Exogenous and Endophytic Fungal Communities of *Dendrobium nobile* Lindl. across Different Habitats and Their Enhancement of Host Plants' Dendrobine Content and Biomass Accumulation

Wenhua Chen, Jingxuan Wang, Jia Song, Qingmei Sun, Bo Zhu,* and Luping Qin*

Read Online

ACCESS | Metrics & More | Article Recommendations | SI Supporting Information

ABSTRACT: Both the biosynthesis and array of bioactive and medicinal compounds in plants can be influenced by interactions with endophytic and exogenous fungi. However, the composition of endophytic and exogenous fungal communities associated with many medicinal plants is unknown, and the mechanism by which these fungi stimulate the secondary metabolism of host plants is unclear. In this study, we conducted a correlative analysis between endophytic and exogenous fungi and dendrobine and biomass accumulation in *Dendrobium nobile* across five Chinese habitats: wild Danxia rock, greenhouse-associated large Danxia stone, broken Danxia stone, broken coarse sandstone, and wood spile. Across habitats, fungal communities exhibited significant differences. The abundances of *Phyllosticta*, *Trichoderma*, and *Hydropus* were higher in wild habitats than in greenhouse habitats. Wild habitats were host to a higher diversity and richness of exogenous fungi than were greenhouse habitats. However, there was no significant difference in endophytic fungal diversity between habitats. The differences between the fungal communities' effects on the dendrobine content and biomass of *D. nobile* were attributable to the composition of endophytic and exogenous fungi. Exogenous fungi had a greater impact than endophytic fungi on the accumulation of fresh weight (FW) and dendrobine in *D. nobile*. Furthermore, *D. nobile* samples with higher exogenous fungal richness and diversity exhibited higher dendrobine content and FW. *Phyllosticta* was the only genus to be significantly positively correlated with both FW and dendrobine content. A total of 86 strains of endophytic fungi were isolated from the roots, stems, and leaves of *D. nobile*, of which 8 strains were found to be symbiotic with *D. nobile* tissue-cultured seedlings. The strain DN14 (*Phyllosticta fallopiae*) was found to promote not only biomass accumulation (11.44%) but also dendrobine content (33.80%) in *D. nobile* tissue-cultured seedlings. The results of this study will aid in the development of strategies to increase the production of dendrobine in *D. nobile*. This work could also facilitate the screening of beneficial endophytic and exogenous fungal probiotics for use as biofertilizers in *D. nobile*.
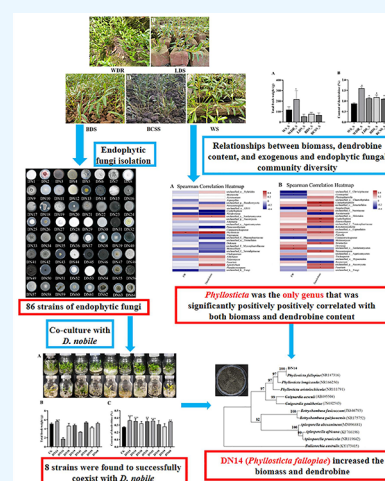
1. INTRODUCTION

Dendrobium nobile Lindl., a member of the Orchidaceae family, is commonly used in traditional Chinese medicine.[1] According to recent research, *D. nobile* exhibits diverse beneficial pharmacological properties, including neuroprotection,[2] cardioprotection,[3] hepatoprotection,[4] nephroprotection,[5] promoting healthy ovarian function,[6] immunomodulation,[7] and protecting against Alzheimer's disease.[8] These diverse beneficial effects are due to the species' complex array of bioactive compounds, including polysaccharides,[7] alkaloids,[9] phenanthrenes,[10] and bibenzyls.[11] Dendrobine, an alkaloid component of *D. nobile* indexed in the 2020 edition of the Chinese Pharmacopoeia,[1] has been specifically found to exert antiosteoporosis,[12] antitumor,[13] and neuroprotective effects,[2] among others. Unfortunately, wild populations of *D. nobile* growing on Danxia landforms (listed on the National Key Protected Wild Medicinal Plants) are becoming increasingly exhausted due to both habitat destruction and increasing

market demand.[14] Cultivated *D. nobile* has been inconsistent in both quality and quantity, which can negatively affect the bioactivity and safety of *D. nobile*-derived medicinal products. Furthermore, because of the difficulty of isolating secondary metabolites present at low levels and the structural complexity of dendrobine, extracts of *D. nobile* often do not meet industry or research requirements.[15,16]
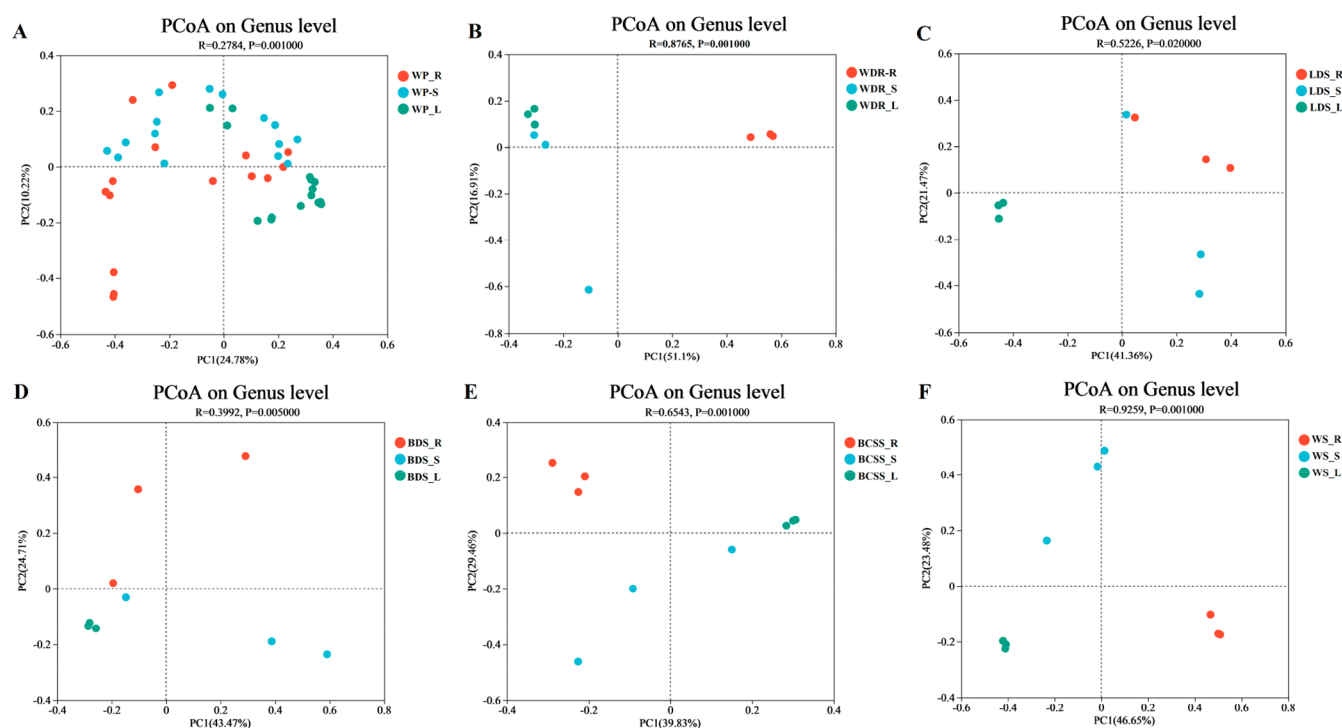
Endophytic fungi, which are present within plant tissues, are among the most crucial components of the plant microecosystem. Endophytic fungi can significantly impact both the quantity and quality of plant-derived medicinal compounds.[17]
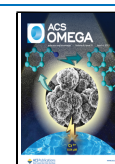
Figure 1. PCoA analysis of fungal communities among three *D. nobile* tissue samples from different habitats. (A) Five tested habitats. (B) Wild Danxia rock: WDR. (C) Large Danxia stone: LDS (>20 cm × 20 cm, greenhouse). (D) Broken Danxia stone: BDS (<2 cm × 2 cm, greenhouse). (E) Broken coarse sandstone: BCSS (<2 cm × 2 cm, greenhouse). (F) Wood spile: WS (greenhouse).

Furthermore, not only can fungal endophytes stimulate the biosynthesis of secondary metabolites in their host plants, but they can also produce their own secondary metabolites.[18] Recently, Li et al. (2017) reported that *Mycena* sp. MF23 was able to promote the production of dendrobine in *D. nobile* through regulating mevalonate (MVA) pathway-related gene expression.[14] In addition, Sarsaiya et al. (2020) found that the *D. nobile*-isolated fungus *Trichoderma longibrachiatum* MD33 was able to produce its own dendrobine.[15] Other work has shown that endophytes can biosynthesize a wide array of bioactive compounds with applications across the food, agricultural, and pharmaceutical industries.[19]

The population dynamics and distribution of endophytic fungi are primarily dependent on the availability of host species-specific habitats, organs, nutrients, and climatic factors.[20] Research on *D. nobile* suggests that endophytic bacterial community structure is dependent on stem length.[21] However, exogenous fungi, growing or originating from outside an organism, are abundant in natural environments, particularly in polluted environments.[22,23] Exogenous fungi can improve the stress resistance of their host plants, including to pathogens, drought, and heavy metals.[24] On rocky surfaces, only exogenous fungi with the ability to digest the rocky substrate may survive.[25] This unique ability to release inorganic nutrients through oxidoreduction, acidolysis, and chelation can aid in the establishment of plants and other organisms in otherwise inhospitable growing environments.[26] In brief, endophytic fungi and exogenous fungi can provide host plants with many benefits, such as promoting plant growth and improving host metabolite profiles.

To date, no comprehensive studies have been reported on the relationships between endophytic and exogenous fungi and dendrobine and biomass accumulation. In this study, gas

chromatography (GC) was performed to determine the dendrobine content in *D. nobile* collected from five habitats (wild Danxia rock, large Danxia stone, broken Danxia stone, broken coarse sandstone, and wood spile). High-throughput DNA sequencing was utilized to explore the community composition and diversity of both endophytic and exogenous fungi in these habitats. Spearman correlation analysis was conducted to investigate the relationships between endophytic and exogenous fungi and dendrobine content and biomass accumulation. Our analysis indicated that one fungal genus, *Phyllosticta*, was significantly positively correlated with both *D. nobile* fresh weight (FW) and dendrobine content. In addition, *Phyllosticta fallopiae* strain DN14 was isolated from leaves of *D. nobile* growing on wild Danxia rock, and its effect on dendrobine and biomass accumulation was evaluated in a coculture trial. The results of this study will aid our understanding of the influence of endophytic and exogenous fungi on the quality of medicinal plant materials. Specifically, these results will benefit efforts to increase the dendrobine content of cultivated *D. nobile*.

2. RESULTS

2.1. Differences in Endophytic Fungal Diversity across Different Plant Organs and Habitats. Both the Shannon and Chao indices indicated that LDS_R exhibited the highest community diversity, and WS_R exhibited the highest community richness, whereas WS_L exhibited the lowest community diversity and richness (Table S1). To examined the differences between fungal communities from different *D. nobile* organs and habitats, PCoA was conducted. The principal coordinate analysis (PCoA) results revealed that there were highly significant differences ($P = 0.001$) in endophytic fungi among different organs in all samples (Figure 1A). The first
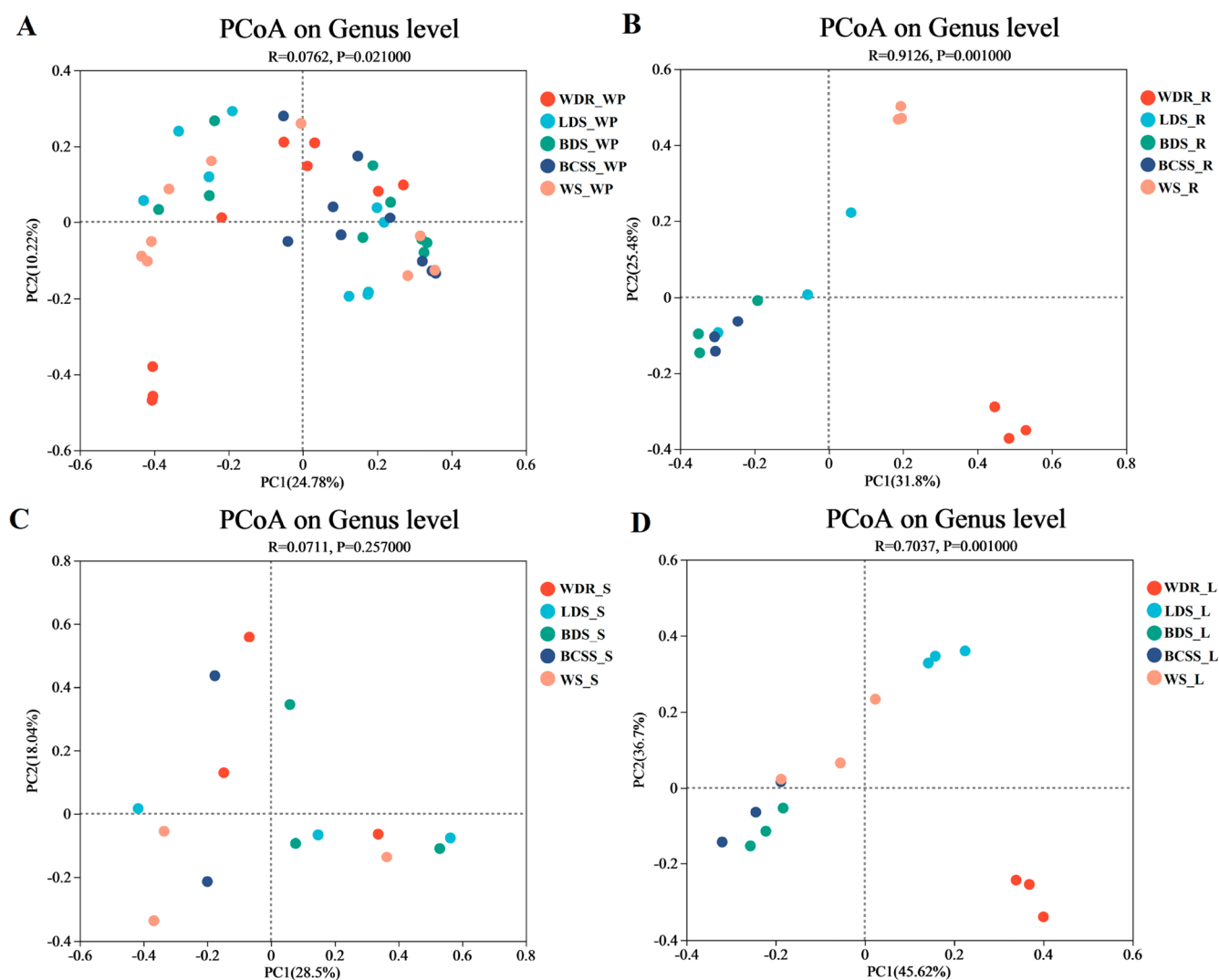
Figure 2. PCoA analysis of fungal communities among different *D. nobile* organ samples from five habitats. (A) Whole plant, WP. (B) Root, R. (C) Stem, S. (D) Leaf, L.
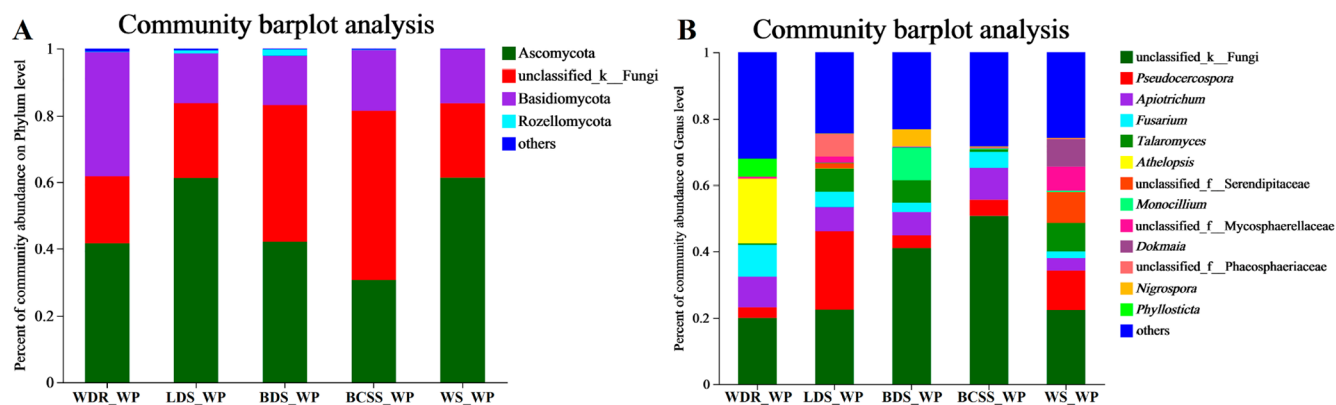
Figure 3. Composition and relative abundance of endophytic fungi at the phylum (A) and genus (B) levels across five habitats. Wild Danxia rock, WDR; large Danxia stone, LDS (>20 cm × 20 cm, greenhouse); broken Danxia stone, BDS (<2 cm × 2 cm, greenhouse); broken coarse sandstone, BCSS (2 cm × 2 cm, greenhouse); and wood spile, WS (greenhouse).

and second principal component axes were found to explain 24.78% and 10.22% of the variation, respectively (Figure 1A). There were also highly significant differences ($P < 0.05$) in the community distribution of endophytic fungi among the three organs (Figure 1B−F). Additionally, there were significant differences ($P = 0.021$) in fungal communities among different
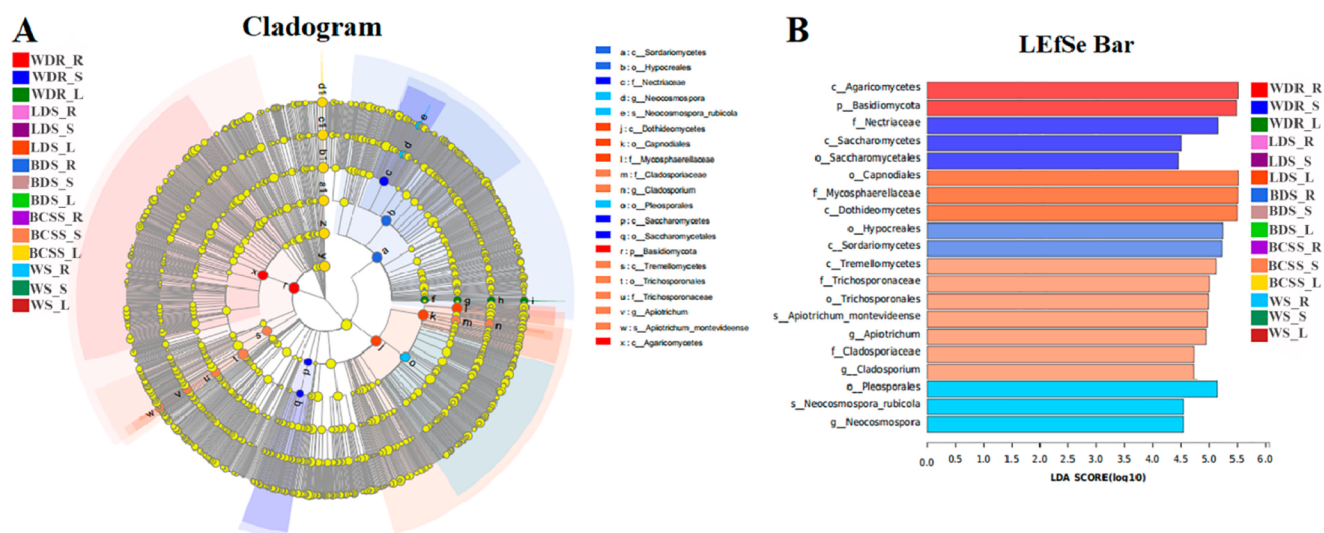
Figure 4. Linear discriminant analysis effect size (LEfSe) and correlation analysis of endophytic fungi from *D. nobile* in different organs and habitats. (A) Cladogram showing significantly enriched fungal taxa of *D. nobile* in different organs and habitats. (B) Indicator fungi with linear discriminant analysis (LDA) scores of 4.0 or greater in fungal communities from *D. nobile* in different organs and habitats. Phylum, class, order, family, and genus levels are listed in order from the inside to the outside of the cladogram. Small circles with different colors in the diagram denote enriched taxa; yellow circles represent taxa with no significant differences between different *D. nobile* organs and habitats. The circle diameter is proportional to the abundance of the taxon. Wild Danxia rock, WDR; large Danxia stone, LDS (>20 cm × 20 cm, greenhouse); broken Danxia stone, BDS (<2 cm × 2 cm, greenhouse); broken coarse sandstone, BCSS (<2 cm × 2 cm, greenhouse); and wood spile, WS (greenhouse).

habitats (Figure 2A). We analyzed the three organs independently and found that the fungal communities of stems were similar and that those of roots and leaves were significantly different (*P* = 0.001) (Figure 2B−D).

Across different habitats, Ascomycota and Basidiomycota were the most dominant endophytic fungal phyla, although the relative abundance of Basidiomycota was significantly greater in the wild habitat than in the greenhouse habitats (Figure 3A). At the genus level, *Apiotrichum*, *Athelopsis*, and *Fusarium* were the top three 3 dominant genera in WDR_WP; *Pseudocercospora*, *Apiotrichum*, and *Talaromyces* were the top three dominant genera in LDS_WP; *Apiotrichum*, *Talaromyces*, and *Mongolism* were the top three dominant genera in BDS_WP; *Pseudocercospora*, *Apiotrichum*, and *Fusarium* were the top three dominant genera in BCSS_WP; *Pseudocercospora*, *Talaromyces*, and *Dokmaia* were the top three dominant genera in WS_WP (Figure 3B). Additionally, 59 common genera of endophytic fungi were identified across the five habitats, while 39, 38, 61, 30, and 30 unique genera were identified in WDR_WP, LDS_WP, BDS_WP, BCSS_WP, and WS_WP, respectively (Figure S1). The three common genera with the greatest relative abundances were *Pseudocercospora*, *Apiotrichum*, and *Fusarium*. The unique genera with the greatest relative abundances were *Phyllosticta*, *Rasamsonia*, and *Bannozyma* in WDR_WP; *Ceratobasidium*, *Leucoagaricus*, and *Neocucurbitaria* in LDS_WP; *Shiraia*, *Coprotus*, and *Minimedusa* in BDS_WP; *Podosordaria*, *Peroneutypa*, and *Zopfiella* in BCSS_WP; and *Glutinomyces*, *Lasiodiplodia*, and *Phlebiopsis* in WS_WP (Figure S2).

We used the Majorbio LDA-LEfSe tool to identify discriminative taxa across *D. nobile* organs and habitats. The enriched taxa (LDA significance threshold of 4.0) are shown in Figure 4A,B. The results of the LEfSe analyses based on the all-against-all test identified 20 biomarkers with significant differences, appearing specifically in WDR_R (two taxa),

WDR_S (three taxa), LDS_L (three taxa), BDS_R (two taxa), BCSS_R (seven taxa), and WS_R (three taxa), from the phylum to the species levels. Kruskal−Wallis ranking was utilized to identify significant differences in WP-associated fungal community composition between different habitats. Subsequently, pairwise multiple comparisons (Tukey−Kramer test) were carried out at the genus level. Significantly different fungal communities were observed in samples from different *D. nobile* habitats (Figure 5). *Phyllosticta*, *Trichoderma*, and *Hydropus* (*P* < 0.05) were concentrated in WDR_WP, but not in the four greenhouse habitats.
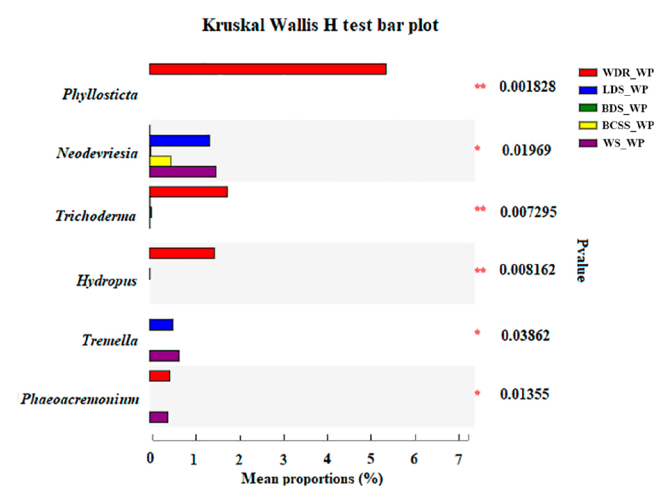
Figure 5. Kruskal−Wallis H-test bar plot of genera with a high relative abundance and statistically significant differences. *$P \leq 0.05$, **$P \leq 0.01$. Wild Danxia rock, WDR; large Danxia stone, LDS (>20 cm × 20 cm, greenhouse); broken Danxia stone, BDS (<2 cm × 2 cm, greenhouse); broken coarse sandstone, BCSS (<2 cm × 2 cm, greenhouse); and wood spile, WS (greenhouse).

2.2. Differences in Exogenous Fungal Diversity Across Different Plant Habitats.

According to the alpha diversity indices, the WDR samples exhibited significantly higher community diversity and richness than the samples from the greenhouse habitats ($P < 0.001$) (Table S2). The PLS-DA analyses revealed distinct fungal profiles between the WDR samples and the greenhouse samples (LDS, BDS, BCSS, and WS) (Figure 6). The COMP1 and COMP2 explained
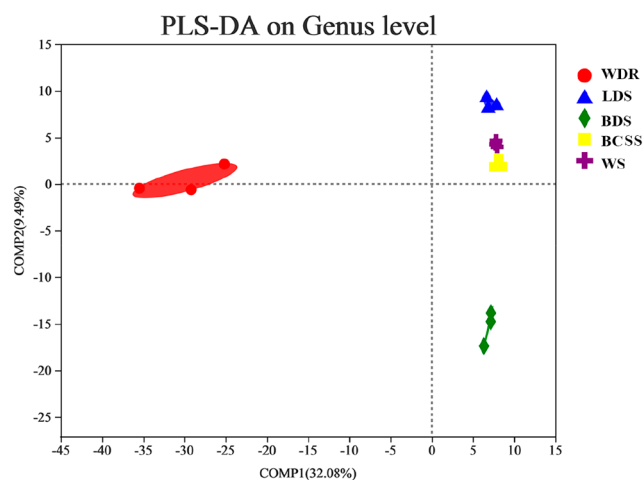
Figure 6. PLS-DA illustrating differences between exogenous fungal communities across five habitats. Wild Danxia rock, WDR; large Danxia stone, LDS (>20 cm × 20 cm, greenhouse); broken Danxia stone, BDS (<2 cm × 2 cm, greenhouse); broken coarse sandstone, BCSS (<2 cm × 2 cm, greenhouse); and wood spile, WS (greenhouse).

32.08% and 9.49% of the variation, respectively, indicating that the fungal communities in the wild (WDR) and greenhouse (LDS, BDS, BCSS, and WS) habitats were significantly different. Among them, the greatest distance was observed for WDR, followed by BDS. By contrast, LDS, BCSS, and WS were separated only by a short distance.

The most dominant exogenous fungal phylum was Ascomycota, with relative abundances of 80.79%, 91.89%, 50.57%, 19.00%, and 43.24% in WDR, LDS, BDS, BCSS, and WS, respectively. By comparison, the relative abundances of Basidiomycota were 10.35%, 4.35%, 10.81%, 21.62%, and 45.06%, respectively (Figure S3 A). The fungal genera with relative abundances greater than 5% included *Hypomyces* (7.09%) and *Sirastachys* (5.67%) in the WDR samples; *Fusarium* (41.54%) and *Neocosmospora* (11.88%) in the LDS samples; *Petriella* (6.44%) in the DXS samples; *Trichosporon* (14.82%) in the CS samples; and *Resinicium* (32.28%), *Meliniomyces* (7.72%), and *Devriesia* (7.44%) in the WS samples (Figure S3B). We identified 35 common genera across the five habitats and 277, 19, 50, 15, and 10 unique genera in WDR, LDS, BDS, BCSS, and WS samples, respectively (Figure S4). The three common genera with the greatest relative abundances were *Cutaneotrichosporon*, *Fusarium*, and *Devriesia*. The unique genera with the greatest relative abundances were *Arthopyrenia*, *Ceratobasidium*, and *Ilyonectria* in the WDR samples; *Fusariella*, *Asterostroma*, and *Subulicystidium* in the LDS samples; *Cochliobolus*, *Hansfordia*, and *Oxyporus* in the BDS samples; *Mucronella*, *Clitocybe*, and *Clavulina* in the BCSS samples; and *Claussenomyces*, *Toxicocladosporium*, and *Geotrichum* in the WS samples (Figure S5).

A total of 47 biomarkers with significant differences (LDA significance threshold of 4.0) were found across the five samples (Figure S6A−C). Among them, 10 biomarkers were identified at the genus level: *Sirastachys* (WDR), *Emericellopsis* (WDR), *Cyphellophora* (WDR), *Cladophialophora* (WDR), *Fusarium* (LDS), *Neocosmospora* (LDS), *Simplicillium* (LDS), *Petriella* (BDS), *Plectosphaerella* (BDS), and *Trichosporon* (BCSS). The Kruskal−Wallis analysis revealed that *Hypomyces* ($P < 0.05$), *Emericellopsis* ($P < 0.01$), and *Cyphellophora* ($P < 0.05$) were concentrated in the wild environment but not in the greenhouse samples. *Sirastachys* ($P < 0.05$) was significantly more abundant in WDR and BDS than in samples from other habitats, whereas the abundance of *Cutaneotrichosporon* ($P < 0.05$) was significantly higher in BDS and BCSS than in samples from other habitats. The relative abundances of *Fusarium* ($P < 0.01$) and *Neocosmospora* ($P < 0.05$) were significantly higher in LDS than in samples. *Resinicium* was significantly more abundant in WS than in other samples, whereas *Trichosporon* was significantly more abundant in BCSS than in other samples (Figure S6C).

2.3. Analysis of Biomass and Dendrobine Content of *D. nobile* Stems.

One-way ANOVA indicated that the biomass and dendrobine content of wild rock-cultivated *D. nobile* (WDR_S) were significantly higher than those of greenhouse wood-cultivated *D. nobile* (WS_S). However, the biomass of wild rock-cultivated *D. nobile* was not significantly higher than that of greenhouse stone-cultivated *D. nobile* (LDS_S, BDS_S, and BCSS_S). The average FW values of whole-plant *D. nobile* samples from WDR, LDS, BDS, BCSS, and WS were 216.73, 54.06, 76.44, 66.45, and 118.43 g, respectively (Figure 7A). WDR_S had the highest dendrobine
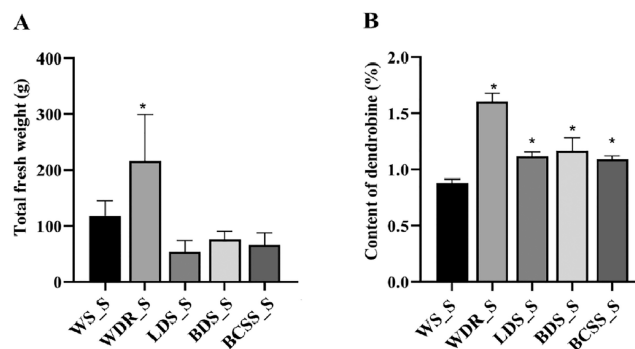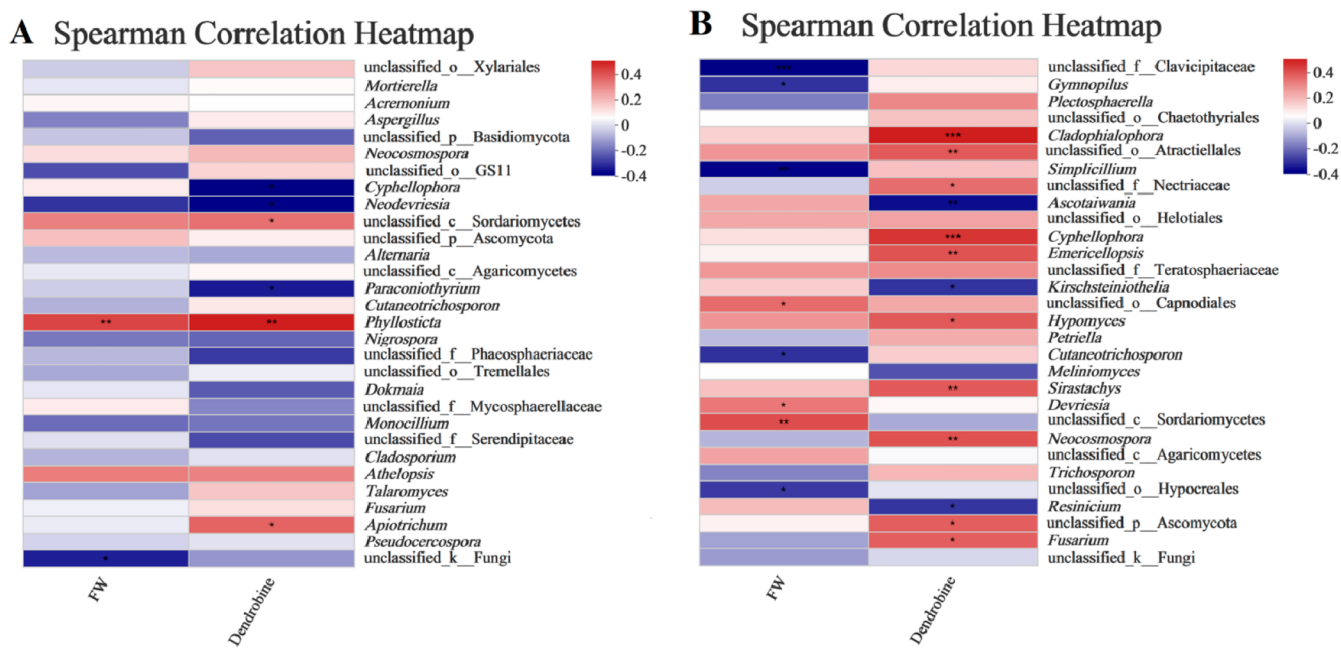

Figure 7. Plant growth parameters and dendrobine content. Total fresh weight (FW) (A) and dendrobine content (B) of *D. nobile* from five different habitats. Asterisks indicate significant differences relative to the control (*$P < 0.05$, Student's *t* test). Wild Danxia rock, WDR; large Danxia stone, LDS (>20 cm × 20 cm, greenhouse); broken Danxia stone, BDS (<2 cm × 2 cm, greenhouse); broken coarse sandstone, BCSS (<2 cm × 2 cm, greenhouse); and wood spile, WS (greenhouse). *$P \leq 0.05$.

content (1.61%), followed by BDS_S (1.17%), LDS_S (1.12%), and BCSS_S (1.09%), while WS_S had the lowest dendrobine content (0.88%) (Figure 7B).

2.4. Relationships between Biomass, Dendrobine Content, and Fungal Community Diversity.

Spearman correlation was utilized to examine the relationships between the top 30 endophytic and exogenous fungal genera, FW, and dendrobine content (Figure 8A,B). The Spearman correlation heatmap indicated that exogenous fungi have a greater impact on biomass and dendrobine content than endophytic fungi.

Figure 8. Top 30 strains of endophytic fungi (A) and exogenous fungi (B) which exhibited significant positive or negative correlations with fresh weight (FW) and dendrobine accumulation. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.
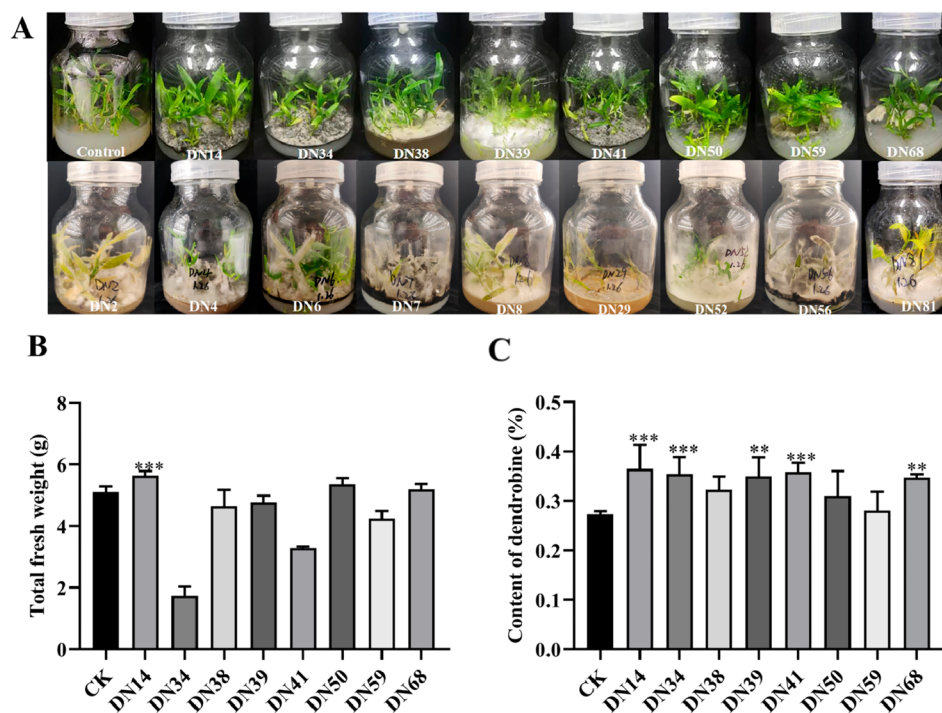
Figure 9. Plant growth parameters and dendrobine content. Shown are the 8 symbiotic strains, as well as other strains that cannot coexist with *D. nobile* tissue culture seedlings (A), total fresh weight (B), and dendrobine content (C) of *D. nobile* culture seedlings. Asterisks indicate significant differences relative to control (***$P < 0.001$, Student's *t* test).

Specifically, *D. nobile* samples with higher exogenous fungal richness and diversity exhibited greater biomass and dendrobine accumulation (Tables S3 and S4). Twenty strains exhibited significant positive or negative correlations with FW or dendrobine accumulation. *D. nobile* samples with higher abundances of *Phyllosticta*, *Apiotrichum*, *Cladophialophora*, *Cyphellophora*, and *Emericellopsis* had higher dendrobine contents. Conversely, *Cyphellophora*, *Neodevriesia*, *Paraconio-*

thyrium, *Ascotaiwania*, *Kirschsteiniothelia*, and *Resinicium* were negatively correlated with dendrobine content (Figure 8A,B). *Phyllosticta* and *Devriesia* were positively correlated with FW, whereas *Gymnnopilus*, *Simplicillium*, and *Cutanetrichosporon* were negatively correlated with FW (Figure 8A,B). Among these fungi, the endophytic fungus *Phyllosticta* was the only genus that was significantly positively correlated with both biomass and dendrobine content ($P < 0.01$) (Figure 8A).

2.5. Enhancement of Plant Growth and Dendrobine Content by Endophytic Fungi.

A total of 86 strains of culturable endophytic fungi were isolated from 450 *D. nobile* tissue samples (Figure S7). Of these, 8 strains were found to successfully coexist with *D. nobile* tissue culture seedlings (Figure 9A), whereas the 78 other strains were found to lack symbiotic ability, including DN2, DN4, DN6, DN7, DN8, DN29, DN52, DN56, and DN81. To determine the effect of these 8 strains on the growth (i.e., biomass) and secondary metabolism (i.e., dendrobine accumulation) of *D. nobile*, tissue culture seedlings were cocultured again for 60 days (Figure 9B,C). Among the 8 strains, DN14 significantly increased the FW of *D. nobile* tissue culture seedlings by 11.44% (Figure 9B). DN14, DN34, DN39, DN41, and DN68 significantly promoted the accumulation of dendrobine in *D. nobile* tissue culture seedlings by 33.80%, 33.09%, 28.37%, 31.56%, and 34.70%, respectively (Figure 9C). These results suggest that DN14 can not only promote the growth of *D. nobile* tissue culture seedlings but also increase the content of dendrobine. The ITS rDNA sequences of this isolate were highly homologous with *Phyllosticta fallopiae* (Figure S8).

3. DISCUSSION

Fungal endophytes play an essential role in promoting the growth of medicinal plants.[16,25] This study revealed significant differences between the diversity and community structure of endophytic and exogenous fungi across different habitats. Overall, both species richness and diversity of exogenous fungi were higher than those of endophytic fungi (Table S2). Because of horizontal endophyte transmission, conspecific plants growing in different environments are likely to host a different group of endophytes.[27] Although seeds can transfer endophytic fungi to host plants through vertical transmission, plants often selectively absorb endophytic fungi during later growth stages.[28] In addition, plants can selectively absorb exogenous fungi and transform them into endophytic fungi, thus preventing other exogenous fungi from invading plant tissues. We also found that the diversity of exogenous fungi from the wild habitat was significantly higher than that of the greenhouse habitats, but the diversity of endophytic fungi did not differ significantly between habitats (Figure S9A−D). The bioweathering of rocks by fungi and other microbes is an important component of biogeochemical nutrient cycling,[29] which is necessary for the growth of exogenous fungi. Wang et al. reported that both the elemental content (N, P, and K) and exogenous fungal diversity were higher in wild habitats than in greenhouse habitats.[30] In another study, we found that both the Si content and *D. nobile*-associated exogenous fungal diversity were highest in a wild habitat, and that the inclusion of Si in the culture medium promoted the growth of some endophytic fungi (unpublished data).

The relative abundance of Basidiomycota was significantly higher in the wild habitat than in the greenhouse habitats (Figure 3A). Ascomycetes tend to grow under harsh environmental conditions, whereas Basidiomycetes tend to grow in resource-rich environments containing many plants.[31] These environmental affinities may explain why we found that Basidiomycetes were significantly more abundant in *D. nobile* from the wild habitat than from the greenhouse habitats. Significant differences in community structure were observed between fungi from different habitats, although several common fungal genera were identified across habitats, including the endophytic genera *Pseudocercospora* and

Apiotrichum and the exogenous genus *Devriesia*. *Pseudocercospora* is reported to cause leaf spot disease in banana, *Ixora coccinea*, and other plants,[32,33] although this is the first study to report this species as an endophyte. *Apiotrichum melanogenum* has been shown to benefit plant growth through the production of ammonia and indole-3-acetic acid (IAA).[34] In American ginseng, a high relative abundance of *Devriesia* in the soil microbial community is associated with a lower incidence of root rot.[35] We also identified several genera which were unique to each habitat (Figures S1 and S2). For example, *Ceratobasidium* was only found in WDR_WP, and *Minimedusa* was only found in BDS_WP. One study reported that IAA secreted by *Ceratobasidium* sp. may promote the growth of *Rehmannia glutinosa*.[36] Soil-borne *Ceratobasidium* sp. AG was shown to promote *Orchis anatolica* seed germination and seedling growth.[37] *Minimedusa polyspora* has been found to stimulate the growth of *Cichorium intybus* by reducing fatty acid and sterol synthesis and promoting 3-OH butyric acid synthesis.[38] Differences in the stimuli and challenges associated with different habitats may be reflected in microbial community differences between habitats. Here, we found that *Trichoderma* and *Emericellopsis* were concentrated in samples from the wild habitat but not in samples from the greenhouse habitats. *Trichoderma asperellum* has been found to increase the tolerance of maize seedlings to saline-alkaline stress by encouraging root growth.[39] *Trichoderma* has been reported to promote soybean plant growth by solubilizing phosphate.[40] *Emericellopsis alkalina* produces a novel lipopeptaibol, emericellipsin A, which acts as an antifungal against the mold *Aspergillus niger*, the yeast *Candida albicans*, and clinical isolates of human pathogens.[23] In another study, indole glucosinolate mutants enriched in *Emericellopsis* suppressed the pathogen *Aspergillus niger*.[41]

Both endophytic and exogenous fungi may act to increase the accumulation of secondary metabolites in medicinal plants.[42] In our study, the dendrobine content and FW were higher in *D. nobile* samples with greater richness and diversity of exogenous fungi (Table S4). Previous studies have found that the diversity of exogenous fungi in different *Panax ginseng* habitats improves productivity and prevents disease.[43] Furthermore, *Panax ginseng* with higher soil microbial community diversity exhibited higher total ginsenoside (TG, Rg1, and Rf) contents.[44] Here, we found that *D. nobile* with a higher abundance of *Phyllosticta*, *Apiotrichum*, *Cladophialophora*, *Cyphellophora*, and *Emericellopsis* exhibited higher dendrobine contents (Figure 8A,B). However, there have been no previous reports of enhanced dendrobine production stimulated by these fungi. We speculate that differences between the endophytic and exogenous fungal communities across different habitats may result in differences in biomass and secondary metabolite accumulation. We also found that exogenous fungi had a greater impact on biomass and dendrobine accumulation than endophytic fungi (Figure 8A,B). A similar result was found in *Eucommia ulmoides*, where redundancy and canonical correspondence analyses (RDA/CCA) revealed that rhizosphere fungi had a greater impact on rosinol, pinoresinol diglucoside, and chlorogenic acid accumulation than endophytic fungi.[45] This may be because rhizosphere fungi can directly and indirectly regulate the growth, development, and secondary metabolite accumulation of host plants, as well as enhance absorption and utilization of nutrients and abiotic and biotic stress resistance of host plants.[24]

In this study, *Phyllosticta* was the only genus to be significantly positively correlated with both FW and dendrobine content (Figure 8A). We isolated 86 endophytic fungal strains from the leaves, stems, and roots of *D. nobile* and found that DN14 (*Phyllosticta fallopiae*) had a significant positive effect on both biomass and dendrobine accumulation (Figure 9B,C). Furthermore, *P. fallopiae* is the first endophytic fungus isolated from *D. nobile*. A previous study reported that *Aloe vera*-isolated *P. fallopiae* L67 exhibited antimicrobial activity against diabetic wound-associated microorganisms.[46] Other studies have also reported that *Phyllosticta* exhibits antimicrobial and neuroprotective properties.[47] In *D. nobile*, DN14 may regulate photosynthesis and thus promote growth.[48] Therefore, the effects of DN14 on the chlorophyll A and B levels, leaf conductivity, stomatal conductance, transpiration rate, and photosynthetic rate of *D. nobile* should be further investigated. Furthermore, because DN14 was found to increase both biomass and dendrobine accumulation, we suggest that research should be conducted on the potential use of DN14 as a biofertilizer for *D. nobile*. Many researchers have reported the growth- and metabolism-promoting effects of endophytic fungi. For example, Zhou et al. (2018) isolated an endophytic fungus, *Alternaria* sp. A13, which markedly enhanced the growth and accumulation of medicinal compounds in *Salvia miltiorrhiza* under both greenhouse and field conditions, demonstrating that *Alternaria* sp. A13 could be used as a potent biofertilizer.[49] Our research group previously reported that D38 (*Chaetomium globosum*) biofertilization significantly promoted the growth of *S. miltiorrhiza* seedlings and increased the contents of tanshinones and phenylpropionic acids in *S. miltiorrhiza* roots.[50,51]

Phyllosticta species are well-known as foliar pathogens as well as foliar endophytes in many plants.[19] However, we found DN14 to be a harmless endophytic fungus which promoted the production of dendrobine in *D. nobile*. Dendrobines are a type of sesquiterpene alkaloid which possess antifungal activity.[52] It is possible that *D. nobile* produced more dendrobine in order to prohibit endophytic *Phyllosticta* from causing disease. According to the correlation analysis and the results of the coculture experiment, the relationship between DN14, biomass, and dendrobine may depend on both direct and indirect mechanisms. As a direct mechanism, fungi colonize plants and produce biomolecules, such as enzymes, which are involved in ligustilide and other secondary metabolite biosynthesis.[17] Furthermore, fungi may produce certain compounds in the host plant, but they can only contribute to dendrobine accumulation when these compounds are absorbed and utilized by the plants.[14] Endophytic fungi produce biochemical signaling molecules, such as unsaturated fatty acids, small peptides, cyclodextrin, chitin, chitosan, and glycoproteins. These biomolecules act to induce gene expression and activate secondary metabolite biosynthetic pathways in the host plant, resulting in the increased accumulation of bioactive compounds.[50] In addition, endophytic fungi synthesize enzymes to convert precursor components into bioactive compounds in medicinal plants.[53] We speculate that DN14 is involved in the accumulation of secondary metabolites and that it synergistically increases the dendrobine content in wildland-associated *D. nobile*. Moreover, the dendrobine content in *Phyllosticta fallopiae* DN14-treated *D. nobile* may be attributed to gene regulation or synthetic biology. The upstream dendrobine biosynthetic pathway is composed of the mevalonate (MVA) and methylerythritol phosphate (MEP) pathways, which provide a basic skeleton for sesquiterpenoid alkaloids in *D. nobile*. For example, the *D. nobile* mycorrhizal fungus *Mycena* sp. MF23 participates in dendrobine biosynthesis by regulating postmodification enzyme (cytochrome P450, aminotransferase and methyltransferase) gene expression.[14,54] Sarsaiya et al. (2020) reported that *D. nobile* endophytic fungus *T. longibrachiatum* MD33 could produce the same dendrobine as the host.[16] They also found that the MVA pathway enzyme-coding genes (hydroxymethylglutaryl-CoA synthase, farnesyl diphosphate synthase and mevalonate kinase) might participate in sesquiterpene alkaloid metabolism in *T. longibrachiatum* isolated from *D. nobile*.[55] Xu et al. (2022) reported that the TPS and CYP450 gene families might also contribute to alkaloid biosynthesis in *D. nobile*.[56] Wang et al. (2022) reported that two dendrobine-type sesquiterpenoid alkaloids (6-hydroxydendrobine and nobilonine) from the *Dendrobium* endophytic bacterium *Pseudomonas protegens* CHA0 were synthesized mainly through the MEP pathway.[21] However, the mechanism through which *Phyllosticta* stimulates dendrobine accumulation is still unclear and requires further exploration. Although the exogenous fungi were found to have generally greater influence on the FW and dendrobine content of *D. nobile*, *Phyllosticta* was the only genus that was significantly positively correlated with both FW and dendrobine accumulation (Figure 8A). In subsequent experiments, we will consider isolating and screening the exogenous fungi in order to determine which strains promote the growth and secondary metabolism of *D. nobile*. In addition, endophytic and exogenous bacteria, such as *Pseudomonas* and *Bacillus*, also have many benefits for medicinal plants, including promoting plant growth, stress resistance, and secondary metabolite accumulation. Future research will be conducted to further screen the endophytic and exogenous bacteria and fungi associated with *D. nobile* and to examine the interaction between bacteria and fungi.

4. MATERIALS AND METHODS

4.1. Sample Collection and Processing. The biennial *D. nobile* samples were collected from the Town of Wanglong (28° 32′ 01″, 105° 51′ 52″, 300 m elevation), Chishui City, Guizhou Province, China. The study site is the main *D. nobile* production area and has a subtropical humid monsoon climate. The site has an annual average temperature of 18.1 °C, an annual rainfall of 1286.8 mm, and 350 frost-free days. The maximum and minimum temperatures occur in July and January, respectively.

The samples were collected from wild Danxia rock (WDR), large Danxia stone (LDS) (>20 cm × 20 cm) in a greenhouse, broken Danxia stone (BDS) (<2 cm × 2 cm) in a greenhouse, broken coarse sandstone (BCSS) (<2 cm × 2 cm) in a greenhouse, and wood spile (WS) grown in a greenhouse (Figure 10A−E). The surfaces of the stones and wood were covered with moss, which was removed at the time of collection. Each *D. nobile* sample was divided into three subsamples, with three biological replicates each: roots (R), stems (S), and leaves (L). Stem samples were divided into two subsamples for further analyses. To prevent contamination from other microbes, the leaves, stems, and roots of *D. nobile* were sterilized as previously described.[57] Successful surface sterilization was confirmed by plating the final rinse solution on potato dextrose agar (PDA) and culturing the plates for 72 h at 28 °C.[58] Each sample was precisely labeled, immediately
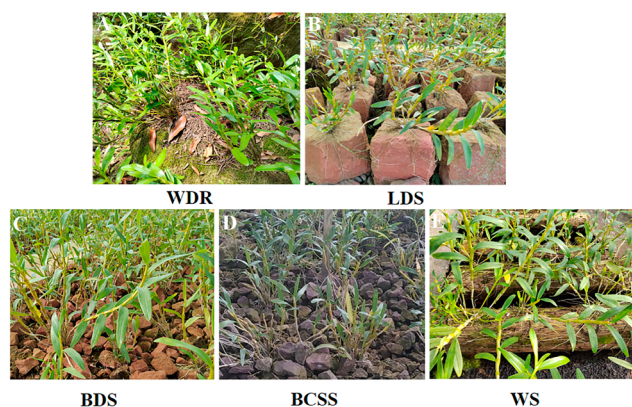
Figure 10. *D. nobile* samples were collected from wild Danxia rock (WDR) (A), large Danxia stone (LDS) (>20 cm × 20 cm) in a greenhouse (B), broken Danxia stone (BDS) (<2 cm × 2 cm) in a greenhouse (C), broken coarse sandstone (BCSS) (<2 cm × 2 cm) in a greenhouse (D), and wood spile (WS) in a greenhouse (E).

placed on ice, and subsequently stored in liquid nitrogen prior to DNA analysis. The surfaces of the rocks and wood posts were swabbed with sterile cotton swabs and stored at −80 °C for subsequent molecular analyses. Other samples were sieved using sterilized 2 mm sieves within 48 h of collection for chemical analysis, and stored within 24 h at 4 °C. In total, the study included 12 rock samples (four rock surfaces with three replicates each) and 45 plant samples (five plant types with three tissue types and three replicates each). The sample abbreviations used throughout the report are WDR roots (WDR_R), WDR stems (WDR_S), WDR leaves (WDR_L), LDS roots (LDS_R), LDS stems (LDS_S), LDS leaves (LDS_L), BDS roots (BDS_R), BDS stems (BDS_S), BDS leaves (BDS_L), BCSS roots (BCSS_R), BCSS stems (BCSS_S), BCSS leaves (BCSS_L), WS roots (WS_R), WS stems (WS_S), WS leaves (WS_L), WDR whole plant (WDR_WP), LDS whole plant (LDS_WP), BDS whole plant (BDS_WP), BCSS whole plant (BCSS_WP), and WS whole plant (WS_WP).

4.2. Determination of Biomass and Dendrobine Content. The total FW (g) of each sample was weighed using an analytical balance ($n = 5$). Each stem was then fully oven-dried for 36−48 h at 60 °C and subsequently ground into powder. GC sample preparation was carried out as specified by the Pharmacopoeia of China (2020). GC was carried out using a Shimadzu 2010 Plus GC−flame ionization detector (FID) using a Shimadzu SH-Rtx-1 capillary column (0.25 μm × 0.25 mm × 30 m), with nitrogen as the carrier gas. Each experiment was performed in triplicate. Each derivatized 1 μL sample was injected, and flame ionization detection was used to extract the components of the total ion chromatogram. The dendrobine concentration was found to be linear ($y = 794.74x − 7191.50$; $r = 0.9990$), with a peak area ranging from 20 to 200 μg/L. The internal standard (naphthalene) and experimental standard (dendrobine) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).

4.3. DNA Analysis of Fungal Communities on Rock and Wood Spile Surfaces and *D. nobile* Leaves, Stems, And Roots. Microbial DNA was extracted from rock and wood spile surfaces and leaves, stems, and roots of *D. nobile* with the FastDNA spin kit (MP Biomedicals, Santa Ana, CA). A NanoDrop 2000 UV−vis spectrophotometer (Thermo

Scientific, Wilmington, MA) was utilized to determine total DNA purity and concentration. Agarose (1%) gel electrophoresis was utilized to determine DNA quality. The internal transcribed spacer region 1 (ITS1 region) of rRNA was amplified using ITS1F-ITS2R primers.[20] The PCR amplification was carried out as follows: 94 °C for 3 min; 35 cycles at 94 °C for 40 s, 52 °C for 50 s, and 72 °C for 1 min; and final extension at 72 °C for 10 min.[20] Sequence library generation, purification, and amplification were performed according to a previously published method.[59] An Illumina MiSeq PE300 platform (Shanghai Majorbio Bio-Pharm Technology Co., Ltd.) was utilized to perform paired-end sequencing of the generated library. Genetic data generated in this experiment were deposited under accession number "PRJNA888816" at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA).

4.4. Isolation and Identification of Culturable Endophytic Fungi. The surfaces of roots and stems were sterilized (3 min in 75% ethanol, 5 min in 5% NaClO, and 30 s in 75% ethanol).[60] Sterilized roots were dissected into 1 cm segments, and leaves were dissected into 0.5 cm × 0.5 cm pieces. The sterilized samples were placed on potato dextrose agar (PDA) plates containing 200 mg L^{-1} ampicillin and incubated for 7−10 days at 25 °C. As the fungal outgrowth appeared, the morphological characteristics of the fungi were observed and recorded.[61] Single colonies were purified at least three times on the PDA medium. The endophytic fungi were identified by morphological and molecular identification following a previously published method.[62] The DNA of isolated endophytic fungi was amplified using the universal primers ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) and ITS5 (5′-TCCTCCGCTTAT TGATATGC-3′), and the amplified products were purified, cloned, and sequenced at Sangon Bioengineering Co., Ltd., Shanghai, China.[63]

4.5. Coculture of Endophytic Fungus with Tissue Culture Seedlings of *D. nobile*. All *D. nobile* seeds used in this study were collected from the Town of Wanglong, Chishui City, Guizhou Province, China. PDA plugs containing one of each of the 86 isolated endophytic fungal strains were used to inoculate *D. nobile* tissue culture seedlings for 60 days. Seedlings were grown in a 25 ± 1 °C greenhouse at 60−70% relative humidity under a 12 h/12 h light/dark cycle. Each treatment consisted of 3 plants. Successful coexistence between fungi and plant was defined according to the following grown characteristics: *D. nobile* plants exhibit tall, straight growth with green stems and leaves; fungal mycelium may or may not exhibit twining around the base of the stem. Unsuccessful coexistence was defined according to the following growth characteristics: *D. nobile* plants exhibit chlorotic stems and leaves, and may lodge or wither; fungal mycelium may exhibit twining around the upper part of the stem or leaves. Finally, we inoculated tissue culture seedlings with a coculture of 8 strains and PDA plug for 60 days. Each treatment consisted of 30 plants. Six plants were randomly selected and used to determine the FW. The method described in section 2.2 was used to determine the dendrobine content.

4.6. Statistical Analysis. Identical analytical procedures were utilized for both plant- and rock-derived fungi. Raw FASTQ files were demultiplexed and filtered with Trimmomatic and then merged with Flash (v1.2.11, https://ccb.jhu.edu/software/FLASH/index.shtml).[64] For endophytic fungi, UPARSE software v7.03 was used to cluster high-quality tags (>97% similarity) into operational taxonomic units (OTUs).[65]

The Ribosomal Database Project (RDP) Classifier v2.11 (https://sourceforge.net/projects/rdp-classifier/) within QIIME[66] was used to evaluate the taxonomy of each ITS sequence through comparison against the Silva databases (Release 119, http://www.arb-silva.de). R software v3.3.1 was used to conduct ANOVA, multidimensional, and Venn diagram analyses on OTUs. Differences in the fungal communities between samples were determined using saMothur software. Mothur was used to calculate the alpha diversity indices at the OTU level. The beta diversity indices were calculated with QIIME software using the weighted UniFrac distances between samples.[67] The normalized genera count table was imported into SIMCA-P+ 12.0 software for partial least-squares discriminant analysis (PLS-DA).[68] Ggplot2 packages of the R software (Version 3.6.0) were applied to draw principal coordinates analysis (PCoA) diagrams.[69] Linear discriminant analysis (LDA) was conducted using linear discriminant analysis effect size (LEfSe), a biomarker discovery algorithm for the identification of taxa and the characterization of metadata class differences.[70] All analyses were performed using the Majorbio Cloud Platform provided by Shanghai Majorbio BioPharm Technology Co., Ltd.

Dendrobine content and growth parameters were analyzed using one-way analysis of variance (ANOVA) with IBM SPSS Statistics 24.0. Graphical results were created using GraphPad Prism 7.0 and were presented as the mean ± standard deviation (SD). The spearman correlation analyses of the top 30 abundant genera, biomass, and dendrobine contents were calculated and performed on a heatmap using R v3.5.1.[71]

5. CONCLUSION

The diversity and composition of the *D. nobile*-associated fungal communities across five habitats were significantly different. The diversity and richness of the exogenous fungal community from the wild habitat were much higher than those from the greenhouse habitats. However, no significant differences were observed in the diversity and richness of endophytic fungi. Many common genera of endophytic and exogenous fungi were identified across habitats, while each habitat was also found to contain distinct fungal strains. The compositions of endophytic and exogenous fungi accounted for the differences between dendrobine content and biomass among different *D. nobile* samples. Exogenous fungi affected the accumulation of biomass and dendrobine more than endophytic fungi. Specifically, *D. nobile* samples with higher richness and diversity of exogenous fungi exhibited higher dendrobine content and FW. The endophyte *Phyllosticta* was the only fungal genus that was significantly positively correlated with both FW and dendrobine content. In the coculture trial, DN14 (*Phyllosticta fallopiae*) was found to enhance the accumulation of both biomass and dendrobine. The results of this study will aid in the development of strategies to increase the production of dendrobine in *D. nobile*. This work could also facilitate the screening of beneficial endophytic and exogenous fungal probiotics for use as biofertilizers in *D. nobile*.

ASSOCIATED CONTENT

Data Availability Statement

The transcriptome data used in this manuscript were submitted to the NCBI, and the Accession number is PRJNA888816.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c00608.

Detailed information on endophytic fungi and exogenous fungi from *D. nobile* across different habitats (PDF)

AUTHOR INFORMATION

Corresponding Authors

Bo Zhu − *School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China;* orcid.org/0000-0003-0281-3585; Email: zhubo@zcmu.edu.cn

Luping Qin − *School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China;* Email: lpqin@zcmu.edu.cn

Authors

Wenhua Chen − *School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China*

Jingxuan Wang − *School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China*

Jia Song − *School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China*

Qingmei Sun − *School of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acsomega.3c00608

Author Contributions

W.C., B.Z., and L.Q. developed the concept and designed the laboratory experiment. W.C., J.W., and J.S. conducted all experiments. W.C. analyzed data. Q.S. collected plant samples. W.C., B.Z., and L.Q. edited the paper. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) *Pharmacopoeia of the People's Republic of China I*; China Pharmacopoeia Committee: Beijing, 2020, pp 195−196.

(2) Pan, H.; Shi, F. G.; Fang, C.; Shi, J. S. Metabolic characterization of a potent natural neuroprotective agent dendrobine *in vitro* and in rats. *Acta Pharmacol. Sin.* **2022**, *43*, 1059−1071.

(3) Ding, S. K.; Wang, L. X.; Guo, L. S.; Luo, P.; Du, J. J.; Zhao, Z. L.; Wang, G. G. Syringic acid inhibits apoptosis pathways via downregulation of p38MAPK and JNK signaling pathways in H9c2 cardiomyocytes following hypoxia/reoxygenation injury. *Mol. Med. Rep.* **2017**, *16*, 2290−2294.

(4) Li, S. Y.; Zhou, J. X.; Xu, S. F.; Li, J.; Liu, J.; Lu, Y. F.; Shi, J. S.; Zhou, S. Y.; Wu, Q. Induction of Nrf2 pathway by *Dendrobium nobile* Lindl. alkaloids protects against carbon tetrachloride induced acute liver injury. *Biomed. Pharmacother.* **2019**, *117*, 109073−109080.

(5) Shin, H. K.; Kim, T. W.; Kim, Y. J.; Park, S. R.; Seo, C. S.; Ha, H.; Jung, J. Y. Protective effects of *Dendrobium nobile* against cisplatin nephrotoxicity both *in-vitro* and *in-vivo*. *Iran. J. Pharm. Res.* **2017**, *6*, 197−206.

(6) Zhang, S.; Tu, H. Y.; Zhu, J. M.; Liang, A. H.; Huo, P.; Shan, K.; He, J. Y.; Zhao, M.; Chen, X.; Lei, X. C. *Dendrobium nobile* Lindl. polysaccharides improve follicular development in PCOS rats. *Int. J. Biol. Macromol.* **2020**, *149*, 826−834.

(7) Fan, Y. J.; Yu, Q. S.; Wang, G.; Tan, J. W.; Liu, S.; Pu, S. R.; Chen, W. C.; Xie, P.; Zhang, Y. X.; Zhang, J.; Liao, Y. X.; Luo, A. X. Effects of non-thermal plasma treatment on the polysaccharide from *Dendrobium nobile* Lindl. and its immune activities *in vitro*. *Int. J. Biol. Macromol.* **2020**, *153*, 942−950.

(8) Li, L. S.; Lu, Y. L.; Nie, J.; Xu, Y. Y.; Zhang, W.; Yang, W. J. *Dendrobium nobile* Lindl alkaloid, a novel autophagy inducer, protects against axonal degeneration induced by Aβ25−35 in hippocampus neurons *in vitro*. *CNS Neurosci. Ther.* **2017**, *23*, 329−340.

(9) Nie, J.; Jiang, L. S.; Zhang, Y.; Tian, Y.; Li, L. S.; Lu, Y. L.; Yang, W. J.; Shi, J. S. *Dendrobium nobile* Lindl. alkaloids decreases the level of intracellular β-amyloid by improving impaired autolysosomal proteolysis in APP/PS1 mice. *Front. Pharmacol.* **2018**, *9*, 1479.

(10) Zhang, Y. Y.; Zhang, Q. Q.; Xin, W.; Liu, N.; Zhang, H. Nudol, a phenanthrene derivative from *Dendrobium nobile*, induces cell cycle arrest and apoptosis and inhibits migration in osteosarcoma cells. *Drug Des. Devel. Ther.* **2019**, *13*, 2591−2601.

(11) Chao, W. H.; Lai, M. Y.; Pan, H. T.; Shiu, H. W.; Chen, M. M.; Chao, H. M. *Dendrobium nobile* Lindley and its bibenzyl component moscatilin are able to protect retinal cells from ischemia/hypoxia by dowregulating placental growth factor and upregulating norrie disease protein. *BMC Complement. Altern. Med.* **2018**, *18*, 193.

(12) Deng, W.; Ding, Z.; Wang, Y.; Zou, B.; Zheng, J.; Tan, Y.; Yang, Q.; Ke, M.; Chen, Y.; Wang, S.; Li, X. Dendrobine attenuates osteoclast differentiation through modulating ROS/NFATc1/MMP9 pathway and prevents inflammatory bone destruction. *Phytomedicine.* **2022**, *96*, 153838.

(13) Song, T. H.; Chen, X. X.; Lee, C. K.; Sze, S. C.; Feng, Y. B.; Yang, Z. J.; Chen, H. Y.; Li, S. T.; Zhang, L. Y.; Wei, G.; Shi, J.; Xu, K.; Ng, T. B.; Zhu, L. L.; Zhang, K. Y. Dendrobine targeting JNK stress signaling to sensitize chemotoxicity of cisplatin against non-small cell lung cancer cells *in vitro* and *in vivo*. *Phytomedicine.* **2019**, *53*, 18−27.

(14) Li, Q.; Ding, G.; Li, B.; Guo, S. X. Transcriptome analysis of genes involved in dendrobine biosynthesis in *Dendrobium nobile* Lindl. Infected with mycorrhizal fungus MF23 (*Mycena* sp.). *Sci. Rep.* **2017**, *7*, 316.

(15) Gong, D. Y.; Chen, X. Y.; Guo, S. X.; Wang, B. C.; Li, B. Recent advances and new insights in biosynthesis of dendrobine and sesquiterpenes. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 6597−6606.

(16) Sarsaiya, S.; Jain, A.; Fan, X. K.; Jia, Q.; Xu, Q.; Shu, F. X.; Zhou, Q.; Shi, J. S.; Chen, J. S. New insights into detection of a dendrobine compound from a novel endophytic *Trichoderma longibrachiatum* strain and its toxicity against phytopathogenic bacteria. *Front. Microbiol.* **2020**, *11*, 337.

(17) Jia, M.; Chen, L.; Xin, H. L.; Zheng, C. J.; Rahman, K.; Han, T.; Qin, L. P. A friendly relationship between endophytic fungi and medicinal plants: A systematic review. *Front. Microbiol.* **2016**, *7*, 906−920.

(18) Liarzi, O.; Bucki, P.; Braun Miyara, S.; Ezra, D. Bioactive volatiles from an endophytic *Daldinia cf. concentrica* isolate affect the viability of the plant parasitic nematode *Meloidogyne javanica*. *PloS ONE* **2016**, *11*, No. e0168437.

(19) Guarnaccia, V.; Gehrmann, T.; Silva-Junior, G. J.; Fourie, P. H.; Haridas, S.; Vu, D.; Spatafora, J.; Martin, F. M.; Robert, V.; Grigoriev, I. V.; Groenewald, J. Z.; Crous, P. W. *Phyllosticta citricarpa* and sister species of global importance to *Citrus*. *Mol. Plant. Pathol.* **2019**, *20*, 1619−1635.

(20) Chen, W. H.; Wu, S. J.; Sun, X. L.; Feng, K. M.; Rahman, K.; Tan, H. Y.; Yu, L. Y.; Xu, L. C.; Qin, L. P.; Han, T. High-throughput sequencing analysis of endophytic fungal diversity in *Cynanchum* sp. *South Afr. J. Bot.* **2020**, *134* (6), 349−358.

(21) Wang, S. S.; Liu, J. M.; Sun, J.; Huang, Y. T.; Jin, N.; Li, M. M.; Liang, Y. T.; Fan, B.; Wang, F. Z. Analysis of endophytic bacterial diversity from different *Dendrobium* stems and discovery of an endophyte produced dendrobine-type sesquiterpenoid alkaloids. *Front. Microbiol.* **2022**, *12*, 775665.

(22) Makadia, T. H.; Adetutu, E. M.; Simons, K. L.; Jardine, D.; Sheppard, P. J.; Ball, A. S. Re-use of remediated soils for the bioremediation of waste oil sludge. *J. Environ. Manage.* **2011**, *92*, 866−871.

(23) Rougeron, A.; Giraud, S.; Alastruey-Izquierdo, A.; Cano-Lira, J.; Rainer, J.; Mouhajir, A.; Le Gal, S.; Nevez, G.; Meyer, W.; Bouchara, J. P. Ecology of *Scedosporium* species: Present knowledge and future research. *Mycopathologia.* **2018**, *183*, 185−200.

(24) Zhu, L.; Yan, H.; Liu, P.; Zhang, Z. Y.; Zhang, S.; Guo, S.; Jiang, S.; Duan, J. A. Research progress on effects of rhizosphere microorganisms on quality formation of medicinal plants and their interaction mechanisms. *Chin. Herb Med.* **2021**, *52*, 4064−4072.

(25) Chen, W.; Wang, Q.; He, L. Y.; Sheng, X. F. Changes in the weathering activity and populations of culturable rock-weathering bacteria from the altered purple silt stone and the adjacent soil. *Geomicrobiol. J.* **2016**, *33*, 724−733.

(26) Wang, Q.; Cheng, C.; Agathokleous, E.; Liu, Y.; Li, X.; Sheng, X. Enhanced diversity and rock-weathering potential of bacterial communities inhabiting potash trachyte surface beneath mosses and lichens-A case study in Nanjing, China. *Sci. Total Environ.* **2021**, *785*, 147357−147367.

(27) Bussey, R. O.; Kaur, A.; Todd, D. A.; Egan, J. M.; El-Elimat, T.; Graf, T. N.; Raja, H. A.; Oberlies, N. H.; Cech, N. B. Comparison of the chemistry and diversity of endophytes isolated from wild-harvested and greenhouse-cultivated *Yerba mansa* (*Anemopsis californica*). *Phytochem. Lett.* **2015**, *11*, 202−208.

(28) Faddetta, T.; Abbate, L.; Alibrandi, P.; Arancio, W.; Siino, D.; Strati, F.; De, F. C.; Fatta, D. B. S.; Carimi, F.; Puglia, A. M.; Cardinale, M.; Gallo, G.; Mercati, F. The endophytic microbiota of *Citrus limon* is transmitted from seed to shoot highlighting differences of bacterial and fungal community structures. *Sci. Rep.* **2021**, *11*, 7078.

(29) Kang, X.; Csetenyi, L.; Gadd, G. M. Colonization and bioweathering of monazite by *Aspergillus niger*: solubilization and precipitation of rare earth elements. *Environ. Microbiol.* **2021**, *23*, 3970−3986.

(30) Wang, Y.; Guo, L. D.; Cheng, H. Y.; Zhao, L.; Chang, F.; Wan, Y.; Wang, H. P. Comparison of endophytic and soil fungi of *Paris polyphylla* diversity from different habitat. *Microbiol. China.* **2020**, *47*, 2867−2876.

(31) Wu, X. F.; Yang, J. J.; Ruan, H.; Wang, S. N.; Yang, Y. R.; Naeem, I.; Wang, L.; Liu, L.; Wang, D. The diversity and co-occurrence network of soil bacterial and fungal communities and their implications for a new indicator of grassland degradation. *Ecol. Indic.* **2021**, *129*, 107989.

(32) Crous, P. W.; Carlier, J.; Roussel, V.; Groenewald, J. Z. *Pseudocercospora* and allied genera associated with leaf spots of banana (*Musa* spp.). *Fungal Syst. Evol.* **2021**, *7*, 1−19.

(33) Alves, J. L.; Barreto, R. W. *Pseudocercospora ixoricola* causing leaf spots on *Ixora coccinea* in Brazil. *Plant Dis.* **2010**, *94*, 278.

(34) Kumla, J.; Nundaeng, S.; Suwannarach, N.; Lumyong, S. Evaluation of multifarious plant growth promoting trials of yeast isolated from the soil of assam tea (*Camellia sinensis* var. assamica) plantations in Northern Thailand. *Microorganisms.* **2020**, *8*, 1168.

(35) Tian, L.; Ou, J.; Sun, X.; Miao, Y.; Pei, J.; Zhao, L.; Huang, L. The discovery of pivotal fungus and major determinant factor shaping soil microbial community composition associated with rot root of *American ginseng*. *Plant Signal Behav.* **2021**, *16*, 1952372.

(36) Beibei, C.; Wang, M.; Hu, Y. L.; Lin, Z. P.; Yu, R. M.; Huang, L. Q. Preliminary study on promoting effects of endophytic fungi to growth of *Rehmannia glutinosa*. *CJCMM* **2011**, *36*, 1137−1140.

(37) Kömpe, Y. O.; Mutlu, V. A. Ex *vitro* symbiotic germination of the seeds of *Anacamptis coriophora* (L.) R.M. Bateman, Pritgeon &

M.W. Chase and *Orchis anatolica* boiss. *Biol. Futur.* **2021**, *72*, 509−516.

(38) Spinelli, V.; Brasili, E.; Sciubba, F.; Ceci, A.; Giampaoli, O.; Miccheli, A.; Pasqua, G.; Persiani, A. M. Biostimulant effects of *Chaetomium globosum* and *Minimedusa polyspora* culture filtrates on *Cichorium intybus* plant: Growth performance and metabolomic traits. *Front. Plant Sci.* **2022**, *13*, 879076.

(39) Fu, J.; Xiao, Y.; Wang, Y. F.; Liu, Z. H.; Yang, K. Saline-alkaline stress in growing maize seedlings is alleviated by *Trichoderma asperellum* through regulation of the soil environment. *Sci. Rep.* **2021**, *11*, 11152.

(40) Bononi, L.; Chiaramonte, J. B.; Pansa, C. C.; Moitinho, M. A.; Melo, I. S. Phosphorus-solubilizing *Trichoderma* spp. from amazon soils improve soybean plant growth. *Sci. Rep.* **2020**, *10*, 2858.

(41) Kudjordjie, E. N.; Sapkota, R.; Nicolaisen, M. *Arabidopsis* assemble distinct root-associated microbiomes through the synthesis of an array of defense metabolites. *PloS One.* **2021**, *16*, No. e0259171.

(42) Hou, Q. Z.; Chen, D. W.; Wang, Y. P.; Ehmet, N.; Ma, J.; Sun, K. Analysis of endophyte diversity of two *Gentiana* plants species and the association with secondary metabolite. *BMC Microbiol.* **2022**, *22*, 90.

(43) Tong, A. Z.; Liu, W.; Liu, Q.; Xia, G. Q.; Zhu, J. Y. Diversity and composition of the *Panax ginseng* rhizosphere microbiome in various cultivation modesand ages. *BMC Microbiol.* **2021**, *21*, 18.

(44) Yu, H.; Zhao, J. X.; You, J.; Li, J. N.; Ma, H. Y.; Chen, X. Factors influencing cultivated ginseng (*Panax ginseng* C. A. Meyer) bioactive compounds. *PLoS One.* **2019**, *14*, No. e0223763.

(45) Yang, J. *Community composition of the related fungi from Eucommia ulmoides Oliv. and its active constituents*. Guizhou University. M., 2019.

(46) Taher, M. A.; Tan, W. N.; Chear, N. J.; Leong, C. R.; Rashid, S. A.; Tong, W. Y. Metabolites haracterization of endophytic *Phyllosticta fallopiae* L67 isolated from *Aloe vera* with antimicrobial activity on diabetic wound microorganisms. *Nat. Prod. Res.* **2022**, *7*, 1−6.

(47) Zhu, X. X.; Liu, Y.; Hu, Y. W.; Lv, X.; Shi, Z. X.; Yu, Y. Y.; Jiang, X. Y.; Feng, F.; Xu, J. Neuroprotective activities of constituents from *Phyllosticta capitalensis*, an endophyte fungus of *Loropetalum chinense* var. *rubrum*. *Chem. Biodivers.* **2021**, *18*, No. e2100314.

(48) Fan, Y. J.; Jiang, T.; Chun, Z.; Wang, G.; Yang, K. G.; Tan, X. Y.; Zhao, J.; Pu, Sr.; Luo, A. X. Zinc affects the physiology and medicinal components of *Dendrobium nobile* Lindl. *Plant Physiol. Biochem.* **2021**, *162*, 656−666.

(49) Zhou, L. S.; Tang, K.; Guo, S. X. The plant growth-promoting fungus (PGPF) *Alternaria* sp. A13 markedly enhances *Salvia miltiorrhiza* root growth and active ingredient accumulation under greenhouse and field conditions. *Int. J. Mol. Sci.* **2018**, *19*, 270−283.

(50) Zhai, X.; Jia, M.; Chen, L.; Zheng, C. J.; Rahman, K.; Han, T.; Qin, L. P. The regulatory mechanism of fungal elicitor-induced secondary metabolite biosynthesis in medical plants. *Crit. Rev. Microbiol.* **2017**, *43*, 238−261.

(51) Rahman, M. H.; Hasan, M. N.; Amin, R.; Setu, M. A. A.; Akter, S.; Nigar, S.; Khan, M. Z. H.; Khan, M. Z. H. Mixed nanocomposite fertilizers influencing endophytic symbiosis and nutritional and antioxidant properties of *Oryza sativa* as a sustainable alternative for commercial fertilizers. *ACS Omega.* **2022**, *7*, 6787−6794.

(52) Hu, X. Y.; Li, X. M.; Yang, S. Q.; Liu, H.; Meng, L. H.; Wang, B. G. Three New Sesquiterpenoids from the algal-derived fungus *Penicillium chermesinum* EN-480. *Mar. Drugs.* **2020**, *18*, 194.

(53) Liu, G.; Lai, D.; Liu, Q. Z.; Zhou, L.; Liu, Z. L. Identifcation of nematicidal constituents of notopterygium incisum rhizomes against *Bursaphelenchus xylophilus* and *Meloidogyne incognita*. *Molecules.* **2016**, *21*, 1276.

(54) Chen, X.; Li, Q.; Xu, X.; Ding, G.; Guo, S.; Li, B. Effects of the endophytic fungus MF23 on *Dendrobium nobile* Lindl. in an artificial primary environment. *ACS Omega.* **2021**, *6*, 10047−10053.

(55) Qian, X.; Jin, H.; Chen, Z. J.; Dai, Q. Q.; Sarsaiya, S.; Qin, Y. T.; Jia, Q.; Jin, L. L. L.; Chen, J. S. Comparative transcriptome analysis of genes involved in sesquiterpene alkaloid biosynthesis in *Trichoderma longibrachiatum* MD33 and UN32. *Front. Microbiol.* **2021**, *12*, 800125.

(56) Xu, Q.; Niu, S. C.; Li, K. L.; Zheng, P. J.; Zhang, X. J.; Jia, Y.; Liu, Y.; Niu, Y. X.; Yu, L. H.; Chen, D. F.; Zhang, G. Q. Chromosome-scale assembly of the *Dendrobium nobile* genome provides insights into the molecular mechanism of the biosynthesis of the medicinal active ingredient of *Dendrobium*. *Front. Genetics.* **2022**, *13*, 844622.

(57) Li, Q.; Ding, G.; Li, B.; Guo, S. X. Transcriptome analysis of genes involved in dendrobine biosynthesis in *Dendrobium nobile* Lindl. Infected with mycorrhizal fungus MF23 (*Mycena* sp.). *Sci. Rep.* **2017**, *7*, 316.

(58) Cui, J. L.; Vinod, V.; Gang, Z. Partitioning of fungal endophyte assemblages in root parasitic plant *Cynomorium songaricum* and its host *Nitraria tangutorum*. *Front. Microbiol.* **2018**, *9*, 666.

(59) Yang, G.; Wang, J. Kinetics and microbial community analysis for hydrogen production using raw grass inoculated with different pretreated mixed culture. *Bioresour. Technol.* **2018**, *247*, 954−962.

(60) Ye, B.; Wu, Y.; Zhai, X.; Zhang, R.; Wu, J.; Zhang, C.; Rahman, K.; Qin, L.; Han, T.; Zheng, C. Beneficial effects of endophytic fungi from the *Anoectochilus* and *Ludisia* species on the growth and secondary metabolism of *Anoectochilus roxburghii*. *ACS Omega.* **2020**, *5*, 3487−3497.

(61) Cui, L.; Noushahi, H. A.; Zhang, Y.; Liu, J.; Cosoveanu, A.; Liu, Y.; Yan, L.; Zhang, J.; Shu, S. Endophytic fungal community of *Huperzia serrata*: Diversity and relevance to the production of huperzine A by the plant host. *Molecules.* **2021**, *26*, 892−910.

(62) Jin, L. R.; Yang, L.; Li, W. J.; Xu, D.; Yang, N. N.; Li, G. Q.; Wan, P. Diversity and biocontrol potential of culturable endophytic fungi in cotton. *Front. Microbiol.* **2021**, *12*, 698930.

(63) Zhang, Q. H.; Zhang, J.; Yang, L.; Zhang, L.; Jiang, D.; Chen, W. D.; Li, G. Q. Diversity and biocontrol potential of endophytic fungi in *Brassica napus*. *Biol. Control.* **2014**, *72*, 98−108.

(64) Magoc, T.; Salzberg, S. L. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* **2011**, *27*, 2957−2963.

(65) Edgar, R. C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* **2010**, *26*, 2460−2461.

(66) Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F. O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic. Acids. Res.* **2012**, *41*, 590−600.

(67) Chen, J.; Bittinger, K.; Charlson, E. S.; Hofmann, C.; Lewis, J.; Wu, G. D.; Collman, R. G.; Bushman, F. D.; Li, H. Z. Associating microbiome composition with environmental covariates using generalized UniFrac distances. *Bioinformatic.* **2012**, *28*, 2106−2113.

(68) Wang, B. H.; Jiang, X. Y.; Cao, M.; Ge, J. P.; Bao, Q. L.; Tang, L. L.; Chen, Y.; Li, L. J. Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. *Sci. Rep.* **2016**, *6*, 32002.

(69) Shen, M. C.; Zhang, Y. Z.; Bo, G. D.; Yang, B.; Wang, P.; Ding, Z. Y.; Wang, Z. B.; Yang, J. M.; Zhang, P.; Yuan, X. L. Microbial responses to the reduction of chemical fertilizers in the rhizosphere soil of flue-cured tobacco. *Front. Bioeng. Biotechnol* **2022**, *9*, 812316.

(70) Huh, J. W.; Roh, T. Y. Opportunistic detection of *Fusobacterium nucleatum* as a marker for the early gut microbial dysbiosis. *BMC Microbiol.* **2020**, *20*, 208.

(71) Feng, W. M.; Liu, P.; Yan, H.; Zhang, S.; Shang, E. X.; Yu, G.; Jiang, S.; Qian, D. W.; Ma, J. W.; Duan, J. A. Impact of bacillus on phthalides accumulation in *Angelica sinensis* (Oliv.) by stoichiometry and microbial diversity analysis. *Front. Microbiol.* **2021**, *11*, 611143.