Leaf and root litter species identity influences bacterial community composition in short-term litter decomposition

Ying Lu, Kun Li, Ruiqiang Ni, Rongchu Han, Chuanrong Li, Caihong Zhang

Abstract

Background: Microorganisms play a crucial role in litter decomposition in terrestrial ecosystems. However, it remains unclear, which effects of leaf litter and root species on bacterial community composition and diversity after one year's decomposition.

Methods: The leaf and fine roots litters of Robinia pseudoacacia, Quercus acutissima, Pinus tabulaeformis and Pinus densiflora, which are the dominant afforestation species in Mount Tai, were analysed using the Nylon litterbag method and Illumina Miseq high-throughput sequencing for the amplification of bacterial 16S rRNA V4-V5. We measured the remaining litter mass and the bacterial community composition and assessed the effects of leaf and root litter species on the bacterial community after one-year decomposition periods.

Results: (1) The remaining masses of leaf and fine roots litters of the four plant species were significantly influenced by organ type and species. The remaining mass of fine root litter was smaller than that of leaf litter for broad-leaved species, and the opposite result was found for coniferous species. (2) The observed species Chao1 and phylogenetic diversity values were significantly lower for leaf litters than for fine root litter. The community richness index was positively correlated with the C

*Corresponding author: Caihong Zhang; E-mail: zhangcaihong78@163.com
Taishan Forest Ecosystem Research Station/Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River, NO. 61, Daizong Road, Taishan District, Taian 271018, China
Full list of author information is available at the end of the article.
content, C:N and lignin content and negatively correlated with N:P, N content and P content. The bacterial community structure differed significantly among leaf and root litter decomposition for the four species \( p<0.05 \). The bacterial community structure in leaf litter was most highly correlated with the initial N content and N:P. The bacterial community structure in fine roots was most highly correlated with the lignin content. (3) The bacterial phyla *Bacteroidetes*, *Acidobacteria* and *Gemmatimonadetes* were significantly affected by litter and species type, and the relative abundances of *Firmicutes* and *Chloroflexi* were only affected by litter type. The relative abundances of *Acidobacteria*, *Firmicutes* and *Chloroflexi* in fine root litter were higher than those in leaf litter, while the opposite result was found for *Bacteroidetes*. The bacterial genera *Burkholderia-Paraburkholderia*, *Sphingomonas* and *Mucilaginibacter* were affected by litter type \( p<0.05 \). The relative abundance of *Burkholderia-Paraburkholderia* in fine root litter was higher than that in leaf litter, while the opposite result was found for *Bradyrhizobium*, *Sphingomonas* and *Mucilaginibacter*. Pearson correlation analysis showed that the average relative abundance of the dominant phyla and genera was affected by the initial litter properties, especially for *Bacteroides*, *Acidobacteria*, *Burkholderia*, and *Sphingomonas*.

**Conclusions:** Litter type, interaction between litter type and species were important than species in shaping the bacterial diversity and community composition in decomposing litter. And this were affected by initial chemical properties of the litter.

**Keywords:** litter decomposition; bacterial community; fine root litter; leaf litter

**Background**
Litter decomposition is the main source of organic matter and nutrients in forest soils and plays an important role in maintaining soil fertility and promoting the normal biological cycle and nutrient balance of forest ecosystems (Manzoni et al. 2008; Pei et al. 2019). In the past decades, many studies on leaf litter decomposition in forest ecosystems have emerged (Chapman and Koch 2007; Mooshammer et al. 2012; Zhang et al. 2016). Previous studies on litter decomposition focused on the aboveground litter decomposition (Mooshammer et al. 2012; Huang et al. 2018; Lin et al. 2019).

Compared to aboveground litter decomposition, research on root litter decomposition has lagged due to the challenges of the location of roots in the soil. However, plant roots, especially fine roots, which account for approximately 33% of the total primary production, have a high turnover rate (Shen et al. 2017). Roots are increasingly regarded as one of the main carbon pools in belowground ecosystems because of their close contact with soil and long residence time during decomposition (Lehmann and Kleber 2015; Huangfu et al. 2019; Zwetsloot et al., 2020). Wang et al (2017) also found that aboveground and belowground litter contribute equally to soil CO₂ emissions. Therefore, the study of root litter decomposition is essential for understanding the formation of soil organic matter and nutrient cycling in forest ecosystems (Cao et al. 2020). Previous studies have involved the comparative study between root decomposition and aboveground litter decomposition (Urcelay et al. 2011; Sun et al. 2018). However, these studies focused on the comparative description of leaf and fine root decomposition and the differences in the initial litter content, and there has been a lack of research on the mechanisms underlying the differences in decomposition. This knowledge gap undoubtedly hinders the understanding of nutrient cycling in ecosystems and the connection between the aboveground and underground parts in ecosystems.
Previous studies have found that litter decomposition was greatly affected by climate on a large scale, but litter decomposition was mainly regulated by the initial chemical properties and microbial community of the litter under consistent environmental conditions (Manzoni et al. 2008 more references). And different types of litter could provide different microenvironments for bacterial community growth, which directly affected the soil bacterial community composition and consequently the litter decomposition rate (Liu et al. 2018; Leloup et al. 2018; Chen et al. 2020). It has indicated that the roots do not mirror the mycorrhizal type-specific decomposition dynamics reported for leaf litter decomposition (Sun et al. 2018). Therefore, we predict that there are differences in the microbial community composition between different leaf and root litter species. At present, little is known about how litter type and litter species shape the microbial community composition during litter decomposition. Therefore, studying the changes in the microbial community composition during litter decomposition based on leaf and root litter species are beneficial for understanding litter decomposition mechanisms.

Microorganisms play an important role during litter decomposition (Manzoni et al. 2008; Otsing et al. 2018; Xiao et al., 2019). At present, research on the microbial community during litter decomposition is mainly concerned with the microbial community structure in the soil (Li et al. 2019), a small number of studies have involved the microbial community in the litter, and those studies mostly focused on fungal communities (Otsing et al. 2018). Otsing et al. (2018) found that litter species richness and especially certain litter species modified fungal community composition both in decomposing leaf and root litter. However, as the largest and most diverse species of microorganisms, bacteria have a relatively high nitrogen content and low carbon content, which promotes the
transformation and decomposition of soil nutrients (Kennedy 1999; Morgan et al. 2005). Studies have shown that bacteria are more resilient than fungi during later periods of litter decomposition (Wardle et al. 2004; Chapman and Koch 2007). In view of the importance of the soil bacterial community structure and diversity in ecosystems, these topics have received increasing attention from researchers (Prescott and Grayston 2013; Guo et al. 2018). Therefore, we analysed the effects of leaf and root litter species on the bacterial diversity and community composition in decomposing litter for four dominant afforestation tree species in Mount Tai using high-throughput sequencing technology, which will provide more comprehensive and complete information on the microbial community structure at a fine resolution (Hong et al. 2015; Sauvadet et al. 2019). We also clarified the effects of microbial activities and initial chemical properties on leaf and root litter and their decomposition, which provides a theoretical basis for the microbe-driven mechanism of litter decomposition. We hypothesized that (1) leaf and root litter species strongly influence the bacterial diversity and community composition of the litter during decomposition, and that these effects influence the decomposition rate. (2) There was a significant correlation between bacterial diversity and community composition and the initial chemical properties of the litter.

Materials and methods

Study site

The study site is located at Mount Tai Forest Ecosystem Observation and Research Station, Shandong province, China (117°05'-117°09'E, 36°17'-36°20'N). The study area has a warm temperate continental monsoon climate. The mean annual temperature is 18.5°C, and the mean annual precipitation is approximately 758 mm, which is mainly concentrated in June-September. The soil
types are neutral to acidic brown soils with a 20-30 cm thin soil layer. The zonal vegetation type is
warm temperate deciduous broad-leaved forest. The current forest coverage rate is 81.57%. Typical
vegetation is evergreen coniferous forest and deciduous broad-leaved forest, dominated by *Pinus*
tabulaeformis, *Platycladus orientalis*, *Pinus densiflora*, *Robinia pseudoacacia*, and *Quercus*
acutissima.

Litter bags were placed in the forest-free area of the Mount Tai Forest Ecosystem Observation and
Research Station. The specific detail of the forest-free area is shown in Table 1. The climate data
including ground surface rainfall, temperature and relative humidity during decomposition are shown
in Fig. 1. Climate data (Monthly averages) were downloaded from the Mount Tai Forest Ecosystem
Observation and Research Station.

**Experimental design and litter bag collection**

In this study, we focused on leaf litter and fine root from four dominant tree species plantations in
Mount Tai, *R. pseudoacacia* (RP), *Q. acutissima* (QA), *P. densiflora* (PD) and *P. tabulaeformis* (PT).
Litter was collected in pure stands of RP, QA, PD and PT. At the beginning of October 2015, when
most of the litter fall occurred, fresh and intact leaf litter was directly collected from the forest floor,
air-dried for 10 d and stored for a week at room temperature (15-25°C). Fine root decomposition was
carried out using live roots with diameters less than 2 mm because it was difficult to separate fresh
roots from those already having decomposed for a period. In October 2015, fine roots (≤2 mm in
diameter) were excavated using shovels from the topsoil (0-20 cm depth) of pure stands where most
fine roots occur. Roots were transported to the laboratory, and the surface soil was removed by washing
in tap water and then in deionized water. To calculate the air-drying/oven-drying ratio of the
decomposition substrate, a small portion of the sample was oven-dried at 65°C to a constant weight. Then, we determined the carbon content (C), nitrogen (N), phosphorus (P), and lignin content in the initial litter.

A field experiment was conducted using the litter bag method. Air-dried litter samples (4 g for fine roots and 6 g for leaf litter) were enclosed in litter bags (15 × 15 cm) made of 1-mm nylon mesh. Subsamples of the initial litter were oven-dried (65°C for 48 h) to calculate the correction factor for converting the air-dried mass to the water-free dry mass. In July 2016, the litter bags were placed in six blocks using a randomized complete block design. Each block included all eight treatments, for a total of 48 samples. The size of the blocks was 10 m × 10 m with 5 m × 5 m isolation zones between blocks. Litter bags were placed in the forest-free area. Litter bags with leaf litter were pinned to the ground surface to prevent movement by wind using U-shaped nails. Litter bags with root litter were inserted into the soil by slicing down through the soil at a 45° angle to a depth of approximately 15 cm and then slipping the litter bags into the incision. In July 2017, we collected the litter bags after one year of decomposition. We took eight litter bags representing the eight treatments from each block and then removed the living plants and soil adhering to the bags with a small brush. Three replicate samples were immediately labelled and placed in liquid nitrogen and immediately transferred to the laboratory to determine the bacterial community structure and diversity. The other three replicate samples were oven-dried for 48 h to a constant weight at 65°C and then weighed.

**Litter chemical analysis**

After determining the dry weight, samples were ground to pass through a 1 mm mesh, and then the total C and N contents in the litter were determined by an elemental analyser (ECS4010, Costech,
Italy). The total P contents were analysed by a continuous flow analyser (PROXIMA, Alliance, France).

We used ultraviolet spectrophotometry colorimetry to determine the lignin contents (Iiyama and Wallis et al. 1988) and determined the ash content by igniting the oven-dried material for 6 hours at 600°C in a muffle furnace to the correct dry weight (Gupta and Singh 1981).

**DNA extraction and sequencing**

Total genomic DNA was extracted using a DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to the manufacturer’s instructions. Three extractions were performed for each sample. DNA concentration and purity were checked by 1% agarose gel electrophoresis, and then DNA was diluted to 1 ng/µL with sterile water. The DNA samples were sent to Novogene (Beijing, China) for analysis using HiSeq sequencing. The V4-V5 region of the 16S rRNA genes was amplified using primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3') with the forward primer modified to contain a unique 6 nt barcode at the 5' end. All PCRs were carried out in 30 µL reactions with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs), 0.2 µM of forward and reverse primers and approximately 10 ng of template DNA. The thermal cycling conditions were as follows: an initial denaturation at 98°C for 1 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 50°C for 30 s, and extension at 72°C for 30 s, followed by 72°C for 5 min. The same volume of 1X loading buffer (containing SYBR green) was mixed with the PCR products, and the mixture was submitted to electrophoresis in a 2% agarose gel. Samples with bright bands between 400 and 450 bp were chosen for downstream analyses. PCR products were mixed in equal density ratios. Then, the mixed PCR products were purified with the Gene JET Gel Extraction Kit (Thermo Scientific). For the generation of sequencing libraries, the NEB Next® Ultra™ DNA
Library Prep Kit for Illumina (NEB, USA) was used, and the index codes were added under the guidance of the manufacturer’s recommendations. A Qubit® 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyser 2100 system were used to assess the quality of the library. Library sequencing was implemented on an Illumina HiSeq platform, and 250 bp/300 bp paired-end reads were generated.

Data analysis

Sequences analyses were performed using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/) (Edgar et al. 2013). Sequences with ≥ 97% similarity were assigned to the same OTUs. A representative sequence for each OTU was screened for further annotation. For each representative sequence, the Silva Database (https://www.arb-silva.de/) (Quast et al. 2013) was used based on the Mothur algorithm to annotate the taxonomic information. Alpha-diversity was used to analyse the richness and diversity within microbial communities. The alpha-diversity indices used were based on the clustered OTUs and included the Chao1, Ace, coverage, PD and Shannon indices. All indices in our samples were calculated with QIIME (Version 1.7.0). Nonmetric multidimensional scaling (NMDS) was used to analyse the bacterial community structure by using Canoco5.0 software. Redundancy analysis (RDA) was used to investigate the relationships between the bacterial community structure and the initial litter chemistry. Two-way ANOVA was used to determine the effects of leaf and root litter species on the initial litter chemistry, remaining litter mass, bacterial alpha-diversity and the relative abundance of the dominant phyla and genera. Pearson correlation analysis was used to determine the correlations between bacterial alpha-diversity, the relative abundance of the dominant phylum and genus, the initial litter chemistry and the remaining mass. SPSS 17.0 software was used for statistical analysis. Graphs were made with Origin 2018 (Origin lab, USA).
Results

Initial litter chemistry and decomposition rate

The initial litter chemistry was controlled by leaf and root litter of different species (Table S1). In addition, there were significant differences in initial chemistry between leaf litter and root litter of the same species, especially for the N and P contents, C: N and N:P (Table 2). For the broad-leaved species (RP and QA), the P content in the leaf litter was lower than that in the fine roots, but opposite results were observed for coniferous species (PD and PT). Interestingly, there were significant differences between leaf and root litter for QA, and the litter quality of the fine roots was significantly higher than that of the leaves (i.e., fine roots had a higher N content and lower C: N). In addition, significant differences were found for the leaf or root of different species (Table 2). Among the four fine root litters, RP had the highest N content and N:P, while QA had the highest P content. The C content, C:N and lignin contents were highest in PD. For the four leaf litters, RP had the highest N:P, while QA had the highest C:N. The C, N and P contents were highest in PD, while the lignin content was highest in PT. Due to the higher N content and lower ratio of C:N, RP was determined to have a higher litter quality than the other four species (Table 2).

Litter type, species and their interaction showed a significant influence on the remaining mass percentage after one year of decomposition in Mount Tai (Table S1). There were significant differences in remaining mass between the leaf and root litter of PD and QA ($p < 0.05$). The remaining mass of fine roots was lower than that of leaf litter for broad-leaved species, and the opposite result was found for coniferous species (Fig. 2). Significant differences were found for the leaf or root of different species (Fig. 2). The fine roots of broad-leaved species had a higher decomposition rate and a lower remaining
mass percentage than those of coniferous species. The decomposition rates of the fine roots ranked as follows: RP>QA>PT>PD. For leaf litter, the decomposition of RP was fastest, while the decomposition of QA was slowest. The decomposition rates of the fine roots ranked as follows: RP>PT>PD>QA (Fig. 2). In addition, there was a marked positive correlation between the remaining mass and the initial C content and C:N (p<0.01), but the remaining mass and N content and N:P showed a significantly negative correlation (p<0.01, Table 5).

**Bacterial alpha-diversity**

The bacterial alpha-diversity indices of the litter were significantly different and were affected by litter type, species and their interactions, except for the Shannon-Wiener index (Table 3, S2). All sample coverage values were higher than 96%, suggesting that the sequence data well reflected the microbial community composition. After one year’s decomposition, the observed species Chao1 and phylogenetic diversity (PD) values for the fine roots were higher than those for the leaf litter (Table 3). In addition, these indices were significantly lower for the fine roots of broad-leaved species than for those of coniferous species (p<0.05); however, opposite results were found for the leaf litter (Table 3, p<0.05). For fine roots, the Shannon-Wiener index of broad-leaved species was smaller than that for coniferous species but was not significantly different. For leaf litter, the Shannon-Wiener index of QA was the lowest, and that of RP was the highest, with significant differences among the four treatments (Table 3).

After one year, significantly positive correlations were observed between the number of observed species and the C:N, lignin content and C content. However, remarkably negative correlations were found between the number of observed species and the N content and N:P (Table 4). The Chao1 index
showed a significantly positive correlation with the C:N and lignin content. The PD index was significantly positively correlated with the C content, C:N and lignin content and was significantly negatively correlated with the N:P, N content and P content (Table 4).

Relative abundance of dominant bacterial phyla and genera

We obtained total 1,299,912 valid sequences from all samples, with a minimum sequence of 181 and a maximum sequence of 73,723 (average = 54,163 sequences), which coordinated with 36 phyla, 100 classes, 129 orders, 261 families, 448 genera, and 251 species. At the phylum level, most of the obtained OTUs belonged to the phyla *Proteobacteria* (62.2%), *Actinobacteria* (14.1%), *Bacteroidetes* (9.0%), and *Acidobacteria* (5.7%), with a relative abundance of more than 5% on average. *Planctomycetes* was the fifth most abundant phylum, followed by *Gemmatimonadetes*, *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes* and *Chloroflexi* (Fig. 3A). At the genus level, the predominant genera in all the samples were *Burkholderia-Paraburkholderia* (6.0%), *Sphingomonas* (3.7%), *Bradyrhizobium* (3.2%) and *Rhizomicrobium* (3.0%) followed by *Rhizobium*, *Mucilaginibacter*, *Caulobacter*, *Chitinophaga*, *Massilia* and *Pseudoxanthomonas* (Fig. 3B).

The bacterial phyla *Bacteroidetes*, *Acidobacteria* and *Gemmatimonadetes* were significantly affected by leaf and root litter species (Table S3, p<0.05). The relative abundance of *Bacteroidetes* and *Acidobacteria* demonstrated a significant response to different leaf and root litter species (Table S3).

The relative abundance of *Bacteroidetes* in fine root litter was lower than that in leaf litter, while the opposite result was found for *Acidobacteria*, especially for QA (Fig. 4A, 4B). The relative abundance of *Gemmatimonadetes* in RP leaf litter was significantly higher than that in the other three leaf litters (p<0.05), but there was no obvious difference among the four fine root treatments (Fig. 4C).
relative abundance of *Firmicutes* and *Chloroflexi* were only correlated with the leaf and root litter (Table S3). The relative abundance of *Firmicutes* in fine root litter was higher than that in leaf litter, but the difference was not significant (Fig. 4D). There was significant difference between the PT leaf and root litter for the abundance of *Chloroflexi* (*p*<0.05, Fig. 4E).

The bacterial genera *Burkholderia-Paraburkholderia* and *Mucilaginibacter* were significantly affected by litter type and species (Table S4, *p*<0.05, Fig. 4F, 4J). The relative abundance of *Bradyrhizobium* and *Rhizomicrobium* had a positive correlation with species only, while *Sphingomonas* had a positive correlation with litter type only (Table S4). The relative abundance of *Burkholderia-Paraburkholderia* in fine root litter was higher than that in leaf litter, while the opposite result was found for *Bradyrhizobium*, *Sphingomonas* and *Mucilaginibacter* (Fig. 4F, 4G, 4H and 4J).

The relative abundance of *Rhizomicrobium* was significantly higher for QA and PT than that for RP and PD (Fig. 4I).

There was no significant correlation between the relative abundances of *Proteobacteria*, *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes* and *Actinobacteria* and the initial litter chemistry (*p*>0.05, Table 5). The relative abundance of *Bacteroidetes* had a significantly positive correlation with N content and N:P (*p*<0.05) and a significantly negative correlation with lignin content (*p*<0.01). A significantly positive correlation was observed between the relative abundance of *Acidobacteria* and the initial lignin content (*p*<0.01), but a significantly negative correlation was observed with the N content and N:P (*p*<0.05). A significantly positive correlation was observed between the relative abundance of *Planctomycetes* with C:N and remaining mass (*p*<0.05), but a significantly negative correlation was observed with N content and N:P (*p*<0.05). The relative abundance of
**Gemmatimonadetes** had a significantly negative correlation with P and C content \((p<0.05)\). The relative abundance of **Chloroflexi** had a significantly positive correlation with C: N \((p<0.05, \text{Table 5})\).

Correlation analysis indicated that the relative abundances of **Burkholderia-Paraburkholderia** and **Rhizomicrobium** had a significantly negative correlation with the initial N content and N:P \((p<0.05, \text{Table 6})\). There was no significant correlation between the relative abundance of **Bradyrhizobium**, **Caulobacter**, **Rhizobium**, **Pseudoxanthomonas**, **Mucilaginibacter** and the remaining mass and the initial litter chemistry \((p>0.05)\). The relative abundance of **Massilia** had a negative correlation with the lignin content \((p<0.05)\). The relative abundance of **Sphingomonas** had a positive correlation with the initial N content and N:P \((p<0.05)\) but a significantly negative correlation with the C:N and initial lignin content \((p<0.05, \text{Table 6})\). A significantly positive correlation was observed between the relative abundance of **Chitinophaga** and the N content \((p<0.05)\) but a significantly negative correlation was observed with the lignin content \((p<0.05, \text{Table 6})\).

**Bacterial community composition**

The NMDS analysis of the bacterial community structure showed that different treatments were clearly distributed in different quadrants, indicating a significant difference in the bacterial community structure (Fig. 5). The results from the ANOSIM nonparametric test also showed that the bacterial community structure in leaf litter was significantly different from that in fine roots \((R=0.5208; p=0.03)\). The redundancy analysis (RDA) of the bacterial community structure and the initial litter chemistry showed that the initial N:P had the greatest impact on the bacterial community structure, followed by the lignin content and N content (Fig. 6). The bacterial community structure in leaf litter was most highly correlated with the initial N content and N:P. The bacterial community structure in fine roots...
was most highly correlated with the lignin content (Fig. 6).

Discussion

Effect of litter type and species on the bacterial diversity and decomposition rate

We found that the bacterial diversity was affected by litter type and species, and the leaf litter bacterial diversity of coniferous species was lower than that of broad-leaved species (Table 3, S2), which agreed with previous findings (Joly et al. 2016). Interestingly, the results were opposite for fine root litter, for which the bacterial diversity of broad-leaved species was significantly lower than that of coniferous species (Table 3). Generally, the composition of microbial communities under broad-leaved forests was radically different from that under coniferous forests (Zhang et al. 2019). These differences could be ascribed mainly to variations in leaf litter chemistry and changes in mycorrhizal communities and colonization (Gunina et al. 2017). Our results showed that initial litter chemistries were different among litter type and species (Table S1). In addition, there were significant differences in the bacterial community structure between leaf and root litter (Fig. 5). The difference of micro-environment in leaf and root litter decomposition significantly affect the microbial community. The higher humidity of the soil environment was beneficial to microbial growth (Banerjee et al. 2016). These may be important reasons for the significant differences in the bacterial community structure and decomposition rates between the leaf and root litter (Fig. 2, 5).

Decomposer activity and the litter decomposition rate are highly dependent on litter quality (Zhang et al. 2016; Lin et al. 2019). There were obvious differences in the remaining mass between leaf and root litter from four dominant afforestation species in Mount Tai (Fig. 2). The remaining mass was significantly positively correlated with the initial C content and C: N and was extremely negatively
correlated with the N content and N:P (Table 5). These results were in agreement with those of previous studies (McLaren and Turkington 2010; Zhao et al. 2017). A large number of studies have found that there is a close correlation between the initial N content and N-related indicators, especially C: N and lignin: N, which are considered as evident indicators for predicting the litter decomposition rate (Mooshammer et al. 2012; Pichon et al. 2020). This may be the reason that the leaf litter of broad-leaved species had a slower decomposition rate than the fine roots, while the opposite result was found for coniferous species (Fig. 2, Table 2). Because of the differences in N and P availability among ecosystems, some researchers have suggested that the relative importance of N and P to litter decomposition may differ (Güsewell et al., 2009). Generally, litter decomposition was limited by N when the N:P <14 but was limited by P when the N:P >16, and N and P were limiting factors when 14<N:P<16 (Güsewell et al. 2009). Therefore, the N content was the main limiting factor for litter decomposition in this study because of the N:P<14 (Table 2).

**Effect of litter type and species on the relative abundances of dominant bacterial phyla**

*Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* were the dominant bacteria, especially *Proteobacteria*, which were the main functional bacteria after one year’s decomposition, accounting for 62.2% of the entire bacterial community (Fig. 3). These results were consistent with previous findings (Gui et al. 2017; Xu et al. 2020). Microbial taxa defined at high taxonomic ranks, such as the phylum, can display ecological coherence of microbial groups due to their responses to environmental changes are predictable (Philippot et al. 2010; Guo et al. 2018). *Proteobacteria* are eutrophic bacteria that are often associated with the addition of labile C (Fierer et al. 2007; Li et al. 2018). *Actinobacteria*, which are saprophytic bacteria, are regarded as less opportunistic and can
produce a wider range of degrading enzymes, some populations can degrade lignin and cellulose (Zhao et al. 2017). However, we found that there was no significant correlation between the relative abundances of Proteobacteria and Actinobacteria and the initial litter chemistry (Table 5). In addition, leaf and root litter species had no effect on the relative abundances of Proteobacteria and Actinobacteria (Table S3). A possible explanation of these results is the “functional breadth hypothesis”, i.e., the ability of soil biota to efficiently decompose all litter types at the same time (Keiser et al. 2014; Fanin et al. 2016). Here, we found no significant difference in the relative abundances of Proteobacteria and Actinobacteria among all litters (Table 5, Fig. 4), suggesting that the decomposer community had a broad functional ability to decompose various litter types (Lin et al. 2019). We found that the relative abundance of Bacteroidetes in fine roots was lower than that in leaf litter, especially for QA and PD (Fig. 4A), and had a significantly negative correlation with the initial lignin content (Table 5). These results were consistent with those of previous works (Lydell et al. 2004; Marie et al. 2016). Marie et al. (2016) found that leaf addition promoted Bacteroidetes and Proteobacteria, but root addition promoted Actinobacteria. Interestingly, the results were opposite for Acidobacteria, with the relative abundance being higher in fine root litter than in leaf litter, especially for QA (Fig. 4B). Moreover, there was a significantly positive correlation between the relative abundance of Acidobacteria and the initial lignin content (Table 5). One possible explanation for this finding is that the abundance of soil microbes is affected by their nutrient preferences and microbial functions (Mau et al. 2015; Banerjee et al. 2016). Acidobacteria can grow in complex polymers, including plant hemicellulose or cellulose and fungal chitin (Eichorst et al. 2011). Several studies indicated that organic amendment could increase the relative abundance of Acidobacteria because
some of the members are present in high abundances in soils with a high organic C content (Li et al. 2017; Guo et al. 2018), despite the general belief that Acidobacteria are oligotrophs. Gemmatimonadetes was frequently detected in environmental 16S rRNA gene libraries and has been identified as one of the top nine phyla found in soils (Janssen et al. 2006; Zhao et al. 2018). Zhao (2018) have demonstrated that soil bacterial taxa such as the phyla Chloroflexi and Gemmatimonadetes were strongly positively correlated with soil C but negatively correlated with Firmicutes. However, we found that the relative abundance of Gemmatimonadetes had a significantly negative correlation with the C content and that the relative abundance of Chloroflexi and Firmicutes had no significant correlation with the C content (Table 5). One potential reason for these results is that our study focused on the bacterial community in the litter of afforestation species, but the other study focused on the bacterial community in the soil after afforestation. Members of Firmicutes include anaerobic bacteria, which can degrade different carbon sources, and some are related to N and denitrification (Aislabie et al. 2013). Chloroflexi is a ubiquitous heterotrophic degrading flora that decomposes carbohydrates (Yamada et al. 2009). We found that the relative abundances of Firmicutes and Chloroflexi were affected by the leaf and root litter (Table 5). One possible explanation for this finding is that the initial litter chemistry and physical positions of the leaf and root were different (Table 2).

Effect of leaf and root litter species on the relative abundance of dominant bacterial genera

At the genus level, the relative abundances of Burkholderia-Paraburkholderia (6.0%), Sphingomonas (3.7%), Bradyrhizobium (3.2%) and Rhizomicrobium (3.0%) were higher than those of other genera and were affected by litter type and species (Fig. 3, S4). Burkholderia-Paraburkholderia and Rhizomicrobium, which were reported to participate in N cycling, are members of denitrifier and...
N$_2$ fixation taxa and require a high N availability (Cheng et al. 2017; Nie et al. 2018). However, there were significantly negative correlations between the relative abundances of *Burkholderia-Paraburkholderia* and *Rhizomicrobium* and the initial litter N content and N:P (Table 6). One possible explanation for this finding is that a high initial N content in the litter could increase N release and then decrease N availability after litter decomposition (Mooshammer et al. 2012). In this study, the relative abundance of *Sphingomonas*, which had a positive correlation with the initial N content and N:P but a negative correlation with the C: N and initial lignin content (Table 6), were affected by leaf and root (Table 6, S4). Members of the genus *Sphingomonas*, which have a widespread distribution in soil and association with plants, have the ability to degrade recalcitrant carbon sources because of the production of proteolytic enzymes or cellulolytic enzymes (Ko et al. 2017).

*Bradyrhizobium* belongs to the nitrogen-fixing bacteria, and the lower relative abundance of *Bradyrhizobium* would significantly reduce nitrogen fixation (Janssens et al. 2010). However, there was no significant correlation between the relative abundance of *Bradyrhizobium* and the remaining mass and initial litter properties (Table 6).

**Conclusions**

By comparing four afforestation trees in Mount Tai, this study revealed the effects of litter type and species on the bacterial diversity and community composition in decomposing litter. In support of our first hypothesis, the bacterial alpha-diversity indices for the litter were significantly different and were affected by litter type, species and their interactions. We found that the community richness in fine roots was higher than that in leaf litter. In addition, these community richness indices in fine roots of broad-leaved species were significantly lower than those in coniferous species; Nevertheless,
opposite results were found for leaf litter. There was a significant correlation between bacterial alpha-diversity, dominant phyla and genera and initial litter chemistries, in agreement with our second hypothesis. Overall, this study suggests that litter decomposition is affected by litter type and species, and the bacterial community plays an important role.

Acknowledgements

We would like to thank the members of Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River for their help with laboratory work; and the Taishan Forest Ecosystem Research Station for all the logistical support during the field work.

Authors’ contributions

Conceived and designed the study: Caihong Zhang. Collected data and samples in the field: Ying Lu, Kun Li, Rongchu Han. Processed samples in the lab: Ying Lu and Kun Li. Analyzed the data: Ying Lu, Kun Li and Ruiqiang Ni. Wrote the paper: Ying Lu, Kun Li, Chuanrong Li and Caihong Zhang. All authors read and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (31500362, 31570705), the Science and Technology Innovation Team of Shandong Province (2019LY005), and the funds of the Shandong “Double Top” Program (SYL2017XTTD03). We are grateful to Professor Bin Zhang at the Nanjing University of Information Science & Technology for his assistance with improving this manuscript, and anonymous reviewers for helpful comments on this manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding
author on reasonable request.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**Author details**

Taishan Forest Ecosystem Research Station / Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River, NO. 61, Daizong Road, Taishan District, Taian 271018, China

**References**

Aislabie J, Deslippe JR, Dymond JR (2013) Soil microbes and their contribution to soil services. Soil Microbial Diversity 112:143-161

Austin AT, Ballaré CL (2010) Dual role of lignin in plant litter decomposition in terrestrial ecosystems. Proceedings of the National Academy of Sciences of the United States of America 107:4618-4622

Banerjee S, Baah-Acheamfour M, Carlyle CN, Bissett A, Richardson AE, Siddique T, Bork EW, Chang SX (2016) Determinants of bacterial communities in Canadian agroforestry systems. Environmental Microbiology 18:1805-1816

Cao JB, He XX, Chen YQ, Chen YP, Zhang YJ, Yu SQ, Zhou LX, Liu ZF, Zhang CL, Fu SL (2020) Leaf litter contributes more to soil organic carbon than fine roots in two 10-year-old subtropical plantations. Science of The Total Environment 704: 135341
Chapman SK, Koch GW (2007) What type of diversity yields synergy during mixed litter decomposition in a natural forest ecosystem? Plant and Soil 299:153-162

Chen Y, Ma S, Jiang H, Hu Y, Lu X (2020) Influences of litter diversity and soil moisture on soil microbial communities in decomposing mixed litter of alpine steppe species. Geoderma 377, 114577

Cheng J, Chen Y, He T, Liao R, Liu R, Yi M, Huang L, Yang Z, Fu T, Li X (2017) Soil nitrogen leaching decreases as biogas slurry DOC/N ratio increases. Applied Soil Ecology 111:105-113

Edgar, Robert C (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods 10:996-998

Fanin N, Fromin N, Bertrand I (2016) Functional breadth and home-field advantage generate functional differences among soil microbial decomposers. Ecology 97:1023–1037

Fierer N, Bradford MA, Jackson RB (2007) Toward an ecological classification of soil bacteria. Ecology 88:1354-1364

Gui H, Purahong W, Hyde KD, Xu JC, Mortimer PE (2017) The arbuscular mycorrhizal fungus *Funneliformis mosseae* alters bacterial communities in subtropical forest soils during litter decomposition. Frontiers in Microbiology 8:1-11

Gunina A, Smith AR, Godbold DL et al. (2017) Response of soil microbial community to afforestation with pure and mixed species. Plant and Soil 412:357-368

Guo J, Liu W, Zhu C et al. (2018) Bacterial rather than fungal community composition is associated with microbial activities and nutrient-use efficiencies in a paddy soil with short-term organic amendments. Plant and Soil 424:335-349
Gupta S R, Singh J S. (1981) The effect of plant species, weather variables and chemical composition of plant material on decomposition in a tropical grassland. Plant and Soil 59: 99-117

Güsewell S, Gessner MO (2009) N: P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology 23:211-219

Hong C, Si Y, Xing Y et al. (2015) Illumina MiSeq sequencing investigation on the contrasting soil bacterial community structures in different iron mining areas. Environmental Science Pollution Research 22:10788-10799

Huang, Y., Ma, K., Niklaus, P. A., & Schmid, B. (2018). Leaf-litter overyielding in a forest biodiversity experiment in subtropical China. Forest Ecosystems, 5(1), 1-9.

Huangfu CH, Hui DF, Qi XX et al. (2019) Plant interactions modulate root litter decomposition and negative plant-soil feedback with an invasive plant. Plant and Soil 437:179-194

Iiyama K, Wallis A F A. (1988) An improved acetyl bromide procedure for determining lignin in woods and wood pulps. Wood Science & Technology 22(3):271-280

Janssen PH (2006) Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology 72:1719-1728

Janssens IA, Dieleman W, Luyssaert S, Subke JA, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G, Papale D, Piao SI, Schulze ED, Tang J, Law BE (2010) Reduction of forest soil respiration in response to nitrogen deposition. Nature Geoscience 3:315-322

Keiser AD, Keiser DA, Strickland MS et al. (2014) Disentangling the mechanisms underlying functional differences among decomposer communities. Journal of Ecology 102:603-609

Kennedy AC (1999) Bacterial diversity in agroecosystems. Agriculture Ecosystems and Environment
Ko Y, Hwang W M, Kim M, et al. (2017) Sphingomonas silvisoli sp. nov. isolated from forest soil. International Journal of Systematic and Evolutionary Microbiology 67(8):2704-2710

Lehmann J, Kleber M (2015) The contentious nature of soil organic matter. Nature 528:60-68

Leloup, J., Baude, M., Nunan, N., Meriguet, J., Dajoz, I., Le Roux, X., & Raynaud, X. (2018). Unravelling the effects of plant species diversity and aboveground litter input on soil bacterial communities. Geoderma, 317, 1-7.

Li F, Chen L, Zhang J, Yin J, Huang S (2017) Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. Front Microbiol 8:187

Li H, Xu Z, Yan Q et al (2018) Soil microbial beta-diversity is linked with compositional variation in aboveground plant biomass in a semi-arid grassland. Plant and Soil 423:465-480

Li, Y., Bezemer, T. M., Yang, J., Lü, X., Li, X., Liang, W., Han, XG., Li, Q. (2019). Changes in litter quality induced by N deposition alter soil microbial communities. Soil Biology and Biochemistry, 130, 33-42.

Lin D, Pang M, Fanin N, Wang H, Qian S, Zhao L, Yang Y, Mi X, Ma K (2019) Fungi participate in driving home-field advantage of litter decomposition in a subtropical forest. Plant and Soil 434:467-480

Liu, J., Dang, P., Gao, Y., Zhu, H., Zhu, H., Zhao, F., & Zhao, Z. (2018). Effects of tree species and soil properties on the composition and diversity of the soil bacterial community following afforestation. Forest ecology and management, 427, 342-349.
Manzoni S, Jackson RB, Trofymow JA, Porporato A (2008) The global stoichiometry of litter nitrogen mineralization. Science 321:684-686

Marie S, Matthieu C, Daniel C, Pierre-Alain M, Cécile V, Isabelle B (2016) The dynamics of soil micro-food web structure and functions vary according to litter quality. Soil Biology and Biochemistry 95:262-274

Mau RL, Liu CM, Aziz M, Schwartz E, Dijkstra P, Marks JC, Price LB, Keim P, Hungate BA (2015) Linking soil bacterial biodiversity and soil carbon stability. The ISME Journal 9:1477–1480

McLaren JR, Turkington R (2010) Plant functional group identity differentially affects leaf and root decomposition. Global Change Biology 16:3075-3084

Mooshammer M, Wanek W, Schnecker J, Wild B, Leitner S, Hofhansl F, Blöchl A, Hämmerle I, Frank AH, Fuchsleger L, Keiblinger KM, Zechmeister-Boltenstern S, Richter A (2012) Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology 93:770-782

Morgan JAW, Bending GD, White PJ (2005) Biological costs and benefits to plant-microbe interactions in the rhizosphere. Journal of Experimental Botany 56:1729-1739

Nie YX, Wang MC, Zhang W et al. (2018) Ammonium nitrogen content is a dominant predictor of bacterial community composition in an acidic forest soil with exogenous nitrogen enrichment. Science of the Total Environment 624:407-415

Otsing E, Barantal S, Anslan S, Koricheva J, Tedersoo L (2018) Litter species richness and composition effects on fungal richness and community structure in decomposing foliar and root litter. Soil Biology and Biochemistry 125:328-339

Pei, G., Liu, J., Peng, B., Gao, D., Wang, C., Dai, W., ... & Bai, E. (2019). Nitrogen, lignin, C/N as
important regulators of gross nitrogen release and immobilization during litter decomposition in a temperate forest ecosystem. *Forest Ecology and Management, 440*, 61-69.

Philippot L, Andersson SG, Battin TJ, Prosser JI, Schimel JP, Whitman WB, Hallin S (2010) The ecological coherence of high bacterial taxonomic ranks. *Nat Rev Microbiol* 8:523–529

Pichon, N. A., Cappelli, S. L., Soliveres, S., Hölzel, N., Klaus, V. H., Kleinebecker, T., & Allan, E. (2020). Decomposition disentangled: a test of the multiple mechanisms by which nitrogen enrichment alters litter decomposition. *Functional Ecology* 1-12

Prescott CE, Grayston SJ (2013) Tree species influence on microbial communities in litter and soil: current knowledge and research needs. *Forest Ecology and Management* 309:19-27

Quast C, Pruesse E, Yilmaz P et al. (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research* 41:590-596

Sauvadet, M., Fanin, N., Chauvat, M., & Bertrand, I. (2019). Can the comparison of above-and below-ground litter decomposition improve our understanding of bacterial and fungal successions?. *Soil Biology and Biochemistry*, 132, 24-27.

Shen YF, Wang N, Cheng RM, Xiao WF, Yang S, Guo Y, Lei L, Zeng LX, Wang XR (2017) Characteristics of fine roots of *Pinus massoniana* in the three gorges reservoir area, China. *Forests* 8:1-13

Sun T, Hobbie SE, Berg B, Zhang HG, Wang QK, Wang ZW, Hättenschwiler S (2018) Contrasting dynamics and trait controls in first-order root compared with leaf litter decomposition. *Proceedings of the National Academy of Sciences* 115: 10392-10397

Urcelay C, Vaieretti MV, Perez M, Diaz S (2011) Effects of arbuscular mycorrhizal colonisation on
shoot and root decomposition of different plant species and species mixtures. Soil Biology and Biochemistry 43:466-468

Wang QK, Yu YZ, He TX, Wang YP (2017) Aboveground and belowground litter have equal contributions to soil CO2 emission: an evidence from a 4-year measurement in a subtropical forest. Plant and Soil 421:7-17

Wardle DA, Bardgett RD, Klironomos JN, Setälä H, VanderPutten WH, Wall DH (2004) Ecological linkages between aboveground and belowground biota. Science 304:1629-1633

Xiao W, Chen HY, Kumar P, Chen C, Guan Q (2019) Multiple interactions between tree composition and diversity and microbial diversity underly litter decomposition. Geoderma, 341: 161-171

Xu M, Lu X, Xu Y, Zhong Z, Zhang W, Ren C, Han XH, Yang GH, Feng Y (2020) Dynamics of bacterial community in litter and soil along a chronosequence of *Robinia pseudoacacia* plantations. Science of The Total Environment 703: 135613.

Yamada T, Sekiguchi Y (2009) Cultivation of uncultured chloroflexi subphyla significance and ecophysiology of formerly uncultured chloroflexi 'Subphylum I' with natural and biotechnological relevance. Microbes and Environments 24:205

Zeng J, Liu X, Song L, Lin X, Zhang H, Shen C, Chu H (2016) Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biology and Biochemistry 92: 41-49

Zhang W, Chao L, Yang Q, Wang Q, Fang Y, Wang S (2016) Litter quality mediated nitrogen effect on plant litter decomposition regardless of soil fauna presence. Ecology 97(10): 2834-2843

Zhang W, Yang K, Lyu Z, Zhu J (2019) Microbial groups and their functions control the decomposition
Zhao BY, Xing P, Wu QLL (2017) Microbes participated in macrophyte leaf litters decomposition in freshwater habitat. FEMS Microbiology Ecology 93:1-15

Zhao FZ, Ren CJ, Zhang L, Han XG, Yang GH, Wang J (2018) Changes in soil microbial community are linked to soil carbon fractions after afforestation: Soil microbial community affects carbon fractions. European Journal of Soil Science 69:370-379

Zwetsloot MJ, Ucros JM, Wickings K, Wilhelm RC, Sparks J, Buckley DH, Bauerle TL (2020) Prevalent root-derived phenolics drive shifts in microbial community composition and prime decomposition in forest soil. Soil Biology and Biochemistry 107797
### Table 1 Specific description of the forest-free areas

|                | Elev. (m) | Slope degree (°) | Slope aspect | Soil layer depth (cm) | pH  | C (%) | N (%) | Soil organic carbon (g/kg) |
|----------------|-----------|------------------|--------------|-----------------------|-----|-------|-------|---------------------------|
| Forest-free area | 730       | 23               | south        | 26.08                 | 5.00| 2.13  | 0.15  | 9.32                      |
Table 2 Initial fine root and leaf litter chemistry of *R. pseudoacacia*, *Q. acutissima*, *P. densiflora* and *P. tabulaeformis*

| Organ | Species | C %     | N %     | P %     | C: N   | N:P   | Lignin % |
|-------|---------|---------|---------|---------|--------|--------|----------|
|       | RP      | 45.57±0.13** | 1.86±0.05** | 0.41±0.01** | 24.58±0.57** | 4.55±0.27** | 28.84±0.36c |
| Leaf  | QA      | 48.23±0.84b  | 1.21±0.03b** | 0.43±0.01c** | 39.91±1.03** | 2.82±0.08c** | 33.63±0.57b  |
|       | PD      | 50.64±0.15e** | 2.02±0.01e** | 0.59±0.01e** | 25.10±0.16b** | 3.42±0.08b** | 22.83±0.54d** |
|       | PT      | 50.34±0.51a  | 1.90±0.08a** | 0.54±0.01b  | 26.64±0.90b** | 3.50±0.16b** | 37.21±0.61a  |
|       | RP      | 48.77±0.33c  | 3.36±0.002a  | 0.56±0.01b  | 14.51±0.09d  | 6.05±0.16a  | 29.59±0.47c  |
| Root  | QA      | 46.39±0.17d  | 1.08±0.01b   | 0.63±0.01a  | 43.02±0.17c  | 1.73±0.04b  | 33.78±0.60b  |
|       | PD      | 54.65±0.17a  | 0.38±0.01d   | 0.50±0.01c  | 142.48±3.72a | 0.76±0.02c  | 38.34±0.30a  |
|       | PT      | 49.96±0.13b  | 0.85±0.003c  | 0.53±0.004bc | 59.04±0.19b  | 1.61±0.01b  | 37.78±0.15a  |

RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different lowercase letters represent significant differences among different species for the same organ (*p*<0.05). Asterisks indicate significant (* *p*<0.05, ** *p*<0.01) differences between leaf litter and fine root for the same species. All data are expressed as the mean ± SE.
**Table 3** Bacterial alpha-diversity indices in litter after fine root and leaf litter decomposition for one year in Mount Tai

| Organ       | Species | RP          | QA          | PD          | PT          |
|-------------|---------|-------------|-------------|-------------|-------------|
| Observed    | Leaf    | 2000±9.8a   | 1946±47.6a  | 1832±10.4b** | 1776±21.0b** |
|             | Root    | 2149±40.7b  | 2155±197.2b | 2759±14.4a  | 2568±22.2a  |
| Chao1       | Leaf    | 2729.5±53.4a** | 2672.9±11a* | 2221.5±86.4b** | 2275.8±88.7b** |
|             | Root    | 3227.5±161.3b | 2824.2±51.1c | 3544.7±29.1a | 3395.0±1.3ab |
| Phylogenetic diversity (PD) | Leaf    | 145.3±0.4** | 142.5±3.4a  | 134.3±2.6b** | 132.8±0.4b** |
|             | Root    | 159.2±2.4b  | 147.8±4.4c  | 198.6±3.0a  | 193.1±1.9a  |
| Shannon     | Leaf    | 9.13±0.06a  | 8.38±0.04d  | 8.97±0.03b  | 8.78±0.03c  |
|             | Root    | 8.43±0.35a  | 8.40±0.28a  | 8.80±0.20a  | 8.76±0.16a  |

RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different lowercase letters in the row represent significant differences among different species for the same organ (*p*< 0.05). Asterisks indicate significant (* *p*<0.05, ** *p*<0.01) differences between leaf litter and fine root for the same species. All data are expressed as the mean ± SE.
Table 4 Correlation analysis between bacterial alpha-diversity and the initial litter chemistry after decomposition for one year

|                        | C %     | N %     | P %     | C: N    | N:P     | Lignin % |
|------------------------|---------|---------|---------|---------|---------|-----------|
| Observed species       | 0.480*  | -0.535**| -0.022  | 0.784** | -0.558**| 0.517**   |
| Chao1                  | 0.305   | -0.294  | -0.077  | 0.618** | -0.303  | 0.499*    |
| Phylogenetic diversity (PD) | 0.526** | -0.500* | -0.056  | 0.769** | -0.521**| 0.561**   |
| Shannon                | 0.114   | -0.038  | -0.227  | 0.028   | 0.053   | -0.229    |

The numbers in the table represent the Pearson’s correlation coefficient (r). **, p<0.01; *, p< 0.05
Table 5 Correlation analysis among the top 10 dominant bacterial phyla, the remaining mass and the initial litter chemistry

| Dominant phylum     | C %     | N %     | P %     | Lignin % | C: N | N:P | Remaining mass % |
|---------------------|---------|---------|---------|----------|------|-----|-----------------|
| **Proteobacteria**  | -0.191  | 0.201   | 0.170   | -0.038   | -0.148 | 0.131 | -0.396          |
| **Actinobacteria**  | 0.095   | -0.229  | -0.195  | 0.115    | 0.159  | -0.147 | 0.278           |
| **Bacteroidetes**   | 0.056   | 0.420*  | -0.043  | -0.542** | -0.313 | 0.425* | 0.113           |
| **Acidobacteria**   | 0.175   | -0.469* | 0.275   | 0.558**  | 0.382  | -0.543** | 0.082          |
| **Planctomycetes**  | 0.278   | -0.477* | -0.201  | 0.356    | 0.408* | -0.440* | 0.482*         |
| **Gemmatimonadetes**| -0.460* | -0.039  | -0.692**| -0.110   | -0.154 | 0.209  | -0.024         |
| **Cyanobacteria**   | 0.251   | -0.028  | 0.051   | 0.314    | 0.051  | -0.049 | 0.061          |
| **Verrucomicrobia** | -0.083  | -0.291  | 0.034   | 0.046    | 0.003  | -0.255 | 0.205          |
| **Firmicutes**      | 0.172   | 0.350   | 0.215   | 0.025    | 0.133  | 0.252  | -0.296         |
| **Chloroflexi**     | 0.174   | -0.377  | -0.179  | 0.275    | 0.427* | -0.331 | 0.085          |
| Mass remaining      | 0.644** | -0.636**| -0.254  | 0.272    | 0.619**| -0.610**| 1              |

The numbers in the table represent the Pearson’s correlation coefficient (r). **, p<0.01; *, p< 0.05
Table 6  Correlation analysis among the top 10 dominant bacterial genera, the remaining mass, and the initial litter chemistry

| Dominant Genus                  | C %  | N %   | P %  | Lignin C: N | N:P  | Remaining mass % |
|--------------------------------|------|-------|------|-------------|------|------------------|
| **Burkholderia-Paraburkholderi** | -0.159 | -0.431* | 0.357 | 0.359 | 0.155 | -0.498* | -0.067 |
| *a                              |      |       |      |             |      |                  |
| **Bradyrhizobium**              | -0.264 | -0.191 | -0.301 | 0.094 | -0.123 | -0.110 | 0.159 |
| **Massilia**                    | 0.074 | 0.122 | 0.139 | -0.433* | -0.171 | 0.089 | 0.128 |
| **Sphingomonas**                | -0.088 | 0.458* | -0.116 | -0.440* | -0.474* | 0.487* | 0.002 |
| **Caulobacter**                 | 0.249 | -0.081 | -0.283 | -0.005 | 0.325 | -0.021 | 0.115 |
| **Rhizomicrobium**              | -0.237 | -0.434* | 0.076 | 0.360 | -0.009 | -0.434* | 0.016 |
| **Rhizobium**                   | 0.104 | 0.322 | 0.140 | -0.310 | -0.239 | 0.268 | -0.091 |
| **Pseudoxanthomonas**           | -0.023 | 0.385 | 0.090 | -0.083 | -0.108 | 0.339 | -0.265 |
| **Chitinophaga**                | 0.118 | 0.470* | 0.201 | -0.459* | -0.156 | 0.381 | -0.065 |
| **Mucilaginibacter**            | 0.171 | -0.100 | 0.051 | 0.035 | -0.114 | -0.109 | 0.353 |

The numbers in the table represent the Pearson’s correlation coefficient (r). **, p<0.01; *, p< 0.05
Figures

**Fig. 1** Monthly variation in rainfall, temperature and relative humidity during the decomposition

**Fig. 2** The remaining mass for leaf and root litters of four species after decomposition for one year in Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different capital letters indicate significant differences between leaf and root for same species. Different lowercase letters signify significant differences among different species for the same organ (*p*<0.05). All data are expressed as the mean ± SE.

**Fig. 3** Relative abundance of the top ten dominant bacterial phyla (A) and genera (B). RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. L: leaf litter, R: fine root

**Fig. 4** Differences in relative abundances of the top ten dominant bacterial phyla (A-E) and genera (F-J) in Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different capital letters represent significant differences among different species for the same organ (*p*<0.05). Asterisks indicate significant (* *p*<0.05, ** *p*<0.01) differences between leaf litter and fine root for the same species. All data are expressed as the mean ± SE.

**Fig. 5** Nonmetric Multidimensional Scaling (NMDS) ordination diagram of the bacterial community structure in the litter after one year of decomposition in Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. The triangles represent the leaf litter, and the circles represent the fine roots.

**Fig. 6** Redundancy analysis (RDA) based on the bacterial community structure and initial litter chemistry. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. The triangles represent the leaf litter, and the circles represent the fine roots.
Fig. 1

[Bar chart showing rainfall, temperature, and relative humidity over time from July 16 to June 17.]
Fig. 2

Remaining mass (%)
Fig. 3

(a) Relative abundance

(b) Relative abundance

Others
Chloroflexi
Firmicutes
Verrucomicrobia
Cyanobacteria
Gemmataceae
Planctomycetes
Acidobacteria
Bacteroidetes
Actinobacteria
Proteobacteria

Others
Mucilaginibacter
Chitinophaga
Pseudoalteromonas
Rhizobium
Rhizomonas
Caulobacter
Sphingomonas
Marsilis
Bradyrhizobium
Burkholderia-Paraburkholderia
Fig. 4
Fig. 6
**Supplementary materials**

**Table S1** Effects of litter type, species and their interaction on initial litter chemistries tested by two-way ANOVA

| df. | C %   | N %   | P %   | C: N | N:P | Lignin | Remaining mass % |
|-----|-------|-------|-------|------|-----|--------|-----------------|
| Species | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Litter type | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.048 |
| Species × Litter type | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

The numbers in the table represent the p values
Table S2 Effects of litter type, species and their interaction on bacterial alpha-diversity indices tested by two-way ANOVA

|                      | df. | Observed species | Coverage % | Chao1 | Ace | Phylogenetic diversity (PD) | Shannon |
|----------------------|-----|------------------|------------|-------|-----|------------------------------|---------|
| Species              | 3   | 0.018            | 0.005      | 0.057 | 0.003 | <0.001                       | 0.074   |
| Litter type          | 1   | <0.001           | <0.001     | <0.001 | <0.001 | <0.001                       | 0.109   |
| Species × Litter type| 3   | <0.001           | <0.001     | <0.001 | <0.001 | <0.001                       | 0.220   |

The numbers in the table represent the p values

43
Table S3 Effects of litter type, species and their interaction on the top ten dominant bacterial phyla tested by two-way ANOVA

| Species          | Species | Litter type | Species ×Litter type |
|------------------|---------|-------------|---------------------|
|                  | F-value | p-value     | F-value             | p-value |
| Proteobacteria   | 1.073   | 0.388       | 0.860               | 0.368   | 0.858 | 0.483 |
| Actinobacteria   | 0.553   | 0.653       | 0.000               | 0.997   | 1.272 | 0.318 |
| Bacteroidetes    | 4.074*  | 0.025       | 16.407**            | 0.001   | 1.059 | 0.394 |
| Acidobacteria    | 3.353*  | 0.045       | 10.647**            | 0.005   | 0.212 | 0.887 |
| Planctomycetes   | 1.430   | 0.271       | 0.124               | 0.729   | 1.506 | 0.251 |
| Gemmatimonadetes | 3.327*  | 0.046       | 14.278**            | 0.002   | 6.698** | 0.004 |
| Cyanobacteria    | 1.067   | 0.391       | 0.107               | 0.748   | 0.691 | 0.571 |
| Verrucomicrobia  | 0.561   | 0.649       | 0.733               | 0.405   | 1.032 | 0.405 |
| Firmicutes       | 2.980   | 0.063       | 16.045**            | 0.001   | 2.036 | 0.149 |
| Chloroflexi      | 0.501   | 0.687       | 4.482*              | 0.050   | 2.165 | 0.132 |
Table S4 Effects of litter type, species and their interaction on the top 10 dominant bacterial genera tested by two-way ANOVA

| Species          | Species | Litter type | Species ×Litter type |
|------------------|---------|-------------|---------------------|
|                  | F-value | p-value     | F-value | p-value     | F-value | p-value |
| Burkholderia-Paraburkholderia | 7.585** | 0.002       | 8.564** | 0.010       | 1.491   | 0.255   |
| Bradyrhizobium   | 3.618*  | 0.036       | 3.848   | 0.067       | 0.891   | 0.467   |
| Massilia         | 0.747   | 0.540       | 2.687   | 0.121       | 0.691   | 0.571   |
| Sphingomonas     | 0.681   | 0.577       | 9.058** | 0.008       | 1.322   | 0.302   |
| Caulobacter      | 1.140   | 0.363       | 0.044   | 0.837       | 0.695   | 0.569   |
| Rhizomicrobium   | 7.034** | 0.003       | 0.246   | 0.627       | 1.143   | 0.362   |
| Rhizobium        | 1.059   | 0.394       | 2.087   | 0.168       | 1.020   | 0.410   |
| Pseudoxanthomonas| 0.902   | 0.462       | 1.442   | 0.247       | 0.829   | 0.497   |
| Chitinophaga     | 3.017   | 0.061       | 0.063   | 0.805       | 1.311   | 0.305   |
| Macilaginibacter | 3.322*  | 0.047       | 13.139**| 0.002       | 0.697   | 0.567   |