Structure and Co-occurrence Network Characteristics of Rhizosphere Soil Fungal Communities of Alsophila spinulosa in Subtropical Chishui River Valley, China

Dongmei Yuan[1], Jin Chen[1], Lifei Yu[1], Huiming Zhang[1], Xiugang Cao[1], Qinqin He[2], Man Yang[1], and Lingbin Yan[1]

[1]Guizhou University
[2]Guizhou

October 16, 2023

Abstract

Aims The co-occurrence of soil microorganisms and plants is of great significance in revealing the material cycle. The study of the community structure and co-occurrence network relationship of rhizosphere soil fungi of the relict plant Alsophila spinulosa can reveal the mechanism of constructing soil fungal communities. Methods The community structure and co-occurrence network characteristics of soil fungi in the rhizosphere of A. spinulosa were analysed using Illumina Miseq sequencing technology and co-occurrence networks. Results The rhizosphere soil fungal communities of A. spinulosa are significantly different from those in the nonrhizosphere soil. The rhizosphere soil fungal phylogeny of A. spinulosa was concentrated in Ascomycota, Mortierellomycota, and Rozellomycota. Aggregation of Cutaneotrichosporon, the main differential species, significantly affected the construction of the rhizosphere fungal community of A. spinulosa. The indicator fungal groups of the rhizosphere soil fungal community of A. spinulosa were significantly influenced by habitat. Saprotrophs are the main fungi responsible for material exchange in A. spinulosa. Increase in the relative abundance of animal pathogens was the main factor affecting the percentage of pathotroph. The rhizosphere soil fungal co-occurrence networks of A. spinulosa had high synergism and network connectivity, and more intense interspecies competition at the order level. Conclusions Overall, the rhizosphere soil fungal community of A. spinulosa altered significantly, with a stable co-occurrence network. Continuous in-depth study on the growth of the key soil fungi can help understand the growth mechanism of A. spinulosa.

Structure and Co-occurrence Network Characteristics of Rhizosphere Soil Fungal Communities of*Alsophila spinulosa* in Subtropical Chishui River Valley, China

Dongmei Yuan [a], Jin Chen[a], Lifei Yu[a], Zhang Huiming [a], Cao Xiugang[a], Qinqin He [b], Man Yang[a], Lingbin Yan [a,*]

[a] Key Laboratory of Plant Resource Conservation and Germplasm Innovation in Mountainous Region (Ministry of Education), College of Life Sciences/Institute of Agro-bioengineering, Gui-zhou University, Guiyang 550025, Guizhou Province, China

[b] Guizhou Chishui Alsophila National Nature Reserve Administration Bureau, Chishui 564700, Guizhou, China

* Correspondence: Lingbin Yan. E-mail:*lbyan@gzu.edu.cn*

Structure and Co-occurrence Network Characteristics of Rhizosphere Soil Fungal Communities of*Alsophila spinulosa* in Subtropical Chishui River Valley, China

Abstract:

Aims The co-occurrence of soil microorganisms and plants is of great significance in revealing the material cycle. The study of the community structure and co-occurrence network relationship of rhizosphere soil fungi of the relict plant *Alsophila spinulosa*can reveal the mechanism of constructing soil fungal communities.

Methods The community structure and co-occurrence network characteristics of soil fungi in the rhizosphere of *A. spinulosa*were analysed using Illumina Miseq sequencing technology and co-occurrence networks.

Results The rhizosphere soil fungal communities of *A. spinulosa* are significantly different from those in the nonrhizosphere soil. The rhizosphere soil fungal phylogeny of *A. spinulosa* was concentrated in Ascomycota, Mortierellomycota, and Rozellomycota. Aggregation of *Cutaneotrichosporon* , the main differential species, significantly affected the construction of the rhizosphere fungal community of *A. spinulosa* . The indicator fungal groups of the rhizosphere soil fungal community of *A. spinulosa* were significantly influenced by habitat. Saprotrophs are the main fungi responsible for material exchange in *A. spinulosa* . Increase in the relative abundance of animal pathogens was the main factor affecting the percentage of pathotroph. The rhizosphere soil fungal co-occurrence networks of *A. spinulosa* had high synergism and network connectivity, and more intense interspecies competition at the order level.

Conclusions Overall, the rhizosphere soil fungal community of*A. spinulosa* altered significantly, with a stable co-occurrence network. Continuous in-depth study on the growth of the key soil fungi can help understand the growth mechanism of *A. spinulosa* .

Keywords: Subtropical river valley; *Alsophila spinulosa* ; Soil fungi; Community structure; Co-occurrence network; Functional prediction

Introduction

Microorganisms play a crucial role in maintaining ecosystem function (Coban et al. 2022). Fungi are the dominant players of the soil microbial community, with a broad scope, high spatial heterogeneity, and crucial ecological functions (Bahram et al. 2015). The soil fungal community structure and co-occurrence network relationships can reveal the construction pattern of soil microbial communities, reflect the functional status of soil ecosystems, and elucidate the material cycling characteristics of plant–soil negative feedback systems (Bever et al. 2012). The soil fungal community structure reportedly responds differently to plants, with higher soil fungal co-occurrence network stability in plant root systems (Wang C et al. 2018; Yuqi Wang et al. 2023; Lei Zhang et al. 2021). Therefore, investigating the structure and co-occurrence network of soil fungal communities is important for revealing the "microbe–plant" relationship.

As an ancient relict tree fern from the Cretaceous age, *A. spinulosa* is a crucial material for studying plant origin and transformation and geographic zones (Qinqin He et al. 2022); the stem is rich in flavonoids, alkaloids, and other active substances, which are of high medicinal value in epidemics treatment and bacterial inhibition (Shuhua Li et al. 2013; Xin Zhang et al. 2018). Harsh breeding conditions, serious habitat destruction, interference by natural enemies, and intense interspecific competition have adversely declined the *A. spinulosa* population and drastically reduced their distribution range. Although recent studies have focused on *A. spinulosa* and developed conservation programs worldwide,*A. spinulosa* is still present on the endangered conservation list. To improve its endangered status, many researchers have performed relevant studies on the population structure and dynamics (Xie et al. 2022), spatial distribution (Yuan 2021), breeding (Lang et al. 2021), natural enemies (Du 2022), community structure (Hui Li et al. 2021; Qin Liu et al. 2019), interspecific relationships (Jiang et al. 2021), and diversity (Zhao 2018) of *A. spinulosa* . Certain relationships between *A. spinulosa* and other plants and animals are known biologically, but studies on *A. spinulosa* –microbial relationships remain relatively scarce. Endophytic fungal diversity reportedly varied in different *A. spinulosa* tissue masses such as pinnae, leaf rachis, petioles, bark, roots, and petiolar apoplasts.*Aspergillus sydowii* and *Dactylonectria pauciseptata* are two endophytic fungi that are widely present in *A. spinulosa*(YongLan Liu et al. 2021; Wei Zang et al. 2020). *Xylaria* ,*Colletotrichum* , and *Pestalotiopsis* constitute the dominant genera of endophytic fungi in the root, stem, and leaf tissues of*A.*

spinulosa (Wenna Zhou 2015). Ectomycorrhizal fungi affect the ecological functions of *A. spinulosa* such as material cycling and nutrient supply (Tedersoo et al. 2020). *Acaulospora* and*Funneliformis* constitute the dominant mycorrhizal fungal genera of *A. spinulosa* . They help improve the resistance of *A. spinulosa* and are of high value for its successful breeding and survival (Lara-Pérez et al. 2014). Studies on *A. spinulosa* –fungus relationship have mainly focused on endophytic fungi, and the effect of fungi on the environment remains unclear, making it difficult to fully understand the relationship between *A. spinulosa* -microorganisms.

Ecological specialization of fungi is reportedly higher than that of bacteria in the soil (Xi 2022). Soil serves as an important site for species exchange, and the soil fungal community structure in the root system of *A. spinulosa* could significantly impact nutrient turnover functions (Lu Han 2022; Wei Li et al. 2022). In the soil, high relative abundances of Basidiomycota are observed in mixed evergreen deciduous broadleaved forests, montane dwarf forests, and deciduous broadleaved forests. Ascomycota was not significantly different and widespread, and Peridiomycota appeared to display a U-shaped variation pattern (Man et al. 2021). It remains unclear whether Ascomycota is similarly widespread in the soil fungal community of the*A. spinulosa* root system. It is important to identify the fungal taxa that differentiate soil fungal communities in the*A. spinulosa* root system based on fungal ecological specialization of fungi. Determination of the ecological functions of soil fungal taxa in the *A. spinulosa* root system would help influence *A. spinulosa* growth. Defining the stability state of the soil fungal community in the inter-root zone of *A. spinulosa*along with the key fungal taxa that critically influence community stability is also crucial. Therefore, an in-depth study on the soil fungal community structure and co-occurrence network in the*A. spinulosa* root system is of great significance to improve*A. spinulosa* –microbial relationships and provide new references for developing *A. spinulosa* conservation strategies.

This study aimed to focus on the rhizosphere soil fungal community of*A. spinulosa* in the subtropical Chishui River Valley, China. Using microbial Illumina Miseq sequencing technology, we revealed the structure of the rhizosphere soil fungal community of *A. spinulosa* and its co-occurrence network to provide rich data and scientific reference for studying this community.

Materials and Methods

Study area

This study was conducted in Chishui City, Guizhou Province (28°16’19’’N, 105deg36’35’’E to 28deg46’0’’N, 106deg15’0’’E, southwestern China), which has a central subtropical humid monsoon climate and is located in the transition zone from the Yunnan–Guizhou Plateau to the Sichuan Basin. This terrain is mainly of the plateau–canyon type and mountain–plain canyon type, with heavy mountains and deep canyons in the southeast, rolling hills in the northwest, and open and gentle river valleys. The terrain is high in the southeast and low in the northwest regions, with the altitude decreasing from the southeast to the northwest. The average annual temperature at the time of this study was 18.1degC, with an annual difference in temperature of 20.1degC–20.5degC. The extreme minimum temperature was -4degC and the extreme maximum temperature was 39degC. The average annual rainfall in this region is 1292.3 mm, mainly concentrated from April to October, accounting for approximately 80% of the annual rainfall. The wind direction is prevalently north, southeast in summer, and north in winter. The soils are mostly neutral and slightly acidic sandy purple soils, and there are many river tributaries. The vegetation type can be classified into four groups: subtropical evergreen broadleaved forest, mixed coniferous forest, coniferous forest, and bamboo forest.

Sample area square setting

The sampling sites were selected in the Chishui area with *A. spinulosa* growth, and the rhizosphere soil was collected within a 4 mm depth from the base of the trunk of each *A. spinulosa* fern with good growth conditions and a similar growth profile. Nonrhizosphere soil samples were also collected from a locality away from the plant root zone. A total of 23 sampling sites were selected, and 46 soil samples were collected. The collected samples were sieved to remove stones, plant remains, roots, and other impurities and then

immediately packed into sterile centrifuge tubes, labeled, and stored in liquid nitrogen. The samples were sent to the laboratory for Illumina Miseq sequencing of soil microorganisms.

DNA extraction and high-throughput sequencing

DNA kit (OMEGA, USA) was used to extract total DNA from the soil fungal flora of each sample, and the extraction quality (1% agarose gel electrophoresis) and DNA quantity (UV spectrophotometry, NanoDrop 2000 Spectrophotometer) were measured. Polymerase chain reaction (PCR) amplification was performed with ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3')_ITS2R (5'- GCTGCGTTCTTCATCGATGC-3') under the following conditions: predenaturation at 95degC for 3 min; followed by 27 cycles of denaturation at 95degC for 30 s, annealing at 55degC for 30 s, and extension at 72degC for 30 s; stable extension at 72degC for 10 min; and final storage at 4degC. The PCR system comprised 4 μL of 5× TransStart FastPfu buffer, 2 μL of dNTPs (2.5 mmol/L), 0.8 μL of upstream primer (5 μmol/L), 0.8 μL of downstream primer (5 μmol/L), 0.4 μL of TransStart FastPfu DNA polymerase, and 10 ng of template DNA, which made up the volume to 20 μL, with three replicates for each sample. The PCR products from the same sample were mixed and detected via 2% agarose gel electrophoresis and then recovered by cutting the gel using AxyPrepDNA Gel Recovery Kit (AXYGEN). The recovered products were eluted using Tris_HCl for 2% agarose electrophoresis detection. Based on the initial quantification via electrophoresis, the PCR products were detected and quantified using the QuantiFluor -ST Blue Fluorescence Quantification System (Promega) and then mixed in appropriate proportions according to the sequencing volume required for each sample.

Based on the Illumina library construction and sequencing results, the obtained PE reads were first spliced according to the overlapping relationship, and the sequence was quality controlled and filtered, after which the samples were differentiated and subjected to OTU clustering analysis. OTU clustering of the nonrepeated sequences (excluding single sequences) was performed at a 97% similarity level, and species annotation analysis was performed using the RDP classifier Bayesian algorithm with the Unite Fungal Database. The community species composition of each sample was determined at each taxonomic level: domain, kingdom, phylum, class, order, family, genus, and species. This technology and sequencing equipment were provided by Shanghai Majorbio Bio-Pharm Technology Co.

Data processing and analysis

Analytical methods

The fungal community in the rhizosphere soil was subjected to corresponding NMDS analyses. LEfSe analysis was performed to compare the species with significant differences among the groups. The construction of evolutionary relationships of soil fungal communities based on microbial phylogenetic evolutionary trees was performed. The functional status of soil fungi was analyzed based on FUNGuild functional prediction. The microbial co-occurrence network visualization was applied to characterize the topology of the soil fungal community network of *A. spinulosa* and screen out the key species. According to the network visualization status, the r and p values of the symbiotic network at different taxonomic levels were as follows: phylum ($r > 0.5$, $p < 0.5$), order ($r > 0.6$, $p < 0.5$), family ($r > 0.5$, $p < 0.5$), genus ($r > 0.68$, $p < 0.05$), and species ($r > 0.68$, $p < 0.05$). Based on the indicator species analysis method of screening the indicator groups of soil fungi at each taxonomic level, the indicator value IndVa was calculated as follows:

$$IndVal = \frac{N_{\mathrm{ij}}}{N_i} \times \frac{M_{\mathrm{ij}}}{M_j} \times 100$$

where N_{ij} is the average abundance value of taxon i in the subgroup j, N_i is the sum of the average abundance values of taxon i in all subgroups; $M_{\mathrm{ij}}$is the number of samples of taxon i occurring in the subgroup j, and M_j is the total number of samples of taxon i in the subgroup j.

Taxa with IndVal of [?]25% were used as indicator taxa, taxa with IndVal of [?]70% were used as character indicator taxa, and taxa with IndVal of 50%–70% were used as habitat indicator taxa. The indicator taxa were analyzed at five taxonomic levels: phylum, order, family, genus, and species.

Data processing

Data analyses were performed using Microsoft Excel 2019, R software, and Shanghai Meguiar's BioCloud platform (*https://www.isanger.com*). Species annotation and assessment were performed using Uparse (version 7.0.1090;*http://drive5.com/uparse/*) software. Dilution curves were constructed using Mothur (version 1.30.1) software to calculate the alpha diversity index for random samples. The beta diversity distance matrix was calculated using Qiime software, NMDS analysis, and graphing in R. Linear discriminant analysis of the samples was performed according to different grouping conditions using LEfSe software (*http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload*) based on the taxonomic composition. Phylogenetic evolutionary trees were constructed using FastTree software (version 2.1.3;*http://www.microbesonline.org/fasttree/*) by selecting the sequences corresponding to the taxonomic information at the species level according to the maximum likelihood method. Taxonomic analysis of fungal communities was performed using FUNGuild via the Megisign cloud platform. Microbial network visualization was performed based on "igraph," "vegan," "tidyverse," "psych'," "ggsci," "magrittr," "ggplot2," "RColorBrewer," and other packages.

Results

Soil fungal community structure

Community composition

The species composition reflects the community structure status. In this study, 259,1494 optimized sequences were obtained for the fungus, with an average sequence length of 227 bp. Clustering at 97% similarity yielded 9986 OTUs belonging to 16 phyla, 65 orders (class), 157 orders, 389 families, 948 genera, and 1624 species.

Dilution curves indicate the rationality of sequencing data. In this study (Fig. 1a), the dilution curves of nonrhizosphere and rhizosphere soil samples of *A. spinulosa* exhibited a gradual rise after the sequencing volume reached a certain depth and then gradually flattened out. The sequencing depth was sufficient to cover most microbial taxa, and increasing the sequencing volume would only lead to the addition of a small number of new species, indicating the rationality of the sequencing volume in this study. According to NMDS analysis, a stress value of 0.083 (Fig. 1b) indicates a significant difference in the fungal community structure between nonrhizosphere and rhizosphere soils of *A. spinulosa* , suggesting that the grouping of nonrhizosphere and rhizosphere soils was well represented.

Note: R indicates soil fungi of *A. spinulosa* ; NO indicates soil fungi of *A. spinulosa* . (a) Dilution curve. (b) NMDS analysis. Points of different colors or traits in the graph represent different sample groups. The closer the points of the two samples, the more similar their species composition. The horizontal and vertical coordinates indicate relative distances and have no practical significance. stress: test for the merit of NMDS analysis. A stress value of <0.2 can be represented by a two-dimensional point plot of NMDS analysis; this graph has some interpretative significance. The stress value of <0.1 indicates a good ranking. When the stress value is <0.05, it is considered well represented.

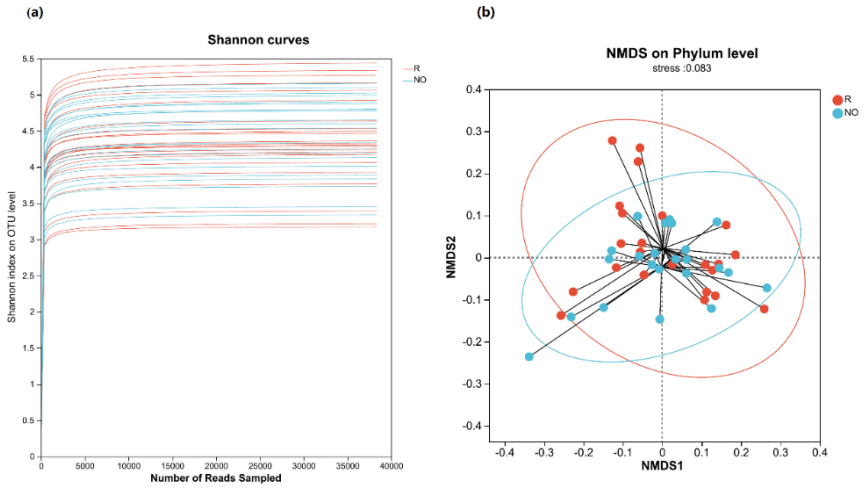


Fig. 1 Soil fungal community dilution curves and community variation characteristics

Phylogenetic and compositional variability

We conducted a phylogenetic analysis of the top 100 species with regard to relative abundance at the species level (Fig. 2a). According to the results, the fungal community phylogeny was highly aggregated between the nonrhizosphere and rhizosphere soils of *A. spinulosa* . The rhizosphere soil of *A. spinulosa* was concentrated in Ascomycota, Mortierellomycota, and Rozellomycota fungi, and the nonrhizosphere soil was concentrated in Ascomycota and Chytridiomycota fungi.

LEfSe multilevel species difference discrimination analysis (Fig. 2b) revealed that the species that differed between the rhizosphere soil community groups of *A. spinulosa* were mainly at the genus level, including *Cutaneotrichosporon* , *Chaetopsina* ,*Dendrosporium* , *Tubulicium* , *Shiraia* ,*Perenniporia* , *Simplicillium* and *Capitofimbria* ,*Tubulicium* , *Shiraia* , *Perenniporia* ,*Simplicillium* , and *Capitofimbria* , with the abundance of species observed in the *Cutaneotrichosporon* group. This was the primary reason for the variation in soil fungi between *A. spinulosa* roots. The main groups of nonrhizospherically differentiated species were the Sordariomycetes group at the phylum level, Chaetothyriales and Trechisporales groups at the order level, and the Herpotrichiellaceae group at the family level.

Note: R indicates *A. spinulosa* rhizosphere soil fungi; NO indicates *A. spinulosa*nonrhizosphere soil fungi. (a) Phylogenetic evolutionary tree of soil fungi based on the species level; (b) LEfSe multilevel species difference discrimination analysis of the soil fungal communities.

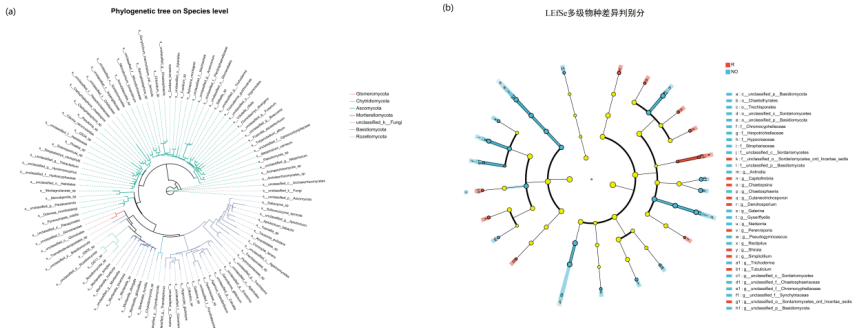


Fig. 2 Phylogeny and LEfSe discrimination

Indicator taxa

The fungal community indicator taxa of nonrhizosphere and rhizosphere soils of *A. spinulosa* were significantly different at different taxonomic levels, with a large proportion of indicator taxa being unclassified soil fungi (Table 1). At the phylum level, 12 rhizosphere indicator taxa with IndVal values of 0.24–0.57 were observed, with the obvious indicator taxon being Chytridiomycota (IndVal = 56.67%). Twelve nonrhizosphere indicator taxa with IndVal values of 0.25–0.53 were also observed at the phylum level, with the obvious indicator taxon being Glomeromycota (IndVal = 52.71%). Only one indicator taxon—Basidiomycota—was common between inter- and nonrhizosphere soils.

At the order level, 84 rhizosphere indicator taxa with IndVal values of 0.21–0.63 were observed, with the obvious indicator taxon being Chantharellales (IndVal = 63.40%). In total, 79 nonrhizosphere indicator taxa with IndVal values of 0.21–0.67 were observed, with the obvious indicator taxon being Orbiliales (IndVal = 67.43%).

At the family level, 154 rhizosphere indicator taxa with IndVal values of 0.21–0.66 were observed, with the obvious indicator taxon being Ceratobasidiaceae (IndVal = 66.43%). Further, 156 nonrhizosphere indicator taxa with IndVal values of 0.20–0.68 were noted, with the obvious indicator taxon being Cordycipitaceae (IndVal = 67.61%).

At the genera level, 241 rhizosphere indicator taxa with IndVal values of 0.20–0.67 were observed, with the obvious indicator taxon being the genus *Tolypocladium* (IndVal = 59.83%). In total, 250 nonrhizosphere indicator taxa with IndVal values of 0.20–0.69 were observed, with the obvious indicator taxon being the genus*Agaricus* (IndVal = 69.12%).

At the species level, 241 rhizosphere indicator taxa with IndVal values of 0.20–0.87 were observed, with the obvious indicator taxa being unclassified species of the genera *Ciliolarina* and*Serendipita* ; both genera had an IndVal value of 86.96%. In total, 283 nonrhizosphere indicator taxa with IndVal values of 0.20–0.91 were observed, with the obvious indicator taxa being*Saitozyma ninhbinhensis* and *Virgatospora echinofibrosa* ; both had an IndVal value of 91.30%.

The number of habitat-indicating taxa in the soil fungal community of*A. spinulosa* was lower at three taxonomic levels, namely, order, family, and genus, than at the nonrhizosphere level. The characteristic indicator taxa were represented only at the species level, with 3 species of soil fungi being characteristic of the *A. spinulosa*rhizosphere and 24 species of soil fungi being characteristic of the nonrhizosphere.

The difference in indicator taxa indicates that soil fungal communities have obvious indicator effects on *A. spinulosa* roots. The unclassified taxa with indicator effects on the *A. spinulosa*rhizosphere soil may be crucial taxa reflecting the soil environmental changes. However, this finding needs to be explored in future research.

Table 1 Indicative values for soil fungal taxa of *A. spinulosa* at different taxonomic levels

	non-rhizosphere	non-rhizosphere	rhizosphere	rhizosphere
taxonomic levels	taxa	IndVal	taxa	IndVal
Phylum	Glomeromycota	52.71%	Chytridiomycota	56.67%
	Unclassified_Fungi	52.62%	Kickxellomycota	55.40%
	Mortierellomycota	51.03%	Zoopagomycota	54.83%
	Ascomycota	50.06%	Rozellomycota	52.15%
	Basidiomycota	49.45%	Basidiomycota	50.55%
Order	Orbiliales	67.43%	Cantharellales	63.40%
	Sordariales	64.71%	Unclassified_Dothideomycetes	60.48%
	Pleosporales	59.32%	Unclassified_Agaricomycetes	60.20%

	non-rhizosphere	non-rhizosphere	rhizosphere	rhizosphere
	Eurotiales	58.97%	GS11	59.63%
	Chaetothyriales	53.71%	Unclassified_Chytridiomycota	59.18%
Family	Cordycipitaceae	67.61%	Ceratobasidiaceae	66.43%
	Agaricaceae	66.79%	Leotiaceae	64.69%
	Orbiliaceae	65.95%	Helotiaceae	64.34%
	Didymellaceae	64.24%	Ophiocordycipitaceae	60.94%
	Aspergillaceae	62.22%	Unclassified_Agaricomycetes	60.20%
Genus	*Agaricus*	69.12%	*Tolypocladium*	59.83%
	Clonostachys	63.07%	Unclassified_GS11	59.63%
	Stilbella	61.12%	Unclassified_Chytridiomycota	59.18%
	Unclassified_Lycoperdaceae	60.61%	Unclassified_Dothideomycetes	57.74%
	Unclassified_Nectriaceae	58.77%	*Lycoperdon*	57.40%
Species	*Saitozyma ninhbinhensis*	91.30%	Unclassified_Ciliolarina	86.96%
	Virgatospora echinofibrosa	91.30%	Unclassified_Serendipita	86.96%
	Aspergillus tamarii	86.96%	Unclassified_Agaricomycetes	73.91%
	Candelabrochaete cirrata	86.96%	Boletaceae_sp	69.57%
	Cetraspora gilmorei	86.96%	*Coprinopsis stercorea*	69.57%

Note: IndVal values of only the top five indicator taxa for each classification level are shown.

Co-occurrence networks of soil fungal communities

Key taxa

Co-occurrence network analysis of soil fungi between Cyathea roots (Fig. 3) demonstrated that the key fungal phyla were Ascomycota, Kickxellomycota, Mortierellomycota, and Rozellomycota. The key fungal groups were Glomerellale, Saccharomycetales, Archaeorhizomycetes (undefined), Capnodiales, Hymenochaetales, and Pleosporales. The key fungal families were Didymosphaeriaceae, Plectosphaerellaceae, Stachybotryaceae, and the undefined family Archaeorhizomycetes. The key fungal genera were *Volutella* , Bionectriaceae undetermined, Arthopyreniaceae undetermined, *Cordana* , and Hypocreales undetermined. The key fungal species were *Neocosmospora rubicola* , Arthopyreniaceae undetermined, Hypocreales undetermined, Clavicipitaceae undetermined, and Plectosphaerellaceae undetermined.

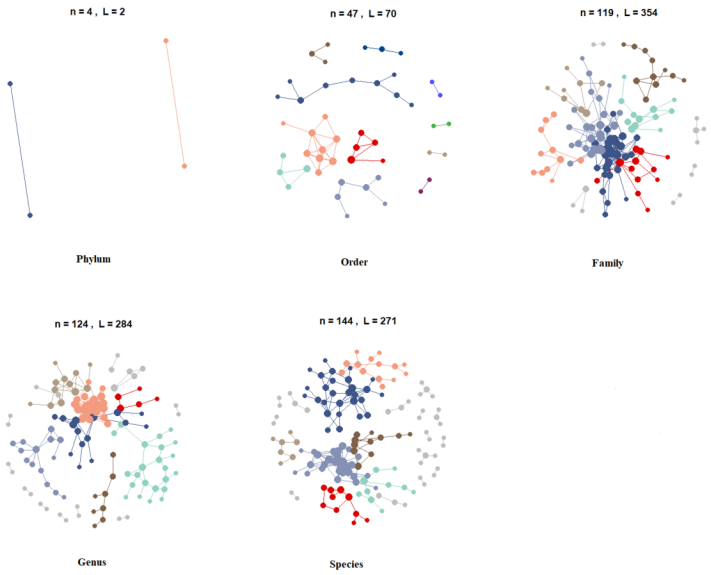


Fig. 3 Co-occurrence network of rhizosphere soil fungi of *A. spinulosa* at different taxonomic levels

Symbiotic network analysis of nonrhizosphere soil fungi of *A. spinulosa* (Fig. 4) revealed that the key fungal phyla were Ascomycota, Glomeromycota, Rozellomycota, Zoopagomycota, Mortierellomycota, and Neocallimastigomycota. The key fungal groups were Hypocreales, the undefined order Chytridiomycota, and Pezizales. The key fungal families were Xylariaceae, Chytridiomycota undetermined, Synchytriaceae, Phaeosphaeriaceae, Didymosphaeriaceae, Thelephoraceae, and Chytridiomycota undetermined. The key fungal genera are *Dokmaia* ,*Fusarium* , *Lycoperdon* , *Phellinus* , *Phialea* ,*Pochonia* , Archaeorhizomycetes (undefined), and *Volutella* . The key fungal species were *Chloridium* undetermined,*Volutella ciliata* , *Fusarium* undetermined, *Dokmaia monthadangi* , and *Metarhizium marquandii* .

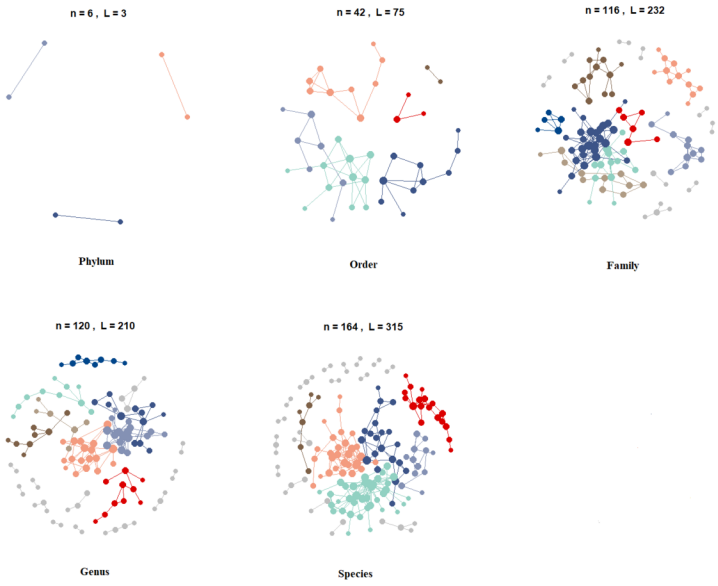
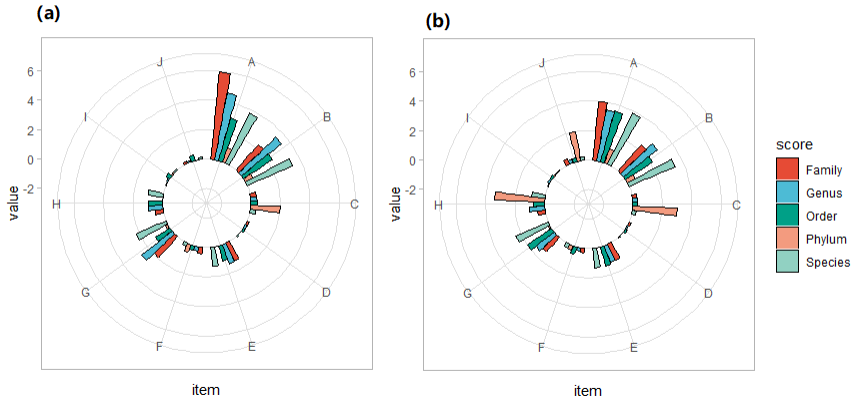


Fig. 4 Co-occurrence network of nonrhizosphere soil fungi of *A. spinulosa* at different taxonomic levels

Analysis of network properties

Further analysis of the network topological properties (Figures 3, 4, and 5) revealed that the number of nodes and connections between the co-occurrence networks of rhizosphere and nonrhizosphere soil fungi of*A. spinulosa* was significantly different. At different taxonomic levels, the ratio of positively correlated margins in the fungal co-occurrence network of the *A. spinulosa* rhizosphere soil was higher than that of the nonrhizosphere soil. The number of negatively correlated margins at the order level was higher in the rhizosphere soil than in the nonrhizosphere soil. This indicates that the rhizosphere soil fungal community of *A. spinulosa* tended to be more stable and that interspecific competition was more intense at the order level. According to network topology analysis, the stability of the fungal co-occurrence network of the *A. spinulosa* rhizosphere soil was the highest at the eye level (network stability = 0.37). The network quality and efficiency of *A. spinulosa* roots were higher at all taxonomic levels than those of non-roots. However, the smaller average network path length and higher network vulnerability also indicated that the co-occurrence network of these soil fungal communities in *A. spinulosa* roots was vulnerable to external environmental disturbances.



Note: (a) Rhizosphere soil fungal topology parameters; (2) nonrhizosphere soil fungal network topology parameters. Different uppercase letters indicate the following: A, average degree; B, average path length; C, clustering coefficient; D, network degree centrality; E, network tight centrality; F, network efficiency; G, network quality; H, information center; I, network vulnerability; J, network stability.

Fig. 5 Topological properties of the soil fungal community networks of*A. spinulosa* at different taxonomic levels

Predictive analysis of fungal function

FUNGuild functional prediction analysis revealed that the soil fungi of*A. spinulosa* included nine main groups: pathotroph, symbiotroph, saprotroph, saprotroph–symbiotroph, pathotroph–symbiotroph, pathotroph–saprotroph, saprotroph–pathotroph–symbiotroph, pathotroph–saprotroph–symbiotroph, and pathogen–saprotroph–symbiotroph. Soil fungi in both *A. spinulosa* rhizosphere and nonrhizosphere were predominantly saprophytic trophic (saprotroph), with the proportion of pathotrophs being higher in the *A. spinulosa* rhizosphere (10.51% vs. 7.76%).

In total, 108 functional groups were detected through functional group identification, and 15 of them had a relatively high abundance (Fig. 6). The main functional groups of symbiotrophs and saprotrophs in the rhizosphere and non-rhizosphere soils of *A. spinulosa* were identical. The main functional groups of symbiotrophs were arbuscular mycorrhizal, ectomycorrhizal, and endophyte fungi. Saprotrophs comprised undefined saprotroph, soil saprotroph, and wood saprotroph as the main functional groups. The main functional groups of pathotrophs between*A. spinulosa* rhizosphere and nonrhizosphere soils were identical: animal pathogen, phytopathogen, and fungal parasite–lichen parasite. However, the status of the functional groups differed, with the animal pathogen predominating the *A. spinulosa* rhizosphere soil and the phytopathogen predominating the nonrhizosphere soil. Compared with fungi in the nonrhizosphere soil, the relative abundance of fungi in the rhizosphere soil of *A. spinulosa* increased for animal pathogens and saprotroph–symbiotroph and decreased for pathotroph–saprotroph.

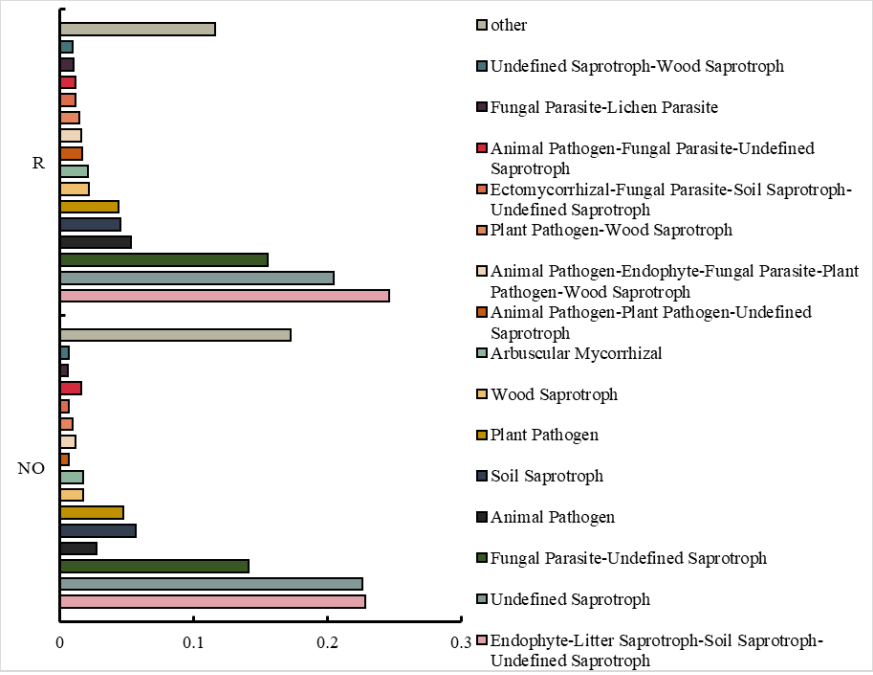


Fig. 6 Predicted functional analysis of soil fungi of *A. spinulosa*

Discussion

Structural and functional characteristics of the fungal community of*A. spinulosa* rhizosphere soil

The plant root zone is defined as the area between 0.5 and 4 mm from the root system. It serves as a medium for the exchange of various substances, such as harmful substances or nutrients, between the soil and plant. This channel is also affected by the activities of and substances secreted from the plant root system itself (Wei Li et al. 2022). The closer the microorganisms are to the plant root system, the higher is their abundance, diversity, and dominance in the soil (Lu Han 2022). An analysis of the phylogenetic and differential structure of the fungal community between the *A. spinulosa* rhizosphere and the*A. spinulosa* nonrhizosphere soils. A high degree of aggregation was observed in the soil fungal phylogeny of the *A. spinulosa*rhizosphere, with the highest aggregation observed in Ascomycota, Mortierellomycota, and Rozellomycota. This is consistent with the findings of most studies on the microbial community structure of rhizosphere soil (Lai et al. 2023; YinYin et al. 2023). LEfSe identifies communities or species that have a significant differential impact on sample delineation (Zewei Zhang et al. 2022). The fungi of the *A. spinulosa* rhizosphere soil differed mainly at the genus level, such as*Cutaneotrichosporon* , *Chaetopsina* , *Dendrosporium* ,*Tubulicium* , *Shiraia* , *Perenniporia* ,*Simplicillium* , and *Capitofimbria* . While investigating the structure of rhizosphere soil microbial communities, a previous study showed that the species contributing more to the differences in the community structure was also concentrated in the bacterial groups at the genus level (Su Liu et al. 2023).

The number and species of indicator taxa were representative and characteristic of the community structure. In the present study, with decreasing taxonomic levels, the number of soil community indicator taxa slightly increased in the *A. spinulosa* nonrhizosphere compared with that the rhizosphere. This may be due to the significant impact of plant roots on the structure of the soil microbial community. Differences in the rhizosphere secretion type and content altered the activity of soil fungi and their ecological niche, leading to the aggregation of specific indicator taxa and reducing the number of indicator taxa (Boyuan Han et al. 2022). In contrast, the fungal community indicator taxa of the *A. spinulosa* rhizosphere soil differed markedly at different taxonomic levels. At the phylum level, Chytridiomycota was the indicator taxon in the *A. spinulosa*rhizosphere, whereas Glomeromycota was the indicator taxon in the*A. spinulosa* nonrhizosphere;

Basidiomycota was a common phylum acting as an indicator taxon. This is in general agreement with the results of a previous study (Zeliang Yang et al. 2020). The microbial decomposition of plant residues affects the input of soil organic matter, and fungi belonging to Chytridiomycota contribute to soil carbon content by providing organic matter to the soil and enhancing soil nutrient cycling through plant residue decomposition (Jinxian Liu et al. 2019). Glomeromycota comprises various crucial symbiotic fungi of the plant root system that influence plant water acquisition and soil stability (YouSan Wang and RunJin Liu 2017). However, their abundance is significantly reduced by plant and nutrient additions (Xiansheng Wang et al. 2022). Basidiomycota fungi are prevalent in soil and are the key fungal degraders of complex wood fibers (Yujie Zhou et al. 2021). In this study, at the order level, the obvious fungal community indicator taxa of the *A. spinulosa* rhizosphere and nonrhizosphere soils were Chantharellales and Orbiliales, respectively. Studies have shown that Cantharellales is closely related to N and P curing rates in fine plant roots (Li et al. 2015). At the family level, the obvious fungal community indicator taxa of the*A. spinulosa*inter-rhizosphere and nonrhizosphere soils were Ceratobasidiaceae and Cordycipitaceae, respectively. Ceratobasidiaceae is the dominant group of mycorrhizal fungi that can easily interact with plant roots to form mycorrhizal associations that affect plant growth (Esposito et al. 2016; Yajuan Fu et al. 2019; Jiayao Li et al. 2021). At the genus level, the obvious fungal community indicator taxa of the *A. spinulosa*rhizosphere and nonrhizosphere soils were *Tolypocladium* and*Agaricus* , respectively. *Tolypocladium* is a basic fungal group that protects plants by inhibiting the pathogenic fungus that causes black pod disease (palm blight) (Wenxia Cui 2017). At the species level, a relatively large number of the unclassified fungal community indicator taxa of the *A. spinulosa* rhizosphere soil were observed. The obvious fungal community indicator taxa of the *A. spinulosa* rhizosphere soil were the unclassified species of the genera*Ciliolarina* and *Serendipita* . The identification of unclassified indicator taxa may be beneficial for elucidating the construction mechanism of the rhizosphere soil fungal community of*A. spinulosa* . Regarding habitat indicator species, the *A. spinulosa* nonrhizosphere and rhizosphere intervals were evident at the family and species levels, respectively. In total, 24 nonrhizosphere and 3 rhizosphere indicator taxa were observed. This indicates that plant habitat influences the soil fungal community structure between plant roots and that this community has a stronger indicator effect.

The functional predictions indicated that saprotrophs were predominant in *A. spinulosa* roots as well as in the nonrhizosphere soil. An increased proportion of pathotrophs was also observed in the *A. spinulosa* roots. Among the main functional groups of pathotrophs, the*A. spinulosa* rhizosphere and nonrhizosphere soils were dominated by animal pathogens. The proportion of pathotrophs among the rhizosphere soil fungi of *A. spinulosa* increased with an increase in the relative abundance of animal pathogens. Simultaneously, the relative abundance of saprotroph–symbiotroph increased and that of pathotroph–saprotroph decreased in the *A. spinulosa* rhizosphere. Saprophytic fungi are the main functional group involved in the decomposition of organic matter such as plant residues and animal manure (MaoSen Li et al. 2022). *A. spinulosa* roots are covered with a thick layer of apomictic material throughout the year, and the high abundance of soil decay fungi is conducive to accelerated material recycling.

Characteristics of the co-occurrence network of soil fungi between the*A. spinulosa* rhizosphere and nonrhizosphere roots

The key taxa play a linking role in the construction of microbial networks. They also influence the construction of ecological networks in biomes. The key fungal taxa of *A. spinulosa* differed at different taxonomic levels between the rhizosphere and non-rhizosphere soils. The key fungal taxa of the *A. spinulosa* rhizosphere soil were Ascomycota and its unclassified groups, whereas those of the nonrhizosphere soil were Chytridiomycota and its unclassified taxa. Ascomycota is positively correlated with total carbon and hemicellulose content and plays a crucial role in plant decomposition (Fuxing Cui et al. 2021). Some fungi belonging to the phylum Chytridiomycota are partial plant parasites. They compete for plant nutrients and produce mobile spores during asexual reproduction. These spores act as a mediator for viral transmission. The endemic key fungal phylum in the*A. spinulosa* rhizosphere soil was Combomycota, whereas those in the nonrhizosphere soil were Glomeromycota, Trapomycota, and Neospora. Some comb fungi belonging to Combomycota are fungal parasites, with their feeding hyphae invading the tissues of other pathogenic fungi and exerting an antagonistic effect (Huang et al. 2022).

Microbial co-occurrence networks indicate the complex interactions between species in microbial communities (Xun Wang et al. 2022). The positive linkage indicates that the fungi are positively correlated with each other and have similar ecological niches or mutualistic symbiosis. In contrast, the negative linkage indicates that fungi are negatively correlated with each other, and an antagonistic or competitive relationship may exist between them (Hou et al. 2020). When the clustering coefficient is higher and the proportion of negative correlation is lower, a higher extent of synergism and stability of the biome are observed (ZhiQi et al. 2021). The clustering coefficients of the fungal community of the *A. spinulosa* inter-rhizosphere soil at the levels of order, family, genus, and species were higher than those of the nonrhizosphere soil. In contrast, the negative correlation ratio was lower in the rhizosphere soil than in the nonrhizosphere soil. This indicates that the fungal community of the *A. spinulosa*rhizosphere soil had a higher extent of network synergy and connectivity than that of the nonrhizosphere soil. Positive correlations were observed between the fungal communities of the *A. spinulosa*rhizosphere and nonrhizosphere soils, indicating that the soil fungal community structure is relatively stable. The proportion of negative correlations between the fungal communities of the *A. spinulosa*rhizosphere and nonrhizosphere soils was high at the levels of order and family, respectively. This indicated that the rhizosphere of *A. spinulosa* plants mainly influences the aggregation of fungal species at the order level. The higher average degree and average clustering coefficient as well as shorter average path length of the fungal community network of the *A. spinulosa* rhizosphere soil indicated higher connectivity and more complex interactions between species (Yang Y et al. 2019). High network centrality is a crucial indicator for assessing the connectivity of the co-occurrence network (Martín González et al. 2010). The highest network center mesocentricity was observed at the species level for both fungal communities of the *A. spinulosa*rhizosphere and nonrhizosphere soils, but it was higher for the rhizosphere soil (0.23) than for the nonrhizosphere soil (0.12). Species such as *Neocosmospora rubicola* , *Microconidia* undetermined,*Archaea* undetermined, *Ascomycota* undetermined, and*Metarhizium carneum* occupy the top five intermediate centralities in the *A. spinulosa* rhizosphere and may play crucial roles in maintaining the structure and function of the rhizosphere fungal community.

Conclusion

Soil fungal phylogeny was more concentrated in the *A. spinulosa*rhizosphere, with *Cutaneotrichosporon* being the main differential group. Basidiomycota was a common phylum in the rhizosphere and nonrhizosphere soils. The rhizosphere soil fungi of *A. spinulosa*were more strongly affected by habitat-indicating effects. The key taxa of the rhizosphere soil fungal community of *A. spinulosa* were Ascomycota and its unclassified group, with complex symbiotic networks and more intense interspecific competition observed at the order level. The increase in the relative abundance of the animal pathogen was the main factor responsible for the increase in the proportion of pathotrophs among the fungi of the *A. spinulosa* rhizosphere soil. The ecological specialization of soil fungi was stronger among the plant roots. Investigating the unclassified taxa would be of great significance for further revealing the construction pattern of soil fungal communities among the *A. spinulosa* roots.

Acknowledgments

We are grateful to the Chishui Alsophila spinulosa National Nature Reserve Administration in Chishui for their help with the field survey.

Conflicts of Interest

The authors declare no conflict of interest.

References

Bahram, M., Peay, K.G., Tedersoo, L., 2015. Local-scale biogeography and spatiotemporal variability in communities of mycorrhizal fungi. New Phytol. 205 (4), 1454-1463.

Baiying Man, Xing Xiang, Yang Luo, Xiaotao Mao, Chao Zhang, Binghua Sun, Xi Wang, 2021. Characteristics and influencing factors of soil fungal community of typical vegetation types in Mount Huangshan, East China. Mycosystema 40(10): 2735-2751

Bever, J.D., Platt, T.G., Morton, E.R., 2012. Microbial population and community dynamics on plant roots and their feedbacks on plant communities. Annu. Rev. Microbiol.

Boyuan Han, Wen Zhang, Fangyu Hu, Qingqing Zhao, Yuan Lu, Xue Kong, Jungang Lu, 2022. Influence of Artificial Root Exudates and Actual Root Exudates on the Microbial Community in Pyrene-contaminated Soil. Environmental Science 43(2): 1077-1088.

Changhong Jiang, Mingyang Chen, Jihai Qi, Dejing Xu, Bangli Wu, Yun Guo, Yuejun He, 2021. The Interspecific Association of the Dominant Xylophyta of Alsophila spinulosa Community of Danxia Landform in North Guizhou, China. Journal of Chongqing Normal University (Natural Science) 38 (03), 114-120.

Coban, O., De Deyn, G.B., van der Ploeg, M., 2022. Soil microbiota as game-changers in restoration of degraded lands. Science 375 (6584).

Chunping Xie, Changling Wang, Houhe Wang, Chaojie Li, Lin Chen, 2022. Population Structure and Dynamic Characteristics ofAlsophilaspinulosa in Diaoluo Mountain Tropic Rain Forest of Hainan. Journal of Xinyang Normal University(Natural Science Edition) 35(4): 1-10.

Esposito, F., Jacquemyn, H., Waud, M., Tyteca, D., 2016. Mycorrhizal fungal diversity and community composition in two closely related platanthera (orchidaceae) species. PLoS One 11 (10), e164108.

Fuxing Cui, Libin Yang, Xin Sui, Qingyang Huang, Daoguang Zhu, 2021. Analysis of differences in fungal composition and diversity during decomposition of cold-temperate larch fallen wood. Journal of Central South University of Forestry Science and Technology 41(8): 36-44.

Hou, F., Zhang, H., Xie, W., Zhou, X., Zhu, X., Zhang, D., 2020. Co-occurrence patterns and assembly processes of microeukaryotic communities in an early-spring diatom bloom. Sci. Total Environ. 711, 134624.

Hui Li, Weikai Bao, Fanglan Li, 2021. Species composition and structure of the tree layer of Alsophila spinulosa community in the Huagaoxi National Nature Reserve, Sichuan. Chin J Appl Environ Biol, 2021, 27 (5): 1399-1404.

Jiayao Li, Zeyu Zhao, Yue Gao, Xiaoke Xing, 2021. Mycorrhizal fungal community composition of medicinal plant Spiranthes sinensis (Orchidaceae). Mycosystema 40(06): 1317-1327.

Jinxian Liu, Cui Li, Zhengming Luo, Xue Wang, Jiabing Tyran, Baofeng Chai, 2019. Distribution Pattern and Diversity Maintenance Mechanisms of Fungal Community in Subalpine Lakes. Environmental Science 40(05): 2382-2393.

Lara-Perez, L.A., Noa-Carrazana, J.C., Hernandez-Gonzalez, S., Alarcon-Gutierrez, E., Sanchez-Velasquez, L.R., Zulueta-Rodriguez, R., Lara-Capistran, L., Andrade-Torres, A., 2014. Diversity and colonization of arbuscular mycorrhizal fungi in the tree fern alsophila firma in rainy and dry season. Symbiosis 62 (3), 143-150.

Lei Zhang, Shuxian Jia, Xiaoling Li, Yuming Lu, Weisheng Lin, Xiaofei Liu, Jianfen Guo, 2021. Effects of Litter and Root Inputs Changing on Soil Microbial Community in a Natural Castanopsis carlesii Forest. Journal of Soil and Water Conservation 35(6): 270-277.

Li, A., Fahey, T.J., Pawlowska, T.E., Fisk, M.C., Burtis, J., 2015. Fine root decomposition, nutrient mobilization and fungal communities in a pine forest ecosystem. Soil Biology and Biochemistry 83, 76-83.

Liangliang Xi, 2022. Soil microbial diversity and its distribution mechanism in temperate deciduous broad-leaved forests of Baiyun Mountains, Henan, China, Henan Agricultural University. MSc.

Lu Han, 2022. Diversity of Soil Microbial Functions in Roots of Exotic Invasive Plant Oat. Molecular Plant Breeding 20(24): 8347-8352.

MaoSen Li, LiYuan Wang, Bo Yang, FuTong Liu, YongJiang Zhang, TianBao Ren, Fujian Zhang, 2022. Effects of biochar on fungal community structure and function on flue cured tobacco. Journal of Agricultural

Resources and Environment 39(5): 1041-1048.

Martin Gonzalez, A.M., Dalsgaard, B., Olesen, J.M., 2010. Centrality measures and the importance of generalist species in pollination networks. Ecol. Complex. 7 (1), 36-43.

Nin Jia, JiMei Shen, ZhongYan Yang, YanLin Wang, 2016. Investigation and analysis of pest and weed species in maize fields in Cangzhou area. Modern Agricultural Science and Technology (23): 141-143.

Qin Liu, Hongping Deng, Zongfeng Li, Sheng Liang, Qiulin Li, Dongping Ni, 2019. Characteristics of plant community in the Guizhou Chishui Alsophila spinulata National Nature Reserve,southwestern China. Journal of Beijing Forestry University 41(1) :19-31.

Qinqin He, Tao Weng, Bangyou Liu, Xiaohong Luo, Jun Mu, Jin Chen, Mingzhou An, Lingbin Yan, 2022. Research Advance in Conservation Strategies of Cyatheaceae Plant Communities based on Interspecific Relationships. Journal of Tropical and Subtropical Botany 30(5): 753-762.

Qijie Yuan, 2021. Simulating the Potential Suitable Habitats of the Relict Plant Alsophila spinulosa in Different Periods Based on MaxEnt Model. Zhejiang University.

Ruibai Zhao, 2018. Study on Population Structure and Distribution Characteristics of Wild Cyatheaceae in Hainan Island. Hainan University.

Sheng Lai, HuiLin Yang, MeiLin Chen, XinYi Long, ShuLi Liu, MinFei Jian, 2023. The analysis of microbial community structure in rhizosphere soil of Carex vegetation in Poyang Lake Nanji Wetland. Journal of Jiangxi Normal University(Natural Science) 47(1): 82-90.

Shuhua Li, Qi Zhao, Ying Cheng, Fang Liu, 2013. Antimicrobial Activities of Vitexin from Alsophila spinutosa. Food Research and Development (14): 4-5, 6.

Suiping Huang, Qili Li, Shaolong Wei, Lihua Tang, Xiaolin Chen, Sumei Huang, Tangxun Guo, 2022. Effects of Streptomyces misionensis TF78 on fusarium wilt of field-grown banana and rhizosphere soil microorganisms. Microbiology China 49(09): 3693-3708.

Su Liu, Haiping Feng, Yandi Cheng, Zhuo Chen, Jianhong Kang, Hongliang Wu, 2023. Diversity characteristics of rhizosphere soil and microbial community under different rotation patterns of baby cabbage. Agricultural Research in Arid Regions 41(2): 105-113.

Tedersoo, L., Bahram, M., Zobel, M., 2020. How mycorrhizal associations drive plant population and community biology. Science 367 (6480).

Wang, C., Liu, D., Bai, E., 2018. Decreasing soil microbial diversity is associated with decreasing microbial biomass under nitrogen addition. Soil Biology and Biochemistry 120, 126-133.

Wei Li, ShiYu Ji, YanBin Yin, YiXuan Liu, PenXi Cao, 2022. Microbial diversity and structural composition of rhizosphere soil of Hordeum vulgare L. and Pisum sativu L.in Tibet. Environmental Ecology 4(7): 62-69.

Wei Zang, L.S.N. KRUA, Chi Shen, Zhenna Du, Xiang Sun, Jianqiu Sun, Jianwei Fu, 2020. Species diversity and community composition of endophytic fungi from Alsophila spinulosa. Mycosystema 39(4)): 731-742.

Wenna Zhou, 2015. Diversity of endophytic fungi from bryophytes, pteridophytes and spermatophytes collected from the Dawei Mountain, Southwest China. Kunming University of Science

Wenxia Cui, 2017. A THE DIVERSITY AND BACTRIOSTASIS OF ENDOPHYTIC FUNGI FROM VIOLA YEDOENSIA MAKINO, Northwestern Agrarian and Forestry University.andTechnology. MSc: 104.

Xiansheng Wang, Yanhao Lian, Hui Guo, Yongzhe Ren, Zeyu Xin, Tongbao Lin, Zhiqiang Wang, 2022. Effects of wheat/safflower intercropping on rhizosphere microbial community function and structure. Chinese Journal of Eco-Agriculture: 1-14.

Xin Zhang, JiYan Sun, Quan Yang, XuanXuan Cheng, XiaoMin Tang, ChunRong Zhang, LiangYun Zhou, LiMin Pan, 2018. Study on Antitussive and Expectorant Effects of Alsophila Spinulosa. China Academic Publishing House 45(07): 1514-1515.

Xun Wang, Qin Liao, Peifang Wang, Qiusheng Yuan, Bin Hu, Xiaolei Xing, Haosen Xu, 2022. Effects of Reservoir Water Depth on Different Plankton Communities and Keystone Species of Network Interaction. Environmental Science: 1-15.

Yajuan Fu, Jian Zhang, Qiyuan Fu, Xiaoqiang Hou, 2019. Diversity of Rhizospheric Fungi and Potential Orchid Mycorrhizas on Cypripedium macranthum Estimated by Clone Library Technique. Southwest China Journal of Agricultural Sciences 32(3): 573-578.

Yang, Y., Gao, Y., Huang, X., Ni, P., Wu, Y., Deng, Y., Zhan, A., 2019. Adaptive shifts of bacterioplankton communities in response to nitrogen enrichment in a highly polluted river. Environ. Pollut. 245, 290-299.

Yi Du, 2022. Study on population dynamics and host selection of phytophagous insects of Alsophila spinulosa. Guizhou Normal University.

YinYin Liu, LiLan Lu, ShuYun Xie, ShiTing Chen, YuPing Wang, WeiBo Yang, 2023. Microbial diversity and community structure characteristics of inter-rooted soils of different coconut varieties under intercropping pineapple pattern. South China Fruits: 1-21.

YongLan Liu, LiNa Zhang, Lu Liang, XiaoLong Liu, PeiYong Song, YaQiang Zheng, 2021. Diversity of endophytic fungi from Alsophila spinulosa in Chishui Alsophila National Nature Reserve, Guizhou Province, Southwest China. Mycosystema 40(10): 2673-2684.

YouSan Wang, RunJin Liu, 2017. A checklist of arbuscular mycorrhizal fungi in the recent taxonomic system of Glomeromycota. Mycosystema 36(07): 820-850.

Yujie Zhou, Xia Jia, Yonghua Zhao, Nannan Chen, Jin Yan, Jianqiu Tang, Xi Wang, Li Liu, 2021. Altitude distribution of fungal community in Huoditang in Qinling Mountains, Northwest China. Chinese Journal of Applied Ecology 32(7): 2589-2596.

Yueting Lang, Changwang Ma, Jing Yu, Rui Gou, Yuehong Yan, Dongmei Yang, 2021. Technology for Rapid Propagation in Vitro of Two Alsophila Species. Chinese Journal of Tropical Crops 42 (06): 1579-1588.

Yuqi Wang, Yurong Ren, Anbang Liao, Qian Dong, Maosong Liu, 2023. Composition and structural characteristics of soil microbial communities in Yancheng typical coastal wetlands. ACTA ECOLOGICA SINICA (06): 1-12.

Zeliang Yang, Pingfang Li, Tao Xue, Shuqin Xiang, Guohong Xiang, 2020. Seasonal Variation Characteristics of Soil Microbial Community Structure and Diversity of Corn in Rhizosphere Soil. Southwest China Journal of Agricultural Sciences 33(5): 1001-1010.

Zewei Zhang, Zhaoying Chen, Tianna Zhang, Shilei Zhou, Jiansheng Cui, Xiao Luo, 2022. Spatial and temporal distribution of aerobic denitrifying bacterial communities in sediments of Gangnan Reservoir. Environmental Science 43(1): 314-328.

ZhiQi Yang, XiaoYu Cheng, HongMei Wang, ZhiLin Zeng, XiaoYan Liu, Jing Cao, ChunTian Su, QiBo Huang, 2021. Environmental driving mechanisms and community building processes in the bacterial community of Luohan Belly Cave, Guilin. Acta Microbiologica Sinica 61(12): 4118-4136.