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Rhizosphere ecological restoration: interactions between nutrient mobilization, core microbial assembly, and phenylalanine metabolism circulation

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Abstract

Continuous cropping obstacles (CCOs) constitute a major threat to agricultural productivity and environmental quality. Despite the recognized significance of rhizosphere restoration for CCOs, the mechanisms underlying rhizosphere restoration are not well understood. We conducted a pot experiment by planting *Panax notoginseng* with the addition of biochar, plant ash, the combination of biochar and plant ash (biochar:plant ash=4:1) or without any addition of biochar and plant ash. We found that rhizosphere soil with planting *P. notoginseng*, without any addition of biochar and plant ash, had a more homogeneous fungal community structure than soils added either with biochar, plant ash or the combination of biochar and plant ash. Among all treatments, the rhizosphere soil added with the combination of biochar and plant ash had the highest fungal and bacterial alpha-diversity. The combination of biochar and plant ash addition also enriched AMF *Rhizophagus* (TOP3 diff genus) in the rhizosphere soil compared to that without any biochar and plant ash addition. The co-occurrence network analysis demonstrated that *Rhizophagus* assembled a core microbial functional circle which helped suppress pathogens by promoting competition for amino acids and improving phenolic acid utilization, nutrient availability, and soil pH. Moreover, the metabolomic analysis revealed that the rhizosphere soil with the combination of biochar and plant ash addition upregulated the phenylalanine-tyrosine-tryptophan biosynthesis pathway, resulting in a decline of phenolic acid production and an increase amino acid accumulation in comparison with that without any biochar and ash addition (OPLS - DA, VIP > 1, FC ≥ 2, FC ≤ 0.5). Our results highlighted the importance of the interplay among keystone microbial species, pH and key metabolites in the rhizosphere soil in mitigating CCOs, building a framework for developing effective management for CCOs.

Highlights

- Biochar reduced soil fungal diversity, beneficial for managing soil-borne fungi-induced continuous cropping obstacles (CCOs).
- Rhizosphere imbalance resulted from the synergistic effects of pathogens and root-secreted phenolic acids in acidic soils.

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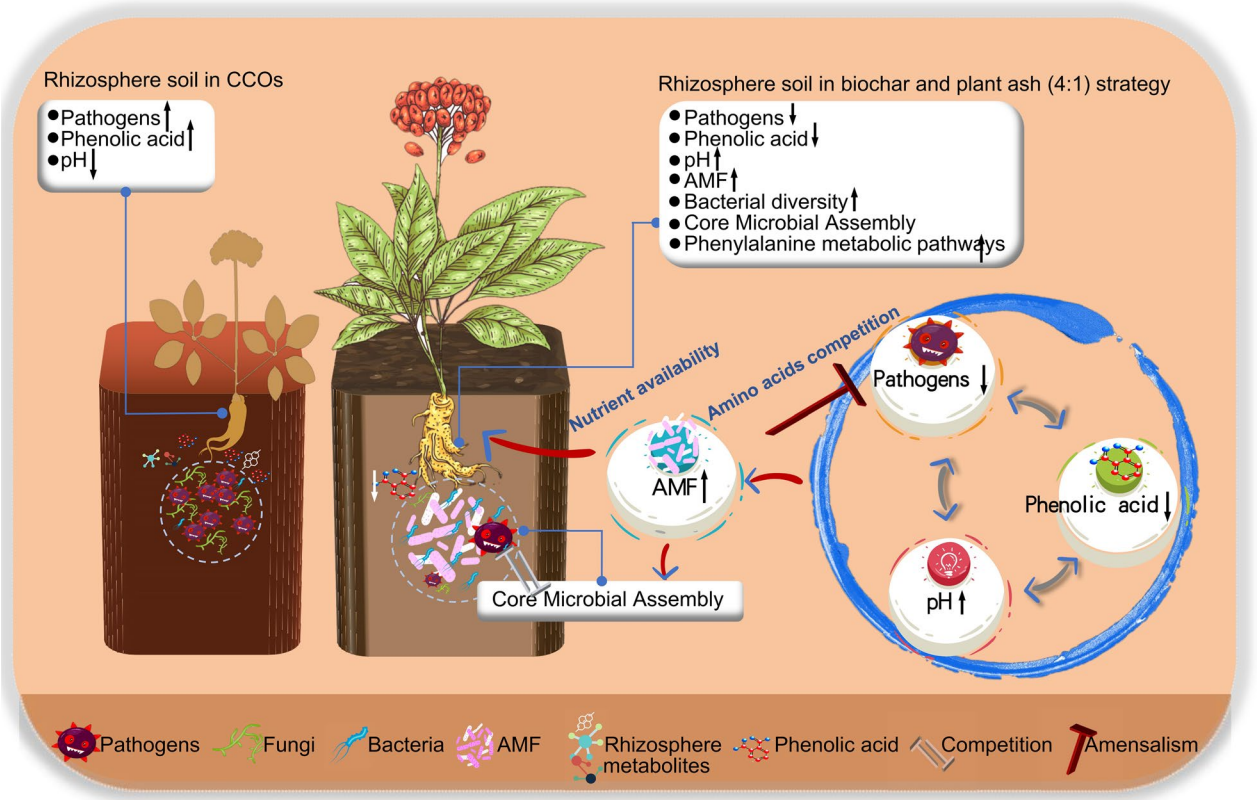
- Improving rhizosphere balance stimulated arbuscular mycorrhizal fungi (AMF), fostering beneficial plant–rhizosphere interactions.
- The phenylalanine–tyrosine–tryptophan pathway was crucial for rhizosphere resistance, lowering phenolic acid production and boosting amino acid.
- A constructed soil properties–microbe–metabolite network framework in the rhizosphere elucidated the intricacies and dynamics of continuous cropping barriers (CCOs).

Keywords Rhizosphere, Continuous cropping barriers, Biochar, AMF, Phenylalanine pathway

Graphical Abstract



1 Introduction

Continuous cropping obstacles (CCOs) characterized by root rot disease, replant diseases, soil sickness, and yield penalties, constitute major threats to plant health and survival, are responsible for significant economic losses in agriculture and threatening the sustainability of ecosystems. Unfortunately, few effective control measures are available, mechanistic understanding of CCOs are largely unknown.

The core issue of CCOs is the destruction of rhizosphere ecology, which perturbs rhizosphere homeostasis. Recent studies have demonstrated that CCOs occur via the destruction of rhizosphere ecology through microbial community imbalance, soil-borne pathogenic fungi invasion, host plant metabolite accumulation and soil degradation.

The rhizosphere serves as the direct interface for energy flow and material exchange between plant roots and soil, that is a hotspot for understanding the mechanisms of CCOs. Clearly, the rhizosphere is home to a diverse microbial community that plays an essential role in promoting plant growth and serves as the first line of defense against plant pathogens, with direct consequences for plant disease outcomes. However, the composition of the rhizospheric microbial community undergoes a pivotal shift towards a fungal-type predominance in the CCOs

soil system, with a proliferation of soil-borne pathogenic fungi and a decline in plant-beneficial microbes (Chen et al. 2022). The pathogen's priority effect triggers host immunosuppression, promoting the colonization of other pathogens and increasing the susceptibility of the subsequent crop (Debray et al. 2022). Aside from such negative consequences, during continuous cropping, the buildup of specific root exudates in the rhizosphere triggers autotoxicity to the host plant roots, damaging the root system and compromising the rhizospheric protective role (van Dam and Bouwmeester 2016). Medicinal plants, in particular, are disproportionately impacted by CCOs due to the specific accumulation of root exudates-allelochemicals that directly affect the health of the host plants and the rhizosphere. Indeed, plants enhance the production and release of allelochemicals in response to self-protection from environmental stresses rather than being detriment to plant development and rhizosphere health (Guerrieri and Rasmann 2024). However, medicinal plants shift from self-protection to auto-toxicity by the accumulation of allelochemicals with continuous cultivation or excessive release of allelochemicals in response to rhizosphere environmental stress (Wang et al. 2022). Accordingly, understanding the effects of continuous cropping on the rhizosphere interface is crucial for developing strategies to alleviate plants' stress responses to environmental challenges that produce autotoxicity. In addition, CCOs lead to the deterioration of soil physicochemical properties (Xu et al. 2021a; b). In particular, soil acidification in the rhizosphere, inhibits nutrient and water uptake in plants, slows crop growth and reduces root biomass, significantly affecting the yield of root-cropped plants, particularly medicinal plants (Yang et al. 2018). Collectively, these findings highlight the critical aspects, rhizosphere ecological restoration is crucial for alleviating the detrimental effects of continuous cropping on plant growth and soil ecological balance. Moreover, CCOs result from a complex system of multiple factors, however, the findings are currently investigated in isolation, yet no single mechanism is likely to be completely responsible for CCOs. The lack of comprehensive and interrelated understanding means that we cannot be certain of the rhizosphere mechanisms involved in overcoming CCOs.

Rhizosphere development is driven by interrelated factors rather than isolated ones. Specifically, the interaction between soil microbial dynamics and root exudate profiles within environments that vary in nutrient status and physicochemical attributes significantly influences rhizosphere function and restoration in continuous cropping, with their dynamics being pivotal to the development of CCOs.

On one hand, increasing the diversity of rhizosphere microbial communities significantly impacts the release of root exudates, soil matter cycling, energy flow, and information transfer, which promotes plant growth and development and enhances the buffering capacity of the root zone against deteriorating ecosystems (Hu et al. 2018). On the other hand, root exudates, which consist of primary metabolites and secondary metabolites, play a crucial role in rhizosphere ecosystem (Yu et al. 2022). These exudates serve as both carbon and nitrogen sources for microbial growth and signaling molecules that attract, stimulate, inhibit, or repel microbes, e.g., by attracting beneficial bacteria that colonize the roots, helping plants resist further infection by pathogens (Trivedi et al. 2021). In turn, soil properties also influence the composition of plant root exudates and drive microbial community structure (Li et al. 2023). However, the plant roots are hidden belowground, and many of the interesting phenomena have remained largely unnoticed. In particular, the role of root exudate in mediating belowground interactions is only beginning to be understood. On the whole, a comprehensive theoretical framework is critical for understanding the multiple factors within the rhizosphere ecosystem, essential for rhizosphere restoration and the mitigation of CCOs.

Root and rhizome-targeted medicinal plants (~ 70%), e.g. *Panax notoginseng* (Tan et al. 2017a), *Panax ginseng*, *Angelica sinensis*, *and Rehmannia glutinosa*, etc. (Yuan et al. 2022), experience significant challenges from CCOs that lead to crop failure. CCOs constitute major threats to the yield and properties of medicinal plants. Biochar holds promise for alleviating CCOs, with evidence suggesting that its application to medicinal plants outperforms chemical and microbial interventions in terms of survival rates (Pervaiz et al. 2020). Our unpublished field data indicate that the biochar strategy is the most effective in mitigating CCOs of *Panax notoginseng*, with the lowest incidence of diseases and the strongest persistence. The efficacy of soil conditioners like biochar is linked to alkaline properties, which enhance soil physicochemical attributes and mitigate acidification (Dong et al. 2016). Nevertheless, the precise role of those efficacy of soil conditioner in bolstering soil microbial communities and restoring rhizosphere is largely unknown. Consequently, advancing our mechanistic understanding of how variations in the assembly of rhizosphere microbiomes influenced by those efficacy of soil conditioners are of paramount importance to provide innovative strategies for repair and regulate rhizosphere to overcome CCOs.

Here, we hypothesized that (1) biochar alters the microbiota and metabolism of the rhizosphere to mitigate the impact of CCOs; (2) the health status of

rhizosphere is determined by the interplay among CCOs scenario. To test those hypotheses, we used medicinal plants—*Panax notoginseng* and Soil conditioners—biochar and plant ash as a model system to (1) investigate the extent to which regular planting with different soil conditioners differentially alters the rhizosphere microbiota and pathogens; (2) determine the effect of the soil conditioner on *Panax notoginseng* rhizospheric metabolism and autotoxic substances; and (3) evaluate how the relationship between microbiota—host plant metabolism -soil properties in the rhizosphere, which factor is more critical in the rhizosphere restoration framework.

2 Materials and methods

2.1 Site description and soil collection and preparation

The potted experiment commenced in a greenhouse in Kunming County, Yunnan Province, China (24° 88′ N, 102° 83′ E). The average humidity ranged from 68 to 75%, with an average temperature of 17 to 21 ℃ in the greenhouse. The continuously cropped soils for the experiment were collected from the harvesting after *P. notoginseng* with the 3-yr continuous cultivation in Wenshan County, Yunnan Province, China (23° 37′ N, 104° 24′ E). The continuous cropped soils in this region are classified as Latosol, the initial physical and chemical properties had a pH of 5.7, and total N, total P, total K and organic matter levels of 0.53, 0.61, 5.80, and 12.436 g/kg, respectively.

2.2 Biochar and plant ash characteristics

Biochar and plant ash were generated by a company in Yunnan, China, whose technology focuses on producing bioenergy. Biochar was formed by pyrolysis of rice straw at 550 ℃ for 7 h under anaerobic conditions in a muffle furnace. After 7 h of heating, the biochar samples were cooled to 18–25 ℃. Biochar samples had a pH of 9.3 (1:10 w/v), and total C, total N, and total ash levels of 68.7%, 0.65%, 10.9%, respectively.

The plant ash was formed by the combustion of rice straw was collected after cooling the sample to 18–20 ℃ and sieving the residue through a 200-mesh screen. The primary constituent of this ash is potassium carbonate, which had a pH of 7.02, with total N, potassium and phosphorus contents of 0.5%, 9% and 2.1%, respectively.

2.3 Plant materials

We conducted an experiment using *P. notoginseng* as the model crop, which is often subject to severe CCOs. The *P. notoginseng* seeds collected from the planting base of Wenshan County, Yunnan Province, China, are robust and complete and free of pests. Soak seeds in 3% hydrogen peroxide for 10 min to disinfect, patted dry with sterile paper, and air-dried before planting. Following disinfection, the seeds were planted in the matrix, subjected to routine management, and subsequently grown for one year (from seeds to seedlings). We conducted an experimental selection of seedlings of *P. notoginseng*.

2.4 Experimental design

We conducted a pot experiment by added biochar, plant ash, and proportional (biochar:plant ash = 4:1). We conducted (i) addition group: biochar strategy; plant ash strategy; proportional strategy. The amount of addition was 5 kg/m^3, both strategy of biochar, plant ash, and their proportion. The continuous cropped soils and the added substances were mixed, and then the P. notoginseng seedlings were planted. The amount of addition is determined based on the experimental results of seedling survival rates in the field. (ii) normal planting: planting was untreated as the control. (iii) non-planting control: to assess potential background soil propertiesl and microbiota. Potted experiments were conducted in a greenhouse, with each treatment group systematically arranged and spatially separated to prevent the spread of soil-borne diseases from affecting the results of soil microbial experiments. Each treatment was replicated in 40 pots.

After ten months of planting, ten soil cores with a depth of 10 cm were randomly selected from each pot, and these ten soil cores were fully mixed into a single composite soil sample as non-rhizosphere soil. Excess-soil on the roots was discarded by gently shaking the plants, and the remaining soil particles attached to the root surface were collected as rhizosphere soil. In total, 9 soil samples (3 addition strategies and 1 normal planting control of rhizosphere and non-rhizosphere soil, 1 non-planting control) were collected.

Soil DNA of rhizosphere soil collected from both rhizosphere and non-rhizosphere soil samples was extracted for microbial community analysis, including bacteria and fungi. All the soil samples were stored at − 80 °C before DNA extraction. The remaining rhizosphere soil was air-dried for determination of soil physicochemical properties.

2.5 High-throughput sequencing of 16S rRNA and ITS regions

Genomic DNA was extracted using a DNA Extraction Kit according to the manufacturer's instructions. To analyze bacterial diversity, the variable regions of 16S rRNA genes (V3–V4 or V4–V5) were amplified using the universal primers 343F and 798R (or 515F and 907R for the V4–V5 region). For fungal diversity analysis, the ITS 1 variable regions were amplified using the universal primers ITS1F and ITS24 (Jiang et al. 2024).

The QIIME software was used for preprocessing and assembly (Caporaso et al. 2010) (version 1.8.0). OTUs were generated using Vsearch software clustering (Rognes et al. 2016). Representative reads were annotated via BLAST searches against the UNITE database (ITS rDNA) (Xu et al. 2020, 2023).

2.6 Comprehensive and precise qualitative and quantitative metabolome profiling

Sample preparation and extraction. The samples were thawed at − 80 °C, vortexed for 10 s. Each treatments soil samples were stored in triplicate 10 ml tubes, and then mixed with 2 mL of each sample. Once completely lyophilized, 200 µL of 70% methanol internal standard extract was added, and the mixture was allowed to cool for 15 min. The mixture was centrifuged at 12,000 r/min at 4 °C for 3 min and stored in a sample flask for LC–MS/MS analysis.

UPLC conditions. A UPLC-ESI–MS/MS system was used to analyze the sample extracts, and SHIMADZU Nexera X2 UPLC and Applied Biosystems 4500 Q TRAP MS were used. Samples stored at low temperatures ranging from 4 ℃ for stability and to prevent degradation. The UPLC system used an Agilent SB-C18 column with a mobile phase of solvent A (pure water with 0.1% formic acid) and solvent B (acetonitrile with 0.1% formic acid). The gradient program began with 95% A and 5% B, transitioned to 5% A and 95% B over 9 min, and then was held for 1 min. The composition was then adjusted to 95% A and 5% B within 1.1 min and maintained for 2.9 min. The flow rate was 0.35 mL/min, the column oven temperature was 40 °C, and the injection volume was 4 µL. The effluent was analyzed using ESI-Triple quadrupole-linear ion trap (QTRAP)-MS.

ESI-Q TRAP-MS/MS. The following ESI operation parameters were used to minimize repetition: source temperature at 550 °C and ion spray voltage (IS) at 5500 V (positive) or − 4500 V (negative). The GSI, GSII, and CUR gases were adjusted to 50, 60, and 25 psi, respectively. The CAD system was set to high. Instrument tuning and mass calibration were conducted using polypropylene glycol solutions in QQQ and LIT modes. QQQ scans were acquired in MRM mode with medium collision gas (nitrogen). DP and CE were optimized for each MRM transition. A unique set of MRM transitions corresponding to the metabolites eluted during that period was monitored during each period.

2.7 Soil physicochemical analysis

We examined and analyzed the nutritional status and physicochemical properties of the rhizosphere soil. We measured the organic matter content via the potassium dichromate method. Total nitrogen and phosphorus were measured with a SEAL-AA3 analyzer and molybdenum-antimony colorimetry, respectively. A flame photometer was used to measure total and available potassium levels. Available nitrogen and phosphorus levels were measured with the alkali-hydrolyzed diffusion method and sodium bicarbonate method, respectively. The cation exchange capacity was using the neutral ammonium acetate method, calculate the CEC of the soil based on the concentration of cations in the extract and the volume of the extract. CEC is commonly expressed in millimoles per kilogram of soil (mmol/kg) or centimoles per 100 g of soil (cmol/100 g) (Shu et al. 2017).

2.8 Statistical analysis

To analyze community structures, a histogram was created to evaluate species richness, uniformity, and alpha diversity within the sample. A boxplot was also generated to visualize the diversity index. To assess diversity indices among groups, a violin plot analysis using the Wilcoxon algorithm was conducted. Beta diversity analysis was used to compare communities across different biological environments. Unsupervised NMDS was performed in R with unit variance scaling. Microbial multivariate statistical analysis identified differentially abundant species between groups using the Wilcoxon algorithm. LEfSe was used to detect up- and down-regulated microbes, and a heatmap was created to display these differences. Evolutionary analysis involved constructing a phylogenetic tree with relative abundance information. PICRUSt2 analysis was conducted using Greengenes 13_5 with Galaxy, and predicted gene family abundances were analyzed using KEGG orthology group count level 3. The Storey FDR in STAMP software was used to avoid Type-I errors. KEGG functional prediction, enzyme classification number (EC), and COG protein prediction were performed, and differences were assessed using the Kruskal–Wallis algorithm. The results were visualized using a heatmap.

To conduct a comprehensive analysis, we utilized R version 3.5.1 to perform redundancy analysis (RDA) and mapping. By leveraging two or three histological quantitative datasets, we generated correlation chord diagrams, cluster heatmaps, and network interaction diagrams to gain deeper insights. To ensure the reliability of our findings, we preceded OPLS-DA (orthogonal partial least squares discriminant analysis) with a permutation test involving 200 permutations to guard against overfitting.

SEM analysis was used to estimate the relative importance of the direct and indirect impacts of microbial, metabolite and soil chemical property factors on the rhizosphere. The latent variable analysis (lavaan) package (version 0.6-17) of R statistical software was used. The SemPlot package was used for data visualization.

3 Results

3.1 Optimizing the assembly of rhizosphere fungal and bacterial communities via added strategies

Fungal community structure composition on various added strategies. We tested the fungal community structure response to various added strategies in the soil of *P. notoginseng*. Besides, we examined microbial community of the rhizosphere and non-rhizosphere soils to comprehensively assess the effects on the fungal community. The results showed that the fungal community structure differed among the proportional (LHG, biochar: plant ash = 4: 1), biochar (LSWTG), and plant ash (LCMHG) strategies (Supplementary Figs. 1A–D). Particularly, the proportional strategy (LHG) altered the fungal community structure at the phylum and genus levels. The proportional strategy(LHG) 'enriched' the *Glomermycota* phylum in the rhizosphere, while the biochar strategy (LSWTG) 'enriched' the *Chytridimicota* and *Basidiomycota* phylum in the non- and rhizosphere soils (Supplementary Fig. 1C, D). Lastly, we found that the fungal community structures differ between the non- rhizosphere and rhizosphere soils at the genus level.

Profiling fungal community diversity on various added strategies. We investigated the degree of the fungal community diversity response to various added strategies in the non- and rhizosphere soils to assess whether mitigating fungal dominance of soil systems in CCOs. We found that planting *P. notoginseng* (LCKG) decreased fungal community diversity in the rhizosphere (T-test, $P < 0.01$; Fig. 1B) when compared with non-planting (LCK). Likewise, the biochar strategy (LSWTG) decreased fungal community diversity.

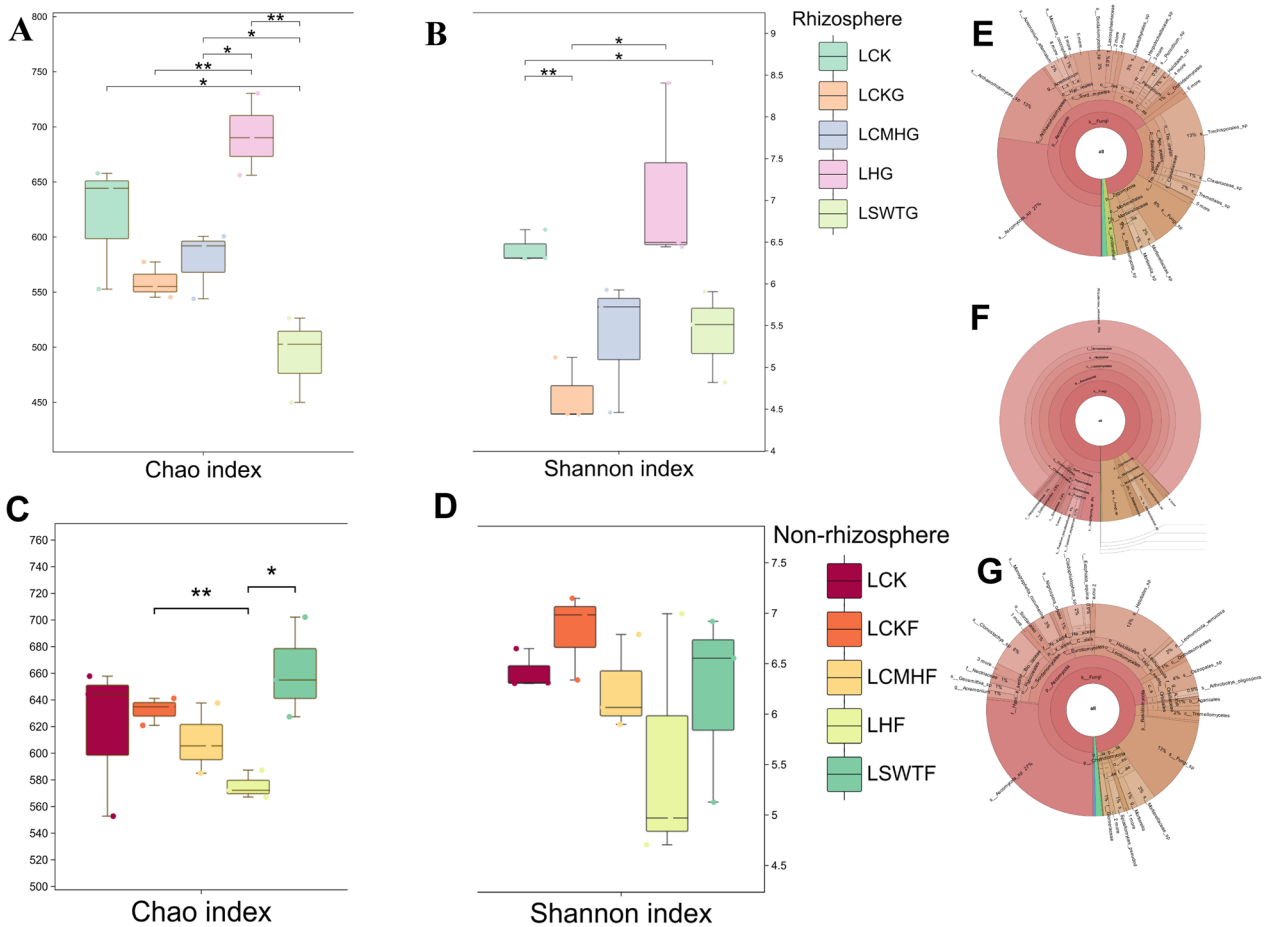


Fig. 1 Fungal community diversity. **A**, **B** Chao1 indices of rhizosphere and non-rhizosphere fungal communities. **C**, **D** Shannon indices of the rhizosphere and non-rhizosphere fungal communities. **E**–**G** Species annotation visualization of Krona graphs of the non-planting control (LCK), normal planting (LCKG) and proportional strategy (LHG). (LCK, non-planting control soil; LCKG and LCKF, rhizosphere and non-rhizosphere soils under normal planting; LCMHG and LCMHF, rhizosphere and non-rhizosphere soils with plant ash addition; LSWTG and LSWTF, rhizosphere and non-rhizosphere soils under the biochar strategy; LHG and LHF, rhizosphere and non-rhizosphere soils of proportional of biochar and plant ash addition)

in the rhizosphere(T-test, $P<0.05$; Fig. 1A, B). Conversely, the proportional strategy (LHG) was significantly increased fungal community diversity compared with normal planting (LCKG) in the rhizosphere(T-test, $P<0.01$; Fig. 1A). Besides, according to the Krona diagram of community structure visualization (Fig. 1E–G), the proportional strategy(LHG) involved rich fungal flora and high diversity (Fig. 1G). However, planting *P. notoginseng* (LCKG) decreased fungal community diversity resulting in a homogeneous fungal community structure (Fig. 1F). Moreover, the fungal community diversity differed between the non- rhizosphere and rhizosphere soils. For example, when compared with normal planting (LCKG), the proportional strategy(LHG) increased the fungal community diversity in the rhizosphere (T test, $P<0.01$; Fig. 1A) but decreased in the non-rhizosphere (T test, $P<0.01$; Fig. 1C). Together, the planting *P. notoginseng* and added biochar strategy were decreased fungal community diversity in the rhizosphere. Notably, the proportional strategy had higher fungal community diversity, with the potential to be greater in mitigating fungal dominance of soil systems in CCOs.

Differential microbes in fungal communities. We identified the differential fungi among the biochar, plant ash, and proportional strategies in the rhizosphere, respectively. Based on the analyses of gene circles and Linear discriminant analysis Effect Size (LEfSe), we found that the biochar strategy (LSWTG) had the highest number of differentially expressed fungal taxa but a considerable fraction of those differential fungi exhibited lower relative abundance when compared with normal planting (LCKG) (Supplementary Fig. 2B). However, the proportional strategy (LHG) exhibited higher relative abundance of a considerable fraction of differential fungi (Supplementary Fig. 2A). Subsequently, we found that the differential fungi differ between the three comparisons, which are the normal planting (LCKG) compared with the biochar, plant ash, and proportional strategies, separately. But the pathogenic *Fusarium* sp. was the only common differential fungus among the three comparisons, and added strategies were decreased the relative abundance of *Fusarium* sp. (Supplementary Fig. 2A–C). The proportional strategy (LHG) 'enriched' the genera *Rhizophagus* belonging to arbuscular mycorrhizal fungi (AMF). Interestingly, the correlation network diagram showed that the soil environment changes caused the interaction network of "key fungus" to change. For instance, the genera *Trichocladium* was the "key fungus" that positively correlated with *Leptodontium* in the proportional strategy (LHG) (Supplementary Fig. 2A) and positively correlated with *Microcera* and *Archaeorhizomyces* in the plant ash strategy (LCMHG) (Supplementary Fig. 2C), but negatively correlated with *Cyhalophora* in the biochar strategy (LSWTG) (Supplementary Fig. 2B). In brief, we identified the differential fungi in the biochar, plant ash, and proportional strategies. Fortunately, all added strategies reduced the abundance of *Fusarium* sp., a critical soil-borne fungal pathogen causing CCOs. The interaction network of "key fungus" varied with dynamic changes in the rhizosphere soils.

Bacterial community structure composition. We used bacterial 16S rRNA gene amplicon sequencing to profile the bacterial community response to various added strategies in the rhizosphere and non-rhizosphere soils(Fig. 2). The results revealed that the added strategy significantly altered the bacterial community structure at the phylum and species level (Fig. 2B–D). For example, the phylum *Acidoberiot* decreased 72.41%, 84.58%, and 80.35% in the proportional (LHG), biochar (LSWTG), and plant ash (LCMHG) strategies, respectively, compared with non-planting (LCK) (Fig. 2A). Moreover, we found that the bacterial species of proportional (LHG) and biochar (LSWTG) strategies in the rhizosphere are still largely unknown, compared to non-rhizosphere bacterial community.

Comparison of the bacterial community diversity and identified differential microbes. We investigated the degree of the bacterial community diversity response to various added strategies in the non- and rhizosphere soils to assess the potential impact on efficient and diverse soil ecological functions. Results showed that the added strategy improved the bacterial community diversity in the non-rhizosphere and rhizosphere soils. Particularly, the proportional strategy (LHG) increased bacterial community diversity in the non- and rhizosphere when compared with normal planting (LCKG) (T-test, $P<0.001$; Supplementary Fig. 4). We further used NMDS analysis and a clustering heatmap revealed that the proportional strategy(LHG) was clustering into a separate class and had a small gap between the non- and rhizosphere (Supplementary Fig. 4E–G). By comparing the interaction networks of "key microbes" in fungi and bacteria, we observed the opposite trend that the interaction network of "key bacteria" remained largely more stable during dynamic changes in the rhizosphere soils. Collectively, the proportional strategy(LHG) significantly increased bacterial community diversity in the non- and rhizosphere, resulting in a distinct bacterial community structure. The interaction network of "key bacteria" was largely stable than that of fungi.

Comparison of the relative abundances of pathogens and plant-beneficial microbes. To evaluate whether the added strategy alters the relative abundance of soil-borne pathogenic fungi and promotes plant-beneficial microbes with the potential to enhance rhizosphere health, we compared the relative abundance of pathogenic and

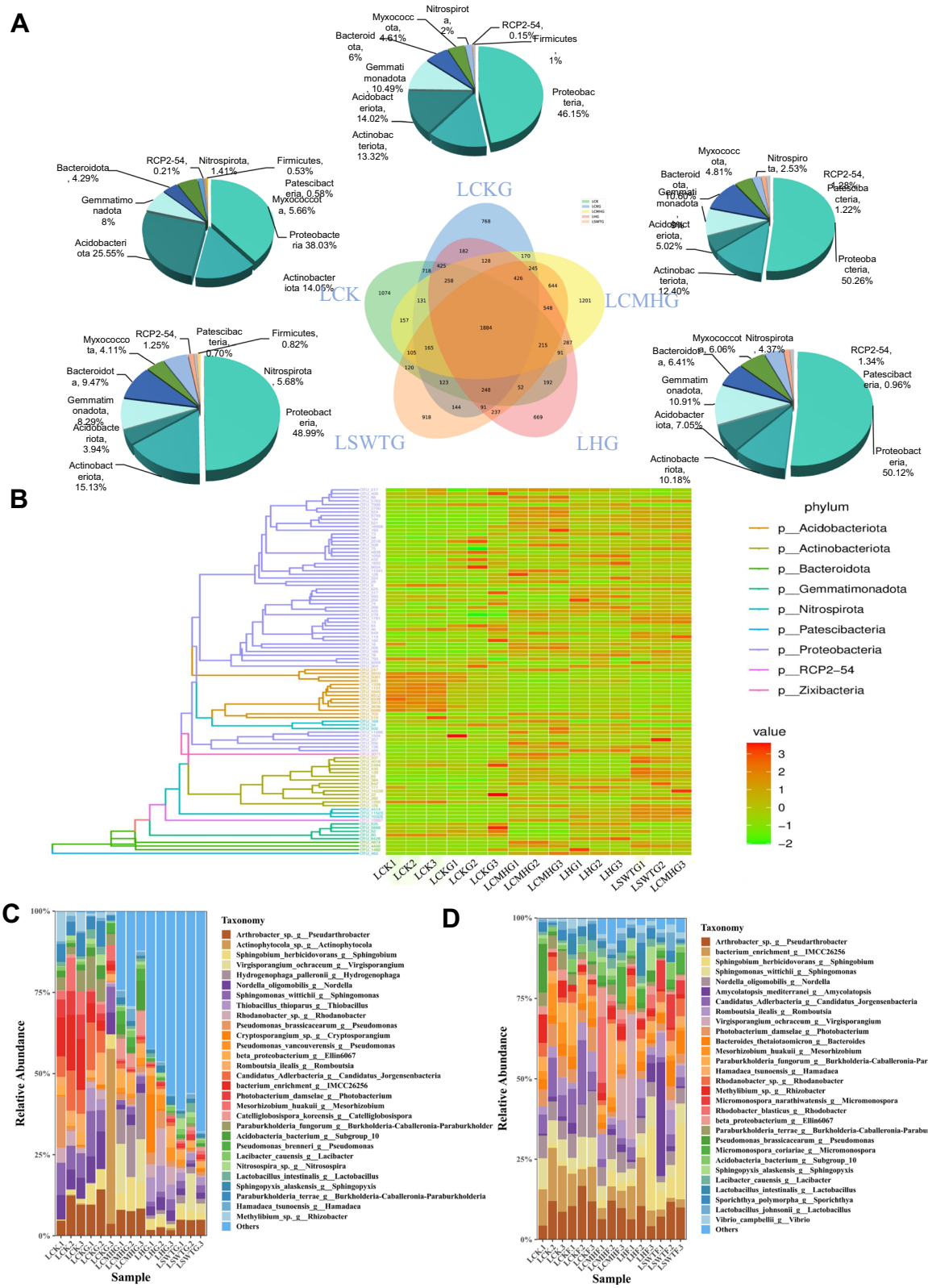


Fig. 2 Demograms of soil bacterial structural evolution and soil bacterial community structure in the non-rhizosphere and rhizosphere soils. **A** Soil bacterial community structure in the rhizosphere at the phylum level. Demogram of soil bacterial structural evolution. **C**, **D** Bacterial microbes in the rhizosphere and non-rhizosphere soils at the species level

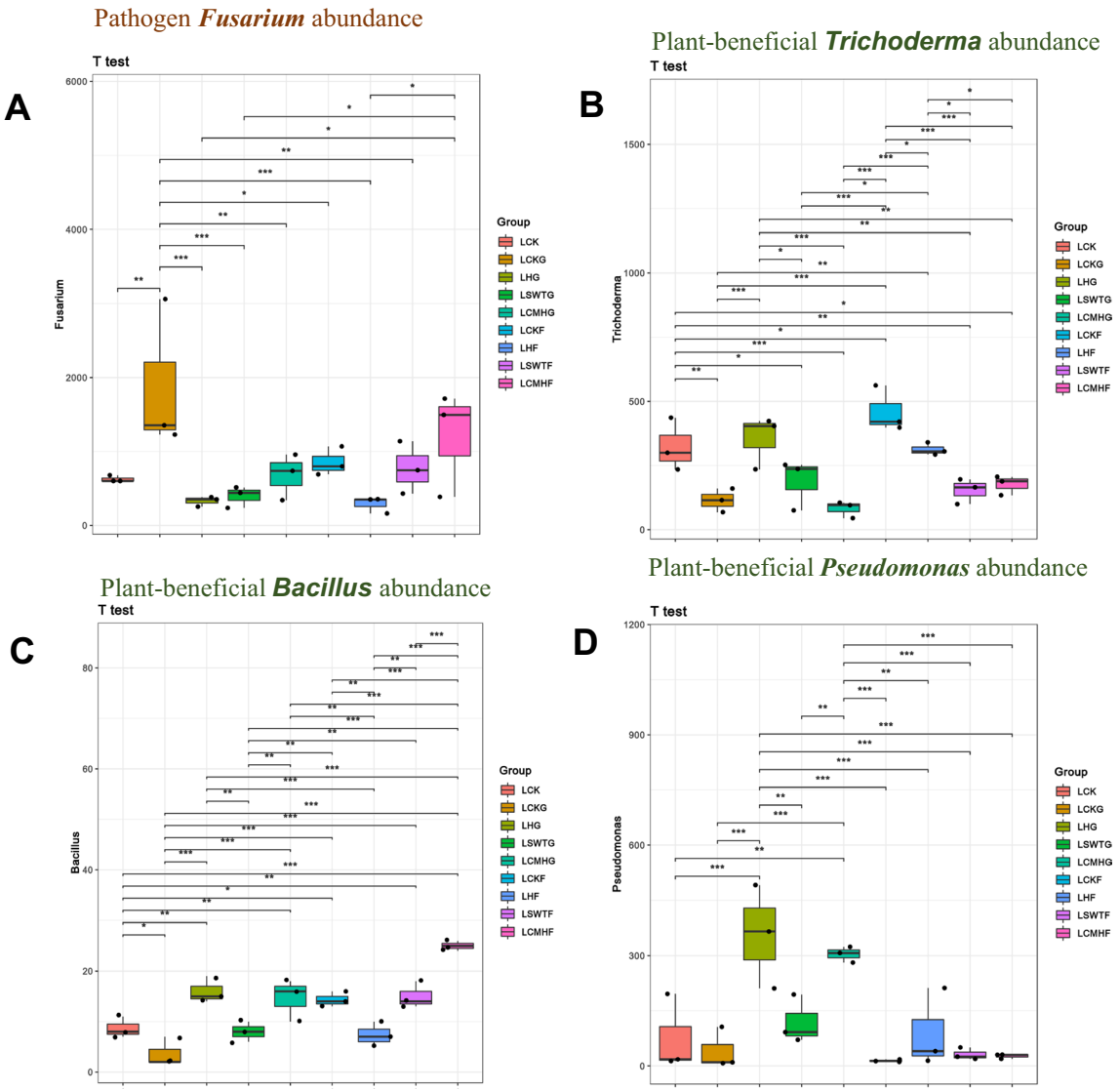


Fig. 3 Differences in the abundance of pathogenic and probiotic microbes. **A** Boxplot of the differences in the abundance of the pathogen *Fusarium*. **B**–**D** Differences in the abundance of the probiotics *Trichoderma, Bacillus,* and *Pseudomonas* in the boxplot

plant-beneficial microbes. The *Fusarium* sp. is a major soil-borne fungal pathogen that directly causes CCOs, we found that the normal planting(LCKG) significantly increased the relative abundance of pathogenic *Fusarium* sp. in the rhizosphere when compared with non-planting (LCK) (T test, $P < 0.01$; Fig. 3A) with potential threats to rhizosphere health and causing CCOs. However, the proportional strategy(LHG) significantly decreased the relative abundance of pathogenic *Fusarium* sp. in the rhizosphere when compared with normal planting (LCKG) (T-test, $P < 0.001$; Fig. 3A)with the potential to suppress *Fusarium* wilt diseases and improve rhizosphere health. Notably, we found that the proportional strategy(LHG) increased the relative abundance of plant-beneficial microbes. Compared with the normal planting(LCKG), the proportional strategy(LHG) significantly increased the relative abundances of *Trichoderma* sp., *Bacillus* sp., and *Pseudomonas* sp. in the rhizosphere (T test, $P < 0.001$; Fig. 3B–E). Together, we found planting *P. notoginseng* increased the relative abundance of soil-borne microbes in the rhizosphere. However, the proportional strategy was decreased pathogens, while increased plant-beneficial microbes in the rhizosphere with the potential to enhance rhizosphere health.

Bacterial community function on the proportional strategy. To explore the microbial function of the proportional strategy, we used a phylogenetic investigation of communities by reconstruction of unobserved states2

(PICRUSt2) to predict the metabolic function (based on Kyoto Encyclopedia of Genes and Genomes, KEGG), COG protein function, and enzyme activity. The results showed that the proportional strategy (LHG) downregulated pathways involved in the biosynthesis of secondary metabolites, phenylalanine metabolism, oxidative phosphorylation, glyoxylate and dicarboxylate metabolism, and glycine-serine-threonine metabolism when compared to the normal planting (LCKG) and non-planting control (LCK) (Supplementary Fig. 5A). Moreover, The proportional strategy (LHG) enhanced key metabolic proteins, specifically by elevating the concentrations of dissimilatory sulfate reductases, which are pivotal for the respiratory process, augmenting the expression of alpha and beta subunits of the metal-dependent hydrolases belonging to beta-lactamase superfamily II (MBL). These hydrolases are instrumental in neutralizing a broad spectrum of beta-lactam antibiotics. Additionally, the proportional strategy (LHG) induced the production of superfamily II helicases, enzymes that are crucial for preserving genomic integrity. They participate in identifying DNA repair sites and facilitate the formation of repair complexes, thereby ensuring the accuracy of DNA repair mechanisms (Supplementary Fig. 5B). Lastly, the proportional strategy (LHG) increased the enzyme activity of the formation of dehydrogenase (NADP (+)) and proton-exporting ATPase, while decreasing the enzyme activity of glutaminyl-tRNA synthase (glutine-hydrolyzing), aspartinyl-tRNA synthase (glutine-hydrolyzing), and acetyl-CoA carboxylase (Supplementary Fig. 5C).

3.2 Metabolism of the rhizosphere soil on added strategy of proportion of biochar and plant ash

Metabolite accumulation distribution of the rhizosphere soil in the proportion strategy. We further tested whether the proportion strategy systemically alters the metabolism of the rhizospheric soil, We used the Ultra Performance Liquid Chromatography-Electrospray Ionization Tandem Mass Spectrometry (UPLC-ESI–MS/MS) system to perform untargeted and targeted metabolomics to profile comprehensive and precise qualitative and quantitative metabolomes. According to the heatmap, the distribution of metabolites accumulation in the rhizospheric soil was dominated by lipids (18.69%), phenolic acids (16.3%), and organic acids (15.31%) (Supplementary Fig. 6A). Notably, the proportional strategy(LHG) reduced the concentration of phenolic acids, lipids, and nucleotides in the rhizospheric soil (Supplementary Fig. 6B).

Differential metabolite screening and distribution in the proportion strategy. We used the orthogonal partial least squares-discriminant analysis(OPLS-DA) model to identify the differential metabolites in the proportional strategy. Comparisons between the proportional strategy(LHG) and normal planting(LCKG) revealed 264 differential metabolites, 92 of which were upregulated but 147 of which were downregulated (Fig. 4A). The differential metabolites classification heatmap showed that the proportional strategy(LHG) downregulated the concentration of phenolic acids, lipids, and nucleotides but increased terpenoids in the rhizosphere soil (Fig. 4B). More specifically, we found that 40% of the downregulated TOP10 differential metabolites belonging to phenolic acids, i.e., 4-hydroxybenzoic acid, caffeic aldehyde, protocatechuic acid, and mandelic acid (Fig. 4C, Supplementary Fig. 7). In addition, according to the correlation cluster heatmap, the results showed that the TOP10 differential metabolites were closely related to one another(correlation coefficient $R \geq 1$; Supplementary Fig. 8A). For example, the phenolic acids (TOP10 differential metabolites), 4-hydroxybenzoic acid was positively correlated with 2-acetyl-2-hydroxybutanoic acid (TOP10 differential metabolites); caffeic aldehyde was positively correlated with L-glycyl-L-isolacine (TOP10 differential metabolites) and *N*-glycyl-L-leucine (TOP10 differential metabolites); and protocatechuic acid (TOP10 differential metabolites) was positively correlated with β-hydroxyisovaleric acid (TOP10 differential metabolites) and vanillic acid (TOP10 differential metabolites) (Supplementary Fig. 8A). Intriguingly, the correlation and chord diagram of the differential metabolites revealed the concentration of interactions within the correlation network of the top 50 differential metabolites, notably around a key metabolite, L-threonine (amino acids) (Fig. 4D). Collectively, our findings indicate that proportional strategy(LHG) reduced the concentration of phenolic acid in the rhizospheric soil with the potential to mitigate autotoxicity on the root of *P. notoginseng* and enhance the rhizospheric health. The differential metabolites primarily belong to the phenolic acid and amino acid categories, and demonstrated a high degree of interconnectedness within the metabolic framework.

KEGG functional annotation and enrichment analysis of differential metabolites in the proportion strategy. To investigate the potential metabolic function difference of rhizosphere soil in the proportion strategy, we used the KEGG database to perform an enrichment analysis of the formation pathways based on the differential metabolites. Here, We found that the proportional strategy(LHG) downregulated pathways of various secondary metabolites biosynthesis (Supplementary Fig. 9, 10), pyrimidine metabolism (Supplementary Fig. 9, 11), starch and sucrose metabolism (Supplementary Fig. 9, 12), and fatty acid degradation (Supplementary Fig. 9). Meanwhile, the proportional strategy(LHG) upregulated biosynthesis pathways of tropane-piperidine-pyridine

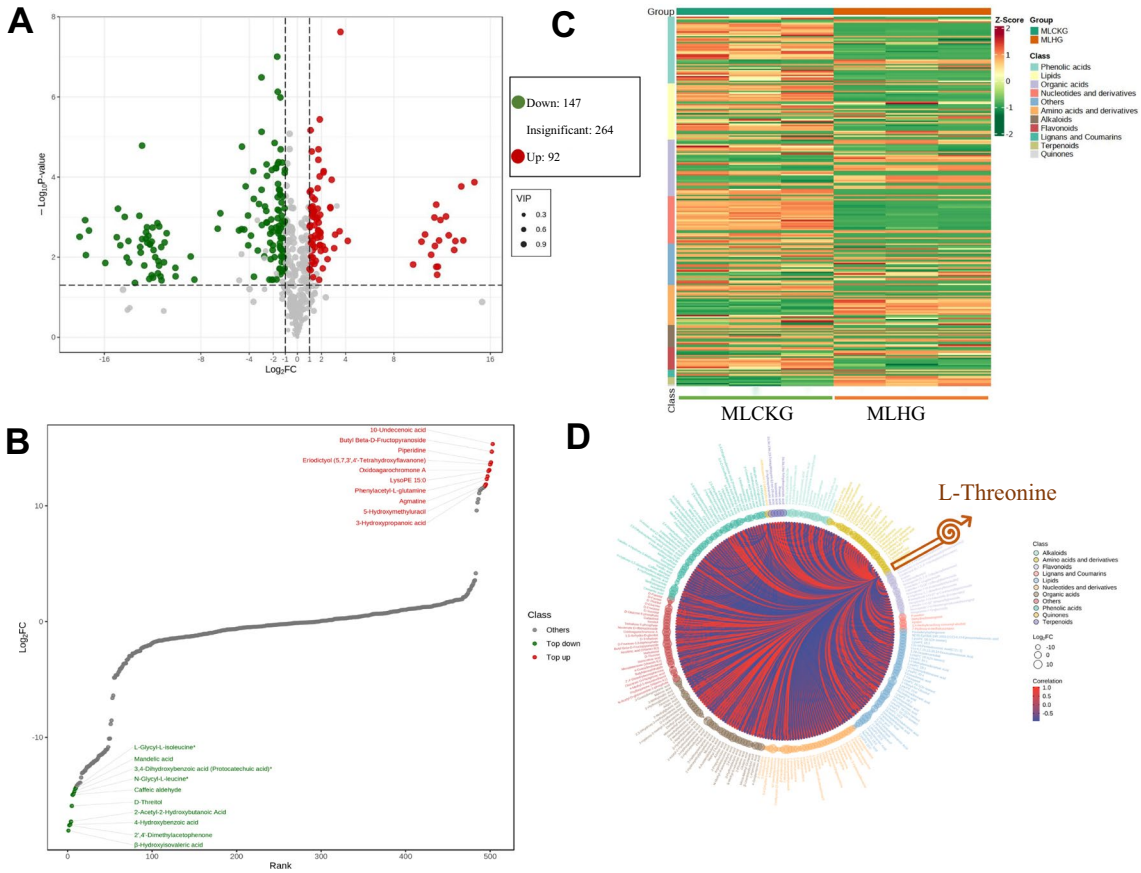


Fig. 4 Differentially metabolite screening and distribution. **A** Volcano plots of the differentially metabolites up and down between the proportional strategy(LHG) and normal planting(LCKG). **B** Heatmap of the differentially metabolite clusters between the proportional strategy (LHG) and normal planting (LCKG). **C** Differences in the distributions of the TOP10 downregulated and upregulated metabolites between the proportional strategy(LHG) and normal planting(LCKG). **D** Chord Diagram of differential metabolite correlations

alkaloid and valine-leucine-isoleucine(Supplementary Fig. 9), resulting in an increase in the differential metabolites of L-tyrosine and L-phenylalanine. Notably, phenylpropanoid biosynthesis and ABC transporters were the enriched differential pathways. The phenylpropanoid biosynthesis pathway involves the differential metabolites of phenolic acid and amino acid categories (Supplementary Fig. 9). The pathway of ABC transporters on environmental information processing (Supplementary Fig. 13) decreased the nucleotides but increased in the differential metabolites of amino acid, e.g., L-threonine, L-phenylalanine, L-isoleucine, leucine, and L-glutamic acid (Supplementary Fig. 13). Lastly, we found that the L-threonine, a key metabolite, participated in the TOP10 differential pathways, including secondary metabolites biosynthesis, ABC transporters, aminoacyl-tRNA biosynthesis, valine-leucine-isoleucine biosynthesis, and amino acids biosynthesis. Together, the analysis pinpointed the phenylpropanoid biosynthesis pathway as a key regulator of amino acid and phenolic acid levels among the primary differential metabolites. L-threonine was identified as a key metabolite correlated with the top 10 differential metabolic pathways, potentially serving as a keystone in the rhizosphere soil's metabolic network.

Profiling the phenylalanine, tyrosine and tryptophan biosynthesis pathways. We further analyzed the differential metabolic pathways that govern the key differential metabolites of phenylalanine and L-threonine, specifically the phenylalanine, tyrosine, and tryptophan biosynthesis pathways to elucidate the pivotal roles of these amino acids within the metabolic landscape of rhizosphere soil system (Fig. 5). First, we found that the proportional strategy(LHG) upregulated the phenylalanine-tyrosine-tryptophan biosynthesis pathways, which involves the phenylpropanoid biosynthesis(TOP10 differential pathways), tyrosine metabolism, glucosinolate biosynthesis (TOP10 differential pathways), tropane-piperidine-pyridine alkaloid biosynthesis (TOP10 differential pathways), and valine-leucine-isoleucine biosynthesis (TOP10 differential pathways), (Fig. 5, Supplementary Fig. 14A).

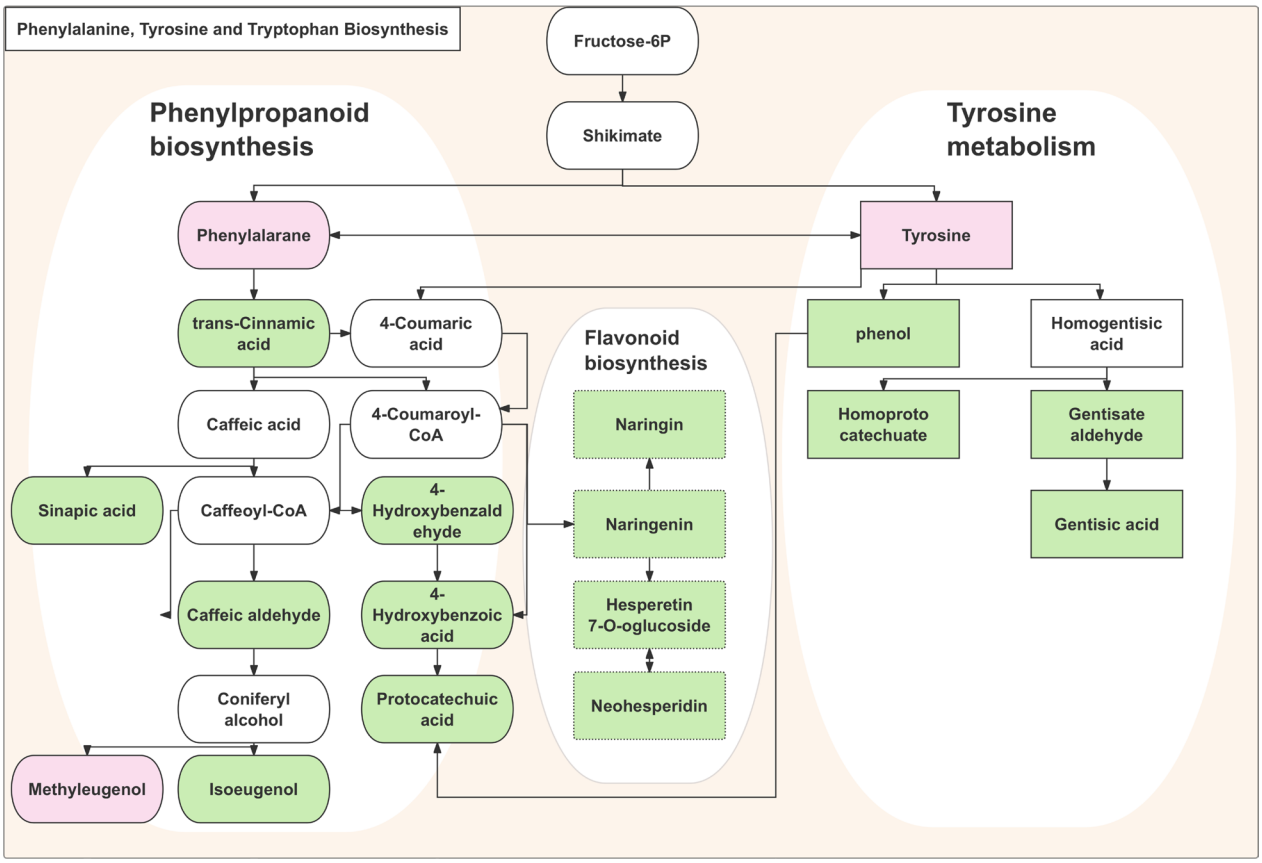


Fig. 5 KEGG functional annotation of the differentially metabolites in the phenylpropanoid biosynthesis and tyrosine metabolism. Red boxes indicate upregulation while green boxes indicate downregulation in metabolic pathways

Moreover, we found that the phenylalanine-tyrosine-tryptophan biosynthesis pathways involves altering the level of phenolic acids and amino acids, which upregulated the differential metabolites of amino acids, e.g., L-phenylalanine, L-tyrosine, L-leucine, and L-isoleucine (Supplementary Fig. 14B, C), and downregulated differential metabolites of phenolic acids, e.g., cinnamic acid, sinapic acid, caffeic aldehyde, isoe, genol, 4-Hydroxybenzoic acid, and protocatechuic acid. Lastly, we found that the proportional strategy (LHG) exerted a downregulatory effect on the flavonoid biosynthesis pathway (Supplementary Fig. 9), intricately linked with the modulation of differential metabolites within the phenylpropanoid biosynthesis. This regulatory shift potentially reduces naringenin levels, a metabolite of crucial precursor in the flavonoid biosynthetic pathway. In summary, the phenylalanine-tyrosine-tryptophan biosynthesis pathway significantly influenced by the proportional strategy (LHG), modulating the concentrations of differential metabolites, particularly phenolic acids and amino acids, playing a critical role in the metabolism function of soil and rhizosphere health.

3.3 Soil physicochemical properties in the rhizosphere soil

To demonstrate whether the proportional strategy(LHG) mitigated soil acidification and affected soil physicochemical properties in the rhizosphere. We assessed the soil's physicochemical properties and nutrient status, including pH (Fig. 6A), organic matter (Fig. 6B), cation exchange capacity (Fig. 6C), total nitrogen (Fig. 6D), total phosphorus (Fig. 6E), total potassium (Fig. 6F), nitric nitrogen (Fig. 6G), available phosphorus (Fig. 6H), and available potassium (Fig. 6I). We found that the proportional strategy(LHG) improved the soil nutrient levels and mitigated soil acidification. Compared with the non-planting (LCK) and normal planting(LCKG), the results showed that the proportional (LHG) strategy significantly increased the soil pH (T-test, $P<0.001$; Fig. 6A), organic matter content (OM)(T-test, $P<0.001$; Fig. 6B), total nitrogen (TN)(T-test, $P<0.001$; Fig. 6D), total phosphorus (TP)(T-test, $P<0.001$; Fig. 6E), available phosphorus (AP)(T-test, $P<0.001$; Fig. 6H), and available potassium (AK) (T-test, $P<0.001$; Fig. 6I). Notably, the proportional strategy(LHG) doubled the increase in organic matter (OM), available phosphorus (AP), and available

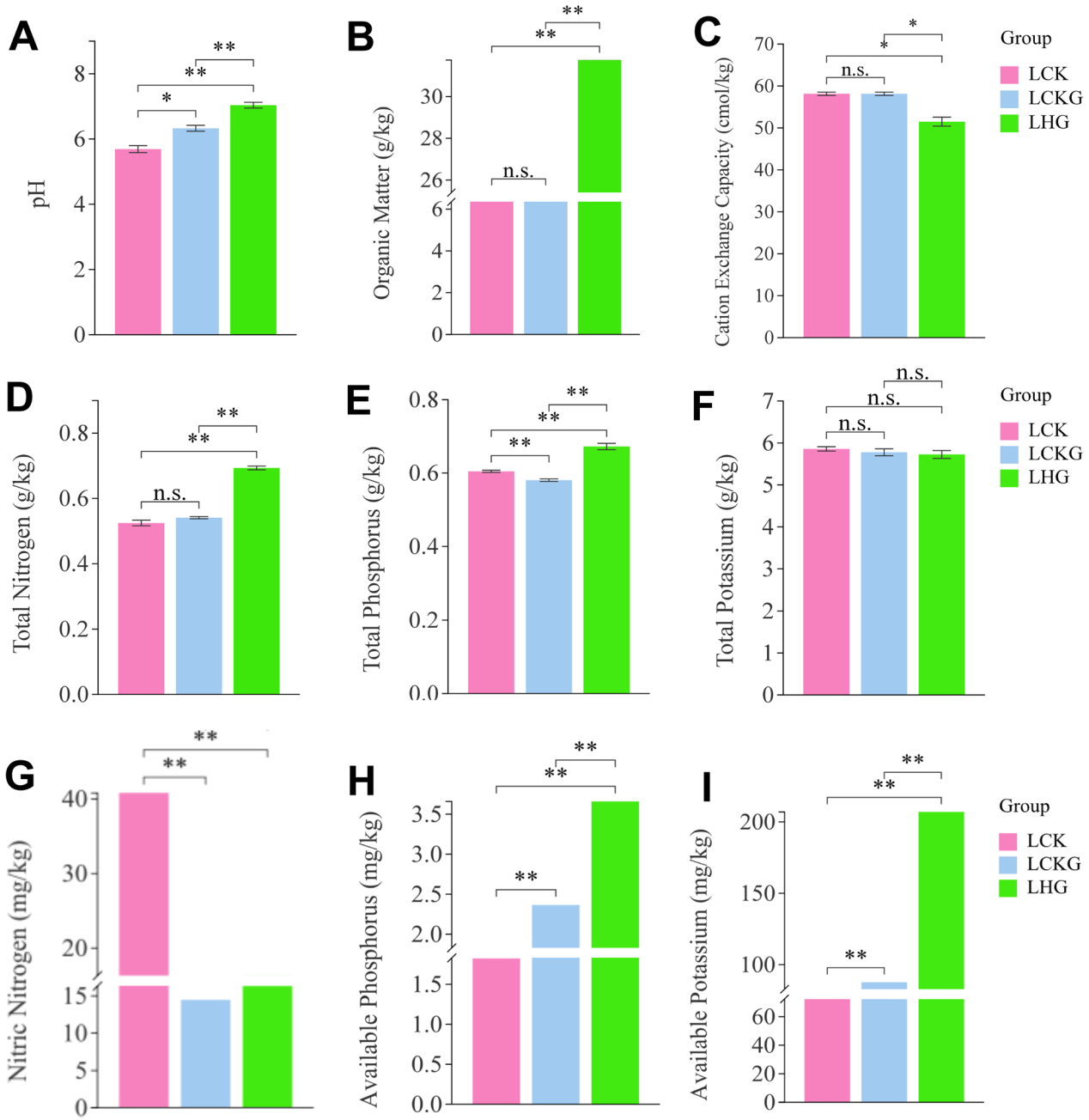


Fig. 6 Soil physicochemical property comparison histogram. Rhizosphere soil physicochemical properties and nutrient status comparison. **A** pH. **B** Organic matter. **C** Cation exchange capacity. **D** Total nitrogen. **E** Total phosphorus. **F** Total potassium. **G** Nitric nitrogen. **H** Available phosphorus. **I** Available potassium

potassium (AK) when compared with the non- planting (LCK) (Fig. 6B, H, I). In brief, the soil physicochemical properties of the rhizosphere demonstrate the efficacy of the proportional strategy in promoting nutrient recirculation and mitigated soil acidification with potential to ameliorate the rhizosphere health.

3.4 Interaction network of the soil environment–microbes–metabolites in the rhizosphere

To test our hypotheses that rhizospheric health status is determined by the interplay of the pathogen attacks, microbial communities, host plant metabolism, and soil properties in the soil systems, we analyze relationship between the pathogenic *Fusarium* sp., differential

microbes, differential metabolites, and soil physicochemical properties of rhizosphere soil. First, the redundancy analysis(RDA) showed that the proportional strategy(LHG) and normal planting(LCKG) had distinctive impact on soil physicochemical properties, microbial communities, and metabolites (Supplementary Fig. 15A–C). The results showed that pathogenic *Fusarium* sp. was positive with normal planting(LCKG). However, the proportional strategy(LHG) was negative with pathogenic *Fusarium* sp, while positive with *Rhizophagus*, *Geosmithia*, and *Leptospirillum* (Supplementary Fig. 15A, B), and positive with soil pH, OM, TN, TP, and AK (Supplementary Fig. 15A).

Moreover, the microbial co-occurrence network analysis revealed that the planting *P. notoginseng*, including all the treatments, decreased the fungal connectivity in the rhizosphere soil when compared with non-planting (LCK) (Fig. 7A–D). In contrast, the bacterial connectivity was only decreased in the biochar strategy (Supplementary Fig. 16A–D). The biochar and proportional strategies resulted in a greater degree of decreased fungal connectivity, segregating and clustering these fungal entities into distinct microbial communities. Notably, within the framework of the proportional strategy(LHG), we observed a clear demarcation between fungal communities associated with arbuscular mycorrhizal fungi (AMF) and another associated with soil-borne pathogens, indicates a distinct ecological niche differentiation between AMF group and soil-borne pathogens group.

We further tested the relationship between AMF and soil-borne pathogenic *Fusarium* sp. The results showed that proportional strategy(LHG) in the rhizosphere soil 'enriched' the genera *Rhizophagus*, *Geosmithia*, and *Leptospirillum* (Supplementary Fig. 2A, 15A, B), which were negative with the pathogenic *Fusarium* sp. in terms of the utilization of amino acids and phenolic acids (Fig. 7E, Supplementary Figs. 17, 18), and positive with enhanced nutrient levels and lower soil pH (Supplementary Fig. 15A, B). Furthermore, the structural equation modeling(SEM) regression analysis revealed that the AMF *Rhizophagus* had a moderate yet direct effect on the pathogenic *Fusarium* sp., rather than the indirect effect of using amino acids to later the pathogenic *Fusarium* sp. In summary, the proportional strategy(LHG) 'enriched' AMF in the rhizosphere directly leading to a decrease in soil-borne pathogenic *Fusarium* sp. (Fig. 8).

Subsequently, we tested the relationship between the pathogenic *Fusarium* sp., soil pH, and phenolic acids, which are significant factors that disrupt rhizosphere ecology. The co- correlation analysis revealed that the pathogenic *Fusarium* sp. was positive with phenolic acids, and negative with soil pH (Fig. 7F). Furthermore, the SEM regression analysis further confirmed that the pathogenic *Fusarium* sp. significantly altered by soil pH rather than phenolic acids, while phenolic acids significantly altered soil pH with an indirect effect on the pathogenic *Fusarium* sp. (Fig. 8).

We used structural equation modeling(SEM) construct the rhizosphere framework under proportional strategy to evaluate the significant factors of microbes, metabolites, and soil physicochemical properties modules on the rhizosphere. Here, we found that the metabolite module, including phenolic acids and amino acids, is significant factor on the rhizosphere, potentially associated with host plant root exudates. The microbe module influence by indirect effects of metabolite module from alterations in soil physicochemical properties rather than direct effects of metabolite module. More Specifically, the composition and function of the fungal and bacterial communities of the rhizosphere under the proportional strategy are determined by indirect effects from the alteration of soil pH and AK via variations in the content of phenolic acids and amino acids. Moreover, the SEM analysis showed the significant factors were amino acids, bacterial community diversity, and soil pH in the rhizosphere, and there was a significant impact of AMF and soil pH on reducing the soil-borne pathogenic *Fusarium* sp. (Fig. 8).

Collectively, we provide a comprehensive analysis of rhizospheric health and construct the rhizosphere framework, revealing that the proportional strategy (LHG) enriches arbuscular mycorrhizal fungi (AMF) and selectively recruits *Geosmithia* and *Leptospirillum*, thereby establishing a symbiotic core microbial network. This network significantly reduces soil-borne pathogenic Fusarium species (Fig. 7F) and is positively associated with increased amino acid levels, improved nutrient availability, elevated soil pH (Supplementary Fig. 15A, B), and efficient phenolic acid utilization. Additionally, we demonstrate that soil pH plays a critical role in pathogen dynamics, suggesting that pH manipulation could be a strategic method for controlling soil-borne diseases. Importantly, we identify the metabolite module as a key component of the rhizosphere framework. Modulating soil metabolites, particularly phenolic acids and amino acids, is essential for rhizosphere restoration and the maintenance of microbial community diversity.

In summary, our findings support the hypothesis that applying the proportional strategy (LHG) restores rhizosphere ecology by facilitating interactions within the "soil environment-microorganism-metabolite" triad. These mechanisms are vital for establishing a beneficial feedback loop, promoting rhizosphere health, and ensuring long-term sustainability.

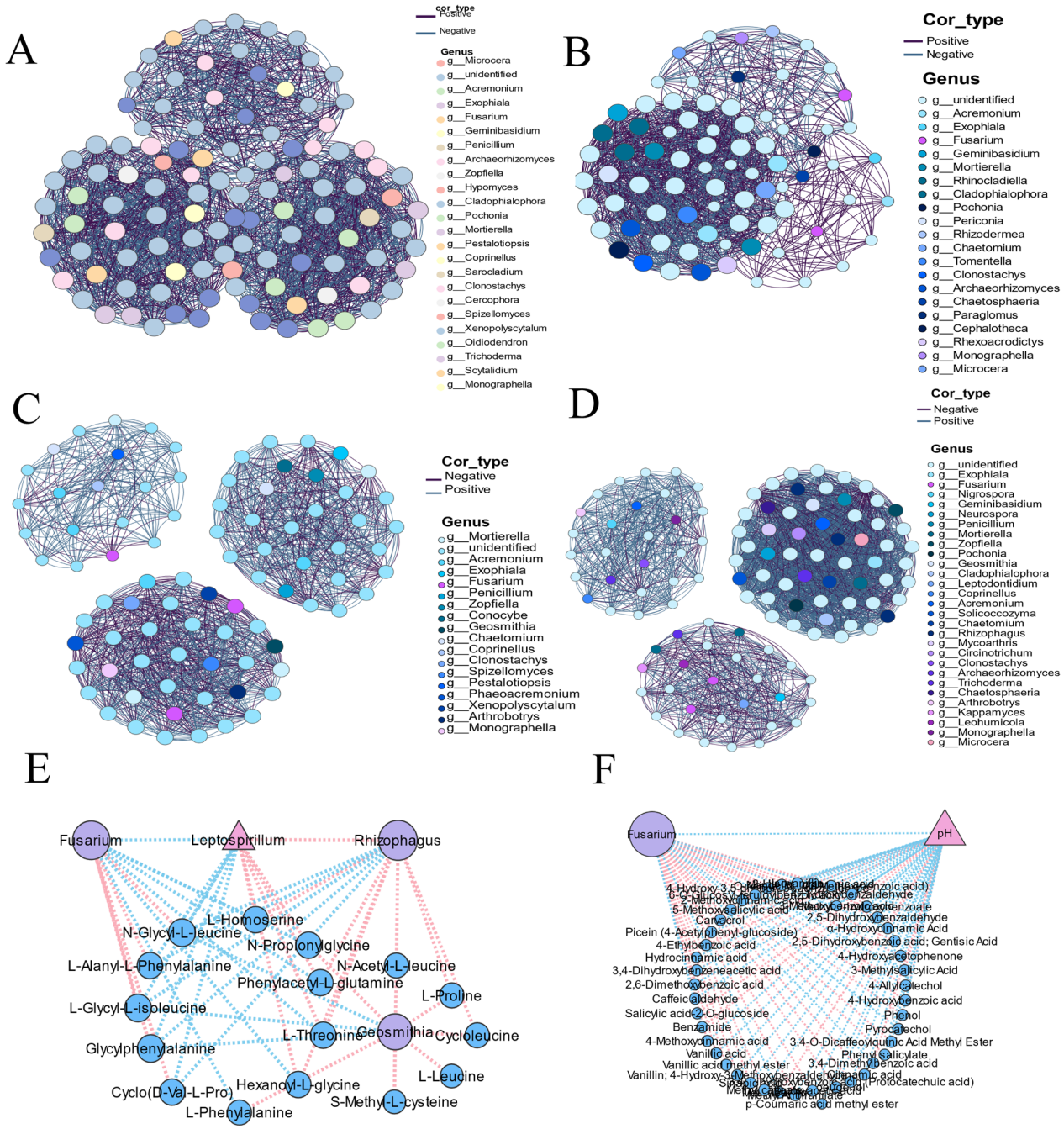


Fig. 7 Co-occurrence network connectivity diagrams for fungal communities. Metabolite–pH interaction network diagrams. **A**–**D** Co-occurrence network connectivity maps of fungal communities for the non-planting control (LCK), normal planting (LCKG), biochar strategy (LSWTG), and proportional strategy (LHG) in rhizosphere soil. **E** Correlation diagram of the interaction network between the pathogen *Fusarium*, the core microbial communities (fungi and bacteria), and amino acids. The red line indicates a positive correlation, and the blue line indicates a negative correlation. **F** Correlation diagram of the interaction network between the pathogen *Fusarium*, phenolic acid content, and pH

4 Discussion

New ecological and sustainable disease management strategies are critical in the context of the rapid evolution of pathogens, global warming, and low-input farming systems. Researchers have studied environmentally friendly methods to control soil-borne diseases, such as biological fungicides (Ma et al. 2021), soil amendments (Li et al. 2019), and microbial inoculants (Mulk et al.

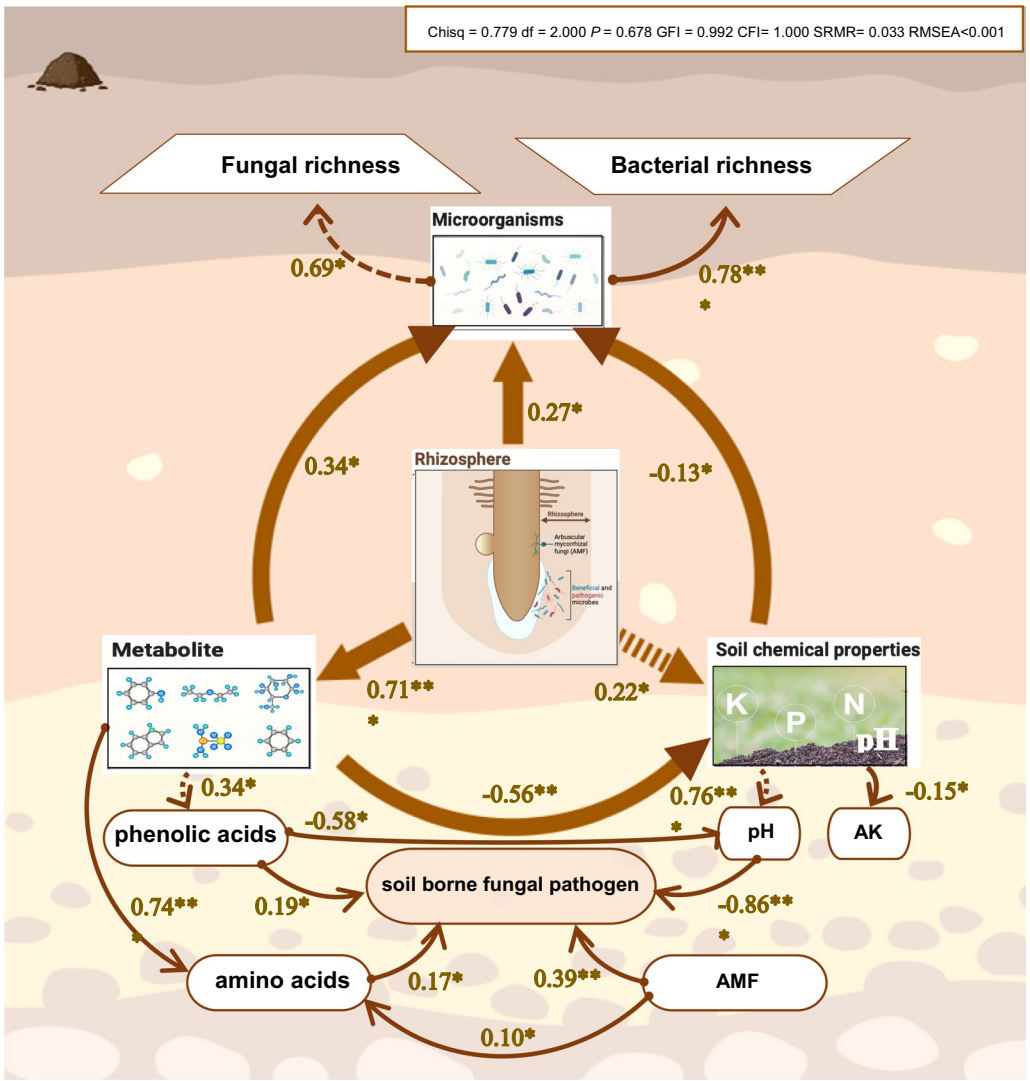


Fig. 8 Structural equation modeling of the proportions of microbes, metabolites and soil chemical properties in the rhizosphere soil correlation network. Single-headed arrows indicate hypothesized directions of causation. The numbers next to the arrows are the standardized path coefficients. The solid and dotted lines represent direct and indirect effects, respectively

2022). Despite these advances, a systematic summary and understanding of the effects and mechanisms of rhizosphere ecology are largely unknown, specifically the metabolic status and regulation of microbes. This knowledge gap limits the availability of effective strategies for managing CCOs. Thus, we need to establish a potential rhizosphere framework to understand and control complex rhizospheric-soil systems using ecosystem methods. It is crucial not only to analyze individual components of the rhizosphere ecosystem in isolation but also to examine the complex interplay among these components. Such an integrated perspective is essential for the effective mitigation of CCOs, offering a pathway towards sustainable agricultural practices and bolstering the resilience of crop systems against diseases.

The rhizosphere assembles different microbial communities to function in response to soil environmental shifts.

Microbial community composition changes and increases in pathogenic fungi in rhizosphere soil are the main factors causing the CCOs of *P. notoginseng*, resulting in the microbial community transforming into a "fungal type," and a decrease in beneficial microbes in CCOs (Tan et al. 2017b). Based on prior research, the abundance of *Fusarium* sp. is a significant factor in causing CCOs of *P. notoginseng*. Re-validated in our study, under the normal planting, *Fusarium* sp. predominantly colonize the rhizosphere, leading to a decline in microbial

diversity, a singular fungal structure, and a potential disruption of the rhizosphere ecological balance. Fortunately, our study confirms that the introduction of biochar and plant ash, either individually or in combination, effectively reduces the prevalence of *Fusarium* sp. in the rhizosphere. However, while biochar significantly diminishes pathogen levels, it also led to a less diverse microbial community. Consistent with our findings, biochar has been shown to reduce *Fusarium*-induced crown and root rot in tomatoes (Jaiswal et al. 2020) and decrease both *Fusarium* abundance (Dror et al. 2022) and fungal diversity (Li et al. 2020) in the soil. This effect may be attributed to biochar's capacity to adsorb pathogenic microbes (Lehmann et al. 2011; Xu et al. 2021a) and produce metabolic compounds that deter them, thereby reducing fungal diversity (Du et al. 2022). While this mechanism contributes to the alleviation of CCOs, the long-term simplification of microbial communities could elicit negative feedback within the rhizosphere ecosystem, underscoring the need for a nuanced approach to biochar application that balances pathogen suppression with the maintenance of microbial diversity.

Notably, the proportional strategy had higher fungal and bacterial community diversity in the rhizosphere, with the potential to be more effective in mitigating fungal dominance of soil systems in CCOs. Soil with higher microbial diversity possesses more ecological functions and greater resistance to environmental stress and enhancing crop productivity. Consistent with the results of this study, the proportional strategy significantly improved the diversity of micrbes, including fungi and bacteria, and in contrast to the biochar strategy, the proportional strategy promoted favorable feedback in the rhizosphere ecosystem. Communities with high diversity exhibit high resource utilization complementarity, obtaining full use of resources in the environment, leaving few resources available to pathogens, and reducing pathogen growth and reproductive ability (Eisenhauer et al. 2013). Moreover, the highly diverse microbial communities produce substances through synergy to inhibit the growth of pathogens. Our results also confirmed that amino acids were positively correlated with AMF and negatively correlated with *Fusarium*. AMF dominate nutrient uptake and undergo nutrient competition, mainly by occupying niches and ultimately reducing the possibility of invasion. Interestingly, bacterial microbes have tighter and firmer rhizosphere associations than fungi, and fungi constantly change their community structure to prevent invasion by pathogens, but they are more easily disrupted.

AMF assemble a core microbial population to increase microbial diversity, dominate amino acids, and occupy niches to resist pathogen invasion. The function of the microbiota, especially resistance to pathogen invasion, is based on interactions between populations rather than the existence of specific traits or species (Saleem et al. 2019). This shift from the perspective of 'resistance genes' to the 'community level' profoundly impacts understanding and guiding microbiome assembly (Gupta et al. 2021). We demonstrated that the proportional strategy recruited AMF to assemble the core microbial flora to resist pathogen attack. However, the assembly of a core microbial flora by AMF is rarely reported in the CCOs of *P. notoginseng*. Our study established an interaction network between the microbiome and metabolome, revealing an indirect negative correlation between AMF and pathogens. We elucidated the central role of the core microbial community network. AMF obtain more soil mineral nutrients for host plants by forming a vast hyphal network in the soil, facilitates to phosphate absorption (Powell and Rillig 2018). In addition, AMF help plants resist various abiotic and biotic stresses and directly or indirectly participate in the soil carbon and nitrogen cycles (Ma et al. 2020). The diversity of AMF determines the productivity and functional diversity of an ecosystem. In line with this, our research showed that the core microbiota establishes microbial interactions between *Rhizophagus* and *Leptospirillum*, *Geosmithia*, PCR2-54, *Polycyclovorans*, and MND1. It reduces the phenolic acid content and increases the amino acid content and protein function. Our results also confirmed that amino acids were positively correlated with AMF and negatively correlated with *Fusarium*. AMF dominate nutrient uptake and undergo nutrient competition, mainly by occupying niches and ultimately reducing the possibility of invasion (Jacoby and Kopriva 2019).

Recent studies have shown that when *Arabidopsis thaliana* leaves are infected with the downy mildew pathogen, the resident species and the pathogen form antagonistic niches to enhance system resistance by recruiting three beneficial microbes (Berendsen et al. 2018). Core microbes form positive feedback loops in rhizosphere ecology through the recruitment of microbial members. Furthermore, AMF-resident species may be more important than other species in resisting pathogen invasion. The association in this study showed that AMF *Rhizophagus* was negatively associated with *Fusarium* in the interaction network. Similarly, studies have shown that AMF have growth-promoting effects and protect against *Fusarium* in host peas (El-Sharkawy et al. 2022). AMF inoculation considerably reduces Fusarium population density and disease severity and plays a significant role in wheat tolerance to *Fusarium* infection by maintaining redox balance and competing for colonization of root sites (Spagnoletti et al. 2021). Similarly, evidence shows

that highly competitive resident species (AMF) help plants resist pathogen invasion through multiple mechanisms, such as niche competition, antibiotic secretion, and induced systemic resistance (ISR) (Beneduzi et al. 2012; Bai et al. 2022a, b). In the future, we will continue to research the mechanisms by which rhizosphere ecological functions are restored, with a focus on the effects of the core microbial community on central AMF.

The phenylalanine–tyrosine–tryptophan biosynthesis pathway decreases phenolic acid levels and promotes amino acid accumulation. Soil metabolites play a pivotal role in plant–microbe interactions in the rhizosphere, influencing pathogenic microbes directly and shaping the microbial community composition indirectly. Our study highlights the metabolite module as a key component of the rhizosphere framework, particularly phenolic acids and amino acids. Phenolic acids, previously shown to be autotoxic to *P. notoginseng* roots and to attract pathogens, lead to altered microbial community structure, increased plant disease susceptibility, and a decline in medicinal value and yield (Bao et al. 2022; Xiang et al. 2022).

We demonstrated that the proportional strategy effectively reduced phenolic acids and their secondary metabolic pathways, thereby disrupting pathogenic signaling and reducing the emission of root-derived stress signals. This reduction decreased release of autotoxic root metabolites and enhanced the rhizosphere health. Additionally, we found that phenolic acids also indirectly modulate pathogen activity by altering soil pH, which may be one of the reasons that biochar alleviates the CCOs (Liu et al. 2022a). Simultaneously, the strategy increased amino acid and terpene levels, which are essential for plant ecological adaptation, defense, and development, enriching the plants' chemical language for communication with microbial communities (Huang and Osbourn 2019). Furthermore, AMF *Rhizophagus* indirectly reduced pathogens by utilizing amino acids.

We performed an enrichment analysis of KEGG pathways, highlighting that the phenylalanine-tyrosine-tryptophan biosynthesis pathway exhibited the most pronounced metabolic shifts, particularly in phenolic acids and amino acids. This pathway, initiating from shikimic acid, is essential for the synthesis of phenylalanine, tryptophan, and tyrosine, precursors to a vast array of phenolic compounds. Our findings indicate that the proportional strategy modulates these biosynthetic pathways, resulting in reduced phenolic acids and increased amino acids within the rhizosphere soil, which is critical for countering fungal dominance and promoting soil ecosystem health.

Moreover, the strategy upregulated the ABC transporter pathway, which is integral for stress resistance in soil pathogens and for maintaining plant homeostasis under various stresses. This upregulation, powered by ATP hydrolysis, involves the regulation of biological processes in plant vacuoles, including detoxification of metal ions, prevention of DNA damage, and support of cell growth. Biochar addition has been shown to enhance gene expression related to plant defense and growth, while downregulating the biosynthesis and signaling of the salicylic acid pathway, which may account for the observed disease suppression and enhancement of plant performance (Jaiswal et al. 2020).

Our work contributes to a comprehensive understanding of the theoretical framework of the rhizosphere ecology of P. notoginseng. The results support our hypothesis that the proportional strategy effectively restores rhizosphere ecology through the interplay of the "soil environment-core microbiota network-metabolic pathway regulation." Specifically, we clarified the direct positive correlations between the soil environment and the core microbiota, the pathogens with phenolic acids and soil pH, the regulation of pathways and the core microbiota network, while highlighting indirect ecological antagonism between core microbiota and pathogens, which generates beneficial feedback for rhizosphere health. The proportional strategy of rhizosphere established a symbiotic network that integrates soil nutrient environment—core microbiota—key metabolites, resulting in decreased pathogenic *Fusarium* and phenolic acids. In future studies, we will institute root metabolomics to analyze the interactions among the "microflora-function-root" and construct a core regulatory network to reveal the rhizosphere ecology—plant feedback mechanism. A larger framework theory for the restoration of rhizosphere ecology has been proposed. Furthermore, an overarching aim of the restoration of rhizosphere ecology is to recombine these core microbiota into rationally designed synthetic microbial communities that exhibit stability and resilience, with genomic data guiding the assembly of high-performing communities to carry out desired functions. The next step is integrating concepts and methods from multiple disciplines, such as plant science, microbial ecology, analytical chemistry, and computational modeling. These in-depth studies address interactions in the rhizosphere microbiome.

5 Conclusion

The added strategy of proportional biochar and plant ash effectively restores the rhizosphere soil ecology, enhancing the soil nutrient status and increasing the soil pH, resulting in the reduction of acidophilic pathogens and mitigating soil acidification. This approach recruits AMF to assemble into core microbial functional groups to promote community diversity and weakening pathogenic niche preemption through competition for nutrients. This core microbial

network was positively associated with increased amino acid levels, improved nutrient availability, elevated soil pH, and efficient phenolic acid utilization. Furthermore, the strategy regulates the phenylalanine-tyrosine-tryptophan biosynthesis and ABC transporter pathways, directly decreasing phenolic acid levels and enhancing ABC transporter and amino acid biosynthesis, promoting the accumulation of L-threonine, L-tyrosine, and L-phenylalanine and providing more nutrients and transport capacity for the core microbial functional circle. Notably, we clarified that rhizosphere ecological restoration and resistance to pathogen invasion is determined by the interplay of networks involving soil environment improvement, core microbial assembly, reduction of phenolic acids and signaling molecules of secondary metabolites, and upregulated phenylalanine metabolism. This review provides a theoretical framework for restoring microbial ecology, designing and constructing synthetic microbial communities, and ultimately offers a solution for continuous cropping obstacles (CCOs).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s42773-024-00402-6.

Supplementary file 1

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Author contributions
All authors contributed to the study conception and design. Jichao Li: Data curation, Writing-review and editing. Zongliang Xu: Conceptualization, Project administration. Tianmei Yang: Investigation, Formal analysis. Jinyu Zhang: Supervision, Project administration, Funding Acquisition. Yingmei Zuo: Preliminary experiments foundation, experimental design. Lei Cheng: Experimental design, Writing-review and editing.

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

Declarations

Ethics approval and consent to participate
The manuscript is approved by all authors for publication. I would like to declare on behalf of my co-authors that the work described is original research that has not been published previously, and not under consideration for publication elsewhere.

Consent for publication
If the manuscript is accepted, we are willing to transfer the publishing rights to this manuscript.

Competing interests
The authors have no competing interests relevant to the content of this article to declare.

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