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Effects of Microbial Inoculants Combined with Maize Straw on Physicochemical Properties and Microbial Community Structure in Black Soil During Soybean Growth

Tingting Hou [1], Chao Jiang [2], Xiangxiang Wang [2], Enyue Fan [2], Tingyu Zhang [2], Jiabao Zhang [3] and Liqiang Meng [2,*]

[1] Institute of Soil and Water Resources and Environmental Science, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China; htt12022023@163.com
[2] Institute of Microbiology, Heilongjiang Academy of Sciences, 68 Zhaolin Street, Harbin 150010, China; jiangchao.80@163.com (C.J.); 18946115901@163.com (X.W.); 13314660731@163.com (E.F.); 18846198063@163.com (T.Z.)
[3] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 211135, China; jbzhang@issas.ac.cn
* Correspondence: mengliqiang83420@163.com

Abstract

Purpose: Black soils in Northeast China are declining in fertility under intensive fertilization, motivating strategies that integrate crop residue return with microbial inoculation. We conducted a field experiment to test whether maize straw return combined with compound microbial inoculants improves soil properties, bacterial communities, and soybean performance. **Methods**: A field experiment compared four treatments: fertilization alone (F), fertilization + inoculants (CF), fertilization and straw (SF), and fertilization and straw with inoculants (CSF). Soil physicochemical properties, enzyme activities, 16S rDNA-based bacterial communities, and soybean agronomic yield were measured across growth stages. **Results**: CSF produced the highest soybean performance, and increased yield by 3.91–5.46% compared with F. CSF increased soil pH, moisture, and nutrient availability (notably available P and K) and enhanced sucrase, urease, catalase, and acid phosphatase activities compared with other treatments. Bacterial communities were dominated by Acidobacteriota and Proteobacteria. CSF increased bacterial abundance and shifted community composition, and pH and available P were key factors associated with community variation. **Conclusions**: Co-applying maize straw and compound microbial inoculants enhances soybean yield while improving soil biochemical functioning and reshaping bacterial communities in black soil.

Keywords: black soil; compound microbial inoculants; physicochemical; enzymes; bacterial community

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1. Introduction

Black soils are among the most fertile agricultural soils worldwide and play a critical role in sustaining crop production in Northeast China [1]. However, decades of intensive cultivation and over-reliance on chemical fertilizers have led to a marked degradation trend in these soils, manifesting as a persistent decline in soil organic matter (SOM) content, increasing acidification, deterioration of physical structure, and weakened biological activity [2]. Growing evidence suggests that these changes result not merely from the depletion of nutrient pools but are closely tied to the disruption of key biogeochemical

processes governing SOM transformation and nutrient cycling, and the consequent breakdown of soil-microbial interaction networks [3]. Therefore, a deeper understanding of how agricultural management practices influence these core ecological functional processes is essential for maintaining the long-term productivity and ecological sustainability of black soil agroecosystems [4].

The return of crop straw to fields is a fundamental conservation agricultural practice widely promoted to replenish soil organic carbon (SOC), improve soil structure, and enhance nutrient retention, aiming to mitigate black soil degradation [5]. In particular, maize straw provides a substantial source of carbon and nutrients that can potentially stimulate microbial activity and soil biochemical processes [6]. However, in the cold–temperate black soil region, straw return alone often leads to inconsistent outcomes due to slow decomposition rates under low-temperature constraints, potentially causing temporary nutrient immobilization during critical crop growth stages [7]. This paradox highlights the critical importance of biological regulation in the straw decomposition process [8].

Microbial inoculants have been increasingly applied to enhance residue decomposition, nutrient mobilization, and plant growth, owing to their capacity to regulate soil biochemical processes and microbial community assembly [9]. Compared to single-strain inoculants, compound microbial inoculants, consisting of multiple taxa with complementary functions, are believed to more effectively mimic or enhance natural microbial synergies [10], thereby providing broader and more stable ecological functions in facilitating straw decomposition, nutrient mobilization, and fostering beneficial microbial networks [11]. Despite their growing application, most studies have focused on short-term effects on crop yield or soil nutrient levels, while the underlying mechanisms linking straw return, soil enzyme activities, and bacterial community structure during crop growth remain insufficiently resolved under field conditions [12]. Moreover, how these interacting processes collectively contribute to crop productivity in black soil systems is still poorly understood [13].

Against this backdrop, we conducted a field experiment in a typical black soil agroecosystem to investigate how the combined application of maize straw and a compound microbial inoculant regulates soil biochemical functioning and bacterial community structure during the soybean growth season. The specific objectives were to (i) quantify the effects of straw return and microbial inoculation on soil physicochemical properties and key enzyme activities, (ii) characterize the dynamic responses of bacterial community composition and diversity to different management practices, and (iii) identify key soil factors driving bacterial community variation and soybean productivity. We hypothesized that the combined application of maize straw and compound microbial inoculant would enhance the coupling between nutrient availability and microbial activity, thereby improving the efficiency of soil biochemical processes and promoting soybean growth more effectively than either practice alone. Our findings are expected to provide a theoretical basis for developing straw-return practices enhanced by microbial regulation for improving soil fertility in black soil regions.

2. Materials and Methods

2.1. Experimental Site

The experimental site is in Sanfu Village, Tongjiang City, Jiamusi City, Heilongjiang Province (47,786° N, 132,701° E). Each plot was 10 m × 5 m wide (50 m^2), with 3 replicates per treatment. It is one of the three major black soil belts worldwide (Soil Taxonomy of the United States Department of Agriculture), with fertile soil and abundant products.

2.2. Experimental Design and Soil Sample Collection

The soybean variety *Heihe 43* was planted in black soil in a 333 m^2 experimental field. Sowing depth and density were consistent across all treatments in each experimental plot. Five points (0–20 cm) were collected and mixed into a single sample using an S-shaped method with a soil auger (diameter 5 cm, depth 0–20 cm; Qingdao Juchuang, Qingdao, China). Each treatment was sampled three times at the seedling, branching, blooming, pod setting, seed-filling, and mature stages of soybean growth. Chemical fertilizer was used at a rate of 60 kg/ha, and maize straw was applied to 4.5 t/ha. The experimental conditions applied were as follows: conventional fertilization (F); compound bacterial inoculant and fertilization (CF); straw returning to the field and fertilization (SF); and compound microbial inoculant, straw returning to the field, and fertilization (CSF). Three replicate soil samples were collected from each sampling site. All soil samples were sealed in a sterile frozen plastic box with roots and immediately divided into two sub-samples, one of which was air dried at 4 °C for soil physicochemical property analysis and the removal of stones, visible plant residues, and insects, such as earthworms. The other soil sample was stored at −20 °C (Haier, Qingdao, China, Model SC-219) for analysis of its microbial diversity and community structure.

2.3. Compound Microbial Inoculants

In the laboratory, a biocontrol bacterium capable of degrading straw and promoting crop growth was prepared. Bacillus subtilis and Bacillus megaterium were mixed in a 1:1 ratio with *Bacillus gelsolicus*. The CFU was 3.88×10^8, and was dipped into the LB liquid culture medium with a pipette gun, agitated for 24 h, fully mixed, and injected into the soil. This inoculant was selected for its N-fixing, P-solubilizing, and stress-resistant functions. It was basally applied and incorporated into 0–20 cm soil. For large-scale production, straw return machines and fertilizer spreaders were used for field operation.

2.4. Physicochemical Analysis

AP was extracted with $NaHCO_3$ and quantified using the molybdenum–antimony colorimetric method. Alkaline nitrogen (AN) was determined by the alkaline hydrolysis diffusion method, and available potassium (AK) was measured using flame photometry. A pore sieve (Shangyu, Shaoxing, China) was used to filter the black soil, filtering off large black soil samples, and the new weight of the black soil was measured using an electronic balance (Yueping, Shanghai, China). The soil sample was then dried in a drying oven (Yiheng, Shanghai, China) at 105 °C for 24 h, weighed, and the water content of the black soil was calculated. Soil water content was determined gravimetrically by oven-drying fresh soil samples at 105 °C for 24 h. A precision pH meter (Sartorius, Gottingen, Germany) was used to measure the pH of each black soil sample in a 1:2.5 soil sample to distilled water ratio. Dry samples were ground and analyzed using soil agrochemical analysis before the evaluation of SOC via dry combustion. The semi-micro Kjeldahl method was used to determine the TN concentration following digestion with concentrated sulfuric acid. After melting with sodium hydroxide, TK was measured using ammonium acetate atomic absorption spectrophotometry. In contrast, TP was determined using acid solubilization/molybdenum–antimony resistance colorimetry at a wavelength of 700 nm. AP was extracted with $NaHCO_3$, and it was determined using molybdenum–antimony colorimetry. AN was determined using the alkali hydrolysis diffusion method, while AK was determined using flame photometry. All the above physicochemical analyses were performed according to the standard methods described by Bao [14].

2.5. Soil Enzyme Activity Determination

Sucrase, urease, acid phosphatase, and catalase are all dynamic soil fertility indicators [15]. Based on soil enzymes and their research methodologies [16], urease activity was determined using sodium phenol colorimetry [17]. The 3,5-dinitrosalicylic acid colorimetry was used to measure the sucrase activity, while the sodium phenyl phosphate colorimetry was used to measure the acid phosphatase activity [18]. Finally, the catalase activity was measured using the potassium permanganate titration method [19].

2.6. Enumeration of Culturable Soil Microorganisms

Culturable soil microorganisms, including bacteria, fungi, and actinomycetes, were quantified using the dilution plate counting method [20]. Fresh soil samples were serially diluted with sterile water, and appropriate dilutions were plated on selective media. Bacteria were cultured on nutrient agar, fungi on Martin's agar medium, and actinomycetes on Gauze's medium. After incubation, colonies with typical morphological characteristics were counted, and microbial abundance was expressed as colony-forming units per gram of dry soil. Three replicate plates were used for each sample.

2.7. DNA Extraction and 16S rDNA Gene Sequencing

Total DNA was extracted from 0.5 g of fresh soil using FastDNA ® SPIN Kit for soil (MP Biomedicals, Irvine, USA) and stored in an environment of −20 °C before further analysis according to the instructions of the manufacturer, Guangdong Magigene Biotechnology Co., Ltd. (Shenzhen, Guangdong, China), to determine the DNA concentration. A method of 1% agarose gel electrophoresis was used to test the purity and quality of the genomic DNA. The PCR products were combined and sequenced in corresponding proportions according to the sequencing criteria of each sample to obtain the target fragment. Quantiflur-ST was used to detect PCR products quantitatively [21]. The PCR amplification products were mixed in equimolar proportions according to the sequencing criteria of each sample, and the Illumina Miseq PE300 platform was used for sequencing analysis. For MiSeq sequencing, the sample DNA served as a template for amplifying bacterial 16S rRNA fragments. The F515 (5′-ACTCCTACGGGAGGCAGCA-3′) and R907 (5′-GACTACHVGGGTWTCTAAT-3′) primers were used to amplify the V3-V4 region of the 16S rRNA gene.

2.8. Statistical Analysis

Data sorting and collation were performed using Microsoft Office Excel 2010. One-way analysis of variance (ANOVA) and Duncan's multiple range test were conducted using SPSS 18.0 to assess significant differences ($p < 0.05$). Pearson correlation analysis, redundancy analysis (RDA), and Mantel test were performed using CANOCO 5.0 to reveal the relationships among soil physicochemical properties, enzyme activities, and bacterial community characteristics. All data were expressed as means ± standard deviations ($n = 3$). Figures were plotted using Origin Pro 8.0 software [22].

3. Results

3.1. Effects of Different Treatments on Soybean Agronomic Traits and Soybean Yield

Soybean growth and yield differed significantly among treatments (Table 1). Compared with F, all amended treatments improved soybean growth performance across growth stages, although the magnitude of the response varied among treatments. SF significantly increased plant height and root length relative to F, whereas CF resulted in higher aboveground and belowground biomass, particularly during the pod-setting and seed-filling stages.

Table 1. Agronomic traits and yield of soybean under different treatments.

Treatments	Plant Height (cm/Plant)	Taproot Length (cm/Plant)	Total Root Length (cm/Plant)	Root Volume (cm^3/Plant)	Aboveground Biomass (g/Plant)
F	45.32 aA	12.23 cB	18.59 aA	9.63 cB	151.53 cB
CF	46.67 aA	14.93 aA	20.70 aA	10.08 bB	148.40 dB
SF	44.71 aA	13.31 bB	19.49 aA	8.80 dC	132.85 eC
CSF	52.56 aA	14.63 aA	18.76 aA	12.95 bB	166.83 aA
	Main Stem Segments (per/Plant)	**Number of Branches (per/Plant)**	**Number of Pods per Plant (per/Plant)**	**Number of Grains per Plant (per/Plant)**	**Soybean Yield (g)**
F	6.4 dC	1.4 dC	42.3 dC	55.2 dD	144.12 cC
CF	12.6 bA	1.9 bA	39.9 eD	57.2 cC	149.76 bB
SF	10.3 cB	2.2 aA	44.7 cB	69.9 aA	151.44 bB
CSF	6.9 dC	1.6 cB	52.8 aA	64.3 bA	166.40 aA

Note: F—fertilization alone; CF—fertilization + compound microbial inoculant; SF—fertilization + maize straw return; CSF—fertilization + maize straw return + compound microbial inoculant. Data are presented as mean ± SD (n = 3). Different lowercase letters within the same growth stage indicate significant differences among treatments at $p < 0.05$, and different uppercase letters indicate extremely significant differences at $p < 0.01$, according to one-way ANOVA followed by Duncan's multiple range test.

Among all treatments, CSF consistently produced the highest stem diameter, plant fresh weight, and total biomass during the later growth stages. Soybean yield under CSF was significantly higher than that under F, with yield under CSF increased by 3.91% (no significant difference) to 5.46% ($p < 0.05$) compared with F treatment. In addition, yield under CSF was significantly higher than that under SF and CF, indicating a stronger positive effect of the combined treatment.

3.2. Effects of Different Treatments on Soil Physicochemical Properties

At the beginning of the test, the initial SOC content in the 0–20 cm soil layer was 26.4 g/kg. Furthermore, TN, total potassium (TK), and total phosphorus (TP) masses were 0.83 g/kg, 16.76 g/kg, and 1.17 g/kg, respectively. Soil physicochemical properties varied significantly among treatments across soybean growth stages (Table 2). Compared with F, CSF, SF, and CF showed different degrees of influence on soil moisture, pH, and nutrient status. Across all growth stages, CSF maintained significantly higher soil moisture content and pH than F ($p < 0.05$). SF and CF also increased soil moisture relative to F, although the magnitude of the increase was smaller than that observed under CSF.

TN, TP, and TK showed relatively small differences among treatments. In contrast, available nutrient fractions responded more strongly to treatment effects. CSF significantly increased AN, AP, and AK compared with F across growth stages ($p < 0.05$). SF notably increased AP, whereas CF showed moderate increases in AN and AK relative to F.

Table 2. Effect of treatment on soil physical and chemical properties.

Stage	Treatment	pH (1:2.5 H_2O)	Organic Matter (g/kg)	Moisture Content (%)	Organic Carbon (g/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	Alkaline Nitrogen (mg/kg)
I	F	5.9 bA	45.6 bA	28.9 aA	26.4 bA	53.9 aA	185.1 aA	7.681 cC
	CF	6.0 aA	38.8 cB	27.6 cB	22.5 cB	54.6 aA	184.9 aA	10.463 bB
	SF	5.8 bA	46.0 bA	27.9 bA	26.7 bA	50.8 bA	173.0 cB	16.052 aA
	CSF	6.1 aA	48.6 aA	29.0 aA	28.2 aA	45.4 cB	182.8 bA	15.346 aA

Table 2. *Cont.*

Stage	Treatment	pH (1:2.5 H_2O)	Organic Matter (g/kg)	Moisture Content (%)	Organic Carbon (g/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	Alkaline Nitrogen (mg/kg)
II	F	6.0 cA	43.7 cB	30.1 bA	25.4 cB	48.8 bB	178.5 bA	14.818 bA
	CF	6.2 aA	45.3 bA	31.3 aA	26.3 bB	49.0 bB	183.9 aA	11.876 cB
	SF	6.1 bA	48.6 aA	29.8 bA	28.2 aA	52.2 aA	164.0 cB	15.014 bA
	CSF	6.0 cA	48.3 aA	30.0 bA	28.0 aA	45.8 cC	185.3 aA	16.558 aA
III	F	5.8 cB	46.2 aA	30.7 aA	26.8 aA	46.7 bB	187.3 aA	14.671 aA
	CF	6.1 bA	44.4 bB	28.2 cC	25.8 bB	50.6 aA	186.9 aA	13.273 bB
	SF	6.2 aA	43.9 cB	29.7 bB	25.5 bB	51.4 aA	173.6 cB	12.556 cB
	CSF	6.1 bA	44.3 bB	28.0 cC	25.7 bB	45.7 bB	185.7 bA	13.247 bB
IV	F	6.0 cB	43.3 bA	31.3 aA	25.1 bA	38.9 bB	169.8 dC	13.972 aA
	CF	6.1 bB	34.4 cB	31.6 aA	20.0 cB	40.1 bB	182.2 bA	9.771 cC
	SF	6.2 bB	46.1 aA	30.9 aA	26.7 aA	48.3 aA	176.7 cB	11.178 bB
	CSF	6.4 aA	43.7 bA	29.7 aA	25.3 bA	39.7 bB	193.7 aA	11.888 bB
V	F	6.4 bB	45.1 aA	31.2 aA	26.2 aA	50.3 aA	182.4 bB	16.052 cC
	CF	6.6 aA	41.9 bB	31.0 aA	24.3 bB	42.1 cC	180.2 cC	41.238 aA
	SF	6.3 cB	38.0 cB	28.8 bB	22.1 cC	41.9 cC	183.4 bB	20.948 bB
	CSF	6.4 bB	44.8 aA	30.4 aA	26.0 aA	44.0 bB	189.6 aA	14.693 cC
VI	F	6.5 bA	40.1 bB	33.2 aA	23.4 bB	44.7 aA	188.2 aA	7.700 cC
	CF	6.8 cB	37.0 cB	29.5 bA	21.9 cB	36.0 bB	181.0 cB	12.589 bB
	SF	6.6 aA	51.4 aA	27.8 cB	29.8 aA	37.7 bB	183.7 bA	24.427 aA
	CSF	6.7 aA	42.7 bB	34.7 aA	24.8 bB	34.0 cB	190.5 aA	16.800 bA

Note: F—fertilization alone; CF—fertilization + compound microbial inoculant; SF—fertilization + maize straw return; CSF—fertilization + maize straw return + compound microbial inoculant. I—seedling stage; II—branching stage; III—flowering stage; IV—pod-setting stage; V—seed-filling stage; VI—mature stage. Data are presented as mean ± SD (n = 3). Different lowercase letters within the same growth stage indicate significant differences among treatments at $p < 0.05$, and different uppercase letters indicate extremely significant differences at $p < 0.01$, according to one-way ANOVA followed by Duncan's multiple range test.

3.3. Effect of Different Treatments on Soil Enzyme Activities

Figure 1a–d depict the activities of four enzymes (sucrase, catalase, urease, and acid phosphatase) at various soybean growth stages. The rhizosphere soil enzyme activities increased as the soybeans grew and developed under varied treatments. Overall, the activities of the four enzymes were higher with composite fertilization than with individual fertilization ($p < 0.05$). The enzyme activities in the CSF treatment showed the highest trend, with an increase in CSF > SF > CF > F, and there were significant differences between treatment groups ($p < 0.05$). In soybean, catalase (Figure 1a) activities increased from 10.19 to 15.71% in the mature stage compared to the seedling stage, with SF > CSF showing insignificant differences at the seedling stage. In sucrase (Figure 1b), the mature stage increased from 25.00 to 30.30% compared to the seedling stage. Among the ureases (Figure 1c), a 23.53–40.62% increase was observed at maturity compared to the seedling stage, with SF > CSF showing significant differences at the soybean outplanting stage. The mature stage of acid phosphatases (Figure 1d) showed a 5.68–14.86% increase compared to the seedling stage, with significant differences across the emergence and branching stages (SF > CSF > CF).

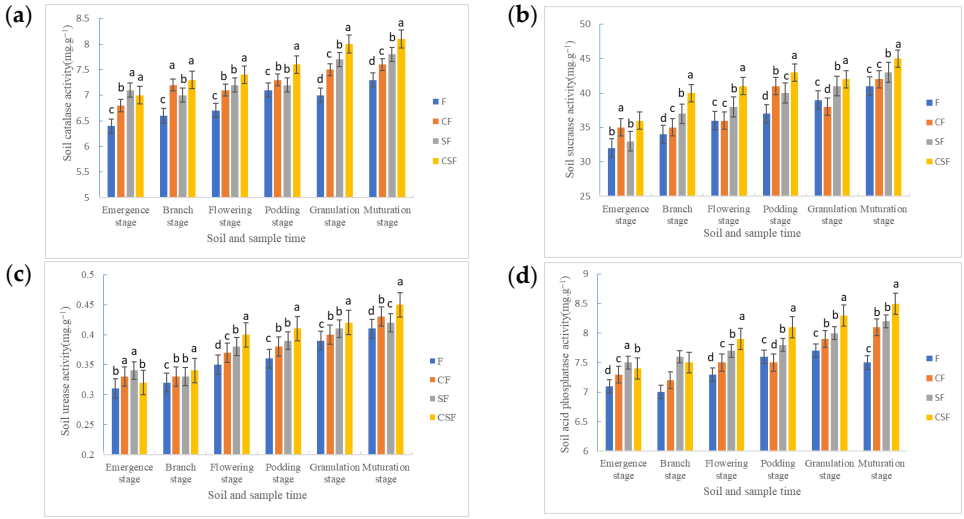


Figure 1. Changes in soil four enzymes during four treatments. (**a**) Catalase; (**b**) Sucrase; (**c**) Urease; (**d**) Acid phosphatase. Effects of fertilization alone (F), fertilization + compound microbial inoculant (CF), fertilization + maize straw return (SF), and fertilization + maize straw return + compound microbial inoculant (CSF) on soil physicochemical properties/enzyme activities during soybean growth. Values are mean ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within the same growth stage according to one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). Error bars represent standard deviations.

3.4. Number of Soil Microorganisms Cultivated Under Laboratory Conditions from Soybeans Under Different Treatments

Supplementary Figure S1 depicts the numbers of soil microorganisms cultured under laboratory conditions for soybeans under various treatments. Under all four treatments, soil bacterial populations increased initially before decreasing. The bacterial numbers in each treatment peaked at the pod stage, where the f treatment had the lowest numbers, the CSF treatment had the highest numbers, SF was approximately the same as the CF treatment, and the mature soybean stage had a lower number than the emergence stage, with a 3.12–5.15% decrease. Except for the CF treatment, all three other treatments showed an initial increase and a decreasing trend in soil fungal populations. The CF treatment showed a decreasing trend, CSF > CF > SF > F; the number of soybean maturity stages was lower than the emergence stage; and the decreasing amplitude was 6.98–11.37%. The various treatments roughly showed a decreasing trend first, followed by an increase and then a decrease, and the number of soybean mature stages was lower than that at the emergence stage, with a decrease of 21.28–46.67%, with the four treatments at anthesis having the highest number. The CSF treatment reached 180 × 105/g.

3.5. Bacterial Community Diversity

For six months, 24 samples were collected from the four soybean treatments, and the soil bacterial coverage (coverage) index ranged from 0.9098 to 1.000. The amount of sequencing data was reasonable, and the sequencing data covered all bacterial taxa in the soil, which can significantly reflect the bacterial properties in the soil environment. Using Illumina MiSeq high-throughput sequencing, all samples yielded 2,075,098 optimized sequences, with 500,737–533,418 optimized sequences obtained per sample. The obtained data were classified at a 97% similarity level, which detected 29,729 out of 1834, 1380, 2019, and 2132 unique to F, CF, SF, and CSF, respectively.

Among them, the bacterial relative abundance in CSF increased by 16.25% compared to that observed in the F treatment (Table 3). In comparison, the relative abundance of bacteria in CF decreased by 32.90% compared to the F treatment. Qime was used to calculate Chao1,

an indicator of flora richness, and Simpson, an indicator of flora diversity. At the phylum level, the lowest Chao1 index was found in SF, which decreased by 45.45% compared to that in F, and the Chao1 index in CSF, which decreased by 15.00% compared to that in CF, all without statistical significance. The lowest Simpson index was found in CF, with a 2.1% decrease compared to F, and the highest was in SF, with no statistical significance. At the genus level, the lowest Chao1 index was found in SF, which decreased by 14.23% compared to that in F, and the Chao1 index of CSF decreased by 3.38% compared to that in CF, with no significant differences.

Table 3. Bacterial alpha diversity indices of black soil in different treatments.

Treatment	OTUs	Richness		Diversity	
		Chao1	ACE	Shannon	Simpson
F	2713.83 ± 120 cC	3114.50 ± 48.33 cB	3321.38 ± 20.8 bB	10.00 ± 0.92 cC	0.995 ± 0.02 cB
CF	2836.00 ± 12.3 cB	2906.33 ± 53.29 dC	3028.33 ± 18.37 cB	10.50 ± 1.38 bB	0.997 ± 0.00 bA
SF	3012.33 ± 34.2 bA	3318.00 ± 102.38 bB	3672.00 ± 21.38 aA	10.33 ± 0.37 bB	0.998 ± 0.01 aA
CSF	3073.50 ± 48.2 aA	3462. 83 ± 72.17 aA	3528.00 ± 36.28 aA	10.83 ± 0.17 aA	0.997 ± 0.03 bA

Note: Treatments: F—chemical fertilization alone; CF—chemical fertilization combined with compound microbial inoculants; SF—chemical fertilization combined with maize straw return; CSF—chemical fertilization combined with maize straw return and compound microbial inoculants; OTUs—operational taxonomic units (97% similarity). Different lowercase letters within the same growth stage indicate significant differences among treatments at $p < 0.05$, and different uppercase letters indicate extremely significant differences at $p < 0.01$, according to one-way ANOVA followed by Duncan's multiple range test.

The CSF had the lowest Simpson index, with a contrast reduction of 9.25%, neither of which was significant. In conclusion, incorporating straw-returned fields into fertilized croplands reduced soil rhizosphere flora richness while increasing community diversity. At the genus level, adding a compound fungicide to fertilized croplands reduced flora diversity while increasing flora richness. Complex fungicides in straw-returned fields significantly reduced community diversity at the phylum level. These findings suggest that compound fungicides enhance straw return while inhibiting soil microbial growth.

3.6. Effects of Different Treatments on Soil Bacterial Community Composition and Soybean Abundance

A bacterial community phylogenetic analysis of genes was performed to identify 20 phyla with the highest relative abundances. At the phylum level, 16 bacterial phyla were detected, with *acidobacter*, *Proteobacteria*, *Chloroflexi*, *Bacteroidetes*, and *Actinobacteria* being the dominant phyla, accounting for 85.79% of all sequences, with relative abundances of 10.79–41.12%, 21.30–36.70%, 2.21–13.91%, 3.46–8.29%, and 2.43–8.90%, respectively (Table 1). Some less abundant phyla, such as *Gemmatimonadetes*, *Firmicutes*, *Verrucomicrobia*, *Wps-2*, *Armatimonadetes*, *Patescribacteria*, *Planctomycetes*, and *Nitrospirae*, were detected in the various treatment samples during the six soybean periods, with relative abundances ranging from 0.014 to 4.8%, with an additional 0.88% being unclassified bacteria (Figure 2a).

The relative abundances of *Acidobacter* and *Chloroflexi* were higher in the CF treatment group than in the other three treatment groups, with *Proteobacteria* in the SF, *Bacteroidetes* in the CSF, and Actinobacteria in the F treatment groups having the highest relative abundances. At the genus level, 18 bacterial genera were detected, with the dominant genera being *Candidatus_Solibacter*, *Sphingomonas*, *Bryobacter*, *Gemmatimonadota*, and *Burkholderia caballeronia paraburkholderia*, accounting for 35.88% of all sequences, and with relative abundances of 7.04–13.10%, 2.24–13.9%, 1.95–15.79%, 0.89–3.68%, and 015–8.14%, respectively. Certain less abundant genera, such as *Mucilaginibacter bacillus*, *Hsb-of53_F07*, and *Granulicella*, were also detected in the various treatments, with abundances ranging from 0.015 to 2.37%. The genus Bacteroidetes contained a high proportion of unclassified bacteria

(15.02–50.80%), resulting in a less dominant flora (Figure 2b). The relative abundance of *Bryobacter* was higher in CF than in the other three treatments. The relative abundance of *Sphingomonas* was the highest in SF, while the relative abundance of *Candidatus_Solibacter* was the highest in CSF.

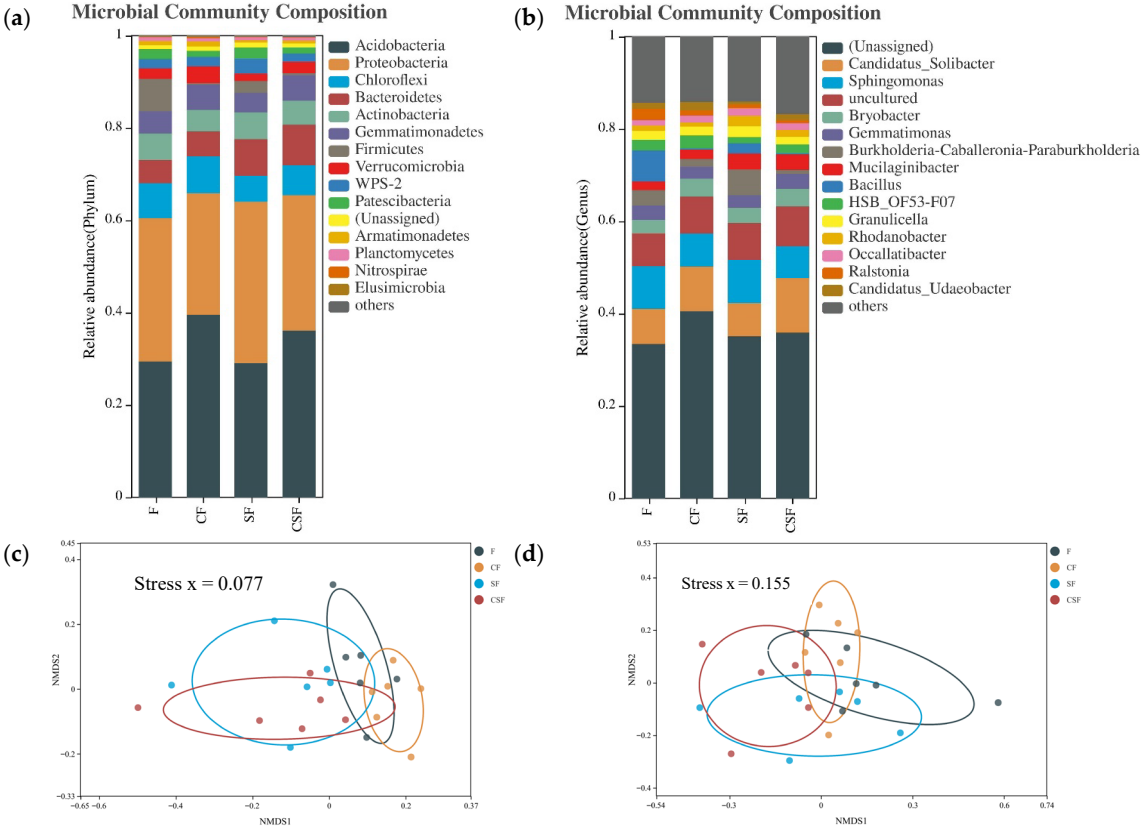


Figure 2. Community composition of soil bacteria at the phylum (**a**) and genus (**b**) level. Primary coordinate analysis of soil bacterial phylum (**c**) and genus (**d**).

3.7. Beta-Diversity of Microbial Community

Non-metric multidimensional scaling (NMDS) analysis of species based on the Bray–Curtis distance algorithm (Figure 2c,d) revealed that the distribution of bacterial communities differed across the treatments. Visual analysis using NMDS led to the classification of the bacterial communities into four distinct groups at the phylum level (Figure 2c), with the stress of 0.077, <0.1 performing better. There were noticeable variations in the community structure between each epoch of each sample, and the nmds1 axis shows a clear distinction between the communities of groups F and CSF, with the CSF > F and SF span containing the F and CF groups.

The NMDS2 axis shows a significant distinction between the three groups, CF, SF, and CSF, with CF > CSF = F > SF. This finding indicates that under fertilization settings, community importance was significantly elevated in the case of the combined and straw-returned fields, with the combined alone being higher than in the straw-returned fields. Thus, the bacterial community structure became unstable and altered due to CSF application. At the genus level (Figure 2d), the bacterial communities were classified into three distinct groups, with stresses of 0.155 and <0.2, indicating the reliability of the data. There was a noticeable differential in the community structure between each sample epoch, as seen from the nmds1 axis. For CF vs. SF, the community of the CSF group was clearly distinguished, where CF > SF = CSF. The CF connected the SF with the CSF along the NMDS2 axis, where CSF > SF. These findings indicate that, under fertilization conditions, the addition of the

composite fungicide modified the soil bacterial community structure, and the composite fungicide mixed with straw functioned better than straw alone.

3.8. Differential Analysis of Microbial Communities of Soybeans Under Different Treatments

Linear Discriminant Analysis (LDA) was performed for the four treatments, revealing that Firmicutes were enriched in treatment F at the phylum level. At the genus level, *bacterium_Eillin5129* was abundant in the SF treatment, Bacillales in the F treatment, *devosia* and *caulobacteraceae* in the CSF treatment, and *camdidatus_Koribacter* in the CF treatment (Figure 3a,b).

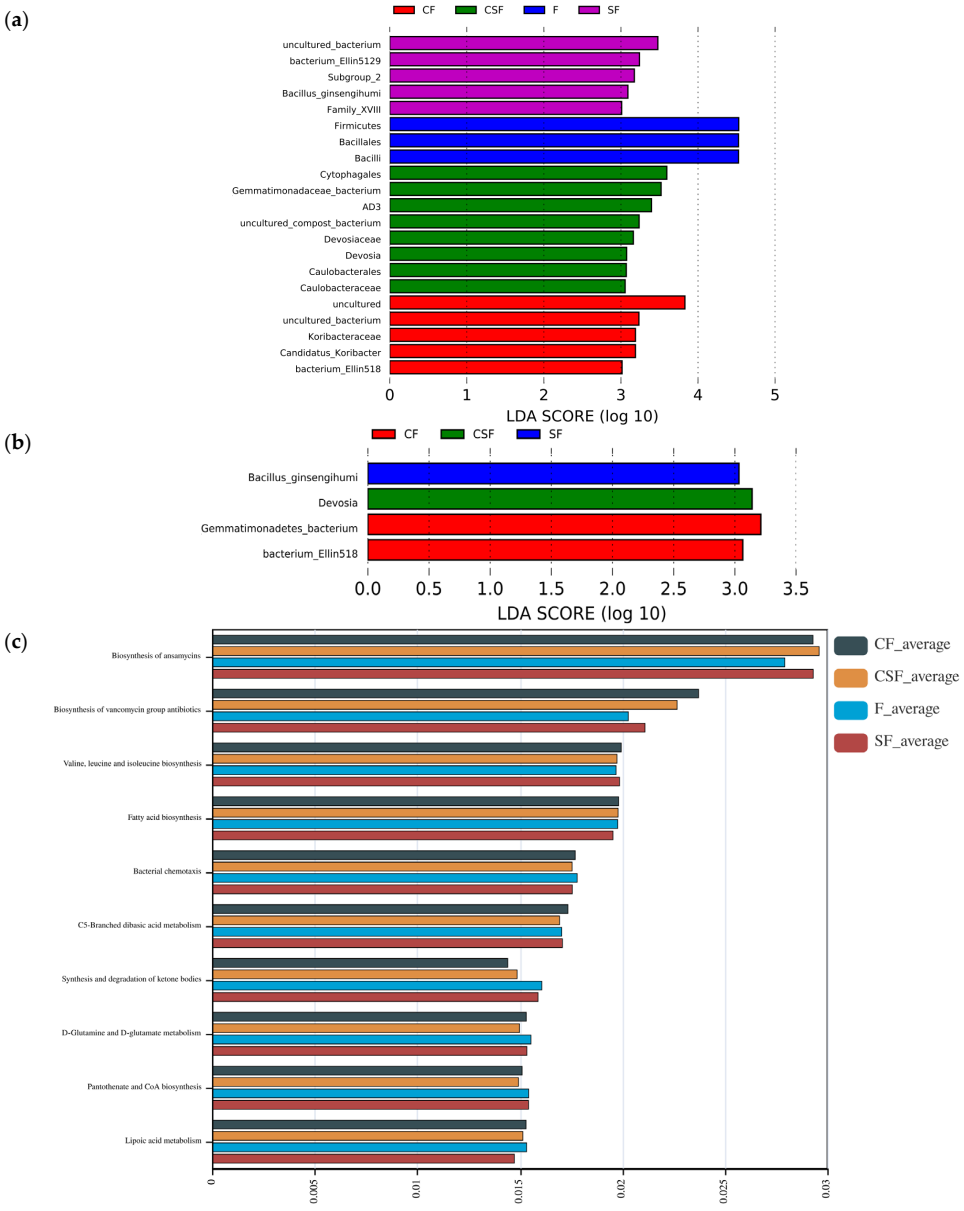


Figure 3. Species difference LEfSE analysis between bacterial phylum (**a**) and genus (**b**) community groups. KEGG multi-group pathway difference analysis. (**c**) Treatments: F—chemical fertilization alone; CF—chemical fertilization combined with compound microbial inoculants; SF—chemical fertilization combined with maize straw return; CSF—chemical fertilization combined with maize straw return and compound microbial inoculants.

3.9. Correlation Analysis Between Soil Physicochemical Properties and Soil Microbial Communities

The Kyoto Encyclopedia of Genes and Genomes (KEGG) was used for detection. Sugar metabolism (carbohydrate metabolism), polyketones (metabolism of terpenoids and polyketides), amino acid metabolism, calcium factor and station compounds, vitamin metabolism (metabolism of cofactors and vitamins), energy metabolism, other acid metabolism (metabolism of other amino acids), lipid metabolism, replication and repair, biodegradation and metabolism (xenobiotic biodegradation and metabolism), and polysaccharide synthesis and metabolism are all part of the second pathway. The content differed slightly between the treatments. Attachments can be seen in the third pathway, affecting sugar metabolism in the second pathway, with the most prevalent being ansamycins, biosynthesis of vancomycin antibiotics, anabolic leucine, and isoleucine biosynthesis.

Changes in bacterial phyla were negatively connected with AN, positively correlated with pH, and negatively correlated with AK ($p < 0.01$), according to a Mantel test examination of the association between bacterial and fungal populations. Changes in bacterial genera correlated positively with SOC, while SOM correlated positively with AN and negatively correlated with AP (Table S1).

Redundancy analysis (RDA) was used to analyze the relationships between various treatments and soil physicochemical factors (pH, SOC, SOM, AN, AK, and AP). Figure 4a,b show the results. Among the phylum-level treatments, different treatments exhibited significant positive or negative correlations with the selected environmental factors: pH (0.00128 of explained total variance), SOC (0.00432 of explained total variance), SOM (0.04289 of explained total variance), AN (0.00294 of explained total variance), AP (0.103 of explained total variance), and AK (0.4588 of explained total variance). The selected factors accounted for 91.6% of the total variations in bacterial community changes. At the genus level, there were significant positive and negative correlations between pH (0.00384 of the total variance explained), SOC (0.00793 of the total variance explained), SOM (0.03976 of the total variance explained), AN (0.02454 of the total variance explained), AP (0.05484 of the total variance explained), and AK (0.15638 of the total variance explained). The factors selected accounted for 73.5% of the total variations in bacterial community changes.

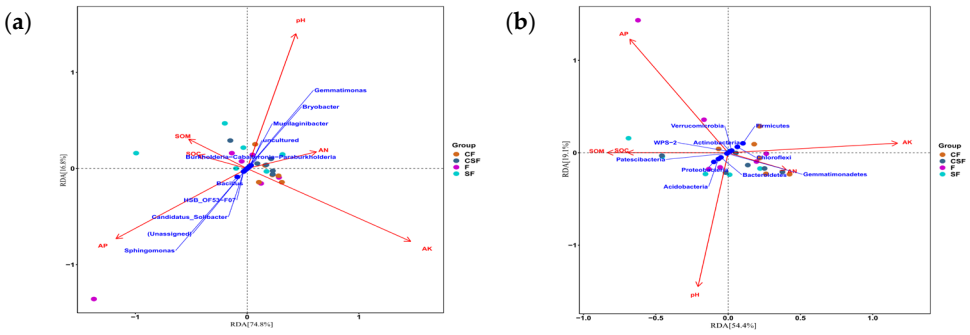


Figure 4. *Cont.*

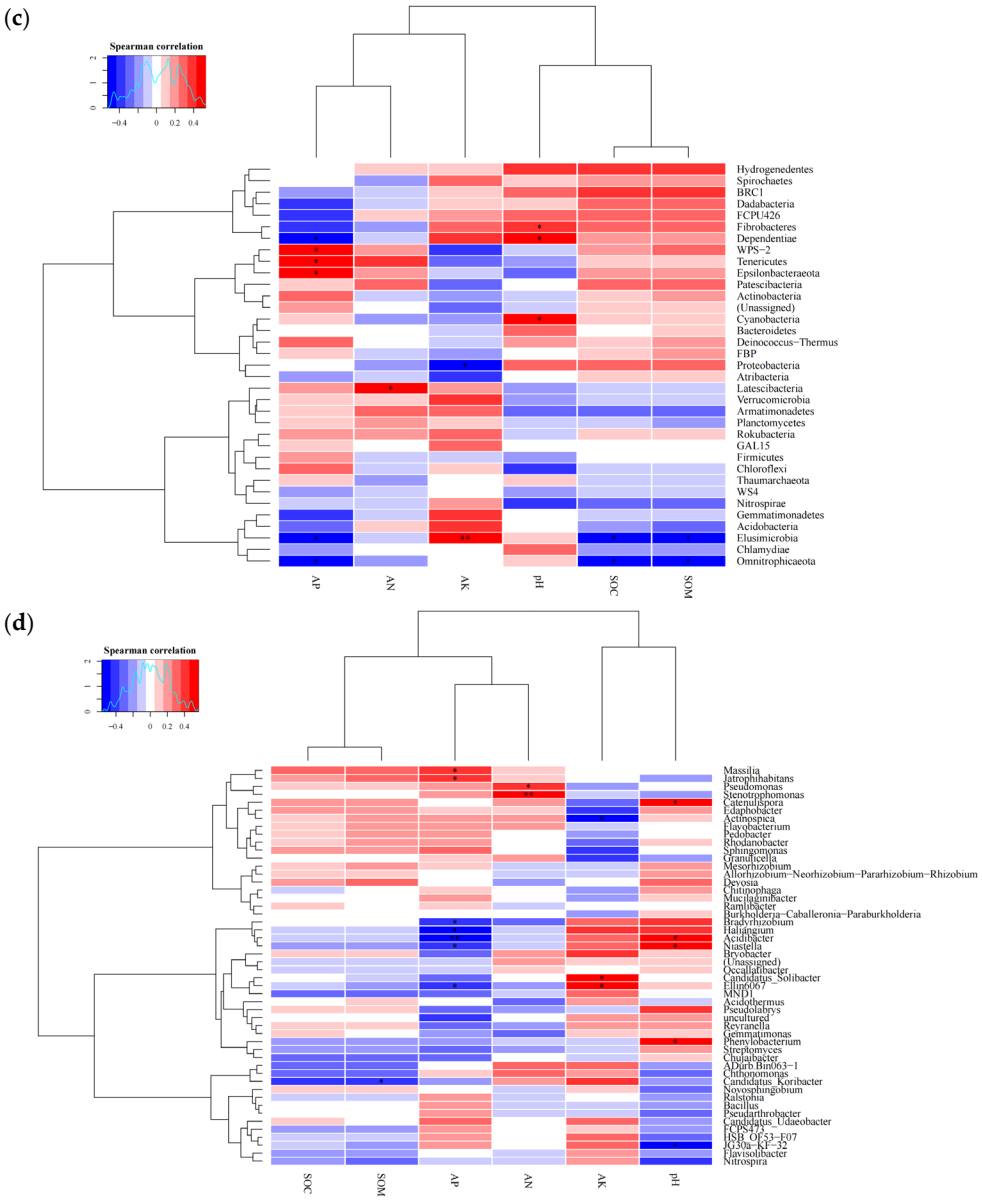


Figure 4. Redundancy analysis (RDA) and heatmap analysis of microbial composition and environmental factors under different treatments. (**a**) Microbial phyla based on RDA; (**b**) Microbial genera based on RDA; (**c**) Microbial phyla based on heatmap; (**d**) Microbial genera based on heatmap. Treatments: F-chemical fertilization alone; CF-chemical fertilization combined with compound microbial inoculants; SF-chemical fertilization combined with maize straw return; CSF-chemical fertilization combined with maize straw return and compound microbial inoculants.* indicates significant correlation at $p < 0.05$, and ** indicates highly significant correlation at $p < 0.01$.

3.10. Relationships Between Soil Properties and Bacterial Community Structure

To explore the effects of soil physicochemical properties on bacterial communities, the link between the physicochemical parameters of black soil and the levels of phyla and genera was examined in this study (Figure 4c,d). The results showed that soil AP at the phylum level was significantly correlated with Dependentiae, *WPS-2*, *tenericutes*, *epsilonbacteraeota*, *elusimicrobia*, and *omnitrophicaeota*, which were significantly correlated with *latescibacteria*. AK was significantly correlated with *Proteobacteria*, and *elusimicrobia* was significantly correlated with pH; *fibrobacteria*, *dependentiae*, *cyanobacteria*, SOC, and SOM were significantly correlated with *elusimicrobia* and *omnitrophicaeota*. At the genus level, soil SOM and *Candidatus_Koribacter* were significantly correlated. AP was significantly corre-

lated with *massilia*, *jatrophihabitans*, *Bradyrhizobium*, *halingum*, *niastella*, *ellin6067*, *acidibacter*, *Pseudomonas*, and *Stenotrophomonas*; AK was significantly correlated with *actinospica*; *Candidatus_Koribacter* and *ellin6067* were significantly correlated; and pH was significantly correlated with *catenulispora*, *acidibacter*, *niastella*, *phylobacterium*, and *jg30a-kf-32.*

Table 4 shows the correlation analysis between black soil physicochemical properties and soybean yield under the four treatments. There was a significant positive correlation between soil pH and F, a significant negative correlation between soil SOM and SF, and soil moisture content, CF, and SOM. Furthermore, there was a significant positive correlation between SOC and CSF and between AK and F, a significant negative correlation with SF, positive correlation between AP and CF. Finally, there was a highly significant negative correlation with CSF but no significant correlation between AN and soybean yield. Pearson's correlation analysis revealed that MC, AP, and AK were significantly correlated with soybean yield, indicating that an appropriate increase in phosphorus and K fertilizers was beneficial to soybean yield and may be the most important environmental factor affecting soybean yield.

Table 4. Pearson correlation analysis between soybean yield and soil physicochemical properties.

	pH (1:2.5 H_2O)	Organic Matter (g/kg)	Moisture Content (%)	Organic Carbon (g/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	Alkaline Nitrogen (mg/kg)
F	0.031 *	0.342	0.612	0.713	0.008 **	0.390	0.125
CF	−0.136 **	0.142	0.026 *	0.618	0.591	0.638 *	0.720
SF	0.701	−0.198 **	0.381 *	0.842	−0.007 *	0.382	0.461
CSF	−0.046	0.655	0.685	0.727 **	0.371	−0.048 **	0.202

Notes: Treatments: F—chemical fertilization alone; CF—chemical fertilization combined with compound microbial inoculants; SF—chemical fertilization combined with maize straw return; CSF—chemical fertilization combined with maize straw return and compound microbial inoculants. *: correlation was significant at 0.05 level; **: correlation was significant at 0.01 level.

4. Discussion

4.1. Effects of Straw and Microbial Inoculants on Soybean Growth and Yield

The experimental results demonstrated that the combined application of CSF exerted a synergistic regulatory effect on the soybean–soil–microbe system, significantly improving soybean agronomic traits and achieving a 5.46% yield increase compared to the solely chemical F condition (the yield increase range was 3.91–5.46%, with the maximum increase reaching 5.46%). In terms of production cost, straw return utilizes agricultural waste without additional cost, and the input of microbial inoculants is low in local farming systems. The 5.46% yield increase under CSF can effectively offset the additional input, showing good economic feasibility in black soil areas. This yield-promoting effect was rooted in the multi-dimensional optimization of soil ecological processes, which aligns with the mechanistic depth and integrative perspective emphasized [23]. The core mechanism underlying the observed benefits involves the cascade amplification of "inoculant-straw-soil-microbe" interactions [24], which comprehensively enhanced soil physicochemical properties, microbial functional diversity, and nutrient cycling efficiency [25]. Notably, straw return has no extra cost, and the low input of inoculants ensures that the yield increase can offset additional production costs in practical farming.

4.2. Improvements in Soil Physicochemical Properties and Nutrient Availability

Long-term excessive chemical fertilization disrupts soil nitrogen cycling equilibrium, leading to the accumulation of ammonium ions and proton release, thereby inducing soil acidification and reducing buffering capacity—key constraints on black soil productivity in

Northeast China [26]. In contrast, the CSF, CF, and SF treatments effectively elevated soil pH from 5.8 to 6.0 (F) to 6.5–7.0, a range optimal for microbial activity and nutrient availability. This pH regulation was primarily driven by cation enrichment from straw decomposition and the metabolic activity of composite inoculants: strains such as Streptomyces and Bacillus in the inoculant secreted organic acids (e.g., citric acid, malic acid) that chelated soil aluminum ions, mitigating acidification stress [27,28]. Meanwhile, their nitrogen transformation capacity (e.g., enhanced nitrate reductase and nitrite reductase activity) reduced ammonia volatilization and nitrogen loss, thereby maintaining soil ion balance [29].

SOM, as the foundational determinant of black soil quality, undergoes severe depletion (by 30–50% within 20 years of reclamation) under conventional farming practices due to accelerated mineralization and inadequate organic input [30]. CSF reversed this trend by fostering a dual pathway of SOM accumulation and activation: straw decomposition replenished labile organic carbon pools in the short term [31]. Meanwhile, bacterial activity in the composite inoculant accelerated the mineralization of recalcitrant fractions (e.g., lignin, cellulose) through the secretion of extracellular enzymes (cellulase, lignin peroxidase), converting them into stable humic substances [32]. Notably, 77% of the increased SOM existed in oxidized form, improving soil aggregate stability [33,34].

SOC sequestration, a critical process linking agricultural management to global carbon cycling, was significantly enhanced in the CSF treatment group [35]. The composite inoculant–straw combination provided continuous exogenous carbon substrates, stimulating the proliferation of carbon-degrading microorganisms (from 0.32 in the F treatment to 0.45 in the CSF treatment) by balancing labile and recalcitrant carbon ratios [36]. Concurrently, root-derived carbon secretion during soybean growth was amplified by improved soil conditions, forming a positive feedback loop between plant growth and SOC accumulation [37]. In terms of soil nutrient availability, the CSF treatment reshaped the soil nutrient pool structure by accelerating straw nutrient release and microbial nutrient transformation [38].

Maize straw, rich in nutrients, underwent sequential decomposition [39]. Labile fractions were rapidly mineralized within 30–40 days of application, releasing AN, AP, and AK to meet the nutrient demands of soybean seedling and flowering stages [40]. Recalcitrant fractions, meanwhile, were gradually converted into humus-bound nutrients under the action of composite inoculants, forming a slow-release nutrient pool that sustained soybean reproductive growth [41]. Specifically, AN, AP, and AK contents increased by 40–45%, 35–40%, and 30–35%, respectively, compared to those in the F treatment, effectively alleviating nutrient limitations on soybean growth [42,43].

4.3. Responses of Soil Nutrient Cycling Enzyme Activities to Different Treatments

Soil enzymatic activity, a sensitive indicator of soil biological function, exhibited coordinated enhancement in the CSF treatment, driven by the optimal carbon-to-nitrogen ratio (C/N = 25:1–30:1) and microbial community optimization [44]. Soybean pod residues, with their high lignocellulose content, induced the secretion of extracellular enzymes involved in carbon, nitrogen, and phosphorus cycling [45]. Notably, the CSF treatment avoided the C/N imbalance caused by excessive straw return, which would otherwise inhibit microbial activity and enzyme secretion [46]. Specifically, cellulase (endoglucanase, cellobiohydrolase) and lignin peroxidase activities increased by 60–70% and 50–55%, respectively, accelerating straw degradation [47]. Meanwhile, urease and acid phosphatase activities rose by 45–50% and 40–45%, promoting organic nitrogen mineralization and inorganic phosphorus activation [48]. These enzymatic responses were tightly coupled with microbial community structure. The enrichment of functional taxa (e.g., Acidobacteriota, Proteobacteria, Actinobacteria) enhanced the synthesis and secretion of extracellular enzymes, forming a

"microbe–enzyme–nutrient" synergy that accelerated soil biogeochemical cycling [49,50]. The combined application of maize straw and compound microbial inoculants (CSF) significantly enhances the activities of key soil enzymes involved in carbon, nitrogen, and phosphorus cycling, including catalase, sucrase, urease, and acid phosphatase, during the soybean growth period [51]. Compared to the F treatment, these enzyme activities in the CSF treatment consistently increased from the seedling to the mature stage, with increments ranging from 5.68% (acid phosphatase) to 30.30% (sucrase). This improvement in soil biochemical functionality effectively promotes soil nutrient transformation and availability, providing robust biogeochemical support for soybean growth and yield formation [52].

4.4. Shifts in Soil Microbial Community Structure and Functional Diversity

Soil microbial communities, as the core drivers of agroecosystem functioning, underwent significant structural and functional optimization under the CSF treatment. Bacterial abundance exhibited a typical "increase–decline–stabilization" trend in response to soil disturbance. That initial surge (1.2–1.5 times higher than the F at 30 days) was followed by a gradual decline as the soil ecosystem reached a new equilibrium, while fungal abundance showed a gradual decrease associated with nutrient release to plants [53]. The CSF treatment significantly altered microbial community composition: the number of unique operational taxonomic units (OTUs) in CSF was 2132, which was 30–35% higher than that in F (1834), and community evenness was enhanced [54]. Dominant phyla such as Acidobacteriota (relative abundance 10.79–41.12%), Proteobacteria (21.30–36.70%), and Chloroflexi (2.21–13.91%), which thrive in carbon-rich habitats, were enriched, while functional groups with phosphorus-solubilizing, nitrogen-fixing, and auxin-secreting capabilities (e.g., Actinobacteria, Firmicutes) maintained stable abundances [55,56]. Notably, the composite inoculant promoted the formation of a more complex and stable microbial co-occurrence network, with core nodes dominated by beneficial taxa (e.g., Bacillus, Rhizobium, Sphingomonas) that enhanced interspecific synergism [57,58]. At the phylum level, the relative abundance of bacteria in CSF increased by 16.25% compared to the F-treated group. At the genus level, the Chao1 index of CSF was 3.38% higher than that of CF. The number of culturable bacteria in CSF peaked at the pod-setting stage, which was the highest among all treatments; the number of fungi in CSF was higher than that in CF, SF, and F, and the number of actinomycetes in CSF reached 180×10^5/g at the flowering stage, which was the highest among all treatments.

Soybean root–soil–microbe interactions not only improved nutrient availability but also suppressed pathogenic taxa (e.g., Xanthomonadaceae abundance reduced by 40–45%) [59]. KEGG functional annotation indicated that amino acid metabolism, energy production, and signal transduction pathways were significantly enriched in the CSF treatment, reflecting enhanced microbial metabolic activity and intercellular communication, which further supported soybean growth and development [60,61].

4.5. Integrated Synergistic Mechanism and Agricultural Application Prospects

Ultimately, the synergistic improvements in soil physicochemical properties, enzymatic activity, and microbial functional diversity created a favorable rhizosphere environment for soybean growth, with the CSF treatment achieving the most significant yield increase [62,63]. This integrated mechanism highlights the potential of combining composite microbial inoculants with straw return when integrated with conservation tillage techniques, such as large-ridge deep scarification, as a sustainable agricultural practice for black soil protection and utilization [64,65]. Beyond yield enhancement, this approach contributes to SOC sequestration, reduces environmental pollution from chemical fertilizers, and improves the ecological resilience of lowland agroecosystems, addressing the dual

challenges of food security and environmental sustainability in intensive agricultural systems [66,67]. The findings provide a mechanistic basis for the development of tailored soil management strategies to maintain black soil fertility and promote long-term agricultural sustainability [68].

4.6. Limitations

This study was a one-year field trial in a cold–temperate Mollisol region. Straw decomposition and microbial colonization may vary with interannual climatic conditions. Long-term multi-year field experiments are required to verify the stability of treatment effects. In addition, the application rates of straw and inoculants in this study followed local recommendations, and dosage optimization should be further explored in future research.

4.7. Conclusions

This study achieved the research objectives and confirmed the hypothesis that straw return combined with microbial inoculants synergistically improves the soybean–black soil system.

The addition of compound microbial inoculants combined with straw return effectively improved soybean agronomic traits and yield, increased soil pH, and promoted SOM accumulation. Meanwhile, this practice enhanced soil nutrient cycling, with significant increases in AP and AK. The CSF treatment significantly elevated the activities of sucrase, urease, catalase, and acid phosphatase, and optimized the soil bacterial community structure, with dominant phyla including Acidobacteriota, Proteobacteria, and Chloroflexi, and enriched beneficial genera such as *Candidatus_Solibacter*, *Sphingomonas*, *Bryobacter*, and *Gemmatimonadota*.

Overall, this combined strategy effectively improves soil fertility and soybean productivity, providing a feasible approach for black soil conservation. Long-term field trials are needed to further verify its stable effects in continuous cropping systems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agronomy16090856/s1: Figure S1: Number of soil microorganisms cultured from soybean under different treatments in the laboratory; Table S1: Correlations between soil microbial communities and soil properties determined by Mantel tests.

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