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Effects of Transplantation and Microhabitat on Rhizosphere Microbial Communities during the Growth of American Ginseng

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Abstract: Transplanting has been widely used in American ginseng (*Panax quinquefolium* L.) cultivation in Northwest China to mitigate the negative effects of continuous cropping obstacles. Because of the accumulation of pathogenic microorganisms and the change in soil properties, transplanting American ginseng to newly cultivated fields after two years of growth has become a major planting pattern. Despite transplanting improving the quality of American ginseng, the effects of soil properties and microbiota on growth during the transplanting process are poorly understood. In the present study, microbial communities, soil physico-chemical properties and morpho-physiological parameters were analyzed to investigate the effects of microbiota and soil characteristics on American ginseng growth in both soil and ginseng root microhabitats. Results indicated that the structure and species of bacterial and fungal communities changed significantly in different microhabitats before and after transplantation. Moreover, the assemblage process of the bacterial community was dominated by deterministic processes. The stochastic process ratio increased and niche breadth decreased significantly after transplanting. While the assembly of the fungal community was dominated by stochastic process, and there was no significant difference in NST, βNTI or niche breadth before and after transplanting. Bacterial co-occurrence networks demonstrated a higher connectivity but a lower aggregation in soil microhabitat, while the fungal community networks remained stable before and after transplantation. Gammaproteobacteria was the biomarker in the soil microhabitat, while Alphaproteobacteria, Betaproteobacteria and Gemmatimonadetes were biomarkers in the ginseng root microhabitat. Sordariomycetes was a biomarker with high relative abundance in the fungal community before and after transplanting. The bacterial functional and important ASVs were significantly correlated with pH, organic matter, total nitrogen, available phosphorus, total potassium root fresh weight, taproot diameter and stem height of American ginseng. Partial least squares path modeling showed that soil properties significantly affected the formation of different microbial specific ASVs. The important functional ASVs in ginseng root microhabitat had a positive effect on American ginseng growth, while the rare taxa had a negative effect. Our results provide a good starting point for future studies of microbial community succession in different microhabitats influenced by the transplantation pattern of American ginseng.

Keywords: American ginseng; transplanting; microhabitats; microbiota; soil properties

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1. Introduction

American ginseng (*Panax quinquefolius* L.) has been used as a herbal medicine with a "cool" property in China for nearly 300 years. It is known for its pharmacological properties

such as anti-oxidation, anti-cancer activity, stimulation of blood flow, and enhancement of the central nervous system [1,2]. Originally native to southeastern Canada and northern United States, American ginseng was introduced to China in the 1980s [3,4]. Because of the imbalance in soil nutrients and continuous cropping obstacles caused by long-term growth in the same field, American ginseng is generally cultivated in Northwest China using a "two-year land-changing planting" pattern. This pattern involves transplanting American ginseng after two years of growth into newly cultivated land for another two years [5,6].

The reduction in crop yield and quality caused by long-term monoculture, known as continuous cropping obstacle, is an pressing challenge to the worldwide cultivation of Chinese herbal medicines, including American ginseng [4]. Transplanting can mitigate, but cannot avoid, the degradation in American ginseng quality and soil deterioration caused by continuous cropping [5,6]. Therefore, it is important to pay attention to the dynamic changes of soil microenvironment during American ginseng planting. Continuous cropping obstacles are influenced by various factors. Previous studies have shown that soil physicochemical properties, such as physical characteristics, nutrient, pH and allelopathic autotoxicity of plants [7–9], can impact the growth of ginseng plants [7,9] and also increase the incidence of diseases [10]. The biotic factors that cause continuous cropping obstacles in the American ginseng soil commonly include the microbiome, protozoa and insect pests [11,12]. While soil-borne pathogenic microorganisms are mainly responsible for biological diseases, other beneficial microbes exhibit resistance to such ailments as well [13,14]. The plant–soil feedback mechanism proposes that alterations in soil properties resulting from plant growth can impact both plant populations and microbial communities [15]. Typically, these mechanisms are usually studied independently; however, given their potential for interaction, it is unlikely that any single mechanism can fully account for plant–soil feedback.

Previous studies have suggested that the rhizosphere bridges the plant–soil relationship and facilitates the exchange of substances between roots and soil [16]. Many rhizosphere activities, including nutrient cycling and bioremediation [6], are mediated by microbiota in the soil–rhizosphere microhabitat, and roots provide a favorable environment for the enrichment of rhizosphere microbiota [17,18]. Moreover, mounting evidence suggests that plant species and sampling time exert significant impacts on the soil and rhizosphere microbial community [19,20]. The advancement of high-throughput sequencing technology and the expansion of bioinformatics methodology [21] has facilitated our comprehension of the interrelationships and interactions among the microbiome, soil properties and plant growth in agricultural practices. However, there is currently no available information on how transplantation pattern affects the rhizosphere microhabitat and microbiota of American ginseng roots. This knowledge is helpful for investigating the causes of obstacles in continuous cropping and their impact on soil environment, thereby promoting the scientific cultivation of American ginseng and improving land use efficiency.

Here, we conducted field trials to investigate the potential impacts of transplanting patterns on soil properties and American ginseng growth by assessing the succession of bacteria and fungi in the soil and rhizosphere microhabitat. We hypothesized that the matter exchange and information transfer between the ginseng root and the soil would greatly disturb the microbiota in the ginseng habitat. We established a randomized experimental transplanting of American ginseng. The changes of bacterial and fungal communities were analyzed, and their interactions with soil properties and the growth of American ginseng was discussed.

2. Materials and Methods

2.1. Experimental Setup and Soil Collection

The whole experiment was conducted in Liuba (latitude 33°40′ N, longitude 106°52′ E) village, Shaanxi province, the only cultivated American ginseng area in Northwest China [5]. The altitude is 1540 m, the mean annual temperature is 11.5 °C, the average annual amount of sunshine is about 1800 h, the annual precipitation is 840–880 mm, the frost-free period is

about 210 days and the soil type is yellow brown soil. In this planting field, the American ginseng was planted in 1.5 m wide and 50 m long seedbeds before and after transplantation. The distance between the seedbeds was approximately 0.5 m, the plant spacing was about 10 cm, and the row spacing was about 20 cm. The field blocks were arranged in a completely randomized block design, with five replicate plots (1.5 × 10 m). American ginseng was cultivated and transplanted on newly cultivated farmland with no previous agricultural tillage. The organic fertilizer used in the experiment was aerobically composted cow manure and mushroom residue, where the organic matter (OM) was 25.10%, total nitrogen (TN) was 11.32 g·kg^{-1}, available phosphorus (AP) was 275.15 mg·kg^{-1}, available potassium (AK) was 2657.13 mg·kg^{-1} and water content (WC) was 1.02%. Organic fertilizer was collected from the experimental base of Shaanxi Institute of Microbiology and was applied as base fertilizer (4.0 kg·m^{-2}) in March before the cultivation of American ginseng, followed by an equal amount of topdressing in March every year. Soil water content was adjusted to a range of 40–50% (*w*/*w*). Good Agricultural Practice (GAP) was followed during the cultivation process [22]. The whole American ginseng plants would be transplanted into new soil blocks after 2 years of cultivation.

Ginseng rhizosphere and non-rhizosphere soil was collected at the beginning of September 2021. The ginseng rhizosphere soil was defined as tightly attached to plant roots. Ten to fifteen healthy plants with green leaves and stems without spots, and ginseng roots without markings and rot were randomly selected. The taproot was vigorously shaken to collect the adhering soil from non-rhizosphere soil samples, followed by vortex oscillation for collection of tightly attached soil for ginseng rhizosphere samples. In this study, ginseng rhizosphere and non-rhizosphere soil was referred as "microhabitats". In total, 20 samples were collected and each sample was subjected to three rounds of liquid nitrogen treatment before being kept on ice and immediately stored at −80 °C until further analysis.

2.2. DNA Extraction, Amplification and Sequence Processing

DNA was extracted from 0.5 g soil using the Fast DNA SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA), and the isolated microbial DNA was used as a template for subsequent sequencing. The yield and quality were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Bacterial 16S rRNA (V3-V4) gene amplifications were amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′) [23], while fungal internal transcribed spacer region ITS2 amplification employed primers ITS1FI2 (5′-GTGARTCATCGAATCTTTG-3′) and ITS2 (5′-TCCTCCGCTTATTGATATGC-3′) [24]. Next-generation sequencing (NGS) preparation, Illumina HiSeq NGS library preparations and Illumina HiSeq sequencing were performed by LC-Bio Technology Co., Ltd, Hangzhou, China. DNA samples were quantified using a Qubit 2.0 fluorometer (Invitrogen, Carlsbad, CA, USA), followed by amplicon generation from 30–50 ng of DNA using the NEBNext® UltraTM DNA Library Prep Kit for Illumina® (New England Biolabs, Beverly, MA, USA), according to the manufacturer's protocol. The total DNA was eluted in 50 µL of Elution buffer and stored at −80 °C until PCR measurement.

The Usearch10 [25] and Vsearch 2.8.1 [26] pipelines were employed for sequence analysis. Forward and reverse sequences were joined, assigned to respective samples based on barcodes and truncated through the removal of the barcode and primer sequences. Quality filtering on joined sequences was performed, and sequences with ambiguous bases and expected errors per base rate ≤0.01 were discarded. Subsequently, the sequences were dereplicated, and singletons (with a minuniquesize < 8) were removed. The sequences were clustered into Amplicon sequence variants (ASVs) using the exact sequence variants algorithm [27,28] (Unoise3), and chimeric sequences were simultaneously removed. The effective sequences were used in the final analysis. The taxonomic identities of the bacterial and fungal ASVs were determined via the clustering program VSEARCH 2.8.1 against the Ribosomal Database Program (RDP, http://rdp.cme.msu.edu/) (accessed on 13 January 2022) and UNITE (https://unite.ut.ee) (accessed on 28 January 2022) at 97% sequence

identity, and the confidence threshold for the RDP classifier for ASVs is 0.8 [29]. The raw data of bacterial and fungal sequences were available at the National Center for Biotechnology Information under BioProject ID PRJNA975712.

2.3. Soil Physico-Chemical Properties and Morpho-Physiological Parameters

Physico-chemical properties of soil were assessed using five replicates per group. For each sample, soil was collected from the top layer (0–30 cm) at five random locations. Then, the soils were thoroughly mixed and transported from field to laboratory in sterile ice containers. In the laboratory, samples were sieved (2 mm mesh) to remove plant debris and air-dried in a designated soil drying room and analyzed for organic matter (OM), total nitrogen (TN), amino nitrogen (AN), total phosphorus (TP), Olsen-P (OP), total potassium (TK) and available potassium (AK) [30]. Dried samples were mixed with deionized water (volume ratio 1:2.5), shaken at 200 rpm for 30 min, and then centrifuged at 12,000 rpm for 5 min to determine pH and electrical conductivity (EC) [31]. OM was quantified through sulfuric acid-potassium dichromate wet oxidation, followed by titration with ferrous sulfate according to the Walkley–Black procedure [30]. TN was determined using the Kjeldahl method [32]. Soil AN was measured via diffusion methods [33]. Soil TP was assessed using the Mo-Sb anti spectrophotometric method [34]. The OP in the soil was measured using the Olsen method [35]. The TK and AK in the soil were determined via ammonium acetate extraction followed by flame photometry [36]. Concurrently, the morpho-physiological parameters of American ginseng in each group were measured. The fresh weight of ginseng root (GW) and stem (SW) was weighed. The length and diameter of taproot (TL, TD), as well as the height and diameter of stem (SH, SD), were measured. The number of branches (BN), stems (SN) and leaves (LN) per plant was recorded.

2.4. Statistical Analysis

Statistical analysis and graphic display were performed using R software version 4.1.2, Auckland, NZ and ImageGP (version 2.0, https://www.bic.ac.cn/ImageGP/ (accessed on 14 July 2022), Chen T., Beijing, China) [37]. Alpha diversity was assessed using the Shannon index with the "vegan" package (version 2.5-6, https://CRAN.R-project.org/package=vegan (accessed on 28 June 2022), Helsinki, Finland) [38], and Faith's phylogenetic diversity index (Faith's PD index) with the "picante" package (version 1.8.2, https://CRAN.R-project.org/package=picante (accessed on 28 June 2022), Eugene, OR, USA) (accessed on 28 June 2022) [39]. Phylogenetic tree analysis of soil was carried out using *cluster_agg* of Usearch10. The alpha diversity indices were calculated through one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests.

The beta diversity of bacteria and fungi was evaluated through principal coordinate analysis (PCoA) based on Bray–Curtis distance. The significance of the effect of transplanting and microhabitats on community dissimilarity was tested using PERMANOVA with the *adonis* function in the "vegan" package. To investigate the assembly processes and multifunctionality of soil and ginseng rhizosphere microbiota, we quantified the Normalized stochasticity ratio (NST) index, beta nearest taxon (βNTI) index and niche breadth under American ginseng transplanting. Nearest-taxon index (NST) [40] and β-nearest taxon index (βNTI) [41] were calculated with the "picante" package (version 1.8.2) to explore the community assembly processes and quantify phylogenetic structure. Niche breadth index was calculated according to Levin's niche breadth [42] equation using the "spaa" package (version 0.2.1, https://CRAN.R-project.org/package=spaa (accessed on 6 July 2022), Hong Kong, China).

To further characterize the impact of transplanting on microbiota in different microhabitats, we evaluated the composition and co-occurrence patterns of bacterial and fungal communities. For taxonomic analysis, a class-level stacking bar chart was generated to check relative abundance of species. The co-occurrence network was estimated using the "igraph" package (version 1.2.5, https://CRAN.R-project.org/package=igraph (accessed on 7 July 2022), Oxford, GB). A valid co-occurrence was considered a statistically robust

correlation between ASVs when the correlation threshold exceeded 0.8 and the p value was below 0.01. The microbial co-occurrence network graphs were visualized using Gephi (version 0.9.2, https://gephi.org/ (accessed on 7 July 2022), Paris, France) [43].

To evaluate the impact of transplanting on microbial community structure and function, we employed two classifications: microbial biomarkers filtered with the random forest algorithm and functional microorganisms selected based on niche breadth. To identify the microbial biomarkers, a random forest classifier (RFC) model was constructed and a 10-fold cross-validation on the RFC model was performed to detect unique ASV-based microbial biomarkers. The filtering criteria for functional microorganisms were as follows: ASVs set with the niche breadth index larger than the mean value were selected; ASVs set with a contribution rate higher than the average and significant p-value according to SIMPER analysis were also selected; The intersection of these two sets of ASVs was considered as the functional microorganism. The random forest algorithm [44] of the "randomForest" package (version 4.6-14, https://CRAN.R-project.org/package=randomForest (accessed on 11 Augest 2022), Berkeley, CA, USA) was used to quantify classification of biomarker ASVs, and the percentage of increase in mean square error (increase in MSE (%)) was used to show the importance of the biomarker ASVs. Additionally, the similarity percentage (SIMPER) algorithm was calculated with the 'vegan' package. To investigate the interrelationships among soil properties and American ginseng growth, correlation analysis was employed to demonstrate the associations between these three variables sets. The correlation between the soil properties and growth variables was evaluated using Spearman's rank correlation test. Prior to correlation analysis, the "Hmisc" package (https://cran.r-project.org/package=Hmisc (accessed on 12 August 2022), Nashville, TN, USA) was used to eliminate collinear factors. To evaluate their associations with ASVs, factors were transformed and normalized before application of the Mantel test [45]. The ASVs with relative abundances below 0.01% of total sequences were defined as "rare" ASVs [46,47]. Partial least squares path modeling (PLS-PM) [48] was employed to quantify the relationships between soil properties, ASVs and American ginseng growth variables.

3. Results

3.1. Soil and Ginseng Rhizosphere Microbiota Harbor Distinct Communities after Transplanting

We first compared the differences in bacterial and fungal communities between soil and ginseng rhizosphere samples pre- and post- transplanting. After transplantation, there was a significant decrease in the phylogenetic diversity, richness and evenness of the bacterial community (Figure 1A, Faith's PD index: $F_{1,18} = 9.527$, $p < 0.01$, Shannon index: $F_{1,18} = 19.2$, $p < 0.001$), while no significant difference was observed in the alpha diversity indices of the fungal community (Figure 1B, Faith's PD index: $p = 0.613$, Shannon index: $p = 0.838$). Moreover, a significant reduction in the alpha diversity of bacterial communities within soil microhabitat was observed after transplantation (Figure 1A, Faith's PD index: $F_{1,8} = 6.19$, $p = 0.0376$, Shannon index: $F_{1,8} = 8.946$, $p = 0.0173$), while no such reduction was detected in ginseng rhizosphere microhabitat (Supplementary data: Table S1). These findings suggested that the transplanting process primarily impacted soil bacterial community diversity, and the bacterial community in ginseng rhizosphere microhabitat remained relatively stable post-transplantation. No discernible differences were detected in soil fungal communities in the transplanting process.

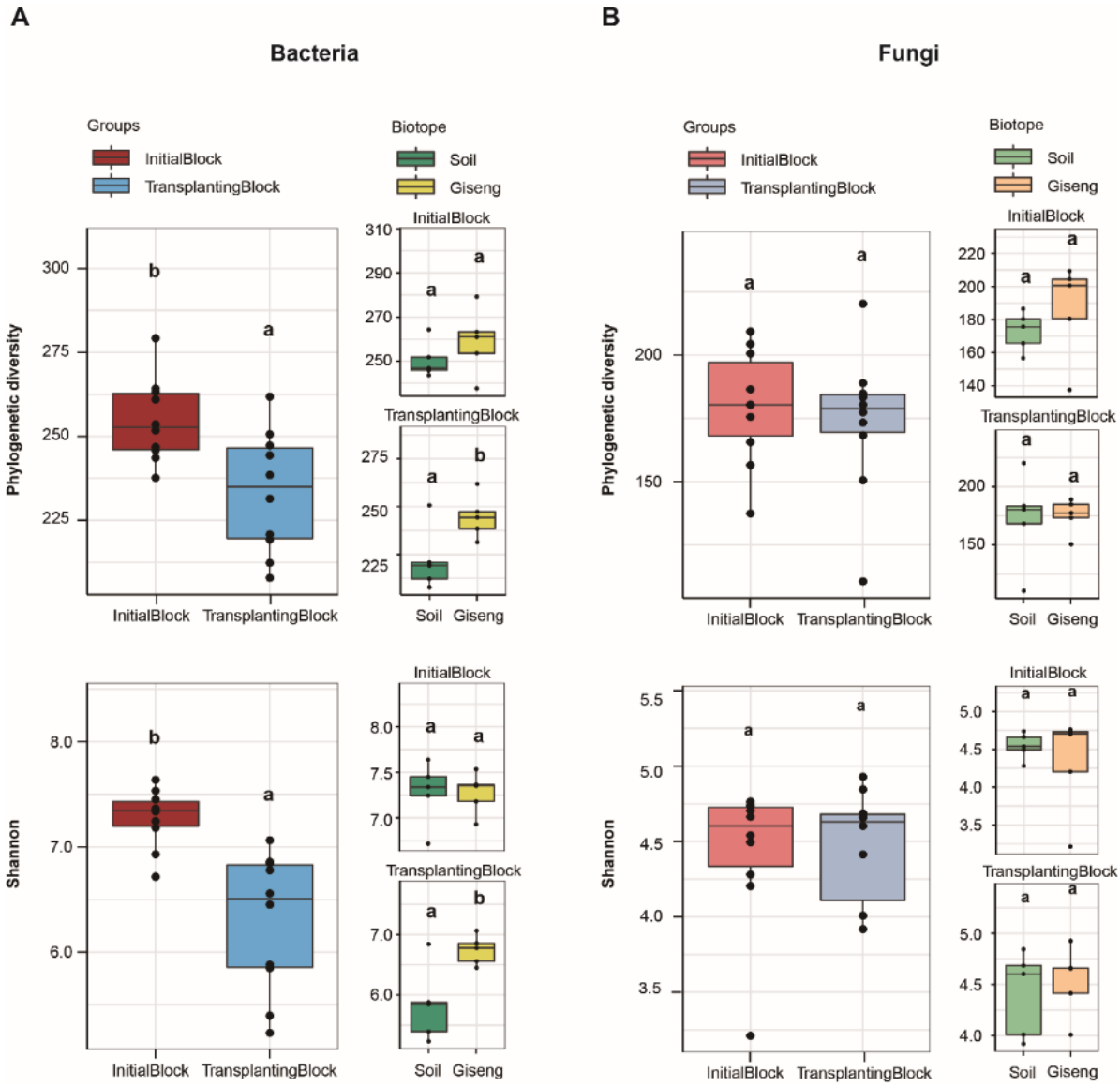

Figure 1. Comparison of microbial diversity and evenness in different microhabitats before and after transplanting. (**A**) Faith's PD and Shannon indices of bacterial community. (**B**) Faith's PD and Shannon indices of fungal community. ANOVA and Tukey's multiple comparison tests were carried out for each group, and the statistical significances ($p < 0.05$) were indicated by different letters.

3.2. Different Structures and Variation of Soil and Rhizosphere Microbiota in Transplanting Process

In principle coordinate analysis (PCoA) of bacterial Bray–Curtis distance across all samples, ginseng rhizosphere samples exhibited clustering while soil samples shifted away from the rhizosphere after transplanting in the second coordinate axis. Additionally, a significant decrease was observed in the Bray–Curtis distance between pre- and post-transplantation samples (Figure 2A). Interestingly, the fungal community structure exhibited the opposite trend, in that a significant separation was observed among ginseng rhizosphere fungi samples along the first coordinate axis after transplantation, while there was no significant alteration in the Bray–Curtis distance of soil samples before and after transplanting. Our study also found that the Bray–Curtis dissimilarity index of the bacterial communities in soil microhabitat decreased significantly after transplanting, as compared to fungal communities (Figure 2B).

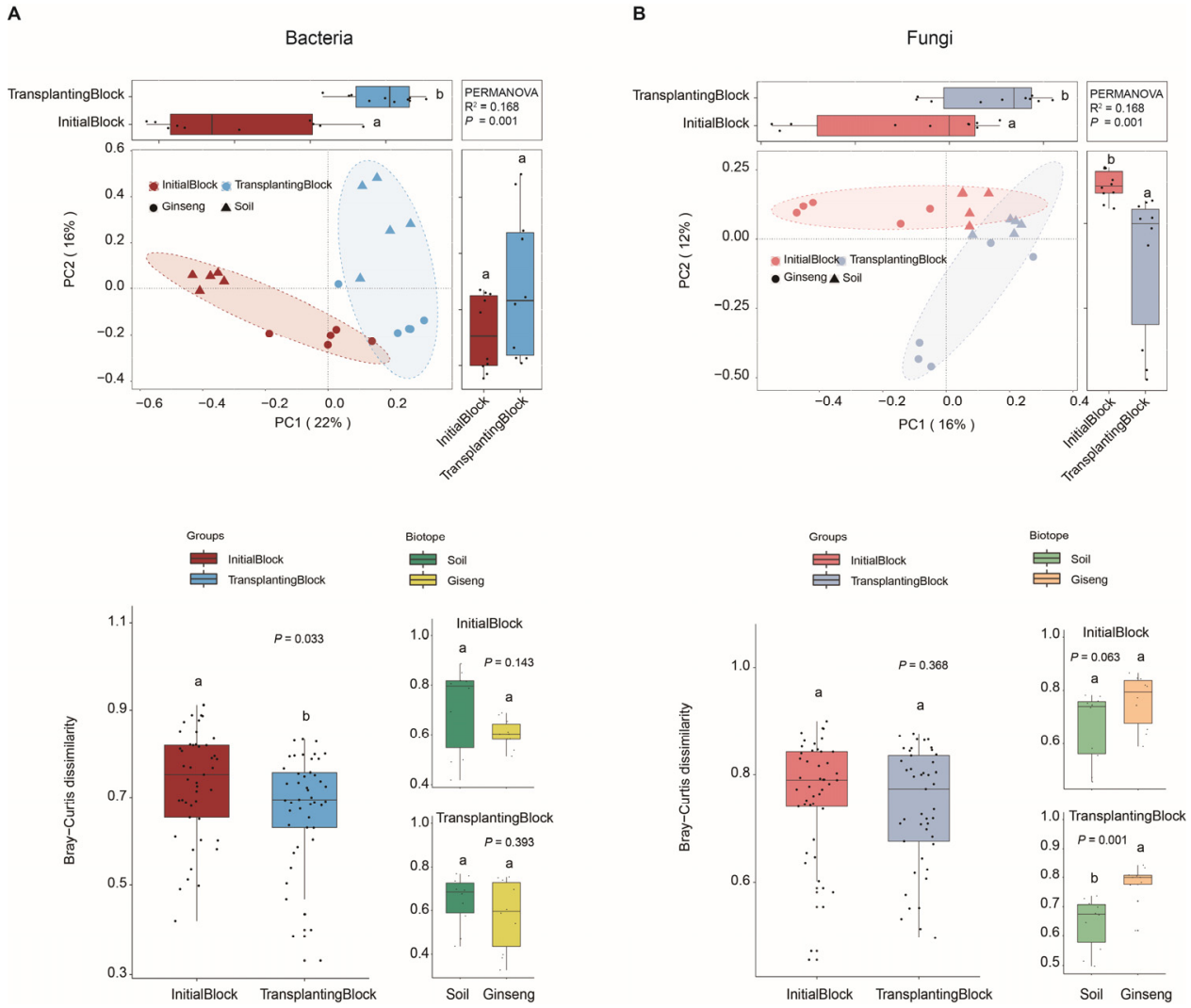

Figure 2. Comparison of community composition and variation degree between soil and ginseng rhizosphere microbiota before and after transplanting based on ASVs. (**A**) Beta diversity measurement with Principal coordinate analysis (PCoA; pairwise comparisons based on PERMANOVA). (**B**) Bray–Curtis distances before and after transplanting between soil and ginseng rhizosphere microbiota.

Generally, significant differences were found in NST, βNTI and niche breadth of bacterial communities before and after transplanting, but not in fungal communities. The NST of the bacterial community increased significantly after transplanting, indicating that the proportion of randomness increased, and the increase was mainly attributed to the contribution of soil microhabitat. The βNTI of the bacterial community was >2 before and after transplantation, suggesting that the community assembly was a deterministic process. However, the βNTI decreased after transplantation, revealing a trend towards stochasticity in the rhizosphere microhabitat bacterial community (Figure 3A). These results suggest that the assembly of the bacterial community after transplanting was stochastic, especially in the ginseng rhizosphere microhabitat. Additionally, the niche breadth of the bacterial community decreased significantly after transplanting, indicating that decreased environmental adaptability caused by this reduction could ultimately lead to a decline in deterministic processes of bacterial community assembly. Although there was no significant difference in the fungal community before and after transplanting, NST significantly decreased and βNTI significantly increased after transplanting in ginseng rhizosphere microhabitat (Figure 3B). This suggests an increase in the deterministic trend of fungal community assembly in ginseng rhizosphere microhabitat after transplanting. In addition,

we also observed that the niche breadth of the fungi community in the ginseng rhizosphere microhabitat was significantly lower compared to that in the soil microhabitat, regardless of transplanting (Figure 3B). This result suggested that the fungi in the ginseng roots were composed of specific taxa and tended to be conservative in function.

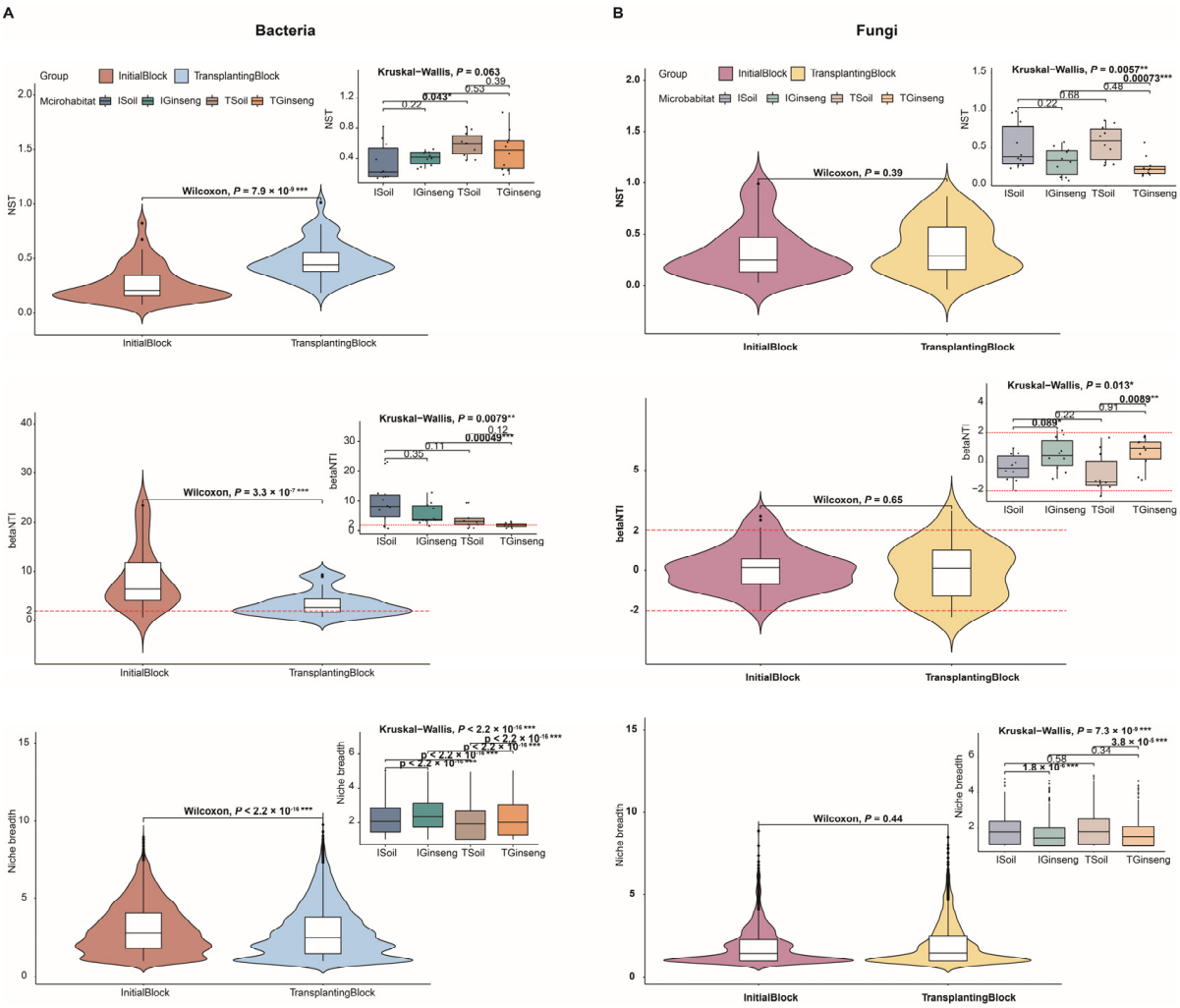

Figure 3. Comparison of assembly processes and multifunctionality of microbiota between soil and ginseng rhizosphere microbiota in transplanting process. (**A**) Normalized stochasticity ratio (NST) index, beta nearest taxon (βNTI) index and niche breadth of bacterial community. (**B**) NST index, βNTI index and niche breadth of fungal community.Black asterisks represented significant differences between groups with Wilcoxon or Kruskal−Wallis test. Asterisk (*) represented $p < 0.05$; double asterisk (**) represented $p < 0.01$; triple asterisk (***) represented $p < 0.001$.

3.3. Composition and Co-Occurrence Network of Microbial Communities in Different Microhabitats before and after Transplanting

Overall, the bacterial community was dominated by Alphaproteobacteria, Gammaproteobacteria, Actinobacteria, Gemmatimonadetes and Betaproteobacteria with the relative abundance exceeding 60% (Figure 4A). Furthermore, there were notable variations in the abundance of certain classes in different microhabitats after transplanting. Gammaproteobacteria and Sphingobacteriia exhibited a significant decrease, whereas Gemmatimonadetes and Betaproteobacteria demonstrated a marked increase in the ginseng rhizosphere microhabitat (Supplementary Materials Table S2). In co-occurrence networks, bacterial classes in soil microhabitat demonstrated a higher network connectivity (as characterized by the degree distribution) but a lower aggregation (as characterized by the clustering

coefficient) than those observed in the ginseng rhizosphere, regardless of transplanting. Additionally, ASVs belonging to Gammaproteobacteria in soil microhabitat were the main hub nodes of the network, whereas classes with high abundance such as Alphaproteobacteria, Gemmatimonadetes and Betaproteobacteria in the ginseng rhizosphere microhabitat were not in the dense area of the network. Meanwhile, we observed a significant increase in the number of nodes (99 to 129) and edges (528 to 932) above the average degree of distribution in the ginseng rhizosphere microhabitat after transplantation, while there was an obvious decrease in the number of edges (1052 to 897) in the soil habitat (Figure 4B).

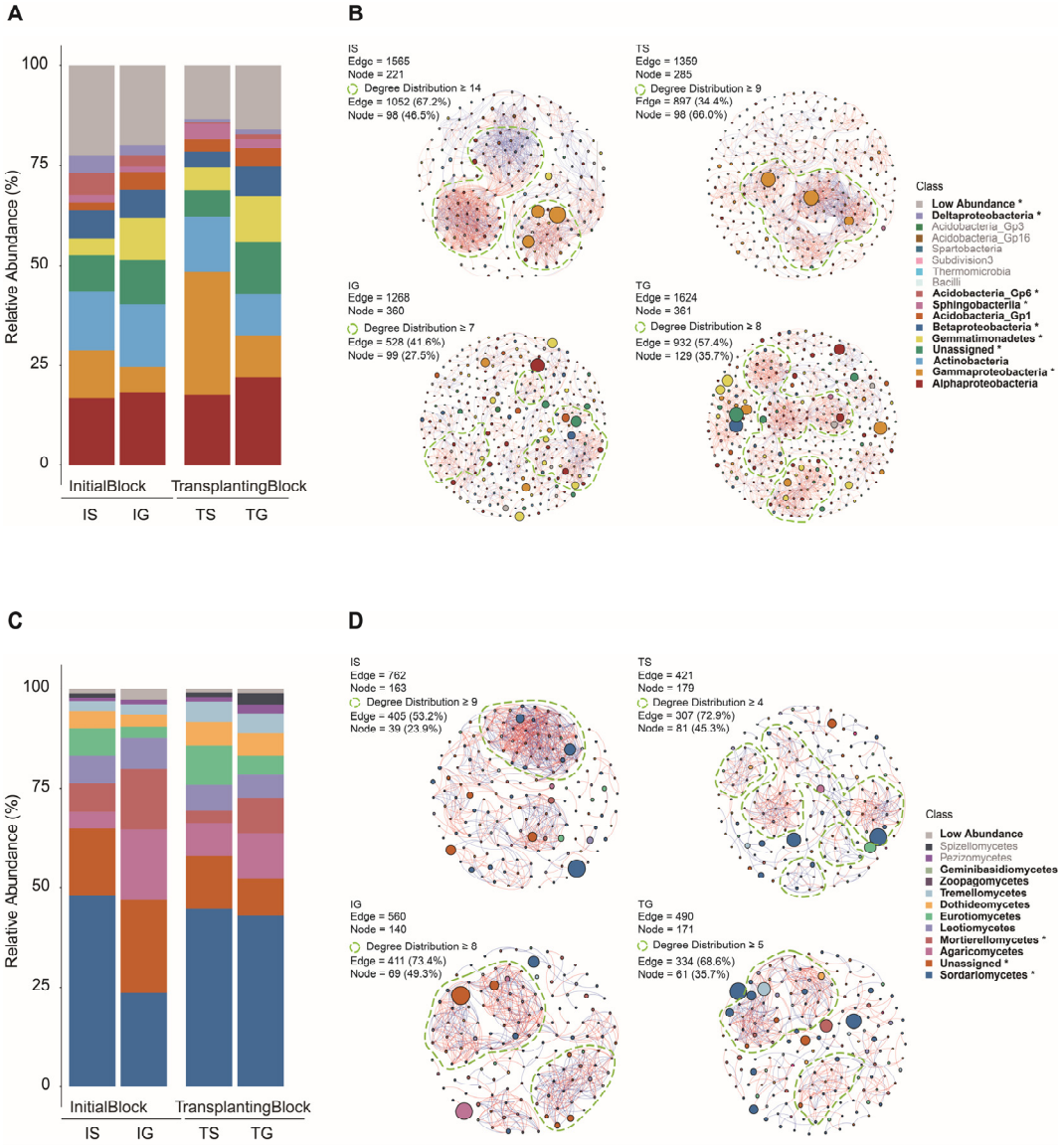

Figure 4. Relative abundance and correlation networks of microbiota before and after transplanting in different microhabitats at class level. (**A**) The relative abundance of the top 10 members of the bacterial community. (**B**) Different effects of transplanting on the networks of bacterial communities in soil and ginseng rhizosphere microhabitat. (**C**) The relative abundance of top 10 members of the fungal community. (**D**) Different effects of transplanting on the networks of fungal communities in soil and ginseng rhizosphere microhabitat. Black asterisks (*) represent classes with significant differences ($p < 0.05$) in relative abundance based on the ANOVA test. The lines between the points represent correlation coefficients (red, positively correlated; blue, negatively correlated). The points enclosed by the green dotted lines represent classes with higher than average degree distribution.

Overall, the fungal community and correlations remained stable before and after transplantation. The combined relative abundance of Sordariomycetes, Agaricomycetes, Mortierellomycetes and Leotiomycetes accounted for over 75% (Figure 4C). Sordariomycetes increased significantly in the ginseng rhizosphere microhabitat after transplanting (Supplementary Materials Table S2). The fungal correlation network had fewer edges and lower distribution after transplanting. Additionally, ASVs belonging to Sordariomycetes in soil microhabitat were the main hub nodes in the network, but were not distributed in the dense network of the ginseng rhizosphere microhabitat. We also found that the proportion of nodes (23.9% to 45.3%) and edges (53.2% to 72.9%) above the average distribution of soil microhabitats increased significantly after transplanting, while those of ginseng rhizosphere microhabitats decreased markedly (Figure 4D, nodes: 49.3% to 35.7%; edges: 73.4% to 68.6%). In summary, these results showed that the transplanting process reduced the connectivity of fungal communities in different microhabitats and weakened the relationship between taxa. Interestingly, our analysis also revealed certain taxa in the microbial correlation network that were not among the top 10 classes, including Bacilli, Thermomicrobia, Spartobacteria, Acidobacteria_Gp16 and Acidobacteria_Gp3 in bacteria, as well as Pezizomycetes and Spizellomycetes in fungi.

3.4. Association of Microbial Biomarkers and Functional Microorganisms with Soil Properties and Growth of American Ginseng

We observed that, although the proportion of ASVs exceeded 50% (55.3% in bacteria and 57.8% in fungi), functional microorganisms exhibited differential distribution before and after transplanting. In the fungal biomarkers screened, we opted for fewer ASVs (156 ASVs) rather than enhancing model accuracy. Finally, through the intersection of microbial markers and functional microorganisms, we identified three subgroups of ASVs (Figure 5): functional ASVs (Bacteria: 232 ASVs, 39.1%; Fungi: 28 ASVs, 15.2%), important ASVs (Bacteria: 255 ASVs, 43.0%; Fungi: 132 ASVs, 71.7%), and important functional ASVs (Bacteria: 106 ASVs, 17.9%; Fungi: 24 ASVs, 13.0%).

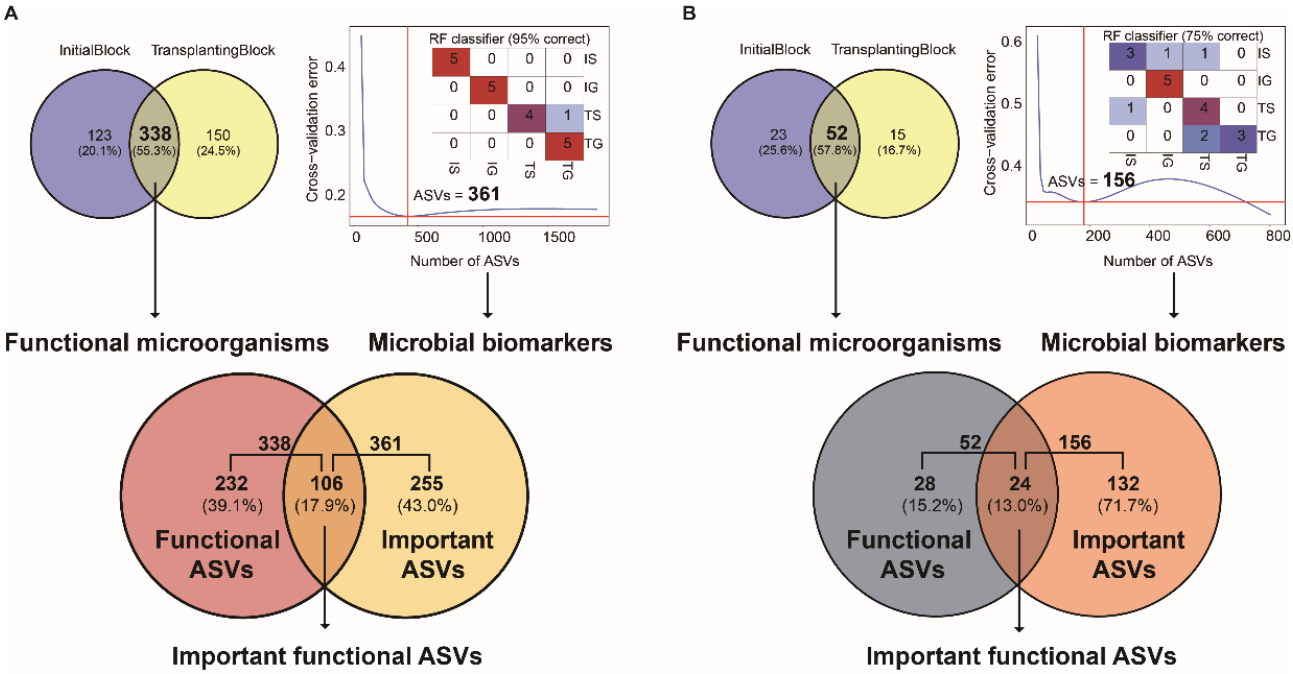

Figure 5. Filtering strategy of microbial biomarkers and functional microorganisms in different microhabitats and definitions of different types of ASVs. (**A**) Bacterial ASV filtering and definition. (**B**) fungal ASV filtering and definition. The diagonal numbers and colors represent the accuracy of the RF classifier with top features. The redder color represented higher correct rate.

Overall, a negative correlation was observed between soil properties and growth variables. In soil properties, pH exhibited a significant positive correlation with organic matter (OM), total nitrogen (TN), and total potassium (TK). Conversely, the growth weight (GW) of American ginseng displayed a significant negative correlation with soil OM, TN and TP. Height of stem (SH) was negatively correlated with both OM and TN. Diameter of taproot (TD) and TN were significantly negatively correlated. Only soil available phosphorus (AP) demonstrated a positive correlation with growth variables (Figure 6).

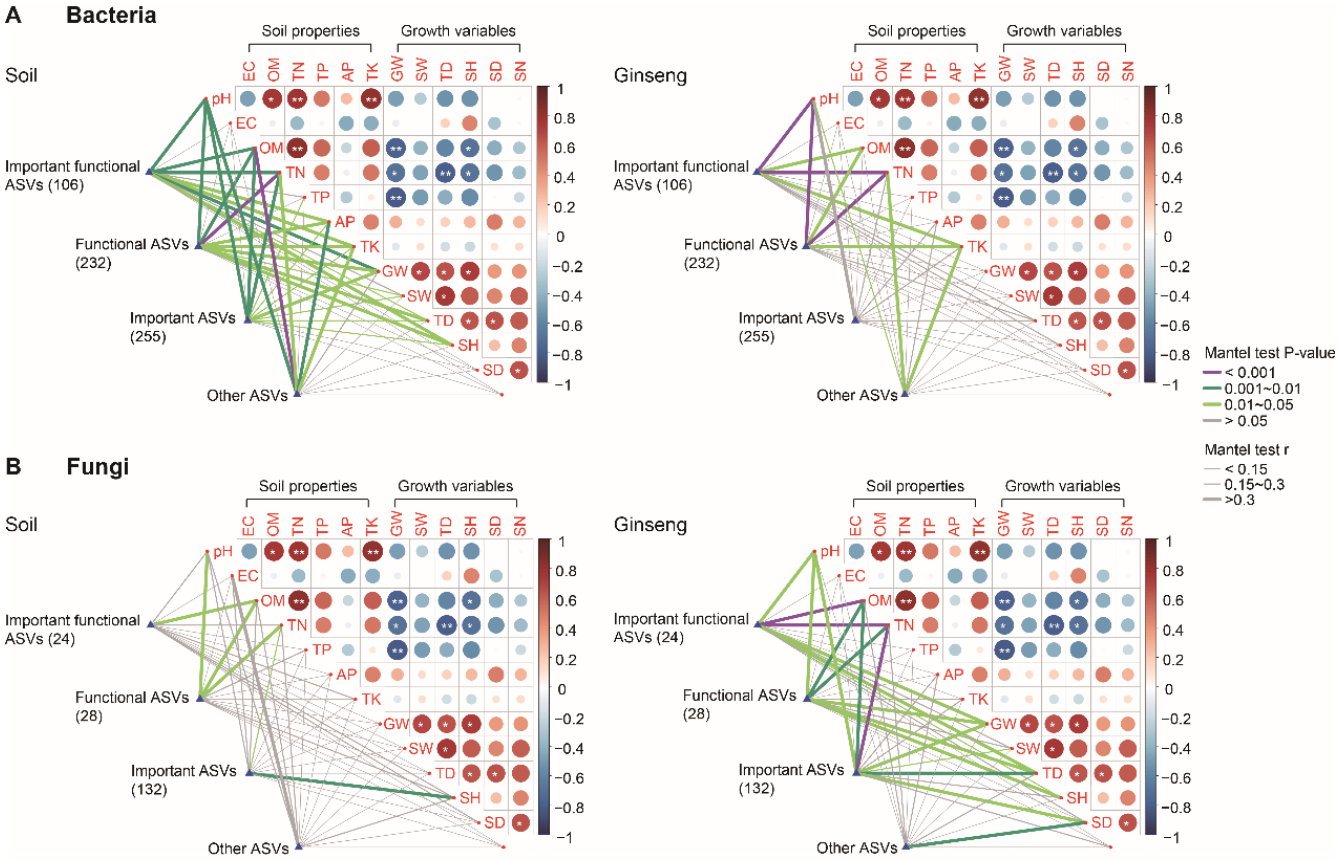

Figure 6. Relationships between soil properties, growth variables and subgroups of important functional ASVs, functional ASVs, important ASVs and other ASVs. (**A**) Bacterial subgroups in soil and ginseng rhizosphere microhabitats. (**B**) Fungal subgroups in soil and ginseng rhizosphere microhabitats. The upper right matrix represented the correlation coefficient matrix diagram between soil properties and growth variables, and the right bar was the correlation coefficient contrast color. Significant Spearman correlation coefficients were marked with an asterisks (*) ($p < 0.05$) and double asterisks (**) ($p < 0.01$).

To determine the relationship between microbiota in different microhabitats and American ginseng growth, we profiled the correlation between ASV subgroups and variables with the Mantel test. We observed that the bacterial ASVs in the soil microhabitat and the fungal ASVs in the ginseng rhizosphere microhabitat were more strongly correlated with soil properties and American ginseng growth. Within the bacterial ASVs subgroups, important functional ASVs and functional ASVs were significantly correlated with soil pH, OM, TN, AP and TK, as well as American ginseng GW and SH in the soil microhabitat. Additionally, functional ASVs were also significantly correlated with TD, and important ASVs were significantly correlated with pH, OM, TN and GW (Figure 6A). Interestingly, although other ASVs subgroup were significantly correlated with some soil properties (pH, OM, TN, AP and TK), this subgroup was not significantly correlated with growth variables of American ginseng. In the ginseng rhizosphere microhabitat, all bacterial subgroups

were not significantly correlated with growth variables of American ginseng. Conversely, important functional ASVs and functional ASVs were strongly significantly correlated with soil pH and OM (Mantel's r > 0.3, *p*-value < 0.001). The results indicated that the bacterial communities in soil microhabitats mainly interacted with soil properties and had little effect on the growth of American ginseng.

In the fungal subgroups, important functional ASVs, functional ASVs and important ASVs were significantly correlated with soil pH, OM and TN as well as American ginseng GW and TD in the ginseng rhizosphere microhabitat (Figure 6B). Notably, OM and TN showed strong correlations with all subgroups of ASVs (Mantel's r > 0.3, *p*-value < 0.01). We observed that important ASVs were significantly strongly correlated with American ginseng TD, while other ASVs were strongly significantly correlated with SD (Mantel's r > 0.3, *p*-value < 0.01). In the soil microhabitat, functional ASVs showed significant correlations with pH, OM and TN, while important functional ASVs were significantly correlated only with OM. Only important ASVs were strongly significantly correlated with American ginseng SH (Mantel's r > 0.3, *p*-value < 0.01). In general, fungal communities mainly interacted with soil properties in the root rhizosphere microhabitat. It was also noteworthy that different subgroups of fungal ASVs, including important ASVs and other ASVs, had a greater impact on the growth of American ginseng.

3.5. Driving Forces for American Ginseng American Growth

Recent studies have increasingly emphasized the importance of rare taxa and studied the responses of rare sub-communities to the cropping process [47,49]. In this study, PLS-PM analysis was conducted to assess the direct and indirect effects of soil properties, bacterial ASVs and fungal ASVs on growth variables in soil and ginseng root microhabitat of American ginseng. According to the previous results, we combined three types of ASV into NW-RF ASVs, and divided the other ASVs into intermediate and rare ASVs (Figure 7). PLS-PM analysis showed that soil properties had significant positive effects on bacterial and fungal ASVs in the soil microhabitat (Figure 7A). In the ginseng root microhabitat, all soil properties had positive effects on bacterial ASVs and negative effects on fungal ASVs (Figure 7B). It was found that bacterial and fungal sub-group ASVs showed different effects in different microhabitat. Bacterial NW-RF ASVs showed negative total standardized effects in soil microhabitat, but a positive one in ginseng root microhabitat, while fungal NW-RF ASVs displayed positive total standardized effects in both microhabitats. Bacterial intermediate ASVs showed positive and rare ASVs showed negative total standardized effects on the American ginseng growth in both microhabitats (Figure 7A). In fungal ASVs, intermediate ASVs exhibited negative effects in soil microhabitats but positive effects in ginseng root microhabitats. Conversely, rare ASVs displayed the opposite trend and had the largest total standardized effects (Figure 7B).

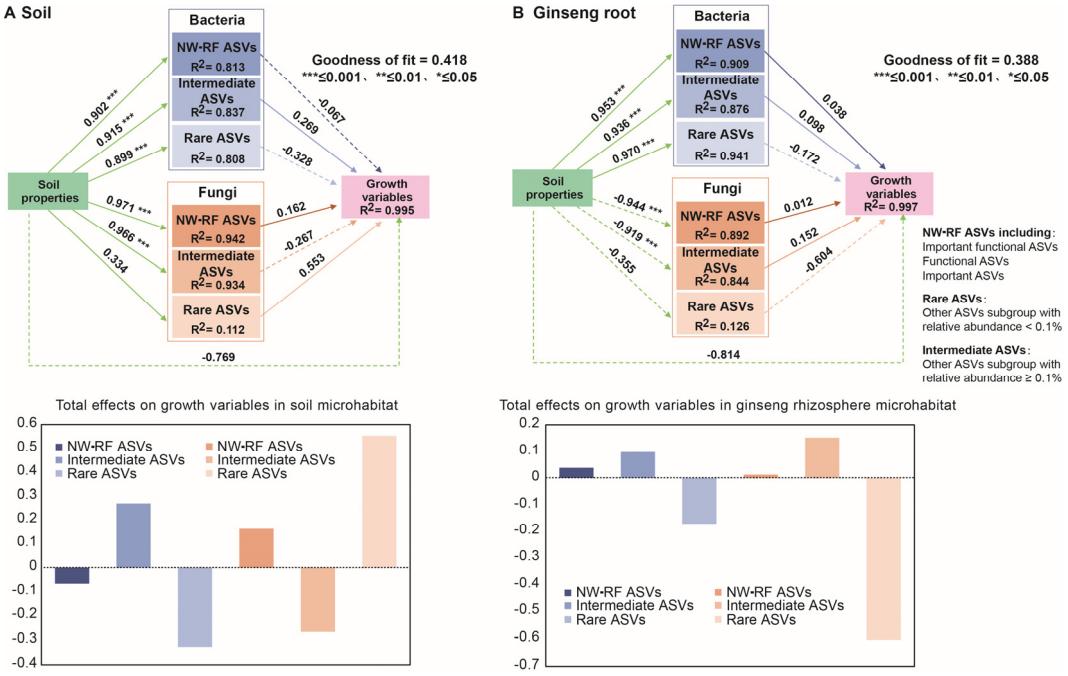

Figure 7. The partial least squares path models showing the effects of soil properties, bacterial ASVs, fungal ASVs on growth variables in soil (**A**) and ginseng root (**B**) microhabitat of American ginseng. Solid and dashed lines indicate positive and negative effects, respectively. Numbers adjacent to each arrow denote partial correlation coefficients (significance codes: *** $\leq$ 0.001, ** $\leq$ 0.01, * $\leq$ 0.05). R^2 values displayed the proportion of variance explained for each factor. The bar chart shows the standardized total effect of each factor on the American ginseng growth variables soil and ginseng root microhabitat.

4. Discussion

Our initial hypothesis was that transplanting pattern was an important factor affecting American ginseng rhizosphere soil properties and microbiota. To adapt to soil environmental disturbances, plants were able to recruit functional microorganisms and alter microbial interactions in the rhizosphere soil by changing chemical conditions and releasing root-derived compounds [18,50]. To verify this hypothesis, the rhizosphere–soil microbial community was investigated before and after transplanting, Further, their effects on soil properties and American ginseng growth was examined. We observed that the transplanting process mainly affected the diversity and structure of the bacterial community, and the fungal community was relatively stable. The bacterial community in soil microhabitat was more active and had a stronger correlation with soil properties, whereas the fungal community of the ginseng rhizosphere microhabitat was more closely related to soil properties and American ginseng growth.

4.1. Response of Soil and Rhizosphere Microhabitat Microbial Communities to Transplanting of American Ginseng

Changes in soil properties of American ginseng have been reported as being the main factor causing disturbance in microbial community structure and composition [51,52]. In this study, significant changes were observed in the structure, variation, assembly process, niche breadth and composition of bacteria compared to fungi before and after transplanting (Figures 1–4). These results suggest that transplanting had little effect on the diversity and structure of fungal communities, consistent with previous studies [10,53]. Moreover, microhabitats had more significant effects on the bacterial community. After transplanting, the Faith's PD and Shannon indices of the soil microhabitat bacterial community decreased significantly, and the Bray–Curtis distances of ginseng root microhabitat were larger (Figures 1 and 2). In agro-ecosystems soils, plant roots established interactions with

rhizosphere microorganisms during growth [16,54]. This effect might contribute to the establishment of a stable bacterial community in the ginseng root microhabitat, while the bacterial community in the soil microhabitat was more dynamic and significantly associated with soil properties and American ginseng growth (Figure 6). Microbial community abundance distributions in the environment could provide a broad reservoir of ecological function and resiliency [55]. Significant changes in soil microhabitat bacteria might be due to the species migration of the adjacent bulk soil microbiota. However, this effect was not observed in fungi. The Bray–Curtis dissimilarity and deterministic process of fungal community in ginseng root microhabitat increased significantly after transplanting (Figures 2 and 3). These results indicated that the ginseng rhizosphere bacterial community structure tended to be conserved after transplantation, while the fungal community structure changed significantly because of the selectivity of American ginseng roots. The composition of plant-colonizing microbial communities was mainly influenced by the selective pressure of plants [56]. In general, these results indicated that the bacterial community was mainly affected by soil properties and mediated rhizosphere activities though soil microhabitats [6]. The fungal community was mainly affected by ginseng root growth, and the root microhabitat provided a favorable environment for the enrichment of rhizosphere fungi [17,18].

4.2. Microbial Composition Exhibited Distinct Correlation Network Patterns in Different Microhabitats before and after Transplanting

The soil physicochemical properties and soil microbial communities interactions shifted with the growth of American ginseng plants [53]. In this study, different microhabitat greatly altered the distribution, the clustering and interactions among hub nodes of different microbial classes of the co-occurrence network (Figure 4; Supplementary data: Table S3). One interesting finding was that Alphaproteobacteria, which exhibited the highest relative abundance, displayed a weak association with the bacterial network (Figure 4). Keystone species belonging to Gammaproteobacteria were more connected with other taxa in soil microhabitat than in ginseng root microhabitat, while Gemmatimonadetes in ginseng root microhabitat showed higher connectivity in the correlation network. The fungal keystone species belonging to Sordariomycetes were more conservative, maintaining high abundance and connectivity in the network (Figure 4), which implied the fungal co-occurrence network was more stable. This study further found that the connectivity of the fungal community network in the ginseng root microhabitat was greatly reduced after transplanting compared with bacteria (Supplementary data: Table S3). After transplanting, soil habitat microbes were more loosely connected, but fungal classes of hub nodes were more closely connected than bacteria (Figure 4). After transplanting, the bacterial network in ginseng root microhabitat was more complex and more connected, while fungal networks had shorter average paths length, more network modules and a higher degree of modularity (Supplementary data: Table S3). The findings suggested that the ginseng rhizosphere microhabitat had a recruitment effect on the bacterial community, promoting the aggregation of bacterial taxa, and maintained this feature even after transplanting. While the fungi in the ginseng root microhabitat showed small-world characteristics after transplanting, which allowed the effects of a disturbance to be transmitted quickly throughout the network, making the fungal system efficient [57].

4.3. Effects of Microbial Communities and Soil Properties on Growth Variables in Soil and Ginseng Root Microhabitats

The similarity percentage (SIMPER) could evaluate the contribution of different species to inter-group variations, while niche breadth was indicative of a species' utilization of environmental resources [58,59]. In this study, we defined functional ASVs based on the niche breadth and contribution rate, and identified important ASVs using a random forest algorithm. Our study revealed that distinct types of ASVs exhibited significant associations with diverse soil properties and growth variables (Figure 6; Supplementary data: Table S4). We observed that important functional bacterial ASVs with high relative abundance were

bASV_11 (*Rhodanobacter*), bASV_31 (*Bradyrhizobium*) and bASV_10 (*Acidobacterium*). *Rhodanobacter* was reported to exhibit antagonistic effects against ginseng root rot pathogen *Fusarium solani* [60]. Additionally, it was previously positively correlated with the soil pH and N, which could impact soil denitrification [61]. *Bradyrhizobium* could protect plants growing in a natural soil against abiotic stress, as it is a known nitrogen-fixing bacteria commonly found in plant soils [62]. As a endophytic and rhizosphere bacterium, *Acidobacterium* could be used as a candidate microorganism to reflect soil fertility and plant health [63]. *Mizugakiibacter* and *Gemmatimonas* was observed in high relative abundance in important (bASV_16, bASV_75) and functional ASVs (bASV_2, bASV_40). *Mizugakiibacter* have commonly been found in abundance at low pH in crop fields [64], and *Gemmatimonas* has been reported to be abundant in healthy Panax ginseng soil, as it has a greater ability to use available C sources [50,65]. In fungal ASVs, *Fusarium*, the main soil-borne pathogen causing root rot disease in American ginseng [10,53], was highly abundant in important functional (fASV_31) and important ASVs (fASV_1), particularly after transplanting. Meanwhile, potential disease-suppressive fungi *Mortierella* was found in high relative abundance in all three types of ASVs (fASV_113 in important functional ASVs, fASV_70 and fASV_121 in important ASVs, fASV_14 and fASV_34 in functional ASVs) [52]. In general, different types of abundant bacterial ASVs primarily regulated soil physiological and biochemical properties like pH, OM and TN, providing a stable growth environment for American ginseng. Fungal ASVs primarily impacted ginseng root growth and played an important role in both the generation and antagonism of soil-borne diseases.

The composition of the microbial community is considered an important component of the plant–soil feedback process [47,66]. Below-ground biomass of plants could affect microbial biomass in soil and further affect microbial diversity and multifunctionality [67]. In our study, soil properties had negative effects on the fungal community in the ginseng root microhabitat. This might be due to the antagonistic effect of American ginseng on pathogenic fungi such as *Fusarium* (fASV_1 and fASV_31; Supplementary data: Table S4), which were highly ranked among NW-RF ASVs, during its growth. Previous studies also showed that root exudates like phenolic acids had antagonistic effects on fungi during the growth of American ginseng [4]. The effect of bacterial NW-RF ASVs on growth was microhabitat-dependent, suggesting that the American ginseng roots were enriched with functional bacteria, including nutrient uptake and pathogen antagonism [6]. Additionally, rare ASVs showed the largest total standardized effects on the growth of American ginseng and the effects of intermediate and rare ASVs varied across different habitats, indicating that fungal communities with low abundance could serve as a reservoir of diversity and function and be enriched in diverse environments [55]. It is worth noting that the influence of microbiota and soil properties on American ginseng growth was not significant, indicating that soil enzyme activity, climate conditions, above-ground plants, and other factors might have an impact on its growth. Overall, this study enhanced our comprehension of the dynamics of rhizosphere microorganisms in American ginseng roots during the transplanting process, thereby providing new insights into the regulation of continuous cropping obstacles in agro-ecosystems under a transplanting pattern.

5. Conclusions

This study revealed the succession of bacterial and fungal communities throughout the transplanting process of American ginseng, which showed different interactions on soil properties and growth in both soil and ginseng root microhabitats. In particular, significant differences were observed in bacterial diversity, NST, βNTI and niche breadth before and after transplantation. However, highly correlated bacterial networks were microhabitat-conserved. Specific bacterial taxa defined by different characteristics appeared to be driving changes in soil properties within the soil microhabitat. The response of fungal Bray–Curtis dissimilarity, NST and βNTI to transplanting was microhabitat-specific, particularly after transplanting. Moreover, specific fungal ASVs mainly affected the growth of American ginseng. Soil properties primarily influenced all microbial ASVs; however,

the total effects on the fungal ASVs were opposite in soil and ginseng root microhabitats, showing the habitat specificity of soil properties on fungal community effects. In addition, it was found that rare ASVs had the largest total effect on American ginseng growth despite their low relative abundance. This study greatly expands our understanding of the interactions between microbial communities and soil properties during the American ginseng transplanting process, which also provides new insights into microbial regulation of rhizosphere microhabitat.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13071876/s1, Table S1: Shannon and Faith's PD indices before and after transplanting in different microhabitats. All values were an average from all replicates ± standard deviations. Black asterisks represent significant differences based on the ANOVA test. Values with the same letters within a column did not significantly differ based on the Tukey's multiple comparisons test. Table S2: The relative abundance of top 10 class level before and after transplanting in different microhabitats. All values were an average from all replicates ± standard deviations. Black asterisks represent significant differences based on the ANOVA test. Values with the same letters within a column did not significantly differ based on the Tukey's multiple comparisons test. Table S3: Properties of the microbiota association networks before and after transplanting in different microhabitats. Table S4 Different types of ASVs, species classification, and average relative abundance in different groups. The bold numbers represented the relative abundance of ASV within the top 30 of the group.

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