

Article

Metagenomic Insights into Corn Stalk Biochar-Mediated Carbon Cycling and Microbial Community Shifts in Black Soil Soybean Rhizosphere

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Abstract

Despite extensive research on biochar's effects on soil properties, the molecular mechanisms linking carbon-cycling functional genes to soil organic carbon dynamics under biochar application remain poorly understood. This study evaluated the effects of corn stalk biochar applied at different rates (0, 300, 600, and 900 kg/hm²) on soil physicochemical properties, microbial community structure, carbon cycling genes, and soybean (HeiNong551) growth in Northeast China's black soil region. Metagenomic analysis and metabolic pathway analysis were also conducted. Biochar application significantly improved soil fertility by increasing pH, moisture content, and various SOC fractions, while modulating the activities of key soil enzymes, including β -glucosidase, alkaline phosphatase, cellulase, and polyphenol oxidase. Metagenomic analysis revealed that biochar substantially enhanced soil microbial richness and diversity, with significant enrichment of beneficial bacterial genera, including *Nocardioides* and *Sphingomicrobium*. Functional gene analysis demonstrated that biochar promoted soil carbon cycling by increasing the abundance of Glycosyltransferases, Glycoside Hydrolases, and Auxiliary Activities, while decreasing the abundance of Carbohydrate-Binding Modules, Carbohydrate Esterases, and Polysaccharide Lyases. Analysis of 22 key carbon cycling pathway genes indicated that biochar enhanced carbon fixation via the reductive tricarboxylic acid cycle (*korA* gene) and promoted the degradation of easily mineralizable SOC components, while suppressing stable carbon degradation pathways.

Keywords: biochar; soybean; soil organic carbon; microbial community; functional gene



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1. Introduction

Soil organic carbon (SOC) represents one of the most fundamental indicators for evaluating soil fertility and ecosystem health [1]. In agricultural systems, SOC influences critical soil functions, including nutrient cycling, water retention, aggregate formation, and habitat provision for soil biodiversity [2]. However, intensive agricultural practices have led to widespread soil degradation globally, with China's farmlands particularly affected by excessive fertilization and substantial SOC losses [3]. The Northeast Black Soil region, despite possessing naturally high organic matter content and inherent fertility, has experienced severe degradation due to intensive cultivation [4]. Historical data reveal the severity of this decline: surface SOC reserves in the Songnen Plain of Heilongjiang Province

decreased by approximately 143.99 million tons, with meadow soils, black calcareous soils, and black soils accounting for 84.55% of the total SOC reduction [5].

Biochar, a carbon-rich solid product obtained through high-temperature pyrolysis of plant and animal residues under anaerobic or oxygen-limited conditions, has emerged as a promising soil amendment for addressing soil degradation while contributing to carbon sequestration [6]. When incorporated into agricultural soils, biochar acts as a multifunctional conditioner, promoting soil aggregate formation, increasing SOC content, and enhancing carbon sequestration capacity [7]. The mechanisms underlying biochar's beneficial effects are multifaceted. Research indicates that biochar reduces carbon dioxide emissions by lowering specific enzyme activities, enhancing microbial carbon use efficiency, strengthening SOC's role in soil aggregate formation, and inhibiting soil carbon mineralization, while simultaneously promoting plant-mediated SOC fixation [8,9]. Additionally, biochar provides essential nutrients and substrates for microbial growth, thereby increasing microbial biomass and activity [10].

The rhizosphere microbiome, often referred to as the plant's "second genome," plays a crucial role in plant growth, development, and nutrient acquisition [10,11]. By indirectly affecting soil microbial metabolic activities, biochar influences root growth and vitality, establishing a sustainable agricultural production system based on the "biochar-microbe-plant" interaction framework [12]. This green and economical approach offers promising solutions for modern agriculture, which faces challenges related to resource efficiency and environmental sustainability [13]. Recent agricultural practices emphasizing crop residue return to fields have gained significant attention for maintaining soil quality and promoting nutrient cycling [14]. With abundant straw resources representing immense development potential, the practice of returning crop residues to farmland not only increases SOC content and replenishes soil mineral nutrients while reducing chemical fertilizer dependency and boosting crop yields, but also supplements soil organic matter and enhances overall soil fertility [15].

Despite biochar's recognized benefits for soil health and crop productivity [16], direct evidence linking specific soil carbon cycling genes to changes in SOC under biochar amendment remains limited. Understanding how different biochar application rates affect soil microbial functional communities and the physiological mechanisms driving key processes in soil carbon cycling is critically important for optimizing biochar use in sustainable agriculture. Microbial-mediated decomposition of organic matter is essential for soil carbon cycling, yet the molecular mechanisms by which biochar influences these processes require deeper investigation. This study addresses these knowledge gaps by examining the effects of varying corn stalk biochar application rates on soil physicochemical properties, enzyme activities, microbial community structure, and carbon-cycling functional genes in the soybean (HeiNong551) rhizosphere soil using metagenomic approaches. The research aims to clarify how biochar application impacts soil fertility, microbial community composition and diversity, and the abundance of genes involved in carbon fixation and degradation pathways. Additionally, the study assesses biochar's effects on soybean growth across different developmental stages. This will elucidate the mechanisms by which soil microorganisms play a role in the global carbon cycle, thereby providing quantifiable scientific evidence to enhance soil potential, ensure the sustainable use of black soil, and promote the sustainable and healthy development of agriculture.

2. Materials and Methods

2.1. Field Experimental Design

The field experiment was carried out at the Research Farm of Harbin Normal University (45°52'10" N, 126°33'20" E) during the 2022 growing season. The soybean variety

HeiNong551 was chosen as the test crop because it is widely cultivated in the Northeast black soil region and has consistent agronomic performance. A randomized complete block design was used with four biochar application treatments, each replicated three times. Before planting, corn stalk biochar was evenly mixed into the soil at rates of 0 kg/hm² (control, CK), 300 kg/hm² (BC300), 600 kg/hm² (BC600), and 900 kg/hm² (BC900). Each plot measured 20 m² (5 m × 4 m) with 1 m buffer zones between plots to prevent cross-contamination and edge effects.

The corn stalk biochar used in this experiment was produced through slow pyrolysis of corn stover at 500 °C under oxygen-limited conditions for 4 h. The biochar was ground and sieved through a 2 mm mesh before application to ensure a uniform particle size. The basic physicochemical properties of the biochar were characterized before field application (Table 1): moisture content of 10.26%, organic carbon content of 510.9 g/kg, total nitrogen of 8.51 g/kg, total phosphorus of 2.34 g/kg (P₂O₅: 5.53 g/kg), and total potassium of 15.76 g/kg (K₂O: 19.15 g/kg). Biochar was manually broadcast on the soil surface and immediately incorporated into the top 0–20 cm soil layer using rotary tillage to ensure thorough mixing. Soybean seeds were sown at a density of 300,000 plants per hectare with a row spacing of 60 cm, following standard local agronomic practices. Standard field management practices, including weed control and pest management, were applied uniformly across all experimental plots throughout the growing season.

Table 1. Basic Physicochemical Properties of Biochar.

Material	Moisture Content %	Organic Carbon g/kg	Total Nitrogen g/kg	Total Phosphorus g/kg	P205 g/kg	Total Potassium g/kg	K20
Corn Stalk	10.26	510.9	8.51	2.34	5.53	15.76	19.15

2.2. Soil Sample Collection

Rhizosphere soil samples were collected during the soybean initial flowering (R1) stage using a shaking method. For each treatment, five soybean plants with consistent growth traits were carefully chosen to form one experimental replicate. Rhizosphere soil was obtained by gently shaking the intact root system to collect soil tightly attached to the roots. The soil from each replicate was thoroughly mixed to create a composite sample, which was then labeled and immediately stored at 4 °C in a refrigerator for later laboratory analysis.

2.3. Methods for Determining the Physicochemical Properties of Soil

With reference to Bao Shidan's Soil Agrochemical Analysis [17], the following parameters were determined for the rhizosphere soil of soybeans under four different treatments: pH, moisture content, total nitrogen, total phosphorus, total potassium, available nitrogen, available phosphorus and available potassium. The following methods were employed: a pH meter (Shanghai INESA Scientific Instruments Co., Ltd., Shanghai, China) to measure pH, the spot method to determine moisture content, an elemental analyser (Thermo Fisher Scientific, Waltham, MA, USA) to determine total nitrogen, the molybdenum–antimony colorimetric method to determine total phosphorus and available phosphorus, a flame photometer (Shanghai Precision Instruments Co., Ltd., Shanghai, China) to determine total potassium, the alkali diffusion method to determine available nitrogen, and the ammonium acetate extraction–flame photometer method to determine available potassium.

2.4. Soil Organic Carbon Fractions

SOC content was determined using the heated oxidation method with potassium dichromate [18]. Microbial biomass carbon (MBC) was determined using the substrate-induced respiration method [19]. ROC content was determined using the potassium permanganate oxidation method [20]. Dissolved organic carbon (DOC) content was determined using the potassium sulphate (0.5 M K₂SO₄) extraction method [21].

2.5. Determination of Soil Enzyme Activity

With reference to Guan Songyin's *Soil Enzymes and Their Research Methods* [22], the 3,5-dinitrosalicylic acid colorimetric method was employed to determine the activity of sucrase and cellulase in soil, the sodium phenolate–sodium hypochlorite colorimetric method was used to determine the activity of urease, and the o-cresol colorimetric method to determine the activity of polyphenol oxidase in soil. The activities of neutral phosphatase, catalase and β -glucosidase in soil were determined in accordance with the instructions provided with the test kits (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China).

2.6. Metagenomic Sequencing and Bioinformatics Analysis

Total microbial genomic DNA was extracted from rhizosphere soil samples using the OMEGA Mag-Bind Soil DNA Kit (M5635-02, Omega Bio-Tek, Norcross, GA, USA) following the manufacturer's instructions. The concentration and purity of the extracted DNA were measured with a Qubit™ 4 Fluorometer (Qubit™4 Fluorometer, with WiFi: Q33238; Qubit™ Assay Tubes: Q32856; Qubit™ 1X dsDNA HS Assay Kit: Q33231) and the Qubit™ 1X dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Metagenomic libraries were prepared using the Illumina TruSeq Nano DNA LT Kit (Illumina, Inc., San Diego, CA, USA) and sequenced on the Illumina NovaSeq platform with paired-end (PE) reads (2 × 150 bp) at Illumina, San Diego, CA, USA. Raw sequencing reads were quality-filtered to eliminate low-quality sequences and adapter contamination. Taxonomic annotation of the high-quality reads was performed using Kaiju software 1.10.2 against the NCBI non-redundant (nr) protein database. Species abundance profiles were created by statistically analyzing the annotation data and the number of reads assigned to each taxonomic unit. Data were organised using Excel 2021, whilst the R software 4.5.3 was used to calculate bacterial community diversity indices and to perform principal component analysis (PCoA) and NMDS analysis based on Bray–Curtis distance. Based on the taxonomic and functional profiles of non-redundant genes, LEfSe (Linear Discriminant Analysis Effect Size) was employed to identify taxonomic groups and functions. One-way analysis of variance (ANOVA) was performed on dominant soil species using SPSS 27; R software was used to plot species abundance clustering heatmaps, and Kruskal–Wallis tests were conducted, with Duncan's post hoc tests and BH multiple comparison correction to analyse differences in bacterial communities between groups. Redundancy analysis (RDA) was used to examine the relationship between soil properties and the composition of the soil bacterial community. Spearman's correlation *p*-values and *R*-values were calculated using SPSS 27, with the analysis of correlation results carried out on the Paiseno Gene Cloud Platform (<https://www.genesccloud.cn/home>, accessed on 10 November 2025) and the Lianchuan Bio Cloud Platform (<https://www.omicstudio.cn/tool>, accessed on 10 November 2025). Functional annotation of carbon-cycling genes was conducted using the CAZyme (Carbohydrate-Active Enzymes) database. Carbon cycle-related genes were classified into six main functional groups: Glycoside Hydrolases (GHs), Glycosyltransferases (GTs), Carbohydrate Esterases (CEs), Auxiliary Activities (AAs), Polysaccharide Lyases (PLs), and Carbohydrate-Binding Modules (CBMs). To identify key genes involved in carbon fixation and degradation, the expression levels of 22 essential enzyme genes

across major carbon cycling pathways were quantitatively analyzed using established protocols. These genes play critical roles in pathways such as the Calvin cycle, the reductive tricarboxylic acid cycle, and various pathways for degrading organic matter, including starch, cellulose, hemicellulose, pectin, and chitin [23,24].

2.7. Determination of Agronomic-Related Traits in Soybean

The agronomic characters of soybean plants in each treatment group were measured at the branching stage, flowering stage and maturity stage, including plant height, fresh and dry weight of stems and leaves, fresh and dry weight of roots and fresh and dry weight of nodules. The number of pods per plant and the number of grains per plant were measured at maturity to evaluate the effect of biochar on soybean agronomic characters.

2.8. Statistical Analysis

All data were analyzed using one-way analysis of variance (ANOVA) with SPSS 26.0. Treatment means were compared with Duncan's multiple range test at a $p < 0.05$ significance level. Pearson correlation analysis was performed to examine relationships between soybean growth parameters, soil properties, enzyme activities, and microbial characteristics. Correlation networks were visualized using R packages, with line thickness indicating correlation strength and statistical significance shown by Mantel test results ($p < 0.01$, $0.01 \leq p < 0.05$, $p \geq 0.05$). Linear discriminant analysis effect size (LEfSe) was used to identify differentially abundant microbial taxa ($\text{LDA} > 3.37$, $p < 0.05$).

3. Results

3.1. Effects of Biochar Application on Soil Physicochemical Properties and Fertility

Application of biochar significantly influenced various soil physicochemical properties in the soybean rhizosphere, as shown in Figure 1. Soil pH gradually increased with higher biochar rates, with all treatments (BC300, BC600, and BC900) exhibiting significantly higher pH than the control. The pH rose from about 7.1 in the control to 7.3, 7.3, and 7.6 in BC300, BC600, and BC900, respectively, with notable differences among treatments. Soil moisture content followed a similar trend, increasing from less than 5% in the control to approximately 15% in all biochar-amended soils.

There was no significant difference in total nitrogen content between the control group and the BC300 treatment; it reached a maximum of 0.91 g kg^{-1} under the BC900 treatment. Total phosphorus content in the rhizosphere exhibited a dose-dependent variation, with the BC900 treatment showing the highest phosphorus content at approximately 1.11 g kg^{-1} , which was significantly higher than the $0.61\text{--}0.69 \text{ g kg}^{-1}$ observed in the control group; no significant differences in total phosphorus content were found between the BC300, BC600 and BC900 treatments. No significant differences were found among BC300, BC600, and BC900 for total potassium. The available nitrogen content remained stable across all treatment groups, ranging from 78.46 to 81.81 mg kg^{-1} , with no significant differences between groups. There were significant differences in available phosphorus content, and the available phosphorus content showed a significant downward trend. The content in the BC300 treatment group (approximately 0.65 g kg^{-1}) was significantly lower than that in the control group (approximately 1.02 g kg^{-1}), whilst the BC600 and BC900 treatment groups were comparable to the BC300 treatment group, with no significant differences. Following the application of biochar, the BC600 treatment group had the highest available potassium content (approximately 1.67 g kg^{-1}); the control group and the BC300 treatment group were at similar levels, whilst the BC600 and BC900 treatment groups were both significantly higher than the control group, and there was no significant difference between the BC600 and BC900 treatment groups.

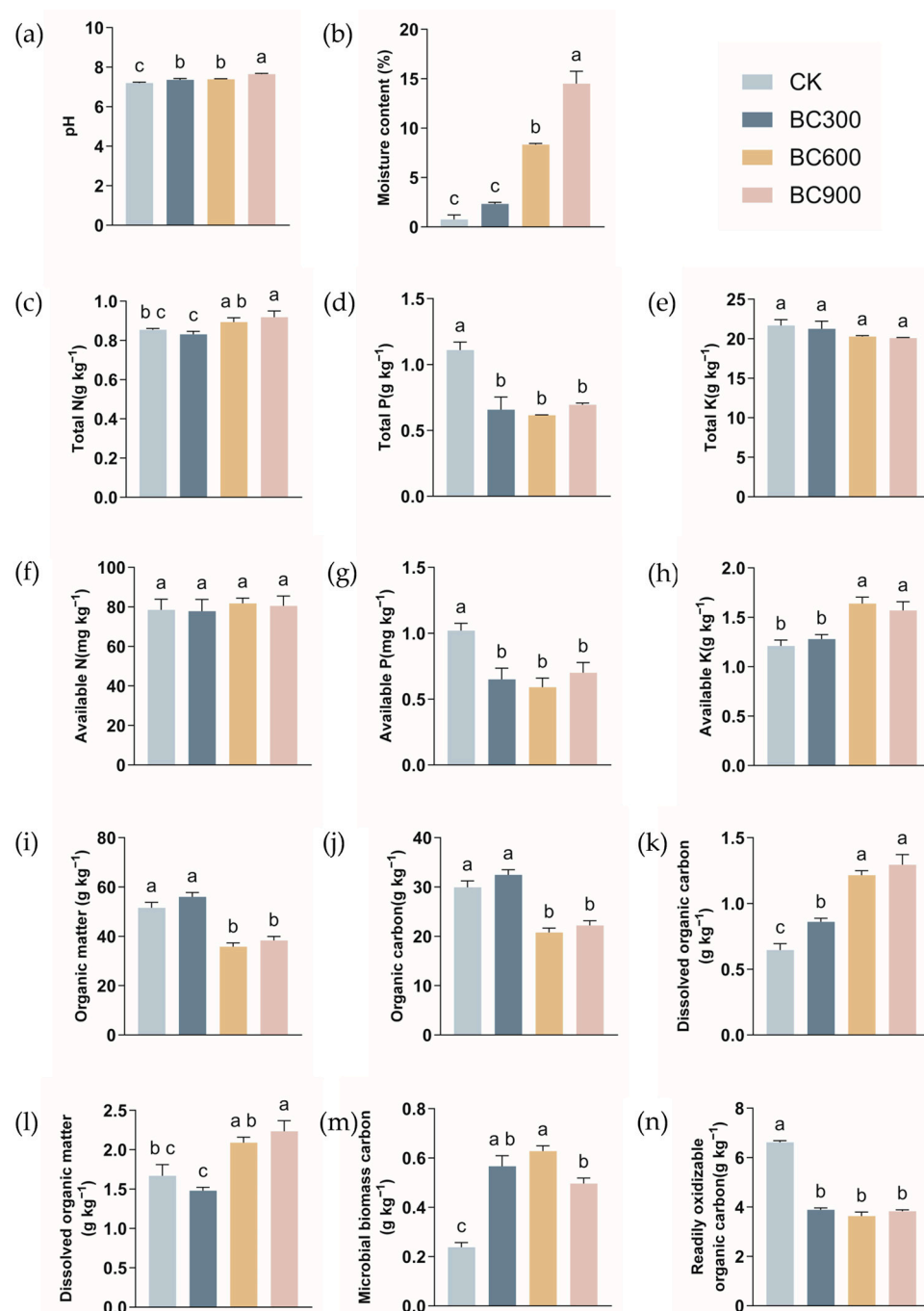


Figure 1. Effects of Graded Biochar Doses on Physicochemical Parameters in Soybean Rhizosphere Soil. The impact of different biochar application rates (CK: 0 kg/hm², BC300: 300 kg/hm², BC600: 600 kg/hm², BC900: 900 kg/hm²) on soil physicochemical properties in the soybean rhizosphere during the initial flowering stage (R1) was examined. Parameters measured include soil pH (a), moisture content (b), total nitrogen (TN) (c), total phosphorus (TP) (d), total potassium (e), available nitrogen (AN) (f), available phosphorus (AP) (g), available potassium (AK) (h), soil organic matter (OM) (i), soil organic carbon (SOC) (j), dissolved organic carbon (DOC) (k), dissolved organic matter (DOM) (l), microbial biomass carbon (MBC) (m), and readily oxidizable organic carbon (ROC) (n). Different letters above bars indicate significant differences among treatments ($p < 0.05$). Data are presented as mean $\pm$ standard error ($n = 3$ replicates per treatment).

Biochar application significantly influenced soil organic carbon content, initially increasing and then declining at higher application rates. The control had the lowest organic carbon, about 28 g kg⁻¹, which notably increased to around 30 g kg⁻¹ with BC300. In

contrast, BC600 and BC900 treatments showed lower levels, roughly 20–22 g kg⁻¹, than BC300, but these differences were not statistically significant. Organic matter content followed a similar pattern, with BC300 reaching about 60 g kg⁻¹—significantly higher than the control at approximately 56 g kg⁻¹. BC600 and BC900 had reduced levels, between 35 and 40 g kg⁻¹, significantly lower than those of both the control and BC300. Dissolved organic carbon varied significantly, with the control around 0.7 g kg⁻¹, and biochar treatments increasing this level. BC300 and BC600 had intermediate values around 0.9 g kg⁻¹, while BC900 reached about 1.2 g kg⁻¹. Significant differences existed between the control and biochar treatments, but BC300 and BC600 did not differ significantly from each other. Microbial biomass carbon was highest in the control at approximately 25 g kg⁻¹. Biochar significantly increased microbial biomass in BC300 (about 0.6 g kg⁻¹) and BC600 (around 0.65 g kg⁻¹) compared to the control, although BC900 showed slightly lower levels (~5 g kg⁻¹), still exceeding the control. Easily oxidizable organic carbon decreased notably with biochar, with the control at around 6.5 g kg⁻¹—significantly higher than all biochar treatments. BC300 dropped sharply to about 3.5 g kg⁻¹, while BC600 and BC900 hovered around 3.0 g kg⁻¹, with no significant difference among these. Overall, these changes suggest that biochar mainly modified soil properties, enhancing certain fertility parameters such as pH, moisture retention, and labile carbon fractions, while also affecting nutrient distribution and availability in the soybean rhizosphere.

3.2. Effects of Biochar Application on Soil Enzyme Activities

Applying biochar significantly affected the activity of various soil enzymes involved in cycling carbon, nitrogen, and phosphorus in the soybean rhizosphere (Figure 2). Sucrase activity decreased in a dose-dependent way with increasing biochar amounts, with the control showing the highest activity at approximately 7.37 mg g⁻¹ 24 h⁻¹, which was much higher than all biochar treatments. The BC300 treatment caused a moderate drop to about 6.5 mg g⁻¹ 24 h⁻¹, while BC600 and BC900 had notably lower activities at around 5.0 and 6.0 mg g⁻¹ 24 h⁻¹, respectively. Statistical tests confirmed significant differences among all treatments, with BC600 exhibiting the lowest sucrase activity. Urease activity stayed relatively steady across treatments, with no significant difference between control and biochar-treated soils, ranging from approximately 0.6 to 0.7 mg g⁻¹ 24 h⁻¹. Acid phosphatase activity showed moderate change, but differences were less obvious than other enzymes. The control and BC300 treatments had similar activities at about 1.3–1.4 mg g⁻¹ 24 h⁻¹, while BC600 was slightly lower at around 1.25 mg g⁻¹ 24 h⁻¹, and BC900 reached approximately 1.5 mg g⁻¹ 24 h⁻¹. Only BC600 was significantly different from BC900; other comparisons were not statistically distinct. Catalase activity varied a lot among treatments: the control had about 320 mg g⁻¹ 24 h⁻¹, while biochar applications significantly increased activity, with BC300 showing the strongest effect, at roughly 445 mg g⁻¹ 24 h⁻¹, notably higher than all other treatments. BC600 and BC900 had intermediate levels at about 336 and 392 mg g⁻¹ 24 h⁻¹, respectively. Most differences were statistically significant. Cellulase activity was especially responsive, it first decreases and then increases again. The control's activity was about 1.84 mg g⁻¹ 72 h⁻¹, while BC300 and BC600 had much lower activities at around 1.1 and 0.9 mg g⁻¹ 72 h⁻¹, respectively, with no difference between these two. BC900 recovered to approximately 1.31 mg g⁻¹ 72 h⁻¹, which was significantly higher than BC300 and BC600 but still below the control. Polyphenol oxidase activity showed the clearest treatment effect, with the control at about 120 mg g⁻¹ 24 h⁻¹. BC300 experienced a moderate increase to around 140 mg g⁻¹ 24 h⁻¹, but this was not statistically different from the control. BC600 showed a large boost to roughly 240 mg g⁻¹ 24 h⁻¹, significantly higher than all other treatments. BC900 had intermediate activity at about 190 mg g⁻¹ 24 h⁻¹, significantly above the control and BC300 but

below BC600. β -glucosidase activity stayed fairly consistent across all treatments, with no significant differences, ranging from about 21 to 25 $\text{mg g}^{-1} \text{h}^{-1}$. These results show that biochar affects soil enzymes differently depending on their role in nutrient cycling. Enzymes involved in breaking down labile carbon, like β -glucosidase, showed little change, while those related to stable organic carbon breakdown, such as cellulase and oxidative enzymes like polyphenol oxidase and catalase, responded in complex ways depending on the biochar rate. These enzymatic changes suggest that biochar influences soil carbon cycling by affecting different decomposition pathways, promoting the formation of stable carbon pools while allowing labile carbon turnover.

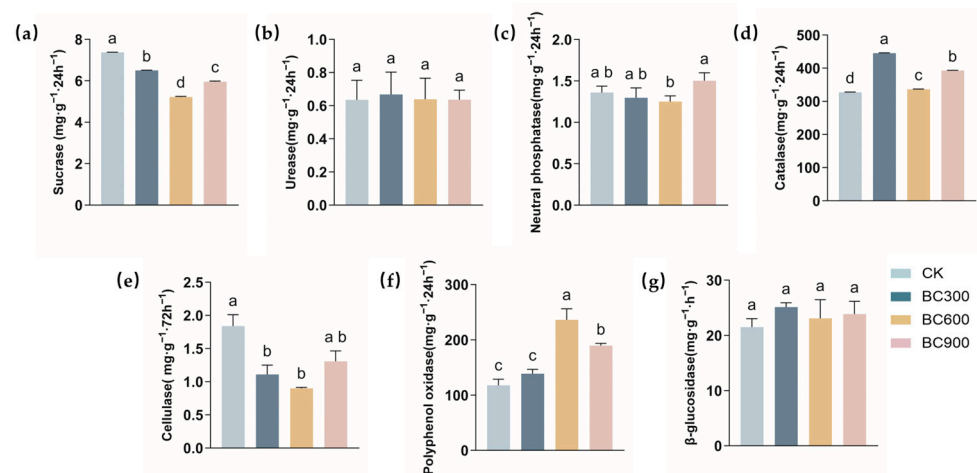


Figure 2. Impacts of Different Biochar Doses on Extracellular Enzyme Profiles in Soybean Rhizospheric Soil. The enzymes analyzed include sucrase (a), urease (b), neutral phosphatase (c), catalase (d), cellulase (e), polyphenol oxidase (f), and β -glucosidase (g). Significant differences between treatments are indicated by different letters above the bars ($p < 0.05$). Data are presented as mean $\pm$ standard error ($n = 3$).

3.3. Effects of Biochar Application on Bacterial Community Composition and Diversity in Soybean Rhizosphere Soil

With regard to α -diversity indices (Figure 1), community richness (Chao 1 and Observed species), community diversity indices (Simpson and Shannon), community species coverage (Goods coverage) and community evenness (Pielou evenness) differed significantly across the treatment groups (Table A2). With regard to β -diversity, this study utilised principal component analysis (PCoA) and NMDS analysis based on Bray–Curtis distances to investigate differences in the β -diversity of soil bacterial communities under different biochar treatment doses. The PCoA results indicated that the soil bacterial community compositions of the CK, BC300, BC600 and BC900 treatments were clearly distinguishable from one another, accounting for 73.3% of the total variation in soil bacterial communities (Figure A1), suggesting that there were certain differences in the soil bacterial communities under different biochar treatment doses. The NMDS analysis results showed that the stress value for the bacterial communities was 0.0000982, all values of which were less than 0.1, indicating that the analysis results were valid and statistically significant. The CK and BC300 groups were located in the same quadrant, indicating a high degree of similarity in bacterial community structure; the BC600 and BC900 groups were situated in different quadrants, indicating substantial differences between them (Figure A2). The results suggest that the application of biochar provided an ample supply of utilisable carbon sources for the growth and reproduction of soil microorganisms, thereby increasing microbial richness and diversity by enhancing both soil α -diversity and β -diversity.

The species difference analysis of soil bacterial community under different doses of biochar treatment showed that each subtype had its own microbial population. The num-

ber of specific independent species treated with CK, BC300, BC600 and BC900 were 328, 510, 536 and 434, respectively, indicating that applying biochar can promote the growth of soil-specific microorganisms (Figure 3a). These findings suggest that moderate- to-high biochar concentrations promote distinct bacterial taxa, thereby increasing diversity. At the genus level (Figure 3c), bacterial communities varied across treatments, with different dominant taxa. The control exhibited a unique structure, with higher abundances of certain genera. As biochar rates increased, the proportions of dominant genera changed, restructuring the bacterial community. LEfSe analysis (Figure 3d) identified significantly enriched biomarker taxa in each group ($LDA > 3.37$, $p < 0.05$). The control had 26 biomarker taxa, mainly Proteobacteria, representing baseline soil bacteria. BC300 had 47 biomarker taxa, with enrichment of Actinobacteria, γ -Proteobacteria, Limnocyndrales, and Actinomycetia, known for organic decomposition and nutrient cycling. BC600 showed 23 biomarkers, notably NC 10 (Methylophilum), involved in methane and nitrogen cycling. BC900 had only 2 biomarkers, primarily the genus AC 69, indicating a more specialized community at high biochar rates. Heatmap analysis of the top 10 genera (Figure 3b) revealed treatment-based clustering, with BC300 and BC600 being similar, while BC900 and the control diverged. Certain genera like Nocardioideae and Sphingomicrobium increased with biochar, contributing to beneficial functions, while UBA 5189 decreased, possibly due to competition. The results suggest that biochar dose selectively enriches or suppresses taxa, shaping community composition and functional potential. Moderate rates (BC300, BC600) enhance diversity and key functional taxa, while very high rates (BC900) may limit diversity.

3.4. Effects of Biochar Application on Carbon Cycling Functional Genes

Based on the CAZyme database, C-loop genes are classified into six categories according to their specific functions, including glycosidases (GHs), glycosyltransferases (GTs), carbohydrate esterases (CEs), auxiliary activities (AAs), polysaccharide lyases (PLs) and carbohydrate-binding modules (CBMs) [25]. The relative abundances of these six gene families, from highest to lowest, are: GTs > GHs > CBMs > CEs > AAs > PLs. Among these, GHs are involved in the hydrolysis of glycosidic bonds. Together with PLs and CEs, these enzymes are typically associated with SOC degradation, whilst AAs are associated with lignin degradation. In contrast, GTs are involved in the formation of glycosidic bonds, creating glycosides associated with SOC biosynthesis. CBMs are non-catalytic protein domains that bind to carbohydrates; although they lack catalytic activity, they are polysaccharide-binding modules involved in carbohydrate degradation. They have been extensively studied in microorganisms and are capable of specifically binding to polysaccharides, thereby effectively enhancing the catalytic efficiency of carbohydrate enzymes [26]. The results of one-way analysis of variance (ANOVA) showed that the application of biochar significantly altered the relative abundances of six functional genes (Figure 4). As the biochar dose increased, the abundance of AAs increased significantly, whilst that of PLs decreased significantly; the abundances of GTs, GHs, CBMs and CEs also showed significant changes across different treatments, indicating that the application of biochar significantly influences the microbial functional genes involved in SOC synthesis and degradation.

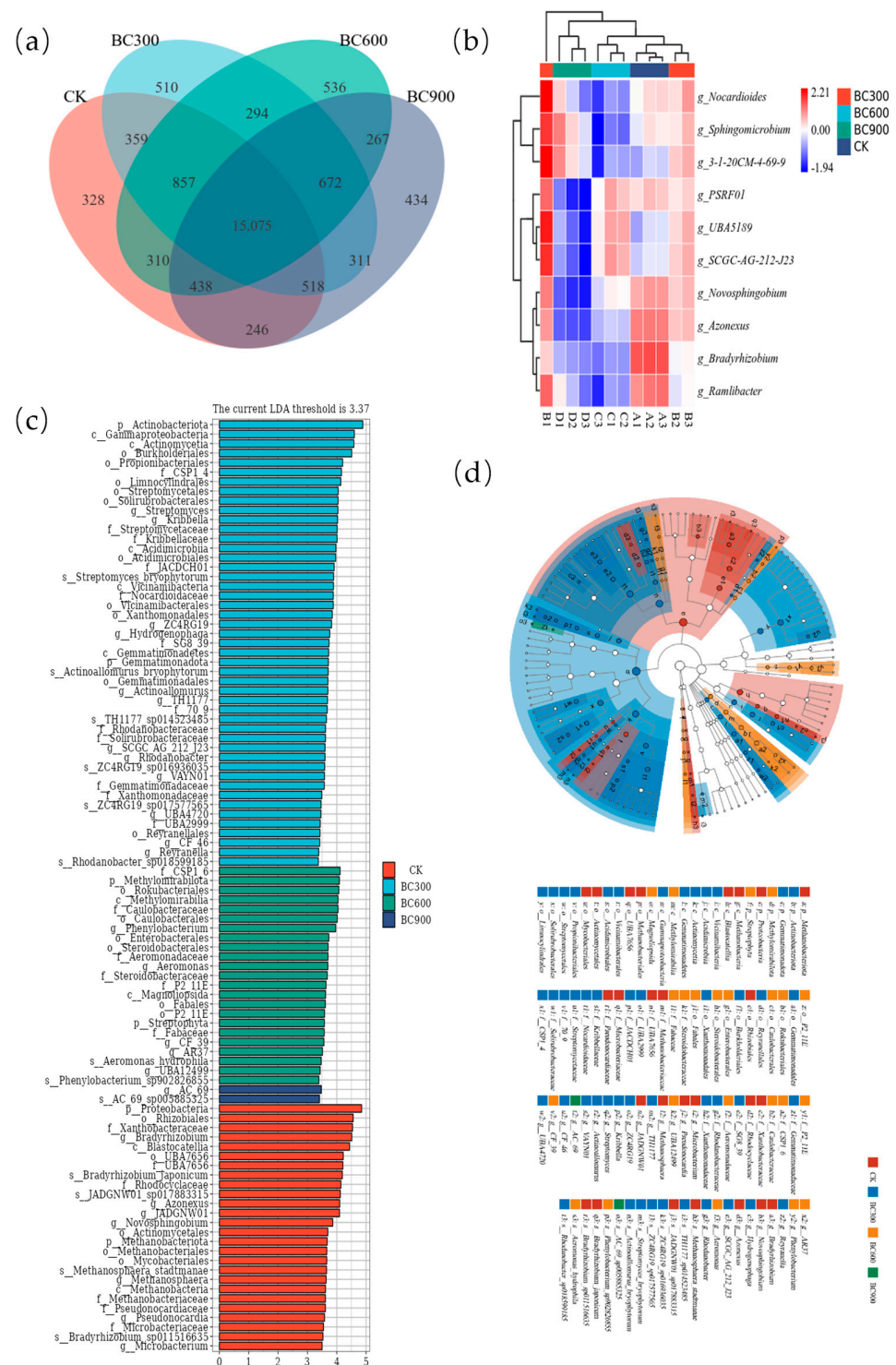


Figure 3. Impacts of Differential Biochar Dosages on Bacterial Community Structure and Diversity in the Rhizospheric Soil of Soybean. (a) Venn diagram showing shared and unique OTUs among treatments (CK: 0 kg/hm², BC300: 300 kg/hm², BC600: 600 kg/hm², BC900: 900 kg/hm²). The core microbiome, comprising 10,072 OTUs, was shared across all treatments. (b) Stacked bar chart displaying bacterial community composition at the genus level, with relative abundance of dominant genera across treatments. (c) LefSe cladogram identifying biomarker taxa significantly enriched in each group (LDA score > 3.37, $p < 0.05$). Colored segments indicate taxonomic levels from phylum to genus, with colors corresponding to treatments. Numbers in parentheses indicate significant biomarker taxa for each treatment. (d) Heatmap of the top 10 bacterial genera's relative abundance across treatments, with hierarchical clustering. Color indicates normalized abundance, with red for higher and blue for lower abundance.

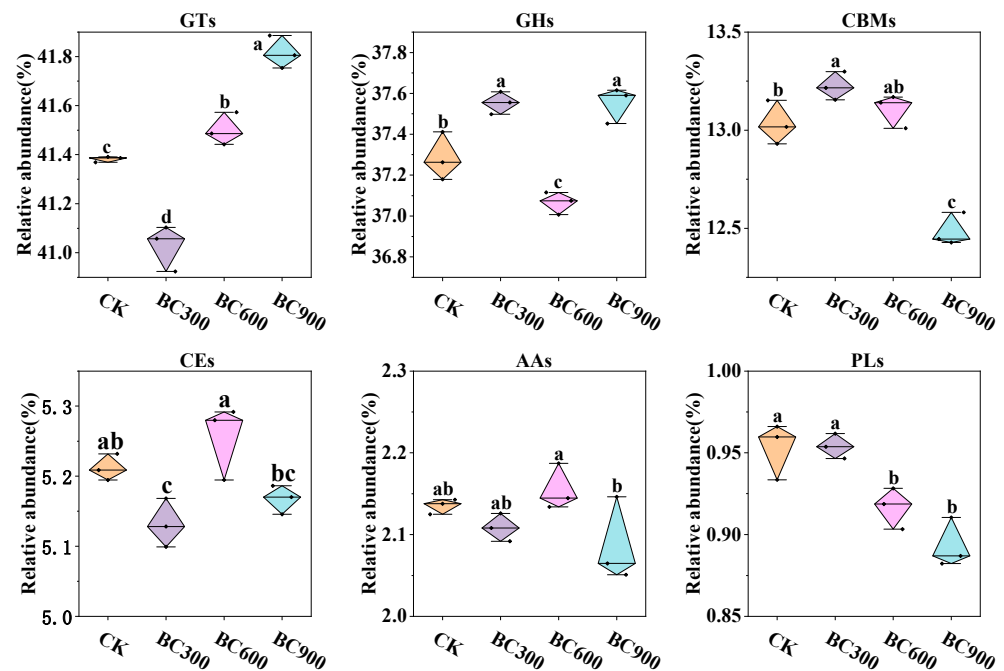


Figure 4. Relative Abundance of Carbon Cycle Genes under Different Doses of Biochar. Boxplot showing the absolute abundance (gene copy numbers) of six major carbohydrate-active enzyme families: Glycosyltransferases (GTs), Glycoside Hydrolases (GHs), Carbohydrate-Binding Modules (CBMs), Carbohydrate Esterases (CEs), Auxiliary Activities (AAs), and Polysaccharide Lyases (PLs). Different letters indicate significant differences among treatments ($p < 0.05$).

Based on the KEGG database, key enzyme genes involved in microbial carbon cycling—including carbon fixation and carbon degradation (starch, pectin, hemicellulose, cellulose, chitin and lignin)—were further identified. Carbon fixation pathways primarily include key enzyme genes in the Calvin cycle (CBB) and the reductive tricarboxylic acid cycle (rTCA): *rbcL*, *rpiA*, *PGK*, *korA*, *korB* and *aclA*. In the process of organic matter decomposition, ranging from easily degradable SOC to difficult-to-degrade SOC, these include key enzyme genes: *amyA*, *pulA*, *malQ* and *malZ*; genes encoding key enzymes involved in the degradation of pectin: *Pectin esterase* and *pel*; genes encoding key enzymes involved in the degradation of hemicellulose: *abfA*, *gmuG*, *xylA* and *xylB*; genes encoding key enzymes involved in the degradation of cellulose: *endoglucanase* and *bglX*; genes encoding key enzymes involved in the degradation of chitin: chitinase and *nagA*; and genes encoding key enzymes involved in the degradation of lignin: *catE* and *laccase*. As shown in Figure 4, the application of biochar significantly influenced the carbon fixation pathways of soil microorganisms. Biochar had no significant effect on the relative abundances of the key genes *rbcL*, *rpiA* and *PGK* involved in the Calvin cycle (CBB) (Figure 5a). However, the application of biochar significantly increased the relative abundance of the *korA* gene (Figure 5b), suggesting that biochar influences carbon fixation pathways primarily by affecting the reductive tricarboxylic acid cycle (rTCA).

One-way analysis of variance revealed that the application of biochar had a significant effect on the degradation of starch, pectin, hemicellulose, cellulose and chitin, but no significant effect on the degradation of lignin (Figure 6). The application of biochar significantly increased the gene abundance of *amyA* and *pulA*, which encode α -amylase and amylopectinase, respectively, involved in starch degradation; however, it had no significant effect on the gene abundance of those encoding 4- α -glucan transferase and α -glucosidase compared with the control (CK) treatment (Figure 6a–d). Consistent with the findings for amylolytic enzymes, the application of biochar significantly increased the gene abundance of *pectin esterase* and *pel*, which encode pectin esterase and pectin lyase,

respectively, during pectin degradation (Figure 6e,f). Conversely, the application of biochar significantly reduced the gene abundance of *xylB* and *xylA*, which encode xylose kinase and xylose isomerase, respectively, in hemicellulose degradation (Figure 6g–j). Similarly, the application of biochar significantly reduced the relative abundance of the *bgIX* gene, which encodes β -glucosidase, in cellulose degradation (Figure 6k,l). In addition, the application of biochar significantly increased the relative abundance of the chitinase gene, which encodes chitinase involved in chitin degradation, and reduced the abundance of the *nagA* gene, which encodes N-acetylglucosamine-6-phosphate deacetylase; however, the differences between the treatment groups were not significant (Figure 6m,n). There were no significant differences in the gene abundance of key enzymes involved in lignin degradation among the various biochar treatment rates (Figure 6o,p). These results indicate that, compared with the degradation of recalcitrant SOC, the application of biochar more effectively promotes the degradation of easily mineralised SOC in soil.

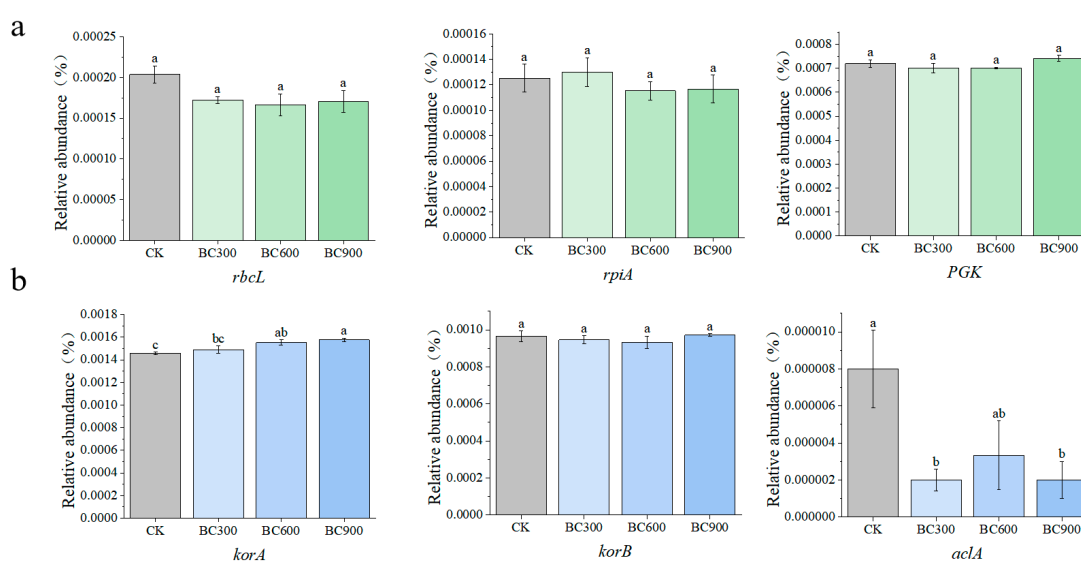


Figure 5. Effects of Different Doses of Biochar on Carbon Fixation. (a) Key genes in the Calvin cycle (CBB); (b) Key genes in the reductive tricarboxylic acid cycle (rTCA). Different letters indicate significant differences among treatments ($p < 0.05$). These functional genes encode key enzymes involved in soil carbon synthesis, degradation, and cycling.

3.5. Effects of Biochar on Agricultural Characters of Soybean in Different Periods

The application of biochar significantly affected the characteristics of soybean in each growth cycle. Biochar significantly promoted the plant height of soybean at all stages, and the plant height of soybean under BC300, BC600 and BC900 treatments was significantly higher than that under CK treatment, and the plant height of soybean under BC900 treatment was the largest (Table A4). The application of biochar had a significant effect on root length only during the pod-setting stage, whilst root nodules increased significantly only under the BC900 treatment. The fresh weights of the soybean stems, leaves and root systems increased markedly compared with the CK treatment at all growth stages; however, the fresh weight of root nodules increased significantly only in the BC900 treatment during the pod-setting stage (Table A5). With the application of biochar, the fresh weight of stems and leaves increased markedly; at all growth stages, the fresh weight of stems and leaves was highest in the BC900 treatment. The fresh weight of the root system and root nodules increased significantly only during the pod-setting stage, with the most significant increase observed in the BC900 treatment. After maturity, the number of pods per plant and the number of seeds per plant increased significantly with the application of biochar, with the most significant increases observed in the BC900 treatment (Table A6).

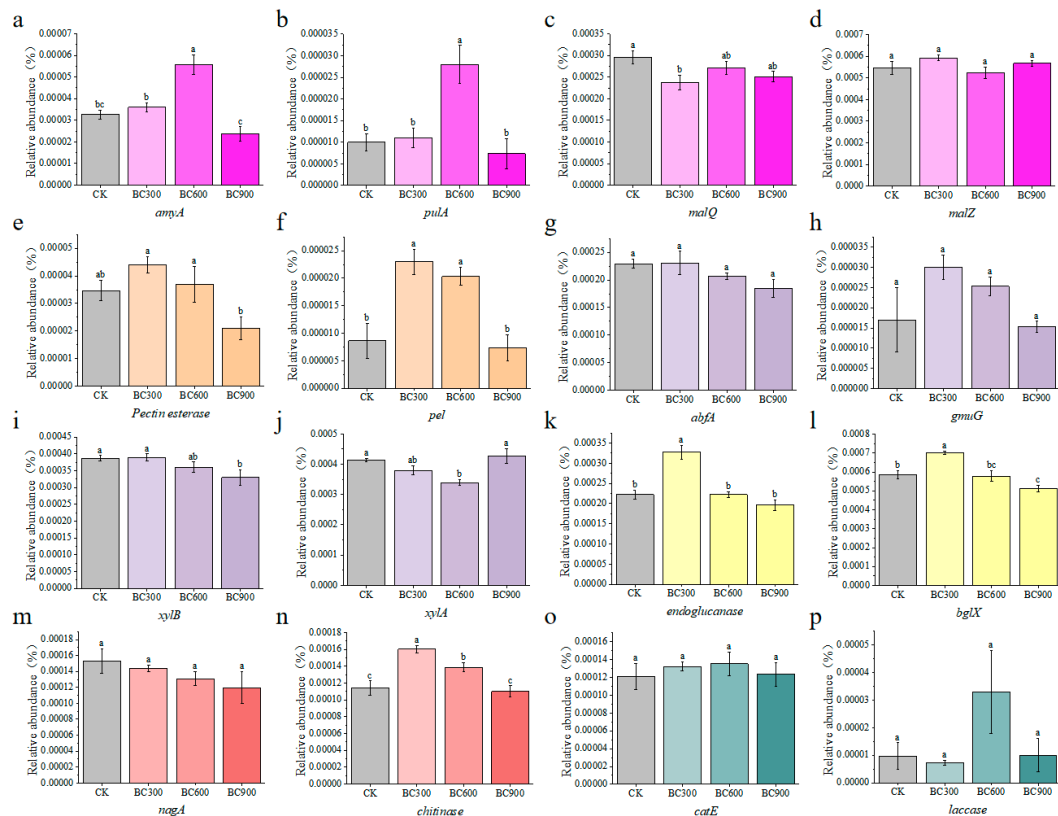


Figure 6. Effects of Different Doses of Biochar on Carbon Degradation Genes. (a–d) Key genes for starch degradation; (e,f) Key genes for pectin degradation; (g–j) Hemicellulose degradation key genes; (k,l) Cellulose degradation key genes; (m,n) Key chitin degradation genes; (o,p) Key genes for lignin degradation. Different letters indicate significant differences among treatments ($p < 0.05$). These functional genes encode key enzymes involved in soil carbon synthesis, degradation, and cycling.

3.6. Correlations Between Soil Properties, Carbon Cycling Genes, and Agronomic Traits of Soybeans

To evaluate the relationships between microbial functional genes involved in soil carbon cycling processes and environmental factors under different doses of biochar treatment, a correlation analysis was conducted between carbon cycling genes and soil physico-chemical properties. Spearman's correlation analysis revealed that GTs were significantly correlated with soil pH, SOC and ROC; GTs were positively correlated with soil pH and negatively correlated with SOC and ROC. However, no significant correlations were detected between GTs and other soil properties. CBMs were positively correlated with SOC. PLs were negatively correlated with soil pH and positively correlated with ROC. CEs and AAs showed no significant correlations with other soil properties (Figure 7).

Spearman's correlation analysis revealed that genes encoding key enzymes involved in carbon fixation pathways and carbon degradation processes exhibited significant responses to environmental factors (Figure 8). Among the carbon fixation pathways, the key gene *rbcL*, which encodes ribulose-1,5-bisphosphate carboxylase (RuBisCO) involved in the Calvin cycle (CBB), showed a negative correlation with soil water content and a positive correlation with soil ROC. The key gene *korA*, which encodes the iron redox enzyme involved in the reductive tricarboxylic acid cycle (rTCA), was positively correlated with soil pH and DOC. In the carbon degradation process, the key enzyme genes encoding the enzymes involved in the degradation of starch, pectin, hemicellulose, cellulose and lignin all showed significant responses to soil properties; however, the key enzyme genes encoding chitin degradation showed no significant correlation with soil properties. Specifically, soil moisture content and pH were negatively correlated with the key enzyme genes *malQ*, *abfA* and *xyiB*, which

are involved in the degradation of starch and hemicellulose. SOC and MBC were positively correlated with the key enzyme genes *Pectin esterase*, *pel*, *endoglucanase* and *catE*, which are involved in the degradation of pectin, cellulose and lignin. The results indicate that soil moisture content, pH, SOC, DOC and ROC are all associated with soil carbon cycling.

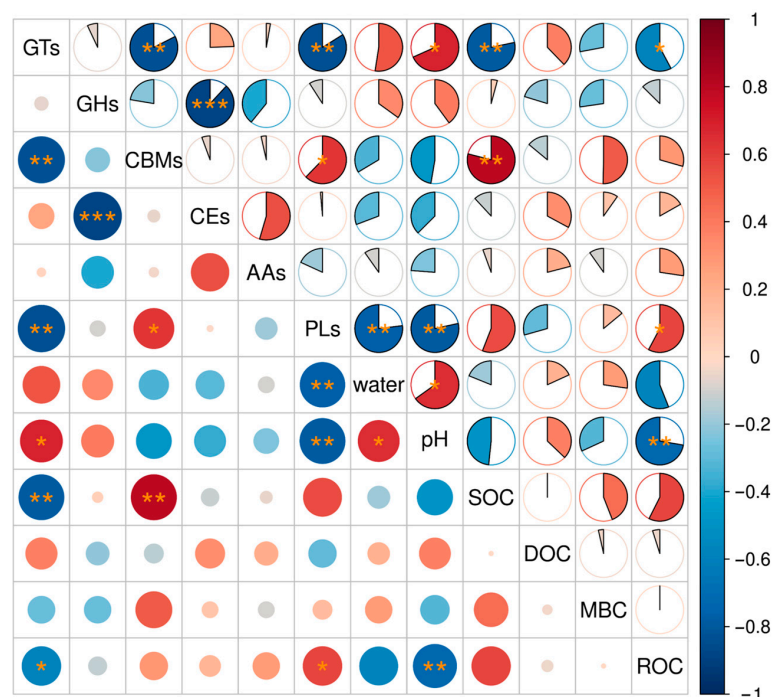


Figure 7. Correlation Analysis between Carbon Cycling Genes and Soil Properties. Asterisks indicate significance levels: none ($p \geq 0.05$), * ($0.01 \leq p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$).

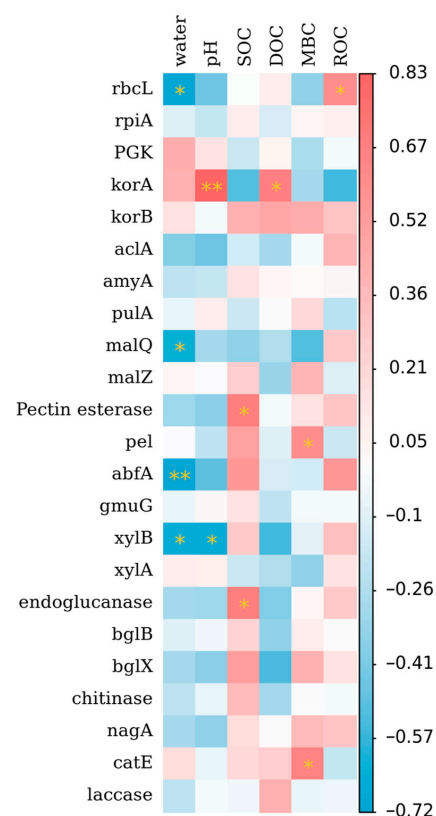


Figure 8. Correlation between Carbon Sequestration and Carbon Degradation Genes and Soil Properties. Asterisks indicate significance levels: none ($p \geq 0.05$), * ($0.01 \leq p < 0.05$), ** ($p < 0.01$).

4. Discussion

4.1. Effects of Biochar Application on Soil Fertility and Function

Biochar is an environmentally friendly soil conditioner with distinctive physicochemical properties, such as a larger specific surface area, increased porosity, and a higher surface charge. These features significantly affect nutrient retention and transformation in soil [27]. Research shows that biochar promotes soil nitrogen turnover and boosts inorganic nitrogen supply by improving nitrogen availability and overall nitrogen conversion rates [28]. Soil moisture and pH levels greatly influence how plants utilize nutrients [29]. This study reveals that adding biochar substantially raises soil pH and moisture content. It also affects soil nitrogen, phosphorus, and potassium levels. With the application of biochar, the total nitrogen content gradually increased, reaching its highest level under the BK900 treatment, whilst the total phosphorus and available phosphorus contents decreased, possibly due to heightened soil phosphatase activity and a rise in microbial community abundance and gene expression linked to phosphorus cycling, which can lead to phosphorus loss [30].

The SOC, a key soil quality indicator, affects chemical, physical, and biological soil properties and is vital for nutrient cycling, soil aggregate formation, water retention, and supporting biodiversity [31,32]. Research shows that biochar application boosts microbial activities linked to SOC breakdown, including SBR, diacetyl fluorescein hydrolysis, and metabolic entropy [33,34]. Its influence on SOC may also depend on soil pH, with higher efficiency in sequestering SOC in acidic and loamy soils, and a lesser effect in alkaline and sandy soils. Findings suggest that biochar application significantly decreases SOC and ROC levels, likely due to increased microbial activity involved in SOC degradation, while soil DOC and MBC levels rise. Since these are the most reactive soil carbon fractions, their changes are driven by microbial processes. At the same time, the reduction in SOC indicates a shift in soil organic matter from resistant to more labile forms, possibly because increased soil DOC supports microbial growth and accelerates SOC breakdown and microbial degradation of SOC [35,36].

4.2. Effects of Biochar Application on Soil Enzyme Activities

Soil enzymes exhibit substrate specificity: proteases target proteins, amylases and cellulases degrade carbohydrates, phosphatases act on organic phosphates, and oxidases and peroxidases work on lignin. Research indicates that soil microorganisms are the main source of soil enzymes. Biochar application influences enzyme activity by affecting microbial metabolism, which can either promote, inhibit, or alter enzyme or substrate reactions through the adsorption of reaction substrates needed by microbes [37]. The results indicate that biochar reduces sucrase activity whilst increasing catalase activity. At the same time, it promotes the activity of enzymes involved in the degradation of stable organic carbon, such as polyphenol oxidase; conversely, biochar significantly reduces the activity of cellulases associated with the degradation of stable SOC. Studies show biochar increases β -1,4-glucosidase activity by decreasing the proportion of SOC in intermediate particle sizes [38]. Similarly, biochar reduces carbon in readily degradable active SOC but increases carbon in refractory active SOC [39], promoting stable carbon build-up and limiting unstable carbon accumulation. It also affects phosphorus availability through chemical and microbial pathways [40] and influences soil nitrogen cycle enzyme activities based on its properties and duration in soil [41]. This study found that biochar markedly affects soil enzymes involved in the phosphorus cycle, with activity levels decreasing, then rising, in response to changes in available soil phosphorus, likely due to biochar raising pH and affecting microbial activity in phosphorus cycling, thus suppressing soil neutral phosphatase.

4.3. Effects of Biochar on the Bacterial Community Structure in the Rhizosphere Soil of Soybean

Soil microorganisms are a major part of the soil environment and are key regulators of soil ecological balance and nutrient cycling [42]. Soil microbial communities are vital in agriculture. Their composition and diversity indicate microbial activity and soil quality, sensitive to environmental changes. Factors like pH, moisture, and SOC content influence their structure and activity [43,44]. In this study, soil moisture, pH, SOC, and ROC were identified as factors affecting changes in soil microbial community composition and function. Among these, soil moisture and pH had consistent effects on the bacterial community structure, while soil ROC had opposite effects. pH, considered one of the most important soil variables, has been identified in many studies as a primary factor shaping soil microbial communities. This is likely because the addition of external materials can alter soil pH, which in turn influences microbial metabolic activity. Likewise, soil moisture indirectly affects microbial activity and community composition by impacting soil electrical conductivity. activity and community composition by affecting soil electrical conductivity [45].

The results of this study show that the application of biochar increased microbial abundance, diversity, and community richness. Biochar provided an accessible carbon source that supported soil microbial growth, thereby boosting their abundance and diversity. Analysis of the dominant bacterial genera in the soil indicated that the relative abundance of the top 10 bacterial genera was significantly affected. Specifically, biochar treatment notably increased the relative abundance of *Nocardia* (Actinobacteria), known for producing various antibiotics, and *Sphingomonas* (α -Proteobacteria). The α -Proteobacteria class encompasses numerous photosynthetic autotrophic bacteria and plant-associated symbionts that are vital to soil nutrient cycling and to promoting plant growth. The use of biochar boosts the growth of key taxa, enhancing the metabolism of unstable SOC. Moreover, ROC, a form of SOC that is easily oxidized and highly reactive, effectively indicates early changes in soil communities and environmental conditions [46].

4.4. Effects of Biochar Application on Soil Carbon Cycling

Carbon cycling is a crucial process in ecosystems, with soil microorganisms playing a key role in vital functions such as carbon fixation and decomposition via various pathways [47]. Known microbial carbon fixation pathways include the Calvin cycle, the reversed tricarboxylic acid cycle, and the reverse glycine cleavage pathway [48]. This study shows that applying biochar significantly affects genes involved in soil carbon synthesis and degradation, indicating that biochar can modify these processes and enhance overall soil carbon cycling [49]. KEGG analysis highlights the reductive tricarboxylic acid cycle as the main microbial carbon fixation pathway during biochar application [50]. Factors like soil moisture, pH, SOC, and ROC drive changes in microbial GHs and GTs genes [51]. These genes help decompose and synthesize soil organic matter. Biochar increases the abundance of carbon-degradation genes, *pulA*, *pel*, *endoglucanase* and *chitinase*, which target easily mineralizable SOC, thereby promoting their breakdown. This process supplies substrates for microbial growth, improving soil microbial carbon utilization efficiency [52].

4.5. Effects of Biochar Application on Soybean Growth

Biochar is rich in essential minerals, including nitrogen, phosphorus, potassium, calcium, and magnesium, which are vital for plant growth. It impacts crop performance by improving soil qualities, water retention, pH, and microbial activity [53,54]. The study shows biochar notably boosted soybean growth at all stages, enhancing both above- and below-ground traits. Increased plant height and biomass indicate better vegetative growth, supported by improved nutrient availability and uptake. Biochar increased soil total

nitrogen, available nitrogen, and pH, creating a more favorable environment for nitrogen uptake, which is crucial for soybean development. Although initially available phosphorus decreased at some levels, overall soil health and nutrient cycling improved plant growth. Increases in root length and weight suggest biochar helped develop a strong root system, enhancing water and nutrient absorption, anchorage, and stress resistance.

The enhancement of nodule formation represents one of the most important biochar effects on soybean growth [55]. Nodules house nitrogen-fixing bacteria (rhizobia) that convert atmospheric nitrogen into ammonia through biological nitrogen fixation, providing the primary nitrogen source for legume crops [56]. The significant increases in both nodule number and weight under biochar application indicate enhanced nitrogen-fixation capacity, thereby reducing dependence on soil- or fertilizer-derived nitrogen. This effect likely results from multiple mechanisms. First, biochar-induced pH increases toward neutral values create optimal conditions for rhizobial survival, infection, and nitrogen fixation, as these processes are pH-sensitive [57]. Second, improved soil moisture retention ensures adequate water supply for nodule function, as nitrogen fixation is inhibited under drought stress [58]. Third, the enhanced abundance and diversity of beneficial rhizosphere bacteria, including potential rhizobial symbionts within α -Proteobacteria, provide larger populations for nodule colonization [59].

Correlation analysis reveals strong positive associations between soybean traits and soil parameters, including nutrients, microbial biomass, and enzymes, confirming biochar's combined effects on soil and plants. Microbial biomass C correlates with plant traits, emphasizing the microbiome's role. Increased microbial activity improves nutrient cycling, soil structure, and pathogen suppression, promote plant growth. These findings demonstrate that biochar enhances soil health, microbial communities, and plant-microbe interactions, thereby increasing productivity. It supplies nutrients, improves soil properties, and fosters beneficial microbes throughout the soybean cycle, supporting sustainable agriculture.

5. Conclusions

This study systematically explored how different application rates of corn stalk biochar (0, 300, 600, and 900 kg/hm²) affect soil properties, microbial communities, carbon cycling, and soybean growth in Northeast China's black soil region. Findings suggest biochar is a promising tool for sustainable farming, fostering beneficial interactions among biochar, microbes, and plants. Biochar notably enhanced soil fertility by increasing pH, moisture, total nitrogen, and organic carbon fractions, while also promoting carbon sequestration through stable pools. It selectively influenced enzyme activities, boosting polyphenol oxidase and decreasing sucrase and cellulase. Activities related to stable carbon decomposition. This differential regulation supports long-term carbon stabilization and nutrient cycling. Metagenomic analysis showed that biochar significantly increased bacterial richness and diversity, enriching beneficial taxa such as *Nocardia* and *Sphingomonas* that assist nutrient cycling and plant growth. Functional gene analysis indicated that biochar systematically modified carbon cycling pathways, elevating GTs, GHs, and AAs, and reducing CBMs, CEs, and PLs—enhancing carbon fixation via the reductive TCA cycle and organic carbon breakdown. These microbial shifts supplied substrates for soil health while safeguarding recalcitrant carbon pools. The synergistic improvements in soil quality and microbial function led to increased soybean growth, including taller plants, improved root development, greater biomass, and more nodules for nitrogen fixation. Overall, applying corn stalk biochar offers a practical approach to developing sustainable, eco-friendly farming systems by promoting carbon sequestration, waste recycling, and higher yields, providing valuable insights for soil management in degraded agricultural regions.

Author Contributions: Conceptualization, J.W. and K.Z.; methodology, J.Z.; validation, J.Z.; data curation, Y.L., R.X. and Y.J.; writing—original draft preparation, J.Z. and K.Z.; writing—review and editing, K.Z. and J.W.; project administration, J.W.; funding acquisition, J.W. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in this study are included in this article. Further inquiries can be directed to the corresponding author.

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

Appendix A

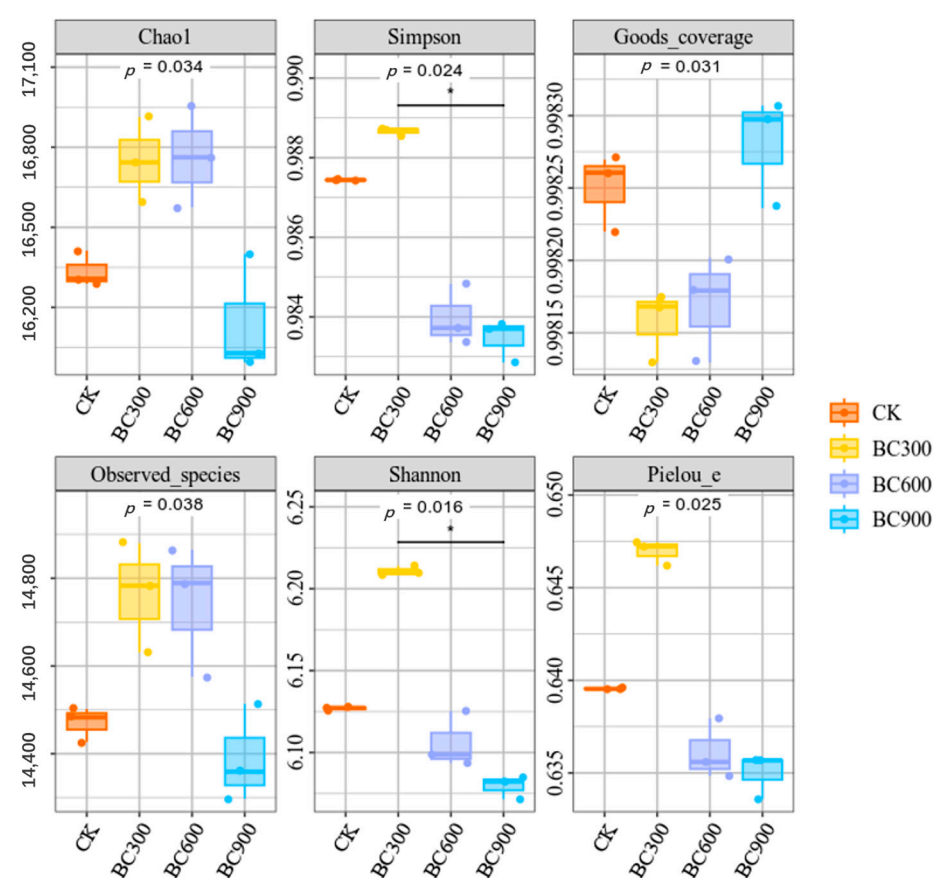


Figure A1. Microbial Community Diversity Index under Different Doses of Biochar. Asterisks indicate significance levels: none ($p \geq 0.05$), * ($0.01 \leq p < 0.05$).

Table A1. Diversity Index of Soil Bacterial Community under Different Doses of Biochar.

Group	Chao Index	Observed Species Index	Simpson Index	Shannon Index	Goods Coverage Index	Pielou Evenness Index
CK	16,336.13 b	14,470.67 b	0.9874 b	6.13 b	0.9983 a	0.6396 b
BC300	16,751.44 a	14,764.67 a	0.9887 a	6.21 a	0.9982 b	0.6470 a
BC600	16,764.63 a	14,743.33 a	0.9840 c	6.11 c	0.9982 b	0.6361 c
BC900	16,140.68 b	14,390.00 b	0.9835 c	6.08 d	0.9983 a	0.6350 c

Note: different lowercase letters indicate significant differences between treatments ($p < 0.05$).

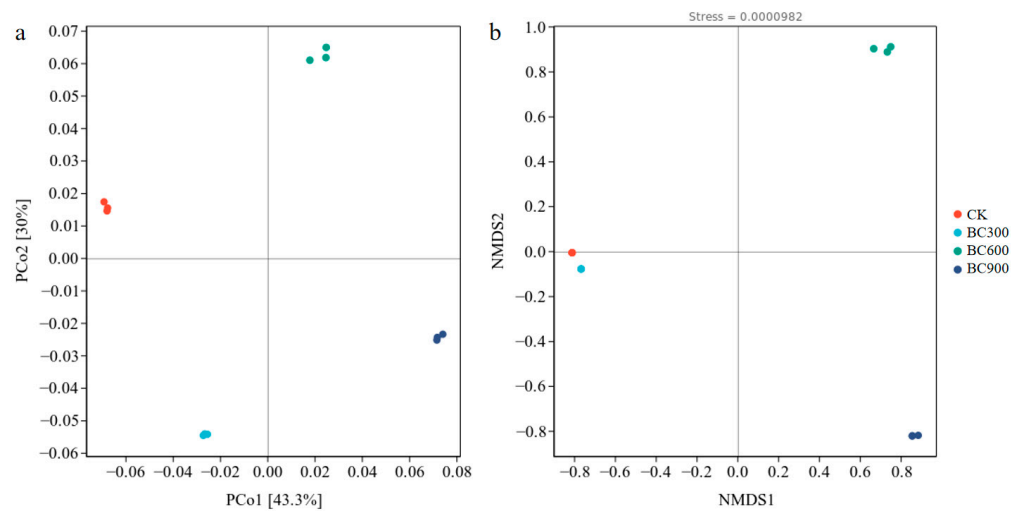


Figure A2. PCoA Analysis and NMDS Analysis of Microbial Community under Different Doses of Biochar. (a) PCoA analysis of microbial community. (b) NMDS analysis of microbial community.

Table A2. The Effect of Biochar on Soil Enzyme Activity.

Group	Sucrase mg g ⁻¹ 24 h ⁻¹	Urease mg g ⁻¹ 24 h ⁻¹	Neutral Phosphatase mg g ⁻¹ 24 h ⁻¹	Catalase mg g ⁻¹ 24 h ⁻¹	Cellulase mg g ⁻¹ 72 h ⁻¹	Polyphenol Oxidase mg g ⁻¹ 24 h ⁻¹	β-Glucosidase mg g ⁻¹ h ⁻¹
CK	7.37	0.64	1.36	327.22	1.84	117.96	21.51
BC300	6.51	0.67	1.30	445.19	1.11	138.86	25.15
BC600	5.22	0.64	1.25	336.23	0.90	236.37	23.09
BC900	5.95	0.64	1.50	392.80	1.31	189.60	23.88

Table A3. Relative Abundance of Functional Genes for Carbon Fixation and Degradation of Microor-ganisms at Different Doses.

Gene	CK			BC300			BC600			BC900		
	1	2	3	1	2	3	1	2	3	1	2	3
<i>rbcl</i>	\	1.94×10^{-4}	2.25×10^{-4}	1.69×10^{-4}	1.81×10^{-4}	1.68×10^{-4}	1.49×10^{-4}	1.57×10^{-4}	1.93×10^{-4}	1.92×10^{-4}	1.46×10^{-4}	1.74×10^{-4}
<i>rpiA</i>	1.20×10^{-4}	1.10×10^{-4}	1.46×10^{-4}	1.51×10^{-4}	1.27×10^{-4}	1.12×10^{-4}	1.03×10^{-4}	1.28×10^{-4}	1.15×10^{-4}	1.18×10^{-4}	9.70×10^{-5}	1.35×10^{-4}
<i>PGK</i>	7.39×10^{-4}	6.87×10^{-4}	7.32×10^{-4}	7.17×10^{-4}	6.62×10^{-4}	7.25×10^{-4}	7.07×10^{-4}	6.97×10^{-4}	7.02×10^{-4}	7.34×10^{-4}	7.25×10^{-4}	7.65×10^{-4}
<i>korA</i>	1.46×10^{-3}	1.47×10^{-3}	1.44×10^{-3}	1.43×10^{-3}	1.54×10^{-3}	1.50×10^{-3}	1.54×10^{-3}	1.52×10^{-3}	1.60×10^{-3}	1.59×10^{-3}	1.54×10^{-3}	1.59×10^{-3}
<i>korB</i>	9.24×10^{-4}	1.02×10^{-3}	9.50×10^{-4}	9.72×10^{-4}	9.63×10^{-4}	9.01×10^{-4}	9.96×10^{-4}	8.89×10^{-4}	9.14×10^{-4}	9.76×10^{-4}	9.57×10^{-4}	9.80×10^{-4}
<i>aclA</i>	1.10×10^{-5}	9.00×10^{-6}	4.00×10^{-6}	1.00×10^{-6}	3.00×10^{-6}	2.00×10^{-6}	2.00×10^{-6}	7.00×10^{-6}	1.00×10^{-6}	1.00×10^{-6}	3.00×10^{-6}	3.00×10^{-6}
<i>amyA</i>	3.60×10^{-5}	2.90×10^{-5}	3.30×10^{-5}	3.20×10^{-5}	3.80×10^{-5}	3.80×10^{-5}	6.40×10^{-5}	5.50×10^{-5}	4.80×10^{-5}	3.00×10^{-5}	2.20×10^{-5}	1.90×10^{-5}
<i>pulA</i>	6.00×10^{-6}	1.20×10^{-5}	1.20×10^{-5}	7.00×10^{-6}	1.50×10^{-5}	1.10×10^{-5}	2.70×10^{-5}	2.10×10^{-5}	3.60×10^{-5}	1.00×10^{-6}	1.30×10^{-5}	8.00×10^{-6}
<i>malQ</i>	3.03×10^{-4}	2.67×10^{-4}	3.18×10^{-4}	2.33×10^{-4}	2.68×10^{-4}	2.11×10^{-4}	2.44×10^{-4}	2.72×10^{-4}	2.98×10^{-4}	2.61×10^{-4}	2.65×10^{-4}	2.28×10^{-4}
<i>malZ</i>	5.33×10^{-4}	6.05×10^{-4}	5.03×10^{-4}	6.19×10^{-4}	5.76×10^{-4}	5.84×10^{-4}	5.28×10^{-4}	5.66×10^{-4}	4.76×10^{-4}	5.47×10^{-4}	5.92×10^{-4}	5.63×10^{-4}
<i>Pectin esterase</i>	3.50×10^{-5}	2.80×10^{-5}	4.10×10^{-5}	5.00×10^{-5}	4.20×10^{-5}	4.00×10^{-5}	4.40×10^{-5}	2.40×10^{-5}	4.30×10^{-5}	2.90×10^{-5}	1.50×10^{-5}	1.90×10^{-5}
<i>pel</i>	6.00×10^{-6}	1.50×10^{-5}	5.00×10^{-6}	2.70×10^{-5}	1.90×10^{-5}	2.30×10^{-5}	2.20×10^{-5}	2.20×10^{-5}	1.70×10^{-5}	4.00×10^{-6}	6.00×10^{-6}	1.20×10^{-5}
<i>abfA</i>	2.45×10^{-4}	2.28×10^{-4}	2.16×10^{-4}	2.54×10^{-4}	2.50×10^{-4}	1.89×10^{-4}	2.11×10^{-4}	1.96×10^{-4}	2.14×10^{-4}	2.16×10^{-4}	1.80×10^{-4}	1.59×10^{-4}
<i>gmuG</i>	1.00×10^{-5}	8.00×10^{-6}	3.30×10^{-5}	2.60×10^{-5}	3.60×10^{-5}	2.80×10^{-5}	2.10×10^{-5}	2.60×10^{-5}	2.90×10^{-5}	1.40×10^{-5}	1.40×10^{-5}	1.80×10^{-5}
<i>xylB</i>	4.02×10^{-4}	3.85×10^{-4}	3.73×10^{-4}	4.01×10^{-4}	3.67×10^{-4}	3.99×10^{-4}	3.31×10^{-4}	3.85×10^{-4}	3.64×10^{-4}	3.56×10^{-4}	3.48×10^{-4}	2.85×10^{-4}
<i>xylA</i>	4.26×10^{-4}	4.11×10^{-4}	4.05×10^{-4}	3.77×10^{-4}	3.56×10^{-4}	4.06×10^{-4}	3.48×10^{-4}	3.49×10^{-4}	3.22×10^{-4}	4.44×10^{-4}	4.60×10^{-4}	3.78×10^{-4}
<i>endoglucanase</i>	2.36×10^{-4}	2.01×10^{-4}	2.30×10^{-4}	3.16×10^{-4}	3.61×10^{-4}	3.06×10^{-4}	2.35×10^{-4}	2.10×10^{-4}	2.23×10^{-4}	1.76×10^{-4}	2.22×10^{-4}	1.91×10^{-4}
<i>bglB</i>	3.41×10^{-4}	4.05×10^{-4}	3.66×10^{-4}	4.08×10^{-4}	4.02×10^{-4}	4.25×10^{-4}	3.27×10^{-4}	3.82×10^{-4}	3.65×10^{-4}	4.03×10^{-4}	3.89×10^{-4}	3.53×10^{-4}
<i>bglX</i>	5.44×10^{-4}	6.19×10^{-4}	5.93×10^{-4}	7.19×10^{-4}	6.99×10^{-4}	6.88×10^{-4}	5.52×10^{-4}	6.37×10^{-4}	5.46×10^{-4}	4.98×10^{-4}	5.47×10^{-4}	4.94×10^{-4}
<i>chitinase</i>	1.11×10^{-4}	1.01×10^{-4}	1.30×10^{-4}	1.64×10^{-4}	1.66×10^{-4}	1.52×10^{-4}	1.28×10^{-4}	1.46×10^{-4}	1.42×10^{-4}	1.22×10^{-4}	1.09×10^{-4}	1.00×10^{-4}
<i>nagA</i>	1.35×10^{-4}	1.83×10^{-4}	1.41×10^{-4}	1.38×10^{-4}	1.51×10^{-4}	1.43×10^{-4}	1.15×10^{-4}	1.45×10^{-4}	1.33×10^{-4}	9.80×10^{-5}	1.01×10^{-4}	1.60×10^{-4}
<i>catE</i>	1.31×10^{-4}	1.40×10^{-4}	9.20×10^{-5}	1.41×10^{-4}	1.32×10^{-4}	1.24×10^{-4}	1.45×10^{-4}	1.52×10^{-4}	1.09×10^{-4}	1.04×10^{-4}	1.18×10^{-4}	1.49×10^{-4}
<i>laccase</i>	1.40×10^{-5}	1.50×10^{-5}	1.50×10^{-5}	9.00×10^{-6}	7.00×10^{-6}	6.00×10^{-6}	6.20×10^{-5}	1.10×10^{-5}	2.60×10^{-5}	2.10×10^{-5}	9.00×10^{-6}	9.00×10^{-6}

Table A4. Effects of Biochar on Plant Height, Root Length and Nodule Number of Soybean at Different stages.

Growth Stage	Treatment	Plant Height (cm)	Root Length (cm)	Root Nodules (pcs)
Branching stage	CK	29.33 d	11.83 a	19.67 a
	BC300	33.80 c	13.60 a	16.00 a
	BC600	37.67 b	11.33 a	18.67 a
	BC900	43.20 a	13.53 a	22.67 a

Table A4. Cont.

Growth Stage	Treatment	Plant Height (cm)	Root Length (cm)	Root Nodules (pcs)
Flowering stage	CK	53.43 d	15.10 a	28.33 a
	BC300	59.97 c	16.10 a	27.33 a
	BC600	72.37 b	14.80 a	24.33 a
	BC900	82.80 a	17.03 a	43.00 a
Pod-setting stage	CK	102.97 c	16.87 b	46.67 b
	BC300	114.77 b	22.37 a	40.00 b
	BC600	121.23 a	20.13 ab	40.33 b
	BC900	121.20 a	22.13 a	94.33 a

Note: different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Table A5. Effect of Biochar on Fresh Weight and Dry Weight of Soybean in Different Periods.

Growth Stage	Treatment	Fresh Weight (g)			Dry Weight (g)		
		Stems and Leaves	Root System	Root Nodules	Stems and Leaves	Root System	Root Nodules
Branching stage	CK	4.77 c	0.83 b	0.07 a	1.51 b	0.34 a	0.04 a
	BC300	6.83 bc	0.91 ab	0.03 a	1.85 b	0.36 a	0.02 a
	BC600	7.64 b	0.95 ab	0.05 a	1.86 b	0.33 a	0.03 a
	BC900	12.48 a	1.39 a	0.07 a	2.99 a	0.51 a	0.04 a
Flowering stage	CK	24.24 b	1.76 b	0.13 a	5.22 b	0.6 a	0.09 a
	BC300	31.05 b	2.95 ab	0.24 a	8.09 b	1.08 a	0.13 a
	BC600	46.49 ab	4.31 a	0.16 a	11.57 a	1.43 a	0.09 a
	BC900	55.62 a	4.25 a	0.16 a	12.39 a	1.37 a	0.12 a
Pod-setting stage	CK	72.54 c	4.16 c	0.43 b	22.51 d	1.49 c	0.16 b
	BC300	98.77 b	5.71 c	0.28 b	28.83 c	2.18 bc	0.13 b
	BC600	120.09 a	6.77 b	0.35 b	35.83 b	2.50 b	0.14 b
	BC900	138.24 a	9.29 a	0.91 a	42.74 a	3.33 a	0.38 a

Note: different lowercase letters indicate significant differences between treatments ($p < 0.05$).

Table A6. Effect of Biochar on the Number of Pods Per Plant and the Number of Seeds per Plant.

Treatment	Pod Number per Plant	Seed Number per Plant
CK	19.25 d	46.65 d
BC300	25.51 c	54.33 c
BC600	30.33 b	68.33 b
BC900	34.19 a	77.06 a

Note: different lowercase letters indicated indicate significant differences between treatments ($p < 0.05$).

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