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

Ying Lu [1,2,†], Liudong Zhang [3,†], Kun Li [1,2], Ruiqiang Ni [1,2], Rongchu Han [1,2], Chuanrong Li [1,2], Caihong Zhang [1,2,*], Weixing Shen [4] and Zhongjun Zhang [5]

1 Key Laboratory of State Forestry Administration for Silviculture of the Lower Yellow River, Taishan Mountain Forest Ecosystem Research Station, Shandong Agricultural University, Tai'an 271018, China
2 Forestry College of Shandong Agricultural University, Tai'an 271018, China
3 Shandong Forestry Protection and Development Service Centre, Jinan 250014, China
4 Mount Tai Scenic Spot Management Committee, Tai'an 271000, China
5 Niushan Forest Farm of Feicheng City, Feicheng 271600, China
* Correspondence: zhangcaihong78@163.com
† These authors contributed equally to this work.

Abstract: Microorganisms play a crucial role in litter decomposition in terrestrial ecosystems. The leaf and fine root litters of *Robinia pseudoacacia* Linn., *Quercus acutissima* Carr., *Pinus tabulaeformis* Carr. and *Pinus densiflora* Sieb. were analysed using the nylon litter bag method and Illumina MiSeq high-throughput sequencing for the amplification of bacterial 16S rRNA V4–V5. We assessed the effects of leaf and root litter species on the bacterial community after one year's decomposition. The results showed that (1) the remaining mass of fine root litter was smaller than that of the leaf litter for *R. pseudoacacia* and *Q. acutissima*, while the opposite result was found for *P. tabulaeformis* and *P. densiflora*. (2) The bacterial community structure in leaf litter was most highly correlated with the initial N content and N:P, while that 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, whereas the relative abundances of *Firmicutes* and *Chloroflexi* were only affected by litter tissues. 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 tissues ($p < 0.05$). 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's correlation analysis showed that the relative abundances of the dominant phyla and genera were affected by the initial litter properties, especially for *Bacteroides*, *Acidobacteria*, *Burkholderia* and *Sphingomonas*. These findings indicate that litter tissues and their interactions with species are more important than the species in shaping the bacterial diversity and community composition, which was affected by the initial chemical properties of the litter.

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

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1. Introduction

Litter decomposition is the main source of organic matter and nutrients in forest soils and plays an important role in maintaining the soil fertility and promoting a regular biological cycle and nutrient balance of forest ecosystems [1,2]. In the past decades, many studies on leaf litter decomposition in forest ecosystems have emerged [3–5]. Previous studies on litter decomposition focused on aboveground litter decomposition [4,6,7]. Compared to aboveground litter decomposition, 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 [8]. 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 [9–11]. Wang [12] 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 [13]. Previous studies have involved the comparative study between root and leaf litter decomposition [14,15]. However, these studies focused on the comparative description of leaf and fine root decomposition and the differences in the initial litter content, and there was a lack of research on the mechanisms underlying the differences of litter decomposition. This knowledge gap undoubtedly hinders the understanding of nutrient cycling and the connection between the aboveground and belowground parts in ecosystems.

Previous studies have found that litter decomposition is greatly affected by climate on a large scale, but litter decomposition is mainly regulated by the litter initial chemical properties and microbial community under consistent environmental conditions [2,16]. Different litter types can provide different microenvironments for bacterial community growth, which directly affect the soil bacterial community composition and, consequently, the litter decomposition rate [16–18]. It has indicated that root decomposition does not mirror the mycorrhizal type reported by leaf litter decomposition [14]. Therefore, we predict that there are differences in the microbial community composition between leaf and root litter decomposition. Until now, little has been known about how litter tissues and litter species shape the microbial community composition during litter decomposition. Therefore, studying the changes of the microbial community composition during leaf and root litter decomposition are beneficial for understanding the litter decomposition mechanisms.

Microorganisms play an important role during litter decomposition [2,19,20]. At present, research on the microbial community during litter decomposition is mainly concerned with the microbial community structure in the soil [21]. Some studies have involved the microbial community in the litter, and those studies mostly focused on fungal communities, and litter species richness and, especially, certain litter species have modified the fungal community composition both in decomposing leaf and root litter [19]. 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 [22,23]. Studies have shown that bacteria are more resilient than fungi during the later periods of litter decomposition [3,24]. In view of the importance of the soil bacterial community structure and diversity in ecosystems, these topics have received increasing attention [25,26]. 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 from Mount Tai using high-throughput sequencing technology, which will provide more comprehensive and complete information on the microbial community structure at a fine resolution [27,28]. 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 hypothesised that (1) litter tissues and litter species strongly influence the bacterial diversity and community composition in litter during decomposition, then influence the litter decomposition rate. (2) There were significant correlations between the bacterial diversity and community composition and the litter initial chemical properties.

2. Materials and Methods

2.1. Study Site

The study site was 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%. The typical vegetation is evergreen coniferous forest and deciduous broad-leaved forest, dominated by *Pinus tabulaeformis* Carr., *Platycladus orientalis* Linn., *Pinus densiflora* Sieb. et Zucc., *Robinia pseudoacacia* Linn. and *Quercus acutissima* Carr.

Litter bags were placed on bare land at the Mount Tai Forest Ecosystem Observation and Research Station. The specific detail of the study site is shown in Table 1. The climate data, including ground surface rainfall, temperature and relative humidity during decomposition, are shown in Figure 1. Climate data (monthly averages) were downloaded from the Mount Tai Forest Ecosystem Observation and Research Station.

Table 1. Specific description of the forest gap.

	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

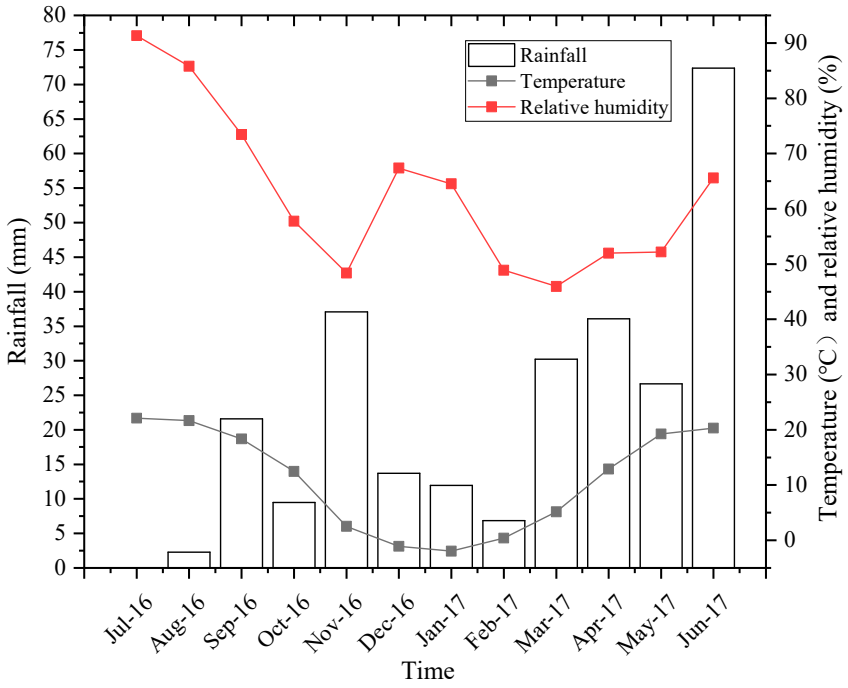

Figure 1. Monthly variation in rainfall, temperature and relative humidity during the decomposition.

2.2. Experimental Design and Litter Bag Collection

In this study, we focused on leaf litter and fine roots from four dominant tree species plantations at Mount Tai: *R. pseudoacacia* (RP), *Q. acutissima* (QA), *P. densiflora* (PD) and *P. tabulaeformis* (PT). The 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 [9]. 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 deionised 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 (C), nitrogen (N), phosphorus (P), and lignin contents 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 randomised complete block design. Each block included all eight treatments (including leaf litter of *R. pseudoacacia* (RP), *Q. acutissima* (QA), *P. densiflora* (PD) and *P. tabulaeformis* (PT) and fine roots of RP, QA, PD and PT), for a total of 48 samples. The size of the blocks was 10 m × 10 m, with 5 m × 5 m isolation zones between the 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.

2.3. 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 [29,30] and determined the ash content by igniting the oven-dried material for 6 h at 600 °C in a muffle furnace to the correct dry weight [31].

2.4. 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, the 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 1× 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 and mixed in equal density ratios. Then, the mixed PCR products were purified with the Gene JET Gel Extraction Kit (Thermo Scientific, Waltham, MA, USA). For the generation of sequencing libraries, the NEB Next® Ultra™ DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) was used, and the index codes were added under the guidance of the manufacturer's recommendations. A Qubit® 2.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) 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.

2.5. Data Analysis

Sequences analyses were performed using Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/, accessed on 1 January 2019) [32]. 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/, accessed on 1 January 2013) [33] 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 (Canoco5.0, Ithaca, NY, USA). A 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's 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 (SPSS 17.0, Chicago, IL, USA) was used for the statistical analysis. The graphs were made with Origin 2018 (Origin lab, Northampton, MA, USA).

3. Results

3.1. Initial Litter Chemistry and Decomposition Rate

The initial litter chemistry was controlled by species and litter tissues (Table S1). There were significant differences in the initial litter chemistry between leaf and root for the same species, especially N-related indicators (N contents, C:N and N:P) (Table 2). For *R. pseudoacacia*, the N content and N:P in the leaf litter were lower than that in the fine roots, and C:N in the leaf litter was higher than that in the fine roots, but the opposite results were observed for the other species (QA, PD and PT). In addition, significant differences were found for different species of the leaf or root (Table 2, $p < 0.05$). Among fine root litter, the N content and N:P for *R. pseudoacacia* were significantly higher than the other species, and the C:N and lignin contents were significantly lower, while the opposite was found for *P. densiflora*. For leaf litter, the C, N and P contents were highest in *P. densiflora*, with lower C:N and lignin contents. *R. pseudoacacia* had the highest N:P, with lower C:N and lignin contents (Table 2).

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%
Leaf	RP	$45.57 \pm 0.13^{c**}$	$1.86 \pm 0.05^{a**}$	$0.41 \pm 0.01^{c**}$	$24.58 \pm 0.57^{b**}$	$4.55 \pm 0.27^{a**}$	28.84 ± 0.36^{c}
	QA	48.23 ± 0.84^{b}	$1.21 \pm 0.03^{b**}$	$0.43 \pm 0.01^{c**}$	$39.91 \pm 1.03^{a*}$	$2.82 \pm 0.08^{c**}$	33.63 ± 0.57^{b}
	PD	$50.64 \pm 0.15^{a**}$	$2.02 \pm 0.01^{a**}$	$0.59 \pm 0.01^{a**}$	$25.10 \pm 0.16^{b**}$	$3.42 \pm 0.08^{b**}$	$22.83 \pm 0.54^{d**}$
	PT	50.34 ± 0.51^{a}	$1.90 \pm 0.08^{a**}$	0.54 ± 0.01^{b}	$26.64 \pm 0.90^{b**}$	$3.50 \pm 0.16^{b**}$	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}

RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. Different lowercase letters represent significant differences among different species for the same organ ($p < 0.05$). Asterisks indicate significant (* $p < 0.05$ and ** $p < 0.01$) differences between leaf litter and fine roots for the same species. All data are expressed as the mean ± SE.

Litter tissues, species and their interaction showed a significant influence on the remaining mass after one year (Table S1). For broad-leaved species (RP and QA), the remaining mass of fine roots was significantly lower than that of leaf litter (Figure 2). However, the opposite result was found for coniferous species (Figure 2). In addition, the remaining mass of leaf or root was significantly different among different species (Figure 2). For fine roots, broad-leaved species had higher decomposition rates and lower remaining masses than those of coniferous species, and the decomposition rates ranked as follows: RP > QA > PT > PD. For leaf litter, the decomposition of *R. pseudoacacia* was the fastest, while the decomposition of *Q. acutissima* was the slowest. The decomposition rates of leaf litter ranked as follows: RP > PT > PD > QA (Figure 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 ($p < 0.01$).

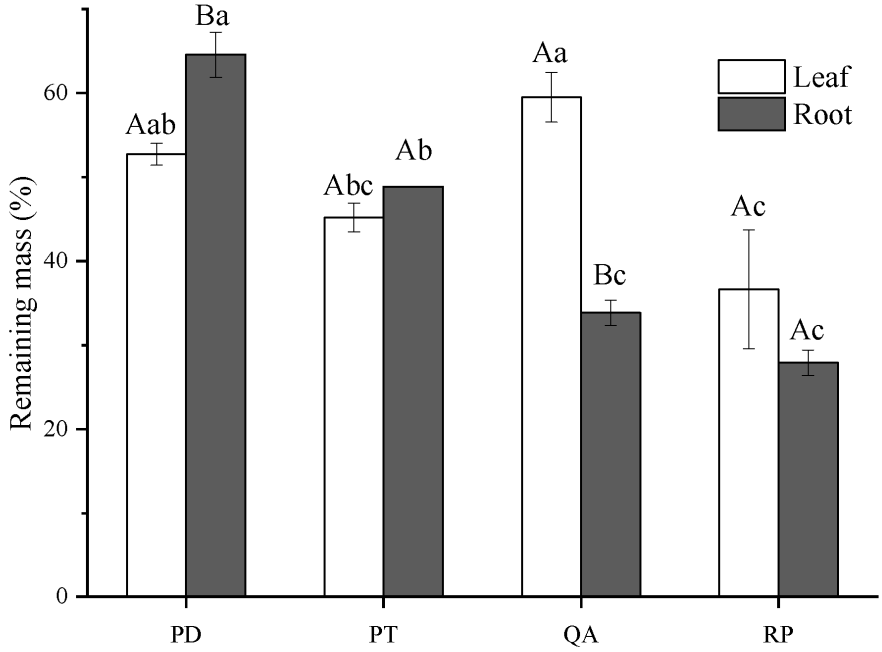

Figure 2. The remaining mass for leaf and root litters of the four species after decomposition for one year at Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. Different capital letters indicate significant differences between leaf and root for the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

3.2. Bacterial Alpha Diversity

All the sample coverage values were higher than 96%, suggesting that the sequence data well-reflected the microbial community composition. The bacterial alpha diversity indices were significantly different and were affected by litter tissues, species and their interactions, except for the Shannon–Wiener index (Table 3 and Table S2). After one year, the observed species, Chao1 and phylogenetic diversity (PD) values for the fine roots were higher than those for the leaf litter (Table 3). For fine roots, three indices of broad-leaved species were significantly lower than those of coniferous species ($p < 0.05$), but the opposite results were found for the leaf litter ($p < 0.05$, Table 3). The Shannon–Wiener index of fine roots were not significantly different among different species ($p > 0.05$). However, the Shannon–Wiener index of leaf litter had significant differences among different species, with the lowest value for *Q. acutissima* and the highest value for *R. pseudoacacia* ($p < 0.05$, Table 3).

Table 3. Bacterial alpha diversity indices in litter after fine root and leaf litter decomposition for one year at Mount Tai.

	Organ	Species				Average Value
		RP	QA	PD	PT	
Observed species	Leaf	2000 ± 9.8 ^{a*}	1946 ± 47.6 ^{a}	1832 ± 10.4 ^{b**}	1776 ± 21.0 ^{b**}	1888 ± 29.1 **
	Root	2149 ± 40.7 ^{b}	2155 ± 197.2 ^{b}	2759 ± 14.4 ^{a}	2568 ± 22.2 ^{a}	2407 ± 90.8
Chao1	Leaf	2729.5 ± 53.4 ^{a*}	2672.9 ± 11 ^{a*}	2221.5 ± 86.4 ^{b**}	2275.8 ± 88.7 ^{b**}	2474.9 ± 258.2 **
	Root	3227.5 ± 161.3 ^{b}	2824.2 ± 51.1 ^{c}	3544.7 ± 29.1 ^{a}	3395.0 ± 1.3 ^{ab}	3247.8 ± 308.4
Phylogenetic diversity (PD)	Leaf	145.3 ± 0.4 ^{a**}	142.5 ± 3.4 ^{a}	134.3 ± 2.6 ^{b**}	132.8 ± 0.4 ^{b**}	138.7 ± 6.4 **
	Root	159.2 ± 2.4 ^{b}	147.8 ± 4.4 ^{c}	198.6 ± 3.0 ^{a}	193.1 ± 1.9 ^{a}	174.7 ± 23.1
Shannon	Leaf	9.13 ± 0.06 ^{a}	8.38 ± 0.04 ^{d}	8.97 ± 0.03 ^{b}	8.78 ± 0.03 ^{c}	8.82 ± 0.30
	Root	8.43 ± 0.35 ^{a}	8.40 ± 0.28 ^{a}	8.80 ± 0.20 ^{a}	8.76 ± 0.16 ^{a}	8.60 ± 0.42

RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. Different lowercase letters in the row represent significant differences among the different species for the same organ ($p < 0.05$). Asterisks indicate significant (* $p < 0.05$ and ** $p < 0.01$) differences between leaf litter and fine roots for the same species. All data are expressed as the mean ± SE.

After one year, significantly positive correlations were observed between the number of observed species and PD values and the C:N, lignin content and the C content. However, remarkably negative correlations were found between the number of observed species and PD values and the N content and N:P ($p < 0.05$, Table 4). The Chao1 index showed a significantly positive correlation with the C:N and lignin content ($p < 0.05$, Table 4).

Table 4. Correlation analysis between the 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). ** $p < 0.01$ and * $p < 0.05$.

3.3. Relative Abundance of Dominant Bacterial Phyla and Genera

We obtained total 1,299,912 valid sequences from all the 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 defined 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%). *Planctomycetes* was the fifth-most abundant phylum, followed by *Gemmatimonadetes*, *Cyanobacteria*, *Verrucomicrobia*, *Firmicutes* and *Chloroflexi* (Figure 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* (Figure 3b).

The bacterial phyla *Bacteroidetes*, *Acidobacteria* and *Gemmatimonadetes* were significantly affected by species and litter tissues (Table S3, $p < 0.05$). 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 *Q. acutissima* (Figure 4a,b). The relative abundance of *Gemmatimonadetes* in *R. pseudoacacia* leaf litter was significantly higher than that in other three leaf litters ($p < 0.05$), but there was no obvious difference among four fine root treatments (Figure 4c). The relative abundance of *Firmicutes* and *Chloroflexi* were only correlated with litter tissues (Table S3). The relative abundance of *Firmicutes* in fine root litter was higher than that in leaf litter, but the difference was not significant (Figure 4d). There was a

significant difference between the *Pinus tabulaeformis* leaf and root litter for the abundance of *Chloroflexi* ($p < 0.05$, Figure 4e).

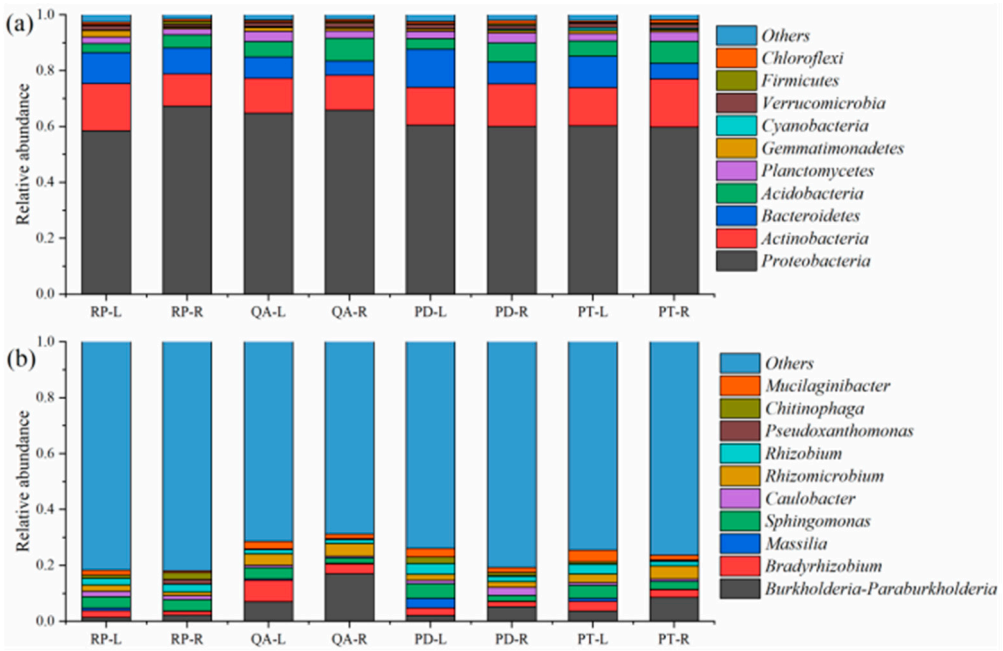

Figure 3. Relative abundance of the top ten dominant bacterial phyla (**a**) and genera (**b**). RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. L: leaf litter and R: fine root.

The bacterial genera *Burkholderia-Paraburkholderia* and *Mucilaginibacter* were significantly affected by litter tissues and species (Table S4, $p < 0.05$, Figure 4f,j). The relative abundance of *Bradyrhizobium* and *Rhizomicrobium* were affected by species only, while *Sphingomonas* was affected by litter tissues 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* (Figure 4f–j). The relative abundance of *Rhizomicrobium* was significantly higher for *Q. acutissima* and *Pinus tabulaeformis* than that for *R. pseudoacacia* and *P. densiflora* (Figure 4i).

There was no significant correlation between the relative abundances of *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Verrucomicrobia* and *Firmicutes* and the initial litter chemistry ($p > 0.05$, Table 5). The relative abundance of *Bacteroidetes* had a significantly positive correlation with the N content and N:P ($p < 0.05$) and a significantly negative correlation with the 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 the N content and N:P ($p < 0.05$). The relative abundance of *Gemmatimonadetes* had a significantly negative correlation with the P and C contents ($p < 0.05$). The relative abundance of *Chloroflexi* had a significantly positive correlation with C:N ($p < 0.05$, Table 5).

The 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 ($p < 0.05$, 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 but a significantly negative correlation with the C:N and initial lignin contents ($p < 0.05$, Table 6). A significantly positive correlation was observed between the relative

abundance of *Chitinophaga* and the N content, but a significantly negative correlation was observed with the lignin content ($p < 0.05$, Table 6).

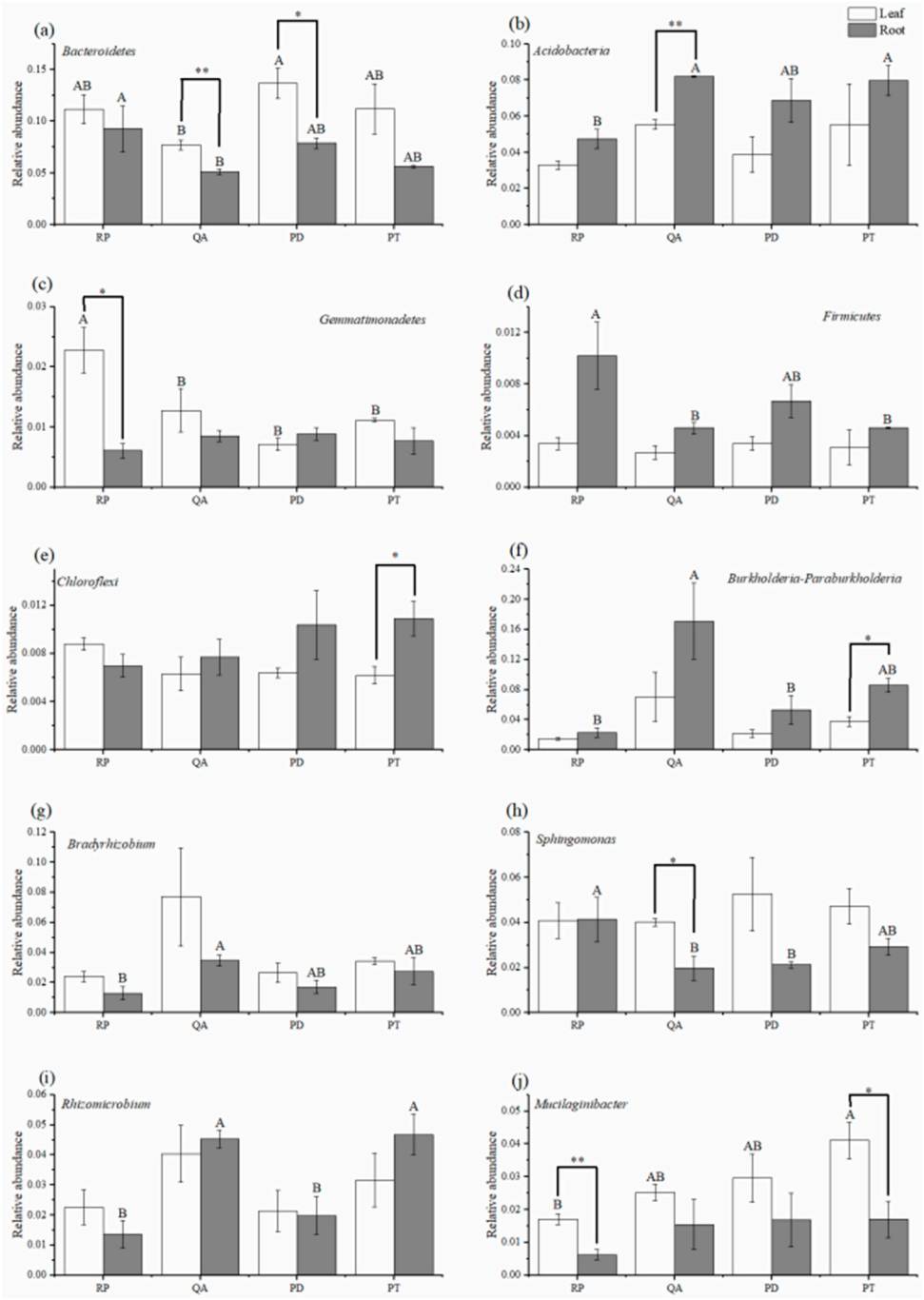

Figure 4. Differences in the relative abundances of the top ten dominant bacterial phyla (**a**–**e**) and genera (**f**–**j**) at Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. Different capital letters represent significant differences among different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

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). ** $p<0.01$ and * $p< 0.05$.

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-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

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

3.4. 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 (Figure 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 results of the redundancy analysis (RDA) showed that the initial N:P had the greatest impact on the bacterial community structure, followed by the lignin content and N content (Figure 6). The bacterial community structure in leaf litter was the most highly correlated with the initial N content and N:P, and the bacterial community structure in fine roots was the most highly correlated with the lignin content (Figure 6).

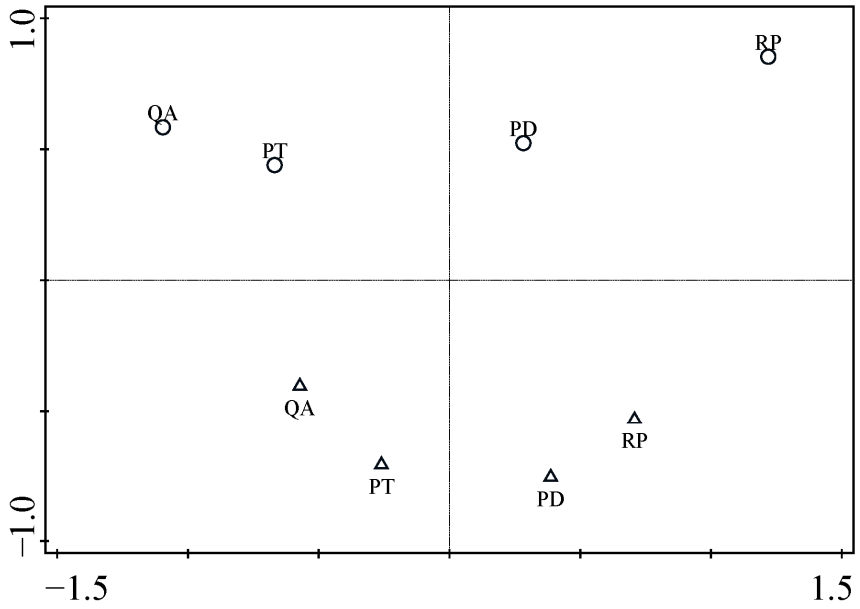

Figure 5. Nonmetric multidimensional scaling (NMDS) ordination diagram of the bacterial community structure in the litter after one year of decomposition at Mount Tai. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. The triangles represent the leaf litter,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

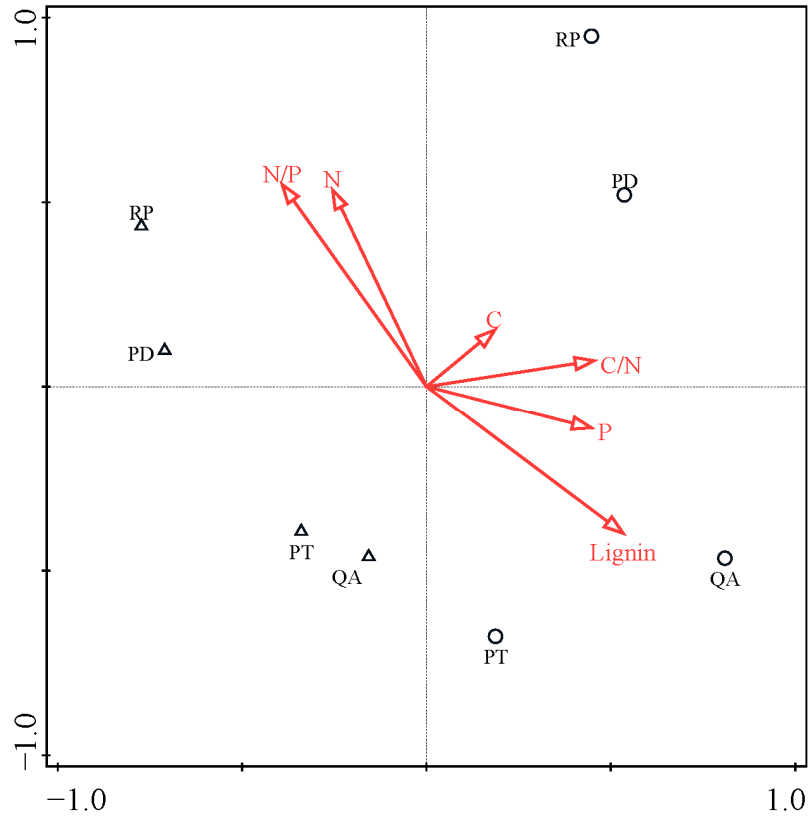

Figure 6. Redundancy analysis (RDA) based on the bacterial community structure and initial litter chemistry. RP: *R. pseudoacacia*, QA: *Q. acutissima*, PD: *P. densiflora* and PT: *P. tabulaeformis*. The triangles represent the leaf litter, and the circles represent the fine roots.

4. Discussion

4.1. Effect of Litter Tissues and Species on Litter Decomposition

Our results showed that the litter decomposition rate was affected by the litter tissues and species (Table S1 and Figure 2). The litter decomposition rates of fine roots were faster than leaf litter for broad-leaved species, while the opposite result was found for coniferous species (Figure 2). Previous studies reached inconsistent conclusions that some studies found a faster degradation for root litter than leaf litter [12], and the opposite trend was also reported [14,34,35]; even both results occurred [36]. In addition, our results found that broad-leaved species had higher decomposition rates than those of coniferous species, and the decomposition rates ranked as follows: RP > QA > PT > PD, except for *Q. acutissima* with slow-decaying leaves (Figure 2). Previous research found that plants possess coordinated nutrient use traits, such that species with fast-decaying leaves also have fast-decaying roots [37]. Our results suggest that the initial litter chemistry (C content, N content, C:N and N:P) emerged as a strong driver of the decomposition rates (Table 5). These results were in agreement with previous studies [34,35,38,39]. A large number of studies found that there was a close correlation between N-related indicators and the litter decomposition rate, especially C:N and lignin:N, which were considered as a key predictor of the decomposition rates [4,40]. The lowest N content and highest C/N of *Q. acutissima* in the four leaf litters may be the main reason that *Q. acutissima* had slow-decaying leave.

4.2. Effect of Litter Tissues and Species on the Relative Abundances of Dominant Bacterial Phyla

Proteobacteria, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* were the dominant bacteria, especially *Proteobacteria*. After one year, *Proteobacteria* were the main functional bacteria, accounting for 62.2% of the entire bacterial community (Figure 3). These results were consistent with previous findings [41,42]. High taxonomy for microorganisms, such as the phylum, can display the ecological coherence of microbial groups because of their responses to environmental changes being predictable [43,44]. *Proteobacteria* are eutrophic bacteria that are often associated with the addition of labile C [45,46]. *Actinobacteria*, which are saprophytic bacteria, are regarded as less opportunistic and can produce a wider range of degrading enzymes, and even some populations can degrade lignin and cellulose [39]. 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, litter tissues had no effect on the relative abundances of *Proteobacteria* and *Actinobacteria* (Table S3). A possible explanation is the "functional breadth hypothesis", i.e., the ability of soil biota to efficiently decompose all litter types at the same time [47,48]. Here, we found no significant difference in the relative abundances of *Proteobacteria* and *Actinobacteria* among all the litters (Table 5 and Figure 4), suggesting that the decomposer community had a broad functional ability to decompose various litter types [6]. Moreover, we found that the relative abundance of *Acidobacteria* in fine roots was higher than that in leaf litter, especially for *Q. acutissima* (Figure 4b), and had a significantly positive correlation with the initial lignin content (Table 5). The reverse result was obtained for *Bacteroidetes* (Figure 4a, Table 5). These results were consistent with previous works that leaf addition promoted *Bacteroidetes* and *Proteobacteria*, but root addition promoted *Actinobacteria* [49]. *Acidobacteria*, an oligotrophic microorganism, can grow in complex polymers, including plant hemicellulose or cellulose and fungal chitin [50]. *Gemmatimonadetes* is frequently detected in environmental 16S rRNA gene libraries and has been identified as one of the top nine phyla in soils [51,52]. Zhao [52] demonstrated that soil bacterial taxa such as the phyla *Chloroflexi* and *Gemmatimonadetes* were strongly positively correlated with the soil C content, but *Firmicutes* was negatively correlated with the C content. However, we found that the relative abundance of *Gemmatimonadetes* had a significantly negative correlation with the C content, and 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. Members of *Firmicutes* include

anaerobic bacteria, which can degrade different carbon sources, and some are related to N and denitrification [53]. *Chloroflexi* is a ubiquitous heterotrophic degrading flora that decomposes carbohydrates [54]. We found that the relative abundances of *Firmicutes* and *Chloroflexi* were affected by litter tissues (Table S3). One possible explanation is that the initial litter chemistry and physical positions of the leaf and roots were different (Table 2).

4.3. 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 tissues and species (Figure 3 and Table S4). *Burkholderia-Paraburkholderia* and *Rhizomicrobium*, which were reported to participate in N cycling, are members of the denitrifier and N_2 fixation taxa and require a high N availability [55,56]. 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 the N release and then decrease the N availability after litter decomposition [4]. 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 litter tissues (Tables 6 and 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 [57]. There was no significant correlation between the relative abundance of *Bradyrhizobium* and the remaining mass and initial litter properties (Table 6). *Bradyrhizobium*, which belongs to nitrogen-fixing bacteria, would be beneficial to nitrogen fixation [58]. In our study, the lower relative abundance of *Bradyrhizobium* (3.2%) may lead to no significant effect on litter decomposition.

4.4. Effect of Litter Tissues and Species on the Bacterial Diversity

As we hypothesised, we found that the bacterial diversity was affected by litter tissues and species, and the leaf litter bacterial diversity of coniferous species was lower than that of broad-leaved species (Tables 3 and S4), which agreed with previous findings [59]. Interestingly, the results were the 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 [60]. These differences could be ascribed mainly to variations in the leaf litter chemistry and changes in the mycorrhizal communities and colonisation [61]. Our results showed that the initial litter chemistries were different among litter tissues and species (Table S1). As we hypothesised, nonmetric multidimensional scaling (NMDS) and the ANOSIM nonparametric test showed that the bacterial community structure in leaf litter was significantly different from that in fine roots (Figure 5; $R = 0.5208$, $p = 0.03$). The difference of the microenvironment in leaf and root litter decomposition significantly affected the microbial community. The higher humidity of the soil environment was beneficial to microbial growth [62]. These may be important reasons for the significant differences in the bacterial community structure and decomposition rates between the leaf and root litter (Figures 2 and 5).

5. Conclusions

By comparing four afforestation trees at Mount Tai, this study revealed the effects of litter tissues 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 tissues, 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 the bacterial alpha diversity, dominant phyla and genera and initial litter chemistries, in agreement with our second hypothesis. Overall, this study suggests that the litter decomposition is affected by litter tissues and species, and the bacterial community plays an important role.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f13091402/s1, Table S1: Effects of litter species, tissue and their interaction on the initial litter chemistries tested by two-way ANOVA; Table S2: Effects of litter species, tissue and their interaction on the bacterial alpha diversity indices tested by two-way ANOVA; Table S3: Effects of litter species, tissue and their interaction on the top ten dominant bacterial phyla tested by two-way ANOVA; Table S4: Effects of litter species, tissue and their interaction on the top ten dominant bacterial genera tested by two-way ANOVA.

Author Contributions: Conceptualisation, C.Z. and C.L.; formal analysis, Y.L. and L.Z.; investigation, K.L., R.N., R.H., C.L., C.Z., W.S. and Z.Z.; writing—original draft preparation, Y.L.; writing—review and editing, Y.L. and L.Z.; supervision, C.Z. and funding acquisition, C.L. and C.Z. All authors have read and agreed to the published version of the manuscript.

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