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Long-term peanut shell biochar application improves soil fertility and bacterial network stability across tobacco-growing regions in China

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Abstract

Soil microorganisms are central to nutrient cycling and soil fertility, and their dynamics are strongly influenced by agricultural management practices. Peanut shell biochar has been widely applied to enhance soil fertility and reduce nutrient loss. However, its long-term effects on soil microbial communities under large-scale field conditions remain poorly understood. To address this knowledge gap, we conducted multi-year field experiments across five major tobacco-growing regions in China. Compared with the control group, long-term addition of peanut shell biochar significantly improved various soil chemical properties in Mudanjiang, Shangluo, Yichun, and Yanshan Town, including pH, available potassium, available phosphorus, organic matter, carbon-to-nitrogen ratio, alkaline hydrolyzable nitrogen, sucrase activity, catalase activity, and urease activity, while reducing the available phosphorus and catalase activity in Xuchang. Soil microbial diversity and community composition exhibited significant variation across sites, primarily shaped by differences in soil chemical properties. Although overall microbial diversity was not significantly altered by biochar addition, specific taxa, such as Firmicutes, Zoopagomycota, and Blastocladiomycota, were enriched, with Bacilli representing 70% of the significantly enriched bacterial taxa. Co-occurrence network analysis revealed that biochar amendment enhanced the complexity and stability of bacterial networks but reduced those of fungal networks. Furthermore, long-term biochar application enhanced soluble sugar content through pathways involving soil organic matter, bacterial community diversity, and specific enriched bacterial taxa. Collectively, these findings underscore the important role of peanut shell biochar in promoting the stability of bacterial networks and enhancing crop quality, providing a sustainable strategy for improving soil health and agricultural productivity.

Highlights

- Peanut shell biochar (PSB) improved soil nutrients and enzyme activities across sites.
- The microbial diversity and composition of rhizosphere soil were significantly influenced by sites and driven by soil chemistry.

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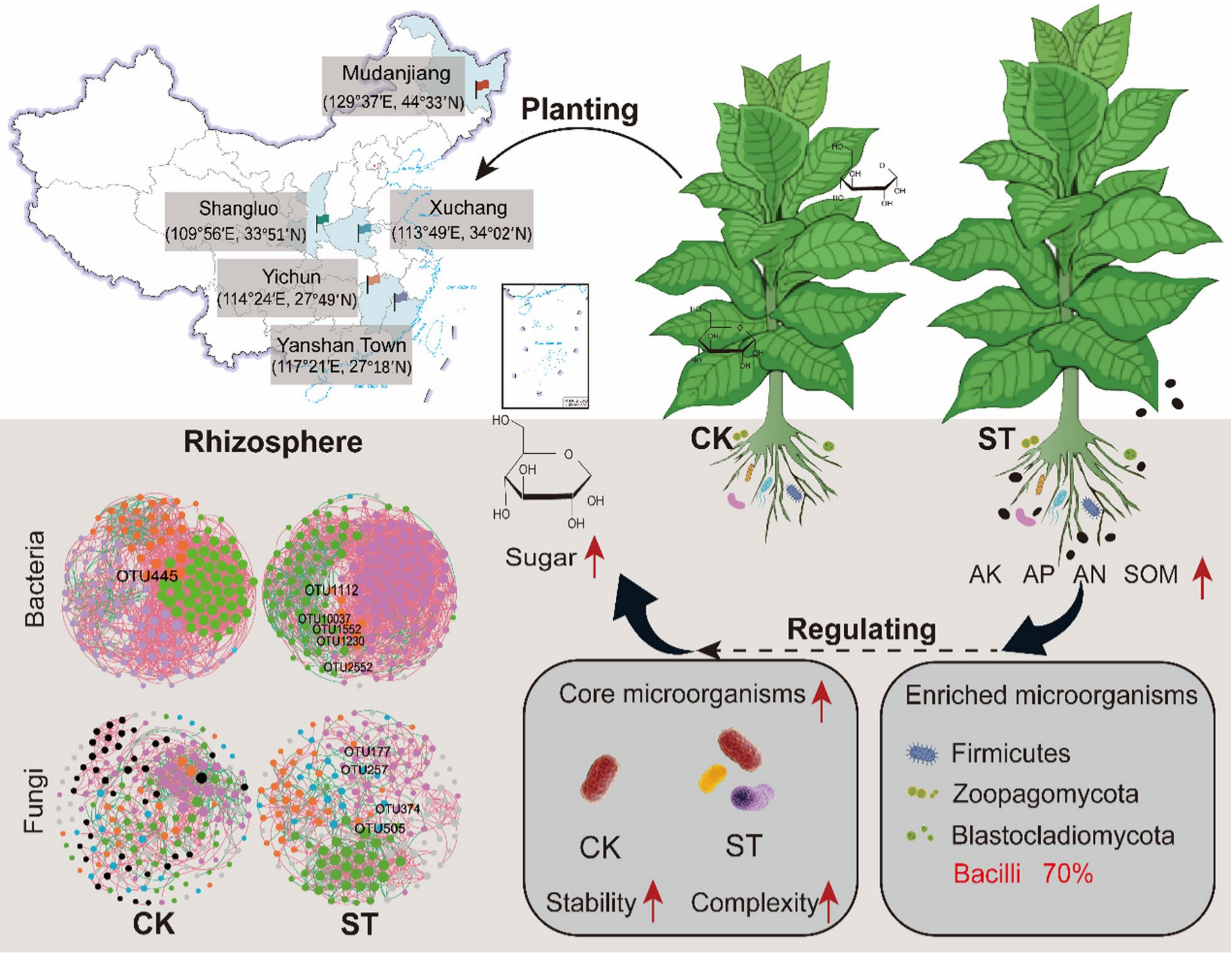
- The relative abundance of Blastocladiomycota, Zoopagomycota, and Firmicutes was significantly increased by PSB, especially the class Bacilli.
- The complexity and stability of the rhizosphere soil bacterial networks were increased by PSB.
- PSB indirectly enhanced tobacco leaf soluble sugar content by altering soil organic matter and bacterial communities.

Keywords Peanut shell biochar, Soil microorganisms, Microbial biogeography, Community stability, Soluble sugars

Graphical Abstract



1 Introduction

Soil microorganisms are the most biologically active components of the soil ecosystem, serving as the fundamental biogeochemical engine that sustains nutrient cycling and drives critical processes such as soil organic matter decomposition, nutrient transformation, pollutant degradation, and the regulation of plant quality (Sokol et al. 2022). In agricultural ecosystems, soil microbial communities enhance the bioavailability of macronutrients such as nitrogen, phosphorus, and potassium through biological nitrogen fixation, phosphorus solubilization, and potassium release, thereby supporting a continuous nutrient supply for plant growth (Bargaz et al. 2018). The stability of these microbial communities is also closely associated with crop growth and quality (Chen et al. 2024a, b). However, modern agricultural practices, including intensive tillage, excessive fertilizer application, and continuous cropping, have caused soil acidification, organic matter depletion, and fertility decline. These changes severely threaten microbial diversity and functional balance, ultimately compromising soil health and the sustainability of crop production (Gupta et al. 2022). Soil microorganisms are highly sensitive to environmental factors, including natural variables (e.g., moisture, temperature, and pH) and anthropogenic influences (e.g., fertilization and grazing) (Wang et al. 2021). Therefore, regulating soil microbial diversity and community composition through improved agricultural practices, such as optimized fertilization strategies, is considered a crucial approach for achieving sustainable and environmentally friendly agricultural production.

Biochar, a carbon-rich material produced through the pyrolysis of biomass, is increasingly recognized as a "super material". Its unique physicochemical properties, including alkaline pH, large specific surface area, surface functional groups, and nutrients, support its capacity to interact synergistically with soil microorganisms, thereby enhancing soil health and crop productivity (Jindo et al. 2014; Tomczyk et al. 2020; Zhu et al. 2017). The mechanisms underlying these interactions are multifaceted (Gorovtsov et al. 2020). Primarily, biochar serves as a crucial microbial refuge. Its extensive surface area and porous architecture provide protective habitats that mitigate predation pressure (Zhang et al. 2023; Pietikäinen et al. 2000). Furthermore, biochar plays a pivotal role in ameliorating soil acidity (Liu et al. 2025a, b). Beyond simply raising soil pH through its inherent alkalinity (Huang et al. 2023), emerging evidence emphasizes that ligand exchange of oxygen-containing functional groups, organic nitrogen mineralization, and adsorption of alkaline cations (NH_4^+) indirectly increase soil pH (Huang et al. 2024). Concurrently, biochar acts as a sustained nutrient source. The gradual release of soluble organic carbon and nutrients provides essential substrates that stimulate microbial growth and activity (Chen et al. 2024a, b). Finally, biochar reduces toxic substances by promoting the formation of humic acid, providing a suitable living environment for microorganisms (Chen et al. 2022a, b, c).

Biochar exerts synergistic effects on soil systems by providing habitats for microorganisms, regulating pH, supplying nutritional supplies, and mitigating toxicity, thereby reshaping both the structural characteristics and interaction patterns of soil microbial communities. The short-term addition of biochar to degraded land has been shown not only to enhance soil nutrient content, microbial diversity, and relative abundance but also to increase the ratio of fungi to bacteria and the ratio of Gram-positive to Gram-negative bacteria (Li et al. 2022; Zhang et al. 2018). Another core advantage of biochar application in soil improvement and microecological regulation is the enrichment of beneficial microorganisms within the plant rhizosphere. For example, biochar addition significantly increases the relative abundance of plant growth-promoting bacteria such as *Bacillus*, phosphate-solubilizing bacteria, nitrogen-fixing bacteria, and rhizobia (Chen et al. 2019; Chen et al. 2022a, b, c; Deinert et al. 2024). Although previous studies have shown that biochar enhances microbial diversity in farmland soils, improves microbial community structure, and regulates microbial interactions, they have mainly focused on short-term effects. However, the long-term impacts of biochar application and the influence of environmental heterogeneity on microbial community succession remain largely overlooked. In particular, systematic investigations into the effects of long-term biochar application on rhizosphere soil microbial communities are still lacking.

To address this gap, this study conducted a six-year field experiment across five major tobacco-growing regions in China, encompassing both temperate and subtropical climate zones (Mudanjiang, Heilongjiang; Shangluo, Shaanxi; Xuchang, Henan; Yichun, Jiangxi; and Yanshan Town, Fujian). The objective was to evaluate the potential of long-term biochar application to regulate rhizosphere soil microbial communities under large-scale field conditions. Specifically, this study aimed to address the following three questions: (1) How does long-term application of peanut shell biochar affect the structure and stability of soil microbial communities on a large scale? (2) What causal relationships exist between biochar-induced changes in soil chemical properties and shifts in microbial communities? (3) Through which molecular or ecological mechanisms does peanut shell biochar regulate soil chemistry properties and specific microbial groups to influence tobacco quality?

2 Materials and methods

2.1 Experimental design and sample collection

This field experiment was carried out over six tobacco-growing seasons from May 2017 to September 2022 at five research sites (Fig. S1): Mudanjiang (MDJ), Heilongjiang (129° 37′ E, 44° 33′ N); Shangluo (SL), Shaanxi (109° 56′ E, 33° 51′ N); Xuchang (XC), Henan (113° 49′ E, 34° 02′ N); Yichun (YC), Jiangxi (114° 24′ E, 27° 49′ N); and Yanshan Town (YSZ), Fujian (117° 21′ E, 27° 18′ N). At each site, two treatments were established: CK (conventional fertilization) and ST (conventional fertilization combined with 900 $kg \cdot ha^{-1}$ peanut shell biochar), resulting in a total of 10 experimental groups, each with at least three replicates (Fig. S2). Given the status of tobacco as a widely cultivated economic crop in China, tobacco seedlings (Yunyan 87, *Nicotiana tabacum*) were selected and planted at all research sites. The biochar applied was peanut shell biochar provided by the Henan

Biochar Engineering Technology Research Center. It was produced through the continuous carbonization of peanut shells for 20 min under low-oxygen conditions at 380–400 ℃, followed by cooling and sieving through a 10-mesh screen. Its key physicochemical properties were as follows: specific surface area, 16.83 $m^2 \cdot g^{-1}$; pH, 8.19; total carbon, 523.20 $g \cdot kg^{-1}$; and total nitrogen, 2.41 $g \cdot kg^{-1}$. The fertilization regime included organic fertilizer (600 $kg \cdot ha^{-1}$), compound fertilizer (1125 $kg \cdot ha^{-1}$), and K_2SO_4 (51% K, 375 $kg \cdot ha^{-1}$), providing nitrogen, phosphorus, and potassium at a ratio of 1.00:1.08:3.08. All fertilizers were applied as basal strip applications before tobacco transplanting. To ensure that treatment effects were attributable solely to biochar and site conditions, both the types and application rates of basal fertilizers were standardized across all sites. The baseline chemical properties of soils from the five sites are presented in Table S1.

Rhizosphere soil samples were collected at a depth of 0–30 cm using the five-point sampling method at the maturity stage (90 days after transplanting). Sampling was performed by first removing surface vegetation and debris using a sterilized stainless-steel shovel, followed by careful excavation of the entire tobacco root system. Loosely adhered soil was removed through vigorous shaking until no further detachment occurred, and remaining soil particles were gently brushed from root surfaces using a pre-sterilized brush. All visible debris, including animal and plant residues, gravel, and other impurities, was removed before sieving the soil through a sterile 2 mm mesh. The obtained rhizosphere soil was divided into two portions. One portion was placed in sterilized 2 mL tubes and stored at − 80 °C for DNA extraction, while the other was naturally air-dried for analyses of soil chemical properties and enzyme activities.

2.2 Determination of soil chemical properties

The activities of soil catalase, urease, and sucrase were determined using spectrophotometric methods, following the protocols provided in the reagent kits (Beijing Solarbio Science & Technology Co., Ltd., China). Extraction was performed using ultrapure water at a soil-to-water ratio of 1.0:2.5, and pH measurement was subsequently conducted using a precision pH meter (IS128C; InsMark, China). Available phosphorus was determined by the molybdenum-antimony ascorbic acid spectrophotometric method following extraction with 0.5 M $NaHCO_3$. Available potassium was quantified by flame spectrophotometry after extraction with 0.5 M NH_4OAc. Alkaline hydrolyzable nitrogen was measured using the alkali diffusion titration method, and soil organic matter was assessed by the potassium dichromate oxidation method (Feng et al. 2021). Detailed operational procedures followed those described by Bao (2000).

2.3 PCR amplification and Illumina sequencing

Microbial DNA was extracted using the E.Z.N.A.® soil DNA kit (Omega Bio-tek, USA) following the manufacturer's instructions. DNA concentration and purity were assessed using a NanoDrop 2000 UV–Vis spectrophotometer (Thermo Fisher Scientific, USA), and DNA integrity was confirmed by agarose gel electrophoresis. The V3–V4 hypervariable regions of bacterial 16S rRNA genes were amplified using primers 338F and 806R, while the V5–V7 hypervariable regions of fungal 18S rRNA genes were amplified with primers SSU0817F and 1196R (Liu et al. 2016) (Table S2). Polymerase chain reaction (PCR) analysis was performed in a 20 μL mixture containing 4 μL of 5× TransStart FastPfu buffer, 2 μL of dNTPs (2.5 mM each), 0.8 μL of each primer (5 μM), 0.4 μL of TransStart FastPfu DNA Polymerase, and 10 ng of template DNA. The PCR amplification protocol consisted of an initial denaturation at 95 °C for 3 min, followed by 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, with a final extension at 72 °C for 10 min. PCR products were separated on 2% agarose gels, purified using a DNA gel recovery and purification kit (Axygen, USA), and quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific, USA).

2.4 Illumina MiSeq sequencing and bioinformatic analysis

Purified PCR products were prepared using the NEXTFLEX rapid DNA-seq kit (Bioo Scientific, USA) and sequenced on the Illumina MiSeq platform (Illumina, USA). Raw FASTQ files were quality-filtered using fastp (version 0.20.0), and sequence reads were merged using the FLASH tool (version 1.2.7) (Chen et al. 2018; Magoč and Salzberg 2011). The sequence splicing procedure involved filtering reads shorter than 50 bp and removing reads containing N bases. Paired-end reads were merged into single sequences using a minimum overlap length of 10 bp and a maximum mismatch ratio of 0.2 in the overlap region. Sequences of each sample were distinguished based on the barcodes and primers at the beginning and end of the sequence (Jiang et al. 2024). High-quality sequences were clustered into operational taxonomic units (OTUs) at 97% similarity using the UPARSE-OTU algorithm in UPARSE (version 7.1) (Tian et al. 2022). Taxonomic classification of OTUs for both bacterial and fungal communities was performed using the Ribosomal Database Project classifier against the Silva database (version 138) (Wang et al. 2007). Alpha diversity indices, including the ACE index and Simpson index, were calculated as described by Amato et al. (2013). Microbial community distribution across sites and treatments was assessed by principal coordinate analysis (PCoA) based on Bray–Curtis distances, with statistical significance evaluated using permutational multivariate analysis of variance (PERMANOVA) (He et al. 2024; Xiao et al. 2025). The representativeness of microbial communities across regions was evaluated using rarefaction curves and Sobs index (Fig. S3). For both bacterial and fungal communities, rarefaction curves plateaued as the number of randomly selected sequences increased, indicating that additional sequencing would yield few new species. Coverage values exceeded 95% for bacterial communities and 98% for fungal communities, confirming that the sequencing data accurately reflected the actual composition of rhizosphere soil microbial communities and were suitable for further analysis.

2.5 Calculation of the response ratio

The natural log-transformed response ratio was used to estimate the effect sizes of peanut shell biochar on soil chemical properties (pH, available phosphorus, available potassium, soil organic matter, alkaline hydrolyzable nitrogen, carbon-to-nitrogen ratio, sucrase activity, catalase activity, and urease activity) as well as on rhizosphere soil microbial communities at the phylum level. The effect size (lnRR) and its variance (Vi) were calculated as follows:

$$\ln(RR) = \ln\left(\frac{m_e}{m_c}\right) \tag{1}$$

$$V_i = \frac{SD_e^2}{n_e m_e^2} + \frac{SD_c^2}{n_c m_c^2} \tag{2}$$

where n_e and n_c represent the sample sizes for the peanut shell biochar treatment and control, respectively; m_e and m_c denote the mean values of the treatment and control; and SD_e and SD_c indicate the corresponding standard deviations. The 95% confidence nterval (CI) of the effect size was estimated using the maximum likelihood method (MLR).

2.6 Construction of rhizosphere soil microbial co-occurrence networks

Rhizosphere soil microbial co-occurrence networks were constructed using the "igraph" package (version 2.1.1) in R (version 4.4.2). To enhance the reliability of the networks, only bacterial OTUs with relative abundances greater than 0.1% and fungal OTUs with relative abundances greater than 0.01% were included (Chen et al. 2022a, b, c). Based on these criteria, 156 bacterial OTUs and 146 fungal OTUs were retained in the control group, while 160 bacterial OTUs and 169 fungal OTUs were retained in the peanut shell biochar treatment group. These OTUs were used to construct the bacterial and fungal co-occurrence networks. False discovery rate (FDR) correction was applied to adjust the p-values of the co-occurrence networks. Based on these adjusted p-values, significant and highly correlated edges were identified with a threshold of $|r| > 0.7$ and $p < 0.01$ (Dai et al. 2024). The networks were visualized, and their topological properties (nodes, edges, average neighbors, modularity, average clustering coefficient, and average path distance) were analyzed using Gephi software (version 0.10) (Jiao et al. 2022; Xiao et al. 2025).

The complexity of each rhizosphere soil microbial co-occurrence network was assessed using indices such as nodes, edges, average neighbors, linkage density, and connectance (Chen et al. 2022a, b, c). Linkage density (LD) refers to the number of links per OTU, while connectance (C) represents the ratio of actual interspecific interactions to the number of possible interspecific interactions within the co-occurrence network (Montoya et al. 2006; Pimm 1984; Wagg et al. 2019). The corresponding formulas were calculated as follows:

$$LD = \frac{E}{N} \tag{3}$$

$$C = \frac{E}{N(N-1)/2} \tag{4}$$

where E and N represent the total number of edges and the total number of nodes in the network, respectively.

To evaluate microbial network stability following peanut shell biochar addition, positive and negative cohesion indices were measured as described by Hernandez et al. (2021). Within-module connectivity (z-score) and among-module connectivity (c-score) were calculated according to the methods described by Guimerà and Nunes Amaral (2005). Based on their topological roles, nodes were classified into four categories: module hubs (z-score > 2.5, c-score ≤ 0.62), connectors (z-score ≤ 2.5, c-score > 0.62), network hubs (z-score > 2.5, c-score > 0.62), and peripherals (z-score < 2.5, c-score < 0.62). In network analysis, module hubs, connectors, and network hubs are considered core microorganisms (Deng et al. 2012; Qiao et al. 2024).

2.7 Structural equation modelling

To investigate the direct and indirect relationships among soil chemical properties, soil microorganisms, and the soluble sugar content of tobacco leaves following biochar amendment, we developed structural equation models (SEMs) according to the approach outlined by Ullman and Bentler (2012). The models were designed to evaluate the following core hypotheses: (1) Biochar directly or indirectly affects the soluble sugar content in tobacco leaves by modifying soil chemical properties; and (2) Biochar indirectly influences the soluble sugar content by shaping specific soil microbial communities. Evidence from Zhang et al. (2025a, b) and Yan et al. (2020) underscores the relevance of soil chemical properties and the microbiome in the soil–plant system. Therefore, we built conceptual models incorporating soil chemical properties, microbial diversity, enriched microbial phyla (represented by the relative abundance of phyla significantly enriched after peanut shell biochar amendment), core microorganisms (represented by the relative abundance of module hubs, connectors, and network hubs in microbial co-occurrence networks), and soluble sugar content of tobacco leaves.

Multiple collinearity tests among predictor variables were assessed using the "car" package (version 3.1.3). The results indicated that the variance inflation factor (VIF) for all variables was below 3, suggesting that collinearity would not substantially affect parameter estimation. Subsequently, the "lavaan" package (version 0.6.19) was employed for parameter estimation via the maximum likelihood method, and chi-square test was used to evaluate the overall model fit (Rosseel 2012). Goodness-of-fit was further assessed using the comparative fit index (CFI) and the root mean square error of approximation (RMSEA). A CFI value closer to 1 and an RMSEA value below 0.05 indicate a satisfactory fit between the model and the data (Yang et al. 2020).

2.8 Data analysis

Significant differences were assessed by analysis of variance (ANOVA) using the aov function, followed by multiple comparisons with the least significant difference (LSD) test from the "agricolae" package (version 1.3-6) (Boitt et al. 2018). Linear mixed models were further applied to evaluate the effects of site-specific environmental conditions on the composition of soil microbial taxa at the phylum level (Liu et al. 2025a, b; Li et al. 2024). Random forest models were constructed using the "randomForest" package (version 4.7-1.2) to quantify the importance of each predictor, and the statistical significance of each predictor was then evaluated using the "rfPermute" package (version 2.5.5) (Shi et al. 2024). Meanwhile, the p-value and R^2 of models were assessed with 1000 permutations of the response variable using "A3" package (version 1.0.0) (Jiao et al. 2018). Correlations between dominant microbial phyla and soil chemical properties were assessed using the Mantel test with the "linkET" package (version 0.0.7.4) (Shi et al. 2025). Enriched microorganisms after peanut shell biochar treatment were analyzed using the "DESeq2" package (Reid et al. 2024), and differentially abundant microorganisms were identified based on the thresholds of $|\log_2 FC| > 1$ and $p < 0.05$ (Gao et al. 2023).

3 Results

3.1 Changes in soil chemical properties following peanut shell biochar addition

The analysis of variance indicated that there were significant differences ($p < 0.001$) in soil chemical properties (pH, available potassium, available phosphorus, organic matter, alkaline hydrolyzable nitrogen, and carbon-to-nitrogen ratio) among the five sites. Specifically, soil samples from MDJ, SL, YC, and YSZ exhibited significantly higher nutrient levels but lower pH values than those from XC (Fig. 1). Compared with the control, peanut shell biochar addition increased soil pH, available potassium, available phosphorus, organic matter, carbon-to-nitrogen ratio, and alkaline hydrolyzable nitrogen, as well as sucrase, catalase, and urease activities in MDJ, SL, YC and YSZ, with average increases of 7.23%, 9.12%, 10.69%, 13.60%, 15.27%, 11.56%, 28.40%, 13.80%, and 53.08%, respectively (Fig. 1 and S4). However, peanut shell biochar application to rhizosphere soil in XC produced a significant negative effect on available phosphorus and catalase activity, while increasing soil pH, alkaline hydrolyzable nitrogen, sucrase activity, and urease activity (Fig. 1).

3.2 Soil microbial diversity and taxon distribution across research sites

PCoA and PERMANOVA were applied to soil microbial data from the five research sites to identify the factors shaping soil microbial community structure at large spatial scales. Significant differences in soil microbial community composition were observed among the research sites (Fig. 2a). The relative abundances of dominant bacterial phyla (Acidobacteriota, Actinobacteriota, Proteobacteria, and Gemmatimonadota) and fungal phyla (Ascomycota, Basidiomycota, and Chytridiomycota) differed significantly among the research sites (Fig. S5 and Table S3). Soil chemical properties, including pH, available potassium, available phosphorus, alkaline hydrolyzable nitrogen, carbon-to-nitrogen ratio, and soil organic matter, significantly influenced the relative abundances of dominant bacterial and fungal phyla across the research sites (Fig. S6 and S7). Furthermore, analysis of variance revealed significant differences in bacterial Ace ($p < 0.001$) and Simpson ($p < 0.01$) indices, as well as the fungal Ace ($p < 0.001$) index, across the research sites. These diversity indices were also significantly influenced by soil chemical properties (Fig. S8).

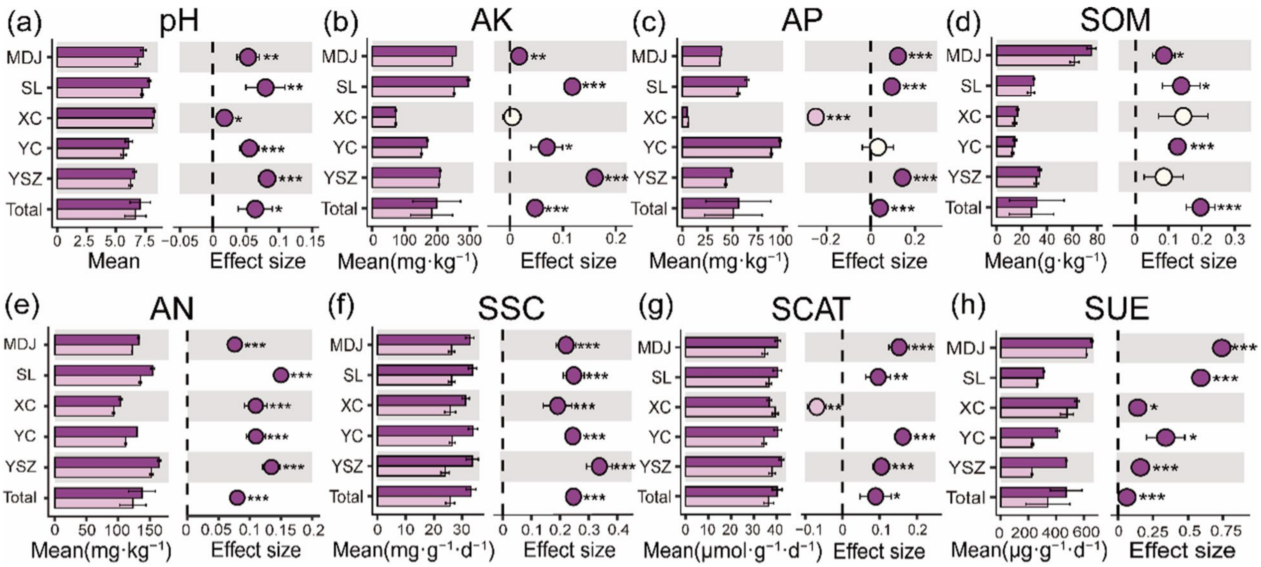


Fig. 1 Changes in soil chemical properties after peanut shell biochar application. The left figure shows the mean values of the control and the peanut shell biochar treatment, represented by light purple and dark purple, respectively. The right panel displays the effect sizes of the peanut shell biochar addition. The dark purple points indicate significant positive effects, the light purple points indicate significant inhibitory effects, and the ivory white points indicate no significant effect. Error bars represent 95% confidence intervals. Symbols "*", "**", and "***" represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. AK: available potassium, AP: available phosphorus, SOM: soil organic matter, AN: alkaline hydrolyzable nitrogen, SSC: sucrase, SCAT: catalase, and SUE: urease

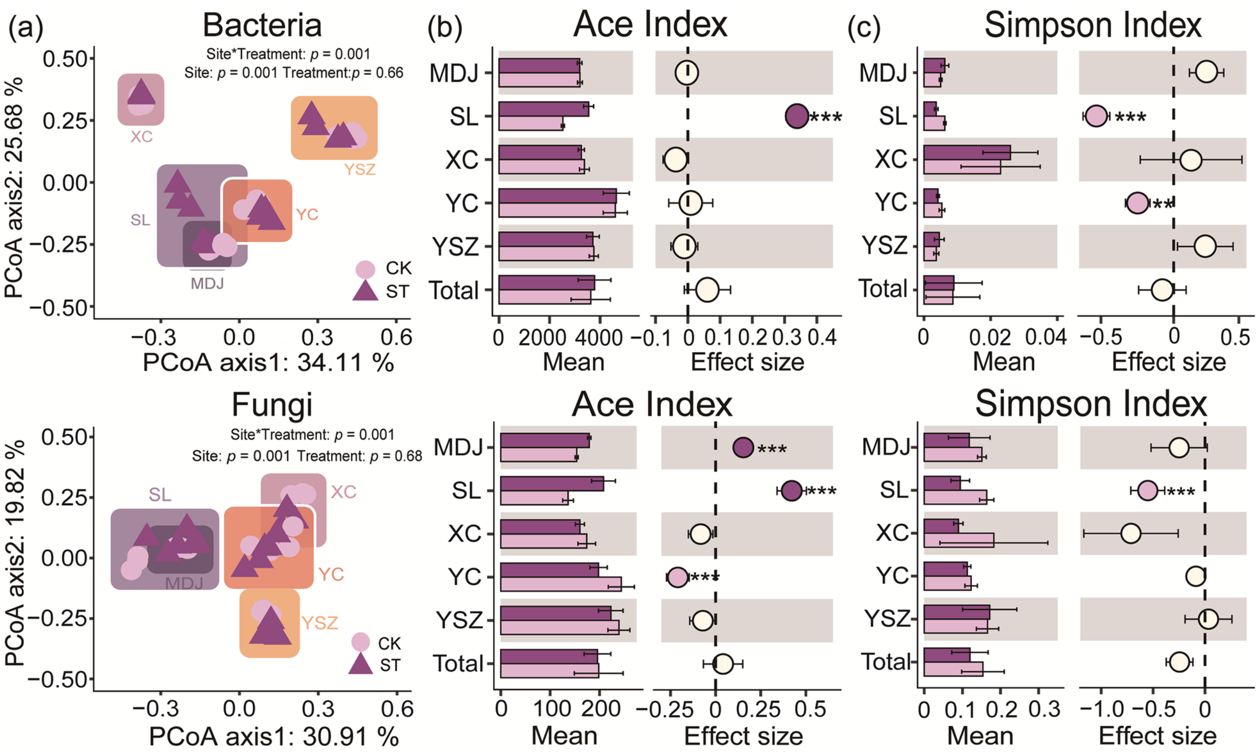


Fig. 2 (**a**) Differences in rhizosphere soil bacterial and fungal community composition across large spatial scales. Effects of peanut shell biochar addition on Ace (**b**) and Simpson (**c**) indices. Color coding is consistent with that in Fig. 1. Error bars represent 95% confidence intervals. Symbols "*", "**", and "***" denote $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

3.3 Correlations between peanut shell biochar addition and rhizosphere soil microbial community composition

Long-term peanut shell biochar application did not significantly alter the Ace and Simpson indices of rhizosphere soil microbial communities across the five major tobacco-growing regions (Figs. 2b, c). However, several microbial taxa were enriched following the peanut shell biochar treatment, including Deinococcota, Bdellovibrionota, Firmicutes, Hydrogenedentes, and Sumerlaeota in bacterial communities, as well as Zoopagomycota, Cavosteliida, Blastocladiomycota, Aphelidea, LKM15, and SAR_k__norank in fungal communities (Fig. 3a, b). The dominant bacterial phyla included Actinobacteriota, Proteobacteria, Chloroflexi, Acidobacteriota, Firmicutes, Gemmatimonadota, Myxococcota, Bacteroidota, Patescibacteria, and Cyanobacteria (Fig. S9a). For fungal communities, the predominant phyla consisted of Ascomycota, Basidiomycota, Mucoromycota, SAR_k__norank, Blastocladiomycota, unclassified_k__Fungi, Zoopagomycota, Chytridiomycota, norank_k__fungi and Aphelidea (Fig. S9b). These groups comprised the vast majority of the microbial community. Notably, the relative abundances of Firmicutes, Blastocladiomycota, and Zoopagomycota significantly increased by 30.63%, 1959.75%, and 126.90%, respectively, compared to those in the control group following peanut shell biochar amendment (Fig. 3; Fig. S9).

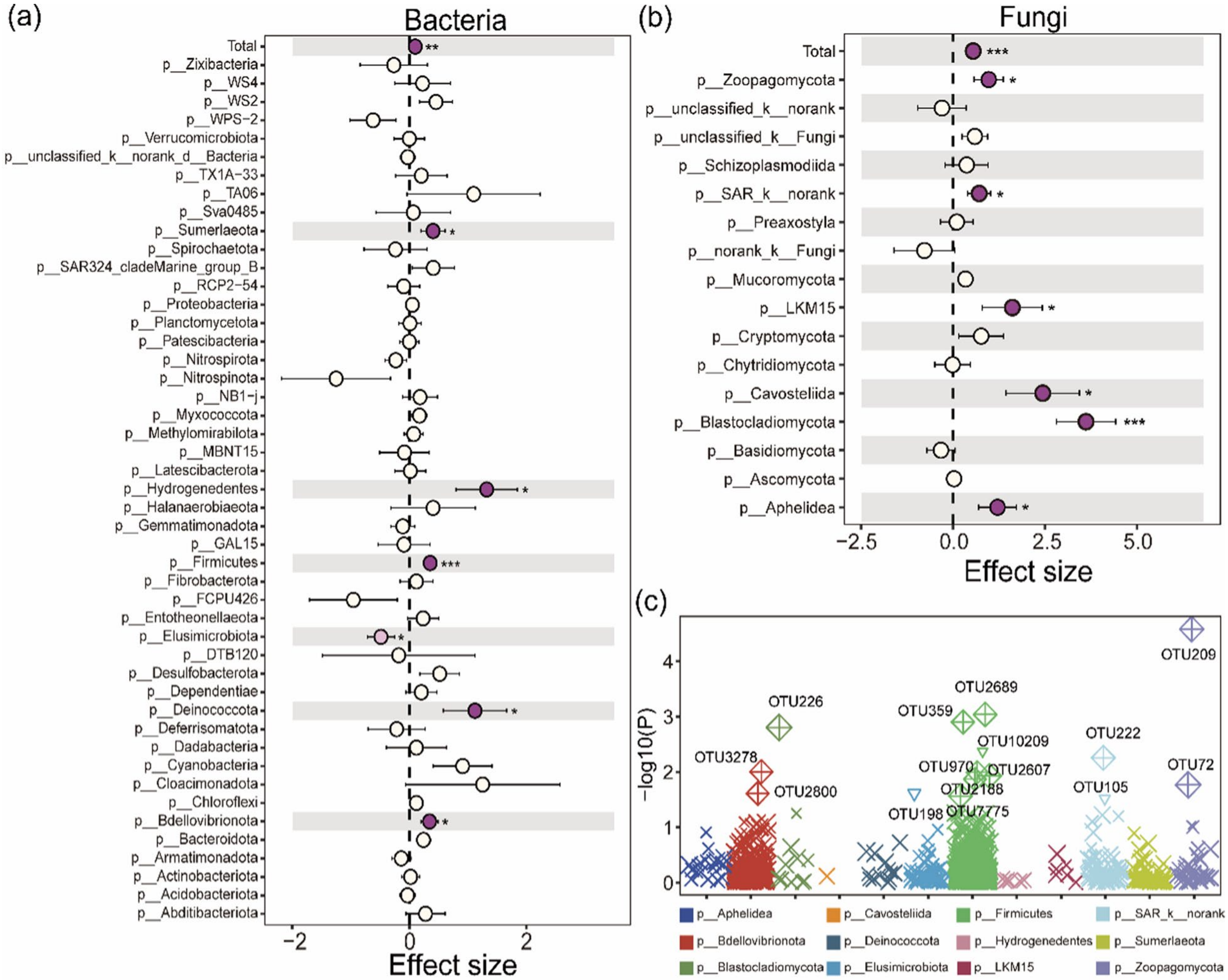


Fig. 3 Effects of long-term peanut shell biochar addition on soil bacterial (**a**) and fungal (**b**) taxa at the phylum level. Color coding is consistent with that in Fig. 1. (**c**) Significantly upregulated and downregulated microbial taxa following peanut shell biochar application. Crosses represent microbial taxa with no significant differences between the control and peanut shell biochar treatment groups, diamonds indicate significantly upregulated taxa, and triangles represent significantly downregulated taxa. Error bars represent 95% confidence intervals. Symbols "*", "**", and "***" represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively

These findings suggest that the significantly enriched microorganisms may play a crucial role in enhancing crop quality following peanut shell biochar application. Based on the criteria of $|\log_2FC|>1$ and $p<0.05$, peanut shell biochar application significantly increased the relative abundances of Bacilli (OTU2188, OTU2689, OTU359, OTU7775, and OTU970), Clostridia (OTU2607), and Bdellovibrionia (OTU2800 and OTU3278) in bacterial communities, with members of Bacilli accounting for 70% of the significantly enriched bacterial flora (Fig. 3c and Table S4). For fungal communities, the addition of peanut shell biochar significantly increased the relative abundance of Zoopagomycota (OTU209 and OTU72) and Blastocladiomycota (OTU226), which accounted for 26.16% and 71.99% of the significantly enriched fungal communities, respectively (Fig. 3c and Table S4).

3.4 Changes in rhizosphere soil microbial co-occurrence networks following peanut shell biochar addition

Co-occurrence networks are a critical tool for elucidating microbial interactions and assessing the complexity and stability of microbial communities. In this study, intra-kingdom networks were constructed for bacteria-bacteria and fungi-fungi interactions across the five research sites, and the results demonstrated variations in soil microbial community co-occurrence networks topological properties (nodes, edges, average neighbors, modularity, average clustering coefficient, and average path distance) among these sites were different (Table S5). To further evaluate the effects of peanut shell biochar addition on the complexity and stability of soil microbial communities, Spearman correlation analysis was employed to construct rhizosphere soil microbial co-occurrence networks for both the peanut shell biochar treatment and control groups (Figs. 4a, b). The addition of peanut shell biochar increased the number of bacterial nodes, edges, average neighbors, linkage density, and connectance, and significantly enhanced bacterial community stability ($p<0.05$) (Fig. 5 and Table S6). However, it had no significant effect on fungal community stability and reduced most of the complexity indices for fungi, including average neighbors, linkage density, and connectance (Fig. 5 and Table S6). Furthermore, peanut shell biochar application decreased the modularity of bacterial communities but increased that of fungal communities (Table S6). The number of core microorganisms in bacterial communities increased

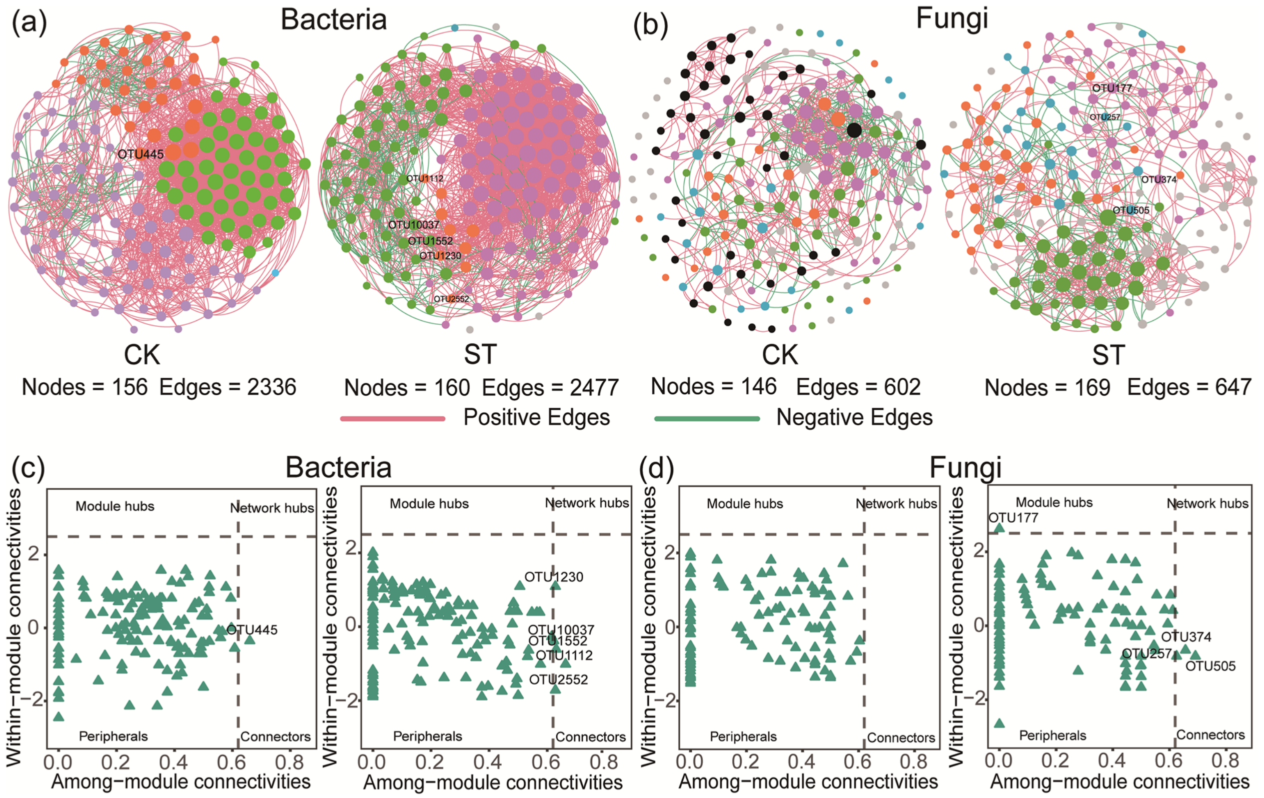


Fig. 4 Co-occurrence networks of bacterial (**a**) and fungal (**b**) communities in the control and peanut shell biochar treatment groups. The color of nodes represents OTUs grouped within the same module in each network. Within- and among-module connectivity patterns of bacterial (**c**) and fungal (**d**) communities were used to identify core microorganisms

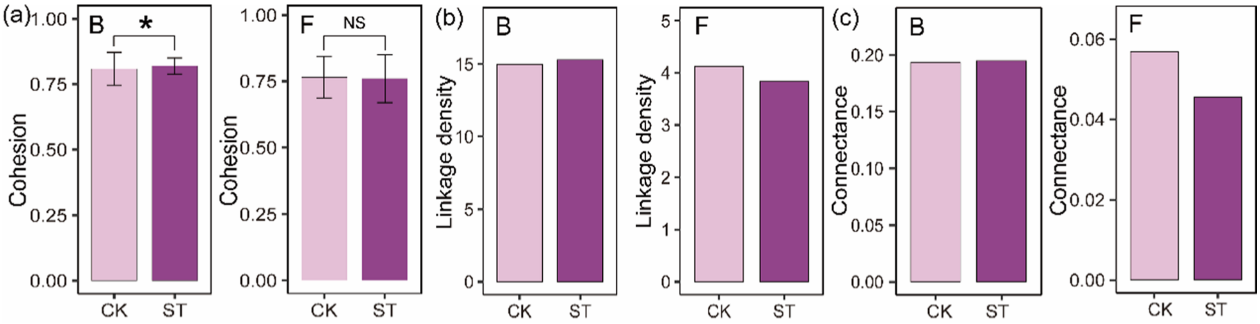


Fig. 5 Complexity and stability of soil microbial communities following peanut shell biochar addition. (**a**) Stability of soil microbial communities. (**b**) Linkage density and (**c**) connectance were used to represent the complexity of soil microbial communities. Light purple represents the control group, and dark purple represents the peanut shell biochar treatment group. The error bars represent the standard deviation (SD). "B" and "F" represent the bacterial and the fungal community, respectively. Symbols "*", "**", and "***" represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. "NS" indicates no significant difference between control and peanut shell biochar treatment groups

from one in the control group (OTU445) to five in the peanut shell biochar treatment group (OTU10037, OTU2552, OTU1552, OTU1112, and OTU1230) (Figs. 4c and Table S7). Similarly, in fungal communities, the number of core microorganisms increased from zero in the control group to four in the peanut shell biochar treatment group (OTU505, OTU374, OTU257, and OTU177) (Figs. 4d and Table S7).

3.5 Potential mechanisms by which peanut shell biochar shaped soil microbial community composition and influenced tobacco sugar content

Peanut shell biochar addition simultaneously enriched the relative abundance of specific microorganisms in rhizosphere soil and increased the number of core microorganisms (Figs. 3 and 4). To investigate the potential roles of these enriched and core microorganisms in regulating tobacco sugar content within bacterial and fungal communities, their relative abundances were incorporated into structural equation models (Fig. S10). The compositions of these enriched and core microorganisms are provided in the caption of Fig. 6. The models demonstrated a good fit with the data, enabling the interpretation of possible mechanisms by which peanut shell biochar promoted soluble sugar accumulation in tobacco leaves (Fig. 6a, b). The structural equation models for bacterial and fungal communities explained 91% and 87% of the variation in soluble sugar accumulation in tobacco leaves, respectively (Fig. 6a, b). Bacterial communities exerted a significant positive effect on soluble sugar accumulation, whereas fungal communities exhibited a significant negative effect (Fig. 6d). Specifically, bacterial diversity and enriched taxa such as Sumerlaeota, Hydrogenedentes, Deinococcota, Bdellovibrionota, and Firmicutes were significantly positively correlated with soluble sugar accumulation (Fig. 6a, d). Fungal diversity and enriched taxa such as Zoopagomycota, SAR_k_norank, Cavosteliida, LKM15, Blastocladiomycota, and Aphelidea were significantly negatively correlated with soluble sugar accumulation (Fig. 6b, d). Furthermore, soil chemical properties, particularly soil organic matter, showed a significant positive correlation with the soluble sugar content in tobacco leaves (Fig. 6d). In contrast, soil pH was negatively correlated with soluble sugar content of tobacco leaves (Fig. 6d). Application of peanut shell biochar significantly enhanced both soil pH and organic matter content (Fig. 6c). Notably, the promotive effect of organic matter on leaf sugar accumulation substantially exceeded the inhibitory effect associated with increased pH (Fig. 6d).

4 Discussion

This study demonstrates that peanut shell biochar amendment exerts multifaceted impacts on the composition of rhizosphere bacterial and fungal communities. Although site-specific variations were observed, the peanut shell biochar treatment consistently enriched specific microbial clusters (Fig. 3). Large-scale field analysis confirmed that long-term peanut shell biochar application strategically modulates the composition of the soil rhizosphere microbial community. These findings align with previous reports (Feng et al. 2021; Yang et al. 2021a, b), further substantiating the role of peanut shell biochar in shaping soil microbial assemblages and promoting soluble sugar accumulation in tobacco leaves. Subsequent sections detail the mechanisms through which peanut shell biochar influences soil physicochemical properties, shapes microbial taxa, and regulates processes contributing to soluble sugar accumulation in tobacco.

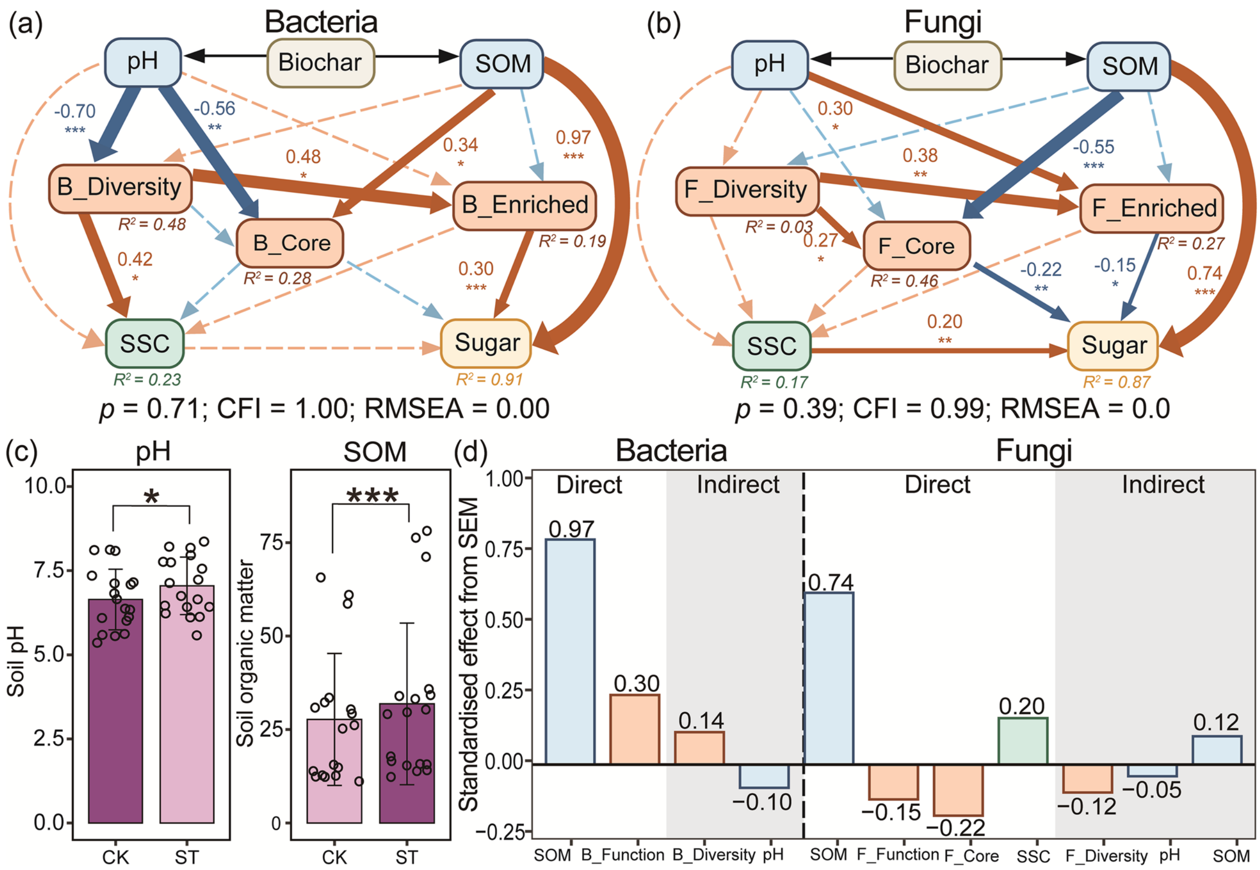


Fig. 6 (**a**) and (**b**) show the structural equation models of the bacterial and fungal communities, respectively, illustrating the direct and indirect effects of soil chemical properties and microbial communities on soluble sugar accumulation in tobacco leaves. Significant positive and negative pathways are represented by orange and blue solid lines, respectively, while non-significant pathways are indicated by orange and blue dotted lines. Line width corresponds to the absolute magnitude of standardized path coefficients. "B_Core" refers to core bacterial microorganisms, including OTU10037, OTU2552, OTU1552, OTU1112, and OTU1230. "F_Core" refers to core fungal microorganisms, including OTU505, OTU374, OTU257, and OTU177. "B_Enriched" denotes enriched bacterial taxa, including Sumerlaeota, Hydrogenedentes, Firmicutes, Deinococcota, and Bdellovibrionota. "F_Enriched" denotes enriched fungal taxa, including Zoopagomycota, SAR_k_norank, Cavosteliida, Blastocladiomycota, LKM15 and Aphelidea. (**c**) Effects of peanut shell biochar amendment on soil pH and organic matter content. (**d**) Direct and indirect effect sizes of peanut shell biochar application on soluble sugar content of tobacco leaves. Symbols "*", "**", and "***" represent $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

4.1 Peanut shell biochar addition substantially influenced soil chemical properties

The analysis of soil chemical properties across the five study sites revealed significant spatial heterogeneity reflecting the complex interaction of pedogenic factors including parent material, climatic conditions, anthropogenic activities, and divergent agricultural management practices (Tan et al. 2023). Notably, the rhizosphere soil of MDJ, SL, YC, and YSZ exhibited markedly higher nutrient concentrations along with lower pH levels compared to those observed at the XC. The relatively impoverished soil conditions at XC are likely due to long-term continuous cropping systems, which have led to the depletion of soil organic matter, increased nutrient loss, and a reduction in buffering capacity (Wu et al. 2025).

The long-term application of peanut shell biochar significantly enhanced the pH, nutrient availability, and enzyme activities in the rhizosphere soils of the MDJ, SL, YC, and YSZ, with these improvements being attributed to the intrinsic physicochemical properties of biochar. Its alkaline nature plays a direct role in neutralizing soil acidity. The alkaline minerals (oxides and soluble carbonates of calcium, magnesium, potassium, and sodium) within biochar dissolve in soil moisture, thereby elevating pH (Houssou et al. 2022; Sun et al. 2022). Biomass pyrolysis endows biochar with high ash content, stable organic carbon, and a well-developed porous structure (Biederman and Harpole 2013; Khan et al. 2024; Li et al. 2018; Yang et al. 2021a, b). These characteristics not only contribute to the direct supplementation of soil nutrients but also mitigate nutrient leaching via adsorption. Furthermore, the large specific surface area and abundant nutrient supply provide favorable habitats and metabolic substrates for soil microorganisms (Zheng et al. 2021). This enhanced microhabitat promotes microbial colonization, stimulates metabolic activity, and accelerates the transformation of mineral nutrients (Rasul et al. 2022). This study showed that the addition of peanut shell biochar significantly enhanced enzyme activities in the rhizosphere soil. Enzyme activities of rhizosphere soil are regulated by pH (Souza and Billings 2022; Zuccarini et al. 2023). Peanut shell biochar amendment elevated the soil pH towards neutral which is the optimal pH of sucrase, catalase, and urease in rhizosphere soil (Liang et al. 2025; Pan et al. 2025; Wang and Kuzyakov 2024; Yang et al. 2024).

However, at the XC research site, which was characterized by an initially higher pH, long-term amendment with the peanut shell biochar resulted in a reduction of available phosphorus in the rhizosphere soil, associated with both chemical reactions and microbial response mechanisms under high-pH conditions. Specifically, alkaline soils typically exhibit elevated concentrations of calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions, which facilitate the precipitation and adsorption of phosphate, thereby decreasing phosphorus availability (Shen et al. 2011). Moreover, the increased pH likely induced a shift in the microbial community composition, such as a reduction in the abundance of acid phosphatase-producing bacteria, which in turn constrained the enzymatic activities of microbial phosphorus mineralization (Deinert et al. 2024).

These findings emphasize that the effects of biochar amendment are strongly contingent on native soil properties, underscoring the importance of developing site-specific application strategies for optimizing its agronomic and environmental benefits.

4.2 Long-term peanut shell biochar addition enriched rhizosphere soil microorganisms

Significant differences in soil microbial community diversity and composition were observed across the five research sites, consistent with previous reports underscoring the dominant role of soil pH and nutrient availability in shaping microbial biogeographic patterns (Zhang et al. 2024). Notably, long-term amendment with peanut shell biochar did not significantly alter the Ace or Simpson indices of the rhizosphere microbial communities across the five major tobacco-growing regions. This finding contrasts with numerous short-term studies that reported a pronounced increase in microbial diversity following biochar application (Nguyen et al. 2018; Zhang and Shen 2022). We propose that short-term biochar amendment acts as a strong environmental perturbation, rapidly introducing abundant bioavailable substrates and improving habitat conditions, thereby inducing a transient increase in microbial diversity. In contrast, long-term application promotes gradual microbial adaptation of the soil microenvironment, leading to a new dynamic equilibrium in which both richness and diversity indices remain stable.

Although long-term biochar amendment did not alter overall microbial diversity, it induced significant functional restructuring within the soil microbial community. Specific enrichment was observed in Firmicutes, Blastocladiomycota, and Zoopagomycota, with the relative abundance of Bacilli comprising 70% of the significantly increased bacterial taxa. This selective enrichment reflects a complex interplay between the physicochemical properties of biochar and the biological traits of these taxa. Biochar establishes distinct microenvironments due to its special physicochemical properties (e.g., porous architecture, toxin adsorption capacity, and sustained nutrient release), imposing strong selective pressures and favoring microorganisms with specialized adaptive strategies (Gao et al. 2024; Wang et al. 2022).

Bacilli thrive under these conditions due to their spore-forming capability and competitive dominance through antibiotic production (Zhang et al. 2020). The extensive surface area of biochar retains readily decomposable organic compounds, including partially carbonized biomass and volatile substances deposited during pyrolysis, such as simple fatty acids, alcohols, phenols, esters, as well as fulvic- and humic acid-like components (Tang and Chen 2018). These substrates provide energy and nutrients for saprophytic fungi like Blastocladiomycota, conferring a competitive growth advantage (Li 2024). Concurrently, the expansion of host populations under nutrient-enriched conditions benefits parasitic Zoopagomycota, which depend on specific soil microorganisms or microfauna for survival and reproduction (Hou et al. 2022). This functionally oriented microbial shift carries important implications for soil health and sustainable agriculture.

Substantial evidence indicates that Bacilli act as multifunctional beneficial microbes, serving as biological agents against pathogens, producing phytohormones, and secreting diverse bioactive compounds that enhance crop growth and disease resistance (Etesami et al. 2023; Keshmirshekan et al. 2024; Khan et al. 2022; Radhakrishnan et al. 2017; Yuan et al. 2013). Blastocladiomycota contribute to organic matter decomposition and nutrient cycling, whereas Zoopagomycota help regulate populations of pathogenic nematodes and other microfauna through parasitism, thereby supporting soil health and biocontrol (Zhao 2024). These results demonstrate that long-term peanut shell biochar amendment enriches microbial taxa with distinct functional traits in both bacterial and fungal communities, providing a theoretical basis for the regulation of soil microflora by long-term biochar amendment and the promotion of crop growth.

4.3 Peanut shell biochar addition alters microbial co-occurrence network stability and complexity

In natural ecosystems, species interactions form intricate networks (Liu et al. 2015). The results reveal differences in the topological structure of microbial co-occurrence networks across research sites, reflecting the pronounced influence of environmental filtering on microbial interactions (Ma et al. 2016). More notably, long-term peanut shell biochar amendment exerted differential effects on bacterial and fungal networks. Peanut shell biochar treatment not only increased the number of keystone microorganisms, complexity, and stability of the bacterial network, but also reduced its modularity. These findings are consistent with recent research indicating that biochar amendment enhances the complexity and stability of bacterial networks in rhizosphere soil by enhancing interactions between soil dissolved organic matter and bacterial communities (Hu et al. 2025; Tian et al. 2025). Additionally, the highly developed porous structure of biochar establishes micro-environmental gradients, ranging from aerobic to anaerobic and eutrophic to oligotrophic, which provide diverse ecological niches (Zhang et al. 2025a, b). It meets the basic conditions for the reproduction of both aerobic and anaerobic bacteria. The pore characteristics of biochar largely determine the community structure of soil microorganisms, and most fungal genera tend to survive in larger pores (Yang et al. 2022). These pores physically compartmentalize microbial communities into discrete microhabitats and smaller bacteria are more likely to establish tight and frequent interactions with neighboring bacteria, forming stronger connections. In contrast, long-term peanut shell biochar modification reduces the complexity of the fungal symbiotic network and increases its modularity. The characteristics of biochar may be incompatible with the survival strategies of some fungal taxa, especially large fungi that rely on vast mycelial networks for connection. Its spatial structure constrains fungal distribution, shifting interaction dynamics among fungi from inter-pore to intra-pore.

4.4 Importance of beneficial bacteria in crop sugar content based on structural equation models

Numerous studies have demonstrated that biochar can significantly enrich beneficial microorganisms in the plant rhizosphere by providing an appropriate physicochemical environment, thereby promoting crop growth (Feng et al. 2024). However, long-term field experiments reveal that peanut shell biochar influences soluble sugar accumulation in tobacco leaves through modifications in soil organic matter, bacterial diversity, and the relative abundance of specific bacterial taxa. Soil organic matter plays a critical role in improving surface soil properties, such as enhanced water retention and air permeability, thereby fostering a more conducive rhizosphere environment for root development (Murphy 2015). Following long-term amendment with peanut shell biochar, distinct ecological functional groups were enriched within both bacterial and fungal communities. Specifically, the bacterial community exhibited significant enrichment of Bacilli, which are closely associated with plant growth promotion, while the fungal community was dominated by taxa involved in nutrient cycling and pathogen resistance. These microbial changes determine that the content of soluble sugars in tobacco leaves is mainly regulated by the bacterial community, a conclusion further supported by structural equation modelling. Moreover, soil pH exhibited a significant negative correlation with leaf soluble sugar content. This is likely attributable to the fact that tobacco thrives optimally within a pH range of 5.5–6.5, whereas the addition of peanut shell biochar elevated the pH of the rhizosphere soil beyond this ideal range (Yang 2023).

However, structural equation models demonstrated that bacterial and fungal communities explained 91% and 87% of the variation in tobacco soluble sugar content, respectively, leaving the remaining 10% unexplained. This unexplained portion may reflect the exclusion of factors such as other soil factors and climatic conditions from the models, highlighting the importance of incorporating additional variables in future analyses. Therefore, further studies should investigate how climatic variability, soil nutrient dynamics, and plant nutrient absorption mechanisms interact with peanut shell biochar to indirectly regulate crop soluble sugar accumulation by reshaping the soil microenvironment.

5 Conclusion

Long-term field experiments conducted across five major tobacco-growing regions in China revealed that peanut shell biochar application significantly enhanced soil fertility by elevating pH, nutrient availability, organic matter content, and enzyme activities. Microbial diversity and community composition exhibited site-specific variations, predominantly influenced by heterogeneities in soil chemical properties. Although overall microbial diversity remained largely unchanged, peanut shell biochar amendment selectively enriched key taxa, including the phyla Firmicutes, Zoopagomycota, and Blastocladiomycota, with the class Bacilli representing 70% of the significantly enriched bacterial taxa. Co-occurrence network analysis indicated that peanut shell biochar enhanced the complexity and stability of bacterial networks but reduced those within fungal communities. Furthermore, peanut shell biochar application indirectly improved soluble sugar content in tobacco leaves by regulating soil organic matter, bacterial diversity, and specific enriched bacterial groups. These findings underscore the potential of long-term peanut shell biochar to promote functionally beneficial microorganisms and enhance crop quality, offering an ecologically sustainable strategy to support green agricultural practices.

Supplementary Information

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Additional file1 (DOCX 52 KB)

Additional file2 (DOCX 1880 KB)

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Author contributions

Tianbao Ren designed the study. Yu Shi provided overall guidance and perspectives on the use of data. Guoshun Liu and Chengwei Li offered additional oversight. Zhuzhu Liao analyzed the data and wrote the manuscript. Quanyu Yin, Zhongke Sun, Peiyan Li, and Zhihong Huang collected the samples. Xianjie Cai, Huilin Feng, and Yaowei Wei performed the experiments and pre-processed the soil samples. Language editing company edited the English text of the manuscript.

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Data availability

All sequencing data have been deposited in the NCBI Sequence Read Archive (SRA) under accession numbers PRJNA1284848 for bacterial samples and PRJNA1284641 for fungal samples.

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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