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Effects of Medicinal Plants on Fungal Community Structure and Function in Hospital Grassplot Soil

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Abstract

Hospital grassplot soil is an important repository of pathogenic fungi exposed to the hospital environment, and the diffusion of these fungi-containing soil particles in the air increases the risk of nosocomial fungal infections. In this study, from the perspective of soil microbes–plant holobiont, four medicinal plants *Mirabilis jalapa*, *Artemisia argyi*, *Viola philippica*, and *Plantago depressa* were used as materials, based on ITS high-throughput amplicon sequencing and simulated pot experiments to explore the effect of medicinal plants on the fungal community in hospital grassplot soil, in order to provide a new exploration for hospital grassplot soil remediation. The results showed that the fungal community ecological guilds in primary test soil was mainly pathogen, and the abundance of animal pathogen with potential threats to human reached 61.36%. After planting medicinal plants, the composition and function of soil fungal community changed significantly. Although this change varied with plant species and growth stages, all samples collected in the pot experiment showed that the pathogen abundance decreased and the saprotroph abundance increased. In addition, 45 of the 46 core fungal genera defined in all potted samples were present in primary test soil, and many of them were human potential pathogens. These findings imply that the idea of enhancing soil quality in hospital grassplot soil by planting specific plants is feasible. However, the initial fungal community of the hospital grassplot soil has a certain stability, and it is difficult to completely eliminate the threat of pathogenic fungi by planting medicinal plants.

Introduction

Humans and the environment share a microbial world. Soil ecosystems, as a common habitat for humans and microorganisms, its microbial composition is particularly related to human health [1]. Local or invasive infections resulting from the inhalation of certain fungal spores in the soil reportedly cause almost 1.4 million globally deaths each year [2]. In particular, hospital grassplot soil is an important species repository of pathogenic fungi exposed to the hospital environment. Airborne transmission of these fungal spore-containing soil particles and dust increases the risk of nosocomial fungal infections and an increasingly serious hidden threat to public health security [3–6]. In addition to the hospitalized patients or their families who are easily infected by inhaling fungal spore-containing soil particles or dust, some potentially pathogenic fungal spores in hospital grassplot soil exhibit resistance to dry air and ultraviolet radiation, and can be transmitted by air currents over large distances, even across continents [7–9]. When they encounter patients who are immunodeficient, or other individuals who are susceptible, such as those with open wounds, these pathogenic fungal spores can cause disease and even epidemiological problems [10]. In addition, pathogenic fungi in hospital grassplot soil can be transported and spread by a surface runoff, humans, insects such as ants, and so on, endangering the surrounding soil and water quality [11]. Therefore, it is urgent to remediate hospital grassplot soil. Using chemical methods, such as spraying chlorine

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containing or quaternary ammonium salts disinfectants, is one of the main strategies to kill pathogenic fungi in the hospital grassplot soil, but this approach often has the risk of secondary environmental pollution and may also lead to resistance in pathogenic fungi [12]. Therefore, exploration and development of novel restoration strategies are necessary to avoid these negative effects.

Fungi are heterotrophs that are highly dependent on exogenous carbon substrates for their growth [13]. Our previous study demonstrated that adding the biological material of chicken feather powder to hospital grassplot soil successfully drove changes in the abundance of potential human pathogenic fungi [14]. Compared with chicken feather powder, plants and soil microorganisms have a closer association, which stems from their relationship from mutualism to antagonism established during long-term coevolution. Based on the concept of symbiosis, plants are no longer simply regarded as an individual, but together with soil microorganisms are considered to be holobionts that interact with each other [15]. Thus, the regulation and management of soil microbial community composition, interaction, and function through plant cultivation to enhance soil ecosystem services may be a hotspot in future ecological research [16]. Numerous studies have shown that certain higher plants, especially medicinal plants, have strong antifungal properties [17–19]. They produce secondary metabolites such as phenolic compounds, saponins, alkaloids, and terpenoids, which are often reported for the treatment of human and animal fungal diseases and are considered a potential source of antifungal drugs [20]. In conclusion, we assumed that planting specific medicinal plants in hospital grassplot soil will drive the transformation of community structure and function of soil fungal, and even successfully reduce the abundance of pathogenic fungi, thereby it will achieve soil ecological restoration and alleviate the impact to public safety.

Four medicinal plants, *Mirabilis jalapa*, *Artemisia argyi*, *Viola philippica*, and *Plantago depressa*, were selected as research materials for their wide ecological adaptation, barren tolerance, fast growth, strong reproduction, easy cultivation, and the ability to produce a variety of antibacterial active ingredients. Among them, isoflavone and dehydrorotenoid produced by *M. jalapa* have inhibitory effects on the human opportunistic pathogen *Candida albicans* [21]. The volatile oil produced by *A. argyi* has a certain lethal effect on human pathogenic fungi *Epidermophyton floccosum* and *Cryptococcus neoformans* [22]. Extracts from *V. philippica* could affect the growth and biofilm formation of *Trichophyton rubrum* [23]. This fungus is the main pathogen of human tinea pedis. The residents of Dong ethnic minority living in southeast of Guizhou Province, China, often use *V. philippica* to treat this fungal disease and obtain significant curative effects. Extracts from *P. depressa* have inhibitory effects on clinical pathogens including *Candida yeast*, *Fusarium oxysporum*, and *Aspergillus niger* [24]. In this study, four medicinal plants were planted in pots containing hospital grassplot soil. Potting soil from planting different plants and different growth stages of the same plant were collected, respectively. Based on ITS high-throughput amplicon sequencing technology, the effects of medicinal plant species and growth stages on the structure and function of fungal community in hospital grassplot soil were studied, in order to provide a new exploration for hospital grassplot soil remediation.

Materials and Methods

Primary Test Soil Collection

A hospital in Guiyang, Guizhou Province, China was selected as the primary test soil collection site. In June 2019, seven plots, measuring 2 × 2 m, were set up on the green soil beside the hospital outpatient department, inpatient department and patient rehabilitation area. Five portions of soil (depth 0–10 cm) were collected from each plot according to the five-point sampling method, each portion of soil was 2 kg, and large particles of impurities such as plant litter, plant roots, and gravel were removed when collecting soils. 35 portions of soil collected from seven plots were transported to the laboratory and mixed evenly. Subsequently, 5 g of mixed soil was taken as the primary test soil sample (PTS) and stored at − 80 °C for total DNA extraction, while the rest of the soil was used for medicinal plant cultivation.

Pot Experiment of Medicinal Plants

We used a pot experiment to study the effects of medicinal plant species and growth stages on the structure and function of fungal communities in hospital grassplot soil. Four treatments of medicinal plant species were set up, including planting *M. jalapa* treatment (MJ), planting *A. argyi* treatment (AA), planting *V. philippica* treatment (VP), and planting *P. depressa* treatment (PD). Rhizosphere soil samples (the part most strongly influenced by plants) were collected at three growth stages for each treatment, including growing stage (G, 40 days after transplanting), flowering stage (F, when the first bunches of buds appear), and withering stage (W, when all the leaves of the plant turn yellow and wilt). A total of 12 samples were collected in the pot experiment, and the information of each sample number is shown in Table 1.

The pot experiment was established at Guizhou University (N 26°44′, E 106°67′), China, from June to December 2019. Plants were grown in the open air, with sunlight through a white plastic membrane roof, which protected the plants against uncontrolled access to rainwater. Soil moisture content was adjusted daily with sterile water, and the

Table 1 Pot experiment samples and their codes

Growth stage of medicinal plants	Potted medicinal plant species	Sample code
Growing stage (G)	*Plantago depressa* (PD)	PD-G
	Artemisia argyi (AA)	AA-G
	Viola philippica (VP)	VP-G
	Mirabilis jalapa (MJ)	MJ-G
Flowering stage (F)	*Plantago depressa* (PD)	PD-F
	Artemisia argyi (AA)	AA-F
	Viola philippica (VP)	VP-F
	Mirabilis jalapa (MJ)	MJ-F
Withering stage (W)	*Plantago depressa* (PD)	PD-W
	Artemisia argyi (AA)	AA-W
	Viola philippica (VP)	VP-W
	Mirabilis jalapa (MJ)	MJ-W

potted soil moisture was maintained in the range of 25–35% (measured by soil moisture meter) based on the moisture condition of the primary test soil (30.56%). Natural temperature was used throughout the experiment, and no exogenous carbon was added. Specific steps are as follows. Seedlings of the four medicinal plants were washed with tap water and sterile water successively to remove the dust, and then transplanted into sterile plastic pots (3.8 L capacity) containing 3.5 kg of mixed primary test soil. Three seedlings were planted in each pot. The rhizosphere soil samples were collected destructively. That is, when sampling plants, surface soil was removed, then the whole plant was collected with a disinfected spoon and the soil around the roots of the plant was shaken into a sterile bag. Therefore, nine pots were set up for each treatment, containing three replicates of three growth stages of medicinal plants. At each growth stage, rhizosphere soil was harvested from nine plants in each treatment replicate, mixed thoroughly and stored at − 80 °C for total DNA extraction.

Total DNA Extraction, PCR Amplification, and Sequencing of Samples

Extraction of metagenomic DNA was performed according to the procedures in the instructions of the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA), and the extracted genomic DNA was detected using 1% agarose gel electrophoresis. Fungal ITS sequences were amplified by PCR using an ABI GeneAmp® 9700 PCR instrument. The 20-μL reaction system contained 4 μL of 5 × Buffer, 2 μL of 2.5 mmol/L dNTPs, 0.8 μL forward primer (5 μmol/L) ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′), 0.8 μL reverse primer (5 μmol/L) 2043R (5′-GCTGCGTTCTTC ATCGATGC-3′), 0.4 μL rTaq polymerase (TaKaRa), 0.2 μL bovine serum albumin, 10 ng template DNA, and ddH_2O to 20 μL. The following thermal cycler program was used for amplification: 3 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C, followed by a final extension for 10 min at 72 °C, then a holding step at 10 °C until the reaction was stopped. The resulting PCR products were subjected to ITS high-throughput amplicon sequencing using an Illumina HiSeq platform at Shanghai Meiji Biomedical Technology Co. (Shanghai, China).

High-Throughput Sequencing Data Analysis

Raw Data Processing

The analysis was conducted by following the "Atacama soil microbiome tutorial" of QIIME2 docs along with customized program scripts (https://docs.qiime2.org/2019.1/). Briefly, raw data FASTQ files were imported into the format which could be operated by QIIME2 system using qiime tools import program. Demultiplexed sequences from each sample were quality filtered and trimmed, de-noised, merged, and then the chimeric sequences were identified and removed using the QIIME2 dada2 plugin to obtain the feature table of amplicon sequence variant (ASV) [25]. The QIIME2 feature-classifier plugin was then used to align ASV sequences to UNITE database to generate the taxonomy table [26]. Any contaminating mitochondrial and chloroplast sequences were filtered using the QIIME2 feature-table plugin. Finally, the data homogenized abundance profile of ASVs and other taxonomic levels was formed. Simultaneously, the Shannon diversity index was calculated for each sample using Diversity in QIIME2 software.

Community Composition and Functional Analysis

Community composition of 13 samples (including one sample from the primary test soil and 12 samples collected from the pot experiment) was analyzed at the genus level, and Excel software was used to generate a bar plot. Simultaneously, the 12 samples collected from the pot experiment were divided into four sample groups of medicinal plant species (each group includes three subgroups of growing, flowering, and wilting stages) and three sample groups of growth stages (each group includes four subgroups of *M. jalapa*, *A. argyi*, *V. philippica*, and *P. depressa*). Subsequently, bars plot of community composition was drawn separately using Excel software to analyze the effect of medicinal plant species and growth stage on fungal community in hospital grassplot soil. The fungal community abundance data for each sample group was the mean value of the fungal community abundance data for their subgroup.

Using the FUNGuild database (https://github.com/UMNFuN/FUNGuild), the fungal communities of 13 samples were parsed into different ecological guilds, such as animal

pathogen, plant pathogen and soil saprotroph [27]. Relative abundance of each guild was calculated based on sequence number, and then a bar plot of the composition of fungal community ecological guilds of 13 samples was drawn by Excel software. To further investigate the effect of medicinal plants on human pathogens in fungal community of the hospital grassplot soil, in addition to unassigned taxa, we classified guilds containing animal pathogen as human potential pathogen group and the rest as non-human potential pathogen group. The sum of the guild abundance containing animal pathogen in each sample was calculated and the value was used as the relative abundance of the human potential pathogen group. Subsequently, Excel software was used to draw a bar plot of human potential pathogen abundance in the fungal community of different sample groups.

Linear Discriminant Analysis

Linear discriminant analysis (LDA) effect size (LEfSe) was performed using the Galaxy platform (https://huttenhower.sph.harvard.edu/galaxy/) to separately determine the fungal taxa that explain the differences between the four sample groups of medicinal plant species (each group includes three subgroups of growing, flowering, and withering stages), and between the three sample groups of growth stages (each group includes four subgroups of *M. jalapa*, *A. argyi*, *V. philippica*, and *P. depressa*). The analysis firstly detected species with significant differences in abundance between groups by the nonparametric Kruskal–Wallis rank sum test. Subsequently, the Wilcoxon rank sum test was used to examine the difference consistency of these differential species across subgroups. Finally, LDA was used to estimate the impact (LDA score, with higher values indicating greater influence) of the different species. In this study, the filter value for the LDA score was set to 4.

Core Taxa Analysis

In this study, the core taxa of fungal community in hospital grassplot soil samples were defined by the membership method described by Dong et al. [28]. Specifically, we used Gephi software to perform coexistence network analysis on the fungal community of 12 samples collected from pot experiment, and defined the genera that coexisted in all samples as core fungi.

Results

Description of Sequencing Results

Fungal ITS gene analysis was carried out by Illumina MiSeq sequencing for one primary test soil sample and other 12 samples collected from pot experiment. Approximately 33,508–61,234 high-quality sequences were detected from each sample, and a total of 4,147 ASVs were obtained from 13 samples. These ASVs belonged to 13 phyla, 35 classes, 91 orders, 199 families, 296 genera, and 305 species (Supplementary Tables S1–S7). Rarefaction curves were plotted for each sample using the Shannon index (Fig. 1). The curves for each sample flattened out, indicating that the sequencing data were reasonable and sufficient to reflect the diversity contained in the samples.

Medicinal Plant Species and Growth Stage Jointly Affect Fungal Community Composition in Hospital Grassplot Soil

At the genus level, the fungal community in primary test soil were dominated by *Metarhizium* (60.94%), *Mortierella* (8.79%), and an unclassified genus of Pleosporales (3.31%) (Fig. 2a). The results of pot experiment showed that medicinal plant species affected the fungal community composition of hospital grassplot soil. In terms of single growth stage, the fungal community composition of hospital grassplot soil planted with different plants differs (Fig. 2a). For example, in the growing stage, the dominant genus in soil samples planted with *P. depressa*, *A. argyi*, *V. philippica*, and *M. jalapa* changed into an unclassified genus of Xylariales (18.37%), an unclassified genus of Pleosporales (12.92%), an unclassified genus of Rozellomycota (29.40%), and *Mortierella* (13.69%), respectively. In terms of three growth stages, there were also significant differences in the fungal community composition of hospital grassplot soil planted with different plants (Fig. 2b). For example, the dominant fungal genus in the *V. philippica* sample group was *Talaromyces* (28.16%), while the dominant fungal genus in the *A. argyi* sample group was *Mortierella* (13.70%).

The growth stage of medicinal plants also influences the fungal community composition of hospital grassplot soil. On the one hand, there were dynamic changes in the fungal community composition of samples from three growth stages growing the same plant (Fig. 2a). For example, the dominant genera in the three growth stage samples of *M. jalapa* were *Mortierella* (13.69%), *Talaromyces* (22.79%), and an unclassified genus of Herpotrichiellaceae (18.74%), respectively. On the other hand, there were differences in fungal community composition and abundance among the three growth stage sample groups (Fig. 2c). For example, the dominant fungal genus in the growing stage sample group was *Mortierella* (9.75%), while the dominant fungal genus in the flowering stage sample group was *Talaromyces* (26.24%).

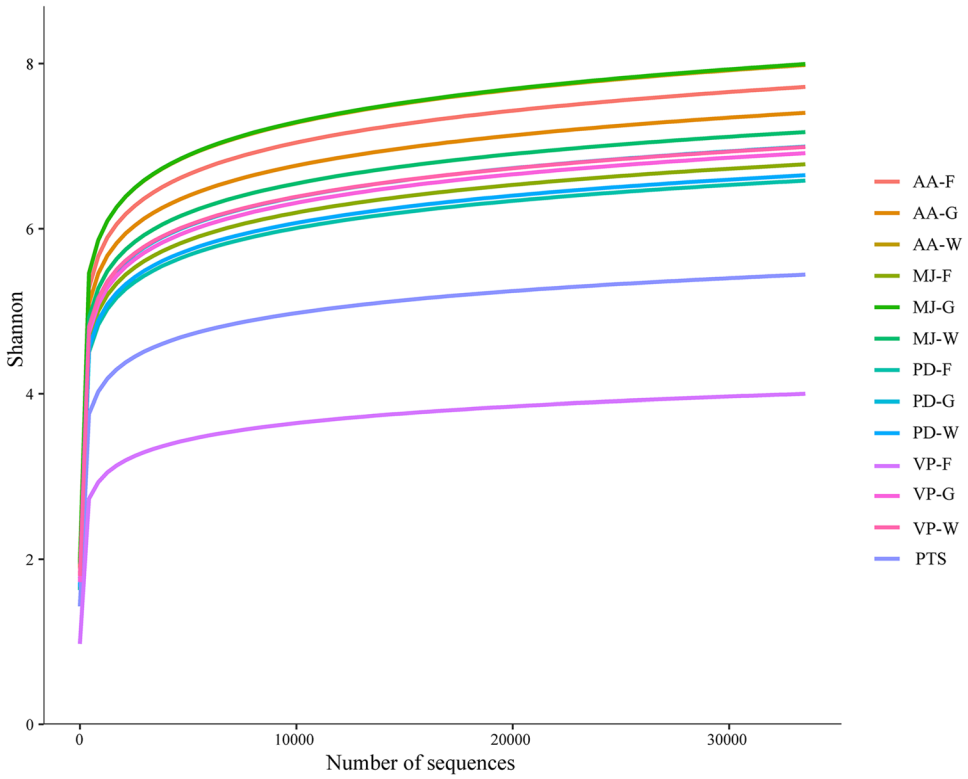

Fig. 1 Shannon index rarefaction curve of fungal community in hospital grassplot soil

Medicinal Plant Species and Growth Stage Jointly Affect Fungal Community Function in Hospital Grassplot Soil

According to parse of FUNGuild platform, except for unassigned taxa, all samples were annotated as 65 ecological guilds (Supplementary Table S8). Analysis based on sequence relative abundance revealed that pathogen dominated in primary test soil fungal community. In particular, the abundance of animal pathogen, a potential threat to human health, was extremely high, reaching 61.36% (Fig. 3a). After potting medicinal plants, the ecological function of fungal community changed markedly. Overall, this change showed that the abundance of pathogen decreased while that of saprotroph increased (Fig. 3a). Except for the dominant ecological guild of *P. depressa* flowering stage sample, which remained associated with animal pathogen (animal pathogen-endophyte-fungal parasite-plant pathogen-wood saprotroph, 27.16%), the dominant guilds of all other samples were undefined saprotroph. Analysis also showed that medicinal plant species and growth stage together influence fungal community function in hospital grassplot soil. Taking the change in animal pathogen abundance as an example, it was lower during the *P. depressa* growing (2.07%) and flowering (1.88%) stages and increased to 7.27% during the withering stage, while it was highest during the *M. jalapa* growing stage (5.12%) and decreased slowly to flowering (4.02%) and withering (3.62%) (Fig. 3a).

Analysis of guilds containing animal pathogen showed that the relative abundance of the human potential pathogen group was as high as 63.78% in primary test soil. After planting medicinal plants in pot experiment, the abundance of the human potential pathogen group in fungal community of hospital grassplot soil decreased significantly. However, there were differences in the abundance of human potential pathogen group among the four plant species sample groups (Fig. 3b). From low to high, it was *A. argyi* (12.29%), *V. philippica* (13.38%), *P. depressa* (19.26%), and *M. jalapa* (22.74%). In addition, the abundance of human potential pathogen group in the three growth stage sample groups differed, with the growing stage (13.30%), the flowering stage (16.47%), and the withering stage (20.98%) in descending order (Fig. 3c). These results indicate that it is feasible to regulate the fungal community function in hospital grassplot soil by changing greenery plant species, and this regulation is also affected by plant growth stage.

Effects Comparison of Medicinal Plant Species and Growth Stage on Fungal Community in Hospital Grassplot Soil

To determine the differences in the effects of medicinal plant species and growth stage on fungal community in hospital grassplot soil, the 12 samples collected from the pot experiment were divided into four medicinal plant species sample groups and three growth stage sample groups for LEfSe

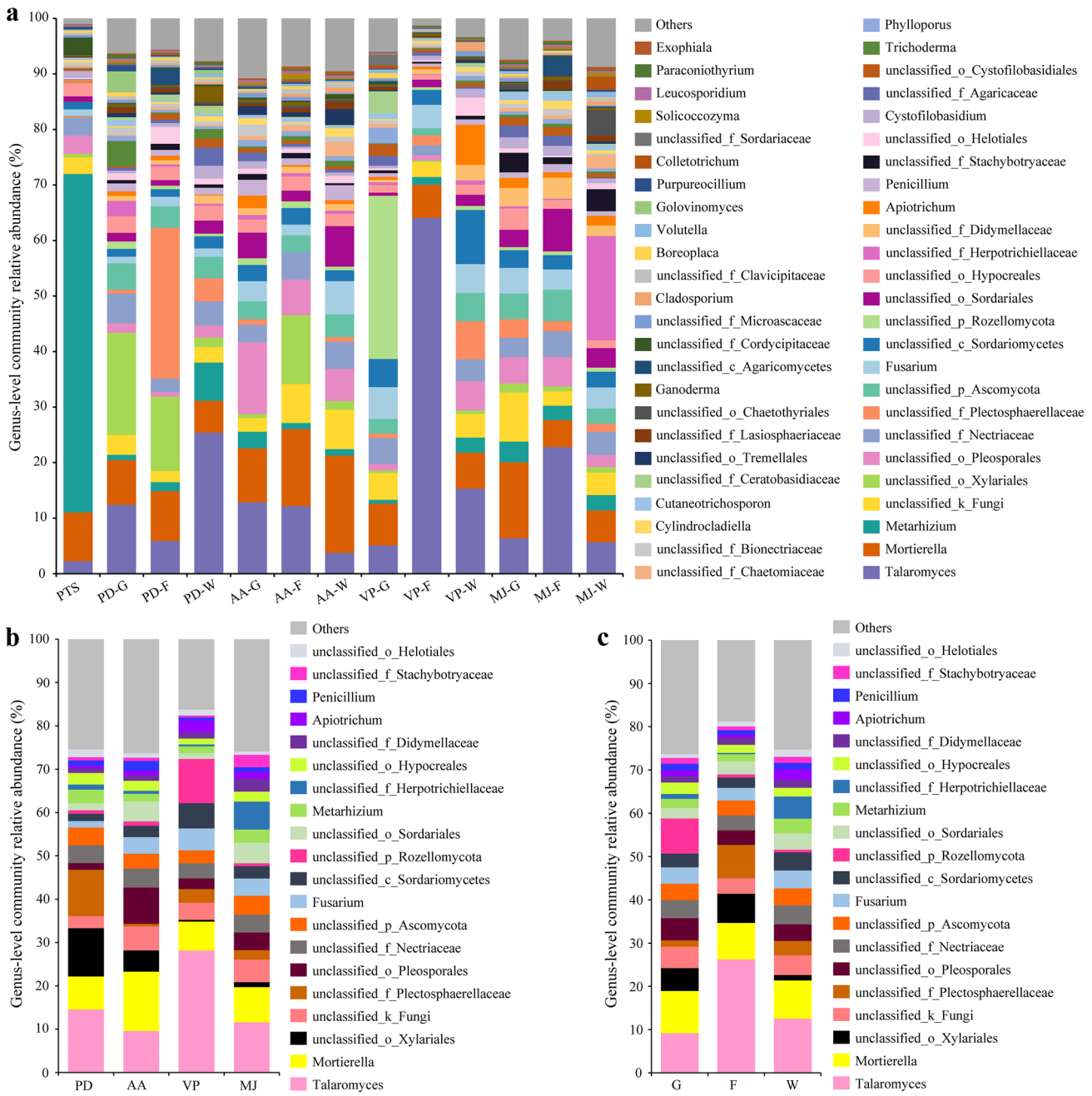

Fig. 2 Bar plot depicting the effect of medicinal plants on fungal community composition in hospital grassplot soil. **a** Bar plot of fungal community composition and relative abundance of 13 samples (including one primary test soil sample and 12 samples collected from pot experiments). Taxa with abundance <2% were combined under "Others". **b** Bar plot of the top 20 fungal genus in abundance in the plant species sample group. **c** Bar plot of the top 20 fungal genus in abundance in the growth stage sample group. PTS (primary test soil sample); see Table 1 for the code information of the 12 potted samples; the plant species sample group includes MJ (*M. jalapa*), AA (*A. argyi*), VP (*V. philippica*), PD (*P. depressa*); the growth stage sample group includes G (growing stage), F (flowering stage), W (withering stage); the same below

analysis. The results showed that the specificity of fungal community in the medicinal plant species sample group was higher than that in the growth stage sample group (Fig. 4). For example, at the genus level, four fungal genera—*Chaetosphaeria*, *Trechispora*, *Coprinopsis*, and *Paraphaeosphaeria*—were significantly correlated with plant species (Fig. 4a, c), while only *Cladosporium* was significantly correlated with growth stage (Fig. 4b, d). Among the four medicinal plant sample groups, the highest number of significant associations with fungal taxa in hospital grassplot soil was recorded with *P.*

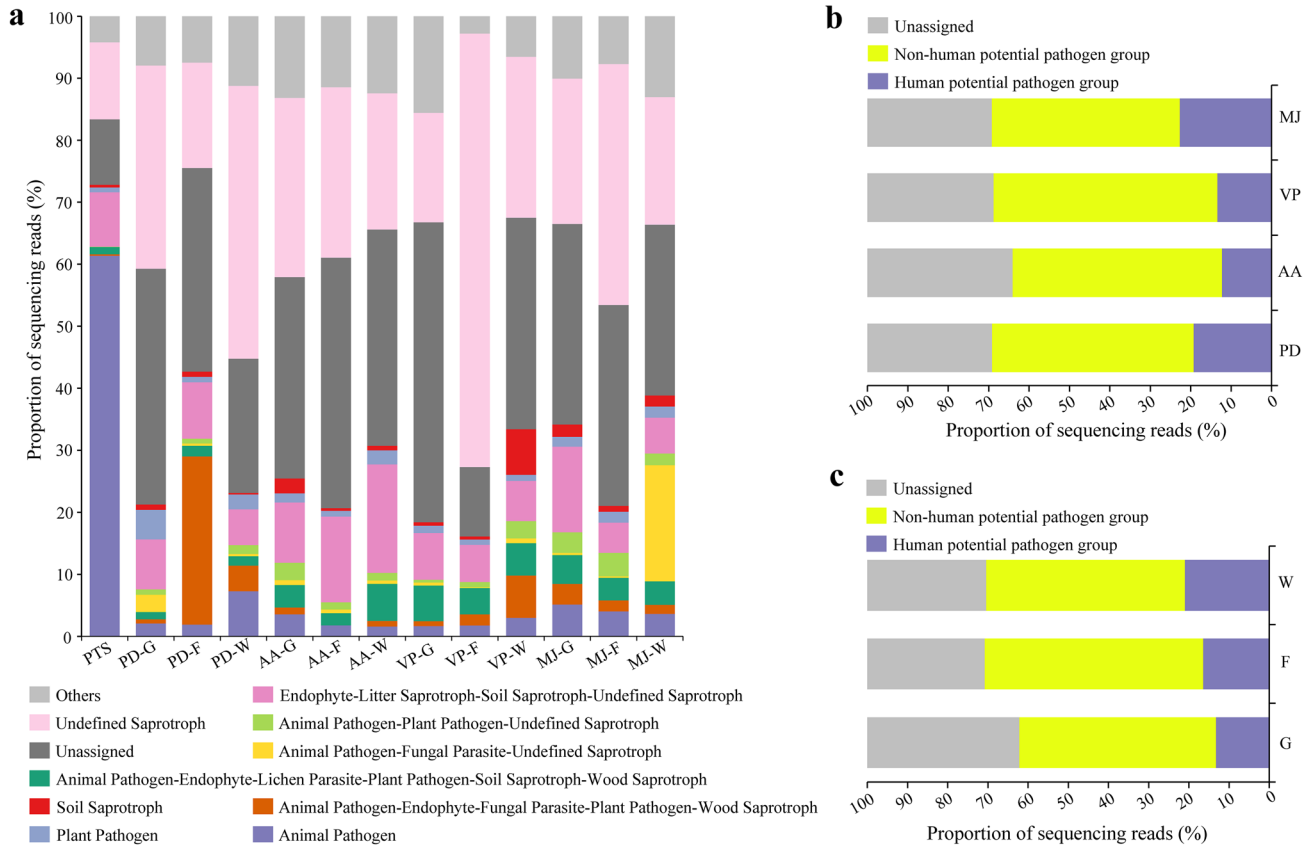

Fig. 3 Bar plot depicting the effect of medicinal plants on fungal community function in hospital grassplot soil. **a** Bar plot of the composition of fungal community ecological guilds (top 10 in abundance) in 13 samples (including one primary test soil sample and 12 samples collected from pot experiments). **b** Bar plot of human potential pathogen abundance in fungal community of the plant species sample group. **c** Bar plot of human potential pathogen abundance in fungal community of the growth stage sample group

depressa, followed by *A. argyi*; the lowest number was with *M. jalapa*, and there were no associations with *V. philippica* (Fig. 4a, c). For example, the fungal taxa enriched in *P. depressa* included three orders (Xylariales, Erysiphales, and Sebacinales), two families (Erysiphaceae and Clavicipitaceae), and one genus (*Chaetosphaeria*), whereas in *M. jalapa* only one genus (*Trechispora*) was present. Furthermore, among the three growth stage sample groups, the withering stage had a significant impact on fungal community in hospital grassplot soil, and the enriched taxa included one family (Cladosporiaceae) and one genus (*Cladosporium*), but not during the growing and flowering stages (Fig. 4b, d). These results indicated that medicinal plant species was a more dominant factor driving changes of fungal community in hospital grassplot soil compared with growth stage.

Core Taxa Analysis of Fungal Community in Hospital Grassplot Soil

In this study, Gephi software was used to conduct coexistence network analysis on the fungal community of all samples collected in the pot experiment, and 46 genera coexisting in 12 samples were defined as core fungi (Fig. 5). These core fungi were unaffected by the medicinal plant species and growth stage in the pot experiment. Further comparative analysis of the core fungal taxa revealed that, all 45 genera were present in primary test soil, except for *Cladosporium* (Supplementary Table: S9). And among them, nine genera (*Chaetomium*, *Cutaneotrichosporon*, *Metarhizium*, *Fusarium*, *Exophiala*, an unclassified genus of Chaetomiaceae, an unclassified genus of Didymellaceae, an unclassified genus of Herpotrichiellaceae, and an unclassified genus of Microascaceae) were annotated as taxa related to animal pathogen (Supplementary Table: S9). These results illustrated that the initial fungal community of hospital grassplot soil had a certain stability, and it was difficult to completely eliminate the threat of pathogenic fungi by planting medicinal plants to restore hospital grassplot soil.

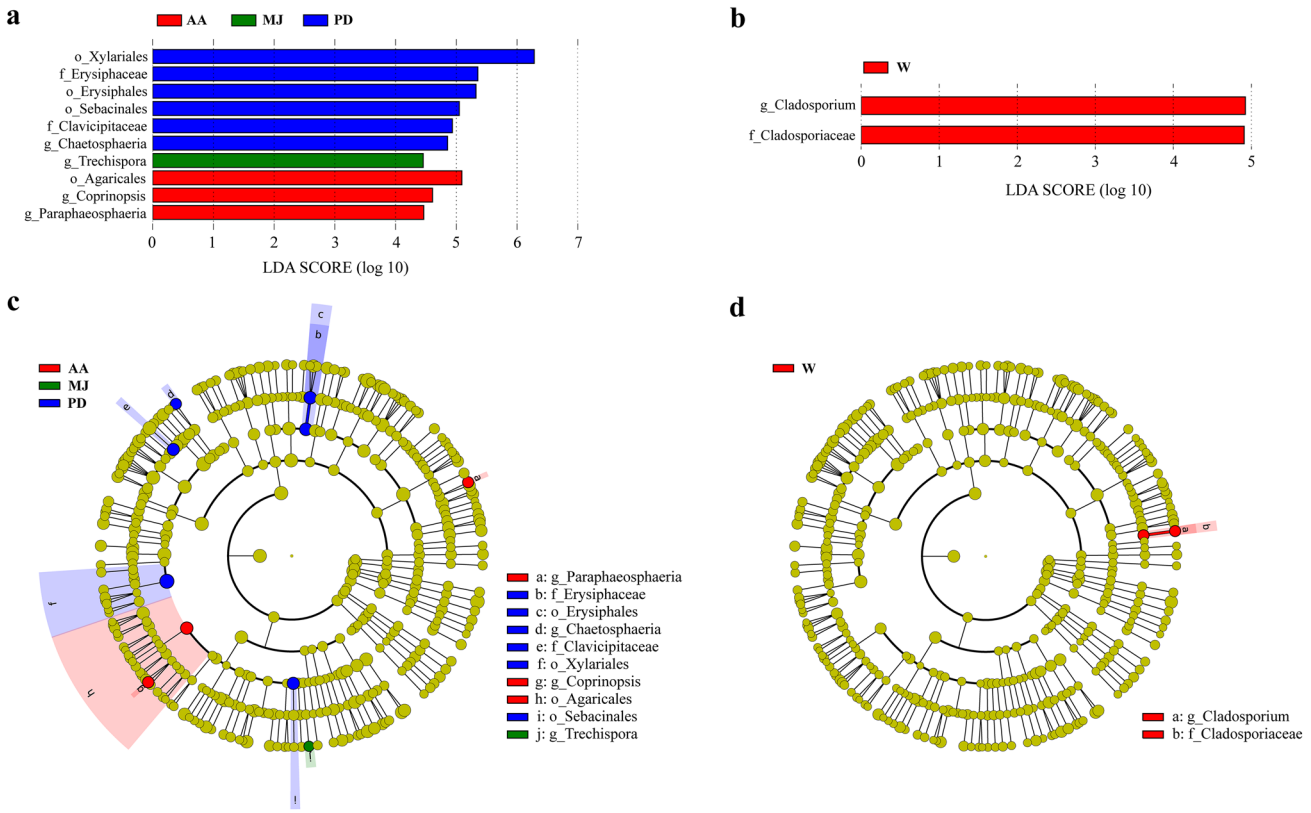

Fig. 4 Linear discriminant analysis of effects (LEfSe) was used to describe the differences in the effects of medicinal plant species and growth stage on fungal community in hospital grassplot soil. **a**, **b** show the bar graph of LDA scores distribution for fungal taxa with significantly different abundance among the plant species sample group and the growth stage sample group, respectively (LDA > 4.0). The degree of influence of a species was expressed by the length of the bar. **c**, **d** Show cladogram of fungal community among the plant species sample group and the growth stage sample group, respectively. From the center outward, the circles represent the kingdom, phylum, class, order, family, and genus, with taxa of significantly different abundance marked by colored shadows (Color figure online)

Discussion

As the number of individuals who are immunocompromised continues to increase globally, there are a greater number of patients with fungal diseases in hospitals [29, 30]. Disinfection precautions in hospital areas where patients come into direct contact, such as medical instruments, ward floors, and hospital beds, are currently highlighted by many public health initiatives [31]. However, the problem of fungal infections caused by hospital grassplot soil, a reservoir of pathogenic fungi, is often overlooked [4]. Based on the perspective that plants and soil microbes are part of a closely linked holobiont, the current study explored the effects of planting medicinal plants on the structure and function of fungal community in hospital grassplot soil, in order to provide a new exploration for hospital grassplot soil remediation.

We observed that the composition of fungal community in hospital grassplot soil changed significantly after planting medicinal plants in pots, and this change differed with medicinal plant species and growth stages. This finding may be related to the preference of fungi for carbon substrates, the variability of root exudates determined by genetic characteristics among medicinal plant species, the dynamic change of soil carbon substrates provided by medicinal plants with growth stages, and abiotic factors (e.g., temperature and light) that change with seasonal variation [32–35].

Functional analysis showed that animal pathogen, a potential threat to human health, was dominant in primary test soil fungal community, with an abundance of 61.36%. This finding was consistent with the particularity of the hospital. After potting medicinal plants, all treatments showed a decrease in pathogen abundance and an increase in saprotroph abundance. This implies that the hypothesis of restoring hospital grassplot soil quality by planting medicinal plants is feasible. Further analysis showed that medicinal plant species and growth stage together influenced the restoration effect. According to the abundance of human potential pathogen group, *A. argyi* was the best restoration species in the tested plants, and the growing stage was the best restoration period among the tested stages. However, this conclusion was reached under the limited species and growth stages of the tested plants in this study. We believe

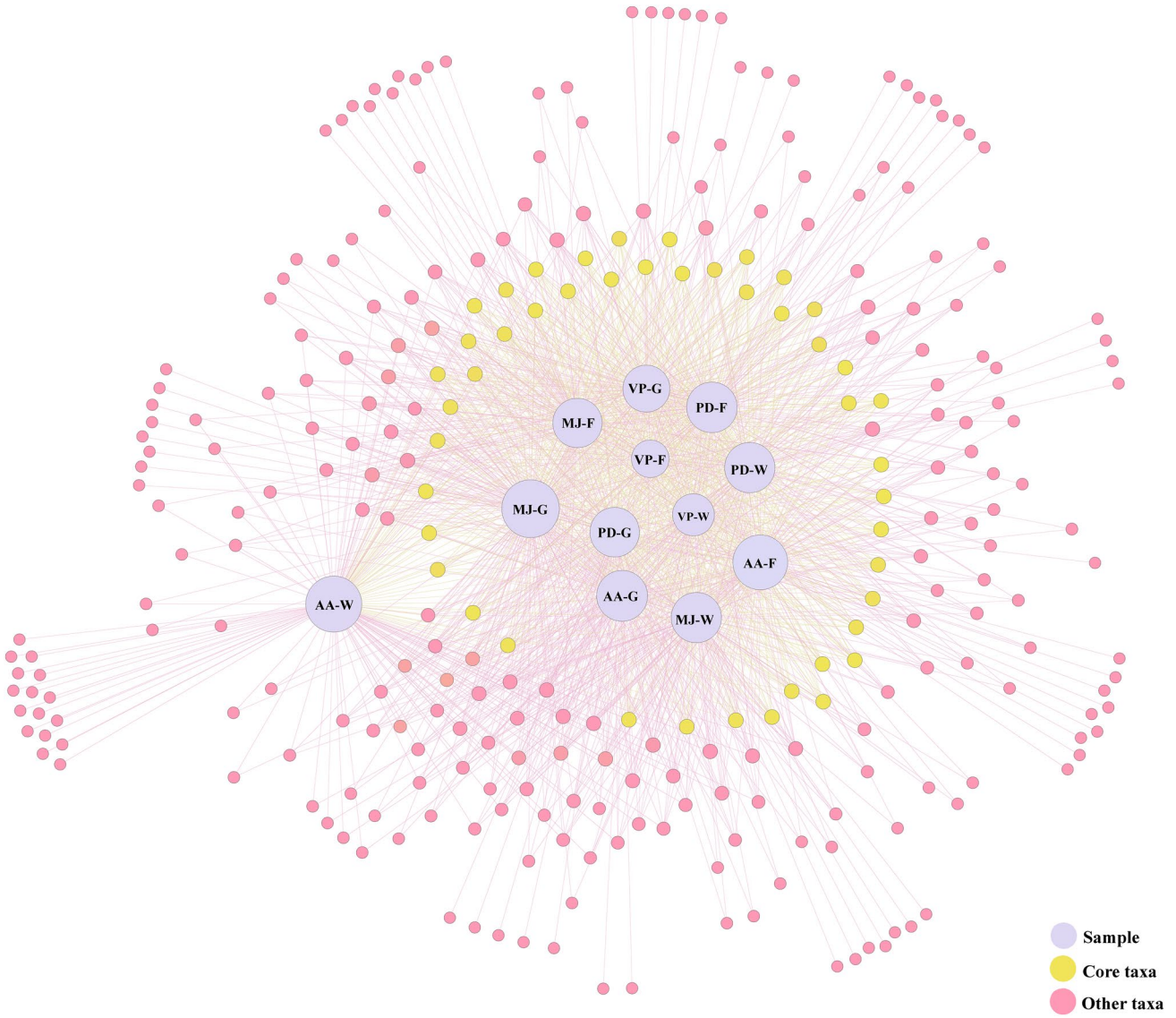

Fig. 5 Coexistence networks define core taxa (genera) of fungal communities in 12 samples collected from pot experiment

that a multi-plant assemblage combining multiple growth stages is more likely to have a long-term, stable, and sustained restorative effect than a single growth stage of a single plant species.

The host plant acts as a filter and can exert a strong selective effect on its associated microbial composition through the immune system, genetic networks, and secretions, which can generate a preference for certain microbial populations [32, 36, 37]. In addition to the influence of host genetic characteristics, plant growth stage also exerts a strong regulatory effect on the composition and function of relevant microbial communities [37–39]. LEfSe analysis revealed that medicinal plant species (host) was the predominant factor causing changes in fungal community in hospital grassplot soil compared with the growth stage. This result was inconsistent with the findings of Li et al. using different genotypes of *Arabidopsis thaliana* as experimental materials [36]. This discrepancy may be related to the fact that the four plants tested in the current study originated from different families and genera.

Microbes that are persistent and widespread in a given environment are known as "core microbiota" [40]. They are important taxa that influence community function and drive community evolution, and are the best predictors of microbial community change [41–44]. In this study, 46 core fungal genera were defined based on coexistence network analysis, which were not affected by medicinal plant species and growth stage. Among them, 45 core taxa were present in primary test soil, and many members were potential human pathogens. For example, *Exophiala* can cause human skin,

subcutaneous tissue, and systemic infections [45]. *F. falciforme* in *Fusarium* can cause meningitis in humans [46]. *Cutaneotrichosporon* can cause invasive infections [47]. *M. anisopliae* in *Metarhizium* can cause keratitis, sinusitis, and skin infections in humans [48, 49]. These findings indicate that the initial fungal community (that is, indigenous microorganisms) of hospital grassplot soil has a certain stability, and it is difficult to completely eliminate the threat posed by pathogenic fungi by planting medicinal plants for restoration.

Conclusion

This is a study to investigate the effect of planting medicinal plants on the structure and function of fungal community in hospital grassplot soil based on the perspective of soil microbes–plant holobiont. Our research indicated that medicinal plant planting could successfully reduce pathogenic fungi abundance in hospital grassplot soil and play a certain restorative role. However, the initial fungal community of hospital grassplot soil has a certain stability, and it is difficult to completely eliminate the threat caused by pathogenic fungi by planting medicinal plants. Nonetheless, our study still provides a new perspective for the future remediation of hospital grassplot soil. The method under this perspective has obvious advantages of green, long-term, and continuous remediation, which may be used as a supplement to chemical remediation measures in hospital environment.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-022-03083-1.

Author Contributions YH and JH: conceptualization and funding acquisition; QS and CD: data acquisition; QS, HH and XZ: formal analysis; QS: writing the first draft; YH and ZL: writing, review, and editing the manuscript.

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Data Availability The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: SAMN21449407; BioProject PRJNA763776).

Code Availability Not applicable.

Declarations

Conflict of interest All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval Not applicable.

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