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ENVIRONMENTAL BIOTECHNOLOGY

The effect of plant compartments on the *Broussonetia papyrifera*-associated fungal and bacterial communities

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Abstract

Plants associate with numerous microbes, but little is known about how microbiome components, especially fungi, adapt to specific plant compartments. The adaptability of microbial function to the plant compartment is also not clear especially for woody species. Here, we characterized the bacterial and fungal communities in root endosphere, stems, and rhizospheres of 33 *Broussonetia papyrifera* seedlings, based on amplification of 16S and ITS rRNA. Results showed that the α-diversity indexes of the bacterial community were significantly different in different plant compartments and they significantly increased from stem to root endosphere to the rhizosphere, whereas those of the fungal community were similar ($p > 0.05$). However, the result of constrained PCoA (CPCoA) and analysis of similarity (ANOSIM) showed that both bacterial and fungal compositions were significantly affected by plant compartments ($p < 0.01$). In detail, the operational taxonomic units (OTUs) distribution of the bacterial community was significantly different, but 249 of 252 fungal OTUs were shared in different plant compartments. Both the bacterial and fungal compositions were significantly influenced by plant compartments, based on the result on phyla, core OTUs, and indicator OTUs level. Further, 40 of 42 enriched KEGG pathways involving the bacteria also differed significantly among plant compartments ($p < 0.01$). This study provides an understanding of the influence of plant compartments on the microbiome and confirms that the disperse limitation of fungal OTUs across different plant compartments is smaller. This study sheds light on how the microbial community adapts to and thrives in different plant compartments.

Keywords *B. papyrifera* · Plant compartment · Fungal community · Bacterial community · Predicted function

Introduction

Along with the whole plant growth stage, the interactions between plant and microbial community including archaea, bacteria, and fungi are very important for plants (Plett and Martin 2018; Tardif et al. 2016). The microbial community is distributed over all plant compartments, from the root zone to the rhizosphere, root endosphere, stem, leaf, and phyllosphere. The plant-associated microbiome enhances various aspects of plant growth, including improving stress responses (de Zelicourt et al. 2013), providing a source of nutrients (Pii et al. 2015), and enhancing disease resistance (Santhanam et al. 2015). Furthermore, the plant-associated microbiome is enriched by plant exudates such as signaling compounds (flavonoid, strigolactones) and organic compounds (amino acids, organic acids, proteins, and fatty acids) and it adapts to various characteristics of their niche, such as UV light (Sundin and Jacobs 1999), temperature conditions, and oxygen levels (Zhalnina et al. 2018). This enrichment process can be influenced by biotic and abiotic factors, especially for the plant compartment which is more tightly connected with the microbial community (Edwards et al. 2015).

Although several studies have highlighted the influence of plant compartments on the microbiome, most of them just focus on the root-associated microbiome. In general, the effect of the root compartment is explored based on the following three aspects: the diversity and operational taxonomic units (OTUs) distribution of the microbiome, microbial

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composition, and the function of the microbial community. For the diversity and OTUs distribution of the microbiome, it has been proved that the plant-associated microbiome originates in the soil and the mechanism underlying colonization of the microbiome from the outside to inside have also been studied in recent years (Edwards et al. 2015). The bacterial community follows a hierarchical filtration mechanism, with diversity decreasing from the rhizosphere to rhizoplane and root endosphere, and the bacterial OTUs distribution is also significantly different (Xiao et al. 2017). Some studies have demonstrated that the influence of root compartment on the diversity indexes and OTUs distribution of fungal community is different from that of the bacterial community, and that the dispersal limitation of arbuscular mycorrhizal fungi is little (Van Geel et al. 2017), but the full understanding about the influence of root compartment on fungal OTUs and diversity indexes is low, especially for the comparison between bacterial and fungal communities.

In addition to the root-associated microbiome, microbial endophytes in the stem are also important to the host plant, especially during the seedling periods. Compared to the adult tree, the microbial community in seedling stem tissue is less diverse and more susceptible to infection by pathogens (Skaltsas et al. 2019), which indicates that the microbial community in a seedling stem is important for plant development. However, the studies which focus on the microbial community in seedling stems are few, how this filtration mechanism in the root-associated compartment applies to the microbial community in stems is poorly understood, and it is important to study microbial colonization of stems through xylem vessels or by aerosols (Bai et al. 2015).

It is also important to understand how the microbial composition and function adapts to a specific habitat plant compartment. Plant compartments have strong effects on bacterial compositions (Hamonts et al. 2018) due to the connection between the characters of a niche and microbial adaptation to that niche (Gottel et al. 2011). Different plant tissues have different physical and chemical properties, so microbial communities need to have different composition to adapt to their niche (Singer et al. 2019). Moreover, microbiomes with different compositions can produce different metabolites with different abilities to help plants grow. In addition, the function of a microbial community also has a close connection with the characteristics of plant compartment, which has been proved in other studies (Beckers et al. 2015). High throughout sequencing has become an important method for the study of the microbial community and makes possible to explore the function of microbial community based on the PICRUSt software (Langille et al. 2013). This software uses the 16S rRNA and database of genomes to predict the function composition in a bacterial community, which elevates our study from composition to microbial function. Although some studies have shown that the predicted KEGG functions of the bacterial community are very different in different sample types (Zarraonaindia et al. 2015), comprehensive studies of the connection between these predicted functions and plant compartments, which would be important for understanding the relationship between the plant and the microbiome, have not yet been performed.

Broussonetia papyrifera (paper mulberry), a member of the *Moraceae* family, belongs to the nitrogen fixing clade in the Angiosperm Phylogeny Group III. Due to its natural distribution in Asia, Europe, and the USA (Chang et al. 2015), this makes it easy to collect seeds. Studies also showed that the *B. papyrifera*-associated microbial community is closely connected with the characters of *B. papyrifera* (Chen et al. 2019; Peng et al. 2019). This indicates that *B. papyrifera* is suitable for studying the effects of plant compartments on the microbial community. In the current study, we investigated the effects of plant compartments on the plant-associated microbiomes for *B. papyrifera*. We collected the rhizosphere soil, roots, and stems of 33 *B. papyrifera* samples and amplified 16S and ITS rRNA to analyze the bacterial and fungal communities in these samples. In this study, we focused on the following three aspects: (1) We compared in depth the influence of root compartments on the fungal community and bacterial community (including diversity indexes and OTUs distribution), and then focused on the microbial difference between root compartment and stem; (2) We explored the effect of plant compartments on the microbial composition; (3) And we studied the relationship between microbial function and plant compartment based on the KEGG predicted data.

Materials and methods

Plant material collection

In order to study the more common effects of the plant compartment on the plant-associated microbiome, we used different genotypes or ecotypes of *B. papyrifera* as the research material. *B. papyrifera* seeds were collected over a 2-week period from July 20, 2015, to August 5, 2015, from 12 cities across most regions of China. Soil samples used to plant *B. papyrifera* were collected from farmland in Beijing (40° 23′ 58″ N, 116° 45′ 39″ E) (total N: 0.71 g/kg, total C: 4.78 g/kg), the soil samples were passed through a 2-mm sieve and dried at 200 °C for 2 h to create a uniform microbial community in all samples. The seeds were soaked in gibberellin and germinated on plates under sterile conditions. The resulting seedlings were transplanted to soil, and soil lacking plants was used as the blank control. A total of 36 pots (33 *B. papyrifera*'s seedings + 3 control soil) were randomly placed in a greenhouse to ensure that the same conditions were used for each plot. The daily maximum temperature set at 28 to 30 °C, and the air moisture was set at 60%. All plots were

watered daily without any supplements and grown for 1 year (November 2015 to November 2016).

Root samples were collected from a depth of 0–20 cm, and we cut 5 to 10 cm of roots for follow-up test; soil for the blank control was sampled from the same depth. Loose soil was vigorously shaken from the roots, leaving approximately 1 mm of soil still attached to the roots, which were stored at 4 °C. The rhizosphere soil was defined as the soil extending up to 1 mm from the root surface (van Elsas et al. 1988). Roots sampled as described above were placed into 50-mL tubes containing 25 mL of sterile phosphate-buffered saline (PBS) solution (Lundberg et al. 2012). After vigorous shaking, the roots were collected and the soil-PBS solution sampled as the rhizosphere soil, and then the soil was pelleted by centrifugation. The rhizosphere soil was stored at − 80 °C. For the root endosphere, the root samples from the last step were washed in fresh PBS to remove the remaining soil (Edwards et al. 2015). The roots were placed into new sterile 50-mL tubes containing 15 mL of phosphate buffer. The tubes were sonicated for 30 s at 50–60 Hz (KQ-50B, ShuMei, Jiangshu, China) and the solution discarded; this step was repeated twice to ensure that all microbes were removed. The roots were stored at − 80 °C as the root endosphere fraction. Stem samples were collected from the plants at 30–35 cm above the soil surface. To avoid the soil and dust interference with the microbial community in stems, we cleared the stems by washing with 70% ethanol (1 min), 2% sodium hypochlorite (30 s) and 70% ethanol (1 min), and washing 2 times with sterile, distilled water (Granzow et al. 2017). The stems were stored at − 80 °C.

DNA extraction from soil and plant tissues

DNA was extracted from 200 mg soil samples using a Rapid Soil DNA Isolation Kit (Sangon Biotech, Shanghai, China) following the manufacturer's instructions. DNA was extracted from 200 mg ground stem and root tissues using the hexadecyltrimethylammonium bromide CTAB method (Chen et al. 2019). The humus acid in the soil and root samples was removed using DNA-EZ Reagent M Humic Acid-Be-Gone B (Sangon Biotech, Shanghai, China) according to the manufacturer's instructions to avoid the inhibitory effect of humus on PCR. DNA concentration and purity were estimated using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and electrophoresis in a 1–2% (w/v) agarose gel.

Analysis of bacterial and fungal communities

For 16S rRNA gene amplification, the V4 region from bacteria was amplified by PCR using the primers 16SF (5′-barcode-TCCTACGGGRSGCAGCAGT-3′) and 16S2 (5′-barcode-GGACTACHVGGGTWTCTAAT-3′), where the barcode is a six-base sequence. The PCR was performed in a total volume of 20 μL containing 10 μL of 2× PCR SuperMix (Phusion®, Thermo Scientific® Phusion), 0.5 μL of each primer (10 μmol/L), 8 μL of ddH_2O, and 1 μL of template DNA. The PCR cycling conditions were as follows: 95 °C for 3 min; followed by 30 cycles each of 95 °C for 30 s, 50 °C for 20 s, and 72 °C for 1 min; a final elongation step of 10 min at 72 °C; and cooling to 4 °C.

For ITS rRNA gene amplification, the ITS1 region from fungi was amplified by PCR using the primers ITSF (5′-barcode-TGAACCTGCGGAAGGATCAT-3′) and ITS2 (5′-barcode-CGATGCGAGAGCCAAGAGAT-3′), where the barcode was a six-base sequence. The PCR was performed in a total volume of 20 μL containing 10 μL of 2× PCR SuperMix (Phusion®, Thermo Scientific® Phusion), 0.5 μL of each primer (10 μmol/L), 8 μL of ddH_2O, and 1 μL of template DNA. The PCR cycling conditions were as follows: 95 °C for 3 min; followed by 37 cycles each of 95 °C for 30 s, 62 °C for 20 s, and 72 °C for 1 min; a final elongation step of 10 min at 72 °C; and cooling to 4 °C.

The PCR products purified using a SanPrep Column PCR Product Purification Kit (Sangon Biotech, Shanghai, China), and the concentration was estimated using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The products were verified via electrophoresis in a 1–2% (w/v) agarose gel. Purified products were pooled in equimolar ratios separately for 12 barcoded samples and combined into a master DNA pool (Peiffera et al. 2013). All pools were purified using a MagBead DNA Purification Kit (CoWin Biosciences, Beijing, China) according to the manufacturer's instructions. To remove the nonspecific amplification products, each pool was verified on a 1.8% agarose gel and purified using a UNIQ-10 Column MicroDNA Gel Extraction Kit (Sangon Biotech, Shanghai, China) and a MagBead DNA Purification Kit according to the manufacturer's instructions. All sequencing was performed on the Illumina platform (Illumina Xten, San Diego, CA, USA) according to standard protocols. All quality sequences related to this project are available at NCBI under the following project IDs: PRJNA600301 and PRJNA600140.

Data processing and statistical analysis

All paired-end reads were analyzed as follows: the reads were merged into raw reads using flash (v.1.2.7) (Magoc and Salzberg 2011), contaminating reads were filtered using Trimmomatic (v.0.33), and chimeric sequences were removed with UCHIME (v.4.2). Operational taxonomic units (OTUs) clustering at 97% was perform with UCLUST (v.1.2.22) (Caporaso et al., 2010; Wang et al. 2012). Taxonomies were assigned to each OTU based on information in the Silva (Release 119) (for bacteria) and UNITE (Release 7.0) (for fungi) databases. Relative abundances of OTUs were

calculated by dividing the abundance of each OTU by the total sequence count per sample analyzed using QIIME (v.1.8.0) (Caporaso et al., 2010). All analyses were conducted in the R (v.3.4.4) environment and the SPSS statistics program (University of Fribourg license, IBM Company, CHI, USA). The normal distribution of the data (include the α-diversity indexes, microbial composition, and KEGG pathways) were tested with Shapiro-Wilk test and the homoscedasticity of variances was tested with Levene's test. The significance of the difference was tested by the ANOVA and the Welch's *t* test. Post hoc comparisons were performed by Tukey's honest significant differences tests or Games–Howell test. *P* values < 0.05 were considered significant. For α-diversity, the Chao1, Ace, Shannon, and Simpson indexes were calculated using Mothur (v.1.30) (Schloss et al. 2009). For beta diversity, the Bray–Curtis distances for all samples were calculated using QIIME. For constrained PCoA analysis (CPCoA), anova.cca from the vegan package in R was used. The ANOSIM (analysis of similarity) was performed with 999 permutations using the function "vegan package" in R (Desgarennes et al. 2014) to explore the effect of plant compartment on the associated microbiome. The KEGG functional pathways of the bacterial community were predicted based on the 16S rRNA data using PICRUSt software (https://picrust.github.com) (Langille et al. 2013). Indicator species analysis was explored with the function "indicspecies package" in R (Cáceres and Legendre 2009).

Results

The different effects of plant compartments on the α- and β-diversity between bacteria and fungi

A total of 2,805,021 high-quality 16S sequences were generated from 102 samples, with a median read count per sample of 22,923 (Supplement Data S1). A total of 19,241,250 high-quality ITS sequences were also generated from the 102 samples, with a median read count per sample of 129,278 (Supplement Data S2). The high-quality reads were clustered based on > 97% sequence identity, and the low abundance operational taxonomic units (OTUs, < 0.005% of all reads) were discarded. We ultimately identified 1878 OTUs for 16S rRNA and 253 OTUs for ITS rRNA. The good's coverage values of bacterial community were higher than 87.9% and the good's coverage values of all fungal communities were higher than 99.9% (Table 1), which showed that the sequencing depth met the requirement of analysis.

To further explore the effect of plant compartments on the microbiome, we calculated the α-diversity indexes of the microbiomes in different plant compartments and control soil. The α-diversity indexes of the bacterial community significantly differed among plant compartments based on the result of ANOVA or Welch's *t* test, followed by Tukey's honest significant differences post hoc tests or Games–Howell test ($p < 0.01$). A similar trend existed for the OTUs number, ACE index, Shannon index and Chao1 index, which significantly increased from stem to root endosphere to the rhizosphere and control soil, but the change of Simpson index was in the opposite direction to them ($p < 0.01$). However, the result of the fungal community was considerably different to the bacterial community, the OTUs number, ACE index, Chao1 index, and Shannon index of the fungal communities in different plant compartments were similar ($p > 0.05$) and all were higher than those of control soil, but the Shannon index in the root endosphere was highest compared with other plant compartments and control soil. The Simpson index of fungal community in plant compartments and control soil was the same (Table 1). These results showed the significant effect of the plant compartment on the α-diversity indexes of the microbiome, and confirmed the notion that the effect of the plant compartment on the bacterial community was different from that of the fungal community.

To visualize the influence of the plant compartment on the microbiome, we compared the differences in the microbiomes through CPCoA based on the Bray–Curtis distance (Fig. 1) and ANOSIM. The influence of plant compartments on the bacterial community was dominant (CPCoA: R^2=0.13, p = 0.001, ANOSIM: $R = 0.682$, $p = 0.001$), PCoA1 explained 51.69% and PCoA2 29.61% of the total variance. In addition, the influence of the plant compartment on the fungal community was also significant (CPCoA: R^2=0.09, $p = 0.001$, ANOSIM: $R = 0.15$, $p = 0.001$), PCoA1 explained 43.19% and PCoA2 34.13% of the total variance. This result revealed that the plant compartment had significant influence on the fungal and bacterial community, and the R^2 of CPCoA and R of ANOSIM were considerably higher in the bacterial community than in the fungal community.

The effect of plant compartments on the distribution of microbial OTUs

To make the effect of plant compartments on microbiome clearer, we examined the distribution of microbial OTUs in difference plant compartments and control soil. The bacterial community in the root endosphere contained 356 OTUs belonging to three phyla, while that in the rhizosphere contained 1750 OTUs belonging to 18 phyla. The bacterial community in soil lacking plants (referred to as control soil) contained 323 OTUs belonging to 17 phyla, and that in stems contained 276 OTUs belonging to three phyla. The rhizosphere contained a much higher OTU number and a greater number of phyla than the other plant compartments and control soil, which is consistent with the result of α-diversity. The bacterial communities in the root endosphere, rhizosphere, control soil, and stem were dominated by *Proteobacteria* (88%, 84%, 56%, and

Table 1 Mean bacterial and fungal α-diversity indexes in different compartment

	Compartment	OTUs number		ACE		Chao1		Simpson		Shannon		Goods coverage
Bacteria	Endosphere	39.94 ± 29.35	b	67.00 ± 39.87	b	56.15 ± 35.11	b	0.09 ± 0.05	b	2.77 ± 0.48	c	0.88
	Rhizosphere	106.97 ± 16.36	a	126.31 ± 20.53	a	124.38 ± 20.90	a	0.07 ± 0.03	c	3.33 ± 0.29	b	
	Stem	17.94 ± 24.43	c	33.53 ± 38.62	c	27.84 ± 33.67	c	0.42 ± 0.38	a	1.57 ± 1.28	d	
	Control soil	90.67 ± 5.13	a	117.92 ± 16.29	a	113.9 ± 11.02	a	0.04 ± 0.00	d	3.64 ± 0.04	a	
Fungi	Endosphere	157.48 ± 18.61	a	181.52 ± 26.58	a	180.54 ± 20.52	a	0.09 ± 0.05	a	3.35 ± 0.37	a	0.99
	Rhizosphere	148.49 ± 14.02	a	174.82 ± 31.82	a	174.60 ± 21.94	a	0.12 ± 0.04	a	3.11 ± 0.24	b	
	Stem	147.55 ± 20.91	a	171.81 ± 25.69	a	168.60 ± 22.14	a	0.13 ± 0.08	a	2.94 ± 0.47	bc	
	Control soil	50.00 ± 13.45	b	58.47 ± 13.76	b	62.71 ± 16.41	b	0.15 ± 0.02	a	2.49 ± 0.06	c	

An OTU was defined as reads with 97% sequence similarity. Values represent average number of α-diversity indexes (± standard error). Statistical significance is indicated by different letters (a, b) as assessed by ANOVA or Welch's *t* test, followed by Tukey's honest significant differences post hoc tests or Games–Howell test ($p < 0.05$)

95%, respectively) at the phylum level (Fig. 2a). The fungal community contained 253 OTUs belonging to four phyla in the root endosphere, 252 OTUs belonging to four phyla in the rhizosphere, 72 OTUs belonging to four phyla in control soil, and 250 OTUs belonging to four phyla in the stems. Therefore, all plant compartments contained four phyla (*Ascomycota*, *Basidiomycota*, *Glomeromycota*, and *Mucoromycota*), and the OTU numbers in different plant compartments were similar and much higher than those in control soil. The fungal communities in stems, control soil, root

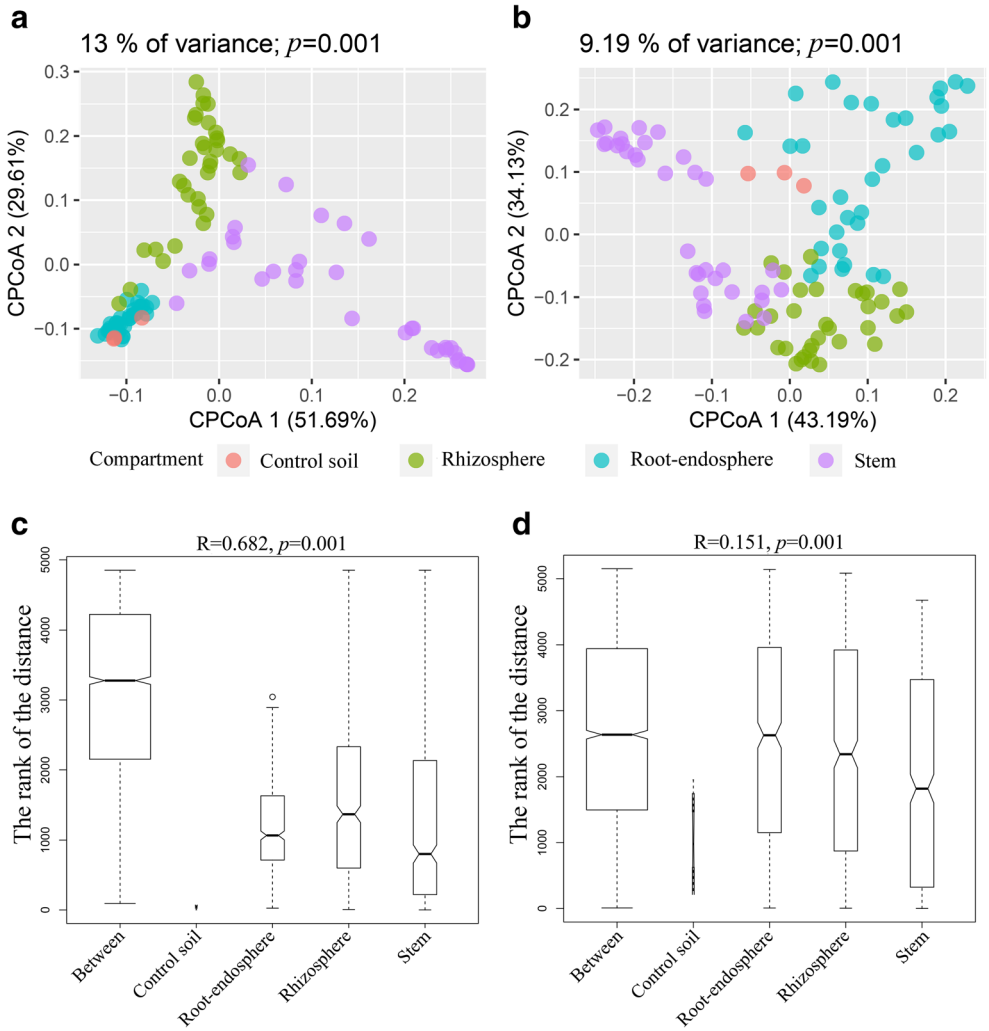

Fig. 1 Comparison of the microbial community across different plant compartments for the bacterial community (**a** and **c**) and the fungal community (**b** and **d**). Constrained principal coordinate analysis (CPCoA) of the microbial community across different plant compartments (rhizosphere, root endosphere, and stem) for the bacterial (**a**) and the fungal (**b**) community based on Bray–Curtis distances. ANOSIM was performed based on the Bray–Curtis distances to compare the bacterial (**c**) and the fungal (**d**) community composition. Calculation of *p* values was performed with 999 permutations

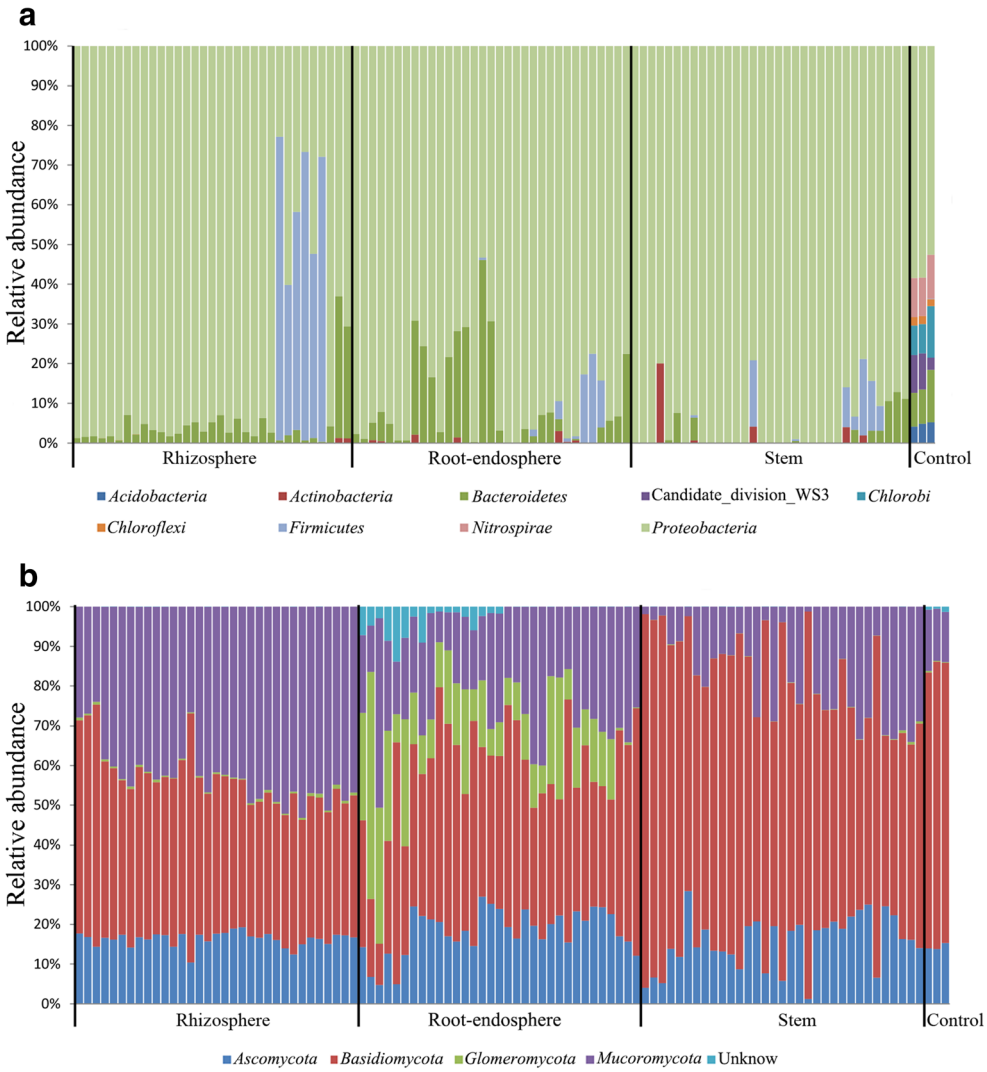

Fig. 2 The composition of the plant-associated microbiome under across different plant compartments. Mean relative abundances (%) of the bacterial community (**a**) and fungal community (**b**) across different plant compartments at the phylum level

endosphere, and the rhizosphere were dominated by *Basidiomycota* (67%, 71%, 40%, and 40%, respectively) (Fig. 2b). This result showed that the effect of plant compartments on the bacterial OTUs was different from that of fungal OTUs.

For the bacterial community, only 88 OTUs were shared among control soil and plant compartments, which were dominated by the *Pseudomonadaceae*, *Chromatiaceae*, *Flavobacteriaceae*, and *Shewanellaceae* at the family level. Only 105 OTUs were shared between the rhizosphere and control soil. The 202 OTUs that were shared among plant compartments were dominated by *Pseudomonadaceae*, *Shewanellaceae*, *Enterobacteriaceae*, *Chromatiaceae*, *Flavobacteriaceae*, and *Lactobacillaceae* at the family level. Most of OTUs in stems and root endosphere were also present in the rhizosphere, except for 4 OTUs which belonged to *Lactobacillaceae*. By contrast, 137 OTUs were found in the root endosphere but not in stems; these were dominated by *Pseudomonadaceae*, *Shewanellaceae*, *Chromatiaceae*, and *Lactobacillaceae*. Finally, 35 OTUs were present in stems but not in the root endosphere; this group was dominated by *Pseudomonadaceae* and *Lactobacillaceae* (Fig. 3a). These results indicated that planting *B. papyrifera* had great influence on the bacterial OTUs in soil, and the plant compartment also had significant effect on the distribution of bacterial OTUs.

The results for the fungal community were different from those of the bacterial community. Only 71 OTUs were shared among control soil and plant compartments. At the phylum level, these OTUs were dominated by the *Basidiomycota* and *Ascomycota* and included small amounts of *Mucoromycota* and *Glomeromycota*. The plant compartments shared 249 OTUs, comprising almost all such fungi. Almost all OTUs in stems and root endosphere were also in the rhizosphere, except one OTU that just appeared in stems and root endosphere and which belonged to the *Glomeraceae*. Only 3 OTUs, belonging to the genus *Conocybe*, were present in the root endosphere but not in stems. All OTUs detected in stem

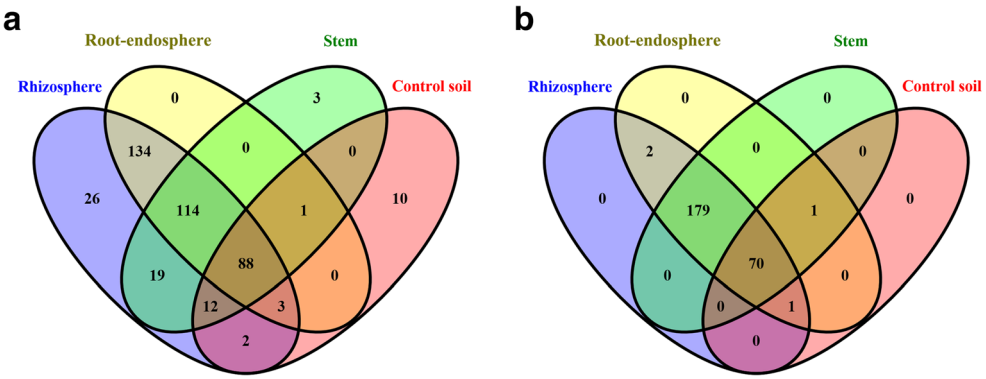

Fig. 3 Venn diagram of OTUs shared between the rhizosphere, stem, root endosphere, and control soil for the bacterial community (**a**) and fungal community (**b**)

could be detected in root endosphere (Fig. 3b). These results indicated that planting *B. papyrifera* also had great influence on the fungal OTUs in soil, but the effect of plant compartment on the fungal OTUs was very little, like also indicated by the results of α-diversity.

Plant compartments had great effects on the microbial composition

To further explore the effect of plant compartments on the microbiome, we compared the microbial compositions in different plant compartments at phylum level and the OTUs. For the individual phyla, the effect of plant compartment was determined based on the result of ANOVA or Welch's *t* test, followed by Tukey's honest significant differences post hoc tests or Games–Howell test. For the bacterial community, the ratio of *Proteobacteria* was high in all bacterial communities (relative abundance = 88.3%), but the content of *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* were significant different across different plant compartments ($p < 0.01$). We observed a significant enrichment ($p < 0.05$) of *Proteobacteria* in plant-associated communities (relative abundance = 89.3%), compared with control soil (56.4%). *Bacteroidetes* was significantly enriched ($p < 0.05$) in root endosphere (9.5%) and control soil (10.1%), compared with rhizosphere (4.7%) and stem (1.8%). In addition, *Nitrospirae*, *Acidobacteria*, *Chlorobi*, Candidate_division_WS3, and *Chloroflexi* were present at higher levels in control soil than in the other plant compartments at the phylum level (Fig. 2a) (Table 2). The core OTUs were defined as the ten most abundant OTUs of each plant compartment and control soil, and we found 33 core OTUs for the bacterial community which accounted for 27.3% (rhizosphere), 49.2% (root endosphere), 67.8% (stem), and 45.2% (control soil) of the total bacterial community. The effect of plant compartments on the core OTUs was tested based on the ANOVA or Welch's *t* test, followed by Tukey's honest significant differences post hoc tests or Games–Howell test. The plant compartment had a significant effect on the most of core OTUs (20/33, $p < 0.05$) (Supplement Table S1). At the family level, *Pseudomonadaceae* and *Shewanellaceae* contents were highest in the rhizosphere. *Chitinophagaceae*, *Xanthomonadales*, *Bradyrhizobiaceae*, *Sphingomonadaceae*, and *Shewanellaceae* were higher in the root endosphere than in other plant compartments and control soil. *Aurantimonadaceae* and *Enterobacteriaceae* contents were higher in stems than in other plant compartments and control soil. Finally, *Nitrosomonadaceae*, *Nitrospiraceae*, BSV26, *Acidobacteriaceae*, *Cytophagaceae*, and *Flavobacteriaceae* contents were highest in control soil (Fig. 4a).

For the fungal community, all the identified phyla showed a significant plant compartment effect ($p < 0.01$). *Basidiomycota* levels were higher in control soil (relative abundance = 70.8%) and stems (67.1%) than in other plant compartments (rhizosphere: 40.4%, root endosphere: 40.4%). *Ascomycota* (18.1%) and *Glomeromycota* (15.5%) levels were higher in the root endosphere versus other plant compartments and control soil (rhizosphere: 16.3%, 0.5%, stem: 15.5%, 0.1%, control soil: 14.3%, 0.3%), whereas *Mucoromycota* levels were higher in the rhizosphere (42.8%) than in other plant compartments and control soil (root endosphere: 23.6%, stem: 17.3%, control soil: 13.8%) (Fig. 2b) (Table 2). For the core OTUs, we found 26 core OTUs across all fungal communities which accounted for 63.8% (rhizosphere), 48.1% (root endosphere), 66.5% (stem), and 83.8% (control soil) of the total fungal community, and most of them (22/26) were significantly influenced by the plant compartment ($p < 0.05$) (Supplement Table S2). *Mortierella*, *Hannaella*, and *Filobasidiales* contents were highest in the rhizosphere. *Conocybe*, *Funneliformis*, *Mortierella*, and *Auriculariales* were more abundant in the root endosphere than in other plant compartments and control soil. The contents of *Hannaella* were higher in stems than in other plant compartments and control soil. *Tremellomycetes*, *Sebacinales*, *Pleosporales*, and *Ceratobasidiaceae* were highest in control soil (Fig. 4b). Together, these results indicated that the plant compartment strongly influenced the composition of bacterial and fungal communities.

Meanwhile, to provide a complete analysis of the effect of plant compartments on the OTUs, we used the species indicator analyses to explore the relationship between whole OTUs and plant compartments. For the bacterial community, the result showed 40 indicator OTUs in control soil, 84 in the root

Table 2 The effect of plant compartment on the microbial phyla

		Rhizosphere		Endosphere		Stem		Control soil		
	Phyla	Relative abundance		Relative abundance		Relative abundance		Relative abundance		Compartment effect
Bacteria	*Acidobacteria*	0.00	b	0.00	b	0.00	b	0.05 ± 0.01	a	
	Actinobacteria	0.00		0.00		0.00		0.00		
	Bacteroidetes	0.05 ± 0.07	ab	0.10 ± 0.12	a	0.02 ± 0.04	b	0.10 ± 0.03	a	$p < 0.01$
	WS3	0.00		0.00		0.00		0.07 ± 0.04		
	Chlorobi	0.00		0.00		0.00		0.09 ± 0.03		
	Chloroflexi	0.00		0.00		0.00		0.02 ± 0.01		
	Firmicutes	0.11 ± 0.24	a	0.02 ± 0.05	a	0.02 ± 0.05	a	0.00	a	$p < 0.01$
	Nitrospirae	0.00		0.00		0.00		0.10 ± 0.00		
	Proteobacteria	0.84 ± 0.24	ab	0.88 ± 0.12	b	0.95 ± 0.07	a	0.56 ± 0.03	c	$p < 0.01$
Fungi	*Ascomycota*	0.16 ± 0.02	ab	0.18 ± 0.06	a	0.15 ± 0.07	ab	0.14 ± 0.01	b	$p < 0.01$
	Basidiomycota	0.40 ± 0.08	b	0.40 ± 0.13	b	0.67 ± 0.17	a	0.71 ± 0.01	a	$p < 0.01$
	Glomeromycota	0.01 ± 0.00	b	0.16 ± 0.12	a	0.00	c	0.00	c	$p < 0.01$
	Mucoromycota	0.43 ± 0.07	a	0.24 ± 0.09	b	0.17 ± 0.11	bc	0.14 ± 0.02	c	$p < 0.01$

Values represent mean relative abundance of phyla (± standard error). Statistical significance is indicated by different letters (a, b) as assessed by ANOVA or Welch's *t* test, followed by Tukey's honest significant differences post hoc tests or Games–Howell test ($p < 0.05$). The compartment effect shows the result of ANOVA or Welch's *t* test

endosphere, four in the rhizosphere, and one in stems (Table 3). To have an in-depth understanding on the indicator OTUs, we focused on the OTUs with an average relative abundance > 1%. The indicator OTUs in control soil belonged to *Nitrosomonadaceae*, *Nitrospiraceae*, BSV26, *Acidobacteriaceae*, *Cytophagaceae*, *Rhodospirillaceae*, *Anaerolineaceae*, *Chromatiaceae*, and *Flavobacteriaceae* ($p < 0.01$). The indicator OTUs in root endosphere belonged to *Pseudomonadaceae* and *Shewanella* ($p < 0.01$). The indicator OTUs in stem belonged to *Aurantimonadaceae* ($p < 0.01$). For the fungal community, the result revealed two indicator OTUs in control soil, 34 in the root endosphere, 35 in the rhizosphere and three in stems (Table 3). The indicator OTUs in control soil belonged to *Sebacinales* and *Tremellomycetes* ($p < 0.05$). The indicator OTUs in the root endosphere belonged to *Hannaella* and *Sordariomycetes* ($p < 0.05$). The indicator OTUs in stems belonged to *Mortierella* ($p < 0.05$). These results also confirmed the finding that the plant compartment affected the plant-associated microbiome.

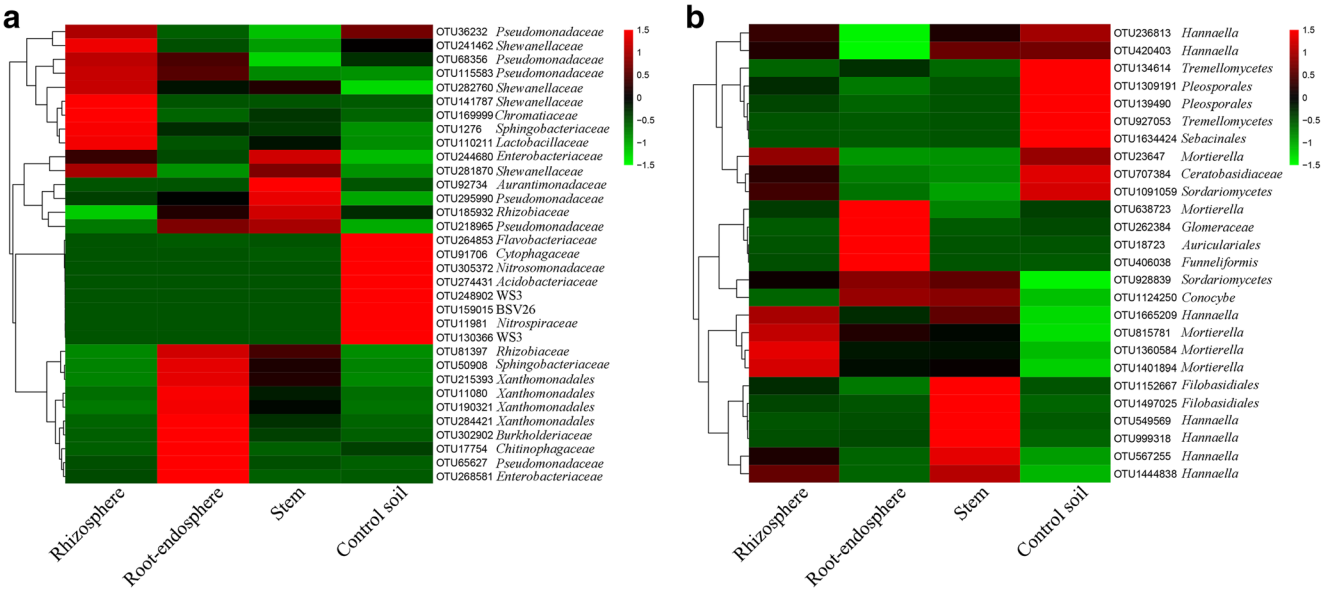

Fig. 4 Heatmap of the average relative abundances of the most abundant OTUs in bacterial community (**a**) and fungal community (**b**) across different plant compartments. Red indicates high abundance and green indicates low abundance. The details of core microbial OTUs of each compartment are showed in the supplement Table S1 and Table S2

Table 3 The enriched indicator OTUs (relative abundance > 0.01) of each compartment

	Compartment		Taxa	Indicator value	*p*	Relative abundance
Bacteria	Control soil	OTU11981	*Nitrospiraceae*	1.00	$p < 0.01$	0.07
	Control soil	OTU24254	*Nitrosomonadaceae*	1.00	$p < 0.01$	0.04
	Control soil	OTU87646	GR-WP33-30	1.00	$p < 0.01$	0.03
	Control soil	OTU130366	Candidate division WS3	1.00	$p < 0.01$	0.04
	Control soil	OTU159015	BSV26	1.00	$p < 0.01$	0.07
	Control soil	OTU174498	GR-WP33-30	1.00	$p < 0.01$	0.02
	Control soil	OTU205836	BSV26	1.00	$p < 0.01$	0.02
	Control soil	OTU248902	Candidate division WS3	1.00	$p < 0.01$	0.04
	Control soil	OTU274431	*Acidobacteriaceae*	1.00	$p < 0.01$	0.05
	Control soil	OTU282848	*Nitrospiraceae*	1.00	$p < 0.01$	0.03
	Control soil	OTU123597	TRA3-20	1.00	$p < 0.01$	0.02
	Control soil	OTU211942	*Xanthomonadales*	1.00	$p < 0.01$	0.04
	Control soil	OTU82457	*Anaerolineaceae*	1.00	$p < 0.01$	0.02
	Control soil	OTU305372	*Nitrosomonadaceae*	1.00	$p < 0.01$	0.04
	Control soil	OTU91706	*Cytophagaceae*	1.00	$p < 0.01$	0.05
	Control soil	OTU19109	*Caulobacteraceae*	0.99	$p < 0.01$	0.02
	Control soil	OTU101502	*Rhodospirillaceae*	0.98	$p < 0.01$	0.03
	Control soil	OTU264853	*Flavobacteriaceae*	0.97	$p < 0.01$	0.04
	Control soil	OTU189823	*Chromatiaceae*	0.96	$p < 0.01$	0.01
	Control soil	OTU126287	*Pseudomonadaceae*	0.96	$p < 0.01$	0.02
	Control soil	OTU59173	*Chromatiaceae*	0.88	$p < 0.01$	0.01
	Control soil	OTU45704	*Chromatiaceae*	0.86	$p < 0.01$	0.01
	Control soil	OTU230465	*Pseudomonadaceae*	0.84	$p < 0.01$	0.02
	Control soil	OTU293643	*Shewanellaceae*	0.76	$p = 0.01$	0.02
	Control soil	OTU89881	*Shewanellaceae*	0.54	$p = 0.03$	0.01
	Endosphere	OTU115583	*Pseudomonadaceae*	0.97	$p < 0.01$	0.02
	Endosphere	OTU241462	*Shewanellaceae*	0.93	$p < 0.01$	0.02
	Endosphere	OTU68356	*Pseudomonadaceae*	0.91	$p < 0.01$	0.01
	Endosphere	OTU36232	*Pseudomonadaceae*	0.88	$p = 0.01$	0.02
	Stem	OTU92734	*Aurantimonadaceae*	0.91	$p < 0.01$	0.47
Fungi	Control soil	OTU1634424	*Sebacinales*	0.81	$p = 0.03$	0.04
	Control soil	OTU927053	*Tremellomycetes*	0.80	$p = 0.01$	0.28
	Endosphere	OTU567255	*Hannaella*	0.92	$p < 0.01$	0.01
	Endosphere	OTU1556376	*Sordariomycetes*	0.77	$p = 0.03$	0.01
	Stem	OTU23647	*Mortierella*	0.74	$p = 0.04$	0.01
	Stem	OTU1360584	*Mortierella*	0.73	$p = 0.05$	0.02

The indicator OTUs were calculated with the Dufrene–Legendre indicator species analysis in R. This result just showed the enriched OTUs (relative abundance > 1%)

Plant compartments had great influence on the functional potential as evidenced through KEGG analysis

To further clarify the relationship between microbiomes and plant compartments, we compared the functional potential of the bacterial community in different plant compartments, as predicted based on 16S rRNA data and PICRUSt software. To determine the functions of members of the bacterial community, we performed KEGG analysis by examining the relationship between the OTUs and KEGG. All OTUs could be assigned to the 215 different KEGG pathways. The most abundant pathways (relative abundance > 1.00% of total pathways) were mainly related to carbohydrate metabolism, amino acid metabolism, energy metabolism, membrane

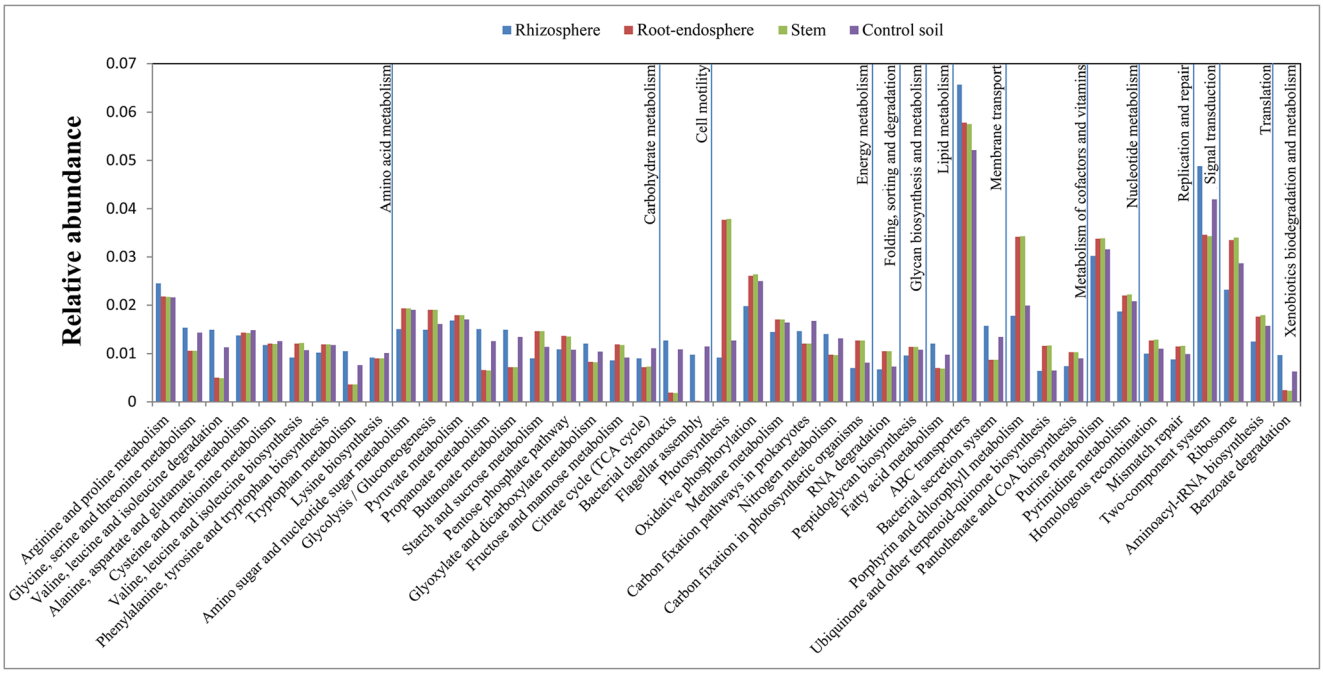

Fig. 5 Relative abundances of dominant KEGG pathways (> 1.00%) of the bacterial community in the root endosphere, rhizosphere, stem, and control soil. The results of the significance test were showed in the supplement Table S3

transport, nucleotide metabolism, and translation (Fig. 5). Most of these pathways were related to basic cellular function, which was similar to the results of other studies (Nacke et al. 2014). In addition, there were significant differences in almost all the most abundant pathways in different plant compartments based on the result of ANOVA or Welch's *t* test ($p < 0.001$) (Supplement Table S3), except for the Cysteine and methionine metabolism. However, half of the most abundant pathways were similar between rhizosphere and control soil, and all the most abundant pathways were similar between root endosphere and stems ($p > 0.01$). The vast majority of KEGG pathways in root endosphere and stems were significantly different from those of the rhizosphere (41 for stems, 41 for root endosphere, $p < 0.01$), except for the Cysteine and methionine metabolism and Lysine biosynthesis. In detail, partial pathways involved in carbohydrate metabolism and amino acid metabolism were more highly enriched in the rhizosphere versus root endosphere and stems. The KEGG pathways belonging to the categories Bacterial Secretion System, Bacterial Chemotaxis, and Flagellar Assembly were more highly enriched in the rhizosphere than in root endosphere and stems. The pathways belonged to the categories energy metabolism, translation and metabolism of cofactors and vitamins were less abundant in the rhizosphere than in stems and root endosphere. These results indicated that the bacterial communities in different plant compartments had different functions in order to adapt to different habitats.

Discussion

It is widely accepted that root compartments strongly influence the root-associated microbiome. In the current study, we confirmed this notion and extended it from root compartment to stem. We also explored the relationship between plant compartment and microbial community in depth, including α-diversity and OTUs distribution of microbial community, composition of microbiome, and the KEGG predicted function. We demonstrated that different plant compartments enrich different fungal or bacterial populations, making them suitable for a specific niche, which could benefit plant growth. For the α-diversity indexes and OTUs distribution of the microbial community, and the bacterial community followed the expectations for a filtration mechanism, which has been confirmed in other studies (Xiao et al. 2017). The diversity of the bacterial community decreased from the rhizosphere to the root endosphere and stems, but for the fungi, this was not the case.

The effect of plant compartments on the α-diversity indexes and OTUs distribution of microbial community

To explore the effects of plant compartments on the microbiome, we examined the α-diversity indexes and OTUs distribution in different plant compartments firstly. The effect of planting *B. papyrifera* on the soil was explored through the comparison between the control soil and rhizosphere. The OTUs number and α-diversity indexes were

higher in the rhizosphere. Although the bacterial community in the rhizosphere could be classified into 18 phyla as in control soil, this community was dominated by the *Proteobacteria* and contained many types of plant growth-promoting bacteria such as *Rhizobiaceae* and *Pseudomonadaceae*, which are closely associated with plants (Neal et al. 2012; Preisig et al. 1993). These results showed a significant influence of planting *B. papyrifera* on the soil. In addition, it had been proved that soil was the primary source of the plant-associated microbiome, and the microbiome in all plant compartments is tightly controlled by an intense process (Philippot et al. 2013; Zarraonaindia et al. 2015). In our study, all OTUs in stems and the root endosphere were also present in the rhizosphere for *B. papyrifera*. Together, these results indicated that planting *B. papyrifera* could alter the microbial composition in the rhizosphere, which served as the source of the plant-associated microbiome.

For the root-associated microbiome, the effect of root compartments on the α-diversity indexes and OTUs of the bacterial community was different to that of the fungal community. For the bacterial community, the α-diversity was much lower in the root endosphere than in the rhizosphere, which was consistent with the bacterial filtration mechanism previously detected in rice (Edwards et al. 2015) and soybean (Xiao et al. 2017), indicating that this mechanism is also suitable for woody plants. This filtration effect between different root compartments was due to niche characteristic difference and selection of the microbial community (Xiao et al. 2017). However, unlike the bacterial community, almost all fungal OTUs on this study were shared among the root compartments, and the α-diversity did not follow the order rhizosphere >root endosphere, as observed in bacteria, which was also similar to other studies (Cregger et al. 2018). Our results demonstrated that the effect of root compartments on the α-diversity indexes and the OTUs distribution of fungal community were different from that of bacterial community, which may be brought by a difference of filtration mechanism. Almost all fungal OTUs could appear in the rhizosphere, root endosphere, and stem, perhaps due to the higher stress tolerance of fungi versus bacteria (Whipps et al. 2008). Our findings indicated that the root compartment had a great influence on the α-diversity indexes and OTUs of bacterial community, but the effects of root compartments on the α-diversity indexes and the OTUs distribution of fungal communities were little as compared with those of the bacterial communities, probably because of the difference of stress tolerance between fungi and bacteria.

The studies about the microbial community in seedling stems were so far few compared to other plant compartments, and whether the filter mechanism of the microbial community in the root compartment is also suitable for that in the stems remains to be explored. For the bacterial community, the α-diversity was lower in stems than in the root endosphere, perhaps due to the exposure of stems to extreme temperatures and UV (Redford et al. 2010). Most bacteria in stems were also in root endosphere, indicating that the root endosphere may be the major source of the bacteria in stems. Perhaps bacteria used the lumens of xylem vessels to reach the stems, as suggested in other studies (Compant et al. 2008). However, this might not be the only pathway for the bacteria in the stems, as there were also some bacteria in stems which were not observed in the root endosphere but were instead present in the rhizosphere. These results also confirmed the notion that the bacteria in soil could enter the stem directly, perhaps using dust as a medium for transport; this pathway also appeared in the fruits (Zarraonaindia et al. 2015) and leaves of other plants (Williams and Marco 2014).

For the fungal community, the OTUs and α-diversity in stems were similar to that in the root compartment. Like the results in the root compartment, almost all fungal OTUs could appear in the root compartment and stems, likely due to the higher stress tolerance (Whipps et al. 2008). In addition, no OTUs were observed in stems but not in the root endosphere, indicating that fungi might not enter the stems directly through dust. Only two OTUs were present in the root endosphere but not in stems: both OTUs belonged to the genus *Conocybe*. The ecological roles of *Conocybe* were currently unclear, but studies have shown that some of them are closely related to plants (Chen et al. 2018) (Malysheva 2017). Together, this finding indicated that the α-diversities of the bacterial community follows the order rhizosphere >root endosphere >stem, and that of the fungal community follows the order rhizosphere = root endosphere = stem. It also showed that the lower disperse limitation of fungi were suitable for them to cultivate the root compartments and the stems.

The composition of fungal and bacterial communities in *B. papyrifera* was highly compartment-specific

Although our result showed that the effects of plant compartments on the α-diversity indexes and the OTUs distribution of fungal communities were different to those of the bacterial communities, in order to have a fully understanding about the relationships between a plant compartment and the microbiome, we investigated the effects of plant compartments on the microbial composition. Consistent with other studies (Tardif et al. 2016), we observed that the microbial composition was different in different plant compartments, as revealed by the result of CPCoA and ANOSIM. This finding indicated that the tissue of *B. papyrifera* strongly controls the microbial community composition. For the bacterial community, as in other plant species, including *Ginkgo biloba* (Leff et al. 2015), grapevine (Zarraonaindia et al. 2015), and *Populus deltoides* (Gottel et al. 2011), *Proteobacteria* were dominant in all *B. papyrifera*-associated compartments, including the root endosphere and rhizosphere; these bacteria

are closely associated with plant growth (Bruto et al. 2014). In detail, *Bradyrhizobiaceae*, *Rhizobiaceae*, and *Pseudomonadaceae* were enriched in the root endosphere. These bacteria are closely associated with nitrogen fixation (Mus et al. 2016) and are enriched by signaling factors such as flavonoids in root exudates (Oldroyd 2013). Members of these families often appeared in the roots of nodule-forming legumes and provided nutrients to the plant (Xiao et al. 2017). In addition, the contents of *Shewanellaceae* and *Pseudomonadaceae* were higher in the rhizosphere than in other plant compartments. These bacteria, which tended to occur in the rhizosphere, could produce secondary metabolites with antagonistic activities (Guan et al. 2016), which could provide these bacteria with competitive advantages (Raaijmakers et al. 2008). In addition, *Aurantimonadaceae* comprised a large portion of the bacterial community in stems. All three OTUs that appeared only in the stems were classified as *Lactobacillaceae*. The anoxic wood environment could harbor bacteria capable of fermentation, as observed in *Populus* (Cregger et al. 2018; Schink et al. 1981). Together, these findings confirmed the notion that plant-associated bacterial communities were enriched via tissue selection based on the character of the niche and adaption of bacteria to habitat conditions.

By contrast, the α-diversity of the fungal community was similar in different plant compartments, but the β-diversity was significantly different, as revealed by CPCoA and ANOSIM. The fungal communities in stems, the root endosphere, and the rhizosphere were dominated by *Basidiomycota* (67%, 40%, and 40%, respectively). By contrast, this was different to other plant species such as wheat, faba bean (Granzow et al. 2017), and *Populus* (Cregger et al. 2018) which were dominated by *Ascomycota*. In this study, the contents of *Ascomycota* and *Glomeromycota* were higher in the root endosphere than in other plant compartments, and the content of *Mucoromycota* was higher in the rhizosphere. At the core OTUs level, the contents of *Hannaella* were higher in stems than in other plant compartments. *Conocybe*, *Funneliformis*, *Mortierella*, and *Auriculariales* contents were also higher in the root endosphere versus other plant compartments; these fungi help roots absorb nutrients from the soil (Bonfante and Genre 2010) and are recruited by signaling factors in root exudates such as strigolactones (Oldroyd 2013). Finally, *Mortierella*, *Hannaella*, and *Filobasidiales* levels were higher in the rhizosphere than in other plant compartments; these fungi are closely related to stress tolerance and are enriched by plant exudates (Wani et al. 2017). Together, these results indicated that although plant compartments had a limited effect on the α-diversity indexes and OTUs distribution of the fungal community compared with the bacterial community, the characters of each niche and plant exudates had a strong influence on the fungal composition. In turn, this adaptability of fungi might improve plant growth or stress tolerance.

The predicted functions of the bacterial community were closely related to their habitat plant compartment

Our study proved that the influence of a plant compartment on the α-diversity indexes and OTUs distribution of the fungal community was different to that of the bacterial community, and confirmed the notion that different plant compartments enrich the abundance of suitable or beneficial microbiota through the enrichment of specific bacteria or fungi. To further explore the relationship between plant compartments and microbial community, we compared the functional potential of the bacterial communities in different plant compartments; few such studies have been performed. The most abundant pathways were related to basic cellular functions such as carbohydrate metabolism, amino acid metabolism, energy metabolism, membrane transport, and nucleotide metabolism and translation, which were similar to the results of other studies (Zarraonaindia et al. 2015). Meanwhile, almost all the most abundant KEGG pathways of the bacterial community were significantly different in different plant compartments. All of the most abundant pathways were similar between root endosphere and stems, and both were different from those of the rhizosphere. In addition, half of the most abundant pathways were similar between rhizosphere and control soil.

The pathways bacterial secretion system, bacterial chemotaxis, and flagellar assembly were much more highly enriched in the rhizosphere than in the root endosphere and stems; these pathways strongly influence the competence of bacteria in the rhizosphere (Compant et al. 2010). Chemotaxis-related genes were enriched in the rhizosphere and could help bacteria invade the plant root surface (Scharf et al. 2016), and flagellar assembly was found closely associated with biofilm formation (Yousef-Coronado et al. 2008). In addition, the pathways involved in energy metabolism, translation, and metabolism of cofactors and vitamins in the rhizosphere were lower than in stems and in the root endosphere, which might be related to the metabolic ability of these plant organs. These results demonstrated that the α-diversity and composition of the bacterial community, as well as the functional potential of the bacterial community, were closely related to the characters of the niche and tend to be suitable for specific habitat plant compartments.

In conclusion, we performed in-depth analysis of the effects of plant compartments on the plant-associated microbiome. This effect extends to all aspects of the microbiome, including the α-diversity indexes and OTUs distribution of the microbial community, the adaptation of the microbial composition to the character of the habitat, and the mutualism between the potential function of the microbiome

and different plant compartments of the plant. We demonstrate that planting *B. papyrifera* has a great influence on the microbial community in soil. The disperse limitation of fungal OTUs across different plant compartments is smaller than that of bacterial OTUs, but both the compositions of bacterial and fungal communities are significantly influenced by the plant compartments. This study sheds light on the relationship between plant compartments and the microbial communities, laying the foundation for studies that aim at improving plant growth by altering the microbiome.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical statement This article does not contain any studies with human participants performed by any of the authors.

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