forests

MDPI

Article

The Effects of Korean Pine and Manchurian Walnut Monocultures and Mixed Plantations on Soil Fungal and Bacterial Communities

Fangyuan Shen [1,†], Ning Liu [1,†], Yujiao Wang [2], Huifeng Liu [3], Haikuan Jia [4] and Lixue Yang [1,*]

[1] Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, Engineering and Technology Research Centre for Northeast Native Tree Species-National Forestry and Grassland Administration, School of Forestry, Northeast Forestry University, Harbin 150040, China; lning0913@163.com (N.L.)
[2] The Third Geological and Mineral Exploration Institute of Gansu Bureau of Geology and Mineral Resources, Lanzhou 730050, China; 18893914939@163.com
[3] Forestry and Agriculture of Academy in Daxing'an Mountains, Jiagedaqi 165000, China; lhf-515@163.com
[4] Honghua Erji Forestry Bureau National Pinus Sylvestris Fine Variety Base, Hulunbuir 021112, China; m15248746298@163.com
* Correspondence: yanglixue@nefu.edu.cn
† These authors contributed equally to this work.

Abstract: (1) Background: Korean pine (*Pinus koraiensis*) and Manchurian walnut (*Juglans mandshurica*) are the main tree species for plantation regeneration in Northeast China, and the mixed plantation of them is one of the typical measures adopted to address the decline in stand productivity in long-term monocultures. However, little is known about the effects of Korean pine and Manchurian walnut monocultures and mixed plantations on soil microbial diversity, composition, and functional groups. (2) Methods: We used ITS and 16S rRNA gene sequencing to detect fungal and bacterial communities and used the FUNGuild, FAPROTAX, and Bugbase databases to predict their functional groups. (3) Results: Fungal and bacterial alpha diversity were always higher in Manchurian walnut monocultures than in Korean pine monocultures. The plantation type had a greater impact on the fungal composition than the bacterial composition. The fungal functional groups were significantly affected by the plantation type ($p < 0.05$), while the bacterial functional groups were barely changed among all plantation types. The soil available nutrient content was the most important soil factor in shaping the microbial community structures and functional groups. (4) Conclusions: Shifts in fungal community compositions and functional groups might play a dominant role in soil nutrient cycling across the different plantation types in Northeast China.

Keywords: Korean pine; Manchurian walnut; fungi; bacteria; community structure; functional groups; soil factors

Citation: Shen, F.; Liu, N.; Wang, Y.; Liu, H.; Jia, H.; Yang, L. The Effects of Korean Pine and Manchurian Walnut Monocultures and Mixed Plantations on Soil Fungal and Bacterial Communities. *Forests* **2023**, *14*, 1594. https://doi.org/10.3390/f14081594

Academic Editor: Lei Deng

Received: 12 June 2023
Revised: 10 July 2023
Accepted: 3 August 2023
Published: 6 August 2023

1. Introduction

The mixed-broadleaf Korean pine (*Pinus koraiensis*) forest makes up the largest forest area in Northeast China. However, large-scale afforestation was implemented half a century ago to meet the economic and ecological needs, since the acreage of natural forest had declined sharply due to historic forest exploitation [1]. Korean pine and Manchurian walnut (*Juglans mandshurica*) are the main tree species for plantation regeneration, and the mixed cultivation of these two species is one of the most common afforestation strategies to cope with the decline in stand productivity in long-term monocultures. Soil microorganisms play important roles in regulating aboveground vegetation dynamics and productivity in forest ecosystems [2]. The relationship between dominant tree species and soil microbial biodiversity is a central question in ecological research [3]. Plantation ecosystems provide convenience for the study of the effects of different plantation types on soil microbial

communities. The changes in the plantation type exert their influence in shaping corresponding microbial communities directly or indirectly through litter decomposition [4], root exudates [5], and soil properties [6,7]. Currently, we still have a limited understanding of the characteristics of the soil microbial communities across Korean pine and Manchurian walnut monocultures and mixed plantations.

As the most abundant soil organisms in soil, fungi and bacteria play both overlapping and disparate roles in soil biogeochemical processes [8], such as the mineralization of soil organic matter [9], nutrient transport [10], and humus decomposition [11]. Fungi-dominated systems are often characterized by slow-growing vegetation, while bacteria-dominated systems usually drive rapid nutrient cycling, supporting the growth of fast-growing vegetation [12,13]. On the one hand, the aboveground dominant tree species plays an important role in determining the distribution of fungal- or bacteria-dominant taxa [14]. Soil conditions, on the other hand, are fundamental factors in shaping the community structures of fungi or bacteria [15]. The physicochemical properties within soil vary widely across different plantation types, affecting the community structures of microbial inhabitants. For example, the soil pH could explain a large proportion of the variance in the soil bacterial composition and diversity at a global scale [16,17]. Bacterial richness might be greatest in midlatitude soils with an approximately neutral pH and relatively high soil carbon (C)-to-nitrogen (N) ratio [15]. In addition, the expression patterns of the fungal functional traits are predominantly linked to differences in the soil C:N ratio and soil moisture [18]. By contrast, the presence of bacterial functions is also mainly driven by the soil pH [15]. At the regional or local scale, however, the identity of the dominant tree species has a crucial effect in determining the quality and quantity of substrates entering the soil [19]. Thus, changes in the microbial community could be driven by the availability of soil nutrients. In particular, the availability of soil C, N, and phosphorus (P) could constrain the number of stably coexisting species of the microbial community based on the principle of competitive exclusion [20], which further affects the microbial functional traits.

Given that different organisms have varying impacts on elemental cycling [13], exploring the changes in microbial functional groups may contribute to revealing the dominant role of fungi or bacteria across Korean pine and Manchurian walnut monocultures and mixed plantations. In this study, we tried to reveal the effects of different plantation types on fungi and bacteria in the community composition, structure, and functional groups. At the same time, we wished to determine the key soil variables that significantly drive the changes in microbial community structures and functional groups. We hypothesized that (1) Manchurian walnut monocultures would promote soil fungal and bacterial diversity given a favorable microenvironment for fungi and bacteria due to easily decomposing litter compared with conifer plantations; (2) fungi and bacteria would show contrasting responses to different plantation types in terms of diversity, community structure, and functional groups; (3) the available nutrient content might be the most important factor in shaping the fungal and bacterial community structures and functional groups among all the soil properties.

2. Materials and Methods

2.1. Study Area

Research was conducted at the National Maoershan Forest Ecosystem Research Station (127°36′~127°39′ E, 45°23′~45°26′ N) of the Northeast Forestry University, Shangzhi city, Heilongjiang Province, China. The area of our study site is characterized by a cold, temperate, continental, and monsoon climate. The study area has a mean annual temperature of 2.8 °C, a minimum temperature of −40.9 °C (January), and a maximum temperature of 34.2 °C (July), with a frost-free period of about 120–140 days. The average annual precipitation and evaporation are 723 mm and 1093 mm, respectively. The average slope in the area is 15°. The secondary forest is mainly composed of *P. koraiensis* mixed with other species, such as *L. gmelinii*, *J. mandshurica*, *Quercus mongolica*, *Fraxinus mandshurica*, and *Betula platyphylla*. The soil is Hap-Boric Luvisol, and the parent material is granite bedrock.

2.2. Experimental Design and Soil Sampling

Mixed cultivation was performed by the alternate mixing of two "belts". One "belt" consisted of five lines of Korean pine, and another "belt" consisted of three lines of Manchurian walnut. In the Spring of 1987, Korean pine and Manchurian walnut mixed plantations and their respective monocultures were established with 2-year-old seedlings. The inter-row and intra-row spaces were set as 2 m × 1.5 m for all three plantation types. All plantation types had the same tending measures after afforestation.

Three representative standard plots (0.06 ha) for each plantation type mentioned above were randomly selected to maximize the inclusion of all herb types in July 2019. We surveyed the diameter at breast height (DBH) and tree height in each plot and calculated the stand volume according to the local standard volume model (Table S1).

After removing the forest floor layer and litter materials, soil cores of 15 cm depth were collected from 20–25 sampling points by a soil auger (5 diameters) along an S-shaped line in each plot. Soil cylinders from the same plot were mixed into a composite sample. Soil samples were placed in a sterilized plastic bag, sealed, and stored in an icebox, and then transported to the laboratory immediately. The fresh soil samples were sieved through a 2 mm sterilized sieve to remove visible roots, rocks, and other residues. After sifting the fresh soil samples, some of the subsamples were freeze-dried and stored at −80 °C before the DNA extraction, while some of the subsamples were stored at 4 °C for the determination of the available nitrogen content, and the rest were air-dried for other physicochemical analyses.

2.3. Soil Physical and Chemical Analysis

We measured the soil pH using a digital pH meter (MT-5000, Shanghai, China) after oscillation in a soil-water (1:5 w/v) suspension for half an hour. The cutting ring method was used to determine the soil moisture (SM), bulk density (BD), and total porosity (STP). After wet digestion with $HClO_4$ and H_2SO_4, we analyzed the content of soil total phosphorus (TP) using a spectrophotometer (TU-1901, Puxi Ltd., Beijing, China) following the method of molybdenum blue colorimetry. We determined the content of soil total nitrogen (TN) and total carbon (TC) by tableting air-dried soil samples with a J200 Tandem laser spectroscopic element analyzer (Applied Spectra, Inc., Fremont, CA, USA). We measured the soil organic carbon (SOC) content following the method of dichromate oxidation. The content of soil ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N) was determined using a continuous flow analytical system (AA3, Seal Co., Freigericht, Germany) after leaching with KCl solution. The content of soil available phosphorus (AP) and available potassium (AK) was determined by the sodium bicarbonate method and flame photometric method, respectively.

2.4. DNA Extraction and PCR Amplification

Soil fungal and bacterial DNA was extracted from soil samples by using an E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, USA) and following the manufacturer's protocols. The concentration and purity of DNA solutions were assessed by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). The quality and quantity of DNA were verified with 1.0% (w/v) agarose gel at a voltage of 5 V and a period of 20 min.

Once the quantity and quality of DNA met the requirements for amplification, the fungal ITS and bacterial 16S genes were amplified. For soil fungi, the primer sets ITS3F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) were used to amplify the ITS2 region. The bacterial 16S rRNA gene was amplified using the primer sets 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reaction was prepared in a 20 μL mixture consisting of 2 μL of 2.5 mM dNTPs, 0.4 μL of FastPfu Polymerase (TransStart FastPfu DNA Polymerase, TransGen, Beijing, China), 0.8 μL of each primer and 10 ng of template DNA (sterile water supplement total system 20 μL), and 4 μL of 5 × FastPfu Buffer. The PCR was performed with the following

procedure: 3 min at 95 °C (denaturation), 30 cycles of 30 s at 95 °C, 30 s at 55 °C (annealing), 45 s at 72 °C (elongation), 10 min at 72 °C (extension).

2.5. Illumina Sequencing and Sequence Data Processing

PCR products were detected by 2% agarose gel electrophoresis. Then, the AxyPrepDNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was applied for polymerization and purification. Next, the PCR products were quantified using a QuantiFluorTM-ST fluorometer (Promega, Madison, WI, USA), following the manufacturer's instructions. The purified amplicons were subjected to paired-end sequencing in equal amounts using the Illumina MiSeq sequencing platform. The sequencing reads were performed based on the standard protocols at Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The raw sequencing reads were deposited into the NCBI database (accession numbers: SRP366084 and SRP366184).

The raw fastq files were demultiplexed by trimmomatic with an in-house perl script and then merged by FLASH after quality filtering by FASTP [21]. The resultant non-chimeric sequences were re-clustered into operational taxonomic units (OTUs) based on 97% similarity with the UPARSE (version 7.1 http://drive5.com/uparse/, accessed on 1 August 2023) algorithm [22]. UCHIME was applied to identify the high-quality chimeric sequences. The taxonomic identities of the sequencing reads for fungi were determined using the UNITE fungal ITS database, while the taxonomy for representative sequences of bacteria was identified by BLAST with the SILVA database. As a flat database hosted by GitHub (https://github.com/UMNFuN/FUNGuild, accessed on 1 August 2023), FUNGuild v1.0 was used to predict fungal functional groups [23]. FAPROTAX v1.1 was used to determine the bacterial ecological functional groups [24]. Bugbase (web platform, https://bugbase.cs.umn.edu, accessed on 1 August 2023) was used to determine bacterial high-level phenotypes.

2.6. Statistical Analysis

We checked the data for normality and homogeneity of variance using the Kolmogorov-Smirnov test and Levene's test, respectively. We used ANOVA to examine the influence of plantation types on soil properties, microbial relative abundance, microbial diversity indices, and microbial functional groups We performed non-metric multidimensional scaling (NMDS) and principal coordinate analysis (PCoA) to determine the dissimilarity of the microbial community compositions and functional groups among different plantation types. We used an analysis of similarities (ANOSIM) and permutational multivariate analysis of variance (Adonis or PERMANOVA) to test the significant differences in the structures of the microbial communities among different plantation types. The ANOSIM and Adonis were performed in R (version 4.2.2) with the 'vegan' package based on 999 permutations. Transformation-based redundancy analysis (tb-RDA) with a Mantel test was used to explain the correlation between soil variables and the structure of the microbial community composition. We conducted a variation partition analysis (VPA) to compare the contributions of different soil variable types to the variations in the fungal and bacterial communities across the three plantation types. To identify the high-dimensional microbial taxa in different plantation types, we followed the method of Segata et al., 2011 [25] using linear discriminant analysis (LDA) effect size (LEfSe) (https://huttenhower.sph.harvard.edu/lefse, accessed on 1 August 2023). We obtained the significantly different taxonomic clades in each plantation type with the criterion of an LDA score greater than 2. All analyses were conducted in R (version 4.2.2, R Core Team, Vienna, Austria), except the LEfSe analysis.

We also constructed co-occurrence networks of the soil fungal and bacterial communities from all soil samples following the method of molecular ecological network analysis (http://ieg4.rccc.ou.edu/mena/, accessed on 1 August 2023), based on the random matrix theory (RMT), as recommended by Deng et al., 2012 [26]. We identified the keystone species in the fungal and bacterial co-occurrence networks by the connectivity intra-module (*Zi*) and inter-module (*Pi*) scores.

3. Results

3.1. Soil Physicochemical Properties

The plantation types had significant effects on the SM, STP, pH, TC, TP, NH_4^+-N, NO_3^--N, AP, AK, C:P, and N:P (Table 1). Notably, mixed plantations significantly increased the soil TP content but significantly decreased the soil NO_3^--N, C:P, and N:P in comparison with both monocultures. In addition, compared with Manchurian walnut monocultures, mixed plantations significantly increased the soil SM, STP, and AP by 47.13%, 13.89%, and 38.70%, respectively, but significantly decreased the soil TC and AK by 2.24% and 13.93%, respectively. Meanwhile, compared with Korean pine monocultures, mixed plantations significantly increased the soil pH and AK by 5.31% and 14.78%, respectively, but significantly decreased the soil NH_4^+-N and AP by 15.14% and 26.10%, respectively.

Table 1. Changes in soil physicochemical properties across the Korean pine and Manchurian walnut monocultures and mixed plantations.

Soil Variable	Plantation Type			ANOVA	
	JM	PK_JM	PK	F	*p*
SM (%)	27.31 ± 0.77 b	40.18 ± 4.11 a	39.52 ± 0.11 a	8.98	0.016 *
BD ($g \cdot cm^{-3}$)	0.78 ± 0.02 a	0.70 ± 0.032 a	0.78 ± 0.02 a	3.35	0.105
STP (%)	75.54 ± 2.98 b	86.03 ± 0.99 a	83.17 ± 2.91 ab	4.82	0.047 *
pH	5.27 ± 0.08 a	5.35 ± 0.09 a	5.08 ± 0.01 b	6.22	0.034 *
TC ($g \cdot kg^{-1}$)	115.94 ± 0.66 a	113.25 ± 0.31 b	114.31 ± 0.35 b	8.47	0.018 *
TN ($g \cdot kg^{-1}$)	6.84 ± 0.24 a	6.83 ± 0.09 a	6.78 ± 0.10 a	0.04	0.960
TP ($g \cdot kg^{-1}$)	0.84 ± 0.01 b	1.15 ± 0.06 a	0.81 ± 0.05 b	17.34	0.003 **
SOC ($g \cdot kg^{-1}$)	60.71 ± 2.44 a	62.98 ± 0.76 a	63.36 ± 0.75 a	0.887	0.460
NO_3^--N-N ($mg \cdot kg^{-1}$)	38.98 ± 1.14 ab	36.32 ± 1.47 b	42.80 ± 0.94 a	7.31	0.025 *
NH_4^+-N ($mg \cdot kg^{-1}$)	5.31 ± 0.22 b	3.88 ± 0.36 c	7.14 ± 0.15 a	40.59	0.000 ***
AP ($mg \cdot kg^{-1}$)	12.14 ± 0.44 c	16.85 ± 0.71 b	22.80 ± 1.98 a	18.61	0.003 **
AK ($mg \cdot kg^{-1}$)	140.38 ± 2.19 a	120.83 ± 1.70 b	105.55 ± 0.90 c	107.71	0.000 ***

Mean ± standard error. BD, bulk density; SM, soil moisture; STP, soil total porosity; TC, total carbon; TN, total nitrogen; TP, total phosphorus; SOC, soil organic carbon; NH_4^+-N, ammonium nitrogen; NO_3^--N, nitrate nitrogen; AP, available phosphorus; AK, available potassium. Different lowercase letters in the same row indicate significant differences ($p < 0.05$) amongst the three plantation types. *, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$. JM, *Juglans mandshurica* monocultures; PK_JM, *Pinus koraiensis* and *J. mandshurica* mixed plantations; PK, *P. koraiensis* monocultures.

3.2. The Sequencing Characteristics and Community Composition of Soil Fungi and Bacteria

Across all soil samples analyzed, we obtained 594,288 soil fungal sequences and 555,472 bacterial sequences. A total of 50,126~72,362 (mean = 66,032) soil fungal and 51,501~72,311 (mean = 61,719) soil bacterial sequences were obtained per sample. The fungal and bacterial average read sequences were 317 bp and 417 bp, respectively, which were >99% of Good's coverage for the ITS and 16S gene regions. The rarefaction curves of the ITS and 16S genes tended to approach the saturation plateau at 97% sequence similarity for all samples (Figure S1), indicating that the sequencing depth was adequate to evaluate the diversity and structure of soil fungi and bacteria across all samples.

A total of 2972 fungal OTUs were identified across all soil samples, distributed among 532 genera, 51 classes, and 17 phyla. At the phylum level, Ascomycota was most abundant, with relative abundances exceeding 60% in each plantation type (Figure 1A). Leotiomycetes and Sordariomycetes were the two most abundant classes, with their total relative abundances exceeding 40% in each plantation type (Figure 1C). Only the top fifteen genera in relative abundance were shown (Figure 1E), and Pseudogymnoascus was the most abundant genus among the three plantation types. The plantation types had a significant influence on the relative abundance of Ascomycota, Basidiomycota, Rozellomycota, and Unclassified K Chromista at the phylum level, and Leotiomycetes, Sordariomycetes, Unclassified P Ascomycota, Rozellomycotina cls Incertae sedis, Unclassified K Chromista, Pezizomycetes, and Tremellomycetes at the class level (Table S2).

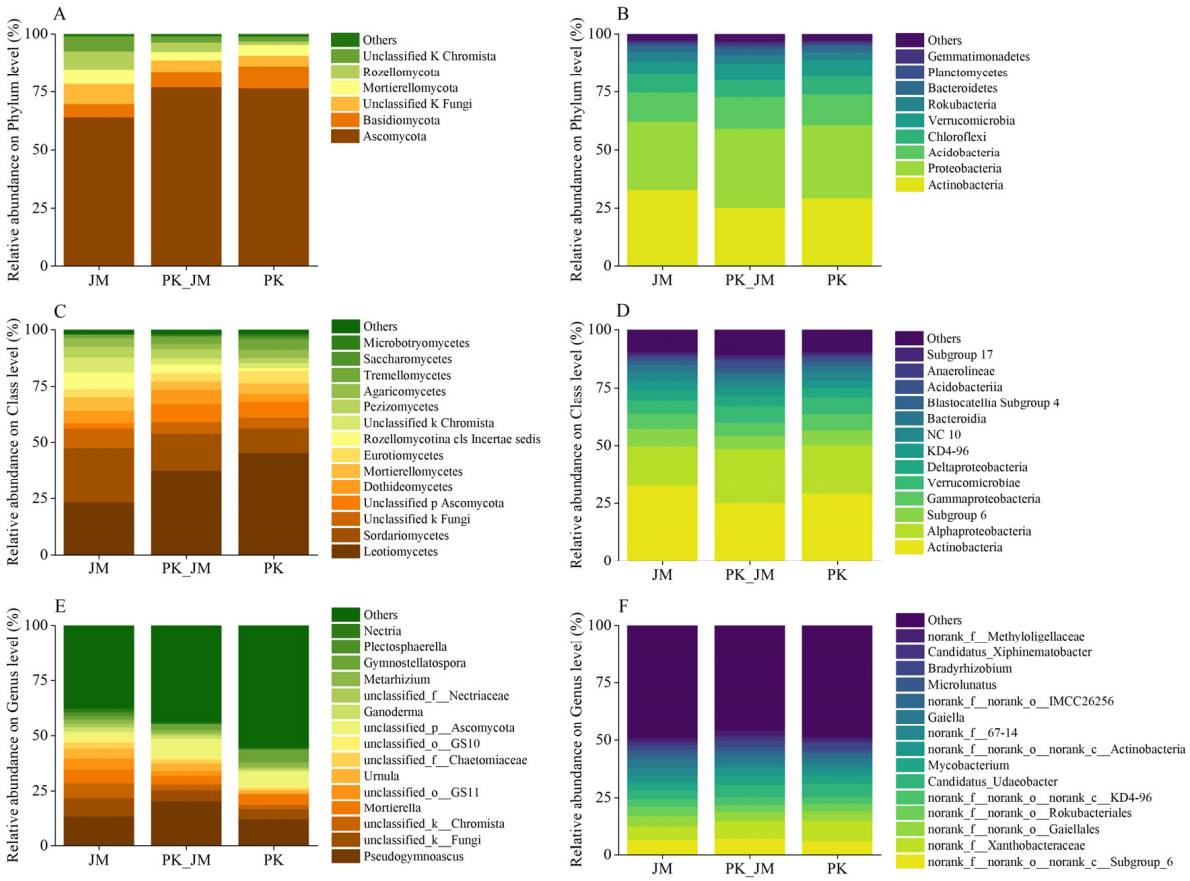

Figure 1. Relative abundances of soil fungal (**A**,**C**,**E**) and bacterial (**B**,**C**,**F**) phyla, classes, and genera across the Korean pine and Manchurian walnut monocultures and mixed plantations. Phyla and classes with relative abundance less than 0.01 were classified as other (**A**–**D**). Only the top fifteen fungal (**E**) and bacterial (**F**) genera in relative abundance are shown. JM, *Juglans mandshurica* monocultures; PK_JM, *Pinus koraiensis* and *J. mandshurica* mixed plantations; PK, *P. koraiensis* monocultures.

For soil bacteria, there were 3622 OTUs identified from all soil samples, distributed among 30 phyla, 82 classes, and 558 genera. At the phylum level, Actinobacteria, Proteobacteria, and Acidobacteria were the three most abundant phyla, with their total relative abundances exceeding 70% across all plantation types (Figure 1B). At the class level, Actinobacteria and Alphaproteobacteria were the two most abundant classes, accounting for half of the total abundance of all classes (Figure 1D). At the genus level, only the top fifteen genera in relative abundance were shown (Figure 1F), and the two unnamed genera (norank_f_norank_o_norank_c_Subgroup_6 and norank_f_Xanthobacteraceae) had the maximum relative abundance among the three plantation types, with total relative abundance of 12.2%, 14.7%, and 14.8% in JM, PK_JM, and PK, respectively. The plantation types had no significant effects on the relative abundances of all bacterial phyla and classes, except for Gemmatimonadetes at the phylum level and Acidobacteriia at the class level (Table S3).

By using LEfSe analysis, we found that the plantation type had a greater impact on the fungal composition than the bacterial composition (Figure 2). Specifically, at the class, order, family, and genus levels, some fungal groups were significantly enriched at different plantation types (Figure 2A). The plantation types barely altered the composition of soil bacteria, since bacteria did not exhibit any enrichments caused by the plantation types at the phylum and class levels, only showing a few enrichments at the order, family, and genus levels (Figure 2B). In addition, the enriched groups caused by the plantation type were mainly found in Manchurian walnut monocultures for both fungi and bacteria, while

the Korean pine and Manchurian walnut mixed plantations had the fewest enriched groups (Figure 2A,B).

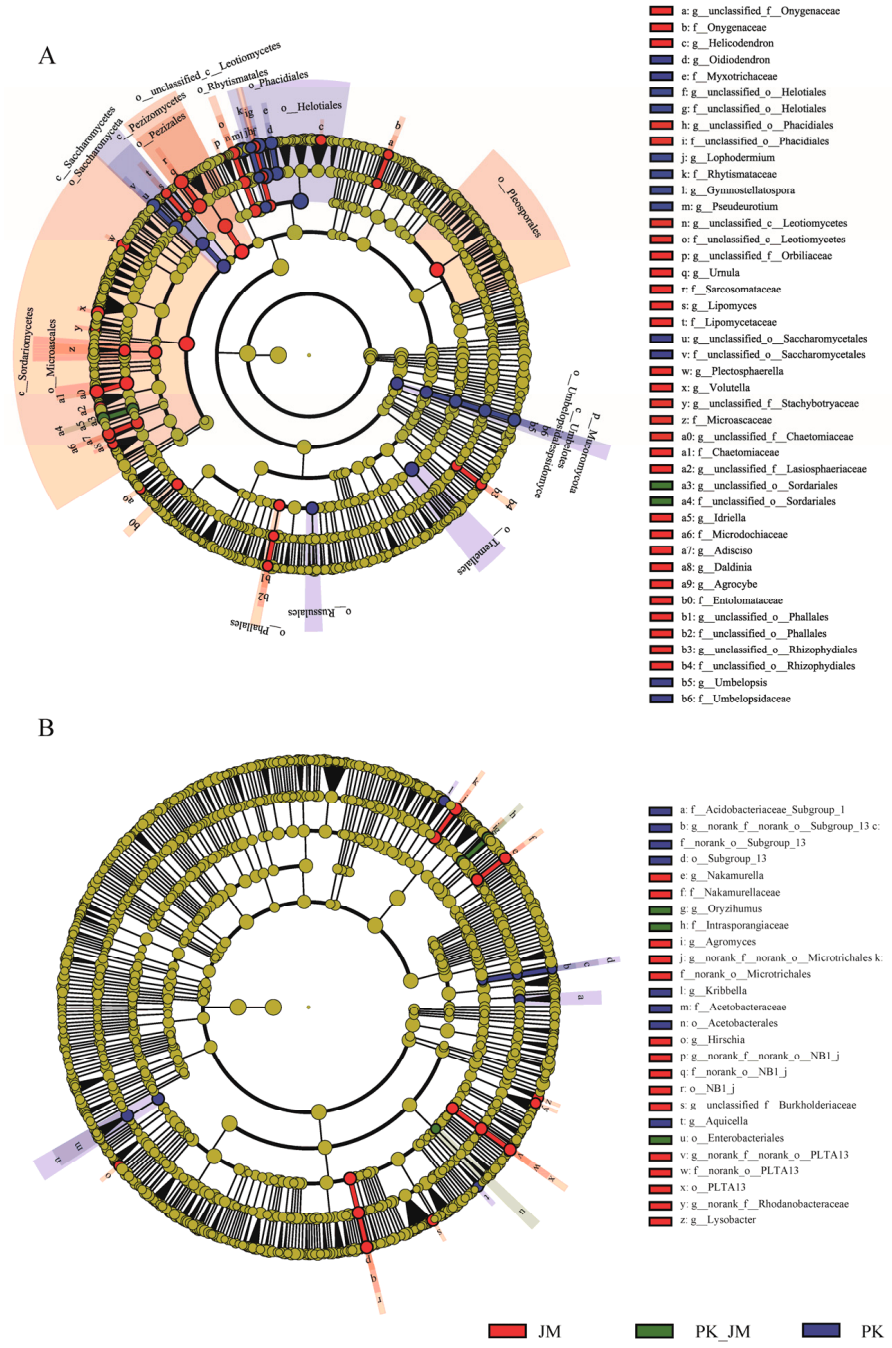

Figure 2. Cladogram showing significant differences between fungal (**A**) and bacterial (**B**) enrichment groups across the Korean pine and Manchurian walnut monocultures and mixed plantations. Taxon nodes with significant differences in relative abundance between different plantation types are represented by colored dots (JM in red, PK_JM in green, PK in blue). If the taxa were not significantly differentially represented among plantation types, the corresponding nodes are colored in yellow. Cladogram circles represent phylogenetic taxa from phylum to genus. The abbreviating labels for fungi range from family to genus level, and for bacteria from order to genus level. Only the LDA scores > 2 for both fungi and bacteria are shown. JM, *Juglans mandshurica* monocultures; PK_JM, *Pinus koraiensis* and *J. mandshurica* mixed plantations; PK, *P. koraiensis* monocultures.

3.3. Fungal and Bacterial Alpha and Beta Diversity

We selected four alpha indices, Sobs, Shannon, Chao1, and Faith's PD, to represent the microbial observed richness, community diversity, community richness, and phylogenetic diversity, respectively (Figure 3). For soil fungi, the plantation type had a significant effect on the Sobs, Shannon, and Faith's PD indices (Figure 3A,B,D), but not the Chao1 index (Figure 3C). Korean pine monocultures had the lowest values for Sobs, Shannon, Chao1, and Faith's PD, while Manchurian walnut monocultures had the highest values for Sobs, Shannon, and Faith's PD (Figure 3A–D). Compared with fungi, the effect of the plantation type on the alpha diversity of bacteria was the opposite. Only the Chao1 index was significantly altered by the plantation type ($p < 0.05$, Figure 3G). Manchurian walnut monocultures had the highest values for Sobs and Shannon, while Korean pine monocultures had the lowest Sobs, Chao1, and Faith's PD values (Figure 3E–H). In addition, the mixed plantations had the largest Chao1 index values across the three plantation types for both fungi and bacteria (Figure 3C,G).

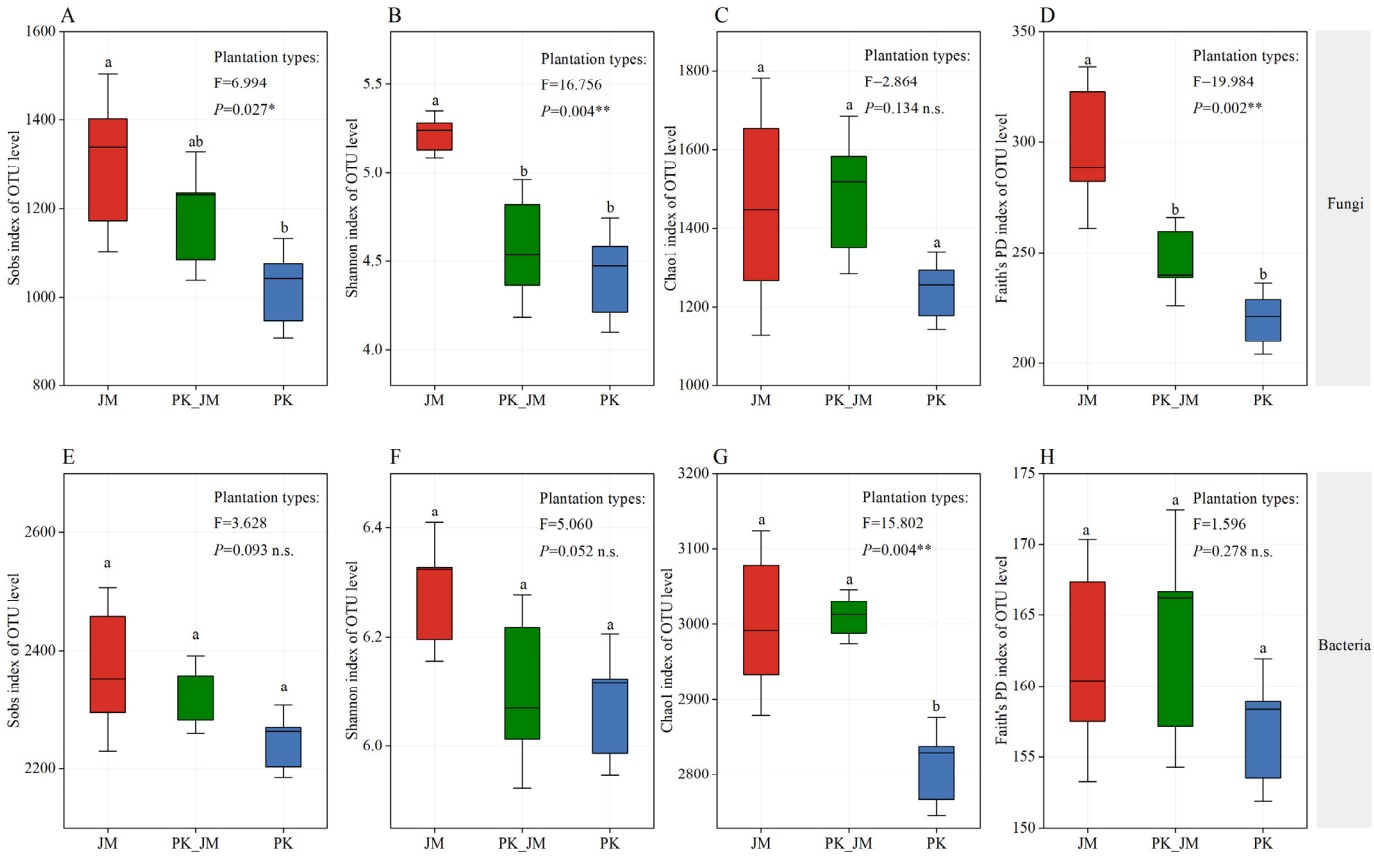

Figure 3. The effects of Korean pine and Manchurian walnut monocultures and mixed plantations on the alpha diversity of fungi (**A**–**D**) and bacteria (**E**–**H**) at the OTU level. Sobs, Shannon, Chao1, Faith's PD represent microbial observed richness, community diversity, community richness, and phylogenetic diversity, respectively. Different lowercase letters indicate significant differences ($p < 0.05$) amongst the three plantation types. ns, not significant; *, $p < 0.05$; **, $p < 0.01$. JM, *Juglans mandshurica* plantations in red color; PK_JM, *Pinus koraiensis* and *J. mandshurica* mixed plantations in green color; PK, *P. koraiensis* plantations in blue color.

The top two principal coordinate axes explained more than 65% of the total variation for the fungal and bacterial communities at the OTU level (Figure 4A,B). The structure of the fungal and bacterial community changed significantly across the Korean pine and Manchurian walnut monocultures and mixed plantations based on the results of ANOSIM (fungi: $p < 0.01$, bacteria: $p < 0.05$) and PERMANOVA (fungi: $p < 0.01$, bacteria: $p < 0.05$) (Figure 4A,B). The structure of the fungal community varied significantly across the differ-

ent plantation types, regardless of the OTU level or genus level. (Figure 4C). However, the bacterial community structure had no significant differences at the genus level across the Korean pine and Manchurian walnut monocultures and mixed plantations (PERMANOVA: $p > 0.05$) (Figure 4D).

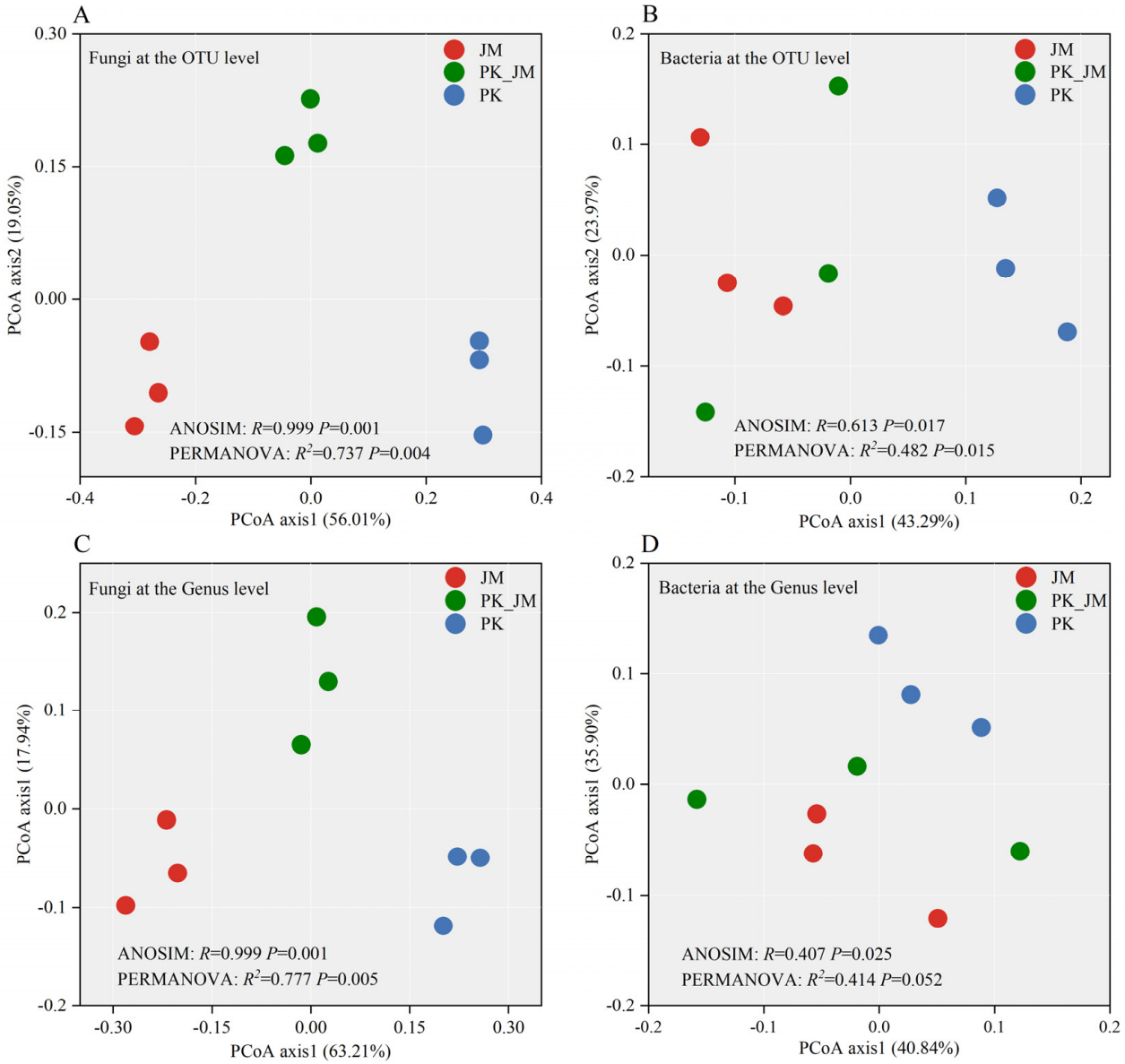

Figure 4. Principal coordinate analysis (PCoA) of the fungal (**A**,**C**) and bacterial (**B**,**D**) community structures at the OTU (**A**,**B**) and genus (**C**,**D**) levels across the Korean pine and Manchurian walnut monocultures and mixed plantations based on the Bray-Curtis distance. JM, *Juglans mandshurica* plantations; PK_JM for *Pinus koraiensis* and *J. mandshurica* mixed plantations; PK, *P. koraiensis* plantations.

3.4. Fungal and Bacterial Functional Groups

The FUNGuild database was used to annotate soil fungi, with three types of fungal trophic modes: pathotroph, saprotroph, and symbiotroph (Figure 5A). Our results showed that pathogen reached its maximum in mixed plantations and was significantly greater than those in Korean pine monocultures (Table S4). Saprotroph reached its minimum in mixed plantations and was significantly lower than those in Korean pine monocultures (Table S4). Symbiotroph did not change significantly across the three plantation types (Table S4). Moreover, there was no significant difference in pathogen and saprotroph abundance between Manchurian walnut monocultures and mixed plantations. Animal

pathogen and undefined saprotroph were the two most abundant fungal functional groups in all plantations.

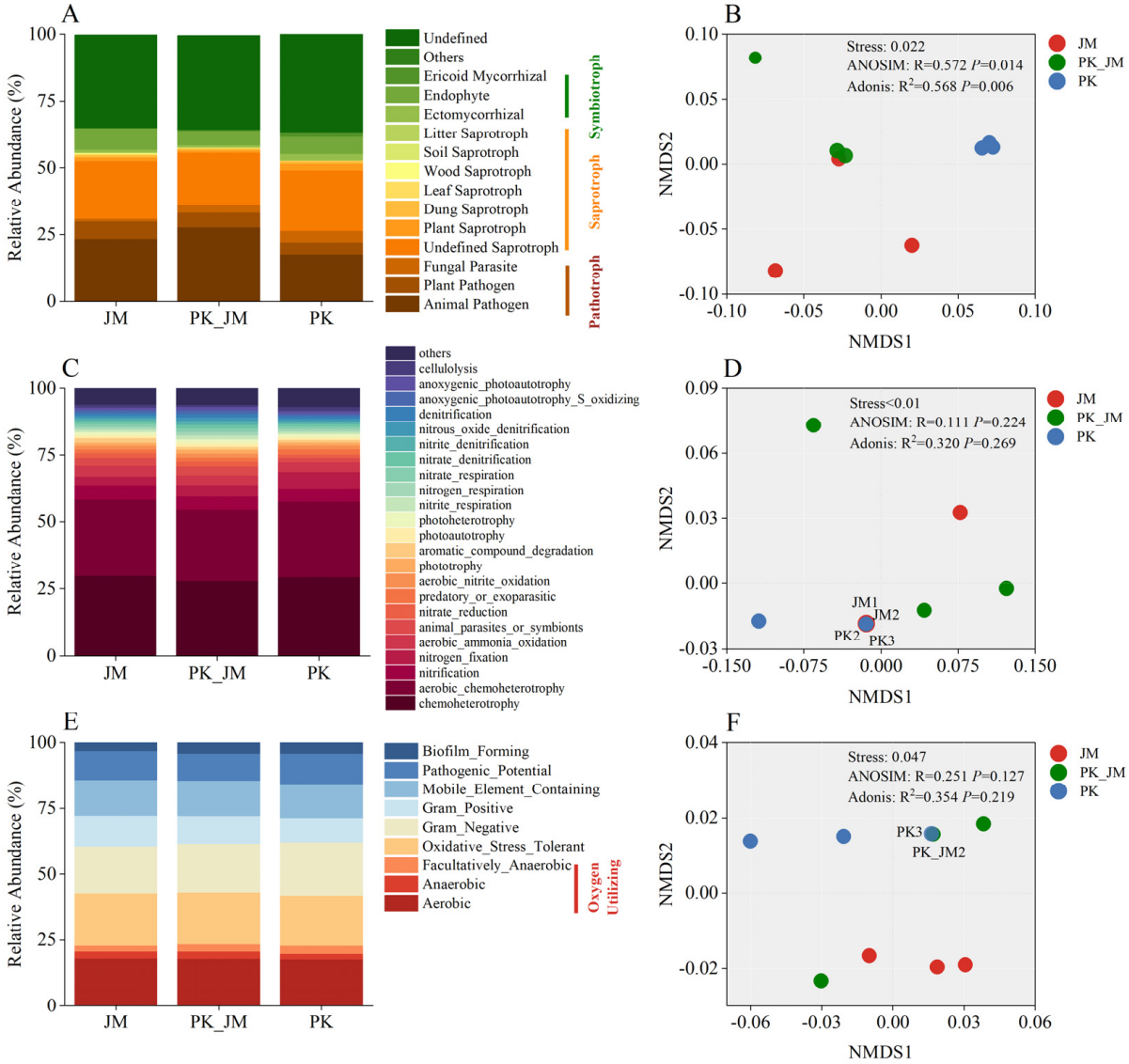

Figure 5. The effects of Korean pine and Manchurian walnut monocultures and mixed plantations on the functional groups of fungal (**A**,**B**) and bacterial (**C**–**F**) communities. Non-metric multidimensional scaling (NMDS) with ANOSIM and Adonis based on Bray-Curtis distance was used to demonstrate the significant effects of plantation types on the structures of fungal (**B**) and bacterial (**D**,**F**) functional groups. The FUNGuild database was used to annotate soil fungal functional groups, while the FAPROTAX and Bugbase databases were used to annotate soil bacterial functional groups. JM, *Juglans mandshurica* plantations; PK_JM, *Pinus koraiensis* and *J. mandshurica* mixed plantations; PK, *P. koraiensis* plantations.

For soil bacteria, we used the FAPROTAX and Bugbase databases to reflect bacterial ecological and high-level phenotype groups, respectively (Figure 5A). Chemoheterotrophy and aerobic chemoheterotrophy were the two most abundant groups, with their total relative abundances exceeding 50% in each plantation type (Figure 5C). Moreover, the relative abundance of aerobic chemoheterotrophy in mixed plantations was visibly lower than that in monocultures. For bacterial high-level phenotype groups, oxygen utilizing, oxidative stress-tolerant, and Gram-negative were the three most abundant groups, with their total relative abundance accounting for more than half of all bacterial phenotypes in each plantation type (Figure 5E).

Further, we observed the changes in the structures of the fungal and bacterial functional groups across the three plantation types through NMDS analysis based on the Bray–Curtis distance (Figure 5B,D,F). The structure of the fungal functional groups varied significantly among the three plantation types (ANOSIM: $p < 0.05$, Adonis: $p < 0.05$, Figure 5B), while the structure of the bacterial functional groups did not change significantly across the different plantation types (ANOSIM: $p > 0.05$, Adonis: $p > 0.05$, Figure 5D,F).

3.5. The Correlations between Soil Variables and Microbial Community Structure

The ordination diagram of RDA showed that the first two axes explained 43.21% and 52.60% of the variation in fungal and bacterial community structure across all plantation types, respectively (Figure 6A,C). For the structures of the fungal and bacterial functional groups, the first two axes explained 49.81%, 49.06%, and 82.63% of the variation in microbial functional groups' structures when annotated by the FUNGuild, FAPROTAX, and Bugbase databases, respectively (Figure 6B,D,E). Available nutrient content was always the greatest contributor to the variations in microbial taxonomy and functional groups based on the results of the variation partition analysis (VPA) (Figure S2). In addition, Mantel tests showed that AP and AK were the most common soil factors significantly affecting the fungal community and function groups ($p < 0.05$, Table S5). pH and NH_4^+-N were the most common soil factors significantly affecting fungal functional groups and bacterial ecological groups (Table S5). AK was the only significant soil factor affecting bacterial phenotype groups (Table S5). Furthermore, the RDA had varying degrees of explanation at different taxonomy levels of fungi and bacteria (Figure S3). Accordingly, the most significant soil factors varied across different taxonomy levels in shaping the microbial community structure (Table S6, Figure S3).

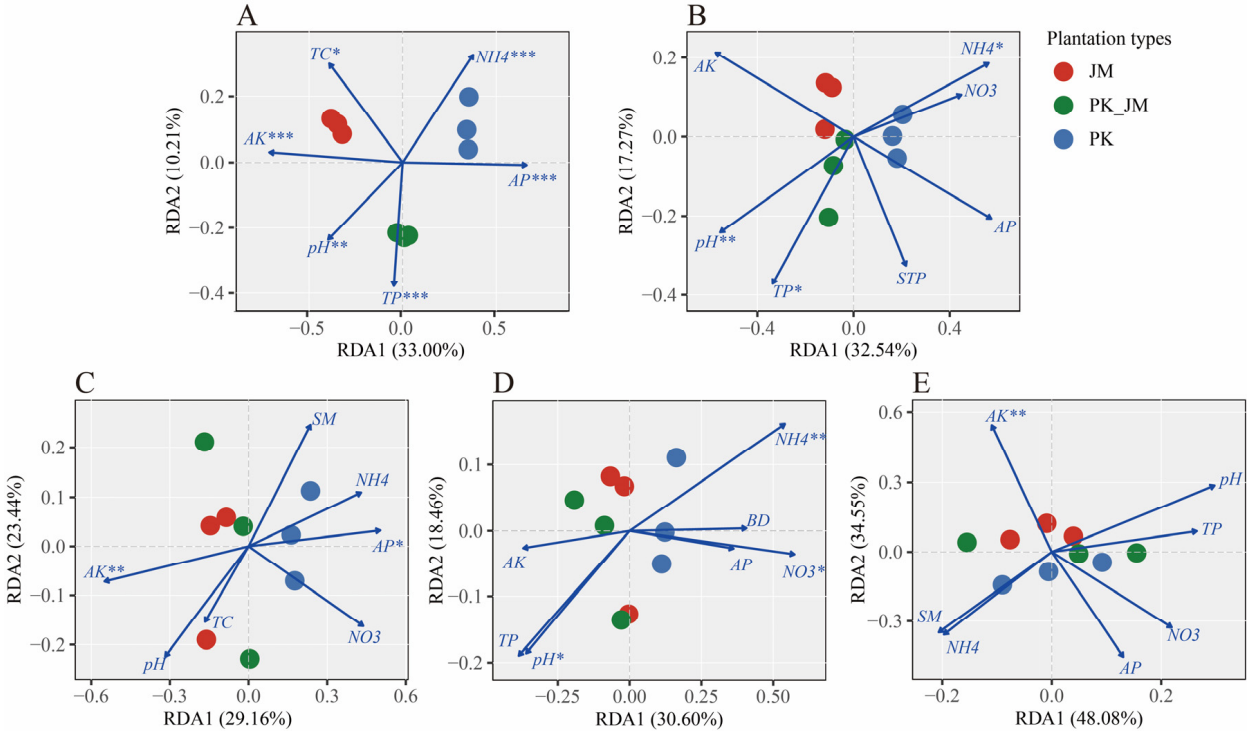

Figure 6. The results of redundancy analysis (RDA) showing the correlations between soil variables and the structure of the microbial community composition in all plantation types, including fungal genus-level taxonomy (**A**) and function categories based on FUNGuild database (**B**), and bacterial genus-level taxonomy (**C**) and function categories based on FAPROTAX (**D**) and Bugbase databases (**E**). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. JM, *Juglans mandshurica* plantations; PK_JM, *Pinus koraiensis* and *J. mandshurica* mixed plantations; PK, *P. koraiensis* plantations.

3.6. The Patterns of Fungal and Bacterial Co-Occurrence Networks

The relationships between the nodes in the networks were significantly correlated because the non-random interactions predominated in the empirical network based on the comparison results of the empirical network and corresponding 100 random networks with the same size and links (Table 2). Across the three plantation types, the bacterial network had more nodes and edges than the fungal one (Figure S4A,B, Table 2). The nodes in the fungal network were dominated by Ascomycota (61.50%), and the nodes in the bacterial network were co-dominated by Actinobacteria (15.62%), Proteobacteria (26.86%), and Acidobacteria (17.52%). Whether in the fungal or bacterial networks, the relationships between nodes were dominated by positive correlations (more than 70%, Table 2). The vast majority of nodes in both the fungal and bacterial networks fell within the peripheral area based on their Zi and Pi scores (Figure S4C). For the fungal network, four nodes (OTU1714, OTU878, OTU1835, OTU2109, all belonging to Ascomycota) were classified as module hubs, and one node (OTU2536, Ascomycota) was specifically classified as a connector. For the bacterial network, two nodes (OTU2281, Chloroflexi and OTU643, Bacteroidetes) were classified as module hubs (Figure S4C). The five largest modules were selected to represent the structures of the sub-networks. The strength of the correlations between the top five fungal modules and soil variables was higher than those in the bacterial sub-networks, based on the results of RDA (Figure S4D).

Table 2. The topological properties of soil fungal and bacterial co-occurrence networks across all the plantation types.

Network Features		Fungi	Bacteria
Empirical network	Similarity threshold	0.94	0.96
	Number of nodes	387	685
	Number of edges	407	553
	R^2 of power law	0.933	0.954
	The percentage of positive correlation	73.71%	75.95%
	The percentage of negative correlation	26.29%	24.05%
	Average degree (AvgK)	2.103	1.615
	Average clustering coefficient (AvgCC)	0.133	0.136
	Average path distance (GD)	8.339	7.394
	Modularity	0.880	0.951
Random network	Average clustering coefficient ± SD	0.004 ± 0.003	0.002 ± 0.001
	Average path distance ± SD	6.520 ± 0.248	9.044 ± 0.727
	Modularity ± SD	0.796 ± 0.008	0.927 ± 0.007

4. Discussion

4.1. Influence of Plantation Type in Shaping Fungal and Bacterial Diversity and Composition

We found that Manchurian walnut monocultures showed relatively higher alpha diversity in both fungi and bacteria than Korean pine monocultures (Figure 3), supporting our first hypothesis. Many studies showed that the fungal alpha diversity was usually higher in broadleaved forests than that in conifer forests [27,28], which was mainly ascribed to microenvironmental changes caused by aboveground vegetation performance. Roots and leaf litters in broadleaved forests contain lower hard-decomposable compounds such as lignin and tannin than conifer forests [29], providing a more suitable condition for soil fungi. However, there is a contradiction in studies of bacterial alpha diversity between broadleaved forests and conifer forests. Some studies found that the bacterial alpha diversity was relatively higher in conifer forests than in broadleaved forests or mixed forests [30,31]. In this study, our results supported the findings of some studies showing that broadleaved forests had higher bacterial alpha diversity than conifer forests [28,32]. The conflicting results for the bacterial alpha diversity in different forest types might be related to forest succession. When a conifer forest is the local climax community, such as *Larix gmelinii* pure forests in some boreal forests, it tends to have high ecosystem stability

with higher bacterial alpha diversity [30,31]. In this study, there was no difference in the succession order across the three plantation types. Similarly to fungal biodiversity, the Manchurian walnut monocultures had a higher rate of litter turnover efficiency than Korean pine monocultures, thus providing more metabolic substrates for bacterial growth and reproduction, which in turn resulted in higher biodiversity [33]. In addition, we found no evidence that the fungal and bacterial species diversity was significantly higher in mixed plantations than monocultures in this study, which was in line with Mommer et al. [34] and Jiang et al. [31].

The plantation types had a significant effect on bacterial Chao1, but not on fungal Chao1 (Figure 3C,G), which supported our second hypothesis regarding microbial diversity. The degree of variation in the bacterial Chao1 across the three plantation types was more significant than that of the OTU richness and Shannon index (Figure 3E–G). Given the nature of the Chao1 index, whose calculation method makes it more sensitive to rare species [35], it suggested that bacteria might have included more rare species than fungi in this study. In addition, we found that mixed plantations could increase the number of rare species because the Chao1 index was the highest in mixed stands for both fungi and bacteria. The plantation types also had different effects on he fungal and bacterial Faith's PD index (Figure 3D,H), indicating that there were significant differences between fungi and bacteria in terms of phylogenetic diversity. Firstly, fungi had greater phylogenetic diversity than bacteria in this study (Figure 3D,H), which is consistent with other temperate forest ecosystems [36]. Secondly, fungal phylogenetic diversity was more likely to be affected by the plantation type (Figure 3D).

The changes in the fungal and bacterial community composition showed inconsistencies across the three plantation types (Figures 1 and 2). Specifically, the fungal-dominant phylum varied significantly among the different plantation types (Figure 1A, Table S2), while the bacterial-dominant phylum did not (Figure 1B, Table S3), supporting our second hypothesis regarding the microbial composition. At the phylum level, the dominant phyla (fungi: Ascomycota and Basidiomycota; bacteria: Actinobacteria and Proteobacteria) were consistent with forests on a global scale [14,37]. Ascomycota, Basidiomycota, Actinobacteria, and Proteobacteria play essential roles in forest ecosystems because they are the fundamental decomposers in regulating nutrient cycling [27]. In this study, the relative abundances of Ascomycota and Basidiomycota in Korean pine monocultures were significantly higher than those in Manchurian walnut monocultures (Figure 1A, Table S2), which might be attributed to differences in litter conditions between plantation types. On the one hand, coniferous litter has higher recalcitrance (lignin, cellulose, and tannin). As the largest group in the fungal kingdom [38], Ascomycota is considered the dominant community in litter residue decomposition [39], and Ascomycota exhibits a strong capacity for decomposition in persistent organic materials such as lignin and keratin [40]. On the other hand, conifer forests have a slower litter decomposition rate than broadleaved forests [29], and Basidiomycota is the dominant decomposer of wood or litter decay residues [41]. Consequently, Korean pine monocultures had higher abundances of Ascomycota and Basidiomycota than Manchurian walnut monocultures. For soil bacteria, our results were inconsistent with studies in tropical or subtropical forest ecosystems [7,42], but were in line with the case in boreal forest ecosystems [31], indicating that the differences in bacterial composition between coniferous and broadleaved forests might not be evident in Northeast China. From the phylum level to the genus level, we found that fungi had more taxa-specific species between Korean pine monocultures and Manchurian walnut monocultures than bacteria (Figure 2). In addition, we also noticed that mixed plantations had very few taxa-specific species based on the result that the taxa-specific species were mainly present in the two monocultures (Figure 2), indicating that the tree identity might have a greater impact on the soil microbial composition than mixed cultivation in the northern region [43].

4.2. Changes in Functional Group Structures of Fungal and Bacterial Communities across Different Plantation Types

Different fungal and bacterial compositions have varying impacts on elemental cycling; thus, exploring microbial functional distribution patterns could be conducive to a more detailed understanding of soil function, as different functional groups are often filtered into distinct environments [13]. In this study, similar to the microbial composition, we found that the plantation types had a significant effect on the fungal functional group structures (Figure 5A,B), but not on the bacterial functional group structures (Figure 5C–F), supporting our second hypothesis regarding microbial functional groups. Fungi-dominated systems are generally associated with the slow decomposition of more chemically recalcitrant organic matter, such as in forest ecosystems [15]. By contrast, bacteria-dominated systems are generally related to rapid nutrient cycling, which promotes the existence of fast-growing plants, as in grassland ecosystems [13].

Distinct from the microbial composition, there was no significant difference between Korean pine monocultures and Manchurian walnut monocultures in the relative abundance of three broad groupings referred to as trophic modes, but there was a significant difference between the mixed plantations and Korean pine monocultures (Figure 5A, Table S4). Mixed plantations had the highest abundance of the pathotroph group, indicating that mixed cultivation could contribute to increased soil animal diversity and activity [44,45]. The saprotroph group had the lowest abundance in the mixed plantations (Table S4), suggesting that there might be rapid nutrient turnover during litter decomposition in mixed broadleaf–conifer forests [46,47]. As one major functional group distinction that has received widespread attention, symbiotroph fungi have a biotrophic relationship with the root systems of plants [48]. Mixed plantations had the lowest abundance of symbiotroph groups among the three plantation types (Figure 5A, Table S4), which might have been related to the tree host specificity of some symbiotroph fungi, such as mycorrhiza and endophyte fungi [49]. However, we found only a few abundant arbuscular mycorrhizal fungi groups in this study (classified as "other" due to their low abundance), which might be because the soil samples that we took avoided the rhizosphere region.

In this study, neither the bacterial ecological groups nor phenotype groups varied significantly across the three plantation types (Figure 5D,F). Groups related to chemoheterotrophy dominated the bacterial ecological groups (Figure 5C), which was consistent with other ecosystems [50], followed by groups associated with nitrogen conversion and groups related to respiration and phototrophy (Figure 5C), supporting the notion that bacteria play crucial roles in the soil carbon and nitrogen cycles [50]. Bacterial phenotype groups by Bugbase analysis showed similar results, namely that most assigned bacteria were predicted to be oxybiotic, as the carbon cycle occurred mostly in the form of respiration [51].

4.3. Soil Factors Play a Key Role in Shaping Soil Microbial Communities

It is well known that bacteria are more diverse than fungi in the species richness of the soil community [13], but the bacterial composition or functional groups had no significant difference across the three plantation types (Figures 4D and 5D,F), which might be closely related to the environmental conditions, such as the climatic conditions, biotic characteristics, and soil properties [27,32]. Importantly, both different climatic conditions and vegetation types could affect soil properties and have further consequences for the microbial composition and functional groups [52,53]. The climatic conditions of our study sites were consistent.

In this study, soil variables could explain more than 40% of the variation in community structures and functional groups across the three plantation types for fungi and bacteria (Figure 6), indicating that soil properties play a vital role in shaping fungal and bacterial community structures and functional groups. Of all the soil variables, the soil pH was one of the most important factors affecting the distribution patterns of fungi and bacteria [15]. Korean pine monocultures had a significantly lower soil pH than the other plantation

types (Table 1). Litters accumulate at the surface soil with a slow decomposition process, producing large amounts of acidic crude humus, which results in an acidic soil pH in the long term. Conifer forests had a slower litter decomposition rate than broadleaved forests [29], forming a lower soil pH than broadleaved forests. On the one hand, a lower soil pH might limit the microbial diversity even more in Korean pine monocultures (Figure 3). On the other hand, most bacterial species are not as acid-tolerant as fungi [54], which might allow the soil pH to override the influences of other soil variables in limiting the bacterial diversity [54]. Soil moisture was another important soil factor in affecting the fungal and bacterial communities (Figures 6, S2 and S3). Soil moisture was significantly higher in Korean pine monocultures and mixed plantations than in Manchurian walnut monocultures (Table 1). Higher soil moisture provides a more conducive habitat for litter decomposition by fungi [55] and beneficial bacteria in the metabolism of substrates [56].

In terms of total nutrient content, it directly determines the nutrient ratios and available nutrient supply. For example, the soil total carbon stock in Manchurian walnut monocultures was significantly higher than those in Korean pine monocultures and mixed plantations (Table 1). Soil total carbon content is an important predictor of fungal and bacterial diversity [19]. Stands characterized by high soil carbon stocks support high soil organic carbon content accordingly, which serves as the fundamental resource for multitudinous fungal and bacterial groups [57].

According to the results of RDA and VPA, the available nutrient content explained the maximum variations in the microbial compositions and functional groups (Figures 6 and S2), which demonstrated the important roles of available nutrients in shaping microbial communities, supporting our third hypothesis. Compared with other soil properties, the available nutrients have a more direct effect on microbial metabolism. In particular, the number of stably coexisting species is expected to be bounded by the available resources based on the principle of competitive exclusion [20]. In this study, the available nutrient content varied significantly across the three plantation types (Table 1). Available nitrogen and phosphorus are the most common elements limiting microbial functioning in various terrestrial ecosystems [58]. The available nitrogen content is also closely related to the phylogenetic diversity of fungi and bacteria [59].

We also found that the soil properties had a different effect on the network structure between fungi and bacteria, suggesting that soil variables not only mediate the microbial composition and functional groups but also the co-occurrence ecological networks among microbial species [60]. There were more soil variables significantly affecting the fungal network structure than the bacterial one (Figure S4D), which might have been related to their different network characteristics [61]. In this study, bacteria had a more complex network, with more nodes and edges, than fungi (Table 2, Figure S4A,B). However, compared to bacteria, the fungal network contained more critical network elements, with more module hubs and connector hubs (Figure S4C), which played a fundamental role in the exchange of energy within different modules, while almost all nodes in the bacterial network were peripherals (Figure S4C), which had little impact on the structure of the bacterial network under external disturbances [62].

5. Conclusions

Compared with Korean pine monocultures, Manchurian walnut monocultures had a positive effect on the alpha diversity of both fungi and bacteria. The plantation type had a more obvious effect on fungi than bacteria in their diversity, community structure, and functional groups. Only at the OTU level did the structures of both the fungal and bacterial communities significantly change across the Korean pine and Manchurian walnut monocultures and mixed plantations. From the genus level to the phylum level, the plantation type only had a significant effect on the fungal community structure. Fungal functional groups varied significantly across the three plantation types, while bacterial functional groups did not change significantly among the three plantation types. The soil physicochemical properties played an important role in shaping the specific fungal and bacteria

communities across the Korean pine and Manchurian walnut monocultures and mixed plantations. Furthermore, the soil available nitrogen, phosphorus, and potassium content were the most important factors in driving both the fungal and bacterial communities.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14081594/s1, Figure S1: The Rarefaction curves of the number of operational taxonomic units (OTUs) for soil fungal (A) and bacterial (B) communities; Figure S2: Venn diagram of variation partition analysis (VPA) showed the contributions of different types of soil variables to the variations in fungal Genus-level taxonomy (A) and function category based on FUNGuild database (B), bacterial Genus-level taxonomy (C) and function category based on FAPROTAX (D) and Bugbase databases (E); Figure S3: The results of Redundancy analysis (RDA) showed the correlation between the soil physicochemical parameters and microbial community composition in different taxonomy levels; Figure S4: The patterns of soil fungal (A) and bacterial (B) co-occurrence networks. Each node represented one type of OTUs and was colored by phylum taxonomic categories; Table S1: The basic situation of the three forest types; Table S2: The effects of plantation types on the relative abundance of soil fungal phyla and classes; Table S3: The effects of plantation types on the relative abundance of soil bacterial phyla and classes; Table S4: The changes of soil fungal functional guilds composition in different plantation types; Table S5: Mantel test results for the correlation between soil microbial community with functional groups and soil variables; Table S6: Mantel test results for the correlation between soil microbial community and soil variables in different taxonomy levels.

Author Contributions: Conceptualization, methodology, formal analysis, writing—original draft, F.S. and N.L.; methodology, investigation, Y.W. and H.L.; investigation, H.J.; conceptualization, writing—review and editing, funding acquisition, L.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Fundamental Research Funds for the Central Universities (2572020DR05; 2572019CP16) and the Heilongjiang Touyan Innovation Team Program (Technology Development Team for Highly efficient Silviculture of Forest Resources).

Data Availability Statement: Data available on request from the authors.

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

References

1. Chen, X.; Li, B.-L.; Lin, Z.-S. The acceleration of succession for the restoration of the mixed-broadleaved Korean pine forests in Northeast China. *For. Ecol. Manag.* **2003**, *177*, 503–514. [CrossRef]
2. Harris, J. Soil Microbial Communities and Restoration Ecology: Facilitators or Followers? *Science* **2009**, *325*, 573–574. [CrossRef]
3. Bastida, F.; Eldridge, D.J.; García, C.; Kenny Png, G.; Bardgett, R.D.; Delgado-Baquerizo, M. Soil microbial diversity–biomass relationships are driven by soil carbon content across global biomes. *ISME J.* **2021**, *15*, 2081–2091. [CrossRef] [PubMed]
4. Liu, R.; Zhang, Y.; Hu, X.-F.; Wan, S.; Wang, H.; Liang, C.; Chen, F.-S. Litter manipulation effects on microbial communities and enzymatic activities vary with soil depth in a subtropical Chinese fir plantation. *For. Ecol. Manag.* **2021**, *480*, 118641. [CrossRef]
5. Badri, D.V.; Vivanco, J.M. Regulation and function of root exudates. *Plant Cell Environ.* **2009**, *32*, 666–681. [CrossRef] [PubMed]
6. Chodak, M.; Klimek, B.; Azarbad, H.; Jaźwa, M. Functional diversity of soil microbial communities under Scots pine, Norway spruce, silver birch and mixed boreal forests. *Pedobiologia* **2015**, *58*, 81–88. [CrossRef]
7. Beugnon, R.; Du, J.; Cesarz, S.; Jurburg, S.D.; Pang, Z.; Singavarapu, B.; Wubet, T.; Xue, K.; Wang, Y.; Eisenhauer, N. Tree diversity and soil chemical properties drive the linkages between soil microbial community and ecosystem functioning. *ISME Commun.* **2021**, *1*, 41. [CrossRef]
8. He, D.; Shen, W.; Eberwein, J.; Zhao, Q.; Ren, L.; Wu, Q.L. Diversity and co-occurrence network of soil fungi are more responsive than those of bacteria to shifts in precipitation seasonality in a subtropical forest. *Soil Biol. Biochem.* **2017**, *115*, 499–510. [CrossRef]
9. Manzoni, S. Flexible Carbon-Use Efficiency across Litter Types and during Decomposition Partly Compensates Nutrient Imbalances—Results from Analytical Stoichiometric Models. *Front. Microbiol.* **2017**, *8*, 661. [CrossRef] [PubMed]
10. Vesala, R.; Kiheri, H.; Hobbie, E.A.; van Dijk, N.; Dise, N.; Larmola, T. Atmospheric nitrogen enrichment changes nutrient stoichiometry and reduces fungal N supply to peatland ericoid mycorrhizal shrubs. *Sci. Total Environ.* **2021**, *794*, 148737. [CrossRef]
11. Wang, W.; Zhang, Q.; Sun, X.; Chen, D.; Insam, H.; Koide, R.T.; Zhang, S. Effects of mixed-species litter on bacterial and fungal lignocellulose degradation functions during litter decomposition. *Soil Biol. Biochem.* **2020**, *141*, 107690. [CrossRef]
12. Fierer, N.; Strickland, M.S.; Liptzin, D.; Bradford, M.A.; Cleveland, C.C. Global patterns in belowground communities. *Ecol. Lett.* **2009**, *12*, 1238–1249. [CrossRef]

13. Crowther, T.W.; van den Hoogen, J.; Wan, J.; Mayes, M.A.; Keiser, A.D.; Mo, L.; Averill, C.; Maynard, D.S. The global soil community and its influence on biogeochemistry. *Science* **2019**, *365*, eaav0550. [CrossRef]
14. Delgado-Baquerizo, M.; Oliverio, A.M.; Brewer, T.E.; Benavent-González, A.; Eldridge, D.J.; Bardgett, R.D.; Maestre, F.T.; Singh, B.K.; Fierer, N. A global atlas of the dominant bacteria found in soil. *Science* **2018**, *359*, 320–325. [CrossRef] [PubMed]
15. Bahram, M.; Hildebrand, F.; Forslund, S.K.; Anderson, J.L.; Soudzilovskaia, N.A.; Bodegom, P.M.; Bengtsson-Palme, J.; Anslan, S.; Coelho, L.P.; Harend, H.; et al. Structure and function of the global topsoil microbiome. *Nature* **2018**, *560*, 233–237. [CrossRef] [PubMed]
16. Leewis, M.-C.; Lawrence, C.R.; Schulz, M.S.; Tfaily, M.M.; Ayala-Ortiz, C.O.; Flores, G.E.; Mackelprang, R.; McFarland, J.W. The influence of soil development on the depth distribution and structure of soil microbial communities. *Soil Biol. Biochem.* **2022**, *174*, 108808. [CrossRef]
17. Zhou, Z.; Wang, C.; Luo, Y. Meta-analysis of the impacts of global change factors on soil microbial diversity and functionality. *Nat. Commun.* **2020**, *11*, 3072. [CrossRef] [PubMed]
18. Talbot, J.M.; Bruns, T.D.; Taylor, J.W.; Smith, D.P.; Branco, S.; Glassman, S.I.; Erlandson, S.; Vilgalys, R.; Liao, H.-L.; Smith, M.E.; et al. Endemism and functional convergence across the North American soil mycobiome. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 6341–6346. [CrossRef] [PubMed]
19. Bardgett, R.D.; van der Putten, W.H. Belowground biodiversity and ecosystem functioning. *Nature* **2014**, *515*, 505–511. [CrossRef] [PubMed]
20. Dal Bello, M.; Lee, H.; Goyal, A.; Gore, J. Resource–diversity relationships in bacterial communities reflect the network structure of microbial metabolism. *Nat. Ecol. Evol.* **2021**, *5*, 1424–1434. [CrossRef]
21. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Huntley, J.; Fierer, N.; Owens, S.M.; Betley, J.; Fraser, L.; Bauer, M.; et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *ISME J.* **2012**, *6*, 1621–1624. [CrossRef] [PubMed]
22. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef] [PubMed]
23. Nguyen, N.H.; Song, Z.; Bates, S.T.; Branco, S.; Tedersoo, L.; Menke, J.; Schilling, J.S.; Kennedy, P.G. FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecol.* **2016**, *20*, 241–248. [CrossRef]
24. Louca, S.; Parfrey Laura, W.; Doebeli, M. Decoupling function and taxonomy in the global ocean microbiome. *Science* **2016**, *353*, 1272–1277. [CrossRef] [PubMed]
25. 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] [PubMed]
26. Deng, Y.; Jiang, Y.-H.; Yang, Y.; He, Z.; Luo, F.; Zhou, J. Molecular ecological network analyses. *BMC Bioinform.* **2012**, *13*, 113. [CrossRef] [PubMed]
27. Xie, L.; Yin, C. Seasonal variations of soil fungal diversity and communities in subalpine coniferous and broadleaved forests. *Sci. Total Environ.* **2022**, *846*, 157409. [CrossRef]
28. Siles, J.A.; Margesin, R. Seasonal soil microbial responses are limited to changes in functionality at two Alpine forest sites differing in altitude and vegetation. *Sci. Rep.* **2017**, *7*, 2204. [CrossRef]
29. Yeung, A.C.Y.; Kreutzweiser, D.P.; Richardson, J.S. Stronger effects of litter origin on the processing of conifer than broadleaf leaves: A test of home-field advantage of stream litter breakdown. *Freshw. Biol.* **2019**, *64*, 1755–1768. [CrossRef]
30. Sui, X.; Li, M.; Frey, B.; Wang, M.; Weng, X.; Wang, X.; Chen, F.; Li, X.; Du, Z.; Yang, L.; et al. Climax forest has a higher soil bacterial diversity but lower soil nutrient contents than degraded forests in temperate northern China. *Ecol. Evol.* **2022**, *12*, e9535. [CrossRef]
31. Jiang, S.; Xing, Y.; Liu, G.; Hu, C.; Wang, X.; Yan, G.; Wang, Q. Changes in soil bacterial and fungal community composition and functional groups during the succession of boreal forests. *Soil Biol. Biochem.* **2021**, *161*, 108393. [CrossRef]
32. Qu, Z.; Liu, B.; Ma, Y.; Xu, J.; Sun, H. The response of the soil bacterial community and function to forest succession caused by forest disease. *Funct. Ecol.* **2020**, *34*, 2548–2559. [CrossRef]
33. Pfeiffer, B.; Fender, A.-C.; Lasota, S.; Hertel, D.; Jungkunst, H.F.; Daniel, R. Leaf litter is the main driver for changes in bacterial community structures in the rhizosphere of ash and beech. *Appl. Soil Ecol.* **2013**, *72*, 150–160. [CrossRef]
34. Mommer, L.; Cotton, T.E.A.; Raaijmakers, J.M.; Termorshuizen, A.J.; van Ruijven, J.; Hendriks, M.; van Rijssel, S.Q.; van de Mortel, J.E.; van der Paauw, J.W.; Schijlen, E.G.W.M.; et al. Lost in diversity: The interactions between soil-borne fungi, biodiversity and plant productivity. *New Phytol.* **2018**, *218*, 542–553. [CrossRef] [PubMed]
35. Wang, X.; Feng, J.; Ao, G.; Qin, W.; Han, M.; Shen, Y.; Liu, M.; Chen, Y.; Zhu, B. Globally nitrogen addition alters soil microbial community structure, but has minor effects on soil microbial diversity and richness. *Soil Biol. Biochem.* **2023**, *179*, 108982. [CrossRef]
36. Peng, W.; Zhu, Y.; Song, M.; Du, H.; Song, T.; Zeng, F.; Zhang, F.; Wang, K.; Luo, Y.; Lan, X.; et al. The spatial distribution and drivers of soil microbial richness and diversity in a karst broadleaf forest. *For. Ecol. Manag.* **2019**, *449*, 117241. [CrossRef]
37. Tedersoo, L.; Bahram, M.; Põlme, S.; Kõljalg, U.; Yorou, N.S.; Wijesundera, R.; Ruiz, L.V.; Vasco-Palacios, A.M.; Thu, P.Q.; Suija, A.; et al. Global diversity and geography of soil fungi. *Science* **2014**, *346*, 1256688. [CrossRef] [PubMed]
38. Blackwell, M. The Fungi: 1, 2, 3 . . . 5.1 million species? *Am. J. Bot.* **2011**, *98*, 426–438. [CrossRef]

39. Zeng, Q.; Liu, Y.; Xiao, L.; An, S. Climate and soil properties regulate soil fungal communities on the Loess Plateau. *Pedobiologia* **2020**, *81–82*, 150668. [CrossRef]
40. Beimforde, C.; Feldberg, K.; Nylinder, S.; Rikkinen, J.; Tuovila, H.; Dörfelt, H.; Gube, M.; Jackson, D.J.; Reitner, J.; Seyfullah, L.J.; et al. Estimating the Phanerozoic history of the Ascomycota lineages: Combining fossil and molecular data. *Mol. Phylogenetics Evol.* **2014**, *78*, 386–398. [CrossRef]
41. Yelle, D.J.; Ralph, J.; Lu, F.; Hammel, K.E. Evidence for cleavage of lignin by a brown rot basidiomycete. *Environ. Microbiol.* **2008**, *10*, 1844–1849. [CrossRef] [PubMed]
42. Chen, X.; Condron, L.M.; Dunfield, K.E.; Wakelin, S.A.; Chen, L. Impact of grassland afforestation with contrasting tree species on soil phosphorus fractions and alkaline phosphatase gene communities. *Soil Biol. Biochem.* **2021**, *159*, 108274. [CrossRef]
43. Chen, X.; Chen, H.Y.H.; Chen, C.; Peng, S. Water availability regulates negative effects of species mixture on soil microbial biomass in boreal forests. *Soil Biol. Biochem.* **2019**, *139*, 107634. [CrossRef]
44. Korboulewsky, N.; Heiniger, C.; De Danieli, S.; Brun, J.J. Effect of tree mixture on Collembola diversity and community structure in temperate broadleaf and coniferous forests. *For. Ecol. Manag.* **2021**, *482*, 118876. [CrossRef]
45. Korboulewsky, N.; Perez, G.; Chauvat, M. How tree diversity affects soil fauna diversity: A review. *Soil Biol. Biochem.* **2016**, *94*, 94–106. [CrossRef]
46. Li, S.; Huang, X.; Shen, J.; Xu, F.; Su, J. Effects of plant diversity and soil properties on soil fungal community structure with secondary succession in the Pinus yunnanensis forest. *Geoderma* **2020**, *379*, 114646. [CrossRef]
47. Chen, Z.; Li, Y.; Chang, S.X.; Xu, Q.; Li, Y.; Ma, Z.; Qin, H.; Cai, Y. Linking enhanced soil nitrogen mineralization to increased fungal decomposition capacity with Moso bamboo invasion of broadleaf forests. *Sci. Total Environ.* **2021**, *771*, 144779. [CrossRef]
48. Dini-Andreote, F.; Pylro, V.S.; Baldrian, P.; van Elsas, J.D.; Salles, J.F. Ecological succession reveals potential signatures of marine–terrestrial transition in salt marsh fungal communities. *ISME J.* **2016**, *10*, 1984–1997. [CrossRef]
49. Uroz, S.; Buée, M.; Deveau, A.; Mieszkin, S.; Martin, F. Ecology of the forest microbiome: Highlights of temperate and boreal ecosystems. *Soil Biol. Biochem.* **2016**, *103*, 471–488. [CrossRef]
50. Wei, G.; Li, M.; Shi, W.; Tian, R.; Chang, C.; Wang, Z.; Wang, N.; Zhao, G.; Gao, Z. Similar drivers but different effects lead to distinct ecological patterns of soil bacterial and archaeal communities. *Soil Biol. Biochem.* **2020**, *144*, 107759. [CrossRef]
51. Johnston, A.S.A.; Sibly, R.M. The influence of soil communities on the temperature sensitivity of soil respiration. *Nat. Ecol. Evol.* **2018**, *2*, 1597–1602. [CrossRef]
52. Pugnaire, F.I.; Morillo, J.A.; Peñuelas, J.; Reich, P.B.; Bardgett, R.D.; Gaxiola, A.; Wardle, D.A.; van der Putten, W.H. Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. *Sci. Adv.* **2019**, *5*, eaaz1834. [CrossRef]
53. Hernández-Cáceres, D.; Stokes, A.; Angeles-Alvarez, G.; Abadie, J.; Anthelme, F.; Bounous, M.; Freschet, G.T.; Roumet, C.; Weemstra, M.; Merino-Martín, L.; et al. Vegetation creates microenvironments that influence soil microbial activity and functional diversity along an elevation gradient. *Soil Biol. Biochem.* **2022**, *165*, 108485. [CrossRef]
54. Fierer, N.; Jackson, R.B. The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 626. [CrossRef]
55. Prescott, C.; Blevins, L.; Staley, C. Litter decomposition in B.C. forests: Controlling factors and influences of forestry activities. *J. Ecosyst. Manag.* **2005**, *5*, 44–57.
56. Shihan, A.; Hättenschwiler, S.; Milcu, A.; Joly, F.-X.; Santonja, M.; Fromin, N. Changes in soil microbial substrate utilization in response to altered litter diversity and precipitation in a Mediterranean shrubland. *Biol. Fertil. Soils* **2017**, *53*, 171–185. [CrossRef]
57. Whalen, E.D.; Lounsbury, N.; Geyer, K.; Anthony, M.; Morrison, E.; van Diepen, L.T.A.; Le Moine, J.; Nadelhoffer, K.; vanden Enden, L.; Simpson, M.J.; et al. Root control of fungal communities and soil carbon stocks in a temperate forest. *Soil Biol. Biochem.* **2021**, *161*, 108390. [CrossRef]
58. Marklein, A.R.; Houlton, B.Z. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol.* **2012**, *193*, 696–704. [CrossRef] [PubMed]
59. Brown, S.P.; Jumpponen, A. Phylogenetic diversity analyses reveal disparity between fungal and bacterial communities during microbial primary succession. *Soil Biol. Biochem.* **2015**, *89*, 52–60. [CrossRef]
60. Tu, Q.; Yan, Q.; Deng, Y.; Michaletz, S.T.; Buzzard, V.; Weiser, M.D.; Waide, R.; Ning, D.; Wu, L.; He, Z.; et al. Biogeographic patterns of microbial co-occurrence ecological networks in six American forests. *Soil Biol. Biochem.* **2020**, *148*, 107897. [CrossRef]
61. Gao, C.; Xu, L.; Montoya, L.; Madera, M.; Hollingsworth, J.; Chen, L.; Purdom, E.; Singan, V.; Vogel, J.; Hutmacher, R.B.; et al. Co-occurrence networks reveal more complexity than community composition in resistance and resilience of microbial communities. *Nat. Commun.* **2022**, *13*, 3867. [CrossRef] [PubMed]
62. 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] [PubMed]