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Biochar

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# Rhizosphere metabolite-mediated soil enhancement: long-term biochar application optimizes continuous soybean production systems

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**Abstract**

Biochar offers promising solutions for agricultural sustainability, yet the intricate mechanisms governing rhizosphere metabolite-microbe-soil interactions remain poorly understood. Through a decade-long field experiment, the effects of sustained biochar application (BC1: 3 t ha$^{-1}$ and BC2: 6 t ha$^{-1}$) versus conventional fertilization (CF) in a continuous soybean system were investigated. The results showed that biochar improved soil properties, especially, BC2, which significantly enhanced porosity (+ 12.71%), pH (+ 11.60%), soil organic carbon (+ 112.45%), enzymatic activities and nutrient content, while reducing bulk density (– 9.92%). Notably, the biochar restructured microbial community networks, increasing beneficial taxa (Firmicutes, *Enterococcus*, *Pseudomonas,* Ascomycota and Mortierellomycota) while suppressing potential pathogens. Meanwhile, the biochar significantly optimized rhizosphere metabolites, including key defensive compounds (di-O-methyl quercetin, capric acid, hypoxanthines, etc.), and optimized the differential metabolites enriched in the isoflavonoid biosynthesis pathway. Multi-omics analysis revealed strong correlations between differential metabolites and improved soil properties under biochar amendment. Accordingly, these improvements manifested in plant performance, including enhanced root development, plant height, biomass accumulation, and yield. Furthermore, the PLS-PM analysis demonstrated that biochar could promote soybean growth in two key pathway mechanisms that directly enhance soil properties, and indirectly improve soil properties by negatively regulating the key metabolites (capric acid, phosphocreatine, beta 1-tomatine, and daidzin). Our findings provide critical theoretical insights for addressing challenges in soybean continuous cropping systems and advancing sustainable farming practices.

## Highlights

- A long-term field experiment revealed biochar effects on continuous soybean cropping through a 10-year study.
- Biochar stimulated beneficial microbes while reducing pathogens in continuous cropping.
- Biochar promoted continuous cropping soybean growth by modulating rhizosphere metabolites and soil properties.

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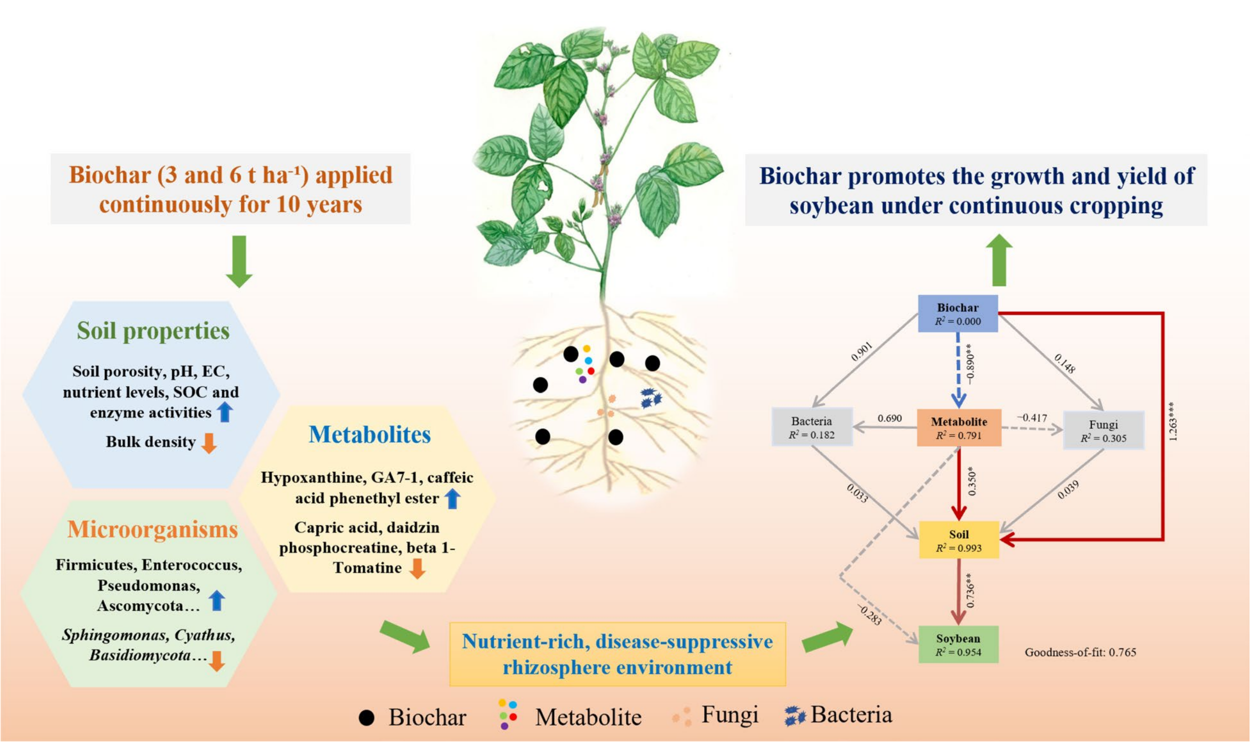
**Keywords** Biochar, Soybean, Soil properties, Soil microorganism, Rhizosphere metabolites

## Graphical Abstract



## 1 Introduction

Soybean (*Glycine max* (L.) Merr.) cultivation represents a significant intersection of global food security and ecosystem sustainability, serving as a vital source of protein and calories for the expanding world population while contributing to agricultural nitrogen cycling (Messina 2022; Yang et al. 2024). However, the widespread adoption of continuous cropping systems in modern agriculture presents complex challenges to sustainability, manifesting as soil structural degradation, chemical imbalances, and biological deterioration, ultimately compromising crop productivity (Lal 1998; Chen et al. 2020a).

The fundamental challenge in continuous soybean cultivation stems from progressive degradation of rhizosphere ecosystem functionality, which encompasses interconnected processes including soil acidification, nutrient imbalance, autotoxic compound accumulation, and altered microbial community dynamics (Sun 2008; Li et al. 2014). These changes promote soil-borne pathogen proliferation while suppressing beneficial microorganisms, severely impacting root development and yield (Gao et al. 2019; Tian et al. 2020). Recent research indicates that yield decline under continuous cropping primarily results from low soil carbon levels and destabilized soil microbial communities, particularly evident in diminishing bacterial populations and diversity (She et al. 2017; Zhang et al. 2021a; Liu et al. 2023). This degradation (i.e., the progressive decline in soil carbon and microbial community stability) intensifies with prolonged monoculture duration (Pang et al. 2021; Zhang et al. 2021a). Moreover, continuous soybean cultivation disrupts the critical bacterial-fungal balance, characterized by decreased bacterial abundance coupled with fungal proliferation (Liu et al. 2020). This shift toward fungal dominance, particularly pathogenic species, compromises essential soil functions including nutrient cycling and disease suppression mechanisms (Chen et al. 2015; She et al. 2017; Gong et al. 2024). Rhizosphere metabolites function as sophisticated ecological mediators orchestrating complex plant-soil-microbe interactions within agricultural ecosystems. These compounds serve dual ecological functions: as microbial resource substrates and as signaling molecules in soil food webs (Hu et al. 2018). While beneficial metabolites can selectively enhance beneficial microorganism recruitment and suppress pathogens, continuous monoculture often leads to detrimental accumulation of specific root exudates (Huang et al. 2013; Zhang et al. 2021b). This accumulation triggers autotoxicity phenomena, directly inhibiting plant growth while inducing adverse shifts in microbial community composition and ecosystem functioning (Chen et al. 2022; He and Wang 2022).

While contemporary agricultural practices addressing continuous cropping constraints primarily depend on cropping system optimization, fertility management, synthetic fungicides, and biological control agents (Ma et al. 2024), these interventions fail to address the fundamental deterioration of rhizosphere microecology. Biochar has emerged as a promising soil amendment, offering unique physicochemical properties that can potentially revolutionize soil quality management (Li et al. 2019; Chen et al. 2020b). Its distinctive characteristics, including porous structure, high specific surface area and abundant surface functional groups, enable multifaceted soil improvements, encompassing pH regulation, enhanced water retention, optimized nutrient availability, and the provision of specialized microbial habitats (Lehmann et al. 2006). This amendment demonstrates remarkable capacity to restructure both soil physicochemical properties and microbial communities, fostering optimal conditions for root development and nutrient acquisition (Sashidhar et al. 2020; Chen et al. 2021). Moreover, biochar application enhances microbial diversity and abundance, particularly beneficial bacterial and fungal populations, thereby optimizing nutrient cycling dynamics, promoting soybean growth, and strengthening resistance against soil-borne pathogens, particularly against prevalent root rot diseases (Kalakodio et al. 2018; Yang et al. 2020; Wu et al. 2022; Zhao et al. 2022).

However, previous research has mostly focused on controlled environments through indoor feeding, such as pot culture, and micro-plot experiments, leaving a significant knowledge gap regarding long-term biochar effects in field conditions. What remains particularly lacking is a comprehensive understanding of biochar influence on the complex networks and modulation pathways connecting soil properties, microbial communities, and rhizosphere metabolites in continuous cropping systems. Based on the understanding that continuous soybean cropping leads to rhizosphere degradation and yield decline, while biochar possesses soil amelioration properties, we hypothesized that: Long-term biochar application in a continuous soybean system mitigates yield constraints through interconnected mechanisms: (1) Improving soil physicochemical properties, thereby creating a more favorable habitat for soil biota; and (2) Modulating the rhizosphere microecology by restructuring microbial community composition towards beneficial taxa and reprogramming rhizosphere metabolite profiles, specifically by suppressing deleterious compounds and enriching beneficial ones, ultimately leading to enhanced soybean growth and yield. Therefore, we conducted a 10-year field experiment investigating the mechanisms by which biochar regulates soil microecology and soybean growth. The study will advance the theoretical foundation for biochar-mediated ecosystem restoration in soybean continuous cropping systems, while providing practical insights for sustainable soybean production management.

## 2 Materials and methods

### 2.1 Experimental site and design

A long-term field experiment was established, situated at the Shenyang Agricultural University Research Base (41.83′N, 123.57′E) in Liaoning Province, China. The study site is characterized by a temperate semi-humid continental climate, exhibiting an average annual temperature of 8.6 degrees Celsius and an average annual precipitation of 715.5 mm. The long-term field experiment started in 2013, and our research sampled in 2022. The test soil was Hapli-Udic Cambisol (FAO classification). The main properties of the soil and biochar used in the experiment are detailed in Table 1.

The long-term field experiment was organized in randomized block design with three treatments: conventional fertilization without biochar (CF) and two different rates of biochar application, 3 t ha$^{-1}$ (BC1) and 6 t ha$^{-1}$ (BC2). Each treatment was replicated thrice in 24 m$^2$ plots. The conventional fertilization regimen followed local government recommendations (N: 36 kg ha$^{-1}$, P: 54 kg ha$^{-1}$, K: 42 kg ha$^{-1}$), with fertilizers consisting of urea (46% N), single superphosphate (18% $P_2O_5$), and potassium

**Table 1** Basic physicochemical properties of the test soil and biochar

| Physicochemical properties | Soil | Biochar |
|---|---|---|
| Raw material | – | Straw |
| Pyrolysis temperature (℃) | – | 400–450 |
| Particle size (cm) | – | 0.30–0.35 |
| pH | 6.50 | 9.24 |
| Total carbon (g kg$^{-1}$) | – | 595.80 |
| Total nitrogen (g kg$^{-1}$) | 1.46 | 8.70 |
| Total phosphorus (g kg$^{-1}$) | 0.58 | 6.60 |
| Total potassium (g kg$^{-1}$) | 18.35 | 10.20 |
| Available nitrogen (mg kg$^{-1}$) | 88.00 | – |
| Available phosphorus (mg kg$^{-1}$) | 18.00 | – |
| Available potassium (mg kg$^{-1}$) | 83.50 | – |

sulfate (50% $K_2O$). The crop follows a single-cropping system per year with continuous cropping. The soybean variety is Tiefeng 40, sown in early May and harvested in early October. Cultivation and management practices including sowing, fertilization, and pest/disease control are consistent with standard local soybean production protocols.

### 2.2 Soil sampling and properties analysis

The soybean roots were dug out of the field, gently shaken, and then the dropped soil was collected as rhizosphere soil at flowering stage. The soil samples were partitioned into three discrete subsets. The first subset was maintained at a temperature of −80 °C to facilitate subsequent analysis of the microbial community structure. The second subset was conserved at 4 °C to enable the assessment of enzyme activities. The third subset was subjected to air-drying for subsequent physico-chemical examinations. Soil enzyme activities were quantified using standardized colorimetric and titrimetric methods: urease activity (indophenol colorimetric), sucrase activity (dinitrosalicylic acid colorimetric), phosphatase activity (disodium phosphate colorimetric), and catalase activity (potassium permanganate titration) (Lin 2010). Soil electrical conductivity (EC) was measured using a portable conductivity meter (S475-uMix, Mettler Toledo, China) at a 5:1 water-soil ratio (Bao 2000). The soil moisture content (SMC) was determined according to the drying method. Soil temperature (Stemp) was determined with a soil thermometer. Subsequent to the initial assessments, a comprehensive suite of soil analyses was conducted, encompassing soil bulk density (SBD), soil porosity (Por), pH, soil organic carbon (SOC), as well as total and available concentrations of nitrogen, phosphorus, and potassium. These analyses were executed in accordance with the procedures outlined by Bao (2000).

### 2.3 Measurements of soybean growth

At flowering stage, representative soybean plants were selected from each treatment. The plant height (from cotyledonary node to vegetative growth pointand) was measured indoor. Then the plants were dried at 60 °C until they reached a constant weight to determine the aboveground and root dry matter weight. At the harvest, the soybean yield per unit area was determined by harvesting the center rows of each plot.

### 2.4 Soil microbial diversity analysis

#### *2.4.1 DNA extraction and sequencing*

Utilizing the CTAB/SDS approach, the complete genomic DNA was isolated from the specimens. The concentration and clarity of the DNA were evaluated on 1% agarose gel matrices. Subsequently, the DNA was adjusted to a concentration of 1 ng $\mu L^{-1}$ using germ-free water. Tailored primers and identifiers were applied to amplify the 16S rRNA and ITS genetic sequences in targeted zones (16S V4: 515F-806R, forward: GTGCCAGCMGCCGCGGTAA; Reverse: GGACTACHVGGGTWTCTAAT)/(ITS1-5F, forward: GGAAGTAAAAGTCGTAACAAGG; Reverse: GCTGCGTTCTTCATCGATGC), which was succeeded by the sequencing process on the NovaSeq6000 system. Sequencing libraries were generated with NEBNext® Ultra™ IIDNA Library Prep Kit (Cat No. E7645). The library quality was evaluated on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Finally, the library was sequenced on an Illumina NovaSeq platform, and 250 bp paired-end reads were generated.

#### *2.4.2 Sequencing data analysis*

Sequencing of the 16S rRNA and ITS1 genes, accompanied by conventional bioinformatics analyses, was performed by Beijing Novozymes Technology Co. Alpha diversity metrics, including Chao1 and Shannon indices, were computed using QIIME2 version (Bolyen et al. 2019). Beta diversity was assessed through the analysis of unweighted UniFrac distances, also within the QIIME2 framework. Cluster analysis was executed utilizing principal component analysis (PCA) to diminish the dimensionality of the initial dataset. This reduction was achieved through the implementation of the ade4 and ggplot2 packages within the R software environment (Version 3.5.3). The Linear Discriminant Analysis (LDA) effect size (LEfSe) software, version 1.0, was employed to conduct LEfSe analysis with an LDA score threshold of 4, aiming to identify differentiating biomarkers.

### 2.5 Rhizosphere soil metabolic profiling analysis

#### *2.5.1 Soil metabolite extraction*

Fifteen days before sampling, five soybean plants were randomly selected in each plot (avoid selecting the soybean plants in the side rows), and the in situ sampling tubes were inserted into the soil at a depth of 20 cm, at an angle of 30 degrees to the ground, and at a distance of 13 cm from the roots of the soybeans. For sampling, the soil was thoroughly moistened, and then a negative-pressure pump was used to extract the liquids from the soil. The five liquids were thoroughly mixed, immediately snap-frozen in liquid nitrogen, and stored at −80 °C.

#### *2.5.2 HPLC–MS/MS analysis*

LC–MS/MS analyses were conducted utilizing an ExionLC™ AD liquid chromatography system integrated with a QTRAP® 6500+mass spectrometer, facilitated by the services of Novogene Co., Ltd. The analytical methodology and experimental conditions were established

**Table 2** Effect of biochar on soil properties

| Treatment | SBD | Por | SMC | Stemp | TN | TP | TK | SOC | pH | EC | AN | AP | AK |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | g cm$^{-3}$ | % | | ℃ | g kg$^{-1}$ | | | | | us cm$^{-1}$ | mg kg$^{-1}$ | | |
| CF | 1.21 ± 0.01$^{a}$ | 49.82 ± 0.55$^{c}$ | 21.44 ± 0.01$^{a}$ | 20.60 ± 0.03$^{a}$ | 0.96 ± 0.00$^{b}$ | 1.34 ± 0.06$^{b}$ | 36.30 ± 1.92$^{b}$ | 10.20 ± 0.05$^{c}$ | 5.69 ± 0.06$^{b}$ | 67.07 ± 6.73$^{b}$ | 75.83 ± 4.04$^{ab}$ | 26.07 ± 0.93$^{a}$ | 78.54 ± 1.99$^{c}$ |
| BC1 | 1.13 ± 0.02$^{b}$ | 53.54 ± 0.60$^{b}$ | 21.58 ± 0.01$^{a}$ | 20.47 ± 0.03$^{b}$ | 1.18 ± 0.01$^{a}$ | 1.47 ± 0.08$^{ab}$ | 37.89 ± 1.26$^{b}$ | 13.22 ± 0.01$^{b}$ | 5.94 ± 0.15$^{b}$ | 68.66 ± 1.13$^{b}$ | 74.67 ± 2.02$^{b}$ | 21.72 ± 3.02$^{b}$ | 83.84 ± 0.58$^{b}$ |
| BC2 | 1.09 ± 0.02$^{b}$ | 56.15 ± 0.67$^{a}$ | 21.86 ± 0.00$^{a}$ | 20.63 ± 0.00$^{a}$ | 1.32 ± 0.12$^{a}$ | 1.63 ± 0.06$^{a}$ | 40.92 ± 1.08$^{a}$ | 21.67 ± 0.10$^{a}$ | 6.35 ± 0.02$^{a}$ | 106.73 ± 4.39$^{a}$ | 82.83 ± 2.02$^{a}$ | 28.63 ± 1.11$^{a}$ | 94.13 ± 0.57$^{a}$ |

Values are expressed as the mean ± standard deviation ($n = 3$), and lowercase letters indicate significant differences between treatments by Tukey's test ($p < 0.05$). The abbreviations are as follows: total nitrogen (*TN*); total phosphorus (*TP*); total potassium (*TK*); available nitrogen (AN); available phosphorus (*AP*); available potassium (*AK*) in accordance with the findings from prior investigations conducted by distinct researchers (Xu et al. 2023).

#### 2.5.3 Metabolite data analysis

These metabolites were annotated to the KEGG database (http://www.genome.jp/kegg/), and their metabolic pathway enrichment was determined based on the KEGG database (Wolthuis et al. 2020). PCA and partial least squares discriminant analysis (PLS-DA) were executed utilizing the metaX software application. Univariate statistical analysis, employing the t-test, was conducted to determine the statistical significance (*p*-value) of the data. Subsequently, volcano plots were generated utilizing the ggplot2 package in the R programming language to identify metabolites of interest, filtered according to their Log2 fold change (FC) and −log10 transformed *p*-values. Pathway enrichment analysis of differential metabolites was conducted, where pathways were considered enriched when $x/n > y/N$ and statistically significant when $p < 0.05$.

### 2.6 Data statistics and analysis

Microsoft Excel 2019 and IBM SPSS Statistics version 19.0 were utilized for the processing and analytical assessment of the dataset. One-factor analysis of variance (ANOVA) and Tukey's test were conducted to assess the statistical significance of the observed variances. Multi-omics correlation analysis is based on multi-omics quantitative data, and Mantel Test is used to calculate the correlation of soil physicochemical properties, bacteria, fungi and metabolites. The color of the line indicates the significance level of the correlation, while the width of the line corresponds to the r-value of Mantel. The Pearson correlation coefficient (r) is represented by the magnitude and intensity of the colors within a single grid cell. The visualization was performed using the function package ggcor 0.9.8.1 (version 4.2.0.) in R (Li et al. 2022). Partial Least Squares Path Models (PLS-PM) were constructed using the pls-pm function package of the R version 4.4.2. Graph Pad Prism version 5.0 was employed for the graphical depiction of the experimental outcomes.

## 3 Results

### 3.1 Effect of biochar on soil properties

Biochar significantly enhanced rhizosphere soil properties, with BC2 treatment demonstrating the most pronounced effects (Table 2). Soil porosity increased by 12.71% and 7.47% in BC2 and BC1 treatments, respectively, while SBD decreased by 9.92% and 6.61% compared to CF. The pH and EC of BC2 increased by 11.60% and 59.13%, respectively, compared with the CF treatment. The BC2 treatment significantly increased SOC, TP, TK, and AK contents of soybean soil, which were 112.45%, 21.64%, 12.73%, and 19.85% higher than the CF treatment.

### 3.2 Effect of biochar on soil enzyme activities

As shown in Fig. 1, soil enzyme activities increased with biochar dose. For BC2 treatment, the soil urease activity (SUE), soil sucrase activity (SSC), soil acid phosphatase activity (SACP), and soil catalase activity (SCAT) increased by 8.07%, 185.52%, 91.87%, and 40.14%, respectively, and for BC1 treatment, the SUE, SSC, and SCAT activities increased by 3.78%, 115.85%, and 20.07%.

### 3.3 Effect of biochar on soil bacteria and fungi

Microbial community analysis revealed substantial shifts in response to biochar application. As shown in Fig. 2A, B, BC2 treatment exhibited the highest bacterial diversity (4601 ASVs) compared to CF (3993) and BC1 (3657), with 1392 shared ASVs across treatments. Fungal communities showed similar trends, with BC2 yielding 854 ASVs compared to 822 and 727 in BC1 and CF, respectively. The principal component analysis (PCA) plot (Fig. 2C, D) illustrated distinct clustering of microbial communities, particularly between BC2 and CF treatments. As shown in Fig. 2E, G and F, H, the mean values of the Chao1 and Shannon indices were higher in the BC2, which demonstrated that applying a higher dose of biochar had a greater potential to improve the community structure and diversity of rhizosphere soil microorganisms in continuously cropped soybean.

At the phylum level (Fig. 3A), Proteobacteria, Acidobacteriota, and Firmicutes dominated the soil bacterial system. BC2 significantly increased the relative abundance of Firmicutes. The abundant bacterial genera (Fig. 3C) were *Ralstonia*, *RB41*, and *Enterococcus*. High-dose biochar application increased the relative abundances of *Enterococcus* and *Pseudomonas*, while the relative abundance of *Sphingomonas* significantly decreased with increasing doses of biochar.

As shown in Fig. 3B, Ascomycota, Mortierellomycota, and Basidiomycota dominated the soil fungal system. Biochar enhanced the relative abundances of Ascomycota and Mortierellomycota compared to CF, and BC1 was more favorable for Ascomycota, while the high-dose biochar treatment BC2 was more favorable for Mortierellomycota. The relative abundance of Basidiomycota was reduced by the dose of biochar. At the genus level (Fig. 3D), *Fusarium* and *Mortierella* dominated the soil fungal system. *Ceratobasidium* and *Cyathus* had the highest relative abundances in the CF compared to the biochar treatments.

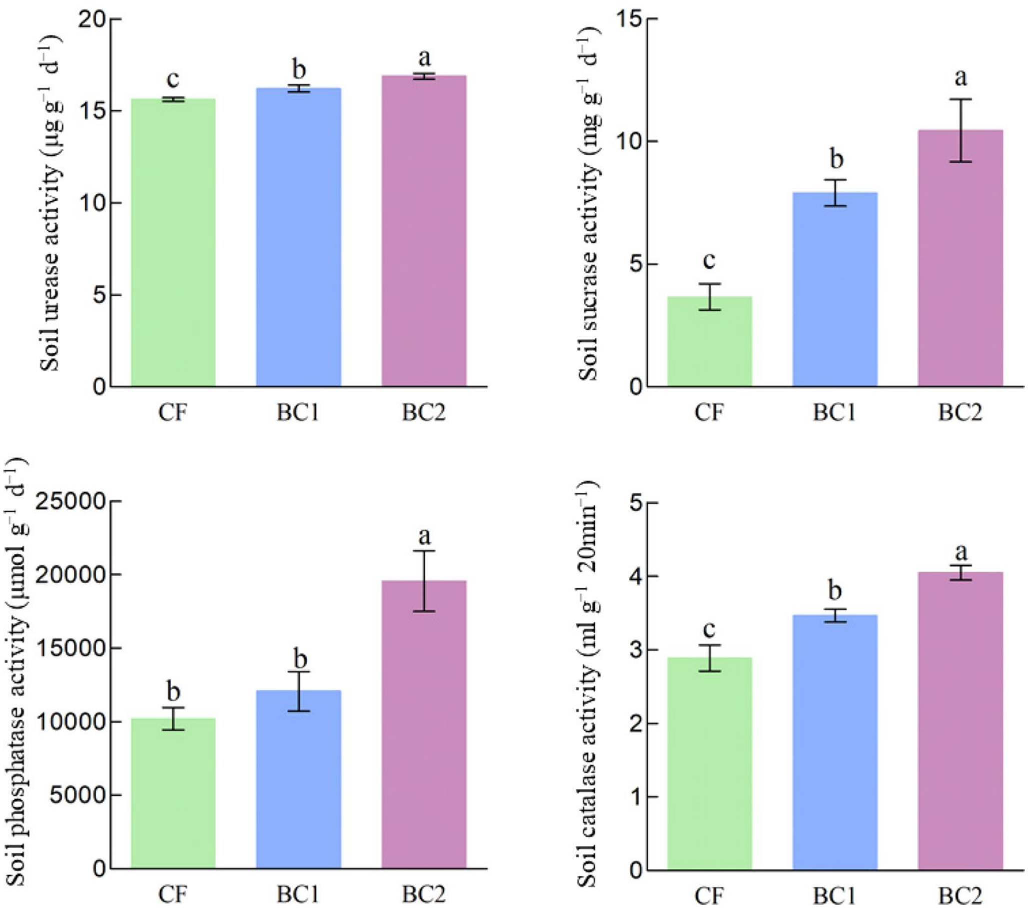


**Fig. 1** Effect of biochar on soil enzyme activities. The graphical representation utilizes bars to depict the mean values, with error bars signifying the standard deviation of the mean ($n = 3$). Distinct lowercase letters denote statistically significant differences among various treatments as determined by a Tukey's test ($p < 0.05$)

Microbial biomarkers in soil were identified through linear discriminant analysis effect size (LEfSe). The results revealed significant differences in the bacterial (Fig. 4A, C) and fungal (Fig. 4B, D) species of soybean continuous cropping soils between the CF and BC2 treatments, with no significant microbial biomarkers in BC1. c_Acidobacteria was enriched in the CF treatment, while p_Bacteroideta, c_Bacteroidia, and c_Bacilli were significantly enriched in the BC2 treatment. Additionally, the soil fungi *g_Cyathus*, *s_Cyathus_stercoreus*, and *f_Nidulariaceae* were enriched under CF, while *s_Mortierella_alpina* was enriched under BC2.

### 3.4 Effect of biochar on rhizosphere metabolites

Principal component analysis (Fig. 5A) revealed significant differences between treatments. BC1 and BC2 showed significant separation from CF, with principal component 1 explaining 27.47% of the total difference. The known differential metabolites among the three treatments were analyzed using Venn's analysis (Fig. 5B), and 66, 107, and 61 differential metabolites were screened.

(See figure on next page.)

**Fig. 2** Effect of biochar on the diversity of soil bacterial and fungal communities. An analytical examination utilizing Venn diagrams was conducted to compare the bacterial and fungal species (denoted as **A** and **B**). PCA was conducted on bacterial and fungal communities utilizing the Bray–Curtis distance matrix (**C** and **D**), to elucidate the underlying patterns of community composition. Investigation into the diversity of bacterial and fungal communities was conducted through the utilization of the Chao1 (**E** and **F**) and Shannon (**G** and **H**) indices to quantify species richness and evenness

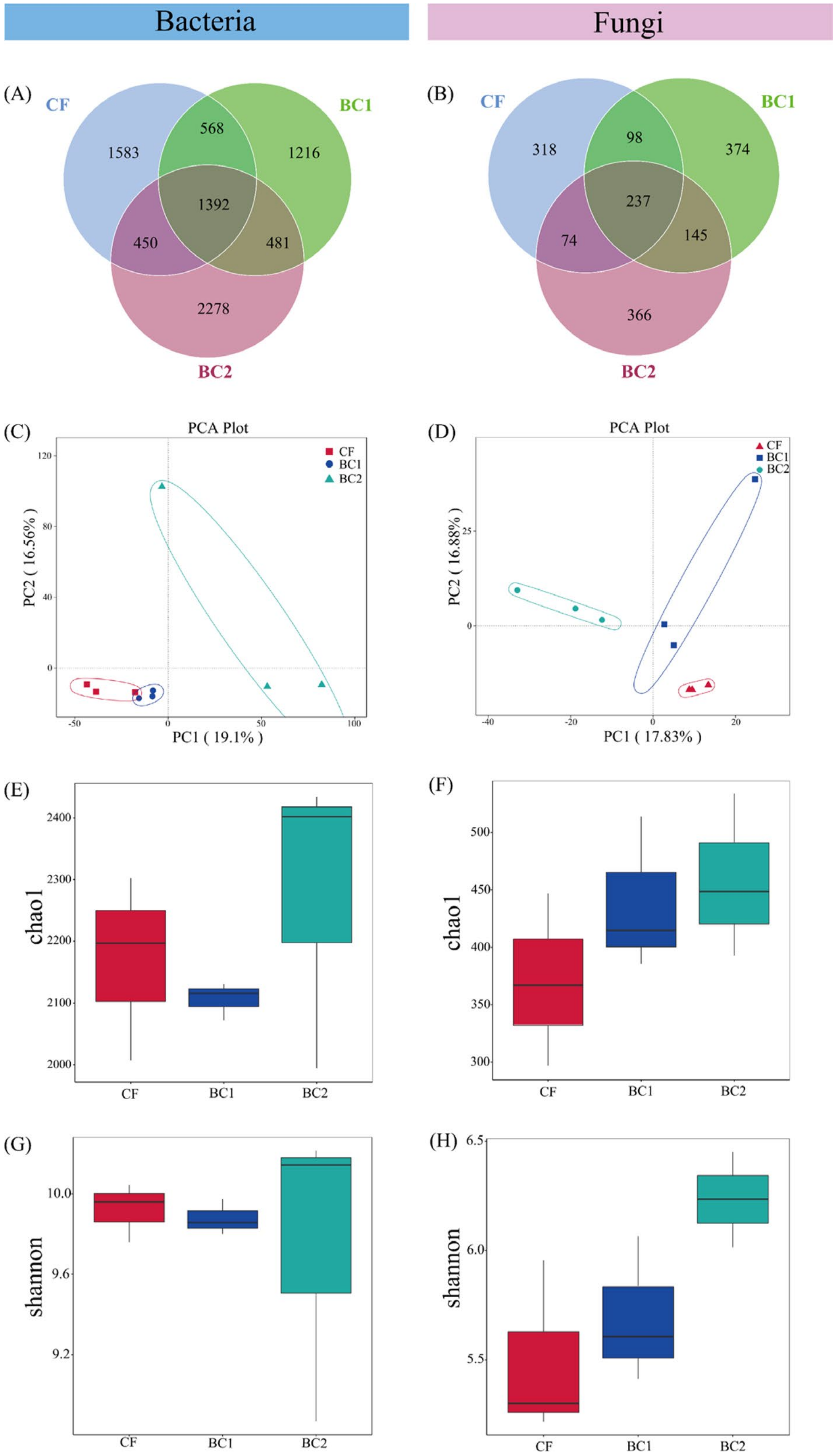


**Fig. 2** (See legend on previous page.)

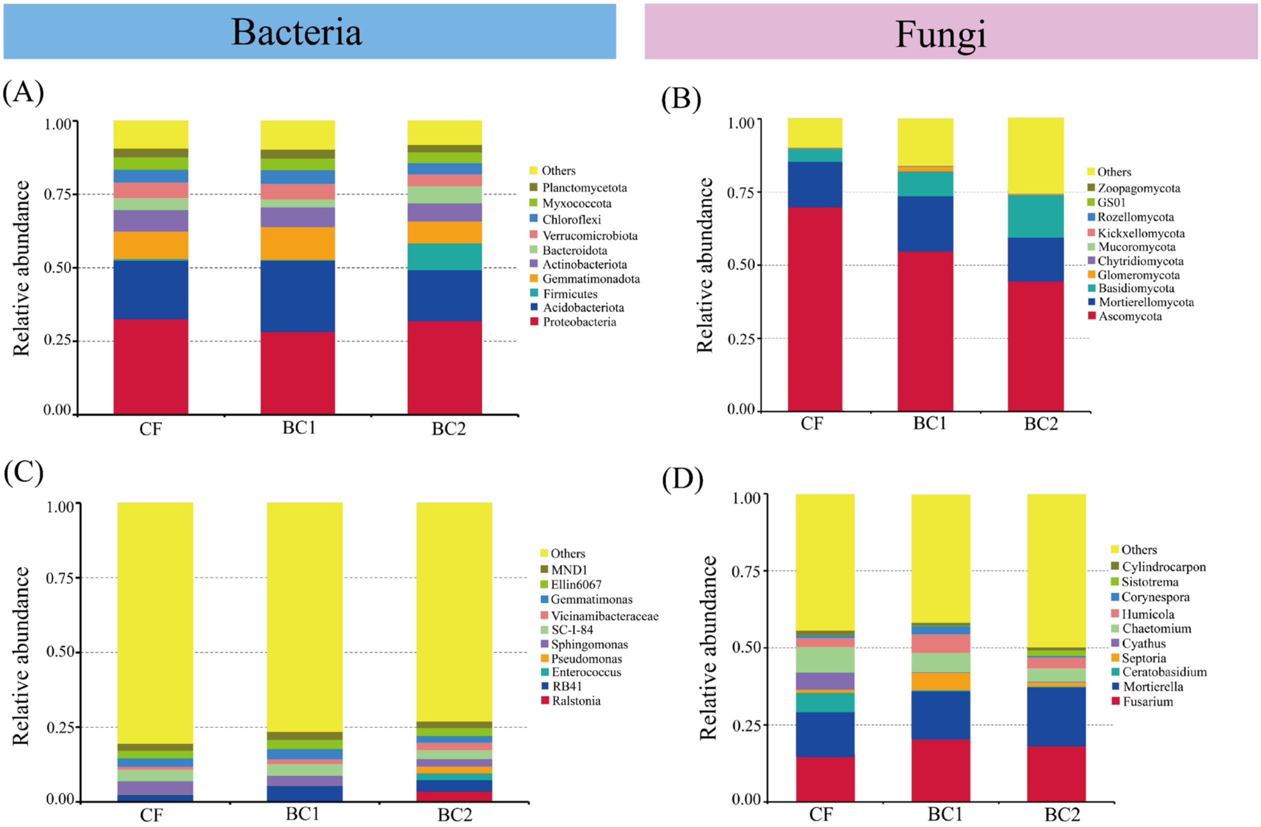


**Fig. 3** Composition of bacterial and fungal communities in soil. Comparative analyses of bacterial and fungal community compositions were conducted at both the phylum (**A** and **B**) and genus (**C** and **D**) taxonomic levels

As shown in Fig. 5C, compared to CF, BC1 significantly up-regulated metabolites including Di-O-methylquercetin and GA7-1, and significantly down-regulated metabolites including phosphocreatine, 4-pyridoxate, and D-pantothenate. Key up-regulated metabolites in BC2 (Fig. 5D) included hypoxanthine and caffeic acid phenethyl ester, while beta 1-tomatine and daidzin were down-regulated. Hypoxanthine and caffeic acid phenethyl ester were significantly down-regulated in BC1 compared to BC2, while differential metabolites including phosphocreatine and 4-pyridoxate were significantly up-regulated (Fig. 5E).

The differential metabolites in CF versus BC1 were mainly enriched in three major pathways: the fructose and mannose metabolism, isoflavonoid biosynthesis, and pentose phosphate pathways (Fig. 6A). Notably, differential metabolites associated with the isoflavonoid biosynthesis pathway were particularly enriched when comparing the BC2 and CF treatments (Fig. 6B). In the comparison between BC1 and BC2 treatments (Fig. 6C), differential metabolites were enriched in three metabolic pathways: pyruvate metabolism, histidine metabolism, and carbon fixation in photosynthetic organisms.

### 3.5 Effect of biochar on soybean growth and yield

Biochar significantly enhanced soybean growth parameters (Fig. 7), with BC2 treatment increasing plant height by 29.95% compared to CF. Additionally, BC2 and BC1 increased aboveground biomass by 51.25% and 29.16%, and root biomass by 26.89% and 13.21%, respectively. BC2 and BC1 achieved a significant increase in yield compared with CF, with an increase of 45.83% and 37.50%.

### 3.6 Correlation relationship between soil properties, microorganisms, and metabolites

Multi-omics correlation analysis (Fig. 8A) revealed that the rhizosphere differential metabolites in continuous cropping soils were closely related to the changes in soil physicochemical properties (porosity, bulk density, pH, SOC, TN, TP, AP, AK, SUE, SSC, SCAT) after biochar application. The correlation analysis between soil environmental factors and rhizosphere differential metabolites (Fig. 8B) showed that hypoxanthine and caffeic acid phenethyl ester positively correlated with most soil environmental factors, and capric acid, phosphocreatine, beta

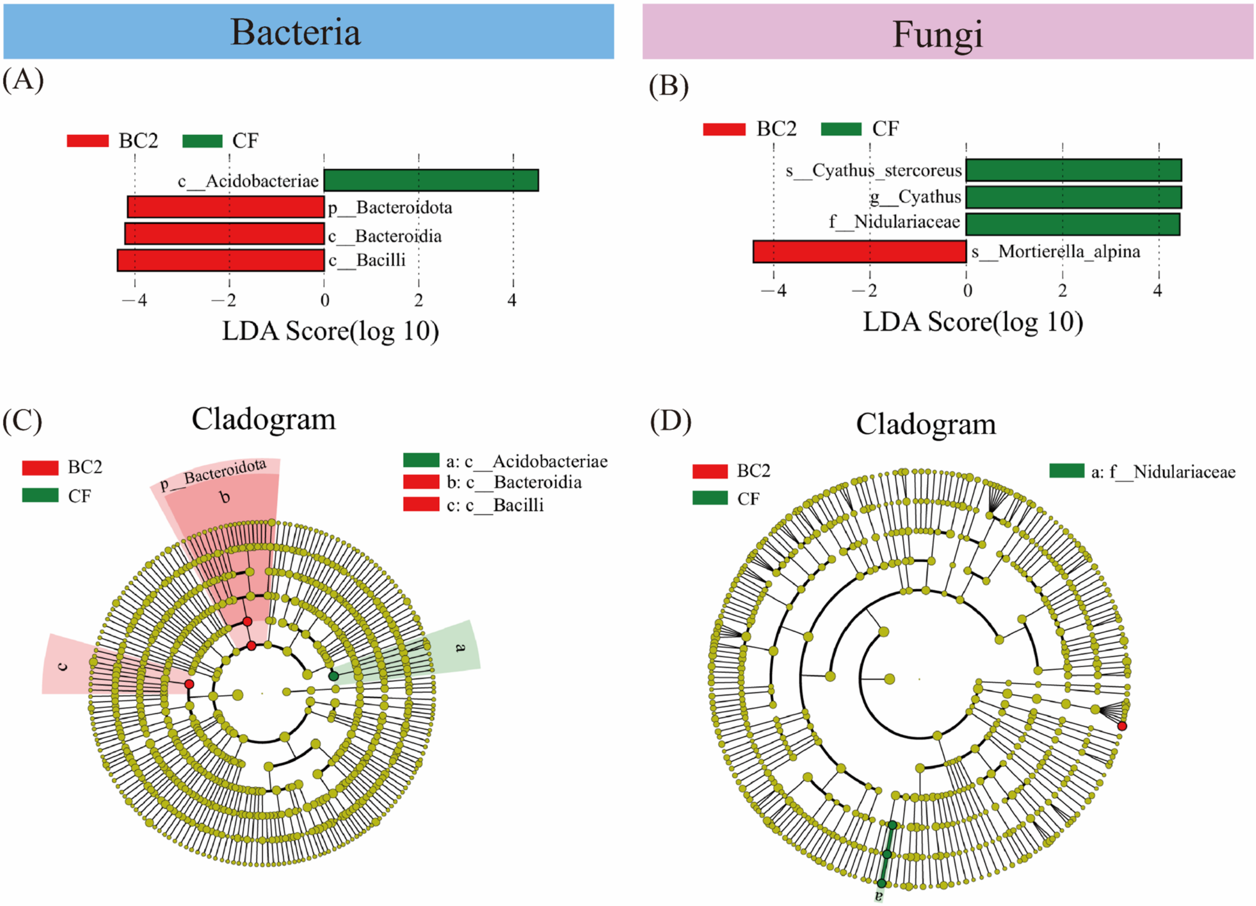


**Fig. 4** Comparative microbiological analysis among distinct groups (CF vs BC1 vs BC2). LEfSe (LDA Effect Size) analysis focusing on bacterial communities (**A** and **C**) and fungal communities (**B** and **D**)

1-Tomatine, and daidzin negatively correlated with most soil factors.

### 3.7 Regulatory pathways of biochar in promoting soybean growth

In PLS-PM (Fig. 9), a goodness of fit (GOF) value greater than 0.600 indicates a good model fit. The PLS-PM demonstrated that under continuous soybean cropping conditions, the long-term application of biochar can directly enhance soil properties, and also can promote soil properties by inhibiting the key rhizosphere metabolites, ultimately facilitating the growth and yield of continuously cropped soybean.

## 4 Discussion

### 4.1 Biochar modulates soil microbial communities

The study demonstrated that biochar effectively enhanced soil physicochemical properties under continuous soybean cropping in a dose-dependent manner. These improvements manifested as increased soil porosity, elevated pH, enriched SOC, amplified enzymatic activities, augmented nutrient profiles, and diminished bulk density. This ameliorated environment created optimal conditions for soil microbiota, as evidenced by our observations of dose-proportional increases in microbial abundance and diversity following biochar application in continuous cropping systems. The underlying mechanisms primarily stem from biochar's distinctive microporous architecture and extensive surface area, which function as protective microhabitats for microbial colonization (Hu et al. 2014; Shi et al. 2022). Furthermore, biochar contains carbon source substances and macro- and micronutrients, it can also increase the contents of soil organic carbon and soil nutrients (Zimmerman et al. 2011; Wang et al. 2016). Consequently, these create favorable nutritional conditions that stimulate microbial proliferation, metabolic activity, and community restructuring (Sohi et al. 2010; Karimi et al. 2020) In particular, our findings demonstrated marked enhancements in soil enzymatic activities—specifically urease, sucrase, phosphatase, and catalase—indicating accelerated microbial metabolic processes that catalyze

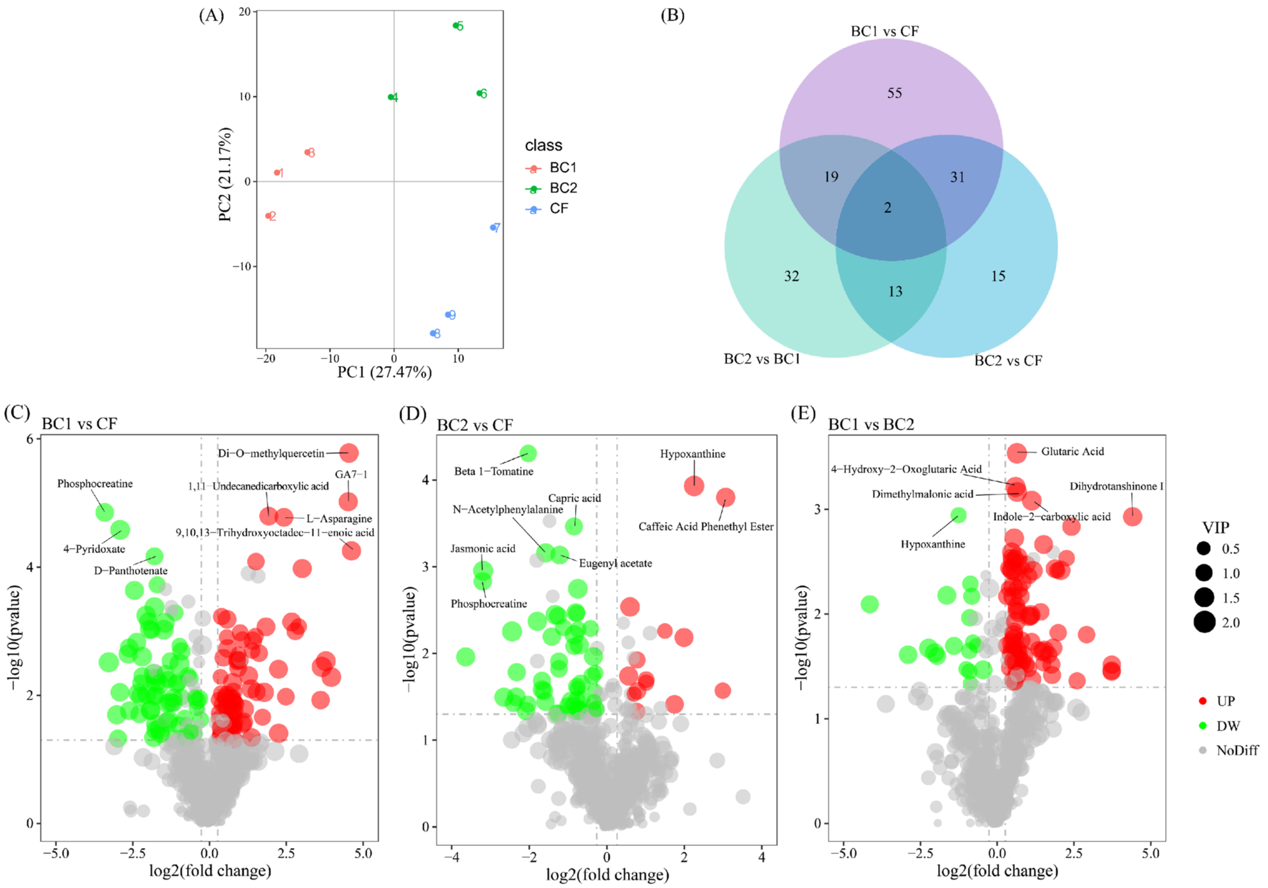


**Fig. 5** Differential analysis of rhizosphere metabolites. PCA graphical representations of soil metabolite profiles (**A**), and a Venn diagram (**B**) illustrating the significant differentially occurring metabolites. Volcano plots depicting the significant differential occurrence of metabolites, BC1 vs CF(**C**); BC2 vs CF (**D**); BC1 vs BC2 (**E**). Red circles denote metabolites that were significantly up-regulated, while green circles signify those that were significantly down-regulated; the diameter of each circle corresponds to the variable importance in the projection (VIP) score

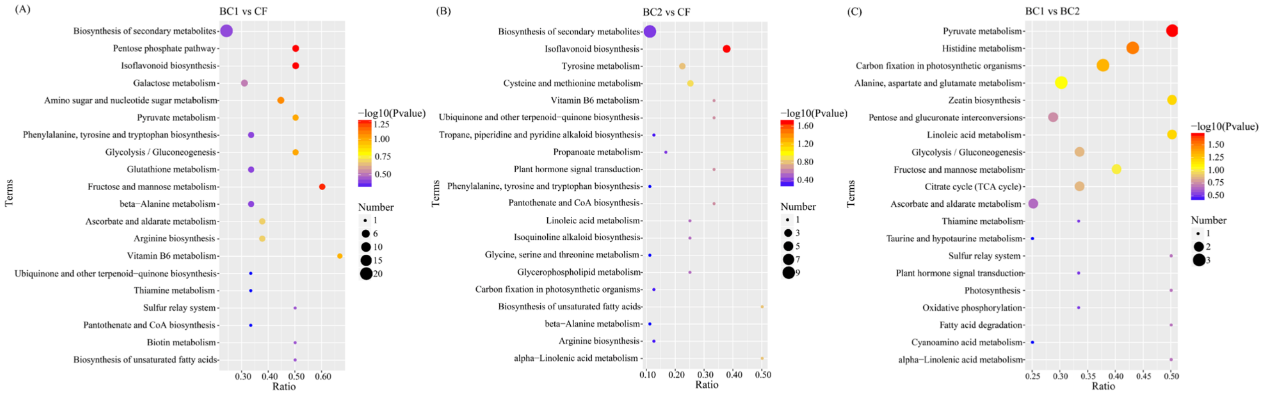


**Fig. 6** Analyses of metabolites pathways for CF vs.BC1 (**A**), CF vs.BC2 (**B**) and BC1 vs.BC2 (**C**). The hue of the dots corresponds to the *p*-value derived from the hypergeometric test. The diameter of the nodes is proportional to the count of distinct metabolites present within the respective metabolic pathway

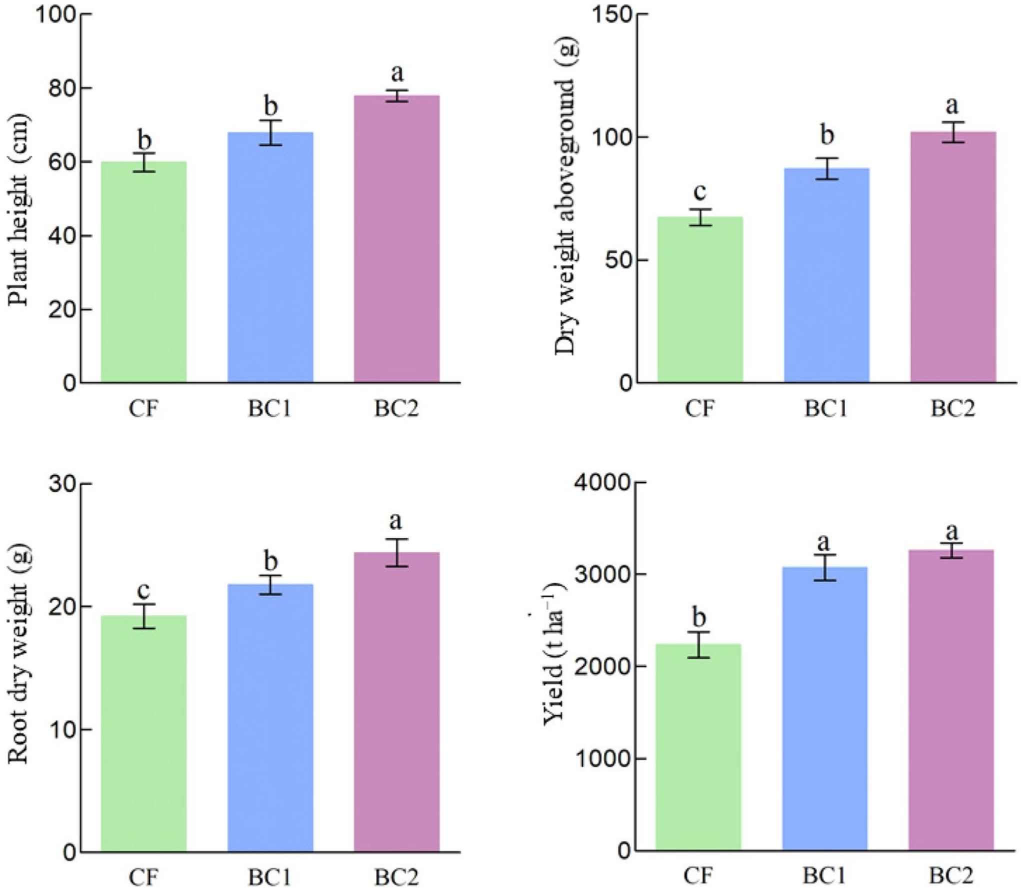


**Fig. 7** Effect of biochar on the growth dynamics and yield of soybean in continuous cropping systems. The graphical representation utilizes bars to depict the mean values, with error bars signifying the standard deviation of the mean ($n = 3$). Distinct lowercase letters denote statistically significant differences among various treatments as determined by a Tukey's test ($p < 0.05$)

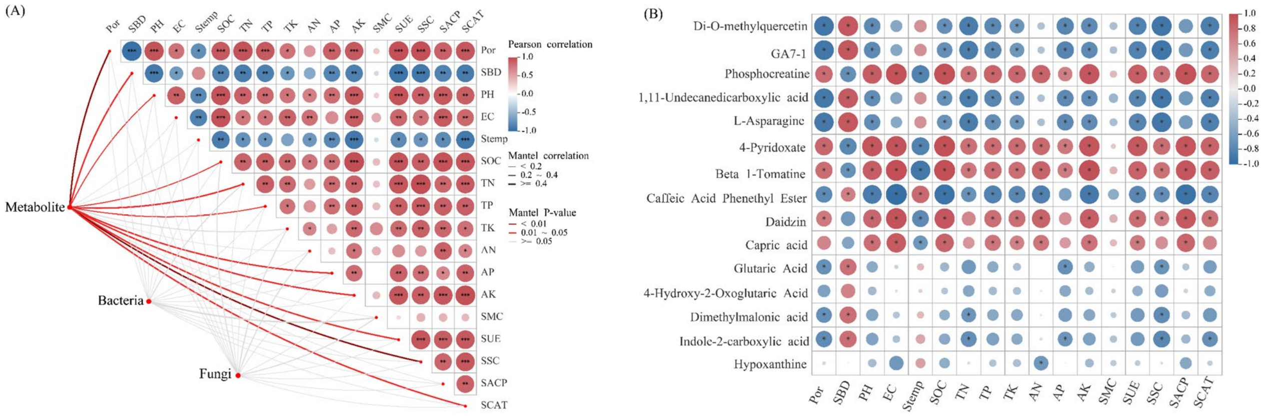


**Fig. 8** Interactions of soil properties, microorganisms and metabolites. Relationship between metabolite, bacteria, fungi and soil properties by Mantel tests (**A**). Correlation analysis of soil properties and rhizosphere differential metabolites (**B**). The red round cake represents a significant correlation between the two traits, blue one is the opposite ($p < 0.05$)

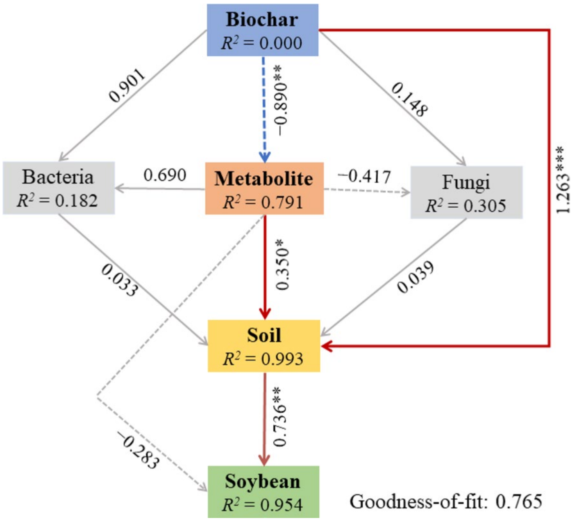


**Fig. 9** PLS-PM on the effect of biochar on the continuous cropped soybean growth. Boxes indicate variables included in the model Variables in the model: biochar, metabolite (capric acid, phosphocreatine, beta 1-Tomatine, and daidzin), bacteria (chao1), fungi (chao1), soil (SOC, TP, TK, AN, AK, Por, PH, EC, SUE, SSC, SACP,and SCAT), and soybean (plant hight, dry weight aboveground, and root dry weight). Red line represents a positive effect; blue line represents a negative effect. The asterisks *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

nutrient transformation pathways critical for plant nutrition (Lehmann et al. 2011; Karimi et al. 2020; Jiang et al. 2021).

Biochar has significant effects on both soil bacteria and fungi. For soil bacteria, biochar increased their abundance and diversity, prevented the imbalance of soil microbial communities, and maintained the health and stability of the soil micro-ecosystem in continuous cropping. In particular, we observed a dose-dependent effect in the long-term application of biochar, wherein high-dose biochar (BC2) significantly increased the relative abundance of Firmicutes while reducing *Sphingomonas* populations. The observed Firmicutes enrichment aligns with previous reports linking this phylum to improved soil structural integrity and nutrient cycling efficiency (Wang et al. 2024). Furthermore, we documented improvements in soil physicochemical parameters consistent with previous investigations (Lehmann 2007; Yao et al. 2012; Chen et al. 2019). These community shifts likely reflect biochar's capacity to alleviate soil compaction, enhance porosity, and increase bioavailability of both macro- and micronutrients (Goldberg 1985; Sohi et al. 2010). While *Sphingomonas* species contribute to pollutant degradation and plant growth promotion (Yu et al. 2013; Asaf et al. 2020), their population decline following biochar amendment may reflect cropping duration effects. Notably, researchers reported substantially lower *Sphingomonas* abundance in long-term continuous cropping systems (11–14 years) compared to shorter durations (2–6 years) post-biochar application, potentially due to rhizosphere metabolite accumulation (Han et al. 2017). Concurrently, the LEfSe analysis revealed BC2 treatment enriched Bacteroidetes (phylum Bacteroidota, class Bacteroidia) and Bacilli populations—taxa critically involved in nutrient cycling and organic matter decomposition (Goodfellow and Williams 1983)—indicating enhanced biogeochemical cycling capacity at higher biochar dosage (BC2). Moreover, the LEfSe analysis revealed Acidobacteria enrichment in control (CF) samples, aligning with previous findings that showed a negative correlation between Acidobacteria abundance and soil pH (Lauber et al. 2008; Chu et al. 2010). Our findings demonstrated that biochar significantly increased soil pH in the continuous cropping, indicating its effectiveness in mitigating acidification. This pH modulation is likely due to the alkaline buffering capacity of biochar's carbonate content and the adsorption of $H^+$ ions by its surface functional groups (Fu et al. 2024), highlighting a key mechanism through which biochar alters microbial communities in continuous cropping.

Regarding fungal communities, our results demonstrated that biochar application significantly increased abundance and diversity metrics while inducing taxonomic compositional shifts. Biochar induced the enrichment of Ascomycota and *Mortierella* species along with the suppression of Basidiomycota, *Ceratobasidium*, and *Cyathus* populations. The increased abundances of Ascomycota and Mortierellomycota—key decomposers of recalcitrant organic matter (Evans and Wallenstein 2012; Beimforde et al. 2014; Egidi et al. 2019)—suggest improved soil fertility through enhanced nutrient mineralization. Conversely, Basidiomycota reduction under biochar amendment aligns with their preference for oligotrophic environments (Ma et al. 2013). Particularly noteworthy was the *Mortierella* enrichment, as this genus exhibits higher abundance in healthy plant rhizospheres compared to root rot-affected soils (Wu et al. 2015), implying potential biochar-mediated disease resistance in soybean. The concurrent suppression of root rot pathogens *Ceratobasidium* and *Cyathus* further supports biochar's phytoprotective potential (Wen et al. 2015; Tian et al. 2020). LEfSe analysis specifically identified *Mortierella alpina* enrichment in BC2—a species documented to mitigate Fusarium-related diseases (Wang et al. 2022)—reinforcing biochar's role in soil-borne pathogen suppression. These biochar-induced shifts in fungal community composition demonstrate its multifaceted role in suppressing phytopathogenic fungi and mitigating disease incidence, thereby highlighting its significance in maintaining rhizosphere health and promoting sustainable productivity in continuous cropping systems.

### 4.2 Biochar modulates rhizosphere metabolites

In this study, biochar application significantly down-regulated several key differential metabolites in soil, including capric acid, beta 1-tomatine, daidzin, and phosphocreatine. Capric acid and beta 1-tomatine are known for their antimicrobial properties (Huang et al. 2014), which, at high concentrations, can inhibit beneficial microbial activity and disrupt the ecological balance of the soil. The reduction of these metabolites following biochar application likely supported the recovery and proliferation of beneficial microbial communities, thereby enhancing the health and functionality of the soil ecosystem. Daidzin, a plant-derived compound with pathogen-inhibitory properties, was also reduced. While daidzin can suppress harmful pathogens (Tan et al. 2022a, b), its excessive presence may negatively affect certain beneficial microorganisms. The decrease in daidzin suggested that biochar application helped regulate its levels, potentially creating a more balanced microbial environment conducive to soil health. Phosphocreatine, primarily recognized for its role in energy storage and transfer in animal systems (Hettling and van Beek 2011), also influences microbial energy metabolism and soil nutrient dynamics (Kitzenberg et al. 2016). Its downregulation suggests that biochar may contribute to stabilizing the soil's energetic environment, reducing the negative effects of excess energy on microbial metabolism. This stabilization likely allowed soil microorganisms to function more efficiently, enhancing overall biological activity and nutrient supply capacity.

In contrast, BC2 significantly up-regulated hypoxanthine and caffeic acid phenethyl ester. Hypoxanthine plays a crucial role in providing nitrogen and energy for plant metabolism, and its regulatory role in plant growth and adaptation to the environment has been documented (Robbins and Kavanagh 1942; Hawkins and Oresnik 2022). Caffeic acid phenethyl ester (CAPE), a plant secondary metabolite, scavenges free radicals, reduces oxidative stress (Ehtiati et al. 2023), and maintains soil ecological balance while protecting root health. CAPE's antimicrobial properties help suppress pathogenic microorganisms (Ehtiati et al. 2023). The significant upregulation of these metabolites under high biochar doses further demonstrated biochar's capacity to suppress soil-borne diseases, maintain healthy soil microecological environments, enhance the disease resistance of soybean plants, and promote the growth of the soybean root system.

Metabolomic pathway analysis revealed significant enrichment of differential metabolites in the isoflavone biosynthesis pathway, highlighting biochar's substantial influence on isoflavonoid metabolism. Isoflavonoids are a major class of antimicrobial compounds in leguminous plants, capable of disrupting the ATPases in the cell membranes of pathogenic bacteria and mitochondrial respiratory electron transport chains, thereby resisting specific pathogenic attacks and fungal infiltration (Wu et al. 2023a). These findings establish biochar as a potential modulator of plant defense mechanisms through selective regulation of isoflavone biosynthesis pathways.

### 4.3 Rhizosphere microecological interactions regulated by biochar

In this study, the integration of multi-omics correlation analysis and PLS-PM offers a mechanistic understanding of how biochar modulates the rhizosphere metabolome to reshape soil physicochemical properties under continuous soybean cropping. The significant correlations between biochar-regulated differential metabolites and improved soil traits highlight the pivotal role of root exudates in mediating soil–plant interactions. For instance, the positive associations of hypoxanthine and CAPE with most soil environmental factors suggest that these metabolites may act as key drivers of soil quality improvement. Conversely, the negative correlations of capric acid, phosphocreatine, β1-tomatine, and daidzin with soil properties imply that these metabolites may represent indicators of soil degradation under continuous cropping. Capric acid, a short-chain fatty acid, has been associated with fungal pathogen proliferation in stressed rhizospheres (Vives-Peris et al. 2020), while β1-tomatine, a glycoalkaloid, could accumulate as a defensive phytotoxin under biotic stress, potentially inhibiting beneficial microbial activities (Zhalnina et al. 2018). Additionally, daidzein released into the soybean rhizosphere may act as allelopathic chemicals in long-term continuous cropping soybean fields (Guo et al. 2011).

The PLS-PM framework (GOF > 0.600) provides empirical evidence for biochar's dual regulatory pathways: direct improvement of soil properties, and indirect modulation through suppressing detrimental rhizosphere metabolites. Research has shown that rhizosphere metabolites are closely related to soil nutrient status and pH (Vives-Peris et al. 2020; Guo et al. 2022; Chen and Liu 2024). In addition, research found that plants release 11–40% of their photosynthetic products as root exudates (Zhalnina et al. 2018). Our previous research found that biochar can enhance soybean photosynthetic capacity (Wu et al. 2023b), suggesting biochar's ability to alter root exudation patterns—likely via enhancing plant photosynthetic capacity and carbon allocation. Such metabolic reprogramming could selectively enrich microbial guilds associated with nutrient cycling (e.g., Bacteroidetes and Bacilli in the study) and suppress pathogens (e.g., *Ceratobasidium* and *Cyathus* in the study), creating a self-reinforcing cycle of soil health improvement. This confirms biochar's role in creating a healthy rhizosphere microenvironment conducive to soybean growth under continuous cropping conditions.

## 5 Conclusion

The 10-year field experiment demonstrated biochar's comprehensive benefits in continuous soybean cultivation, encompassing improved soil structure, fertility, and optimized microbial communities. The biochar also significantly shifted rhizosphere metabolic profiles, particularly enhancing compounds crucial for plant defense and growth regulation, which are closely associated with the improvement of soil properties. Ultimately, the biochar led to a significant improvement in soybean growth. Furthermore, pathway analyses showed that biochar could directly ameliorate soil properties, or indirectly enhance soil properties by inhibiting the key metabolites and ultimately promote soybean growth. These findings advance our understanding of rhizosphere metabolite-microbe-soil interactions, expand the theoretical framework for biochar-mediated soil management in continuous cropping systems, and present a viable strategy for optimizing sustainable soybean production.

### Author contributions

Di Wu: Conceptualization, Methodology, Validation, Investigation, Data curation, Writing—Original draft, Visualization. Yuxue Zhang: Investigation, Validation, Formal analysis. Wenqi Gu: Investigation, Formal analysis. Yuanyuan Sun: Investigation. Zifan Liu: Investigation. Wenjia Wang: Investigation. Liqun Xiu: Supervision. Weiming Zhang: Conceptualization, Methodology, Resources, Writing—Review & Editing, Project administration, Funding acquisition. Wenfu Chen: Conceptualization, Resources, Project administration, Funding acquisition.

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### Data availability

The data can be obtained from the authors upon request.

## Declarations

### Competing interests

Wenfu Chen is the EiC of the journal Biochar, but he was not involved in the peer-review or handling of the manuscript. The authors have no other competing interests to disclose.

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