

Article

Metabolomics and Microbiomics Reveal the Cultivation-Dependent Divergence in Ginsenoside Biosynthesis and Rhizosphere Ecology of *Panax ginseng*

Siqi Liu ^{1,†}, Dehua Wu ^{2,†} , Wenqi Ma ¹, Tielin Wang ¹, Binbin Yan ¹ , Yang Ge ¹, Feng Xiong ¹, Hongyang Wang ^{1,*} and Chuanzhi Kang ^{1,2,*} 

¹ Key Laboratory for Quality Assurance and Sustainable Use of Dao-di Herbs, National Resource Center for Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China; lsikeycong@163.com (S.L.); mwq1224@163.com (W.M.); wtl82@163.com (T.W.); yb51598@126.com (B.Y.); geyang@nrc.ac.cn (Y.G.); xfzyzy@163.com (F.X.)

² Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China; w3495305651@163.com

* Correspondence: whybio@126.com (H.W.); kangchuanzhi1103@163.com (C.K.)

† These authors contributed equally to this work.

Abstract

Background: Cultivation environments impose distinct abiotic and biotic stresses that act as primary drivers reshaping the metabolic profile and microbiome assembly of medicinal plants. This study investigates the impact of simulative habitat versus arched greenhouse cultivation on the synthesis of bioactive ginsenosides and the associated root microbiome structure in *Panax ginseng*. **Methods:** A combined metabolomics and microbiomics approach was applied to compare ginsenoside accumulation and rhizosphere microbial community composition under the two cultivation modes. **Results:** Ginseng from simulative habitat cultivation exhibited significantly higher ginsenoside content, particularly ginsenoside Re, compared to arched greenhouse cultivation, with this advantage being more pronounced in long-term cultivation. Microbiome profiling revealed that specific taxa, including *Bradyrhizobium*, were strongly enriched in simulative habitats and positively correlated with enhanced ginsenoside accumulation, suggesting a microbiome-mediated mechanism for metabolic plasticity. In contrast, arched greenhouse cultivation was associated with a more complex microbial structure characterized by increased negative interactions, which may compromise metabolic quality. **Conclusions:** These findings, utilizing multi-omics correlations, provide a theoretical basis for optimizing *Panax ginseng* quality through ecological cultivation strategies that leverage stress-responsive microbe–metabolite interactions.

Keywords: *Panax ginseng*; stress-induced metabolism; microbe-metabolite interaction; simulative habitat cultivation; rhizosphere microbe



Academic Editor: Wolfgang Eisenreich

Received: 13 January 2026

Revised: 4 February 2026

Accepted: 12 February 2026

Published: 18 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Panax ginseng, a rare and valuable traditional Chinese medicinal herb, has a long history of medicinal use and a huge international market. Ginseng has the effects of tonifying qi, regulating qi, curing deficiency, strengthening the spleen and benefiting the lungs. It is mainly used for symptoms such as weakness, cold extremities with a faint pulse, and spleen deficiency with a reduced appetite [1]. Ginsenosides are considered

to be the main active chemical components for the pharmacological effects of ginseng; thus, ginsenoside content is considered a standard for evaluating ginseng quality. To date, more than 50 ginsenosides have been isolated from ginseng roots and rhizomes, and have a wide range of biological activities [2,3], such as anti-inflammatory, anti-cancer, and immune-boosting effects [4–6].

Panax ginseng is native to the Far East and North America, with major production areas in China and Korea [7]. A total of 70% of the world's ginseng is produced in China, and mainly cultivated in the northeastern regions of Jilin, Liaoning, and Heilongjiang [8]. There are two primary cultivation methods for ginseng: simulated wild cultivation and greenhouse cultivation. Simulated wild cultivation refers to a farming method in the process of growing Chinese medicinal herbs that aims to replicate the wild habitat of medicinal plants as closely as possible [9], particularly imitating the original ecological environment of authentic medicinal materials, to complete the entire growth and development cycle of the plant. In ginseng cultivation, this is also known as forest farming. It involves sowing ginseng seeds in forests that resemble the natural habitat of wild ginseng, with minimal or no human intervention, allowing the ginseng to grow under natural conditions [7]. Habitat-mimicking cultivation of ginseng usually takes more than 15 years for the plants to reach maturity, resulting in a longer growth cycle. However, forest-produced ginseng is closer in appearance and quality to wild ginseng and is therefore popular among consumers [10].

Greenhouse cultivation is a method of growing ginseng on farmland, usually using blue plastic film as a shade canopy over the soil, rather than being placed directly on the soil. This shading method alters the spectral quality of the light that reaches the ginseng, increases planting density, and relies heavily on fertilizers and pesticides. As a result, ginseng grown under greenhouse conditions is prone to soil-borne diseases and needs to be harvested within approximately 5 years to avoid infections by pathogens such as *Fusarium cerealis* and *Ilyonectria robusta*, which respectively cause root rot [11] and rusty root disease [12]. These infections will decrease the yield losses and quality.

Various microorganisms exist in soil and are a crucial component of the soil ecosystem [13]. Long-term monoculture of ginseng can easily lead to an imbalance in the soil microbial community, especially the invasion and establishment of pathogenic microorganisms causing soil-borne diseases, ultimately resulting in outbreaks of these diseases [14]. *Fusarium* species are the primary pathogens of soil-borne diseases in ginseng [15]. The severity of soil-borne diseases in ginseng increases with the length of the cultivation period, leading to reduced survival rates. This situation occurs mainly in arched-greenhouse-cultivated ginseng, whereas simulative-habitat-cultivated ginseng can grow healthily for 15–20 years or even longer. Studies have shown that the accumulation levels of soil-borne pathogens such as *Ilyonectria*, *Fusarium*, *Gibberella*, and *Cylindrocarpon* do not increase with the extension of the cultivation period of simulative-habitat-cultivated ginseng [10]. In order to reduce the incidence of disease and improve survival rates and yields in arched-greenhouse-cultivated ginseng, growers often use large amounts of fertilizers, pesticides, and growth-promoting agents. This practice leads to increased pesticide residues, insufficient levels of active medicinal components, and significant differences in appearance from wild ginseng [16].

In this study, we hypothesize that the cultivation mode dictates ginseng quality through microbiome-mediated metabolic reprogramming. By selecting *P. ginseng* samples grown in simulative habitats and arched greenhouses within geographically comparable locations, we applied a combined metabolomics and microbiomics approach. This research aims to characterize the associations between rhizosphere microbial community structure and ginsenoside accumulation profiles, and to identify microbial taxa that are consistently associated with ginsenoside-related quality traits. The findings provide a data-driven

basis for ecological cultivation strategies and for prioritizing candidate microbial taxa for future validation. However, because this study is observational and correlation-based, targeted functional experiments will be required before microbial interventions can be recommended to enhance ginsenoside accumulation and ginseng quality.

2. Materials and Methods

2.1. Sample Collection

In September 2021, samples of well-grown, uniformly sized *Panax ginseng* were randomly collected from mixed coniferous forests and agricultural fields in Caiyuan Town, Ji'an City, Jilin Province, China, to investigate 2 distinct cultivation regimes: simulative habitat cultivation (SH) located in mixed coniferous forests mimicking wild environments, and arched greenhouse cultivation (AG) situated in agricultural fields. The study design employed a stratified random sampling strategy, selecting three independent fields for each cultivation pattern and harvesting four samples per field ($n = 12$ per group; Supplementary Table S1). The experimental groups consisted of 5-year and 15-year ginseng from the SH regime (designated as SH5 and SH15, respectively) and 5-year ginseng from the AG regime (designated as AG5). Each biological replicate was fractionated into three compartments: rhizomes (R), rhizosphere soil (RS, defined as soil adhering within 0–3 mm of the rhizome surface), and bulk soil (BS). Immediately following collection, soil samples were preserved in sterile bags containing dry ice, while plant tissues were stored in ice boxes at low temperatures; all samples were transported to the laboratory on ice and processed subsequently.

2.2. Sample Preparation and DNA Extraction

Bulk soil and rhizosphere samples were transported to the laboratory on dry ice and immediately stored at -80°C . The *P. ginseng* rhizomes were first rinsed thoroughly with tap water. A 1 cm segment was cut 2 cm below the shoot base for endophytic bacteria analysis (Supplementary Figure S1). The remaining portion was freeze-dried for ginsenoside content analysis. Under sterile conditions, the 1 cm segments were rinsed three times with sterile water and soaked in 70% ethanol for 1 min; the ethanol was discarded, and then the sample was soaked in 12% sodium hypochlorite solution for 3 min, followed by five washes with sterile water [17]. The FastDNA[®] Spin Kit for Soil (MP Biomedicals, Solon, OH, USA) was used to extract total DNA from soil and plant tissues. The DNA extracts were verified on a 1% agarose gel, and DNA concentration and purity were determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Waltham, MA, USA). The samples were then sent to Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China) for sequencing.

2.3. Determination of Ginsenosides Content

The lyophilized *P. ginseng* samples were pulverized into fine powder using a ball mill. Precisely 0.15 g of the powder, sieved through a 60-mesh screen, was combined with 1.5 mL of 70% methanol. The mixture was weighed and then extracted ultrasonically for 60 min (250 W, 40 kHz). We allowed it to cool naturally to room temperature; the weight was compensated with an additional 70% methanol. Following centrifugation at 13,000 rpm for 10 min, the supernatant was passed through a 0.22 μm membrane filter and diluted fivefold to obtain the final test solution.

Liquid chromatography was carried out on a Waters ACQUITY UPLC HSS T3 column (2.1 mm $\times$ 100 mm, 1.8 μm) (Milford, MA, USA). The mobile phase comprised 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B), with the following gradient program: 0–2.5 min, 28–31% A; 2.5–3 min, 31–35% A; 3–4.5 min, 35–36% A; 4.5–6 min,

36–37% A; 6–7 min, 37–50% A; 7–9 min, 50–98% A; 9–11 min, 98% A; 11–12 min, 98–28% A; and 12–15 min, 28% A.

Mass spectrometric detection was performed with an Ion source: Turbo V; Ionization mode: ESI (−); Source temperature: 550 °C; Data acquisition: MRM. The contents of ginsenosides Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2, and Ro were quantified using external standards. All reference standards (purity $\geq$ 98%) were supplied by Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

2.4. 16S/ITS rRNA Amplification and Sequencing

The V5–V7 hypervariable regions of the 16S rRNA genes from endophytic and rhizospheric bacteria were amplified via a two-step PCR approach. In the initial round, amplification was performed using primers 779F (5'-AACMGGATTAGATACCCCKG-3') and 1392R (5'-ACGGGCGGTGTGTRC-3') [18,19]. A subsequent nested PCR employed the forward primer 779F and the reverse primer 1193R (5'-ACGTCATCCCCACCTTCC-3') [20].

Each PCR reaction mixture (25 μ L total volume) comprised 0.4 μ L of FastPfu DNA Polymerase (5 U/ μ L), 0.8 μ L of each primer (10 μ M), 4 μ L of 5 $\times$ buffer, 2 μ L of dNTPs (2.5 mM), 1 μ L of template DNA, and 17.5 μ L of ddH₂O. Following purification, the amplicons were pooled in equimolar amounts and subjected to paired-end sequencing (2 $\times$ 300 bp) on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA). All library preparation and sequencing procedures were conducted by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) following established protocols.

2.5. Data Processing and Statistical Analyses

All data processing was conducted on the Majorbio Cloud Platform. Raw sequencing reads were quality-filtered and dereplicated. High-quality sequences were clustered into Operational Taxonomic Units (OTUs) at 97% sequence similarity using UPARSE (v11), generating an OTU abundance table [21]. Taxonomic assignment was performed against the SILVA 16S rRNA database (v138) with a confidence threshold of 0.7. OTUs annotated as chloroplast or mitochondria were removed prior to downstream analyses. For consistency with this OTU-based workflow, between-group differential abundance and co-occurrence network analyses were conducted using the OTU table. We note that 97% OTU clustering has lower resolution than ASV-based denoising approaches and may merge closely related sequence variants; this limitation is acknowledged in the revised manuscript.

Microbial community composition was visualized at the phylum and genus levels using bar plots, and Rank-Abundance curves were generated, both with R software (v3.3.1). Alpha diversity indices (Shannon and Sobs) were computed using *mothur* (v1.30.2) [22]. Beta diversity was assessed via principal coordinates analysis (PCoA) based on Bray–Curtis distances in *QIIME* (v1.9.1), with statistical significance tested by ANOSIM.

For intergroup comparisons, the Kruskal–Wallis rank-sum test was applied using R (stats package, v3.3.1) and Python (scipy package, v1.0.0), with False Discovery Rate (FDR) correction for multiple comparisons. Species interaction networks were constructed through univariate analysis using *Networkx* (v1.11) [23]. Spearman's rank correlations and corresponding *q*-values were calculated with the R *psych* package [24], and correlation heatmaps were visualized using the *pheatmap* package in R [25].

Statistical analysis was conducted using SPSS 19.0. One-way ANOVA followed by Tukey's test ($p < 0.05$) was applied to compare ginsenoside contents. Figures were prepared with GraphPad Prism 8.0.1.

3. Results

3.1. Comparison of Ginsenoside Profiles Across Cultivation Modes and Ages

The Chinese Pharmacopoeia (2020 edition) stipulates that ginsenoside Rb1 in dried ginseng products shall not be less than 0.20% (2 mg/g), and the sum of ginsenoside Rg1 and ginsenoside Re shall not be less than 0.30% (3 mg/g) [1]. In this study, all *P. ginseng* samples collected under different cultivation modes and ages met the requirements of the Chinese Pharmacopoeia for $Rg1 + Re \geq 3 \text{ mg/g}$ (Supplementary Table S2). However, there were significant differences in the content of Rb1. A total of 66.7% and 50.0% of the samples in the AG5 and SH5 groups satisfied $Rb1 \geq 2 \text{ mg/g}$, respectively, while all of the SH15 group satisfied $Rb1 \geq 2 \text{ mg/g}$. According to the requirements of the Chinese Pharmacopoeia, the qualification rate of the SH15 samples reached the highest level, followed by AG5, while the qualification rate of SH5 samples was the lowest level.

In addition, except for ginsenosides Rb3, Rc, Re, Rg2, and Ro, the content of the other five ginsenosides, Rg1+Re, and the sum of 10 ginsenosides in SH15 were significantly higher than those in AG5 and SH5 (Figure 1C–N). The content of ginsenoside Re in SH5 and SH15 was significantly higher than that in AG5 (Figure 1H). Although no statistically significant differences were detected between SH5 and AG5 regarding mean Rb1 and Rg1+Re concentrations ($p > 0.05$), the SH5 group displayed numerically higher values (Figure 1C,M). Collectively, these chemical profiles suggest that SH15 represents the superior quality phenotype, followed by SH5 and AG5. However, in addition to the planting pattern, the length of cultivation could also be responsible for such results.

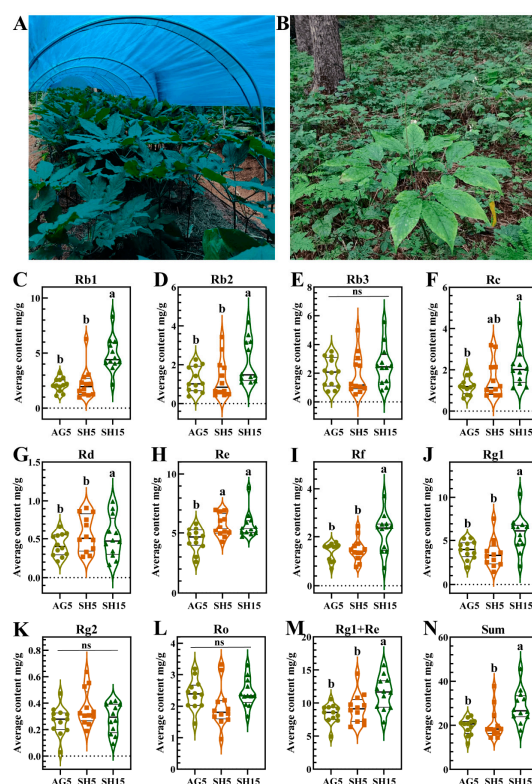


Figure 1. Growth environment and ginsenoside content of *P. ginseng*. Arched greenhouse cultivation (A) and simulative habitat cultivation (B) of *P. ginseng*. (C–L): The content of ginsenoside Rb1, Rb2, Rb3, Rc, Rd, Re, Rf, Rg1, Rg2 and Ro. (M): The sum of the contents of ginsenoside Rg1 and Re. (N): The sum of the 10 ginsenoside contents mentioned above. AG5: *P. ginseng* cultivated in an arched greenhouse for 5 years. SH5: *P. ginseng* cultivated in the forest for 5 years. SH15: *P. ginseng* cultivated in the forest for 15 years. Significance was calculated using ANOVA ($n = 12$). a and b represent $p < 0.05$.

3.2. Alpha Diversity Analysis of *P. ginseng* Rhizosphere and Rhizome Endophytic Bacteria

To investigate the link between the microbial communities (both rhizospheric and endophytic) of *P. ginseng* and its quality, high-throughput sequencing of bacterial conserved regions was performed on a total of 108 samples. These comprised 36 bulk soil samples, 36 rhizosphere soil samples, and 36 rhizomes from *P. ginseng*. After quality control, 16,413 bacterial effective sequences were normalized to the smallest sample size, yielding a final dataset of 1,772,604 high-quality sequences from the V5-V7 region of the bacterial 16S rRNA gene. Rarefaction curves, which approached plateaus, confirmed that the sequencing depth was sufficient to capture the majority of microbial diversity across all samples (Supplementary Figure S1A,B).

The α -diversity of the microbial communities was assessed using the Sobs richness index and the Shannon diversity index at the OTU level (Figure 2). Under arched greenhouse cultivation, both the richness and diversity of bacteria in the rhizosphere soil of *P. ginseng* were significantly lower than those in the unplanted bulk soil (Figure 2A–D), suggesting that planting activity exerted the strongest influence on rhizosphere bacterial community structure. No significant differences were observed in bulk soil bacterial richness or diversity among the AG5, SH5, and SH15 conditions. In contrast, the rhizosphere soil bacterial richness and diversity in SH5 and SH15 were markedly higher than those in AG5 (Figure 2A,B).

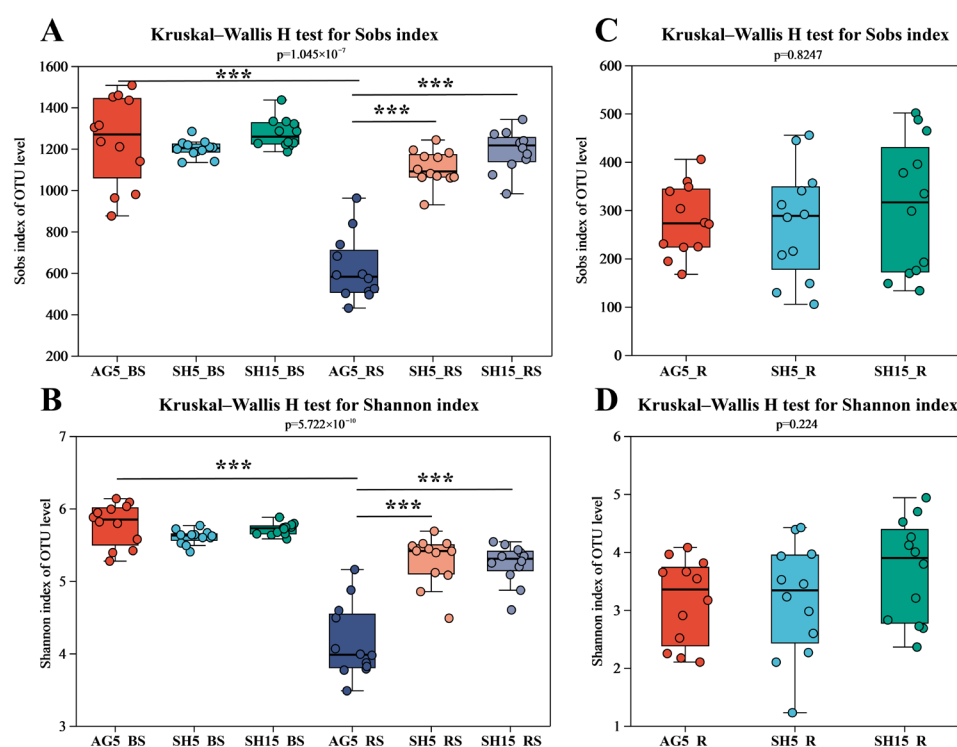


Figure 2. Estimation of the microbial community by α -diversity. (A–D): α -diversity of bacterial communities. AG5: *P. ginseng* cultivated in an arched greenhouse for 5 years. SH5: *P. ginseng* cultivated in the forest for 5 years. SH15: *P. ginseng* cultivated in the forest for 15 years. BS: Bulk soil. RS: Rhizosphere soil. R: Endophytic rhizomes. Estimation of α -diversity representing 12 biological replicates (***) $p < 0.001$.

3.3. Composition and Structure of the Bacterial Community

At the phylum level, the top five phyla of rhizosphere bacteria in ginseng roots are mainly composed of *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Acidobacteriota*, and *Myxococcota* (Figure 3A). Compared to SH5 and SH15, the rhizosphere bacteria of AG5 had fewer *Myxococcota*, with *Chloroflexi* being the fifth most abundant phylum. The

top five phyla of endophytic bacteria in the roots of AG5 and SH5 treatment groups were Proteobacteria, Actinobacteriota, Firmicutes, Bacteroidota, and Myxococcota, while in SH15, the fifth most abundant phylum was Acidobacteriota instead of Myxococcota (Figure 3A, Supplementary Table S3). At the genus level, there were significant differences in rhizosphere and endophytic bacteria among the three groups (Figure 3B, Supplementary Table S3). The top three genera of rhizosphere soil bacteria in AG5 were *Burkholderia-Caballeronia-Paraburkholderia* (21.24%), *Rhodanobacter* (9.75%), and *Mycobacterium* (8.81%). In SH5, the top three genera were *Bacillus* (11.56%), *Mycobacterium* (5.90%), and unclassified lineages within *Xanthobacteraceae* (5.32%). In SH15, the top three genera were unclassified lineages within *Xanthobacteraceae* (10.44%), *Bradyrhizobium* (8.79%), and *Mycobacterium* (5.24%). The top three genera of endophytic bacteria in the roots of AG5 were *Delftia* (21.09%), *Amycolatopsis* (14.06%), and *Mycobacterium* (12.51%). In SH5, the top three genera were *Delftia* (29.95%), *Pseudomonas* (10.88%), and *Sphingomonas* (6.32%). In SH15, the top three genera were *Delftia* (18.52%), *Sphingomonas* (13.25%), and *Stenotrophomonas* (5.46%). *Delftia* was the most abundant genus among the endophytic bacteria in ginseng roots across all three groups, indicating that *Delftia* is specifically enriched in ginseng roots regardless of cultivation mode and age.

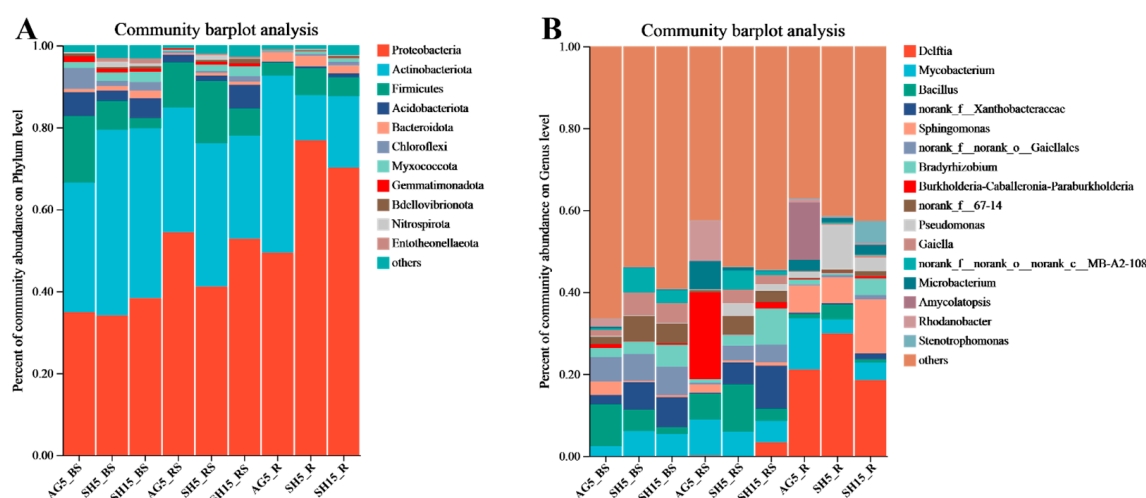


Figure 3. The composition of bacterial (A,B) communities at the phylum and genus levels. Phyla making up less than 1% of the total composition were classified as “others”. Genera making up less than 5% of the total composition were classified as “others”. There were 12 biological replicates per group. AG5: *P. ginseng* was cultivated in an arched greenhouse for 5 years. SH5: *P. ginseng* was cultivated in the forest for 5 years. SH15: *P. ginseng* was cultivated in the forest for 15 years. BS: Bulk soil. RS: Rhizosphere soil. R: Rhizomes endophytic.

To examine the community structures of rhizosphere and endophytic bacteria in ginseng roots at the OTU level, principal coordinates analysis (PCoA) based on Bray–Curtis distances was performed. The results revealed a clear separation in rhizosphere bacterial communities between ginseng grown under arched greenhouse conditions (AG5) and those under simulative habitat cultivation (SH5 and SH15), with the first principal component (PC1) explaining 41.17% of the variation (Figure 4A). In contrast, the rhizosphere communities of SH5 and SH15 clustered closely together. This pattern mirrored the differences observed among the corresponding bulk soils, supporting the view that the soil environment plays a key role in shaping the rhizosphere bacterial community of ginseng.

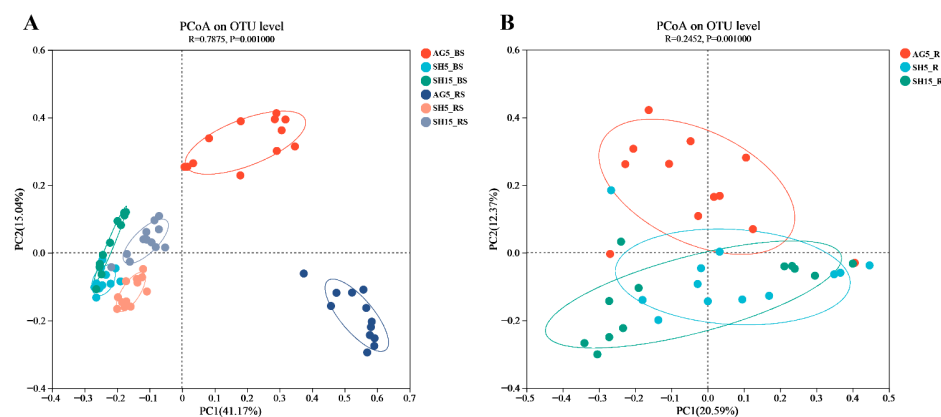


Figure 4. PCoA plot of ginseng microbial OTUs for BS and RS groups (A), and PCoA plot for R group (B). The Bray–Curtis distance algorithm was used to calculate the distance matrix between all 72 samples based on their OTU abundance tables. This distance matrix was then used to perform principal coordinates analysis (PCoA) to visualize the relationships among samples. Group differences were assessed using ANOSIM with 999 permutations to evaluate the significance of inter-group differences. AG5: *P. ginseng* cultivated in an arched greenhouse for 5 years. SH5: *P. ginseng* cultivated in the forest for 5 years. SH15: *P. ginseng* cultivated in the forest for 15 years. BS: Bulk soil. RS: Rhizosphere soil. R: Endophytic rhizomes.

Conversely, the endophytic bacterial communities in ginseng roots showed much greater similarity across the AG5, SH5, and SH15 groups, with PC1 accounting for only 20.59% of the variance (Figure 4B). These findings further indicate that the assembly of the endophytic bacterial structure in ginseng roots is relatively stable and less influenced by external growing conditions.

3.4. Differential Microbial Analysis of Arched-Greenhouse-Cultivated Ginseng and Simulative-Habitat-Cultivated Ginseng

The composition of the rhizosphere bacterial community under simulative habitat cultivation (SH5 and SH15) differed markedly from that under arched greenhouse conditions (AG5) at the genus level, mirroring the pattern observed in the bulk soil. Specifically, the relative abundances of the majority of the top 15 bacterial genera were significantly greater in SH5 and SH15 compared to AG5 (Figure 5A,C). Specifically, the relative abundances of unclassified lineages within *Xanthobacteraceae*, *Bradyrhizobium*, unclassified lineages within *Gaiellales*, unclassified lineages within *Methylobacteriaceae*, unclassified lineages within 67–14, unclassified lineages within MB-A2-108, *Gaiella*, *Pseudomonas*, *Paenibacillus*, and unclassified lineages within SC-I-84 were significantly higher in SH5 and SH15 compared to AG5. Additionally, *Bacillus* was significantly more abundant in SH5 than in AG5. Only four genera, *Burkholderia*-*Caballeronia*-*Paraburkholderia*, *Mycobacterium*, *Rhodanobacter*, and *Microbacterium*, had significantly higher relative abundances in AG5 compared to SH5 and SH15. This is consistent with the significantly higher Shannon index of rhizosphere bacteria in SH5 and SH15 compared to AG5.

Among the top 15 bacterial genera with significant differences in the roots of simulative-habitat-cultivated ginseng (SH5 or SH15), the relative abundances of 10 genera were higher compared to AG5. These genera include *Pseudomonas*, *Bradyrhizobium*, *Bacillus*, *Sphingobium*, unclassified lineages within *Xanthobacteraceae*, unclassified lineages within *Enterobacteriaceae*, unclassified lineages within 67–14, unclassified lineages within *Gaiellales*, *Vibrio*, and *Allorhizobium*-*Neorhizobium*-*Pararhizobium*-*Rhizobium*. In contrast, the relative abundances of *Mycobacterium*, *Amycolatopsis*, *Lechevalieria*, *Mesorhizobium*, and *Rhodanobacter* were significantly higher in AG5 than in SH5 and SH15 (Figure 5B).

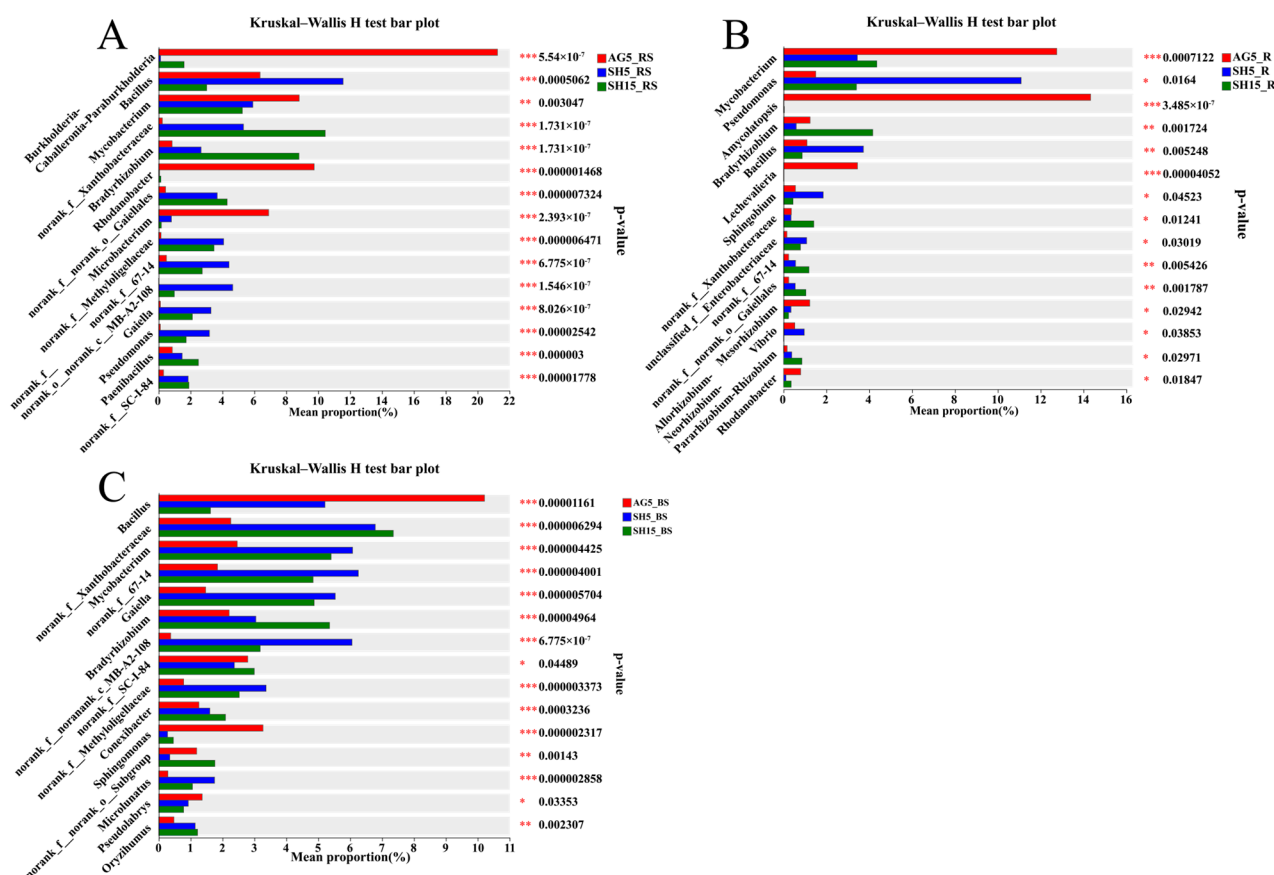


Figure 5. Differential average abundance of the top 15 bacterial genera among RS (A), R (B), and BS (C) groups identified using the Kruskal–Wallis H test. The bar plots illustrate the bacterial genera with the most significant differences in abundance across the RS, R, and BS groups. Each bar represents the mean relative abundance of a bacterial genus. AG5: *P. ginseng* cultivated in an arched greenhouse for 5 years. SH5: *P. ginseng* cultivated in the forest for 5 years. SH15: *P. ginseng* cultivated in the forest for 15 years. BS: Bulk soil. RS: Rhizosphere soil. R: Endophytic rhizomes. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

3.5. Network Analysis of Rhizosphere and Endophytic Microbes in Greenhouse-Cultivated and Simulative-Habitat-Cultivated Ginseng

The overall rhizosphere co-occurrence network exhibited higher connectivity than the root endophyte network. The number of edges in the rhizosphere network (188–391) was higher than that in the endophyte network (111–159), while the number of nodes was comparable (48–50), indicating a denser association structure among rhizosphere taxa (Supplementary Table S4). Within the rhizosphere, AG5 showed the highest number of edges (391), followed by SH5 (220) and SH15 (188), suggesting the most interconnected rhizobacterial network under arched greenhouse cultivation (AG5) (Figure 6A–C). In the endophyte network, SH15 had the highest number of edges (159), followed by SH5 (129) and AG5 (111), indicating increased network connectivity with cultivation years in the endophytic compartment (Figure 6D–F).

The proportion of negative edges varied among treatments. In the rhizosphere network, AG5 and SH15 had similar proportions of negative edges (approximately 42.5% and 42.0%, respectively), whereas SH5 showed a lower proportion (20.0%). In the endophyte network, SH15 exhibited the highest proportion of negative edges (about 22.0%), while SH5 and AG5 were lower (approximately 12.4–14.5%).

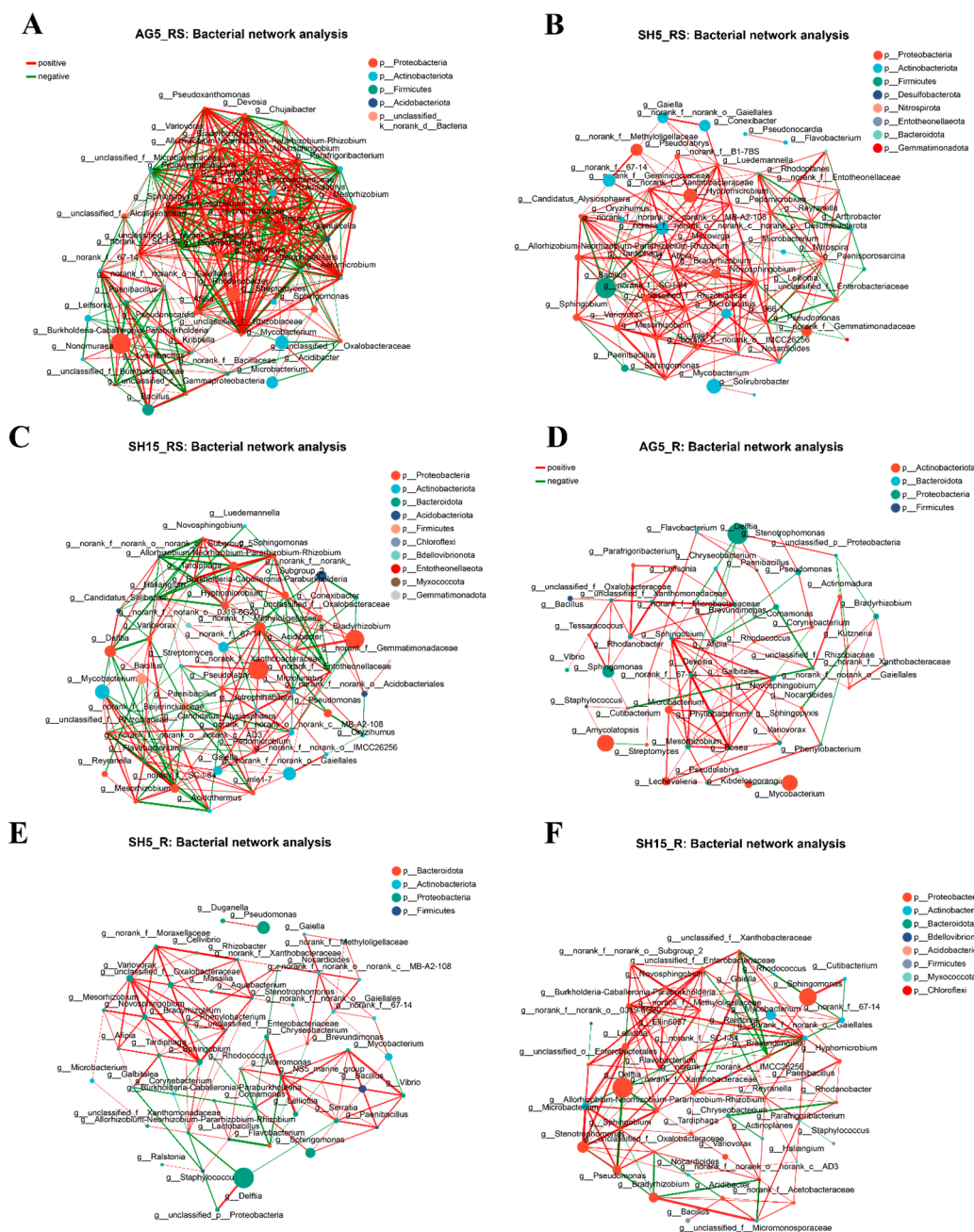


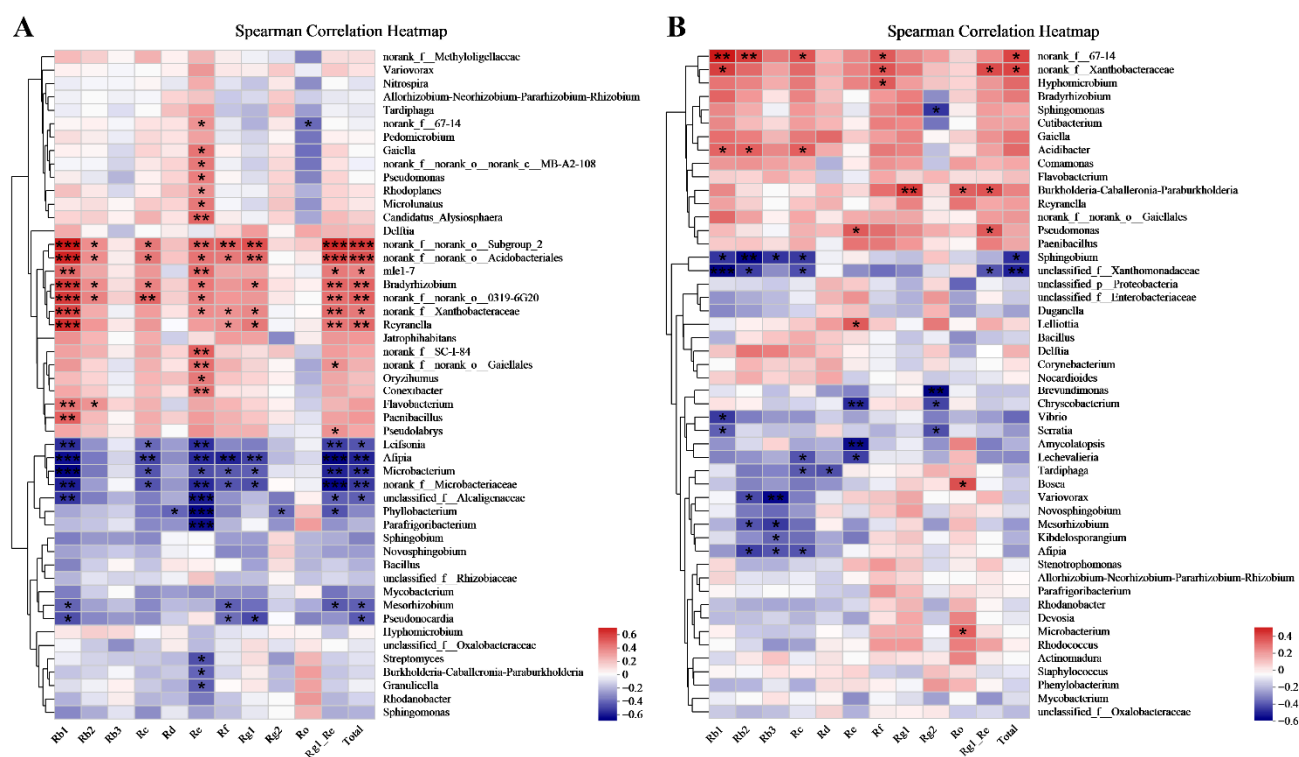
Figure 6. Network analysis of rhizosphere bacterial genera (A–C) and endophytic bacterial genera (D–F). The network structures display significant correlations (both positive and negative) among the top 50 relatively abundant bacterial genera in the AG5 (A,D), SH5 (B,E), and SH15 (C,F) samples. Red edges indicate positive correlations, whereas green edges indicate negative correlations. AG5: *P. ginseng* cultivated in an arched greenhouse for 5 years. SH5: *P. ginseng* cultivated in the forest for 5 years. SH15: *P. ginseng* cultivated in the forest for 15 years. BS: Bulk soil. RS: Rhizosphere soil. R: Rhizome endophyte.

Consistent with the edge patterns, the average degree in the rhizosphere network (7.83–16.29) was generally higher than that in the endophyte network (4.53–6.63), indicating that rhizosphere nodes were connected to more neighbors on average. AG5 had the highest average degree in the rhizosphere network (16.29), exceeding SH5 (8.80) and SH15 (7.83). Transitivity was higher in the rhizosphere networks than in the endophyte networks, indicating a greater tendency for nodes to form closed triplets in the rhizosphere. In

contrast, transitivity in the endophyte networks was lower and showed smaller differences among treatments (Supplementary Table S4).

3.6. Correlation Between Ginsenoside Content and Microbial Relative Abundance

Spearman's correlation analysis indicated that numerous bacterial genera in the ginseng rhizosphere were significantly associated with ginsenoside content. Several genera—including unclassified lineages within Subgroup_2, unclassified lineages within Acidobacteriales, mle1-7, *Bradyrhizobium*, unclassified lineages within 0319-6G20, unclassified lineages within *Xanthobacteraceae*, and *Reyranelia*—showed a significant positive correlation with all three ginsenoside indices. These taxa were present at higher, and in some cases significantly higher, relative abundances in the SH5 and SH15 samples compared to AG5 (Figure 7A; Supplementary Figure S2A–G).



between endophytic bacterial communities and ginsenoside content was less pronounced than that observed for rhizosphere microbes. This suggests a more subtle role of endophytes in directly influencing ginsenoside accumulation within the plant.

4. Discussion

4.1. Impact of Cultivation Methods on Ginseng Quality

It is well known that the growth duration is one of the crucial factors determining ginseng quality [26]. This point can be supported by the quantitative analysis of ginsenosides. As can be observed from Figure 1, compared to AG5, SH5 has significantly higher levels of ginsenoside Re among the 12 ginsenoside indices measured, while the other indices were not significantly different. This suggests that simulative habitat cultivated ginseng is only slightly superior to arch greenhouse cultivated ginseng in terms of quality formation when grown over the same period. However, when the growth period is extended to 15 years, SH15 exhibits significantly higher levels in 9 out of the 12 measured ginsenoside indices compared to AG5, including the primary pharmacologically active components ginsenoside Rd, Re, and Rg1. As a consequence, growth duration is indeed a critical factor in determining ginseng quality. Over time, simulative-habitat-cultivated ginseng accumulates more active components, leading to a significant improvement in quality.

It is evident that continuous cropping obstacles severely limit the formation of high-quality ginseng. In the arched greenhouse cultivation environment, the growth period of ginseng is restricted to within five years. If the cultivation period exceeds five years, diseases will break out extensively, thereby causing a sharp decline in yield [27]. In contrast, the soil bacterial Sobs index and Shannon index of simulative-habitat-cultivated ginseng are more similar to those of bulk soil (Figure 2A,B). This indicates that it is closer to natural growing conditions with high levels of microbial diversity and can provide a wider range of ecological functions such as nutrient cycling, disease resistance and environmental adaptation [28]. This result demonstrated the potential of simulative-habitat-cultivated ginseng in improving soil health and crop quality. It may help reduce the incidence of diseases, enhance the resistance of ginseng to stress, and increase survival rates. These characteristics make simulative habitat cultivation a viable way to achieve long-lived, high-quality ginseng cultivation [29]. This characteristic makes simulative habitat cultivation a feasible approach for achieving long-cycle, high-quality ginseng cultivation.

More efforts can be invested into the accumulation mechanisms of ginsenosides and the complex relationships between environmental factors and their impact. This can provide scientific guidance for improving ginseng quality and yield. Additionally, the comprehensive effects of different cultivation methods on ginseng growth and quality can be investigated to optimize cultivation techniques, enhance the medicinal value, and increase the economic benefits of ginseng. By integrating modern agricultural technology with existing cultivation techniques, more efficient cultivation models can be innovated, promoting the sustainable development of the ginseng industry.

4.2. Differences in Rhizosphere Bacteria Among Various Cultivation Systems

By combining the genus-level relative abundance (Figures 3B and 5) with the heatmap of the correlation between rhizosphere microorganisms and ginsenoside content (Figure 7A), it can be observed that *Bradyrhizobium*, which has a relatively high abundance in rhizosphere soil, shows a potential promoting trend on the 12 ginsenoside content indicators measured. In accordance with further analysis of its relative abundance in different groups using a mixed-effects model (Supplementary Table S5), time had a more significant impact on *Bradyrhizobium* abundance compared to cultivation mode. The gradual increase in abundance with time was also observed in another *Panax* plant [30]. *Bradyrhizo-*

bium is well-known for its nitrogen-fixing capabilities and its symbiotic relationship with legumes [31]. In addition, it has been shown to produce IAA, ACC deaminase, iron carriers and phosphate solubilizers to promote plant growth [32–34]. This is the first report of a significant positive correlation between *Bradyrhizobium* and ginsenoside content. In a study on ginseng plants, the abundance of *Bradyrhizobium* was significantly higher in healthy *Panax notoginseng* than in root rot-infected plants [35], a trend also observed in previous studies on *Panax ginseng* [36]. In this study, *Bradyrhizobium*, as a beneficial microorganism, was progressively enriched over the years, aligning with previous discussions highlighting the importance of growth duration for ginseng quality. This further confirms the critical role of simulative habitat cultivation in the formation of high-quality ginseng.

However, not all bacteria strongly associated with ginseng quality were affected by plant age. The mixed-effects model results showed that the abundance of unclassified lineages within *Xanthobacteraceae* was more influenced by the cultivation mode (Supplementary Table S5). *Xanthobacteraceae*, a common family in rhizosphere soils of plants, belongs to the Proteobacteria phylum. High relative abundance was found in *Matricaria chamomilla* L. and *Panax notoginseng* [37,38]. The genus exhibits diverse biological functions, primarily related to nitrogen fixation [39]. *Xanthobacteraceae* population abundance may be dependent on nitrogen concentration, decreasing at higher nitrogen levels and increasing when nitrogen is scarce [40]. This explains the phenomenon observed in current study and the results of the mixed-effects model: the abundance of *Xanthobacteraceae* in the bulk soil of arched-greenhouse-cultivated ginseng is lower than in simulative-habitat-cultivated ginseng. This may be due to the fact that nitrogen fertilizers are frequently applied to farmland to ensure ginseng yield, and these fertilizers, through leaching and weathering, increase nitrogen levels in the bulk soil, inhibiting *Xanthobacteraceae* growth. However, nitrogen levels in the rhizosphere soil are much higher than in the bulk soil, further suppressing *Xanthobacteraceae* abundance. In the simulative-habitat-cultivated ginseng system, the situation is different. Under natural forest conditions, soil nitrogen content decreases with depth [41]. The root zone of 5-year-old simulative-habitat-cultivated ginseng is closer to the surface, which is nitrogen-rich. As the roots grow deeper over time, they selectively enrich nitrogen-fixing microorganisms to ensure adequate nitrogen supply. Therefore, simulative-habitat-cultivated ginseng more easily increases the abundance of unclassified lineages within *Xanthobacteraceae*, which is strongly positively correlated with ginsenoside content, improving ginseng quality. This demonstrates the advantage of this cultivation mode, even without considering growth duration.

Although not ranked highly in relative abundance, some microorganisms exhibit strong correlations with ginsenoside in the correlation analysis. For example, unclassified lineages within Subgroup_2 and unclassified lineages within Acidobacteriales. Currently, there are few reports linking unclassified lineages within Subgroup_2 to plant secondary metabolites. As for unclassified lineages within Acidobacteriales, studies on *Panax ginseng* [42], *Panax quinquefolius* [43], and *Panax notoginseng* [44] have shown that its abundance increases with cultivation years or as soil acidification intensifies, a common phenomenon during ginseng cultivation [45]. These findings suggest that the strong correlation between unclassified lineages within Acidobacteriales and ginsenoside production may be due to soil pH decrease over time, rather than any direct influence on ginsenoside synthesis. There is also evidence that Acidobacteriales abundance decreases as pH drops [46]. However, in this study, the use of phosphate-solubilizing bacteria in ginseng pot experiments might interact with Acidobacteriales, leading to its reduction, rather than a direct effect from pH.

4.3. Differences in Endophytic Bacteria in Ginseng Roots Under Different Cultivation Modes

Among endophytic bacteria, the relative abundance of unclassified lineages within 6–14 in ginseng roots showed a clear and consistent positive correlation with multiple ginsenosides. In contrast, its relative abundance in the rhizosphere exhibited only weak associations with ginsenoside content. This compartment-specific pattern suggests that the relationship is more likely linked to successful colonization of internal root tissues, where direct microbe–host interactions can influence plant specialized metabolism. Once established inside roots, endophytes may act as biotic stimuli that activate host defense-related metabolic programs, shift physiological stress status, and improve nutrient availability, thereby favoring carbon allocation toward triterpenoid biosynthesis and ultimately promoting ginsenoside accumulation. Previous work reported that unclassified lineages within 6–14 are also abundant in the roots of *Mirabilis himalaica* [47], a plant rich in triterpenoid secondary metabolites structurally similar to ginsenoside aglycones [48]. This cross-host enrichment in triterpenoid-rich roots supports the idea that this lineage is linked to triterpenoid-associated root environments. At the same time, the correlation we observed can be interpreted conservatively in two plausible ways. The lineage may promote ginsenoside accumulation by stimulating host triterpenoid biosynthesis after colonization, or roots with higher ginsenoside pools may provide a selective niche that favors this lineage, leading to its enrichment where ginsenosides are already abundant. These interpretations are not mutually exclusive and point to the need for follow-up inoculation experiments combined with host pathway expression and metabolite profiling to clarify directionality.

While *Burkholderia*–*Caballeronia*–*Paraburkholderia* does not represent a dominant taxon within the endophytic bacterial community, it demonstrated a significant association with specific ginsenoside levels. This pattern does not necessarily indicate direct stimulation of *de novo* biosynthesis. A more conservative interpretation is that this group preferentially colonizes ginseng roots where ginsenosides and related glycosides are abundant, potentially utilizing these compounds as substrates or benefiting from the physiological state of high-ginsenoside plants [49]. Another possibility is that it contributes to shifts in ginsenoside composition through biotransformation, which would alter particular ginsenoside levels without increasing total synthesis. Either scenario provides a mechanistic basis for why a taxon can show strong metabolite associations despite modest relative abundance.

Hyphomicrobium also showed a strong positive correlation with ginsenoside content, consistent with reports from other studies [50]. Beyond ginseng, *Hyphomicrobium* has been identified in the endophytic microbiota of various perennial plants such as *Panax notoginseng* [51], Apple Trees [52], *Pulsatilla tongkangensis* [53], and *Dendrobium* [54], where its abundance often increases with plant age. This trend aligns with our observations (Supplementary Figure S3H), in which the genus was markedly more abundant in 15-year-old simulated-habitat-cultivated ginseng (SH15) compared with the other groups. Functionally, *Hyphomicrobium* contributes to the nitrogen cycle as a denitrifying bacterium, helping to maintain nitrogen equilibrium in plants and prevent the accumulation of toxic nitrites [55]. Because nitrogen status and stress regulation are tightly coupled to carbon allocation and secondary metabolism, improved nitrogen homeostasis and reduced nitrosative stress provide a plausible physiological link between *Hyphomicrobium* enrichment and sustained ginsenoside accumulation during long-term cultivation.

The unclassified taxon lineages within Xanthobacteraceae were present at high relative abundance in both the rhizosphere and ginseng root tissues and exhibited significant positive correlations with multiple ginsenosides. As noted earlier, known functions of Xanthobacteraceae are largely related to nitrogen fixation [39]. The recruitment of nitrogen-fixing bacteria from the rhizosphere into root tissues, establishing a symbiotic relationship, represents a common mutualistic strategy [56]. In ginseng, enhanced nitrogen

acquisition and a more stable nutrient environment could indirectly favor ginsenoside accumulation by supporting long-term growth while maintaining the metabolic capacity to invest in specialized metabolites. Thus, the strong colonization ability of unclassified lineages within Xanthobacteraceae in ginseng roots suggests its potential as a microbial candidate for enhancing ginsenoside accumulation. Moreover, its relative abundance was significantly greater in simulated-habitat-cultivated ginseng (SH5 and SH15) than in arched greenhouse-grown ginseng (AG5), pointing to a possible advantage of the simulated-habitat cultivation method.

5. Conclusions

This study provides a comprehensive comparative analysis of *Panax ginseng* quality and its associated microbiome under simulative habitat versus arched greenhouse cultivation regimes. The findings demonstrate that simulative habitat cultivation significantly outperformed arched greenhouse cultivation in terms of ginsenoside content, particularly ginsenoside Re. The 15-year-old ginseng grown in simulative habitat cultivation had significantly higher levels of multiple ginsenosides compared to the 5-year-old samples, highlighting the critical role of growth duration in ginseng quality. Microbiome profiling revealed that this quality improvement is intrinsically linked to a distinct rhizosphere configuration: the SH environment maintains high bacterial diversity and specifically enriches beneficial taxa such as *Bradyrhizobium* and *Xanthobacteraceae*. The robust positive correlations observed between these taxa and key ginsenoside indices suggest that these microbes are associated with improved plant performance, potentially through nutrient cycling (e.g., nitrogen fixation), and may be linked to variation in host secondary metabolism via microbe–plant signaling. In arched greenhouse cultivation, the rhizosphere microbial co-occurrence network showed higher connectivity and a higher proportion of negative edges, indicating a shift in association structure compared with simulative habitat cultivation. Notably, taxa such as *Burkholderia*–*Caballeronia*–*Paraburkholderia* were more abundant under arched greenhouse cultivation. Given that members of this group include both beneficial and potentially pathogenic lineages, their enrichment may reflect altered rhizosphere conditions associated with greenhouse management; however, the specific roles of these taxa and their links to soil-borne disease susceptibility require targeted functional validation. Collectively, our results suggest that cultivation mode and duration are associated with substantial changes in rhizosphere/endophytic community composition and network topology, which co-vary with ginsenoside-related quality traits. Future work should prioritize isolation and functional characterization of candidate taxa, as well as controlled inoculation or microbiome-manipulation experiments, before translating these associations into microbiome-informed strategies for sustainable production of high-quality ginseng.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/metabo16020138/s1>. Supplementary files, Supplementary Methods [57–62]. Supplementary Figure S1, Rarefaction curves of bacteria (A and B) and in rhizosphere soil and endophytic of *P. ginseng*, and bulk soil. AG5: *P. ginseng* cultivated in arched greenhouse for 5 years. Supplementary Figure S2, Relative abundance of rhizosphere bacterial genera significantly correlated with ginsenoside components. Supplementary Figure S3, Relative abundance of endophytic bacterial genera significantly correlated with ginsenoside components. Supplementary Figure S4, Redundancy Analysis (RDA) Plot of Ginsenoside Components and Soil Factors. Supplementary Table S1, Sampling Information. Supplementary Table S2, Ginsenoside content of *P. ginseng*. Supplementary Table S3, Composition and Structure of The Bacterial and Fungal Community. Supplementary Table S4, Network analysis parameters of bacteria and fungi in the rhizosphere and rhizome of *P. ginseng* cultivated in arched greenhouse and forest. Supplementary

Table S5, Results of the Mixed-Effects Model. Supplementary Table S6, Results of Background Soil Physical and Chemical Property Determination.

Author Contributions: Conceptualization, W.M.; methodology, T.W. and F.X.; software, B.Y. and Y.G.; writing—original draft preparation, S.L.; writing—review and editing, D.W.; supervision, H.W.; funding acquisition, C.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the National Key Research and Development Program of China (2023YFC3503802), Scientific and technological innovation project of China Academy of Chinese Medical Sciences (CI2024E003, CI2024C010YNL), Fundamental Research Funds for the Central public welfare research institutes (ZXKT25043).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Chinese Pharmacopoeia Editorial Board. *Pharmacopoeia of the People's Republic of China*, 1; China Medical Science Press: Beijing, China, 2020.
- Chen, Y.; Zhao, Z.; Chen, H.; Yi, T.; Qin, M.; Liang, Z. Chemical differentiation and quality evaluation of commercial asian and american ginsengs based on a UHPLC–QTOF/MS/MS metabolomics approach. *Phytochem. Anal.* **2015**, *26*, 145–160. [\[CrossRef\]](#)
- Lee, C.H.; Kim, J.-H. A review on the medicinal potentials of ginseng and ginsenosides on cardiovascular diseases. *J. Ginseng. Res.* **2014**, *38*, 161–166. [\[CrossRef\]](#)
- Niu, Z.; Liu, Y.; Shen, R.; Jiang, X.; Wang, Y.; He, Z.; Li, J.; Hu, Y.; Zhang, J.; Jiang, Y.; et al. Ginsenosides from *Panax ginseng* as potential therapeutic candidates for the treatment of inflammatory bowel disease. *Phytomedicine* **2024**, *127*, 155474. [\[CrossRef\]](#)
- Biswas, T.; Mathur, A.K.; Mathur, A. A literature update elucidating production of panax ginsenosides with a special focus on strategies enriching the anti-neoplastic minor ginsenosides in ginseng preparations. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 4009–4032. [\[CrossRef\]](#)
- Jang, W.Y.; Hwang, J.Y.; Cho, J.Y. Ginsenosides from *Panax ginseng* as key modulators of NF- κ B signaling are powerful anti-inflammatory and anticancer agents. *Int. J. Mol. Sci.* **2023**, *24*, 6119. [\[CrossRef\]](#)
- Huang, J.; Wu, Y.; Gao, Q.; Li, X.; Zeng, Y.; Guo, Y.; Zhang, H.; Qin, Z. Metagenomic exploration of the rhizosphere soil microbial community and their significance in facilitating the development of wild-simulated ginseng. *Appl. Environ. Microbiol.* **2024**, *90*, e02335-23. [\[CrossRef\]](#)
- Fan, J.-W.; Xu, X.-T.; Cheng, H.; Sang, Z.; Shi, Y.-H. Standardization of *Panax ginseng*: Current status of global trade, demands, and development. *Am. J. Chin. Med.* **2023**, *51*, 909–927. [\[CrossRef\]](#)
- Yi, S.-Y.; Kang, C.-Z.; Wang, W.; Song, X.-W.; Xu, T.; Lu, H.-B.; Luo, S.-L.; Liu, D.; Guo, L.-P.; Han, B.-X. comparison of planting modes of dendrobium huoshanense and analysis of advantages of simulated cultivation. *China J. Chin. Mater. Med.* **2021**, *46*, 1864–1868. [\[CrossRef\]](#)
- Aizi, T.; Lijuan, L.; Lihua, L.; Wei, L.; Jiamei, Q. Comparative analysis of microbial community structure in different times of *Panax ginseng* rhizosphere microbiome and soil properties under larch forest. *BMC Genom. Data* **2023**, *24*, 51. [\[CrossRef\]](#)
- Gao, J.; Wang, Y.; Guan, Y.M.; Chen, C.Q. *Fusarium cerealis*, a new pathogen causing ginseng (*Panax ginseng*) root rot in China. *Plant Dis.* **2014**, *98*, 1433. [\[CrossRef\]](#)
- Li, Q.; Zhan, Y.; Xu, Y.; Zhang, L.; Di, P.; Lu, B.; Chen, C. Deciphering the transcriptomic response of *ilyonectria robusta* in relation to ginsenoside Rg1 treatment and the development of ginseng rusty root rot. *FEMS Microbiol. Lett.* **2022**, *369*, fnac075. [\[CrossRef\]](#)
- Li, L.; Zhao, C.; Chen, Q.; Liu, T.; Li, L.; Liu, X.; Wang, X. Study on microbial community structure and soil nitrogen accumulation in greenhouse vegetable fields with different planting years. *Agronomy* **2022**, *12*, 1911. [\[CrossRef\]](#)
- Fujii, T.; Minami, M.; Watanabe, T.; Sato, T.; Kumaishi, K.; Ichihashi, Y. Characterization of inter-annual changes in soil microbial flora of *Panax ginseng* cultivation fields in shimane prefecture of western Japan by DNA metabarcoding using next-generation sequencing. *J. Nat. Med.* **2021**, *75*, 1067–1079. [\[CrossRef\]](#)
- Strange, R.N.; Scott, P.R. Plant disease: A threat to global food security. *Annu. Rev. Phytopathol.* **2005**, *43*, 83–116. [\[CrossRef\]](#)

16. Lu, Z.; Fang, N.; Liu, Y.; Zhang, Z.; Pan, H.; Hou, Z.; Li, Y.; Lu, Z. Dissipation and residues of the diamide insecticide chlorantraniliprole in ginseng ecosystems under different cultivation environments. *Environ. Monit. Assess.* **2017**, *189*, 1–10. [CrossRef]
17. Fan, Z. Endophytic Bacterial and Fungal Community Compositions in Different Organs of Ginseng (*Panax ginseng*). *Arch. Microbiol.* **2022**, *204*, 1–11. [CrossRef]
18. Bulgarelli, D.; Rott, M.; Schlaeppli, K.; Ver Loren van Themaat, E.; Ahmadinejad, N.; Assenza, F.; Rauf, P.; Huettel, B.; Reinhardt, R.; Schmelzer, E.; et al. Revealing structure and assembly cues for arabidopsis root-inhabiting bacterial microbiota. *Nature* **2012**, *488*, 91–95. [CrossRef]
19. Lundberg, D.S.; Lebeis, S.L.; Paredes, S.H.; Yourstone, S.; Gehring, J.; Malfatti, S.; Tremblay, J.; Engelbrektson, A.; Kunin, V.; Del Rio, T.G.; et al. Defining the core arabidopsis thaliana root microbiome. *Nature* **2012**, *488*, 86–90. [CrossRef]
20. Bulgarelli, D.; Garrido-Oter, R.; Münch, P.C.; Weiman, A.; Dröge, J.; Pan, Y.; McHardy, A.C.; Schulze-Lefert, P. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host Microbe* **2015**, *17*, 392–403. [CrossRef]
21. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef]
22. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef]
23. Hagberg, A.; Conway, D. Networkx: Network Analysis with Python. 2020. Available online: <https://github.com/networkx> (accessed on 11 February 2026).
24. Revelle, W. *An Overview of the Psych Package*; Department of Psychology, Northwestern University: Evanston, IL, USA, 2011; 76p.
25. Kolde, R.; Kolde, M.R. Package ‘pheatmap’. *R Package* **2015**, *1*, 790.
26. Chen, K.; Tong, Y.; Wang, T.; Zhou, X.; Zhou, J.; Ge, Y.; Zheng, H.; Yu, M.; Luo, Y.; Ji, R. Challenges of continuous cropping obstacles in *Panax ginseng*: Formation and response mechanisms. *Sci. Tradit. Chin. Med.* **2025**, *3*, 8–14. [CrossRef]
27. Haq, M.Z.U.; Yu, J.; Yao, G.; Yang, H.; Iqbal, H.A.; Tahir, H.; Cui, H.; Liu, Y.; Wu, Y. A systematic review on the continuous cropping obstacles and control strategies in medicinal plants. *Int. J. Mol. Sci.* **2023**, *24*, 12470. [CrossRef]
28. Maron, P.-A.; Sarr, A.; Kaisermann, A.; Lévêque, J.; Mathieu, O.; Guigue, J.; Karimi, B.; Bernard, L.; Dequiedt, S.; Terrat, S.; et al. High microbial diversity promotes soil ecosystem functioning. *Appl. Environ. Microbiol.* **2018**, *84*, e02738-17. [CrossRef]
29. Guo, N.; Yang, Y.; Yang, X.; Guan, Y.; Yang, J.; Quan, J.; Yan, H.; Hou, W.; Zhang, G. Growth age of mountain cultivated ginseng affects its chemical composition. *Ind. Crops Prod.* **2021**, *167*, 113531. [CrossRef]
30. Luo, L.; Guo, C.; Wang, L.; Zhang, J.; Deng, L.; Luo, K.; Huang, H.; Liu, Y.; Mei, X.; Zhu, S.; et al. Negative Plant-Soil Feedback Driven by Re-assembly of the Rhizosphere Microbiome With the Growth of *Panax notoginseng*. *Front. Microbiol.* **2019**, *10*, 1597. [CrossRef]
31. Brechenmacher, L.; Kim, M.-Y.; Benitez, M.; Li, M.; Joshi, T.; Calla, B.; Lee, M.P.; Libault, M.; Vodkin, L.O.; Xu, D.; et al. Transcription profiling of soybean nodulation by *Bradyrhizobium japonicum*. *Mol. Plant-Microbe Interact.* **2008**, *21*, 631–645. [CrossRef]
32. Antoun, H.; Beauchamp, C.J.; Goussard, N.; Chabot, R.; Lalande, R. Potential of rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativus* L.). *Plant Soil.* **1998**, *204*, 57–67. [CrossRef]
33. Hakim, S.; Naqqash, T.; Nawaz, M.S.; Laraib, I.; Siddique, M.J.; Zia, R.; Mirza, M.S.; Imran, A. Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. *Front. Sustain. Food Syst.* **2021**, *5*, 617157. [CrossRef]
34. Timofeeva, A.; Galyamova, M.; Sedykh, S. Prospects for Using Phosphate-Solubilizing Microorganisms as Natural Fertilizers in Agriculture. *Plants* **2022**, *11*, 2119. [CrossRef]
35. Cui, X.; Liu, Y. Structure and Function of Rhizosphere Soil and Root Endophytic Microbial Communities Associated With Root Rot of *Panax notoginseng*. *Front. Plant Sci.* **2022**, *12*, 15. [CrossRef]
36. Chen, F.; Xie, Y.; Jia, Q.; Li, S.; Li, S.; Shen, N.; Jiang, M.; Wang, Y. Effects of the Continuous Cropping and Soilborne Diseases of *Panax ginseng* C. A. Meyer on Rhizosphere Soil Physicochemical Properties, Enzyme Activities, and Microbial Communities. *Agronomy* **2023**, *13*, 210. [CrossRef]
37. Xia, F.; Hao, H.; Qi, Y.; Bai, H.; Li, H.; Shi, Z.; Shi, L. Effect of salt stress on microbiome structure and diversity in chamomile (*Matricaria chamomilla* L.) rhizosphere soil. *Agronomy* **2023**, *13*, 1444. [CrossRef]
38. Li, M.; Chen, Z.; Qian, J.; Wei, F.; Zhang, G.; Wang, Y.; Wei, G.; Hu, Z.; Dong, L.; Chen, S. Composition and function of rhizosphere microbiome of *Panax notoginseng* with discrepant yields. *Chin. Med.* **2020**, *15*, 85. [CrossRef]
39. Yang, Y.; Xu, N.; Zhang, Z.; Lei, C.; Chen, B.; Qin, G.; Qiu, D.; Lu, T.; Qian, H. Deciphering microbial community and nitrogen fixation in the legume rhizosphere. *J. Agric. Food Chem.* **2024**, *72*, 5659–5670. [CrossRef]
40. Ren, W.; Zhang, L.; Maness, N.; Wang, X.; Tang, M.; Xu, T. Changes in the diversity of pecan (*Carya illinoensis*) rhizosphere microbial community with different nitrogen fertilization, a case study in oklahoma pecan orchard. *Sci. Hortic.* **2023**, *321*, 112365. [CrossRef]

41. Gholizadeh, A.; Saberioon, M.; Pouladi, N.; Ben-Dor, E. Quantification and depth distribution analysis of carbon to nitrogen ratio in forest soils using reflectance spectroscopy. *Int. Soil Water Conserv. Res.* **2023**, *11*, 112–124. [\[CrossRef\]](#)
42. Shi, Z.; Yang, L.; Yang, M.; Li, K.; Yang, L.; Han, M. Temporal heterogeneity of the root microbiome in *Panax ginseng* soils across ecological compartments under mild soil disturbance. *Front. Microbiol.* **2024**, *15*, 1340575. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Dong, L.; Xu, J.; Zhang, L.; Yang, J.; Liao, B.; Li, X.; Chen, S. High-throughput sequencing technology reveals that continuous cropping of american ginseng results in changes in the microbial community in arable soil. *Chin. Med.* **2017**, *12*, 18. [\[CrossRef\]](#)
44. Hei, J.; Li, Y.; Wang, Q.; Wang, S.; He, X. Effects of exogenous organic acids on the soil metabolites and microbial communities of panax notoginseng from the forest understory. *Agronomy* **2024**, *14*, 601. [\[CrossRef\]](#)
45. Matsumoto, S.; Doi, H.; Kasuga, J. Changes over the years in soil chemical properties associated with the cultivation of ginseng (*Panax ginseng meyer*) on andosol soil. *Agriculture* **2022**, *12*, 1223. [\[CrossRef\]](#)
46. Sun, H.; Shao, C.; Liang, H.; Qian, J.; Jin, Q.; Zhu, J.; Zhang, G.; Lv, B.; Zhang, Y. Bacterial community response in ginseng rhizosphere soil after pseudomonas P1 inoculation integrating intracellular non-targeted metabolomics analysis. *Environ. Technol. Innov.* **2024**, *35*, 103633. [\[CrossRef\]](#)
47. Zhang, E.; Lu, Y.; Zhao, R.; Yin, X.; Zhang, J.; Yu, B.; Yao, M.; Liao, Z.; Lan, X. Endophytic bacterial community structure and diversity of the medicinal plant mirabilis himalaica from different locations. *Braz. J. Microbiol.* **2023**, *54*, 2991–3003. [\[CrossRef\]](#)
48. Zhang, G.L.; Xing, Q.Y.; Zhang, M.Z. Glycolipids from mirabilis himalaica. *Phytochemistry* **1997**, *45*, 1213–1215. [\[CrossRef\]](#)
49. Luo, L.; Wang, L.; Deng, L.; Mei, X.; Liu, Y.; Huang, H.; Du, F.; Zhu, S.; Yang, M. Enrichment of Burkholderia in the Rhizosphere by Autotoxic Ginsenosides to Alleviate Negative Plant-Soil Feedback. *Microbiol. Spectr.* **2021**, *9*, e0140021. [\[CrossRef\]](#)
50. Shi, Z.; Yang, L.; Yang, M.; Li, K.; Yang, L.; Han, M. Temporal patterns of endophytic microbial heterogeneity across distinct ecological compartments within the *Panax ginseng* root system following deforestation for cultivation. *Front. Microbiol.* **2024**, *15*, 1402921. [\[CrossRef\]](#)
51. Wei, G.; Li, M.; Zhang, G.; Chen, Z.; Wei, F.; Jiao, S.; Qian, J.; Wang, Y.; Wei, J.; Wang, Y.; et al. Temporal dynamics of rhizosphere communities across the life cycle of *panax notoginseng*. *Front. Microbiol.* **2022**, *13*, 853077. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Pan, J.; Dong, Q.; Wen, H.; Liu, Y.; Wang, X.; Liu, Y.; Zhang, X.; Shi, C.; Zhao, D.; Lu, X. Composition and diversity of endophytic rhizosphere microbiota in apple tree with different ages. *Mol. Biotechnol.* **2023**, *66*, 2219–2229. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Dutta, S.; Na, C.S.; Lee, Y.H. Features of bacterial microbiota in the wild habitat of pulsatilla tongkangensis, the endangered “long-sepal donggang pasque-flower plant,” endemic to karst topography of korea. *Front. Microbiol.* **2021**, *12*, 656105. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Wang, S.-S.; Liu, J.-M.; Sun, J.; Huang, Y.-T.; Jin, N.; Li, M.-M.; Liang, Y.-T.; Fan, B.; Wang, F.-Z. Analysis of endophytic bacterial diversity from different dendrobium stems and discovery of an endophyte produced dendrobine-type sesquiterpenoid alkaloids. *Front. Microbiol.* **2022**, *12*, 775665. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Liu, S.; Liu, Y.; Cai, Y. Incubation study on remediation of nitrate-contaminated soil by *Chroococcus* sp. *Environ. Sci. Pollut. Res.* **2023**, *30*, 117637–117653. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Cocking, E.C. Endophytic colonization of plant roots by nitrogen-fixing bacteria. *Plant Soil* **2003**, *252*, 169–175. [\[CrossRef\]](#)
57. Bao, S.D. *Agricultural and Chemistry Analysis of Soil*; Agric Press: Beijing, China, 2005.
58. Egli, M.; Sartori, G.; Mirabella, A.; Favilli, F.; Giaccari, D.; Delbos, E. Effect of north and south exposure on organic matter in high Alpine soils. *Geoderma* **2009**, *149*, 124–136. [\[CrossRef\]](#)
59. Bardelli, T.; Gómez-Brandón, M.; Ascher-Jenull, J.; Fornasier, F.; Arfaioli, P.; Francioli, D.; Egli, M.; Sartori, G.; Insam, H.; Pietramellara, G. Effects of slope exposure on soil physico-chemical and microbiological properties along an altitudinal climosequence in the Italian Alps. *Sci. Total Environ.* **2017**, *575*, 1041–1055. [\[CrossRef\]](#)
60. Murphy, P.N.C.; Stevens, R.J.; Christie, P. Long-term application of animal slurries to grassland alters soil cation balance. *Soil Use Manag.* **2005**, *21*, 240–244. [\[CrossRef\]](#)
61. Liu, Y.; Zhang, L.; Lu, J.; Chen, W.; Wei, G.; Lin, Y. Topography affects the soil conditions and bacterial communities along a restoration gradient on Loess-Plateau. *Appl. Soil Ecol.* **2020**, *150*, 103471. [\[CrossRef\]](#)
62. Fageria, N.K.; Baligar, V.C.; Clark, R.B. Micronutrients in crop production. *Adv. Agron.* **2002**, *77*, 185–268. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.