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Spatial differences in *Casuarina equisetifolia* L. endophyte community structure

Qi Lin, Zhixia Xu, Miaomiao Li, Ying Wang and Lei Li*

Abstract

Purpose: *Casuarina equisetifolia*, a fast-growing, abundant tree species on the southeastern coast of China, plays an important role in protecting the coastal environment, but the ecological processes that govern microbiome assembly and within-plant microorganism transmission are poorly known.

Methods: In this paper, we used ITS and 16S amplification techniques to study the diversity of fungal and bacterial endophytes in critical plant parts of this species: seeds, branchlets, and roots. Additionally, we examined the litter of this species to understand the process of branchlets from birth to litter.

Result: We uncovered a non-random distribution of endophyte diversity in which branchlets had the greatest and seeds had the lowest endophytic fungal diversity. In contrast, litter endophytic bacteria had the highest diversity, and branchlets had the lowest diversity. As for fungi, a large part of the seed microbiome was transmitted to the phyllosphere, while a large part of the bacterial microbiome in the seed was transmitted to the root.

Conclusion: Our study provides comprehensive evidence on diversity, potential sources, and transmission pathways for non-crop microbiome assembly and has implications for the management and manipulation of the non-crop microbiome in the future.

Keywords: *Casuarina equisetifolia*, Amplification technique, Plant endophyte, Microbial diversity, Hainan

Background

There are many types of endophytes, and they have an extremely broad distribution, as microorganisms are present inside the tissues of almost every plant (Aly et al. 2011). Endophytes may grow in roots, stems, and leaves, in which they have been interacting and evolving with each other and form a “holobiont” with the plant (Vandenkoornhuys et al. 2015). Harnessing the plant microbiome to maximize management and manipulation of non-crop microbiome is increasingly viewed as a viable sustainable approach in the future. Therefore, it is necessary for us to understand the ecological processes that govern microbiome assembly.

Plant-associated microorganisms are diverse and not only change plant physiology and metabolism but also increase tolerance towards biotic and abiotic stresses (Yao et al. 2019), such as increasing defense responses to pathogens (Herre et al. 2007; Tian et al. 2014), and they can also promote plant growth (Santoyo et al. 2016; Xing 2018), thereby increasing environmental adaptability (Hoveland 1993; Rodriguez et al. 2008). Furthermore, endophytes can produce spores during plant tissue senescence for reproductive purposes, which are better able to mutualize with plants (Carroll 1988; Sherwood and Carroll 1974).

The above studies have showed that plants and their microbiomes have co-evolved for millions of years, and most of these interactions are reciprocal, increasing the fitness of both parties (Foster et al. 2017). However, it is unclear where plant-associated microorganisms originate. Some researchers believe that the similarity in

*Correspondence: lei-li@126.com

Ministry of Education Key Laboratory for Ecology of Tropical Islands, Hainan Normal University, Haikou 571158, China



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microbial composition between plants of the same species, such as maize, is largely due to selective supplementation from the surrounding environment and controlled by the physiology, morphology, and genetic characteristics of the plant species (Bouffaud et al. 2014; Hassani et al. 2018; Yeoh et al. 2017), that is, endophytes in different ecological niches in the same plant may enter the plant from the soil through horizontal transmission. However, this explanation is just valid when all environments contain all (or most) microorganisms required for the assembly of the plant microbiome. Moreover, this viewpoint overlooks the potential contribution of seed to plant microbiome assembly. After plant seeds have germinated, endophytes in the seeds may be vertically transmitted to the roots, stems, and leaves during plant growth and development. Thus far, many studies on vertical transmission in plants have focused on the composition and assembly of seed microbiomes, while few studies have considered the dynamic processes of establishment and transmission during plant development. However, the latter is a critical step in maintaining plant microbiome continuity and also a potential bottleneck for vertical transmission (Hodgson et al. 2014; Shahzad et al. 2018; Wassermann et al. 2019).

At the end of the 1950s, China introduced *Casuarina equisetifolia* to Guangdong province for the first time (Li et al. 2015). *C. equisetifolia* is currently an important coastal tree in southeastern China (Liu et al. 2020; Ye and Yu 1997). The tree *C. equisetifolia* is a species with abundant endophytes, and the endophytes promote plant growth and increase disease and pest resistance (Kang and Zhong 1999; Zhang et al. 2003), which facilitates plant capacity for salt and drought resistance, all of which are important in ecological restoration and resistance to natural disasters in coastal regions (Lin et al. 2008; Liu et al. 2013). Our previous studies showed that endophytes were involved in allelopathy, hindering the natural regeneration of forestland. Hitherto, many studies on *C. equisetifolia* endophytes have focused on the traditional separation, identification, and allelopathy of endophytic

bacteria and fungi (Huang 2019; Wang 2017; Zhang et al. 2020; Zuo et al. 2020). However, there are very few studies examining the composition and assembly of endophytes in different ecological niches, potential sources, and transmission paths. This is an important knowledge gap because how the host shapes microbiome assemblies and transmission patterns across the different parts of the tree remains largely unknown.

In this study, high-throughput sequencing was used to study the endophytic biome structure and transmission paths in *C. equisetifolia* seeds, branchlets, roots, and litter. The objectives of this study were to (1) determine what the diversity and composition of the microbial community are in *C. equisetifolia* tissues and (2) identify the relationship among the microbiota in seeds, branchlets, roots, and litters for further understanding of the physiological and ecological role of endophytes in *C. equisetifolia*. We expected to find a high microbial diversity in *C. equisetifolia*. Given differences in structure and niches that exist between roots and branchlets, we expected to find spatial compartmentalization of the microbial community between the two tissues. We also hypothesized that the majority of seed-associated microbes would be transient and that only a small fraction of the microbial taxa would be transmitted to other parts of the plant.

Results

Endophytic diversity in different ecological niches

From the Sobs Index of Abundance in Table 1, it could be seen that fungal abundance in *C. equisetifolia* tissues showed a trend of branchlets > litter > roots > seeds. The endophytic fungal abundance in branchlets and litter was 2–3 times that of seeds and roots. From the Shannon Index of Diversity (Table 1), the result showed that there were no significant differences in endophytic fungal diversity among various *C. equisetifolia* tissues.

Endophytic bacterial abundance and diversity showed a trend of litter > roots > seeds > branchlets (Table 1), and there were significant differences in endophytic bacterial abundance and diversity among litter, roots, and

Table 1 Abundance and Diversity Index of endophytes in seeds, branchlets, roots, and litters of *Casuarina equisetifolia*

Sample	Endophytic fungi		Endophytic bacteria	
	Sobs	Shannon	Sobs	Shannon
Seed	89.33 ± 5.84 c	2.18 ± 0.18 a	62.67 ± 9.49 c	0.51 ± 0.05 c
Branchlet	295.33 ± 19.81 a	3.18 ± 0.11 a	27.33 ± 6.36 c	0.31 ± 0.10 c
Root	103.33 ± 23.31 c	2.74 ± 0.33 a	211.67 ± 14.77 b	3.59 ± 0.22 b
Litter	233.33 ± 20.19 b	2.21 ± 0.43 a	487.33 ± 12.81 a	5.06 ± 0.03 a

Sobs index represents species richness

Shannon index represents species diversity

Different letters indicate significant differences ($P < 0.05$)

branchlets. However, there were no significant differences in endophytic bacterial abundance and diversity between seeds and branchlets.

Assembly of endophytes in different ecological niches

There were nine fungal phyla, 29 classes, 72 orders, 158 families, 248 genera, and 324 species and 24 bacterial phyla, 50 classes, 132 orders, 232 families, 410 genera, and 647 species in *C. equisetifolia* seeds, branchlets, roots, and litter.

As seen in the community bar chart (Fig. 1a), endophytic fungi in *C. equisetifolia* seeds, branchlets, roots, and litter mainly consisted of four phyla. The dominant fungal phyla in seeds, branchlets, and roots were Ascomycota, accounting for 98.24%, 67.04%, and 58.43%, respectively. The dominant fungal phyla in litter were Basidiomycota, accounting for 69.59%.

The endophytic fungi in seeds, branchlets, roots, and litter mainly consisted of 36 genera (Fig. 1b). The fungal genus with the highest abundance in seeds was *Phaeophleospora*, accounting for 25.34%. This genus accounted for 4.35% and 0.13% of genera in branchlets and litter and was not found in roots. The fungal genus with the highest abundance in branchlets was unclassified_p_Ascomycota, accounting for 29.54%. This genus accounted for 5.93%, 19.18%, and 1.87% of genera in branchlets, roots, and litter. The genus with the highest abundance in roots was *Trichaptum*, accounting for 27.88%, and this genus was not found in seeds, branchlets, and litter. The genus with the highest abundance in litter was unclassified_o_Agaricales, accounting for 32.20%. The proportion of this genus in branchlets was 0.01%, and it was not found in seeds and roots. The main endophytic fungal genera common to seeds, branchlets, roots, and litter were unclassified_p_Ascomycota accounting for 5.93%, 29.54%, 19.18%, and 1.87%, respectively, and unclassified_k_Fungi accounting for 1.30%, 16.95%, 9.39%, and 2.97%, respectively.

As seen in Fig. 1c, endophytic bacteria in *C. equisetifolia* seeds, branchlets, roots, and litter mainly consisted of eight phyla. The dominant bacterial phylum in seeds and branchlets was Cyanobacteria, accounting for 88.29% and 92.79%, respectively. This phylum accounted for a lower ratio in roots and litter. The dominant bacterial phylum in roots and litter was Proteobacteria, accounting for 41.30% and 49.03%, respectively, and this phylum accounted for a lower ratio in seeds and branchlets.

The endophytic bacteria in *C. equisetifolia* seeds, branchlets, roots, and litter mainly consisted of 44 genera (Fig. 1d). The bacterial genus with the highest abundance in seeds and branchlets was norank_f_norank_o_Chloroplast, accounting for 88.29% and 92.79%, respectively. The bacterial genus with the highest abundance in roots

was *Pseudomonas*, accounting for 13.12%. The bacterial genus with the highest abundance in litter was *Sphingomonas*, accounting for 8.95%.

Endophytic community structure and spatial distribution in different ecological niches

A principal component analysis (PCoA) of endophytic fungi in *C. equisetifolia* tissues showed that the contribution of the two greatest differential characteristics, PC1 and PC2, was 58.97%. Branchlet and litter endophytic fungi were extremely close in the PC1 axis. Litter endophytic fungal samples were further away from seeds, branchlets, and roots in the PC2 axis (Fig. 2a). As seen in Fig. 2b, the contribution of PC1 and PC2 was 83.92%. The bacteria in the seed and branchlet were more similar.

Heatmap analysis was carried out on the top 30 microbial genera by abundance. Branchlet and root endophytic fungal communities were clustered into the same group, and there were differences compared with seed and litter endophytic fungal communities (Fig. 3a). However, endophytic bacteria in litter and root were clustered into one group, while those in seed and branchlet were clustered into another group (Fig. 3b). These results were consistent with those of PCoA analysis.

Transmission of seed microbiome to different ecological niches

Venn diagram analysis of OTUs at the genus level in fungi showed that there were 49 OTUs common in seeds and branchlets, accounting for 80.33% of seeds. Eight OTUs were common in seeds and roots, accounting for 13.11% of roots. There were 78 common OTUs in branchlets and litter, accounting for 51.66% of branchlets (Fig. 4a and c). As observed in Fig. 4b and d (bacterial genus level), 17 OTUs were common in seeds and branchlets, accounting for 22.97% of seeds. There were 49 OTUs common in seeds and roots, accounting for 66.22% of roots. There were 15 OTUs that are common in branchlets and litter, accounting for 55.56% of branchlets. Figure 4c and d is a more intuitive reaction to Fig. 4a and b, respectively.

Dynamic transmission of the core microbiome in aboveground and belowground tissues from different ecological niches

Microbiomes with a relative abundance of more than 1% were collected from Fig. 1. Five fungal genera and 3 bacterial genera in seeds were transmitted to branchlets, namely *Parateratosphaeria*, etc. Two fungal genera and 3 bacterial genera were transmitted to roots: *Pseudomonas*, etc. Five fungal genera in branchlets were transmitted to litter: *Phaeophleospora*, etc. Interestingly, no transmission of bacteria between branchlets and litter was found (Fig. 5).

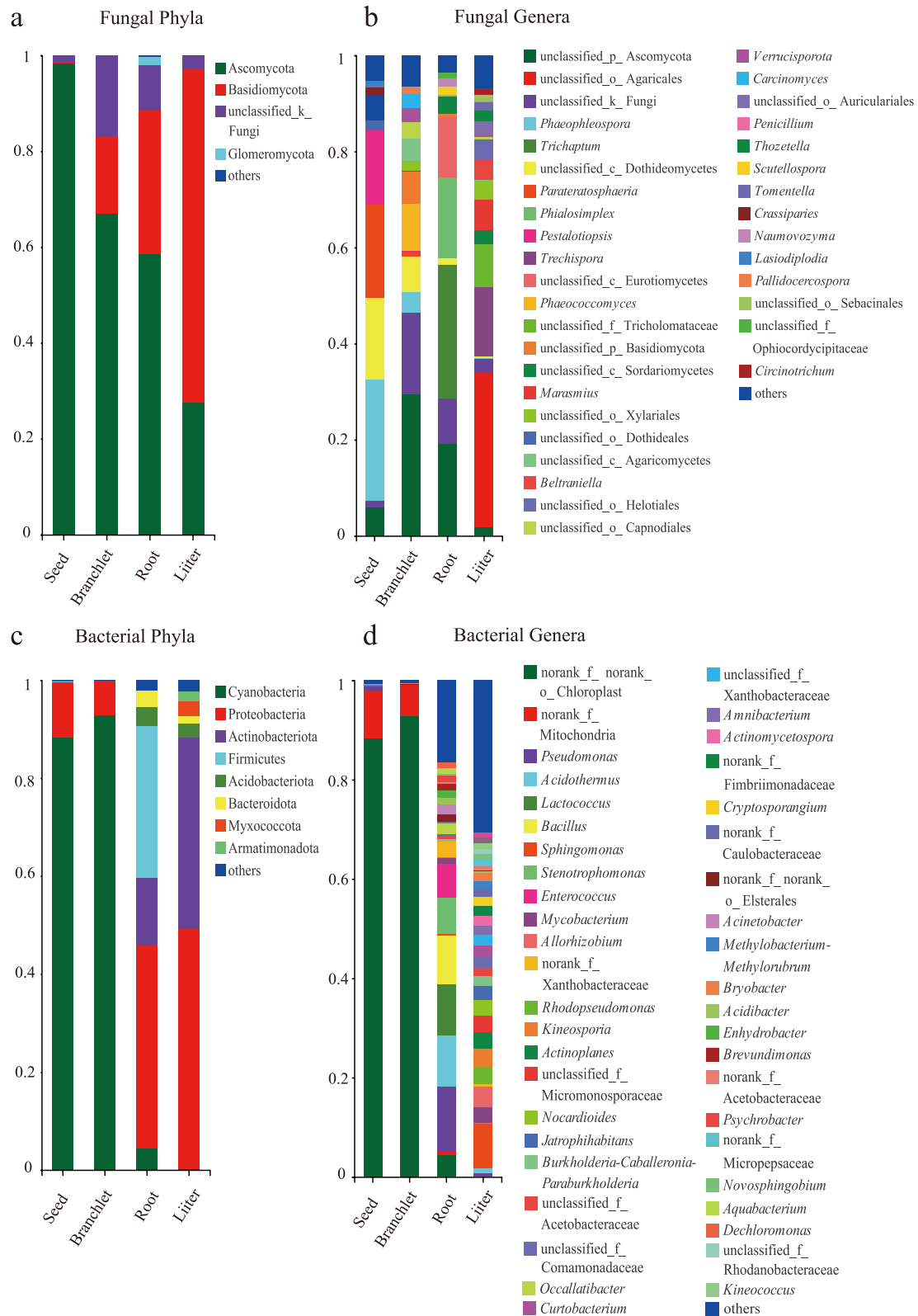
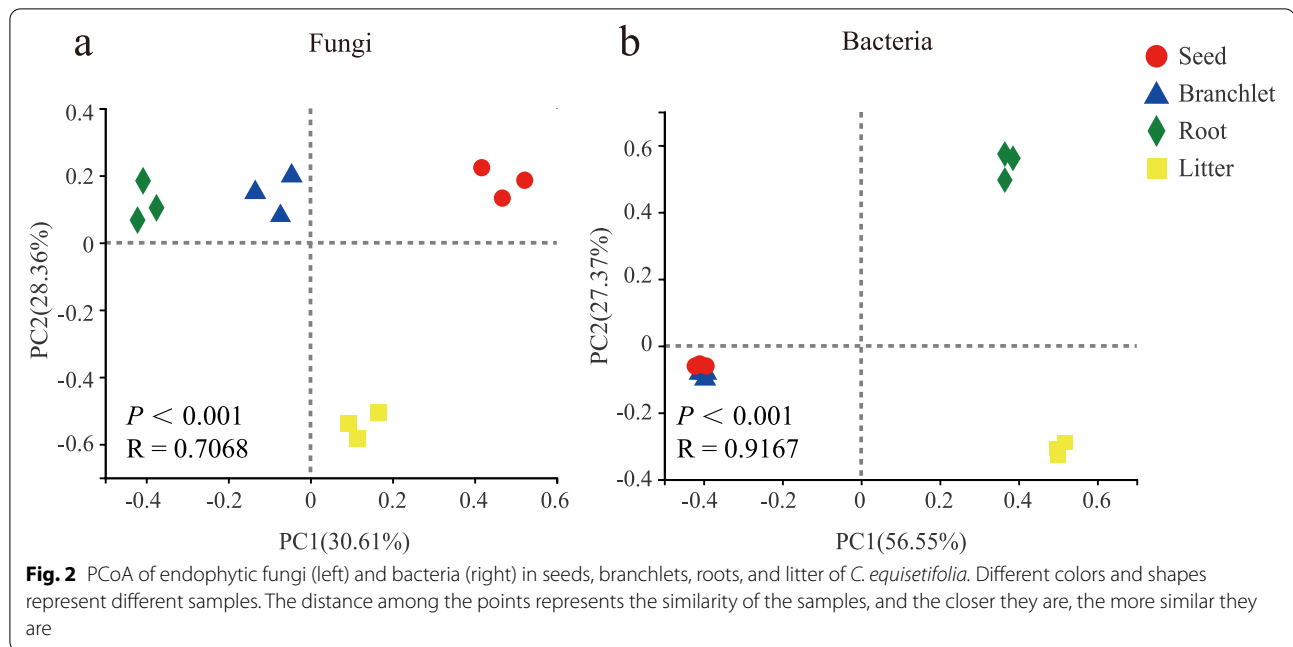


Fig. 1 Endophytic fungi (top) and bacteria (bottom) community composition of *Casuarina equisetifolia* seeds, branchlets, roots, and litter. The abscissa represents different ecological niches and the ordinate represents the relative abundance of microbial genera



Discussion

While the diversity of the plant microbiome is increasingly recognized, the assemblages and transmission pathways of the plant microbiome are poorly known. In the present study, a scenario survey on the diversity of fungal and bacterial endophytes in seeds, branchlets, roots, and litter demonstrated that branchlets had the greatest and seeds had the lowest endophytic fungal diversity. In contrast, litter endophytic bacteria had the highest diversity, and branchlets had the lowest diversity. Moreover, we identified the potential sources and transmission pathways of the non-crop microbiome through multiple machine-learning methods. Collectively, these results provide clear evidence of microbial inheritance in plants, niche differentiation of the inherited microbiome, and divergent transmission routes from the seeds to the phyllosphere and roots. These findings shed new light on the potential source and transmission pathway of plant-associated microbes and increase our knowledge of the distribution of microorganism and dispersal within natural environments.

Microbiome composition and assembly

This study is to examine endophyte community diversity in *C. equisetifolia* seeds, branchlets, roots, and litter, and many types of endophytes were found. We found that there were differences in endophytes among tissues of *C. equisetifolia*. The abundance and diversity of the endophytic bacteria in branchlet were the lowest (Table 1). A study on the microbiome in strawberries and *Alsophila spinulosa* also obtained similar results (da Silva et al. 2020; Zang et al. 2020). We inferred the reasons that might be due to selective pressure from the host immune system and plant secretions on the leaves. As a result, only a few bacteria could colonize branchlets (Guttman et al. 2014; Hacquard et al. 2015; Kembel et al. 2014).

We further found that the abundance and diversity of endophytic fungi were lower in litter than in branchlets (Table 1), and the composition of microorganisms in litter was different from other ecological niches (Fig. 2). When the branchlets are detached from plants, becoming litter, some endophytic fungi may change their ecological strategy and survive by saprophytic method, while

(See figure on next page.)

Fig. 3 Heatmap of abundance of endophytic fungi (top) and bacteria (bottom) of *C. equisetifolia* seeds, branchlets, roots, and litter at genus taxonomic levels. The abscissa is the sample name, and the ordinate is the species name. The clustering tree above the Heatmap represents the differences between samples, and the clustering tree on the left of the Heatmap represents the differences between microbes, with clustering into one group meaning that the differences are small. The variation of the abundance of different species in the sample is displayed through the color gradient of the color block. The value represented by the color gradient is on the right of the figure

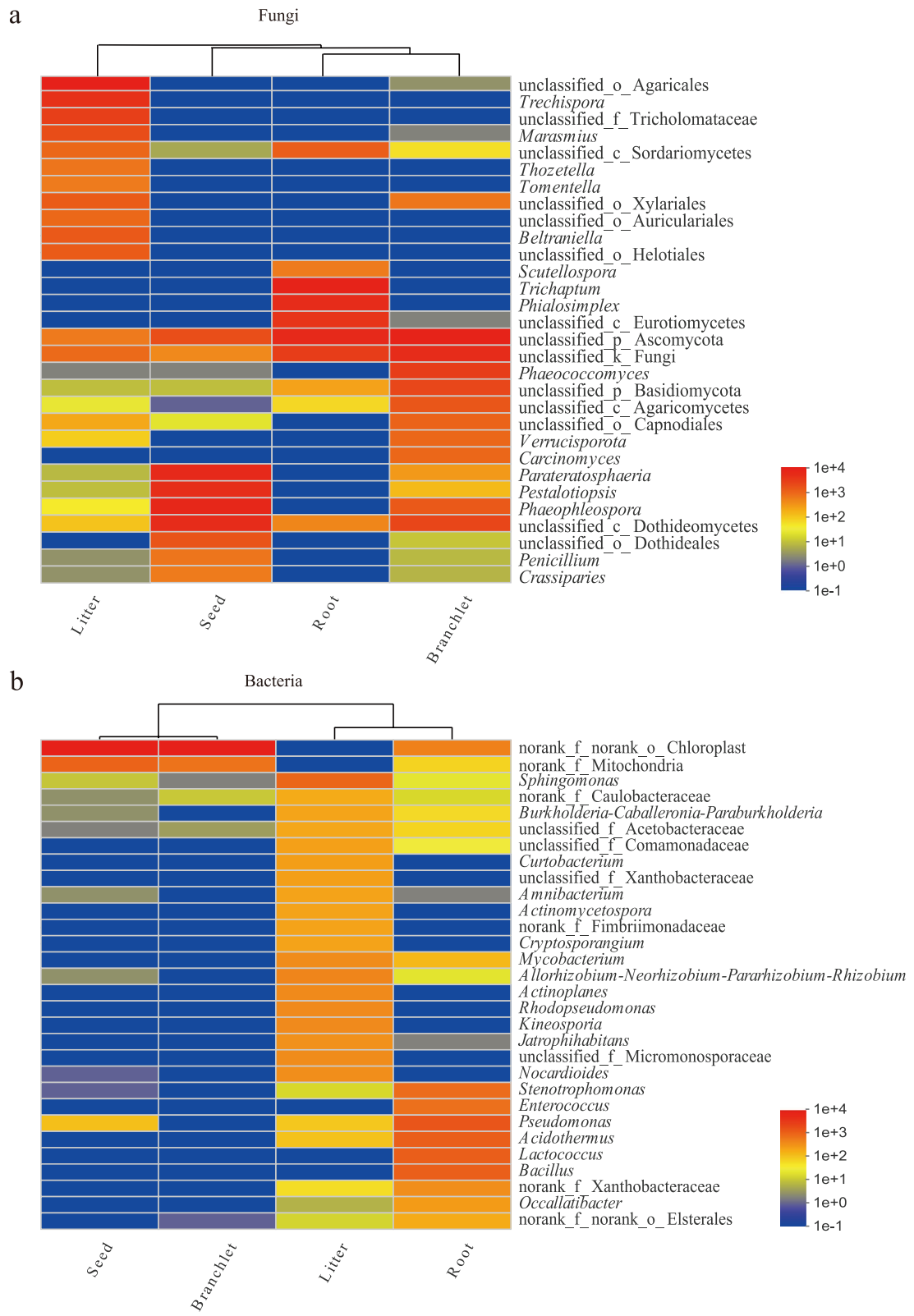
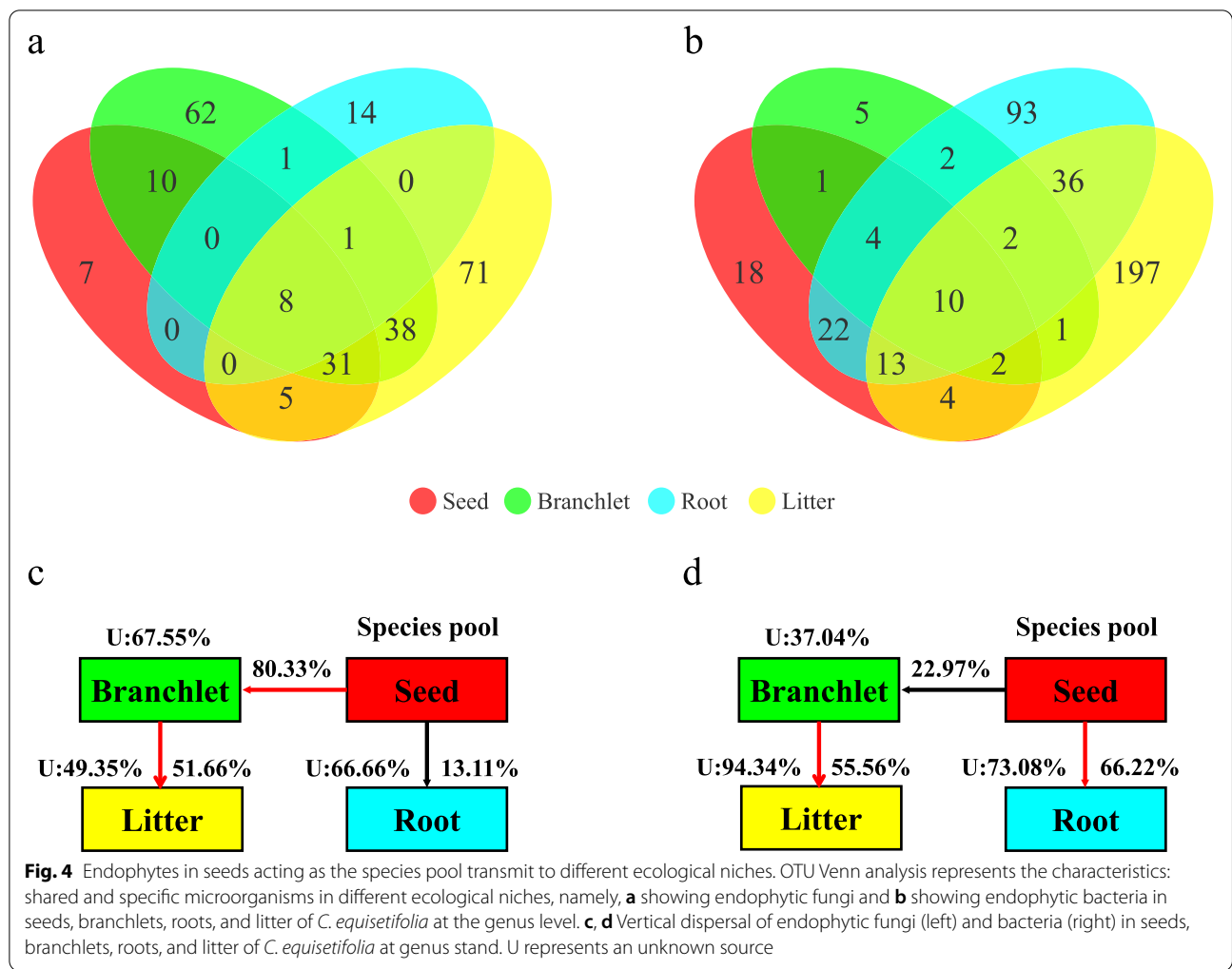


Fig. 3 (See legend on previous page.)



some endophytic fungi that cannot change their ecological strategy may die (Promputtha et al. 2007; Selosse et al. 2008). These results imply that bacteria and fungi have different tolerance in different ecological niches.

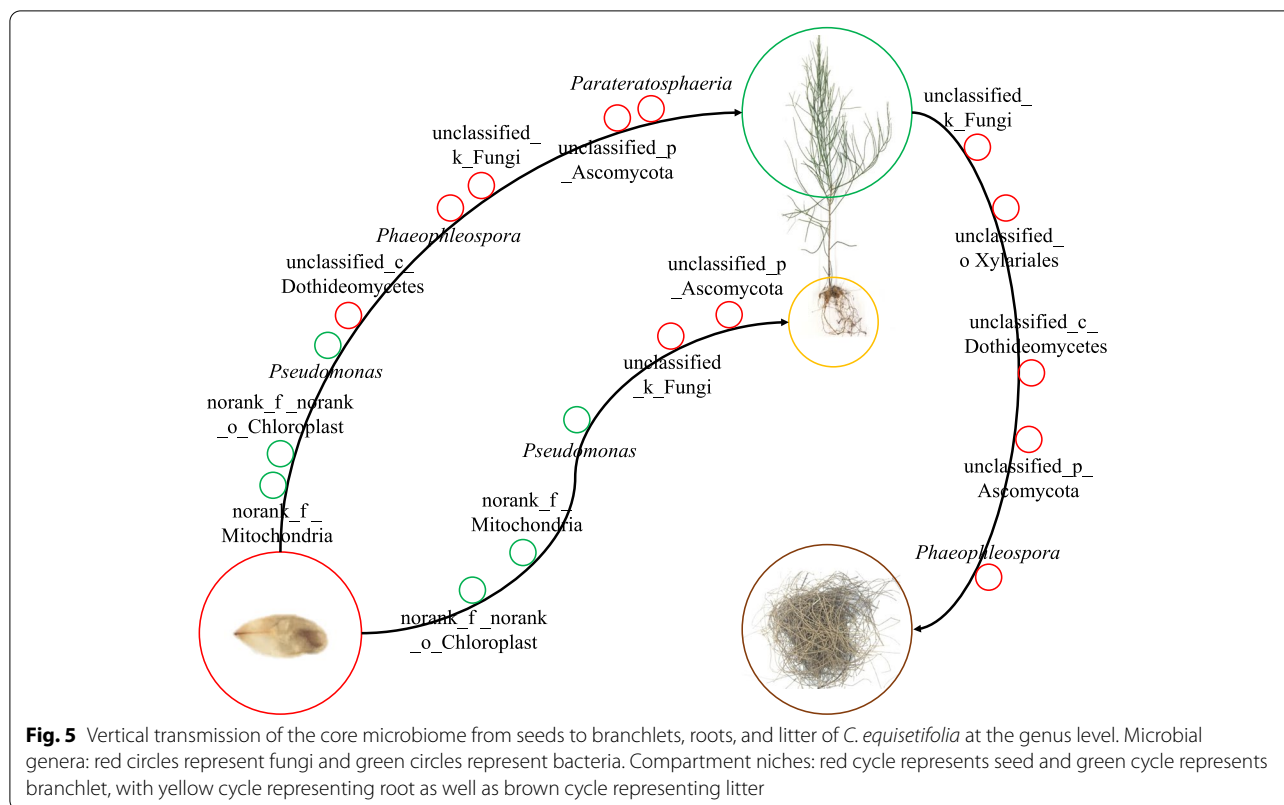
As seen in Table 1, the abundance and diversity of endophytes in seeds were low, which might be due to the limited resources in seeds. Several unconfirmed endophyte taxa were found in seeds (Fig. 1), showing that further studies on seed microorganisms are needed to discover the potential of new species in this ecological niche.

Dominant and keystone taxa of microbiomes

Dominant and biomarker taxa are considered potential keystone taxa, and they have important ecological functions in microbiome assembly and ecosystem functioning (Banerjee et al. 2018; Delgado-Baquerizo et al. 2018). The major endophytic fungi in *C. equisetifolia* seeds, branchlets, roots, and litter were Ascomycota and Basidiomycota. In addition, the major phylum of endophytic

bacteria in seeds and branchlets was Cyanobacteria, and Proteobacteria was the most dominant phyla of endophytic bacteria in roots and litter (Fig. 1). Liu et al. obtained similar conclusions (Liu et al. 2021). Shahzad et al. and Guo et al. found that most rice seed endophytic fungal phyla were Ascomycota and Basidiomycota (Guo et al. 2020; Shahzad et al. 2017). Ren et al. indicated that Proteobacteria was highly enriched in Jingbai Pear trees (Ren et al. 2019).

Although highly diverse microorganisms colonize ecological niches in plant tissues, few taxa occupy dominant positions. Our study revealed that *Pseudomonas* was significantly enriched in the roots (Fig. 1). This finding was backed up by previous research (Weller 1988; Weller 2007). *Pseudomonas* grows in different habitats and usually is regarded as a plant and seed pathogen, but it can promote plant growth and some species have inhibitory effects on soil pathogens (Burr et al. 1978; Kumar and Dube 1992). Therefore, we speculated that *Pseudomonas* might protect *C. equisetifolia* from soil pathogens



and stimulate its development though this hypothesis required direct evidence. Similarly, we found that *Bacillus* was also significantly enriched in roots (Fig. 1). Some *Bacillus* spp., such as *Bacillus cereus*, have been reported to be antagonistic to soil-transmitted diseases (Fira et al. 2018). Yaish et al. isolated *Bacillus* strains from date palm seedlings and found that they can promote the growth and development of date palm trees under salinity stress (Yaish et al. 2015). Our results also revealed that Dothideomycetes were present in the branchlets (Fig. 1), which were saprophytic fungi associated with nutrient cycling (Adams et al. 2013). All these findings suggest that plant tissues may create different ecological niches for specific microbiomes, and these microbiomes may recognize signaling molecules and adapt to the environment unique to each ecological niche (Cordovez et al. 2019; Foster et al. 2017). The determination of these dominant and keystone taxa can provide essential information to control microbiomes.

Potential sources and transmission routes of microbiomes

Although researchers have focused on the diversity of plant microbiomes and plant-microbe interactions, previous studies have reported that a large proportion of microbial taxa are shared between belowground and aboveground organs in plants (Bai et al. 2015; Wagner

et al. 2016). However, our knowledge of the transmission routes of microorganisms in different plant tissues is limited. In this paper, we studied the transmission processes of *C. equisetifolia* endophytes from seeds to litter, which is an important but overlooked aspect of vertical transmission.

As seen in Fig. 4c, 80.33% of endophytic fungi were transferred to branchlets from seeds. This result shows that the diversity of transmitted fungi in seeds is far higher than in transient fungi, i.e., seeds may be a store for branchlets endophytes, further emphasizing the ecological roles of seeds in endophytic microbiome aggregation and sources (Hardoim et al. 2015; Rahman et al. 2018). These results suggest that vertical transmission occurs through seeds (Hodgson et al. 2014). Our study also found that the diversity of the endophytic fungi in branchlets was the highest (Table 1), many endophyte-specific fungal genera in branchlets, but the abundance of these genera was low (Fig. 1). This is not surprise due to the fact that fungi in the leaves may arise from horizontal transmission (Bayman et al. 1998; Frohlich et al. 2000; Kaneko and Kaneko 2004), with rainfall and wind transmitting spores (da Silva et al. 2020). As duration increases, the opportunities for spore deposition increase, i.e., fungi attached to leaf surfaces enter the leaves through the stomata or cuticle, and leaves are

infected after germination (Bayman et al. 1998). These outcomes further show that leaf microorganisms are affected by the dual effects of the host and external environment due to the fact that the leaf surface is considered as an interface between the host and the environment (Lindow and Brandl 2003; Remus-Emsermann and Schlechter 2018). In the present study, 13.11% of endophytic fungi were transferred from seeds to roots, while 66.22% of endophytic bacteria were transferred to roots (Fig. 4c, d), signifying that there are large differences in the tendency for root migration of fungal and bacterial genera, and/or that some genera proliferate more rapidly once they have migrated to the roots. Although endophyte communities in roots and branchlets have the same source, there are differences. We inferred that the reasons for these differences included the life history of microorganisms and regulatory factors that limited the migration of microorganisms to plant roots. Changes were found to occur in fungal communities from oak trees after falling to the ground, making these communities more similar to soil communities (Fort et al. 2019). Our previous studies showed that the distribution of endophytic fungi in roots was significantly affected by total phosphorous (Huang et al. 2020), and high phosphorus content was detrimental to the growth of endophytic fungal communities (Zuo et al. 2020), thereby enabling root endophytes to form a unique subset. We also found that more than half of the fungi (51.66%) and bacteria (55.56%) were transferred from branchlets to litter, and 94.34% of endophytic bacteria in litter were from unknown sources (Fig. 4c, d), suggesting that they may be more affected by the external environment. These results show that ecological niches in different spaces have dominant effects on the assembly of plant microbiomes, providing comprehensive evidence for a theoretical framework of ecological niches selection. Host plants filter and enrich microbial taxa with specific functions in different ecological niches (Müller et al. 2016), and competition for these resources by microorganisms drives evolutionary radiation, thereby differentiating and adapting to different ecological niches to reduce competition (Foster et al. 2017).

Although the relative abundance of certain taxa was low, they were only present in branchlets, roots, or litter (Fig. 1). Examples include *norank_f_norank_o_Elsterales*, which was only present in branchlets (0.01%), *unclassified_f_Ophiocordycipitaceae*, which was only present in roots (1.37%), and *norank_f_Xanthobacteraceae*, which was only present in litter (0.48%). Although it is difficult to explain the specific causes for this phenomenon, there are several reasonable hypotheses: (1) natural differences exist between *C. equisetifolia* individuals, leading to taxon differences in microbiomes between seeds and various plant tissues;

(2) some taxa initially below the level of detection are transmitted to branchlets, roots, and litter, and rapidly proliferate there; and (3) this is an effect of horizontal transmission.

In summary, our results provide some theoretical basis for ecological niches differentiation and transmission route differentiation of *C. equisetifolia* seed, branchlet, root, and litter endophytes. In the following research, we will focus on the influence of the external environment on plant microorganisms.

Materials and methods

Sampling site

The Guilinyang Coastal Development Area in Haikou, Hainan, was selected as the site of the *C. equisetifolia* forest. This region (20°01'02" N, 110°31'20" E) has a tropical maritime monsoon climate. The annual mean temperature is 23.8 °C, with abundant heat and a long sunshine duration. The annual mean precipitation is 1500–2000 mm, and the mean relative humidity is 85%. There are distinct wet and dry seasons, of which precipitation is the highest in May–October. Most of the wind throughout the year is from the east and northeast, and the annual mean wind speed is 3.4 m/s. During the typhoon activity period, typhoons are relatively concentrated and higher in quantity with diverse routes in April–October (Cai et al. 2010; Wan et al. 2016).

Sample collection and surface sterilization

In September–October, seeds, branchlets, roots, and litter were collected in the *C. equisetifolia* forest (stand age: 15–20 years) in 2020. For seed, branchlet, and root sampling, about five individual plants were randomly selected, and litter with a thickness of 0–5 cm around the same plants was collected, with five subsamples thoroughly mixed as a biological sample (25–50 g for each sample), and each sample had three biological replicates. A total of 12 samples were stored in a sterile plastic bag and placed in a cooler box with ice packs for transport. The epiphytic microorganisms of samples were removed according to the previous methods (Bodenhausen et al. 2013; Chen 2021), with some modifications. Briefly, 10–15 g of branchlets and litter as well as 3–5 g roots (rhizosphere soils were firstly removed by brush carefully) tissues were submerged in 0.1 M Potassium phosphate buffer (pH 8.0) and subjected to sonication at 40 kHz for 1 min. Considering seeds, 3–5 g were immersed into 0.1 M Mercury dichloride for 60 s. Sterilization was checked by plating the last washing water on Luria-Bertani (LB) used for bacteria and Potato Dextrose Agar (PDA) used for fungi and incubating at 28 °C.

Endophytic DNA extraction, microbial rRNA gene amplification, and sequencing

Total genomic DNA was extracted from collected samples following FastDNA[®] Spin Kit for Soil (MP Biomedicals, U.S) after surface sterilization. ITS1F (5'-CTTGGT CATTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCG TTCTTCATCGATGC-3') were used for PCR amplification, and 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used for PCR amplification. The PCR reaction mixture contained 12.5 µL KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA, USA), 1.5 µL (10 µM) each primer, and 10ng template DNA, followed by PCR-grade water added to a final volume of 25 µL. The PCR amplification cycling conditions were as follows: initial denaturation at 98 °C for 3 min, 30 cycles of denaturing at 95 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, with a final 10 min elongation at 72 °C. Sequencing was performed on an Illumina Miseq PE300 platform (Majorbio, Shanghai, China).

Data processing and diversity analysis

The overlapping relationship of paired-end (PE) reads was used to merge reads into a sequence, and quality control and filtration was carried out based on the raw sequence. OTU clustering was carried out based on 97% similarity (Edgar 2013), and chimeras were removed. The ribosomal database project (RDP) classifier (Wang et al. 2007) (<http://rdp.cme.msu.edu/>, version 2.2) was used for species classification and annotation of each sequence. Statistical analyses were performed based on various taxonomic levels. Uparse (version 7.1 <http://drive5.com/uparse/>) software was used for OTU clustering and bioinformatics analysis (Chen et al. 2016; Rogers et al. 2016; Ye et al. 2017); Mothur (version v.1.30.1 http://www.mothur.org/wiki/Schloss_SOP#Alpha_diversity) was used for the analysis of the alpha index (Sobs richness and Shannon diversity), and OTU similarity was assessed at 97% (Caporaso et al. 2010; Youssef et al. 2009). Community histogram (bar chart) analysis was carried out based on R (version 3.3.1), and R was used for PCoA statistical analysis and graph plotting (Ling et al. 2014). The vegan package in R was used for Heatmap analysis (Mitter et al. 2017). R was used for the Venn diagram analysis of all OTUs.

Conclusions

This study provides evidence for the assembly and transmission pathways of *C. equisetifolia* microbiomes. Our results showed that branchlets had the greatest and seeds had the lowest endophytic fungal diversity. In contrast, litter endophytic bacteria had the highest diversity, and

branchlets had the lowest diversity. In different ecological niches, the composition of endophytic fungi in branchlets and roots was more similar, and the composition of endophytic bacteria in seeds and branchlets was more similar, but they were different in litter from other ecological niches whether endophytic bacteria or fungi. Further, we revealed that as for fungi, a large part of the seed microbiome was transmitted to the phyllosphere, while a large part of the bacterial microbiome in seed was transmitted to the root. The endophytic fungi that transmitted to the branchlet were related to nutrient cycling, and endophytic bacteria to the root were involved in inhibiting soil-borne microorganisms. These results further deepen our understanding of microbiomes in different ecological niches and transmission processes and highlight the important effect on the vertical transmission during the assembly of the plant microbiome. From an ecological perspective, the results of this study have implications for the distribution, dispersal, persistence, and function of plant-associated microbes in the natural environment.

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Authors' contributions

Lei Li conceived the idea and designed the experiment; Qi Lin performed the experiment, analyzed the data, and wrote the paper; Miaomiao Li, Ying Wang, and Zhixia Xu performed the experiment. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Declarations

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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