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Effects of sweet pepper straw biochar on soil microbial communities and growth of continuously cropped cucumber

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Abstract

Purpose This study evaluates biochar from crop residues as a solution to soil degradation in continuous monoculture within greenhouse agriculture, focusing on its impact on soil microbial communities and cucumber plant growth.

Methods We analyzed biochar derived from tomato straw (TSB), sweet pepper straw (SPSB), and eggplant straw (ESB), assessing their nutrient content, cation exchange capacity, and adsorption rates. This study examined the effects of three concentrations (2.5%, 5%, and 7.5% w/w) of the more promising SPSB on soil properties and cucumber growth.

Results SPSB showed significantly higher levels of nitrogen, phosphorus, and potassium, along with superior adsorption capacity compared to TSB and ESB. The 5% w/w SPSB concentration notably improved cucumber growth, increasing plant height by 13.01%, stem thickness by 20.79%, leaf area by 50.26%, and dry weight by 58.56% relative to the control. High-throughput sequencing revealed this concentration significantly altered soil microbial community structure, enhancing bacterial and fungal diversity. It increased beneficial bacterial groups (Firmicutes, Actinobacteria, Bacillus) and modified fungal communities, with a decrease in Ascomycota and *Aspergillus* and shifts in *Penicillium* abundance. Functional genomic analysis indicated enrichment in bacterial metabolic pathways and fungal replication and expression genes.

Conclusion SPSB, especially at a 5% w/w concentration, emerges as an effective soil amendment in greenhouses affected by continuous monoculture. This approach represents a sustainable method to enhance soil health and crop productivity.

Keywords Biochar, Continuous cropping obstacle, Cucumber, Soil microflora, Sweet pepper

Introduction

Cucumber (*Cucumis sativus* L.) is a valuable vegetable crop widely cultivated in greenhouses. Yet, continuous monoculture has an adverse effect on its yield and quality (Xiao et al. 2019). For this reason, reducing the negative impact of continuous cropping has attracted widespread attention from domestic and foreign scholars. Current practice in agricultural production involves soil improvement and chemical control measures to maintain healthy soil for high-quality yield. Soil fumigation or replacement can provide healthy soil (Xiong et al. 2022) but at a high cost and could disturb the ecological balance. Meanwhile,

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chemical control approaches involve using phosphorus and potassium fertilizers (Zhang et al. 2022), antibiotics, or insecticides. However, these methods have potential ecological and environmental risks (Mirzaei et al. 2020).

Biochar, a carbon-rich material produced from plant residues by pyrolysis or oxidation–reduction reactions, has attracted attention recently (Li et al. 2017). Biochar is often prepared from agricultural wastes such as straw and is therefore cost-effective and environmentally friendly. Studies have shown that biochar can improve soil quality, optimize microbial communities (Zheng et al. 2022), and promote pollutant removal in specific environments (Fu et al. 2022). Biochar applications in greenhouse crop production, including tomatoes (Calcan et al. 2022), peppers (Wang et al. 2019a, b), and cucumbers (Liu et al. 2022), improve growth and yield by increasing nutrient availability and limiting soil-borne diseases. However, studies have also reported the detrimental effects of biochar on the greenhouse cultivation of crops. These effects depend on the application concentration, the physical and chemical characteristics of the biochar used, and the properties of the target soils (Xiang et al. 2021). Accordingly, the highly intensive production mode in greenhouse agriculture should determine special cultivation management measures, including the wise application of biochar.

In recent years, there has been an increasing demand for vegetables, so their cultivation in greenhouses has expanded. Vegetables such as tomatoes, sweet peppers, and eggplants are common crops with high nutritional and medicinal values. Their planting areas and yields have remained high globally. Notably, in the highly intensive production mode of greenhouses, a large amount of waste is generated, including vegetable straw and residual parts (Chen et al. 2022), which often causes environmental problems due to the inability to timely process. Therefore, converting greenhouse vegetable straws into biochar is of great economic value. Using biochar for soil applications can enhance resource utilization and improve the soil quality for the continuous cultivation of crops such as cucumbers.

The present study aims to investigate the type and dosage of biochar suitable for improving the continuous cultivation of cucumber in greenhouses We hypothesize that appropriate concentration of sweet pepper straw biochar (SPSB) may improve soil quality and microbial diversity, enhancing nutrient availability and cycling and promoting plant growth.

Materials and methods

Experimental materials

The cucumber scion used in this study was Hong qiang Xin xiu, and the rootstock was Ba tu 430 pumpkin. The used soil sample was collected from the surface layer (10–20 cm) of a greenhouse in Linyi County, Shandong Province, China (35°06′′N, 117°41′′E), where cucumber has been continuously grown for over 10 years. The three types of tested biochar, namely tomato straw biochar (TSB), sweet pepper straw biochar (SPSB), and eggplant straw biochar (ESB), were prepared by pyrolysis at 365 °C for 104 min, 489 °C for 95 min, and 347 °C for 83 min, respectively.

Biochar characterization methods

Various characterization techniques were employed to analyze the prepared biochar samples. The methylene blue adsorption capacity (MBAC) served as a quantitative measure of the biochar's adsorptive properties, adhering to a prescribed standard protocol (Liu et al. 2021). The adsorption capacity was ascertained using the equation: (A0–A1)/A0 × 100%, where A0 represents the initial absorbance of the methylene blue standard solution and A1 is the absorbance of the supernatant subsequent to the addition of the methylene blue standard solution to the specimen. The cation exchange capacity (CEC) of the biochar was determined through the sodium acetate exchange technique (Wang et al. 2019a, b). The total nitrogen content within the prepared biochar was quantified utilizing the Kjeldahl method (Hardy 2009). Estimation of the total phosphorus content was conducted via the molybdenum blue spectrophotometric procedure (Felgentreu et al. 2018). The total potassium content was ascertained in accordance with the flame photometry method, following the methodology delineated by Banerjee and Prasad (2020). Each analytical determination was replicated a minimum of three times to ensure accuracy.

Pot experiment design

The pot experiment was conducted in a greenhouse at the Horticultural Experimental Station of Shandong Agricultural University (36°11′30′′N, 117°7′2′′E) from February to June 2022. The study involved four treatments, with varying proportions of biochar mixed with soil. The biochar-soil mixtures in each pot had a total mass of 1 kg. The pots measured 15 cm in top diameter, 9 cm in bottom diameter, and 12 cm in height. The treatments were designated as follows: CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), and S3 (7.5% w/w biochar + soil). Each treatment consisted of three replicates, with 10 pots per replicate. In every pot, a two-leaf stage cucumber seedling was planted, and the plants were managed following standard cultural practices.

Plant sampling and analysis methods

At 40 days old, five cucumber plants with similar growth statuses were selected from each treatment for measuring growth indicators. The dry weight of the aboveground

and underground parts was measured, the plant height was determined by a ruler, the stem thickness was estimated by a caliper, and the first true leaf of the plant was measured using the benchtop leaf area meter (LI-3100C, Beijing Li Gao Tai Ke Technology Co., Ltd., Beijing, China).

Root-zone soil sampling and measurement methods

Forty days subsequent to planting, a trio of pots from each treatment were selected for both plant and soil sampling. The soil specimens were meticulously sieved through a 2-mm mesh to eliminate plant residues and extraneous impurities, subsequently being bifurcated into two distinct portions. The first portion was allocated for the assessment of the soil's chemical attributes, while the second was preserved at a temperature of − 80 °C for subsequent microbial community analysis. These soil samples were then diluted with water at a ratio of 1:5. The soil's pH level was gauged utilizing a pH meter (PHS-3E, Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China), and the electrical conductivity (EC) was ascertained with a conductivity meter (DDSJ-319L, Shanghai INESA Scientific Instrument Co., Ltd., Shanghai, China). The content of soil organic matter was determined through the potassium dichromate volumetric technique, in accordance with the methodology delineated by Zhao et al. (2019). The available nitrogen (TN) was quantified via the alkali diffusion method (Zhang et al. 2021), while the available phosphorus (TP) was estimated by employing carbonate-bicarbonate extraction coupled with the molybdenum antimony colorimetric procedure (Lutz et al. 1971). The available potassium (TK) was assessed through ammonium acetate extraction, followed by the flame photometric technique (Zhu et al. 2019). To ensure statistical robustness, each determination was replicated thrice.

DNA extraction and PCR amplification

In this study, we utilized the MP Reagent Kit (Fast DNA® Spin Kit for Soil, MP Biomedicals, USA) to extract total DNA from microbial communities, following the manufacturer's guidelines. The extracted DNA was quantified using 1% agarose gel electrophoresis, in accordance with established protocols. To determine the optical density (OD) values of the DNA solutions from both control and biochar-treated soil samples, spectrophotometric analysis (NanoDrop2000) was performed at 260-nm and 280-nm wavelengths. The OD values obtained, ranging from 1.8 to 2.0, indicated an absence of protein contamination and validated the samples for further PCR amplification steps. For bacterial 16S rRNA gene amplification of the V3–V4 variable region, primers 338F (5′-ACTCCT ACGGGAGGCAGCAG-3′) and 806R (5′-GGACTA CHVGGGTWTCTAAT-3′) were used. In addition, the primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were employed for fungal ITS rDNA amplification. The PCR amplification was performed using a PCR machine (ABI Gene Amp® 9700) as follows: 95 °C for 3 min of initial denaturation, 27 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min and storage at 4 °C. Each sample was amplified in triplicate.

16S amplicon sequencing and raw letter analysis

The V3–V4 region of the 16S rRNA gene was sequenced using MiSeq amplicon sequencing. The acquired sequencing data underwent assembly, quality control, and adapter trimming to generate optimized sequences. These optimized sequences were subsequently submitted to the Sequence Read Archive (SRA) under BioProject IDs PRJNA961072 and PRJNA962855.

Microbial community diversity and functional assessment methods

In assessing the richness and diversity of microbial communities, the Chao1 and Shannon indices were employed. The Bray–Curtis distance algorithm facilitated the execution of nonmetric multidimensional scaling (NMDS), serving to scrutinize the disparities in microbial community structures among various samples. Furthermore, the Adonis test was invoked to contrast the degrees of variation interposed between different groups. For the purpose of predicting microbial functionality, a comparative analysis was conducted with the Kyoto Encyclopedia of Genes and Genomes (KEGG), an established gene function database, developed under the auspices of the Bioinformatics Laboratory at Kyoto University, Japan.

$$H_{shannon} = -\sum_{i=1}^{Sobs} \frac{n_i}{N} ln \frac{n_i}{N}$$

where Sobs represents the count of observed OTUs, n_i denotes the number of OTUs encompassing i sequence, and N signifies the total number of sequences present.

$$S_{chao1} = Sobs + \frac{n_1(n_1 - 1)}{2(n_2 + 1)}$$

where S_{chao1} denotes the count of estimated OTUs, S_{obs} signifies the number of observed OTUs, n_1 corresponds to the number of OTUs containing only a single sequence, and n_2 represents the number of OTUs that encompass precisely two sequences.

Data analysis

Analysis of the high-throughput sequencing data pertaining to soil microorganisms was conducted utilizing a cloud service furnished by Majorbio Biomedical Technology Co., Ltd. (Shanghai, China; https://www.majorbio.com/). For the merging of pair-end sequences, Flash software (version 1.2.11) was employed (https://ccb.jhu.edu/software/FLASH/index.shtml). Operational taxonomic unit (OTU) counting was facilitated through the use of USEARCH software (version 11) (http://www.drive5.com/usearch/), while OTU clustering was performed with UPARSE software (version 11) (http://www.drive5.com/uparse/). Alpha diversity analysis was carried out using mothur software (version 1.30.2) (https://www.mothur.org/wiki/Download_mothur), and the QIIME software (version 1.9.1) was utilized to create taxonomic abundance tables and compute beta-diversity distances (http://qiime.org/install/index.html). KEGG functional prediction of 16S rRNA gene sequences was achieved through the PICRUSt software (version 1.1.0). Fungal function was predicted by comparing the FungalTraits database, and the SPSS software (version 21.0) was utilized for statistical evaluations and the assessment of differences between groups in soil property experiments. A *p*-value of less than 0.05 was deemed to be indicative of statistical significance.

Results

Physicochemical properties of biochar

To characterize the different types of biochar, we conducted a series of physical and chemical tests. The MBAC and CEC tests were employed to assess the negative charge surface area and the porosity of the biochar, while the EC test was utilized to determine the concentration of the conducting molecules or ions in the biochar. The TN, TP, and TK were used to evaluate the nutrient content of the biochar. Table 1 illustrates the physicochemical properties of TSB (tomato straw biochar), SPSB (sweet pepper straw biochar), and ESB (eggplant straw biochar). The results revealed that the SPSB significantly exhibited the highest MBAC, CEC, TN, and TK compared to the other tested biochar. Notably, compared to SPSB, there were no significant differences in the EC and TP values of ESB. In general, the SPSB demonstrated superior nutrient content and adsorption capacity (Table 1).

Chemical properties of SPSB-treated soil

Our findings revealed that soil pH, available potassium (AK), available hydrolyzable nitrogen (AHN), available phosphorus (AP), and soil organic matter (SOM) all saw significant increases in the three treatments (S1, S2, S3) compared with the control (CK). As we escalated the application of SPSB, AK, AP, and SOM demonstrated a steady uptrend. Meanwhile, soil pH manifested a pattern of an initial rise followed by a decline, and conversely, electrical conductivity (EC) exhibited a trend of first decreasing and subsequently increasing (Fig. 1).

Effect of SPSB on cucumber growth

An examination was conducted into the influence of SPSB on cucumber growth by assessing various growth attributes. As delineated in Table 2, the impact of distinct SPSB dosages on plant development is detailed. The findings revealed that the S2 treatment exhibited a mean height of 99 cm, stem diameter of 7.61 mm, and a dry weight of 15 g, metrics that were appreciably greater than those recorded in the alternative treatment. The S1 treatment was characterized by the most expansive leaf area, averaging 189.27 cm2, a figure that surpassed those in the remaining treatments. In a comprehensive assessment, the growth of cucumber in the S2 treatment emerged as the most favorable, succeeded by the S1, S3 treatments, and CK.

The effects of SPSB on microbial α-diversity

A Venn diagram provides a lucid depiction of the distinct operational taxonomic units (OTUs) within a sample community. Through high-throughput sequencing analysis, we identified a total of 1879 bacterial OTUs across all the samples (Fig. 2A). The number of unique OTUs associated with the CK, S1, S2, and S3 treatments was determined to be 167, 238, 268, and 167, respectively. These data suggest an initial increase followed by a decrease in the number of unique bacterial OTUs as the application

Table 1 Physicochemical properties of biochar from different feedstocks

Biochar	MBAC (%)	CEC ($cmol(+)\cdot kg^{-1}$)	EC ($mS\cdot cm^{-1}$)	TN ($mg\cdot g^{-1}$)	TP ($mg\cdot g^{-1}$)	TK ($mg\cdot g^{-1}$)
TSB	89.04 ± 0.09c	35.62 ± 0.31b	3.22 ± 0.10b	19.00 ± 0.04b	4.80 ± 0.44b	25.30 ± 0.62b
SPSB	99.45 ± 0.29a	45.58 ± 0.42a	3.89 ± 0.80a	27.50 ± 0.34a	8.20 ± 0.53a	32.80 ± 0.53a
ESB	92.02 ± 0.17b	35.53 ± 0.28b	3.77 ± 0.07a	16.50 ± 0.03c	7.80 ± 0.26a	17.70 ± 0.56c

MBAC Methylene blue adsorption capacity, *CEC* Cation exchange capacity, *EC* Electrical conductivity, *TN* Total nitrogen content, *TP* Total phosphorus content, *TK* Total potassium content. The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)

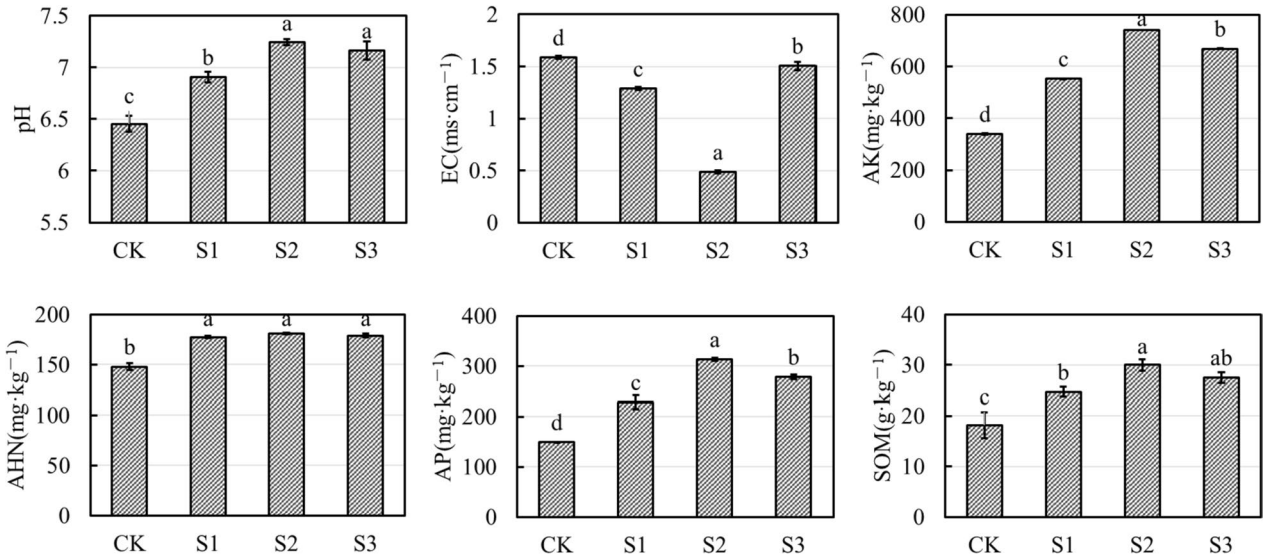

Fig. 1 Effects of different concentrations of SPSB on soil chemical properties in a continuous cropping system. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil). AK, available potassium; AHN, alkali-hydrolyzable nitrogen; AP, available phosphorus; SOM, soil organic matter. The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)

Table 2 The impact of SPSB application on the growth of greenhouse continuous cropping cucumber

Treatment	Plant height (cm)	Stem diameter (mm)	Leaf area (cm^2)	Dry weight (g)
CK	87.6 ± 4.67 cd	6.30 ± 0.16b	111.37 ± 6.11d	9.46 ± 0.46 cd
S1	98.6 ± 1.95a	7.58 ± 0.21a	189.27 ± 8.45a	10.84 ± 1.16bc
S2	99 ± 3.24a	7.61 ± 0.21a	167.35 ± 2.59c	15.00 ± 1.66a
S3	93.6 ± 1.67b	7.35 ± 0.15a	184.22 ± 5.47ab	10.94 ± 1.22bc

CK Control without SPSB. S1, S2, and S3 represent SPSB concentrations of 2.5%, 5%, and 7.5% w/w, respectively. The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)

of SPSB in soil escalates. In parallel, analysis of the sequencing data revealed 256 fungal OTUs (Fig. 3B). The CK, S1, S2, and S3 treatments harbored 115, 122, 141, and 154 unique OTUs, respectively. These observations indicate that akin to the bacterial community, the count of soil-specific fungal species exhibits a pattern of initial rise followed by a decline in response to SPSB treatment.

In analyzing the soil microbial α-diversity for the bacterial community (Fig. 3A), both the Shannon and Chao indices for the S1, S2, and S3 treatments were significantly higher than those of the control (CK). Notably, the S2 treatment exhibited the peak values. This trend suggests that as the SPSB concentration increases, bacterial community diversity and abundance first rise and then decline.

For the fungal community (Fig. 3B), the Shannon index in the S1, S2, and S3 treatments was significantly elevated compared to the control. However, the Chao index showed no significant difference, indicating that the fungal community's response to SPSB differs from that of the bacterial community.

The effects of SPSB on microbial β-diversity

Nonmetric multidimensional scaling (NMDS) was employed to simplify the analysis of bacterial community structure variations between samples. The results revealed a broad distribution of sample sites across treatments. The Adonis test confirmed that these intergroup differences were statistically significant (Fig. 4A). This suggests not only that SPSB significantly modified the bacterial community structure in the soil but also that these modifications were positively associated with the SPSB concentration.

Conversely, the NMDS analysis for the fungal community structure indicated that the distances between data points within the community expanded as the SPSB dosage increased. However, the test for differences between communities revealed no statistical significance (Fig. 4B). This implies that the impact of SPSB on the diversity of fungal species in the soil was limited.

The effects of SPSB on microbial composition

Figure 5 lists the top 10 bacterial phyla by relative abundance. Firmicutes, largely comprising gram-positive bacteria, dominated in all treatments, accounting

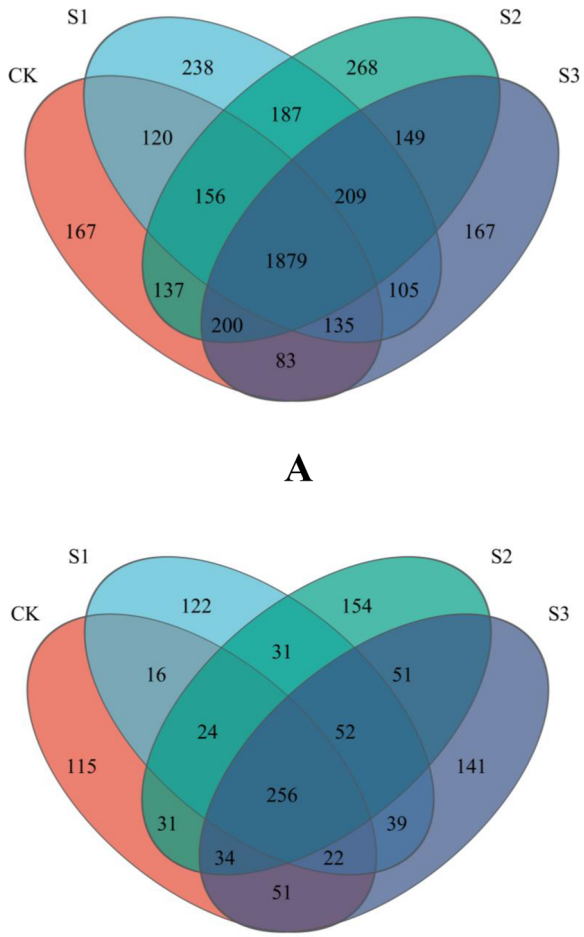

Fig. 2 Venn analysis of the bacterial (**A**) and fungal (**B**) communities influenced by different SPSB concentrations. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil)

for over 27% of the total, followed by Actinobacteriota (> 17%) and Proteobacteria (> 12%). The escalation in biochar dosage instigated a general pattern of an initial ascent followed by a descent in the relative abundance of principal bacterial phyla. Compared to the control (CK), the relative abundance of Firmicutes in the S1, S2, and S3 treatments increased by 25.15%, 33.8%, and 15.66%, respectively. Meanwhile, the relative abundance of Proteobacteria decreased by 16.21%, 18.06%, and 10.51% for the same treatments. Notably, the relative abundance of Actinobacteriota decreased by 4.89% in S1 and 4.84% in S3, while in the S2 treatment, it increased by 25.21% (Fig. 5A).

At the genus level, *Bacillus* was predominantly present (> 13%). Compared to the CK, its relative abundance increased by 22.72% in S1, 51.16% in S2, and 23.74% in S3. However, statistical tests revealed no significant differences between the S1 and S3 treatments compared to CK (Fig. 5B).

In the soil's fungal community, Ascomycota was the dominant phylum, comprising over 68% of the relative abundance. Other prominent phyla included Olpidiomycota, Basidiomycota, Rozellomycota, Mortieretiomycota, Chytridiomycota, Mucoromycota, Glomeromycota, and unclassified fungi (Fig. 6A). Compared to the CK, the relative abundance of Ascomycota saw a slight increase in the S1 treatment, while it decreased significantly in the S2 and S3 treatments by 26.14% and 10.42%, respectively.

The 10 most abundant fungal genera were *Aspergillus*, *Mycothermus*, *Penicillium*, *Cephaliophora*, *Olpidium*, *Melanocarpus*, and unclassified fungi (Fig. 6B). The fungi, *Aspergillus* and *Penicillium*, significantly influenced the quality of cucumber plants and fruits. Compared to the CK treatment, the content of *Aspergillus* decreased by 26.29% in S1, 79.83% in S2, and 45.65% in S3. Conversely, the relative abundance of *Penicillium* increased by 35.4%, 237.7%, and 43.91% in the S1, S2, and S3 treatments, respectively.

Microbial function predictive analysis

The classification of functional genes within the bacterial community was achieved through the utilization of the KEGG database. At the foundational functional tier, six distinct categories of biological metabolic pathway functions were identified, encompassing cellular processes, environmental information processing, genetic information processing, human diseases, metabolism, and organismal systems. Furthermore, 36 sub-functions were identified at the secondary functional level, of which 18 had a relative abundance exceeding 1% (Fig. 7A).

At the metabolic level, biological activities such as carbohydrate metabolism, amino acid metabolism, energy metabolism, metabolism of cofactors and vitamins, nucleotide metabolism, and lipid metabolism were amplified in the bacterial community due to SPSB. This implies that SPSB could enhance these pathways' metabolic activities in the microbial community. Similarly, metabolic activity in membrane transport and signal transduction pathways at the environmental information processing level increased with SPSB application. This suggests that SPSB may boost the microbial community's response to environmental signals. In addition, the introduction of SPSB amplified metabolic activities related to translation, DNA replication, and repair, indicating that SPSB might stimulate these functions in the microbial community at the genetic information processing level.

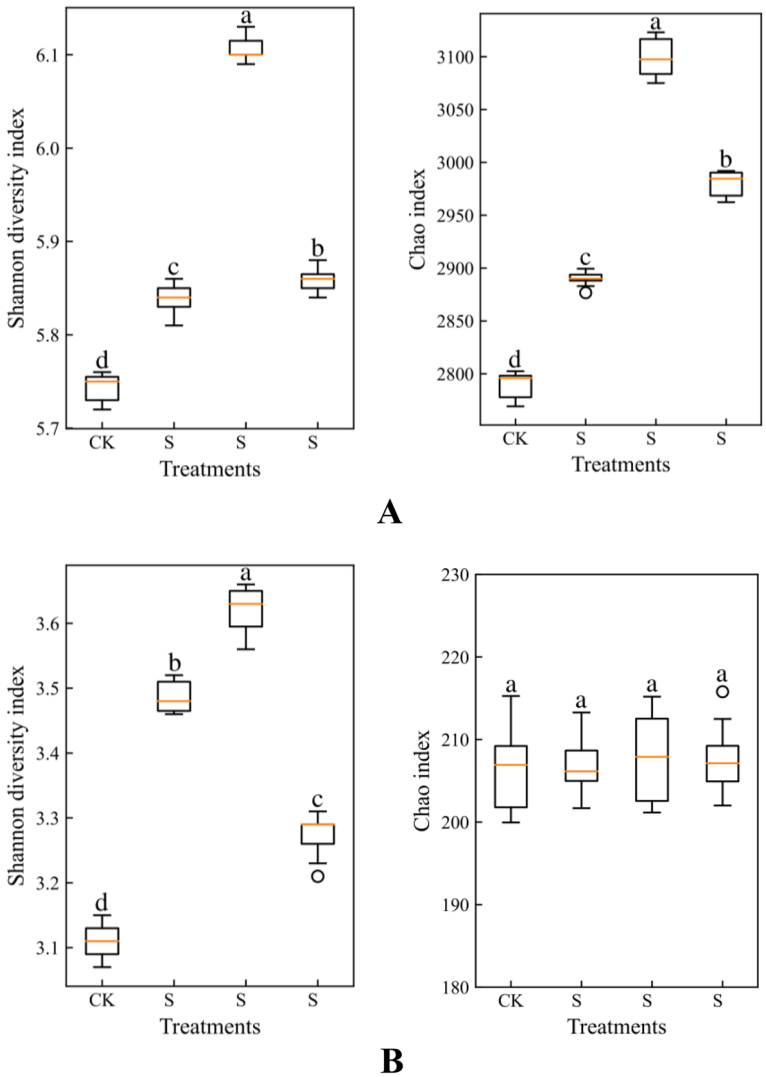

Fig. 3 Alpha diversity index of bacterial (**A**) and fungal (**B**) communities affected by SPSB concentrations. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil). The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)00

The KEGG database was utilized for the annotation of fungal enzyme functions, facilitating the identification of the top 20 enzymes in fungi based on the abundance of their gene expression (Fig. 7B). Enzymes such as adenosine triphosphatase, DNA-directed RNA polymerase, glucan 1,4-alpha-glucosidase, L-arabinose isomerase, and exo-alpha-sialidase exhibited peak activity levels in the S2 treatment group. These observations suggest that a medium concentration of SPSB might influence a broad range of biochemical reactions, thereby impacting the synthesis and transformation of metabolites and ultimately affecting the growth and metabolism of fungal communities.

Functional classification of fungi, as per the FungalTraits database, revealed that unspecified saprotrophs, soil saprotrophs, and algal parasites constituted nearly 50% of all fungal operational taxonomic units (OTUs). Notably, the S1 treatment exhibited a significantly higher relative abundance of algal parasites compared to CK. Additionally, the abundance of plant pathogens was lower across all four treatments (Fig. 7C).

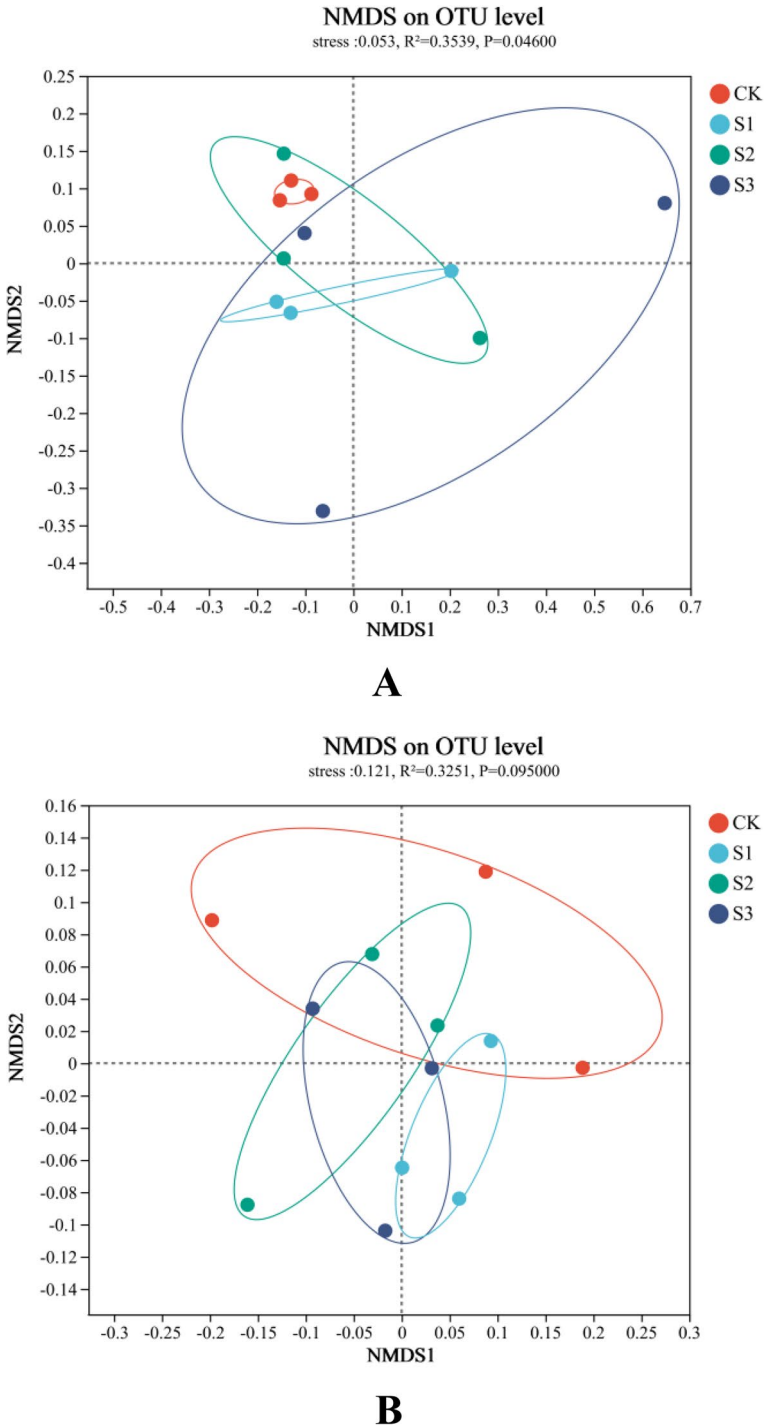

Fig. 4 NMDS analysis of the bacterial (**A**) and fungal (**B**) communities influenced by different SPSB concentrations. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil). Stress < 0.2, the graph is interpretable; $P < 0.05$, the difference between groups is significant

Discussion

SPSB boasts a superior inorganic nutrient profile and distinct adsorption properties

While many studies have highlighted biochar's capabilities in adsorbing pollutants (Rehman et al. 2023), modulating soil attributes (Zhang et al. 2023a, b), and

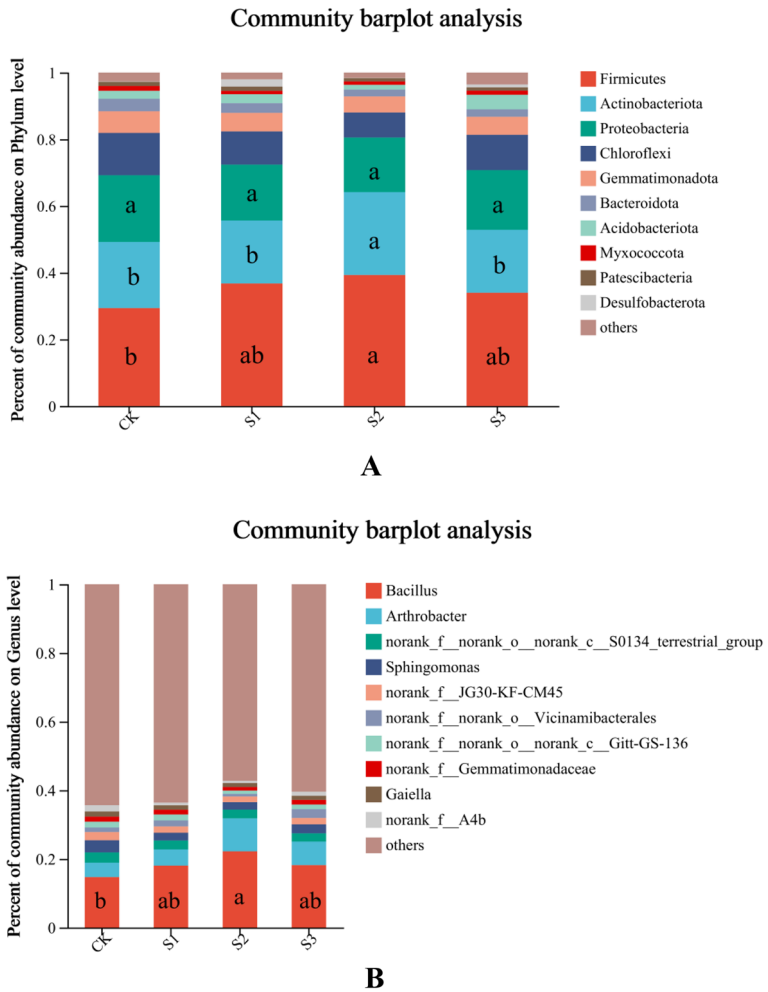

Fig. 5 Bacterial community composition at phylum (**A**) and genus (**B**) levels influenced by SPSB concentrations. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil). The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)

promoting crop growth (Zhang et al. 2023a, b), the influence of the raw materials used in biochar preparation remains underexplored. In our study, we prepared biochar from three vegetable straws commonly found in northern China's greenhouses. A comparative analysis of their physicochemical properties showed that sweet pepper straw biochar (SPSB) contains higher levels of total nitrogen, phosphorus, and potassium than tomato straw biochar (TSB) and eggplant straw biochar (ESB). This enhanced nutrient profile might result from the thermal decomposition of chain-structured cellulose during biochar production, leading to dehydrogenation and deoxygenation, thereby forming carbon radicals. The thicker stem and greater degree of lignification in sweet pepper plants could contribute to a more nutrient-rich content after carbonization.

Biochar's adsorptive capacity is attributed to its intricate porous structure and the diverse functional groups on its surface. This capacity is also influenced by the pyrolysis temperature. Previous research indicates that as pyrolysis temperatures rise, biochar's total pore volume tends to expand, leading to the creation of finer micropores. However, this can concurrently diminish the surface functional groups (Cong et al. 2022). In our study, variations in pyrolysis temperatures likely explain the observed disparities in the adsorptive capacities of the biochars.

Moderate SPSB application enhances cucumber growth

The notable positive effects of SPSB on cucumber growth parameters provide compelling evidence of its

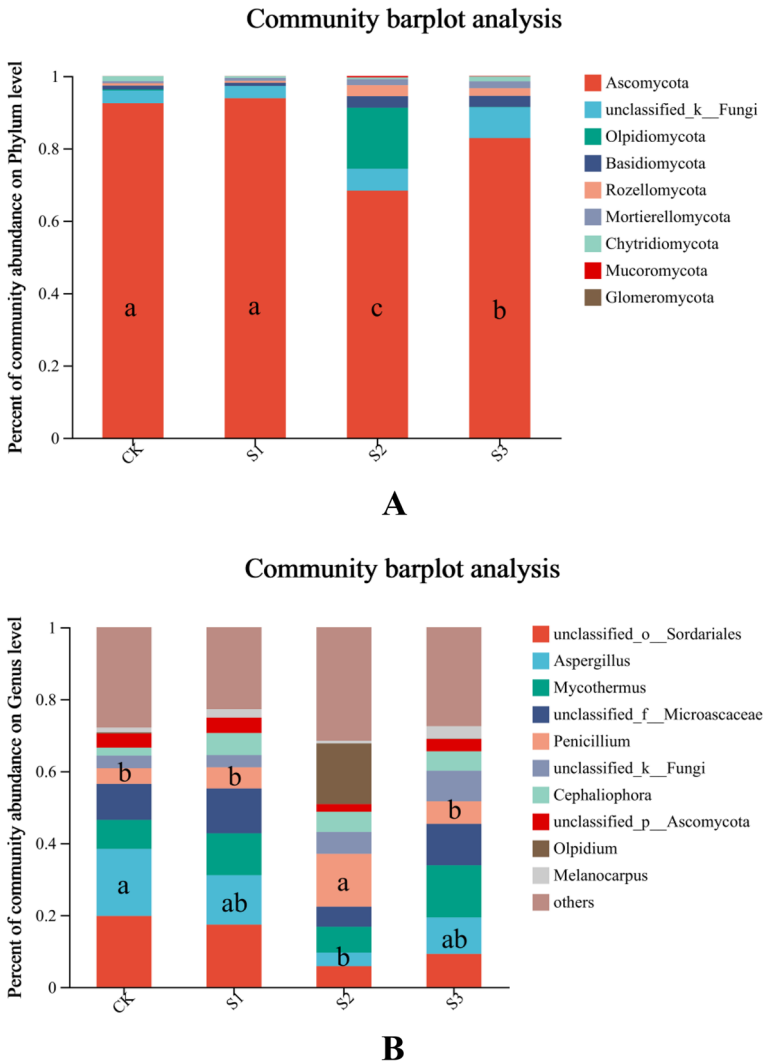

Fig. 6 Fungal community composition at phylum (**A**) and genus (**B**) levels with varying SPSB treatment concentrations. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil). The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)

beneficial influence on plant development. When compared to the control (CK), all SPSB treatments exhibited significant enhancements in plant growth metrics. Specifically, the S2 treatment outperformed the others, registering the highest values for plant height, stem thickness, and dry weight. This suggests that the SPSB concentration used in S2 is particularly effective in fostering plant growth. However, it's intriguing that the leaf area index for the S2 treatment was less than that of S1 and S3, a variation that might stem from differences in the growth stages of the plants. These insights emphasize the need to fine-tune SPSB concentrations to maximize different facets of plant growth. They also reinforce the potential of SPSB as a valuable soil amendment to bolster plant growth and yield.

SPSB alters the diversity of the soil microbial community

Soil microbial diversity plays a crucial role in maintaining ecosystem stability by driving essential functions such as nutrient cycling, plant productivity, and soil functionality (Romero et al. 2023; Wagg et al. 2019; Delgado-Baquerizo et al. 2020). However, continuous cropping often leads to changes in the ecosystem of the root zone due to the prolonged accumulation of the same crop's root exudates, which limits microbial diversity (Xu et al. 2021). According to a recent study, the diversity of the soil bacterial community significantly decreased with increasing

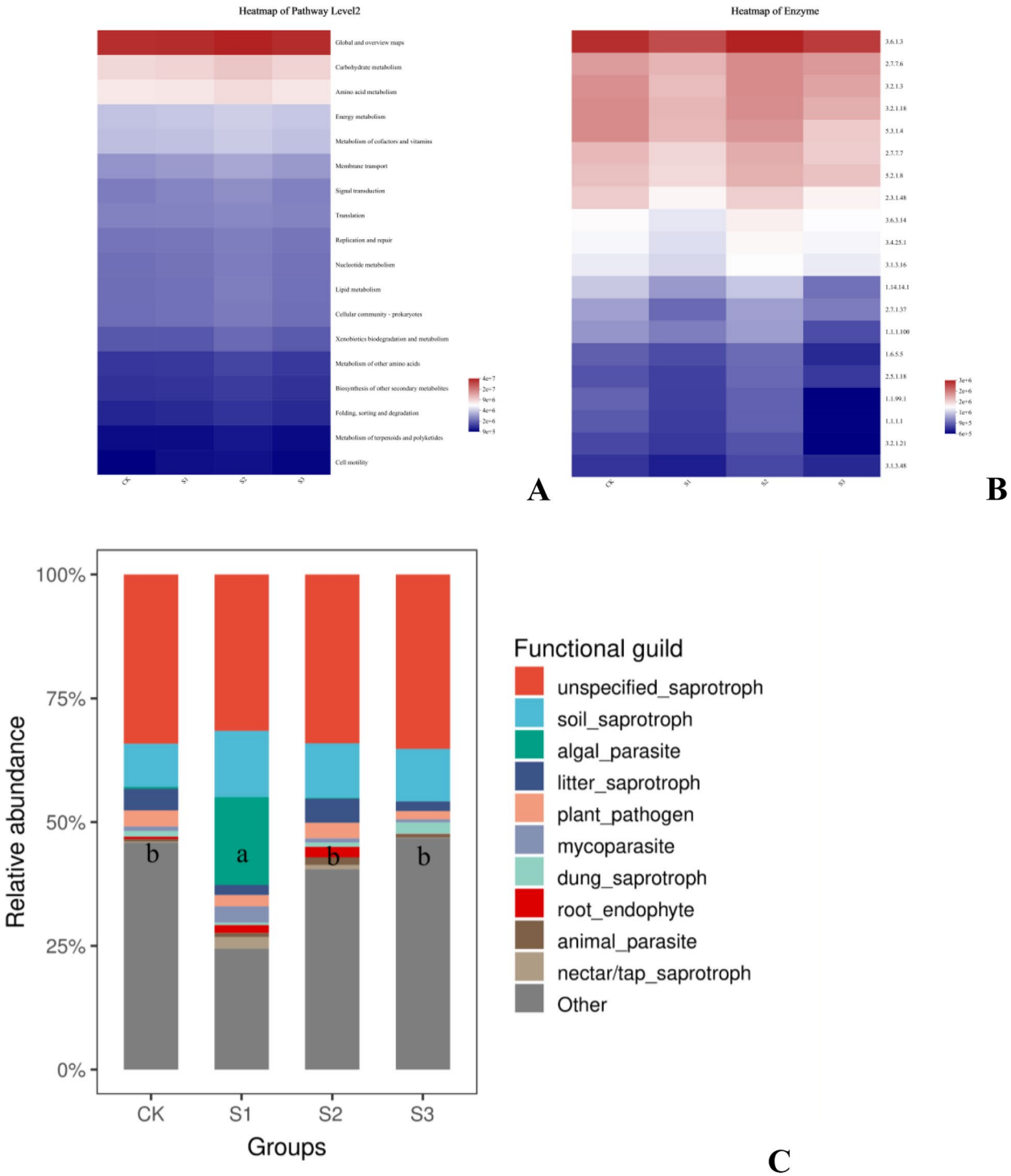

Fig. 7 Predictive analysis of bacterial (**A**) and fungal (**B**, **C**) function affected by SPSB treatment. CK (no biochar), S1 (2.5% w/w biochar + soil), S2 (5% w/w biochar + soil), S3 (7.5% w/w biochar + soil). The significant differences are determined by the Tukey test, with different lowercase letters indicating significant differences between groups ($P < 0.05$)

continuous sweet potato cropping years, resulting in an increased risk of diseases (Gao et al. 2021). In this study, the microbial community diversity was higher in the SPSB treatment compared to CK, indicating that biochar could promote the emergence of unique microbial species and widen community heterogeneity and individual allocation uniformity. This biochar effect encourages the restoration of the soil's ecological function and reinforces resistance stability. The soil treated with a medium concentration of SPSB had the highest microbial diversity. This data suggests that high concentrations of SPSB could exhibit the risk of reducing microbial diversity. Furthermore, the SPSB treatment had a more pronounced effect on bacterial diversity in the soil compared to its impact on fungi, a finding that aligns with results from a previous study (Awasthi et al. 2017). This behavior could be due to the rich cellular functions and complex network structures of fungi compared to bacteria. Another explanation is that the porous biochar provides more space for the attachment of fungal mycelia due to its large surface area. These features allow fungal communities to be more stable and reduce the impact of root zone soil environment on fungal community richness.

SPSB enriched beneficial bacteria and reduced the relative abundance of some fungi

Microbial imbalances in soil are identified as significant challenges to continuous cropping (Cui et al. 2022). Thus, maintaining a harmonious balance between beneficial and pathogenic microorganisms can mitigate these challenges. In alignment with our observations, a recent study on greenhouse soils from prolonged continuous cucumber cultivation revealed a dominance of Actinobacteriota in the bacterial community. Actinobacteriota, a distinct group of aerobic bacteria, are ubiquitous across various soil types. They are adept at decomposing complex compounds like aromatic compounds, cellulose, and lignin, playing a pivotal role in organic matter turnover and carbon cycling (Bao et al. 2021). In our study, Actinobacteriota's relative abundance in the S2 treatment saw a significant rise, while in the S1 and S3 treatments, it experienced a slight decline, albeit not statistically significant. It is documented that Actinobacteriota thrive in nutrient-rich soils, but an excess of organic matter can hinder their growth and reproduction (Bhatti et al. 2017). The heightened soil organic matter resulting from a high concentration of SPSB might account for the relative decline of Actinobacteriota in the S3 group.

At the genus level, *Bacillus*, a quintessential biocontrol bacterium, saw an increased relative abundance with SPSB treatment and demonstrated that most *Bacillus* strains curtail the proliferation of soilborne pathogenic microorganisms, thereby reducing the likelihood of soilborne plant diseases (Dimkić et al. 2022). Notably, certain *Bacillus* strains exhibit strong antagonism towards *Fusarium*, a notorious plant pathogen in continuous cropping systems, especially for cucumbers. The surge in *Bacillus* abundance underscores the potential of SPSB in bolstering soil's biological defense in continuous cropping.

In the realm of mycology, it is remarkable to note that nearly 83,000 species of the Ascomycota phylum have been identified and described to this day (Senanayake et al. 2022). This phylum exhibits a vast array of interactions with plant life. Many of these interactions are pathogenic in nature, leading to various plant diseases. Notable examples include the notorious rice blast (Wang and Valent 2017) and the widespread occurrence of powdery mildews (Li et al. 2023). These instances underscore the critical ecological and agricultural impact of Ascomycota species and powdery mildews. Our study honed in on the *Penicillium* and *Aspergillus* genera within the Ascomycota phylum. Some *Penicillium* species have shown efficacy against nematode infestations, a prevalent concern in greenhouse farming. SPSB treatments, particularly S2, amplified the abundance of the *Penicillium* genus while diminishing that of the *Aspergillus* genus, thereby potentially reducing the risk of cucumber fruit rot.

SPSB affected the functionality of soil microorganisms

Utilizing PICRUSt, we forecasted the functions of bacteria within soils subjected to varying SPSB dosages. The findings indicated that the predominant relative abundances were associated with the metabolism of carbohydrates, amino acids, energy, and cofactors and vitamins. Among them, carbohydrate metabolism is closely linked to soil carbon–nitrogen cycling and phosphorus dissolution. The amino acid metabolism primarily facilitates the removal and conversion of soil NH_4^+ (Xie et al. 2020). Energy metabolism aids in the resistance of cells to iron-mediated oxidative damage, and the metabolism of cofactors and vitamins is necessary for withstanding adverse environmental conditions. At the primary classification level using the KEGG database, the relative abundances of genes involved in metabolism, environmental information processing, cellular processes, and organismal systems were significantly upregulated in the S2 group. This upregulation indicated that SPSB improved the capacity of the soil bacterial community to adjust to environmental changes driven by continuous cropping, accelerated bacterial community succession, and actively engaged the microbial community in basic metabolic processes. The enzyme annotation using the KEGG database showed that the relative abundance of ATP synthase, DNA-mediated DNA polymerase, and

RNA polymerase increased in the S2 treatment group. These findings suggested that medium-concentration biochar promoted fungal gene replication and expression in the treated soil. The 1,4-alpha-glucosidase directly participates in the metabolic pathway of starch and glycogen (Ram and Venkatasubramanian 1982), indicating that the sugar metabolism of the soil fungal community is dynamic. However, the abundance of these enzymes decreased in the S1 and S3 treatment groups, indicating that the soil fungal communities responded differently to different amounts of SPSB biochar. Currently, there are limitations to the prediction of fungal function by PICRUSt. Therefore, further analysis and verification of fungal metabolic functions using techniques such as metagenomic sequencing are necessary.

Conclusion

In this study, we explored the efficacy of biochars derived from vegetable straws in addressing the challenges of successive cucumber cultivation in greenhouses. Our findings indicated that sweet pepper straw biochar (SPSB) ameliorated soil quality in a concentration-responsive manner. Notably, a moderate concentration of SPSB (5% w/w) bolstered soil fertility and microbial diversity by attracting beneficial microorganisms to the root zone, subsequently boosting cucumber growth. These results underscore the potential of moderate SPSB concentrations as a soil amendment, highlighting their capacity to rejuvenate a balanced microbial ecosystem, enhance soil attributes, and facilitate the continuous cultivation of cucumbers in greenhouses.

Supplementary Information

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Supplementary Material 1.

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Not applicable

Authors' contributions
JL designed research and experiments. XC and YD assisted in the completion of the study. HL wrote the main manuscript text. DZ revised the manuscript. MW performed critical reading and revising suggestions. All authors read, reviewed, and approved the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Consent for publication
All the authors have approved the manuscript that is enclosed.

Competing interests
The authors declare that they have no competing interests.

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