

## Article

# Seasonal Diversity of Endophytic Fungi in *Camphora bodinieri* with Citral Chemotype

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## Abstract

To investigate the influence of seasonal dynamics on the endophytic fungal community of *Camphora bodinieri* with citral chemotype, we employed standardized sampling and high-throughput sequencing to analyze the diversity, composition, and structure of endophytic fungi across different seasons. A total of 1825 Amplicon Sequence Variants (ASVs) were obtained, belonging to 12 phyla, 43 classes, 104 orders, 239 families, 449 genera, and 663 species. Among them, 23 ASVs were shared across all four seasons, suggesting the presence of a core fungal community. The number of season-specific ASVs was highest in autumn (730), followed by summer (336), spring (325), and winter (202). The results showed that season significantly affected the Shannon ( $F_{3,9} = 5.927$ ,  $p = 0.016$ ) and Simpson ( $F_{3,9} = 4.892$ ,  $p = 0.026$ ) indices, with the diversity and species richness of endophytic fungi being highest in autumn and lowest in winter. Across different tissues, the similarity of endophytic fungal communities between leaves and stems was comparatively higher than that with roots. Furthermore, different fungal taxa exhibited differential distribution patterns along temperature–moisture gradients. This study explored the seasonal diversity of endophytic fungi in *C. bodinieri* with citral chemotype, providing a theoretical basis for understanding host–endophyte interactions.

**Keywords:** chemotype; species richness; high-throughput sequencing; community structure; community similarity

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## 1. Introduction

Endophytic fungi refer to fungi that inhabit the internal tissues or intercellular spaces of healthy plants during part or all of their life cycle. Their interactions with host plants are mutualistic, encompassing an ecological continuum ranging from mutualism and commensalism to latent pathogenicity, depending on environmental conditions, host physiological status, and the fungal genetic background [1]. Endophytic fungi play indispensable roles in promoting plant growth, enhancing stress resistance (e.g., against diseases, drought, and pests), assisting in nutrient uptake, and synthesizing bioactive secondary metabolites [2].

*Camphora bodinieri* (H.Lév.) Y. Yang, Bing Liu & Zhi Yang, as a *Cinnamomum* species endemic to Central Asia, is particularly notable for being rich in a specific chemotype of citral, namely *C. bodinieri* with citral chemotype (CBCC) [3]. Citral is a mix of the isomeric monoterpenoid aldehydes, geranial and neral [4], and serves as an essential component for the synthesis of vitamins A, E and ionones, with wide-ranging applications in medicine, food, flavors, and fragrances [5]. Therefore, an in-depth analysis of the composition, structure, and dynamics of the endophytic fungal community in CBCC aims to characterize its microbial diversity. This study provides a crucial theoretical basis and data support for the future mining of fungal strains with specific metabolic functions, as well as for further investigating the potential impacts of these fungi on host physiology and secondary metabolites (such as essential oil components).

Endophytic fungal communities are not static; their structure and diversity are influenced by various factors across different spatial scales [6]. Among these, seasonal variation is a key dynamic driver. Through significant changes to environmental parameters including temperature, light, humidity, and precipitation, seasonality directly affects host physiological status (e.g., growth stage, metabolic activity, defense responses) as well as the growth and reproductive conditions of the microorganisms themselves [7]. Environmental factors play a critical role in shaping the structure and diversity of microbial communities [8,9], and numerous studies have demonstrated that seasonal changes can significantly reshape the diversity and composition of endophytic fungal communities across a wide range of plant species [10,11]. However, for specific plant varieties such as *C. bodinieri* with a specific chemotype (e.g., citral type), the question of how their endophytic fungal communities respond to seasonal dynamics remains poorly understood.

In various plant systems, seasonal variation has been established as a key driver of endophytic fungal community dynamics [7]. Against this background, this study aims to explore whether and how seasonal changes affect the endophytic fungal communities in CBCC. To this end, we employed high-throughput ITS amplicon sequencing to systematically analyze the community composition and structure of endophytic fungi in the roots, stems, and leaves of *C. bodinieri* across different seasons. This study will elucidate the spatiotemporal distribution patterns of these fungal communities, providing baseline data and a scientific basis for the future targeted mining of functional endophytic resources, as well as for further investigation of the potential links between microbiota and host secondary metabolism.

## 2. Materials and Methods

### 2.1. Experimental Materials and Sampling Methods

The experimental material consisted of one-year-old cuttings of CBCC cultivated in the Camphor Tree Excellent Clone Cutting Orchard of the Jiangxi Provincial Engineering Technology Center for Camphor Tree Breeding, Development and Utilization (Nanchang City, Jiangxi Province, China; 28°41'47" N, 116°1'49" E). The four sampling dates were April, July, October, and January, selected as the representative month for each quarter, respectively (spring, Sp; summer, Su; autumn, Au; winter, Wi). Four healthy clones with consistent growth vigor were selected as replicates. For each plant, 5–8 intact mature leaves, 5–8 healthy stem segments, and 5–8 fine healthy roots were collected. This resulted in a total of 48 samples nested within the four clonal individuals. Each tissue from each replicate was processed separately. Sampling tools were sterilized by soaking in 75% alcohol followed by flaming. Samples were placed and labeled in sterile self-sealing bags. Immediately after collection, the samples were placed in an insulated container with ice packs and transported to the laboratory for microbial DNA extraction.

## 2.2. Sample DNA Extraction and PCR Amplification

### 2.2.1. Sample Surface Sterilization

First, any soil attached to the surface of *C. bodinieri* was shaken off, and then the soil remaining on the roots, stems, and leaves was washed away with sterile water. After the samples were blotted dry with absorbent paper, surface sterilization was performed: the samples were rinsed with sterile water for 30 s, transferred to 75% ethanol (Xilong Scientific Co., Ltd., Shantou, China) for 1 min, and then soaked in 2% sodium hypochlorite solution (Tianjin Hengxing Chemical Reagent Manufacturing Co., Ltd., Tianjin, China) for 3 min [12]. After this treatment, the samples were transferred back to 75% ethanol for 1 min and finally rinsed with sterile water for 30 s to complete surface sterilization [13]. Rinse controls, imprint controls, and environmental controls were established following [14]. After surface sterilization of the root, stem, and leaf samples, 100 µL of the final rinse solution was spread onto potato dextrose agar (PDA) plates, and plates spread with sterile water served as the negative control. Meanwhile, surface-sterilized tissues of *C. bodinieri* were repeatedly pressed onto blank plates and then incubated as the imprint control. A set of blank culture media was left open throughout the procedure and processed following blank instrument manipulation as the environmental control. All plates were incubated at 26 °C for 7–10 days to verify the completeness of disinfection [15].

Although the culture-based controls described above can effectively assess viable, culturable surface-contaminating microorganisms, they have inherent limitations: they cannot rule out residual DNA from non-culturable microorganisms, nor can they detect potential molecular-level contamination introduced during subsequent DNA extraction, PCR amplification, and library construction. Therefore, the fungal ASVs explored in this study via sequencing should be cautiously interpreted as “fungal communities potentially associated with the internal tissues of *C. bodinieri*” rather than strictly defined absolute endophytic communities. All related conclusions are discussed within this exploratory framework.

### 2.2.2. DNA Extraction, PCR Amplification and Sequencing

After freezing in liquid nitrogen, approximately 0.5 g of tissue per sample was used for DNA extraction with the D1500-50T, Solarbio (Beijing, China) [16]. The DNA concentration of each sample was quantified with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Model 2000, Waltham, MA, USA), and quantified DNA samples were stored at −20 °C. PCR amplification was performed using TransStart® FastPfu DNA Polymerase (TransGen Biotech, AP221-02; Beijing, China), with primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC) targeting the ITS1F-ITS2R region. Four replicate reactions were set up for each sample. The PCR reaction conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min; annealing at 50 °C for 1 min; extension at 72 °C for 1 min; and a final extension at 72 °C for 10 min [17]. The PCR reaction mixture (25 µL) consisted of 8.5 µL ddH<sub>2</sub>O, 2 µL template DNA, 1 µL each of primers ITS1F and ITS2R, and 12.5 µL of the master mix. Barcode-specific primers were synthesized based on the target sequencing region. To improve the concentration and quality of the sequencing template and reduce random amplification bias from individual PCR runs, PCR products from the same sample were pooled and detected via 2% agarose gel electrophoresis. The target bands were then excised and purified using the AxyPrep DNA Gel Extraction Kit (Shanghai Meiji Yuhua Biomedical Technology Co., Ltd., Shanghai, China). The purified DNA was eluted with Tris-HCl buffer and verified again via electrophoresis. The qualified PCR products were subjected to high-throughput sequencing on the Illumina MiSeq platform by Shanghai Majorbio Bio-pharm Technology Co., Ltd. (www.majorbio.com) (Shanghai, China).

### 2.3. Bioinformatic and Statistical Analyses

#### 2.3.1. Data Optimization

In this study, Amplicon Sequence Variant (ASV) denoising was applied for data processing. Raw sequencing reads were quality-controlled using fastp [18] (<https://github.com/OpenGene/fastp>, v.0.23.4, accessed on 4 August 2026) and merged using FLASH [19] (<https://ccb.jhu.edu/software/FLASH/>, v.1.2.7, accessed on 4 August 2026). The processing steps were as follows: bases with a quality value below 20 at the read tail were trimmed; a 10 bp sliding window was adopted, where if the average quality value within the window dropped below 20, all bases from that window to the end of the read were truncated; reads shorter than 50 bp after quality control were discarded; and reads containing ambiguous bases (N) were removed. Based on the overlap between paired-end reads, reads were merged into a single consensus sequence with a required minimum overlap length of 10 bp. The maximum allowed mismatch ratio in the overlap region of merged sequences was set to 0.2, and sequences that did not meet this threshold were filtered out. Samples were sorted according to the barcodes and primers at both ends of the sequences, and the orientation of each sequence was adjusted. The number of allowed mismatches was 0 for barcodes and 2 for primers, respectively.

#### 2.3.2. ASV Denoising and Taxonomic Analysis

Based on default parameters, the quality-controlled and merged optimized sequences were denoised using the DADA2 plugin within the QIIME2 (2025.4) pipeline [20,21]. The sequences obtained after DADA2 (or Deblur) denoising are generally referred to as ASVs. Sequences annotated as chloroplasts or mitochondrial in all samples were removed. To minimize the impact of sequencing depth on subsequent alpha diversity and beta-diversity analyses, the number of sequences per sample was rarefied to 20,000. After rarefaction, the average Good's coverage for each sample remained 99.09%. Taxonomic analysis of ASVs was performed using the Blast classifier in QIIME2 based on the UNITE database (Release 9.0) [22].

#### 2.3.3. Data Analysis

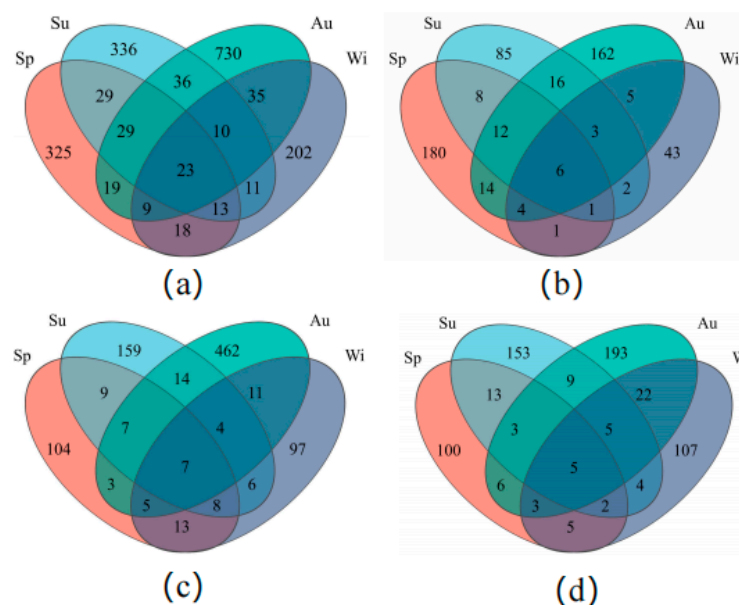
All data obtained from high-throughput sequencing were analyzed on the Majorbio Cloud Platform [23] (<https://cloud.majorbio.com>). Seasonal differences in alpha diversity indices (Shannon, Simpson, Sobs, and Chao) were analyzed using linear mixed-effects models (LMMs). In the models, season was treated as a fixed factor, and plant identity (PlantID) was included as a random intercept to account for repeated sampling from the same individuals across seasons. Post-hoc pairwise comparisons were performed using Tukey's HSD adjustment. All analyses were performed in R 4.5.1 (<https://www.r-project.org/>, accessed on 4 August 2026). Principal coordinate analysis (PCoA) based on the Bray–Curtis distance algorithm was applied to assess the similarity of microbial community structures among samples, and PERMANOVA based on Bray–Curtis distances was performed using the adonis2 function to test the effects of tissue type, sampling time, and their interaction on endophytic fungal community structure. To account for repeated sampling from the same individual plants across different seasons, permutations were restricted within each plant individual (strata = PlantID), with 999 permutations. The abundance proportions of fungal taxa from phylum to genus levels among different groups were analyzed and determined using Python 2.7 (<https://www.python.org/>, accessed on 4 August 2026) [24]. Based on Spearman's correlation ( $|r| > 0.6$ ,  $p < 0.05$ ), taxa were selected for correlation heatmap analysis [25].

### 3. Results

#### 3.1. Analysis of Community Composition

The sequence data were derived from the NCBI SRA database under BioProject number PRJNA1427898. Based on taxonomic analysis of the ASVs (Figure 1), a total of 1825 ASVs belonging to 12 phyla, 43 classes, 104 orders, 239 families, 449 genera, and 663 species of fungi were obtained from 48 tissue samples of CBCC. The samples were grouped into four categories according to collection season (Figure 1a). The distribution of ASVs differed significantly across seasons: autumn (Au) had the highest ASV count at 891, of which 730 were unique to autumn; spring (Sp), summer (Su), and winter (Wi) had 465, 487, and 321 ASVs, respectively, with 325, 336, and 202 unique ASVs, respectively. Twenty-three ASVs were shared across all four seasons, and additional ASVs were shared between two or three seasons.

When the 48 samples were divided into three tissue types (12 samples per tissue) and further grouped into four subsets by season, ASV counts showed that stem tissue had the highest number of ASVs (909) (Figure 1c), followed by leaf tissue with a total of 630 ASVs (Figure 1d), and root tissue with 542 ASVs (Figure 1b). Across different seasons, root tissue had six shared ASVs, accounting for only 1.1% of the total. In the root tissue, spring had the highest number of unique ASVs (180), while winter had the lowest (43). In stem tissue, seven ASVs were shared across all seasons, representing only 0.8% of the total. In this tissue, autumn had the highest number of unique ASVs (462) and winter had the lowest (93). In leaf tissue, five ASVs were shared across seasons, accounting for 0.8% of the total. Autumn had the highest number of unique ASVs (193), and spring had the lowest (100).

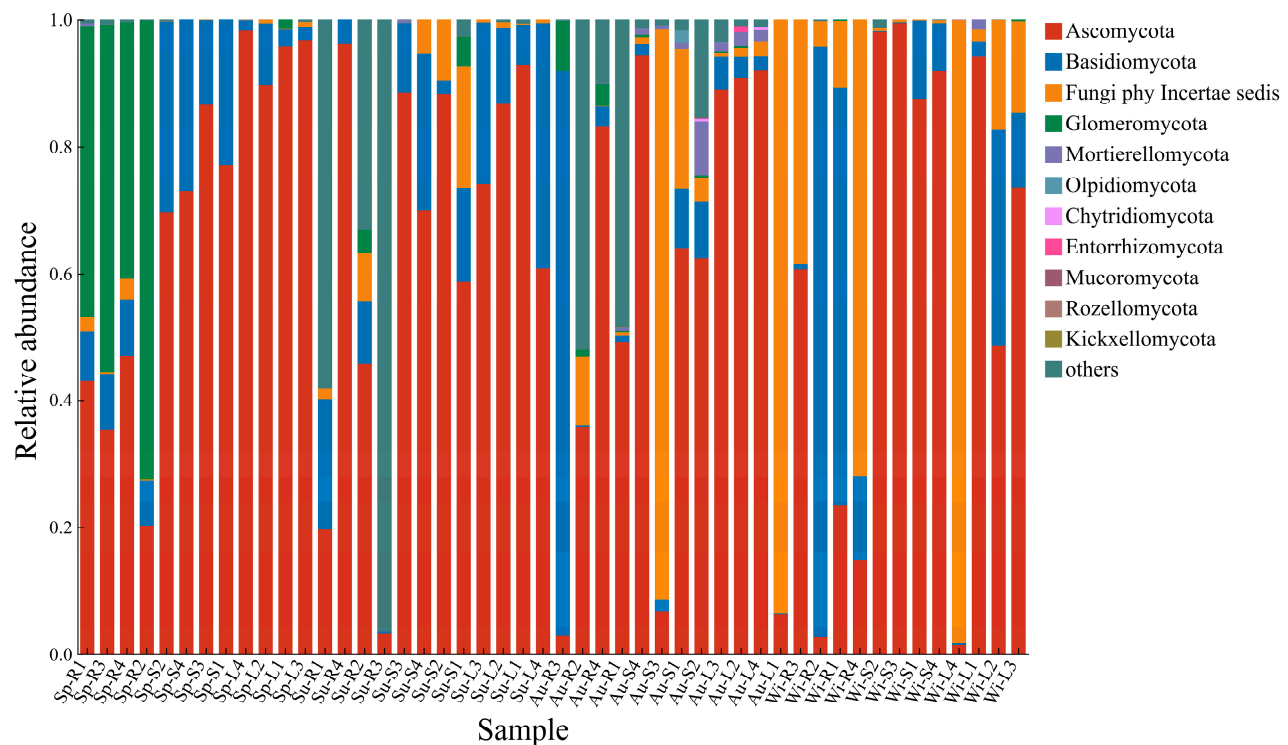


**Figure 1.** Effects of different seasons on the community composition of CBCC. Venn diagrams corresponding to ASV counts of fungal communities from (a) all tissues, (b) roots, (c) stems, (d) leaves. (Sp) spring, (Su) summer, (Au) autumn, and (Wi) winter.

#### 3.2. Analysis of Endophytic Fungal Community Structure

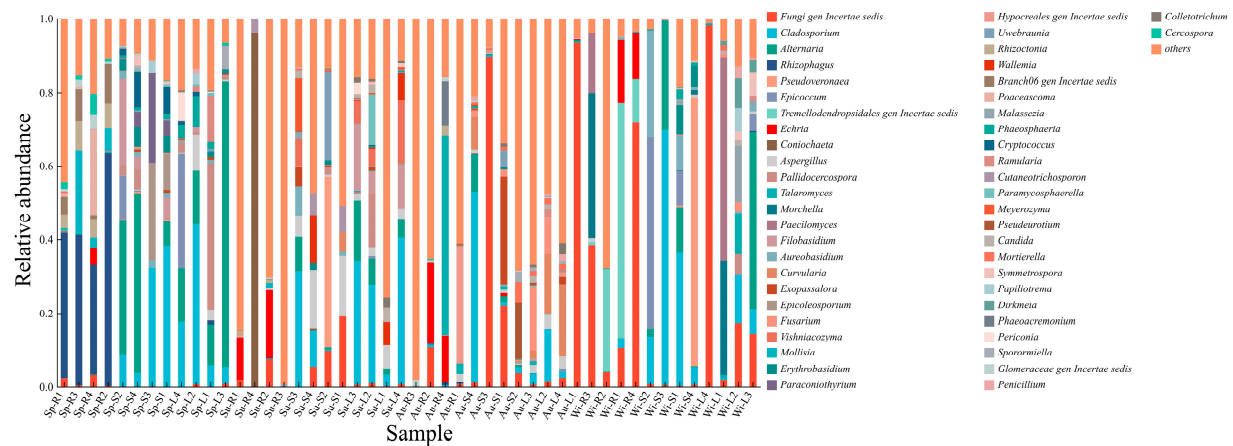
At the phylum level (Figure 2), the top three phyla in the spring samples of CBCC were Ascomycota, Glomeromycota, and Basidiomycota, which constituted the core fungal community in spring. Ascomycota was the dominant phylum in most samples across all seasons, with a relative abundance generally exceeding 50%, indicating that it is the pre-dominant group of endophytic fungi in CBCC. The overall structure of endophytic fungal

communities at the phylum level was highly consistent across different samples. Glomeromycota was only highly enriched in root samples in spring; its abundance decreased sharply in summer (Su), recovered slightly in autumn (Au), and was almost undetectable in winter (Wi). These results demonstrate that sequences assigned to Glomeromycota had the highest relative abundance in spring samples. Basidiomycota showed a significant increase in abundance in summer, autumn, and winter samples, and became the secondary dominant phylum in some summer and autumn samples, exhibiting a seasonal dynamic opposite to that of Glomeromycota.



**Figure 2.** Bar chart of fungal relative abundance at the phylum level. Note: (Sp) spring; (Su) summer; (Au) autumn; (Wi) winter; (R) roots; (S) stems; (L) leaves.

At the genus level (Figure 3), differences in genus distribution were observed across seasons and tissue types. For instance, dominant genera varied even within the same plant: *Rhizophagus* dominated root sample R4 in spring, accounting for 29.77%; *Coniochaeta* became overwhelmingly abundant in root R4 in summer, reaching 96.08%; *Talaromyces* prevailed in root R4 in autumn with a relative abundance of 54.09%; and *Fungi gen Incertae sedis* was the dominant taxon in root R4 in winter at 71.96%. Stem tissues harbored a richer diversity of fungal genera. *Alternaria* made up a relatively high proportion of the community in spring stems; summer and autumn stems possessed comparatively complex fungal assemblages, while *Cladosporium* predominated in winter stems. Fungal communities in leaves displayed pronounced seasonal fluctuations: *Alternaria* accumulated in spring leaves, declined in relative abundance in summer, was undetectable in autumn, and rebounded in winter. In terms of seasonal dynamics, large spatial differences in fungal communities existed among different plant tissues in spring. Summer saw a marked increase in the relative abundance of rare taxa across all tissues, accompanied by weakened dominance of core genera and elevated community diversity. In autumn, root fungal communities differed significantly from those in stems and leaves. Winter was characterized by relatively low overall community diversity.

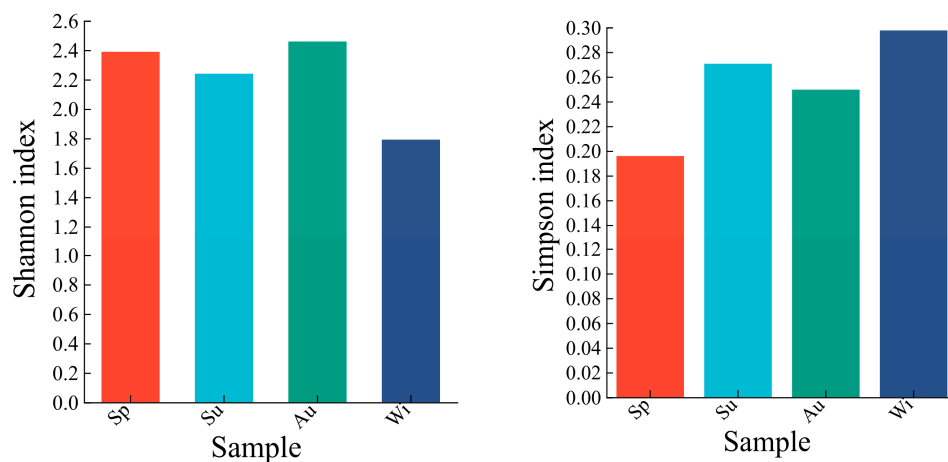


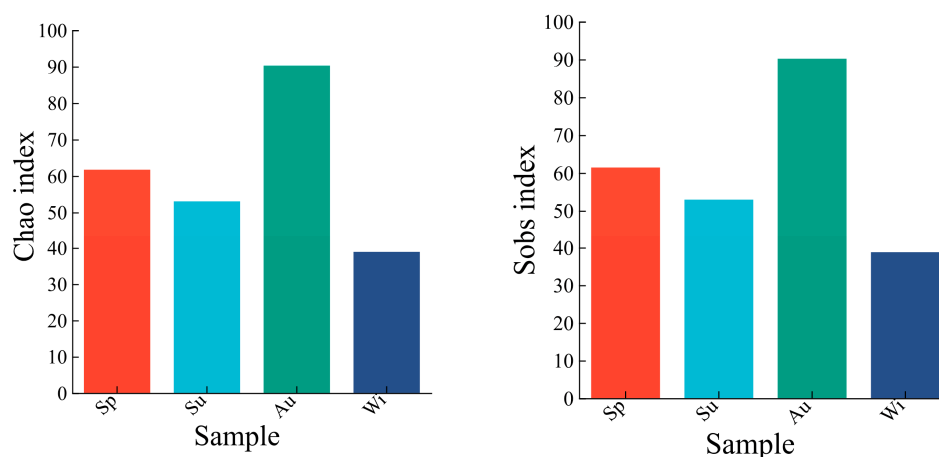
**Figure 3.** Bar chart of fungal relative abundance at the genus level.

### 3.3. Alpha Diversity Index Analysis

Alpha diversity of endophytic fungi in CBCC was analyzed using the Shannon, Simpson, Sobs, and Chao indices across seasons (Figure 4). The Shannon index was positively correlated with community diversity and evenness, whereas the Simpson dominance index was negatively correlated with community diversity. The Shannon index values followed the order autumn > spring > summer > winter, indicating that fungal community diversity and evenness were highest in autumn and lowest in winter. Consistent with the ecological interpretation of the Simpson index, its values ranked winter > summer > autumn > spring, showing that community diversity was lowest in winter and highest in spring. The Sobs and Chao richness indices exhibited consistent seasonal variation trends (autumn > spring > summer > winter), with endophytic fungal species richness peaking in autumn and dropping to the minimum in winter.

To account for the non-independence of repeated sampling from the same plant individuals, linear mixed-effects models with PlantID as a random intercept were used to analyze the effect of season on alpha diversity. The results showed that season significantly affected the Shannon ( $F_{3,9} = 5.927$ ,  $p = 0.016$ ) and Simpson ( $F_{3,9} = 4.892$ ,  $p = 0.026$ ) indices. Tukey's post-hoc pairwise comparisons revealed that the Shannon index was significantly higher in autumn than in summer ( $p = 0.045$ ) and winter ( $p = 0.006$ ); the Simpson index was significantly higher in winter than in autumn ( $p = 0.012$ ), corroborating the Shannon results. Although the Sobs and Chao richness indices did not reach statistical significance ( $p = 0.051$  and  $p = 0.058$ , respectively), they exhibited a similar trend, with the highest values in autumn and the lowest in winter (Table 1).





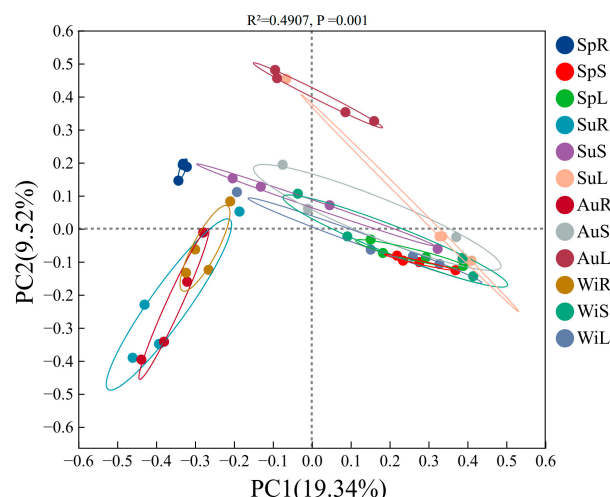
**Figure 4.** Bar chart of the intergroup distribution of Alpha diversity indices. Note: (Sp) spring; (Su) summer; (Au) autumn; (Wi) winter.

**Table 1.** Linear mixed-model results for seasonal effects on alpha diversity indices.

| Index   | NumDF | DenDF | F-Value | p-Value |
|---------|-------|-------|---------|---------|
| Shannon | 3     | 9.00  | 5.927   | 0.016   |
| Simpson | 3     | 9.00  | 4.892   | 0.026   |
| Sobs    | 3     | 9.00  | 3.801   | 0.051   |
| Chao    | 3     | 9.00  | 3.615   | 0.058   |

### 3.4. Beta-Diversity Analysis

Principal coordinate analysis (PCoA) at the ASV level was performed to explore the beta-diversity characteristics of endophytic fungal communities in CBCC (Figure 5). The contribution rates of PC1 and PC2 were 19.34% and 9.52%, respectively, with a cumulative contribution of 28.86%. Samples from different seasons and tissues overlapped considerably in the ordination space, indicating high similarity in endophytic fungal community composition across season and tissue groups. Root samples were mostly distributed in the negative region of PC1, and root samples collected in summer, autumn, and winter clustered closely in the ordination space. This indicated that root fungal communities from these three seasons shared higher compositional similarity with each other than with other samples. Leaf samples were concentrated in the positive interval of PC1 and were clearly separated from root samples in the ordination space, indicating that the community composition between roots and leaves only showed a differentiation trend, rather than extremely low similarity. Stem samples fell between root and leaf samples, with some points overlapping with leaf samples, suggesting that the composition of stem endophytic fungal communities was intermediate between that of roots and leaves, and shared partial compositional overlap with leaf communities. Within the same tissue type, seasonal samples exhibited positional shifts in the ordination space, with autumn samples showing relatively higher dispersion. This implied that seasonal factors have a potentially driving effect on community composition.



**Figure 5.** PCoA scatter plot of beta diversity. (Sp) spring; (Su) summer; (Au) autumn; (Wi) winter; (R) roots; (S) stems; (L) leaves.

PERMANOVA further quantified the effects of these factors (Table 2). The results showed that sampling time ( $R^2 = 0.118$ ,  $p = 0.001$ ), tissue type ( $R^2 = 0.181$ ,  $p = 0.001$ ), and their interaction ( $R^2 = 0.191$ ,  $p = 0.001$ ) all had highly significant effects on community structure. Together, these factors explained approximately 49.1% of the total variation, with the interaction term accounting for the largest proportion (19.1%), indicating that the effect of tissue type on community composition varied with sampling time.

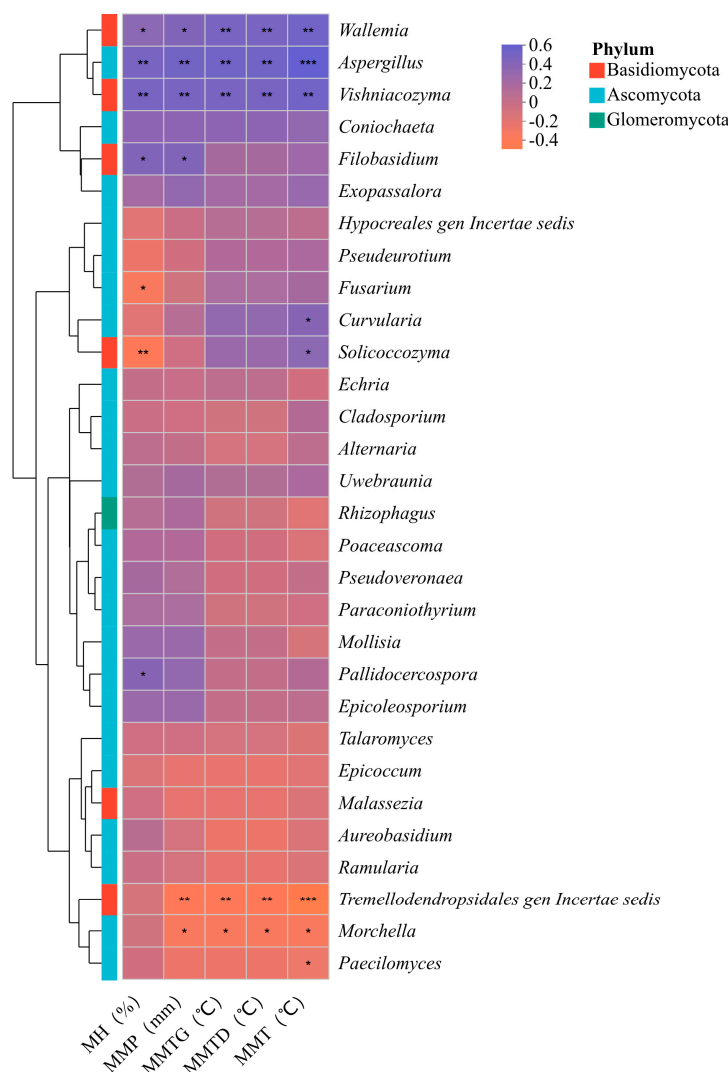
**Table 2.** PERMANOVA analysis of beta diversity for endophytic fungal communities.

| Source                      | Df | SumsOfSqs | MeanSqs | F.Models | R <sup>2</sup> | Pr (>F) |
|-----------------------------|----|-----------|---------|----------|----------------|---------|
| Sampling Time               | 3  | 2.29682   | 0.76561 | 2.78583  | 0.11824        | 0.001   |
| Tissue Type                 | 2  | 3.51638   | 1.75819 | 6.39754  | 0.18102        | 0.001   |
| Sampling Time × Tissue Type | 6  | 3.71907   | 0.61984 | 2.25543  | 0.19145        | 0.001   |
| Residuals                   | 36 | 9.89362   | 0.27482 | -        | 0.5093         | -       |
| Total                       | 47 | 19.42589  | -       | -        | 1              | -       |

### 3.5. Correlation Analysis Between Endophytic Fungi and Environmental Factors

From the response patterns shown in the ordination plot (Figure 6), it can be seen that different fungal genera exhibit differential distribution trends along environmental gradients: *Wallemia*, *Aspergillus*, and *Vishniacozyma* tend to be more concentrated in the direction of higher temperature, higher humidity, and higher precipitation; *Filobasidium* is biased towards areas with higher humidity and precipitation; and *Curvularia* and *Solicoccozyma* show some correspondence with higher temperature. On the other hand, *Solicoccozyma* is less distributed in the direction of higher humidity; *Tremellodendropsidales* gen *Incertae sedis* and *Morchella* are relatively less distributed in the direction of higher temperature and precipitation; and *Fusarium* is less distributed in the direction of higher humidity. The only representative genus of *Glomeromycota*, *Rhizophagus*, is more distributed in the direction of higher precipitation and humidity, but less so in the direction of higher temperature. In addition, most of the dominant genera, such as *Alternaria*, *Cladosporium*, and *Uwebraunia*, do not show obvious correspondence with environmental gradients in the plot. At the phylum level, the response patterns of genera within Ascomycota vary considerably, while within Basidiomycota, a differentiation between moisture-preferring taxa and low-temperature-preferring taxa is observed. It should be emphasized again that the above observations are based solely on the visual distribution in

the ordination plot and given the insufficient replication of environmental data (with only four independent seasonal values), they cannot be taken as evidence that temperature, humidity, or precipitation influence the growth or relative abundance of specific fungal genera. These preliminary exploratory results await further validation through independent multi-year or multi-site sampling data.



**Figure 6.** The heatmap of correlations between endophytic fungal genera and environmental factors. Note: MMP (mm): mean precipitation; MH (%): relative humidity; MMT (°C): mean temperature; MMTG (°C): mean maximum temperature; MMTD (°C): mean minimum temperature. \*  $p < 0.05$ : significant; \*\*  $p < 0.01$ : very significant; \*\*\*  $p < 0.001$ : extremely significant.

## 4. Discussion

### 4.1. Effects of Different Seasons on the Community Composition of Citral-Type *C. bodinieri*

As seasons changed, endophytic fungal communities exhibited certain successional patterns [26]. Only 23 ASVs were shared across all seasonal groups. The high abundance of season-specific ASVs was identified (most abundant in autumn, and least abundant in winter). The higher richness of autumn-specific ASVs may be associated with changes in tissue microenvironments caused by nutrient remobilization as the plant enters the late growth stage [27]. The reduction in winter-unique species and diversity (in line with the Shannon and Simpson indices) coincided with lower environmental temperatures [28],

which is consistent with the findings of [29]. Similarly, Mishra [30] reported that season and tissue type significantly influence endophytic fungal communities.

In the analysis of endophytic fungal community structure, Ascomycota was found to be the dominant phylum in most samples, which is consistent with the general characteristics of endophytic fungal communities in most woody plants. This is usually ascribed to the potent cell-wall-degrading enzymes and varied spore dispersal mechanisms of Ascomycota, making them more suited to the intricate microhabitats of woody tissues. Studies by Cheng and Liu showed that the dominant endophytic fungi in the leaves of *Cinnamomum camphora* and *Cinnamomum longepaniculatum* were both Ascomycota, which is similar to the results of the present study [31,32]. There were seasonal differences in the growth of endophytic fungi belonging to the same phylum. Glomeromycota was highly enriched only in root samples collected in spring (Sp); its relative abundance decreased sharply in summer (Su), rebounded slightly in autumn (Au), and was barely detected in winter (Wi) samples. These results demonstrate that sequences assigned to Glomeromycota exhibited the highest relative abundance in spring samples. This suggests that seasonal changes in environmental conditions may alter the growth status of endophytic fungi [33].

The composition of dominant fungal genera in plants varies across different seasons. For example, *Alternaria* was enriched in spring (29.40%) but was not detected in autumn. From a seasonal dynamics perspective, the spatial variation in the community across different tissue types was relatively large in spring. Deng reported seasonal differences in the colonization of endophytic fungi in the roots of *Dasiphora parvifolia*, with higher fungal diversity in spring roots, which is consistent with our results [34]. The number of unique species across all tissue types remained low in winter, which aligns with the established pattern that low winter temperatures inhibit the growth and activity of most fungi.

#### 4.2. Effects of Different Seasons on the Community Diversity of Citral-Type *C. bodinieri*

In this study, the  $\alpha$ -diversity of endophytic fungi associated with CBCC exhibited significant seasonal variation. The Shannon index showed the highest diversity in autumn and the lowest in winter, consistent with previous findings [35]. The Simpson index further corroborated this trend, with significantly higher dominance in winter than in autumn, reflecting lower community diversity during the cold season. However, an earlier study on olive tree endophytes reported higher diversity in spring than in autumn [36], which contrasts with our observation of higher diversity in autumn than in spring. This discrepancy may be attributed to differences in host plant species or environmental conditions.

Regarding species richness, both Chao and Sobs indices showed numerical trends with the highest values in autumn and the lowest in winter, although these differences did not reach statistical significance ( $p = 0.051$  and  $0.058$ , respectively). This pattern partially aligns with the report of lowest diversity in winter [37], but that study also found highest diversity in spring, whereas in our study the Shannon index in spring was intermediate between autumn and summer, not fully matching [37]. These inconsistencies may stem from variations in host plant identity, geographical location, or climatic factors, and the underlying causes merit further investigation.

Among different tissues and seasons, the diversity and species richness of endophytic fungi in the stems of CBCC were comparatively higher, whereas those in the roots were comparatively lower. This observation is consistent with the view that different tissue types provide heterogeneous microenvironments for endophytic fungi [38]. The PCoA analysis showed that leaf and stem samples were relatively clustered in ordination space, whereas root samples were more dispersed. The PERMANOVA analysis further identified tissue type as a significant factor driving community differentiation.

Regarding the pronounced differences in root community structure compared to other tissues, we speculate that these may be attributable to the distinct microenvironments of aboveground versus belowground tissues (e.g., light exposure, aeration, and soil physicochemical properties). However, this hypothesis remains to be tested with integrated environmental data in future studies. Davide et al. [39] suggested that soil environmental factors may exert selective effects on root-associated fungal communities; nevertheless, their specific contribution to our study on plant systems remains unclear and warrants further experimental investigation.

#### 4.3. Driving Role of Environmental Factors on Seasonal Diversity

This study described the concomitant variation patterns of temperature and precipitation in the community differentiation of dominant endophytic fungal genera. Different fungal taxa exhibited certain distributional differences in response to environmental factors: genera such as *Wallemia*, *Aspergillus*, and *Vishniacozyma* showed higher relative abundance under warm and humid conditions, whereas *Solicoccozyma*, *Tremellodendropsidales*, *Morchella*, and *Fusarium* showed higher relative abundance under cooler and drier conditions. These differential patterns are consistent with previous studies: endophytic fungal richness is negatively correlated with seasonal temperature [40], and weather conditions during the growing season drive fungal richness and community structure. In addition, the abundances of *Venturia*, *Cladosporium*, and *Podosphaera* are influenced by weather and differ between southern and northern regions [41]. Moreover, most dominant genera, including *Alternaria* and *Cladosporium*, showed weak correlations with environmental factors, and their relative abundances did not exhibit obvious changes along climatic gradients under the conditions of this study. It should be noted that this study is based on survey data from a specific region and sampling time points and is therefore a descriptive observational study. The associations between environmental factors and fungal taxa abundances are correlations rather than causal relationships, and the effects of individual factors may be confounded by unmeasured variables (e.g., soil physicochemical properties, host physiological status). Nevertheless, these results provide baseline data on the seasonal distribution patterns of endophytic fungal communities in this region and suggest that temperature and precipitation may play a potential role in community assembly; however, the underlying driving mechanisms require further verification through controlled experiments or long-term observational studies.

## 5. Conclusions

Through high-throughput sequencing, a total of 12 phyla, 43 classes, 104 orders, 239 families, 449 genera, and 663 species of fungi were detected. Between different seasons, the community composition and diversity of endophytic fungi differed, with comparatively higher species richness in autumn and lower diversity in winter. The relative abundances of fungal phyla varied among seasons, but Ascomycota remained dominant in most seasonal samples. Differences were also observed among tissue types, with leaf and stem communities showing greater similarity to each other than to root communities. Under the observational conditions of this study, the diversity of endophytic fungal communities showed a co-varying trend with temperature and precipitation. Different fungal taxa exhibited differential distribution patterns along temperature–moisture gradients: some taxa showed higher relative abundances in warm and humid samples, while others showed higher relative abundances in cooler and drier samples, and most dominant genera did not display obvious changes in relative abundance along climatic gradients. It should be emphasized that this study is a descriptive observation based on a specific region and four sampling time points. The above associations are merely descriptive findings derived from the current dataset, and their potential underlying relationships require

further experimental verification. I believe a future perspective on this type of study is appropriate, one that relates to the results obtained.

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/horticulturae12080981/s1>, Table S1: ASV table.full; Table S2: Taxonomy assignment table.

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**Data Availability Statement:** Data supporting the findings of this work, including the raw data, are available in this paper and its Supplementary Information Files: ASV table (Supplementary Table S1); Taxonomy assignment table (Supplementary Table S2). The raw sequencing data were deposited in the NCBI SRA database under BioProject number PRJNA1427898.

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

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