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# Rhizosphere bacterial and fungal communities succession patterns related to growth of poplar fine roots

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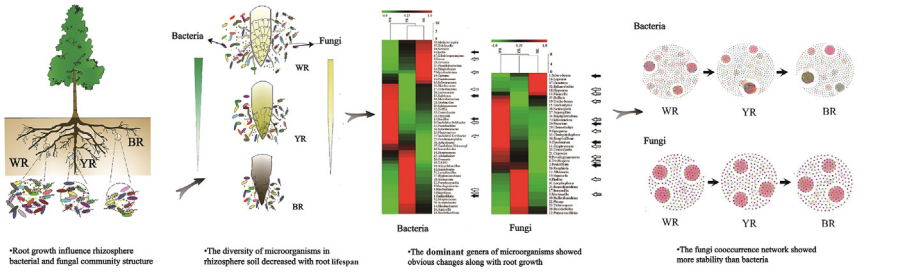
## HIGHLIGHTS

- The rhizosphere microbial communities are related to root lifespan.
- Soil carbon drives the succession of rhizosphere microbial community.
- Bacteria appeared more sensitive than fungi along root growth.
- The microbial community succession showed a close relationship with soil nutrients.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Understanding the succession patterns of microbial community along root growth provides deep insights into interaction between fine roots and microbes. In the study, we investigated this issue using fine roots from poplar trees and grouped these fine roots into three growth stages: newborn white roots (WR), mature yellow roots (YR) and aging brown roots (BR). Root surface traits were observed under a scanning electron microscopy (SEM). Adhered soils on roots of the three growth stages were grouped into the three soil compartments, correspondingly. The 16S rRNA and ITS1 region were sequenced for bacteria and fungi inhabiting rhizosphere soils, respectively. Phospholipid fatty acid (PLFA) technology was employed to examine the biomass of bacterial and fungal communities. The anatomical traits of fine roots show apparent differences among the WR, YR and BR. Both bacteria and fungi have 25 dominant genera with a relative abundance over 1%, of which, four genera of the bacteria (*Bacillus, Burkholderia, Ralstonia* and *Dyella*) differ in abundance among the WR, YR and BR soil compartments and four genera of the fungi (*Fusarium, Chaetomium*, *Penicillium* and *Scleroderma*) differ in abundance among these soil compartments. The operational taxonomic units (OTUs) showed the highest richness in the WR soil compartment for bacteria and in the YR soil compartment for fungi, indicating a different succession pattern between the bacterial and fungal communities. Furthermore, the biomass of bacterial community is larger than the fungal community according to PLFAs, and both decreased along fine root growth. The total carbon (TC) in the soil increases along root growth while the dissolved organic carbon (DOC) decreases. Redundancy analysis (RDA) shows a close correlation between twelve dominant bacteria genera and the total organic carbon (TOC), the readily oxidizable organic carbon (ROC) and DOC and ten dominant fungi genera with the TOC and ROC. In conclusion, our results indicate that fine roots growth has shaped the composition and structure of root associated bacterial and fungal communities.



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## 1. Introduction

Fine roots, despite accounting for only 0.1–3% of tree biomass (Vogt et al., 1986), play a disproportionately important role in the cycling of nutrients in terrestrial ecosystems (Finér et al., 2011). Carbon allocation pattern in forest ecosystems can be markedly influenced by fine root turnover (Xia et al., 2015). The first and second order fine roots, having a short life and quick turnover, transfer large quantities of carbohydrates into soils (Xia et al., 2010), resulting in a loss of net primary productivity (NPP) (Odelade and Babalola, 2019) and change in the NPP allocation between above and below ground in forests (Zhu et al., 2018). Therefore, more attention has been paid to the biotic and abiotic factors affecting fine root survival and lifespan (Iversen, 2014). The interaction between roots and soils needs to be urgently revealed in root ecology research (Paradiso et al., 2019).

The growth and activity of fine roots are modified by the surrounding soil environment (Lladó et al., 2017; Wang et al., 2018; Williams and de Vries, 2020) and many ecological processes between roots and microbes (Andreote and Silva, 2017; Lladó et al., 2017; Hartman and Tringe, 2019). First, fine roots can release carbon components into the surrounding soils, which are essential for microbial community recruitment (Hassan et al., 2019). In turn, microbial community shows beneficial or harmful effects on roots growth (Zarraonaindia et al., 2015; Hashem et al., 2019). Hence, the interaction between fine roots and microbial community is important in regulating plant growth.

The structure of microbial community not only depends on the root activity and the available niches in rhizosphere soils (Bulgarelli et al., 2013; Edwards et al., 2015) but also on the competition among microbes and their capacity of microbes to use organic carbon (Li et al., 2019; Compant et al., 2010). Bacteria use dissolved organic carbon (DOC) more efficiently whereas fungi tend to use complex organic carbon, such as cellulose (Eichlerová et al., 2015). As a result, bacteria usually cannot dominate nutrient-rich rhizosphere niches, and fungi such as *Ascomycota* and *Glomeromycota* can respond rapidly to carbon rhizodeposits (Vandenkoornhuyse et al., 2007; Hannula et al., 2012). In addition, the difference between bacterial and fungal communities may be associated with their interactions under rhizosphere microenvironment conditions (Saleem et al., 2019).

Despite the importance of root-associated microbial communities to ecological processes, the interaction of roots and soil microbes is still less clear. Different patterns between bacterial and fungal communities in rhizosphere soils can be affected by the state of plant development (Mougel et al., 2006; Schlemper et al., 2017) and rooting depth (Hao et al., 2020). Furthermore, root traits (i.e., root types, root zones, root order, root diameter) may affect soil microbes assembling in the rhizosphere (Philippot et al., 2013; Wang et al., 2017; Saleem et al., 2018; Ulbrich et al., 2020). Therefore, the composition and structure of the rhizosphere microbial communities may be shaped by the growth and development of roots and the soil stoichiometric properties (Delgado-Baquerizo et al., 2017). However, the succession pattern and driving mechanism of rhizosphere microbial community along fine root growth need to be further explored.

In this study, we collected distal fine roots at three growth stages in a ten-year poplar plantation. The morphological and anatomical traits of the fine roots and the ultrastructure of the root surface were observed. The high-throughput sequencing technology and phospholipid fatty acid (PLFA) method were employed to quantify the bacterial and fungal communities in the rhizosphere soil. We hypothesize that the root-associated microbial communities would be correlated with the fine root lifespan. The objective of our study is to (1) clarify the succession patterns of rhizosphere bacterial and fungal communities along fine root growth, (2) identify the relationship between carbon components and microbial community assembly, and (3) uncover the interactions of soil carbon (C), nitrogen (N) and phosphorous (P) stoichiometry with microbial community along fine root growth.

## 2. Materials and methods

### 2.1. Study site, roots and soil sampling

We chose the study site in *Gaoqiao* national forest farm (N35°53′, E116°50′), which is located on the tidal land near the *Dawenhe* river in central Shandong province, China. The soil has a coarse texture, which means a low capacity in water retention and low content of organic matter. The study site lies in continental monsoon region in warm temperate zone with an annual average temperature, extreme maximum and minimum temperature being 13.4 °C, 40.7 °C and − 19.4 °C, respectively. The annual frost-free period is about 200 days, and the annual average precipitation is approximately 690 mm. A one-hectare plot was established in a 10-year poplar plantation (*Populus* × *euramericana* 'Neva') without irrigation or fertilization since enclosed in 2012. The understory vegetation in the plot consists of annual and perennial herbs.

We set up three quadrates in the plot and selected three trees within each quadrate. Then, we sampled five soil blocks (50 cm in length and width and 20 cm in depth) at a distance of 0.5 m around the tree according to the distribution of poplar fine roots (Zhu et al., 2018), resulting in a total of 45 soil blocks (5 soil blocks/tree × 3 trees/quadrate × 3 quadrates). Prior to collecting the roots, we removed all herbaceous vegetation on the soil blocks using a flat blade. Poplar roots were distinguished from those of herbaceous roots based on colour and flexibility. Poplar roots were collected from the soil blocks and quickly placed into an icebox. In the laboratory, we only collected the first and second order fine roots and categorized them into three groups based on surface colour (Fig. S1): white new born roots (WR), yellow mature roots (YR) and brown aging roots (BR) (Zhu et al., 2018) (Fig. S1). We mixed fine roots from the same root group into one sample and obtained nine samples (3 samples/quadrate × 3 quadrates). Soils adhered on root surface were collected with a small brush for C, N and P stoichiometry analysis. Each group of fine roots was placed in a 50 mL centrifuge tube, and the adhered soil particles were rinsed into the tube using 30 mL phosphate buffer solution (PBS). After ultrasonic treatment for 10 min, the roots were removed for anatomical trait observation under a SEM. Root-adhering soil pellets were collected after centrifuge (Li et al., 2014) and stored at −80 °C for genomic DNA extraction and the phospholipid fatty acid analysis (PLFA) of soil microbes.

### 2.2. Fine root characteristics analysis

#### 2.2.1. The anatomical traits of fine roots

Ten fresh and clean fine roots were used for anatomical traits analyses. The roots were dissected into 4–6 mm segments and stained with safranine-fast green for 12 h. These segments were dehydrated using a series of 70, 85, 95, and 100% alcohol (Dong et al., 2015), and then placed into paraffin slices for subsequent photographing under a Nikon E200 digital microscope (Nikon Corporation Company, Tokyo City, Japan).

#### 2.2.2. Scanning electron microscopy (SEM)

Ten fresh and clean fine roots of each group were randomly selected, and 5–8 roots with a length of approximately 0.8 cm were selected for SEM observation. The protocols are described in Wang et al. (2017).

### 2.3. Soil bacterial and fungal community analysis

#### 2.3.1. DNA extraction and amplification of soil bacterial and fungal community

Microbial DNA was extracted from approximately 100 mg soil using Soil DNA Extraction Kits (Omega, USA) and the quality of DNA was checked using an RS232G UV spectrophotometer (Eppendorf Company, Hamburg, Germany). The double V region (V4–V5) of bacteria 16S rDNA was amplified using primers 515F (5-GTGCCAGCMGCCGCG

GTAA-3) and 907R (5-CCGTCAATTCMTTTRAGTTT-3). The ITS1 region of fungal community was amplified using ITS5F (5-GGAAGTAAAAGTCGTA ACAAGG-3) and ITS1R (5-GCTGCGTTCTTCATCGATGC-3). Amplicons were sequenced with an Illumina MiSeq using pair-end 250 bp sequencing.

*2.3.2. Sequence data filtration*

The raw data were trimmed using Trimmomatic (Bolger et al., 2014) in paired-end mode. A SLIDING-WINDOW method was performed to filter the pair-ended sequences, with a window size of 10 bp and a step size of 1 bp (Jiang et al., 2019). A MINLENGTH step was used to discard reads shorter than 150 bp. FLASH v1.2.7 (http://ccb.jhu.edu/software/FLASH/) (Magoč and Salzberg, 2011) was used to merge the pair-ended sequences with the overlapping region ≥10 bp. Finally, the valid sequence was identified based on the barcode sequence corresponding to each sample. The chimera sequence was identified and removed using USEARCH v. 5.2.236 (http://www.drive5 .com/usearch/) implemented in QIIME ver. 1.8.0 (http://qiime.org/) (Caporaso et al., 2010).

*2.3.3. OTUs clustering and annotation*

The high-quality sequences with a minimum similarity value of 97% were identified (Edgar, 2010), and the longest sequence within each category was selected to blast the bacterial annotation database Greengenes (Release 13.8, http:// greengenes. seconggenome.com/) (DeSantis et al., 2006) and the fungal annotation database UNITE (Release 5.0, https://unite.ut.ee/) (Koljalg et al., 2013) to obtain taxonomic information of each representative OTU sequence. To ensure the accuracy, OTUs with a relative abundance less than 0.001% of the total number of sequences was discarded (Bokulich et al., 2013). After filtering, a total of 227,534 and 313,419 sequences were retained in bacterial and fungal communities, respectively, representing 27,967 bacteria and 31,939 fungi in white root (WR), 23,071 bacteria and 36,874 fungi in yellow root (YR), and 24,806 bacteria and 35,659 fungi in brown root (BR) (Table S1). All sequences were deposited in the NCBI Sequence Read Archive (SRA) database under the accession number of SRP237999 for bacteria and SRP234058 for fungi.

*2.3.4. Analyses of OTUs*

Rarefaction curves were generated using Sigmaplot 12.5 (Systat Software Inc., San Jose, CA, USA) and used to compare the relative richness of bacteria and fungi OTUs across the soil compartments. Venn diagrams, microbial co-occurrence networks and a PLS-DA diagram were generated based on the obtained OTU abundance by R version 3.6.1 (R Core Team, 2020). In addition, Ace index, Chao1 index, Shannon index and Simpson index were calculated for each soil compartment using MOTHUR ver. 1.8.0. Redundant analysis (RDA) was performed using Canoco for Windows (version 5.0, Microcomputer Power, USA). The heatmaps were generated using MEV 4.9.0. A one-way analysis of variation (ANOVA) and a least significant difference (LSD) multiple comparison ($\alpha = 0.05$) were used to assess the difference in the relative abundance of soil bacteria and fungi at genus level among the soil compartments.

*2.3.5. The PLFA of the soil compartments*

The soil bacterial and fungal communities were quantified by phospholipid fatty acids (PLFAs). PLFAs were extracted following the method of Bossio et al. (2006) and the composition was analyzed using a gas chromatography (GC) system (Agilent 6890, Agilent Technologies, Inc., USA). The PLFA components were identified by the Sherlock Microbial Identification System (MIS) 4.5 (MIDI, Newark, Delaware, USA). Each component was converted to units of $nmol \cdot g^{-1}$ dry soil from an internal standard FAME 19:0 (Matreya Inc., State College, PA, USA) reaction value. PLFAs were named according to Frostegard et al. (1993).

*2.4. Rhizosphere soil stoichiometry characteristics*

*2.4.1. Soil C, N, P analysis*

The total carbon (TC) and total nitrogen (TN) were measured after dry combustion using an elemental analyzer (Vario macro cube, Elementar, Germany). The total phosphorus (TP) and available phosphorus (AP) were measured using an automatic intelligent chemistry analyzer (Smartchem 200, AMS/Westco, Italy). Soil nitrate ($NO_3^-$-N) and ammonium ($NH_4^+$-N) were measured using an automatic flow analyzer (SEALAA3, Germany).

*2.4.2. Soil carbon component analysis*

The total organic carbon (TOC) and readily oxidizable organic carbon (ROC) were determined by potassium dichromate volumetric and external heating methods (Anderson and Domsch, 1989), and 333 mmol·L-1 potassium permanganate oxidation-colorimetry (Blair et al., 1995), respectively. The soil microbial biomass carbon (SMBC), particulate organic carbon (POC) and dissolved organic carbon (DOC) were determined by the fumigation incubation method (Vance et al., 1987), Garten's method (Garten et al., 1999) and TOC Analyzer (Elementar Vario TOC, Germany) (Gregorich et al., 2003), respectively.

## 3. Results

*3.1. The anatomical and morphological traits of fine roots at different growth stages*

WR showed compacted and arranged cortex tissues and a small stele area at cross-section whereas YR and BR showed the developed vascular bundle, vessels and obviously detached cortical tissues (Fig. 1). SEM showed dense root hairs and orderly-arranged epidermal cells on the surface of the WR and withered root hairs and shriveled epidermal cells on the surface of both the YR and BR (Fig. 2). These morphological traits indicate the likely change in physiological and functional activity with root growth and development.

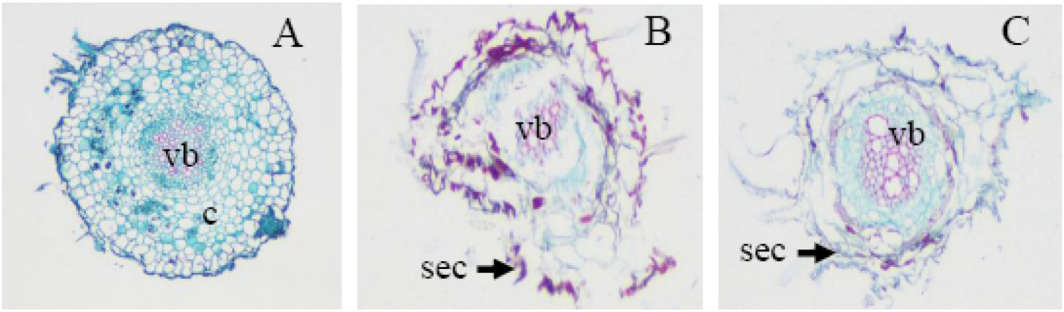


**Fig. 1.** The root anatomical traits at different growth stages. Letters A, B and C represent white new born root (WR), yellow mature root (YR) and brown aging root (BR), respectively. vb and sec represent vascular bundle and sloughed-off cortical tissues, respectively.

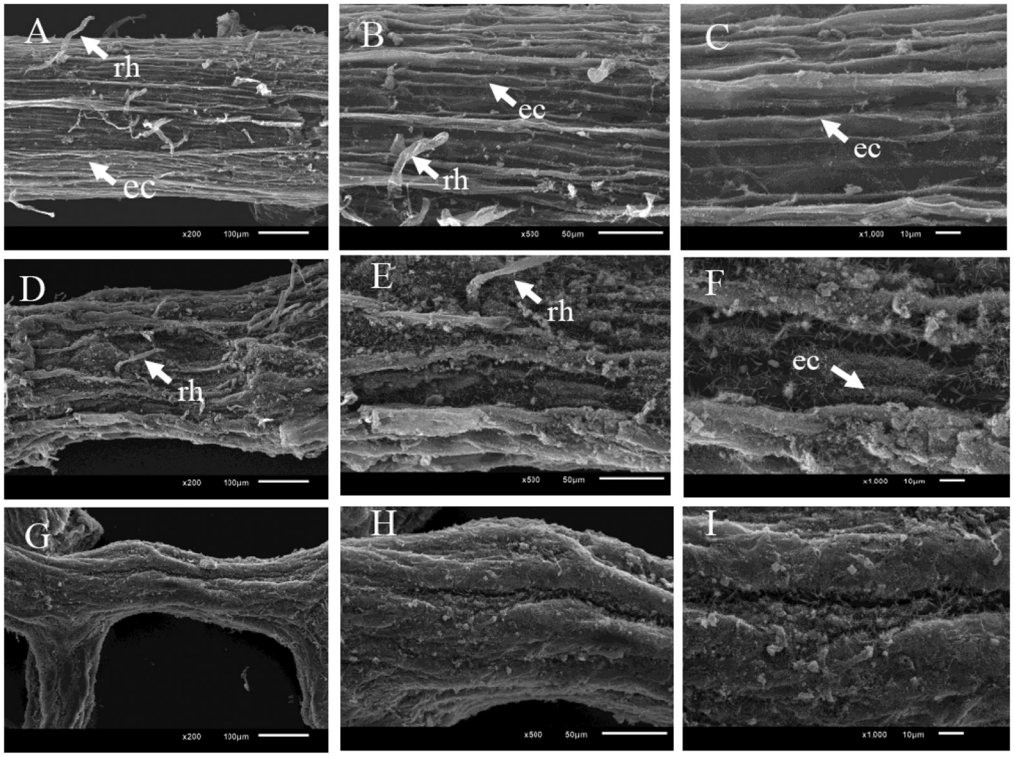


**Fig. 2.** The scanning electric microscopy (SEM) images at different growth stages of fine root. A–C, SEM images of the WR. D–F, SEM images of the YR. G–I, SEM images of the BR. rh and ec represent root hairs and cortical cells, respectively. The bar in A, D and G is 100 μm; in B, E and H is 50 μm; in C, F and I is 10 μm.

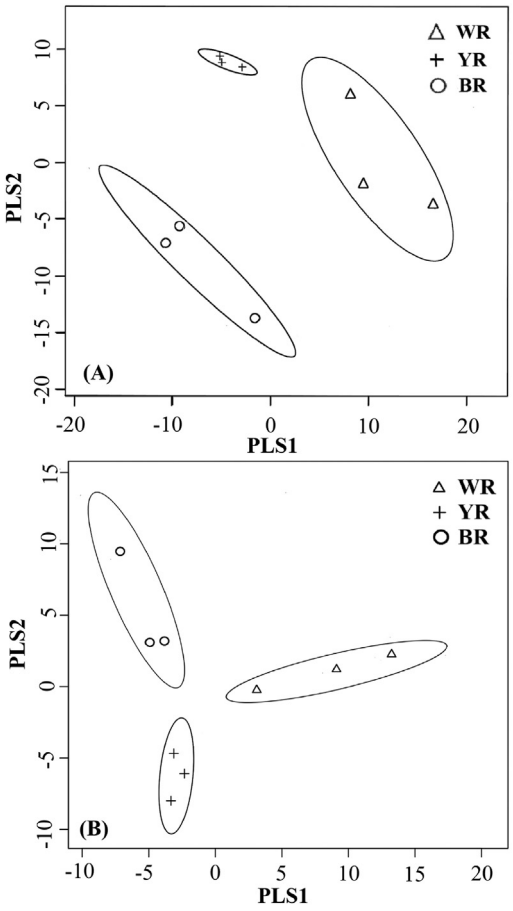


**Fig. 3.** Partial least square discriminant analysis (PLS-DA) on root-inhabiting bacterial (A) and fungal (B) communities at different growth stages of fine root.

### 3.2. The soil C, N and P stoichiometry in different soil compartments

The WR soil compartment showed less content of TC but more DOC. The content of SMBC was 2.1– 2.6 times greater in the BR soil compartment than in the WR and YR soil compartments (Table S2). However, the content of TOC, ROC and POC did not differ significantly among the three soil compartments ($P > 0.05$). Meanwhile, the content of TN, TP and $NH_4^+$-N also showed significant differences among the three soil compartments (Table S3). For example, the YR soil compartment consisted of more TN (0.85 $mg \cdot g^{-1}$) but less TP (0.23 $mg \cdot g^{-1}$), while the WR soil compartment had more $NH_4^+$-N (0.037 $mg \cdot g^{-1}$), indicating a close relationship between the rhizosphere soil stoichiometric characteristics with root growth and development.

### 3.3. The OTU-based composition and structure of bacterial and fungi communities

PLS-DA analysis showed that the OTUs from the nine soil samples formed three clusters for both bacteria and fungi, corresponding to the three stages of root growth (Fig. 3). However, the OTU richness of microbial communities did not show significant differences among the three soil compartments (Fig. S2). The number of shared OTUs among the three soil compartments was 2546 for bacteria and 424 for fungi (Fig. S3). In addition, the richness and diversity indices of microbial community did not show a significant difference among the three soil compartments (Table S4). Heatmap analysis on 52 genera of bacteria and 38 genera of fungi (with an relative abundance >0.1%) showed a very close relationship between some microbes and root growth (Fig. 4). Furthermore, some dominant bacteria (i.e. *Bacillus, Burkholderia, Ralstonia* and *Dyella*) and fungi (i.e. *Fusarium, Chaetomium* and *Scleroderma*), with a relative abundance >1%, showed significant differences in the relative abundance among the three root groups (Table S5). The co-occurrence networks of bacteria showed that *Chloroflex* and *Actinobacteria* exhibited obvious interactions in the YR

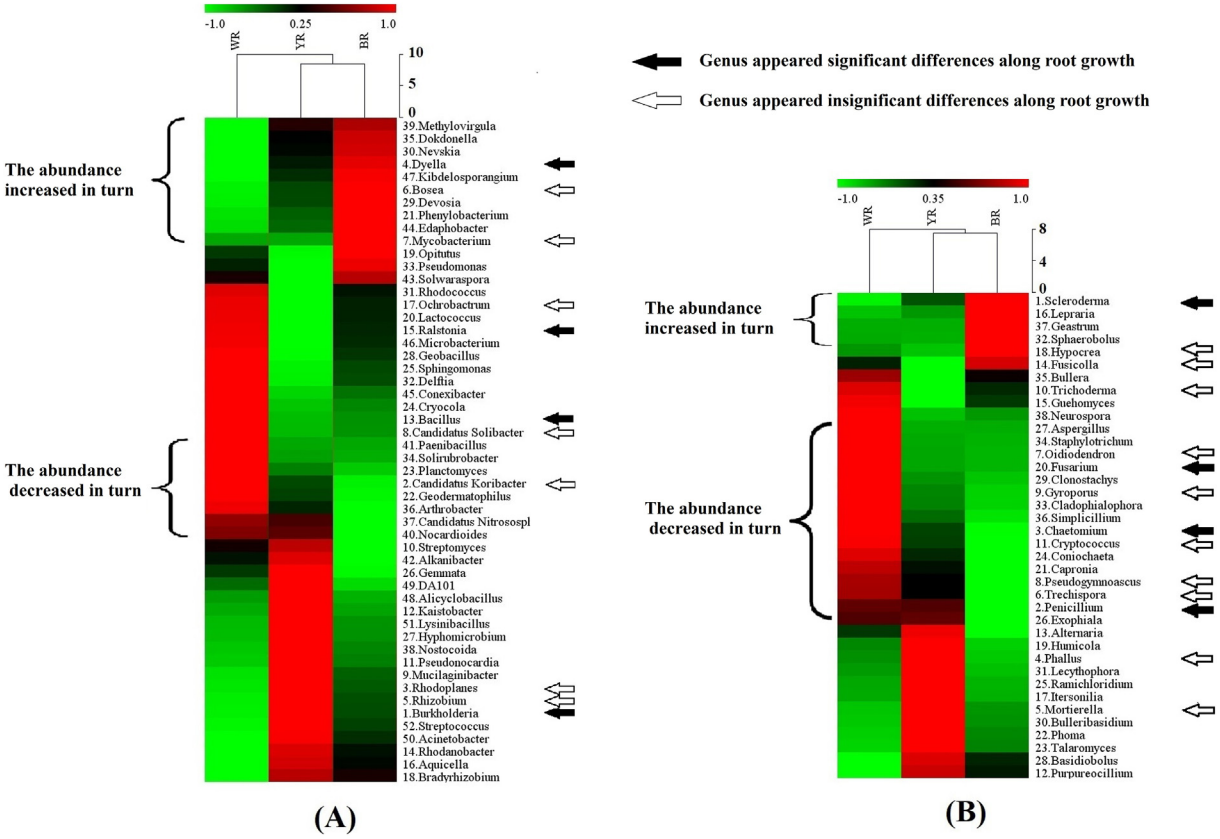


**Fig. 4.** The heatmap of root-associated bacteria (A) and fungi (B) with the relative abundance of above 0.1%. WR, YR and BR represent the corresponding soil compartments for each root group. Colored red and green represent the genus with higher abundance and lower abundance, respectively.

soil compartment and the BR soil compartment, respectively (Fig. 5A). However, the interaction of fungi phyla was relatively stable compared with bacteria (Fig. 5B).

### 3.4. The PLFA-based abundance of bacterial and fungal communities

In total, 53 PLFA biomarkers were detected in the three soil compartments (Table 1). The abundance of Gram Negative ($G^-$) bacteria was higher in the WR soil compartment than the YR and BR soil compartments. However, Gram Positive ($G^+$) bacteria were most abundant in the YR soil compartment and least abundant in the BR soil compartment. Both the bacteria-fungi and $G^+$-$G^-$ ratios peaked in the YR soil, indicating the close association between the activity of fine root and recruitment of bacteria community at the stage of mature fine roots.

### 3.5. The correlation between the microbial community and soil carbon components

Both dominant genera of the bacterial and fungal communities showed a close relationship with the soil carbons, with RDA1 and RDA2 explained 70.2% and 4.5% of variation for the bacterial community and 50.4% and 10.1% of variation for the fungal community (Fig. 6). *Mycobacterium* and *Dyella* of the bacterial community showed a positive correlation with ROC and *c. Solibacter* and *c. Koribacter* showed a positive correlation with DOC. Meanwhile, *Burkholderia* and *Rhodoplanes* revealed a positive correlation with TOC ($P < 0.05$) (Fig. 6A). *Trechispora*, *Penicillium*, *Fusarium* and *Chaetomium* of the fungal community showed a positive correlation with both DOC and TOC. In contrast, *Scleroderma* was significantly positively related to ROC ($P < 0.05$) (Fig. 6B).

### 3.6. The correlation between the microbial community and soil N and P

Bacterial groups *Ochrobactrum*, *Ralstonia*, *Rhizobium* and *Bosea* were positively related to TN and TP, and *Bacillus* and *c. solibacter* showed a positive relationship with available nitrogen ($NH_4^+$-N, $NO_3^-$-N) and AP ($P < 0.05$) (Fig. 7A). Fungal groups *Trechispora*, *Oidiodendron*, *Trichoderma* and *Mortierella* were positively correlated with TP. *Pseudogymnoascus* was positively related to AP ($P < 0.05$), and *Fusicolla*, *Hypocrea* and *Gyroporus* were significantly positively related to TN ($P < 0.05$) (Fig. 7B). Based on PLFAs, the bacterial and fungal communities were significantly positively correlated with AP ($P < 0.05$) and TN ($P < 0.01$), respectively (Fig. S4).

## 4. Discussion

### 4.1. The changes of soil carbon components with fine root growth

Soil carbon is one of the most important substrates for microbes, of which, active soil organic carbon (active SOC), is the most important component of SOC. Despite its low content, active SOC is more sensitive to environmental factors (Haynes, 2005), and thus showed a more profound influence on plants and microorganisms (Wang et al., 2015). Active SOC could be characterized by DOC, ROC and SMBC (Chen et al., 2017). DOC and ROC can be utilized by most microorganisms and are closely related to the soil microbial community (Nyawade et al., 2019). In this study, DOC decreased significantly in the YR and BR soil compartments (Table S2) and the absolute abundance of microbial communities decreased as well (Table 1), indicating that DOC has shaped the microbial community. Plant root growth can affect soil carbon components, especially in rhizosphere soil. According to the

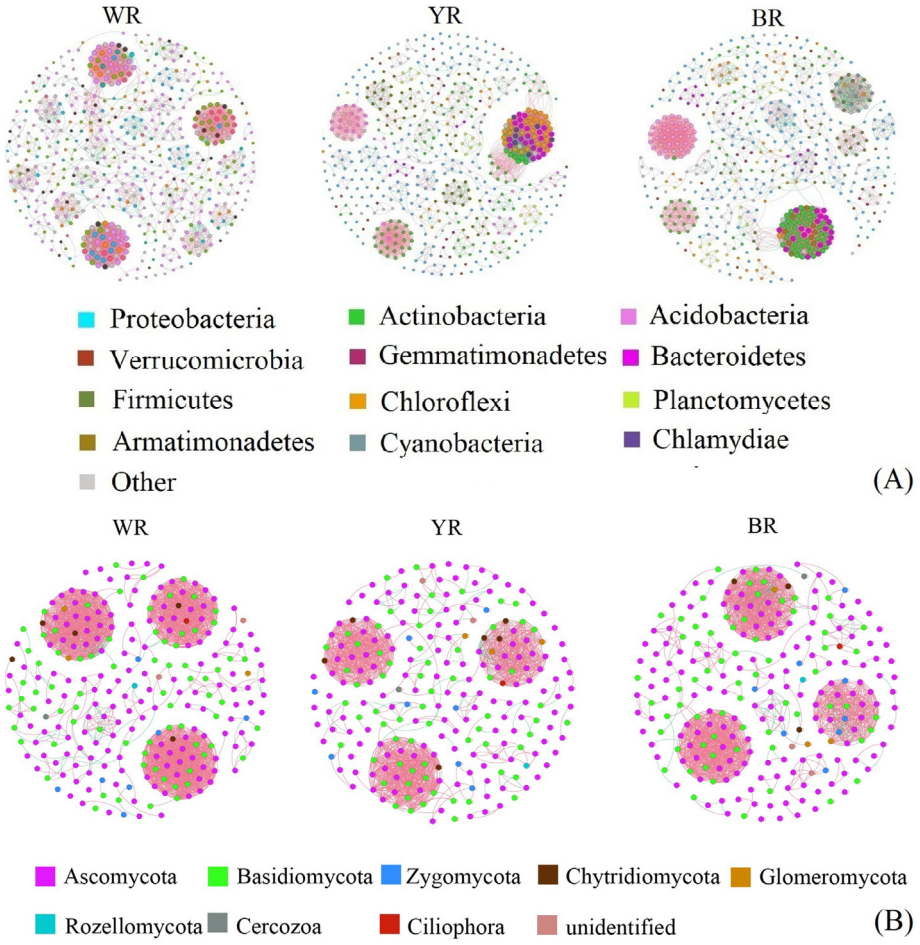


**Fig. 5.** The co-occurrence network of bacteria (A) and fungi (B) at three growth stages of fine root. Nodes represent the dominant genera represented by different colors. The red and green line connecting the nodes indicates positive correlation and negative correlation, respectively ($P < 0.01$).

anatomical traits and SEM, poplar fine roots showed obvious morphological differences along root growth (Figs. 1 and 2), reflecting their different physiological and metabolic functions. For example, young roots can release various root exudates into soils (Keiluweit et al., 2015), providing dissolved carbon substrates for microorganisms. With the secondary growth of fine roots, sloughed cortex, root crown and root hairs (Fig. 2) become other important sources to SOC (Bingham, 2007). All these carbon inputs greatly changed the utilization substrates

**Table 1**
Soil microbial diversity and abundance at the three stages of fine root growth (mean ± SE).

| Microbial groups | PLFA biomarkers | WR ($nmol \cdot g^{-1}$) | YR ($nmol \cdot g^{-1}$) | BR ($nmol \cdot g^{-1}$) |
|---|---|---|---|---|
| Bacteria[a] | 11:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 24:0, 10:0 3OH, 14:1 w5c, 15:1 w6c, 16:0 2OH, 16:1 w7c, 16:1 w7c alcohol, 16:1 w8c, 16:1 w9c, 17:0 cyclo w7c, 17:1 w8c, 18:1 w5c, 18:1 w7c, 19:0 cyclo w7c, 20:1 w9c. 11:0 iso, 12:0 anteiso, 13:0 iso, 13:0 anteiso, 14:0 iso, 15:0 iso, 15:0 anteiso, 15:1 iso w6c, 16:0 iso. | 25.55 ± 3.83a | 18.90 ± 3.38b | 13.13 ± 3.31c |
| Fungi[b,c] | 18:1 w9c, 18:2 w6c. | 9.30 ± 1.26a | 8.79 ± 7.12a | 3.85 ± 2.60b |
| Bacteria/fungi | | 2.78 ± 0.31b | 9.08 ± 6.09a | 7.47 ± 3.49a |
| Gram negative[d] | 10:0 3OH, 14:1 w5c, 15:1 w6c, 16:0 2OH, 16:1 w7c, 16:1 w7c alcohol, 16:1 w8c, 16:1 w9c, 17:0 cyclo w7c, 17:1 w8c, 18:1 w5c, 18:1 w7c, 19:0 cyclo w7c, 20:1 w9c. | 7.85 ± 2.70a | 2.72 ± 0.93b | 3.35 ± 0.83b |
| Gram positive[e] | 11:0 iso, 12:0 anteiso, 13:0 iso, 13:0 anteiso, 14:0 iso, 15:0 iso, 15:0 anteiso, 15:1 iso w6c, 16:0 iso. | 8.93 ± 1.24a | 10.34 ± 0.64a | 5.29 ± 0.99b |
| $G^+/G^-$ | | 1.40 ± 0.43b | 5.41 ± 2.41a | 1.64 ± 0.13b |

G+/G- Ratio of Gram-positive bacteria to Gram-negative bacteria.
[a] Bossio et al. (2006).
[b] Olsson (1999).
[c] Kaiser et al. (2010).
[d] Gutknecht et al. (2012).
[e] Moche et al. (2015).

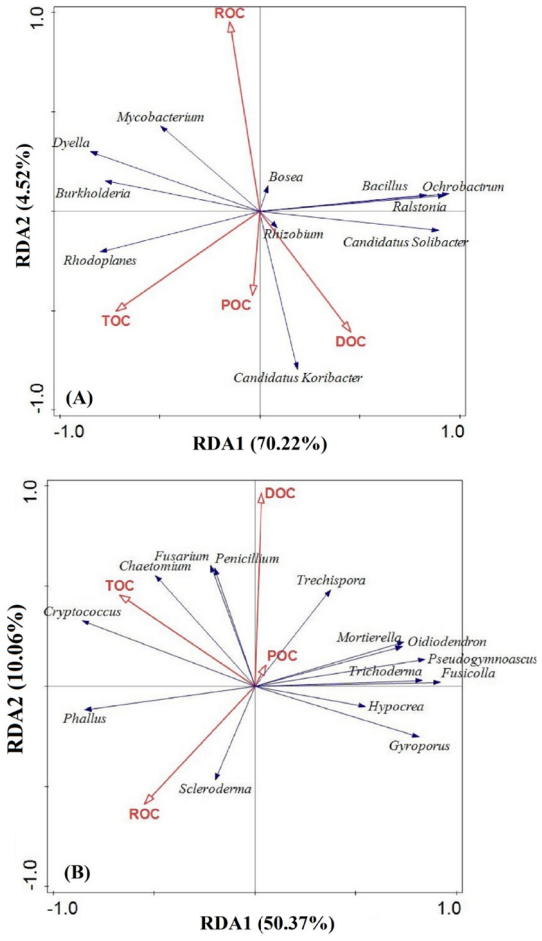


**Fig. 6.** The relationships between the dominant genera of bacteria (A) and fungi (B) and the soil carbon components at different growth stages of fine roots. Red vectors represent trajectories of soil chemical parameters as independent variables; black vectors represent dominant genera of bacteria (A) and fungi (B). The acute and obtuse angles represent positive and negative relationships, respectively. Abbreviations: total organic carbon (TOC), dissolved organic carbon (DOC), readily oxidizable organic carbon (ROC) and particulate organic carbon (POC).

of microbes (Tardy et al., 2015). The different carbon components in the rhizosphere suggest that the microbial community colonized in the rhizosphere may demonstrate succession along the growth of fine roots.

### 4.2. Response of microbial community to soil carbon components

The α diversity of microbial communities in rhizosphere soil decrease along the fine roots growth despite shows no significant differences. PLFA shows a decrease in the biomass of bacteria and fungi in rhizosphere soils along the fine root growth. Based on these, we speculated that the fine root metabolic activity may affect the microbial structure. The PLFA and SMBC of microbial communities appeared positively related to SOC and soil N (Lu et al., 2019; Nyawade et al., 2019), however, PLFAs extracted from the cell wall of living bacteria and fungi may be different from SMBC based on chloroform fumigation during the microorganism biomass estimation. Previous studies showed that resource distribution (especially SOC) plays an important role in shaping the rhizosphere microbial communities (Waring et al., 2013). Generally, when the active SOC is limited, the microbial biomass would decrease (Wang et al., 2015). In the study, the active SOC decreases along root growth (Table S2), affecting the structure of microbial community. Meanwhile, the active SOC is a mixture of carbon compounds, providing resources for various microbes and shaping the structure and biomass of microbial communities (Saleem et al., 2019). Soil nitrogen usually plays a more important role in affecting SMBC than the soil carbon does in most of ecosystems, and demonstrates a positive relationship with SMBC (Wardle, 1992). In the study, we detected more TN and available nitrogen in the YR and BR soil compartment (Table S3). Therefore, the results support that SMBC may be influenced by multiple soil factors and demonstrated different changes from PLFA.

In this study, the PLFAs of bacterial and fungal communities were observed to have different succession patterns. For instance, bacteria community shows differences in abundance among the three soil compartments while fungi significantly are less abundant only in the BR soil compartment. The results indicated that the bacterial community is more sensitive to root activity compared with the fungal community, possibly due to their different metabolic activity for SOC between bacteria and fungi (Sommer et al., 2017). For instance, the DOC in root exudates is rich and beneficial to bacteria in the WR soil compartment (Mougel et al., 2006). By contrast, fungi can utilize the complex organic compounds and exfoliated cortical tissues (Soares and Rousk, 2019). Hence, the changes of carbon resources along the root growth can promote the ecological niche differentiation between root-associated bacterial and fungal communities at a specific stage of root growth. The different succession patterns between bacterial and fungal communities are also likely due to the available carbon substrates and the different ecological strategies for carbon utilization, especially in the rich-SOC environment (Winkler et al., 2017). However, the utilization of easily available carbon is limited for bacteria due to their fixed niche in the soil. By contrast, fungi with mycelium can utilize carbon more efficiently

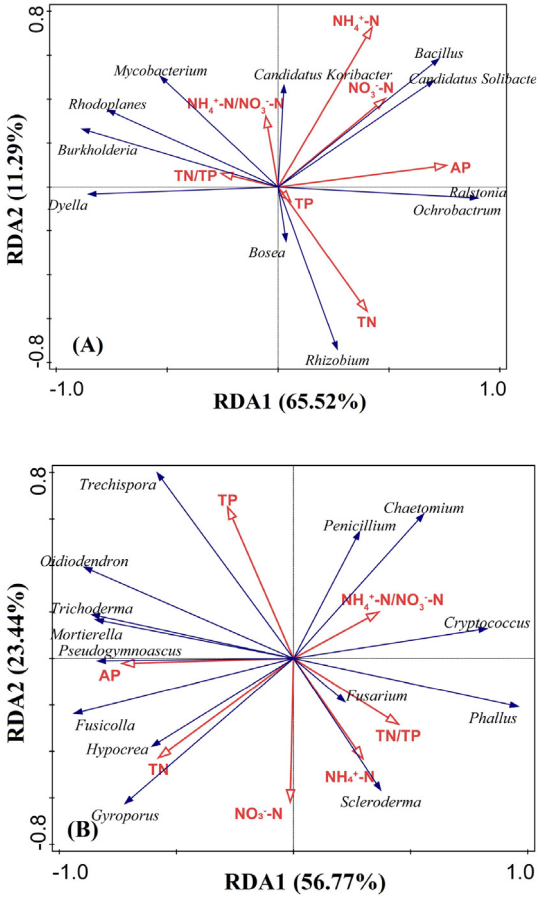


**Fig. 7.** The correlation between soil bacterial (A) and fungal (B) communities with soil nutrients. Abbreviations: total nitrogen (N), available nitrogen ($NO_3^-$-N and $NH_4^+$-N), total phosphorus (P), available phosphorus (AP), the ratio of $NH_4^+$-N to $NO_3^-$-N ($NH_4^+$-N/$NO_3$-N), and the ratio of N to P (N:P).

from a much larger spatial scale (Otten et al., 2001; Zhou et al., 2017). The co-occurrence network also shows that the composition of fungal community is more stable compared with the bacterial community (Fig. 4).

In this study, twenty-five dominant microbial groups inhabiting poplar fine roots have a relative abundance over 1%, including 11 genera of bacteria and 14 genera of fungi. Among these, four genera of each bacteria and fungi show a significantly different abundance among the three growth stages, demonstrating a close relationship with root function and activity. For example, bacteria *Bacillus* and *Ralstonia* are abundant in the WR soil compartment and *Burkholderi* in the YR soil compartment with the former two reported to be closely-associated with phosphate solubilization (Alori et al., 2017) and the latter promoted biodegradation, biological control and plant growth (Peix et al., 2001; Bevivino et al., 2000). Highly abundant *Dyella* in the BR soil compartment facilitates litter decomposition (Gui et al., 2017). Some fungi (i.e., *Scleroderma*) increase with root growth, and can inhibit the proliferation of phytopathogenic fungi (Soytong et al., 2014), indicating that the functional recruitment of the fungal community in the BR soil compartment is mainly for biodegradation and disease prevention. Together, these indicate that microbes in the primary stage of fine root growth (in the WR soil compartment) were related to nutrients assimilation whereas those in the old stage (in the BR soil compartment) mainly exerted biological control and disease prevention.

### *4.3. The relationships between dominant microbial groups and soil nutrients*

Some studies have proposed that the difference in microbial community structure is driven by soil stoichiometry (Delgado-Baquerizo et al., 2017). Our study indicates that the bacterial community showed a different relationship with soil nutrients from the fungal community (Figs. 6, 7), supporting the importance of soil C, N, and P stoichiometry in shaping the bacterial and fungal communities. According to the growth-rate hypothesis (Sterner and Elser, 2002), young roots usually release more carbon into the rhizosphere soil to facilitate the rapid growth and colonization of microbes, which demands for phosphorus (Peñuelas and Sardans, 2009). Hence, the high level of available phosphorus in soil facilitates recruitment of some microbes for the young roots (WR), such as the dominant bacteria *Bacillus* and *Ralstonia* and fungi *Penicillium, Fusarium* and *Chaetomium*. At the mature stage of fine root growth, roots and microorganisms would compete for phosphorus, resulting in decrease of microbial diversity (Delgado-Baquerizo et al., 2017) and the senescence of fine roots. Our study supports the plant-microbe competition hypothesis (Dijkstra et al., 2013) that plants and soil microorganisms would compete for mineral nutrients when available nutrients are deficient (Cheng, 1999).

The aggregate destruction hypothesis suggests that roots could change soil structure and promote soil organic matter (SOM) decomposition (Lu et al., 2019). The hypoxic soil macro- and micro-aggregates reduce the activity of natural SOM (Six et al., 2002). In the study, $NH_4^+$-N decreases along root growth and is much higher in the WR soil compartment than the YR and BR soil compartments (Table S3), indicating its relation to the positive rhizosphere priming effects (RPE). First, the WR exudates many DOCs and stimulated the growth and activity of microorganisms (Table S2), further leading to the increase of co-metabolic decomposition of SOM. However, TN in the WR soil compartment is lower (Table S3), indicating that N might be limiting for fine root growth. Thus, the plant roots may release exudates into rhizosphere, promoting microbial growth and extracellular enzyme production for N mineralization from SOM and thereby increasing SOM decomposition (Yin et al., 2018). Meanwhile, the WR can promote the destruction of soil aggregates, and expose unstable SOM to the microorganisms, thus resulting in the positive rhizosphere priming effects (RPE) (Lu et al., 2019; He et al., 2020) and the increase in $NH_4^+$-N content (Six et al., 2000).

## 5. Conclusion

Our study demonstrated the different succession patterns of bacterial and fungal communities among the three stages of fine roots. Bacteria appeared more sensitive than fungi to root functional activities. However, both bacteria and fungi are dependent on the quantity and quality of rhizosphere soil carbon components. Meanwhile, the succession of the microbial community showed a close relationship with soil nutrients in the rhizosphere. The recruitment of bacterial community driving by rich DOC at the early stage of fine roots would promote the mineralization of SOM and improve available nutrients. Collectively, these findings strengthen the connection between the fine root growth and the soil microbial community, and provide deep insights into the relationships between the microbial community succession and the fine root lifespan. However, more details about the niche differentiation mechanism of bacterial and fungal communities along fine root growth need to be further studied.

## CRediT authorship contribution statement

**Qiliang Zhu:** Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Nian Wang:** Data curation, Writing - review & editing. **Baoli Duan:** Data curation. **Qingkui Wang:** Data curation. **Yanping Wang:** Conceptualization, Methodology, Funding acquisition, Writing - original draft, Writing - review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143839.

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