Leaf and Root Litter Species Identity Influences Bacterial Community Composition in Short-Term Litter Decomposition

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Research

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

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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

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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 CO_2 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.

63 Previous studies have found that litter decomposition was greatly affected by climate on a large
64 scale, but litter decomposition was mainly regulated by the initial chemical properties and microbial
65 community of the litter under consistent environmental conditions (Manzoni et al. 2008 more
66 references). And different types of litter could provide different microenvironments for bacterial
67 community growth, which directly affected the soil bacterial community composition and consequently
68 the litter decomposition rate (Liu et al. 2018; Leloup et al. 2018; Chen et al. 2020). It has indicated that
69 the roots do not mirror the mycorrhizal type-specific decomposition dynamics reported for leaf litter
70 decomposition (Sun et al. 2018). Therefore, we predict that there are differences in the microbial
71 community composition between different leaf and root litter species. At present, little is known about
72 how litter type and litter species shape the microbial community composition during litter
73 decomposition. Therefore, studying the changes in the microbial community composition during litter
74 decomposition based on leaf and root litter species are beneficial for understanding litter decomposition
75 mechanisms.

76 Microorganisms play an important role during litter decomposition (Manzoni et al. 2008; Otsing et
77 al. 2018; Xiao et al., 2019). At present, research on the microbial community during litter
78 decomposition is mainly concerned with the microbial community structure in the soil (Li et al. 2019),
79 a small number of studies have involved the microbial community in the litter, and those studies mostly
80 focused on fungal communities (Otsing et al. 2018). Otsing et al. (2018) found that litter species
81 richness and especially certain litter species modified fungal community composition both in
82 decomposing leaf and root litter. However, as the largest and most diverse species of microorganisms,
83 bacteria have a relatively high nitrogen content and low carbon content, which promotes the

84 transformation and decomposition of soil nutrients (Kennedy 1999; Morgan et al. 2005). Studies have
85 shown that bacteria are more resilient than fungi during later periods of litter decomposition (Wardle et
86 al. 2004; Chapman and Koch 2007). In view of the importance of the soil bacterial community
87 structure and diversity in ecosystems, these topics have received increasing attention from researchers
88 (Prescott and Grayston 2013; Guo et al. 2018). Therefore, we analysed the effects of leaf and root litter
89 species on the bacterial diversity and community composition in decomposing litter for four dominant
90 afforestation tree species in Mount Tai using high-throughput sequencing technology, which will
91 provide more comprehensive and complete information on the microbial community structure at a fine
92 resolution (Hong et al. 2015; Sauvadet et al. 2019). We also clarified the effects of microbial activities
93 and initial chemical properties on leaf and root litter and their decomposition, which provides a
94 theoretical basis for the microbe-driven mechanism of litter decomposition. We hypothesized that (1)
95 leaf and root litter species strongly influence the bacterial diversity and community composition of the
96 litter during decomposition, and that these effects influence the decomposition rate. (2) There was a
97 significant correlation between bacterial diversity and community composition and the initial chemical
98 properties of the litter.

99 **Materials and methods**

100 **Study site**

101 The study site is located at Mount Tai Forest Ecosystem Observation and Research Station,
102 Shandong province, China (117°05'-117°09'E, 36°17'-36°20'N). The study area has a warm temperate
103 continental monsoon climate. The mean annual temperature is 18.5°C, and the mean annual
104 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℃). 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

126 decomposition substrate, a small portion of the sample was oven-dried at 65℃ to a constant weight.
127 Then, we determined the carbon content (C), nitrogen (N), phosphorus (P), and lignin content in the
128 initial litter.

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

144 **Litter chemical analysis**

145 After determining the dry weight, samples were ground to pass through a 1 mm mesh, and then
146 the total C and N contents in the litter were determined by an elemental analyser (ECS4010, Costech,

147 Italy). The total P contents were analysed by a continuous flow analyser (PROXIMA, Alliance, France).
148 We used ultraviolet spectrophotometry colorimetry to determine the lignin contents (Iiyama and Wallis
149 et al. 1988) and determined the ash content by igniting the oven-dried material for 6 hours at 600℃ in a
150 muffle furnace to the correct dry weight (Gupta and Singh 1981).

151 **DNA extraction and sequencing**

152 Total genomic DNA was extracted using a DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA,
153 USA) according to the manufacturer's instructions. Three extractions were performed for each sample.
154 DNA concentration and purity were checked by 1% agarose gel electrophoresis, and then DNA was
155 diluted to 1 ng/μL with sterile water. The DNA samples were sent to Novogene (Beijing, China) for
156 analysis using HiSeq sequencing. The V4-V5 region of the 16S rRNA genes was amplified using
157 primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3')
158 with the forward primer modified to contain a unique 6 nt barcode at the 5' end. All PCRs were carried
159 out in 30 μL reactions with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs),
160 0.2 μM of forward and reverse primers and approximately 10 ng of template DNA. The thermal
161 cycling conditions were as follows: an initial denaturation at 98℃ for 1 min, followed by 30 cycles of
162 denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and extension at 72℃ for 30 s, followed by
163 72℃ for 5 min. The same volume of 1X loading buffer (containing SYBR green) was mixed with the
164 PCR products, and the mixture was submitted to electrophoresis in a 2% agarose gel. Samples with
165 bright bands between 400 and 450 bp were chosen for downstream analyses. PCR products were mixed
166 in equal density ratios. Then, the mixed PCR products were purified with the Gene JET Gel Extraction
167 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 35 181 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). The

252 relative abundance of *Firmicutes* and *Chloroflexi* were only correlated with the leaf and root litter
253 (Table S3). The relative abundance of *Firmicutes* in fine root litter was higher than that in leaf litter, but
254 the difference was not significant (Fig. 4D). There was significant difference between the PT leaf and
255 root litter for the abundance of *Chloroflexi* (p<0.05, Fig. 4E).

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

264 There was no significant correlation between the relative abundances of *Proteobacteria*,
265 *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes* and *Actinobacteria* and the initial litter chemistry (p>0.05,
266 Table 5). The relative abundance of *Bacteroidetes* had a significantly positive correlation with N
267 content and N:P (p<0.05) and a significantly negative correlation with lignin content (p<0.01). A
268 significantly positive correlation was observed between the relative abundance of *Acidobacteria* and
269 the initial lignin content (p<0.01), but a significantly negative correlation was observed with the N
270 content and N:P (p<0.05). A significantly positive correlation was observed between the relative
271 abundance of *Planctomycetes* with C:N and remaining mass (p<0.05), but a significantly negative
272 correlation was observed with N content and N:P (p<0.05). The relative abundance of

273 *Gemmatimonadetes* had a significantly negative correlation with P and C content ($p<0.05$). The relative
274 abundance of *Chloroflexi* had a significantly positive correlation with C: N ($p<0.05$, Table 5).
275 Correlation analysis indicated that the relative abundances of *Burkholderia-Paraburkholderia* and
276 *Rhizomicrobium* had a significantly negative correlation with the initial N content and N:P ($p<0.05$,
277 Table 6). There was no significant correlation between the relative abundance of *Bradyrhizobium*,
278 *Caulobacter*, *Rhizobium*, *Pseudoxanthomonas*, *Mucilaginibacter* and the remaining mass and the initial
279 litter chemistry ($p>0.05$). The relative abundance of *Massilia* had a negative correlation with the lignin
280 content ($p<0.05$). The relative abundance of *Sphingomonas* had a positive correlation with the initial N
281 content and N:P ($p<0.05$) but a significantly negative correlation with the C:N and initial lignin content
282 ($p<0.05$, Table 6). A significantly positive correlation was observed between the relative abundance of
283 *Chitinophaga* and the N content ($p<0.05$) but a significantly negative correlation was observed with the
284 lignin content ($p<0.05$, Table 6).
285 **Bacterial community composition**
286 The NMDS analysis of the bacterial community structure showed that different treatments were
287 clearly distributed in different quadrants, indicating a significant difference in the bacterial community
288 structure (Fig. 5). The results from the ANOSIM nonparametric test also showed that the bacterial
289 community structure in leaf litter was significantly different from that in fine roots ($R=0.5208$; $p=0.03$).
290 The redundancy analysis (RDA) of the bacterial community structure and the initial litter chemistry
291 showed that the initial N:P had the greatest impact on the bacterial community structure, followed by
292 the lignin content and N content (Fig. 6). The bacterial community structure in leaf litter was most
293 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

315 correlated with the N content and N:P (Table 5). These results were in agreement with those of previous
316 studies (McLaren and Turkington 2010; Zhao et al. 2017). A large number of studies have found that
317 there is a close correlation between the initial N content and N-related indicators, especially C: N and
318 lignin: N, which are considered as evident indicators for predicting the litter decomposition rate
319 (Mooshammer et al. 2012; Pichon et al. 2020). This may be the reason that the leaf litter of
320 broad-leaved species had a slower decomposition rate than the fine roots, while the opposite result was
321 found for coniferous species (Fig. 2, Table 2). Because of the differences in N and P availability among
322 ecosystems, some researchers have suggested that the relative importance of N and P to litter
323 decomposition may differ (Güsewell et al., 2009). Generally, litter decomposition was limited by N
324 when the N:P <14 but was limited by P when the N:P >16, and N and P were limiting factors when
325 14<N:P<16 (Güsewell et al. 2009). Therefore, the N content was the main limiting factor for litter
326 decomposition in this study because of the N:P<14 (Table 2).

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

328 *Proteobacteria* , *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* were the dominant bacteria,
329 especially *Proteobacteria* , which were the main functional bacteria after one year's decomposition,
330 accounting for 62.2% of the entire bacterial community (Fig. 3). These results were consistent with
331 previous findings (Gui et al. 2017; Xu et al. 2020). Microbial taxa defined at high taxonomic ranks,
332 such as the phylum, can display ecological coherence of microbial groups due to their responses to
333 environmental changes are predictable (Philippot et al. 2010; Guo et al. 2018). *Proteobacteria* are
334 eutrophic bacteria that are often associated with the addition of labile C (Fierer et al. 2007; Li et al.
335 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.

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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.

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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.

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578 **Tables**

579 **Table 1** Specific description of the forest-free areas

	Elevation (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

580

581

582 **Table 2** Initial fine root and leaf litter chemistry of *R. pseudoacacia*, *Q. acutissima*, *P. densiflora* and *P.*

583 *tabulaeformis*

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

584 RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different lowercase

585 letters represent significant differences among different species for the same organ (p<0.05). Asterisks

586 indicate significant (* p<0.05, ** p<0.01) differences between leaf litter and fine root for the same

587 species. All data are expressed as the mean ± SE.

588

589 **Table 3** Bacterial alpha-diversity indices in litter after fine root and leaf litter decomposition for one

590 year in Mount Tai

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

591 RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different lowercase

592 letters in the row represent significant differences among different species for the same organ ($p< 0.05$).

593 Asterisks indicate significant (* $p<0.05$, ** $p<0.01$) differences between leaf litter and fine root for the

594 same species. All data are expressed as the mean ± SE.

595

596

597 **Table 4** Correlation analysis between bacterial alpha-diversity and the initial litter chemistry after

598 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

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

600

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). **, $p<0.01$; *, $p< 0.05$

605 **Table 6** Correlation analysis among the top 10 dominant bacterial genera, the remaining mass, and the

606 initial litter chemistry

Dominant Genus	C %	N %	P %	Lignin %	C: N	N:P	Remaining mass %
Burkholderia-Paraburkholderia	-0.159	-0.431*	0.357	0.359	0.155	-0.498*	-0.067
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

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

608

Figures

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

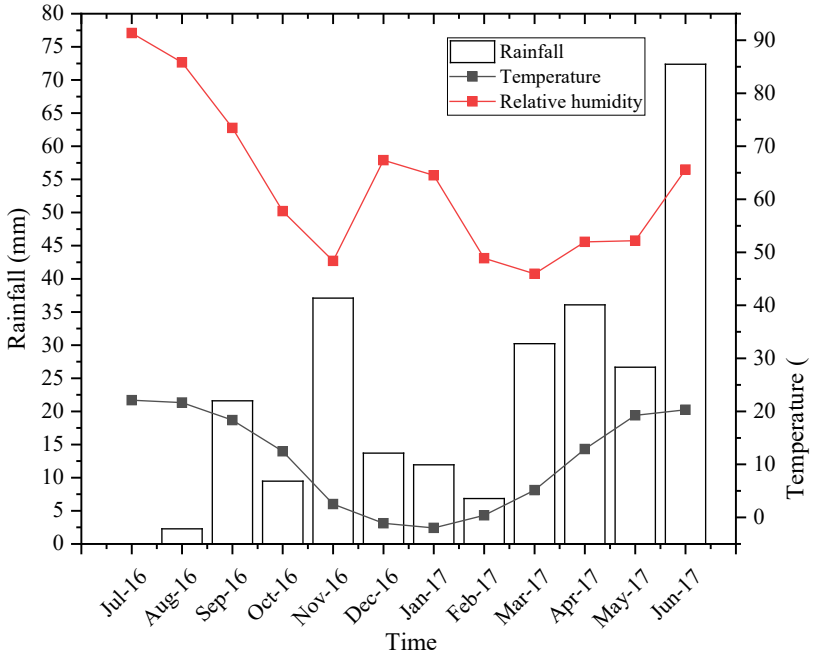
Fig. 2 The remaining mass for leaf and root litters of four species after decomposition for one year in Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora*, PT: *P. tabulaeformis*. Different capital letters indicate significant differences between leaf and root for same species. 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.



631

632 **Fig. 1**

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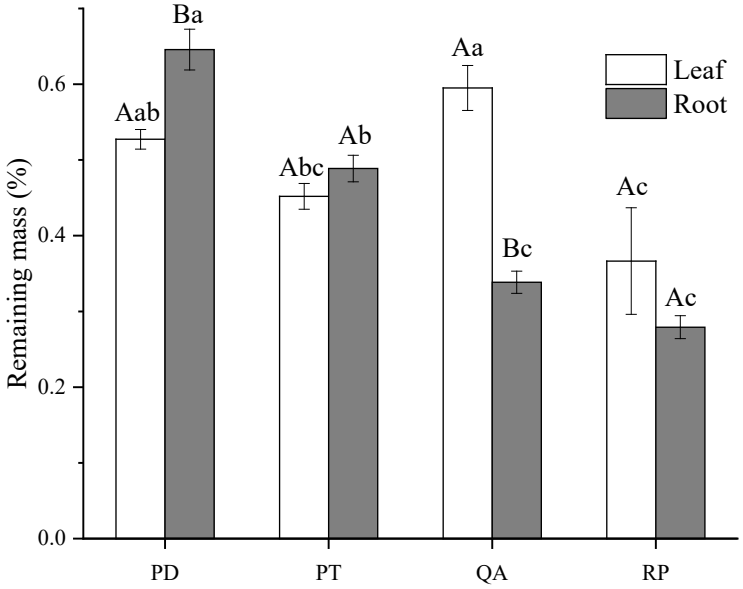

635

636 **Fig. 2**

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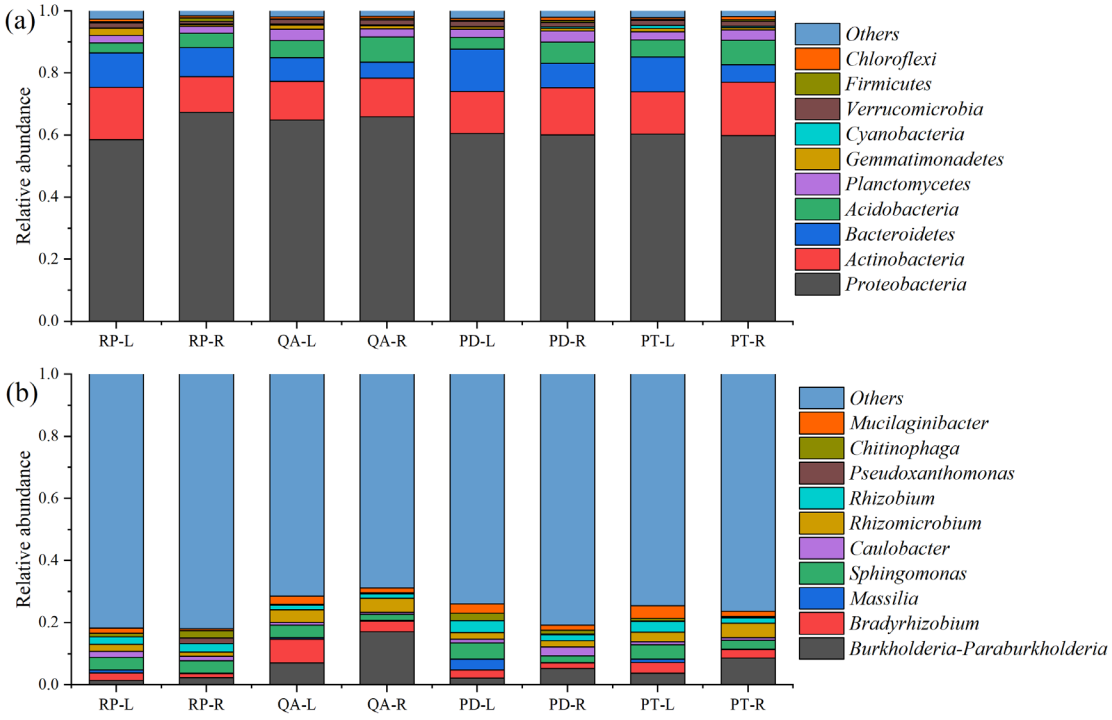
(a)
Relative abundance
1.0
0.8
0.6
0.4
0.2
0.0
RP-L
RP-R
QA-L
QA-R
PD-L
PD-R
PT-L
PT-R
Others
Chloroflexi
Firmicutes
Verrucomicrobia
Cyanobacteria
Gemmatimonadetes
Planctomycetes
Acidobacteria
Bacteroidetes
Actinobacteria
Proteobacteria
(b)
Relative abundance
1.0
0.8
0.6
0.4
0.2
0.0
RP-L
RP-R
QA-L
QA-R
PD-L
PD-R
PT-L
PT-R
Others
Mucilaginibacter
Chitinophaga
Pseudoxanthomonas
Rhizobium
Rhizomicrobium
Caulobacter
Sphingomonas
Massilia
Bradyrhizobium
Burkholderia-Paraburkholderia

Fig. 3

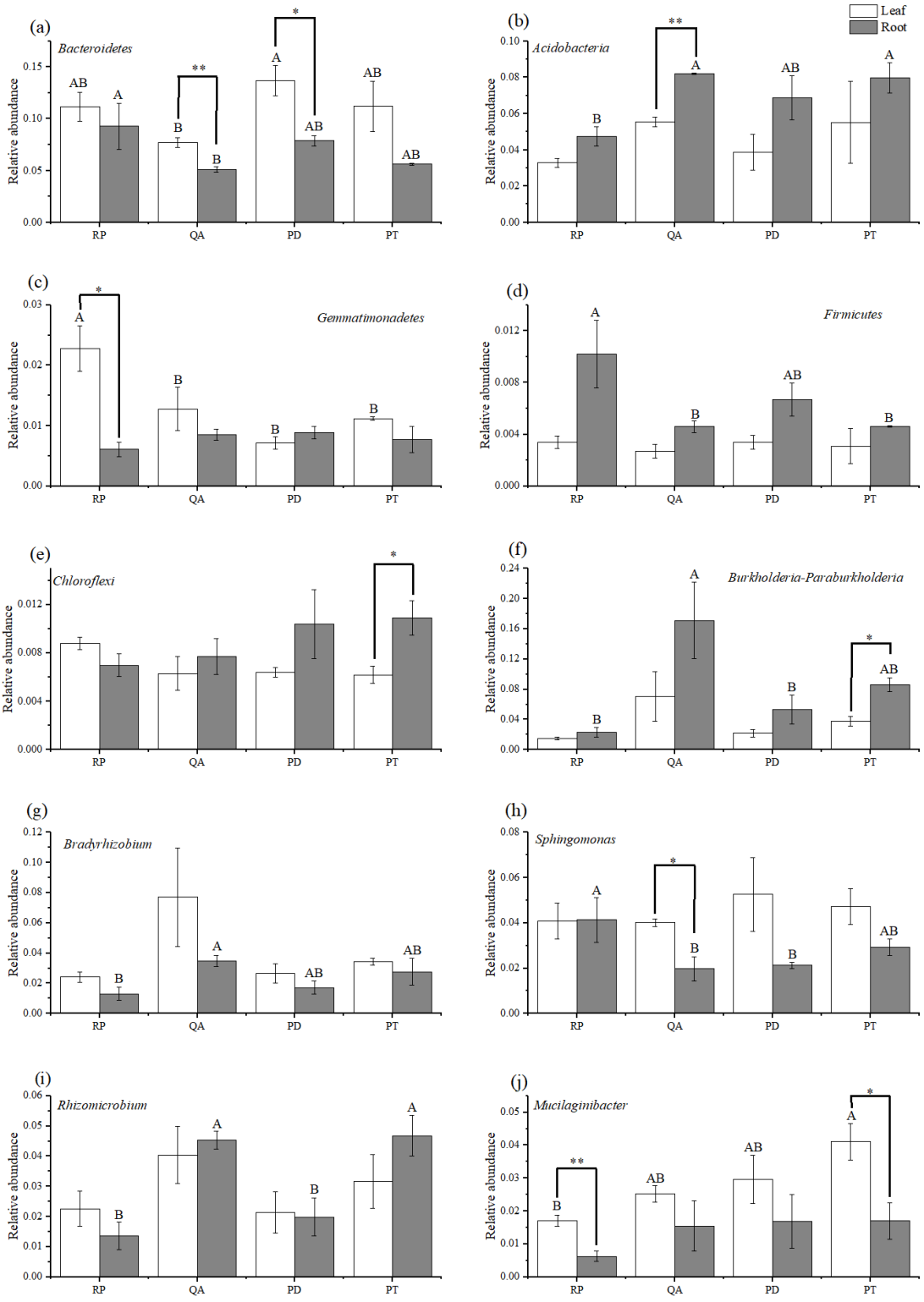
(a)
Bacteroidetes
(b)
Acidobacteria
Leaf
Root
(c)
Gemmatimonadetes
(d)
Firmicutes
(e)
Chloroflexi
(f)
Burkholderia-Paraburkholderia
(g)
Bradyrhizobium
(h)
Sphingomonas
(i)
Rhizomicrobium
(j)
Mucilaginibacter
Relative abundance
RP
QA
PD
PT

Fig. 4

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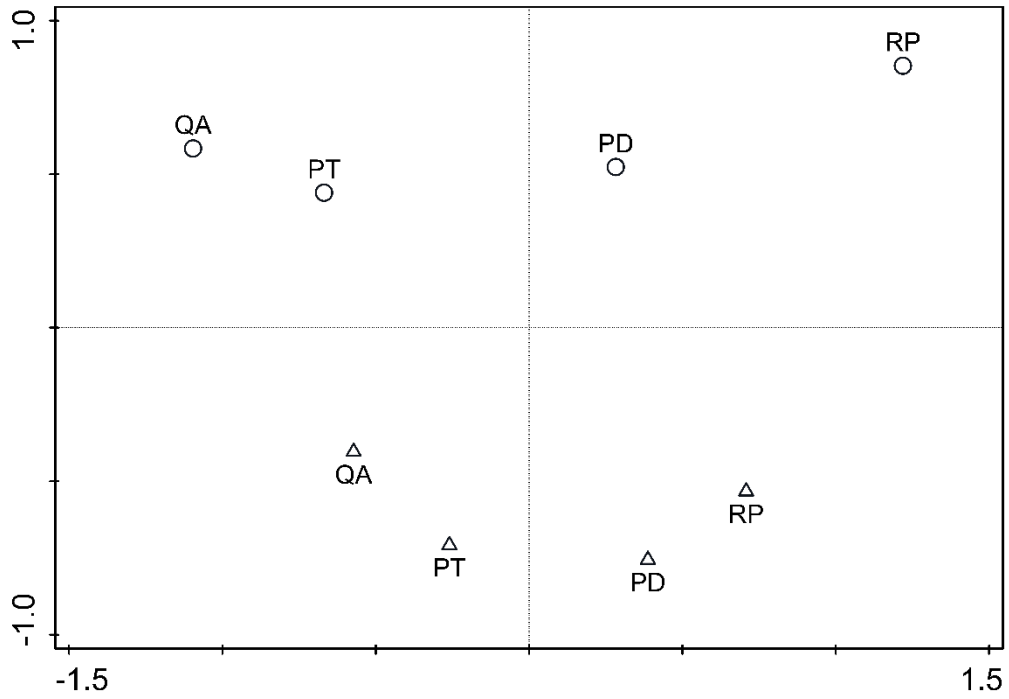

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648 **Fig. 5**

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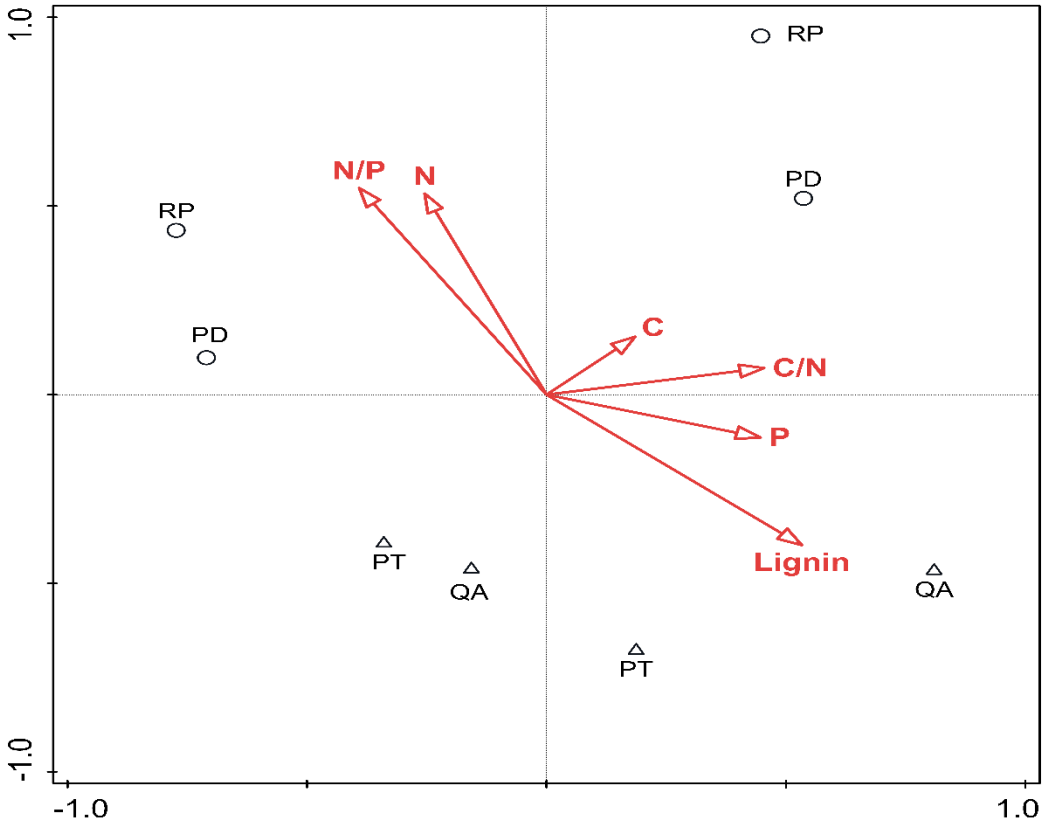

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652 **Fig. 6**

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654 **Supplementary materials**

655 **Table S1** Effects of litter type, species and their interaction on initial litter chemistries tested by

656 two-way ANOVA

	df.	C %	N %	P %	C: N	N:P	Lignin %	Remaining mass %
Species	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Litter type	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.048
Species ×Litter type	3	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

657 The numbers in the table represent the *p* values

658

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

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

	Species		Litter type		Species ×Litter type	
	F-value	*p*-value	*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

667 **Table S4** Effects of litter type, species and their interaction on the top 10 dominant bacterial genera

668 tested by two-way ANOVA

	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
Mucilaginibacter	3.322*	0.047	13.139**	0.002	0.697	0.567

669

Figures

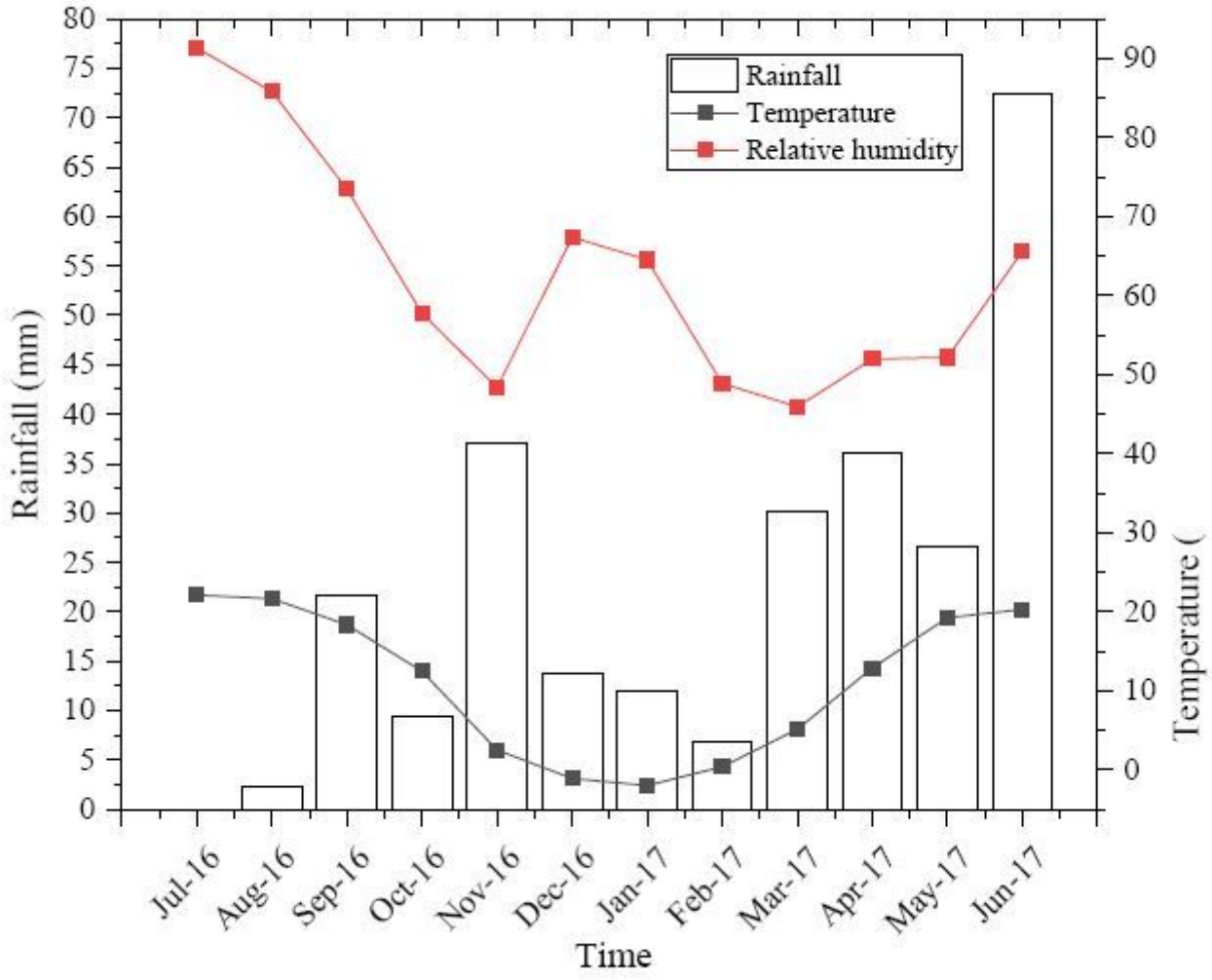

Figure 1

Monthly variation in rainfall, temperature and relative humidity during the decomposition

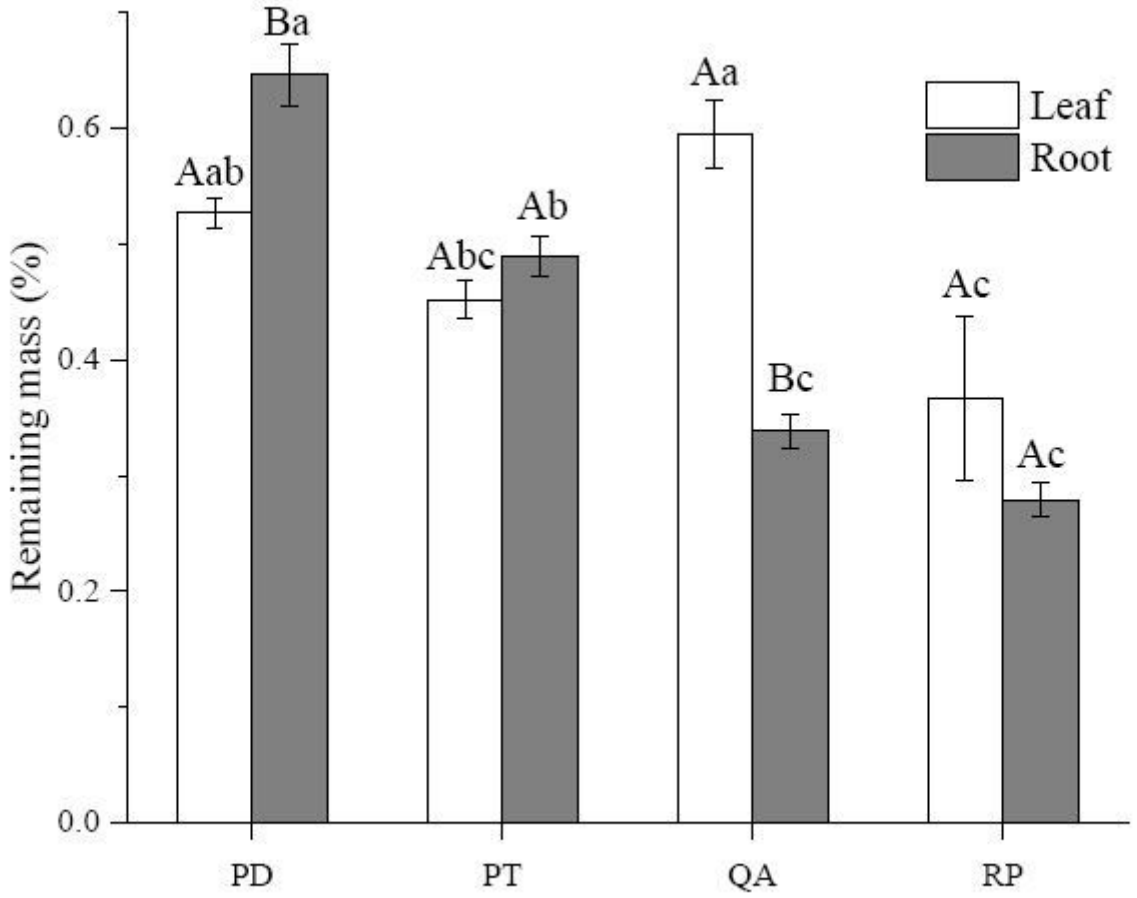

Figure 2

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

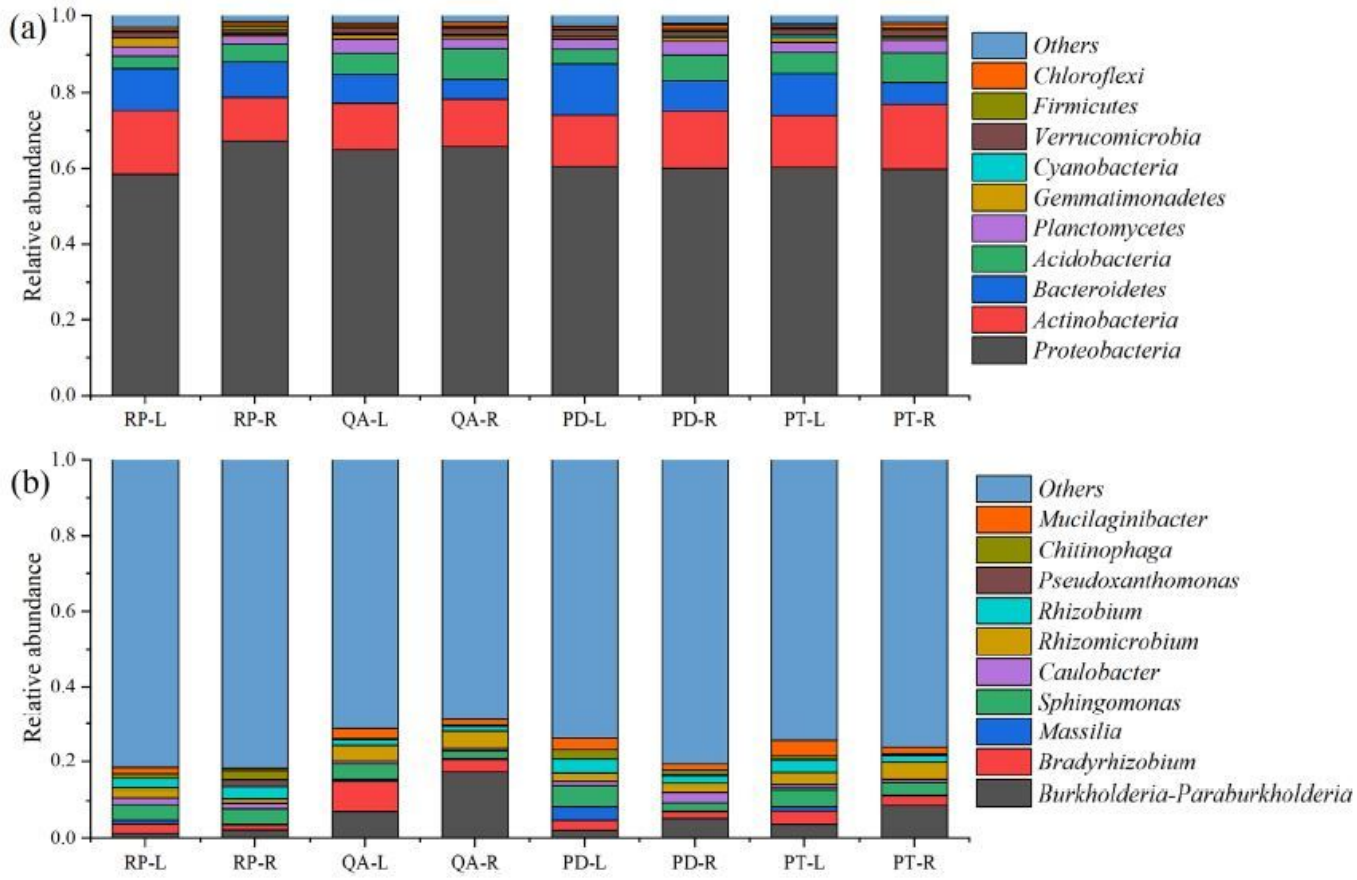

Figure 3

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

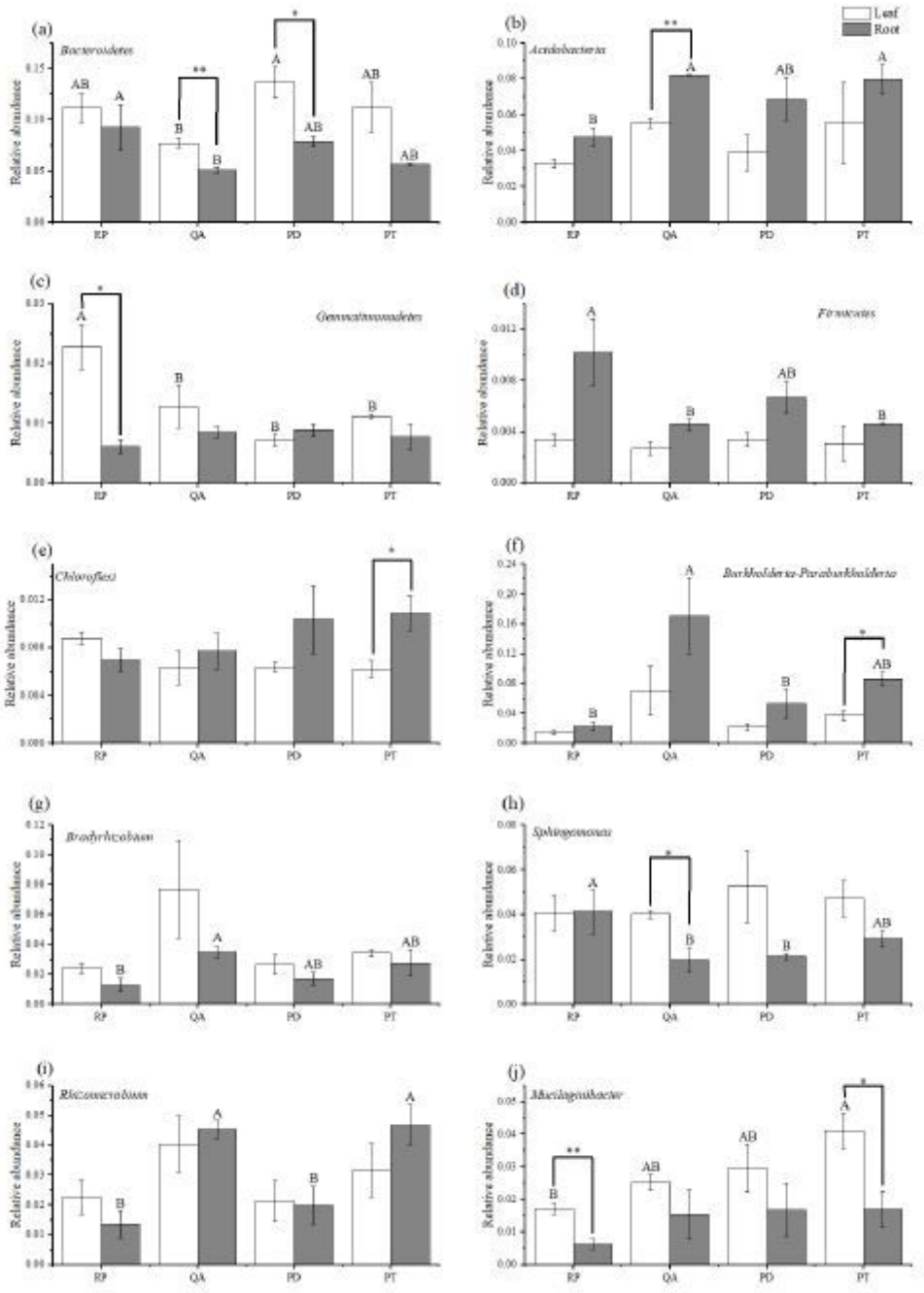

Figure 4

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.

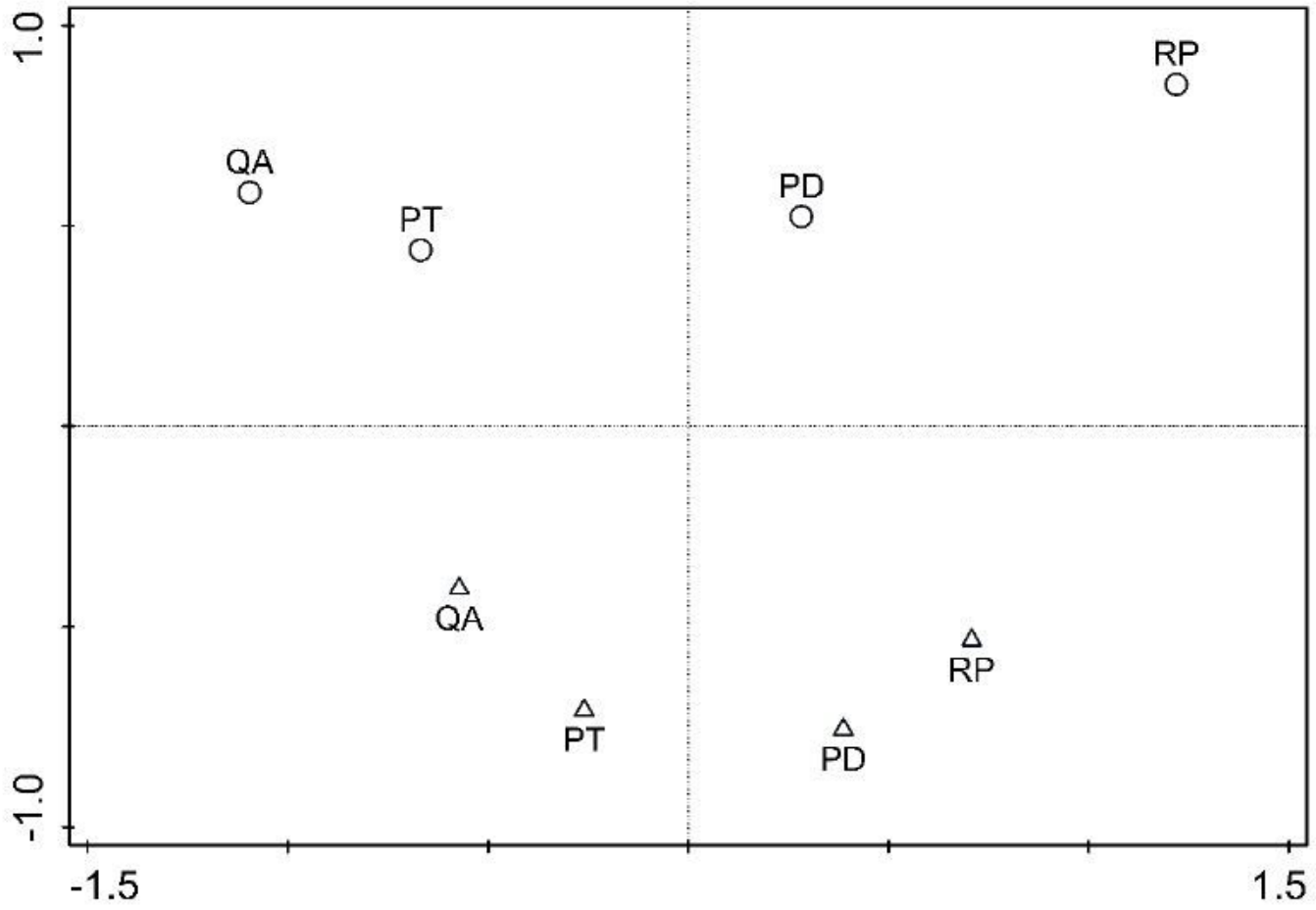

Figure 5

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.

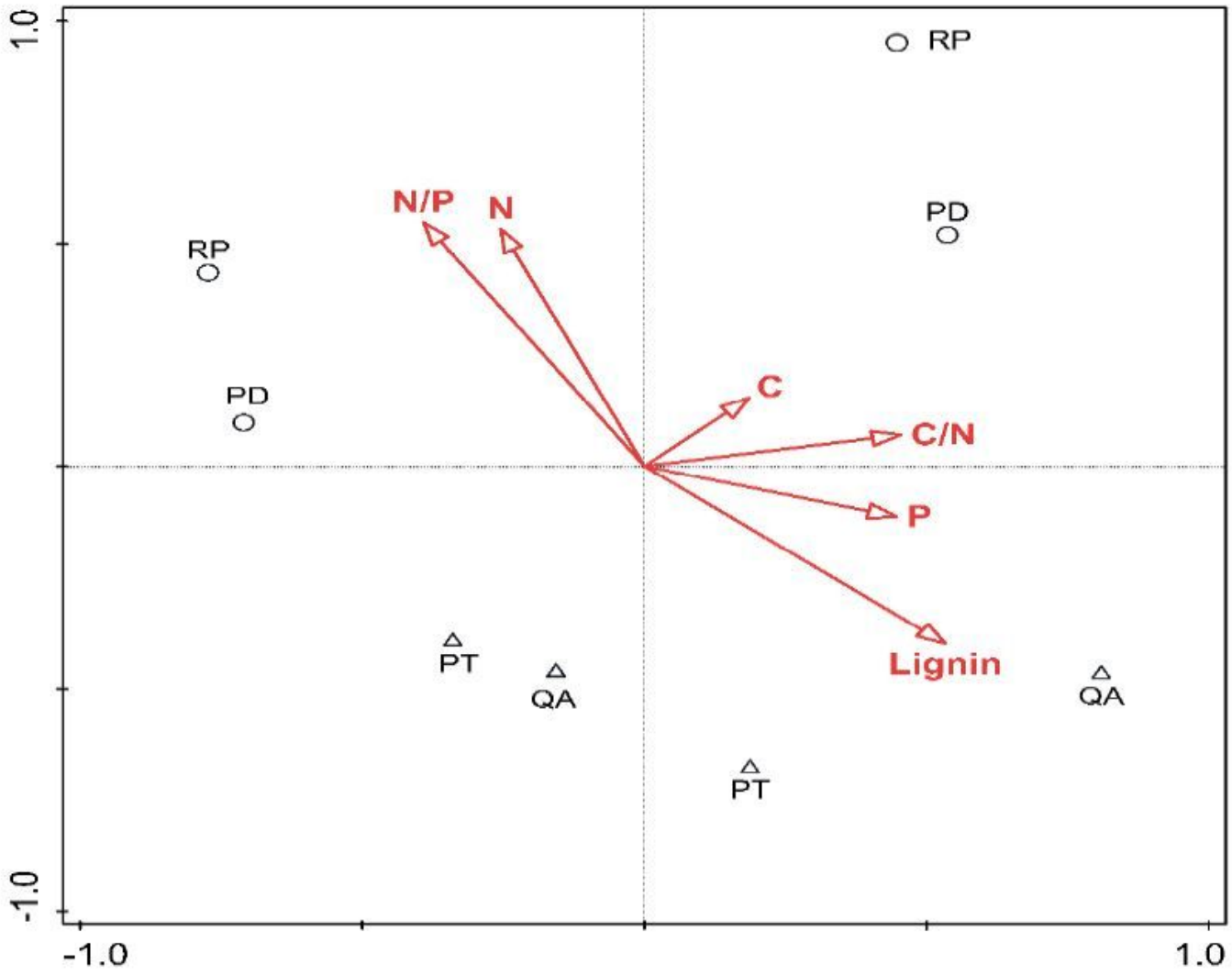

Figure 6

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.