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Changes in Soil Chemistry and Microbial Communities in Rhizospheres of Planted *Gastrodia elata* on a Barren Slope and under a Forest

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Abstract: Continuous cropping of the important achlorophyllous medicinal orchid *Gastrodia elata* Blume causes an imbalance in soil microecology leading to soil-borne diseases. However, the impacts on different land covers remain largely unknown. Hence, this study aimed to investigate changes in the soil nutrient composition and the global microbial community structure in rhizospheres of *G. elata* cultivated on a barren slope (HPGJ) and under a forest (LXT) using integrated shotgun metagenomics and an analysis of soil chemical properties. High-throughput sequencing revealed an increase in the abundance of Proteobacteria, Actinobacteria, Mucoromycota, Basidiomycota, and Ascomycota, which drive N- and C-cycling genes in HPGJ and LXT. Notably, the fungal community was significantly improved in the HPGJ (from 0.17% to 23.61%) compared to the LXT (from 0.2% to 2.04%). Consequently, mineral cycling was enhanced in the HPGJ, resulting in a more improved soil nutrient composition than in the LXT. The soil chemical properties analysis unveiled a significant increase in the contents of the total nitrogen, NO_3^--N, organic matter, total carbon, organic carbon, total sulfur, and total phosphorus in the HPGJ, while no changes were recorded in the LXT. It was noteworthy that the abundance of pathogenic microorganisms increased significantly in the HPGJ compared to the LXT. Our results provide supporting data to optimize *G. elata* cultivation on slopes.

Keywords: *Gastrodia elata*; metagenomics; soil chemical properties; mineral element cycling; soil microecology; pathogenic microbes

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1. Introduction

The *G. elata* Blume is a perennial herbaceous achlorophyllous orchid mainly distributed in Eastern Asia, particularly in China, Korea, and Japan [1,2]. Its rhizomes (mature tubers) are of great value to health care, medicine, and food [3–5]. It is one of the most expensive traditional Chinese medicines and is widely used to treat many diseases, such as polio, convulsions, epilepsy, headaches, tetanus, numbness of the limbs, dizziness, rheumatic arthralgia, hand and foot failure, etc. [3,6]. Pharmacological studies have shown that the plant has a neuroprotective ability and may represent an essential resource for the treatment of chronic diseases, including Alzheimer's, epilepsy, dementia, brain aging-related diseases, hypertension, depression, and for the improvement of blood flow in the brain and heart [7–11]. Accordingly, the demand for high-quality *G. elata* is increasing, and the reliance on wild *G. elata* from mountainous regions is leading to its scarcity and an inability to meet market demand [6,11]. As a mycoheterotrophic plant, the artificial cultivation of *G. elata* requires forest resources and is associated with serious ecological problems, including the imbalance of the soil microecology and triggering an increase in the abundance of pathogenic microbes [6,12–14]. However, few studies have focused on the

impacts of *G. elata* cropping on the diversity, composition, and structure of the global rhizosphere soil microbiome. Moreover, slope soils have not been investigated, and the effects of different land covers remain elusive.

Soils are some of the most diverse ecosystems on our planet with dynamic and interacting communities of bacteria, fungi, archaea, protozoa, and viruses, collectively referred as the 'soil microbiome' [15]. The soil microbiome drives the biogeochemical cycling of micronutrients and macronutrients, which provides essential elements for plant growth that are also critical for animal life [15–17]. It also performs the dichotomous functions of solid organic carbon (SOC) mineralization and the stabilization of carbon inputs into organic forms [15]. Bacteria and fungi control critical soil processes related to the cycling of N (nitrogen), C (carbon), and P (phosphorus) [15,18,19]. Particularly, bacteria decompose dead plant organs and are essential for the decomposition of dead fungal hyphae [16]. They also interact with mycorrhizal fungi and plant roots in the rhizosphere as mycorrhizal helpers or commensals [16]. The cultivation of *G. elata* relies on its symbiotic relationship with Armillaria (Basidiomycota) fungi, and its rhizosphere is rich in diverse microorganisms, including other Basidiomycota, Actinobacteria, Firmicutes, Ascomycota, Proteobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes, Acdobacteriota, etc. [11,13,20,21]. We then hypothesized that the cultivation of *G. elata* could promote mineral element cycling and thus improve soil chemical properties. However, local biogeochemical environments strongly influence microbial metabolic responses even within specific biomes [15]. Therefore, it is of great importance to evaluate the impacts of *G. elata* cultivation on the mineral nutrient cycling and chemical properties of rhizospheres of different land covers.

Metagenomics is a novel approach to investigating microorganisms extracted from a specific environment through the screening of functional genes or sequencing analysis [22,23]. Soil metagenomics involves the isolation and sequencing of soil DNA to analyze their microbial diversity, community composition, functional activities, genetic and evolutionary relationships, and interactions with the environment [22,24]. It has been widely applied to elucidate the effects of the soil microbial metabolism on C and N cycling and to explore the dynamic changes in microbiome diversity and composition in the rhizospheres of many crop and heterotrophic plants [13,17,20,25–29]. Therefore, the main objective of this study was to investigate the impacts of *G. elata* cultivation on the diversity of barren slope (HPGJ) and under forest (LXT) soils' microbiota, mineral elements cycling, and chemical properties using shotgun metagenomics and a chemical properties analysis. We hypothesize that *G. elata* cropping will have a positive effect on the abundance of rhizosphere microbiome and soil nutrient dynamics and compositions.

2. Materials and Methods

2.1. Collection of Soil Samples

The control (HPT, barren slope soil and FLXT, understory soil) and rhizosphere (HPGJ, rhizosphere slope soil of wild *G. elata* and LXT, rhizosphere soil of *G. elata* under forest) soil samples were collected in January 2022 from Yangjiahaiba, Zhaotong, Yunnan Province, the main *G. elata* production region in China [30]. The altitude and geographical coordinates of the sites were 1949.5 m, 27°47′50″ N, and 104°17′22″ E, respectively. Sampling was carried out using conventional methods. The control sites had no history of *G. elata* planting. At each sampling site, an area of 100 m^2 was selected, and five individual samples were taken using the five-point sampling method [31]. For the rhizosphere soil sampling, the *G. elata* rhizome was removed from the soil, the topsoil was shaken off, and the soil near the rhizome was gently scraped with cotton swabs (at least 5 g). All soil samples were placed in pre-labeled sterile bags and then transported to the laboratory at low temperatures. They were then sieved through a 2 mm mesh to discard residues. We randomly selected three samples (out of the five) of each group for all analyses. Each sample was further divided into two subsamples. One was immediately air-dried and used for the determination of the total organic content, total nitrogen, organic matter, total carbon, etc. The other subsamples were stored at −80 °C for sequencing and the determination of the $NH4^+$-N, $NO3^-$-N, etc.

2.2. Soil Chemical Properties Analysis

Soil water extracts were used for the pH determination using a pH meter. The other soil properties, including the total nitrogen, total carbon, organic carbon, total sulfur, total phosphorus, $NO3^-$-N, organic matter, $NH4^+$-N, effective boron, and total potassium contents were evaluated according to the NY/T 1121.2-2006 Soil Testing methods [32,33].

2.3. DNA Extraction, Library Preparation and Sequencing

Microbial DNA extraction from the twelve soil samples was performed using the E.Z.N.A.® stool DNA kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's protocols. Metagenomic shotgun sequencing libraries were constructed and sequenced at Shanghai Biozeron Biological Technology Co. Ltd., Shanghai, China as per Tong et al. [31]. Briefly, 1 μg of genomic DNA from each sample was trimmed using a Covaris S220 Focused-ultrasonicator (Woburn, MA, USA), followed by library construction (fragment length of approximately 450 bp) and Illumina HiSeq X sequencing using pair-end 150 bp (PE150) mode. The quality of the raw sequence reads was checked to remove adapter contaminants and low quality reads using Trimmomatic (http://www.usadellab.org/cms/uploads/supplementary/Trimmomatic) [34]. The reads were then mapped to the human genome (version: hg19) using the BWA mem algorithm (parameters: -M -k 32 -t 16, http://bio-bwa.sourceforge.net/bwa.shtml). Clean reads were obtained for further analyses after discarding host genome contaminations and poor quality data.

2.4. Reads-Based Phylogenetic Annotation

The customized Kraken database (Kraken2) was used to perform the taxonomic analysis of the clean reads [35]. This database contains all genome sequences of bacteria, archaea, fungi, viruses, protozoa, and algae per the NCBI RefSeq database (release number: 90). Read classification was conducted at seven phylogenetic levels (domain, phylum, class, order, family, genus, species). Reads for which no information was available were grouped as unclassified. We estimated the abundance of each taxon using Bracken (https://ccb.jhu.edu/software/bracken/), which can accurately generate species- and genus-level abundances even for multiple nearly identical species. The Bray–Curtis distance algorithm was used to calculate the distance between samples for the beta diversity analysis. The principal coordinates analysis (PCoA) was conducted using ANOSIM.

2.5. Metagenomic De Novo Assembly, Gene Prediction and Annotation

MegaHit with "--min-contig-len 500" parameters was used to generate contigs [36]. The ORFs (open reading frames) of assembled contigs were predicted using Prodigal (v2.6.3) [37] and sets of unique genes were generated by clustering using CD-HIT (parameters: -n 9 -c 0.95 -G 0 -M 0 -d 0 -aS 0.9 -r 1) [38]. The longest sequence of each cluster was chosen as the representative sequence of each gene in the unique gene set, and the number of reads for each gene was calculated using the salmon software [39]. Finally, gene abundance was calculated according to the methods of Li et al. [40]. The functional annotation of unique-gene sets was carried out by Blast against the KEGG databases using BLASTX.

2.6. Statistical Analyses

A one-way analysis of variance was used to differentiate between the soils' physico-chemical properties. The significance level was set at $p < 0.05$. The statistical analysis and bar graphs were performed in GraphPad Prism v9.0.0121 (GraphPad 159 Software Inc., La Jolla, CA, USA). TBtools software was used to construct heatmaps [41].

3. Results

3.1. Impacts of G. elata Cropping on Barren Slope and under Forest Soils' Chemical Properties

To reveal the effects of *G. elata* cropping on the rhizosphere soil of a barren slope (HPGJ) and rhizosphere soil of under forest cover (LXT), we evaluated eleven soil chemical characteristics of these soil samples and their respective controls, including barren slope soil (HPT) and understory soil (FLXT) (Figure 1). Compared to the HPT, the *G. elata* cropping induced a

significant increase in the pH and the contents of the total nitrogen, NO_3^--N, organic matter, total carbon, organic carbon, total sulfur, and total phosphorus of the HPGJ (Figure 1A–C,E–I). In contrast, no significant differences in these chemical parameters were found between the FLXT and LXT (Figure 1A–C,E–I). Compared to the controls (the HPT and FLXT, respectively), the NH_4^+-N content of the HPGJ and the LXT decreased significantly, while the effective boron and total potassium content increased significantly (Figure 1D,J,K).

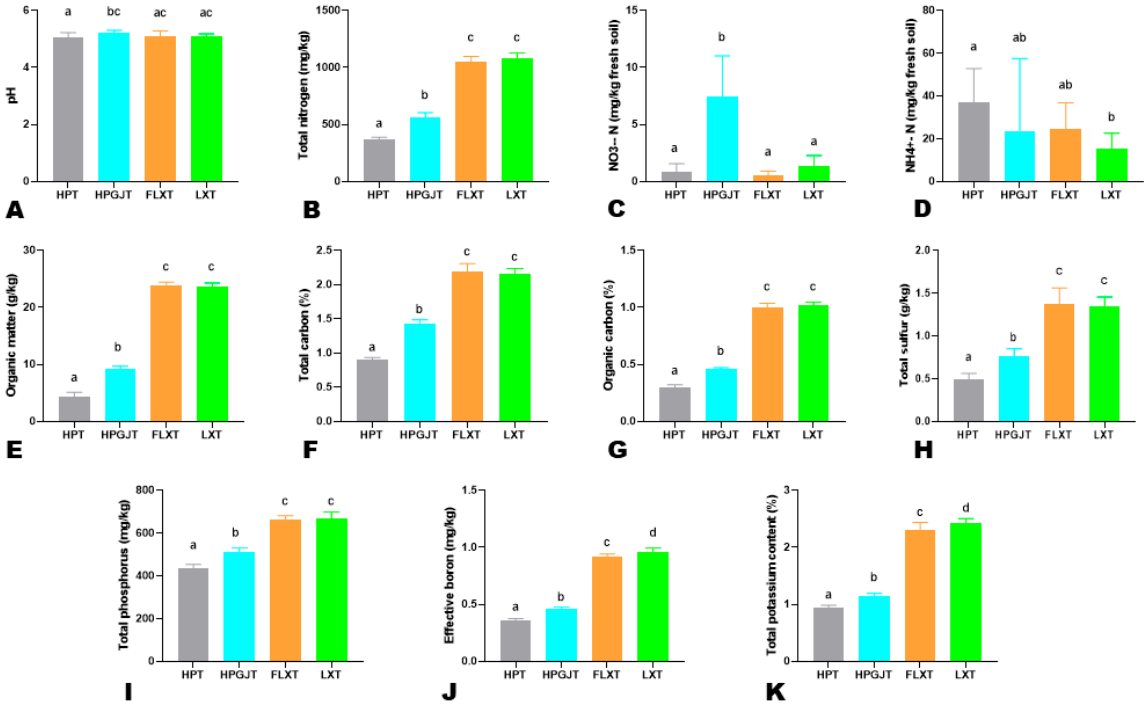

Figure 1. Chemical properties of the four soil groups. (**A**) pH; (**B**) Total nitrogen; (**C**) NO_3^-—$^-$N; (**D**) NO_4^+—N; (**E**) Organic matter; (**F**) Total carbon; (**G**) Organic carbon; (**H**) Total sulfur; (**I**) Total phosphorus; (**J**) Effective boron; (**K**) Total potassium. Different letters above bars indicate statistical differences at $p < 0.05$. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

3.2. Shotgun Metagenome Sequencing Results

To characterize *G. elata* cropping-induced microbiome changes in the HPT and FLXT, twelve samples (including three replicates for each soil type) were sequenced using Illumina HiSeq X. Shotgun metagenome sequencing yielded approximately 968.9 million total sequences, with an average of about 80.8 million per sample (Table S1). After the quality controls, a total of 952,074,876 (average of 79,339,573 per sample) high-quality reads were obtained. The average length of the microbes was 483 bp (Table S1).

3.3. Composition and Diversity of the Microbial Community

To explore changes in the microbial communities in terms of both the taxa and functions, we classified the sequences into Bacteria (93.95%), Eukarya (4.35%), Archaea (1.51%), and Viruses (0.19%) based on their similarity to entries in the MG-RAST database. The petal map analysis revealed that 824,198 genes were shared between the twelve samples (Figure 2A). The HPGJ showed a higher number of specific genes than the HPT, while the number of specific genes of the LXT was lower than that of the FLXT (Figure 2A).

In order to discriminate between the different soils, we estimated the beta diversity based on the Bray–Curtis distance matrix (Figure S1). Based on Bray–Curtis dissimilarities, we performed hierarchical clustering, and the result showed that the microbial community structures of the FLXT and HPT and their respective *G. elata* rhizosphere soils were different (Figure 2B). We also performed an NMDS and a principal coordinate analysis (PCoA), and the results were

supportive of the hierarchical clustering (Figure 2C,D). The PCoA analysis showed that PC1 (41.52%) was the major contributor to the differences in microbial community composition between the barren slope and under forest soil samples (Figure 2D). There were no major differences in the microbial community structure within the FLXT and LXT (Figure 2C,D). In contrast, the HPGJ and HPT exhibited significant differences in microbial community composition and structure, indicating that *G. elata* cropping on the barren slope strongly influenced the soil microecology compared to that of the under forest (Figure 2C,D).

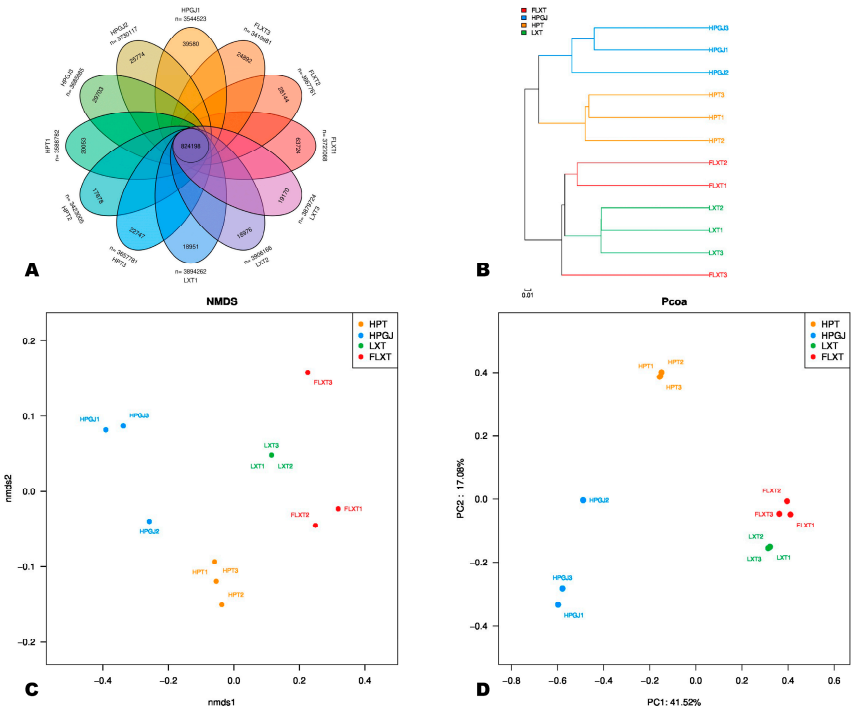

Figure 2. Estimation of similarity and distance between soil groups. (**A**) Homology analysis; (**B**) Hierarchical clustering tree of the samples (the length of the branches represents the distance between samples); (**C**) NMDS plot; (**D**) Principal coordinate analysis (PCoA). The closer the two samples are on the NMDS and PCoA plots, the more similar the species composition of the two samples. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

3.4. Changes in the Microbial Community at the Phylum and Genus Levels

To clarify the changes in microbial community structure induced by *G. elata* in the FLXT and the HPGJ, we analyzed two taxonomic levels, including phylum and genus. The dominant microbial phyla within the four soil groups were Actinobacteria, Proteobacteria, Actinobacteria, and Chloroflexi (Figures 3A and S2). As shown in Figure 3B,C, *G. elata* cropping on barren slope soil caused a decrease in the abundance of Acidobacteria (from 39.58% to 21.48%) and Chlorofexi (from 7.97% to 4.63%), and an increase in the abundance of Protobacteria (from 28.69% to 30.56%), Actinobacteria (from 12.06% to 13.33%), Mucoromycota (from 0.06% to 13.84%), Basidiomycota (from 0.06% to 6%), and Ascomycota (from 0.05% to 3.77%), indicating a significant increase in the fungal community. Except for the Chloroflexi (which decreased from 15.23% to 11.04%), the cultivation of *G. elata* in the under forest induced a slight increase in the abundance of Acidobacteria (from 33.4% to 34.73%), Proteobacteria (from 24.75% to 28.14%), Actinobacteria (from 9.41% to 14.07%), Mucoromycota (from 0.06% to 0.15%), Basidiomycota (from 0.05% to 1.38%), and Ascomycota (from 0.09% to 0.51%) (Figure 3D,E). There was a marked increase in the fungal community in the rhizosphere of the barren slope soil (from 0.17% to 23.61%) compared to the rhizosphere of the under forest soil (from 0.2% to 2.04%) (Figure 3B–E).

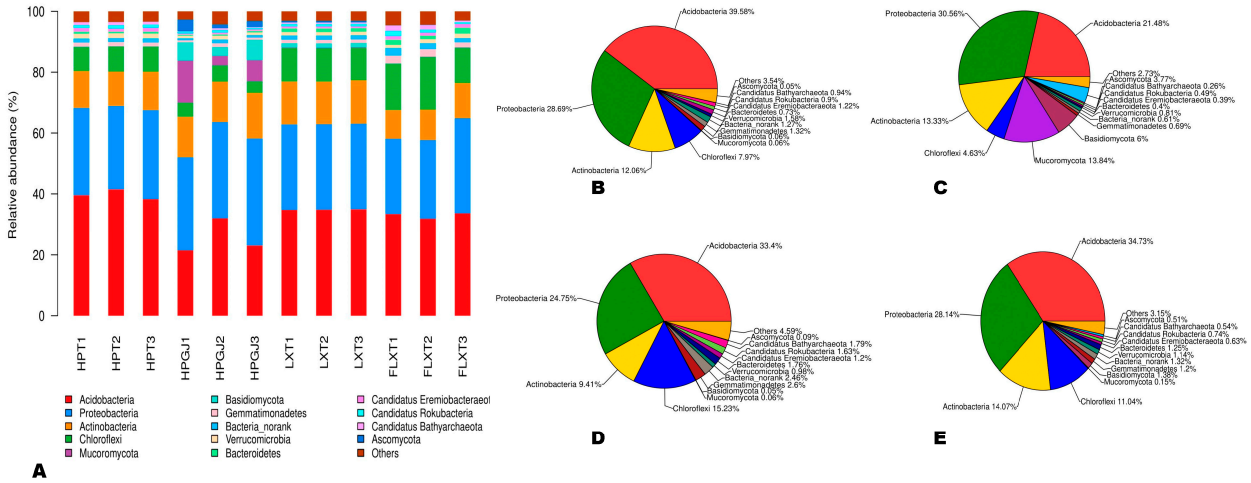

Figure 3. The composition and structure of the microbial community at the phylum level. (**A**) Relative abundances of the microbial phyla in different samples. (**B**,**C**) Pie plots for microbial community analysis at the phylum level in HPT and HPGJ, respectively. (**D**,**E**) Pie plots for microbial community analysis at the phylum level in FLXT and LXT, respectively. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

The predominant microbial genera were Acidobacteria_norank, Chloroflexi_norank, Actinobacteria_norank, Bradyrhizobium, and Alphaproteobacteria_norank (Figures 4A and S3). Compared to the under forest soil, *G. elata* cropping induced significant changes in the microbial community structure at the genus level (Figure 4B–E). The abundance of many genera, including Paraburkholderia, Arthrobacter, Mucor, Saitozyma, Trichoderma, Rhizopus, etc., increased significantly in the rhizosphere of the barren soil (Figure 4B,C). Meanwhile, the abundance of Acidobacteria_norank and Chloroflexi_norank decreased considerably from 30.67% and 5.8% to 16.99% and 3.2%, respectively (Figure 4B,C). Changes in the abundance of genera in the rhizosphere of the under forest were slight (Figure 4D,E).

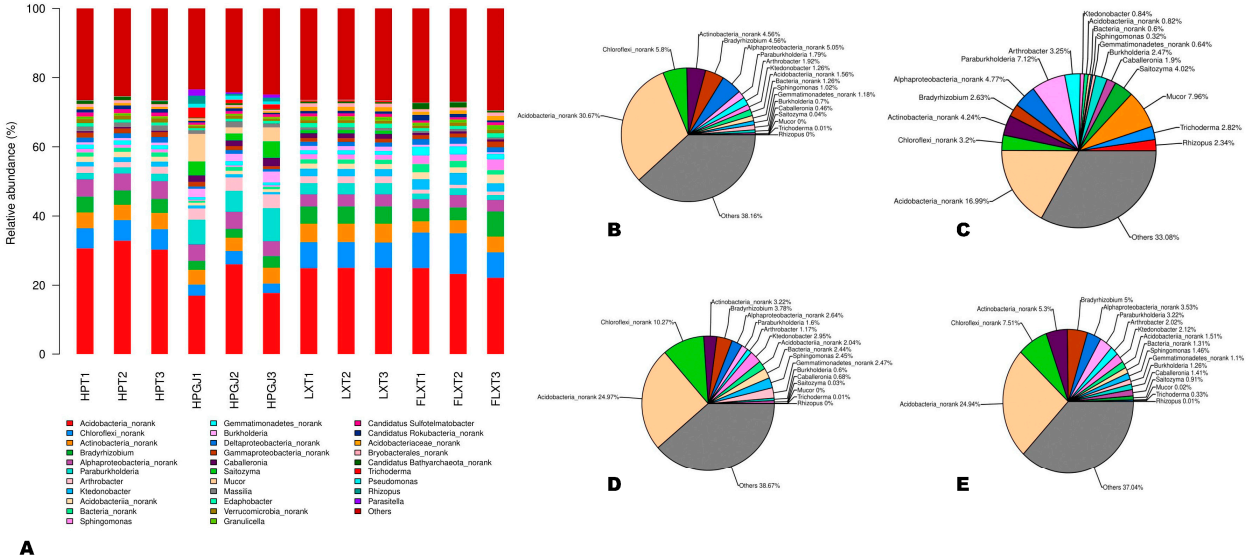

Figure 4. The composition and structure of the microbial community at the genus level. (**A**) Relative abundances of the microbial genera in different samples. (**B**,**C**) Pie plots for microbial community analysis at the genus level in HPT and HPGJ, respectively. (**D**,**E**) Pie plots for microbial community analysis at the genus level in FLXT and LXT, respectively. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

3.5. Patterns of Functional Changes

To investigate functional changes in rhizosphere soils from the barren slope and under forest, we performed a KEGG analysis. In general, the functional genes were mainly involved in the metabolism of carbohydrates, amino acids, energy, cofactors, and vitamins (Figure S4). *G. elata* cropping induced significant changes in the microbial metabolism in the barren slope soil compared to the under forest soil (Figure 5). The RNA processing and modification, nuclear structure, extracellular structures, chromatin structure and dynamics, and cytoskeleton were highly induced in the HPGJ compared to the LXT. Carbohydrate transport and metabolism and secondary metabolite biosynthesis, transport, and catabolism were induced in the LXT, whereas they were down-regulated in the HPGJ (Figure 5). No major changes in amino acid, inorganic ions, and lipid transport and metabolism were observed in the LXT, in contrast to the HPGJ, where they were down-regulated (Figure 5).

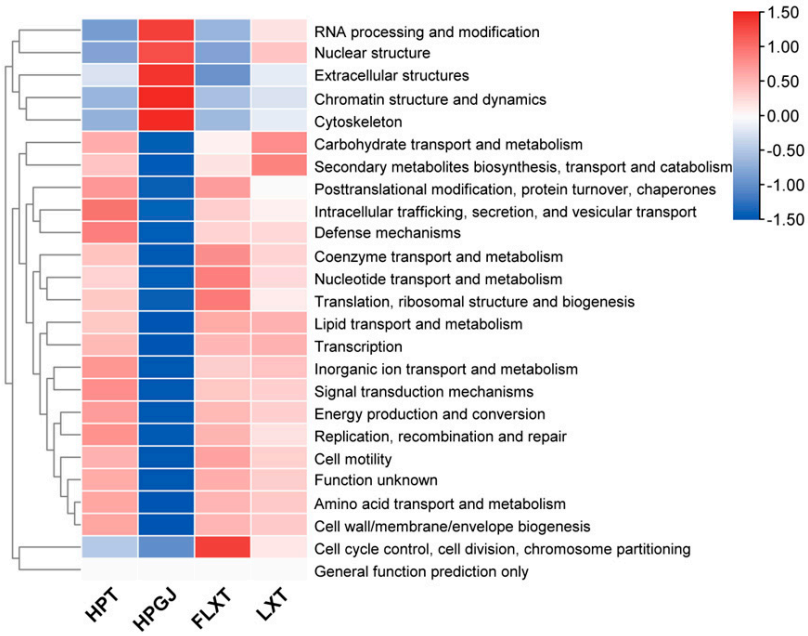

Figure 5. KEGG annotation of functional genes in different samples. The scaled Log2 fold change of each annotaion is plotted in a blue-white-red color scale. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

3.6. Changes in Mineral Elements Cycling in Barren Slope and under Forest Soils

To explore the effects of *G. elata* cropping on mineral element cycling, we examined the expression of genes involved in N (nitrogen), C (carbon), S (sulfur), and P (phosphorus) cycling. Regarding the N cycling, our analysis showed that the genes involved in assimilatory nitrate reduction (nitrate to nitrite) and dissimilatory nitrate reduction (nitrite to ammonia) were more highly induced in the HPGJ than in the LXT (Figure 6A,B). The genes involved in nitrate reduction (nitrite to ammonia) and denitrification (nitric oxide to nitrous oxide) were down-regulated in the HPGJ, whereas they were slightly induced in the LXT. Genes related to N fixation, assimilation, and mineralization were down-regulated in both the HPGJ and LXT (Figure 6A,B).

Regarding the C cycling, aerobic respiration and fermentation-related genes were significantly induced in the rhizosphere of the barren slope (HPGJ), whereas no difference in the aerobic respiration and down-regulation of fermentation was observed in the LXT (Figure 7A,B). Aerobic and anaerobic C fixation were down-regulated in both the HPGJ and LXT (Figure 7A,B). Many genes involved in the S cycle were down-regulated in both the HPGJ and LXT (Figure 7C,D). Except for sulfate uptake, genes related to assimilatory sulfate reduction (sulfite to sulfide), sulfur mineralization, and sulfite uptake were induced in the HPGJ in contrast to the LXT (Figure 7C,D). Meanwhile, assimilatory sulfate reduction (PAPS to sulfite) and sulfide cycling (sulfide to sulfur) were induced in the LXT in contrast to the HPGJ (Figure 7C,D). Organic P mineralization was slightly induced in both the HPGJ and LXT (Figure S5A,B).

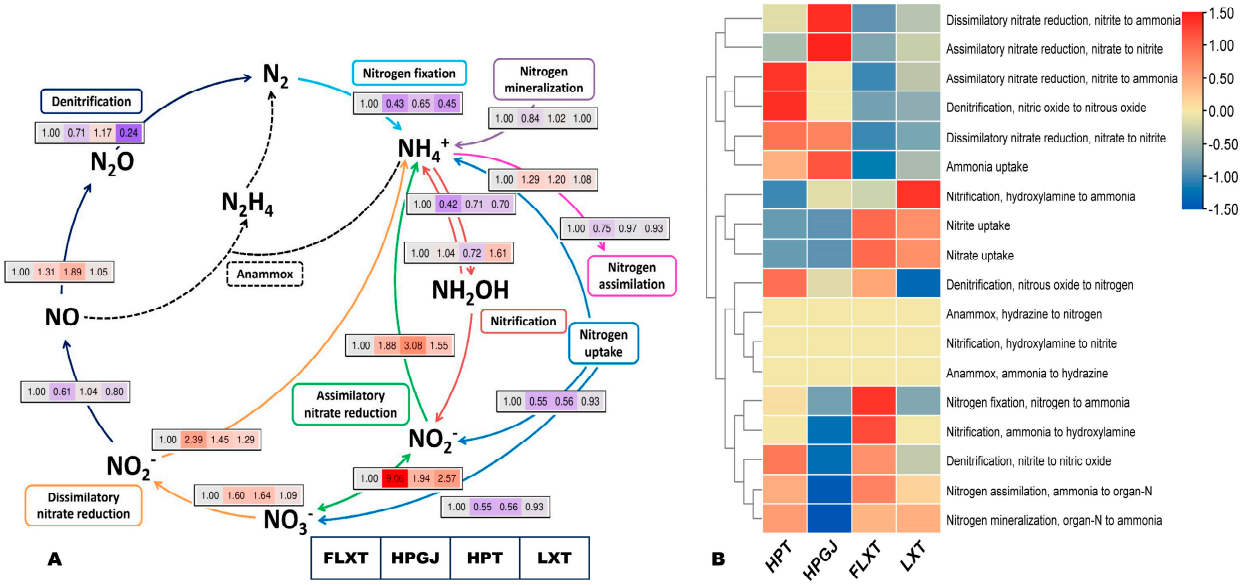

Figure 6. Effects of *G. elata* cultivation on N cycling. (**A**) Expression fold change of N cycling-related genes taking FLXT as the control. (**B**) Abundance of N cycling-related genes in different samples. The scaled Log2 fold change of the abundance of genes in each process is plotted in a blue-yellow-red color scale. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

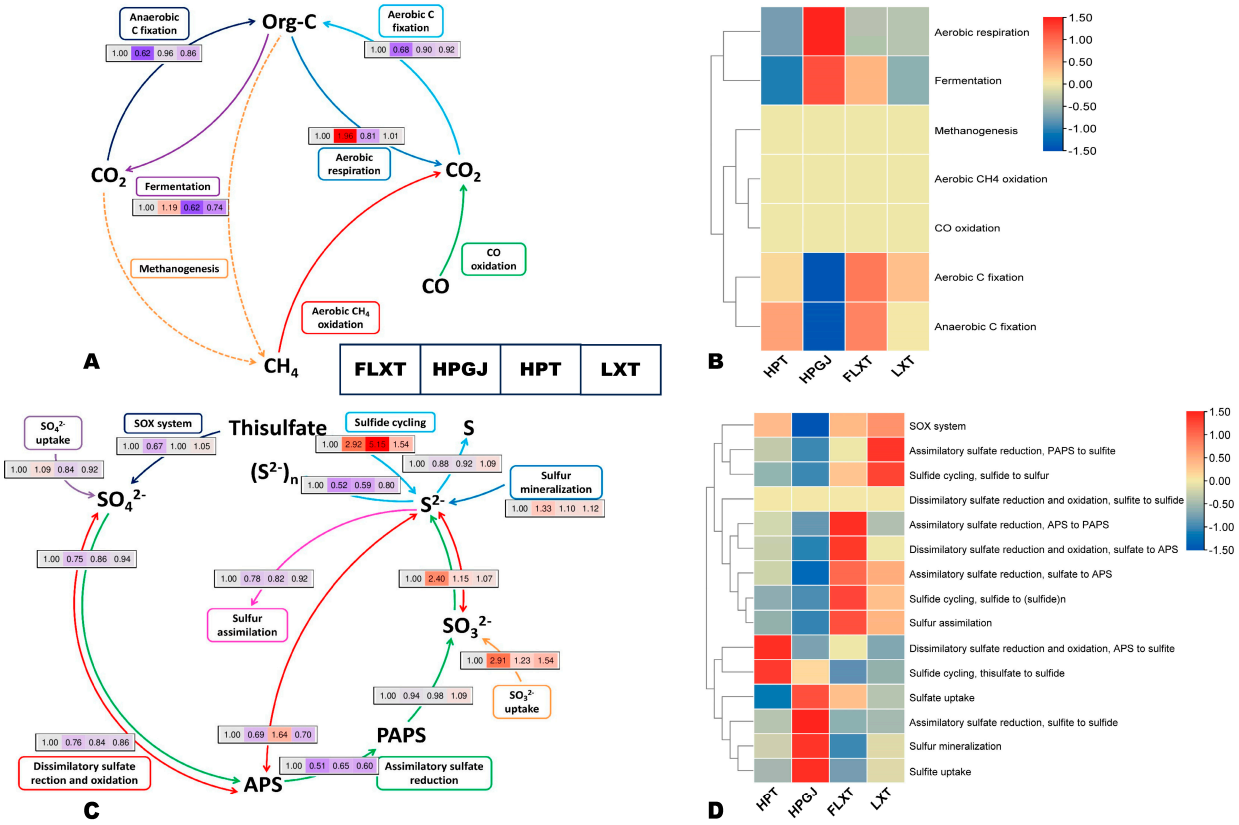

Figure 7. Effects of *G. elata* cultivation on C and S cycling. (**A**,**C**) Expression fold change of C and S cycling-related genes taking FLXT as the control, respectively. (**B**,**D**) Abundance of C and S cycling-related genes in different samples, respectively. The scaled Log2 fold change of the abundance of genes in each process is plotted in a blue-yellow-red color scale. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

3.7. Impacts of G. elata Cropping on Pathogenic Microorganisms' Community in Barren Slope and under Forest Soils

Previous studies have shown that *G. elata* cropping causes a significant increase in pathogenic microbes in rhizosphere soils [6,11–13]. To compare the impact of *G. elata* cropping on pathogenic microbial communities in barren slope and under forest soils, we examined the abundance of the 50 most abundant pathogen species found in the four soil groups. As shown in Figure 8, 38 pathogenic species were more abundant in the HPGJ and LXT than in their respective controls. However, 94.76% (36 out of 38) of these species were significantly more abundance in the HPGJ than in the LXT (Figure 8).

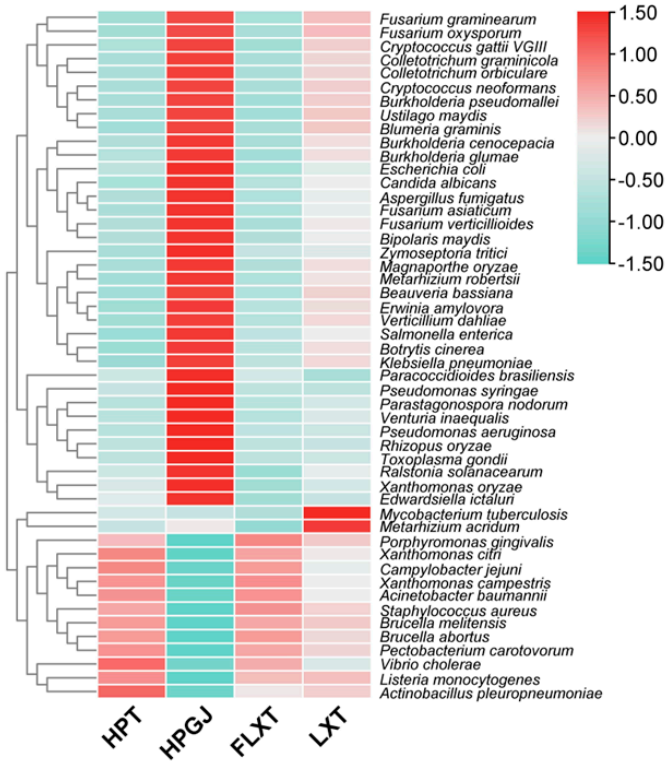

Figure 8. Abundance of top 50 pathogenic microbes in different samples. The scaled Log2 fold change of the abundance of each pathogen is plotted in a light blue-white-red color scale. HPT, barren slope soil; HPGJ, rhizosphere slope soil of wild *G. elata* rhizosphere; FLXT, understory soil; and LXT, rhizosphere soil of *G. elata* under forest.

4. Discussion

G. elata is a very important medicinal orchid with documented therapeutic properties [3–5,7–10]. It is, therefore, essential to improve its production. Unfortunately, as a mycoheterotrophic achlorophyllous plant, its artificial cultivation requires primary forest resources [11,12]. Furthermore, its continuous cropping leads to significant ecological problems with a low yield and quality [6,11,12]. Therefore, understanding the impacts of *G. elata* cultivation on the microecology and properties of different soil types is a prerequisite for its sustainable production. In the present study, soil metagenomics and a chemical properties analysis were used to characterize the effect of *G. elata* cropping on the global microbial composition and structure, mineral element cycling, and mineral nutrient composition of rhizosphere soils of a barren slope (HPGJ) and under forest (LXT). Soil metagenomics is an advanced method widely used to decipher the microecology of rhizosphere soils [26,29,42,43].

Our analysis showed that Bacteria (93.95%) were the predominant taxa identified in the soil groups, followed by Eukarya (4.35%), Archaea (1.51%), and Viruses (0.19%), indicating that Bacteria are the major microbial community in *G. elata* rhizosphere zones. The

sequencing of reforested mined soils and their controls has identified 97.64% Bacteria, 0.59% Archaea, 1.61% Eukarya, and 0.02% Viruses [44]. Beta diversity analyses showed that the microbial community composition and structure of the HPGJ was very different from that of the HPT (control), while the LWT showed no major difference from its control (FLXT). These results show that cultivating *G. elata* on slope soil may cause greater microecological changes than its cultivation under forests. The abundance of Proteobacteria, Actinobacteria, Mucoromycota, Basidiomycota, and Ascomycota increased significantly in the HPGJ compared to a slight increase in the LXT. Notably, the abundance of the fungal community increased from 0.17% to 23.61% in the HPGJ and from 0.2% to 2.04% in the LXT compared to their respective controls. These results are consistent with the previous study by Yuan et al., who found that the cultivation of *G. elata* improved the diversity and richness of the microbial community in the mycorrhizosphere and rhizosphere of the soil [21]. However, the authors reported a significant reduction in the abundance of the Basidiomycota community [21]. Taken together, these results infer that the development of the fungal community during *G. elata* growth is very complex, and that planting *G. elata* on barren slope soils may result in better growth and production than under forests. *G. elata* is a mycoheterotrophic orchid with a strong preferential association with various mycorrhizal fungi from which it obtains nutrients and carbon during its growth and development [13,14,29]. Further studies on various other soil types are required to determine the optimal soil conditions for the microecological improvement and higher yield of *G. elata*. A previous study has revealed a significant variation in the diversity of the endophytic bacterial community of *G. elata* in different regions of China [20]. In addition, the slope aspect was found to have a strong influence on the microbial community characteristics and soil nutrient composition [45].

Soils are reservoirs of the most diverse microbial communities, which drive biogeochemical cycles, particularly the cycling and storage of C, N, and other nutrients [19,46,47]. Diverse bacteria and fungi are involved in mineral nutrient cycling, and soil biodiversity is associated with vital ecological functions [17,48]. Notably, the abundance of N- and C-cycling gene carriers, such as Proteobacteria and Planctomycetes, improves the soil mineral nutrient composition [18,27]. Concordantly, we found that the increase in abundance of Proteobacteria, Actinobacteria, Mucoromycota, Basidiomycota, and Ascomycota was associated with improved N and C cycling, particularly in the HPGJ. The abundance of the functional genes involved in nitrification (hydroxylamine to ammonia), assimilation (nitrate to nitrite) and dissimilation (nitrite to ammonia), ammonia uptake, aerobic respiration, and fermentation increased, notably in the HPGJ. These results denote the potential beneficial effects of *G. elata* on mineral nutrient mineralization and the improvement of soil the chemical properties, especially in barren slope soils. In support of this, the analysis of soil chemical properties revealed a significant increase in the contents of the total nitrogen, NO_3^--N, organic matter, total carbon, organic carbon, total sulfur, and total phosphorus in the HPGJ, while no changes were recorded in the LXT. This suggests that planting *G. elata* could be an effective biological approach to improve the chemical properties and microbiome diversity of slope soils.

The One Health concept establishes the interconnectedness of human, plant, animal and environmental (soil) health through the cycling of subsets of microbial communities [48,49]. Soils are the most critical element of One Health, serving as a source and reservoir of beneficial and pathogenic microbes [48,49]. Previous studies have shown that *G. elata* cropping causes soil-borne diseases and a significant reduction in yield and quality by greatly increasing the abundance of pathogenic microorganisms in rhizosphere zones [6,11–13]. In agreement with previous findings, we examined the abundance of pathogenic microbes and found that most of them increased in the HPGJ and LXT. Notably, the increase in the abundance of pathogenic microorganisms was significantly higher in the HPGJ than in the LXT. The high increase in pathogens in the HPGJ could be attributed to inadequate drainage and its greater exposure to warmer temperatures [50]. These results indicate that although *G. elata* cultivation could improve the microbial diversity and chemical properties of slope soils, it may also lead to serious soil-borne diseases and

health problems. The development of efficient biological solutions to mitigate or eliminate pathogens associated with *G. elata* heterotrophy is required to sustain its production and quality for the food and health security perspective.

5. Conclusions

In summary, this study combined the analysis of soil chemical properties and metagenomics and unraveled the impacts of *G. elata* cultivation on the rhizosphere soils of a barren slope and under forest microecology, mineral cycling, and mineral nutrient composition. *G. elata* cropping caused a marked difference in the microbial composition, diversity, and structure in the rhizosphere of barren slope soil, while a slight difference was noticed in the rhizosphere under the forest. The abundance of microbes involved in mineral element cycling increased with *G. elata* cultivation. Notably, the abundance of fungal phyla (Mucoromycota, Basidiomycota, and Ascoomycota) and genera was greatly increased in the rhizosphere of the barren slope. Correspondingly, N, C, and S cycling was enhanced (high abundance of their functional genes), particularly in the rhizosphere soil of the barren slope, resulting in a significant improvement in the content of soil mineral elements (total nitrogen, NO_3^--N, organic matter, total carbon, organic carbon, total sulfur, and total phosphorus). Furthermore, we found a significant increase in the abundance of pathogenic microbes in the rhizosphere soil of the barren slope compared to that under the forest. Our findings show the potential of *G. elata* cropping for improving the microecology and mineral nutrient content and composition of slope soils. However, further studies are needed to identify specific approaches to limit the development of pathogenic microorganisms that can occasion serious soil-borne disease problems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15020331/s1, Figure S1: Bray-Curtis distance between samples; Figure S2: Heatmap of relative abundances of the microbial phyla in different samples; Figure S3: Heatmap of relative abundances of the microbial genera in different samples; Figure S4: General KEGG annotation of functional genes in all samples; Figure S5: Effects of *G. elata* cultivation on P cycling; Table S1: Summary of the high-quality soil sequencing data.

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References

1. Yang, J.; Li, P.; Li, Y.; Xiao, Q. GelFAP v2.0: An Improved Platform for Gene Functional Analysis in *Gastrodia elata*. *BMC Genom.* **2023**, *24*, 164. [CrossRef] [PubMed]
2. Yuan, Y.; Jin, X.; Liu, J.; Zhao, X.; Zhou, J.; Wang, X.; Wang, D.; Lai, C.; Xu, W.; Huang, J.; et al. The *Gastrodia elata* Genome Provides Insights into Plant Adaptation to Heterotrophy. *Nat. Commun.* **2018**, *9*, 1615. [CrossRef] [PubMed]

3. Zhan, H.D.; Zhou, H.Y.; Sui, Y.P.; Du, X.L.; Wang, W.H.; Dai, L.; Sui, F.; Huo, H.R.; Jiang, T.L. The Rhizome of *Gastrodia elata* Blume—An Ethnopharmacological Review. *J. Ethnopharmacol.* **2016**, *189*, 361–385. [CrossRef] [PubMed]
4. Wu, Y.N.; Wen, S.H.; Zhang, W.; Yu, S.S.; Yang, K.; Liu, D.; Zhao, C.B.; Sun, J. *Gastrodia elata* Bl.:A Comprehensive Review of Its Traditional Use, Botany, Phytochemistry, Pharmacology, and Pharmacokinetics. *Evid.-Based Complement. Altern. Med.* **2023**, *2023*, 5606021. [CrossRef] [PubMed]
5. Wu, J.; Wu, B.; Tang, C.; Zhao, J. Analytical Techniques and Pharmacokinetics of *Gastrodia elata* Blume and Its Constituents. *Molecules* **2017**, *22*, 1137. [CrossRef] [PubMed]
6. Long, L.l.p.; Luo, L.f.l. Effects of Different Years of Natural Recovery of *Gastrodia elata* on the Community Structure of Bacteria and Fungi in Rhizosphere Soil. *Res. Sq.* **2021**. [CrossRef]
7. Heese, K. *Gastrodia elata* Blume (Tianma): Hope for Brain Aging and Dementia. *Evid.-Based Complement. Altern. Med.* **2020**, *2020*, 8870148. [CrossRef] [PubMed]
8. Manavalan, A.; Ramachandran, U.; Sundaramurthi, H.; Mishra, M.; Sze, S.K.; Hu, J.-M.; Feng, Z.W.; Heese, K. *Gastrodia elata* Blume (Tianma) Mobilizes Neuro-Protective Capacities. *Int. J. Biochem. Mol. Biol.* **2012**, *3*, 219–241.
9. Matias, M.; Silvestre, S.; Falcão, A.; Alves, G. *Gastrodia elata* and Epilepsy: Rationale and Therapeutic Potential. *Phytomedicine* **2016**, *23*, 1511–1526. [CrossRef]
10. Shi, X.; Luo, Y.; Yang, L.; Duan, X. Protective Effect of *Gastrodia elata* Blume in a Caenorhabditis Elegans Model of Alzheimer's Disease Based on Network Pharmacology. *Biomed. Rep.* **2023**, *18*, 37. [CrossRef]
11. Yu, E.; Gao, Y.; Li, Y.; Zang, P.; Zhao, Y.; He, Z. An Exploration of Mechanism of High Quality and Yield of *Gastrodia elata* Bl. f. Glauca by the Isolation, Identification and Evaluation of Armillaria. *BMC Plant Biol.* **2022**, *22*, 621. [CrossRef] [PubMed]
12. Jiang, W.K.; Zhang, J.Q.; Guo, L.P.; Yang, Y.; Xiao, C.H.; Yuan, Q.S.; Wang, X.; Zhou, T. Thoughts and Suggestions on Ecological Cultivation of *Gastrodia elata*. *Zhongguo Zhongyao Zazhi* **2022**, *47*, 2277–2280. [PubMed]
13. Chen, L.; Wang, Y.C.; Qin, L.Y.; He, H.Y.; Yu, X.L.; Yang, M.Z.; Zhang, H.B. Dynamics of Fungal Communities during *Gastrodia elata* Growth. *BMC Microbiol.* **2019**, *19*, 158. [CrossRef]
14. Chen, L.; Xiang, W.; Wu, H.; Ouyang, S.; Lei, P.; Hu, Y.; Ge, T.; Ye, J.; Kuzyakov, Y. Contrasting Patterns and Drivers of Soil Fungal Communities in Subtropical Deciduous and Evergreen Broadleaved Forests. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 5421–5433. [CrossRef] [PubMed]
15. Jansson, J.K.; Hofmockel, K.S. Soil Microbiomes and Climate Change. *Nat. Rev. Microbiol.* **2020**, *18*, 35–46. [CrossRef] [PubMed]
16. Lladó, S.; López-Mondéjar, R.; Baldrian, P. Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change. *Microbiol. Mol. Biol. Rev.* **2017**, *81*, e00063-16. [CrossRef] [PubMed]
17. Bastida, F.; Eldridge, D.J.; García, C.; Kenny Png, G.; Bardgett, R.D.; Delgado-Baquerizo, M. Soil Microbial Diversity–Biomass Relationships Are Driven by Soil Carbon Content across Global Biomes. *ISME J.* **2021**, *15*, 2081–2091. [CrossRef] [PubMed]
18. Hu, X.; Gu, H.; Liu, J.; Wei, D.; Zhu, P.; Cui, X.; Zhou, B.; Chen, X.; Jin, J.; Liu, X.; et al. Metagenomics Reveals Divergent Functional Profiles of Soil Carbon and Nitrogen Cycling under Long-Term Addition of Chemical and Organic Fertilizers in the Black Soil Region. *Geoderma* **2022**, *418*, 115846. [CrossRef]
19. Isobe, K.; Ohte, N. Ecological Perspectives on Microbes Involved in N-Cycling. *Microbes Environ.* **2014**, *29*, 4–16. [CrossRef]
20. Zheng, H.; Zhang, P.; Qin, J.; Guo, J.; Deng, J. High-Throughput Sequencing-Based Analysis of the Composition and Diversity of Endophytic Bacteria Community in Tubers of *Gastrodia elata* f.Glauca. *Front. Microbiol.* **2023**, *13*, 1092552. [CrossRef]
21. Yuan, Q.S.; Xu, J.; Jiang, W.; Ou, X.; Wang, H.; Guo, L.; Xiao, C.; Wang, Y.; Wang, X.; Kang, C.; et al. Insight to Shape of Soil Microbiome during the Ternary Cropping System of Gastradia Elata. *BMC Microbiol.* **2020**, *20*, 108. [CrossRef]
22. Zhang, L.; Chen, F.X.; Zeng, Z.; Xu, M.; Sun, F.; Yang, L.; Bi, X.; Lin, Y.; Gao, Y.J.; Hao, H.X.; et al. Advances in Metagenomics and Its Application in Environmental Microorganisms. *Front. Microbiol.* **2021**, *12*, 766364. [CrossRef] [PubMed]
23. Vieira, A.F.; Moura, M.; Silva, L. Soil Metagenomics in Grasslands and Forests—A Review and Bibliometric Analysis. *Appl. Soil Ecol.* **2021**, *167*, 104047. [CrossRef]
24. Daniel, R. The Metagenomics of Soil. *Nat. Rev. Microbiol.* **2005**, *3*, 470–478. [CrossRef]
25. Xie, Z.; Yu, Z.; Li, Y.; Wang, G.; Liu, X.; Tang, C.; Lian, T.; Adams, J.; Liu, J.; Liu, J.; et al. Soil Microbial Metabolism on Carbon and Nitrogen Transformation Links the Crop-Residue Contribution to Soil Organic Carbon. *npj Biofilms Microbiomes* **2022**, *8*, 14. [CrossRef]
26. Ye, L.; Wang, X.; Wei, S.; Zhu, Q.; He, S.; Zhou, L. Dynamic Analysis of the Microbial Communities and Metabolome of Healthy Banana Rhizosphere Soil during One Growth Cycle. *PeerJ* **2022**, *10*, e14404. [CrossRef] [PubMed]
27. Mosley, O.E.; Gios, E.; Close, M.; Weaver, L.; Daughney, C.; Handley, K.M. Nitrogen Cycling and Microbial Cooperation in the Terrestrial Subsurface. *ISME J.* **2022**, *16*, 2561–2573. [CrossRef]
28. Li, W.; Lei, X.; Zhang, R.; Cao, Q.; Yang, H.; Zhang, N.; Liu, S.; Wang, Y. Shifts in Rhizosphere Microbial Communities in Oplopanax Elatus Nakai Are Related to Soil Chemical Properties under Different Growth Conditions. *Sci. Rep.* **2022**, *12*, 11485. [CrossRef]
29. Liu, T.; Li, C.M.; Han, Y.L.; Chiang, T.Y.; Chiang, Y.C.; Sung, H.M. Highly Diversified Fungi Are Associated with the Achlorophyllous Orchid Gastrodia Flavilabella. *BMC Genom.* **2015**, *16*, 185. [CrossRef]
30. Shi, Z.-W.; Ma, C.-J.; Kang, C.-Z.; Wang, L.; Zhang, Z.-H.; Chen, J.-F.; Zhang, X.-B.; Liu, D.-H. Ecological suitability regionalization for *Gastrodia elata* in Zhaotong based on Maxent and ArcGIS. *China J. Chin. Mater. Medica* **2016**, *41*, 3155–3163. [CrossRef]

31. Tong, A.Z.; Liu, W.; Liu, Q.; Xia, G.Q.; Zhu, J.Y. Diversity and Composition of the Panax Ginseng Rhizosphere Microbiome in Various Cultivation Modesand Ages. *BMC Microbiol.* **2021**, *21*, 18. [CrossRef]
32. Ruirui, C.; Xiaoting, W. BOOK REVIEW: Analytical Methods for Soil and Agro-Chemistry (in Chinese). Edited by H. Z. Zhu, P. A. He, C. Z. Chen, H. M. Zhou, D. C. Su, J. M. Xu, H. Y. Qin, S. D. Bao, R. K. Lu, S. H. Jiang Soil Science Society of China Beijing, China Agricultural Science and Technology Press, 2000, pp. 638. ISBN: 9787801199256. *Eur. J. Soil Sci.* **2022**, *73*, 221–248. [CrossRef]
33. Majumdar, S.; Muruganantham, L.; Karmakar, K.; Nagabovanalli Basavarajappa, P. BOOK REVIEW: Soil Analysis. Edited by S. K. Singh, D. R. Biswas, C. A. Srinivasamurthy, S. P. Datta, G. Jayasree, P. Jha, S.K. Sharma, R. N. Katkar, K. P. Raverkar, A. K. Ghosh, Indian Society of Soil Science, New Delhi, India, 2019, pp. INR1400, ISBN 81-903797-8-X. *Eur. J. Soil Sci.* **2022**, *73*, 37–41. [CrossRef]
34. Bolger, A.M.; Lohse, M.; Usadel, B. Trimmomatic: A Flexible Trimmer for Illumina Sequence Data. *Bioinformatics* **2014**, *30*, 2114–2120. [CrossRef] [PubMed]
35. Wood, D.E.; Salzberg, S.L. Kraken: Ultrafast Metagenomic Sequence Classification Using Exact Alignments. *Genome Biol.* **2014**, *15*, R46. [CrossRef]
36. Li, D.; Liu, C.-M.; Luo, R.; Sadakane, K.; Lam, T.-W. MEGAHIT: An Ultra-Fast Single-Node Solution for Large and Complex Metagenomics Assembly via Succinct de Bruijn Graph. *Bioinformatics* **2015**, *31*, 1674–1676. [CrossRef] [PubMed]
37. Hyatt, D.; Chen, G.-L.; Locascio, P.F.; Land, M.L.; Larimer, F.W.; Hauser, L.J. Prodigal: Prokaryotic Gene Recognition and Translation Initiation Site Identification. *BMC Bioinform.* **2010**, *11*, 119. [CrossRef] [PubMed]
38. Fu, L.; Niu, B.; Zhu, Z.; Wu, S.; Li, W. CD-HIT: Accelerated for Clustering the next-Generation Sequencing Data. *Bioinformatics* **2012**, *28*, 3150–3152. [CrossRef]
39. Patro, R.; Duggal, G.; Love, M.I.; Irizarry, R.A.; Kingsford, C. Salmon Provides Fast and Bias-Aware Quantification of Transcript Expression. *Nat. Methods* **2017**, *14*, 417–419. [CrossRef]
40. Li, J.; Jia, H.; Cai, X.; Zhong, H.; Feng, Q.; Sunagawa, S.; Arumugam, M.; Kultima, J.R.; Prifti, E.; Nielsen, T.; et al. An Integrated Catalog of Reference Genes in the Human Gut Microbiome. *Nat. Biotechnol.* **2014**, *32*, 834–841. [CrossRef]
41. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef] [PubMed]
42. White, H.J.; León-Sánchez, L.; Burton, V.J.; Cameron, E.K.; Caruso, T.; Cunha, L.; Dirilgen, T.; Jurburg, S.D.; Kelly, R.; Kumaresan, D.; et al. Methods and Approaches to Advance Soil Macroecology. *Glob. Ecol. Biogeogr.* **2020**, *29*, 1674–1690. [CrossRef]
43. Shi, R.; Gu, H.; He, S.; Xiong, B.; Huang, Y.; Horowitz, A.R.; He, X. Comparative Metagenomic and Metabolomic Profiling of Rhizospheres of Panax Notoginseng Grown under Forest and Field Conditions. *Agronomy* **2021**, *11*, 2488. [CrossRef]
44. Sun, S.; Badgley, B.D. Changes in Microbial Functional Genes within the Soil Metagenome during Forest Ecosystem Restoration. *Soil Biol. Biochem.* **2019**, *135*, 163–172. [CrossRef]
45. Huang, Y.M.; Liu, D.; An, S.S. Effects of Slope Aspect on Soil Nitrogen and Microbial Properties in the Chinese Loess Region. *Catena* **2014**, *125*, 135–145. [CrossRef]
46. Liu, M.; Sui, X.; Hu, Y.; Feng, F. Microbial Community Structure and the Relationship with Soil Carbon and Nitrogen in an Original Korean Pine Forest of Changbai Mountain, China. *BMC Microbiol.* **2019**, *19*, 218. [CrossRef] [PubMed]
47. Bahram, M.; Hildebrand, F.; Forslund, S.K.; Anderson, J.L.; Soudzilovskaia, N.A.; Bodegom, P.M.; Bengtsson-Palme, J.; Anslan, S.; Coelho, L.P.; Harend, H.; et al. Structure and Function of the Global Topsoil Microbiome. *Nature* **2018**, *560*, 233–237. [CrossRef]
48. Banerjee, S.; van der Heijden, M.G.A. Soil Microbiomes and One Health. *Nat. Rev. Microbiol.* **2023**, *21*, 6–20. [CrossRef]
49. van Bruggen, A.H.C.; Goss, E.M.; Havelaar, A.; van Diepeningen, A.D.; Finckh, M.R.; Morris, J.G. One Health—Cycling of Diverse Microbial Communities as a Connecting Force for Soil, Plant, Animal, Human and Ecosystem Health. *Sci. Total Environ.* **2019**, *664*, 927–937. [CrossRef]
50. Delgado-Baquerizo, M.; Guerra, C.A.; Cano-Díaz, C.; Egidi, E.; Wang, J.-T.; Eisenhauer, N.; Singh, B.K.; Maestre, F.T. The Proportion of Soil-Borne Pathogens Increases with Warming at the Global Scale. *Nat. Clim. Chang.* **2020**, *10*, 550–554. [CrossRef]

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