

# Interactions between Endophytic Fungal Community Succession and Fruit Quality Components in *Siraitia grosvenorii* at Different Developmental Stages

**Qiuling Pang**

Guangxi Academy of Specialty Crops

**Yuqing Su**

Guangxi Academy of Specialty Crops

**Yuan Xi**

Guangxi Academy of Specialty Crops

**Zhengmin Mei**

Guangxi Academy of Specialty Crops

**Xiaoxiao Wu**

Guangxi Academy of Specialty Crops

**Jingcheng Wu**

Guangxi Academy of Specialty Crops

**Mufan Hu**

China Agricultural University

**Tailiang Lu**

Guangxi Academy of Specialty Crops

**Cuina Fu**

Guangxi Academy of Specialty Crops

**Cheng Zeng**

595473900@qq.com

Guangxi Academy of Specialty Crops

---

## Research Article

**Keywords:** *Siraitia grosvenorii*, endophytic fungi, fruit quality, community assembly

**Posted Date:** October 21st, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-11147798/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.  
[Read Full License](#)

**Additional Declarations:** No competing interests reported.

---

# Abstract

## Background

*Siraitia grosvenorii* is a medicinal and edible plant species endemic to China. Its fruit quality is regulated jointly by developmental stage, cultivation environment, and symbiotic microorganisms, and endophytic fungi are recognized as key factors influencing the accumulation of bioactive medicinal components. However, how endophytic fungal communities succeed during fruit development and how this succession relates to fruit quality components such as mogroside V and total flavonoids remain poorly understood.

## Results

Fruits at four developmental stages (young fruit, RG; early expansion, DG; green fruit, QG; mature fruit, CSG) were used to determine the contents of total flavonoids, water-soluble sugars, soluble proteins, and mogroside V, and high-throughput sequencing was used to profile the diversity, composition, and assembly of the endophytic fungal communities. Total flavonoid content was highest at the young fruit stage (0.72%) and declined gradually with development. Soluble protein and mogroside V contents peaked at the mature stage (5.92 mg/g and 21.85 mg/g, respectively). The fungal  $\alpha$ -diversity indices were relatively low at the young fruit and early expansion stages but increased significantly at the mature stage, indicating higher species diversity, richness, and phylogenetic diversity at maturity. Analyses of  $\beta$ -diversity and community composition revealed significant differences in community structure and composition among different developmental stages, with the early expansion stage being a key turning point for community decline and subsequent reconstruction; *Cladosporium delicatulum* was the dominant species throughout the entire developmental period. Community assembly was governed mainly by stochastic drift (contributing > 66%), whereas the contribution of dispersal limitation rose from 3.19% at RG to 31.33% at CSG, and deterministic environmental filtering weakened markedly at maturity. Mantel test and redundancy analysis further confirmed that soluble protein and mogroside V were significant explanatory factors for community variation at the mature stage, while the correlation heatmap indicated that both were highly significantly positively correlated with the abundances of dominant fungal genera such as *Cladosporium*.

## Conclusions

These results indicate that endophytic fungal community assembly in *S. grosvenorii* fruit shifts from early flavonoid-mediated processes to later protein- and mogroside V-mediated nutrient recruitment. The synergy between metabolites and the core microbiota established at maturity is likely a key mechanism underlying the high specialization of the mature endophytic fungal community.

## Introduction

*Siraitia grosvenorii* (Swingle) C. Jeffrey, a perennial vine of the family Cucurbitaceae and genus *Siraitia*, is native to the subtropical region of Guilin, Guangxi, China, and is a medicinal and edible plant resource endemic to China [1]. The mature fruit contains mogroside V as its primary active component, which is roughly 300 times sweeter than sucrose and virtually calorie-free. As a result, the fruit is widely used as a natural sugar substitute and has become an important raw material in the international functional sweetener market [2]. Mogroside V also displays a range of pharmacological activities, including heat-clearing and lung-moistening effects, hypoglycemic, antioxidant, anti-inflammatory, and gut microbiota-modulating properties [3], and it therefore holds considerable application potential in medicine and functional foods. Fruit quality in *S. grosvenorii* is not determined solely by genetic factors, however. It is regulated by cultivation environment, developmental stage, harvesting and processing, and microbial communities. Among these, endophytic fungi that reside within plant tissues and co-evolve with their host, acting as the plant's "second genome," have been shown in recent years to be key regulators of quality formation in medicinal plants [4].

Endophytic fungi are defined as fungal taxa that complete all or part of their life cycle within healthy plant tissues without causing obvious disease symptoms [5]. They are ubiquitous in plants, and each host species harbors a specific fungal microbiome. Through molecular interactions, these microorganisms confer multiple ecological benefits on their hosts, including growth promotion, enhanced adaptability, and increased stress tolerance [6]. In medicinal plants, endophytic fungi regulate host growth and development directly by secreting plant hormones, producing volatile organic compounds, and releasing polysaccharide elicitors, and they also influence the synthesis and accumulation of bioactive medicinal components by activating key enzyme genes in secondary metabolic pathways [7]. Liu et al. [8] showed that the endophytic fungus *Sarocladium kiliense* DF2 promoted the accumulation of dihydrotanshinone and tanshinone IIA in *Salvia miltiorrhiza* through the salicylic acid signaling pathway, and pharmacological inhibition confirmed that salicylic acid signaling is an indispensable mediator of this plant–fungus interaction. The endophytic fungus *Trichoderma atroviride* D16 promotes tanshinone accumulation by enhancing abscisic acid and salicylic acid signaling [9], whereas *Penicillium steckii* DF33 promotes tanshinone biosynthesis by regulating the expression of CYP450 genes [10]. Notably, the interaction between endophytic fungi and medicinal plants is not unidirectional but rather a bidirectional dynamic interplay. A study by Zhao et al. on *Dendrobium nobile* identified a dual regulatory mechanism involving phytohormone signaling and endophytic fungal communities, revealing the key role of the hormone–microbiome–metabolite network in dendrobine accumulation and offering a new perspective on how endophytic fungi coordinate with host plants to regulate secondary metabolite biosynthesis [11]. Collectively, these studies demonstrate that endophytic fungi, as important regulators of active ingredient accumulation in medicinal plants, play a critical role in improving the quality of Chinese medicinal materials.

Research on endophytic fungi of *S. grosvenorii* has advanced considerably in recent years. Tamang et al. [12] examined the endophytic microbial diversity of dioecious *S. grosvenorii* and found that male and female plants harbor distinct endophytic microbial communities with pronounced tissue-specific distribution patterns. Predictive functional analysis further indicated that root endophytic communities

are enriched in enzymes involved in secondary metabolite biosynthesis, particularly those related to mogrosides, a finding with important implications for mogroside biosynthesis. Ma et al. [13] systematically isolated endophytic fungi from seeds, seedlings, mature plants, and fruits of *S. grosvenorii* grown in Canadian greenhouses and found that they comprised 60 genera and more than 150 species across 20 orders, with fungal abundance and diversity highest in leaves, confirming that *S. grosvenorii* is a rich host of endophytic fungi. Xu et al. [14] isolated 150 endophytic fungal strains from male and female *S. grosvenorii* plants using morphological characterization combined with rDNA-ITS sequence analysis and assigned them to nine genera, with *Colletotrichum* and *Fusarium* dominant. These fungi showed clear preference and tissue specificity in their distribution between sexes and across roots, stems, and leaves. Chen et al. [15] further revealed that endophytic fungi from *S. grosvenorii* possess a rich capacity for mogroside biotransformation; the isolated strains *Diaporthe angelica* and *Fusarium solani* showed significant transforming activity toward mogroside V. Together, these studies have laid an important foundation for understanding the resource distribution, community composition, and metabolic functions of endophytic fungi in *S. grosvenorii*.

Most existing studies have nevertheless focused on mature *S. grosvenorii* plants, examining endophytic fungal diversity, tissue distribution, and microbial community differences between male and female plants. They have not systematically elucidated how endophytic fungal communities succeed during fruit development, nor how this succession is coupled to fruit quality formation. During the progression from fruit set to maturation, host physiological status (for example, sugar accumulation, pH changes, and hormonal fluctuations), the tissue microenvironment, and the substantial accumulation of secondary metabolites inevitably restructure the endophytic fungal community. Nguyen et al. [16] found that during development of *Vaccinium myrtillus* L. fruit from the green stage through veraison to maturity, endophytic fungal diversity increased gradually and community composition changed markedly, with host phenolic compounds substantially shaping community structure, indicating that host secondary metabolites play a key role in fungal community assembly. Xie et al. [17] reported that endophytic microbial communities of the medicinal plant *Rubus chingii* Hu undergo phased succession during fruit ripening, closely linked to the accumulation of bioactive components. Specifically, certain bacterial genera (*Geodermatophilus* and *Brevundimonas*) and yeast genera (*Metschnikowia* and *Starmerella*) were significantly positively correlated with key secondary metabolites, including ellagic acid, flavonoids, and phenolic acids, and the authors concluded that fruit ripening results from the synergistic interplay of host development, microbial succession, and metabolic reprogramming. Similarly, during Fengtang plum fruit development, endophytic microbial communities displayed stage-specific succession, with  $\alpha$ -diversity increasing significantly at later stages and the dynamics of dominant fungal genera closely associated with fruit disease resistance and quality formation [18]. During *Gastrodia elata* development, different stages selectively enriched specific fungi, driving continuous succession and reshaping of the community [19]. From an ecological perspective, the assembly of endophytic fungal communities is a multi-tiered selection process driven jointly by host genotype, developmental stage, tissue type, and environmental factors, and this selective effect can differ markedly across fruit developmental stages [20].

In summary, quality formation in medicinal plants reflects a multi-layered interplay among host development, microbial community succession, and metabolic regulatory networks. Previous studies have revealed the resource abundance and tissue distribution of endophytic fungi in *S. grosvenorii* and have begun to investigate changes in mogroside content during fruit development. However, studies that integrate the dynamic succession of endophytic fungal diversity across developmental stages with the accumulation patterns of fruit functional components remain rare. To address this gap, the present study used *S. grosvenorii* fruits at different developmental stages. High-throughput sequencing was used to systematically analyze the composition and diversity of endophytic fungal communities, and high-performance liquid chromatography (HPLC) and other methods were used to determine the contents of major functional components, including mogroside V, total flavonoids, and water-soluble sugars. By integrating bioinformatics and multivariate statistical analyses, we explored the potential correlations between endophytic fungal community succession and fruit quality formation. These findings provide a scientific basis for understanding the ecological functions of endophytic fungi in *S. grosvenorii* and the microbial regulatory mechanisms underlying fruit quality formation, and they lay a theoretical foundation for microbiome-based cultivation and targeted quality regulation of *S. grosvenorii*.

## Results

### Dynamic metabolite accumulation across fruit developmental stages in *Siraitia grosvenorii*

The fruit morphology of *S. grosvenorii* at different fruit developmental stages is shown in Fig. 1A. Changes in total flavonoid, water-soluble sugar, soluble protein, and mogroside V contents in *S. grosvenorii* fruits at different fruit ages are shown in Fig. 1(B ~ E), and the corresponding detailed data are presented in Table S1. Total flavonoid content was highest in the RG group, with a mean of approximately 0.72%, which was significantly higher than in the CSG, QG, and DG groups ( $P < 0.05$ ); no significant differences were detected among the other three groups. For water-soluble sugars, no significant difference was detected between the CSG and DG groups, whereas significant differences were observed among all other pairwise comparisons. For soluble proteins, no significant difference was detected between the QG and RG groups, whereas significant differences were observed among all other pairwise comparisons; the CSG group had the highest content, with a mean of 5.92 mg/g, significantly higher than the other three groups. For mogroside V, no significant difference was detected between the DG and RG groups; the CSG group had the highest content, with a mean of 21.85 mg/g, significantly higher than the other three groups, and the QG group ranked second, with a mean of 3.93 mg/g, significantly higher than the DG and RG groups. Across the whole growth period, total flavonoid content was highest at the young fruit stage and then declined, with no significant changes after the early expansion stage. Water-soluble sugar content first increased, then decreased, and then increased again; soluble protein content first decreased and then increased; and mogroside V content increased progressively with fruit age, reaching its maximum at maturity.

# Developmental stage-mediated diversity and structural differentiation of endophytic fungal communities in fruits

The  $\alpha$ -diversity indices were used to characterize the microbial diversity and species richness of *S. grosvenorii* fruits at different fruit ages (Fig. 2A and Table S2). The Shannon, Sobs, Chao1, ACE, and PD indices of the CSG group were all significantly higher than those of the QG, DG, and RG groups ( $P < 0.05$ ), indicating greater species diversity, richness, and phylogenetic diversity, and a more balanced and stable community structure. The Shannon index of the DG group was significantly lower than those of the CSG and RG groups, reflecting relatively low species diversity. The Sobs, Chao1, ACE, and PD indices of the DG and RG groups were all at their lowest levels, indicating markedly reduced community richness and phylogenetic complexity with a tendency toward community homogenization. The  $\alpha$ -diversity of the QG and RG groups fell between those of the CSG and DG groups, with the RG group showing slightly lower community richness than the QG group. Collectively, the CSG group harbored a microbial community characterized by high diversity and high richness, whereas the DG and RG groups exhibited comparatively low overall community complexity and ecological functional potential.

Principal coordinate analysis (PCoA) based on the Bray-Curtis distance was performed to characterize the overall differentiation of microbial community structure across fruit ages (Fig. 2B). The first two principal coordinates (PC1 and PC2) together explained 53.96% of the total community variation (PC1, 46.38%; PC2, 7.58%), indicating that the first coordinate mainly distinguished the core structural differences among communities. The analysis revealed significant separation of microbial communities among groups ( $R^2 = 0.5151$ ,  $P = 0.001$ ), demonstrating that developmental stage exerted highly significant remodeling effects on community structure. Samples from the CSG and QG groups clustered tightly on the left side of the coordinate axis, indicating highly similar endophytic microbial community structures. This cluster was clearly separated from the RG and DG groups, implying that the microbial communities at the green fruit and mature stages possessed distinctive core compositions that differed significantly from those at fruit ages of less than 25 days. RG samples were distributed near the midpoint of the PC1 axis, occupying a transitional zone between the CSG and DG groups with a slight upward shift along PC2. DG samples were concentrated on the right side of the PC1 axis, forming a fungal niche markedly distinct from that of the CSG and QG groups and thus representing the group with the most pronounced community structural differentiation.

## Succession dynamics of endophytic fungal community composition and dominant taxa over time

According to the Venn diagram (Fig. 3A), the CSG group harbored the highest number of unique OTUs (59 OTUs assigned to 15 known genera and 30 known species), followed by the QG group (1 OTU assigned to 1 known species) and the RG group (1 OTU assigned to 1 known species). A total of 85 OTUs were shared across all four fruit age groups, assigned to 52 known genera including *Cladosporium*, *Cercospora*, and *Pseudocercospora*. Among three-way overlaps, the CSG, QG, and RG groups shared the

most OTUs (50 OTUs assigned to 33 known genera and 46 known species), whereas the QG, DG, and RG groups shared the fewest (7 OTUs assigned to 2 known genera and 5 known species). For two-way overlaps, the CSG and QG groups shared 68 OTUs assigned to 32 genera including *Neodeighonia*, *Uwebraunia*, and *Pallidocercospora*, together with some unclassified genera.

At the family level, Cladosporiaceae (comprising the genera *Cladosporium* and *Toxicocladosporium*) exhibited the highest relative abundance in the CSG group, following a pattern of initial decrease and later increase with fruit age (RG, 21.38%; DG, 5.67%; QG, 32.08%; CSG, 40.50%) (Fig. 3B). Pleosporaceae (including *Curvularia*, *Exserohilum*, *Alternaria*, *Cochliobolus*, *Dendryphiella*, *Bipolaris*, and *Setosphaeria*) was most abundant in the RG group (17.26%), which also harbored the highest abundances of Ustilaginaceae (3.71%) and Sporidiobolaceae (4.71%). Nectriaceae was most abundant in the DG group (6.05%). The QG group was characterized by relatively high abundances of Didymellaceae (13.16%), Mycosphaerellaceae (6.86%), Bulleribasidiaceae (4.78%), and Didymosphaeriaceae (2.94%), followed by the CSG group at 4.90%, 4.62%, 3.85%, and 2.44%, respectively. Trichosphaeriaceae (containing the genus *Nigrospora*) consistently displayed higher abundance in the CSG group than in the other groups.

The results of the Kruskal-Wallis H test are shown in Fig. 3C. Among the 11 fungal genera examined, 10 showed statistically significant differences among groups ( $P < 0.05$ ), of which 9 showed highly significant differences ( $P < 0.01$ ), indicating that during fruit development the relative abundances of these taxa were subject to clear selection or regulation. In the CSG group, *Cladosporium* and *Nigrospora* were significantly more abundant than in the other groups. The QG group was characterized by significant enrichment of *Cercospora* and *Exserohilum*. In the RG group, the dominant genera were *Curvularia* and *Sporobolomyces*, which were significantly more abundant than in the other groups.

Across fruit development, the dominant species displayed distinctive dynamic changes (Fig. 3D). The abundances of *Cladosporium delicatulum* and *Curvularia intermedia* first decreased and then increased, whereas the abundance of *Gibberella intricans* followed a pattern of initial increase, subsequent decrease, and final increase. Overall, the DG group represented a critical turning point in community composition, as both the total abundance of the three species and the abundance of each individual species were significantly reduced at this stage. This indicates that the environmental conditions at this time point exerted a clear inhibitory effect on these three taxa, and that the community structure differed significantly from those at other fruit ages. The RG group exhibited specific changes in species composition, with *Curvularia intermedia* showing a significantly increased abundance and becoming the second most dominant species after *Cladosporium delicatulum*. This feature distinguished the RG group from the other three groups and corroborated the specific enrichment of *Curvularia* in the RG group.

### **Ecological drivers of endophytic fungal community assembly in *S. grosvenorii* fruits**

Null model analysis (Fig. 4) revealed that fruit developmental stage significantly altered the relative contributions of deterministic and stochastic processes to community assembly. Drift (DR) was the overwhelmingly dominant process across all groups, contributing more than 66% in each of the four groups and approaching nearly 90% in the DG and RG groups, indicating that stochastic events

constituted the core driver of fungal community composition, whereas deterministic environmental filtering (HoS/HeS) was relatively weak overall. At the early stages (RG and DG), the contribution of homogeneous selection (HoS) to within-fruit community assembly was higher than at later stages (QG and CSG), decreasing from 4.03% and 6.91% to less than 1%. The contribution of dispersal limitation (DL) showed the opposite trend, increasing from 3.19% to 31.33% with fruit age. Heterogeneous selection (HeS) and homogeneous dispersal (HD) only affected the RG, DG, and QG groups, with HeS contributing most in the QG group (6.18%), suggesting that the habitat at this stage exhibited slight environmental heterogeneity that drove community differentiation. The contribution of HD increased significantly with fruit age but remained below 3% across all groups. Collectively, stochastic processes exerted the most pronounced influence on fungal community assembly throughout fruit development.

## Regulatory effects of metabolites on endophytic fungal community differentiation and their interaction mechanisms

The results of the Mantel test and correlation analysis are shown in Fig. 5A. The fungal community in the CSG group was the primary responder to metabolic factors, with a significant association detected only between the fungal community and metabolic factors in this group, whereas community changes in the other groups showed no significant correlation with these factors. This indicates that the CSG community was most strongly regulated by metabolic factors. Total flavonoids (TF), water-soluble sugars (WSS), and soluble proteins (SP) were significantly positively correlated with the CSG fungal community, corroborating the high abundance of dominant genera such as *Cladosporium* revealed by the Kruskal-Wallis test and suggesting that these factors may be primary drivers of dominant taxon enrichment in the CSG group. Significant antagonistic and synergistic relationships were observed among the factors: TF showed a highly significant negative correlation with WSS and mogroside V, whereas SP showed a highly significant positive correlation with mogroside V, indicating that these factors interact in a complex manner to influence CSG community assembly.

The correlation heatmap illustrates the relationships between metabolic factors and fungal genera (Fig. 5B). TF showed a highly significant positive correlation with *Curvularia*, *Sporobolomyces*, and *Exserohilum*. WSS showed a significant positive correlation with *Gibberella* and a significant negative correlation with *Exserohilum*, *Dirkmeia*, and *Moesziomyces*. SP and mogroside V showed highly significant positive correlations with nine genera, including *Gibberella*, *Nigrospora*, *Pseudopithomyces*, *Cercospora*, *Phaeosphaeriopsis*, *Cladosporium*, *Hannaella*, *Saitozyma*, and *Epicoccum*.

The relationships between fungal community composition and metabolic factors are presented in the redundancy analysis (RDA) ordination plot (Fig. 5C). The first two axes (RDA1 and RDA2) together explained 52.42% of the total community variation (RDA1, 50.69%; RDA2, 1.73%), indicating that metabolic factors accounted for a substantial proportion of community variation. SP, mogroside V, TF, and WSS were all identified as significant factors influencing community composition, with SP and mogroside V exhibiting longer arrows that suggest stronger explanatory power. The arrow of

*Cladosporium delicatulum* was oriented in a direction highly consistent with those of SP and mogroside V, forming an acute angle and indicating that high contents of these two factors significantly promoted the enrichment of this species. The arrows of *Curvularia intermedia* and *Gibberella intricans* were oriented consistently with those of TF and WSS, also forming acute angles and suggesting significant positive correlations with these two genera. Collectively, the selective effects of SP, mogroside V, WSS, and other factors may be the core reason driving the separation of the CSG group from the other three groups. This finding is consistent with the null model analysis, which indicated that although stochastic drift predominated overall, deterministic environmental filtering directly shaped the unique community structure of the CSG group.

## Discussion

Fruit ripening in medicinal plants involves concurrent physiological changes in the host and interactions with endophytic fungi, and the host–fungus symbiosis can synchronously regulate plant stress tolerance and metabolic capacity. This study examined the dynamic co-evolutionary relationship between endophytic fungal community composition and metabolite accumulation during fruit development in the medicinal cash crop *S. grosvenorii*. The results showed that the signature metabolic differences distinguishing immature from mature fruit are closely coupled with the stage-specific succession of endophytic fungal communities.

The dynamic accumulation of primary and secondary metabolites during fruit development is a critical indicator of physiological maturation and quality formation, and it also constitutes the material basis for establishing the endophytic microenvironment and regulating microbial colonization and community succession. The four categories of metabolites measured here exhibited distinctly different accumulation curves across the developmental cycle. Total flavonoid content peaked at the young fruit stage and then declined continuously, consistent with the established view that flavonoids accumulate abundantly in young tissues to constitute chemical defense [21]. However, the extent and rate of flavonoid decline in *S. grosvenorii* were notably pronounced, differing markedly from the peak accumulation patterns reported in fleshy fruits such as grape during rapid growth and ripening [22, 23]. This discrepancy may reflect the fact that cucurbitane-type triterpenoid glycosides (mogroside V) serve as the predominant secondary metabolites at the mature stage, accompanied by a substrate competition-driven shift of the carbon skeleton from the phenylpropanoid pathway to the mevalonate pathway [24, 25]. This shift in metabolic flux offers a physiological explanation for the rapid decline in flavonoid content at maturity, and it suggests that the host may transition from broad-spectrum flavonoid-based defense to a targeted chemical ecological defense mediated by triterpenoid glycosides during development. Mogroside V is highly accumulated at the mature stage, yet its ecological function has long been overlooked. Recent studies have revealed that plant glycosides, in addition to their roles in storage and defense, can serve as selective growth substrates for rhizosphere and endophytic microorganisms [26]. Moreover, the strong positive correlation between proteins and mogroside V at maturity suggests a possible synergistic effect: proteins provide a nitrogen source for microbial proliferation, while the deglycosylated metabolites of mogroside V release carbon signals, together

establishing a symbiotic microenvironment highly favorable to specific microorganisms. This hypothesis provides a clear entry point for subsequent functional validation. Compared with other metabolites, water-soluble sugars fluctuated relatively little throughout development, with a transient increase at the DG stage reflecting a temporary imbalance between polysaccharide hydrolysis and sucrose accumulation [27]. As a general basal carbon source, water-soluble sugars have weaker indicative value for distinguishing community characteristics across stages than proteins and mogroside V; nevertheless, their marked accumulation at maturity indirectly supports the view that the endophytic fungal community of *S. grosvenorii* is finely regulated by carbon and nitrogen metabolites.

The structural succession of endophytic fungal communities along the developmental timeline is not merely a passive response to changes in the host environment, but a composite ecological process driven jointly by active host selection, intra-community assembly, and interspecific interactions [28, 29]. Here, endophytic fungal diversity followed a dynamic pattern of initial decrease and later increase during development, peaking at maturity, a phenomenon also observed in *Rubus idaeus* and *Malus domestica* fruit [30, 31]. This may reflect the abundant nutrients in mature fruit, such as sugars, proteins, and amino acids, which provide favorable conditions for microbial colonization and the accumulation of community diversity [32]. It also indicates that high concentrations of specific secondary metabolites can override the conventional relationship between developmental stage and microbial diversity [30]. From an ecological perspective, the host does not passively allow random microbial colonization during development, but actively modulates microbial interactions to meet growth requirements, a process facilitated by core microbiota hubs that maintain plant health and mediate nutrient metabolism [33]. At the early expansion stage, the endophytic fungal community declined, which may be associated with rapid fruit enlargement during which carbon–nitrogen metabolic flux is substantially redirected, total flavonoid content declines markedly, and soluble proteins and mogroside V have not yet been extensively synthesized. This creates a temporary niche vacuum arising from a gap in nutrient substrates. The colonization and persistence of host-selected endophytic fungi are co-determined by deterministic mechanisms such as environmental filtering and by stochastic processes. Plant developmental stage is a key factor governing microbial community assembly; plant-secreted signaling molecules can recruit and enrich functional microorganisms, while seasonal environmental factors such as air and rainfall also influence colonization [33]. Here, stochastic processes exerted the most pronounced influence on fungal community assembly throughout fruit growth, whereas deterministic processes had a relatively strong impact only before maturation. This may reflect the fact that fruit defense barriers are not yet fully established during early development, leaving the fruit susceptible to external environmental influences while internal metabolites are still accumulating. Nevertheless, the available data cannot quantitatively delineate the specific contributions of environmental factors to within-fruit community assembly, and this warrants further investigation.

The association between metabolites and endophytic fungal communities is a key link in deciphering how host physiological state translates into microbial community structure. Plants can actively shape the composition and function of their symbiotic microbial communities through chemical pathways, including root exudates, volatile organic compounds, and tissue-accumulated metabolites [16, 34, 35].

Here, correlation analyses explicitly linked specific microbial taxa to the accumulation of key metabolites. *Gibberella*, *Nigrospora*, *Phaeosphaeriopsis*, *Epicoccum* and *Cladosporium* showed significant positive correlations with soluble proteins and mogroside V, while *Curvularia* showed significant positive correlations with total flavonoid content, suggesting that these fungi may participate in promoting or stabilizing the accumulation of these bioactive compounds. For instance, Zhang et al. isolated two novel bicyclic sesquiterpenes from *Nigrospora* sp. E121 [36], and Yuan et al. isolated four unprecedented tremulane-type sesquiterpenes from the mangrove-derived endophytic fungus *Phaeosphaeriopsis* sp. P11 [37]. These terpenoid-producing genera synthesize terpene precursors via the mevalonate (MVA) pathway, which are then catalyzed by modifying enzymes such as terpene synthases (TPS) and cytochrome P450 monooxygenases (CYP450) to generate structurally diverse terpenoids [38–40]. *Gibberella*, *Nigrospora*, *Phaeosphaeriopsis*, and *Cladosporium* represent key endophytic fungal taxa in *S. grosvenorii*, and because mogroside V, the primary bioactive component, is a triterpenoid, its transformation and biosynthesis may be associated with endophytic fungi. Furthermore, genera such as *Curvularia* and *Exserohilum* can transform or produce flavonoids: Harwoko et al. demonstrated that *Epicoccum nigrum* can biotransform plant flavonoids such as kaempferol [41], and Liu confirmed that *Curvularia inaequalis* can produce flavonoids [42]. In addition to their roles in terpenoid and flavonoid transformation and synthesis, these endophytic fungi produce polyketides, alkaloids, lactones, cytochalasans, isocoumarins, and other pharmaceutically relevant compounds [43–45], and some function as biocontrol agents against plant pathogens [46]. The high abundance and enrichment of these core fungal genera in fruit therefore suggest that they may play an important functional supporting role in the accumulation of host terpenoids and flavonoids through structural modification or homeostatic maintenance.

## Conclusion

In summary, this study clarified the core associations between metabolites and endophytic fungal communities during *S. grosvenorii* fruit development. The temporal progression of fruit development regulates the stage-specific accumulation of total flavonoids, water-soluble sugars, soluble proteins, and mogroside V, producing differentiated microenvironments that drive dynamic succession in the diversity, structure, and dominant taxa of endophytic fungal communities. Stochastic drift predominates in community assembly, while the balance between deterministic and stochastic processes is modulated by fruit development, with metabolites likely serving as key mediators of this regulation. Soluble proteins and mogroside V, as core metabolic factors, directionally regulate the fungal community structure at maturity and constitute the central linking hubs of the metabolite–microbiome interplay. These findings refine the theoretical framework of fruit–microbe interactions in *S. grosvenorii*, clarify the mechanisms by which metabolites drive fungal community succession, and provide a theoretical basis for improving fruit quality through the regulation of fruit development and optimization of metabolite accumulation. This work also serves as a reference for future studies of endophytic microorganisms in other vine-borne fruits.

# Methods

## Plant material and sampling

The *Siraitia grosvenorii* samples used in this study were derived from a single virus-free tissue-cultured plantlet selected by the Institute of Chinese Medicinal Materials, Guangxi Academy of Specialty Crops. The plants were trial-planted in Lingui District, Guilin City, Guangxi Zhuang Autonomous Region, China (25°23'57"N, 110°2'50"E), which is one of the major production areas for *S. grosvenorii*. To ensure randomness, samples were collected during the summer and autumn of 2022 at four developmental stages: young fruit (RG, 3–5 days), early expansion (DG, 20–25 days), green fruit (QG, 50–55 days), and mature fruit (CSG, 80–85 days). Four biological replicates were collected from each of three plots within the production area. For each replicate, fruits were sampled from five individual plants, with several fruits taken per plant. Adjacent sampling areas were separated by a minimum distance of 3 m, and all collections were conducted between 9:00 and 10:00 a.m. at approximately the same location. Fruits were picked with sterile gloves, placed into sterile bags, and stored on ice (+ 4°C) for transport and subsequent processing.

Within 24 h of harvest, fruits were surface-sterilized following the protocol described by Nguyen et al. [47] to remove contaminants from the peel. For each replicate, approximately 60 fruits were pooled and processed together. After surface sterilization, fruits were sampled with a sterile biopsy punch and placed into sterile 50 mL conical tubes, then stored at – 80°C until lyophilization. Lyophilized samples were ground into fine powder under cold sterile conditions using a mortar and pestle. From each pooled sample, 1 g aliquots were taken for DNA extraction and subsequent sequencing, and the remainder was used for metabolite analysis. For metabolite analysis, the pooled samples (excluding those intended for lyophilization) were oven-dried and ground into powder.

## DNA extraction, library construction, sequencing, and data processing

Total genomic DNA of the microbial communities was extracted using the E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. After quality assessment, purity determination, and concentration measurement, qualified DNA samples were used as templates for PCR amplification of the ITS region with the primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3'). The PCR reaction mixture (20 µL) consisted of 10 µL of 5× Pro Taq, 0.8 µL of each primer (5 µmol·L<sup>-1</sup>), and template DNA at 10 ng·µL<sup>-1</sup>, adjusted to 20 µL with sterile water. The amplification program was: initial denaturation at 95°C for 3 min; 27 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; a final extension at 72°C for 10 min; and a hold at 4°C.

PCR products were used to construct sequencing libraries with the NEXTFLEX® Rapid DNA-Seq Kit. Library construction included: (1) adapter ligation; (2) removal of adapter-self-ligated fragments by magnetic bead-based selection; (3) enrichment of library templates by PCR amplification; and (4) recovery of PCR products with magnetic beads. Sequencing was performed on the Illumina PE300 platform (Shanghai Majorbio Bio-pharm Technology Co., Ltd., China).

## Chemical profiling of metabolites

Water-soluble sugar content was determined by the anthrone colorimetric method [48], soluble protein content by the Coomassie Brilliant Blue method [48], total flavonoid content by the  $\text{AlCl}_3$  colorimetric method [49], and mogroside V content by high-performance liquid chromatography (HPLC) [50]. Each sample was assayed in triplicate, and the means were calculated.

## Statistical analysis

Statistical processing of the measured metabolite data was performed using Microsoft Excel 2016, and graphical representations were generated with GraphPad Prism 10.1.2 and Raincloud-shiny. Partial data analyses were performed on the Majorbio Cloud Platform (<https://cloud.majorbio.com>). Operational taxonomic units (OTUs) were clustered at a 97% similarity threshold using UPARSE [51] (<http://drive5.com/uparse/>, version 7.1). Alpha diversity indices, including Chao1 and Shannon, were calculated with mothur [52] (<http://www.mothur.org/wiki/Calculators>). Principal coordinate analysis (PCoA) based on the Bray-Curtis distance was used to examine the similarity of microbial community structures among samples, and permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences among groups. The Kruskal-Wallis rank sum test was used to analyze differences in fungal communities among groups. Phylogenetic-bin-based null model analysis (iCAMP) was used to infer community assembly mechanisms. The Mantel test, based on the Bray-Curtis distance, was used to investigate the correlation between community distance matrices and metabolites. Species for correlation network analysis were selected based on Spearman correlation coefficients ( $|r| > 0.6$ ,  $p < 0.05$ ) [53]. Redundancy analysis (RDA), which combines correspondence analysis with multiple regression, was used to investigate the relationships between metabolites and fungal communities.

## Declarations

### Ethics approval and consent to participate

The study protocol complied with relevant institutional, national, and international guidelines and legislation.

### Consent for publication

Not applicable.

# Competing interests

The authors declare that they have no competing interests.

# Funding

This work was supported by the Guangxi Natural Science Foundation (2024GXNSFBA999268) and the Guangxi Longsheng Blueberry Experiment Station (Gui TS2022007).

# Author Contribution

Q.P. and C.Z. initiated and designed the research. Q.P., Y.S., Y.X., Z.M., X.W., J.W., M.H., T.L. and C.F. performed the experiments. Q.P., Y.S., and Y.X. wrote the manuscript. C.Z. revised the manuscript. All authors contributed to the article, reviewed the manuscript, and approved the submitted version.

# Acknowledgements

We thank the anonymous reviewers for their critical readings and constructive comments on the manuscript.

# Data Availability

The microbiome data generated during the current study are available in the NCBI SRA repository under Bioproject No. PRJNA1525095.

# References

1. GaoWQ, Wen ZL. Anti-exercise Fatigue Effect of Active Components of Medicinal Plant *Siraitia grosvenorii* (Swingle). *Mol Plant Breed*. 2022;20(23):7923–30. 10.13271/j.mpb.020.007923.
2. Shivani, Thakur BK, Mallikarjun CP, Mahajan M, Kapoor P, Malhotra J, Dhiman R, Kumar D, Pal PK, Kumar S. Introduction, adaptation and characterization of monk fruit (*Siraitia grosvenorii*): a non-caloric new natural sweetener. *Sci Rep*. 2021;11:6205. <https://doi.org/10.1038/s41598-021-85689-2>.
3. Li C, Lin LM, Sun F, Wang ZM, Huo HR, Dai L, Jiang TL. Chemistry and pharmacology of *Siraitia grosvenorii*: A review. *Chin J Nat Med*. 2014;12(02):89–102. [https://doi.org/10.1016/S1875-5364\(14\)60015-7](https://doi.org/10.1016/S1875-5364(14)60015-7).
4. Jia M, Chen L, Xin HL, Zheng CJ, Rahman K, Han T, Qin LP. A Friendly Relationship between Endophytic Fungi and Medicinal Plants: A Systematic Review. *Front Microbiol*. 2016;7:906.

- 10.3389/fmicb.2016.00906.
5. Petrini O, Fisher PJ. Occurrence of fungal endophytes in twigs of *Salix fragilis* and *Quercus robur*. *Mycol Res*. 1990;94(8):1077–80. [https://doi.org/10.1016/S0953-7562\(09\)81336-1](https://doi.org/10.1016/S0953-7562(09)81336-1).
  6. Rodriguez RJ, White JF Jr, Arnold AE, Redman RS. Fungal endophytes: diversity and functional roles. *New Phytol*. 2009;182:314–30. <https://doi.org/10.1111/j.1469-8137.2009.02773.x>.
  7. Wang Y, Chen N, Deng K, Zhong X, Li Z, Li L, Xu D. Effects and molecular mechanism of endophytic elicitors on the accumulation of secondary metabolites in medicinal plants. *Front Microbiol*. 2025;16:1558567. <https://doi.org/10.3389/fmicb.2025.1558567>.
  8. Liu ZY, Wang Q, Yang SR, Zheng SP, Liang ZS, Chen HM. *Sarocladium kiliense* DF2 enhances tanshinone biosynthesis in *Salvia miltiorrhiza* roots via salicylic acid signaling. *Ind Crops Prod*. 2025;238:122372. <https://doi.org/10.1016/j.indcrop.2025.122372>.
  9. Xie XG, Sheng KX, Lu WL, Wang HR, Rahman K, Feng KM, Han T. Endophytic fungus *Trichoderma atroviride* D16 increases tanshinone accumulation in *Salvia miltiorrhiza* plantlets by enhancing abscisic acid and salicylic acid signaling. *Symbiosis*. 2023;90:171–81. <https://doi.org/10.1007/s13199-023-00927-7>.
  10. Lv XM, Zhang WY, Chu SY, Zhang HH, Wu YQ, Zhu Y, Yang DF, Zhu YH, Mans RA, Chen D, Liang HM. Endophytic fungus *Penicillium steckii* DF33 promoted tanshinones biosynthesis in *Salvia miltiorrhiza* by regulating the expression of CYP450 genes. *Gene*. 2024;899:148094. <https://doi.org/10.1016/j.gene.2023.148094>.
  11. Zhao Y, Xiong N, Ji X, Zhang D, Jia Q, Qin L, Wu X, Tan D, Xie J, He Y. A Dual Regulatory Mechanism of Hormone Signaling and Fungal Community Structure Underpin Dendrobine Accumulation in *Dendrobium nobile*. *Biomolecules*. 2025;15(10):1366. <https://doi.org/10.3390/biom15101366>.
  12. Tamang A, Kaur A, Thakur D, Thakur A, Thakur BK, Shivani, Swarnkar M, Pal K, Hallan P, Pandey V. Unraveling endophytic diversity in dioecious *Siraitia grosvenorii*: implications for mogroside production. *Appl Microbiol Biotechnol*. 2024;108:247. <https://doi.org/10.1007/s00253-024-13076-8>.
  13. Ma L, Elmhirst JF, Darvish R, Wegener LA, Henderson D. Abundance and diversity of fungal endophytes isolated from monk fruit (*Siraitia grosvenorii*) grown in a Canadian research greenhouse. *Plant-Environment Interact*. 2024;5(2):e10142. <https://doi.org/10.1002/pei3.10142>.
  14. Xu XL, Luo HY, Yan XJ, Lu FL, Li DP. Diversity of endophytic fungi isolated from *Siraitia grosvenorii*. *Guihaia*. 2020;40(5):618–27. 10.11931/guihaia.gxzw201901057.
  15. Chen B, Yu FM, Jiang Z. Mogroside V-producing Endophytic Fungi Isolated from *Siraitia grosvenorii*. *Planta Med*. 2020;86(13–14):983–7. 10.1055/a-1102-1168.
  16. Nguyen MP, Lehosmaa K, Martz F, Koskimäki JJ, Toth K, Ahonen SHK, Häggman H, Pirttilä AM. Dynamics of fungal endophytic communities in bilberry (*Vaccinium myrtillus* L.) fruits through development is shaped by host phenolic compounds. *FEMS Microbiol Ecol*. 2025;101(1):fae168. <https://doi.org/10.1093/femsec/fae168>.
  17. Xie Y, Dai D, Zeng H, Tian Y, Zou C, Meng Y, Wu Z, Li J. Endophyte community shifts in *Rubus chingii* during fruit ripening are associated with key metabolites. *Front Plant Sci*. 2025;16:1727436.

<https://doi.org/10.3389/fpls.2025.1727436>.

18. Lei JQ, Shi YN, Li H, Wang R. Characterizing the Endophytic Microbiome and Microbial Functional Assemblages Associated with Fengtang Plum (*Prunus salicina* Lindl.) Development and Resistance. *Horticulturae*. 2025;11(5):483. <https://doi.org/10.3390/horticulturae11050483>.
19. Chen L, Wang YC, Qin LY, He HY, Yu XL, Yang MZ, Zhang HB. Dynamics of fungal communities during *Gastrodia elata* growth. *BMC Microbiol*. 2019;19:158. <https://doi.org/10.1186/s12866-019-1501-z>.
20. Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol Mol Biol Rev*. 2015;79(3):293–320. <https://doi.org/10.1128/membr.00050-14c>.
21. Ren S, Cui J, Wang L, Lu Z, Jin B. Plant Flavonoids: Biosynthesis, Regulation, and Roles in Biotic and Abiotic Stresses. *Plant Cell Environ*. 2026;49(8):5664–84. <https://doi.org/10.1111/pce.70540>.
22. Yang Q, Li C, Wang Y, Pei X, Wang A, Jin L, Fang L. Transcriptome and Metabolome Analyses of Flavonoid Biosynthesis During Berry Development of Muscadine Grape (*Vitis rotundifolia* Michx). *Plants*. 2025;14:2025. <https://doi.org/10.3390/plants14132025>.
23. Fang F, Tang K, Huang WD. Changes of flavonol synthase and flavonol contents during grape berry development. *Eur Food Res Technol*. 2013;237:529–40. <https://doi.org/10.1007/s00217-013-2020-z>.
24. Dong H, Qi XQ. Biosynthesis of triterpenoids in plants: Pathways, regulation, and biological functions. *Curr Opin Plant Biol*. 2025;85:102701. <https://doi.org/10.1016/j.pbi.2025.102701>.
25. Thimmappa R, Geisler K, Louveau T, O'Maille P, Osbourn A. Triterpene biosynthesis in plants. *Annu Rev Plant Biol*. 2014;65:225–57. <https://doi.org/10.1146/annurev-arplant-050312-120229>.
26. Zhong Y, Xun WB, Wang XH, Tian SW, Zhang YC, Li DW, Zhou Y, Qin YX, Zhang B, Zhao GW, Cheng X, Liu YG, Chen HM, Li LG, Osbourn A, Lucas J, Huang W, Ma SW, Shang YS. Root-secreted bitter triterpene modulates the rhizosphere microbiota to improve plant fitness. *Nat Plants*. 2022;8:887–96. <https://doi.org/10.1038/s41477-022-01201-2>.
27. Koch K. Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. *Curr Opin Plant Biol*. 2004;7(3):235–46. <https://doi.org/10.1016/j.pbi.2004.03.014>.
28. Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS Biol*. 2016;14(1):e1002352. <https://doi.org/10.1371/journal.pbio.1002352>.
29. Dastogeer KMG, Tumpa FH, Sultana A, Akter MA, Chakraborty A. Plant microbiome—an account of the factors that shape community composition and diversity. *Curr Plant Biology*. 2020;23:100161. <https://doi.org/10.1016/j.cpb.2020.100161>.
30. Xie Y, Dai D, Zeng H, Tian Y, Zou C, Meng Y, Wu Z, Li J. Endophyte community shifts in *Rubus chingii* during fruit ripening are associated with key metabolites. *Front Plant Sci*. 2025;16:1727436. <https://doi.org/10.3389/fpls.2025.1727436>.

31. Kithan-Lundquist R, McMillan HM, Gdanetz K, Liber J, He SY, Sundin GW. Microbial community succession and dynamics during the season-long development of apple fruit (*Malus domestica*). *Microbiol Spectr*. 2026;14(4):e0283625. <https://doi.org/10.1128/spectrum.02836-25>.
32. Luo X, Sun K, Li HR, Zhang XY, Pan YT, Luo DL, Wu YB, Jiang HJ, Wu XH, Ma CY, Dai CC, Zhang W. Depletion of protective microbiota promotes the incidence of fruit disease. *ISME J*. 2024;18(1):wrae071. <https://doi.org/10.1093/ismejo/wrae071>.
33. Xiong C, Singh BK, He JZ, Han YL, Li PP, Wan LH, Meng GZ, Liu SY, Wang JT, Wu CF, Ge AH, Zhang LM. Plant developmental stage drives the differentiation in ecological role of the maize microbiome. *Microbiome*. 2021;9:171. <https://doi.org/10.1186/s40168-021-01118-6>.
34. Fan X, Ge AH, Qi S, Guan Y, Wang R, Yu N, Wang E. Root exudates and microbial metabolites: signals and nutrients in plant-microbe interactions. *Sci China Life Sci*. 2025;68:2290–302. <https://doi.org/10.1007/s11427-024-2876-0>.
35. Zhou DY, Li Y, Xing YM, Guo SX, Chen J. Microbiome of medicinal plants and its effect on medicinal plant secondary metabolites: a review. *Microbiol China*. 2022;49(9):3989–4003. 10.13344/j.microbiol.china.211188.
36. Zhang JC, Yang XQ, Zhou H, Yan YB, Ding ZT. The new analogues of  $\beta$ -trans-bergamotene from endophytic fungus *Nigrospora* sp. E121 with yam culture medium. *Nat Prod Res*. 2025;39(20):5761–7. <https://doi.org/10.1080/14786419.2024.2353910>.
37. Yuan YC, Li JS, Shen L, Wu J. Four Tremulanes and Two Pyroglutamate-Containing Dipeptides from the Mangrove Endophytic Fungus *Phaeosphaeriopsis* sp. P11. *Chem Biodivers*. 2025;22:e202402556. <https://doi.org/10.1002/cbdv.202402556>.
38. Lopes AA, Pupo MT. Biosynthesis of aphidicolin proceeds via the mevalonate pathway in the endophytic fungus *Nigrospora sphaerica*. *J Braz Chem Soc*. 2011;22(1):80–5. <https://doi.org/10.1590/S0103-50532011000100010>.
39. Ye K, Ai HL. Pimarane Diterpenes from Fungi. *Pharmaceuticals*. 2022;15(10):1291. <https://doi.org/10.3390/ph15101291>.
40. Wang BX, Si W, Wu YF, Zhang XQ, Wang SY, Wu CF, Lin HP, Yin LH. Research progress in biosynthesis and metabolism regulation of gibberellins in *Gibberella fujikuroi*. *Chin J Biotechnol*. 2020;36(2):189–200. 10.13345/j.cjb.190194.
41. Harwoko H, Hartmann R, Daletos G, Ancheeva E, Frank M, Liu Z, Proksch P. Biotransformation of Host Plant Flavonoids by the Fungal Endophyte *Epicoccum nigrum*. *ChemistrySelect*. 2019; 4, 13054. <https://doi.org/10.1002/slct.201903168>
42. Liu ZY. Endophytic fungi producing flavonoids from *Spiranthes sinensis*. *Mycosystema*. 2011;30(1):133–7. 10.13346/j.mycosystema.2011.01.022.
43. Wang B. Isolation and Identification of Secondary Metabolites from Endophytic Fungi *Nigrospora chinensis* GGY-3 and *Aspergillus versicolor* 347 and Their Biological Activities Research. Nanjing Agricultural Univ. 2023. 10.27244/d.cnki.gnjnu.2023.000132.

44. Wang Y, Zhou LM, Leng K, Wang C, Wang P. A new neuroprotective isocoumarin from the marine-derived fungus *Phaeosphaeriopsis* sp. WP-26. *Acta Pharm Sinica*. 2026;61(4):1294–300. 10.16438/j.0513-4870.2025-0951.
45. Luo LL, Li WL, Du XH, Chen YL. Research progress on secondary metabolites from *Curvularia* species and their bioactivities. *Nat Prod Res Dev*. 2025;37(2):375–91. 10.16333/j.1001-6880.2025.2.019.
46. Braga RM, Padilla G, Araújo WL. The biotechnological potential of *Epicoccum* spp.: diversity of secondary metabolites. *Crit Rev Microbiol*. 2018;44(6):759–78. <https://doi.org/10.1080/1040841X.2018.1514364>.
47. Nguyen MP, Lehosmaa K, Martz F, Koskimäki JJ, Koskimäki AM, Häggman H. Host species shape the community structure of culturable endophytes in fruits of wild berry species (*Vaccinium myrtillus* L., *Empetrum nigrum* L. and *Vaccinium vitis-idaea* L.). *FEMS Microbiol Ecol*. 2021;97(8):fiab097. <https://doi.org/10.1093/femsec/fiab097>.
48. Wang XK, Huang JL. Principles and Techniques of Plant Physiological and Biochemical Experiments. Beijing: Higher Education Press; 2015.
49. Long Y, Kuo X, Cheng SX, Wang WP, Wang C, Pan HS. AlCl<sub>3</sub>Colorimetry for Determination of Total Flavonoids *Leptopus Chinensis* Chinese Herbal Medicine. *J Zhengzhou University(Engineering Science)*. 2010;31(4):113–5.
50. Zhou XL, Wang S, Chen L, Yuan JQ, Mo CM, Ma XJ, Liao JH. Determination of mogroside v in different variety *siraitia grosvenorii* by HPLC. *Mod Prev Med*. 2011;38(05):924–6.
51. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods*. 2013;10:996–8. <https://doi.org/10.1038/nmeth.2604>.
52. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Van Horn DJ, Weber CF. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*. 2009;75(23):7537–41. <https://doi.org/10.1128/AEM.01541-09>.
53. Barberán A, Bates ST, Casamayor EO, Fierer N. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J*. 2012;6:343–51. <https://doi.org/10.1038/ismej.2011.119>.

## Figures

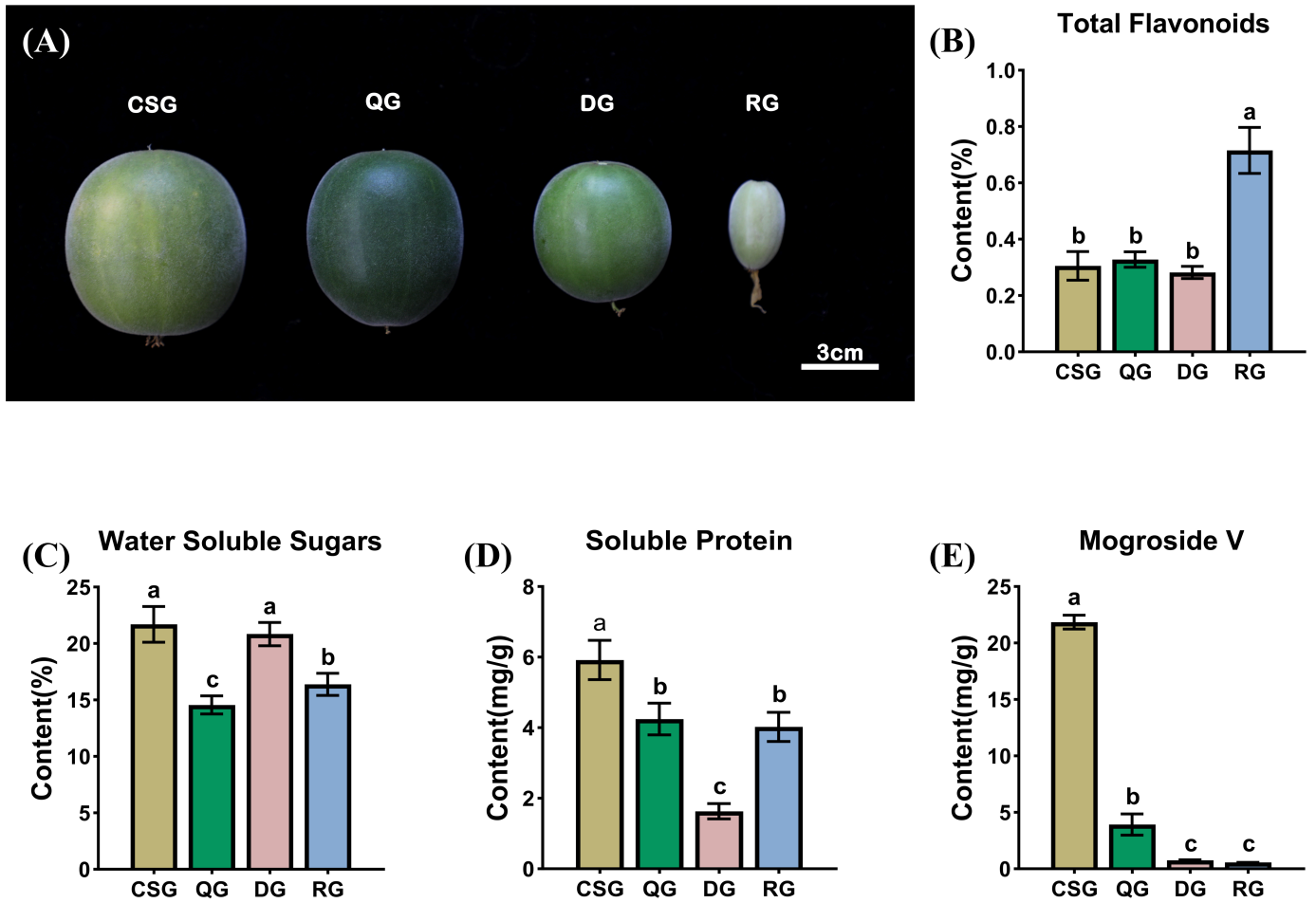


**Figure 1**

Fruit Morphology and Metabolite Contents of *S.grosvenorii* at Different Developmental Stages. A, Fruit morphology at different developmental stages. B, Total flavonoid content. C, Water-soluble sugar content. D, Soluble protein content. E, Mogroside V content.

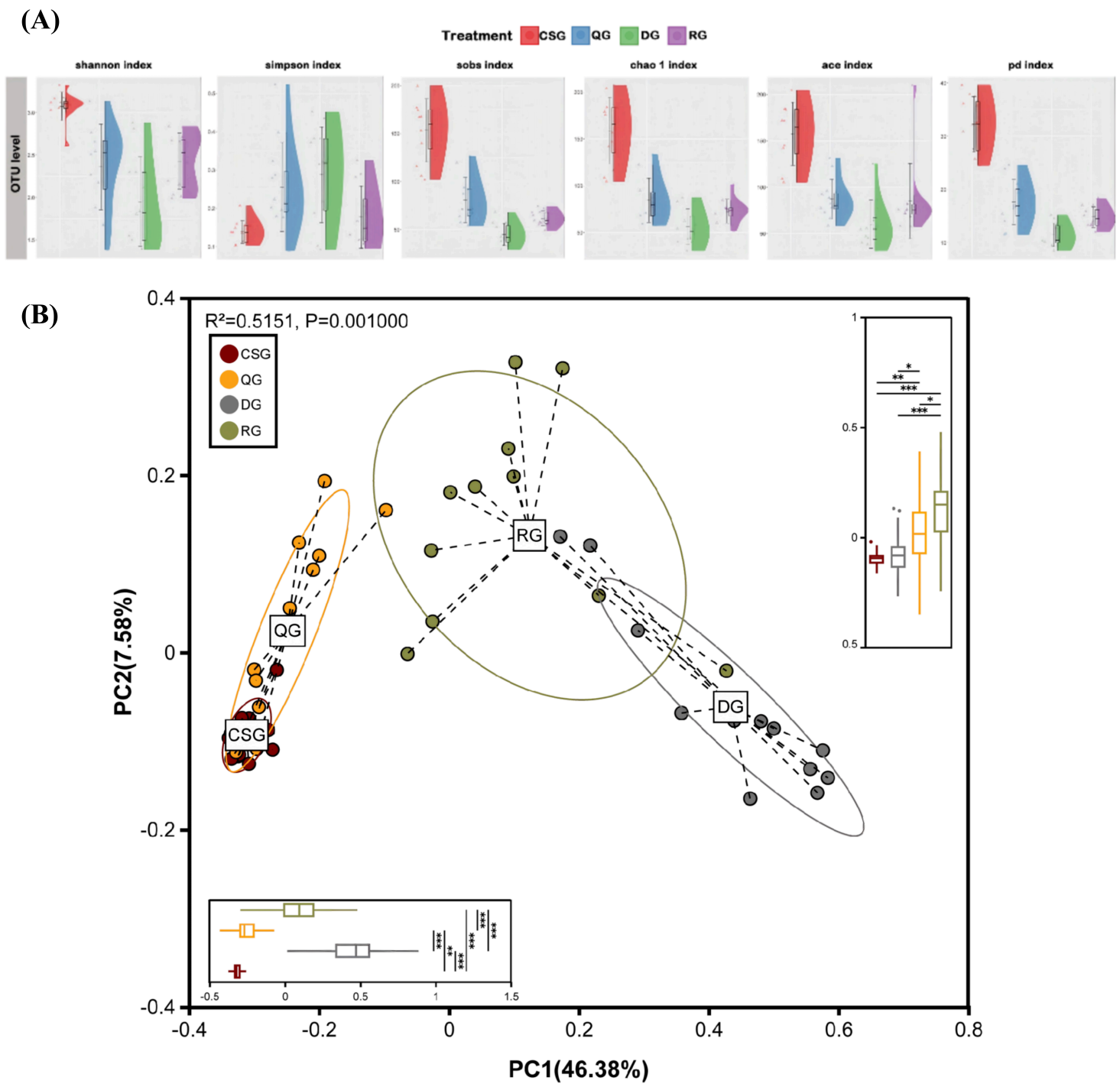


**Figure 2**

Endophytic fungal diversity in *S. grosvenorii* at different developmental stages. A,  $\alpha$ -diversity index analysis. B,  $\beta$ -diversity analysis: principal coordinate analysis (PCoA). Asterisks indicate statistically significant differences in pairwise comparisons among developmental stages; the same applies below.

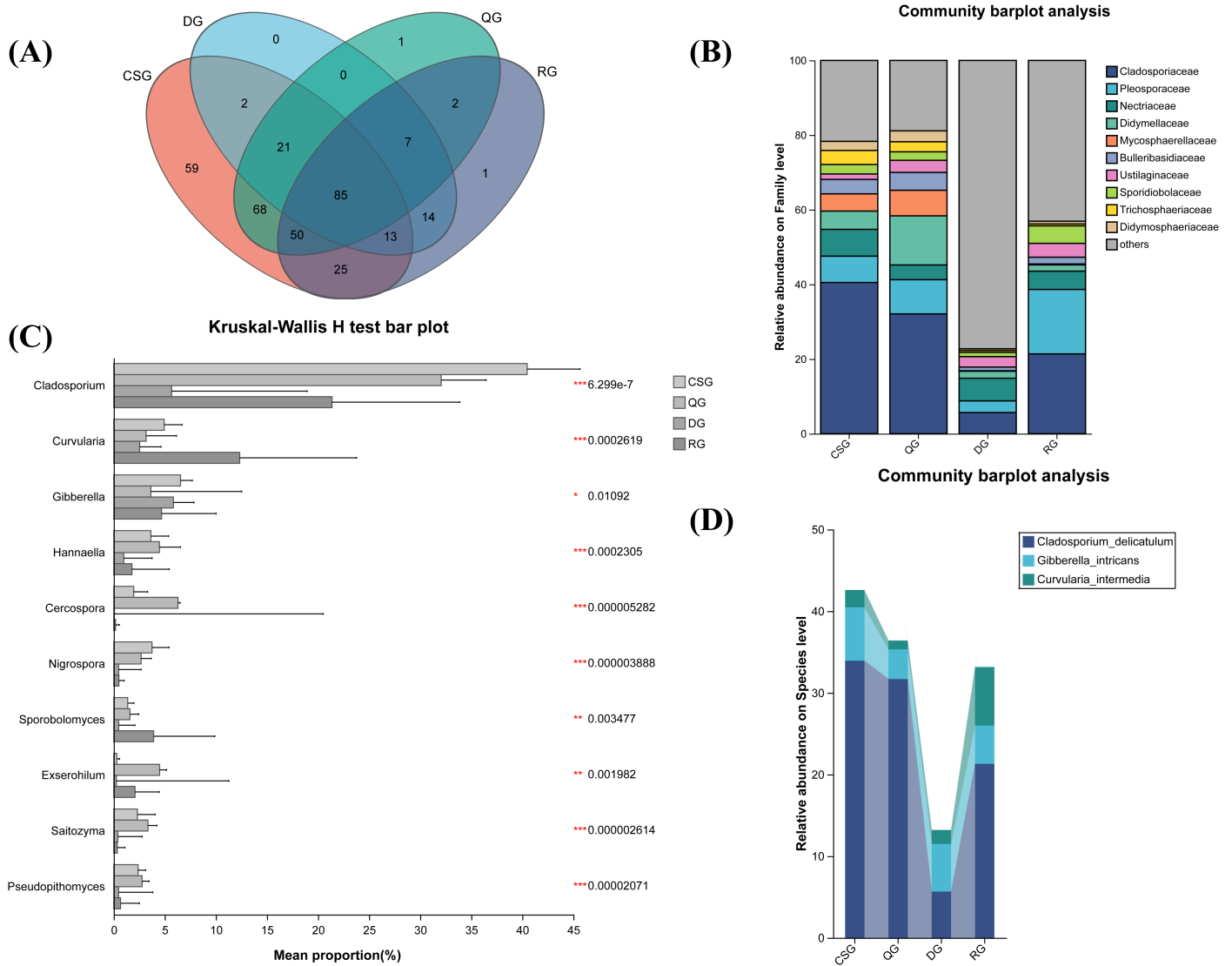


**Figure 3**

Composition analysis of endophytic fungal communities in *S. grosvenorii* at different developmental stages. A, Venn diagram of shared and unique OTUs. B, stacked bar chart of dominant fungal families. C, bar chart of the Kruskal-Wallis H test for dominant fungal genera. D, alluvial diagram of dominant fungal species.

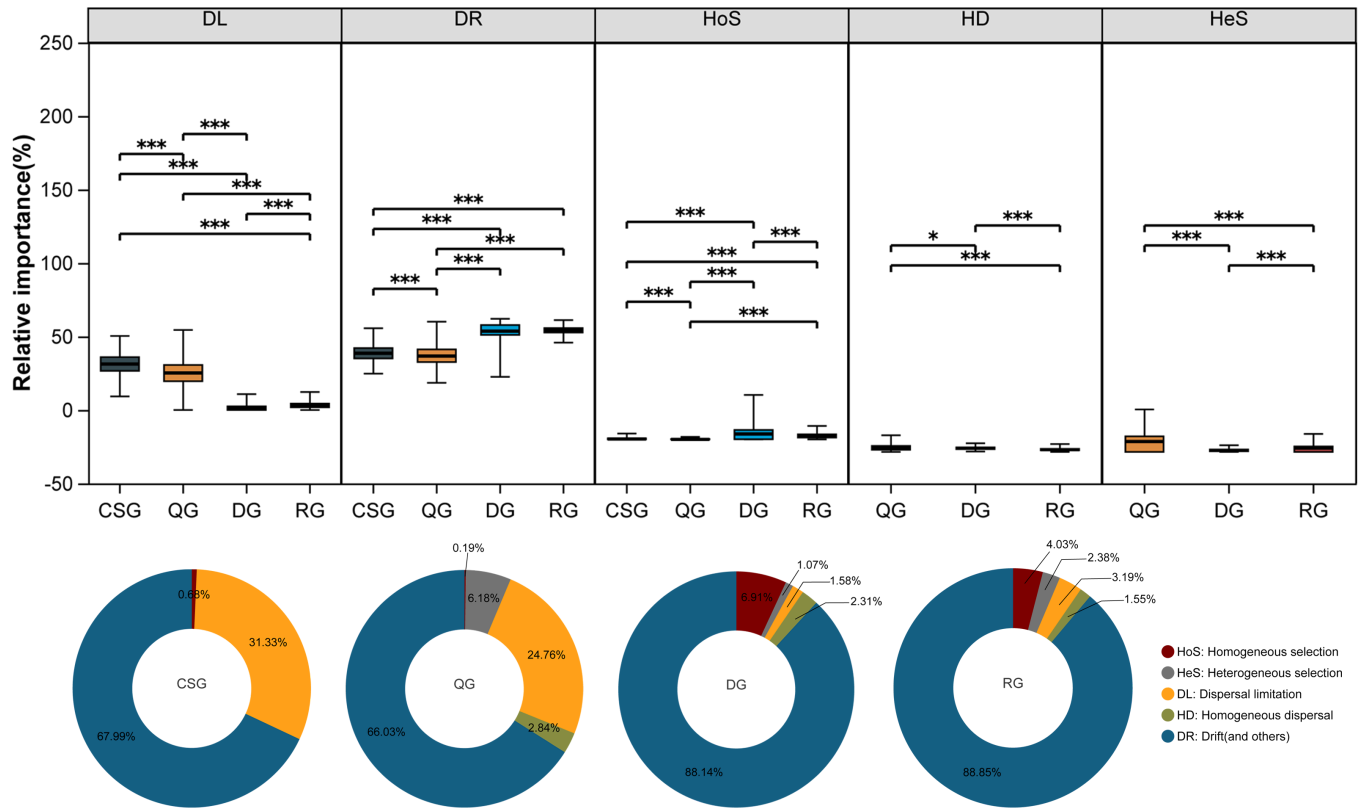


**Figure 4**

iCAMP analysis at the fungal genus level across developmental stages of *S. grosvenorii*.

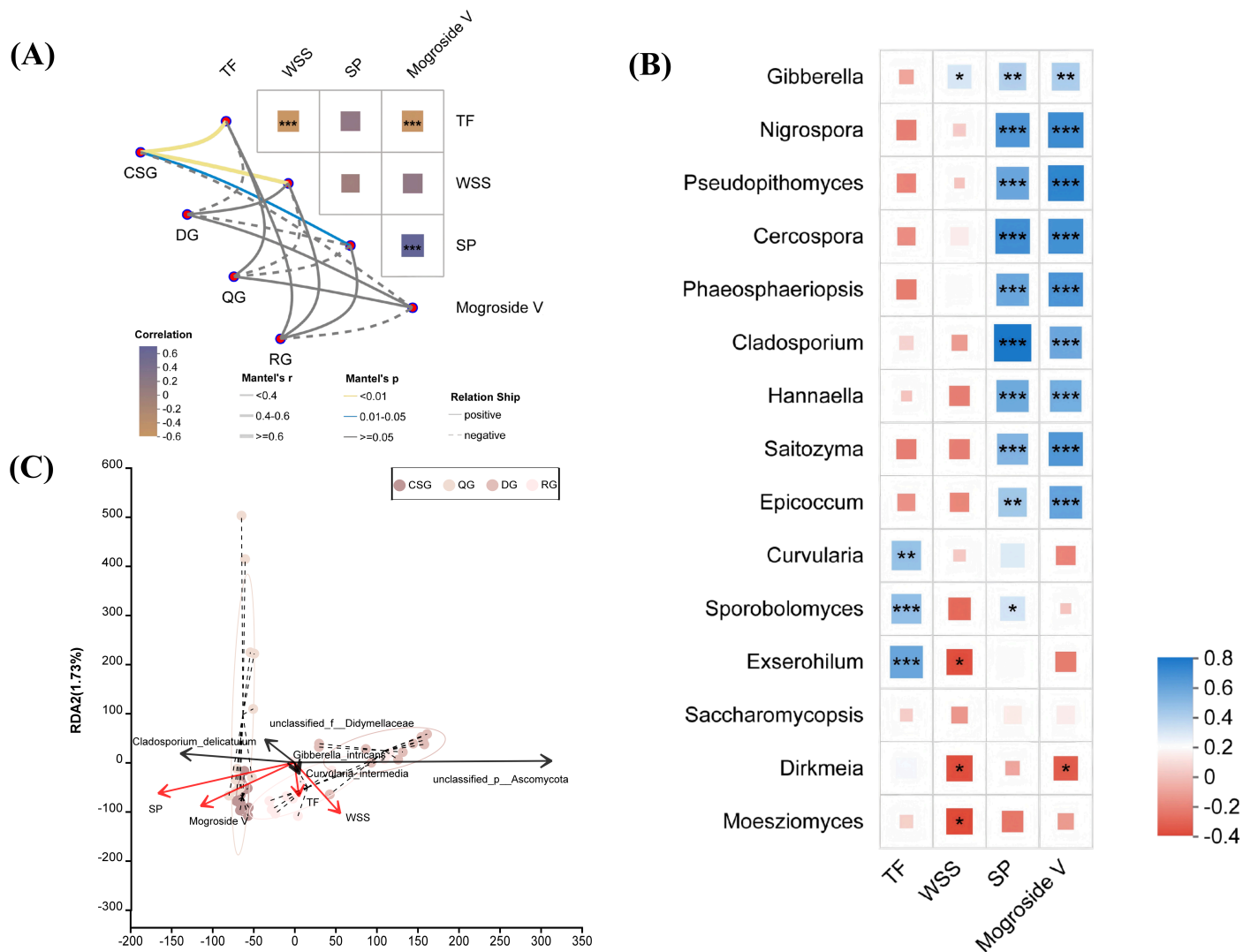


**Figure 5**

Association analysis between metabolites and fungal communities in *S. grosvenorii* at different developmental stages. A, Mantel test and combined correlation analysis. B, correlation heatmap between fungal genera and metabolites. C, redundancy analysis (RDA) ordination plot between fungal species and metabolites. TF, total flavonoids; WSS, water-soluble sugars; SP, soluble proteins.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1..xlsx](#)
- [TableS2..xlsx](#)