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Artificial Cultivation Reshapes Soil Nutrient Heterogeneity, Microbial Community Structure, and Multi-Nutrient Cycling Drivers of the Endangered Medicinal Plant *Sinopodophyllum hexandrum*

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Abstract

Artificial cultivation of the endangered medicinal plant *Sinopodophyllum hexandrum* is a key strategy for resource protection and supply, yet cultivation can cause soil degradation and microbial disorder, while the effect of cultivation on the microbial community and its relationship with soil nutrients remains unclear. This study aimed to explore the effects of artificial cultivation on the soil–microorganism–nutrient cycling system of *Sinopodophyllum hexandrum*, a rare medicinal plant. We compared three groups (Native-wild, Mix-wild, Mix-cultivated) by analyzing soil physicochemical properties, microbial diversity, community structure, co-occurrence networks, and multi-nutrient cycling drivers. Geographic position drove spatial (landscape scale) heterogeneity of soil nutrients, while cultivation shaped its vertical (soil depth) counterpart. Cultivation altered the natural vertical nutrient pattern via surface fertilization, causing nutrient surface retention. Microbial communities exhibited wild-specific/cultivation-specific responses, bacteria were slightly more sensitive to cultivation effect than fungi. Cultivation altered microbial network complexity depending on the host and increased instability, with only bacterial network associations correlating with soil factors. Fungal diversity and specific taxa became core drivers of multi-nutrient cycling. This study clarifies cultivation's regulatory mechanism on *S. hexandrum*'s soil–microorganism system, providing a basis for optimizing cultivation management and protecting this endangered species.

Keywords: *Sinopodophyllum hexandrum*; artificial cultivation; microbial community

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1. Introduction

Sinopodophyllum hexandrum (Royle) Ying is an endangered medicinal plant belonging to the Berberidaceae family which is native to the high-altitude Himalayan regions between 2000 and 4500 m above sea level [1,2]. Its roots and rhizomes are rich in podophyllotoxin, a key precursor for synthesizing anti-cancer drugs, making it of high medicinal and economic value [3]. Due to over-harvesting and habitat destruction, wild *S. hexandrum* populations have sharply declined, and artificial cultivation has become the primary approach to meet market demand and protect wild resources [4]. However, large-scale cultivation often leads to changes in soil ecological processes (such as nutrient imbalance and microbial community degradation), which directly affect the growth and medicinal quality of *S. hexandrum* [5].

Thus, clarifying the response of the soil–microorganism–nutrient cycling system to cultivation disturbance is critical for optimizing cultivation management and ensuring sustainable utilization of this plant species.

Soil nutrients exhibit spatial and vertical heterogeneity shaped by both geographic factors and human activities. For wild plants, geographic drivers like altitude and climate create distinct spatial patterns and natural vertical gradients [6–8]. However, artificial cultivation disrupts these patterns through surface fertilization (causing nutrient retention in the 0–20 cm layer) and tillage (reducing vertical differentiation) [8,9]. While such cultivation-induced changes are reported in other medicinal plants [10,11], comparative studies on wild and cultivated *S. hexandrum* are scarce. Critical gaps remain in understanding how cultivation transforms nutrient heterogeneity in this species.

Soil microorganisms play a pivotal role in regulating soil nutrient cycling, and their community structure and function are highly sensitive to environmental changes [12,13]. Previous studies have shown that cultivation can alter microbial diversity and community composition in *Panax ginseng* and *Glycyrrhiza uralensis* [12,14]. Nevertheless, most studies focus on a single microbial group or ignore the specific responses of bacteria and fungi to cultivation. Additionally, microbial co-occurrence networks reflect the complexity of interspecific interactions and ecosystem stability [15], but how cultivation affects the network structure of *S. hexandrum* soil microorganisms and their associations with soil factors has not been reported. Soil multi-nutrient cycling is a comprehensive process driven by both abiotic (soil physicochemical properties) and biotic (microorganisms) factors [16]. Traditionally, bacteria are considered the main drivers of nutrient cycling, while the role of fungi (especially under cultivation conditions) has been underestimated [17]. For *S. hexandrum*, the key microbial taxa regulating multi-nutrient cycling, and whether their effects differ between wild and cultivated populations, remain unknown.

To address these knowledge gaps, this study compared three *S. hexandrum* groups (Native-wild, Mix-wild, Mix-cultivated) to investigate (1) the differences in soil nutrient spatial and vertical heterogeneity between wild and cultivated populations; (2) the group-specific responses of bacterial and fungal communities to cultivation; (3) the variation in microbial co-occurrence network structure and its association with soil factors; and (4) the core microbial drivers of soil multi-nutrient cycling. We hypothesized that artificial cultivation would reshape soil nutrient spatial and vertical distributions by disrupting natural processes (e.g., leaching, decomposition) via surface fertilization and tillage, and alter soil microbial community structure and network stability as cultivation-driven nutrient enrichment and habitat disturbance favor stress-tolerant over native symbiotic taxa. Cultivation would also shift multi-nutrient cycling's core drivers from bacteria to fungi, as fungi better adapt to artificial high-nutrient environments (e.g., decomposing fertilizer-derived labile organics). This study aims to reveal the regulatory mechanism of cultivation on the *S. hexandrum* soil-microorganism system, providing a theoretical basis for the scientific cultivation and conservation of this endangered medicinal plant.

2. Materials and Methods

2.1. Study Area

The Taizi Mountain National Nature Reserve (hereinafter referred to as the "Reserve"), located in Gansu Province, lies in the transitional zone between the Qinghai–Tibet Plateau and the Loess Plateau of China. It spans from 102°43′ E to 103°42′ E longitude and 35°02′ N to 35°36′ N latitude, extending approximately 100 km from east to west and about 10 km from north to south, covering a total area of 84,700 hectares (hm^2). The elevation ranges from 2200 to 4636 m, characterized by high terrain, deep incision, and a west-to-east incline, forming part of the marginal uplift zone of the eastern Qinghai–Tibet Plateau.

The Reserve experiences a temperate continental monsoon climate. Influenced by its topography, the climate exhibits significant horizontal (landscape scale) and vertical (soil depth) variations. It is characterized by cool and short summers, long and cold winters, a slow spring warm-up, and a rapid autumn cool-down. The climate is also dry in winter and humid in autumn. The annual average temperature is 5.1 °C, with a frost-free period of about 110 days. The average annual precipitation is around 660 mm, peaking at 1030.4 mm [18]. According to the FAO classification, the soil types of all sampling sites are Humic Cambisols.

2.2. Sample Collection

Soil samples were collected in September 2023, with a distance exceeding 100 m between each sampling site, and root soil samples of both wild and artificially cultivated *S. hexandrum* were then collected. We randomly selected three plots (5 × 5 m) as replicates for wild plant (each of three) and cultivated plant (each of three) in one site, and randomly selected three plots (5 × 5 m) as replicates for wild plant in five sites. Therefore, three plant type of *S. hexandrum* were selected in this study (Figure 1A, Table 1): (1) wild plant in native habitat (Native-wild); (2) wild plant in mixed habitat with wild plants and cultivated plants by artificial cultivation (Mix-wild); (3) cultivated plant in mixed habitat (Mix-cultivated). Four sampling points (front, back, left, and right) around each plant were chosen to collect soil samples from three distinct depth layers: A (0–10 cm), B (10–20 cm), and C (20–30 cm depths), along with rhizosphere soil (D). Our protocol adhered to the ethical principles of minimizing disturbance to the collection area while ensuring the uniformity, scientific validity, and comparability of the sample sites [19].

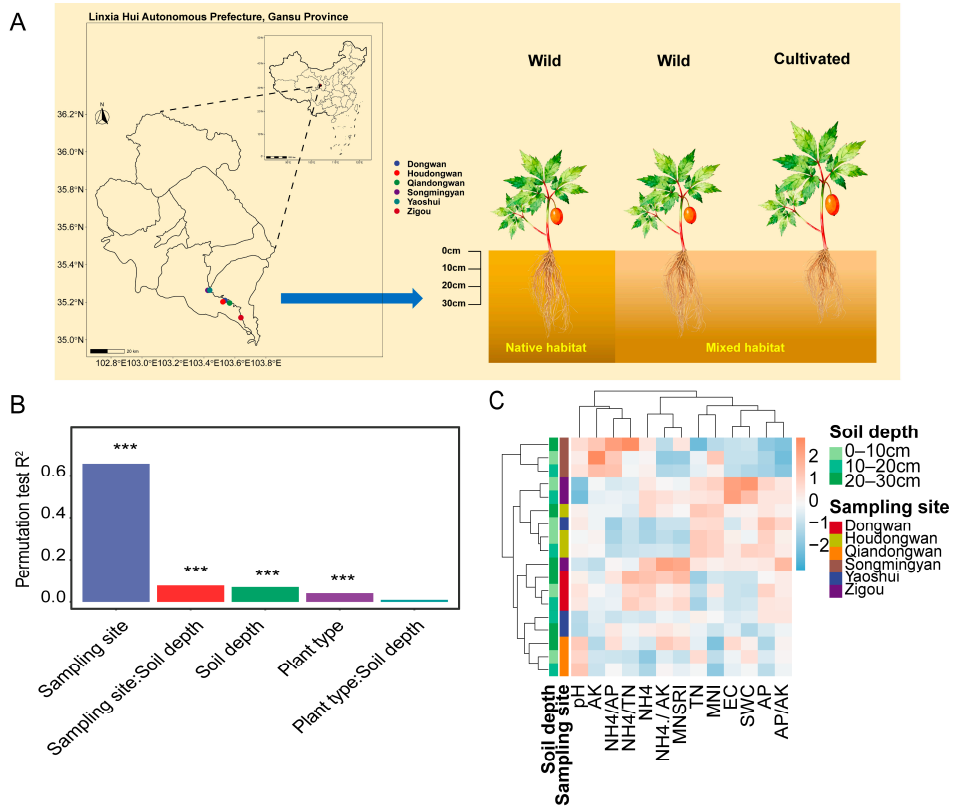


Figure 1. (**A**) Sample collection location and experimental setting and the sketch of three *Sinopodophyllum hexandrum* groups in native habitat and mixed habitats. (**B**) The effect of Sampling site, soil depth, plant type on the bacterial communities across all samples (PERMANOVA test, *** $p < 0.001$). (**C**) Soil properties in different sampling sites with different soil depths. Color intensity represents the standardized value of nutrient indicators (light = low, dark = high).

Table 1. Basic sampling information.

Sample Number	Coordinate		Elevation/m
	Longitude/E	Latitude/N	
Mix-wild	103°32′4″	35°12′59″	2426
Mix-wild	103°31′11″	35°12′34″	2412
Mix-wild	103°32′11″	35°12′34″	2469
Mix-cultivated	103°32′35″	35°13′32″	2425
Mix-cultivated	103°31′44″	35°13′10″	2408
Mix-cultivated	103°31′45″	35°13′10″	2405
Hou Dongwan (Native-wild)	103°31′13″	35°12′33″	2419
Zigou (Native-wild)	103°38′15″	35°7′32″	2369
Yaoshui (Native-wild)	103°26′5″	35°16′14″	2340
Qian Dongwan (Native-wild)	103°33′51″	35°12′11″	2507
Song Mingyan (Native-wild)	103°25′21″	35°16′12″	2391

Note: Samples 1 to 3 are from wild *S. hexandrum*, while samples 4 to 6 are from artificially propagated *S. hexandrum*, all collected from the same site. The five sampling points of native wild *S. hexandrum* were Hou Dongwan, Zigou, Yaoshui, Qian Dongwan, and Songmingyan.

The four samples from each soil depth layer were combined into one composite sample and subdivided to approximately 1 kg using the quadrant method. Immediately after collection, the collected soil samples were labeled and stored in dedicated sample bags, stored in insulated containers with ice packs, then transported to the laboratory for subsequent analysis. Processed samples were either frozen in foam boxes and sealed immediately with 30 kg of dry ice for analysis or stored in a −80 °C freezer for future analysis. The remaining samples were air-dried in a cool, ventilated location, ground finely with porcelain mortars, sieved through a 0.15 mm nylon mesh to remove debris, and finally stored in sealed bags for physicochemical property assessments. Sampling details are summarized in Table 1. In total, 99 samples (Native-wild × five sampling sites × three soil layers × three replicates + Mix-wild × one sampling site × three plants × three soil layers × three replicates + Mix-cultivated × one sampling site × three plants × three soil layers × three replicates) were obtained for further processing.

2.3. Determination of Soil Physicochemical Properties

Soil water content (SWC) was measured by the oven-drying method. Soil available phosphorus (AP) content was determined using the Sodium Bicarbonate Extraction-Molybdenum Antimony Anti-Spectrophotometric Method. Soil Available Potassium (AK) content was measured using the Ammonium Acetate Extraction-Flame Photometry Method [20]. Total nitrogen (TN) was quantified using the Kjeldahl method. Soil Ammonium Nitrogen (NH_4) content was measured with 2 M KCl using a Soil Nutrient Rapid Tester (Model OK-Q, Zhengzhou Oukexi Instruments Manufacturing Co., Ltd., Zhengzhou, China). The determination of soil indicators was performed according to conventional methods [21]. To derive a quantitative soil multi-nutrient cycling index value (MNI), we standardized each of the five nutrients' properties (TN, AP, AK, NH_4) using the Z score transformation. These standardized ecosystem functions were then averaged to obtain this index. To derive a quantitative multi-nutrient stoichiometric ratio index (MNSRI), we standardized each of the five soil properties (NH_4/AK, NH_4/AP, NH_4/TN, AP/AK) using the Z score transformation. These standardized ecosystem functions were then averaged to obtain this index.

2.4. High-Throughput Sequencing and Processing

DNA was extracted from samples using the MagPure Stool DNA KF Kit B (Magen Biotech, Guangzhou, China) according to the manufacturer's instructions. Library

preparation was performed using 2× Phanta Max Master Mix (polymerase, VAZYME, Nanjing, China) with degenerate primers 338F (ACTCCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT) to amplify the V3–V4 hypervariable region of bacterial 16S rDNA, and degenerate primers ITS3 (GCATCGATGAAGAACGCAGC) and ITS4 (TCCTCCGCTTATTGATATGC) to amplify the ITS2 hypervariable region of fungal ITS rDNA. The PCR reaction mixture (50 μL) contained 30 ng template DNA and universal PCR primers. The thermal cycling protocol was as follows: Initial denaturation at 95 °C for 3 min; 30 cycles of (denaturation at 95 °C for 15 s, annealing at 56 °C for 15 s, extension at 72 °C for 45 s), final extension at 72 °C for 5 min. PCR products were purified using DNA sorting magnetic beads (BGI, LB00V60). PCR products were processed using high-density DNA nanochip technology and subjected to paired-end (PE300) sequencing on the DNBSEQ-G400 platform (BGI, Shenzhen, China).

Reads containing primers were trimmed using cutadapt v2.6 to remove primer and adapter contamination. Low-quality reads were filtered by window-based trimming (window length = 30 bp; average quality score < 20 led to removal of downstream sequence). Reads shorter than 75% of the original length, those containing "N," or those with low complexity (e.g., >10 consecutive A/T/C/G bases) were discarded, yielding clean data. OTUs were clustered using Usearch after sequence assembly. Paired-end reads were merged with FLASH (v1.2.11) to generate high-variable region tags. These were clustered into OTUs using USEARCH (v7.0.1090), at a 97% sequence similarity threshold, with de novo chimera detection via uchime_denovo and singleton removal before clustering; the minimum retained read length after merging and trimming was 220 bp. Representative OTU sequences were taxonomically annotated via the RDP Classifier at a confidence threshold of 0.6 OTUs; sequences without annotations or outside the study scope were excluded. Sequences matching "Chloroplast" and "Mitochondria" were removed from the datasets. To minimize the impact of sequencing artifacts, singletons were removed from the datasets. The number of fungal reads per sample ranged from 25,262 to 89,221 with an average of 75,643 reads. The number of bacterial reads per sample ranged from 49,551 to 114,579 with an average of 80,445 reads. The sequencing data used in this study has been uploaded to NCBI with the accession number PRJNA1279783.

2.5. Statistical Analysis

All statistical analyses were conducted in R version 4.5.0 (http://www.r-project.org, accessed on 25 December 2025). We validated normality (Shapiro–Wilk test) and variance homogeneity (Levene's test) for all ANOVA analyses. Normal data used ANOVA with Tukey's HSD post-hoc tests; non-normal data used non-parametric Kruskal–Wallis tests with Fisher's LSD. Constrained principal coordinates analysis (CAP) based on Bray–Curtis distances was employed to visualize inter-sample relationships. To analyze the main effects of plant type, site, soil depth and their interactions on soil nutrients and microbial communities, permutational multivariate analysis of variance (PERMANOVA) was implemented with the "vegan" package [22]. The significance of soil properties was assessed via one-way analysis of variance (ANOVA), only applied for pairwise comparisons after confirming no significant three-way interactions, using the "stats" package in R. The alpha diversity index was computed using the "microeco" package, Phylogenetic Diversity (PD) was calculated based on the phylogenetic tree constructed from OTU representative sequences; significant differences in this index were identified via a multiple non-parametric Kruskal–Wallis test, followed by a post-hoc test that applied Fisher's least significant difference criterion (using the "agricolae" package). Linear Discriminant Analysis Effect Size (LEfSe) was applied to identify group-specific microbial biomarkers (Kruskal–Wallis p-value < 0.05, logarithmic LDA score > 3.5) [23].

To investigate potential interspecific interactions, we estimated associations among all members of microbial communities in *S. hexandrum* samples. To reduce rare OTUs in the dataset, we removed OTUs with a relative abundance < 0.01%. Robust correlation networks were constructed using the "igraph" package [24], with the following criteria: Spearman's correlation coefficients (ρ) > 0.8 and false discovery rate (FDR)-corrected *p*-values < 0.01. A series of network topological features were evaluated, including degree, number of nodes, positive links, negative links, average degree, average path length, graph density, clustering coefficient, and modularity. Network visualization was performed using the interactive Gephi platform version 0.9.7 [25]. The network robustness test—an effective approach for measuring network stability (more specifically, resistance)—was conducted by assessing changes in natural connectivity after removing nodes from the static network. The topological characteristics of each sample's subnetwork were analyzed using the "igraph" package, following the method previously described [26]. Subsequently, Spearman correlation coefficients between network topological features and environmental factors were computed.

Key soil variables affected by parasitism were identified via random forest analysis. To evaluate variable importance, the percentage increase in mean square error (MSE) for each variable was analyzed using the "rfPermute" package [27] with 1000 permutations, a higher MSE% value denotes greater importance of the corresponding variable. Significance of the models and cross-validated R^2 values were assessed with 1000 permutations of the response variable, using the "A3" package [28].

3. Results

3.1. Variation in Soil Physicochemical Properties Between Wild and Cultivated Sinopodophyllum hexandrum

Permutational Multivariate Analysis of Variance (PERMANOVA) showed that the overall difference among sampling sites was the most significant (R^2 = 0.658, $p < 0.001$), followed by the interaction between sampling site and depth (R^2 = 0.078, $p < 0.001$), and then plant type (R^2 = 0.071, $p < 0.001$) (Figure 1B). Based on nutrient data from five wild sampling sites, a distinct "hotspot–coldspot" pattern was observed (Figure 1C, Tables 2 and 3). Qiandongwan had a higher pH; Songmingyan was enriched in available potassium (AK) and NH_4/available phosphorus (AP); Dongwan showed higher levels of NH_4^+/total nitrogen (TN), NH_4^+, NH_4/AK, and multi-nutrient stoichiometric ratio index (MNSRI); while Houdongwan and Zigou exhibited enrichment in TN, multi-nutrient index (MNI), electrical conductivity (EC), soil water content (SWC), AP, and AP/AK. This pattern was attributed to geographic isolation-induced differences in climate and vegetation, which regulated nutrient distribution.

For the vertical (soil depth) heterogeneity driven by cultivation, further analysis was conducted on six sampling sites in the same region. Among all nutrient indicators, only MNI, AK, and AP/AK showed no significant differences across plant types; the remaining indicators (especially EC, NH_4, NH_4/TN, and MNSRI) exhibited extremely significant vertical differentiation (Figure 2A). Specifically, in the 20–30 cm layer, NH_4/AP, NH_4, NH_4/TN, NH_4^+/total potassium (TK), and MNSRI were significantly enriched in the Mix-wild group; in the 0–10 cm and 10–20 cm layers, these indicators were concentrated in the Mix-cultivated group, which was attributed to the surface retention effect; and SWC, EC, TN, AK, and MNI were mainly enriched in the Native-wild group across all 0–30 cm layers. These results confirmed that cultivation significantly disrupted the natural vertical nutrient gradient of wild *S. hexandrum*. Further comparison of inter-layer nutrient differences revealed that the Mix-cultivated group had only five indicators (AK, MNI, AP, NH_4/AP, NH_4) with significant inter-layer variation, far fewer than the Native-wild

and Mix-wild groups (Figure 2B). Additionally, significant inter-layer differences in AK were only detected in the Mix-wild and Mix-cultivated groups. Across all groups, AK, AP, and MNI decreased significantly with soil depth. NH_4, MNSRI, NH_4/TN, and NH_4/AP increased with depth in the Native-wild and Mix-wild groups, whereas these indicators decreased with depth in the Mix-cultivated group.

Table 2. Distribution characteristics of soil nutrients in *S. hexandrum* under different propagation regimes within the mixed habitat.

Sample Number	Soil Depth/cm	NH_4^+-N /(mg/kg)	Available *p*/(mg/kg)	Available K/(mg/kg)	Total N/(g/kg)	pH	EC/(μS/cm)	SWC/(%)
Mix-wild	0~10	49 ± 1^{cC}	86 ± 1^{bB}	174 ± 8	2.95 ± 0.01^{dD}	5.77 ± 0.01	78.00 ± 1.15^{bB}	13.1 ± 0.06^{dD}
	10~20	79 ± 1^{aA}	74 ± 1^{bB}	145 ± 1	1.94 ± 0.01^{eE}	6.11 ± 0.01	58.00 ± 1.15^{eD}	15.00 ± 0.06^{eE}
	20~30	99 ± 1^{aA}	62 ± 0^{dD}	132 ± 4	1.94 ± 0.01^{dD}	6.11 ± 0.01	58.00 ± 1.15^{eD}	15.00 ± 0.06^{dD}
Mix-wild	0~10	51 ± 2^{cC}	86 ± 0^{bB}	167 ± 8	4.27 ± 0.01^{aA}	6.21 ± 0.01	94.00 ± 1.15^{aA}	22.97 ± 0.03^{aA}
	10~20	59 ± 1^{cC}	69 ± 1^{cC}	144 ± 3	2.88 ± 0.01^{bB}	6.28 ± 0.00	64.00 ± 1.15^{dCD}	22.97 ± 0.03^{aA}
	20~30	99 ± 3^{aA}	63 ± 1^{dCD}	144 ± 3	2.88 ± 0.01^{bB}	6.28 ± 0.00	64.00 ± 1.15^{bB}	20.33 ± 0.03^{bB}
Mix-wild	0~10	50 ± 1	96 ± 0	164 ± 6	3.67 ± 0.01^{bB}	6.44 ± 0.01	82.00 ± 1.15^{bB}	19.57 ± 0.03^{bB}
	10~20	57 ± 2	90 ± 0	160 ± 8	3.52 ± 0.02^{aA}	6.48 ± 0.01	87.33 ± 0.67^{aA}	19.57 ± 0.03^{bB}
	20~30	57 ± 0	74 ± 0	144 ± 0	3.52 ± 0.02^{aA}	6.48 ± 0.01	87.33 ± 0.67^{aA}	23.93 ± 0.03^{cC}
Mix-cultivated	0~10	90 ± 5	81 ± 1^{cC}	154 ± 2^{cA}	2.22 ± 0.00^{eE}	6.67 ± 0.01	66.00 ± 1.15	11.57 ± 0.03^{eE}
	10~20	69 ± 1	74 ± 1^{bB}	152 ± 4^{abA}	2.1 ± 0.01^{dD}	6.77 ± 0.00	70.00 ± 1.15	11.57 ± 0.03^{eE}
	20~30	68 ± 3	65 ± 1^{cC}	149 ± 5^{aA}	2.1 ± 0.01^{dD}	6.77 ± 0.00	70.00 ± 1.15	16.47 ± 0.03^{cC}
Mix-cultivated	0~10	98 ± 3^{aA}	76 ± 0^{dD}	180 ± 3^{aA}	2.21 ± 0.00^{eE}	8.90 ± 1.00	42.00 ± 1.15^{dD}	17.50 ± 0.12^{cC}
	10~20	84 ± 2^{aA}	75 ± 0^{bB}	142 ± 1^{bA}	2.06 ± 0.04^{dD}	7.91 ± 1.00	38.00 ± 1.15^{fE}	17.50 ± 0.12^{cC}
	20~30	74 ± 2^{bB}	74 ± 1^{aA}	116 ± 8^{cB}	2.06 ± 0.04^{cC}	7.91 ± 1.00	38.00 ± 1.15^{dD}	15.30 ± 0.06^{eE}
Mix-cultivated	0~10	83 ± 3^{bB}	70 ± 0^{eE}	157 ± 0	3.17 ± 0.02^{cC}	6.79 ± 0.02	68.67 ± 1.76^{cC}	10.80 ± 0.06^{fF}
	10~20	67 ± 2^{bB}	70 ± 0^{cC}	152 ± 2	2.65 ± 0.01^{cC}	6.89 ± 0.00	78.67 ± 1.76^{bB}	10.80 ± 0.06^{fF}
	20~30	49 ± 4^{dC}	68 ± 1^{bB}	140 ± 1	2.65 ± 0.01^{bB}	6.89 ± 0.00	78.67 ± 1.76^{bcBC}	15.40 ± 0.06^{aA}

Note: Different lowercase letters within a column indicate significant differences ($p < 0.05$) in soil nutrient indices among sampling sites within the same soil layer, while different uppercase letters denote highly significant differences ($p < 0.01$).

Table 3. Spatial distribution of soil nutrients in Native-wild *S. hexandrum* populations across conservation stations within the native habitat.

Sample Number	Soil Depth/cm	NH_4^+-N /(mg/kg)	Available *p*/(mg/kg)	Available K/(mg/kg)	Total N/(g/kg)	pH	EC/(μS/cm)	SWC/(%)
Hou Dongwan (Native-wild)	0~10	46.26 ± 0.73^{cC}	84.32 ± 0.02^{bB}	172.53 ± 0.07^{bB}	6.12 ± 0.01^{aA}	6.47 ± 0.01^{cC}	196.00 ± 1.15^{bAB}	22.50 ± 0.06^{dD}
	10~20	52.63 ± 0.68^{cC}	82.30 ± 0.07^{aA}	165.60 ± 0.78^{bB}	6.12 ± 0.01^{aA}	6.57 ± 0.00^{cC}	209.00 ± 1.15^{bB}	33.97 ± 0.03^{bB}
	20~30	68.26 ± 0.93^{bB}	74.81 ± 0.02^{aA}	155.20 ± 1.13^{bB}	6.14 ± 0.01^{aA}	6.62 ± 0.02^{cC}	186.67 ± 2.4^{abA}	25.43 ± 1.37^{aA}
Zigou (Native-wild)	0~10	64.37 ± 0.49^{aA}	81.63 ± 0.11^{cC}	159.80 ± 0.26^{cC}	5.45 ± 0.01^{bB}	5.57 ± 0.02^{dD}	459.00 ± 99.00^{aA}	48.53 ± 0.15^{aA}
	10~20	68.52 ± 1.11^{aA}	75.53 ± 0.27^{aA}	144.90 ± 0.06^{cC}	5.12 ± 0.01^{bB}	5.44 ± 0.01^{eE}	466.67 ± 1.76^{aA}	38.80 ± 0.65^{aA}
	20~30	72.93 ± 0.47^{aA}	73.73 ± 0.03^{cAB}	105.00 ± 2.00^{dD}	5.10 ± 0.01^{bB}	6.19 ± 0.01^{dD}	209.00 ± 1.15^{abA}	26.97 ± 0.12^{aA}
Yaoshui (Native-wild)	0~10	45.44 ± 0.80^{cC}	91.93 ± 1.04^{aA}	156.33 ± 1.05^{dD}	5.46 ± 0.01^{bB}	6.70 ± 0.01^{bB}	80.67 ± 1.76^{bB}	26.80 ± 0.06^{cC}
	10~20	53.11 ± 0.18^{cC}	74.81 ± 0.22^{aA}	142.00 ± 0.40^{cC}	4.81 ± 0.00^{cC}	5.98 ± 0.01^{dD}	100.67 ± 0.67^{dD}	20.47 ± 0.03^{dD}
	20~30	62.97 ± 1.57^{cC}	57.23 ± 1.38^{bAB}	135.80 ± 1.65^{cC}	4.14 ± 0.01^{cC}	5.96 ± 0.01^{eE}	124.00 ± 1.15^{abA}	12.43 ± 0.03^{cC}
Qian Dongwan (Native-wild)	0~10	42.11 ± 0.41^{dD}	58.11 ± 0.21^{dD}	125.30 ± 0.46^{eE}	5.44 ± 0.00^{bB}	6.97 ± 0.01^{aA}	140.00 ± 1.15^{bB}	29.70 ± 0.06^{bB}
	10~20	47.01 ± 0.05^{dD}	49.43 ± 0.44^{bB}	115.17 ± 0.23^{dD}	4.26 ± 0.02^{dD}	7.15 ± 0.01^{aA}	120.67 ± 1.76^{cC}	23.20 ± 0.06^{cC}
	20~30	53.47 ± 0.63^{dD}	45.23 ± 0.10^{dC}	97.04 ± 0.32^{eE}	3.96 ± 0.01^{dD}	7.10 ± 0.00^{aA}	262.00 ± 99.00^{aA}	25.90 ± 0.06^{aA}
Song Mingyan (Native-wild)	0~10	60.92 ± 0.45^{bB}	44.11 ± 0.34^{eE}	344.67 ± 0.90^{aA}	4.10 ± 0.00^{cC}	6.67 ± 0.01^{bB}	56.00 ± 1.15^{bB}	15.93 ± 0.09^{eE}
	10~20	61.93 ± 0.09^{bB}	43.45 ± 0.06^{cC}	259.80 ± 2.81^{aA}	3.99 ± 0.00^{eE}	6.78 ± 0.01^{bB}	44.67 ± 0.67^{eE}	12.50 ± 0.06^{eE}
	20~30	65.12 ± 0.11^{cBC}	37.44 ± 0.02^{eD}	244.97 ± 0.35^{aA}	1.41 ± 0.03^{eE}	6.87 ± 0.01^{bB}	50.00 ± 1.15^{bA}	16.77 ± 0.18^{bB}

Note: Within each column, values followed by different lowercase letters indicate statistically significant differences ($p < 0.05$) in soil nutrient parameters among sampling sites for the same soil depth, while different uppercase letters denote highly significant differences ($p < 0.01$).

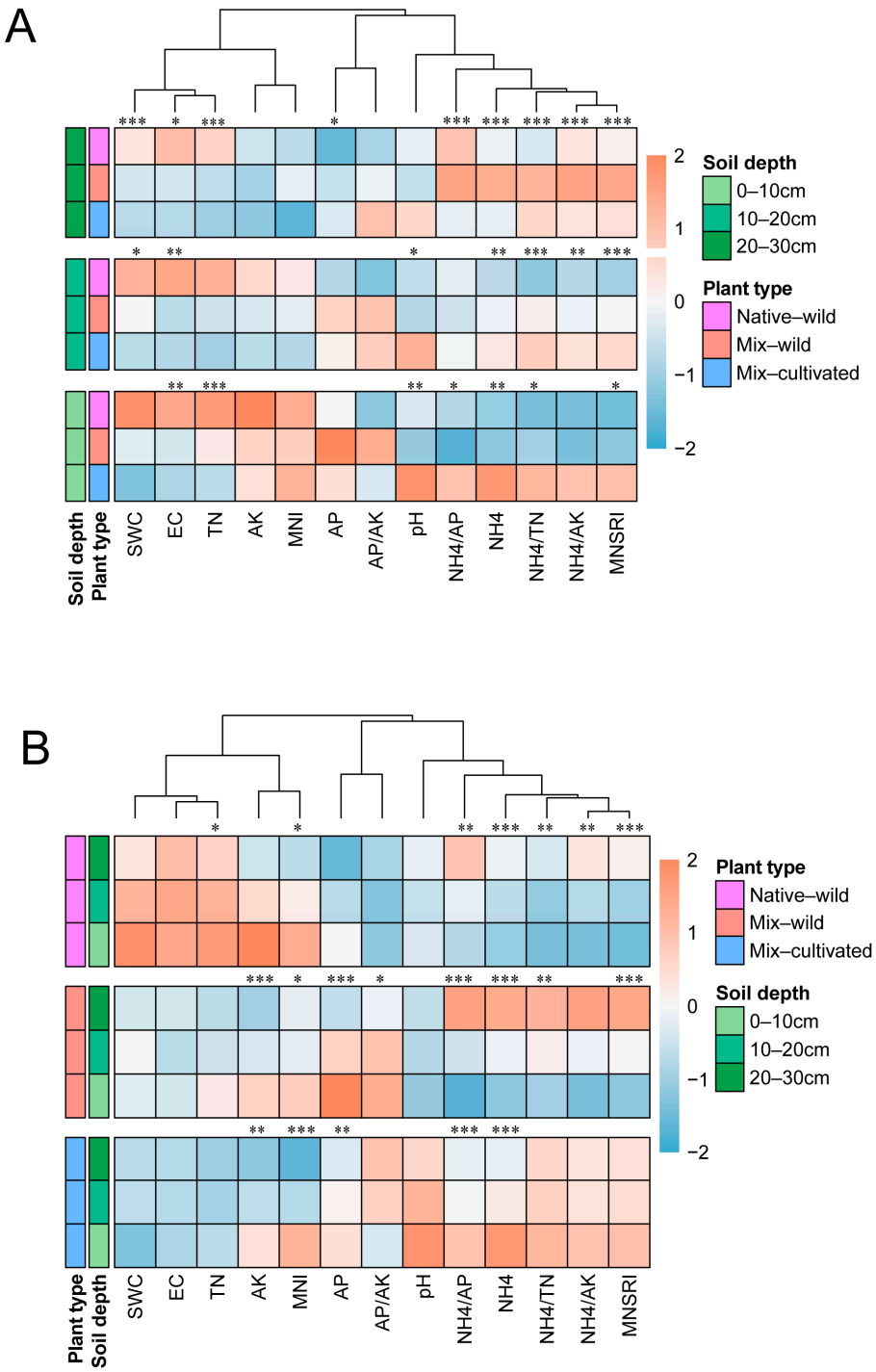


Figure 2. Soil properties of *Sinopodophyllum hexandrum* with different soil depths (**A**) and plant types (**B**) ($n = 3$). Values within the same column followed by asterisks indicate significant differences (ANOVA, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

3.2. Spatial Dynamics of Soil Microbial Diversity in Wild and Cultivated Sinopodophyllum hexandrum

To dissect how cultivation affects microbial communities across soil layers, microbial diversity analysis was performed. In terms of α-diversity (Shannon index, Richness index, and Phylogenetic Diversity (PD) index), the Mix-cultivated group showed significantly higher values than the Native-wild and Mix-wild groups (Figure 3A). Notably, no significant differences in α-diversity were observed across soil layers, indicating that cultivation had a stronger impact on microbial diversity than soil depth. Principal Coordinate Analysis (PCoA) further revealed distinct community separation patterns (Figure 3B). For bacterial

communities, the Native-wild group clustered with both the Mix-cultivated and Mix-wild groups, while the Mix-cultivated and Mix-wild groups were completely separated. PERMANOVA confirmed that plant type (R^2 = 0.087, $p < 0.001$) had a far greater effect on bacterial community composition than soil depth (R^2 = 0.008, $p > 0.05$). In terms of fungal communities, the Native-wild group clustered with the Mix-wild group, while the Mix-cultivated group was completely isolated from the two wild groups. PERMANOVA also showed that plant type (R^2 = 0.052, $p < 0.001$) exerted a stronger influence than soil depth (R^2 = 0.007, $p > 0.05$). The cumulative explanatory variation of the bacterial community (PCoA1 + 2 = 35.2%, plant type R^2 = 0.087) was higher than that of the fungal community (30.61%, plant type R^2 = 0.052). These results collectively demonstrated that plant type is the core driver of microbial community differentiation, and bacteria are significantly more sensitive to cultivation disturbance than fungi.

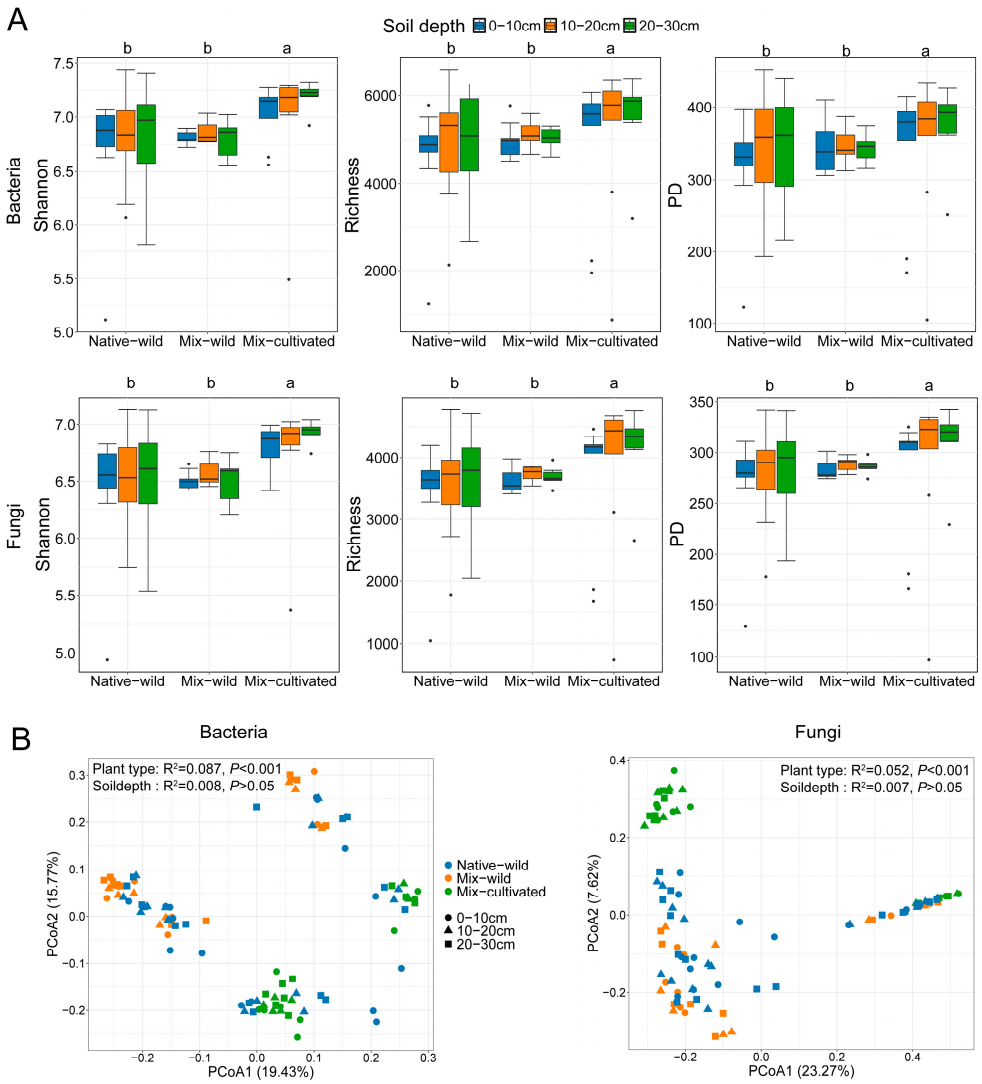


Figure 3. General response patterns of the diversities in the soil microbial communities of *Sinopodophyllum hexandrum* by plant type. (**A**) The boxplot of the differences of the alpha diversity indices of the bacterial and fungal communities between plant types (multiple comparison with Kruskal–Wallis tests, different letters above the plot indicate significant differences). (**B**) The plots of principal coordinates analysis (PCoA) based on Bray–Curtis distances showing all the bacterial and fungal compositional variation explained by plant type. Variance values were examined via PERMANOVA test.

3.3. Compositional Characteristics in Wild and Cultivated Sinopodophyllum hexandrum

The dominant taxa of bacterial and fungal communities were consistent across all groups, but their relative abundances varied significantly among the Native-wild, Mix-wild, and Mix-cultivated groups (Figure 4A). For bacteria, *Proteobacteria*, *Acidobacteriota*, and *Verrucomicrotiota* served as the core dominant phyla. *Proteobacteria* was most abundant in the Native-wild group (30%), lowest in the Mix-wild group (23%), and slightly recovered in the Mix-cultivated group (25%); *Acidobacteriota* showed a wild-low, cultivation-high trend, with the lowest abundance in the Native-wild group (21%) and similarly high levels in the Mix-wild (26%) and Mix-cultivated (26%) groups; *Verrucomicrotiota* was most concentrated in the Mix-wild group (12%), least in the Mix-cultivated group (6%), and intermediate in the Native-wild group (10%), indicating its high sensitivity to cultivation disturbance. Amongst fungi, the dominant phyla were *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*, with more pronounced inter-group differences in relative abundance. Ascomycota was highest in the Mix-cultivated group (62%), lowest in the Mix-wild group (45%), and intermediate in the Native-wild group (57%), suggesting artificial cultivation promotes its enrichment; *Basidiomycota* exhibited a wild-high, cultivation-low pattern, with the highest proportion in the Mix-wild group (38%), followed by the Native-wild group (22%), and only 11% in the Mix-cultivated group (making it the most distinctly differentiated fungal phylum); *Mortierellomycota* was most abundant in the Mix-cultivated group (21%), while the Native-wild (14%) and Mix-wild (12%) groups had similar abundances, reflecting its adaptation to artificially cultivated soil environments.

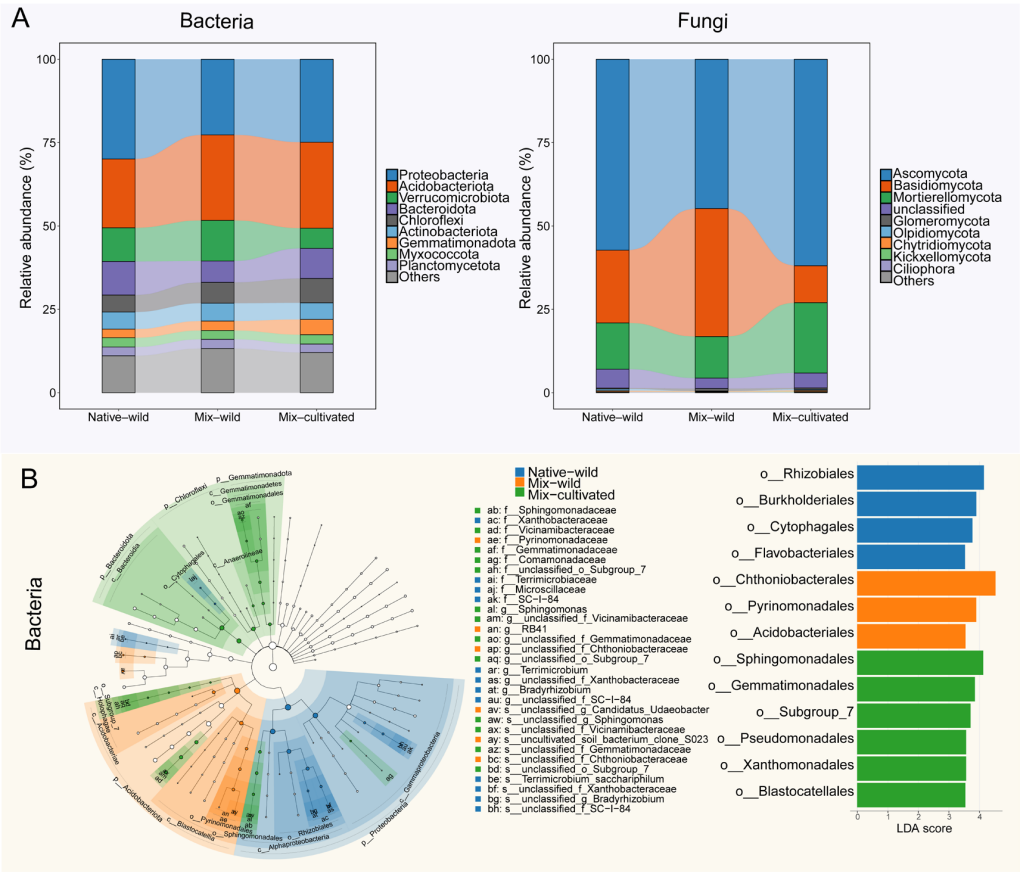


Figure 4. *Cont.*

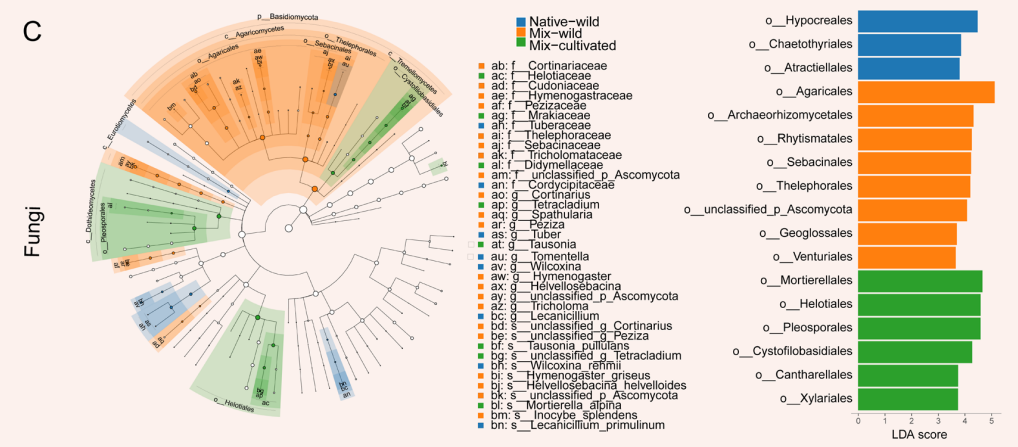


Figure 4. Phylogenetic tree, taxonomic composition, and distribution patterns of *Sinopodophyllum hexandrum*. (**A**) Taxonomic composition of the microbial communities among *Sinopodophyllum hexandrum* groups at the phylum level. LEfSe identifying the biomarker taxa of bacteria (**B**) and fungi (**C**) associated with three plant type groups.

Linear Discriminant Analysis Effect Size (LEfSe) analysis further clarified group-specific taxonomic enrichment patterns (Figure 4). At the phylum level, the Native-wild group was significantly enriched in *Proteobacteria* and *Basidiomycota*, the Mix-wild group in *Acidobacteriota* and *Basidiomycota*, and the Mix-cultivated group in *Bacteroidota* and *Gemmatimonadota*; at the bacterial order level (Figure 4B), *Rhizobiales*, *Chthoniobacterales*, and *Sphingomonadales* were the dominant enriched taxa in the Native-wild, Mix-wild, and Mix-cultivated groups, respectively; at the fungal order level (Figure 4C), the Native-wild group was enriched in *Hypocreales*, the Mix-wild group in *Agaricales*, and the Mix-cultivated group in *Mortierellales*.

3.4. Co-Occurrence Patterns of the Microbial Communities in Wild and Cultivated Sinopodophyllum hexandrum

Network analysis was used to explore differences in microbial interspecific interactions across groups. Topological properties showed that cultivation induced distinct changes in microbial network structure (Figure 5A). In terms of bacterial networks, compared with the Native-wild group, the Mix-wild group had a higher average degree (indicating more complex interspecific interactions), while the Mix-cultivated group had a lower average degree (simpler interactions). This suggested cultivation drove bacterial network complexity and depended on the host. In fungal networks, a similar trend was observed—Mix-wild networks were more complex, and Mix-cultivated networks were simpler. Node removal experiments further revealed network instability (Figure 5B): the natural connectivity of bacterial networks in the Mix-wild group and fungal networks in the Mix-cultivated group decreased more sharply than in other groups, indicating cultivation increased network vulnerability.

Correlation analysis between network properties and soil factors showed that only bacterial networks were significantly associated with soil variables ($p < 0.05$) (Figure 5C). pH and SWC were positively correlated with the average degree (AD), positive link number (PL), and negative link number (NL) of the Mix-wild bacterial network; AP/AK was positively correlated with AD, PL, and NL of the Native-wild bacterial network; and AK was negatively correlated with AD, PL, and NL of the Native-wild bacterial network. Fungal networks showed no significant correlations with soil factors, further confirming bacterial sensitivity to environmental changes.

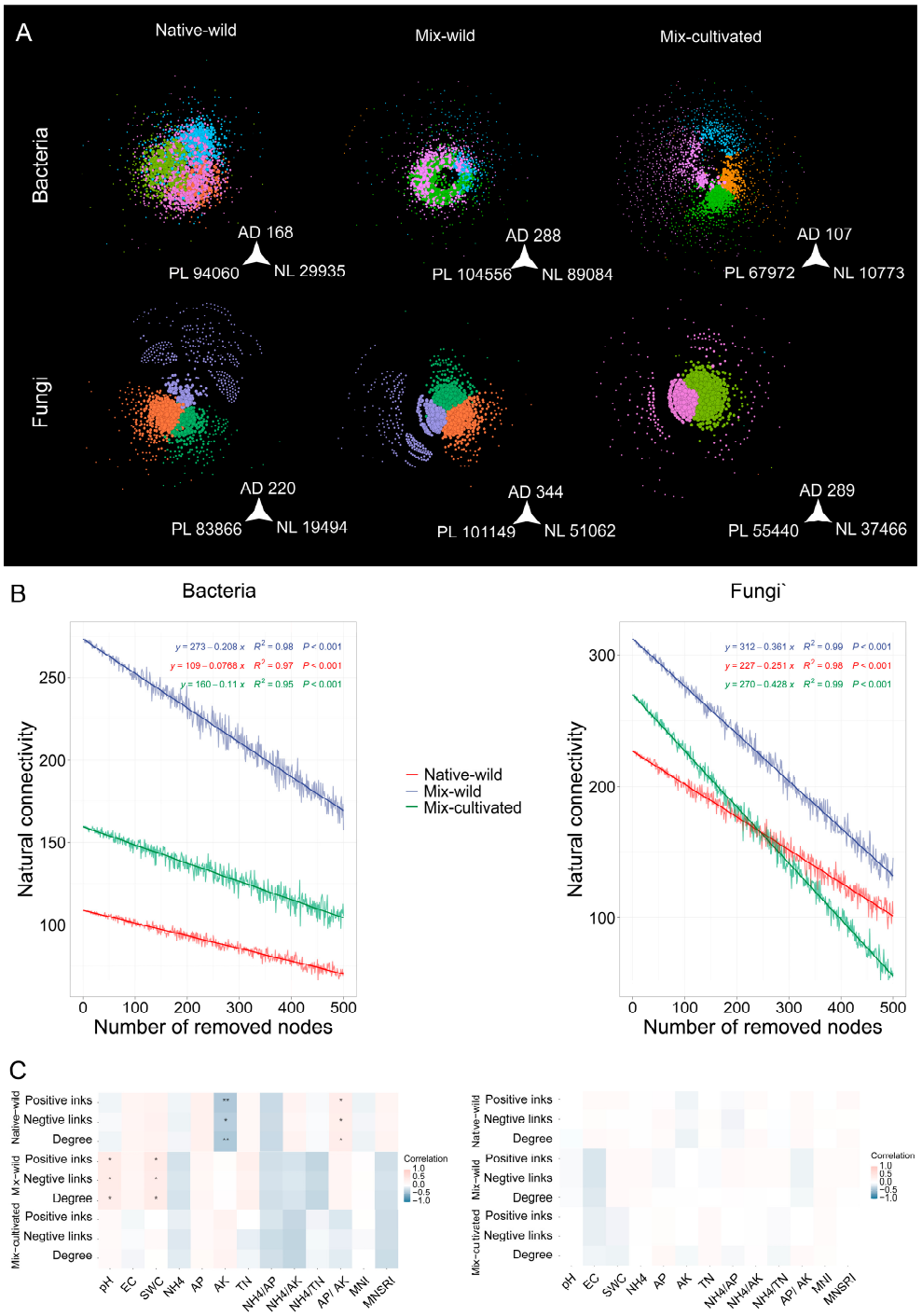


Figure 5. Variations in the co-occurrence patterns of soil microbial communities of *Sinopodophyllum hexandrum* by plant type. (**A**) The network diagram showing the relationship pattern of the bacterial and fungi communities with three plant types. The relationships between species showed that there was a strong correlation (the Spearman's correlation coefficient was 0.8) and significant (adjusted $p < 0.01$). The node size indicates the degree of the node. The average degree (AD), positive associations (PL) and negative associations (NL) in each network are displayed. (**B**) Network robustness of the bacterial and fungal communities with three plant types. Lines denote the ordinary least squares linear regression. (**C**) The Spearman correlations between the soil properties and the positive associations in the bacterial and fungal networks. * $p < 0.05$, ** $p < 0.01$.

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3.5. Potential Drivers of Soil Multi-Nutrient Cycling in Wild and Cultivated Sinopodophyllum hexandrum

To identify the key drivers of soil multi-nutrient cycling in wild and cultivated *Sinopodophyllum hexandrum*, Random Forest (RF) analysis was conducted (Figure 6). For the soil multi-nutrient cycling index across the vertical soil profile, the fungal Shannon index emerged as the primary predictor, followed by the bacterial Shannon index. However, the drivers of the multi-nutrient stoichiometric ratio index (MNSRI) varied among the three groups: fungal β-diversity was the key predictor in the Native-wild group, while the bacterial Shannon index and bacterial phylogenetic diversity (PD) dominated as drivers in the Mix-wild and Mix-cultivated groups, respectively.

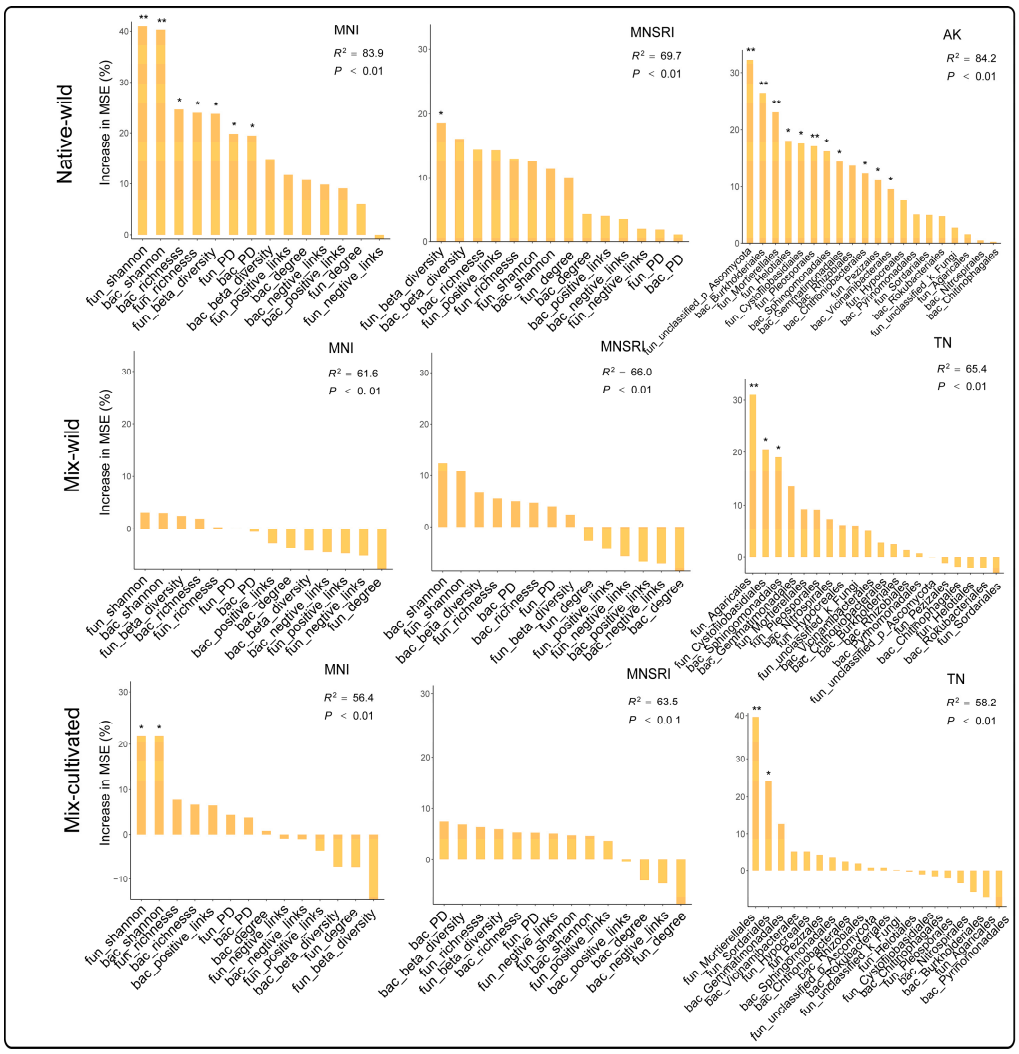


Figure 6. Potential drivers of variation in soil multi-nutrient cycling in *Sinopodophyllum hexandrum*. Random forest (RF) mean predictor importance (percentage of increase of mean square error) of microbial alpha-diversity, beta-diversity and network association indices as drivers for the soil multi-nutrient cycling index (MNI) and multi-nutrient stoichiometric ratio index (MNSRI) in the whole profile of three plant types. RF mean predictor importance (percentage of increase of mean square error) of dominant order as drivers for soil properties in the whole profile. Percentage increases in the MSE (mean squared error) of variables were used to estimate the importance of these predictors, and higher MSE% values imply more important predictors. Significance levels are as follows: * $p < 0.05$ and ** $p < 0.01$.

At the order level, distinct taxonomic drivers were observed for soil nutrients across groups ($p < 0.05$). Evidently, not all microbial orders contributed alike to the various edaphic variables among all three plants. For the soil nutrient associated with the most microorganisms, unclassified Ascomycota was the most important variable for predicting AK in Native-wild, while Agaricales and Mortierellales were the most important variable for predicting TN in Mix-wild and Mix-cultivated, respectively, indicating fungal importance in soil nutrient cycling after artificial cultivation. Other important variables for predicting soil properties (Figure S1) in Native-wild were the *Sphingomonadales*, *Pezizales* and *Helotiales* for AP, NH_4 and TN; in Mix-wild, the *Nitrospirales*, *Pleosporales* and *Pleosporales*, for AK, AP and NH_4, respectively; in Mix-cultivated, the *Rhizobiales*, *Vicinamibacterales* and *Pyrinomonadales*, for AK, AP and NH_4. These results confirm that under cultivation conditions, fungi became the dominant drivers of soil multi-nutrient cycling, with bacteria playing a supplementary role.

4. Discussion

4.1. Cultivation Disturbance Reshapes the Spatial and Vertical Heterogeneity Patterns of Soil Nutrients

The heterogeneous distribution of soil nutrients is the basis for plant–microbe interactions [29]. This study found that geographic factors and cultivation methods respectively drive the spatial and vertical heterogeneity of nutrients, which is highly consistent with the environmental resource filter [30]. In wild *S. hexandrum*, the adonis test confirmed that differences among sampling sites are the core source of spatial heterogeneity of soil nutrients. Different sampling sites form a "hotspot–coldspot" pattern through differentiated nutrient regulation. This may be related to differences in climate and vegetation types caused by geographical isolation. Similar studies have also found that geographic factors such as altitude and precipitation shape the spatial distribution pattern of nutrients by altering soil leaching [31,32].

Cultivation methods significantly disrupted the natural vertical nutrient pattern of wild *S. hexandrum*. This result is consistent with the general consensus that cultivation disturbance changes the vertical distribution of soil nutrients [33,34], but *S. hexandrum* exhibits unique responses. Notably, only five nutrient indicators showed significant differences among soil layers in the Mix-cultivated group, far fewer than in the wild groups. Additionally, high-nutrient indicators were enriched in Mix-wild in the 20–30 cm layer and in Mix-cultivated in the 0–20 cm layer. This indicates that cultivation not only changes the vertical distribution ratio of nutrients but also weakens the degree of vertical differentiation of soil nutrients under natural conditions. This may be caused by the surface retention effect of artificial fertilization, where compound fertilizers are difficult to migrate downward, leading to nutrient accumulation in the surface layer and insufficient nutrient supply in the deep layer [35]. In contrast, soils in uncultivated plots rely on plant residue decomposition and microbial transformation to form a more stable vertical gradient [36].

4.2. Specific Response of Microbial Communities to Cultivation Disturbance

Changes in microbial diversity and community structure are direct responses of soil ecosystems to disturbances. This study found that the response of *S. hexandrum* microbial communities to cultivation exhibits specificity that bacteria are significantly more sensitive than fungi. This differential sensitivity can be attributed to their distinct ecological strategies and physiological adaptations. Bacteria, as typical r-strategists, respond rapidly to changes in labile nutrient availability, leading to consistent shifts in community composition. In contrast, fungi, often as K-strategists with diverse trophic modes and resilient hyphal networks, possess a broader ecological niche and greater buffer capacity against disturbance.

This result provides a new perspective for understanding microbial regulation in the cultivation of medicinal plants.

In terms of α-diversity, the Shannon, Richness, and PD indices of the Mix-cultivated group were significantly higher than those of the wild groups, which is consistent with the results of studies on crop plants [37]. Artificial fertilization increases soil nutrient availability, providing sufficient carbon and nitrogen sources for heterotrophic microorganisms, thereby increasing community richness [38]. There was no significant difference in α-diversity among different soil layers, further confirming that the impact of cultivation methods on microorganisms is far greater than that of shallow soil depth. In addition, β-diversity analysis showed that the variation of the bacterial community was higher than that of the fungal community, suggesting that the response of bacterial communities to cultivation disturbance is more consistent. In contrast, fungi have a wider ecological niche (multiple trophic modes such as saprophytism, symbiosis, and parasitism), leading to more scattered responses to disturbances. This is similar to the findings in cultivated *Astragalus membranaceus*: bacterial community structure is more susceptible to artificial disturbances such as fertilization and tillage, while fungal communities are more affected by natural environments [39,40].

4.3. Adaptive Differentiation of Microbial Community Structure: From Natural Symbiosis to Artificial Adaptation

Wild and cultivated *S. hexandrum* have significant differences in soil microbial community structure and function, reflecting the functional adaptive evolution of communities to different habitats [41]. PCoA results showed complete separation of rhizosphere bacterial and fungal communities between wild and cultivated types, with a higher degree of differentiation in bacteria. LEfSe analysis revealed wild groups (Native-wild, Mix-wild) were mainly enriched with taxa adapted to natural environments, while the cultivated group was enriched with taxa adapted to artificial environments. At the bacterial order level, enrichment patterns further clarified habitat adaptation. Native-wild enriched Rhizobiales, containing nitrogen-fixing taxa like Ensifer, a key driver of nitrogen supply in soils in uncultivated plots [42]; Mix-wild enriched Chthoniobacterales, mostly involved in organic carbon decomposition, matching the plant residue-dominated carbon cycle in its soil [43]; Mix-cultivated enriched Sphingomonadales, tolerant to fertilizer-induced pH changes and increased available nutrients, with nitrogen release to adapt to cultivated nutrient demands [44,45]. At the fungal order level, Native-wild enriched Hypocreales, some taxa of which have biocontrol functions, helping wild *S. hexandrum* resist pathogens and enhance stress resistance [46]; Mix-wild enriched Agaricales, mostly ectomycorrhizal fungi, echoing Basidiomycota enrichment and emphasizing mycorrhizal symbiosis [47]; Mix-cultivated enriched Mortierellales, mainly saprophytic, relying on easily decomposable organic matter in artificial soils, consistent with the fungal functional shift from symbiosis to saprophytism [48,49].

LEfSe analysis clearly demonstrated a habitat–taxa–function correspondence, in which wild groups enriched nitrogen-fixing and mycorrhizal-related taxa adapted to natural oligotrophic environments, while the cultivated group enriched organic matter-degrading and fertilizer-tolerant taxa adapted to artificial high-nutrient environments. This specific enrichment not only confirms cultivation's role in remodeling microbial communities but also provides clear targets for analyzing microbial functional differentiation and screening dominant taxa for *S. hexandrum* cultivation.

4.4. Cultivation Disturbance Aggravates Microbial Network Instability

The topological structure of microbial networks reflects the complexity and stability of interspecific interactions within communities. This study found that artificial cultiva-

tion caused opposite changes in the complexity of microbial networks between wild and cultivated *S. hexandrum*, and fungal networks showed a similar trend. This result reveals the in-depth impact of cultivation disturbance on microbial interaction relationships [50]. Compared with Native-wild, the bacterial network of Mix-wild had a higher average degree (more complex interactions), while that of Mix-cultivated had a lower average degree (simpler interactions). This indicates that moderate disturbance, such as artificial protection or mild management of wild populations, may promote synergistic interactions between microorganisms, while severe disturbance, such as large-scale cultivation, simplifies the network structure [51,52]. This is partially consistent with the intermediate disturbance hypothesis, which states that moderate disturbance can increase community complexity, but excessive disturbance will break the balance [52].

In addition, node removal experiments showed that the natural connectivity of the bacterial network in Mix-wild and the fungal network in Mix-cultivated decreased more sharply, indicating that cultivation disturbance increases network vulnerability. This may be related to the replacement of dominant taxa, and stable symbiotic taxa in wild networks are replaced by taxa susceptible to environmental fluctuations in cultivated networks, leading to a decrease in network disturbance resistance [53]. At the same time, only bacterial networks were significantly correlated with soil factors (pH, SWC, AP/AK), further confirming that bacteria are more sensitive to environmental changes than fungi. This forms a causal echo with the previous result that bacterial diversity is more sensitive to cultivation, bacterial networks are more dependent on soil nutrient supply, while fungal networks may be regulated by other factors such as plant residue types and root exudates.

4.5. Fungi Become the Core Biological Drivers of Soil Multi-Nutrient Cycling Under Cultivation Conditions

The driving mechanism of soil multi-nutrient cycling is the key to soil–microbe interactions. Through random forest analysis, this study found that fungal diversity and specific taxa are the core predictors of multi-nutrient cycling, and cultivation significantly enhances the regulatory role of fungi. This finding breaks the traditional understanding that bacteria dominate soil nutrient cycling [54,55]. Specifically, the fungal Shannon index was the primary predictor of the soil vertical profile multi-nutrient cycling index of wild and cultivated *S. hexandrum*. This may be related to the unique role of fungi in organic matter decomposition and nutrient transformation: in soils in uncultivated plots, ectomycorrhizal fungi *Tomentella* promote nitrogen and phosphorus absorption through symbiosis with plants [56]; in cultivated soils, saprophytic fungi *Mortierella* accelerate carbon and nitrogen release by decomposing easily soluble organic matter remaining from fertilization, thereby becoming the engine of nutrient cycling [57]. In addition, there were differences in nutrient-driving taxa among different cultivation types: unclassified *Ascomycota* predicted AK in Native-wild, *Agaricales* predicted TN in Mix-wild, and *Mortierellales* predicted TN in Mix-cultivated. This indicates that cultivation disturbance reshapes the biological driving mechanism of nutrient cycling.

Notably, bacterial taxa such as *Rhizobiales* and *Vicinamibacterales* in the cultivated group had significant predictive effects on AK and AP, indicating that bacteria are still indispensable in specific nutrient transformations. For example, the nitrogen-fixing function of *Rhizobiales* can supplement nitrogen consumption in cultivated soils [58], and *Vicinamibacterales* are involved in phosphorus activation [44]. Together with fungi, they form a nutrient cycling network in cultivated soils. This result provides targets for improving nutrient use efficiency of cultivated *S. hexandrum* through microbial regulation.

5. Conclusions

This study investigated the effects of artificial cultivation on *Sinopodophyllum hexandrum* by comparing wild (Native-wild, Mix-wild) and cultivated (Mix-cultivated) groups. Key findings are as follows: Geographic factors and cultivation respectively drove soil nutrients' spatial and vertical heterogeneity. Cultivation broke the wild plants' natural vertical nutrient pattern via surface fertilization, causing nutrient surface retention and reduced vertical differentiation. Microbial communities showed cultivation-specific responses (bacteria more sensitive than fungi). Cultivation caused obvious changes in microbial network complexity and increased instability. For multi-nutrient cycling, fungal diversity and taxa became core drivers, breaking the notion that bacteria dominate nutrient cycling. Overall, this study clarifies cultivation's regulatory mechanism on the *S. hexandrum* soil–microorganism–nutrient system, providing a basis for optimizing cultivation and protecting this endangered medicinal plant. Future research should focus on long-term system dynamics and in situ verification of microbial functions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d18010024/s1. Figure S1: Potential drivers of variation in soil multi-nutrient cycling in *Sinopodophyllum hexandrum*.

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

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References

1. Sharma, N.; Thakur, M.; Sharma, P.; Dutt, B.; Sharma, Y.P. In vitro propagation from seeds and enhanced synthesis of podophyllotoxin from root callus of *SinoPodophyllum hexandrum* Royle TS Ying (*Himalayan mayapple*)—An endangered medicinal plant. *Ind. Crops Prod.* **2022**, *186*, 115300. [CrossRef]
2. Zhao, Q.; Dong, M.; Li, M.; Jin, L.; Paré, P.W. Light-induced flavonoid biosynthesis in *Sinopodophyllum hexandrum* with high-altitude adaptation. *Plants* **2023**, *12*, 575. [CrossRef]
3. Wang, Y.; Zhang, G.; Chi, X.; Chen, S. Green and efficient extraction of podophyllotoxin from *Sinopodophyllum hexandrum* by optimized subcritical water extraction combined with macroporous resin enrichment. *Ind. Crops Prod.* **2018**, *121*, 267–276. [CrossRef]
4. Xiao, M.; Li, Q.; Wang, L.; Guo, L.; Li, J.; Tang, L.; Chen, F. ISSR analysis of the genetic diversity of the endangered species *Sinopodophyllum hexandrum* (Royle) Ying from Western Sichuan Province, China. *J. Integr. Plant Biol.* **2006**, *48*, 1140–1146. [CrossRef]
5. Pang, Z.; Tayyab, M.; Kong, C.; Liu, Q.; Liu, Y.; Hu, C.; Huang, J.; Weng, P.; Islam, W.; Lin, W.; et al. Continuous Sugarcane Planting Negatively Impacts Soil Microbial Community Structure, Soil Fertility, and Sugarcane Agronomic Parameters. *Microorganisms* **2021**, *9*, 2008. [CrossRef] [PubMed]

6. Zhang, S. Heterogeneity of soil nutrients: A review of methodology, variability and impact factors. *J. Environ. Earth Sci.* **2019**, *1*, 6–28. [CrossRef]
7. Wu, Z.; Zhou, Y.; Wang, M. Spatial differentiation and influencing factors of effective phosphorus in cultivated soil in the water source area of the mid-route of South-to-North water transfer project. *Front. Microbiol.* **2024**, *15*, 1463291.
8. Sun, R.; Li, W.; Dong, W.; Tian, Y.; Hu, C.; Liu, B. Tillage Changes Vertical Distribution of Soil Bacterial and Fungal Communities. *Front. Microbiol.* **2018**, *9*, 699. [CrossRef]
9. Singh, M.; Sidhu, H.; Humphreys, E.; Thind, H.; Jat, M.; Blackwell, J.; Singh, V. Nitrogen management for zero till wheat with surface retention of rice residues in north-west India. *Field Crops Res.* **2015**, *184*, 183–191. [CrossRef]
10. Zhang, Y.; Zheng, Y.; Xia, P.; Xun, L.; Liang, Z. Impact of continuous *Panax notoginseng* plantation on soil microbial and biochemical properties. *Sci. Rep.* **2019**, *9*, 13205. [CrossRef] [PubMed]
11. Xu, X.; Ouyang, H.; Cao, G.; Richter, A.; Wanek, W.; Kuzyakov, Y. Dominant plant species shift their nitrogen uptake patterns in response to nutrient enrichment caused by a fungal fairy in an alpine meadow. *Plant Soil* **2011**, *341*, 495–504. [CrossRef]
12. Xiao, C.; Yang, L.; Zhang, L.; Liu, C.; Han, M. Effects of cultivation ages and modes on microbial diversity in the rhizosphere soil of Panax ginseng. *J. Ginseng Res.* **2016**, *40*, 28–37. [CrossRef]
13. Dong, L.; Li, Y.; Xu, J.; Yang, J.; Wei, G.; Shen, L.; Ding, W.; Chen, S. Biofertilizers regulate the soil microbial community and enhance Panax ginseng yields. *Chin. Med.* **2019**, *14*, 20. [CrossRef] [PubMed]
14. Zhong, C.; Chen, C.; Gao, X.; Tan, C.; Bai, H.; Ning, K. Multi-omics profiling reveals comprehensive microbe–plant–metabolite regulation patterns for medicinal plant *Glycyrrhiza uralensis* Fisch. *Plant Biotechnol. J.* **2022**, *20*, 1874–1887. [CrossRef]
15. Barberán, A.; Bates, S.T.; Casamayor, E.O.; Fierer, N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J.* **2012**, *6*, 343–351. [CrossRef]
16. Nguyen, B.-A.T.; Chen, Q.-L.; Yan, Z.-Z.; Li, C.; He, J.-Z.; Hu, H.-W. Distinct factors drive the diversity and composition of protistan consumers and phototrophs in natural soil ecosystems. *Soil Biol. Biochem.* **2021**, *160*, 108317. [CrossRef]
17. 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*, e00074-18. [CrossRef]
18. Xu, S.; Rong, Z.; Ma, H.; Xie, J.; Wang, F. Study on Terrestrial Wild Vertebrate Diversity and Geographical Fauna in Qinghai Area of Qilian Mountain National Park, China. *Sustainability* **2022**, *14*, 16452. [CrossRef]
19. Wei, T.; Bu, L.; Wu, D.; Chen, H.; Gao, D.; Liu, H.; Xu, H.; Cai, Y.; Yang, J.; Guo, P. Temporal and spatial characteristics of soil nutrients under different artificial vegetation types in Yuzhong Basin, Gansu Province. *J. Henan Agric. Sci.* **2021**, *50*, 79. [CrossRef]
20. Zhang, Z.; Hu, B.; Hu, G. Spatial heterogeneity of soil chemical properties in a subtropical karst forest, Southwest China. *Sci. World J.* **2014**, *2014*, 473651. [CrossRef]
21. Bao, S. *Soil Agrochemical Analysis*; China Agriculture Press: Beijing, China, 2000.
22. Dixon, P. VEGAN, a package of R functions for community ecology. *J. Veg. Sci.* **2003**, *14*, 927–930. [CrossRef]
23. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic biomarker discovery and explanation. *Genome Biol.* **2011**, *12*, R60. [CrossRef]
24. Csardi, G.; Nepusz, T. The igraph software. *Complex Syst.* **2006**, *1695*, 1–9.
25. Bastian, M.; Heymann, S.; Jacomy, M. Gephi: An open source software for exploring and manipulating networks. In Proceedings of the International AAAI Conference on Web and Social Media, Los Angeles, CA, USA, 17–20 May 2009; pp. 361–362.
26. Ma, B.; Wang, H.; Dsouza, M.; Lou, J.; He, Y.; Dai, Z.; Brookes, P.C.; Xu, J.; Gilbert, J.A. Geographic patterns of co-occurrence network topological features for soil microbiota at continental scale in eastern China. *ISME J.* **2016**, *10*, 1891–1901. [CrossRef] [PubMed]
27. Archer, E. *rfPermute: Estimate Permutation p-Values for Random Forest Importance Metrics*; Version 2.5.5; NOAA: Washington, DC, USA, 2016; Volume 1.
28. Fortmann-Roe, S. Consistent and clear reporting of results from diverse modeling techniques: The A3 method. *J. Stat. Softw.* **2015**, *66*, 1–23. [CrossRef]
29. Wang, L.; Liao, D.; Rengel, Z.; Shen, J. Soil–plant–microbe interactions in the rhizosphere: Incremental amplification induced by localized fertilization. *Front. Agric. Sci. Eng.* **2025**, *12*, 57–68. [CrossRef]
30. Laliberté, E.; Zemunik, G.; Turner, B.L. Environmental filtering explains variation in plant diversity along resource gradients. *Science* **2014**, *345*, 1602–1605. [CrossRef]
31. Cui, Y.; Fang, L.; Deng, L.; Guo, X.; Han, F.; Ju, W.; Wang, X.; Chen, H.; Tan, W.; Zhang, X. Patterns of soil microbial nutrient limitations and their roles in the variation of soil organic carbon across a precipitation gradient in an arid and semi-arid region. *Sci. Total Environ.* **2019**, *658*, 1440–1451. [CrossRef] [PubMed]
32. Wang, H.; Shi, X.; Yu, D.; Weindorf, D.C.; Huang, B.; Sun, W.; Ritsema, C.J.; Milne, E. Factors determining soil nutrient distribution in a small-scaled watershed in the purple soil region of Sichuan Province, China. *Soil Tillage Res.* **2009**, *105*, 300–306. [CrossRef]

33. Massey, P.; Creamer, R.; Schulte, R.; Whelan, M.; Ritz, K. The effects of earthworms, botanical diversity and fertiliser type on the vertical distribution of soil nutrients and plant nutrient acquisition: A mesocosm study. *Biol. Fertil. Soils* **2013**, *49*, 1189–1201.
34. Jobbagy, E.G.; Jackson, R.B. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry* **2001**, *53*, 51–77. [CrossRef]
35. Lei, N.; Zhang, J.; Zhang, J.; Sun, Z.; Wang, Z. Nutrient accumulation and fertilizer potential of terrace section soils in loess areas. *Sci. Rep.* **2025**, *15*, 21229. [CrossRef]
36. Thorup-Kristensen, K.; Halberg, N.; Nicolaisen, M.; Olesen, J.E.; Crews, T.E.; Hinsinger, P.; Kirkegaard, J.; Pierret, A.; Dresbøll, D.B. Digging deeper for agricultural resources, the value of deep rooting. *Trends Plant Sci.* **2020**, *25*, 406–417. [CrossRef]
37. Shenton, M.; Iwamoto, C.; Kurata, N.; Ikeo, K. Effect of Wild and Cultivated Rice Genotypes on Rhizosphere Bacterial Community Composition. *Rice* **2016**, *9*, 42. [CrossRef]
38. Wang, Q.; Jiang, X.; Guan, D.; Wei, D.; Zhao, B.; Ma, M.; Chen, S.; Li, L.; Cao, F.; Li, J. Long-term fertilization changes bacterial diversity and bacterial communities in the maize rhizosphere of Chinese Mollisols. *Appl. Soil Ecol.* **2018**, *125*, 88–96. [CrossRef]
39. Ai, C.; Zhang, S.; Zhang, X.; Guo, D.; Zhou, W.; Huang, S. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma* **2018**, *319*, 156–166. [CrossRef]
40. Liang, J.-P.; Xue, Z.-Q.; Yang, Z.-Y.; Chai, Z.; Niu, J.-P.; Shi, Z.-Y. Effects of microbial organic fertilizers on *Astragalus membranaceus* growth and rhizosphere microbial community. *Ann. Microbiol.* **2021**, *71*, 11. [CrossRef]
41. Arnold, B.J.; Huang, I.-T.; Hanage, W.P. Horizontal gene transfer and adaptive evolution in bacteria. *Nat. Rev. Microbiol.* **2022**, *20*, 206–218.
42. Pongsilp, N.; Nimnoi, P. Inoculation of *Ensifer fredii* strain LP2/20 immobilized in agar results in growth promotion and alteration of bacterial community structure of Chinese kale planted soil. *Sci. Rep.* **2020**, *10*, 15857. [CrossRef] [PubMed]
43. Rakitin, A.L.; Kulichevskaya, I.S.; Beletsky, A.V.; Mardanov, A.V.; Dedysh, S.N.; Ravin, N.V. *Verrucomicrobia* of the family *Chthoniobacteraceae* participate in xylan degradation in boreal peat soils. *Microorganisms* **2024**, *12*, 2271. [CrossRef]
44. Ackerly, D.D. Community assembly, niche conservatism, and adaptive evolution in changing environments. *Int. J. Plant Sci.* **2003**, *164*, S165–S184. [CrossRef]
45. Yan, C.; Zhao, S.; Wu, H.; Yang, D.; Hou, Z.; Shan, F.; Yan, S.; Lyu, X.; Ma, C.; Gong, Z. Nitrogen release and microbial community characteristics during the decomposition of field-incorporated corn straw in Northeast China. *Land Degrad. Dev.* **2024**, *35*, 3897–3910. [CrossRef]
46. Ownley, B.H.; Gwinn, K.D.; Vega, F.E. Endophytic fungal entomopathogens with activity against plant pathogens: Ecology and evolution. *BioControl* **2010**, *55*, 113–128. [CrossRef]
47. Cai, Q.; Codjia, J.E.I.; Buyck, B.; Cui, Y.-Y.; Ryberg, M.; Yorou, N.S.; Yang, Z.L. The evolution of ectomycorrhizal symbiosis and host-plant switches are the main drivers for diversification of *Amanitaceae* (*Agaricales*, *Basidiomycota*). *BMC Biol.* **2024**, *22*, 230. [CrossRef]
48. Li, F.; Chen, L.; Redmile-Gordon, M.; Zhang, J.; Zhang, C.; Ning, Q.; Li, W. Mortierella elongata's roles in organic agriculture and crop growth promotion in a mineral soil. *Land Degrad. Dev.* **2018**, *29*, 1642–1651.
49. Tamayo-Vélez, Á.; Osorio, N.W. Soil fertility improvement by litter decomposition and inoculation with the fungus *Mortierella* sp. in avocado plantations of Colombia. *Commun. Soil Sci. Plant Anal.* **2018**, *49*, 139–147. [CrossRef]
50. Li, W.; Xie, L.; Xu, Y.; Yang, M. Effect of mixed planting on soil nutrient availability and microbial diversity in the rhizosphere of *Parashorea chinensis* plantations. *Front. Microbiol.* **2024**, *15*, 1464271. [CrossRef] [PubMed]
51. Vera, A.; Moreno, J.; Siles, J.; López-Mondejar, R.; Zhou, Y.; Li, Y.; García, C.; Nicolás, E.; Bastida, F. Interactive impacts of boron and organic amendments in plant-soil microbial relationships. *J. Hazard. Mater.* **2021**, *408*, 124939. [CrossRef] [PubMed]
52. Mai, S.; Mao, H.; Jiang, Y.; Huang, T.; Yang, Q.; Xing, G.; Wang, X.; Yang, H.; Liu, W. Characteristics of the soil arbuscular mycorrhizal fungal community along succession stages in tropical forest and its driving factors. *Front. Environ. Sci.* **2023**, *10*, 1110655. [CrossRef]
53. Agler, M.T.; Ruhe, J.; Kroll, S.; Morhenn, C.; Kim, S.-T.; Weigel, D.; Kemen, E.M. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *PLoS Biol.* **2016**, *14*, e1002352.
54. Zhou, X.; Chen, X.; Qi, X.; Zeng, Y.; Guo, X.; Zhuang, G.; Ma, A. Soil bacterial communities associated with multi-nutrient cycling under long-term warming in the alpine meadow. *Front. Microbiol.* **2023**, *14*, 1136187.
55. Dai, Z.; Su, W.; Chen, H.; Barberán, A.; Zhao, H.; Yu, M.; Yu, L.; Brookes, P.C.; Schadt, C.W.; Chang, S.X.; et al. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of *Actinobacteria* and *Proteobacteria* in agro-ecosystems across the globe. *Glob. Change Biol.* **2018**, *24*, 3452–3461.
56. Tang, P.; Ning, C.; Liu, T.; Tan, Z.; Wang, X.; Liu, S.; Yan, W. Impact of nitrogen enrichment on the functions of extracellular enzymes in ectomycorrhizal community and nutrient status of pine seedlings. *Appl. Soil Ecol.* **2024**, *202*, 105576. [CrossRef]

57. Ozimek, E.; Hanaka, A. *Mortierella* species as the plant growth-promoting fungi present in the agricultural soils. *Agriculture* **2020**, *11*, 7. [CrossRef]
58. Hu, W.; Wang, X.; Xu, Y.; Wang, X.; Guo, Z.; Pan, X.; Dai, S.; Luo, Y.; Teng, Y. Biological nitrogen fixation and the role of soil diazotroph niche breadth in representative terrestrial ecosystems. *Soil Biol. Biochem.* **2024**, *189*, 109261.

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