Plant Soil
DOI 10.1007/s11104-017-3449-9

REGULAR ARTICLE

Differences in root-associated bacterial communities among fine root branching orders of poplar (*Populus* × *euramericana* (Dode) Guinier.)

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Received: 29 March 2017 / Accepted: 28 September 2017
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Abstract
Background and Aims Root branching leads to morphological and functional heterogeneity of fine roots. However, the structure of soil microbiota associated with root branching orders has never been investigated. Deep insights into rhizosphere microbial community could provide a better understanding of the plant-microbe relationship.
Methods Fine roots of poplar (*Populus* × *euramericana* (Dode) Guinier.) were sampled and sorted into three groups according to their branching orders. Scanning electron microscopy (SEM) was used to observe the surface features of different orders of fine roots. Illumina MiSeq was employed to analyze the bacterial community structure of soil compartments from different root orders (i.e., R1, R2, and R3) and bulk soil compartment (NR).
Results SEM showed that the first-order root with smaller diameter had dense coverage of vigorous root hairs, whereas higher order roots with larger diameter had sloughed-off cortical tissues on the rhizoplane. The diversity of bacterial communities was higher in the R1 and R2 compartment than in R3 or NR. There were 80 genera with a relative abundance above 0.05% in soils. Ternary plot revealed that bacterial genera were significantly enriched in R1 than in R2 or R3. Redundant analysis (RDA) showed that 12 dominant bacterial genera with a relative abundance over 1% were significantly correlated with P and NH_4^+-N content of soils.
Conclusions Root orders could influence the inhabitation of bacterial communities. The core groups of bacterial communities inhabiting the rhizosphere were correlated with soil nutrients. The root order-dependent interactions of plant-microbe provided a new model about the association between roots and soils.

Keywords Bacterial community · High-throughput sequencing · Poplar tree · Rhizoplane feature · Root orders

Qitong Wang and Nian Wang contributed equally to this work.

Responsible Editor: Stéphane Compant

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11104-017-3449-9) contains supplementary material, which is available to authorized users.

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Introduction

Fine roots (≤2 mm in diameter) play an important role in global biogeochemical cycling of carbon and nitrogen

Published online: 05 October 2017

(Jackson et al. 1997; Caldwell et al. 1998; Osmont et al. 2007; Kong et al. 2014). They transfer much carbon, nitrogen, and some molecular substances into soils via the sloughed-off cells and dead cortical tissues (Barlow 1975; Gordon and Jackson 2000; Jones et al. 2009; Badri and Vivanco 2009). These transferred materials proliferate specific microbes to colonize the rhizosphere (Grayston et al. 1998, 2001), resulting in great difference in community structure and abundance of rhizosphere bacteria from that of the bulk soil (Uroz et al. 2010; Bulgarelli et al. 2012; Li et al. 2014). This spatial differentiation of microbiota partly determines various plant-microbe interactions (Berg and Smalla 2009; Haichar et al. 2014) and even influences soil nutrient cycling (Eisenhauer et al. 2012). Therefore, insights into rhizosphere microbial community could provide a better understanding of the plant-microbe relationship.

Plant roots can be ordered based on branching. Plant roots have different orders with the first-order roots being distal and tip-ended; whereas the second-order roots connect first-order roots, whose parent roots are the third-order root (Pregitzer et al. 2002). Such order reflected the development of a single root into a system. Therefore, roots of different orders have different morphological characteristics, ages and chemical properties (Hajek et al. 2014; Wells and Eissenstat 2002; Guo et al. 2008b), which may influence microorganisms around it (Watteau et al. 2002). In addition, root order reflects the relationship between anatomical traits and physiological function. Lower-order roots with primary structures and cortical cells are responsible for nutrient and water uptake (Guo et al. 2008a), and higher-order roots with secondary xylems mainly transport water and nutrients (Hishi and Takeda 2005). For decades, many studies have focused on the morphological aspects of fine roots; however, the morphological and functional heterogeneity of root orders within a root system was less studied (Pregitzer et al. 1997; Iversen 2014; McCormack et al. 2015). The functional heterogeneity among root orders implies that they may affect the rhizosphere soil environment differently. To the best of our knowledge, few studies have addressed root order-dependent relationships among roots and bacterial communities, or investigated whether root-associated soil bacterial communities differ among root orders. The fact that mycorrhizal fungi only infect the first-order fine roots to enhance water and nutrients absorption rather than the second or higher-order roots of trees (Guo et al. 2008b; Xia et al. 2010) indicates that root order is closely related to the establishment of root-associated microbial communities.

The structure of microbial communities is influenced by biotic and abiotic factors. Soil properties (e.g. pH, temperature, and moisture) have an impact on the temporal and spatial distributions of microbial communities in a soil profile. However, we know very little about the biotic factors. Currently, sources of carbon in soils, such as root exudates that contain large quantities of carbohydrates, are considered important in shaping the structure of microbial communities in the rhizosphere (Paterson et al. 2007; Hartmann et al. 2009). Approximately 5–10% of the photosynthates are transported into soils through root exudates (Farrar et al. 2003). In addition, carbon sources, such as sloughed-off cells or tissues, and dead roots constitute a major fraction of carbon rhizodeposition in an ecosystem (Jones et al. 2009). Therefore, physiological activities of roots during growth and development greatly affect bacterial community structure in rhizosphere soils. Studies on rhizosphere and endosphere microbiome of poplar (*Populus deltoides*) roots (Gottel et al. 2011; Shakya et al. 2013) have provided insights into the microbial communities present inside and on the surface of fine roots. However, poplar roots consist of multiple orders (Hajek et al. 2014). In addition, the anatomical traits revealed functional differentiation between low and high-order roots with first and second-order roots absorbing water and nutrients, and fourth and fifth-order roots carrying out transportation and storage (Xu et al. 2015). Therefore, understanding rhizosphere interaction is necessary to investigate the bacterial communities associated with poplar root orders.

In the present study, we studied the association of diversity and composition of rhizosphere bacterial communities with poplar root morphological features and soil nutrient conditions. First, we observed the surface features of fine roots of different orders in poplar using a scanning electron microscope (SEM). Then, we conducted a detailed examination on bacterial community structures in rhizosphere soils associated with different orders of fine roots using Illumina MiSeq (Michael et al. 2012; Rutvisuttinunt et al. 2013; Williams et al. 2014; Yoon-seong et al. 2015). We hypothesized that the structure of rhizosphere bacterial communities would

differ significantly among root orders; we expected that poplar would provide a root order-dependent model for analyzing microbial community structure and provide deeper insights into the interaction between plant roots and soils.

Materials and methods

Study site

The study site was within the Gaoqiao National Forest Farm (35°53′N, 116°50′E), which is situated on the alluvial plain of the *Dawen He* River in eastern China. It has a typical warm temperate climate, with an average rainfall of 800 mm and a mean annual air temperature of 10 °C. The lowest and highest temperatures recorded were −17.8 and 35.2 °C in 2001 and 2006, respectively. Soil there was sandy alluvial loam with lower contents of organic matter and available nutrients (Wang et al. 2016).

Fine root sampling

The poplar (*Populus* × *euramericana* (Dode) Guinier.) plantation used for roots sampling were established in the spring of 2009, with a density of 500 trees per hectare, and no fertilization or irrigation had been applied since then. Complete root systems were obtained using the soil monolith sampling methods (Vogt et al. 1998; Fan and Guo 2010; Zadworny et al. 2015). Briefly, three 10× 10 m plots were established within the plantation with plots separated from each other by 5–10 m to avoid interactions (Fig. S1a). Three trees were selected for root sampling from each plot whose height, diameter at breast height (DBH), and crown size were close to that of the average value of poplar trees within each plot. We selected trees from different locations within the plot given the soil heterogeneity. All herbaceous vegetations (i.e., *Setaria viridis*, *Digitaria sanguinalis*, *Chenopodium serotinum*, and *Amaranthus tricolor*) and their roots present within a 1-m radius of the sampling tree were completely removed. A soil block (50 cm in length, 50 cm in width and 20 cm in depth) was excavated in each of the four directions (east, south, west and north) to account for uneven vertical distribution of poplar fine roots at the soil profile (Fig. S1b).

To avoid a root fracture during excavation, we cut the roots along the border of soil block and removed the soil around it. Thus, the whole soil block was isolated and excavated for root sampling. A 2-mm sieve was used for removing soil and collecting living roots of poplar. Poplar roots were easily distinguished from the residual roots of other herbs in the soil block due to their multiple branches and hard texture (Hajek et al. 2014). Only live roots were selected, because those dead ones are usually colonized by saprotrophic microorganisms and undergo decomposition (Bagniewska-Zadworna et al. 2014) which would affect the relationship between roots and microbes. Live roots of poplar were distinguishable from dead ones due to their pale- and brown-color and elastic texture. The sampled live roots were immediately stored in a cooler at approximately 5 °C. All fresh roots from one tree were pooled and sorted into groups according to their branching order (Fig. S2). In this study, we divided poplar roots into three groups: roots of the first two orders, roots of the third order, and roots of the fourth and fifth order due to the similar primary structures in the first two order roots and obvious secondary structure in the third to fifth order roots (Fig. S3).Thus, 27 root groups were obtained for further analyses (3 plots × 3 trees × 3 groups).

Scanning electron microscopy (SEM) of root surface

Ten fresh and clean fine roots of the same order were randomly selected and 5–8 root fragments with a length of ~0.8 cm were used for morphological observation using SEM. They were saturated in 3.5% glutaraldehyde and fixed for 24 h at 4 °C. The fixed root fragments were then washed five times (20 min each time) with 5% phosphate-buffered saline solution (PBS; 140 mM NaCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4; pH 7.0) and fixed in 1% osmium tetroxide for 5 h. Subsequently, the fixed root fragments were washed with 5% PBS again in the same manner and dehydrated at 25 °C using a graded ethanol series (45, 55, 70, 85, 95, and 100% ethanol). The specimens were kept overnight in absolute ethanol at 4 °C. Specimens were dried using a critical point dryer (K850, Quorum, East Sussex, UK). The dried specimens were pasted on the sample stage subjected to ion spraying process. Finally, the specimens were observed and photographed with a scanning electron microscope (JSM-6610LV, JEOL, Tokyo, Japan).

Root-adhering soil collection

Another subset of roots sorted into groups was used for root-adhering soil collection from each order roots. Large and visible soil particles were removed from fresh roots. Each group of fine roots was placed in a 50 mL centrifuge tube, and tightly attached soil particles from their surface were rinsed into the tube using 30 mL PBS. After removing the roots, the tubes were centrifuged at 7000 *g* relative centrifugal force (RCF) for 10 min, and root-adhering soil pellets on the tubes were collected (Li et al. 2014) and stored at −80 °C for genomic DNA extraction and nutrient analysis. To avoid the loss of water-soluble nutrients, the supernatant was stored at 5 °C for nutrient analysis, combined with the below supernatant. The root-adhering soil pellets collected from fine roots of each group were defined as one rhizosphere soil compartment (i.e., R1 was from first and second order roots, R2 from third order roots, and R3 was from fourth and fifth order roots). Soils from the 27 root groups were merged into nine soil compartments using the three plots as replicates. In addition, three bulk soil samples from each plot were pooled, and served as a control (NR).

Soil nutrients analysis

The content of NO_3^--N and NH_4^+-N of the soil samples were measured by an automatic flow analyzer (SEAL AA3, SEAL Analytical GmbH, Deutschland, Germany) and the available phosphorus (P) was measured by an automatic intelligent chemistry analyzer (Smartchem 200, AMS-Westco, Rome, Italy). Briefly, the mixture of 2.0 mg soil, 5 mL of the above supernatant (see "Root-adhering soil collection" section) and 15 mL 0.01 mol L^{-1} $CaCl_2$ was used to analyze the content of NO_3^--N and NH_4^+-N and the mixture of 1.25 mg soil and 5 mL of the above flushed soil solution (see "Root-adhering soil collection" section) and 20 mL 0.5 mol L^{-1} $NaHCO_3$ (pH = 8.5) was used to analyze the content of the P. These mixtures were vibrated for 1 h and centrifuged for 10 min at 500 *g* RCF, and the supernatant was obtained to determine the content of these nutrients, with the reference to the standard signals of the analyzer. The stoichiometry of soil N and P was expressed by NO_3^-:NH_4^+ ratio and N:P ratio.

High-throughput sequencing of soil bacteria

DNA extraction

Soil genomic DNA was extracted from approximately 50 mg soil using a soil DNA kit (Mag-Bind, Omega Inc., Norcross, GA, USA). The quality of DNA was checked using a spectrophotometer (NanoDrop-ND 2000, Thermo-Scientific, Wilmington, DE, USA) and the quantity was measured with a Qubit 2.0 Fluorometer (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA) using Broad-range assay reagents.

Sequencing

In this study, the V4 region of bacterial 16S rRNA genes was amplified in a PCR machine (Bio-rad T100, Bio-Rad Laboratories, Hercules, CA, USA) from soil microbial genomic DNA (Gottel et al. 2011), using the universal barcoded primers (forward primers 520F: 5′-AYTGGGYDTAAAGNG-3′, reverse primers 802R: 5′-TACNVGGGTATCT AATCC-3′) (Meng et al. 2014; Pan et al. 2015). The oligonucleotides included A or B sequencing adapter ligated to the 5′ end of forward and reverse primers. A unique 10-mer barcode sequence was added between the sequencing adapter and the forward primer to differentiate between samples. V4 amplicons were sequenced on Illumina platform (Illumina MiSeq Sequencer, Illumina Inc., San Diego, CA, USA). The sequence data have been deposited in the Sequence Read Archive (SRA) of the NCBI (accession number: SRP093800).

Sequence data filtration

The raw data were trimmed using QIIME software (Caporaso et al. 2010) in paired end mode using the following steps. First, sliding window method was performed for quality screening each sequence (window size of 5 bp, the step of 1 bp, starting from the first base position of 5′ end). We used fastq_quality_filter program of UPARSE (Edgar 2013) to remove bases with a QC value below 20. Sequences with a length below 150 bp after trimming were discarded. After quality filtration, reads with at least 10 bp overlap between the two contiguous reads were connected, using the

FLASH version 1.2.7 (Magoc and Salzberg 2011). Chimera sequences were removed, using UCHIME method of MOTHUR software version 1.31.2 (Schloss et al. 2009; Edgar et al. 2011).

Sequences clustering and annotation

The high-quality sequences were clustered, using UCLUST method in the QIIME software (Edgar et al. 2011). The operational taxonomic units (OTUs) were defined at a similarity level of 97% (Ling et al. 2015). The most abundant sequences in each OTU were selected as the representative sequences. The sequences were aligned using reference sequences in Greengenes database Release 13.8 (Desantis et al. 2006), to obtain the taxonomic information of each OTU in QIIME software. These OTUs with very low abundant sequences (relative abundance <0.001%) were discarded (Table S1), because they cannot reflect the true composition of soil bacterial communities (Bokulich et al. 2013). The relative abundance of each phylogenetic group was expressed as the number of sequences affiliated with that group divided by the total number of sequences per sample.

Data analysis

The soil bacterial communities of four soil compartments were categorized at the genus level. According to the abundance of OTUs, three non-parametric estimators (i.e., Ace index, Chao1 index and Shannon index) were calculated for each compartment using MOTHUR ver. 1.31.2 (Edgar et al. 2011). Rarefaction curves were generated using Sigmaplot 12.5 (Systat Software Inc., San Jose, CA, USA) to compare the relative number of bacterial OTUs across soil compartments (Zhao et al. 2014). One-way ANOVA was conducted to test the difference in the relative abundances of soil bacteria at the genus level among soil compartments. The least significant difference (LSD) was tested among branching orders. A heat map was prepared using the package ggplot in R software (Wickham 2009). Ternary plots were prepared using the function "ternaryplot" from Origin 8.0 (OriginLab Corporation, Northampton, MA, USA). The relative contribution of each bacterial genus was assessed according to the location of the point in the ternary plot (Bulgarelli et al. 2012; Pires et al. 2012). Principal component analysis (PCA) was used for the ordination of bacterial communities, and redundant analysis (RDA) was used to link soil bacterial communities to the soil available N and P (Ling et al. 2015) using Canoco for Windows 4.5 software (Braak and Šmilauer 2002).

Results

Differences in rhizoplane among root orders

Consistent with distinct anatomical traits of root orders (Fig. S3), the SEM revealed clear morphological differences in rhizoplane among root orders (Fig. 1), with the first-order fine roots having smaller diameters, dense vigorous root hairs (Fig. 1a) and crystal-like substances on the rhizoplane (Fig. 1c). In contrast, the third-order fine root has larger diameters, no root hairs, and intact cortical tissues (Fig. 1d). Some cortical cells of the third-order roots began to slough off (Fig. 1e) whereas most of the cortical tissues sloughed off and cork formed from the fourth-order fine roots (Fig. 1h). All these features show morphological heterogeneity of rhizoplanes among poplar root orders.

Soil available N and P content

The NH_4-N content was significantly higher in the rhizosphere than in the bulk soil samples, whereas the NO_3^--N content was similar between rhizosphere soils and bulk soils (Table 1). The content of available P ranged from 0.026 to 0.032 g kg^{-1} in poplar plantation soils. However, it did not differ among rhizosphere soils and bulk soils. The ratios of NH_4^+:NO_3^- ranged from 0.46 to 1.20 whereas N:P ratios ranged from 1.41 to 1.99, with a significant difference observed only between the rhizosphere and bulk soils. The NH_4^+-N content differed significantly among root orders, with the highest in R3 and the lowest in R1.

The overall structure of bacterial communities

A total of 967,248 high quality sequences were obtained from the four soil compartments (Table S1).

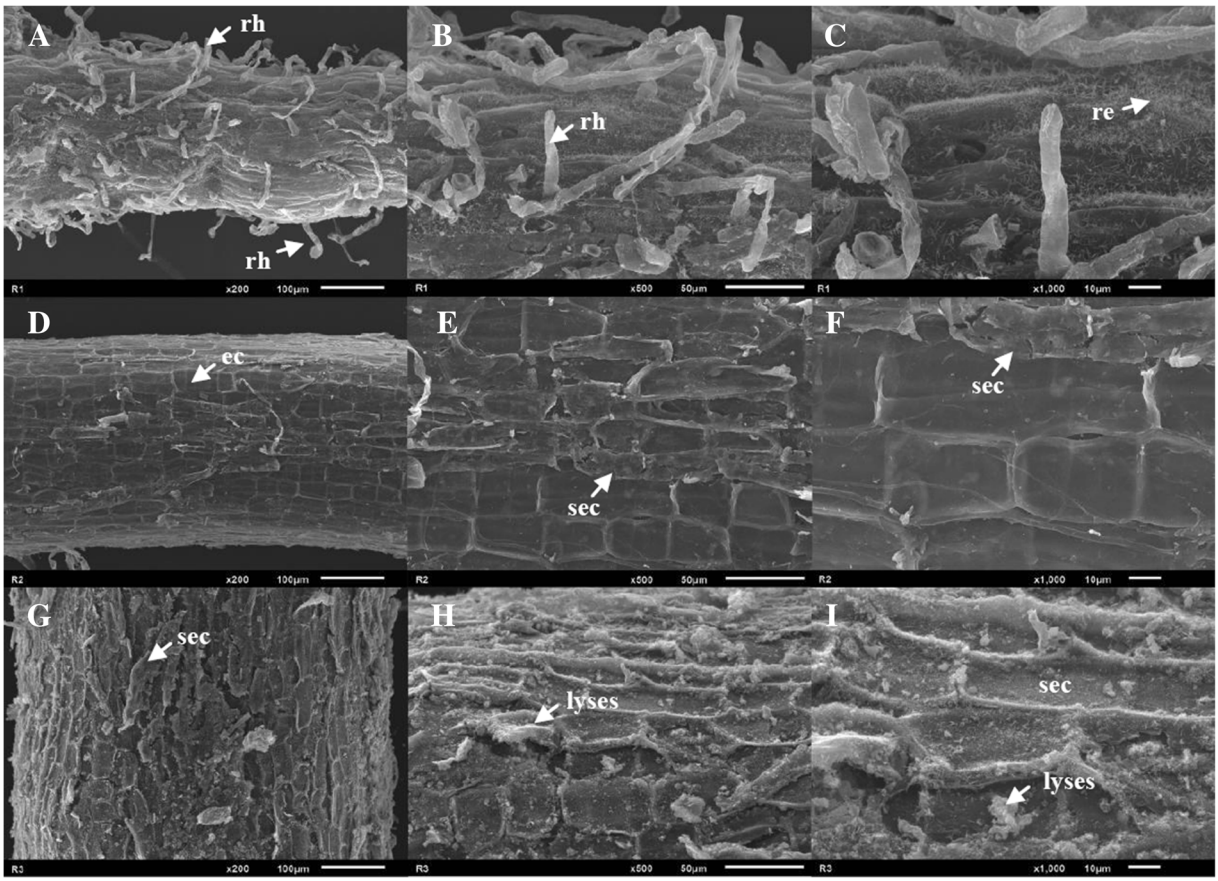

Fig. 1 Scanning electron micrographs of fine roots' surfaces in different root orders. **a**–**c** SEM monographs of the first-order fine root rhizoplane. rh and re represent root hairs and root exudates-like substances, respectively. **d**–**f** SEM monographs of the third-order fine root rhizoplane. ec and sec represent cortical cells and sloughed-off cortical tissues, respectively. **g**–**i** SEM monographs of the fourth-order fine root rhizoplane. The bar in A, D and G is 100 μm; in B, E and H is 50 μm; in C, F and I is 10 μm

The estimated number of OTUs increased with the number of sequences, and the number of OTUs in R1 and R2 compartments were higher than that in R3 and NR (Fig. 2). The diversity of soil bacterial community in the rhizosphere soil was higher than that in bulk soil (Table 2). The lowest diversity of bacterial communities occurred in the R3 compartment, indicating that the composition of bacterial community were significantly reduced in higher order roots ($P < 0.05$). The overlap among OTUs was maximum

Table 1 The N and P stoichiometry of the four soil compartments (mean ± SE)

Soil compartments	NH_4-N ($g \cdot Kg^{-1}$)	NO_3-N($g \cdot Kg^{-1}$)	P($g \cdot Kg^{-1}$)	NH_4:NO_3	N:P
R1	0.0243 ± 0.002c	0.0270 ± 0.0005a	0.0261 ± 0.0012a	0.8980 ± 0.064c	1.9933 ± 0.343a
R2	0.0291 ± 0.008b	0.0273 ± 0.0001a	0.0321 ± 0.0010a	1.0650 ± 0.024b	1.7627 ± 0.487a
R3	0.0327 ± 0.007a	0.0272 ± 0.0002a	0.0305 ± 0.0016a	1.1999 ± 0.082a	1.9542 ± 0.267a
NR	0.0123 ± 0.001d	0.0271 ± 0.0001a	0.0284 ± 0.0024a	0.4555 ± 0.049d	1.4143 ± 0.228b

NR represents bulk soil; R1, R2 and R3 represent rhizosphere soils surrounding the first two order roots, the third order roots and the fourth and fifth order roots, respectively

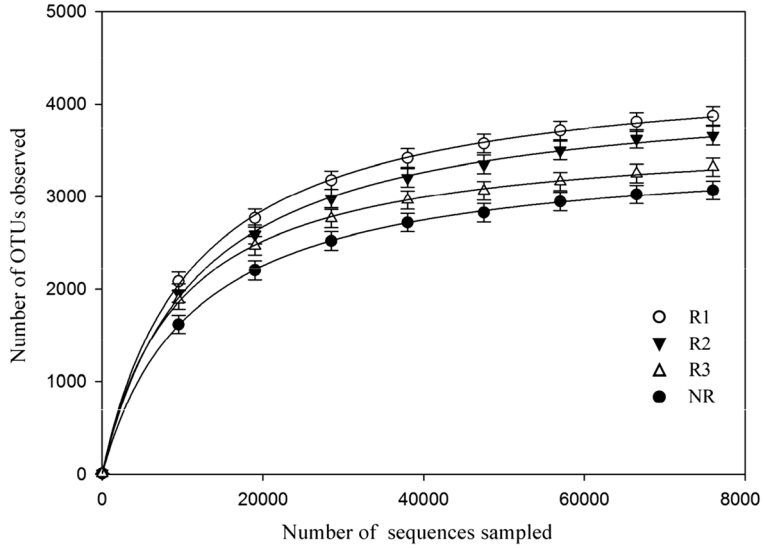

Fig. 2 Rarefaction curves of OTUs in four soil compartments (mean ± standard error). NR represents bulk soil; R1, R2 and R3 represent rhizosphere soils surrounding the first two order roots, the third order roots and the fourth and fifth order roots, respectively

between R1 and R2, and minimum between R3 and NR (Fig. S4), indicating that more similar bacteria inhabited near lower order roots. PCA showed that the first and second axis account for 64.8 and 22.7% of the total variation in soil bacterial communities in poplar plantation soils, respectively. The three rhizosphere soil samples from the same order roots were clustered together (Fig. 3). The bacterial communities in R1, R2, and R3 were separated from those in the NR by PC1.

The composition of soil bacterial communities

The OTUs consisted of 607, 593, 582, and 432 bacterial genera in R1, R2, R3, and NR, respectively. Among these genera, 80 genera had a relative abundance above 0.05%. The heatmap showed that these bacterial genera were distributed differentially among rhizosphere soil compartments (Fig. 4). PCA revealed that the first and second axis accounted for 43.6 and 39.8% of the total variation in soil bacterial communities, respectively. The bacterial communities in rhizosphere soil compartments were separated from those in NR by PC1, and the bacterial communities in the R1 compartment were separated by PC2 (Fig. S5), indicating that the bacterial genera differed between rhizosphere and bulk soil samples. Similarly, the bacterial genera varied along root orders, with R2 and R3 sharing more similar bacterial communities, which is consistent with the results PCA. Ternary plot revealed that some genera (i.e. *Rhizobium*, *Aquicella*, *Rhodanobacter*) were significantly enriched in the R1 compartment, whereas others (i.e. *Mycobacterium*, *Hydrogenophaga*, *Hylemonella*) were enriched in R3 (Fig. S6).

Table 2 The bacterial community diversity in the four soil compartments (mean ± SE)

Soil compartments	Diversity index of bacterial community			
	Ace	Chao1	Simpson	Shannon
R1	4079.12 ± 21.58a	4142.79 ± 191.93a	0.0046 ± 0.00024b	6.67 ± 0.037a
R2	4078.45 ± 95.77a	4146.91 ± 99.09a	0.0045 ± 0.00012b	6.64 ± 0.024a
R3	3505.53 ± 18.47b	3586.22 ± 23.55b	0.0066 ± 0.00038a	6.44 ± 0.044b
NR	3493.91 ± 47.87b	3555.61 ± 41.62b	0.0053 ± 0.00048b	6.48 ± 0.037b

The four indices are non-parametric estimators calculated according to the OTUs abundance of bacterial community by MOTHUR software

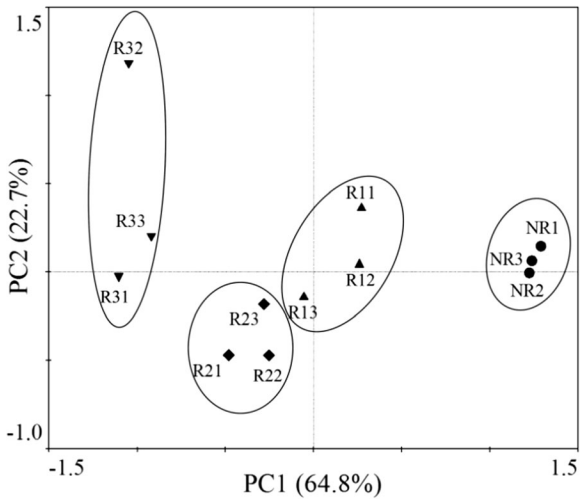

Fig. 3 PCA on bacterial communities in soil samples from the four soil compartments. R11/R12/R13, R21/R22/R23, R31/R32/R33 and NR1/NR2/NR3 represent the three parallel samples of R1, R2, R3 and NR compartment, respectively

Fig. 4 The heatmap diagram of 80 bacteria genera with the relative abundance of above 0.05%. NR represents bulk soil; R1, R2 and R3 represent rhizosphere soils surrounding the first two order roots, the third order roots and the fourth and fifth order roots, respectively

Correlation between core groups of bacterial communities and soil nutrients

There were 12 common genera with a relative abundance above 1% (Fig. 5). Of these, seven (*Bacteroides*, *Bifidobacterium*, *Haemophilus*, *Lactobacillus*, *Parabacteroides*, *Streptococcus*, and *Veillonella*) were only distributed in rhizosphere, whereas the remaining five occurred both in the rhizosphere and bulk soil. *Burkholderia* and *Bacteroides* were most abundant in R1 and R2 compartments, respectively whereas seven (*Bacteroides*, *Bifidobacterium*, DAI 01, *Lactobacillus*, *Lactococcus*, *Streptococcus*, and *Veillonella*) were most abundant in R3.

The 12 core abundant groups in the bacterial community demonstrated close relationship with soil nutrients conditions of poplar plantation. The first two axes accounted for 51.0 and 47.2% of the bacterial communities, respectively (Table S2). Available P and NH_4^+-N showed large loadings on axis 1 (Table S3), and significantly correlated with most of bacterial communities (Table S4). RDA biplot showed that *Bacteroides*, *Bifidobacterium*, *c. Koribacter*, DA101, *Haemophilus*, *Lactobacillus*, *Parabacteroides*, *Streptococcus*, and *Veillonella* were located in the negative direction of axis 1 (Fig. 6); however, all these genera were positively correlated with available P and NH_4-N (Table S4). In contrast, *Burkholderia* and *c. Koribacter* were located in the positive direction of axis 1 and negatively related to the soil nutrients. Additionally, though NO_3^--N and N:P ratio showed large loadings on axis 2, *Lactococcus* was significantly positively related to NO_3^--N ($r = 0.321$, $P < 0.05$).

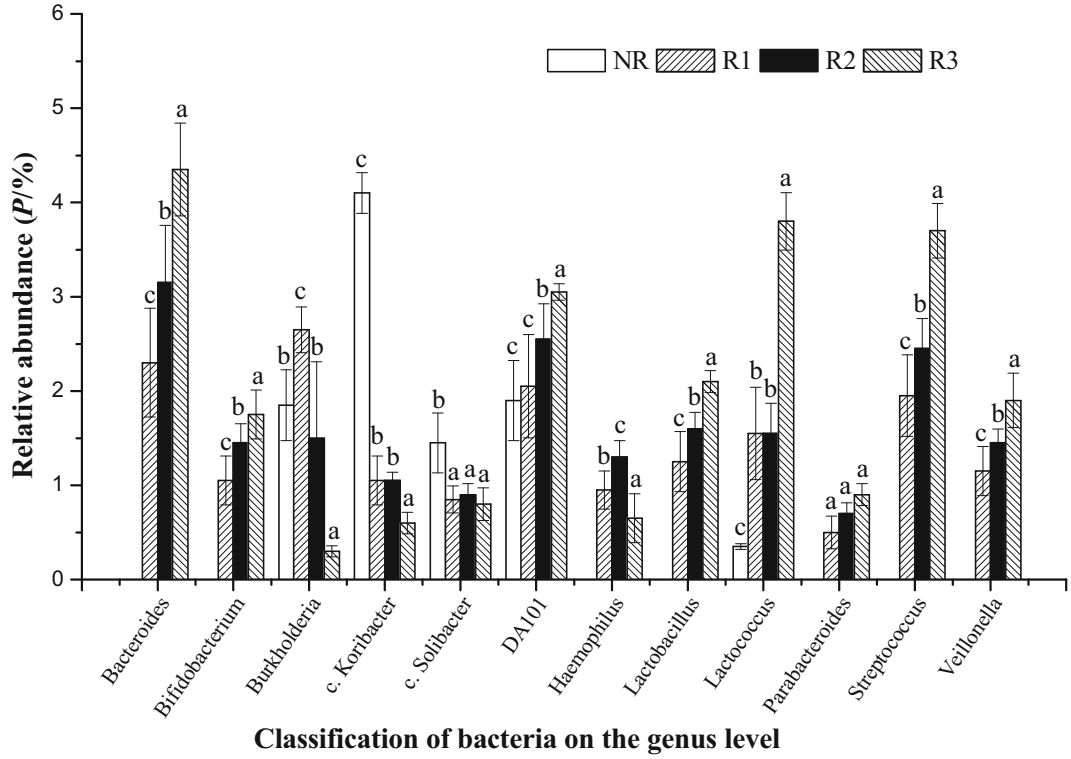

Fig. 5 The relative abundance of the 12 core groups of soil bacteria with the relative abundance above 1% (mean ± standard error). Letters a, b, c and d represent difference among soil compartments at the significance level ($P < 0.05$). There is no significant difference in the relative abundance if different categories share the same letter

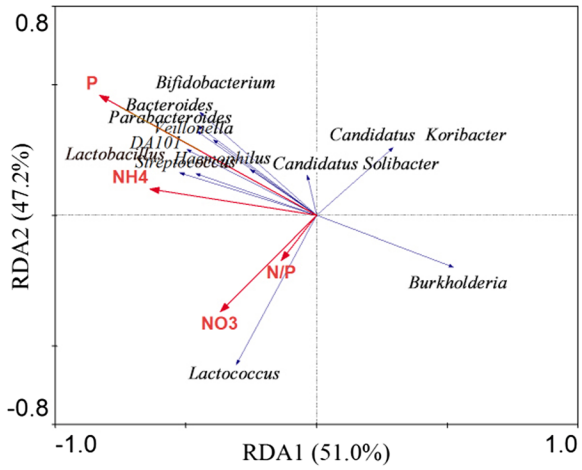

Fig. 6 The correlation between soil microbial communities and soil nutrients as determined by redundancy analysis (RDA). The 12 bacteria genera with a relative abundance above 1% are included as biological variables and the available nitrogen (NO_3-N and NH_4-N), available phosphorus and N:P ratio are included as environmental variables

Discussion

Differences in bacterial communities among root orders

Previous studies have shown significant differences in bacterial communities between rhizosphere and bulk soils (Uroz et al. 2010; Bulgarelli et al. 2012; Li et al. 2014). In the present study, we further elucidated the differentiation of bacterial communities present in root-adhering soil; the decreased diversity of bacterial communities along root orders indicated that the colonization by bacterial communities is closely associated with root orders. SEM revealed that first-order roots have vigorous root hairs on their rhizoplanes, whereas root hairs are absent and cortical tissues sloughed-off in higher order roots. These observations are consistent with other studies showing that diverse bacteria-like structures on the rhizoplane of *Arabidopsis* are closely associated with plant cell-wall features (Bulgarelli et al. 2012). These morphological differences largely determine variations in carbon sources among fine

root orders. Therefore, we had predicted that the morphological differences of the root surface among different orders might determine the bacterial communities associated with different root orders. Our study supports the theory that the rhizosphere microorganism community structure is shaped by plant roots (Hartmann et al. 2009).

We further defined the core bacterial microbiome in rhizosphere soils of poplar tree at the genus level. *Burkholderia* was observed to be one of the core genera of the lower-order roots (Fig. 5). In many tree species, *Burkholderia* was enriched in rhizosphere soils (Berg et al. 2005; Timonen and Hurek 2006). They could promote biodegradation, bio-control, and serve as plant growth promoting rhizosphere bacteria (PGPR) in agriculture. In the present study, the high abundance of *Burkholderia* around poplar lower-order roots implies that this compartment of rhizosphere soils may be a hotspot zone for PGPR selection. In contrast, *Bacteroides* with a high relative abundance only distributed in rhizosphere soils (Fig. 5). It belongs to an important carbohydrate-degrading bacterium that can degrade carbohydrate macromolecules into glucose and other small molecules. The high abundance of *Bacteroides* indicates that substantial amounts of macromolecular carbohydrates are deposited around the higher-order roots. The lower-order roots may deposit soluble carbon sources into rhizosphere soils to proliferate bacteria that help in solubilization of soil nutrients. Further, several other carbohydrate-degrading bacteria would be present around the higher-order roots because more dead cortical tissues would be produced by secondary growth of these parts involved in the transportation of nutrients and water. The core bacterial groups determine specific root-soil interactions, and reflect functional differentiation of roots in their compartments.

Variation in soil nutrients along root orders

The concentration of NH_4-N is higher in the rhizosphere soil than that in the bulk soil, and it increases from lower to higher root orders, whereas NO_3^--N and available P do not show significant differences (Table 1). These results indicate that the soil chemistry differs among root orders. In the present study, the lowest concentration of NH_4-N was investigated in the rhizosphere of first-order roots, which might be attributed to the uptake of NH_4-N by fine roots or the decrease of soil organic nitrogen ammonization around these roots. However, *P.* × *euramericana* showed higher preference for NO_3^--N than NH_4^+-N uptake (unpublished data), indicating that the decreased mineralization of organic nitrogen might be responsible for the lowest concentration of NH_4-N around lower order roots. Other studies have shown that, with rhizodeposition of carbon into surrounding soils, bacteria utilize these carbon resources, thereby leading to a priming effect during soil organic matter (SOM) decomposition (Kuzyakov 2010; Kuzyakov and Xu 2013; Cheng et al. 2014). The microbial biomass and extracellular enzyme activities surrounding roots increase with the exudation of carbon from roots. A significant positive rhizosphere priming effect (RPE) on soil nitrogen (10–52%) mineralization in soybean and sunflower (Zhu et al. 2014) is consistent with the decrease of NH_4-N in rhizosphere soils of lower order roots.

The structure of soil bacterial community may be responsible for the differences in soil nutrient concentrations among soil compartments, owing to RPE. Three hypotheses describing the effect of RPE on soil nutrient mineralization have been put forward; microbial mining hypothesis in soils characterized by low nutrient availability, preferential substrate utilization hypothesis in soils with high nutrient availability, and plant-microbe competition hypothesis (Dijkstra et al. 2013). Within the framework of these three hypotheses, the relationships between soil bacterial community and soil nutrient conditions appear complicated. First, bacteria tend to directly utilize exudates from lower-order roots for their energy requirement rather than SOM decomposition. In contrast, the high-order roots only perform water and nutrient transportation, thereby reducing the competition between bacteria and plants. Hence, less mineralized nitrogen was released into rhizosphere soils around higher-order roots due to decreased decomposition of SOM. When energy-rich carbon compounds from sloughed-off periderm cells are added into the soil, an increasing level of extracellular enzymes is produced around higher order roots, which is used for SOM decomposition and conversion of soil nitrogen into available NH_4-N.

In our study, soil bacterial genera demonstrate a clear root order-dependent community structure (Fig. S5), and the abundance of nitrite bacteria and denitrifying bacteria (e.g., *Bacteroides*) shows a close correlation with NH_4-N concentration (Fig. 6), indicating that they may play an important role in promoting ammonization. Due to the differing concentration of soil available

nitrogen with root orders, poplar roots may provide an excellent model to test the hypotheses about RPE on soil nutrient mineralization. We predict that preferential substrate utilization and root-microbe competition may dominate in rhizosphere soils around different order roots. However, further investigation on CO_2 production and N mineralization using isotope-labeling technology could be helpful in testing this model.

Conclusions

In this study, significant differentiation in rhizosphere bacterial communities has been found among root orders. The abundance and diversity of bacterial communities decrease from lower to higher root orders, indicating that the presence of bacterial communities in rhizosphere soil is closely associated with root order. The soil nutrient content varies significantly among root orders. The core groups of bacterial communities are closely correlated with soil nutrient conditions. Therefore, the root order-dependent interaction between roots and soil microorganisms provides a new domain of research on plant-microbe relationships.

Acknowledgments This work was funded by the National Basic Research Program of China (2012CB416904) and the National Natural Science Foundation of China (31570618). Additionally, this study was supported by the funds of the Shandong Double Tops Program (Grant No. SYL2017XTTD03). We thank the anonymous reviewers for the helpful comments.

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