



OPEN ACCESS

EDITED BY
Rajib Bandopadhyay,
University of Burdwan, India

REVIEWED BY
Vivek Kumar,
Swami Rama Himalayan University, India
Rui Xing,
Chinese Academy of Sciences (CAS),
China

*CORRESPONDENCE

Jianli Liu
✉ jl7523@126.com

RECEIVED 29 December 2025
REVISED 11 May 2026
ACCEPTED 18 May 2026
PUBLISHED 02 June 2026

CITATION

Yang J, Lu J, Wang Y, Wu M, Yang R,
Liu T, Lu L, An Y and Liu J (2026)
Succession patterns of foliar endophytic
fungal communities during different leaf
growth stages in *Lycium barbarum*.
Front. Ecol. Evol. 14:1777264.
doi: 10.3389/fevo.2026.1777264

COPYRIGHT

© 2026 Yang, Lu, Wang, Wu, Yang, Liu,
Lu, An and Liu. This is an open-access
article distributed under the terms of the
[Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/)
(CC BY). The use, distribution or
reproduction in other forums is
permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication
in this journal is cited, in accordance
with accepted academic practice. No
use, distribution or reproduction is
permitted which does not comply with
these terms.

Succession patterns of foliar endophytic fungal communities during different leaf growth stages in *Lycium barbarum*

Jingjing Yang, Jie Lu, Yanzhe Wang, Manmei Wu, Rui Yang,
Tianci Liu, Liangsi Lu, Yajing An and Jianli Liu*

School of Biological Science and Engineering, North Minzu University, Key Laboratory of Ecological
Protection of Agro-Pastoral Ecotones in the Yellow River Basin, School of Biological Science and
Engineering, North Minzu University, National Ethnic Affairs Commission of the People's Republic of
China, Ningxia Key Laboratory of Microbial Resources Development and Applications in Special
Environment, Yinchuan, Ningxia, China

Lycium barbarum (Goji) is an important traditional Chinese medicinal plant widely distributed across Northwest China. Its young leaves are usually developed into tea or vegetables. Foliar endophytic fungi respond to leaves development, growth and health. Herein, high-throughput amplicon sequencing was used to assess dynamic changes in foliar endophytic fungal communities in young, mature, and old *L. barbarum* leaves. Results revealed that as leaf growth progressed, a decrease was observed in the diversity and evenness of foliar endophytic fungal communities, whereas an initial increase followed by a decrease was observed in community richness and the number of total and specific amplicon sequence variants (ASVs). The potential pathogen *Alternaria* was dominant genus in young and mature leaves, another potential pathogen *Arthrocladiella* was dominant genus in old leaves. The foliar endophytic fungal co-occurrence networks were initially complex but simplified with leaf growth. The function prediction stated the potential plant pathogen of foliar endophytic fungal communities gradually increased with leaf growth. Except for the leaf soluble sugar content, all other factors, including average temperature, average humidity, leaf transpiration rate, leaf net photosynthetic rate, leaf ammonium nitrogen content, and leaf ammonia nitrogen content, exhibited a significant correlation with the structure of foliar endophytic fungal communities at different growth stages of *L. barbarum* leaves ($p < 0.05$). Altogether, this study reveals the dynamics and function of foliar endophytic fungal communities during different growth stages of *L. barbarum* leaves, providing novel insights into the composition, diversity, and function of these communities and their relationship with host plants.

KEYWORDS

community characteristics, dynamic changes, foliar endophytic fungi, leaf growth stages, *Lycium barbarum*

1 Introduction

Lycium barbarum (Goji) is a perennial woody plant of the genus *Lycium* in the family Solanaceae (Zhang et al., 2024a). It is an important traditional Chinese medicinal plant, as its fruit (lycii fructus, Gojizi) contains abundant bioactive compounds such as polysaccharides, flavonoids, and betaine (Ma et al., 2023; Yu et al., 2023). *L. barbarum* is widely cultivated across Northwest China, particularly in Ningxia, Gansu, Qinghai, and Xinjiang, and is an important economic crop for local farmers. Ningxia is one of the most important production regions of *L. barbarum*, with a cultivation history of more than 600 years (Wang et al., 2019; Lu et al., 2021). Studies have shown that nutritional components in the leaves and fruits of *L. barbarum* are similar, whereas ash, crude protein, calcium, iron, thiamine, and niacin contents are higher in leaves than in fruits (Ni et al., 2024). In addition, the amino acid content in *L. barbarum* leaves (12 mg/100g sample) is higher than that in fruits (9 mg/100g sample). The amount of free amino acids is relatively high in *L. barbarum* leaves, accounting for 24% of the total amino acid content, which is convenient for direct absorption by humans (Huang et al., 2024). The young leaves of *L. barbarum* are usually developed into tea or eaten as fresh seasonal vegetables (Lei et al., 2022). In addition, *L. barbarum* is a native ecological tree used for wind prevention, sand fixation, and reclamation of saline-alkali soils (Dong et al., 2021; Wu et al., 2023).

Endophytic fungi are found in various tissues and organs of healthy plants during a certain growth stage or throughout their life cycle. They can be detected through isolation from strictly surface-disinfected plant tissues or direct polymerase chain reaction (PCR)-based amplification of microbial DNA from plant tissues (Stone et al., 2000, 2004; López-Mencheró et al., 2024). As important members of the micro-ecosystem within plants, endophytic fungi have a close relationship with host plants (Hassani et al., 2018; Li et al., 2025). They can promote plant growth (Narayan et al., 2021; Su et al., 2024; Ramachandran et al., 2025); prevent and control plant diseases and pests (Zhang et al., 2024b; Kushwaha et al., 2025); enhance plant stress tolerance (Kamyab et al., 2024; Usman et al., 2024; Waqar et al., 2024; Kharwar et al., 2025); influence the ecological adaptability, evolutionary process, and diversity of host plants (Aamir et al., 2020; Harrison et al., 2021; Patyal et al., 2024). Leaves are important organs for photosynthesis and are increasingly becoming a major focus area in research on foliar endophytic fungi (FEEs) (Arnold, 2007).

In this study, high-throughput amplicon sequencing was used to analyze foliar endophytic fungal communities at different leaf growth stages in *L. barbarum* and assess the response of dynamic changes in these communities to different leaf growth stages. In addition, we investigated the impact of climatic factors (e.g., average temperature and humidity), leaf physiological states (e.g., transpiration and net photosynthetic rates), and leaf nutrients used by microorganisms (e.g., ammonium nitrogen [NH₄⁺], ammonia nitrogen [NO₃-N], and soluble sugar) on foliar endophytic fungal

communities and identified the factors influencing the structure of these communities in *L. barbarum* leaves.

2 Materials and methods

2.1 Experimental site

The experimental site is an *L. barbarum* planting field located in Luhuatui, Xixia, Yinchuan City, Ningxia Hui Autonomous Region (106°16'39" E, 38°64'72" N), at an altitude of 1072m. The study site is a typical farmland with a temperate continental climate, with an average annual temperature of 9 C–11 C and an annual average accumulated temperature of 3250 C–3300 C. The average annual sunshine duration is approximately 2600–3000 hours, and the average annual precipitation is 130–200 mm, mostly concentrated from June to September. The average annual evaporation is 1600–2000 mm, with the highest evaporation occurring from July to August. The soil type is classified as light grey calcareous soil and anthropogenic alluvial soil from the Yellow River. The study site receives abundant sunshine and is one of the regions in China with the highest solar radiation and sunshine duration. Surface water sources are abundant, with the Yellow River being the primary source ensuring the irrigation of numerous farmlands.

Three 10m × 10m communities of *L. barbarum* were selected as sample plots, with a spacing of 100m between the plots.

2.2 Leaf sampling

In each sample plot with 5 *L. barbarum* plants with uniform growth, a well-growing branch was selected from the four directions of east, south, west, and north. To assess the photosynthetic characteristics of leaves, 6–8 healthy leaves were collected from the top of each branch and mixed as one sample. The samples were placed in a low-temperature preservation box and sent to the laboratory. Sampling covered a complete growth and development cycle of *L. barbarum* leaves. Leaves samples were collected on May 17, 2024 (young leaf stage [young leaves]), July 10, 2024 (full leaf stage [mature leaves]), and October 15, 2024 (old leaf stage [old leaves]) (Figure 1). Three replicates were prepared for each leaf growth stage. Overall, total nine samples were collected. Each sample was divided into two portions, with one portion being surface-sterilized and stored at –80 C for sequencing and the other portion being naturally dried for the measurement of nutrient content.

2.3 Nutrients used by microorganisms in leaves

The collected leaves were oven-dried at 70 C until they reached a constant weight. To determine the stoichiometric characteristics of leaves, the leaves were ground into powder using a ball mill. Leaf

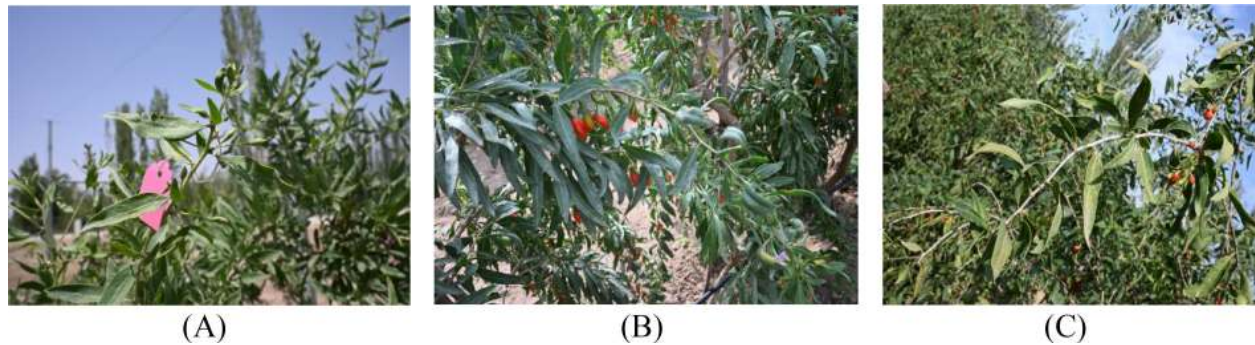


FIGURE 1

Morphology of *L. barbarum* leaves at different growth stages. (A) young leaf stage (young leaves). (B) full leaf stage (mature leaves). (C) old leaf stage (old leaves).

NH_4^+ (A), NO_3^- (X), and soluble sugar (K) contents were measured through visible spectrophotometry using corresponding test kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, Jiangsu, China).

2.4 Climatic factors and photosynthetic characteristics of leaves

Climatic factors such as average temperature (T) and average humidity (SD) were measured using a small weather station on the field (WX-QC6, Shandong Wanxiang Environmental Technology Co., Ltd., Weifang, Shandong, China). The transpiration rate (Z) and net photosynthetic rate (JQ) of leaves were assessed using a portable photosynthesis-measuring instrument (LI-6400P, LI-COR Co., Ltd., Lincoln, Nebraska, USA). The average value of 10 leaves from each tree was measured.

2.5 DNA extraction, library preparation, and sequencing

To remove surface debris, all collected leaves were rinsed under running water. After the leaves were sequentially sterilized with 75% ethanol and sodium hypochlorite (0.5% available chlorine) for 3 and 5 minutes, respectively, they were washed with sterile water three times and dried using a sterile filter paper. To assess whether the sterilization of the leaf surface was successful, 200 μL of the last used sterile water and sterilized leaves were cultured on potato dextrose agar (PDA) at 25 C for 7 days. No fungal or bacterial colonies appeared on the medium, validating that the leaves were thoroughly sterilized.

A DNasecure Plant Kit (DP320, Tiangen Biotech [Beijing] Co., Ltd., Beijing, China) was used to extract total DNA from leaf samples. After the concentration and purity of the extracted genomic DNA was assessed through 1% agarose gel electrophoresis, the fungal ITS1 regions of the rRNA gene were amplified through PCR using the primers ITS1F/ITS2R (Adams et al., 2013). PCR products were recovered, purified using an AxyPrep DNAGel Extraction Kit (Axygen Biosciences, Union City, CA, USA), and quantified using a Quantus Fluorometer (Promega Corporation,

Madison, USA). Subsequently, an Illumina DNA Prep Kit (Illumina, San Diego, CA, USA) was used to construct libraries, and sequencing was performed on an Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA). All samples were sent to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for total DNA extraction, PCR, and library construction. The sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) database under the accession number PRJNA1219372.

2.6 Bioinformatic and statistical analyses

Demultiplexed sequencing reads had primers and adapters removed by cutadapt (v1.18) (Martin, 2011). After demultiplexing, the resulting sequences were quality filtered with fastp (0.19.6) and merged with FLASH (v1.2.11). Then the high-quality sequences were de-noised using DADA2 (v1.12.1) pipeline (Callahan et al., 2016) plugin in the Qiime2 (version 2024) pipeline with recommended parameters, which obtains single nucleotide resolution based on error profiles within samples. DADA2 denoised sequences are usually generate a table of amplicon sequence variants (ASVs). Each sample obtained averagely 50,000 reads. ASVs were classified and annotated using the UNITE database (Nilsson et al., 2019). Bioinformatic analysis was performed using the Majorbio Cloud platform (<https://cloud.majorbio.com>) (Ren et al., 2022). Significant differences among groups were estimated using one-way analysis of variance (ANOVA).

3 Results

3.1 Diversity of foliar endophytic fungal communities at different growth stages in *L. barbarum* leaves

The α -diversity of foliar endophytic fungal communities varied with the leaf growth stage in *L. barbarum*. The Shannon and Simpson indices gradually decreased and reached their lowest

TABLE 1 α -diversity indices (Mean $\pm$ SD) of foliar endophytic fungal communities during different leaf growth stages in *L. barbarum*.

Samples	Sobs	Shannon	Simpson	Ace	Chao	Heip	Coverage
young	40.33 $\pm$ 4.04 ^b	2.07 $\pm$ 0.11 ^a	0.79 $\pm$ 0.03 ^a	41.24 $\pm$ 3.55 ^c	40.53 $\pm$ 3.87 ^b	0.18 $\pm$ 0.00 ^a	1.00 $\pm$ 0 ^a
mature	116.33 $\pm$ 3.01 ^a	1.91 $\pm$ 0.29 ^a	0.71 $\pm$ 0.08 ^a	124.84 $\pm$ 6.06 ^a	125.37 $\pm$ 5.04 ^a	0.05 $\pm$ 0.01 ^b	1.00 $\pm$ 0 ^a
old	51.00 $\pm$ 9.04 ^b	1.02 $\pm$ 0.17 ^b	0.49 $\pm$ 0.09 ^b	70.49 $\pm$ 17.58 ^b	64.4 $\pm$ 14.12 ^b	0.04 $\pm$ 0.00 ^b	1.00 $\pm$ 0 ^a

Different lowercase letters indicate significant differences between different leaf growth stages at the 0.05 level.

values in old leaves, which were 49.27% and 62.02% of their highest values in young leaves, respectively. With regard to richness, the Sobs, Chao, and Ace indices showed an initial increase followed by a decrease, with mature leaves showing the highest values. The evenness index Heip gradually decreased, with the highest value in young leaves being 4.5 times the lowest value in old leaves. Community coverage did not differ significantly among the three leaf growth stages. Overall, as leaf growth progressed, the diversity and evenness of foliar endophytic fungal communities in *L. barbarum* decreased, whereas the richness initially increased and subsequently decreased (Table 1).

The foliar endophytic fungal communities in all leaf samples were clustered based on weighted UniFrac distances at the ASV level. The fungal communities at the three leaf growth stages were clearly distinguished. Analysis of similarities (Anosim) indicated significant differences between groups ($R = 0.9095$, $p = 0.001$). In addition, permutational multivariate analysis of variance (PERMANOVA) validated that the differences between groups were relatively significant ($R^2 = 0.7966$, $p = 0.008$). The old group was significantly separated from the young and mature groups on the PC1 axis, whereas the young group was significantly separated from the mature and old groups on the PC2 axis. Altogether, the foliar endophytic fungal communities at different leaf growth stages of *L. barbarum* were clearly distinguished (Figure 2).

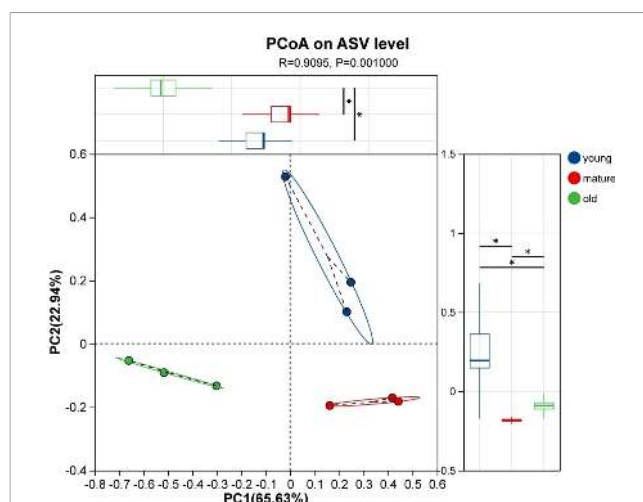


FIGURE 2
PCoA of endophytic fungal communities at different growth stages of *L. barbarum* leaves based on weighted UniFrac distances at the ASV level. Young, foliar endophytic fungal communities during the young leaf stage; mature, foliar endophytic fungal communities during the full leaf stage; and old, foliar endophytic fungal communities during the old leaf stage.

3.2 Composition of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*

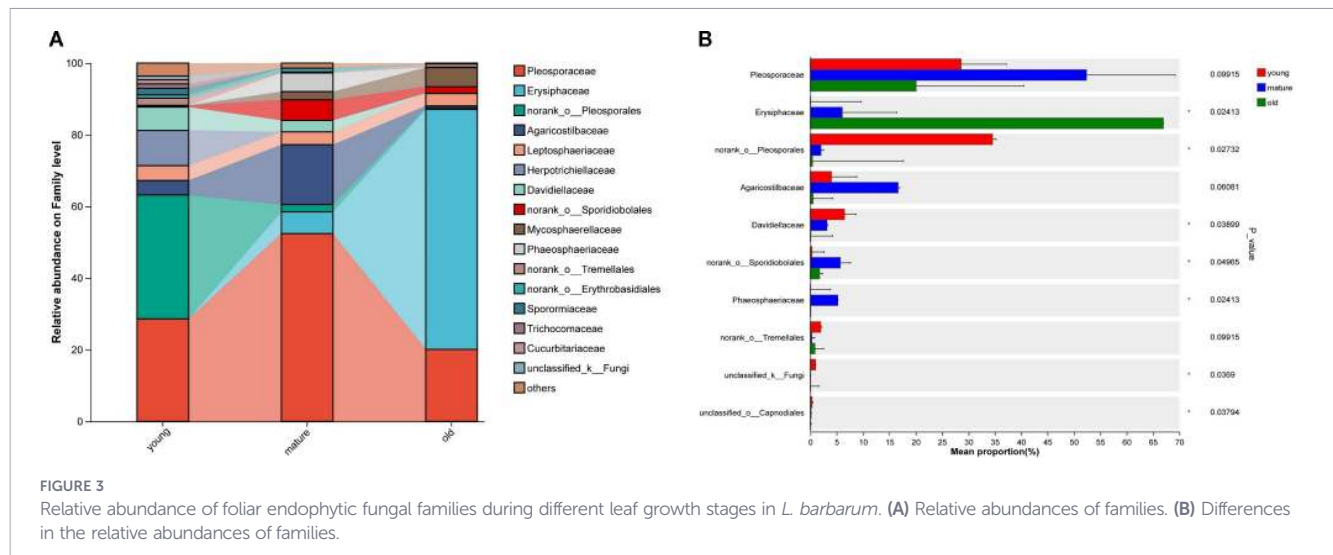
Overall, three phyla of foliar endophytic fungi were found at the three leaf growth stages of *L. barbarum*, with Ascomycota being the dominant phylum across all stages. However, the relative abundance of Ascomycota varied at different growth stages: 89.60% in young leaves, 75.33% in mature leaves, and 96.56% in old leaves. In addition, the relative abundance of Basidiomycota was 9.37%, 24.64%, and 3.44% in young, mature, and old leaves, respectively (Supplementary Figure 1A).

The proportions of Ascomycota ($p = 0.039$), Basidiomycota ($p = 0.039$), and unclassified_k_Fungi ($p = 0.037$) showed significant differences among the three leaf growth stages. The relative abundance of Ascomycota decreased initially but increased subsequently, being the highest in old leaves, and that of Basidiomycota increased initially but decreased subsequently, being the highest in mature leaves (Supplementary Figure 1B).

Overall, 51 families of foliar endophytic fungi were found at the three leaf growth stages of *L. barbarum*, with the dominant families being different at the three stages. In young leaves, the dominant family was norank_o_Pleosporales, with a relative abundance of 34.59%, followed by Pleosporaceae (28.6%) and Herpotrichiellaceae (9.86%). In mature leaves, Pleosporaceae had the highest relative abundance of 52.4%, followed by Agaricostilbaceae (16.68%) and Erysiphaceae (6.08%). In old leaves, Erysiphaceae had the highest relative abundance of 66.98%, followed by Pleosporaceae (20.11%) (Figure 3A).

Differential analysis revealed that among the top 10 families, the relative abundances of Erysiphaceae ($p = 0.024$), norank_o_Pleosporales ($p = 0.027$), Davidiellaceae ($p = 0.039$), norank_o_Sporidiobolales ($p = 0.049$), Phaeosphaeriaceae ($p = 0.024$), unclassified_k_Fungi ($p = 0.037$), and unclassified_o_Capnoidiales ($p = 0.038$) were significantly different among the three leaf growth stages. The relative abundances of norank_o_Sporidiobolales and Phaeosphaeriaceae initially increased but subsequently decreased with leaf growth, with the lowest abundances observed in young leaves. The relative abundances of norank_o_Pleosporales, Davidiellaceae, and the potential phytopathogen Erysiphaceae gradually decreased with leaf growth (Figure 3B).

The dominant genera of foliar endophytic fungal communities were different at the three leaf growth stages of *L. barbarum*. In young leaves, *Alternaria* had the highest relative abundance of 28.59%, followed by *Phoma* (20.29%), *unclassified_f_norank_o_Pleosporales* (13.88%), *Exophiala* (9.86%), and *Cladosporium* (6.52%). In mature leaves, *Alternaria* had the highest relative abundance of 49.94%, followed by *Bensingtonia* (16.68%), *Arthrocladiella* (6.08%), *Sporobolomyces* (5.71%), and *unclassified_f_Phaeosphaeriaceae*



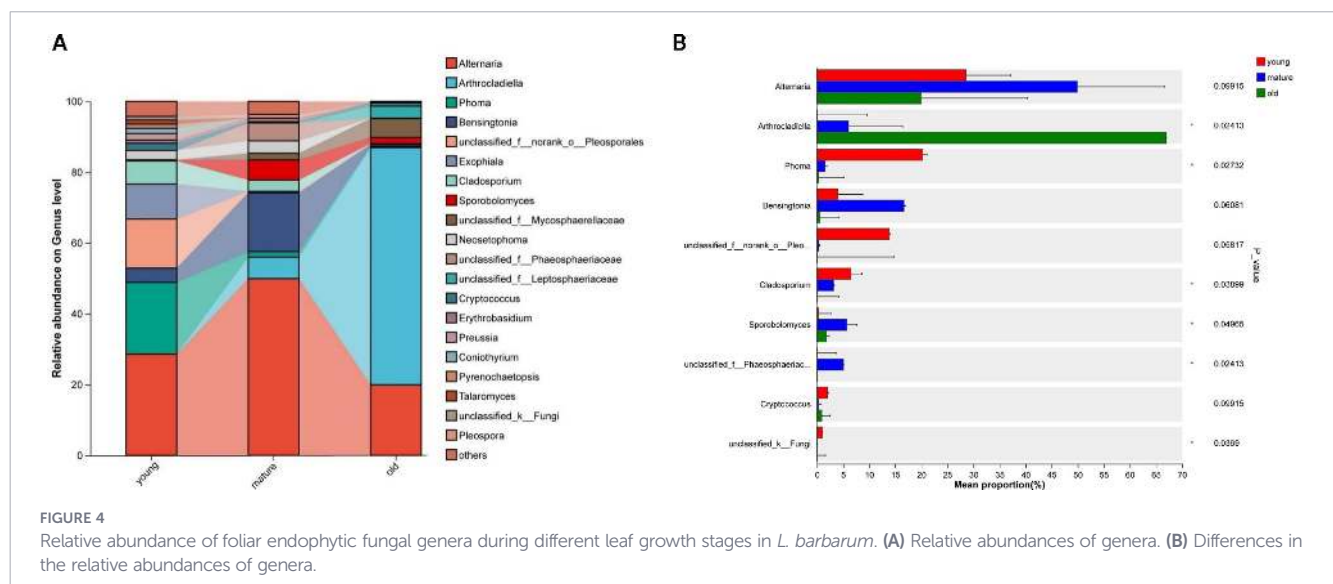
(5.07%). In old leaves, *Arthrocladiella* had the highest relative abundance of 66.98%, followed by *Alternaria* (19.98%) (Figure 4A).

Differential analysis indicated that among the top 10 genera, the relative abundances of *Arthrocladiella*, *Phoma*, *Cladosporium*, *Sporobolomyces*, and *unclassified_f_Phaeosphaeriaceae* were significantly different across the three leaf growth stages. The relative abundance of the potential phytopathogen *Arthrocladiella* increased with leaf growth, whereas that of the potential phytopathogens *Phoma* and *Cladosporium* decreased with leaf growth. An initial increase followed by a decrease was observed in the relative abundance of *Sporobolomyces*, with the lowest abundance observed in young leaves (Figure 4B).

Linear discriminant analysis effect size (LEFse) analysis (LDA value ≥ 3.0 as the criterion) indicated that at the genus level, *Phoma*, *Cladosporium*, and *unclassified_o_Capnodiales* were biomarkers for young leaves; *Sporobolomyces*, *Pleosporea*, *Epicoccum*, *Dissoconium*, *Neocamarosporium*, *Stemphylium*, *Phaeosphaeria*, *unclassified_f_Phaeosphaeriaceae*, *unclassified_c_Dothideomycetes*, and *unclassified_c_Exobasidiomycetes* were biomarkers for mature leaves; and *Arthrocladiella* was a biomarker for old leaves (Figure 5).

Overall, 206 ASVs were found for foliar endophytic fungal communities at the three leaf growth stages of *L. barbarum*. The number of total ASVs initially increased and subsequently decreased with leaf growth. Overall, young, mature, and old leaves showed 69, 164, and 76 ASVs, respectively, with mature leaves showing >2 times more ASVs than young and old leaves. Similarly, the number of specific ASVs initially increased but subsequently decreased. Overall, 37, 91, and 3 specific ASVs were identified in young, mature, and old leaves. However, their percentages decreased. Specifically, the percentage of specific ASVs was 53.62% in young leaves, 55.49% in mature leaves, and 3.95% in old leaves. The number and percentage of common ASVs between stages increased with leaf growth. A total of 30 common ASVs were

Overall, 206 ASVs were found for foliar endophytic fungal communities at the three leaf growth stages of *L. barbarum*. The number of total ASVs initially increased and subsequently decreased with leaf growth. Overall, young, mature, and old leaves showed 69, 164, and 76 ASVs, respectively, with mature leaves showing >2 times more ASVs than young and old leaves. Similarly, the number of specific ASVs initially increased but subsequently decreased. Overall, 37, 91, and 3 specific ASVs were identified in young, mature, and old leaves. However, their percentages decreased. Specifically, the percentage of specific ASVs was 53.62% in young leaves, 55.49% in mature leaves, and 3.95% in old leaves. The number and percentage of common ASVs between stages increased with leaf growth. A total of 30 common ASVs were



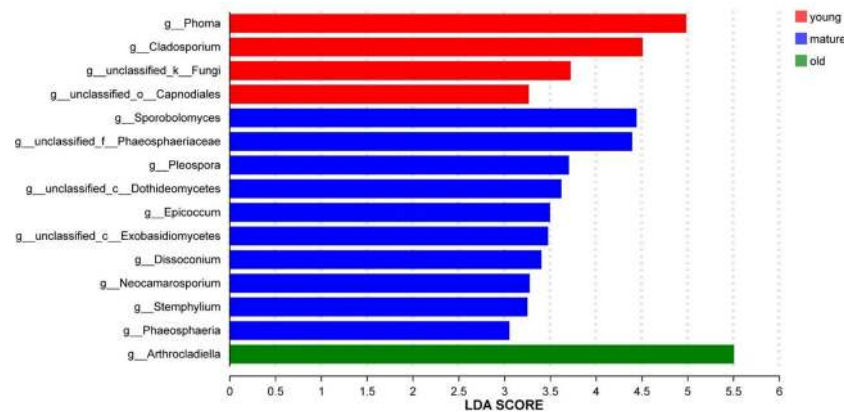


FIGURE 5

LEFse analysis of foliar endophytic fungal genera at different leaf growth stages of *L. barbarum*.

identified between young and mature leaves, accounting for 43.48% and 18.29% of young and mature leaves, respectively. A total of 68 common ASVs were identified between mature and old leaves, accounting for 41.46% and 89.47% of mature and old leaves, respectively. A total of 25 common ASVs were identified among young, mature, and old leaves, accounting for 36.23%, 15.24%, and 32.89% of young, mature, and old leaves, respectively (Figure 6).

The number of total and specific ASVs initially increased and subsequently decreased with leaf growth. On the contrary, the percentage of common ASVs among the three stages initially decreased and subsequently increased with leaf growth. The number and percentage of common ASVs between two stages increased, and the percentage of specific ASVs at each stage decreased.

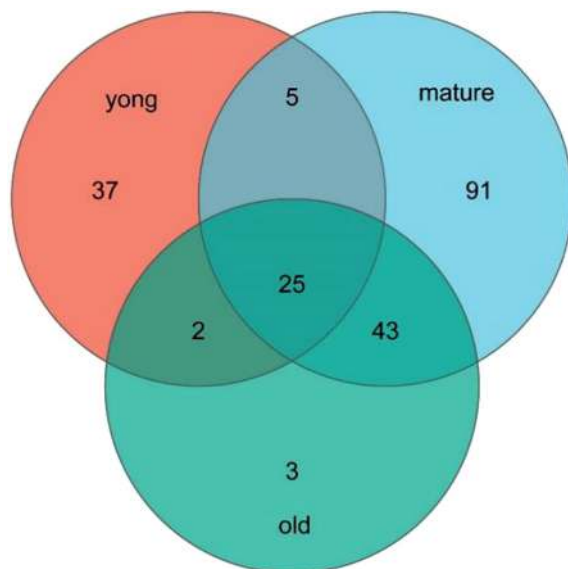


FIGURE 6

Venn diagram of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum* at the ASV level.

3.3 Co-occurrence networks of foliar endophytic fungal communities during the three leaf growth stages in *L. barbarum*

An interaction network of the top 200 ASVs was constructed based on the following criteria: correlation coefficient ≥ 0.5 and $p < 0.05$. As leaf growth progressed, the number of nodes, edges, positive edges, and negative edges; average degree; and the percentage of negative edges increased initially and decreased subsequently, with mature and young leaves exhibiting the highest and lowest values, respectively. However, the percentage of positive edges initially decreases and subsequently increased. Density gradually increased with leaf growth, showing the highest value in old leaves. On the contrary, modularity gradually decreased with leaf growth, showing the lowest value in old leaves. Diameter, average path distance, and average clustering coefficient were similar across the three growth stages (Figure 7, Supplementary Table 1).

3.4 Function of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*

The functions of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum* were predicted using the FunGuild tool. In both young and mature leaves, the most abundant function of endophytic fungi was Animal Pathogen-Endophyte-Plant Pathogen-Wood Saprotroph, with an abundance of 28.59% and 49.59%, respectively. In old leaves, the most abundant function of endophytic fungi was Plant Pathogen, with an abundance of 66.99%, followed by Animal Pathogen-Endophyte-Plant Pathogen-Wood Saprotroph (20%). Endophyte-Dung Saprotroph-Lichen Parasite-Litter Saprotroph-Plant Pathogen-Soil Saprotroph-Wood Saprotroph (20.3%), Unknown (16.38%), Animal Pathogen-Undefined Saprotroph (9.86%), and Undefined Saprotroph (9.76%) also had relatively high abundances in young leaves, whereas Undefined Saprotroph had a relatively high abundance in mature leaves (Figure 8A). Old leaves showed a significantly high relative abundance of Plant Pathogen. These findings

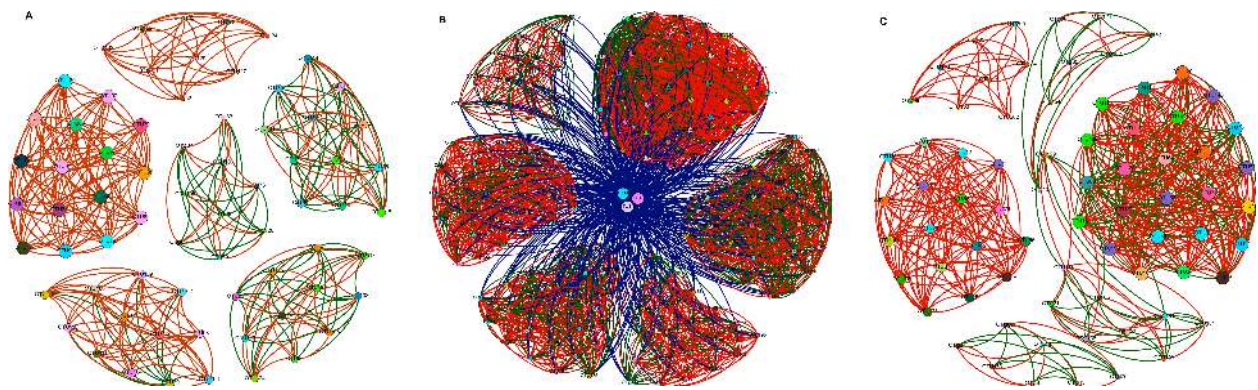


FIGURE 7

Co-occurrence networks of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum* based on Spearman's correlation coefficient ($|r| \geq 0.5$, $p < 0.05$). Positive correlations are represented by red lines, and negative correlations are represented by green lines. (A–C) Fungal co-occurrence networks in young, mature, and old leaves.

indicate that the function of potential plant pathogens gradually increases with leaf growth in *L. barbarum* (Figure 8B).

3.5 Assembly process of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*

The endophytic fungal communities in young, mature, and old *L. barbarum* leaves showed different ecological processes of community assembly. Mature leaves showed drift ($\beta\text{NTI} < 2$ and $\text{RCBray} < 0.95$), young leaves showed drift ($\beta\text{NTI} < 2$) and heterogeneous selection ($\beta\text{NTI} > 2$), and old leaves showed drift ($\beta\text{NTI} < 2$) with heterogeneous selection ($\beta\text{NTI} > 2$) and homogeneous selection ($\beta\text{NTI} < -2$) (Figure 9).

3.6 Environmental factors driving foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*

Canonical Correlation Analysis (CCA) was used to examine the effects of average temperature (T), average humidity (SD), leaf transpiration rate (Z), leaf net photosynthetic rate (JQ), leaf NH_4^+ content (A), leaf NO_3^- content (X), and leaf soluble sugar content

(K) on the composition of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum* (Supplementary Table 2). PERMANOVA indicated that all factors except for K were strong drivers of community composition ($R^2 = 0.824$, $p < 0.05$; $R^2 = 0.626$, $p < 0.05$; $R^2 = 0.808$, $p < 0.01$; $R^2 = 0.719$, $p < 0.05$; $R^2 = 0.771$, $p < 0.05$; and $R^2 = 0.775$, $p < 0.05$, respectively). In old leaves, A and X showed a positive correlation with community composition; however, this correlation was negative in young and mature leaves. On the contrary, T, JQ, and Z were positively correlated with community composition in mature and young leaves and negatively correlated with community composition in old leaves. In mature leaves, SD showed a positive correlation with community composition; however, this correlation was negative in young and old leaves (Figure 10).

3 Discussion

Foliar endophytic fungi play an indispensable role in plant–microbe interactions, thereby playing an important role in plant leaf health. This study analyzed the foliar endophytic fungal communities of *L. barbarum* at different leaf growth stages. Results revealed

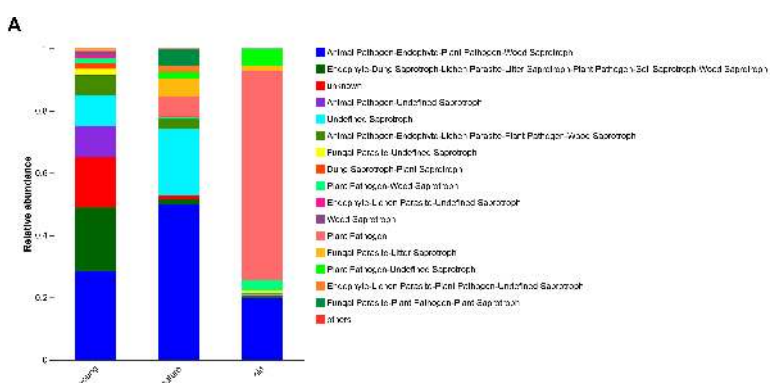
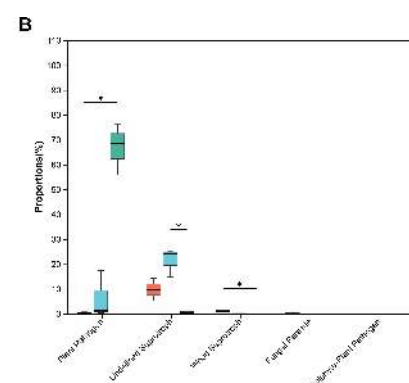


FIGURE 8

Predicted functions of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*. (A) Functions of foliar endophytic fungal communities predicted using FunGuild. (B) Differences in the functions. * indicate significant differences at the 0.05 level.



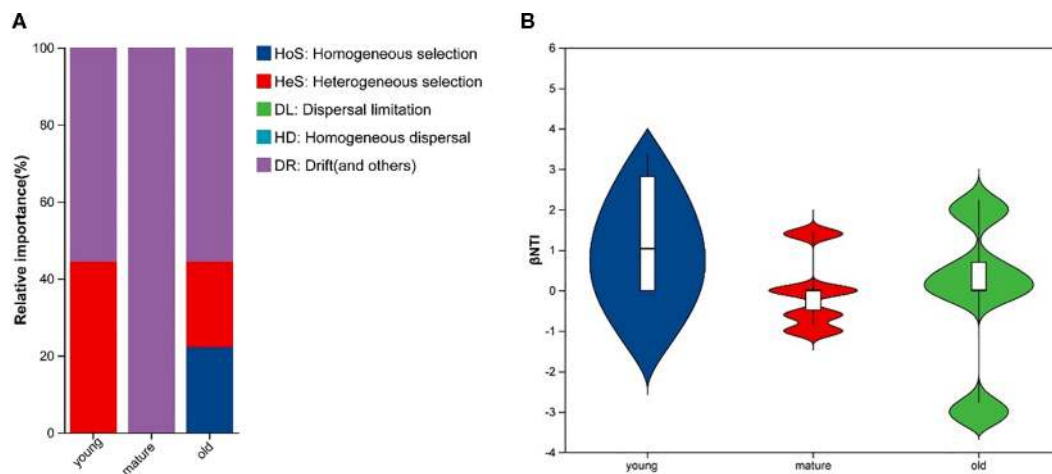


FIGURE 9

Assembly process of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*. (A) Assembly process of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum*. (B) β NTI values of different leaf samples.

that the composition of foliar endophytic fungal communities varied with the leaf growth stage. In particular, diversity and evenness decreased with leaf growth.

The dominant group is characteristics of fungal communities. This study showed that the dominant genus of foliar endophytic fungal communities was *Alternaria* in young and mature *L. barbarum* leaves and *Arthrocladiella* in old leaves. Ren et al (Ren et al., 2019). reported that *Alternaria* was the dominant genus of foliar endophytic fungal communities in Jingbai pear trees. *Russula* has been identified as the dominant genus of foliar endophytic fungal communities in *Rheum palmatum*, which is widely used in traditional Chinese medicine (Chen et al., 2023). In addition, *Lasiodiplodia* (7.2%) has been identified as the most abundant genus of endophytic fungal communities in *Theobroma cacao*

leaves (Wemheuer et al., 2020). Therefore, the different host plants have different dominant foliar fungi group. A host-caused filter appears to play a major role in shaping foliar fungal communities (Liu et al., 2021). found strong evidence supporting phylogenetic congruence between host plants and foliar fungi in both asymptomatic and symptomatic leaves (Whitaker et al., 2020). found that the structure of foliar endophytic fungal communities was significantly related to species and spatial distance between host plants but did not exhibit a significant correlation with phylogenetic distance between host species. The functions of host species, such as metabolic traits or phenology, can drive dissimilarity in foliar endophytic fungal communities. These results are consistent with our findings.

This study showed that the number of total ASVs of foliar endophytic fungal communities at each leaf growth stage initially increased and subsequently decreased with leaf growth, with the number of total ASVs in mature leaves being >2 times higher than that in young and old leaves. These total ASVs originate from two pathways: transmission from leaves at the previous growth stage and entry of external environment into the leaves. Furthermore, the number of specific ASVs initially increased and subsequently decreased with leaf growth, with mature leaves showing the highest number of specific ASVs. However, their percentage decreased with leaf growth, especially in old leaves. The number and percentage of common ASVs between two stages increased with leaf growth. These findings indicated that the transmission from leaves at the previous growth stage was stronger and the entry of external pathogens into the leaves was weaker. Regarding the assembly process of foliar endophytic fungal communities at different leaf growth stages, mature leaves showed drift, whereas old leaves showed drift combined with heterogeneous and homogeneous selection. These results are consistent with each other. Although mature leaves showed the highest number of total ASVs of foliar endophytic fungal communities, only the changes in community richness corresponded to changes in total ASVs, whereas diversity and evenness decreased with leaf growth. The fungal co-occurrence network was initially complex and subsequently simple. Mature

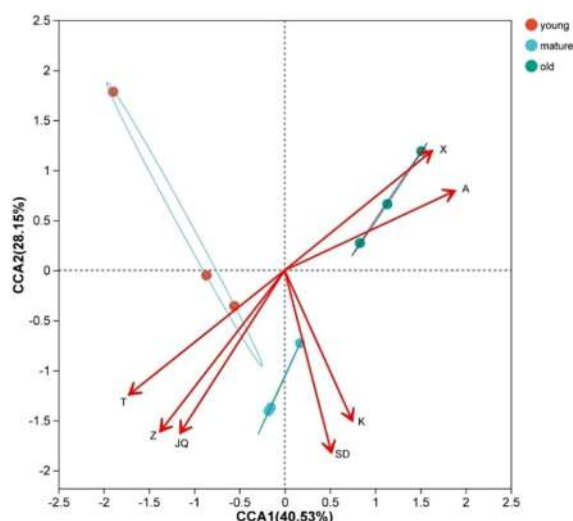


FIGURE 10

CCA of the correlation between the structure of foliar endophytic fungal communities and environmental factors at different leaf growth stages in *L. barbarum*. T, average temperature; SD, average humidity; JQ, leaf net photosynthetic rate; Z, leaf transpiration rate; A, leaf NH_4^+ content; X, leaf NO_3^- content; K, leaf soluble sugar content.

leaves showed the highest number of nodes, edges, positive edges, and negative edges and average degree. They also had the highest percentage of negative edges but the lowest percentage of positive edges. These findings indicated that an increase species abundance in mature leaves enhanced species interactions but does not entirely led to an increase in community diversity.

Furthermore, CCA was used to analyze the effects of climatic factors, physiological states of leaves and leaf nutrients utilized by microorganisms on the endophytic fungal community structure in *L. barbarum* leaves. Except for K, all other factors substantially influenced the endophytic fungal community structure in *L. barbarum* leaves at different leaf growth stages (Jumpponen and Jones, 2010). reported that foliar macronutrients (e.g., nitrogen, potassium, and sulfur), micronutrients (e.g., boron, manganese, and selenium), and trace elements (e.g., cadmium, lead, and zinc) should not be considered drivers of community composition (Oono et al., 2020). found that plants with higher leaf N-to-phosphorus ratios had lower endophyte richness; however, an increase in sodium content, which may be toxic to fungi at high concentrations, led to an increase in endophyte richness (Liu et al., 2019). identified 11 leaf traits (e.g., specific leaf area, leaf N content, leaf pH, toughness, latex alkaloid content, and latex volume per leaf area) as important abiotic factors shaping the endophytic fungal community structure in *Ficus* leaves (Oita et al., 2021). found a negative correlation between the richness of endophytic fungal communities and temperature seasonality. González-Teuber et al. found that among leaf traits associated with resistance against pathogens and/or herbivores (e.g., cell wall polysaccharides, leaf toughness, flavonoids, anthocyanins, terpenoids, and chitinases). The increase in the content of cell wall polysaccharides decreased fungal read counts, whereas endophyte diversity showed a decrease with increasing anthocyanin levels. Moreover, a positive correlation was found between fungal read counts and terpenoid levels (González-Teuber et al., 2020). These findings indicate that environmental factors influencing microbial community structure may vary depending on the specific environmental conditions used in the experiment.

Herein, the main functions of foliar endophytic fungal communities at different leaf growth stages in *L. barbarum* (predicted using FUNGuild) were found to be Animal Pathogen-Endophyte-Plant Pathogen-Wood Saprotroph and Plant Pathogen. A study showed that the dominant functions of endophytic fungi in *Rheum palmatum* among different tissues and ages were saprotroph, pathotroph-symbiotroph, and symbiotroph (Chen et al., 2023). Maybe, the function of plant pathogen gradually increased with leaf growth. Furthermore, the relative abundance of the potential phytopathogen *Arthrocladiella* increased with leaf growth, likely owing to the aging of leaves. The risk of powdery mildew outbreak in later stages of leaf growth is relatively high; therefore, plants should be carefully monitored during these stages to prevent and control the disease.

4 Conclusion

This study investigated the diversity and structure of foliar endophytic fungal communities during different leaf growth stages

in *L. barbarum* through amplicon sequencing. Results revealed that the composition of foliar endophytic fungal communities varied greatly among young, mature, and old leaves. The number of total and specific ASVs of foliar endophytic fungal communities initially increased and subsequently decreased with leaf growth, with mature leaves showing the highest number of total ASVs. Changes in community richness corresponded to the number of total ASVs; however, diversity and evenness decreased with leaf growth. The fungal co-occurrence network was initially complex but simplified with leaf growth. The dominant genus of foliar endophytic fungal communities was *Alternaria* in young and mature leaves and *Arthrocladiella* in old leaves. The abundance of the predicted function of plant pathogen gradually increased with leaf growth. Climatic factors, physiological states of leaves, and nutrients utilized by microorganisms positively regulate the structure of foliar endophytic fungal communities. These findings provide novel insights into the composition, diversity, and function of foliar endophytic fungal communities in *L. barbarum* and their relationships with the host plant.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://www.ncbi.nlm.nih.gov/PRJNA1219372>.

Author contributions

JY: Investigation, Writing – original draft, Writing – review & editing. JLu: Investigation, Writing – original draft, Writing – review & editing. YW: Investigation, Writing – original draft, Writing – review & editing. MW: Investigation, Writing – original draft, Writing – review & editing. RY: Investigation, Writing – original draft, Writing – review & editing. TL: Investigation, Writing – original draft, Writing – review & editing. LL: Investigation, Writing – original draft, Writing – review & editing. YA: Formal analysis, Writing – original draft, Writing – review & editing. JLi: Conceptualization, Formal analysis, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declared that financial support was received for this work and/or its publication. The High-level Talent Program of North Minzu University (No. 2025BG303), the National Natural Science Foundation of China (No. 31460212), the Innovation Team for Genetic Improvement of Economic Forests (No. 2022QCXTD04), and the Science and Technology Leading Talent Project of Ningxia (No. 2022GKLRLX06).

Acknowledgments

The authors appreciate the insightful feedback and recommendations provided by the editors and reviewers. The authors also thank KetengEdit (www.ketengedit.com) for linguistic assistance.

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial

intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fevo.2026.1777264/full#supplementary-material>

References

- Aamir, M., Rai, K. K., Zehra, A., Kumar, S., Yadav, M., Shukla, V., et al. (2020). "Fungal endophytes: classification, diversity, ecological role, and their relevance in sustainable agriculture," in *Microbial endophytes prospects for sustainable agriculture*. Ed. A. K. V. K. Singh (Cambridge, UK: Elsevier).
- Adams, R. I., Miletto, M., Taylor, J. W., and Bruns, T. D. (2013). Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances. *ISME J.* 7, 1460–1460. doi: 10.1038/ismej.2013.84
- Arnold, A. E. (2007). Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol. Rev.* 21, 51–66. doi: 10.1016/j.fbr.2007.05.003
- Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* 13, 581–583. doi: 10.1038/nmeth.3869
- Chen, D. W., Wang, Y. H., Shi, W. J., Zhao, J., Hou, Q. Z., Zhang, H., et al. (2023). Analysis of endophyte diversity of *Rheum palmatum* among different tissues and ages. *Arch. Microbiol.* 205, 14. doi: 10.1007/s00203-022-03349-z
- Dong, S. D., Wan, S. Q., Kang, Y. H., Miao, J. X., and Li, X. B. (2021). Different mulching materials influence the reclamation of saline soil and growth of the *Lycium barbarum* L. under drip-irrigation in saline wasteland in northwest China. *Agric. Water Manage.* 247, 106730. doi: 10.1016/j.agwat.2020.106730
- González-Teuber, M., Vilo, C., Guevara-Araya, M. J., Salgado-Luarte, C., and Gianoli, E. (2020). Leaf resistance traits influence endophytic fungi colonization and community composition in a South American temperate rainforest. *J. Ecol.* 108, 1019–1029. doi: 10.1111/1365-2745.13314
- Harrison, J. G., Beltran, L. P., Buerkle, C. A., Cook, D., Gardner, D. R., Parchman, T. L., et al. (2021). A suite of rare microbes interacts with a dominant, heritable, fungal endophyte to influence plant trait expression. *ISME J.* 15, 2763–2778. doi: 10.1038/s41396-021-00964-4
- Hassani, M. A., Durán, P., and Hacquard, S. (2018). Microbial interactions within the plant holobiont. *Microbiome* 6, 58. doi: 10.1186/s40168-018-0445-0
- Huang, H., Ni, Z. J., Wu, Z. F., Ma, Y. L., Hu, F., Thakur, K., et al. (2024). Comparison of polyphenols in Goji (*Lycium barbarum* L.) leaves at different leaf positions under different extraction methods. *Ind. Crop Prod.* 209, 117982. doi: 10.1016/j.indcrop.2023.117982
- Jumpponen, A., and Jones, K. L. (2010). Seasonally dynamic fungal communities in the phyllosphere differ between urban and nonurban environments. *New Phytol.* 186, 496–513. doi: 10.1111/j.1469-8137.2010.03197.x
- Kamyab, A., Samsampour, D., Ahmadinasab, N., and Bagheri, A. (2024). Lamiaceae family-derived endophytic fungi: induced tolerance to drought stress in plants. *BMC Plant Biol.* 24, 1104. doi: 10.1186/s12870-024-05764-4
- Kharwar, R. N., Prajapati, P., and Sinha, S. (2025). Endophytic fungi mediated alleviation of salinity stress in crop plants: an insight and future prospect. *Symbiosis*. 96, 1–28. doi: 10.1007/s13199-025-01060-3
- Kushwaha, K., Pal, J., Banyal, D. K., Paul, S., and Katoch, G. (2025). Unlocking the biocontrol and plant growth potential of endophytic fungi against pea root rot complex in Himachal Pradesh, India. *Eur. J. Plant Pathol.* 171, 691–708. doi: 10.1007/s10658-024-02977-z
- Lei, Z. L., Chen, X. Q., Cao, F. L., Guo, Q. R., and Wang, J. H. (2022). Phytochemicals and bioactivities of Goji (*Lycium barbarum* L. and *Lycium chinense* Mill.) leaves and their potential applications in the food industry: a review. *Int. J. Food Sci. Technol.* 57, 1451–1461. doi: 10.1111/ijfs.15507
- Li, Q. X., Lin, F. C., and Su, Z. Z. (2025). Endophytic fungi-Big player in plant-microbe symbiosis. *Curr. Plant Biol.* 42, 100481. doi: 10.1016/j.cpb.2025.100481
- Liu, J. W., Zhao, J., Wang, G., and Chen, J. (2019). Host identity and phylogeny shape the foliar endophytic fungal assemblages of *Ficus*. *Ecol. Evol.* 9, 10472–10482. doi: 10.1002/ecs3.5568
- Liu, X., Jia, P., Cadotte, M. W., Zhu, C., Si, X. F., Wang, Y. Q., et al. (2021). Host plant environmental filtering drives foliar fungal community assembly in symptomatic leaves. *Oecologia* 195, 737–749. doi: 10.1007/s00442-021-04849-3
- López-Menchero, J. R., Andrés-Yeves, M. F., González-Coloma, A., and González-García, V. (2024). "Endophytic fungi: taxonomy, isolation, and current applications," in *The diversity of the fungal world*. Ed. J. Putzke (London, UK: IntechOpen).
- Lu, Y. Y., Guo, S., Zhang, F., Yan, H., Qian, D. W., Shang, E. X., et al. (2021). Nutritional components characterization of Goji berries from different regions in China. *J. Pharm. Biomed. Anal.* 195, 113859. doi: 10.1016/j.jpba.2020.113859
- Ma, R. H., Zhang, X. X., Ni, Z. J., Thakur, K., Wang, W., Yan, Y. M., et al. (2023). (Goji) as functional food: a review of its nutrition, phytochemical structure, biological features, and food industry prospects. *Crit. Rev. Food Sci. Nutr.* 63, 10621–10635. doi: 10.1080/10408398.2022.2078788
- Narayan, O. P., Verma, N., Jogawat, A., Dua, M., and Johri, A. K. (2021). Sulfur transfer from the endophytic fungus improves maize growth and requires the sulfate transporter SiSulT. *Plant Cell* 33, 1268–1285. doi: 10.1093/plcell/koab006
- Ni, Z. J., Ma, R. H., Wang, W., Thakur, K., Ma, Y. L., Khan, M. R., et al. (2024). Potential biosurfactant and methanol extraction for phenolic active substances from *Lycium barbarum* fruits and leaves. *Ind. Crop Prod.* 212, 118333. doi: 10.1016/j.indcrop.2024.118333
- Nilsson, R. H., Larsson, K.-H., Taylor, A. F. S., Bengtsson-Palme, J., Jeppesen, T. S., Schigel, D., et al. (2019). The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res.* 47, D259–D264. doi: 10.1093/nar/gky1022

- Oita, S., Ibáñez, A., Lutzoni, F., Miadlikowska, J., Geml, J., Lewis, L. A., et al. (2021). Climate and seasonality drive the richness and composition of tropical fungal endophytes at a landscape scale. *Commun. Biol.* 4, 313. doi: 10.1038/s42003-021-01826-7
- Oono, R., Black, D., Slessarev, E., Sickler, B., Strom, A., and Apigo, A. (2020). Species diversity of fungal endophytes across a stress gradient for plants. *New Phytol.* 228, 210–225. doi: 10.1111/nph.16709
- Patyal, U., Kaur, M., Faizan, M., Đalović, I., and Trivan, G. (2024). A review of endophytic fungi and their applications in different fields of biotechnology. *J. Microbiol. Biotechnol.* 9, 1–17. doi: 10.23880/oajmb-6000299
- Ramachandran, S. P., Jayanthikumari, V. P., Kodavanthodi, F., and Saraladevi, R. M. (2025). Characterization of plant growth-promoting endophytic fungi from *Aegle marmelos* Corr. and their role in growth enhancement and yield in rice. *Folia Microbiol.* 71, 93–105. doi: 10.1007/s12223-025-01254-8
- Ren, F., Dong, W., Sun, H., and Yan, D. H. (2019). Endophytic mycobiota of jingbai pear trees in north China. *Forests* 10, 260. doi: 10.3390/f10030260
- Ren, Y., Yu, G., Shi, C. P., Liu, L. M., Guo, Q., Han, C., et al. (2022). Majorbio Cloud: A one-stop, comprehensive bioinformatic platform for multiomics analyses. *Imeta* 1, e12. doi: 10.1002/imt2.12
- Stone, J. K., Bacon, C. W., and White, J. F. (2000). “An overview of endophytic microbes: endophytism defined,” in *Microbial endophytes*. Eds. C. W. Bacon and J. F. White (M Dekker Inc, New York).
- Stone, J. K., Polishook, J. D., and White, J. F. (2004). “Endophytic fungi,” in *Biodiversity of fungi: inventory and monitoring methods*. Eds. B. G. Mueller and M. S. Foster (Elsevier, Burlington, MA, USA).
- Su, H. Y., Guo, Y. T., Gu, L., Shi, X. M., Zhou, Y. Y., Wu, F. L., et al. (2024). A novel growth-promoting dark septate endophytic fungus improved drought tolerance in blueberries by modulating phytohormones and non-structural carbohydrates. *Tree Physiol.* 44, tpae105. doi: 10.1093/treephys/tpae105
- Usman, M., Shah, I. H., Sabir, I. A., Malik, M. S., Rehman, A., Murtaza, G., et al. (2024). Synergistic partnerships of endophytic fungi for bioactive compound production and biotic stress management in medicinal plants. *Plant Stress* 11, 100425. doi: 10.1016/j.stress.2024.100425
- Wang, Y. J., Liang, X. J., Guo, S. J., Li, Y. K., Zhang, B., Yin, Y., et al. (2019). Evaluation of nutrients and related environmental factors for wolfberry (*Lycium barbarum*) fruits grown in the different areas of China. *Biochem. Syst. Ecol.* 86, 103916. doi: 10.1016/j.bse.2019.103916
- Waqar, S., Bhat, A. A., and Khan, A. A. (2024). Endophytic fungi: Unravelling plant-endophyte interaction and the multifaceted role of fungal endophytes in stress amelioration. *Plant Physiol. Biochem.* 206, 108174. doi: 10.1016/j.plaphy.2023.108174
- Wemheuer, F., Berkelmann, D., Wemheuer, B., Daniel, R., Vidal, S., and Daghela, H. B. (2020). Agroforestry management systems drive the composition, diversity, and function of fungal and bacterial endophyte communities in *Theobroma Cacao* Leaves. *Microorganisms* 8, 405. doi: 10.3390/microorganisms8030405
- Whitaker, B. K., Christian, N., Chai, Q., and Clay, K. (2020). Foliar fungal endophyte community structure is independent of phylogenetic relatedness in an Asteraceae common garden. *Ecol. Evol.* 10, 13895–13912. doi: 10.1002/ece3.6983
- Wu, R. J., Liu, B. Q., Xue, B., Gao, R. L., Ndzana, G. M., Liu, R. T., et al. (2023). Changes in soil organic carbon and nutrient pools in aggregate-sized fractions along a chronosequence of wolfberry (*Lycium barbarum* L.) plantations in arid areas of Northwest China. *Soil Use Manage.* 39, 1109–1124. doi: 10.1111/sum.12926
- Yu, J., Yan, Y. M., Zhang, L. T., Mi, J., Yu, L. M., Zhang, F. F., et al. (2023). A comprehensive review of goji berry processing and utilization. *Food Sci. Nutr.* 11, 7445–7457. doi: 10.1002/fsn3.3677
- Zhang, L. H., Xu, W., Zhao, Z. B., Long, Y. H., and Fan, R. (2024b). Biocontrol potential and growth-promoting effect of endophytic fungus SD1–4 against potato leaf spot disease caused by. *BMC Microbiol.* 24, 255. doi: 10.1186/s12866-024-03411-4
- Zhang, L., Zhang, E. R., Wei, Y. Q., and Zheng, G. Q. (2024a). Phylogenetic analysis and divergence time estimation of species in China based on the chloroplast genomes. *BMC Genomics* 25, 569. doi: 10.1186/s12864-024-10487-9