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Effects of cultivation duration of the crop and growth stages on rhizosphere soil physicochemical properties, enzyme activities, and microbial communities of ginseng under forest

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Abstract: In this study, Illumina MiSeq sequencing of 16S and ITS2 rRNA genes were used to determine the dynamic changes in bacterial and fungal communities and soil properties and enzyme activities in rhizosphere soil of ginseng under forest after 5, 10 and 15 years of cultivation and different growth stages. Results showed that the changes were particularly prominent in 10-year-old ginseng under forest, and the trends of organic carbon, alkaline hydrolysed nitrogen, and available potassium were extremely similar in different duration of the crop, especially in the middle stage of rapid root growth, when soil nutrient consumption was severe, and soil enzyme activities of rhizosphere were significantly reduced. The observed changes in soil properties and enzyme activities caused by the cultivation duration of the crop and growth stage could be explained by the variations in the microbiome. The microbial composition of 10-year-old ginseng under forest has undergone significant changes, at the genus level, both *Acinetobacter* bacteria and *Kazachstania* fungi exhibited a higher abundance; the abundance of Bacillota (Firmicutes), and *Candidatus udaeobacter* with significantly lower abundance. This study initially revealed the changes in nutrient utilisation of ginseng under forest at different cultivation duration of the crop and different growth stages, as well as the regulatory role played by microbes in this process preliminarily. We consider 10 years to be a critical stage for the long-term cultivation of ginseng in the forest, during which it is more sensitive to environmental factors and may exhibit special dynamic changes affecting its growth and quality. This provides a reference for further precision planting and harvesting of ginseng under the forest.

Keywords: *Panax*; nutrient cycling; microbial diversity; micro-ecology; microbiota

Panax ginseng C. A. Meyer, a perennial herbaceous plant in the Araliaceae family, is a traditional precious Chinese herbal medicine with important economic value (Aizi et al. 2023). Wild ginseng germplasm resources are scarce due to excessive land exploitation and disruption of the environment; thus, wild ginseng has been gradually replaced by cultivated ginseng in the market (Wu et al. 2013). The cultivation mode can be divided into the cultivation of ginseng under the forest, the cultivation of ginseng in deforestation, and the cultivation of ginseng in the farmland (Zhang et al. 2020). With the development of ginseng cultivation and strengthening arable land protection, ginseng under forest has gradually become one of the main ways of ginseng cultivation. Planting ginseng in the forest can realise the full use

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of forest land, which is conducive to its stereoscopic management, improve the ecological effect, and promote the sustainable development of agriculture.

The rhizosphere is an active area of soil where plant roots and microorganisms interact and is of great importance for plant health, development, productivity as well as nutrient cycling (Atkinson and Watson 2000, Zhalnina et al. 2018). As the most active component in the soil ecosystem, rhizosphere microbial diversity and abundance can be affected by many factors (Goodwin 2022). Several studies have shown, soil physicochemical properties including soil pH could directly or indirectly affect the structure and function of rhizosphere microbiota (Wei et al. 2018, Xun et al. 2019, Zhang et al. 2022). Soil environment is one of the most important factors affecting ginseng's growth, development and quality. Soil physicochemical properties, soil enzyme activities and microbial communities have significant effects on the growth, yield and quality of ginseng roots (Jin et al. 2022, Chen et al. 2023). In addition, different cropping years of ginseng will also alter the soil microbial community (Dong et al. 2017, Tong et al. 2021).

All processes and functions taking place in the rhizosphere are dominated by the activities of plant roots, rhizosphere microorganisms and root-microorganism interactions, and enzymes are recognised as the main actors of all activities occurring in rhizosphere environments (Gianfreda 2015). Soil enzyme activity and soil microorganisms are two important components that make up the soil microbiological environment, which is an important factor in determining soil quality and plays an irreplaceable role in agroecosystems.

Research on ginseng under forest mostly focuses on cultivation techniques, species selection, and pest control. However, the dynamic changes of rhizosphere soil microorganisms, soil functionality and the mechanism of microbial action of ginseng under forest during the cultivation duration of the crop and root rapid growth stages are still unclear. By using Illumina MiSeq sequencing of the 16S and ITS2 rRNA genes, we tested the effect of three cultivation duration of the crop (5, 10, and 15 years) and different stages of rapid growth of ginseng roots, respectively (the early stage of rapid root growth, the middle stage of rapid root growth, and the late stage of rapid root growth) between the rhizosphere soil microorganisms of ginseng under forest and the soil environment. We expect to elucidate the differences in the dynamic change in soil nutrient patterns in the rhizosphere soil of ginseng under forest at different cultivation duration of the crop, and different stages of rapid growth of ginseng roots, as well as the regulatory role played by microbes in the process, and to explore the critical stage of long-term cultivation of ginseng under forest, so as to provide references for the further precise cultivation and harvesting of ginseng under forest.

MATERIAL AND METHODS

Site description and soil sampling. The experiment was conducted in 2022 at the ginseng under forest plantation and breeding base of Dayang Ginseng Industry in Helong City, Yanbian Korean Autonomous Prefecture, Jilin Province, China (41°59'44"N, 128°22'42"E; 650 m a.s.l.). The test area has a frost-free period of about 120 days, the average annual precipitation was 573.6 mm, the average annual temperature was 5.6 °C, and the average annual humidity was about 70%. The soil type was dark-brown earth (Chromic Luvisols, FAO Soil Classification System), with a measured pH of 5.6. The organic carbon content of soil is 35.8 g C/kg, the cation exchange capacity is 25 $cmol_+$/kg, the content of alkaline hydrolysed nitrogen is 204.22 mg/kg, the content of available potassium is 161.89 mg/kg, and the content of available phosphorus is 12.39 mg/kg.

The rapid growth of ginseng root is mainly in the stage after the red fruit stage, when ginseng enters into the rapid growth stage, with rapid accumulation of saponin content, higher amount of starch, and plump ginseng firmness. The rapid growth of ginseng roots after the red-fruit stage was divided into three stages: the early stage of rapid root growth, the middle stage of rapid root growth, and the late stage of rapid root growth. This experiment collected the rhizosphere soil of ginseng under forest for 5, 10 and 15 years during stages A (the early stage of rapid root growth for which the sample was collected in July); B (the middle stage of rapid root growth for which the sample was collected in August), and C (the late stage of rapid root growth for which the sample was collected in September). A three-point sampling method was adopted. The 10 cm surface soil layer of the selected areas was removed, and 4–10 cm of soil was taken at a distance of 3 cm in diameter around the ginseng and fibrous roots. The rhizosphere soil of 30 ginseng was collected and evenly mixed randomly from each sample site, and

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all soil samples were transported to the laboratory in ice boxes. A portion of the soil was naturally air-dried and successively through an 18-mesh, 60-mesh and 100-mesh sieve to determine soil properties. A portion of soil was stored at 4 °C for soil enzyme activity within a week. Rhizosphere soil was collected using the root shaking method and stored at –80 °C to detect rhizosphere microorganisms. The samples were labelled as TA5, TA10, TA15, TB5, TB10, TB15, TC5, TC10, and TC15 according to the cultivation duration of the crop and growth stages. Each experimental treatment was analysed using three biological replicates.

Soil physicochemical analysis. Soil pH was measured using a pH meter in a 1:2.5 soil-water suspension. Soil organic carbon (SOC) was measured using the potassium dichromate oxidation-external heating method (Fan et al. 2017). Soil total nitrogen (TN) was determined using the Kjeldahl nitrogen process (Jones and Willett 2006). Soil total phosphorus (TP) was determined by the molybdenum antimony colourimetric method (Shen et al. 2019). Soil total potassium (TK) and available potassium (AK) were extracted from the soil with NH_4OAc and determined by a flame photometer (Bilias and Barbayiannis 2019). Soil alkaline hydrolysed nitrogen (AHN) was determined using the alkali hydrolysis diffusion method (Gianello and Bremner 1986). Soil-available phosphorus (AP) was determined by sodium bicarbonate extraction-molybdenum antimony spectrophotometry (Miranda et al. 2001).

Soil enzyme activity. The activity of soil urease (UE) was determined by sodium phenol-sodium hypochlorite colourimetry (Li et al. 2019). The soil phosphatase (ALP) activity was determined by disodium phenyl phosphate colourimetry (Tan et al. 2014). The activity of soil sucrase (E1103) was determined by 3,5-dinitrosalicylic acid colourimetry (Gao et al. 2013). The activity of soil catalase (CAT) and cellulase (CEN/EG) was determined using potassium permanganate titration (Johansson and Borg 1988, Chen et al. 2023).

Soil DNA extraction. The total DNA was extracted from 0.25–0.5 g of fresh soil using a Magnetic Soil and Stool DNA Kit (Tiangen Biotech, Beijing, China). The DNA quality was tested by 1.2% agarose gel electrophoresis and DNA Analyser System SMA 1000 (Merinton, Beijing, China).

Genome amplification and sequencing. The statistical analysis in the biological section was conducted using BMKCloud (www.biocloud.net), and microbial diversity was sequenced based on the Illumina Novaseq platform. We utilised paired-end sequencing to construct short fragment libraries for sequencing. By assembling, filtering, clustering or denoising reads and conducting species annotation and abundance analysis, we could uncover the microbial composition of the samples. Further analysis, such as redundancy and correlation analysis, helps to explore differences between samples.

Statistical analysis. A two-way analysis of variance (ANOVA) with Duncan's test was performed using SPSS 20.0 (IBM Corporation, Armonk, USA) to analyse the differences in the soil properties and soil enzyme activity. Significant differences among treatments were indicated by distinct letters at $P < 0.05$ by Duncan's multiple range test. Redundancy analysis (RDA) assessed the relationship between soil properties and microbial community composition. The RDA was performed using the "vegan" package in R (V3.6.2, Bell Laboratories, USA).

RESULTS

Soil physicochemical properties. The results in Figure 1 indicate that the content of organic carbon, alkaline hydrolysed nitrogen and available phosphorus in 5-year-old ginseng under forest rhizosphere soil was significantly reduced in the C stage ($P < 0.05$). The contents of organic carbon, alkaline hydrolysed nitrogen and available potassium in the rhizosphere soil of 10-year-old ginseng under forest showed a trend of decreasing first and then increasing with increasing period, with stage B significantly lower than the other stages ($P < 0.05$). The contents of organic carbon, alkaline hydrolysed nitrogen and available phosphorus in the rhizosphere soil of 15-year-old ginseng under forest showed a trend of increasing first and then decreasing with increasing period, with stage B significantly higher than the other stages ($P < 0.05$).

In stages A and C, the organic carbon content of the soil showed a trend of increasing first and then decreasing with increasing cultivation duration of the crop ($P < 0.05$). In stage B, the content of organic carbon, alkaline hydrolysed nitrogen, and available phosphorus in the soil showed a trend of decreasing first and then increasing with increasing cultivation duration of the crop, and the 10-year-old ginseng under forest was significantly lower than that of other cultivation duration of the crop ($P < 0.05$).

Soil enzyme activity. The results in Figure 2 indicate that the activities of urease and catalase in the

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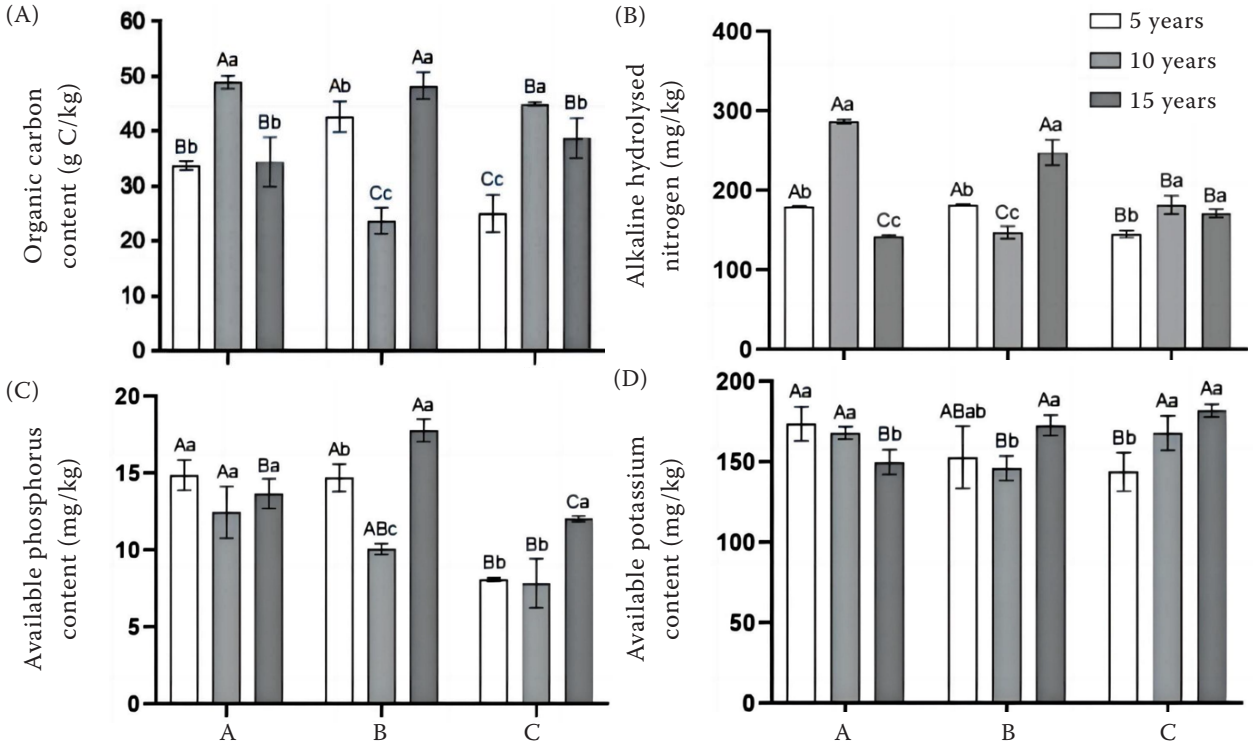

Figure 1. Soil physicochemical properties in soil under different cultivation durations of the crop and growth stages of ginseng under forest. (A) Organic carbon; (B) alkaline hydrolysed nitrogen; (C) available phosphorus; and (D) available potassium. Capital letters indicate significant differences between different growth stages of the same cultivation duration of the crop ($P < 0.05$); lowercase letters indicate significant differences between different cultivation duration of the crop during the same growth stage ($P < 0.05$). A – early stage of rapid root growth for which the sample was collected in July; B – middle stage of rapid root growth for which the sample was collected in August; C – late stage of rapid root growth for which the sample was collected in September

rhizosphere soil of 5-year-old and 15-year-old ginseng under forest showed a trend of increasing first and then decreasing with increasing period and were significantly higher in stage B than in stage A and C ($P < 0.05$), while phosphatase activity was the lowest in stage A. The activities of sucrase and catalase in the rhizosphere soil of 10-year-old ginseng under the forest showed a trend of decreasing first and then increasing with increasing periods, with significant differences ($P < 0.05$) between different growth stages.

In stages, A and C, the activities of urease and catalase in the rhizosphere soil of ginseng under forest showed a trend of increasing first and then decreasing with increasing cultivation duration of the crop, and the 10-year-old ginseng under forest was significantly higher than the other cultivation duration of the crop ($P < 0.05$). In stage B, urease, phosphatase, sucrase, and catalase activities in the rhizosphere soil of 10-year-old ginseng under forest were significantly lower than those of 15-year-old ginseng under forest ($P < 0.05$). The sucrase and cellulase activities increased from the cultivation duration of the crop during the C stage, with significant differences ($P < 0.05$) between the cultivation duration of the crop.

Venn diagrams of bacterial and fungal OTUs. To characterise the microbial community in the rhizosphere soil of ginseng under forest with different cultivation duration of the crop and growth stages, Illumina MiSeq sequenced nine samples. A total of 329 bacterial OTUs (Figure 3A) and 686 fungal OTUs (Figure 3B) were found in nine different ginseng under forest rhizosphere soils, with 310 shared bacterial OTUs and 658 shared fungal OTUs.

Alpha diversity indices of the bacterial and fungal communities. The alpha diversity reflects the abundance and diversity of microorganisms in soil. The diversity and richness indices of bacterial communities are listed in Table 1, and fungal in Table 2. The coverage indices between the groups of sequenced bacterial and fungal samples were all above 0.989, indicating that the amount of sequenc-

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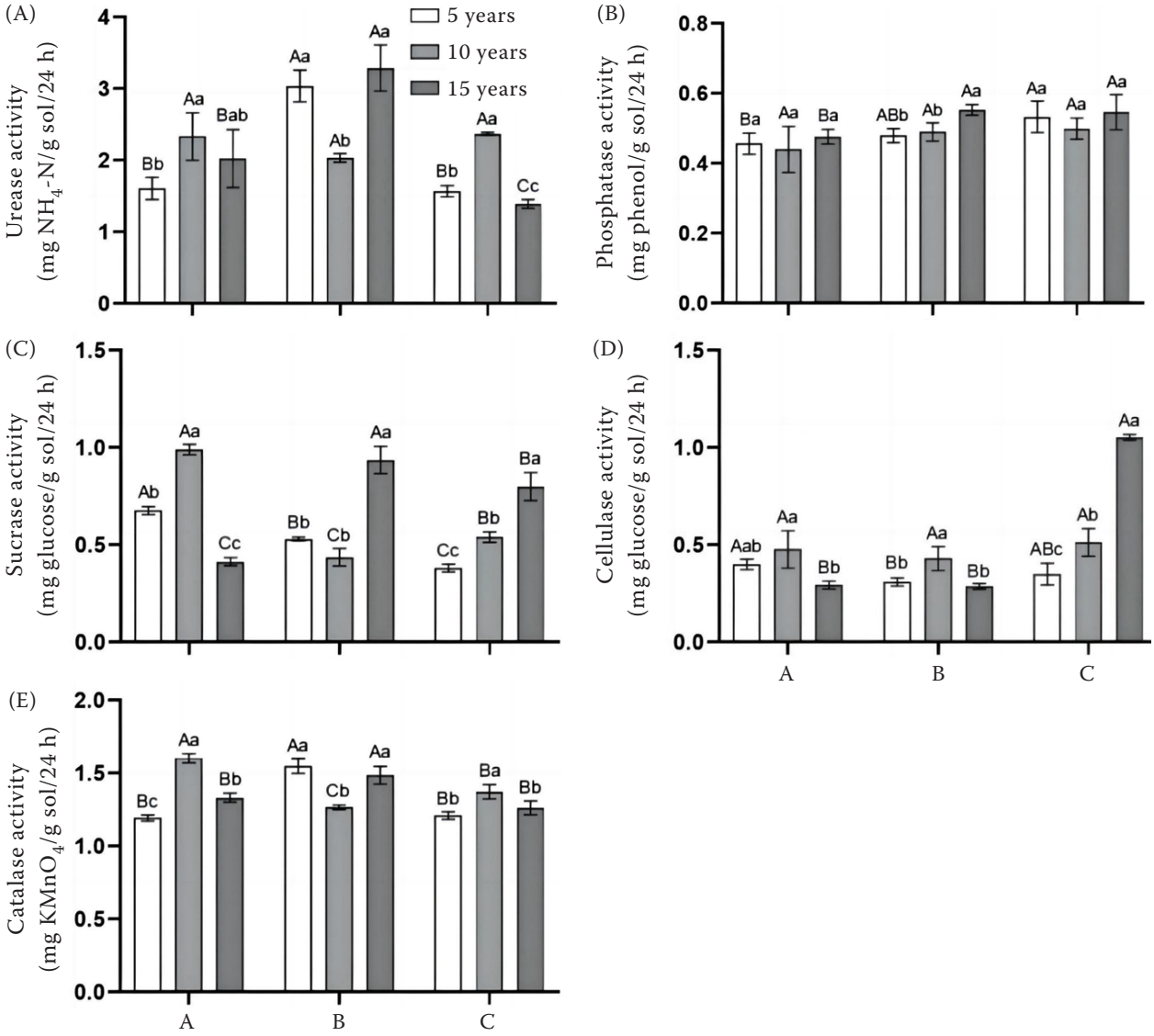

Figure 2. Soil enzyme activities in soil under different cultivation durations of the crop and growth stages of ginseng under forest. (A) Urease; (B) phosphatase; (C) sucrase; (D) cellulase, and (E) catalase. Capital letters indicate significant differences between different growth stages of the same cultivation duration of the crop ($P < 0.05$); lowercase letters indicate significant differences between different cultivation duration of the crop during the same growth stage ($P < 0.05$). A – early stage of rapid root growth for which the sample was collected in July; B – middle stage of rapid root growth for which the sample was collected in August; C – late stage of rapid root growth for which the sample was collected in September

ing was sufficient to cover the microbial composition of these samples.

The results showed that the bacterial Chao1 and Shannon indices of 10-year-old ginseng under forest showed a trend of decreasing first and then increasing with increasing period, and the lowest bacterial diversity was found in TB10. In stage A, the Chao1 and Shannon indices of bacteria showed a trend of increasing first and then decreasing with increasing cultivation duration of the crop, and TA10 had the highest bacterial diversity and abundance (Table 1). In stage B, the fungal Chao1 and Shannon indices gradually decrease with the increase in the cultivation duration of the crop (Table 2).

Soil bacterial and fungal abundance. The results showed that the dominant bacterial phylum in the nine soil treatments belonged to the Proteobacteria. The relative abundance of Proteobacteria and Firmicutes in the TA5 was significantly higher than that of other bacteria, accounting for about 56% and 15% of the

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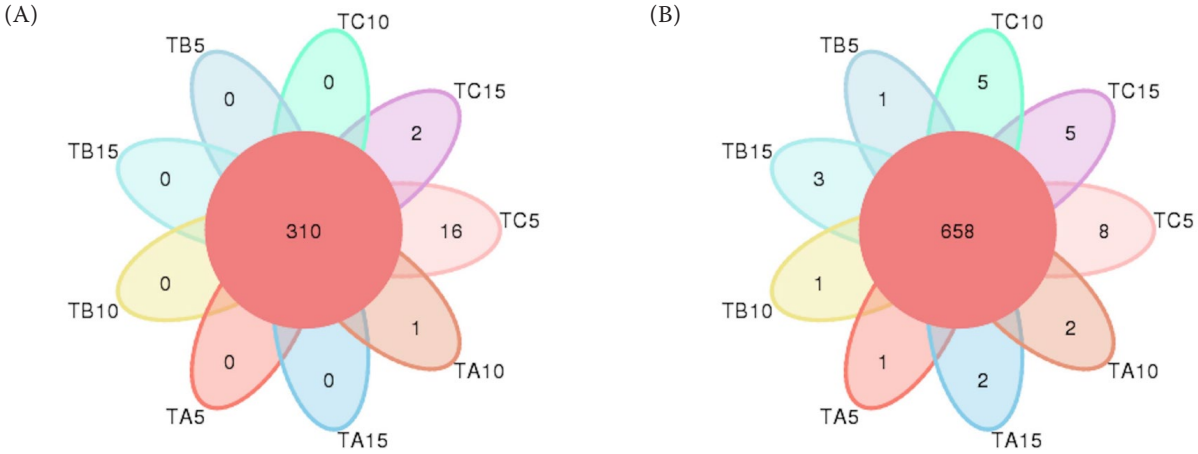

Figure 3. Venn diagrams of (A) bacterial and (B) fungal operational taxonomic units (OTUs) in the nine soil samples. TA5 – soil from ginseng under forest planted for 5 years collected in July; TA10 – soil from ginseng under forest planted for 10 years collected in July; TA15 – soil from ginseng under forest planted for 15 years collected in July; TB5 – soil from ginseng under forest planted for 5 years collected in August; TB10 – soil from ginseng under forest planted for 10 years collected in August; TB15 – soil from ginseng under forest planted for 15 years collected in August; TC5 – soil from ginseng under forest planted for 5 years collected in September; TC10 – soil from ginseng under forest planted for 10 years collected in September; TC15 – soil from ginseng under forest planted for 15 years collected in September

total number of bacteria. The relative abundance of Proteobacteria was significantly higher, and the relative abundance of Firmicutes, Acidobacteria, and Verrucomicrobia was significantly lower in TB10 compared to that in TA10 and TC10 (Figure 4A).

Further analysis of the classification information of species at the genus level indicated that the most notable bacterial dominant genus was the *uncultured_bacterium_f_Enterobacteriaceae*, which accounted for about 29% of the total number of bacteria. The relative abundance of the *uncultured_bacterium_f_Enterobacteriaceae* genus decreased gradually with increasing period, and it was highly enriched in TA5 and TA15. The abundance of *Candidatus udaeobac-*

Table 1. The alpha diversity of bacteria in the rhizosphere soil of the nine samples

Sample ID	Feature	ACE	Chao1	Simpson	Shannon	PD_whole_tree	Coverage
TA5	515	754.6929	728.6947	0.6362	2.5046	32.2971	0.9972
TA10	966	992.9586	1010.6667	0.9777	7.0517	49.2089	0.9990
TA15	566	804.7771	802.0194	0.5817	2.2298	33.5347	0.9970
TB5	914	956.8742	965.1875	0.9661	6.4863	46.0546	0.9987
TB10	929	978.9987	992.9241	0.8991	5.7316	47.8027	0.9986
TB15	904	983.2419	1004.1236	0.9662	6.5294	46.4842	0.9979
TC5	1023	1047.8496	1064.5909	0.9913	8.1627	51.7991	0.9990
TC10	978	994.1147	1006.2857	0.9858	7.8677	49.2994	0.9993
TC15	892	925.8084	935.3333	0.9883	7.9030	45.8503	0.9989

ACE – index abundance based coverage estimator; PD_whole_tree – index phylogenetic diversity whole tree. TA5 – soil from ginseng under forest planted for 5 years collected in July; TA10 – soil from ginseng under forest planted for 10 years collected in July; TA15 – soil from ginseng under forest planted for 15 years collected in July; TB5 – soil from ginseng under forest planted for 5 years collected in August; TB10 – soil from ginseng under forest planted for 10 years collected in August; TB15 – soil from ginseng under forest planted for 15 years collected in August; TC5 – soil from ginseng under forest planted for 5 years collected in September; TC10 – soil from ginseng under forest planted for 10 years collected in September; TC15 – soil from ginseng under forest planted for 15 years collected in September

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Table 2. The alpha diversity of fungi in the rhizosphere soil of the nine samples

Sample ID	Feature	ACE	Chao1	Simpson	Shannon	PD_whole_tree	Coverage
TA5	1284	1306.3027	1341.973	0.9863	8.1471	177.7352	0.9991
TA10	1334	1374.5658	1387.602	0.9531	7.2233	180.7904	0.9986
TA15	1312	1332.6276	1354.9773	0.9898	8.2863	175.994	0.9992
TB5	1332	1351.7598	1363.0526	0.9883	8.2759	185.0849	0.9991
TB10	1290	1303.864	1322.2368	0.9851	8.1356	175.996	0.9993
TB15	1217	1276.6612	1275.5532	0.9668	6.6610	1701221	0.9982
TC5	1319	1335.1073	1337.1316	0.9823	7.9111	180.9524	0.9993
TC10	1335	1352.0234	1366.7885	0.9868	8.0374	180.5606	0.9992
TC15	1235	1275.0106	1279.0110	0.9626	7.2928	166.5613	0.9988

ACE – index abundance based coverage estimator; PD_whole_tree – index phylogenetic diversity whole tree. TA5 – soil from ginseng under forest planted for 5 years collected in July; TA10 – soil from ginseng under forest planted for 10 years collected in July; TA15 – soil from ginseng under forest planted for 15 years collected in July; TB5 – soil from ginseng under forest planted for 5 years collected in August; TB10 – soil from ginseng under forest planted for 10 years collected in August; TB15 – soil from ginseng under forest planted for 15 years collected in August; TC5 – soil from ginseng under forest planted for 5 years collected in September; TC10 – soil from ginseng under forest planted for 10 years collected in September; TC15 – soil from ginseng under forest planted for 15 years collected in September

ter genus was decreased significantly in TB10. The relative abundance of the *Acinetobacter* genus was highly enriched in TB10, while the *Serratia* genus was enriched in TB15. The percentage of other bacterial groups was 48% (Figure 4C).

The dominant fungal phyla in the rhizosphere soil of ginseng under the forest were Ascomycota and Basidiomycota. The relative abundance of Ascomycota was 67%, with relative abundance decreasing with the cultivation duration of the crop in stage B. The relative abundance of Basidiomycota was highly abundant in TC15. The relative abundance of Mortierellomycota was significantly increased in TB15 compared to that in TB5 and TB10 (Figure 4B).

The dominant fungal genus was successively *Aspergillus*, *Fusarium*, *Mortierella*, *Cladosporium*, *Kazachstania*, *Russula*, *Trichosporon*, *Trichoderma*, *Penicillium*, and *Botryotrichum*. The *Fusarium* genus was significantly enriched in TA10 but abruptly decreased afterwards. The relative abundance of 10-year-old ginseng under forest *Kazachstania* presented a trend of increasing first and then decreasing with increasing periods, with the highest relative abundance of *Kazachstania* in TB10. The relative abundance of *Mortierella* was significantly higher in TB15 than in TB5 and TB10. The relative abundance of *Trichoderma* and *Russula* genus were highly enriched in TB15 and TC15, respectively (Figure 4D).

Heatmap of the Cluster correlation analysis. The correlation heat map showed that at the bacterial phylum level (Figure 5A), TC15 was extremely significantly positively correlated with WPS-2 and Dependentiae; TC10 was significantly positively correlated with Fusobacteria and Spirochaetes, and TC5 was significantly positively correlated with Epsilonbacteraeota, Tenericutes, and Deferribacteres. TA5 and TA15 were mainly significantly negatively correlated with the dominant microorganisms at the level of soil phylum. At the bacterial genus level (Figure 5B), TC15 was extremely significantly positively correlated with *Singulisphaera*; TB15 was significantly positively correlated with *Serratia* and *Burkholderia-Caballeronia-Paraburkholderia*. TB10 was significantly positively correlated with *Acinetobacter*, and TB5 was significantly positively correlated with *Sphingobium*. TA5 and TA15 were mainly significantly negatively correlated with the dominant microorganisms at the level of the soil genus.

At the fungal phylum level (Figure 5C), TA10 was significantly positively correlated with Zoopagomycota; TC15 was significantly positively correlated with Basidiomycota and Olpidiomycota; TB15 was significantly positively correlates with Mucoromycota, and TC5 was significantly positively correlated with Mortierellomycota and Kickxellomycota.

At the fungal genus level (Figure 5D), TC10 was significantly positively correlated with *Amanita*; TC5 was significantly positively correlated with *Papiliotrema* and *Wallemia*; TC15 was significantly

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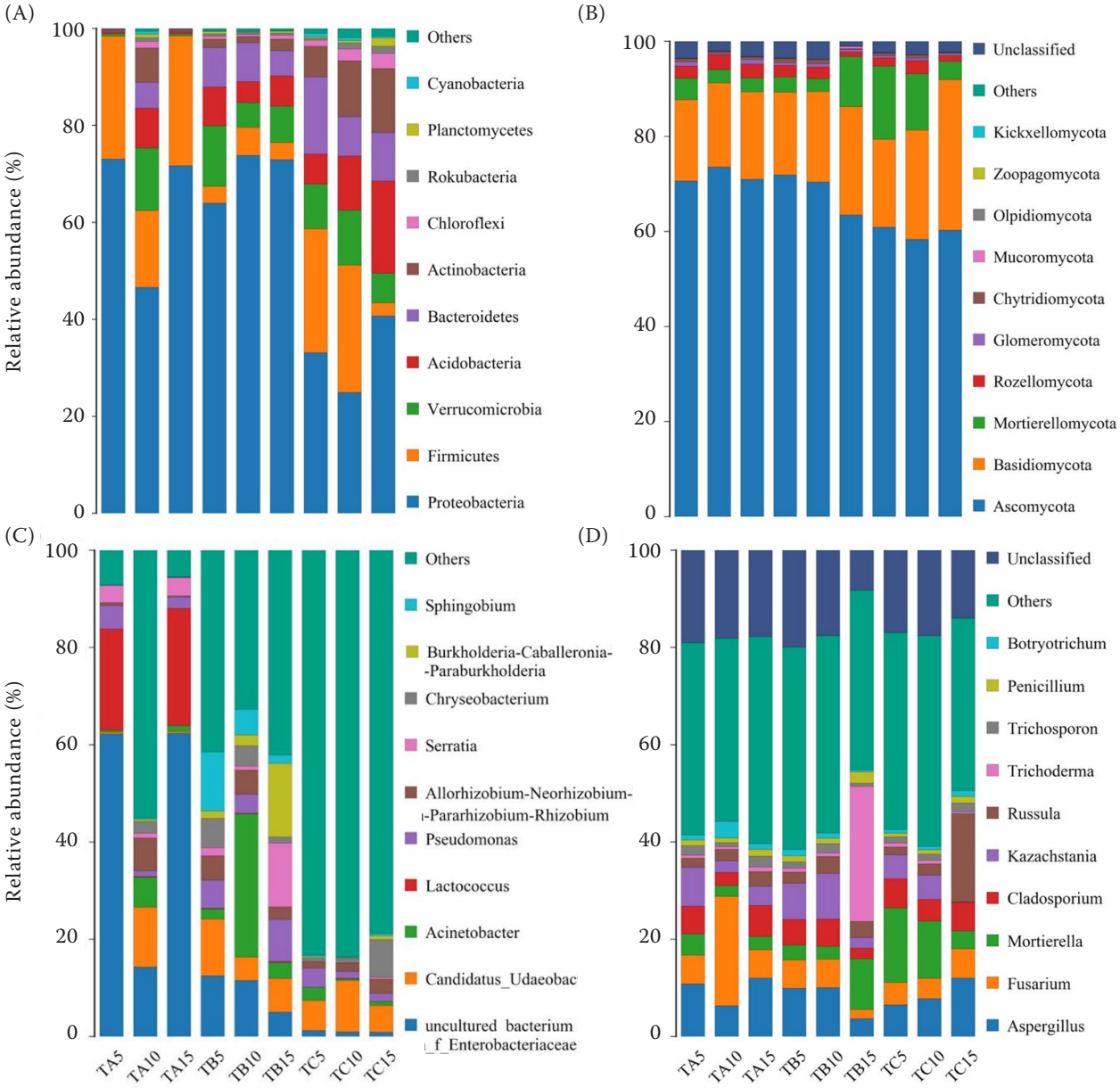

Figure 4. Relative abundance of bacterial and fungal taxa at (A, B) phyla and (C, D) genera levels in different soil samples. TA5 – soil from ginseng under forest planted for 5 years collected in July; TA10 – soil from ginseng under forest planted for 10 years collected in July; TA15 – soil from ginseng under forest planted for 15 years collected in July; TB5 – soil from ginseng under forest planted for 5 years collected in August; TB10 – soil from ginseng under forest planted for 10 years collected in August; TB15 – soil from ginseng under forest planted for 15 years collected in August; TC5 – soil from ginseng under forest planted for 5 years collected in September; TC10 – soil from ginseng under forest planted for 10 years collected in September; TC15 – soil from ginseng under forest planted for 15 years collected in September

positively correlated with *Russula*; TA10 was significantly positively correlated with *Cladophialophora*, *Erysiphe*, *Fusarium* and *Botryotrichum*, and TB15 was significantly positively correlated with *Penicillium*, *Solicoccozyma* and *Trichoderma*.

Relationships between the microbial community and soil properties. The pH, AK, and AP were the main factors that changed the bacterial community structures in soil (Figure 6). pH was positively correlated with Actinobacteria and Chloroflexi but negatively

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(A)

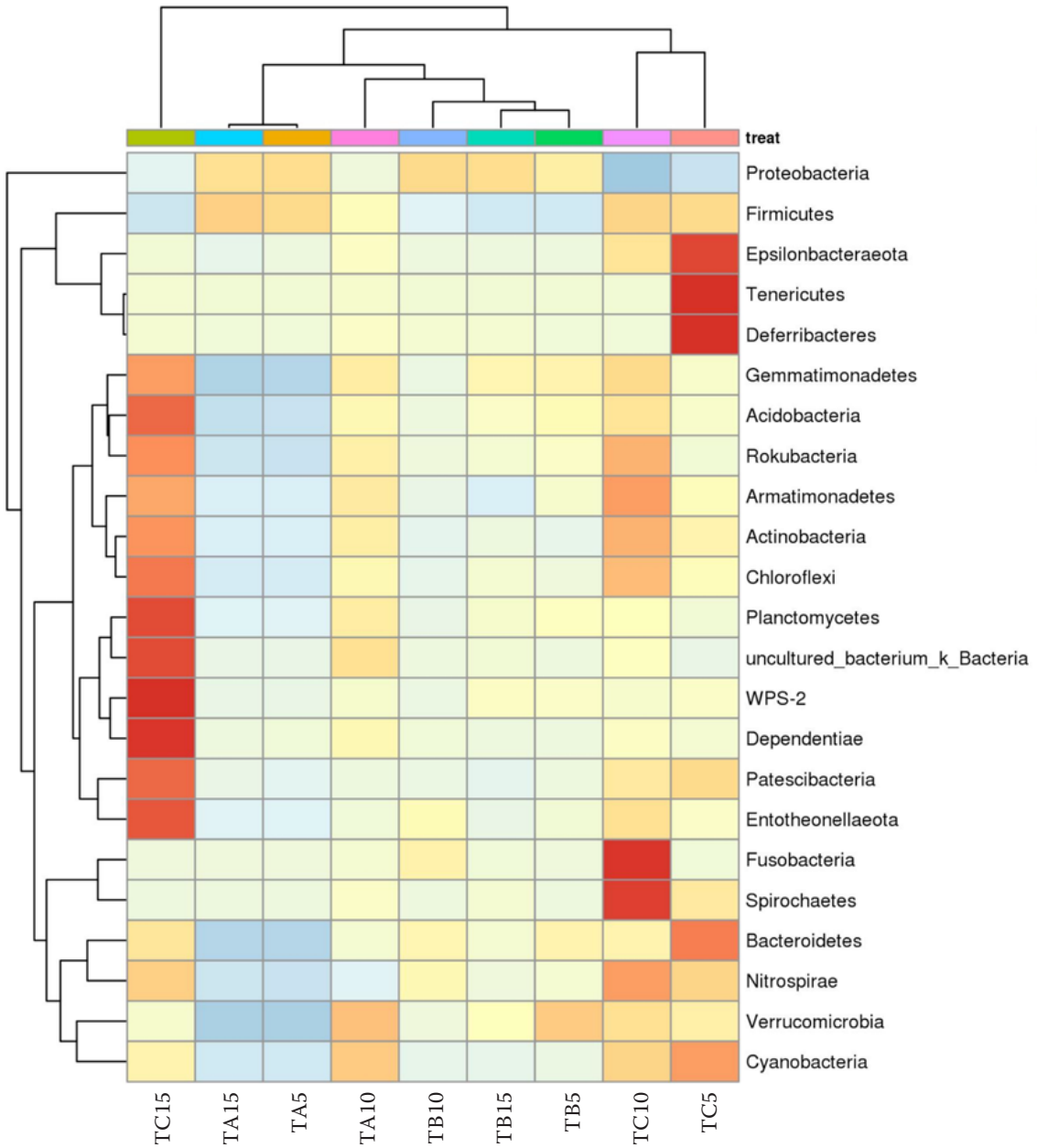

Figure 5. Heatmaps of soil bacterial and fungal communities at (A, C) phyla and (B, D) genera levels in the different soil samples. TA5 – soil from ginseng under forest planted for 5 years collected in July; TA10 – soil from ginseng under forest planted for 10 years collected in July; TA15 – soil from ginseng under forest planted for 15 years collected in July; TB5 – soil from ginseng under forest planted for 5 years collected in August; TB10 – soil from ginseng under forest planted for 10 years collected in August; TB15 – soil from ginseng under forest planted for 15 years collected in August; TC5 – soil from ginseng under forest planted for 5 years collected in September; TC10 – soil from ginseng under forest planted for 10 years collected in September; TC15 – soil from ginseng under forest planted for 15 years collected in September

correlated with Proteobacteria. AK was positively correlated with Planctomycetes and Acidobacteria but negatively correlated with Firmicutes. AP was positively correlated with Proteobacteria but negatively correlated with Firmicutes (Figure 6A).

The pH was positively correlated with *Candidatus udaeobacter* but negatively correlated with *Pseudomonas*, *Lactococcus* and *Serratia*; AK was positively correlated with *Chryseobacterium* and *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*, and AP was

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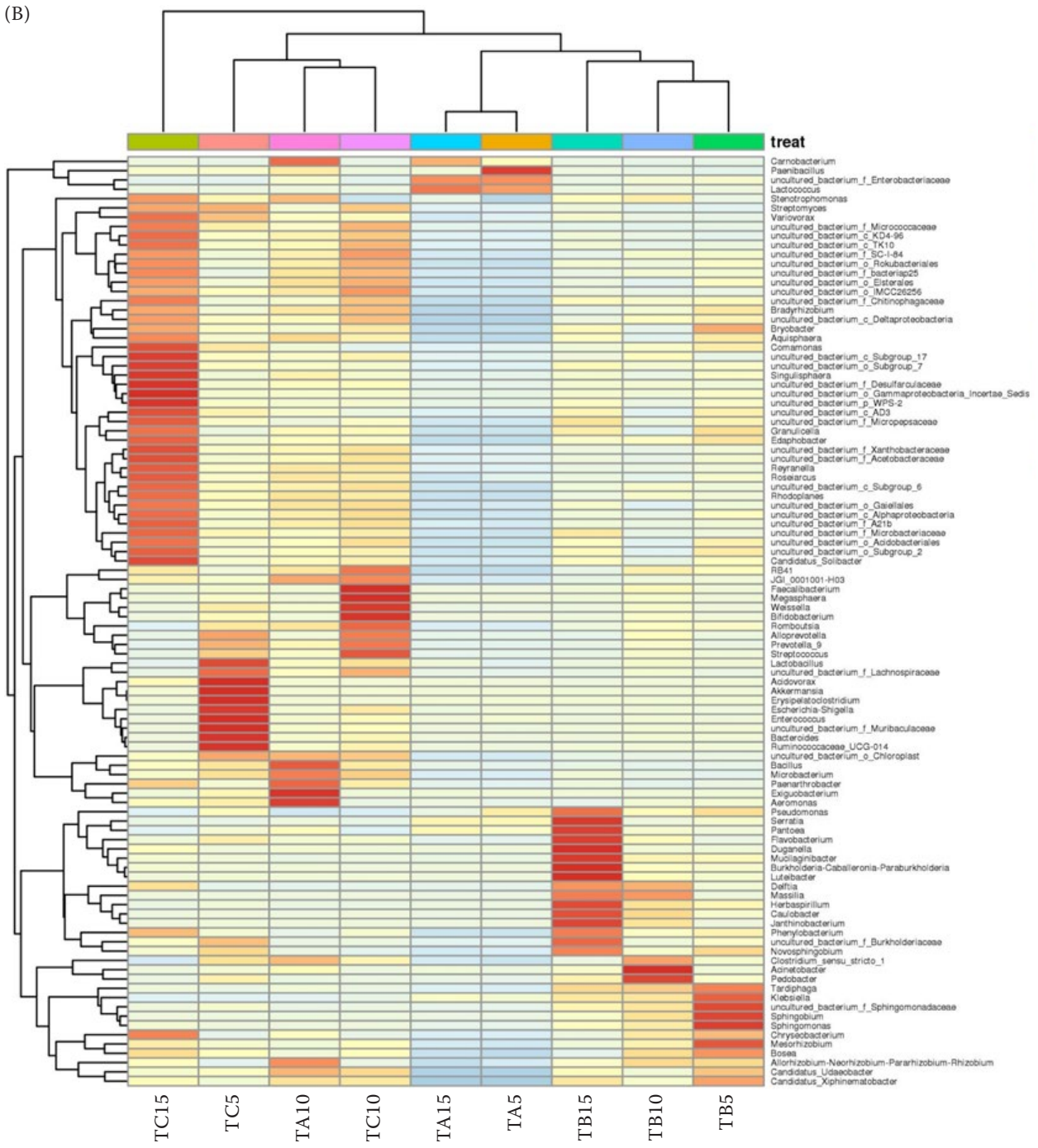

Continued Figure 5. Heatmaps of soil bacterial and fungal communities at (A, C) phyla and (B, D) genera levels in the different soil samples

positively correlated with *Burkholderia-Caballeronia-Paraburkholderia* and *Sphingobium* (Figure 6B).

The pH, AK and TK were the main factors that changed the fungal community structures in the soil. The pH and AK were positively correlated with Basidiomycota and Olpidiomycota but negatively correlated with Ascomycota, Chytridiomycota, Rozellomycota and Zoopagomycota. Total potassium was positively correlated with Zoopagomycota and Kickxellomycota but negatively correlated with Olpidiomycota (Figure 7A).

The TK and AHN were the main factors that changed the fungal community structures in the soil. TK, AHN, TN, TP, SOC and AK were significantly positively correlated with *Penicillium*; AP was positively correlated with *Aspergillus* but negatively correlated with *Mortierella* and *Trichoderma* (Figure 7B).

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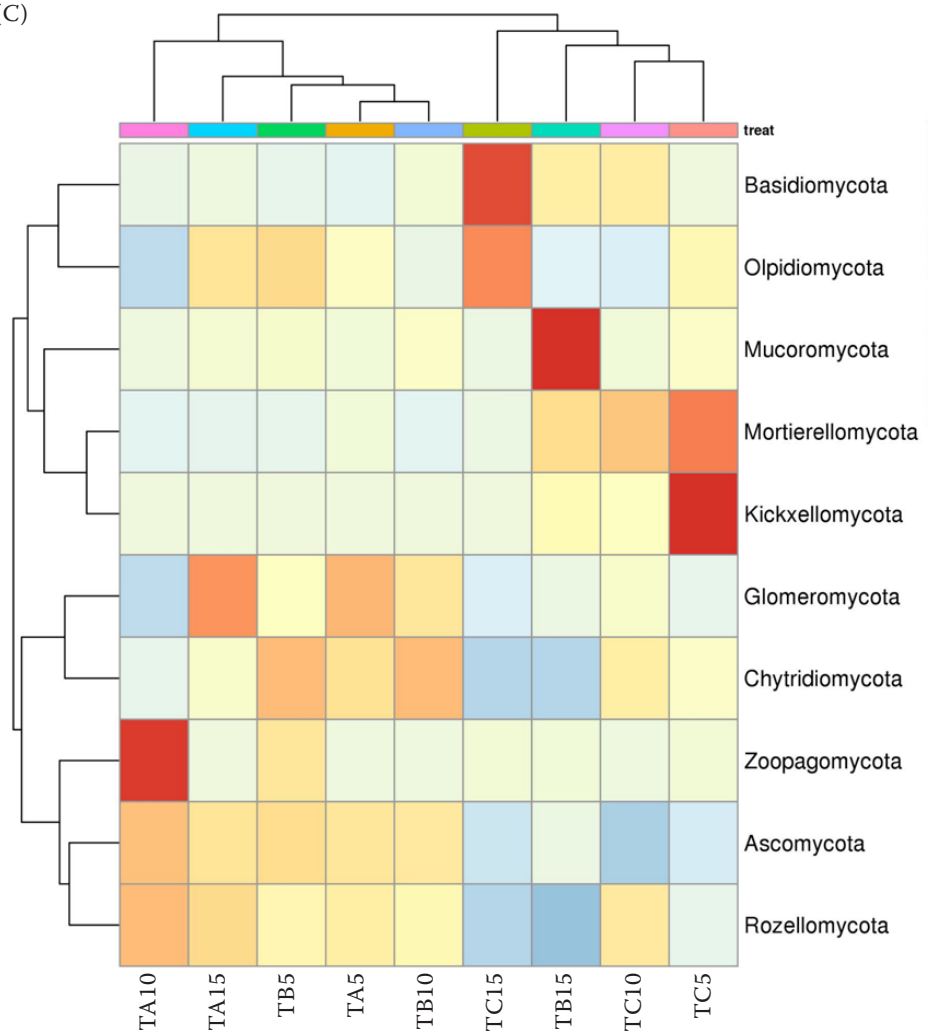
(C)



Continued Figure 5. Heatmaps of soil bacterial and fungal communities at (A, C) phyla and (B, D) genera levels in the different soil samples

Relationships between the microbial community and soil enzyme. ALP was positively correlated with Actinobacteria and Chloroflexi but negatively correlated with Proteobacteria. CEN was positively correlated with Planctomycetes and Acidobacteria but negatively correlated with Firmicutes. E1103 was negatively correlated with Firmicutes, and UE was positively correlated with Proteobacteria (Figure 8A).

Lactococcus, *Acinetobacter*, *Pseudomonas*, *Serratia*, *Burkholderia-Caballeronia-Paraburkholderia* and *Sphingobium* were negatively correlated with ALP but positively correlated with UE. CEN, E1103 and CAT were positively correlated with *Bradyrhizobium*, *Chryseobacterium*, *Allorhizobium-Neorhizobium-Parahizobium-Rhizobium* and *Candidatus Udaeobacter* (Figure 8B).

ALP was positively correlated with Mortierellomycota, Kickxellomycota, Olpidiomycota and Mucoromycota but negatively correlated with Rozellomycota and Ascomycota. EG and E1103 were positively correlated with Basidiomycota but negatively correlated with Glomeromycota and Chytridiomycota. UE and CAT were significantly positively correlated with Zoopagomycota (Figure 9A).

ALP was positively correlated with *Trichoderma*, *Mortierella* and *Penicillium* but negatively correlated with *Aspergillus*. EG, E1103, UE and CAT were positively correlated with *Russula*, *Fusarium* and *Botryotricichum* but negatively correlated with *Kazachstania*, *Cladosporium* and *Trichosporon* (Figure 9B).

DISCUSSION

Changes in physicochemical properties and enzyme activities of ginseng under forest rhizosphere

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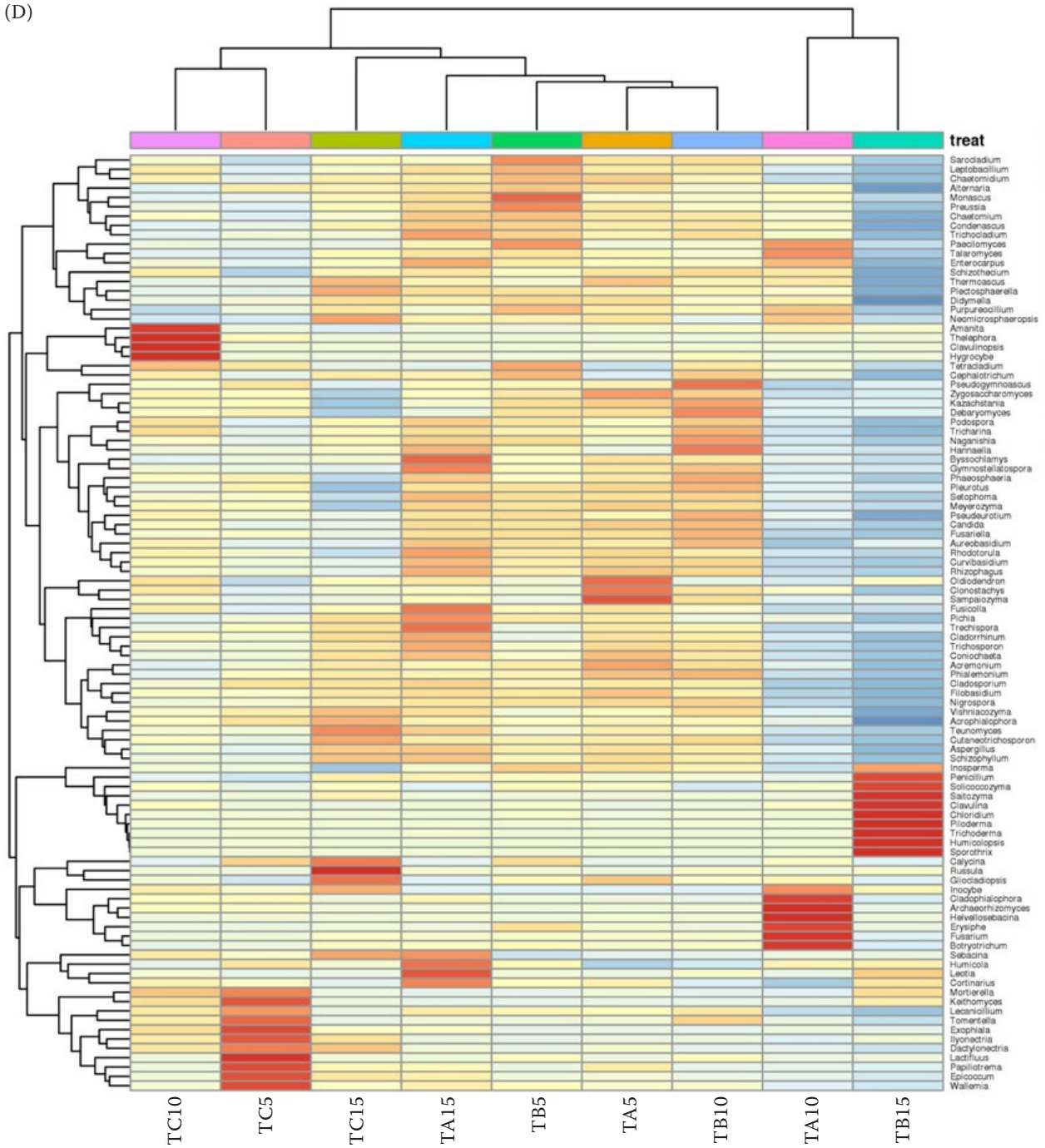

Continued Figure 5. Heatmaps of soil bacterial and fungal communities at (A, C) phyla and (B, D) genera levels in the different soil samples

soil in different cultivation duration of the crop and growth stages. Soil physicochemical properties and soil enzyme activities reflect soil fertility and are important indicators for assessing soil fertility (Xu et al. 2022). In this study, the results showed that soil nutrient content and soil enzyme activities showed generally consistent trends with the cultivation duration of the crop and growth stage. In stages A and C, soil organic carbon and alkaline hydrolysed nitrogen content increased to varying degrees in the short-term successive cultivation years (T5 and T10) and declined after 15 years of successive cultivation. These were compatible with the results of Niu et al. (2015) and Chen et al. (2018), who found that soil-available nitrogen, phosphorus and organic carbon decreased significantly after 25 years of continuous

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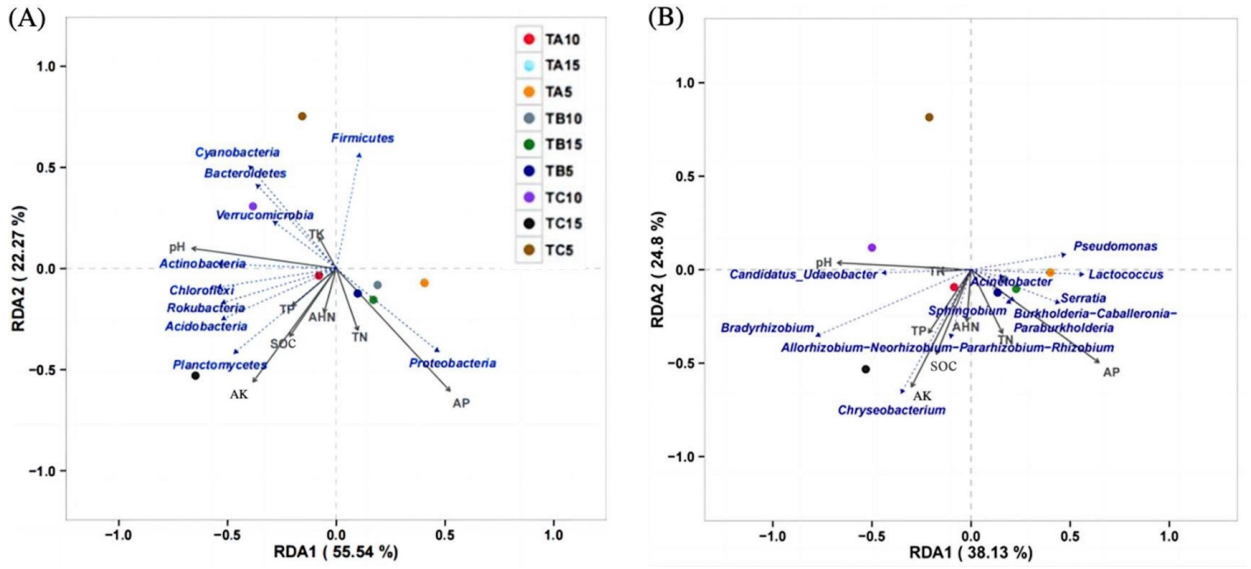

Figure 6. Redundancy analysis (RDA) of soil properties and community composition of bacteria at (A) phyla and (B) genera levels in the different soil samples. TN – total nitrogen; TP – total phosphorus; TK – total potassium; SOC – soil organic carbon; AHN – alkaline hydrolysed nitrogen; AP – available phosphorus; AK – available potassium; TA5 – soil from ginseng under forest planted for 5 years collected in July; TA10 – soil from ginseng under forest planted for 10 years collected in July; TA15 – soil from ginseng under forest planted for 15 years collected in July; TB5 – soil from ginseng under forest planted for 5 years collected in August; TB10 – soil from ginseng under forest planted for 10 years collected in August; TB15 – soil from ginseng under forest planted for 15 years collected in August; TC5 – soil from ginseng under forest planted for 5 years collected in September; TC10 – soil from ginseng under forest planted for 10 years collected in September; TC15 – soil from ginseng under forest planted for 15 years collected in September

planting. The contents of organic carbon, alkaline hydrolysed nitrogen and available potassium in the rhizosphere soil of 10-year-old ginseng under forest were significantly lower ($P < 0.05$) during the B stage, indicating that the 10-year-old ginseng under forest consumed more soil nutrients during this stage.

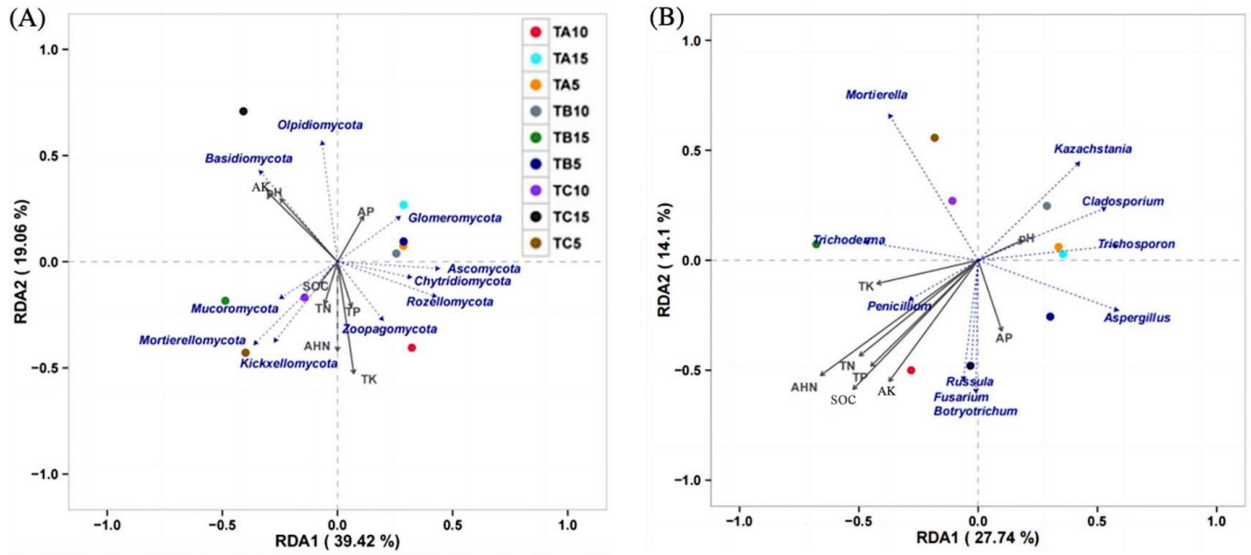

Figure 7. Redundancy analysis (RDA) of soil properties and community composition of fungi at (A) phyla and (B) genera levels in the different soil samples. TN – total nitrogen; TP – total phosphorus; TK – total potassium; SOC – soil organic carbon; AHN – alkaline hydrolysed nitrogen; AP – available phosphorus; AK – available potassium

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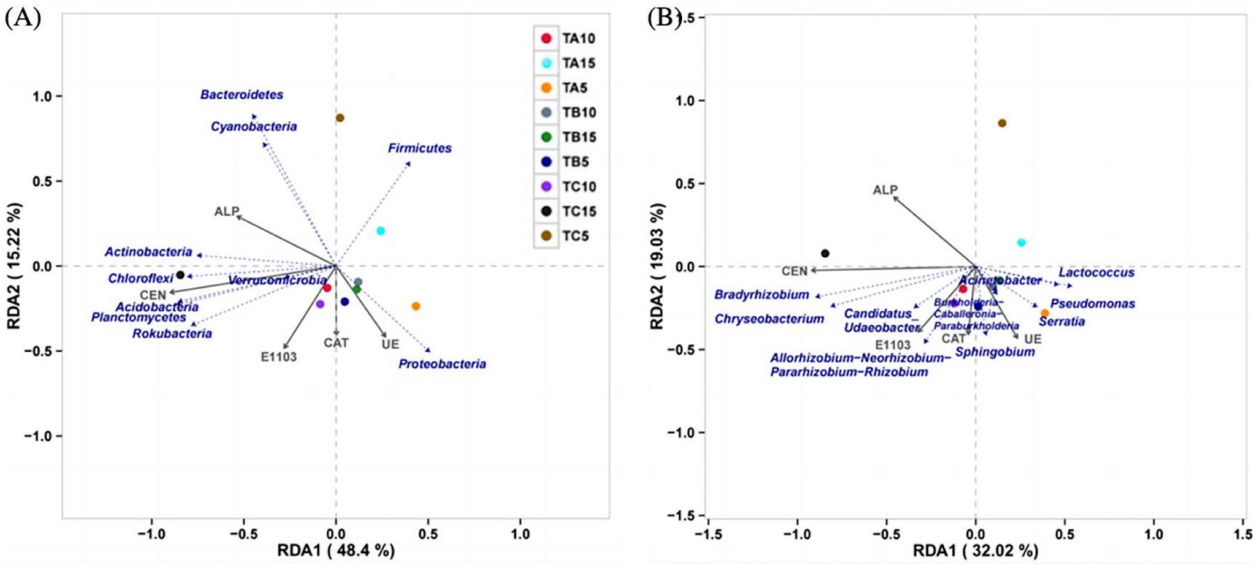

Figure 8. Redundancy analysis (RDA) of soil enzyme and community composition of bacteria at (A) phyla and (B) genera level in the different soil samples. ALP – phosphatase; CEN – cellulase; E1103 – sucrase; CAT – catalase; UE – urease

Because the growth stages after fruiting are a stage of rapid accumulation of saponin content, we speculate that the consumption of soil nutrients by 10-year-old ginseng in forests is related to saponin accumulation. The results of Liu et al. (2017) align with our conjecture, as they found that the average ginsenoside concentration was lowest in July, then increased in August and slightly decreased in September.

Soil enzymes are products of plant and animal residue decomposition, exudation from plant roots and soil microbial metabolism, which involves many crucial biochemical functions in the soil (Floch et al. 2009). Our study found that the activities of sucrase and catalase in the rhizosphere soil of 10-year-old ginseng under forest were significantly decreased ($P < 0.05$) in stage B, which was consistent with the trend of changes in soil physicochemical properties. In stages, A and C, the activities of urease and catalase in the rhizosphere soil of ginseng under the forest showed a trend of increasing first and then

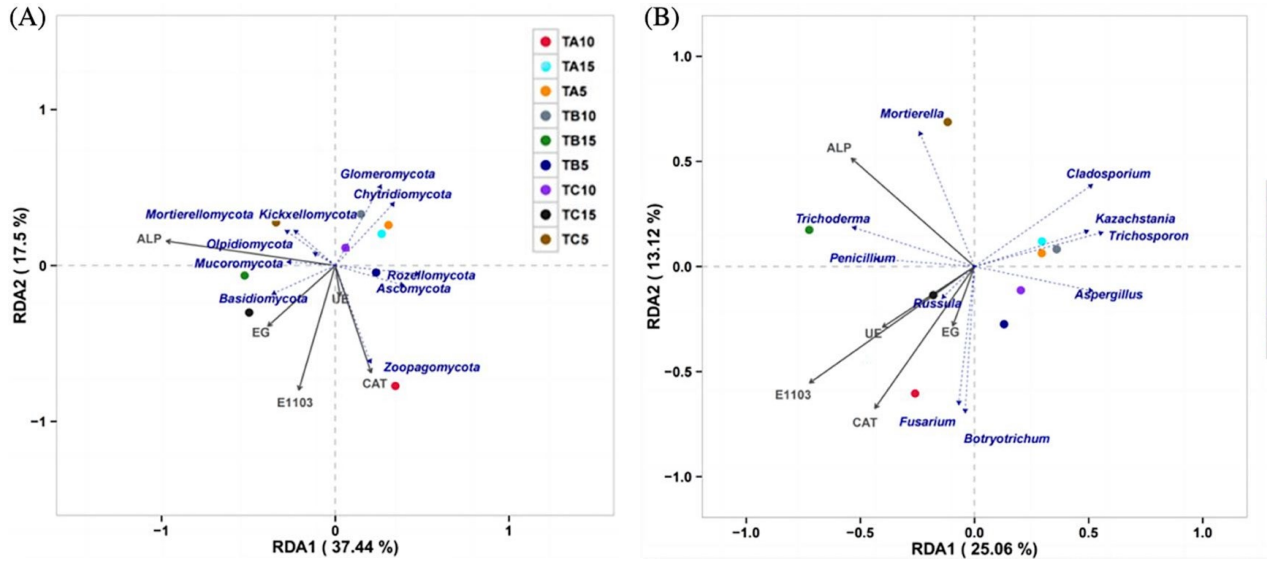

Figure 9. Redundancy analysis (RDA) of fungi's soil enzyme and community composition at (A) phyla and (B) genera levels in the different soil samples. ALP – phosphatase; EG – cellulase; E1103 – sucrase, CAT – catalase; UE – urease

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decreasing with increasing cultivation duration of the crop. In particular, enzyme activities increased after 5 and 10 years of continuous planting but decreased after 15 years. This result is similar to the findings of Wu et al. (2022) when studying the activities of urease, peroxidase, and phosphatase in the soils under different water oat planting years. We speculated that the high increase in the activities of urease and catalase in 5–10 years of continuous cropping could be explained by the increase in soil properties and microbial diversity indexes, which is also consistent with the findings of Wang et al. (2022).

Changes in microbial community composition of ginseng under forest rhizosphere soil in different cultivation duration of the crop and growth stages. Microbial communities can serve as sensitive biomarkers of soil health and function, and they play a crucial role in maintaining soil function and ecosystem sustainability (Zuppinger-Dingley et al. 2014, Zhang et al. 2021). Previous research has found that ginseng cultivation, particularly across cultivation years and for varying periods, affects the abundance and organisation of ginseng rhizosphere microbial populations (Vendan et al. 2012, Aizi et al. 2023). In this study, 10-year-old ginseng under forest bacterial diversity and abundance decreased significantly during stage B (Table 1), which we believe is related to changes in physicochemical properties and enzymatic activities in TB10. Chen et al. (2022) showed that barriers to sustained cultivation of many crops were associated with changes in the structure and composition of soil microbial communities. In stage B, the Chao1 and Shannon indexes of fungi decreased gradually with increasing cultivation duration of the crop (Table 2). These findings are consistent with recent studies on different crop species (Liu et al. 2020a, b), which found that long-term successive cropping reduced the diversity and richness of bacteria and fungi in the rhizosphere soil. In long-term continuous cropping systems, the same type of root secretions are continuously secreted in the rhizosphere, which can stimulate the colonisation of certain microbiota; this may be a possible reason for the decrease in rhizosphere biodiversity under long-term continuous cropping (Li et al. 2019, Liu et al. 2021).

At the phylum level, we found a total of ten dominant phyla, with Proteobacteria and Firmicutes being the dominant bacterial phyla (Figure 4A). Firmicutes contain broad-spectrum biocontrol bacteria such as Bacillus (Fira et al. 2018). A total of ten dominant bacterial genera were found in this study. The relative abundance of *Acinetobacter* in TB10 was significantly higher than in the other samples (Figure 4C). *Acinetobacter* is a broad-spectrum pathogen, and increases in its relative abundance can cause ginseng disease. By analysing the dynamic changes of soil properties, enzyme activities and microbial communities of ginseng under forest at three cultivation duration of the crop and different stages of rapid growth of ginseng roots, we speculate that 10 years is a critical stage for long-term ginseng cultivation under forest. Different from 5- and 15-year-old ginseng under forest, 10-year-old ginseng under forest is more sensitive to changes in environmental factors and may experience specific dynamic changes that severely affect its growth and quality.

Ascomycota and Basidiomycota were the dominant fungal phyla in the ginseng under forest rhizosphere soil samples (Figure 4B). Ascomycota and Basidiomycota are the two main groups of fungi in the soil that have the function of maintaining the stability of the soil microhabitat (Li et al. 2022); most of the Ascomycota are saprophytic fungi which degrade organic substances in the soil such as lignin and keratin (Beimforde et al. 2014). The relative abundance of Mortierellomycota was significantly higher in the TB15 samples. It has been suggested that Mortierellomycota may potentially contain a pathogen that could cause soilborne diseases in ginseng.

The relative abundance of *Fusarium* in TA10 was significantly higher than in other samples but gradually decreased with increasing period (Figure 4D), suggesting that it has a certain self-regulation ability itself, which is consistent with the results of the Fuqua and Winans (1994) study. The relative abundance of *Mortierella* in TB15 was significantly higher than that in TB5 and TB10. Ratledge confirmed that *Mortierella* could produce a variety of polyunsaturated fatty acids; these fatty acids are rich in carbon sources, which may affect the soil microbial community indirectly by altering nutrient uptake and soil microbial habitats (Ratledge and Wynn 2002). The RDA results showed that phosphatase was positively correlated with *Mortierella* (Figure 9B), so we suggest that the increase in the relative abundance of *Mortierella* may be responsible for the significant increase in phosphatase activity in TB15. The results of Kanse et al. (2015) showed that the increase of *Mortierella* can dissolve soil phosphorus by releasing organic acids in the soil to significantly increase phosphatase activity, which in turn affects the available phosphorus con-

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tent in the soil, which is consistent with the results of the present study. Furthermore, it is evident that the samples correlate more strongly with bacteria than fungi (Figure 5), suggesting that changes in cultivation duration of the crop and growth stage have a greater impact on soil bacteria than fungi.

Correlation analysis of physicochemical properties and soil microbial communities and enzyme activities of rhizosphere soil of ginseng under a forest of different cultivation duration of the crop and growth stages. Soil properties and enzyme activities are closely related to soil microbial community composition, and long-term continuous cultivation leads to degradation of soil properties and unbalance of soil nutrient content (Maguire et al. 2020, Bian et al. 2022). There were significant differences in the enzyme activity changes of ginseng under forest different cultivation duration of the crop and growth stages. Among which the changes of urease and catalase were consistent with the trend of organic carbon changes. It indicates that the organic carbon content can affect most of the enzyme activities in the rhizosphere soil of ginseng under forest, which has a significant effect on maintaining the growth and development of ginseng under forest. Changes in soil properties and enzyme activities caused by the cultivation duration of the crop and growth stage could be explained by the variations in the microbiome. The relative abundance of *Acinetobacter* bacteria and *Kazachstania* fungi in TB10 was significantly higher than that in TA10 and TC10, while that of Firmicutes and *Candidatus udaeobacter* was significantly lower than that in TA10 and TC10. This indicates that there is a complex interrelationship between the changes in the rhizosphere soil microbial community of ginseng under forest and the soil physicochemical properties and enzyme activities in different cultivation duration of the crop and growth stages. This is consistent with the study by Shi et al. (2024), who found that soil physicochemical properties such as pH, TN, AN, TP, OP and SOC had significant effects on ginseng soil bacteria and fungi.

The RDA results showed significant segregation between samples from different cultivation durations of the crop and growth stages, suggesting that cultivation durations of the crop and growth stages influence bacterial and fungal community structure to some extent. Furthermore, the results of this study showed that pH, AP, and AK were the main factors that changed the bacterial community structure in rhizosphere soil (Figure 6). William et al. (2014) found that the pH of the soil significantly affects the composition of soil microbial communities and also significantly affects the accumulation of effective elements in the soil, which is consistent with the results of the present study. The RDA results at the genus level of bacteria showed that *Lactococcus*, *Acinetobacter*, *Pseudomonas*, *Serratia*, *Burkholderia-Caballeronia-Paraburkholderia* and *Sphingobium* were negatively correlated with phosphatase but positively correlated with urease (Figure 8B). The function of soil urease involves making urea available to plants by converting it to ammonia, while soil phosphatase is catalysing soil organic P compounds to be mineralised into inorganic P and directly affect the biological effectiveness of soil P, and urease and phosphatase activities are closely related to soil N and P contents and microbial species abundance (Cenini et al. 2016, Kumar and Garkoti 2022).

In conclusion, the soil properties and enzyme activities change with different durations of crop and root rapid growth stages of ginseng under forest. The contents of organic carbon, alkaline hydrolysed nitrogen, and available potassium in the rhizosphere soil of 10-year-old ginseng under forest decreased significantly in the middle stage of rapid root growth. The community structure of bacteria and fungi in rhizosphere soil was shifted under the cultivation duration of the crop and growth stage. Changes in the cultivation duration of the crop and growth stage have a stronger effect on soil bacteria than fungi. In addition, pH, AP, and AK were the main factors that changed the bacterial community, while pH, AK, AHN and SOC significantly shifted the fungal community structure. Based on the above results, we speculate that the microbial community and function in rhizosphere soil of ginseng under forest depend on the dynamic changes in soil nutrients. The results provide a reference for further precision cultivation and harvesting of ginseng in the forest. Nevertheless, we only conducted 16S rDNA and ITS studies on the soil microbiome of ginseng under forests. In the future, we plan to collect samples from the cultivation duration of the crop and conduct metagenomics and macro metabolomic studies to understand better microbial resources for healthy and sustainable ginseng cultivation under forests.

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