

Article

Intercropping Shapes the Metabolome and Microbiome of Medicinal Giant Lily (*Cardiocrinum giganteum*) in Bamboo, Chinese Fir, and Mixed Forests

Jie Zhang ^{1,2}, Yilin Ning ^{1,2}, Haoyu Wu ³, Guibin Gao ^{1,2}, Zhizhuang Wu ^{1,2}, Yuwen Peng ^{1,2}, Zhiyuan Huang ^{1,2} and Xiaoping Zhang ^{1,2,*} 

- ¹ China National Bamboo Research Center, Key Laboratory of State Forestry and Grassland Administration on Bamboo Forest Ecology and Resource Utilization, Hangzhou 310012, China; zhangjie@caf.ac.cn (J.Z.); ningyl@caf.ac.cn (Y.N.); anshu998@caf.ac.cn (G.G.); wzzcaf@126.com (Z.W.); pywpyw54321@caf.ac.cn (Y.P.); zhiyuanhuang@caf.ac.cn (Z.H.)
- ² National Long-Term Observation and Research Station for Forest Ecosystem in Hangzhou-Jiaxing-Huzhou Plain, Hangzhou 310012, China
- ³ Research Institute of Forestry, Chinese Academy of Forestry, Beijing 100091, China; haoyuwu@caf.ac.cn
- * Correspondence: xiaopingzhang@caf.ac.cn

Abstract: Intercropping is a promising strategy for sustainable medicinal plant cultivation, but its impact on plant–microbe interactions remains poorly understood. This study investigated the influence that intercropping giant lily (*Cardiocrinum giganteum*) with bamboo (BG), Chinese fir (FG), and mixed forests (MG) had on the giant lily metabolome and microbiome compared to a monoculture control (GG). Metabolomic analysis revealed that BG significantly increased the accumulation of terpenoids (e.g., yucalexin B22, 19.39-fold), alkaloids (e.g., anabasine, 2.97-fold), and steroids (e.g., O-acetyl-lariciresinol, 4.49-fold), while MG induced the production of stress-related metabolites (e.g., aflatoxin G2, 128.62-fold), and FG enhanced nitrogen metabolism (e.g., putrescine, 2.47-fold). Intercropping altered the rhizosphere and endophytic microbial communities, with BG enriching beneficial bacteria (e.g., Acidobacteria and Alphaproteobacteria) and FG promoting symbiotic fungi (e.g., Serendipita and Xylariales). Network analysis revealed strong correlations between specific microbial taxa (e.g., *Bacillus* and Ceratobasidiaceae) and key metabolites (e.g., norpandamarilactonine A, methylgingerol), indicating their potential roles in shaping the metabolic profiles of giant lily. These findings highlight the complex interplay between intercropping systems, microbial communities, and medicinal plant metabolism and provide a basis for developing targeted cultivation strategies to enhance the production of bioactive compounds in giant lily and other medicinal plants.

Keywords: giant lily; intercropping; agroforestry; rhizosphere microbiome; endogenous microbiome; secondary metabolites



Citation: Zhang, J.; Ning, Y.; Wu, H.; Gao, G.; Wu, Z.; Peng, Y.; Huang, Z.; Zhang, X. Intercropping Shapes the Metabolome and Microbiome of Medicinal Giant Lily (*Cardiocrinum giganteum*) in Bamboo, Chinese Fir, and Mixed Forests. *Forests* **2024**, *15*, 2201. <https://doi.org/10.3390/f15122201>

Academic Editor: Marco Landi

Received: 14 November 2024

Revised: 6 December 2024

Accepted: 9 December 2024

Published: 13 December 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The intricate and dynamic relationships between plants and their associated microorganisms play a pivotal role in the formation of terrestrial ecosystems, in the promotion of global biodiversity, and in the enhancement of agricultural productivity [1,2]. This dynamic relationship has evolved over millions of years, and the complex interactions between plants and microorganisms have a profound impact on plant health. Rhizosphere microorganisms and endogenous bacteria community involved in plant nutrient cycling and access enhance resistance, adjusting the synthesis of secondary metabolites [3]. Certain rhizobia facilitate phosphorus solubilization and nitrogen fixation, whereas endophytic fungi enhance the synthesis of plant defense compounds [4]. It is therefore imperative to gain an understanding of these complex interactions if sustainable agricultural practices are to be developed and the cultivation of economically important plants, in particular medicinal species, is to be optimized. The complicated relationship between plants and

microbes in the context of medicinal plants is of special significance given the critical importance of the production of bioactive compounds in these crops. Microbes can promote the growth of medicinal plants and their resistance through a variety of mechanisms, the most important of which is to promote the synthesis of therapeutic compounds, including the production of plant hormones, thereby enhancing dissolved nutrients and regulating plant gene expression [5,6]. The endophytic association between *Dictyophora indusiata* and *Bacillus aryabhattai* stimulated production of growth-promoting metabolites (lysoPC 18:1 and dihydrobenzofuran) in sugarcane roots, enhancing both crop yield and flavonoid metabolism during endophytic phenotype development [7]. *Arabidopsis* root triterpenes (thalianin, thalianyl fatty acid esters, and arabidin) exert a selective influence on the root microbiome, providing carbon sources for specific bacteria through metabolic breakdown (e.g., thalianyl fatty acid esters to palmitic acid) or inhibiting the growth of other bacteria [8]. Understanding and using plant and microbial interactions can significantly increase the yield and quality of medicinal plants and enhance their therapeutic potential.

Intercropping, the practice of growing multiple crops in a single field, has emerged as an effective way to harness plant–microbe interactions to increase agricultural productivity and sustainability. The benefits of intercropping have been demonstrated for several farming systems. For example, research shows that a corn, soybean, peanut, and cotton intercropping system can strengthen the soil nutrient cycle, promote ecosystem diversity, and promote crop growth and soil health by altering the microbial community [9]. Intercropping can enhance the interaction between plants and microbes, resulting in increased crop yields and soil quality, thereby reducing the need for fertilizer and pesticide inputs.

Recent studies have indicated that intercropping medicinal plants in agroforestry systems has the potential to improve the yield and quality of the target species. For instance, Wang et al. [10] illustrated that intercropping *Panax notoginseng* with tree species not only promoted biomass accumulation and reduced disease occurrence but also increased saponin concentration through alterations in soil metabolites. Similarly, Duan et al. [11] reported that intercropping *P. notoginseng* with ryegrass and red clover resulted in a notable increase in ginsenoside content and facilitated the access of beneficial bacteria to the rhizosphere. It has been proven that intercropping legumes can improve soil quality and tea quality in tea plantations by regulating rare bacterial communities [12]. These findings highlight the potential of intercropping as an optimization strategy for medicinal plant cultivation in agroforestry.

Giant lily (*Cardiocrinum giganteum*) is a cash crop with dual medicinal and edible uses. Revered for their massive stature and showy flowers, giant lily has been used to treat a variety of diseases, including respiratory, cardiovascular, and inflammatory conditions. Thus, it is an optimal candidate for integration into agroforestry systems. Recent phytochemical studies have revealed that giant lily is a rich source of bioactive compounds, including steroidal saponins, polysaccharides, and flavonoids, with a range of pharmacological activities [13–15]. However, the effects of intercropping on the giant lily metabolome and microbiome remain to be fully elucidated, particularly in the context of agroforestry systems.

As a comprehensive database of features, the metabolome effectively reflects disturbances to plants due to their environment and plants' adaptive response to intercropping conditions [16]. Microbes influence key processes, including nutrient cycling, disease resistance, and specialized metabolite biosynthesis, and these microbial–metabolite interactions are critical for optimizing medicinal plant cultivation in agroforestry systems [17]. This is particularly true for giant lily because the impact of understory intercropping on its microbiome, metabolome, and medicinal potential in different forest systems remains unknown. It is imperative to fill this knowledge gap to facilitate the development of sustainable cultivation methods that can optimize the production of bioactive compounds in giant lily and other valuable medicinal plants.

This study had three objectives: (1) to characterize the rhizosphere and endophytic microbial communities associated with giant lily in different intercropping systems; (2) evaluate

the response of the giant lily to different systems of intercropping; (3) to explore the potential interactions between the microbiome and metabolome of giant lily under different intercropping systems.

2. Materials and Methods

2.1. Study Area and Sample Collection

The research site is situated in Jianxin Village, Jiangdi Township, Longsheng Autonomous County, Guilin City, Guangxi Zhuang Autonomous Region, China (110°24' E, 25°80' N) (Figure S1). The region is characterized by a subtropical monsoon climate, with an altitudinal range of 845–850 m above sea level. The area receives an average annual precipitation of 1500–2400 mm and enjoys 1670 h of sunshine per year. The average annual temperature is 18.1 °C, with extreme temperatures of 4.8 °C and 39.5 °C recorded. The frost-free season extends for approximately 314 days. The terrain exhibits a typical karst topography, and the soil is classified as Haplic Lixisols according to the World Reference Base for Soil Resources, 4th edition (2022). To investigate the influence of different intercropping systems on the rhizosphere microbial communities, endogenous bacteria, and bulb metabolites of the medicinal plant giant lily, four intercropping arrangements were established within 400 m² experimental plots: giant lily intercropped with natural stands of Moso bamboo (*Phyllostachys edulis*) (BG), with a Chinese fir (*Cunninghamia lanceolata*) forest stand (FG), and with a bamboo-fir mixed forest (MG). Additionally, a 400 m² forest gap was used for giant lily intercropping (GG) as a control treatment, simulating a monoculture system. Each intercropping system was designed to ensure consistent sampling and comparison conditions, with giant lily planted at a density of three plants per square meter. The giant lily plants were three years old at the time of sampling. The experimental plots were situated on slopes with inclinations ranging from 23° to 29°, and the study area has been under uniform forest management practices for over a decade.

In August 2024, when the giant lily reached maturity, six three-year-old plants were randomly chosen from each plot and pooled into a single sample. This process resulted in a total of 24 individual samples (4 systems × 6 replicates). Rhizosphere samples were collected by carefully removing the soil clinging to the roots and passing it through a 2.0 mm sieve to remove plant debris and rocks. The samples were subsequently stored at −80 °C for later biodiversity analysis. The harvested bulbs were preserved in liquid nitrogen to facilitate metabolite detection and endophytic microbial diversity analysis.

2.2. DNA Extraction and Sequencing

DNA extraction and quantification were performed on rhizosphere soil and plant bulb samples using the Magnetic Soil and Stool DNA Kit (DP712-02, TIANGEN, Beijing, China) and the TIANamp Bacteria DNA Kit (DP302-02, TIANGEN, Beijing, China), respectively, following the manufacturer's instructions. The extracted DNA was quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). PCR amplification targeted the V3-V4 region of the 16S rRNA gene in rhizosphere soil bacteria using the primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTATCTAATCC-3') [18], the V4 region of the 16S rRNA gene in plant bulb endophytic bacteria using the primers 515F (5'-GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [19], and the ITS2 region in both rhizosphere soil and plant bulb fungi using the primers ITS1FI2 (5'-GTGARTCATCGAATCTTTG-3') and ITS2 (5'-TCCTCCGCTTATTGATATGC-3') [20]. The PCR conditions included pre-denaturation at 98 °C for 30 s, followed by 32 cycles of denaturation at 98 °C for 10 s, annealing at 54 °C for 30 s, extension at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The amplified PCR products were purified using AMPure XT Beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified using a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). The quality and quantity of the amplicon libraries were assessed using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA) and the Library Quantification Kit for Illumina (Kapa Biosciences, Wilmington,

MA, USA). Finally, the libraries were pooled in equimolar amounts and sequenced on an Illumina NovaSeq PE250 platform (Illumina, San Diego, CA, USA).

2.3. Metabolite Extraction and LC-MS Detection

The plant bulb samples were snap-frozen in liquid nitrogen and swiftly ground into a fine powder to facilitate metabolite extraction. A 100 mg aliquot of the powdered sample was subjected to extraction using 1 mL of pre-cooled 50% methanol, following the manufacturer's operating manual. The extraction mixture was vortexed for 1 min, allowed to stand at room temperature for 10 min, and then stored overnight at -20°C . The mixture was centrifuged at $4000\times g$ for 20 min, and the resulting supernatant was transferred to a new tube and stored at -80°C until LC-MS analysis. Metabolite detection was carried out using a Vanquish Flex UHPLC system (Thermo Fisher Scientific, Bremen, Germany) coupled with high-resolution mass spectrometers, including Q-Exactive, Q-Exactive plus, and OE 120 (Thermo Fisher Scientific, Bremen, Germany). Chromatographic separation was performed using an ACQUITY UPLC T3 column ($100\text{ mm} \times 2.1\text{ mm}$, $1.8\text{ }\mu\text{m}$, Waters, Milford, MA, USA) maintained at 40°C . The mobile phase consisted of solvent A (water containing 5 mM of ammonium acetate and 5 mM of acetic acid) and solvent B (acetonitrile), with a flow rate of 0.3 mL/min. The gradient elution conditions were optimized for each sample type to ensure optimal separation and detection of metabolites.

2.4. Data Analysis

The acquired mass spectrometry data were preprocessed using XCMS software (version 1.9) for peak picking, grouping, retention time correction, and annotation of isotopes and adducts. Metabolite annotation was achieved by matching the exact molecular mass data (m/z) of the samples with those from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB), with a mass difference threshold of less than 10 ppm. Further validation of the annotated metabolites was performed using isotopic distribution measurements. Statistical analysis was conducted in R 4.4.1, with raw metabolite intensities normalized using the median normalization method. Hierarchical clustering was carried out using the pheatmap packages (version 1.0.12). Partial least squares discriminant analysis (PLS-DA) was performed using the ropls package (version 1.30.0), and variable importance in projection (VIP) values were calculated for each variable. Pearson's correlation coefficient from the cor package was used for correlation analysis. Differentially abundant metabolites were identified based on the following criteria: $p < 0.05$, fold change (FC) > 1.2 , and $\text{VIP} > 1$, with all three conditions being satisfied simultaneously. Hypergeometric test-based KEGG pathway enrichment analysis was conducted on the differentially abundant metabolites ($p \leq 0.05$).

The 16S rRNA gene and ITS sequencing data were analyzed using the QIIME2 pipeline, which involved quality filtering, merging of paired-end reads, removal of chimeras, and generation of amplicon sequence variants (ASVs) using the DADA2 algorithm. Taxonomic assignment was carried out using the QIIME2 feature-classifier plugin with the SILVA (Release 138) and NT-16S (Release 20230718) databases for bacteria and the UNITE database for fungi. Alpha diversity indices were calculated using QIIME2, and differences between groups were determined using the Wilcoxon rank-sum test ($p < 0.05$). Beta diversity was assessed using Bray–Curtis distances, followed by principal component analysis (PCA) visualization using R package “ggplot2” (version 3.4.2). Linear discriminant analysis effect size (LEfSe) was performed to identify differentially abundant taxa (Linear Discriminate Analysis (LDA) score > 4.0 , $p < 0.05$). Redundancy analysis (RDA) was selected based on the gradient lengths (<3) of the first detrended correspondence analysis axis for both bacterial and fungal communities to explore the relationships between the differentially abundant metabolites and the microbial communities. Procrustes analysis was conducted to correlate microbial community structure (Bray–Curtis distances) with metabolite profiles (Euclidean distances) using R package “vegan” (version 2.6.4), with significance assessed by Monte Carlo permutation test (999 permutations). The co-occurrence network anal-

ysis was conducted by calculating Spearman's rank correlations, retaining only robust ($|r| > 0.9$) and significant ($p < 0.05$) associations [21]. Networks were visualized using Gephi 3.10.3 [22].

3. Results

3.1. Secondary Metabolites of Giant Lily Bulbs Under Different Intercropping Systems

PLS-DA revealed distinct metabolic profiles among the intercropping systems ($p < 0.001$), with the first two components capturing 27.90% and 22.98% of the total variance, respectively (Figure 1a,b). Heatmap analysis demonstrated clear segregation of metabolic profiles across the intercropping systems. BG showed the greatest divergence from the GG control system, while MG exhibited distinct patterns characterized by strong metabolite upregulation, particularly of carbohydrates and organic acids. FG showed the most focused metabolic response, with selective upregulation of amino acids and nitrogen-rich compounds such as guanylylurea and L-tyrosine. Each system displayed characteristic metabolic signatures, with BG showing notable upregulation of glycoside-containing compounds and specialized metabolites. Metabolomic analysis of 356 metabolites revealed 62 differentially abundant metabolites ($p < 0.05$) among the GG, MG, BG, and FG systems, suggesting distinct physiological adaptations (Figure 1c). GG exhibited the highest levels of lipid-related compounds and octanol, while MG demonstrated the most extensive metabolic alterations. These alterations in MG were characterized by increases in stress-related compounds such as aflatoxin G2 (FC = 128.62), yucalexin B22 (FC = 54.43), and kaurenic acid (FC = 19.54), as well as enhanced carbohydrate metabolism, with increased levels of trehalose (FC = 6.32) and melibiose (FC = 4.29). BG also displayed substantial changes, with increases in stress-related compounds, including yucalexin B22 (FC = 19.39), kaurenic acid (FC = 14.13), and azoxy-2-procarbazine (FC = 12.14), along with enhanced lipid and amino acid metabolism and moderate increases in carbohydrate metabolism. In contrast, FG exhibited balanced metabolic reprogramming, with moderate changes in organic acid metabolism, amino acid accumulation, and stress-related compounds accompanied by reduced lipid-related metabolites. Secondary metabolism showed coordinated changes alongside primary metabolic shifts, with system-specific accumulation patterns. Phenylpropanoids were highest in MG, with increased levels of isoferulic acid (FC = 2.83), and they were reduced in FG (FC = 0.18). β -D-glucopyranoside derivatives exhibited variable responses across systems. Nitrogen-containing compounds such as putrescine (FC = 2.47) were highest in BG. Specific metabolic suppressions differed among systems, with MG showing decreases in nucleosides such as 2'-deoxyadenosine (FC = 0.07). BG displayed reduced aromatic compounds, including 2-phenylethanol (FC = 0.13) and methylgingerol (FC = 0.23), while FG exhibited decreased lipid-derived metabolites such as SL 17:0;O/17:0;O (FC = 0.07) and DGDG O-9:0_2:0 (FC = 0.30).

Pairwise comparisons revealed additional metabolic differences, with the number of differential metabolites ranging from 64 to 97 (Figure 2a–c; Table S1). Notable differences included increased alkaloid (narcotoline, FC = 2.97; hupehenine, FC = 3.83) and steroid metabolism (O-acetylsolasodine, FC = 4.49; 7a,12a-dihydroxy-cholestene-3-one, FC = 2.89) in BG compared to GG. FG showed significant increases in amino acid derivatives (ornithine, FC = 5.41; pipecolate, FC = 4.45) compared to GG. In contrast, phenylpropanoids (isoferulic acid, FC = 0.18) and some steroid derivatives (O-Acetylsolasodine, FC = 0.16; solasodine, FC = 0.46) were decreased in FG compared to GG. KEGG pathway enrichment analysis of the differential metabolites revealed ATP-binding cassette (ABC) transporters as the most significantly enriched pathway, followed by biosynthesis of plant secondary metabolites and central carbon metabolism (Figure 2d). The enriched pathways encompassed metabolism, environmental information processing, cellular processes, and genetic information processing categories. Primary metabolic pathways included the tricarboxylic acid (TCA) cycle and carbon fixation, while secondary metabolism predominantly involved alkaloid biosynthesis, with notable representation of membrane transport systems.

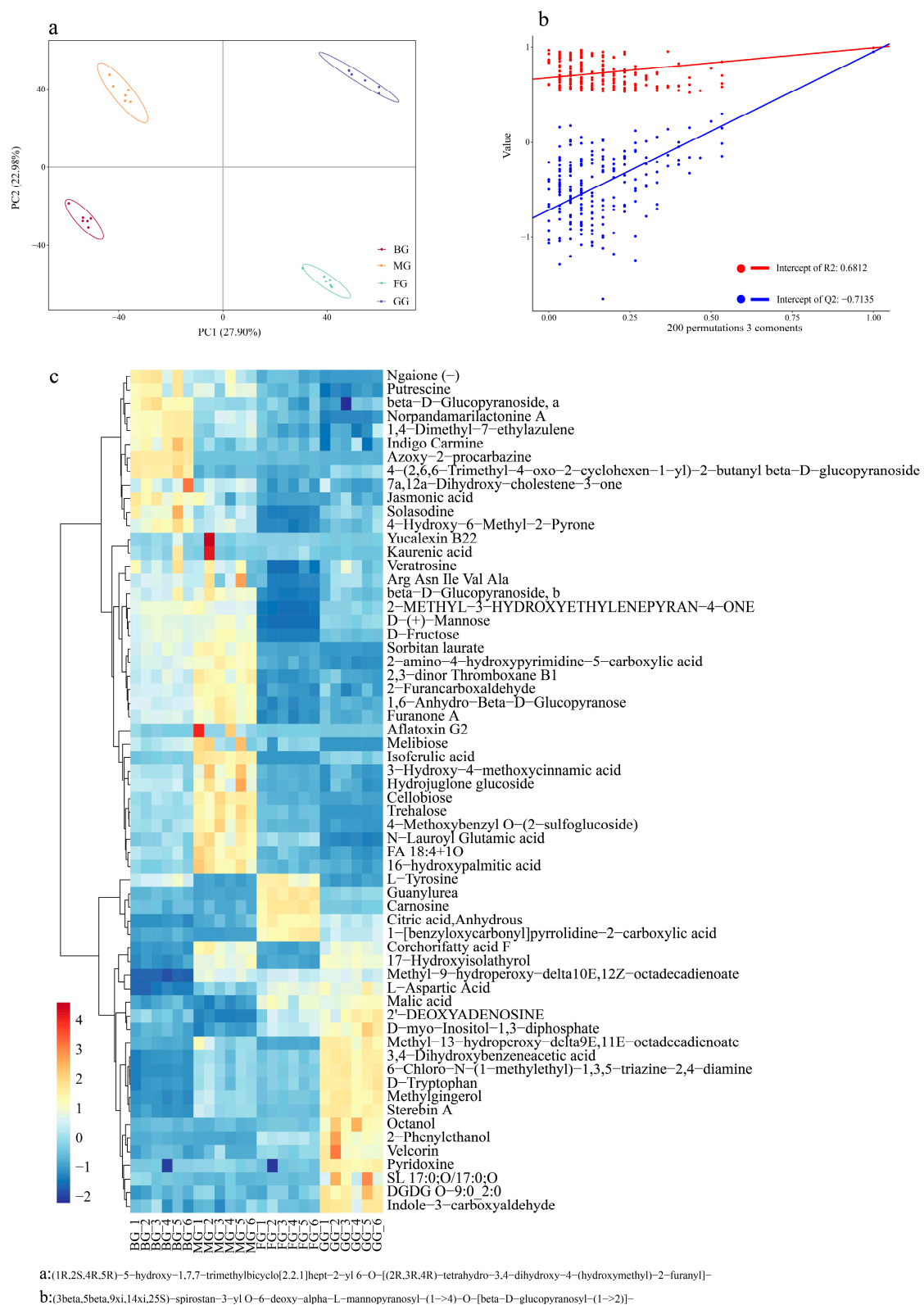


Figure 1. Effects of different intercropping systems on the secondary metabolism of giant lily bulbs. **(a)** Partial least squares discriminant analysis (PLS-DA) score plot showing the metabolic profiles of giant lily bulbs under different intercropping systems: bamboo-giant lily (BG), Chinese fir-giant lily (FG), mixed forest-giant lily (MG), and forest gap-giant lily (GG). **(b)** Permutation test for PLS-DA model validation. **(c)** Heatmap displaying the relative abundance of differentially accumulated metabolites among the four intercropping systems.

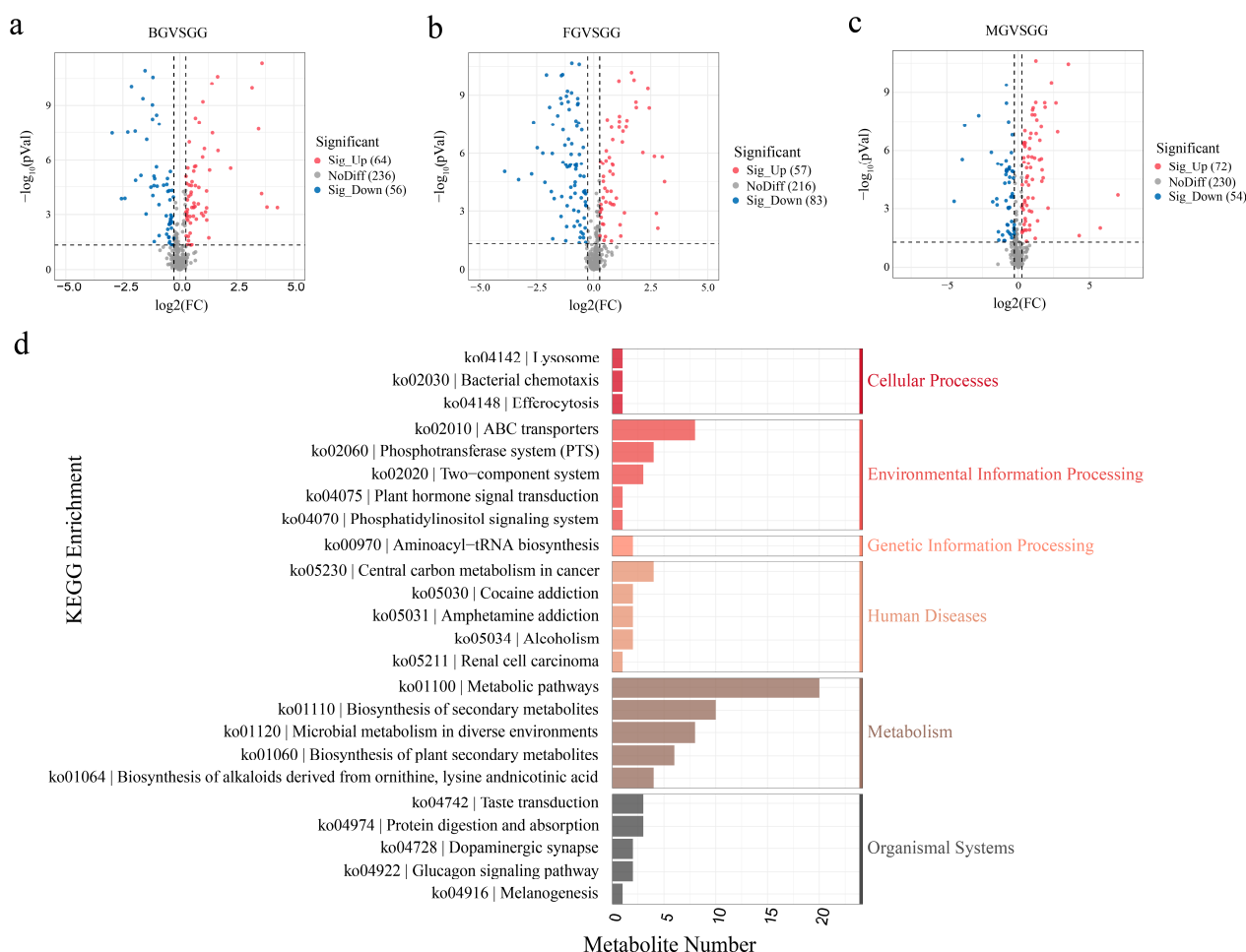


Figure 2. Effects of different intercropping systems on the secondary metabolism of giant lily bulbs. (a–c) Volcano plots illustrating the differentially abundant metabolites. (d) KEGG pathway enrichment analysis of the differentially abundant metabolites.

3.2. Effects of Intercropping Systems on Rhizosphere and Endophytic Microorganisms of Giant Lily

High-throughput sequencing of 96 samples generated 7,993,949 raw reads (average 83,270 reads/sample). Analysis at a 97% sequence similarity threshold identified 3665 fungal and 8328 bacterial endophytic ASVs. Rhizosphere soil exhibited higher microbial diversity, containing 40,624 bacterial and 7001 fungal ASVs, representing 4.88-fold and 1.91-fold increases compared to endophytic communities, respectively.

3.2.1. Variations in Microbial Community Diversity

Alpha diversity analysis revealed higher Shannon indices in the rhizosphere compared to endophytic communities (Figure 3a–d). In the rhizosphere, bacterial and fungal diversity peaked in GG and MG systems, respectively, while endophytic bacteria showed the highest diversity in FG. All between-system differences were significant ($p < 0.05$) except for those of endophytic fungi. PCA demonstrated distinct clustering patterns in bacterial and fungal communities across systems ($p = 0.001$). Principal coordinate analysis revealed distinct microbial community compositions between systems (Figure 3e,f). The bacterial communities showed clear separation (77.85% variation explained), with BG and GG assemblages clustering together but distinct from FG, while MG exhibited the greatest compositional divergence. Similarly, fungal community ordination (49.29% variation explained) demonstrated that GG assemblages were clearly differentiated from other systems, whereas FG and BG shared similar community structures, and MG occupied an intermediate position.

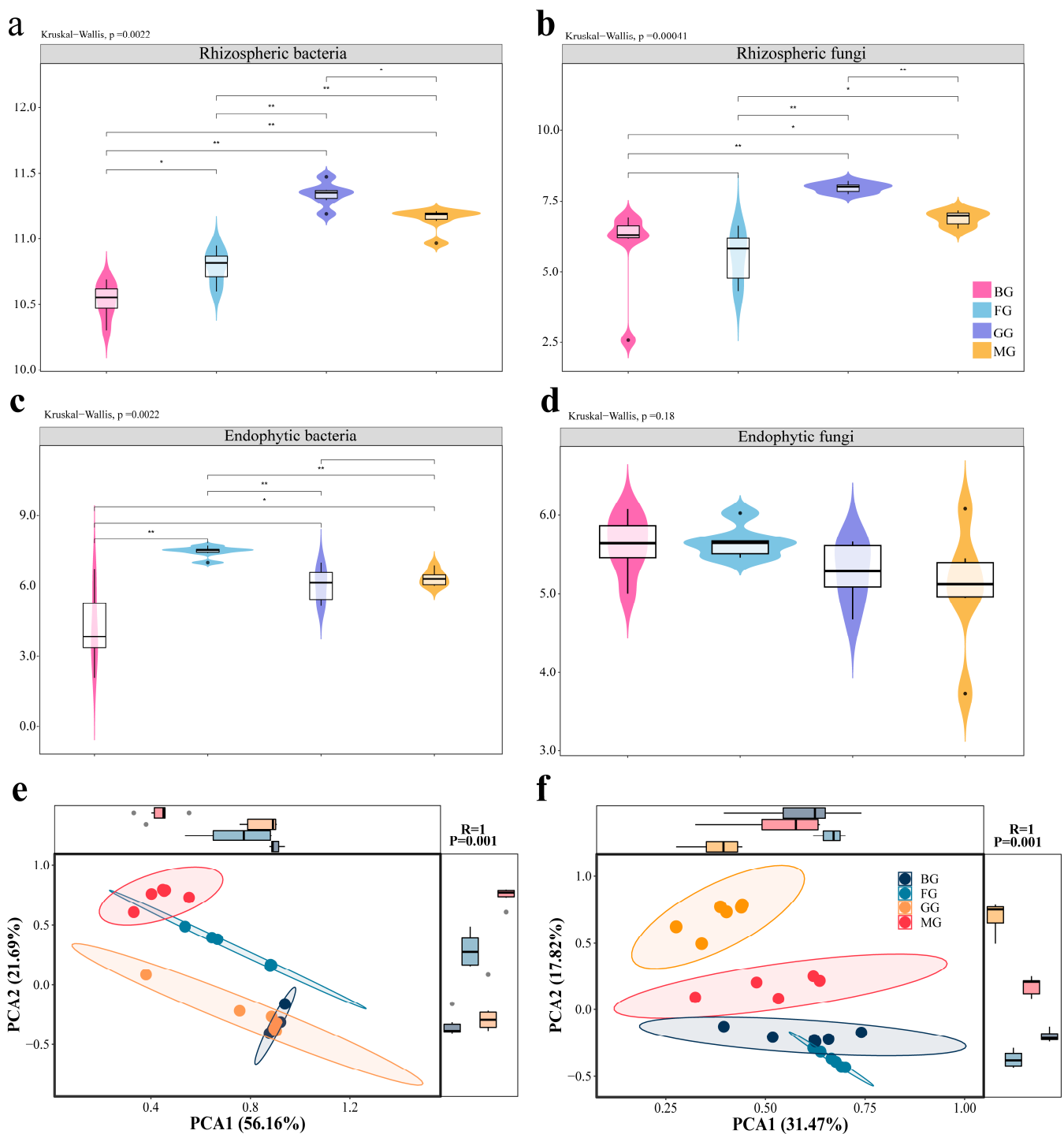


Figure 3. Microbial diversity analysis. (a–d) Alpha diversity (Shannon index) comparisons of rhizosphere bacteria (a), rhizosphere fungi (b), endophytic bacteria (c), and endophytic fungi (d) among the four intercropping systems. (e,f) Principal coordinates analysis plots depicting the beta diversity of bacterial (e) and fungal (f) communities in the rhizosphere and endosphere of giant lily. “*” shows at $p \leq 0.05$, “**” shows at $p \leq 0.01$.

3.2.2. Dominant Microbial Communities of Giant Lily in Different Intercropping Systems

Taxonomic profiling revealed compartment-specific microbial compositions (Figure 4). The rhizosphere bacterial community comprised three major phyla: Proteobacteria (25.45%), Planctomycetota (19.79%), and Acidobacteriota (16.37%), with *unclassified Gemmataceae* representing the most abundant genus (9.19%). Rhizosphere fungi were predominantly

Ascomycota (59.79%) and Basidiomycota (32.33%), with *Cryptococcus* (13.91%) and *unclassified Ascomycota* (7.28%) as the dominant genera. Endophytic bacterial communities showed strong dominance of Proteobacteria (68.62%) and Cyanobacteria (22.35%), with *unclassified Chloroplast sequences* (22.33%) and *Burkholderia-Caballeronia-Paraburkholderia* (13.59%) as the major genera. The endophytic fungal community was dominated by Ascomycota (68.40%) and Basidiomycota (30.51%), with four abundant genera: *Gibberella* (14.88%), *Ceratobasidium* (10.95%), *unclassified Xylariales* (10.55%), and *unclassified Ascomycota* (10.46%).

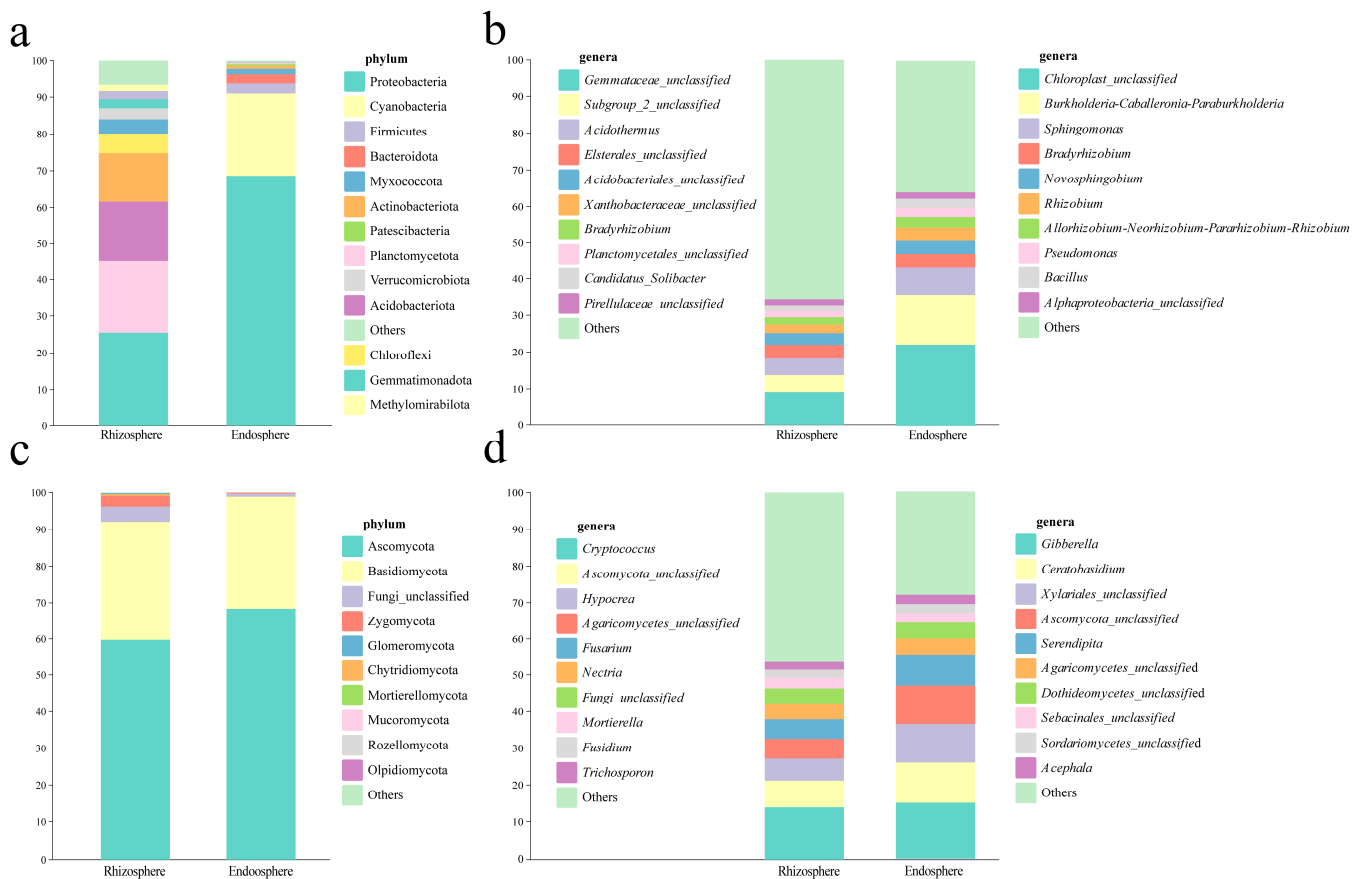


Figure 4. Microbiota of rhizosphere and endophytes. (a) Relative abundance of bacterial phyla in the rhizosphere and endosphere. (b) Relative abundance of bacterial genera in the rhizosphere and endosphere. (c) Relative abundance of fungal phyla in the rhizosphere and endosphere. (d) Relative abundance of fungal genera in the rhizosphere and endosphere.

3.2.3. Differences in the Microbiomes of Giant Lily in Different Intercropping Systems

Kruskal–Wallis testing identified system-specific differences in microbial abundance at the genus level ($p < 0.05$), with the endosphere containing 113 bacterial and 49 fungal genera and the rhizosphere harboring 148 bacterial and 79 fungal genera with differential abundance (Table S2). LEfSe analysis (LDA > 4.0) revealed distinct microbial signatures across systems and compartments (Figure 5). In the rhizosphere, bacterial communities showed system-specific enrichments. In the case of BG, these enrichments were in Acidobacteriota (Acidobacteriales) and Alphaproteobacteria (Elsterales, Rhizobiales with *Acidotherrmus*); for GG, these were Methylomirabilota (Rokubacteriales), Latescibacterota, and Nitrospirota (Nitrospiraceae); for MG, these were Planctomycetota (Gemmatales); and for FG, Actinobacteriota was enriched. Fungal communities were distinguished by the following: GG by Ascomycota (Nectriaceae: *Fusarium*, *Nectria*; Pleosporaceae: *Dendryphion*); FG by Basidiomycota (Tremellomycetes: *Cryptococcus*, *Trichosporon*); MG by Zygomycota (Mortierellaceae) and Nectriaceae (*Ilyonectria*); and BG by Ascomycota (Hypocreaceae, Microascaceae). Endosphere bacterial communities were characterized by the follow-

ing: MG by Burkholderiaceae (*Burkholderia*–*Caballeronia*–*Paraburkholderia*); GG by Sphingomonadaceae (*Sphingomonas*), Rhizobiaceae (*Rhizobium* complex), and Oxalobacteraceae (*Duganella*, *Herbaspirillum*); FG by Bacillaceae (*Bacillus*), Bradyrhizobiaceae, and Xanthobacteraceae; BG by Pseudomonadaceae and Chitinophagaceae. Fungal communities showed distinct patterns: GG showed a pattern of Cantharellales (*Ceratobasidium*); FG of Sebaciales (*Serendipita*) and Xylariales; MG of Sordariomycetes (*Gibberella*) and Dothideomycetes; BG of Helotiales (*Acephala*), Glomerellaceae, and unclassified Ascomycota.

3.3. Interactions Between Differential Microorganisms and Differential Metabolites

The Procrustes analysis reveals distinct patterns of microbial–metabolite relationships across root compartments and crop systems (Figure 6). In the rhizosphere, FG and GG systems consistently demonstrate stronger correlations between both bacterial and fungal communities and metabolites, as evidenced by shorter distances and more parallel configurations. Conversely, MG consistently shows the weakest coupling in the rhizosphere, with longer distances and more divergent angles, indicating greater dissimilarity between microbial and metabolite profiles. BG shows moderate coupling strength, with intermediate distances and varying angles between microbial and metabolite configurations. In the endosphere, the patterns of microbial–metabolite relationships are more variable across crop systems. BG exhibits the strongest correlation in bacterial communities, with shorter distances and more parallel configurations, while showing moderate coupling with fungal communities. GG maintains consistent coupling in both bacterial and fungal communities, as indicated by stable distances and parallel configurations across microbial types. However, FG shows a notable shift in the endosphere, with longer distances and more divergent angles for both bacterial and fungal analyses compared to its rhizosphere results. MG maintains weak correlations in the endosphere, particularly in fungal communities, suggesting a persistent trend of decoupling between microbes and metabolites. Across all comparisons, GG uniquely displays the most stable patterns, maintaining consistent distances and parallel configurations in both compartments and microbial types. In contrast, MG consistently shows weaker correlations in most comparisons, with longer distances and more divergent angles, indicating generally looser microbe–metabolite associations across both compartments and microbial types. FG demonstrates compartment-specific patterns, with stronger coupling in the rhizosphere but weaker associations in the endosphere. BG shows microbial type-specific patterns, with stronger bacterial–metabolite coupling compared to fungal–metabolite associations, particularly in the endosphere.

Rhizosphere bacteria–metabolite analysis revealed 3085 significant correlations (33.6% of 9176 pairs; $p < 0.05$, FDR-corrected) comprising 1190 positive (38.6%) and 1895 negative (61.4%) associations (Figure S2). Correlations spanned $\rho = 0.405$ – 0.923 (median = 0.584 , IQR = 0.488 – 0.692). Glucopyranoside conjugate showed the most correlations (140; 42 positive, 98 negative) and the highest average strength ($\rho = 0.752$), including 10 very strong positive correlations ($\rho > 0.900$) between *Pajaroellobacter*, on the one hand, and both *WPS-2_unclassified* (both $\rho = 0.923$) and *Elsterales_unclassified* ($\rho = 0.911$). Solasodine followed with 124 correlations (31 positive, 93 negative), while citric acid displayed a unique pattern (91 positive, 26 negative).

Among rhizosphere fungi–metabolite pairs (4898), 2608 (53.3%) showed significant correlations (1,603 positive, 1,005 negative), with an ρ ranging from 0.405 to 0.930 (median = 0.655 , IQR = 0.540 – 0.741) (Figure S3). Sordariomycetes–norpandamarilactonine A exhibited the strongest correlation ($\rho = 0.930$), with 12 correlations exceeding $\rho > 0.900$. 2-METHYL-3-HYDROXYETHYLENOPYRAN-4-ONE and β -D-Glucopyranoside each correlated with 52 fungi, while Clavulinaceae and Sordariomycetes showed 55 correlations each. Highest average correlations were observed for *Helicobasidium* ($\rho = 0.772$), *Platyglloeales* ($\rho = 0.767$), *Ngaione* (–) ($\rho = 0.702$), and norpandamarilactonine A ($\rho = 0.699$).

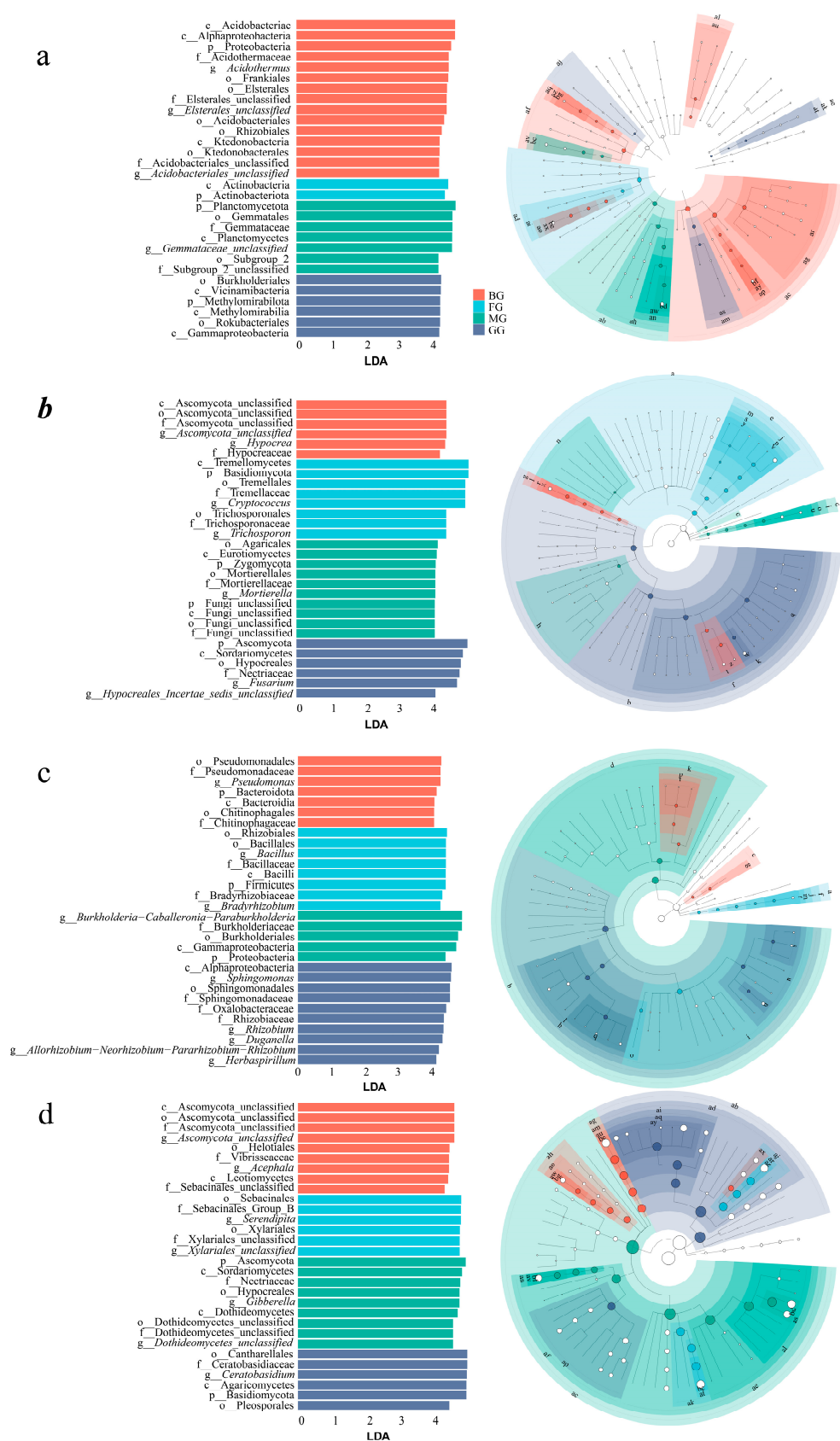


Figure 5. Linear discriminant analysis effect size (LEfSe) results of microbial groups in the rhizosphere and endophytic bacteria of different intercropping systems. (a) Rhizosphere bacteria; (b) rhizosphere fungi; (c) endophytic bacteria; (d) endophytic fungi.

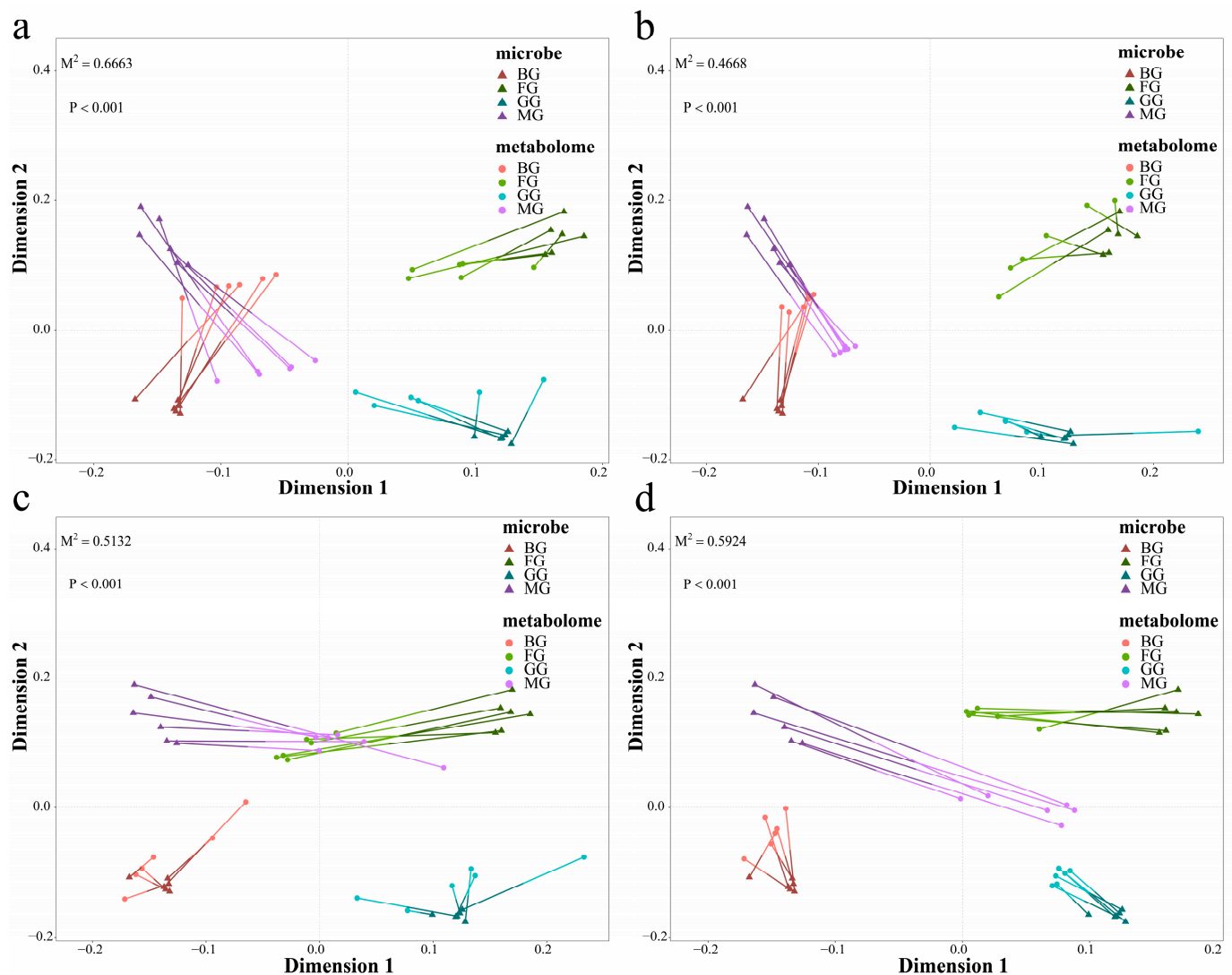


Figure 6. Procrustes analysis of microbial community composition and metabolic profiles in giant lily across intercropping systems. (a) Rhizosphere bacteria; (b) rhizosphere fungi; (c) endophytic bacteria; (d) endophytic fungi. The connecting line represents the vector residuals of the two sorted configurations, which can be used to evaluate the variation between the two. A shorter connecting line indicates a higher level of consistency between the two datasets.

Endophytic bacteria–metabolite analysis yielded 3043 significant correlations (43.4% of 7006 pairs; 1427 positive, 1616 negative) spanning $\rho = 0.405\text{--}0.947$ (median = 0.630, IQR = 0.523–0.730) (Figure S4). Eleven very strong negative correlations emerged, with *Bacillus*–norpandamarilactonine A strongest ($\rho = -0.947$). Pyridoxine and L-tyrosine showed the most correlations (82 and 81 bacteria; 56/26 and 27/54 positive/negative ratios), while *Bacillus* and *Klebsiella* each correlated with 58 metabolites. The highest average correlations were observed for *Sandaracinaceae* and *Niveibacterium* (both $\rho = 0.702$), Methyl-13-hydroperoxy- $\Delta^9E,11E$ -octadecadienoate ($\rho = 0.692$), and L-tyrosine ($\rho = 0.689$).

Among 3038 endophytic fungi–metabolite pairs, 1593 (52.4%) correlated significantly (794 positive, 799 negative), spanning $\rho = 0.405\text{--}0.969$ (median = 0.642, IQR = 0.540–0.734) (Figure S5). Sixteen correlations exceeded $\rho > 0.900$ (14 negative), with *Ceratobasidiaceae*–methylgingerol the strongest ($\rho = -0.969$). β -D-Glucopyranoside derivative and Ngaione (-) each correlated with 32 fungi (9/23 and 8/24 positive/negative ratios), while *Campylospora* and *Chaetothyriales* showed the highest correlation frequencies (57 each; 24/33 and 23/34 positive/negative ratios). *Pezizaceae* demonstrated the highest average corre-

lation strength ($\rho = 0.756$), followed by Solasodine ($\rho = 0.729$), citric acid, anhydrous ($\rho = 0.725$), and Dendryphon ($\rho = 0.718$).

To further investigate the organization and potential interactions among the strongly correlated microbial taxa and metabolites, we conducted a network analysis focusing on the 52 strong associations ($|\rho| > 0.90$) (Figure 7a). The resulting scale-free network consisted of 56 nodes comprising 25 metabolites, 20 rhizosphere microbes, and 11 endosphere microbes. The network exhibited a clear compartment-specific organization, with distinct correlation patterns and modularity. The rhizosphere sub-network was characterized by a predominance of positive correlations (76%, 19/25 edges) centering around specialized glycosides. In particular, 4-(2,6,6-trimethyl-4-oxo-2-cyclohexen-1-yl)-2-butanyl beta-D-glucopyranoside emerged as the primary hub, with a degree of 11. Conversely, the endosphere sub-network displayed a majority of negative correlations (81.5%, 22/27 edges). Betweenness centrality analysis identified norpandamarilactonine A and endophytic *Ceratobasidiaceae* as key bridging components potentially playing crucial roles in mediating interactions between the rhizosphere and endosphere communities. The network exhibited significant modularity (11 communities, modularity = 0.68) and compartment-specific correlation patterns ($\chi^2 = 24.56$, $p < 0.001$), suggesting a highly organized and specialized structure of microbial–metabolite interactions within the medicinal plant.

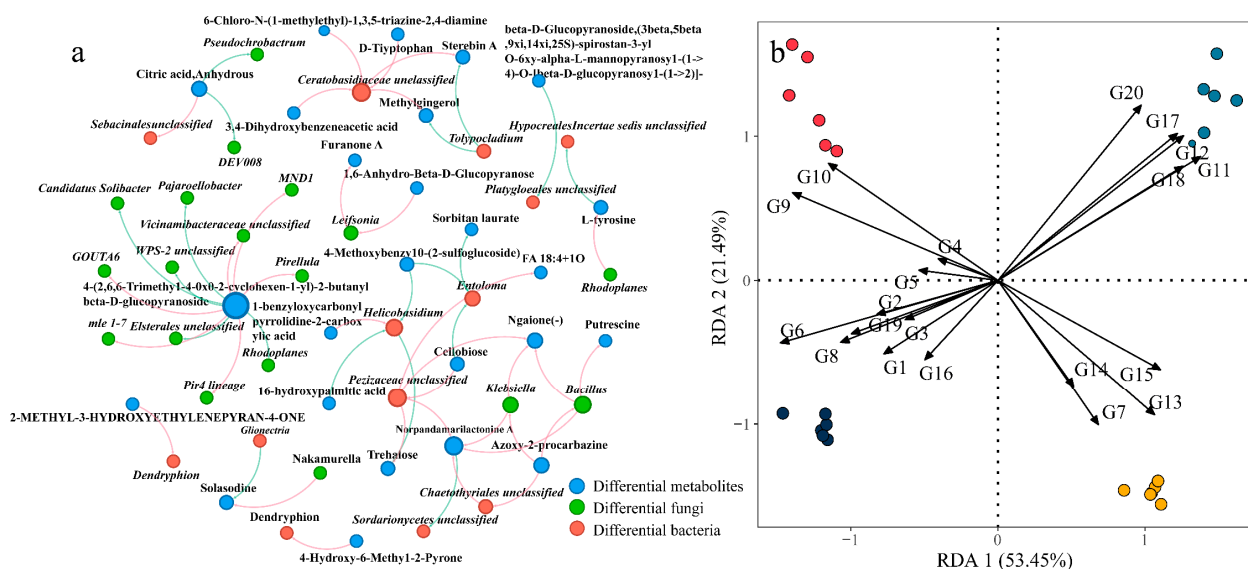


Figure 7. Interactions between differentially abundant microorganisms and differentially abundant metabolites. (a) Co-expression network of differentially abundant microorganisms and differentially abundant metabolites ($|\rho| > 0.90$); (b) Redundancy analysis results of typical differentially abundant microorganisms and differentially abundant metabolites.

Based on the network analysis, we selected 20 key microbial taxa for further investigation using RDA analysis (Table S3). These taxa included five each from rhizosphere bacteria (e.g., *Candidatus Solibacter*, *Rhodoplanes*), rhizosphere fungi (e.g., *Sordariomycetes*, *Helicobasidium*), endosphere bacteria (e.g., *Bacillus*, *Klebsiella*), and endosphere fungi (e.g., *Ceratobasidiaceae*, *Tolypocladium*). RDA analysis revealed that these 20 microorganisms collectively explained 74.94% of the total variance in metabolite profiles across the investigated systems (Figure 7b). Intriguingly, system-specific associations emerged, indicating distinct microbial assemblages and their potential functional roles under different conditions. The BG system was characterized by the prevalence of soil-dwelling *Sordariomycetes* and endophytic *Sebacinales*, while the FG system was dominated by endophytic *Bacillus* and *Klebsiella*. The GG system exhibited strong associations with soil Proteobacteria, particularly *Pseudochrobactrum* and *Rhodoplanes*. In contrast, the MG system was primarily linked to specialized Basidiomycetes such as *Helicobasidium* and *Entoloma*. Fitting analysis confirmed

significant correlations between 16 of the 20 differential microorganisms and the observed metabolite profiles, underlining the potential importance of these microbial taxa in shaping the metabolome of the medicinal plant under different system conditions.

3.4. Functional Analysis of Microorganisms and Secondary Metabolites

Functional analysis revealed compartment-specific metabolic pathway associations between differential microorganisms and metabolites. While fungal communities in both compartments showed no significant pathway overlap with metabolites, rhizosphere bacteria shared 19 significant metabolic pathways, and endophytic bacteria shared 21 pathways, with metabolites (Table S4). Both compartments exhibited common pathways involved in fundamental metabolic processes, including ABC transporters, carbon metabolism (citrate cycle, fructose and mannose metabolism, galactose metabolism, starch and sucrose metabolism), and amino acid metabolism (alanine, aspartate and glutamate metabolism, beta-alanine metabolism, histidine metabolism, phenylalanine metabolism, tyrosine metabolism). The endophytic bacteria uniquely displayed bacterial chemotaxis and phosphotransferase system (PTS) pathways, suggesting enhanced cellular communication and substrate transport in the endosphere compartment, while sharing other basic metabolic processes, such as aminoacyl-tRNA biosynthesis, cyanoamino acid metabolism, glyoxylate and dicarboxylate metabolism, and methane metabolism, with the rhizosphere bacteria.

4. Discussion

4.1. Effects of Intercropping Systems on the Secondary Metabolites of Giant Lily

The practice of intercropping has been demonstrated to exert a pronounced influence on the metabolic profiles and bioactive compounds of medicinal plants [23]. The findings of this study demonstrate that the metabolic responses of giant lily in different intercropping systems exhibit notable distinctions.

The BG system triggered specific terpenoid biosynthesis in the giant lily bulbs, with dramatic increases in yucalexin B22 (FC = 19.39) and kaurenic acid (FC = 14.13) levels. The increased accumulation of terpenoids under intercropping conditions may be a defense response to biotic and abiotic stresses [24]. Moreover, the allocation of resources for terpenoid biosynthesis in the giant lily may represent an adaptive trade-off between growth and defense, as observed in other plant species under resource-limited conditions [25]. The BG system exhibited a significant increase in alkaloid content, with anabasine and lupinine showing fold changes of 2.97 and 3.83, respectively, and enhanced steroid metabolism, as evidenced by the accumulation of O-acetylilariciresinol (FC = 4.49) and 7a,12a-dihydroxycholest-3-en-26-one (FC = 2.89) compared to the GG system. This particular accumulation is ascribed to considerable enhancement of the MVA-mediated terpenoid backbone biosynthesis and steroid biosynthesis pathways [26]. Research on *Fritillaria hupehensis* shows that intercropping significantly increases steroidal alkaloid content, primarily through the upregulation of key genes in alkaloid biosynthetic pathways and through enhanced energy metabolism via oxidative phosphorylation [27]. These findings suggest that increased steroid metabolism in the giant lily may contribute to its adaptation to the specific ecological challenges posed by bamboo forests.

The MG system elicited a complex stress response, as indicated by the substantial accumulation of aflatoxin G2 (FC = 128.62) and compatible solutes such as trehalose (FC = 6.32) and melibiose (FC = 4.29). The enhanced accumulation of trehalose in giant lily under the MG system is consistent with observations for *P. notoginseng* intercropped with *Taxus chinensis*, where significant upregulation of trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase was observed, leading to increased trehalose content and improved stress tolerance [10]. These findings suggest that metabolic adaptations in giant lily under the MG system are driven by the integrated effects of light and nutrient stress mediated by the coordinated regulation of carbohydrate and secondary metabolite pathways [28].

The FG system induced a marked upregulation of nitrogen metabolism in giant lily, as evidenced by a 2.47-fold increase in putrescine levels and significant enrichment of

the alanine, aspartate, and glutamate metabolism pathways. This metabolic response is likely driven by elevated soil nitrogen availability under the coniferous canopy, similar to how intercropping of peanuts with maize significantly increases the activities of nitrogen metabolism-related enzymes and the content of different amino acids in peanut roots compared to monocropped controls [29]. Polyamines such as putrescine interact with various metabolic pathways and influence plant growth and stress responses. For instance, Shi et al. [30] found that the manipulation of arginase expression, which is involved in putrescine biosynthesis, modulates ROS accumulation and abiotic stress tolerance in *Arabidopsis*. The coordinated upregulation of nitrogen metabolism and putrescine accumulation in giant lily highlights the intricate regulatory networks governing nitrogen utilization efficiency and stress tolerance in medicinal plants under nitrogen-rich conditions [31].

The GG system exhibited a distinct metabolic profile characterized by the highest levels of lipid-related compounds and octanol. As fundamental components of the plasma membrane, lipids play a critical role in facilitating plant adaptation to abiotic stresses, and studies have shown that these elevated levels may be due to competition for water and nutrients in the intercropping environment [32]. The elevated octanol content may enhance plant uptake and transport processes through its high octanol-water partition coefficient, allowing binding to non-specific lipid transfer proteins and accumulation in membrane lipids, thereby facilitating transport and storage of lipophilic compounds as part of stress response mechanisms in competitive environments [33].

KEGG pathway enrichment analysis revealed that ABC transporters were the most significantly enriched pathways in giant lily under different intercropping systems. ABC transporters play crucial roles in the transport of secondary metabolites across membranes and contribute to the accumulation and distribution of these compounds in plants [34]. The biosynthesis of plant secondary metabolites was also significantly enriched in giant lily. Duan et al. [27] observed a notable enhancement in the flavonoid and terpene biosynthetic pathways in *Fritillaria hupehensis* as a consequence of intercropping with *Magnolia officinalis*. The upregulation of these pathways was associated with an increased accumulation of bioactive compounds, such as salvianolic acids and tanshinones, in intercropped *S. mil-tiorrhiza*. Furthermore, the enrichment of central carbon metabolism pathways, such as the TCA cycle and carbon fixation, highlights the importance of primary metabolism in supporting the biosynthesis of secondary metabolites in giant lily under intercropping systems. In a study on the effect of intercropping on the metabolic profile of *P. notoginseng*, Wang et al. [10] observed significant upregulation of citrate synthase and malate dehydrogenase, key enzymes in the TCA cycle, in the roots of *P. notoginseng* intercropped with *Taxus chinensis*. This upregulation was positively correlated with increased accumulation of ginsenosides, suggesting that enhanced central carbon metabolism supports the biosynthesis of these bioactive compounds.

4.2. Differences in Diversity and Composition of the Microbial Community in Giant Lily

The composition and diversity of microbial communities associated with giant lily varied significantly among the different intercropping systems, consistent with previous research that revealed the significant influences of intercropping combinations on rhizosphere fungal communities [35]. A comparison of microbial diversity between compartments consistently showed higher Shannon indices in the rhizosphere than in endophytic communities, consistent with the findings of Chen et al. [36] in the medicinal plant *Rheum palmatum*. They attributed the higher rhizosphere diversity to abundant nutrients and complex ecological interactions in the rhizosphere niche, supporting a more diverse microbial community than the selective endophytic compartment.

The PCA demonstrated that bacterial communities exhibited stronger responses to intercropping types than fungal communities, mirroring previous research findings [37]. In the giant lily rhizosphere, bacterial diversity was highest in the GG system, and fungal diversity in the rhizosphere was highest in the MG system, which may be related to soil properties and enzyme activity [38]. Proteobacteria, Planctomycetes, and Acidobacteria

were the dominant bacterial phyla in the giant lily rhizosphere. Additionally, Zhu et al. [39] discovered that Proteobacteria is the most abundant phylum in Rhizobium, and several genera promote plant growth and are positively correlated with bioactive compound content. The substantial presence of Planctomycetes and Acidobacteria suggests their potential roles in nutrient cycling and organic matter decomposition, which may influence carbon and nitrogen levels in the intercropping system [40]. Further evidence comes from Delgado-Baquerizo et al. [41], who demonstrated positive correlations between these phyla and soil organic carbon and total nitrogen contents in their global study of soil microbiomes.

The endophytic bacterial community exhibited a distinct taxonomic structure, with Proteobacteria and Cyanobacteria being the dominant phyla. In many plants, the most abundant phylum was Proteobacteria [42]. Previous studies have demonstrated that cyanobacteria can facilitate plant photosynthesis and the conversion of atmospheric nitrogen dioxide into nitrogen compounds that plants can absorb and utilize [43]. Fungal communities displayed distinct compositional patterns between compartments, with Ascomycetes and Basidiomycetes dominating both the rhizosphere and the endosphere, aligning with previous studies [44]. The marked differences in bacterial community composition between the rhizosphere and endosphere compartments indicate strong host selection processes, as demonstrated by You et al. [45] in their study on *Schisandra sphenanthera*, where endospheric communities showed reduced diversity and distinct compositional profiles. In contrast, the relative consistency in fungal community proportions between compartments suggests lower host specificity, which may be due to the differential response of microorganisms to environmental factors [46]. These contrasting patterns in bacterial and fungal host specificity align with broader ecological observations; Li and Wu documented greater bacterial community differentiation between intercropping systems than between fungal communities [35]. This pattern was further substantiated by Song et al. [47], who attributed differential responses to the selective influence of cultivation practices on bacterial communities and the broader environmental adaptability of fungi.

The LEfSe analysis revealed distinct microbial enrichment patterns across different intercropping systems in both the rhizosphere and endosphere compartments of giant lily. In the rhizosphere, the BG system resulted in the enrichment of Acidobacteria and Alphaproteobacteria. Previous studies have demonstrated that Acidobacteria are involved in the degradation of complex organic compounds and nutrient cycling in the soil [48], whereas Alphaproteobacteria includes many taxa capable of nitrogen fixation, phosphate solubilization, and plant growth regulator production [49]. The FG system rhizosphere was enriched with Chloroflexi and Actinobacteria. Research has shown that Chloroflexi participate in organic matter degradation and carbon cycling in soil [50], and Actinobacteria are known to produce various bioactive compounds, including antibiotics and plant growth regulators [51]. In the MG system, Planctomycetes and Verrucomicrobia were enriched in the rhizosphere. Studies have found that Planctomycetes are involved in ammonia oxidation and nitrogen cycling [52], while Verrucomicrobia participate in polysaccharide degradation and carbon cycling [53]. Rhizosphere fungal communities also showed system-specific enrichment patterns. The BG system resulted in the enrichment of Ascomycetes and Mortierellomycetes. Previous research has shown that while Ascomycetes includes both pathogenic and beneficial fungi, many members can promote plant growth and suppress soil-borne pathogens [54], and Mortierellomycetes are involved in organic matter degradation and nutrient cycling [55]. The FG system resulted in the enrichment of Basidiomycetes and Glomeromycetes. Studies have demonstrated that Basidiomycetes includes many ectomycorrhizal fungi that can form symbiotic associations with plants [56], whereas Glomeromycetes forms arbuscular mycorrhizal associations that can enhance nutrient acquisition and stress tolerance [57].

In the endophytic compartment, the BG system showed enrichment of Firmicutes and Deltaproteobacteria. Studies on *Phlomis rotata* have found that abundance of these endophytic bacterial systems correlates with flavonoid biosynthesis content [58]. The FG system resulted in the enrichment of Gammaproteobacteria and Bacteroidetes. Previous

research has indicated that an expansion in the population of these bacterial systems is conducive to enhancing nutrient uptake by plants [59]. The MG system showed enrichment of Actinobacteria and Planctomycetes, whereas the GG system showed enrichment of Chloroflexi and Verrucomicrobia. Endophytic fungal communities also exhibited system-specific patterns. The BG system enriches Ascomycota and Basidiomycota, and previous studies have shown that these fungal systems can effectively produce exopolysaccharides with multiple biological activities [60]. The FG system resulted in the enrichment of Mucoromycetes and Mortierellomycetes. Research on *Atractylodes macrocephala* has found negative correlations between these fungal systems and disease incidence and positive correlations with plant biomass [61].

4.3. Potential Mechanism of Interaction Between Giant Lily Microbes and Metabolites

The plant-associated microbiome plays a crucial role in modulating medicinal plant metabolism through various mechanisms such as nutrient acquisition, gene expression regulation, and the production of signaling molecules [62]. For instance, the endophytic fungus *Cladosporium tenuissimum* DF11 induces the accumulation of tanshinones in *S. miltiorrhiza* by upregulating key biosynthetic genes [63], while the rhizosphere bacterium *Pseudomonas brassicacearum* subsp. *neoaureantiaca* enhances tanshinone production in *S. miltiorrhiza* hairy roots by increasing the expression of biosynthetic genes and the activities of key enzymes [64].

Procrustes analysis revealed distinct patterns of microbial–metabolite correlations in the rhizosphere and endosphere of giant lily. In the rhizosphere, the FG and GG systems showed stronger correlations between the bacterial and fungal communities and metabolites. Liu et al. [65] reported that intercropping *B. chinense* with maize increased the diversity and abundance of beneficial rhizosphere bacteria, such as *Bacillus* and *Pseudomonas*, which positively correlated with saikosaponin accumulation. In contrast, the MG system had the weakest correlations, possibly due to increased competition for resources or changes in soil properties that are less favorable for specific microbial–metabolite interactions [66]. Glucopyranoside conjugates, solasodine, and citric acid emerged as key metabolites in the rhizosphere bacterial–metabolite associations. Kudjordjie et al. [67] demonstrated that maize-synthesized benzoxazinoids affect rhizosphere microbiome assembly and composition. Hou et al. [68] found that inoculation of *Gentiana* plants with endophytic fungi led to significant changes in the root metabolome, including alterations in secoiridoid and iridoid glycoside levels. Duan et al. [7] reported that intercropping sugarcane with *Dictyophora indusiata* increases the accumulation of lysoPC 18:1 and dihydrobenzofuran in the sugarcane root system, thereby promoting sugarcane growth by activating flavonoid metabolism. These studies demonstrate the complex interplay between plant species, microbial communities, and environmental factors in shaping rhizosphere microbial–metabolite associations.

The endosphere microbial–metabolite associations exhibit distinct patterns compared to the rhizosphere, suggesting that the endosphere represents a unique niche for microbial–metabolite interactions with potentially different driving factors and functional implications [69]. Mastan et al. [70] found that the endophytic fungi *Fusarium redolens*, *Phialemoniopsis cornearis*, and *Macrophomina pseudophaseolina* increased the forskolin content in *Coleus forskohlii* by modulating key biosynthetic genes. Further evidence of endophyte–metabolite relationships was demonstrated in *Ephedra sinica*, where distinct endophytic communities between wild and cultivated plants were significantly correlated with the differential production of bioactive compounds, including ephedrine, pseudoephedrine, and flavonoids such as kaempferol and rhamnetin [71]. These findings underscore the potential of endophytic microorganisms to produce unique bioactive compounds that influence plant metabolism.

Network analysis revealed compartment-specific patterns in the organization and modularity of microbial–metabolite interactions. The rhizosphere subnetwork showed predominantly positive correlations centered around specialized glycosides as primary hubs, which is consistent with previous findings [72]. In contrast, the endosphere sub-network

was dominated by negative correlations, with norpandamarilactonine A endophytic Cera-tobasidiaceae as key bridging components potentially mediating rhizosphere–endosphere interactions, similar to observations by Ren et al. [73] in *Scrophularia ningpoensis*. These findings provide insights into the factors that influence medicinal plant quality and bioactivity, suggesting potential synergistic effects on the rhizosphere and competitive interactions or the ability of endophytic microbes to modulate plant gene expression in the endosphere [68].

RDA and fitting analysis revealed the significant influence of specific microbes on the giant lily metabolome and the potential for targeted microbial intervention. The differential metabolites in the BG system were more closely associated with Sordariomycetes and Sebaciniales, which are known for their diverse secondary metabolite production and roles in plant nutrient acquisition and stress tolerance [45]. The unique metabolic profile of the FG system was strongly correlated with the presence of *Bacillus* and *Klebsiella*, microorganisms recognized for their plant growth-promoting effects and roles in nitrogen fixation and phosphate solubilization [38]. In the GG system, the differential metabolites exhibited a close relationship with Proteobacteria, particularly *Pseudochrobactrum* and *Rhodoplanes*, which are known for their metabolic versatility and roles in organic matter decomposition and nutrient cycling [36]. However, functional redundancy among microbial taxa should also be considered because some microorganisms may share similar traits or occupy similar niches [74].

Functional analyses revealed compartment-specific metabolic pathway associations between different microorganisms and metabolites in giant lily, with bacterial communities exhibiting a closer functional link to plant metabolism than fungal communities [7]. Both compartments exhibited common pathways involved in fundamental metabolic processes, suggesting a functional overlap and complementarity between rhizosphere and endosphere bacteria [75]. Endophytic bacteria display unique chemotaxis and PTS pathways, reflecting their adaptation to the endospheric microenvironment [46]. Significant associations between bacterial communities and pathways related to central carbon metabolism, amino acid biosynthesis, and secondary metabolite production suggest that they play important roles in modulating primary and specialized plant metabolism [46]. The lack of significant pathway associations in fungal communities may reflect a more generalist or complementary role for fungi in medicinal plant microbiomes [76].

5. Conclusions

This study revealed the significant influence of intercropping systems on the metabolic and microbial profiles of giant lily in an agroforestry context. The metabolomic analysis identified 62 differentially abundant metabolites shared among the four intercropping systems. The BG system notably increased the accumulation of bioactive compounds such as terpenoids, alkaloids, and steroids. The MG system induced a complex stress response characterized by the accumulation of stress-related metabolites and the upregulation of carbohydrate metabolism. The FG system enhanced nitrogen metabolism, as evidenced by the increased accumulation of compounds such as putrescine and the enrichment of alanine, aspartate, and glutamate metabolic pathways. The GG system contained the highest levels of lipid-related compounds and octanol. These metabolic changes were accompanied by compartment-specific shifts in bacterial and fungal communities. In the rhizosphere, bacterial diversity peaked in the GG system, whereas fungal diversity peaked in the MG system. The BG system enriched beneficial bacteria, whereas the FG system promoted symbiotic fungal growth in the endosphere. Network analysis revealed strong correlations between specific microbial taxa and key metabolites, suggesting their potential roles in shaping the metabolic profiles of giant lily. Furthermore, our results suggest that intercropping with bamboo could be a promising strategy for enhancing the production of medicinally important compounds, such as terpenoids, alkaloids, and steroids, in giant lily. Our findings highlight the need for future research to elucidate the functional roles of the key microbial taxa identified in this study through isolation, culture, and inoculation experiments. This will be crucial for developing effective microbial inoculant formulations

that can be used to modulate the giant lily microbiome and enhance bioactive compound production in agricultural settings.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f15122201/s1>, Table S1: Differentially abundant metabolites identified in pairwise comparisons among the four intercropping systems (BG, FG, MG, and GG). Table S2: Differentially abundant microbial genera in the rhizosphere and endosphere of giant lily under different intercropping systems. Table S3: Microbial taxa selected for redundancy analysis (RDA) based on their contributions to the overall metabolite profile differences among intercropping systems. Table S4: Significantly enriched metabolic pathways shared between differentially abundant microorganisms and metabolites in the rhizosphere and endosphere of giant lily. Figure S1: Map indicating the location of the study site. Figure S2: Correlation analysis of rhizosphere differential bacterial genera with differential metabolites. Figure S3: Correlation analysis of rhizosphere differential fungal genera with differential metabolites. Figure S4: Correlation analysis of endophytic differential bacteria genera with differential metabolites. Figure S5: Correlation analysis of endophytic differential fungal genera with differential metabolites.

Author Contributions: Conceptualization, J.Z. and X.Z.; methodology, J.Z. and Y.N.; software, H.W.; validation, J.Z., Y.N. and X.Z.; formal analysis, H.W. and X.Z.; investigation, J.Z., Y.N. and G.G.; resources, Y.P. and Z.H.; data curation, J.Z. and Y.N.; writing—original draft preparation, J.Z.; writing—review and editing, J.Z., X.Z., Y.N., H.W., G.G., Y.P. and Z.H.; visualization, H.W. and X.Z.; supervision, X.Z. and Z.W.; project administration, X.Z. and Z.W.; funding acquisition, X.Z. and Z.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Fundamental Research Funds of CAF: CAFYBB2022XE002 and by the People’s Government of Zhejiang Province–Chinese Academy of Forestry cooperative project: 2023B04.

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials; further inquiries can be directed to the corresponding author.

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

References

1. Trivedi, P.; Leach, J.E.; Tringe, S.G.; Sa, T.; Singh, B.K. Plant–Microbiome Interactions: From Community Assembly to Plant Health. *Nat. Rev. Microbiol.* **2020**, *18*, 607–621. [\[CrossRef\]](#)
2. Zhou, J.; Tang, C.; Kuzyakov, Y.; Vancov, T.; Fang, Y.; Song, X.; Zhou, X.; Jiang, Z.; Ge, T.; Xu, L.; et al. Biochar-Based Urea Increases Soil Methane Uptake in a Subtropical Forest. *Geoderma* **2024**, *449*, 116994. [\[CrossRef\]](#)
3. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; Van Der Putten, W.H. Going Back to the Roots: The Microbial Ecology of the Rhizosphere. *Nat. Rev. Microbiol.* **2013**, *11*, 789–799. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Compant, S.; Samad, A.; Faist, H.; Sessitsch, A. A Review on the Plant Microbiome: Ecology, Functions, and Emerging Trends in Microbial Application. *J. Adv. Res.* **2019**, *19*, 29–37. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Egamberdieva, D.; Wirth, S.J.; Alqarawi, A.A.; Abd_Allah, E.F.; Hashem, A. Phytohormones and Beneficial Microbes: Essential Components for Plants to Balance Stress and Fitness. *Front. Microbiol.* **2017**, *8*, 2104. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Pagnani, G.; Pellegrini, M.; Galieni, A.; D’Egidio, S.; Matteucci, F.; Ricci, A.; Stagnari, F.; Sergi, M.; Lo Sterzo, C.; Pisante, M.; et al. Plant Growth-Promoting Rhizobacteria (PGPR) in Cannabis Sativa ‘Finola’ Cultivation: An Alternative Fertilization Strategy to Improve Plant Growth and Quality Characteristics. *Ind. Crop. Prod.* **2018**, *123*, 75–83. [\[CrossRef\]](#)
7. Duan, M.; Li, X.; Wu, X.; Long, S.; Huang, H.; Li, Y.; Liu, Q.-H.; Zhu, G.; Feng, B.; Qin, S.; et al. Dictyophora Indusiata and Bacillus Aryabhattai Improve Sugarcane Yield by Endogenously Associating with the Root and Regulating Flavonoid Metabolism. *Front. Plant Sci.* **2024**, *15*, 1326917. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Huang, A.C.; Jiang, T.; Liu, Y.-X.; Bai, Y.-C.; Reed, J.; Qu, B.; Goossens, A.; Nützmann, H.-W.; Bai, Y.; Osbourn, A. A Specialized Metabolic Network Selectively Modulates *Arabidopsis* Root Microbiota. *Science* **2019**, *364*, eaau6389. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Shu, D.; Banerjee, S.; Mao, X.; Zhang, J.; Cui, W.; Zhang, W.; Zhang, B.; Chen, S.; Jiao, S.; Wei, G. Conversion of Monocropping to Intercropping Promotes Rhizosphere Microbiome Functionality and Soil Nitrogen Cycling. *Sci. Total Environ.* **2024**, *949*, 174953. [\[CrossRef\]](#)
10. Wang, C.; Mao, G.; Li, Y.; Zi, W.; Wang, Q.; Huang, H.; Yang, M.; Du, F.; Mei, X.; Deng, W.; et al. Tree Root-Mediated Soil Metabolome in Agroforestry Enhancing the Growth and Quality of *Panax notoginseng*. *Plant Soil.* **2024**. [\[CrossRef\]](#)
11. Duan, W.; Chen, X.; Ding, Y.; Mao, X.; Song, Z.; Bao, J.; Fang, L.; Guo, L.; Zhou, J. Intricate Microbe-plant-metabolic Remodeling Mediated by Intercropping Enhances the Quality of *Panax quinquefolius* L. *Physiol. Plant.* **2024**, *176*, e14499. [\[CrossRef\]](#)

12. Wang, S.; Zhang, X.; Li, X.; Shen, J.; Sun, L.; Zaman, S.; Wang, Y.; Ding, Z. Different Changes of Bacterial Diversity and Soil Metabolites in Tea Plants-Legume Intercropping Systems. *Front. Plant Sci.* **2023**, *14*, 1110623. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Li, Y.-F.; Song, J.; Guan, W.-L.; Li, F.-R. Seed Dormancy and Germination in *Cardiocrinum Giganteum* Var. *Yunnanense*, a Perennial Herb in China with Post-Dispersal Embryo Growth. *Seed Sci. Technol.* **2020**, *48*, 303–314. [\[CrossRef\]](#)
14. Zhang, R.-R.; Lin, Z.-X.; Lu, X.-Y.; Xia, X.; Jiang, R.-W.; Chen, Q.-B. CGY-1, a Biflavonoid Isolated from *Cardiocrinum Giganteum* Seeds, Improves Memory Deficits by Modulating the Cholinergic System in Scopolamine-Treated Mice. *Biomed. Pharmacother.* **2019**, *111*, 496–502. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Shou, J.-W.; Zhang, R.-R.; Wu, H.-Y.; Xia, X.; Nie, H.; Jiang, R.-W.; Shaw, P.-C. Isolation of Novel Biflavonoids from *Cardiocrinum Giganteum* Seeds and Characterization of Their Antitussive Activities. *J. Ethnopharmacol.* **2018**, *222*, 171–176. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Walker, T.W.N.; Alexander, J.M.; Allard, P.; Baines, O.; Baldy, V.; Bardgett, R.D.; Capdevila, P.; Coley, P.D.; David, B.; Defosse, E.; et al. Functional Traits 2.0: The Power of the Metabolome for Ecology. *J. Ecol.* **2022**, *110*, 4–20. [\[CrossRef\]](#)
17. Jansson, J.K.; Hofmockel, K.S. Soil Microbiomes and Climate Change. *Nat. Rev. Microbiol.* **2020**, *18*, 35–46. [\[CrossRef\]](#) [\[PubMed\]](#)
18. Logue, J.B.; Stedmon, C.A.; Kellerman, A.M.; Nielsen, N.J.; Andersson, A.F.; Laudon, H.; Lindström, E.S.; Kritzberg, E.S. Experimental Insights into the Importance of Aquatic Bacterial Community Composition to the Degradation of Dissolved Organic Matter. *ISME J.* **2016**, *10*, 533–545. [\[CrossRef\]](#)
19. Walters, W.; Hyde, E.R.; Berg-Lyons, D.; Ackermann, G.; Humphrey, G.; Parada, A.; Gilbert, J.A.; Jansson, J.K.; Caporaso, J.G.; Fuhrman, J.A.; et al. Improved Bacterial 16S rRNA Gene (V4 and V4-5) and Fungal Internal Transcribed Spacer Marker Gene Primers for Microbial Community Surveys. *mSystems* **2016**, *1*, e00009-15. [\[CrossRef\]](#) [\[PubMed\]](#)
20. Karlsson, L.; Friberg, H.; Steinberg, C.; Persson, P. Fungicide Effects on Fungal Community Composition in the Wheat Phyllosphere. *PLoS ONE* **2014**, *9*, e111786. [\[CrossRef\]](#)
21. Liu, L.; Chen, H.; Liu, M.; Yang, J.R.; Xiao, P.; Wilkinson, D.M.; Yang, J. Response of the Eukaryotic Plankton Community to the Cyanobacterial Biomass Cycle over 6 Years in Two Subtropical Reservoirs. *ISME J.* **2019**, *13*, 2196–2208. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Bastian, M.; Heymann, S.; Jacomy, M. Gephi: An Open Source Software for Exploring and Manipulating Networks. *Proc. Int. AAAI Conf. Web Soc. Media* **2009**, *3*, 361–362. [\[CrossRef\]](#)
23. Kutos, S.; Bennett, R.E.; Rao, M.V.; Fleischer, R.C.; Rice, R.A.; Muletz-Wolz, C.R. Farm Management and Shade Tree Species Influence Coffee Soil Microbiomes in Central and South America. *Appl. Soil Ecol.* **2024**, *202*, 105571. [\[CrossRef\]](#)
24. Mahajan, M.; Kuiry, R.; Pal, P.K. Understanding the Consequence of Environmental Stress for Accumulation of Secondary Metabolites in Medicinal and Aromatic Plants. *J. Appl. Res. Med. Aromat. Plants* **2020**, *18*, 100255. [\[CrossRef\]](#)
25. Karasov, T.L.; Chae, E.; Herman, J.J.; Bergelson, J. Mechanisms to Mitigate the Trade-off between Growth and Defense. *Plant Cell* **2017**, *29*, 666–680. [\[CrossRef\]](#) [\[PubMed\]](#)
26. Li, Y.; Li, Z.; Zhang, F.; Li, S.; Gu, Y.; Tian, W.; Tian, W.; Wang, J.; Wen, J.; Li, J. Integrated Evolutionary Pattern Analyses Reveal Multiple Origins of Steroidal Saponins in Plants. *Plant J.* **2023**, *116*, 823–839. [\[CrossRef\]](#)
27. Duan, Y.; Liu, X.; Wu, J.; You, J.; Wang, F.; Guo, X.; Tang, T.; Liao, M.; Guo, J. Transcriptomic and Metabolic Analyses Reveal the Potential Mechanism of Increasing Steroidal Alkaloids in *Fritillaria Hupehensis* through Intercropping with *Magnolia officinalis*. *Front. Plant Sci.* **2022**, *13*, 997868. [\[CrossRef\]](#)
28. Shameer, S.; Ratcliffe, R.G.; Sweetlove, L.J. Leaf Energy Balance Requires Mitochondrial Respiration and Export of Chloroplast NADPH in the Light. *Plant Physiol.* **2019**, *180*, 1947–1961. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Jiang, Y.; Khan, M.U.; Lin, X.; Lin, Z.; Lin, S.; Lin, W. Evaluation of Maize/Peanut Intercropping Effects on Microbial Assembly, Root Exudates and Peanut Nitrogen Uptake. *Plant Physiol. Biochem.* **2022**, *171*, 75–83. [\[CrossRef\]](#)
30. Shi, H.; Ye, T.; Chen, F.; Cheng, Z.; Wang, Y.; Yang, P.; Zhang, Y.; Chan, Z. Manipulation of Arginase Expression Modulates Abiotic Stress Tolerance in Arabidopsis: Effect on Arginine Metabolism and ROS Accumulation. *J. Exp. Bot.* **2013**, *64*, 1367–1379. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Winter, G.; Todd, C.D.; Trovato, M.; Forlani, G.; Funck, D. Physiological Implications of Arginine Metabolism in Plants. *Front. Plant Sci.* **2015**, *6*, 534. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Wang, W.; Li, M.-Y.; Zhou, R.; Zhu, S.-G.; Tao, H.-Y.; Khan, A.; Uzamurera, A.G.; Wang, B.-Z.; Jin, J.-M.; Ma, Y.; et al. Effects of Interspecific Interactions on Soil Carbon Emission and Efficiency in the Semiarid Intercropping Systems. *Soil Tillage Res.* **2023**, *234*, 105857. [\[CrossRef\]](#)
33. Wang, Y.; Chen, R.; Zhang, Z.; Fu, Z.; Zhang, L.; Tan, F. Kinetics of Uptake, Translocation, and Metabolism of Organophosphate Esters in Japonica Rice (*Oryza Sativa* L.): Hydroponic Experiment Combined with Model. *Sci. Total Environ.* **2024**, *951*, 175838. [\[CrossRef\]](#)
34. Hwang, J.-U.; Song, W.-Y.; Hong, D.; Ko, D.; Yamaoka, Y.; Jang, S.; Yim, S.; Lee, E.; Khare, D.; Kim, K.; et al. Plant ABC Transporters Enable Many Unique Aspects of a Terrestrial Plant's Lifestyle. *Mol. Plant* **2016**, *9*, 338–355. [\[CrossRef\]](#)
35. Li, S.; Wu, F. Diversity and Co-Occurrence Patterns of Soil Bacterial and Fungal Communities in Seven Intercropping Systems. *Front. Microbiol.* **2018**, *9*, 1521. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Chen, D.; Jia, L.; Hou, Q.; Zhao, X.; Sun, K. Analysis of Endophyte Diversity of *Rheum Palmatum* from Different Production Areas in Gansu Province of China and the Association with Secondary Metabolite. *Microorganisms* **2021**, *9*, 978. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Sterkenburg, E.; Bahr, A.; Brandström Durling, M.; Clemmensen, K.E.; Lindahl, B.D. Changes in Fungal Communities along a Boreal Forest Soil Fertility Gradient. *New Phytol.* **2015**, *207*, 1145–1158. [\[CrossRef\]](#) [\[PubMed\]](#)

38. Gong, X.; Liu, C.; Li, J.; Luo, Y.; Yang, Q.; Zhang, W.; Yang, P.; Feng, B. Responses of Rhizosphere Soil Properties, Enzyme Activities and Microbial Diversity to Intercropping Patterns on the Loess Plateau of China. *Soil Tillage Res.* **2019**, *195*, 104355. [\[CrossRef\]](#)
39. Zhu, B.; Wu, J.; Ji, Q.; Wu, W.; Dong, S.; Yu, J.; Zhang, Q.; Qin, L. Diversity of Rhizosphere and Endophytic Fungi in *Atractylodes Macrocephala* during Continuous Cropping. *PeerJ* **2020**, *8*, e8905. [\[CrossRef\]](#)
40. Zhang, X.; Gao, G.; Wu, Z.; Wen, X.; Zhong, H.; Zhong, Z.; Yang, C.; Bian, F.; Gai, X. Responses of Soil Nutrients and Microbial Communities to Intercropping Medicinal Plants in Moso Bamboo Plantations in Subtropical China. *Environ. Sci. Pollut. Res.* **2020**, *27*, 2301–2310. [\[CrossRef\]](#)
41. Delgado-Baquerizo, M.; Reich, P.B.; Trivedi, C.; Eldridge, D.J.; Abades, S.; Alfaro, F.D.; Bastida, F.; Berhe, A.A.; Cutler, N.A.; Gallardo, A.; et al. Multiple Elements of Soil Biodiversity Drive Ecosystem Functions across Biomes. *Nat. Ecol. Evol.* **2020**, *4*, 210–220. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Kim, H.-S.; Lee, S.-H.; Jo, H.Y.; Finneran, K.T.; Kwon, M.J. Diversity and Composition of Soil Acidobacteria and Proteobacteria Communities as a Bacterial Indicator of Past Land-Use Change from Forest to Farmland. *Sci. Total Environ.* **2021**, *797*, 148944. [\[CrossRef\]](#)
43. Singh, J.S.; Kumar, A.; Rai, A.N.; Singh, D.P. Cyanobacteria: A Precious Bio-Resource in Agriculture, Ecosystem, and Environmental Sustainability. *Front. Microbiol.* **2016**, *7*, 529. [\[CrossRef\]](#)
44. Terhonen, E.; Blumenstein, K.; Kovalchuk, A.; Asiegbu, F.O. Forest Tree Microbiomes and Associated Fungal Endophytes: Functional Roles and Impact on Forest Health. *Forests* **2019**, *10*, 42. [\[CrossRef\]](#)
45. You, C.; Qin, D.; Wang, Y.; Lan, W.; Li, Y.; Yu, B.; Peng, Y.; Xu, J.; Dong, J. Plant Triterpenoids Regulate Endophyte Community to Promote Medicinal Plant *Schisandra Sphenanthera* Growth and Metabolites Accumulation. *J. Fungi* **2021**, *7*, 788. [\[CrossRef\]](#)
46. Li, Y.; Liu, Y.; Zhang, H.; Yang, Y.; Wei, G.; Li, Z. The Composition of Root-Associated Bacteria and Fungi of *Astragalus Mongholicus* and Their Relationship with the Bioactive Ingredients. *Front. Microbiol.* **2021**, *12*, 642730. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Song, P.; Liu, J.; Huang, P.; Han, Z.; Wang, D.; Sun, N. Diversity and Structural Analysis of Rhizosphere Soil Microbial Communities in Wild and Cultivated *Rhizoma Atractylodis Macrocephalae* and Their Effects on the Accumulation of Active Components. *PeerJ* **2023**, *11*, e14841. [\[CrossRef\]](#) [\[PubMed\]](#)
48. Kalam, S.; Basu, A.; Ahmad, I.; Sayyed, R.Z.; El-Enshasy, H.A.; Dailin, D.J.; Suriani, N.L. Recent Understanding of Soil Acidobacteria and Their Ecological Significance: A Critical Review. *Front. Microbiol.* **2020**, *11*, 580024. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Peng, G.; Zhang, W.; Luo, H.; Xie, H.; Lai, W.; Tan, Z. *Enterobacter Oryzae* Sp. Nov., a Nitrogen-Fixing Bacterium Isolated from the Wild Rice Species *Oryza Latifolia*. *Int. J. Syst. Evol. Microbiol.* **2009**, *59*, 1650–1655. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Speirs, L.B.M.; Rice, D.T.F.; Petrovski, S.; Seviour, R.J. The Phylogeny, Biodiversity, and Ecology of the Chloroflexi in Activated Sludge. *Front. Microbiol.* **2019**, *10*, 2015. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Ventura, M.; Canchaya, C.; Tauch, A.; Chandra, G.; Fitzgerald, G.F.; Chater, K.F.; Van Sinderen, D. Genomics of *Actinobacteria*: Tracing the Evolutionary History of an Ancient Phylum. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 495–548. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Fuerst, J.A.; Sagulenko, E. Beyond the Bacterium: Planctomycetes Challenge Our Concepts of Microbial Structure and Function. *Nat. Rev. Microbiol.* **2011**, *9*, 403–413. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Fierer, N.; Ladau, J.; Clemente, J.C.; Leff, J.W.; Owens, S.M.; Pollard, K.S.; Knight, R.; Gilbert, J.A.; McCulley, R.L. Reconstructing the Microbial Diversity and Function of Pre-Agricultural Tallgrass Prairie Soils in the United States. *Science* **2013**, *342*, 621–624. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Zeilinger, S.; Gupta, V.K.; Dahms, T.E.S.; Silva, R.N.; Singh, H.B.; Upadhyay, R.S.; Gomes, E.V.; Tsui, C.K.-M.; Nayak, S. Friends or Foes? Emerging Insights from Fungal Interactions with Plants. *FEMS Microbiol. Rev.* **2016**, *40*, 182–207. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Wani, Z.A.; Mirza, D.N.; Arora, P.; Riyaz-Ul-Hassan, S. Molecular Phylogeny, Diversity, Community Structure, and Plant Growth Promoting Properties of Fungal Endophytes Associated with the Corms of Saffron Plant: An Insight into the Microbiome of *Crocus sativus* Linn. *Fungal Biol.* **2016**, *120*, 1509–1524. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Tedersoo, L.; May, T.W.; Smith, M.E. Ectomycorrhizal Lifestyle in Fungi: Global Diversity, Distribution, and Evolution of Phylogenetic Lineages. *Mycorrhiza* **2010**, *20*, 217–263. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Smith, S.E.; Read, D.J. *Mycorrhizal Symbiosis*, 3rd ed.; Academic Press: Amsterdam, The Netherlands; Boston, MA, USA, 2008; ISBN 978-0-12-370526-6.
58. Li, Z.; Geng, G.; Xie, H.; Zhou, L.; Wang, L.; Qiao, F. Metabolomic and Transcriptomic Reveal Flavonoid Biosynthesis and Regulation Mechanism in *Phlomis rotata* from Different Habitats. *Genomics* **2024**, *116*, 110850. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Hortal, S.; Bastida, F.; Moreno, J.L.; Armas, C.; García, C.; Pugnaire, F.I. Benefactor and Allelopathic Shrub Species Have Different Effects on the Soil Microbial Community along an Environmental Severity Gradient. *Soil Biol. Biochem.* **2015**, *88*, 48–57. [\[CrossRef\]](#)
60. Osińska-Jaroszuk, M.; Jarosz-Wilkolazka, A.; Jaroszuk-Ścisiel, J.; Szałapata, K.; Nowak, A.; Jaszek, M.; Ozimek, E.; Majewska, M. Extracellular Polysaccharides from Ascomycota and Basidiomycota: Production Conditions, Biochemical Characteristics, and Biological Properties. *World J. Microbiol. Biotechnol.* **2015**, *31*, 1823–1844. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Zhu, B.; Zhang, Q.; Hua, J.; Cheng, W.; Qin, L. The Traditional Uses, Phytochemistry, and Pharmacology of *Atractylodes Macrocephala* Koidz.: A Review. *J. Ethnopharmacol.* **2018**, *226*, 143–167. [\[CrossRef\]](#) [\[PubMed\]](#)
62. Wu, W.; Chen, W.; Liu, S.; Wu, J.; Zhu, Y.; Qin, L.; Zhu, B. Beneficial Relationships between Endophytic Bacteria and Medicinal Plants. *Front. Plant Sci.* **2021**, *12*, 646146. [\[CrossRef\]](#) [\[PubMed\]](#)

63. Chen, H.; Chen, J.; Qi, Y.; Chu, S.; Ma, Y.; Xu, L.; Lv, S.; Zhang, H.; Yang, D.; Zhu, Y.; et al. Endophytic Fungus *Cladosporium Tenuissimum* DF11, an Efficient Inducer of Tanshinone Biosynthesis in *Salvia Miltiorrhiza* Roots. *Phytochemistry* **2022**, *194*, 113021. [[CrossRef](#)] [[PubMed](#)]
64. Yan, Y.; Zhang, S.; Zhang, J.; Ma, P.; Duan, J.; Liang, Z. Effect and Mechanism of Endophytic Bacteria on Growth and Secondary Metabolite Synthesis in *Salvia Miltiorrhiza* Hairy Roots. *Acta Physiol. Plant.* **2014**, *36*, 1095–1105. [[CrossRef](#)]
65. Liu, L.; Cao, H.; Geng, Y.; Zhang, Q.; Bu, X.; Gao, D. Response of Soil Microecology to Different Cropping Practice under *Bupleurum Chinense* Cultivation. *BMC Microbiol.* **2022**, *22*, 223. [[CrossRef](#)]
66. Dong, Q.; Zhao, X.; Zhou, D.; Liu, Z.; Shi, X.; Yuan, Y.; Jia, P.; Liu, Y.; Song, P.; Wang, X.; et al. Maize and Peanut Intercropping Improves the Nitrogen Accumulation and Yield per Plant of Maize by Promoting the Secretion of Flavonoids and Abundance of *Bradyrhizobium* in Rhizosphere. *Front. Plant Sci.* **2022**, *13*, 957336. [[CrossRef](#)] [[PubMed](#)]
67. Kudjordjie, E.N.; Sapkota, R.; Steffensen, S.K.; Fomsgaard, I.S.; Nicolaisen, M. Maize Synthesized Benzoxazinoids Affect the Host Associated Microbiome. *Microbiome* **2019**, *7*, 59. [[CrossRef](#)] [[PubMed](#)]
68. Hou, Q.; Chen, D.; Wang, Y.; Ehmet, N.; Ma, J.; Sun, K. Analysis of Endophyte Diversity of *Gentiana Officinalis* among Different Tissue Types and Ages and Their Association with Four Medicinal Secondary Metabolites. *PeerJ* **2022**, *10*, e13949. [[CrossRef](#)]
69. Zhang, J.; Liu, Y.-X.; Zhang, N.; Hu, B.; Jin, T.; Xu, H.; Qin, Y.; Yan, P.; Zhang, X.; Guo, X.; et al. NRT1.1B Is Associated with Root Microbiota Composition and Nitrogen Use in Field-Grown Rice. *Nat. Biotechnol.* **2019**, *37*, 676–684. [[CrossRef](#)] [[PubMed](#)]
70. Mastan, A.; Bharadwaj, R.; Kushwaha, R.K.; Vivek Babu, C.S. Functional Fungal Endophytes in *Coleus Forskohlii* Regulate Labdane Diterpene Biosynthesis for Elevated Forskolol Accumulation in Roots. *Microb. Ecol.* **2019**, *78*, 914–926. [[CrossRef](#)] [[PubMed](#)]
71. Zhang, H.; Xia, Y.; Cui, J.-L.; Ji, X.; Miao, S.-M.; Zhang, G.; Li, Y.-M. The Composition Characteristics of Endophytic Communities and Their Relationship with Metabolites Profile in *Ephedra Sinica* under Wild and Cultivated Conditions. *Environ. Sci. Pollut. Res.* **2023**, *30*, 95648–95659. [[CrossRef](#)] [[PubMed](#)]
72. Tang, X.; He, Y.; Zhang, Z.; Wu, H.; He, L.; Jiang, J.; Meng, W.; Huang, Z.; Xiong, F.; Liu, J.; et al. Beneficial Shift of Rhizosphere Soil Nutrients and Metabolites under a Sugarcane/Peanut Intercropping System. *Front. Plant Sci.* **2022**, *13*, 1018727. [[CrossRef](#)] [[PubMed](#)]
73. Ren, D.; Guo, K.; Sun, Q.; Zhu, B.; Qin, L. Variations in Rhizospheric and Endophytic Root Fungal Communities of *Scrophularia Ningpoensis* in Different Producing Areas. *Curr. Microbiol.* **2023**, *80*, 323. [[CrossRef](#)]
74. Liu, J.; Zhang, W.; Teng, C.; Pang, Z.; Peng, Y.; Qiu, J.; Lei, J.; Su, X.; Zhu, W.; Ding, C. Intercropping Changed the Soil Microbial Community Composition but No Significant Effect on Alpha Diversity. *Front. Microbiol.* **2024**, *15*, 1370996. [[CrossRef](#)] [[PubMed](#)]
75. Zhu, L.; Yan, H.; Zhou, G.; Jiang, C.; Liu, P.; Yu, G.; Guo, S.; Wu, Q.-N.; Duan, J. Insights into the Mechanism of the Effects of Rhizosphere Microorganisms on the Quality of Authentic *Angelica Sinensis* under Different Soil Microenvironments. *BMC Plant Biol.* **2021**, *21*, 285. [[CrossRef](#)] [[PubMed](#)]
76. Fu, H.; Song, Z.; Li, S.; Lan, S.; Zeng, X.; Huang, W. Effects of Bamboo Forest Type and Density on the Growth of *Bletilla Striata* and Root Endophytic Fungi. *Australas. J. Min. Met.* **2022**, *14*, 391. [[CrossRef](#)]

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