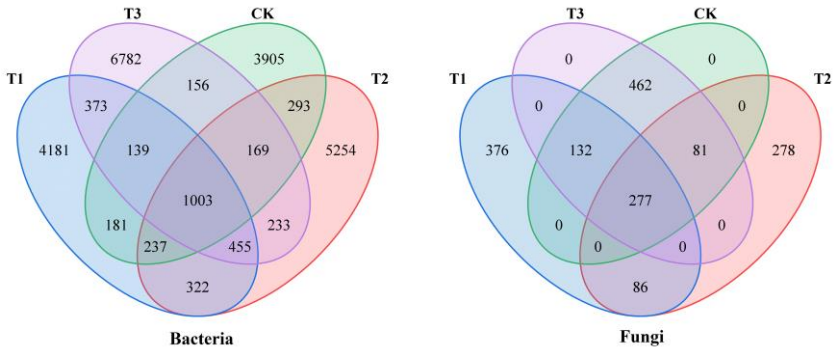

Figure 3. Venn diagrams of bacterial and fungal OTUs detected in soil from different treatments.

3.4. Effects of Different Treatments on Bacterial and Fungal Communities

Principal component analysis (PCA) was used to compare and determine the structure and differences between microbial communities. The closer the distance in the PCA chart, the more similar the samples were. Principal Component 1 (PC1) and Principal Component 2 (PC2) explained 65.97% and 16.36% of the total bacterial variation, respectively (Figure 4a). Compared with CK, the T1, T2, and T3 treatments significantly differed in bacterial analyses (Figure 4a), indicating that plastic mulch films as well as the addition of microbial agents can alter soil bacterial community composition, especially after microbial agents are added. Principal Component 1 (PC1) and Principal Component 2 (PC2) explained 39.12% and 15.16% of the total fungal variation, respectively (Figure 4b). A free separation of the T3 treatment from other treatments was observed based on the results of the fungal analyses (Figure 4b), indicating that the addition of microbial agents could significantly change the fungal community composition.

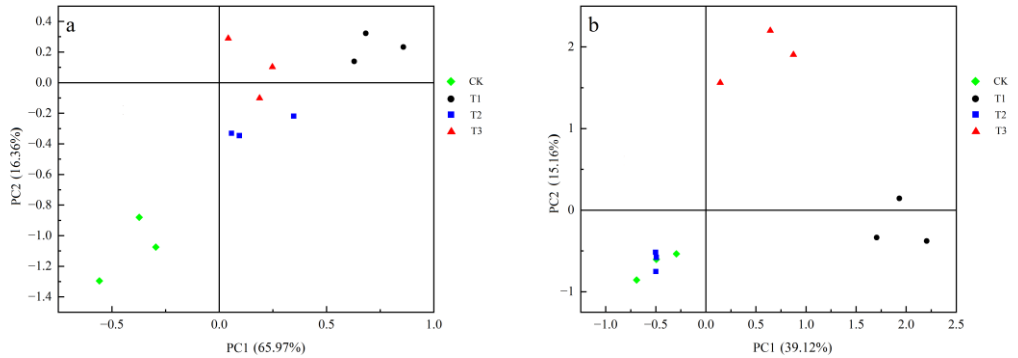

Figure 4. Principal component analysis of (**a**) bacterial and (**b**) fungal communities in the soil sample.

3.5. Relationship between Predominant Soil Environmental Factors and Soil Microbial Community

Redundancy analysis (RDA) was used to examine the relationship between the soil microflora and various environmental factors. There is a cumulative explanatory variable for bacterial abundance distribution on the first and second axes that accounts for 46.11% and 22.29% of the variance, respectively, of the total variance (Figure 5a). The TOC content explained 40.8% of the total variation, suggesting that it was an important factor in the soil microbiome structure (Figure 5a). In the first and second axes, the cumulative explanatory variables in the fungal abundance distribution represent 37.68% and 9.63% of the total variation, respectively (Figure 5b). Among the fungal samples, water content (WC) correlated most strongly with community composition, which explained 19.5% of the total variation (Figure 5b). There is a significant positive correlation between soil temperature and the abundance of *Candidatus_Solibacter* and a significant negative correlation between soil water content and the abundance of *Acidothermus* (Figure 6). The soil pH is significantly positively correlated with the abundance of *Gemmatimonas*, *Haliangium*, and *Cryptococcus* and significantly negatively correlated with the abundance of *Candidatus_Udaeobacter* (Figure 6). Soil TOC and TN content are significantly positively correlated with the abundance of *Gemmatimonas*, *Bryobacter*, and *Haliangium* and significantly negatively correlated with the abundance of *Acidothermus* (Figure 6).

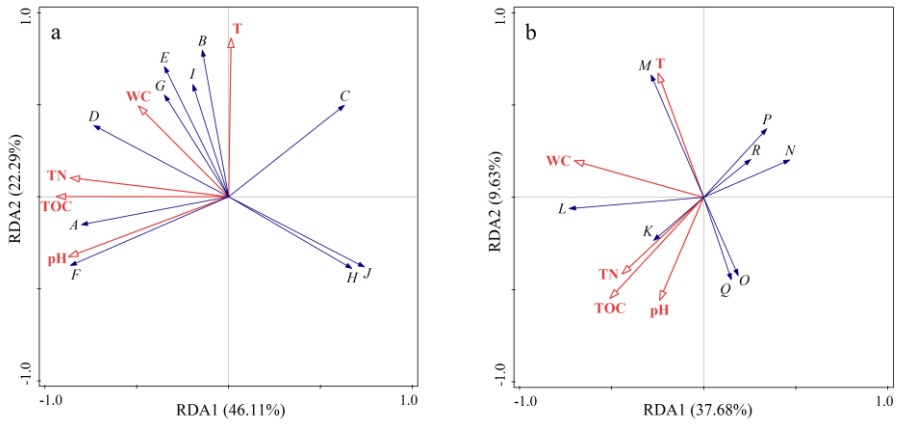

Figure 5. Redundancy analysis indicating the impacts of various environmental factors on the (**a**) bacterial and (**b**) fungal community structures. For (**a**), the blue lines represent the bacterial genus, and the red lines represent soil environmental factors. For (**b**), the blue lines represent the fungal genus, and the red lines represent soil environmental factors. A: *Gemmatimonas*, B: *Candidatus_Solibacter*, C: *Candidatus_Udaeobacter*, D: *Bryobacter*, E: *Candidatus_Koribacter*, F: *Haliangium*, G: *ADurb.Bin063-1*, H: *Acidothermus*, I: *Geobacter*, J: *Acidibacter*, K: *Mortierella*, L: *Parasola*, M: *Trechispora*, N: *Pseudaleuria*, O: *Gibberella*, P: *Chaetomium*, Q: *Cryptococcus*, R: *Thanatephorus* (the same in the figure below).

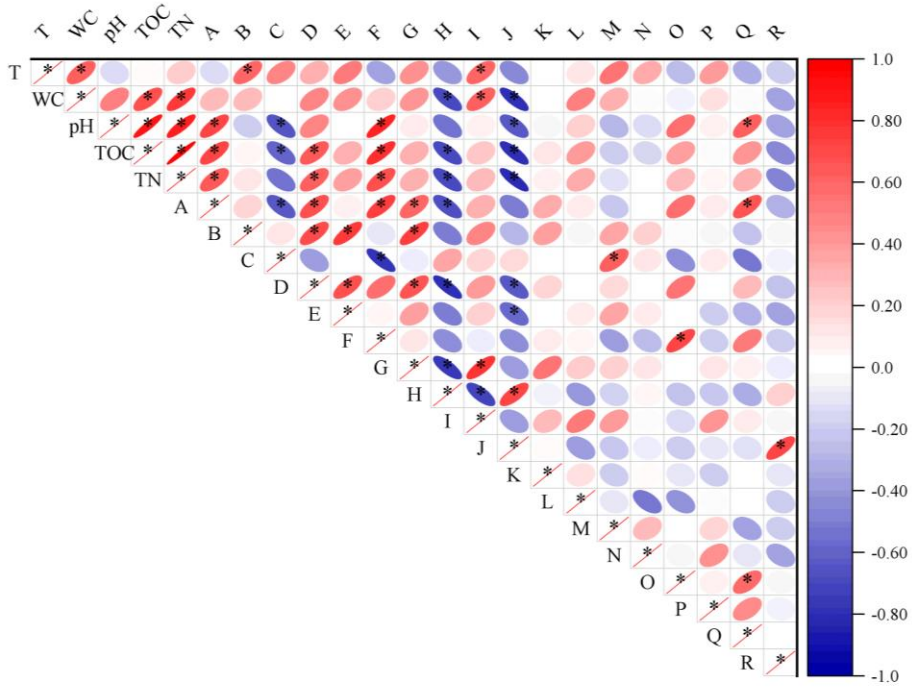

Figure 6. Correlation heatmap between environmental factors and dominant bacterial and fungal phyla. * $p < 0.05$.

4. Discussion

4.1. Effect of Microbial Agents and Mulch Film Covering on the Physicochemical Properties of Soil

In this study, we aimed to investigate the effect of measures to promote rice straw decomposition (including microbial agents and plastic mulch films) on soil physicochemical properties and the structure of the microbial community. Microorganisms not only play an important role in straw decomposition [12] but also have an impact on soil physicochemical properties. The results of this study indicate that the addition of microbial agents mainly composed of *Bacillus*, filamentous fungi, and lactic acid bacteria significantly increased the soil pH from 5.83 to 6.42. This may be related to microorganisms like *Bacillus* subtilis producing alkaline metabolites such as ammonia and carbonate, which can neutralize acidic substances in the soil and increase the pH value [33,34]. Luo et al. [35] showed that film mulching can improve the physical and chemical properties of soil, such as by increasing soil moisture. This is consistent with the results of this study. Temperature has a significant impact on straw decomposition, and the in situ decomposition rate of straw is often slow under low-temperature conditions [36]. It has been reported that the microbial straw decomposition additive can raise the environmental temperature, which improves the decomposition effect of straw [37,38]. This study found that the application of microbial agents and plastic mulch films increased the soil temperature significantly, likely due to the activity of the microbial agents, while the mulch film reduced heat loss. The results show that the measures to promote straw decomposition could also increase TOC and TN content, with these increases potentially being the result of the positive role of organic matter decomposition and the nitrogen cycling of the microbial agents [39] (Figure 1). The return of straw to the soil can enhance soil microorganism activities and N fixation by altering soil C/N ratios [40]. Additionally, mulch film covering provided a high-humidity soil environment, which was more conducive to promoting straw decomposition.

4.2. Effects of Microbial Agents and Mulch Film Covering on the Bacterial and Fungal Communities

Soil microbial communities are important indicators of soil quality [32] and are affected by agricultural measures [41]. Our results show that the application of microbial agents and mulch film significantly increased the number of clean tags, number of OTUs,

Chao1 index, and Shannon index of the bacteria and fungi in the rhizosphere soil of tobacco plants (Table 1). *Bacillus* subtilis is a type of probiotic that produces enzymes and can secrete many types of degradative enzymes, including cellulase [42]. The study results indicate that the addition of microbial agents mainly composed of *Bacillus*, filamentous fungi, and lactic acid bacteria significantly increased the relative abundance of *Acidobacteria* and *Gemmatimonadetes* (Figure 2a). *Acidobacteria* and *Gemmatimonadetes* are two bacterial phyla that are commonly found in soil environments. *Acidobacteria* has been identified as one of the dominant phyla in various soil types, including sod-podzolic, dark-gray, typical chernozem, brown soil, and meadow–chestnut soil [43]. They play a role in nutrient cycling, and their abundance decreases with soil depth [43]. *Gemmatimonadetes* bacteria, on the other hand, are abundant in agricultural soils and have been shown to reduce N_2O gas emissions [44]. They have also been detected in various types of soil samples and are believed to be involved in N_2O reduction in agricultural soils [45]. The incorporation of straw with plastic film mulch significantly affects soil bacterial community structures [15,46]. Plastic film mulch alone has a negative effect on soil bacterial richness [47]. However, straw incorporation has a positive effect on bacterial diversity [13]. The combination of straw and plastic film mulch increases the abundance of *Gemmatimonas*, *Bryobacter*, and *Parasol* at the genus level in the soil. Plastic film mulching has been found to have varying effects on the abundance and composition of fungal communities. *Ascomycota* has been observed to decrease with plastic film mulching [48], while *Basidiomycota* has been observed to increase [49].

4.3. Relationship between Bacterial and Fungal Communities and Soil Physicochemical Properties

It is important to note that the structure of a soil microbiome is heavily influenced by its environment, and interactions within a microbiome and with the environment are crucial to shaping its diversity [50,51]. The results of this study showed a correlation between the main microbial community structure after the application of microbial agents with mulching and the environmental factors related to these samples. In addition, further analysis revealed a significant correlation between microbial abundance and soil pH, organic matter, moisture, and other physicochemical characteristics. Soil organic carbon has significant effects on bacterial community structure. Changes in the composition of organic carbon during forest succession also contribute to shifts in the soil bacterial community structure [52]. Straw amendment increases the TOC content and alters the soil bacterial community composition [53]. Using RDA and correlation heatmaps, we found that soil TOC content had the greatest impact on the bacterial communities, particularly *Gemmatimonas*, and water content had a profound effect on fungi (Figures 5 and 6). This is because *Gemmatimonas* bacteria are involved in the degradation and cycling of organic matter in the soil [54]. An increased soil water content promotes fungal growth and reproduction, as moist environments are more conducive to fungal survival [55]. In addition to affecting enzyme activity, microbial respiration rate, and substrate availability, temperature also affects the heterotrophic respiration of microorganisms [56,57].

5. Conclusions

The application of microbial agents and mulching to late rice straw in China tobacco–rice rotation areas can promote the straw's return to the field and improve soil physicochemical properties. The results demonstrated that straw return with microbial agents (T1) improved the pH of acidic soils, while straw return with mulching (T2) increased the soil moisture content. Additionally, straw return with microbial agents and mulching (T3) increased the soil temperature and soil TOC and TN content. The dominant phyla in the bacterial community structure were *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, *Actinobacteria*, *Gemmatimonadetes*, and *Planctomycetes*. The T1, T2, and T3 treatments increased *Acidobacteria* abundance but decreased *Chloroflexi* abundance compared to the CK treatment. For the fungal community, the T2 treatment significantly increased the relative abundance of *Basidiomycota* but decreased the relative abundance of *Ascomycota*. Moreover, the application

of microbial agents and mulching can significantly increase the richness and diversity of the bacterial and fungal communities. By employing redundancy analysis (RDA), our study determined that soil microorganisms are significantly influenced by the TOC content, pH, and water content. This study promotes the return of late rice straw to the field and provides a theoretical basis and technical support for improving crop planting soil.

Author Contributions: Conceptualization, X.D. and X.W.; methodology, J.H.; software, X.W.; formal analysis, B.X.; investigation, H.L.; resources, X.D.; data curation, J.H.; writing—original draft preparation, X.W.; writing—review and editing, L.Y. and Y.L.; supervision, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

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

References

1. Laudicina, V.A.; Ruisi, P.; Badalucco, L. Soil Quality and Crop Nutrition. *Agriculture* **2023**, *13*, 1412. [CrossRef]
2. Huang, J.; Li, R.; Chen, Z.; Zhang, B.; Lei, H.; Pan, R.; Yang, M.; Pan, M.; Tang, L. Effects of Straw Returning Techniques on Soil Nutrients, Organic Carbon and Microbial Diversity in Tobacco-rice Rotation System. *Chin. J. Rice Sci.* **2023**, *37*, 415–426.
3. Li, C.; Zhang, C. Effects of Straw Returning on Paddy Soil Environmental Remediation. In Proceedings of the ICESE 2022: Proceedings of the 2022 12th International Conference on Environment Science and Engineering, Beijing, China, 2–5 September 2022.
4. Xia, L.; Lam, S.; Wolf, B.; Kiese, R.; Chen, D.; Butterbach-Bahl, K. Trade-offs between soil carbon sequestration and reactive nitrogen losses under straw return in global agroecosystems. *Glob. Chang. Biol.* **2018**, *24*, 5919–5932. [CrossRef]
5. Yang, H.; Xu, M.; Li, Y.; Xu, C.; Zhai, S.; Liu, J. The impacts of ditch-buried straw layers on the interface soil physicochemical and microbial properties in a rice-wheat rotation system. *Soil Tillage Res.* **2020**, *202*, 104656. [CrossRef]
6. Hou, X.; Wu, P.; Wang, Y.; Li, P.; Wang, X.; Li, R. Effects of returning straw with nitrogen application on soil water and nutrient status, and yield of maize. *Chin. J. Appl. Ecol.* **2018**, *29*, 1928–1934.
7. Li, P.; Tong, H.; Han, Y.; Jiang, Y.; Wu, C. Effect of Straw Return, Directly or as Biochar, on Nitrifying Microbes in Fluvo-aquic Soil. *Acta Pedol. Sin.* **2019**, *56*, 1471–1481.
8. Martínez-Eixarch, M.; Alcaraz, C.; Viñas, M.; Noguerol, J.; Aranda, X.; Prenafeta-Boldú, F.-X.; Català-Forner, M.; Fennessy, M.S.; Ibáñez, C. The main drivers of methane emissions differ in the growing and flooded fallow seasons in Mediterranean rice fields. *Plant Soil* **2021**, *460*, 211–227. [CrossRef]
9. Chen, L.; Yao, B.; Peng, Y.; Qin, T.; Zhou, Y.; Sun, C. Effects of straw return and straw biochar on soil properties and crop growth: A review. *Front. Plant Sci.* **2022**, *13*, 986763. [CrossRef]
10. Teng, K.; Zhang, Q.; Peng, J.; Chen, Q.; Tian, M.; Chao, J. Effects of reductive soil disinfestation on chemical properties and microbial community structure of soils before and after tobacco planting. *Tob. Sci. Technol.* **2022**, *55*, 9–19.
11. Allsup, C.M.; George, I.; Lankau, R.A. Shifting microbial communities can enhance tree tolerance to changing climates. *Science* **2023**, *380*, 835–840. [CrossRef]
12. Gao, X.; Liu, W.; Li, X.; Zhang, W.; Bu, S.; Wang, A. A novel fungal agent for straw returning to enhance straw decomposition and nutrients release. *Environ. Technol. Innov.* **2023**, *30*, 103064. [CrossRef]
13. Li, Y.; Hu, Y.; Song, D.; Liang, S.; Qin, X.; Siddique, K.H. The effects of straw incorporation with plastic film mulch on soil properties and bacterial community structure on the loess plateau. *Eur. J. Soil Sci.* **2021**, *72*, 979–994. [CrossRef]
14. Tong, W.; Yang, M.; Wang, H.; Feng, X.; Zhang, L.; Zhou, B. Effects of tillage methods on fungal community structure in rhizosphere soil of flue-cured tobacco in mountainous tobacco fields. *Acta Tabacaria Sin.* **2021**, *27*, 56–63.
15. Liu, B.; Dai, Y.; Cheng, X.; He, X.; Bei, Q.; Wang, Y.; Zhou, Y.; Zhu, B.; Zhang, K.; Tian, X.; et al. Straw mulch improves soil carbon and nitrogen cycle by mediating microbial community structure and function in the maize field. *Front. Microbiol.* **2023**, *14*, 1217966. [CrossRef]
16. Cong, P.; Wang, J.; Li, Y.; Liu, N.; Dong, J.; Pang, H.; Zhang, L.; Gao, Z. Changes in soil organic carbon and microbial community under varying straw incorporation strategies. *Soil Tillage Res.* **2020**, *204*, 104735. [CrossRef]
17. Liu, G.; Bai, Z.; Shah, F.; Cui, G.; Xiao, Z.; Gong, H.; Li, D.; Lin, Y.; Li, B.; Ji, G.; et al. Compositional and structural changes in soil microbial communities in response to straw mulching and plant revegetation in an abandoned artificial pasture in Northeast China. *Glob. Ecol. Conserv.* **2021**, *31*, e01871. [CrossRef]
18. Bodenhausen, N.; Hess, J.; Valzano, A.; Deslandes-Hérold, G.; Waelchli, J.; Furrer, R.; Marcel, G.; van der Heijden, A.; Schlaeppi, K. Predicting soil fungal communities from chemical and physical properties. *J. Sustain. Agric. Environ.* **2023**, *2*, 225–237. [CrossRef]
19. Abid, A.A.; Zou, X.; Gong, L.; Castellano-Hinojosa, A.; Afzal, M.; Di, H.; Zhang, Q. Physicochemical variables better explain changes in microbial community structure and abundance under alternate wetting and drying events. *Agriculture* **2022**, *12*, 762. [CrossRef]

20. Gao, J.; Zhao, Q.; Chang, D.; Ndayisenga, F.; Yu, Z. Assessing the effect of physicochemical properties of saline and sodic soil on soil microbial communities. *Agriculture* **2022**, *12*, 782. [CrossRef]
21. Zou, X.; Yao, K.; Zeng, F.; Zhang, C.; Zeng, Z.; Zhang, H. Diversity and Assembly of Bacteria Community in Lime Soil under Different Karst Land-Use Types. *Forests* **2023**, *14*, 672. [CrossRef]
22. Li, J.; Bates, K.A.; Hoang, K.L.; Hector, T.E.; Knowles, S.C.; King, K.C. Experimental temperatures shape host microbiome diversity and composition. *Glob. Chang. Biol.* **2023**, *29*, 41–56. [CrossRef]
23. Yuan, X.; Li, C.; Tang, Y.; Chen, Z.; Huang, C. The effects of functional microbial agents on the soil microbial communities of high-frigid grassland under desertification in Northwest Sichuan. *Bot. Serbica* **2022**, *46*, 259–268. [CrossRef]
24. Xu, Z.; Wallach, R.; Song, J.; Mao, X. Effect of Plastic Film Colours and Perforations on Energy Distribution, Soil Temperature, and Evaporation. *Agronomy* **2023**, *13*, 926. [CrossRef]
25. Yang, B.; Feng, L.; Li, X.; Yang, G.; Ma, Y.; Li, Y. Effects of plastic film mulching on the spatiotemporal distribution of soil water, temperature, and photosynthetic active radiation in a cotton field. *PeerJ* **2022**, *10*, e13894. [CrossRef]
26. Zhang, Z.; Li, N.; Sun, Z.; Yin, G.; Zhang, Y.; Bai, W.; Feng, L.; Yang, J. Fall Straw Incorporation with Plastic Film Cover Increases Corn Yield and Water Use Efficiency under a Semi-Arid Climate. *Agriculture* **2022**, *12*, 2151. [CrossRef]
27. Shao, J.; Gao, C.; Afi Seglah, P.; Xie, J.; Zhao, L.; Bi, Y.; Wang, Y. Analysis of the available straw nutrient resources and substitution of chemical fertilizers with straw returned directly to the field in China. *Agriculture* **2023**, *13*, 1187. [CrossRef]
28. Bao, S.D. *Soil Agro-Chemistry Analysis*, 3rd ed.; China Agriculture Press: Beijing, China, 2000; pp. 14–114.
29. Karlsson, I.; Friberg, H.; Steinberg, C.; Persson, P. Fungicide effects on fungal community composition in the wheat phyllosphere. *PLoS ONE* **2014**, *9*, e111786. [CrossRef]
30. Schloss, P.D.; Gevers, D.; Westcott, S.L. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA-based studies. *PLoS ONE* **2011**, *6*, e27310. [CrossRef]
31. Kou, Z.; Zhou, X. Variation of soil bacterial community in tobacco field after different years of continuous monocropping. *J. Plant Nutr. Fertil.* **2020**, *26*, 511–521.
32. Chen, D.; Wang, X.; Carrion, V.J.; Yin, S.; Yue, Z.; Liao, Y.; Dong, Y.; Li, X. Acidic amelioration of soil amendments improves soil health by impacting rhizosphere microbial assemblies. *Soil Biol. Biochem.* **2022**, *167*, 108599. [CrossRef]
33. Mahapatra, S.; Yadav, R.; Ramakrishna, W. Bacillus subtilis impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. *J. Appl. Microbiol.* **2022**, *132*, 3543–3562. [CrossRef] [PubMed]
34. Hanafiah, A.S.; Haniv, Y.; Hidayat, B. Increased soil pH due to the application of sulfate-reducing bacteria, rhizobia and amendments to acid sulfate soils planted with soybeans. *IOP Conf. Ser. Earth Environ. Sci.* **2022**, *977*, 012011. [CrossRef]
35. Luo, C.L.; Zhang, X.F.; Duan, H.X.; Mburu, D.M.; Ren, H.X.; Kavagi, L.; Dai, R.; Xiong, Y.C. Dual plastic film and straw mulching boosts wheat productivity and soil quality under the El Nino in semiarid Kenya. *Sci. Total Environ.* **2020**, *738*, 139808. [CrossRef] [PubMed]
36. Zhang, X.; Borjigin, Q.; Gao, J.; Yu, X.; Zhang, B.; Hu, S.; Han, S.; Liu, R.; Zhang, S. Community succession and straw degradation characteristics using a microbial decomposer at low temperature. *PLoS ONE* **2022**, *17*, e0270162. [CrossRef] [PubMed]
37. Song, K.; Zhou, Z.; Leng, J.; Fang, S.; Zhou, C.; Ni, G.; Kang, L.; Yin, X. Effects of rumen microorganisms on the decomposition of recycled straw residue. *J. Zhejiang Univ.-Sci. B* **2023**, *24*, 336–344. [CrossRef] [PubMed]
38. Li, Y.; Zhang, G.; Xu, P.; Zhou, S.; Li, Y.; Ma, L.; Yang, Z.; Wu, Y. Effects of Exogenous Bacterial Agents on Material Transformation and Microbial Community Composition during Composting of Tomato Stalks. *Sustainability* **2022**, *14*, 16284. [CrossRef]
39. Zuber, S.M.; Behnke, G.D.; Nafziger, E.D.; Villamil, M.B. Carbon and nitrogen content of soil organic matter and microbial biomass under long-term crop rotation and tillage in Illinois, USA. *Agriculture* **2018**, *8*, 37. [CrossRef]
40. Wang, Y.; Qin, M.; Zhan, M.; Liu, T.; Yuan, J. Straw return-enhanced soil carbon and nitrogen fractions and nitrogen use efficiency in a maize–rice rotation system. *Exp. Agric.* **2024**, *60*, e5. [CrossRef]
41. Bakker, P.A.; Berendsen, R.L.; Van Pelt, J.A.; Vismans, G.; Yu, K.; Li, E.; Bentum, S.V.; Poppeliers, S.W.; Gil1, J.S.; Zhang, H.; et al. The soil-borne identity and microbiome-assisted agriculture: Looking back to the future. *Mol. Plant* **2020**, *13*, 1394–1401. [CrossRef]
42. Su, Y.; Liu, C.; Fang, H.; Zhang, D. Bacillus subtilis: A universal cell factory for industry, agriculture, biomaterials and medicine. *Microb. Cell Factories* **2020**, *19*, 173. [CrossRef]
43. Oshiki, M.; Toyama, Y.; Suenaga, T.; Terada, A.; Kasahara, Y.; Yamaguchi, T.; Araki, N. N_2O reduction by Gemmatimonas aurantiaca and potential involvement of Gemmatimonadetes bacteria in N_2O reduction in agricultural soils. *Microbes Environ.* **2022**, *37*, ME21090. [CrossRef] [PubMed]
44. Muneer, M.A.; Hou, W.; Li, J.; Huang, X.; Kayani, M.; Cai, Y.; Yang, W.; Wu, L.; Ji, B.; Zheng, C. Soil pH: A key edaphic factor regulating distribution and functions of bacterial community along vertical soil profiles in red soil of pomelo orchard. *BMC Microbiol.* **2022**, *22*, 38. [CrossRef] [PubMed]
45. Zeng, Y.; Koblížek, M. Phototrophic Gemmatimonadetes: A new "purple" branch on the bacterial tree of life. In *Modern Topics in the Phototrophic Prokaryotes: Environmental and Applied Aspects*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 163–192.
46. Dou, Y.; Wen, M.; Yang, C.; Zhao, F.; Ren, C.; Zhang, N.; Liang, Y.; Wang, J. Effects of straw and plastic film mulching on microbial functional genes involved in soil nitrogen cycling. *Front. Microbiol.* **2023**, *14*, 1205088. [CrossRef]
47. Muñoz, K.; Thiele-Bruhn, S.; Kenngott, K.G.; Meyer, M.; Diehl, D.; Steinmetz, Z.; Schaumann, G.E. Effects of plastic versus straw mulching systems on soil microbial community structure and enzymes in strawberry cultivation. *Soil Syst.* **2022**, *6*, 21. [CrossRef]

48. Hu, Z.E.; Xiao, M.L.; Ding, J.N.; Ji, J.H.; Chen, J.P.; Ge, T.D.; Lu, S.B. Response characteristics of soil microbial community under long-term film mulching. *Huan Jing Ke Xue* **2022**, *43*, 4745–4754. [PubMed]
49. Zhang, W.; Ma, J.; Cui, Z.; Xu, L.; Liu, Q.; Li, J.; Wang, S.; Zeng, X. Effects of Biodegradable Plastic Mulch Film on Cabbage Agronomic and Nutritional Quality Traits, Soil Physicochemical Properties and Microbial Communities. *Agronomy* **2023**, *13*, 1220. [CrossRef]
50. Fox, A.; Widmer, F.; Lüscher, A. Soil microbial community structures are shaped by agricultural systems revealing little temporal variation. *Environ. Res.* **2022**, *214*, 113915. [CrossRef] [PubMed]
51. El Amrani, B. Aspects of the rhizospheric microbiota and their interactions with the soil ecosystem. *Vavilov J. Genet. Breed.* **2022**, *26*, 442.
52. Zhang, Q.; Wang, X.; Zhang, Z.; Liu, H.; Liu, Y.; Feng, Y.; Yang, G.; Ren, C.; Han, X. Linking soil bacterial community assembly with the composition of organic carbon during forest succession. *Soil Biol. Biochem.* **2022**, *173*, 108790. [CrossRef]
53. Fan, W.; Yuan, J.; Wu, J.; Cai, H. Effects of straw maize on the bacterial community and carbon stability at different soil depths. *Agriculture* **2023**, *13*, 1307. [CrossRef]
54. Mujakić, I.; Piwosz, K.; Koblížek, M. Phylum Gemmatimonadota and its role in the environment. *Microorganisms* **2022**, *10*, 151. [CrossRef] [PubMed]
55. Długosz, J.; Piotrowska-Długosz, A.; Breza-Boruta, B. The effect of differences in soil water content on microbial and enzymatic properties across the soil profiles. *Ecohydrol. Hydrobiol.* **2023**, *6*, 10. [CrossRef]
56. García, F.C.; Clegg, T.; O'Neill, D.B.; Warfield, R.; Pawar, S.; Yvon-Durocher, G. The temperature dependence of microbial community respiration is amplified by changes in species interactions. *Nat. Microbiol.* **2023**, *8*, 272–283. [CrossRef]
57. Kumar, S.; Najar, I.N.; Sharma, P.; Tamang, S.; Mondal, K.; Das, S.; Sherpa, M.T.; Thakur, N. Temperature–A critical abiotic paradigm that governs bacterial heterogeneity in natural ecological system. *Environ. Res.* **2023**, *234*, 116547. [CrossRef]