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Biochar-induced microbial and metabolic reprogramming enhances bioactive compound accumulation in *Panax quinquefolius* L.

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Abstract

Panax quinquefolius L., with a history of over 300 years in traditional Chinese medicine, is notably rich in ginsenosides—its primary bioactive components. Although our previous study found that biochar application could enhance the content of ginsenoside Re, Rg and other contents in *P. quinquefolius*, its effect on the overall secondary metabolism of *P. quinquefolius* and its mechanism are still unclear. In this paper, the correlation between plant microbiome and secondary metabolites was studied from the perspective of plant rhizosphere microorganisms and endophytes, and the mechanism of biochar-induced metabolic reprogramming of *P. quinquefolius* was revealed. The results showed that biochar treatment significantly increased the accumulation of various substances in *P. quinquefolius*, including nucleosides, glycerophosphocholines, fatty acyls, steroidal glycosides, triterpenoids, and other bioactive compounds. Additionally, biochar treatment significantly enriched beneficial rhizosphere microorganisms such as *Bacillus*, *Flavobacterium*, and *Devosia*, while reducing the relative abundance of harmful fungi like *Fusarium*. Furthermore, it promoted endophytic *Flavobacterium*, *Acaulospora*, and *Glomus*, and suppressed pathogenic genera such as *Plectosphaerella*, *Cladosporium*, and *Phaeosphaeria*. These shifts in rhizosphere microbial community and endophytes structure and function were closely linked to the accumulation of secondary metabolites (e.g. ginsenosides Rg_3, F2) in *P. quinquefolius*. Overall, our findings suggest that biochar may influence key endophytes and rhizosphere microorganisms to regulate the accumulation of secondary metabolites in *P. quinquefolius*. Therefore, this study provides valuable insights into the potential application of biochar in Chinese medicine agriculture.

Keywords *Panax quinquefolius* L., Biochar, Rhizosphere microorganism, Endophyte, Active metabolites

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Introduction

Panax quinquefolium L., also known as American ginseng, has been a staple in traditional Chinese medicine (TCM) for over three centuries [1]. TCM theory posits that *P. quinquefolius* replenishes qi, nourishes yin, clears heat, and promotes body fluid production [2–4]. Modern clinical studies have validated these uses, confirming its anti-cancer, anti-diabetes, immunomodulatory, and neuroprotective effects [5–7]. *P. quinquefolius* contains a wealth of active ingredients, including saponins, organic acids, sugars, and volatile oils [8–10]. Among these, ginsenoside is the primary active component and the key indicator of its medicinal quality [11, 12]. In recent years, *P. quinquefolius* has gained widespread use in health foods, cosmetics, and other industries across Europe, America, and Asia [13]. In 2023, China officially recognized *P. quinquefolius* as both a medicine and a food. With the increasing market demand, ensuring the herb's quality becomes crucial. Currently, most *P. quinquefolius* on the market is cultivated, with China being a major producer, accounting for 30% of global production - approximately 2100 tonnes [14]. Therefore, it is essential to implement effective measures to enhance the content of active metabolites in *P. quinquefolius*.

Biochar is a carbon-rich, porous, and alkaline solid produced from the pyrolysis of biomass waste [15]. It possesses a large specific surface area, abundant pores, and various physical properties, which enhance its adsorption capacity and improve soil microbial community structure [16–18]. Studies have shown that biochar can boost crop yield and the accumulation of secondary metabolites in medicinal plant, such as maize, tobacco, *Aloe vera* (L.) Burm. f., *Fritillaria thunbergii* Miq.and so on [19–22]. Our team discovered that biochar increases the accumulation of ginsenosides Re, Rg in *P. quinquefolius* [23], although its effect on the overall secondary metabolism of *P. quinquefolius* and its mechanism are still unclear, and further research is needed.

Recently, the role of rhizosphere microorganisms and endophytes in remodeling plant metabolism has gained significant attention. These microorganisms are crucial for the accumulation of host metabolites induced by various environmental factors [24, 25]. Our group previously found that rhizosphere arbuscular mycorrhizal fungi (AMF) promote the accumulation of ginsenosides in *P. quinquefolius* and elucidated the signal transduction mechanism [26–28]. Biochar, when introduced into the soil, can influence rhizosphere microorganisms [17, 29], potentially affecting the accumulation of secondary metabolites. Further study is needed to determine if biochar can regulate the accumulation of these metabolites by modifying the rhizosphere microecological environment.

Additionally, endophytes are microorganisms that live harmlessly within the healthy tissues or organs of plants [30]. They originate from the rhizosphere or are influenced by rhizosphere microorganisms [31]. Endophytes significantly impact the quality of medicinal materials [32], by altering the metabolite composition of their host plants [33, 34]. Our team discovered correlations between endophytes in *P. quinquefolius* and their secondary metabolites, indicating that endophytes influence accumulation of secondary metabolites [35]. Further study is needed to determine if biochar's effect on *P. quinquefolius*'s secondary metabolite accumulation is related to endophytes. It can be seen that endophytes and rhizosphere microorganisms play an important role in the accumulation of secondary metabolites in plants. Therefore, it is worth studying whether biochar regulates secondary metabolism by modulating endophyte communities and rhizosphere microorganisms. To investigate this, ultra high-performance liquid chromatography (UPLC-MS/MS) was employed to detect metabolites in *P. quinquefolius* roots, and 16 S and ITS microbial sequencing was used to analyze *P. quinquefolius* rhizosphere microorganisms and endophyte.

By integrating microbiome and metabolomics analyses, we elucidated the correlation between rhizosphere microorganisms, endophytes, and secondary metabolites. Our objectives were twofold: (1) To determine the effects of biochar application on metabolic reprogramming in *P. quinquefolius*. (2) To reveal how biochar influences the rhizosphere microbial community and endophyte structure composition in *P. quinquefolius*, as well as their relevance to secondary metabolism. This study aims to uncover the mechanism behind biochar's regulation of *P. quinquefolius* secondary metabolism from the perspective of rhizosphere microorganisms and endophytes. Furthermore, it also provides a reference for biochar application in plant production.

Materials and methods

Experimental design

The experiment was carried out at a test station in Wendeng District, Shandong Province, China (121°49 'E, 37°05' N), a major production area for *P. quinquefolius*. Biochar provided by Jiangsu Huafeng Agricultural Bioengineering Co., Ltd., was applied at a rate of 1.8%. Prior to the experiment, *P. quinquefolius* seeds were surface-disinfected following the method detailed in Yang et al. [23]. A pot experiment was conducted outdoors from March 2022 to October 2022. Before initiating the pot experiment, surface soil (0–20 cm) was collected from the experimental site, air-dried, ground, and sieved through a 2 mm screen. Biochar was then mixed with the soil at concentrations of 0% (CK) and 1.8% (biochar), with the selected concentration based on previous laboratory

optimizations. Both the soil and biochar were autoclaved at 121 °C and 0.10 MPa for 2 h before the experiment to eliminate microorganisms. Six *P. quinquefolius* seeds were evenly placed on the surface of 2.0 kg of the soil-biochar mixture in each pot. An additional 100 g of the mixture was spread over the seeds to ensure uniform depth. Thirty pots were prepared for each treatment. In October 2022, all potted plants were watered to 70% of the field water volume and then buried in the same soil at the test site. Each treatment was replicated thrice.

Sample collection

Plant and soil samples were collected in October 2022. Each plant sample was divided into two sub-samples. One was extracted with liquid nitrogen and stored in a refrigerator at -80 ℃ for endophytic bacteria and plant metabolism analysis. The other sub-sample was stored in dry ice and immediately shipped back to the laboratory for saponin analysis. Soil samples were collected from a depth of 0–11 cm, approximately 2 cm from the roots of each plant, using a sterile spatula. Excess soil was vigorously shaken off the roots, leaving about 1 mm of soil. The root surface was then carefully wiped with a sterile brush, and the collected soil was stored in a disinfected bag. Soil samples were treated with liquid nitrogen and stored at -80 ℃ for microbiological analysis.

Microbial community analysis

For soil samples, DNA was extracted using the Magnetic Soil and Stool DNA Kit. For plant root samples, DNA was extracted using the CTAB extraction method. DNA purity and concentration were measured by agarose gel electrophoresis. Suitable DNA samples were placed into a centrifuge tube and diluted to 1 ng/μL with sterile water. The following primers were used for PCR amplification:

- Bacterial 16 S v4 region: 515 F (GTGCCAGCMGCCGCGGTAA) and 806 R (GGACTACHVGGGTWTCTAAT).
- ITS1-5 F region: ITS5-1737 F (CTTGGTCATTTAGAGGAAGTAA) and ITS2-2043R(GCTGCGTTCTTCATCGATGC).
- Plant bacterial 16S gene: 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3').
- Plant fungal ITS genes: ITS1-1 F-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS1-1 F-R (5'-GCTGCGTTCTTCATCGATGC-3').

The library was constructed using the TruSeq® DNA PCR-Free Sample Preparation Kit, and the constructed library was quantified by Qubit and Q-PCR. The qualified libraries were sequenced on the Illumina NovaSeq6000 platform at Novogene Biotechnology Co., Ltd., Beijing, China. Final data presentation: T1 is CK and T2 is biochar treatment. The same below.

Metabonomics analysis

100 mg plant root samples (six replicates per group) were weighed, frozen in liquid nitrogen, and the homogenate was resuspended with prechilled 80% methanol by well vortex. The samples were incubated on ice for 5 min and then were centrifuged at 15,000 g, 4 °C for 20 min. Some of supernatant was diluted to final concentration containing 53% methanol by LC-MS grade water. The samples were subsequently transferred to a fresh Eppendorf tube and then were centrifuged at 15,000 g, 4 °C for 20 min. Finally, the supernatant was injected into the LC-MS/MS system analysis. UHPLC-MS/MS analyses were conducted using a Vanquish UHPLC system coupled with an Orbitrap Q Exactive HF-X mass spectrometer (Thermo Fisher, Germany) at Novogene Co., Ltd. (Beijing, China). The compounds were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.kegg.jp/kegg/compound/). The differentially ab undant metabolites (DAMs) were identified with a variable importance in project (VIP) value ≥ 1 and an absolute log2 (fold change [FC]) ≥ 1. VIP values were obtained from the orthogonal partial least squares discriminant analysis (OPLS-DA) results using the R package MetaboAnalystR (https://www.metaboanalyst.ca/).

Determination of ginsenoside content

The ginsenoside content was determined as described previously [36]. Each root sample (0.5 g) was crushed and extracted using 70% methanol. The suspension was then subjected to ultrasonic treatment at 50 °C for 30 min, followed by filtration. The combined filtrate was dried at 45 °C using a rotary evaporator, and the residue was redissolved in 5 mL of methanol. After further filtration through a 0.22 μm membrane filter, ginsenosides were analyzed using an Agilent 1200 HPLC system (Agilent Technologies Co., Ltd.). Separation was achieved on a YMC-PACK ODA-A column (250 mm × 4.6 mm, 5 μm, YMC Co., LTD., Kyoto, Japan) with a mobile phase comprising acetonitrile (A) and 0.1% phosphoric acid solution (B). The gradient elution procedure was as follows: 0–25 min, A: 19–20%; 25–60 min, A: 20–40%; 60–90 min, A: 40–55%; 90–100 min, A: 55–60%. The mobile phase flow rate was set to 1 mL·min^{-1}. The detection wavelength was 203 nm, and the sample size was 20 μL. Ginsenoside standards (Rg1, Re, Rf, Rb1, Rb2, Rd, Rh1, Rh2) were obtained from Chengdu Manset Biotechnology Co., Ltd.

Statistical analysis

Statistical analysis was performed using SPSS 26.0. Tukey's test was used to assess differences in means, with

data expressed as mean ± standard deviation. Differences between the two groups were analyzed using Student's t-test with a significance level of < 0.05.

Result

Effects of Biochar on the metabolome of *P. quinquefolius* roots

To assess the impact of biochar on the metabolome of *P. quinquefolius* roots, a partial least squares discriminant analysis (PLS-DA) was performed on the differential metabolites (DAMs). The PLS-DA scores indicated significant metabolic differences between treatments (Fig. 1A). Compared to the CK, biochar treatment up-regulated 98 metabolites and down-regulated 101 metabolites (Fig. 1B).

Biochar application resulted in differences in the relative abundance of 199 DAMs (Tab S1). To analyze the response to biochar, heat maps of two sets of metabolite classes were generated (Fig. 1C). These included mainly 37 lipids and lipid-like molecules, 15 nucleosides, nucleotides, and analogues, and 8 organoheterocyclic compounds (Fig. 1D). First, lipids, which are key components of cell membranes and act as energy reserves [37], showed significant up-regulation. This includes glycerophospholipids, fatty acyls, steroids, steroid derivatives, and prenol lipids. Membrane lipid remodeling is an important adaptation strategy for plants under abiotic stress [38]. Notably, many of the up-regulated lipids have biological and medicinal activity, such as oleic acid, prohydrojasmon, jervine, brassinolide, timosaponin A1, vitamin A, (20R) ginsenoside Rg3, ginsenoside F2, poricoic acid B, notoginsenoside Fe, ginsenoside Rb1, protopanaxadiol, and ziyuglycoside II. Secondary nucleotides, crucial for energy metabolism and plant physiological processes [39], were also up-regulated. Examples include cytidine, isoguanosine, guanosine, adenosine, and uridine. Among these, purine nucleotides are precursors of cytokinin biosynthesis, which regulate plant growth and development and enhance abiotic stress tolerance. Finally, organoheterocyclic compounds were up regulated after biochar application. These include adenine, cytosine, guanine, 5-hydroxymeloxicam, 4-methylaminoantipyrine, (-)-chimonanthine, and indole-3-carboxylic acid. Additionally, biochar application increased other bioactive metabolites like targinine, schisandrin B, and galanthamine.

Using the KEGG database and previous studies, a metabolic pathway was constructed for the top 20 differential metabolites, highlighting the relationship between these compounds in the metabolic spectrum of differently treated *P. quinquefolius*. Figure 1E displays the significance of each pathway, determined by *p*-value and abundance factors; larger, darker bubbles indicate more significant pathways. Key pathways included purine and pyrimidine metabolism, zeatin biosynthesis, valine, leucine, and isoleucine degradation, propanoate metabolism, terpenoid backbone biosynthesis, brassinosteroid biosynthesis, and plant hormone signal transduction. These pathways help explain the differences between biochar and the root metabolites of *P. quinquefolius*. Notably, among the detected saponins, the relative content of ginsenosides increased with biochar treatment.

Ginsenoside is the primary active component of *P. quinquefolius* and a key indicator of its medicinal quality. As shown in Table 1, biochar treatment significantly increased the contents of ginsenosides Re, Rb_1, Rb_2, and Rh_2 by 22.20%, 16.97%, 44.00%, and 24.78% respectively, compared to the CK ($P < 0.05$). While the contents of ginsenosides Rg_1, Rh_1, and Ro also increased, these changes were not statistically significant ($P > 0.05$). Additionally, the content of ginsenoside Rd decreased significantly by 12.8% ($P < 0.05$).

Effects of Biochar on rhizosphere soil microorganisms of *P. quinquefolius*

The effects of biochar application on the rhizosphere soil microorganisms of *P. quinquefolius* are shown in Fig. 2. Figure 2A displays the top 10 amplicons of 16 S rRNA detected in all samples, mainly Proteobacteria, Acidobacteriota, and Bacteroidota. Compared to the CK, biochar treatment significantly increased the relative abundance of Bacteroidota by 60.55% ($P < 0.05$). The relative abundance of Acidobacteriota, Verrucomicrobiota, and Actinobacteria also increased. Figure 2B showed the top 10 amplicons of ITS detected in all samples, with Basidiomycota, Ascomycota, and Mortierellomycota being the main phyla.

Heat maps were used to describe the trend, distribution and abundance of the first 35 bacteria genera (Fig. 2C). Compared to the CK, biochar treatment increased the relative abundance of *Steroidobacter*, *Sphingomonas*, *Dongia*, *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, *Bacillus*, *Hirschia*, *Flavobacterium*, *Sphingobium*, *Terrimonas*, *Devosia*, and *Subgroup_10*. Conversely, it decreased the relative abundance of *Massilia*, *MND1*, *Candidatus_Koribacter*, *Burkholderia-Caballeronia-Paraburkholderia*, *Mucilaginibacter*, *Candidatus_Nitrosotalea*, and *Duganella*. The heat map described the trends, distribution, and abundance of the top 35 fungi genera (Fig. 2D). Compared to the CK, biochar treatment significantly enhanced the levels of *Tausonia*, *Solicoccozyma*, *Naganishia*, *Thanatephorus*, and *Pseudogymnoascus* but significantly reduced the levels of *Mortierella*, *Fusarium*, *Pseudaleuria*, and *Trichoderma* ($P < 0.05$).

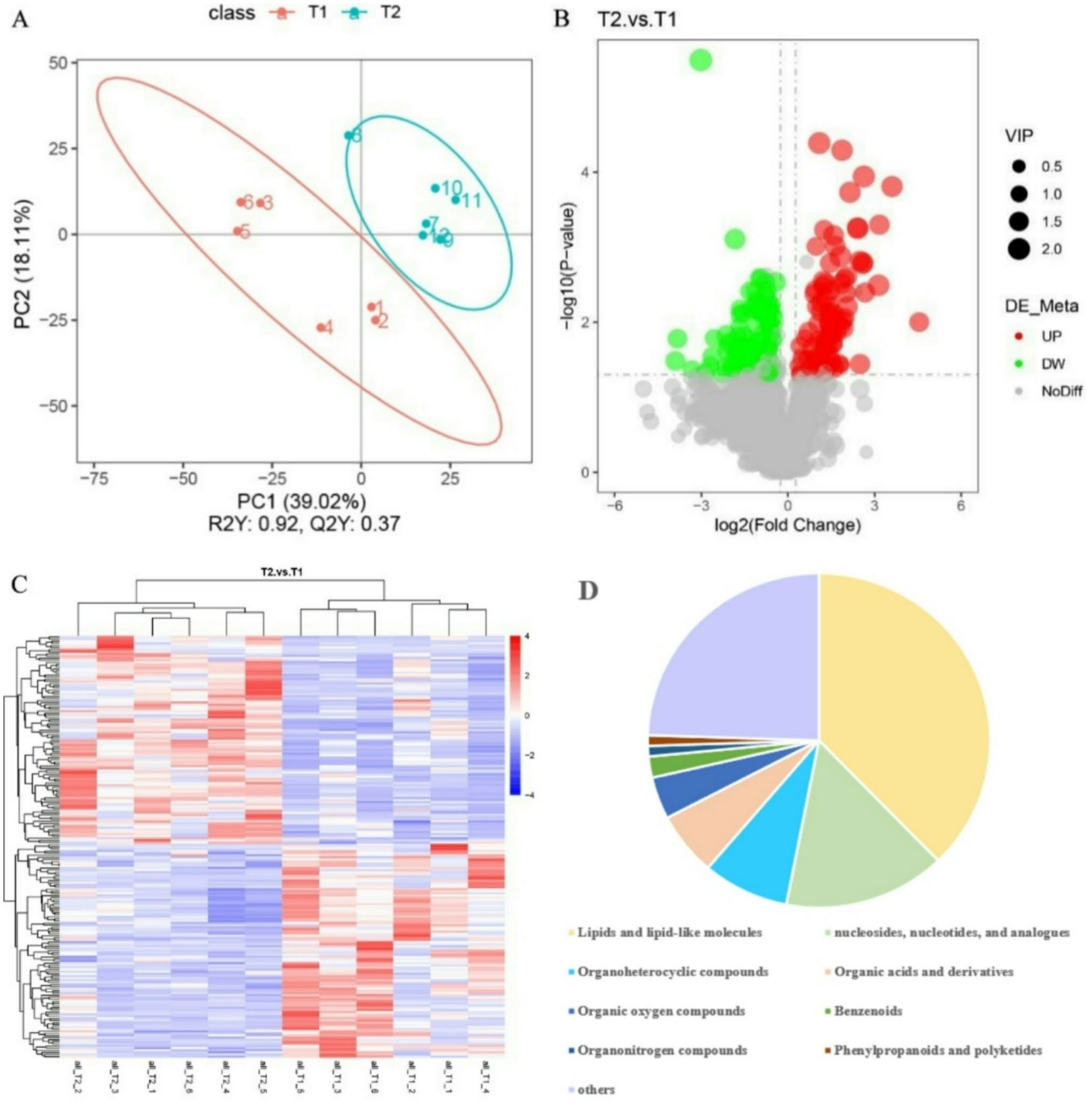

Fig. 1 Effects of biochar on rhizome metabolomics of *P. quinquefolius* roots. **A**: PLS-DA score chart. Each point in the diagram represents a sample, with samples from the same group represented by the same color. **B**: Volcano map. A dot in the figure represents a metabolite, with red and green colors indicating up-regulation and down-regulation, respectively. **C**: Heatmap analysis between different treatments (T2 vs. T1). **D**: Classification of 98 upregulated differential metabolites between different treatments (T2 vs. T1). **F**: KEGG enrichment analysis (T2 vs. T1). T1 is CK, T2 is biochar treatment

Effects of Biochar on endophytic diversity of *P. quinquefolius* roots

The effects of biochar on the endophytic diversity of *P. quinquefolius* are shown in Fig. 3. After clustering sample sequences with 97% similarity, the OTUs sparse curve becomes sufficiently saturated to reveal the diversity of microbial communities (Figs. 3A, B). A Venn diagram was constructed at the OTU level to analyze the composition of species in the samples. As shown in Figs. 2D and 3C, the number of bacterial and fungal OTUs in plant tissues was relatively large after biochar treatment, with a relatively small number of shared OTUs. This indicates that the composition of endophytic bacteria and fungi differed greatly between treatments.

The analysis of the sample species' abundance and diversity was conducted using the α diversity index

Table 1 Effect of Biochar on ginsenoside content of *P. quinquefolius* root

	T1	T2
Rg_1	1.73 ± 0.14	1.80 ± 0.13
Re	6.63 ± 0.26	5.42 ± 0.28**
Rb_1	5.81 ± 0.16	6.79 ± 0.25**
Rh_1	1.64 ± 0.09	1.90 ± 0.15
Ro	1.47 ± 0.05	1.68 ± 0.21
Rd	2.31 ± 0.12*	2.01 ± 0.05
Rb_2	0.27 ± 0.01	0.40 ± 0.05*
Rh_2	0.58 ± 0.06	0.73 ± 0.03*

T1 is CK, T2 is biochar treatment. Data were expressed as mean ± SD of three replicates. Different symbols indicated significant difference between different treatments (*$P < 0.05$,**$P < 0.01$, Student's *t*-test)

(Table 2). The Chao1 richness index estimated species richness, and the Shannon (H') index quantitatively described biodiversity based on species richness. Table 2 showed that, after applying biochar, the α diversity index (Chao1 and Shannon) of endophytic bacteria in *P. quinquefolius* increased. However, the Shannon index for fungi decreased significantly ($P < 0.05$). Beta diversity was evaluated at the OTU level to compare the endophytic community structures under different treatments. NMDS analysis was used to illustrate the between- and within-group differences of the samples (Figs. 3E, F). The results indicated that the *P. quinquefolius* samples could be well separated, and the endophytic bacterial communities were significantly different ($P < 0.05$).

Effect of Biochar on endophytes composition of *P. quinquefolius* roots

The effect of biochar on the endophyte composition of *P. quinquefolius* roots is shown in Fig. 4. The top 10 amplicons of 16 S rRNA detected in the two treatments are predominantly Proteobacteria, followed by Cyanobacteria, accounting for over 84% (Fig. 4A). The endophytic fungal communities of two treated *P. quinquefolius* samples were analyzed at the phylum, order, family, and genus levels. As illustrated in Fig. 4B, the primary phyla were Glomeromycota, Ascomycota, and Basidiomycota. Notably, the abundance of Glomeromycota significantly increased with biochar addition.

Heat maps describe the trend, distribution, and abundance of the top 35 bacteria genera (Fig. 4C). After biochar addition, the abundance of endophytic bacteria in *P. quinquefolius* roots, including *Neochlamydia*, *Subgroup_10*, *Bosea*, *Shinella*, *Reyranella*, *Polaromonas*, and *Flavobacterium*, increased significantly ($P < 0.05$). The heat map in Fig. 4D displays the trends, distribution, and abundance of the top 35 fungi genera. Compared to the

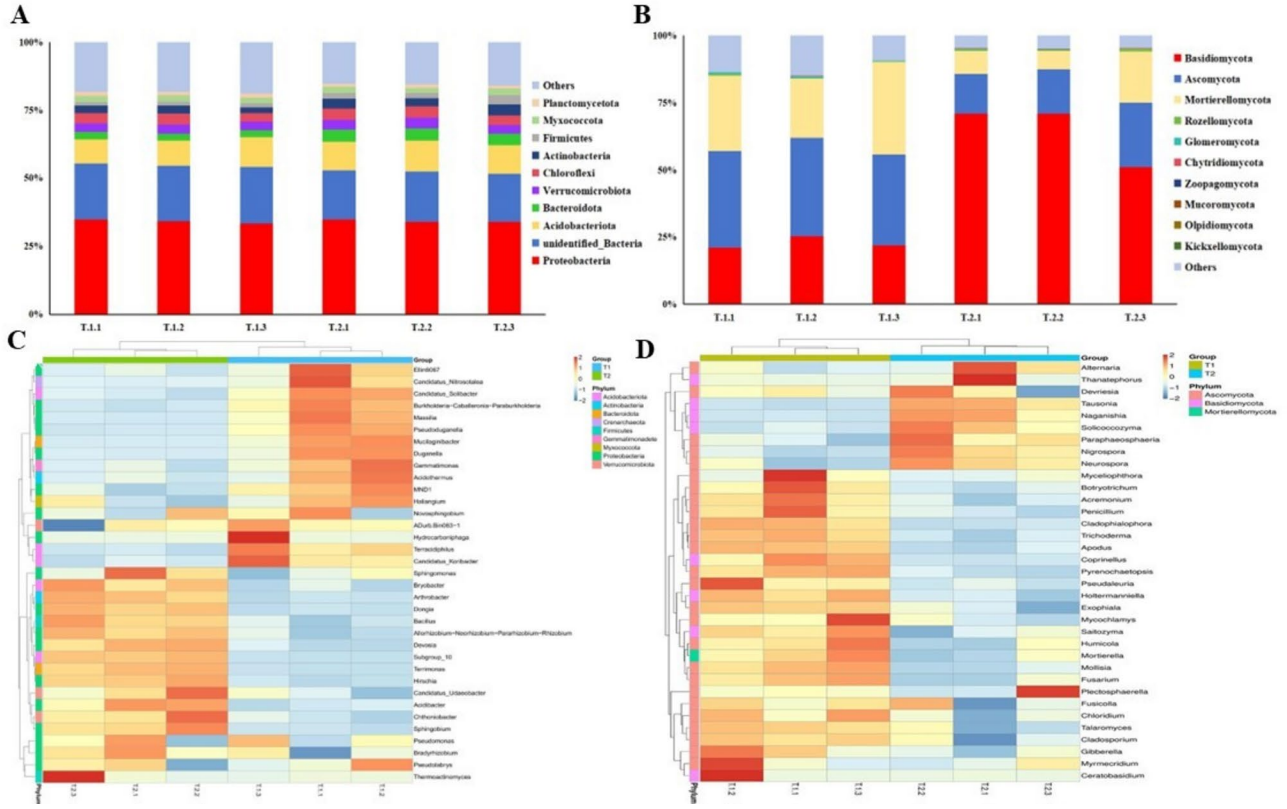

Fig. 2 Relative abundance of bacteria (**A**) and fungi (**B**) in the rhizosphere soil samples of *P. quinquefolius* at the phylum level. Heat maps of soil bacterial (**C**) and fungal (**D**) microbial communities at the genus level

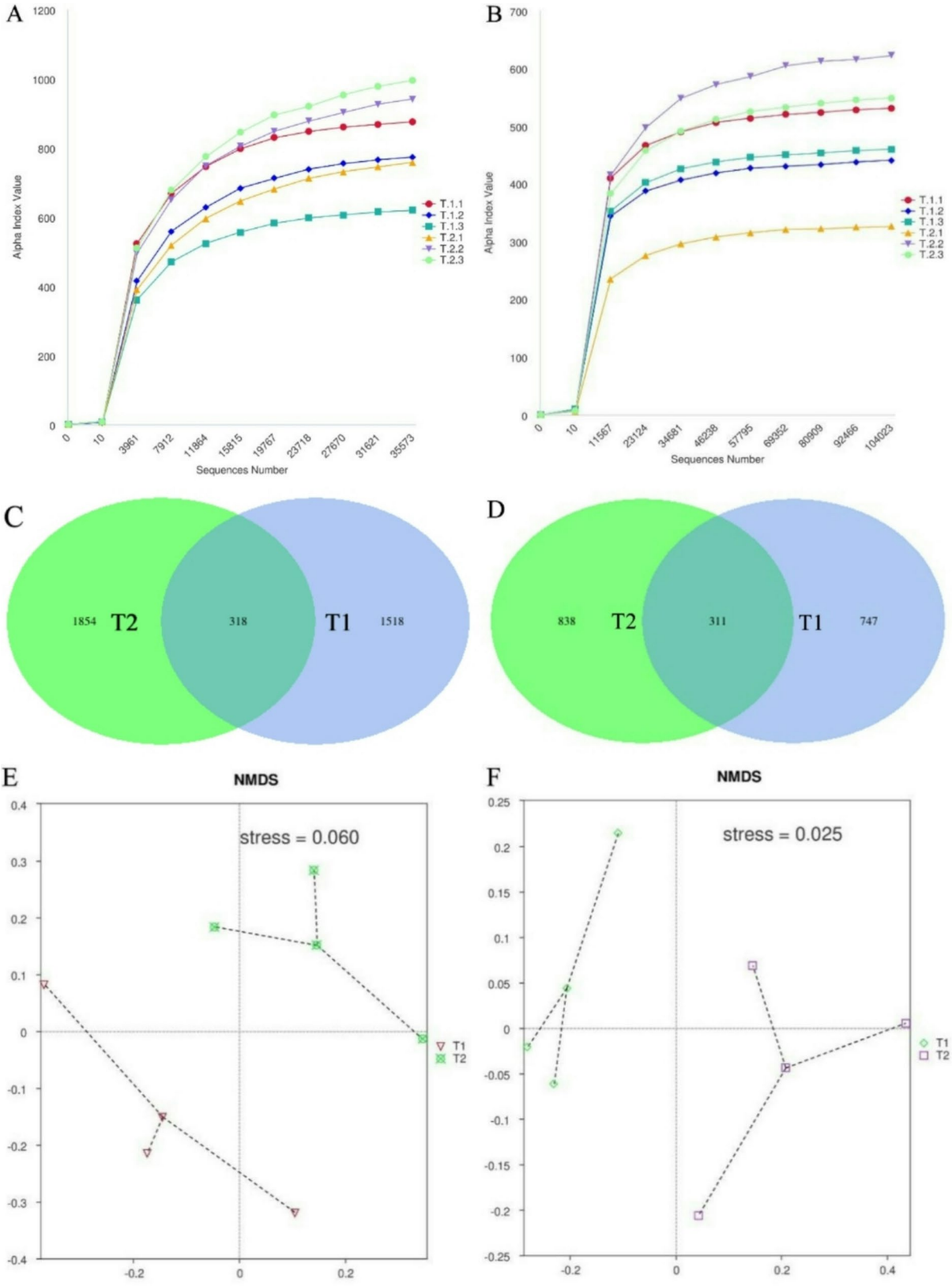

Fig. 3 Effects of biochar on endophytic bacterial diversity in *P. quinquefolius* roots. Rarefaction curves of endophytic bacteria (**A**) and fungi (**B**); Venn diagram of OTU distribution of endophytic bacteria (**C**) and fungi (**D**) in the different tissues of *P. quinquefolius*; Multi-sample NMDS analysis of endophytic bacteria (**E**) and fungi (**F**) in *P. quinquefolius* samples. T1 is control, T2 is biochar treatment. Each treatment has three replicates

Table 2 Effect of Biochar on α diversity of endophytic bacteria of *P. quinquefolius* root

	Bacteria		Fungi	
	Chao1	Shannon	Chao1	Shannon
T1	808.74 ± 150.30	5.46 ± 0.85	480.30 ± 48.85	5.91 ± 0.13
T2	1066.53 ± 136.24	6.32 ± 0.23	502.85 ± 89.83	5.08 ± 0.32*

T1 is CK, T2 is biochar treatment. Data were expressed as mean ± SD of three replicates

CK, biochar treatment significantly increased the relative abundance of beneficial fungi such as *Rhizophagus* and *Glomus* ($P < 0.05$). Conversely, it significantly reduced the relative abundance of certain pathogenic fungi, including *Plectosphaerella*, *Cladosporium*, and *Phaeosphaeria* ($P < 0.05$).

Correlation analysis between soil microorganism diversity and metabolites of *P. quinquefolius*

To study the impacts of soil microorganisms on the quality of *P. quinquefolius*, we performed a correlation analysis between soil microorganisms and plant metabolites. Figure 5 showed that fatty acyls, steroids and their derivatives, and prenol lipids positively correlated with *Mycobacterium*, *Shinella*, *Reyranella*, *Polaromonas*, and *Corymbiglomus*, while *Chloroplast* and *Sphingomonas* significantly and negatively correlated with these metabolites. Additionally, steroids and their derivatives positively correlated with *Sphingobium*, *Novosphingobium*, *Neochlamydia*, *Bosea*, *Rozellomycota_gen_Incertae_sedis*, *Acremonium*, and *Alloleptosphaeria*. Schisandrin B and galanthamine showed significant positive correlations with *Subgroup_10*, *Polaromonas*, *Methylotenera*, *Thanatephorus*, and *Zopfiella* ($P < 0.05$).

Correlation analysis between endophytic diversity and metabolites of *P. quinquefolius* roots

To understand the response of *P. quinquefolius* root metabolites to endophytic bacteria, we identified key differential secondary metabolites and conducted a correlation analysis with endophytic bacteria and fungi. Figure 6 reveals that prenol lipids positively correlated with *Chloroplast*, *Prevotella*, *Acidibacter*, *Sphingomonas*, *Armatimonadales*, *Burkholderia-Caballeronia-Paraburkholderia*, and *Pyrenochaetopsis*. Fatty acids and conjugates positively correlated with *Chloroplast*, *Sphingomonas*, *Armatimonadales*, and *Burkholderia-Caballeronia-Paraburkholderia*, but negatively correlated with *Pyrenochaetopsis*. Steroids and their derivatives positively

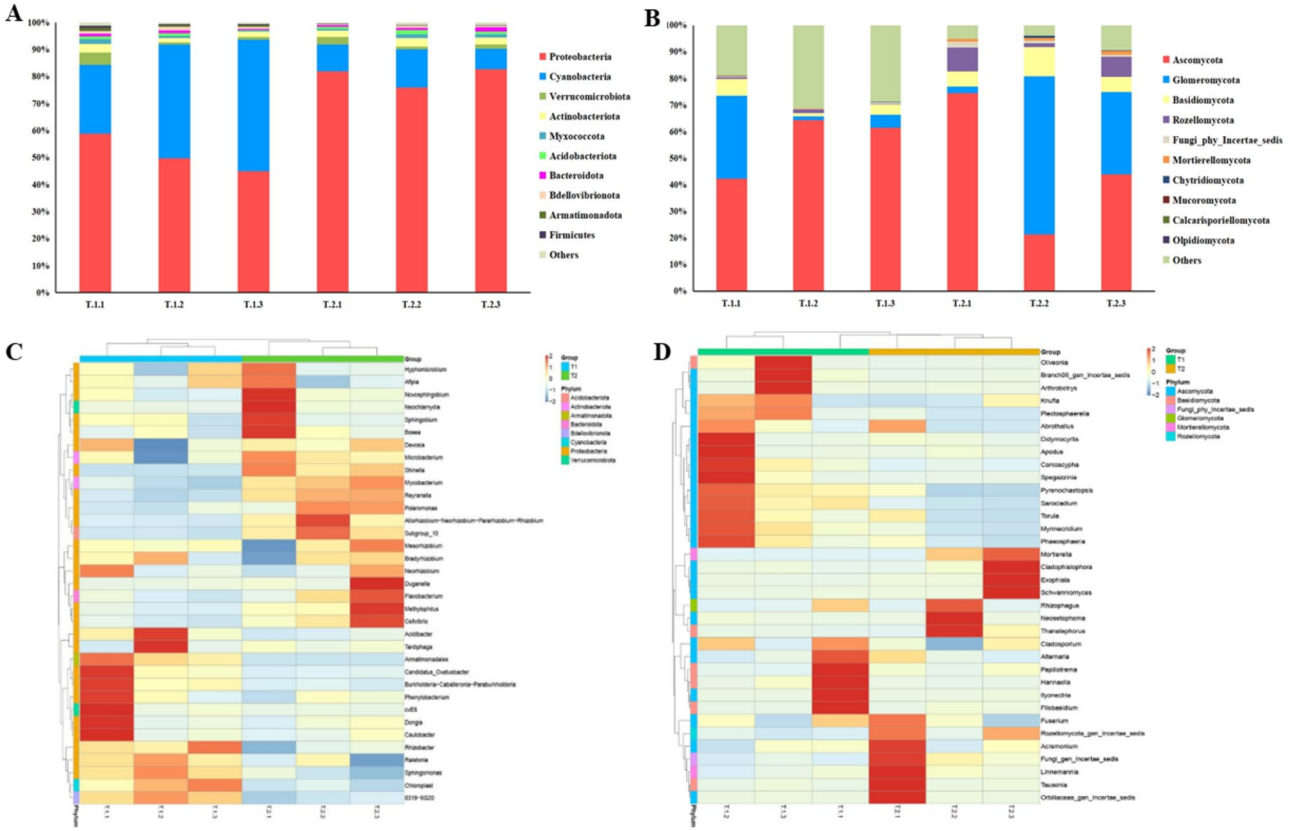

Fig. 4 Relative abundance of endophytic bacteria (**A**) and fungi (**B**) in *P. quinquefolius* samples at the gate level, and heat map of endophytic bacterial (**C**) and fungal (**D**) communities

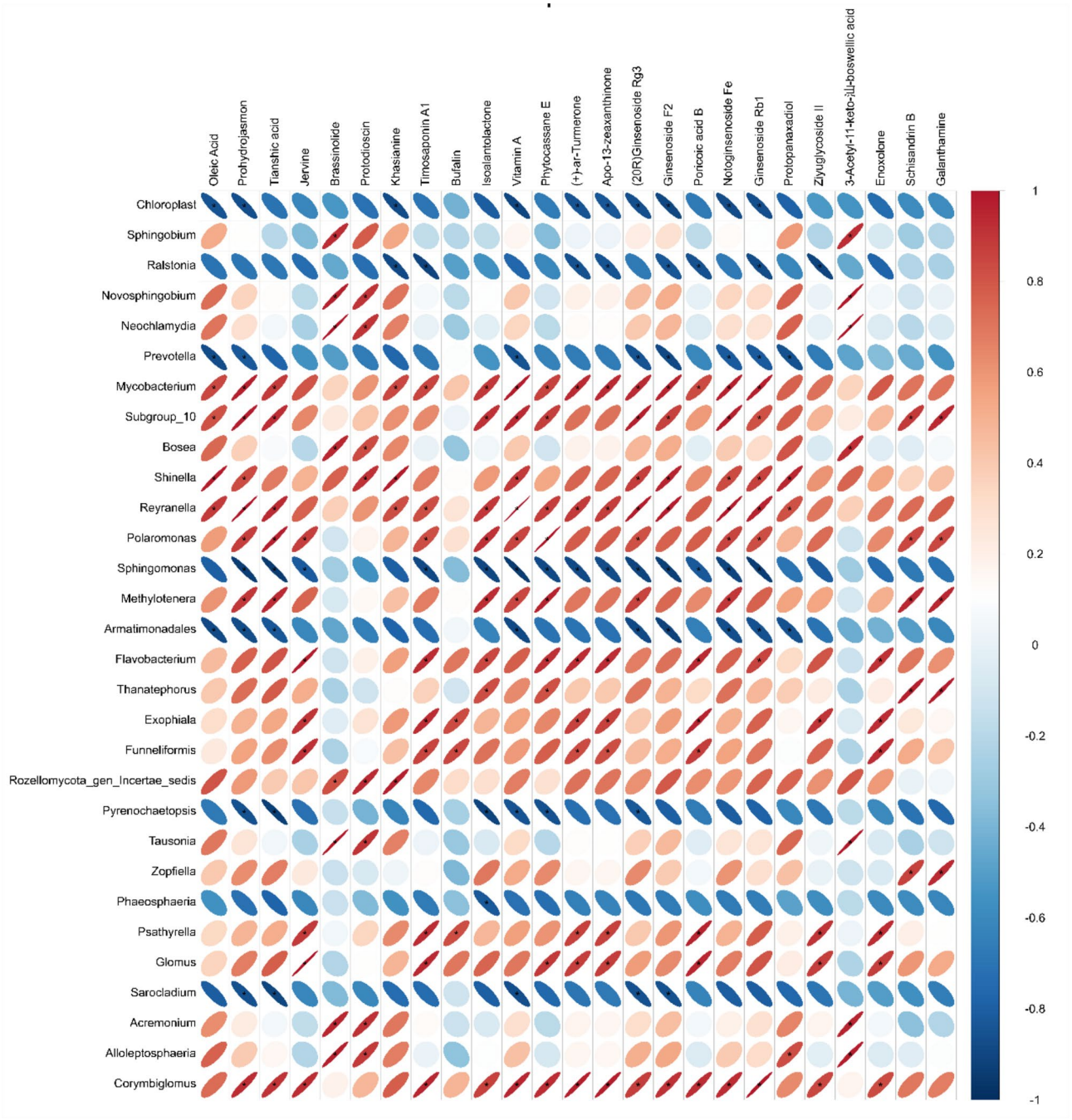

Fig. 5 Heat map of the correlation between rhizosphere microorganisms of *P. quinquefolius* and active secondary metabolites. Positive and negative correlations are marked in red and blue, respectively. Color intensity indicates the degree of correlation: significant ($P < 0.05$)

correlated with *Chloroplast*, *Phenylobacterium*, *Prevotella*, *Armatimonadales*, *Burkholderia-Caballeronia-Paraburkholderia*, *Tausonia*, and *Acremonium*. Notably, these bacteria and fungi (*Chloroplast*, *Prevotella*, *Sphingomonas*, *Armatimonadales*, *Burkholderia-Caballeronia-Paraburkholderia*, *Psathyrella*, *Glomus*) significantly related to most metabolites in the plants, suggesting their role in the synthesis and accumulation of metabolites in *P. quinquefolius*.

Discussion

The application of biochar was found to promote the reprogramming of secondary metabolites in *P. quinquefolius*. Notably, ginsenosides, which are key components for evaluating the quality of *P. quinquefolius* due to their immune-boosting, anti-tumor, and antioxidant properties [13], were generally up-regulated. Specific ginsenosides that showed increased levels include (20R) ginsenoside Rg3, ginsenoside F2, notoginseng saponin Fe,

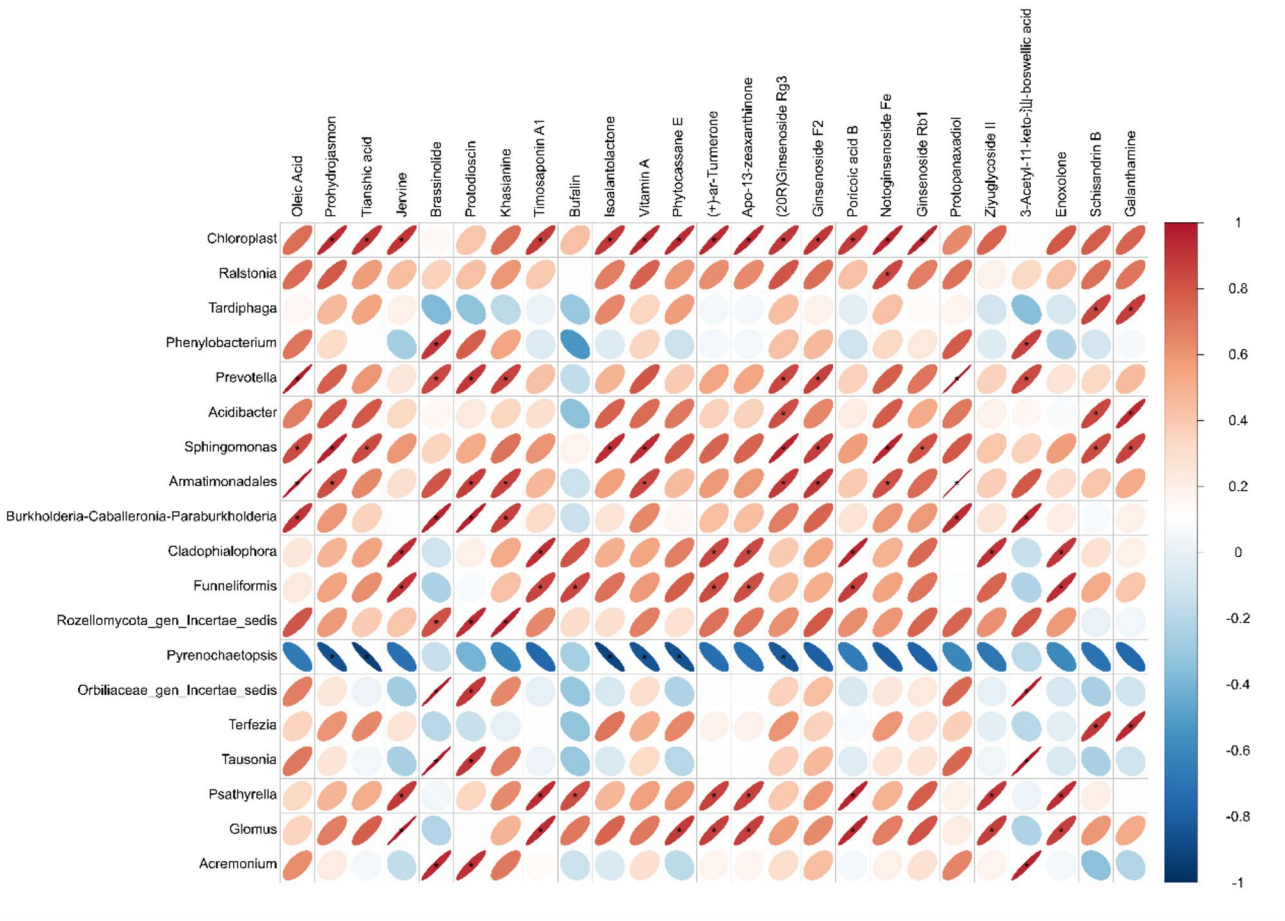

Fig. 6 Heat map of the correlation between *P. quinquefolius* endophytic and active secondary metabolites. Positive and negative correlations are marked in red and blue, respectively. Color intensity indicates the degree of correlation: significant ($P < 0.05$)

and ginsenoside Rb1. Besides ginsenosides, the relative contents of vitamin A, brassinolide, and galantamine also increased. Brassinolide plays a crucial role in enhancing plant stress resistance and quality by regulating the contents of unsaturated fatty acids, proline, phenolic compounds, and flavonoids [40, 41]. Galantamine is a primary ingredient in medications used to treat Alzheimer's disease, helping to reduce some of its symptoms [42]. In conclusion, biochar treatment can reprogram secondary metabolites of *P. quinquefolius* and improve the quality of *P. quinquefolius*.

The microbiome is a critical component for maintaining plant biomass production, soil fertility, and overall plant health [43]. Following biochar treatment, the soil bacterial community changed significantly. The relative abundance of *Sphingomonas*, *Bacillus*, and *Flavobacterium* increased, while *Bacteroides* and *Solicoccozyma* increased significantly.They can enhance soil health by improving the soil environment. Specifically, *Sphingomonas* and *Solicoccozyma* can degrade chemical pesticides like glyphosate [44, 45], and *Bacteroides* thrives in conditions of lower salinity and soil moisture [46], indicating an improved cultivation environment for *P. quinquefolius*. *Bacillus* and *Flavobacterium* promote plant growth, inhibit plant diseases, and enhance plant resistance to abiotic stress [47–50]. In addition, the addition of biochar also decreased significantly the relative abundance of *Fusarium.* The decline of *Fusarium*, a major cause of soil-borne diseases in *P. quinquefolius*, can reduce the incidence of plant diseases [20]. Correlation studies reveal that *Mycobacterium*, *Reyranella*, *Flavobacterium*, and *Corymbiglomus* in soil are positively correlated with active secondary metabolites. Manero et al. found that rhizosphere probiotics can enhance the secondary metabolism of the host plant *Hypericum perforatum*, increasing its medicinal components [51]. After biochar treatment, rhizosphere microbial community changes were significantly correlated with the accumulation of secondary metabolites in *P. quinquefolius*. This suggests that shifts in the rhizosphere microecology may be a key mechanism by which biochar stimulates the secondary metabolism of *P. quinquefolius*.

Endophytes are initially adapted by soil bacteria to the plant rhizosphere [52] and then colonize the plant root surface and some rhizodermal cells [53]. Changes in soil microorganisms may induce structural changes in endophytes. Biochar treatment increased the abundance of certain endophytic bacteria (*Pseudomonas* and *Flavobacterium*) and fungi (*Rhizophagus*, *Cladophialophora*, *Acaulospora*, and *Glomus*) in *P. quinquefolius*. *Pseudomonas* and *Flavobacterium* effectively colonize the plant environment, promoting growth and antagonizing plant pathogens [54, 55]. *Cladophialophora* enhances seedling and plant growth [56]. Arbuscular mycorrhizal fungi (AMF), including *Rhizophagus*, *Acaulospora*, and *Glomus*, promote plant growth and development, and affect the biosynthesis of plant secondary metabolites like phenols, flavonoids, and terpenes [28, 57]. Conversely, the abundance of *Plectosphaerella* and *Phaeosphaeria* decreased significantly. The reduction of these pathogens suggests improved plant health after biochar treatment. Correlation studies reveal that *Chloroplast*, *Sphingomonas*, *Cladophialophora*, and *Glomus* are positively correlated with ginsenosides, while *Tausonia* and *Acremonium* show significant correlation with brassinolide. During the co-evolution of endophytes and plants, endophytes can produce unique secondary metabolites and induce their synthesis in host plants, as demonstrated in *Salvia miltiorrhiza* Bunge, *Bletilla striata*, and *Codonopsis pilosula* [58–60]. After biochar treatment, changes in endophytes were significantly related to the accumulation of secondary metabolites in *P. quinquefolius*. This suggests that alterations in endophytes may be a crucial mechanism by which biochar influences the secondary metabolism of *P. quinquefolius*.

Conclusion

In this study, it was found that biochar application upregulated the accumulation of key metabolites such as terpenoids and nucleosides, glycerophosphocholines, fatty acyls, steroidal glycosides. In addition, the structure and function of rhizosphere microorganisms were changed after biochar treatment, especially *Flavobacterium* showed a positive correlation with secondary metabolites such as ginsenoside Rb1. Moreover, the diversity structure and function of endophytes were also changed with biochar treatment, especially *Sphingomonas* was positively correlated with secondary metabolism such as ginsenosides Rb_1, Rg_3, F_2. In summary, biochar facilitates a complex microbe-plant metabolic reprogramming that improves the overall quality of medicinal materials. This finding provides valuable insights into the potential use of biochar in traditional Chinese medicine agriculture.

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Author contributions
XL.C. performed the experiments, drafted the manuscript's main sections and contributed to data analysis and interpretation of results. XY.M., Y.D., T.C., Y.W., J.B., and L.F. conducted data analysis, provided technical support for experimental methods, and reviewed the data for accuracy. LP.G. and J.Z. conceived the research topic, conducted the overall planning and supervision of the study.

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Data availability
The sequencing data generated in the study are deposited to the NCBI SRA database under Bioproject No. PRJNA1254450, PRJNA1254922, PRJNA1255382 and PRJNA1255842.

Declarations

Ethics approval and consent to participate
Plant materials (*P. quinquefolius*) used in the experiments were from Weihai Wendeng District Dao-di ginseng industry Development Co. LTD (Weihai, China). All plant materials (not endangered materials or species) were provided free of charge, and comply with local institutional guidelines and legislation.

Consent for publication
The authors have approved of publication, and there is no conflict of interest. All the authors equally approve of publication.

Competing interests
The authors declare no competing interests.

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