The Temporal and spatial endophytic fungal community of *Huperzia serrata*: diversity and relevance to huperzine A production by the host

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Abstract

Background

Plants maintain the steady-state balance of the mutually beneficial symbiosis relationship of their endophytic fungi through secondary metabolites. Meanwhile endophytic fungi can serve as biological inducers to promote the biosynthesis and accumulation of valuable secondary metabolites in host plants through a variety of ways. The composition and structure of endophytic fungal community are affected by many factors, including tissues, seasons and so on. We studied the community diversity, temporal and spatial pattern of endophytic fungi isolated from the roots, stems and leaves of *Huperzia serrata* in different seasons. The correlation between endophytic fungi and huperzine A content in plants was analyzed.

Results

A total of 7005 operational taxonomic units were isolated, and all strains were identified as 14 phyla, 54 classes, 140 orders, 351 families, 742 genera. Alpha diversity analysis showed that the diversity of endophytic fungi in stem and leaf was higher than that in root, and the diversity in summer (August) was lower than that in other months. NMDS analysis showed that the endophytic fungal communities of leaves, stems and roots were significantly different, and the root and leaf communities were also different between four seasons. Through correlation analysis, it was found that 33 genera of the endophytic fungi of *Huperzia serrata* showed a significant positive correlation with the content of huperzine A ($p < 0.05$), of which 13 genera (*Strelitziana*、*Devriesia*、*Articulospora*、*Derxomyces*、*Cyphellophora*、*Trechispora*、*Kurtzmanomyces*、*Capnobotryella*、*Erythrobasidium*、*Camptophora*、*Stagonospora*、*Lachnum*、*Golubevia*) showed a highly significant positive correlation with the content of huperzine A ($p < 0.01$). These endophytic fungi may have the potential to promote the biosynthesis and accumulation of huperzine A in plant.

Conclusions

This report is the first time to analyze the diversity of endophytic fungi in tissues of *Huperzia serrata* from different seasons, which proves that there is variability in different tissues and seasonal distribution patterns. These findings provide references to the study of Endophytic Fungi of *Huperzia serrata.*

Introduction

Huperzia serrata (Thunb. ex Murray) Trev., also known as Qian Ceng Ta, is a perennial medicinal fern of Huperzia in Lycopodiaceae. It has the curative effects of hemostasis, removing blood stasis, detoxification and treatment of schizophrenia[1–4]. Alkaloids, triterpenoids and flavonoids are the main effective ingredients of *H. serrata*[5].

Huperzine A (HupA), isolated from *H. serrata* in 1986, has been proved to be a highly selective and reversible acetylcholinesterase inhibitor with a new chemical structure, and has a strong efficacy in the treatment of Alzheimer disease (AD) and myasthenia gravis[4, 6, 7]. HupA has been approved as a drug for the treatment of AD in China[8] and is used as a supplement to prevent further memory degradation in the United States[9]. At present, there are about 50 million AD patients in the world, and the aging population of society will further lead to a continuous increase in the number of AD patients[10]. However, the content of HupA in the wild *H. serrata* is low (0.0046%-0.0133%)[11], and the plants grow slowly, which is difficult to meet the market demand. Overexploitation and habitat fragmentation have made *H. serrata* an endangered plant in China[12, 13]. At present, the chemical synthesis of HupA is not suitable for industrial production[14], as is the artificial cultivation technology and tissue culture technology of *H. serrata*[15].

Endophytic fungi are considered to be microorganisms in cells or intercellular spaces at a certain life stage or throughout the life cycle in healthy plants, inducing hidden and asymptomatic infections in plant tissues, without causing disease symptoms. Among them, fungi that have a dormant or latent period in plant tissues before causing disease symptoms on the host plants are still endophytic fungi, although these fungi are clearly pathogenic; pathogenic fungi that are parasitic in plants but where disease symptoms do not appear after infection are also endophytic fungi[16]. Endophytic fungi obtain most or all of the nutrients from the host plants and provide ecological benefits to the plants in return. Apparent host benefits include improved tolerance to heavy metals, increased drought resistance, reduced herbivory, systemic resistance against pathogens, and generally enhanced growth[17]. At present, researchers have isolated some endophytic fungi, which produce HupA, from *H. serrata* and other Phlegmariurus by using conventional methods of isolation and purification of endophytic fungi[18–29]. In addition, endophytic fungi also promote the production of secondary metabolites of host plants by stimulating key genes in the plant biosynthesis pathway and synthesizing enzymes that can convert precursors to active compounds or their analogues[30–32]. Therefore, it is necessary to study the diversity and composition of Endophytic Fungi of *H. serrata*. It is reported that the content of HupA produced by plants varies from different tissues and seasons[11]. Several studies have also shown that endophytic fungal community composition is correlated with plant tissues and seasons[33–35].

In this study, we explored the temporal and spatial diversity of endophytic fungal community of *H. serrata*. Furthermore, endophytic fungi with significant positive correlation between HupA content of *H. serrata* were screened. It provides more reference to a clearer understanding of the fungal community ecology of this important medicinal plant, and helps to uncover endophytic fungi with important functions.

Results

Classification and distribution of Endophytic Fungi

A total of 7005 operational taxonomic units (OTUs) were detected in 36 samples(4 seasons × 3 tissues × 3 replicates)of *H. serrata*. In all samples, the rarefaction curves tended to be flat, indicating that the sequencing depth was sufficient (Fig. 1). According to ITS sequence classification, all strains were identified as 14 phyla, 54 classes, 140 orders, 351 families, 742 genera (including unclassified and unidentified groups). At the phylum level, Ascomycota and Basidiomycota were dominant phyla in each sample, accounting for 54% and 25% of the sequence, respectively (Fig. 2). The main classes of Ascomycota were Eurotiomycetes (20%), Dothideomycetes (12%) and Leotiomycetes (11%). The main classes of Basidiomycota were Agaricomycetes (11%) and Tremellomycetes (8%) (Fig. S1).

At the genus level, *Cladophialophora* (8%), *Sebacina* (3%), *Cladosporium* (2%), *Russula* (2%), *Tausonia* (2%), *Trichomerium* (2%) and *Cypellophora* (2%) were the top 7 genera with high abundance, and they were distributed among different tissues in different seasons (Fig. S1). The dominant endophytic fungal groups (genera with relative abundance > 2%) varied from different tissues and seasons. In tissue distribution, the dominant genera in leaf were *Cladosporium, Cladophialophora, Tausonia, Cypellophora* and *Endophora*. The dominant genera in the stem were *Cladophialophora, Trichomerium* and *Cypellophora*. The dominant genera in the root were *Sebacina, Cladophialophora, Russula, Cystofilobasidium, Chloridium* and *Oidiodendron*. *L*atifluus was a genus of fungi endemic to roots (Fig. 3).

In terms of seasonal distribution, the dominant fungal genera in February were *Cladophialophora, Tausonia, Leucosporidium*, and *Russula*. In May, the dominant fungal genera were *Cladophialophora、Chloridium、Russula、Trichomerium、Cyphellophora* and *Mortierella*. In August, the dominant fungal genera were *Sebacina、Cladosporium、Cystofilobasidium、Cladophialophora、Dioszegia、Endophoma、Lactifluus* and *Tausonia.* In November, the dominant fungal genera were *Cladophialophora、Russula* and *Cyphellophora. Latifluus* was a genus of fungi endemic to August. *Chloridium* was not detected in the samples of August (Fig. 4).

Diversity of Endophytic Fungi

A total of 7005 OTUs were detected in all ITS libraries, of which 2547, 2888 and 3490 OTUs were detected in root, stem and leaf samples respectively. Of these, only 432 OTUS were common to the three tissues (Fig. 5a). The number of unique and common OUTs for the three tissues was leaf > stem > root. The endophytic fungal communities in three tissues were calculated alpha diversity values. The depth index (Goods coverage) of each sample library was more than 99.9%, indicating that the sampling was reasonable. Shannon, Simpson's and Pielou's evenness indexes of stems were significantly higher than those of roots (Fig. 6a). In general, all diversity indexes showed that the highest richness, diversity and evenness of endophytic fungal community was the stem, followed by the leaf, and the lowest was the root.

The detected OUTs were clustered in four months. 1724, 2182, 3003 and 2608 OTUs were detected in M8, M11, M2 and M5 respectively. Of these, only 280 OTUS were common to the four months (Fig. 5b).

Overall, the total and unique OTUs in 4 months were M2 > M5 > M11 > M8. The endophytic fungal communities in four months were calculated alpha diversity values. The Simpson's index and Pielou's evenness index in M5 were significantly higher than those in M8 (Fig. 6b). This indicates that the richness, diversity and evenness of endophytic fungi were highest in M5 and lowest in M8.

The Temporal and spatial patterns of endophytic fungal community

The similarity between endophytic fungi in different tissues and seasons was analyzed by Non-metric multidimensional scaling (NMDS). When the stress value of NMDS result is less than 0.2, it is considered that the result of NMDS analysis is more reliable.

The results showed that there were highly significant differences ($R^2 = 0.137$, $p = 0.001$) in endophytic fungi between different tissues (Fig. 7a). The results of four months' analysis showed that there were highly significant differences ($p < 0.01$) in the community distribution of endophytic fungi between the three tissues in August and November (Fig. 7b, 7c). In February and May, the endophytic fungal communities of leaf and stem were similar, and they were significantly different from those of root ($p < 0.05$) (Fig. 7d, 7e).

The grouping test results showed that there were significant differences ($R^2 = 0.0983$, $p = 0.024$) in fungal communities of endophytic fungi between different months, but NMDS analysis showed that stress > 0.2 (Fig. 8a). Therefore, we analyzed the three tissues independently, and the endophytic fungal communities of the three tissues were significantly different ($p < 0.05$) between four months. The results of NMDS analysis showed that the grouping results of roots and leaves were more reliable (stress < 0.2) (Fig. 8b, 8d), while the fungal communities of stems were not reliable for grouping between four months (Fig. 8c). The fungal communities of roots were similar in November and May. The fungal communities of leaves were similar in February, August and November. The fungal communities of stems in August and November were similar, those in February and May were similar.

Spatial and temporal distribution of endophytic fungi producing HupA

Using conventional methods of isolation and purification of endophytic fungi, researchers have isolated many endophytic fungi producing HupA from *H. serrata*. In this study, we detected 13 genera of HupA-producing endophytic fungi. The relative abundance of these 13 genera of endophytic fungi in different tissues and seasons were analyzed. In different tissues, there were 7 genera in leaf(*Acremonium*、*Alternaria*、*Arthrinium*、*Botrytis*、*Cladosporium*、*Colletotrichum*、*Podospora*), 4 genera in stem(*Aspergillus*、*Leptosphaeria*、*Mucor*、*Penicillium*) and 2 genera in root(*Fusarium*、*Trichoderma*)with higher relative abundance (Fig. 9a). In general, the endophytic fungi producing HupA had the highest relative abundance in leaf, followed by stem, and the lowest relative abundance in root.

The relative abundance of these 13 genera of HupA-producing endophytic fungi varied in four months, with 6 genera higher in August (*Alternaria*、*Cladosporium*、*Colletotrichum*、*Fusarium*、*Leptosphaeria*、*Mucor*), 4 genera higher in November (*Acremonium*、*Arthrinium*、*Aspergillus*、*Penicillium*), 2 genera higher in February (*Botrytis*、*Podospora*), and 1 genus higher in May(*Trichoderma*) (Fig. 9b). The relative abundance of endophytic fungi producing HupA were the highest in August, followed by November, and lower in February and May.

Correlation between endophytic fungal communities and HupA content of H. serrata

The contents of HupA in 36 samples of *H. serrata* (4 seasons × 3 tissues × 3 replicates) were determined respectively (Fig. 10). In the same month, HupA content was the highest in leaves, followed by stems and roots. The HupA content of leaves in November was the highest.

The correlation between the contents of HupA with endophytic fungal communities and species was further analyzed and the Spearman correlation index was calculated. It was found that 33 genera of the endophytic fungi of *H. serrata* showed a significant positive correlation with the content of HupA ($p < 0.05$), of which 13 genera showed a highly significant positive correlation with the content of HupA ($p < 0.01$) (Table 1).

Table 1 The correlation of endophytic fungi with HupA content within *H. serrata* ($p<0.05$).

Genus	Spearman's correlation coefficient	Significance（p）
Strelitziana	0.776542	0
Devriesia	0.644937	0.000022
Articulospora	0.643924	0.000023
Derxomyces	0.640771	0.000026
Cyphellophora	0.579162	0.000215
Trechispora	0.556435	0.000425
Kurtzmanomyces	0.474750	0.003438
Capnobotryella	0.453266	0.005500
Erythrobasidium	0.448894	0.006030
Camptophora	0.446763	0.006304
Stagonospora	0.445764	0.006436
Lachnum	0.440435	0.007181
Golubevia	0.435129	0.007996
Phyllosticta	0.421009	0.010558
Taphrina	0.409584	0.013113
Colacogloea	0.395349	0.017009
Bannoa	0.393780	0.017492
Lepiota	0.393608	0.017546
Arachnopeziza	0.391924	0.018079
Helminthosporium	0.384183	0.020705
Marasmius	0.381949	0.021519
Cryptocoryneum	0.379425	0.022472
Pestalotiopsis	0.375937	0.023845
Pseudeurotium	0.371951	0.025498
Meira	0.361083	0.030493
Alternaria	0.357805	0.032148
Herpotrichia	0.349738	0.036536
Carlosrosaea	0.349643	0.036590
Proliferodiscus	0.347363	0.037917
Lactarius	0.341633	0.041423
Tylospora	0.339846	0.042568
Arthrinium	0.339309	0.042918
Moesziomyces	0.339130	0.043034

Discussion

This study is the first to identify and compare endophytic fungi from three tissues of *H. serrata* in four seasons. A total of 7005 OTUs were identified, belonging to 14 phyla, 54 classes, 140 orders, 351 families, 742 genera. Ascomycota and Basidiomycota were the dominant phyla, and their abundance accounted for 79% of the communities (Fig. S1). At the genus level, the dominant genera of endophytic fungi in each sample were different. *Cladophialophora* (8%) was a dominant genus in different seasons and different tissues. *Latifluus* was a unique genus of root samples of August. (Figs. 3 and 4)

The number of strains isolated from the three tissues was leaf > stem > root (Fig. 5a). The highest richness and diversity of endophytic fungal community was the stem, followed by leaf and root (Fig. 6a). This result is different from previous reports[36, 37], which may be due to the fact that endophytic fungi were isolated from *H. serrata* in different seasons, and the differences of endophytic communities in plant tissues are shaped by the factors such as plant species, soil type, geographic, and environmental conditions[38]. NMDS analysis showed that there were significant differences between endophytic fungal communities in root, stem and leaf. However, in February and May, the endophytic fungal communities of leaf were similar to that of stem (Fig. 7). This was consistent with the reported results[36, 37].

Alpha diversity analysis showed that the richness and diversity of endophytic fungi was the lowest in summer (August) and the highest in spring (May) (Fig. 6b). NMDS analysis showed that there were significant differences between endophytic fungal communities in the four seasons. The analysis of the same tissue in different months revealed that there were significant differences in the communities of root and leaf among the four seasons, but the grouping effect of the stem communities in the four seasons was not well (Fig. 8). Since the fungal diversity of stem was higher than that of leaf and root, the grouping effect of whole plant communities in four months was influenced by the distribution of endophytic fungal communities in stem.

The variation in a seasonal pattern of colonization might be notably associated with the seasonal behavior of endophytic fungi. In seasonal studies, some endophytic fungi isolated from summer or autumn had high diversity. It was suggested that the high humidity and high temperature in the rainy season are conducive to the growth and diffusion of fungal spores[35, 39]. However, some studies have reported that the diversity of endophytic fungi is higher in winter. The variation in the diversity of endophytic fungi in different season might be due to the fact that the levels of secondary metabolites varies throughout the year[40–42]. The climate of August where the plant materials were harvested is suitable for the growth of endophytic fungi[43–45]. In the four months of this study, the number and diversity of strains isolated from *H. serrata* collected at the end of August were the least. *Sebacina* was the most dominant community in August. *Sebacina* has been reported to promote plant growth while weakening plant resistance to herbivores[46]. HupA produced by *H. serrata* is an alkaloid that can resist herbivores[47, 48]. In this study, *Sebacina* showed a highly significant negative correlation with the content of HupA in plant. It can be speculated that *Sebacina* reduces the content of HupA and damages the herbivorous resistance of plant. At the same time, there is a well-developed symbiotic relationship between plants and their endophytic fungi. Some endophytic fungi producing HupA also have antifungal activity, and their relative abundance was higher in August (*Colletotrichum*、*Fusarium*)[49, 50], which may be the reason for the low diversity of endophytic fungi in August.

In this study, we found 13 verified genera that produce HupA. Their relative abundance was the highest in leaves, followed by stems, and the lowest in roots (Fig. 9a). In the distribution of months, there were 10 genera with higher relative abundance in August and November (Fig. 9b). The relative abundance of most HupA-producing bacteria in summer and autumn is higher than that in spring and winter. *Cladosporium*, which produces HupA, was the dominant genus in the leaf tissues and August plant of *H. serrata*. Therefore, when the strains producing HupA are isolated in the future, the probability of successful isolation from leaves in summer and autumn is higher.

The accumulation of alkaloids is an important chemical defense strategy for plants to adapt to environmental stress [51–53], and the defense response of plants is usually triggered by the perception of endophytic fungi[54, 55]. The content of HupA in plants was consistent with the law of the number of fungus isolated (Figs. 5 and 10). In every month, the content of leaf was the highest, followed by stem, root was the lowest, and the lowest content of leaf and stem were in August. Thus, the existence of some special fungal species might be one key factor that induced the accumulation of HupA in *H. serrata*.

Through correlation analysis, it was found that 33 genera of the endophytic fungi of *H. serrata* showed a significant positive correlation with the content of HupA ($p < 0.05$), of which 13 genera showed a highly significant positive correlation with the content of HupA ($p < 0.01$) (Table 1). Correlation analysis is a feasible and convenient method to identify fungal endophytes that promote the accumulation of secondary metabolites in plants[56]. These endophytic fungi may have the potential to promote the biosynthesis and accumulation of HupA in plant. Endophytic fungi can serve as biological inducers biological inducers to promote the production of secondary metabolites of host plants by stimulating key genes and synthesizing enzymes in plant biosynthesis pathway[57, 58]. Endophytic fungus *Mucor circinelloides* DF20 promote tanshinone biosynthesis and accumulation in *Salvia miltiorrhiza* root by upregulating the key enzyme genes expression levels of the biosynthesis pathway[31]. Endophytic fungi that promote the biosynthesis and accumulation of HupA in plants and its mechanism need to be further studied.

Conclusions

In this study, the endophytic fungal composition of different tissues and four seasons of *H. serrata* was identified to verify the variability in the spatial and temporal distribution patterns. In addition, we also analyzed the correlation between endophytic fungi and plant HupA. It provides a data reference to the promotion of plant HupA biosynthesis.

Materials And Methods

Collection and sterilization of plant materials

The material is *H. serrata* grown in the field (Ningqiang County, Hanzhong City, Shaanxi Province, latitude 32°55'N / longitude 105°55'E, with an altitude of about 840 meters). According to the local climate, March-May is spring, June-August is summer, September-November is autumn, and December-the following February of the year as winter[43]. *H. serrata* was collected at the end of four months, August 2017, November 2017, February 2018, and May 2018. The whole plants were dug up with the roots and soil, kept the plants moist, placed in sterile self-sealing bags at 4℃, and the material was processed within 24 h. The surface of the fresh *H. serrata* tissues were washed with water. It was divided into three parts: root, leaves, and stems, with the roots and stems divided into small 2 cm segments. The material was surface sterilized: sterilized distilled water rinse for 30 s, 75% alcohol sterilize for 2 min, sterilized distilled water rinse 3 times for 1 min each, and then sterilize 0.1% $HgCl_2$ for 8 min, sterile water rinse 5 times for 2 min each, finally blotting dry the tissue surface with sterile filter paper. The material was divided into two parts, one for DNA extraction of endophytic fungi and the other for HupA content detection.

Extraction of endophytic fungal DNA and construction of ITS clone libraries

Extraction of total DNA from plant materials by Cetyltrimethylammonium Bromide (CTAB) method[59]. Using primers ITS1F (5′–CTTGGTCATTTAGAGGAAGTAA–3′) and ITS2 (5′–GCTGCGTTCTTCATCGATGC–3′), the ITS region was amplified（TransStart® FastPfu Fly DNA Polymerase）. The amplification products were purified and recovered by magic DNA clean beads (Vazyme VAHTSTM DNA Clean Beads). The sequencing libraries were prepared with TruSeq Nano DNA LT Library Prep Kit of Illumina company. Then, the construction and sequencing of the ITS clone libraries were performed by Personalbio (Shanghai Personal Biotechnology Co., Ltd., China.) using an Illumina NovaSeq6000 platform.

Sequence processing and data analysis

Qiime2 software was used for data analysis. Call dada2 method for quality control, denoising, splicing, and chimerism removal[60]. The above steps were analyzed for each library. After the denoising of all libraries was completed, the ASVs feature sequences and ASV tables were merged, and singletons ASVs were removed (in all samples, the total number of sequences is only 1 ASV). We used UNITE database (Release 8.0, https://unite.ut.ee/)[61] to annotate species taxonomy.

In the previous analysis steps, the abundance table of ASV/OTU has been generated, and some subsequent analysis steps need to be carried out at the same sequencing depth level. Therefore, the table needs to be transformed. The rarefaction method is adopted, and the flattening depth is set to 95% of the minimum sample sequence size[62, 63].

Python language tools are used to visualize the composition and distribution of samples at a specific classification level. We used Krona software (https://github.com/marbl/Krona/wiki) to make an interactive display of the taxonomic composition of the community[64].

In order to comprehensively evaluate the alpha diversity of endophytic fungal communities, Chao1[65], observed species, Shannon[66], Simpson's[67], faith's PD[68], Pielou's evenness[69] and Good's coverage[70] indexes were evaluated using qiime2 analysis software. Subgroup samples were plotted as box plots using the Python language tool alpha diversity index to visualize the differences in alpha diversity between sample groups. Kruskal Wallis rank sum test and Dunn' test were used as post test to verify the significance of the difference.

Beta diversity analysis was used to compare the similarity between sample communities. Jaccard distance were calculated using QIIME2 analysis software, and then NMDS analysis was done on these distance matrices using Python[71], and the results were plotted as two-dimensional scatter plots.

Extraction and content detection of HupA in plants

The extraction method of HupA from plants has been improved on the basis of traditional methods[72].

The plant materials were dried at 40°C for 24h, then ground into powder using liquid nitrogen and dried again. The material was weighed, mixed with 0.5% HCl at a ratio of 1:20 (g/ml), sonicated (500W for 15 min), then left to stand for 12-14h. The supernatant was obtained by centrifugation and filtration, and the

above extraction steps were repeated twice (without standing), and the supernatants of the three extractions were combined. The supernatant was adjusted to pH 9.0-9.5 with NH_4OH, allowed to stand for 2–3 h, extracted three times using equal volumes of chloroform (2 min of standing and 15 min of sonication each time), and the chloroform of the three times were combined. Evaporate the solvent with a rotary evaporator, and dissolve the crude alkaloid extract with 2ml chromatographic methanol. Filtered with 0.22μm organic microporous membrane and stored in the injection vial for standby.

HPLC detection: The detection of HupA was performed on a Shimadzu LC-20AT (Shimadzu, Tokyo, Japan) high performance liquid chromatograph with a C18 column (250 mm × 4.6 mm, 5μm; Thermo Scientific, Waltham, MA, USA). The mobile phase was ammonium acetate (0.08 mol/L)–methanol–acetonitrile (60:30:10, v/v), the flow rate was 0.8 mL/min, the injection volume was 10μL, and the detection wavelength was 308 nm.

Declarations

Authors' contributions

All authors contributed to the study conception and design. ZHS analyzed the data of this project; XBL and YLW extraction of DNA and prepared the PCR; JY, KY and QMH helped the sample collection and gave instructions for analysis of data; ZHS, BG, YHW, YPF, XBL, JY, KY, QMH and YLW drafted the manuscript. All the authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available

from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

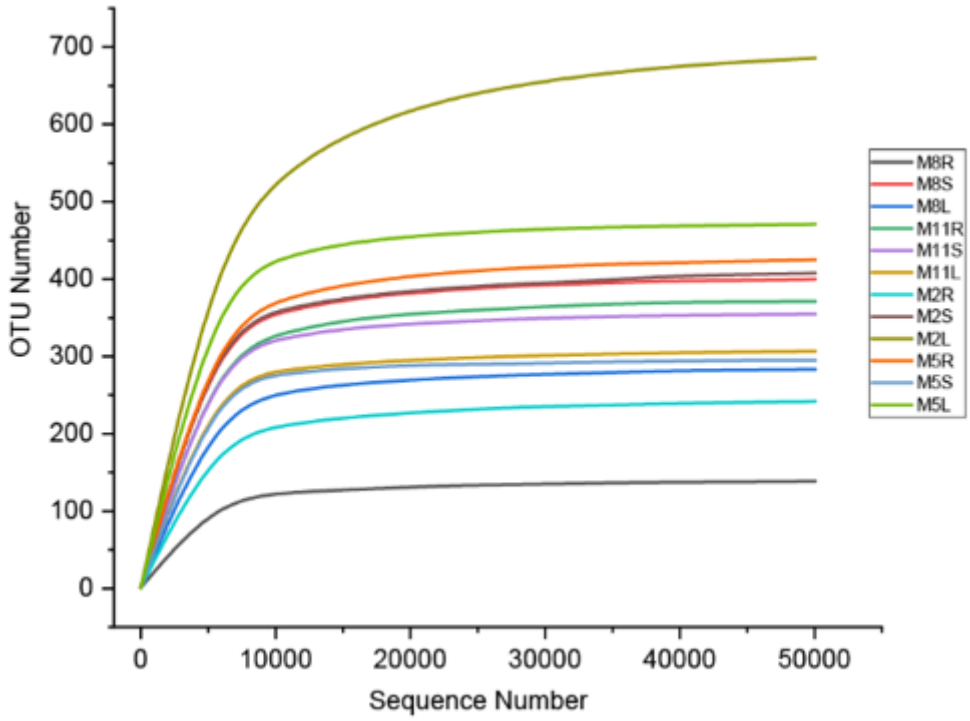

Figure 1

Rarefaction curves of OTUs in different samples (M8, August; M11, November; M2, February; M5, May; R, root; S, stem; L, leaf).

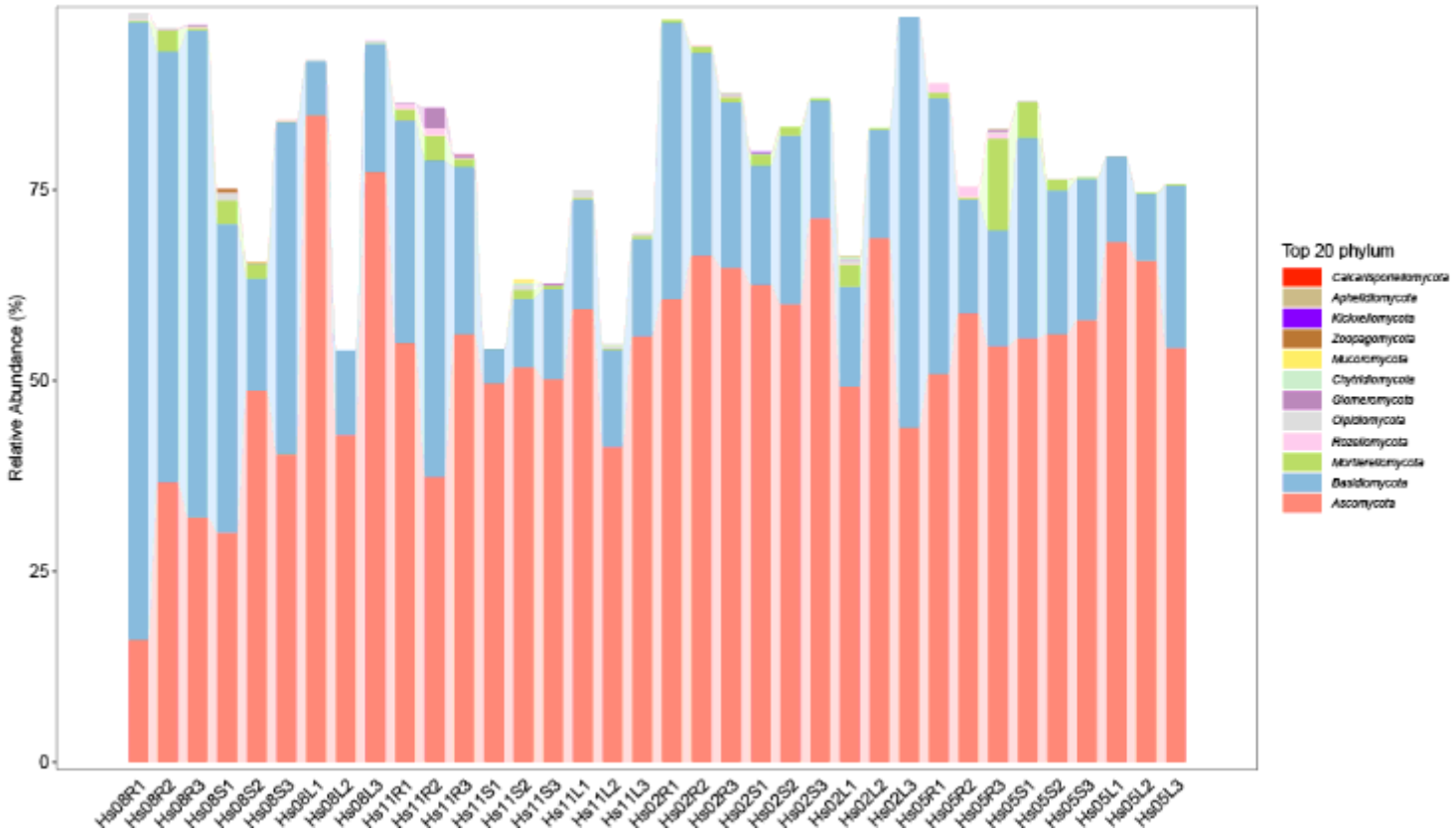

Figure 2

Relative abundance of endophytic fungi at the phylum level in all samples of *H. serrata.*

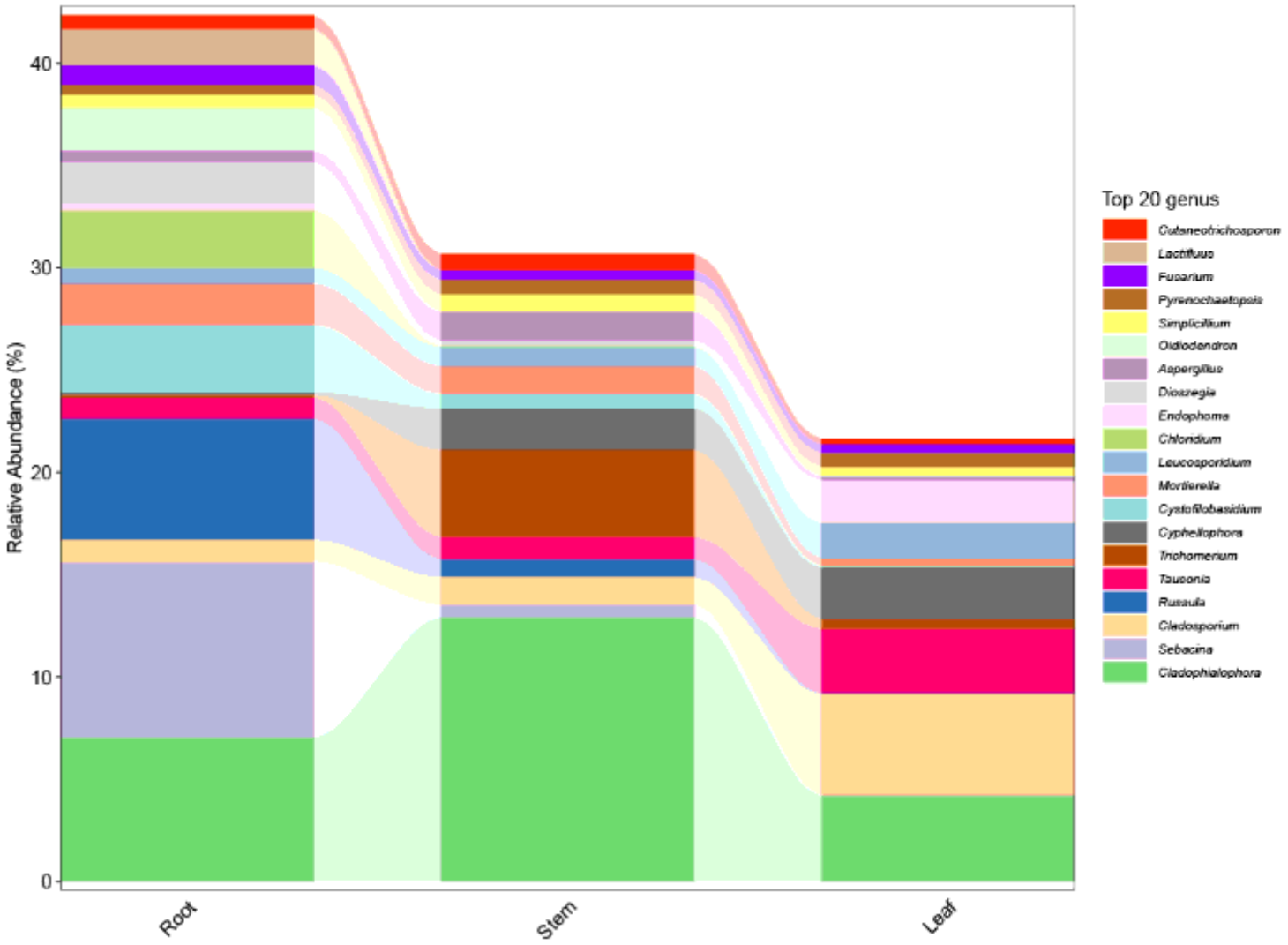

Figure 3

Relative abundance of endophytic fungi in the root, stem and leaf of *H. serrata.*

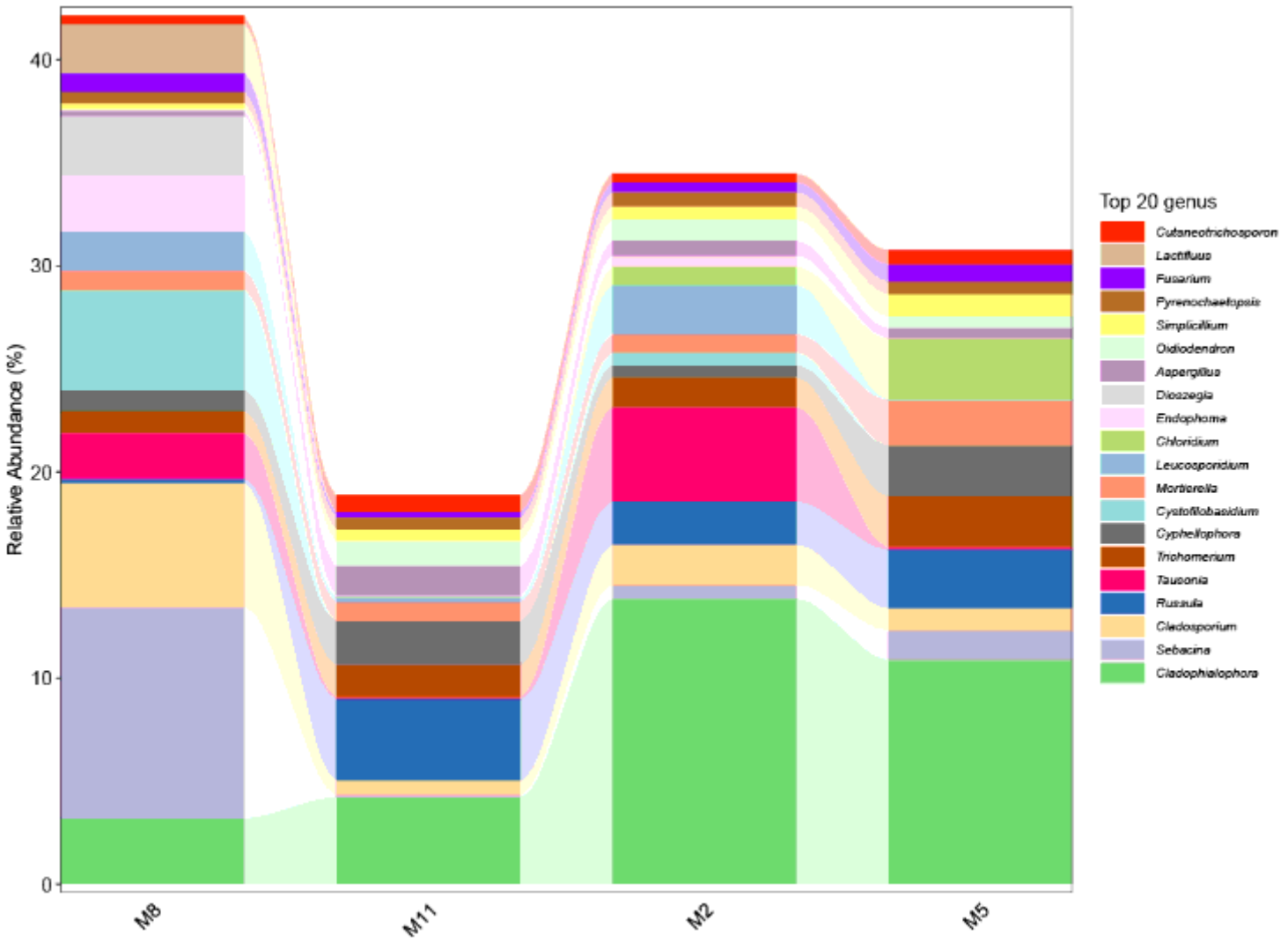

Figure 4

Relative abundance of endophytic fungi in August (M8), November (M11), February (M2) and May (M5).

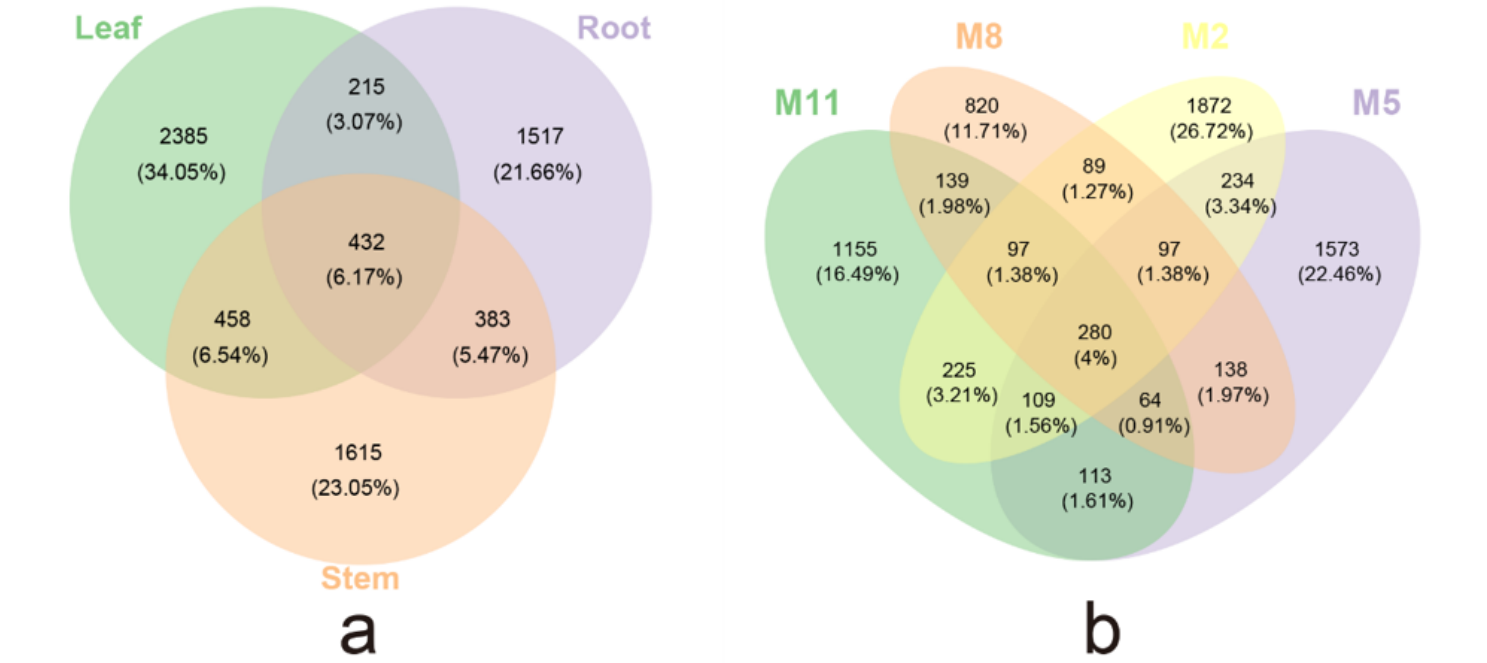

Figure 5

Venn diagrams showing number of shared OTUs among sample groups. **a** Number of shared OTUs among the root, stem and leaf samples associated with *H. serrata*. **b** Number of shared OTUs among the August (M8), November (M11), February (M2) and May (M5) samples.

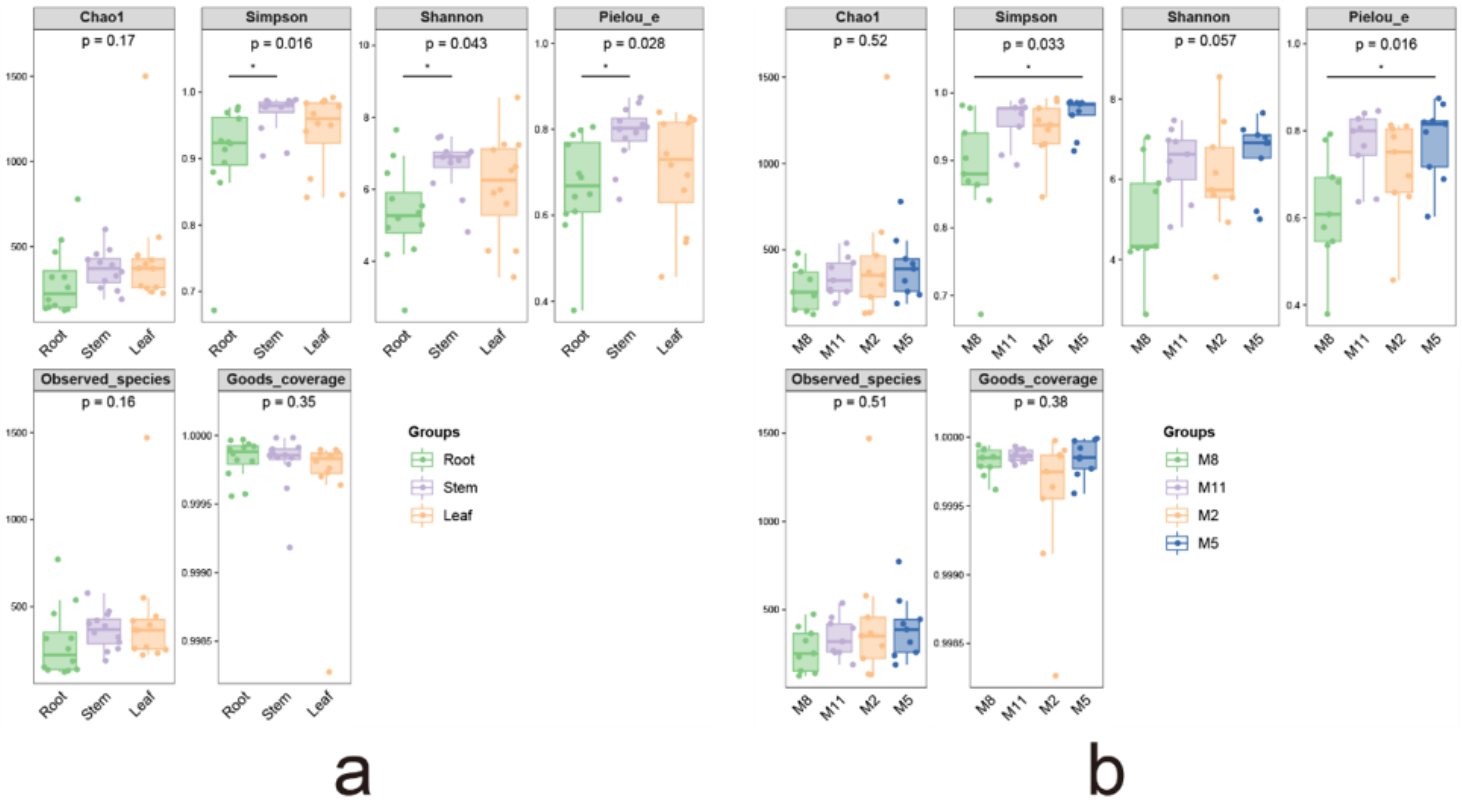

Figure 6

a Alpha diversity values of endophytic fungi in different tissue samples. **b** Alpha diversity values of endophytic fungi in different season samples (M8, August; M11, November; M2, February; M5, May). The number under the diversity index label is the p value of Kruskal Wallis test. The asterisk on the line in the figure indicates that there is a significant difference between the two groups ($p < 0.05$).

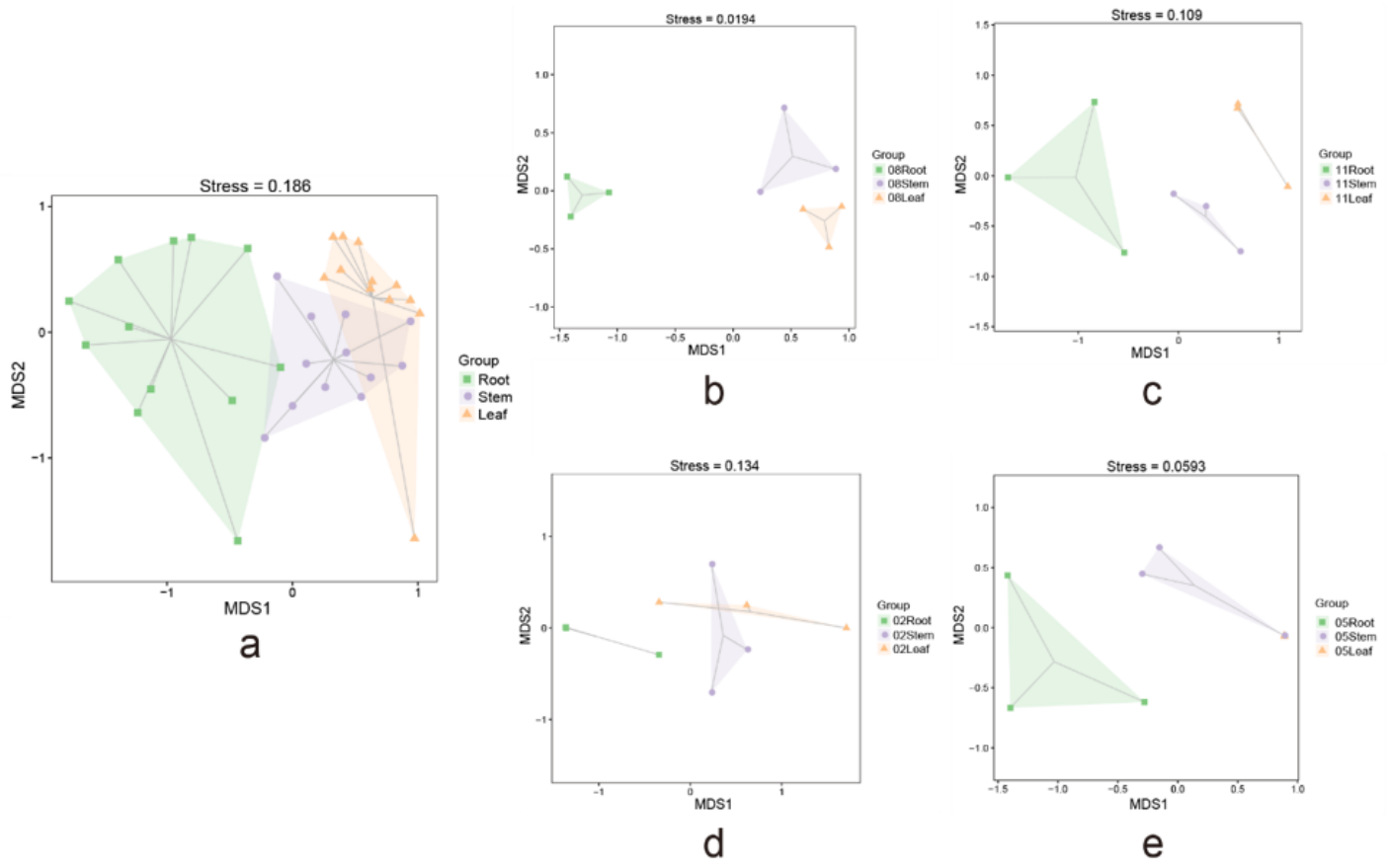

Figure 7

Comparison of fungal communities among three tissues of *H. serrata* in different seasons (**a** four seasons; **b** August; **c** November; **d** February; **e** May).

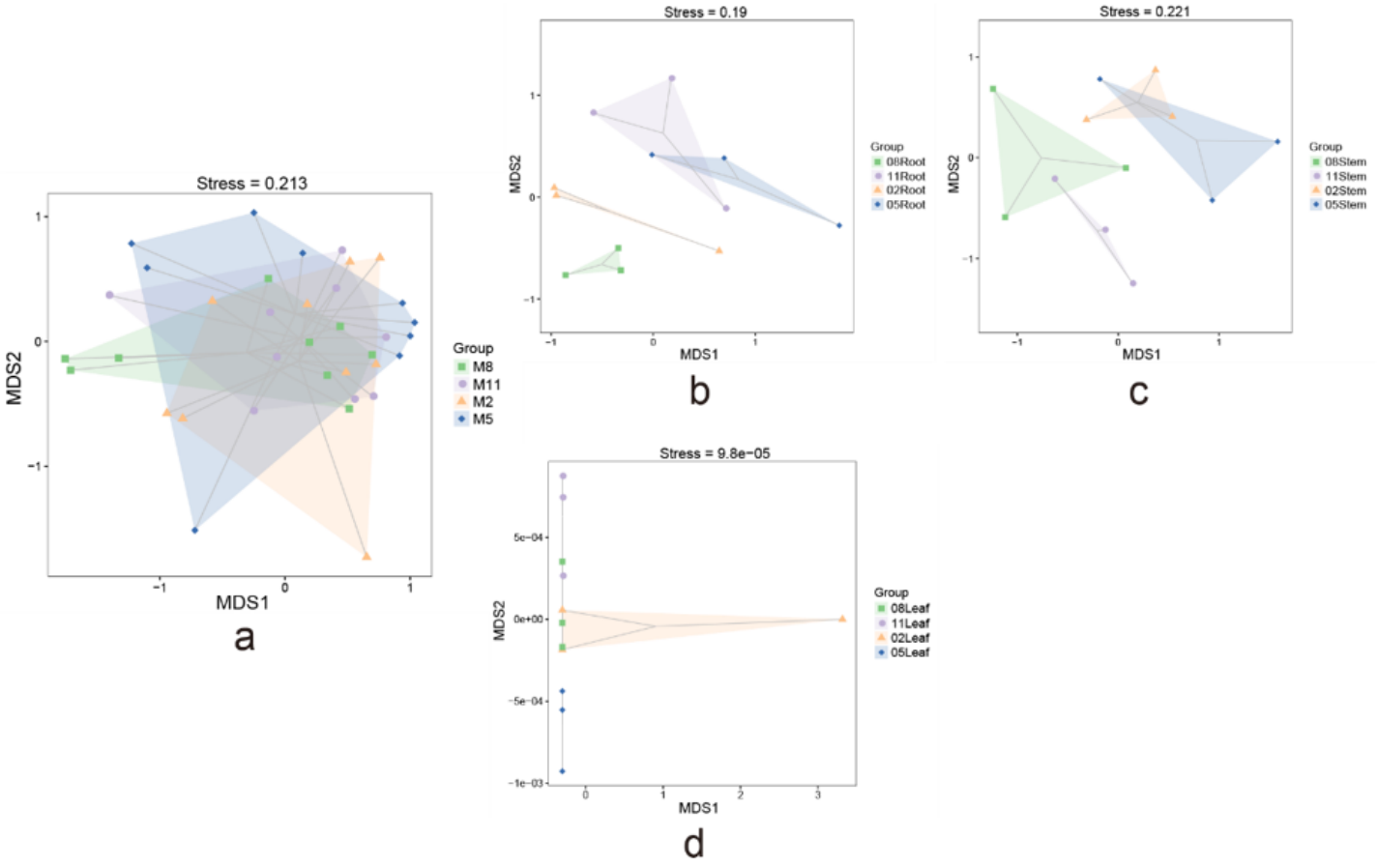

Figure 8

Comparison of microbial communities among four seasons samples of *H. serrata* in different tissues (**a** three tissues; **b** root; **c** stem; **d** leaf).

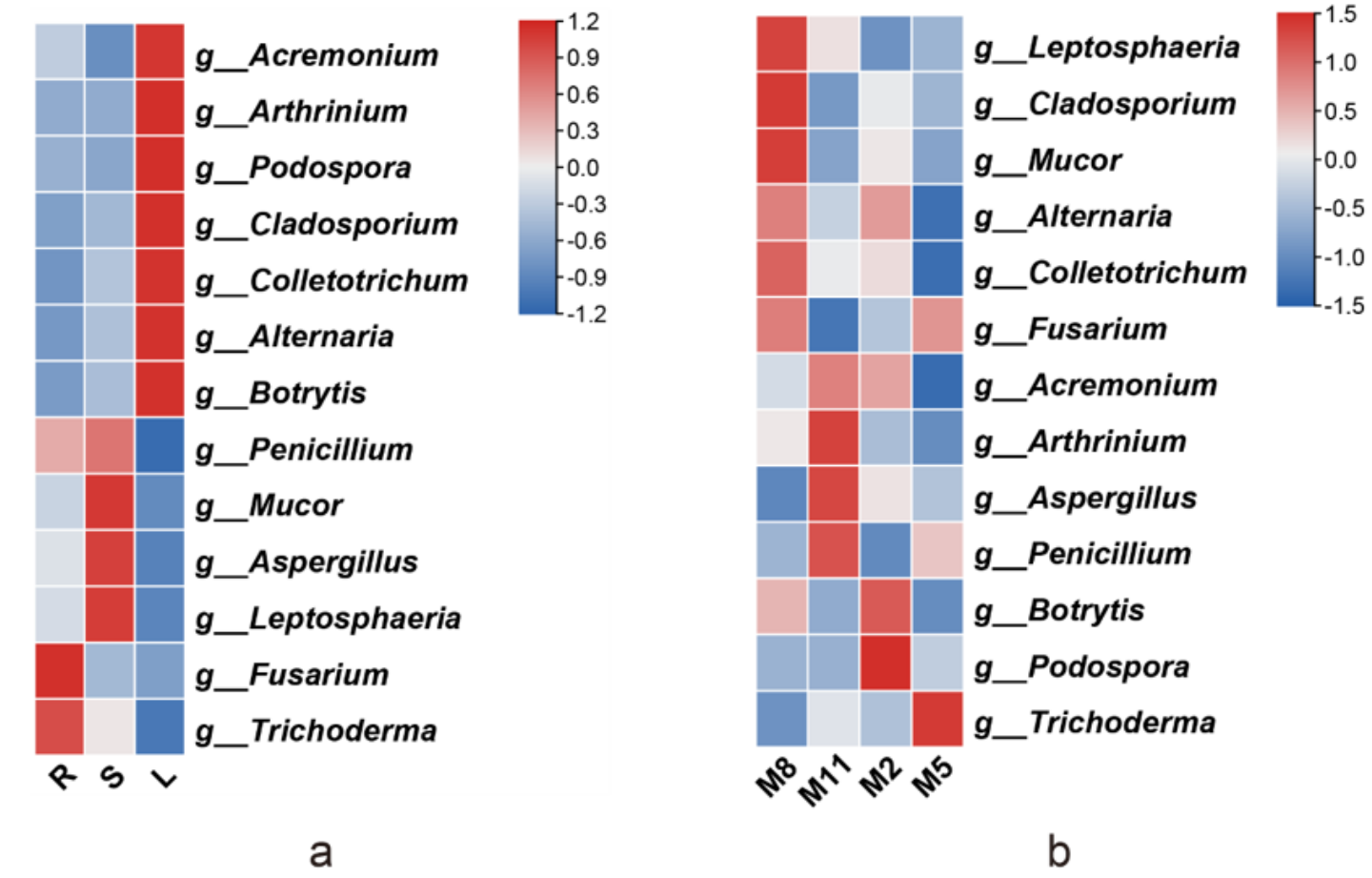

Figure 9

Heat map of relative abundance of 13 genera of HupA-producing endophytic fungi (**a**) in different tissues (R, root; S, stem; L, leaf) and (**b**) seasons (M8, August; M11, November; M2, February; M5, May).

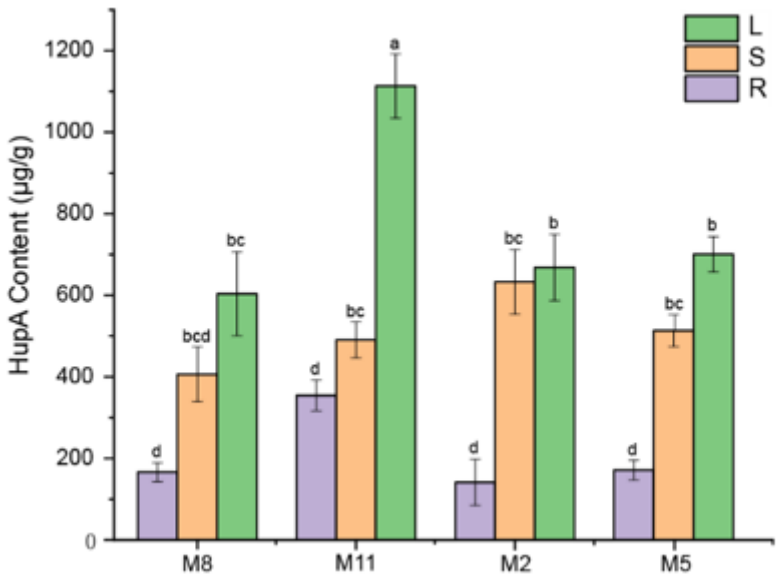

Figure 10

Content of HupA in *H. serrata* (M8, August; M11, November; M2, February; M5, May; R, root; S, stem; L, leaf). Different letters above the bars indicate statistically significant ($p < 0.05$) differences according to Tukey's Method tests. Error bars represent SE.

Supplementary Files

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