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The Influence of Forest Litter Characteristics on Bacterial and Fungal Community Diversity in the *Picea crassifolia* Ecosystem on the Qinghai–Tibet Plateau

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Abstract: The biodiversity and activity of microorganisms are crucial for litter decomposition, but how litter traits at different stages of decomposition drive changes in microbial communities has yet to be thoroughly explored. In the typical alpine hilly area of the Qinghai–Tibet Plateau, three types of litter at different decomposition stages were selected under a natural *Picea crassifolia* (*Picea crassifolia* Kom.) forest: undecomposed (A-1), partially decomposed (A-2), and fully decomposed (A-3). By measuring physicochemical indicators, microbial diversity, and the composition of the litter at different decomposition stages, this study investigates the community changes and responses of bacteria to litter characteristic changes at different decomposition levels. The results show that with the increase in decomposition level, bacterial diversity increases, community structure changes, and network complexity gradually increases, while the changes in fungal communities are insignificant. Structural equation modeling indicates that the first principal component (PC1) of litter properties is significantly negatively correlated with bacterial diversity and positively correlated with bacterial community composition. There is no significant correlation between fungal diversity and community composition, indicating a closer relationship between bacteria and litter characteristics than fungi. In summary, with an increase in litter decomposition level, the diversity and network complexity of bacterial and fungal communities will significantly increase, which is related to the changes in various litter characteristics. This study provides a scientific basis for the regulatory mechanism of litter decomposition and turnover in the alpine hilly area of the Qinghai–Tibet Plateau, specifically in *Picea crassifolia* forests.

Keywords: bacteria; fungi; network complexity; litter traits; Qinghai–Tibet Plateau

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1. Introduction

Forest litter, as an important link between aboveground vegetation and underground soil in forests, plays a crucial role in the growth and development of forest vegetation. On the one hand, it drives the material circulation and energy flow in forest ecosystems and is the most active part of these systems. On the other hand, as a source of nutrients for forest soil, it has an extremely important impact on climate change. Microorganisms, as the primary decomposers in the process of litter decomposition within forest ecosystems, are capable of returning nutrients such as nitrogen, phosphorus, and potassium from plants back to the soil and atmosphere through decomposition. They are also the main producers

of enzymes that break down lignin and cellulose, the two most abundant plant materials in forest soil [1,2]. Therefore, microorganisms play a crucial role in litter decomposition [3,4]. Soil microorganisms involved in litter decomposition are mainly fungi and bacteria [5,6]. Due to their intrinsic properties, they exhibit different response mechanisms and potential functions in adapting to the environment. Generally, fungi have a broader tolerance range for pH values compared to bacteria, which have a narrower tolerance [7,8]. Additionally, fungi can penetrate dead plant tissues through enzyme secretion, utilizing their nutrients for reproduction and growth, thereby degrading the dead plant material. Smaller bacteria can survive in the spaces created by fungal activity. When the nutrient supply is ample, they can grow and reproduce rapidly, accelerating the decomposition of dead plant materials [5]. Studies have reported that litter fragments exposed to air are mainly decomposed by fungi [9]. At the same time, those submerged in water are primarily decomposed by bacteria [10]. It is important to note that the role of bacteria and fungi in litter decomposition depends on various factors. For example, changes in surface and soil moisture content can alter the quantity of fungi and bacteria, affecting litter decomposition. Moreover, the number and types of decomposers determine the rate of litter decomposition [11]. A rise in the number of decomposers escalates the breakdown of organic matter, whereas a greater diversity of decomposer species improves the synergy between them—for instance, arthropods and earthworms break down large organic materials for the benefit of soil microorganisms [11]. Multiple microorganisms can provide a variety of decomposing enzymes, compensating for the insufficiency of a single type of enzyme, thus facilitating the decomposition process [12]. Additionally, recent research has indicated that changes in litter traits might affect microbial communities. However, how litter traits at different decomposition stages drive changes in microbial communities has yet to be fully explored.

Reports suggest that litter quality and environmental factors explain about 60–70% of the decomposition rate globally and regionally [13]. Recent studies have shown that environmental factors related to various climatic conditions are the most important determinants of litter decomposition [14,15]. In contrast, some studies report that plant functional traits, such as litter quality, have a major impact on the large-scale decomposition rate [16]. These findings suggest that the impact of litter quality might be underestimated in decomposition studies compared to climatic parameters [17]. According to previous research, the quality of litter substrate changes with the decomposition stage [18]. In the initial stages of decomposition, microorganisms effectively utilize unstable plant components [19]. Early in the decomposition process, the decomposition rate correlates positively with the nitrogen content in the litter, while in the later stages, it is negatively correlated [20,21]. This is possibly because the decomposition rate of litter at different stages may be limited by different chemical components, such as nitrogen content in the early stages and lignin content in the later stages [22]. Due to structural and compositional differences, various types of litter also decompose at different rates [23]. Generally, conifer litter, with higher lignin, fiber, and secondary metabolite contents, decomposes slower than angiosperm litter [24]. The composition and content of litter material not only determine its decomposition process and relationship with environmental factors, such as litter with a high soluble component possibly having a stronger leaching effect of moisture, but also the decomposition of litter with high lignin content may have a stronger dependence on light [25,26].

The composition and content of litter determine its relationship with environmental factors and the preferences of decomposing microorganisms, thus influencing the microbial decomposition rate [27]. Variables commonly used to evaluate litter quality include C, N, lignin content, the C/N ratio, the lignin/N ratio, the N/P ratio, etc. Generally, a high rate of decomposition is associated with high N and P contents, low lignin content, or low lignin/N and C/N ratios in the litter [26]. Generally, high nitrogen (N) and phosphorus (P) content, low lignin content, or low lignin/N and low carbon/nitrogen (C/N) ratios are considered indicators of high-quality litter. Existing research reports a positive correlation between nitrogen content in litter and the rate of litter decomposition, while lignin content negatively impacts litter decomposition [28]. Studies have shown that higher N and P

contents in global litter correlate with faster decomposition rates, while the relationships of lignin/N and C/N ratios with the decomposition rate are negative [29]. Scholars have proposed that the decisive role of litter chemical characteristics in the decomposition process depends on the decomposer's sensitivity to these chemical traits. Unique microbial groups might prefer certain chemical components or ratios between them while being indifferent or even averse to others. Hence, high-quality litter does not necessarily imply a high decomposition rate [27]. However, it remains to be seen how chemical properties such as litter composition drive the changes in microbial communities at different stages of decomposition.

The Qinghai–Tibet Plateau, the most extensive plateau in Asia and the highest in the world, is known as the "Third Pole" and the "Roof of the World" [30]. It is also one of China's most important forest regions and contains some of its few remaining primary forests, with unique ecosystem types such as alpine hilly areas. Common tree species in this region include *Picea crassifolia* (*Picea crassifolia* Kom.) and Qilian juniper, and the forest type is a subalpine dark coniferous forest. The mountain forest ecosystems in this region are characterized by coniferous trees whose needles contain high amounts of oily components, leading to a similarly high oil content in the litter. This characteristic makes the litter hard to decompose and slows down its degradation speed. Additionally, the turnover of forest litter in the cold, hilly areas of the Tibetan Plateau is influenced by the region's high altitude and cold climate [31,32]. The combined effect of stand characteristics and environmental factors results in the slow decomposition of forest floor litter. This study, therefore, focuses on the cold, hilly areas of the Tibetan Plateau, chosen for their typical ecosystem traits, to analyze dominant microbial species involved in litter composition and decomposition processes. Through a detailed examination of microbial community structure differences during litter decomposition and the effects of varying chemical compositions of litter on these microbial communities, this study aims to unveil the processes of material cycling and energy flow within the plateau's ecosystems. It seeks to thoroughly understand the interactions between litter microbial communities and traits and the responses of these microbes to changes in litter properties. This insight is crucial for clarifying the mechanisms that regulate litter decomposition and turnover in the area, holding significant scientific relevance.

2. Materials and Methods

2.1. Study Area

The study site is located in Datong Hui and Tu Autonomous County, Xining City, Qinghai Province (100°51′–101°56′ E, 36°43′–37°23′ N), typifying an alpine hilly area. The altitude ranges from 2280 to 4622 m with slopes of 30–40°. It features a plateau continental climate with an annual average temperature of 4.9 °C, a maximum of 35.6 °C, and a minimum of −26.1 °C. The frost-free period lasts 45–60 days. The annual precipitation is 549.9 mm, concentrated between July and September. The area enjoys more than 2500 h of sunshine annually, with a long, cold winter and a spring season characterized by strong winds and sandstorms. Climatic features include low atmospheric pressure, long sunlight duration, high solar radiation, a large diurnal temperature variation, a long freezing period, a short frost-free period, low precipitation, and high evaporation. The primary forest type in the region is the temperate coniferous forest dominated by *Picea crassifolia*, an intermediate succession stage of natural Qinghai coniferous forests. The dominant shrubs and herbs in the understory are *Rosa sweginzowii Koehne (Rosaceae)* and *Aconitun sinomontanum*, respectively. The average forest coverage in the area is about 79%, with soil types including mountain brown soil and chestnut soil developed on loess parent material.

2.2. Experimental Design

In May 2023, *Picea crassifolia* leaf litter was collected at 3100 m above sea level in the study area. Forest cover in forest soil science is categorized into three layers: the L layer consists of recently fallen leaves, branches, bark, and other nearly undecomposed plant remains; the F layer is composed of partially decomposed plant remains, where the

original plant tissue is still identifiable; and the H layer comprises further decomposed plant remains, with the original plant tissue no longer recognizable (referenced from the book *Forest Soil Science* [33].

The sampling method involved setting up three 50 m × 50 m plots within a forest stand as replicates, with each plot being 80–100 m apart. Within each selected plot, sampling was conducted at five points arranged diagonally across a 1 m^2 area, mixing samples from the litter layer. The collected litter was categorized into three types corresponding to the three soil layers: undecomposed A-1 for the L layer (fresh needles with clear shapes of fallen leaves), partially decomposed A-2 for the F layer (leaves in a state of decomposition, with incomplete shapes and containing fragments and other impurities, distinctly different from fresh leaves), and decomposed A-3 for the H layer (the humus layer, where leaf shapes are indistinguishable), with debris being removed from each category. After collection, the litter from the three types at the five sampling points within the same large plot was mixed evenly to obtain a total of nine samples.

First, the mixed samples were divided into two portions on-site. From one portion, 10 g was taken and placed into a sterilized freezing tube, which was then stored in a liquid nitrogen container for temporary preservation. Upon returning to the laboratory, this portion was quickly placed into a −80 °C freezer for microbial analysis in the litter. The remaining samples were taken back to the laboratory in sterile sealed polyethylene bags. After air-drying at room temperature, the samples were ground using a vibration ball mill (GT-200, Geruideman Instrument Equipment, Beijing, China) and sieved through a 100-mesh sieve to determine the litter's properties and chemical components.

2.3. Litter Properties

The pH value of the litter was determined using a pH electrode (PHS-3E, Leici, Shanghai) with a deionized water-to-soil ratio of 2.5:1 [32]. Litter components such as pectin, starch, cellulose, hemicellulose, lignin, and total sugars were measured using the microanalysis method [34]. Total carbon (TC) was determined using photometry [35]. Total nitrogen (TN) was measured using the Kjeldahl method with an Italian VELP automatic Kjeldahl apparatus (UDK149, Ensoul Technology Ltd., Beijing, China) [36]. All physicochemical parameters were measured in triplicate and averaged.

2.4. Soil Bacteria and Fungi Analysis

First, microbial genomic DNA was extracted from 0.5 g of litter using HiPure Soil DNA Kits (D3142, Guangzhou Meiji Biological Technology Co., Ltd., Guangdong, China). The quality and concentration of extracted DNA were quantified using a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) by measuring absorbance ratios at 260/280 nm and 260/230 nm. An amplicon survey of the 16S and ITS rRNA was performed to determine soil bacterial and fungal community composition and diversity. The V3 + V4 hypervariable regions of the 16S rRNA gene were amplified using primers 341F and 806R. The ITS2 region of fungal rRNA was amplified using primers ITS3_KYO2 and ITS4. Both sets of primers were tagged with adaptors, pads, linkers, and unique barcode sequences for each sample.

The PCR amplification used a KOD enzyme amplification system to ensure successful and accurate amplification, with each sample undergoing three PCR reactions. The first-round PCR products were purified using AMPure XP Beads, then quantified with Qubit3.0 and diluted to 1 ng/μL with sterile water. A second round of amplification was then performed using the KOD enzyme amplification system, and the products were quantified with an ABI StepOnePlus Real-Time PCR System (Life Technologies, Carlsbad, CA, USA). All PCR reactions used AMPure XP Beads (Beckman Coulter, Brea, CA, USA) and were sequenced using the Hiseq2500 PE250 mode.

Low-quality bases, Ns, and linker contamination sequences were filtered out from the sequencing data. The demultiplexing and quality filtering of raw fastq observations were performed using Trimmomatic [37]. Paired-end clean reads of original DNA fragments

were combined using FLASH software (version 1.2.11) [38]. High-quality sequences with a quality score above 20 and a length greater than 50 bp were retained. The maximum mismatch ratio for merged sequence overlap was set at 0.2, with a minimum overlap length of 10 bp. Unassembled reads were discarded. Unprocessed sequences were processed using QIIME 1.9.1 software. After quality checking on USEARCH to remove chimeric sequences, all effective tags sequences from all samples were clustered into operational taxonomic units (OTUs) using UPARSE (version 7.0.1090) software (https://drive5.com/contact.html (accessed on 1 November 2023), with an identity of 97% [39]. Representative sequences for each OTU were selected and classified using the RDP Classifier (version 2.11) based on the UNITE database [40]. A total of 15,945 bacterial sequences and 1919 fungal sequences were obtained from all litter samples.

In summary, the number of OTUs in the litter of each sample at different decomposition layers ranged from 701 to 9470 (Supplementary Tables S1 and S2), sufficient for subsequent data analysis. The number of sequences in each sample was rarefied to a uniform sequencing depth based on the sample with the lowest sequence read count (Supplementary Figure S1).

2.5. Data Processing

First, microbial (bacteria and fungi) α-diversity was measured, including richness, chao1, Shannon, Simpson, sob, and Ace. Next, the normality of the data was tested using the Shapiro–Wilk test. Data that did not follow a normal distribution were log-transformed to improve normality. The physicochemical properties of different litter decomposition layers, microbial diversity, and network attributes were tested using one-way ANOVA and Fisher's least-significant-difference (LSD) multiple comparison test. Principal coordinate analysis (PCoA) with Bray–Curtis distance measurements was used to assess changes in the bacterial and fungal community structure (β diversity). The statistical significance of differences between litter decomposition layers was assessed by permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis distance metrics with 999 permutations, using the "vegan" package.

Linear Discriminant Analysis Effect Size (LEfSe) software (version 1.1) was used to analyze differences in microbial communities between groups and identify potential microbial biomarkers. Using LEfSe, we first conducted a Kruskal–Wallis rank-sum test across all group samples. The differentially abundant species were then compared pairwise using the Wilcoxon rank-sum test. Finally, the differences were ranked using results obtained from linear discriminant analysis (LDA) (Supplementary Figure S2), and an evolutionary branch diagram was created by mapping the differences onto a known hierarchical classification tree (Supplementary Figure S3).

OTUs with values less than 0.01% were discarded to explain the relationships among OTUs in bacterial and fungal taxa. Spearman's rank correlation coefficients were determined using the "psych" package. Statistically significant correlations (determined using Spearman's coefficient, ρ, $p < 0.001$) with ρ values greater than 0.6 or less than -0.6 were retained for the network analysis [41]. Network topological characteristics (including node number, edge number, the proportion of negative edges, and the proportion of positive edges) for each soil sample were extracted using the subgraph function in the "igraph" package. Higher numbers of nodes and edges suggest a more connected network, reflecting more potential complexity of soil networks.

This passage describes a method for establishing a piecewise structural equation model in the R environment using the "piecewiseSEM" package. This model was used to identify potential pathways through which abiotic and biotic factors influence soil microbial communities under different layers of litter decomposition. Before constructing the model, an RDA (redundancy analysis) was conducted to screen for physicochemical factors that significantly affect the microbial community (Supplementary Tables S3 and S4). The significance of these factors was then assessed using the envfit function in R.

To avoid collinearity among variables, a principal component analysis (PCA) was performed on the physicochemical factors that significantly influence the microbial community. For bacteria, the first two axes explain 89.8% and 5% of the variance, respectively, while for fungi, they explain 86.8% and 8.4% (Supplementary Figure S4). Additionally, two-dimensional nonmetric multidimensional scaling (NMDS) was performed to summarize the microbial taxonomic composition. The fitted models were examined using Fisher's C statistic, and a model was considered to have an adequate fit to the data if it had a Fisher's C statistic with a p-value greater than 0.05. Path coefficients were used to quantify the connections between these block variables. All data analyses were carried out using R software (version 4.0.4; R Development Core Team, 2021).

3. Results

3.1. Chemical and Compositional Components of Different Litter Layers

In our study, we examined the vertical variation of 15 properties of litter under different litter layers (Figure 1). The properties, including cellulose, hemicellulose, lignin, total sugars, the C/N ratio, and the cellulose/N ratio, revealed that as the degree of litter decomposition increased, there was a gradual decrease in the content of insoluble polysaccharides (ISPs), lignin, cellulose, hemicellulose, starch, total sugars, and total carbon. Notably, the contents of cellulose, hemicellulose, and starch showed significant differences ($p < 0.05$) across the layers.

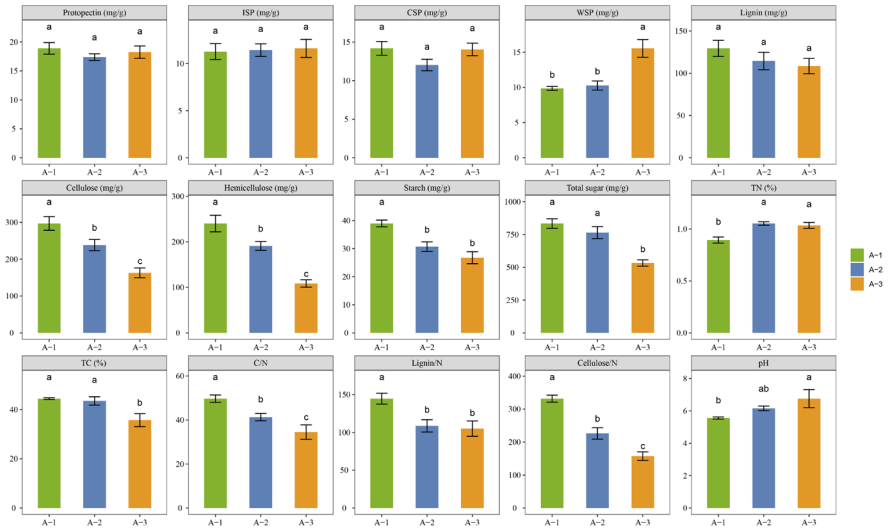

Figure 1. Main variation referring to litter properties on the natural *Picea crassifolia* forest in the Qinghai–Tibet Plateau. (Data are means ± SD. Bars (n = 3) with different lowercase letters indicate significant differences as revealed by Tukey's HSD tests ($p < 0.05$). ISP, ion-bound pectin; CSP, covalently bonded pectin; WSP, soluble pectin; TN, total nitrogen; TC, total carbon. A-1, A-2, and A-3 represent the litter layers of undecomposed litter layer, decomposed litter layer, and humus layer, respectively. Note: different lowercase letters indicate differences between groups).

It is important to note that the pH of the litter increased with the degree of decomposition. The ratios of C/N, cellulose/N, and lignin/N all decreased as decomposition progressed, with the C/N and cellulose/N ratios showing statistical significance ($p < 0.05$). The content of original pectin and CSP (carbohydrate sulfate polymers) in the three different decomposition layers first increased and then decreased. Similarly, the total carbon content showed an initial increase followed by a decrease across the different decomposition layers.

3.2. Analysis of Litter Microbial Community Diversity

The alpha diversity of the bacterial community, represented by five common indices, showed significant differences across different layers of litter decomposition (Figure 2a).

These indices include Shannon, Simpson, Sobs, Chao1, and ACE. It was observed that these diversity indices significantly increased with the progression of litter decomposition. Notably, there were no significant differences between layers A-2 and A-3 ($p < 0.05$).

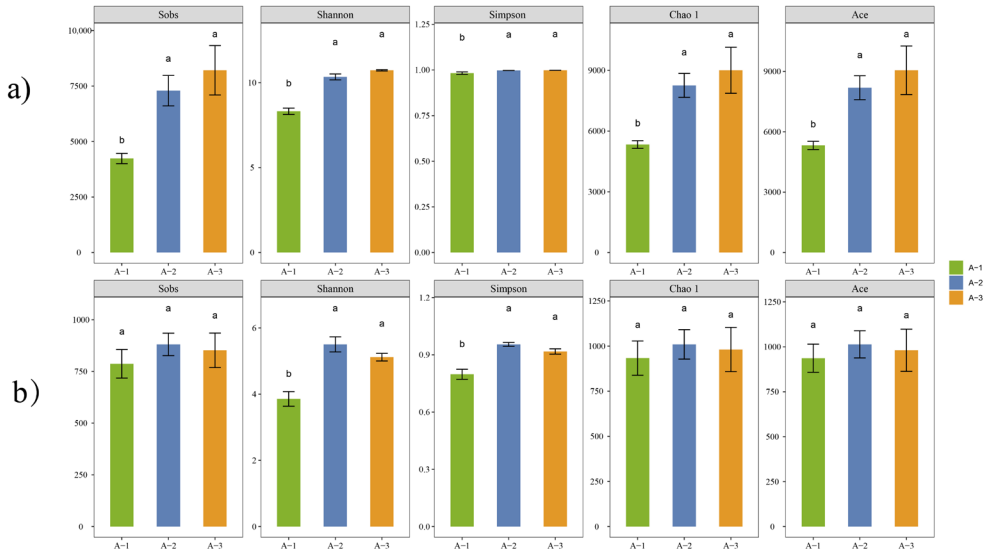

Figure 2. Main variation referring to different layers of bacterial (**a**) and fungal (**b**) of litter microbial diversity on the natural *Picea crassifolia* forest in the Qinghai–Tibet Plateau. (Data are means ± SD. Bars (n = 3) with different lowercase letters indicate significant differences as revealed by Tukey's HSD tests ($p < 0.05$). A-1, A-2, and A-3 represent the litter layers of undecomposed litter layer, decomposed litter layer, and humus layer, respectively. Note: different lowercase letters indicate differences between groups).

In contrast, the alpha diversity of the fungal community did not show significant differences across the litter decomposition layers (Figure 2b). The indices used to measure fungal diversity were also Shannon, Simpson, Sobs, Chao1, and ACE. In this case, the diversity initially increased and then decreased as decomposition progressed. It is important to note that, except for the Shannon and Simpson indices, the trends in the other indices were not statistically significant ($p < 0.05$).

3.3. Litter Microbial Composition

The beta diversity of bacterial communities in different litter layers showed significant differences ($p < 0.01$) when analyzed by PCoA (principal coordinates analysis) and PERMANOVA. The first and second principal components accounted for 57% and 13% of the variability in litter samples, respectively. Notably, layers A-2 and A-3 displayed greater divergence, especially within the A-3 group, where there was a greater distance between individual samples (Figure 3a).

For fungal communities, PCoA analysis indicated significant differences in beta diversity across different decomposition stages of litter layers ($p < 0.01$). The contributions of the first and second principal components to sample variation were 48% and 20%, respectively. Additionally, there was good clustering within the A-1 group, and the variation in fungal communities within each group increased with the degree of litter decomposition (Figure 3b).

Microbial composition data revealed that at the phylum level, the top three dominant bacterial species in terms of relative abundance were Proteobacteria, Actinobacteria, and Bacteroidetes, with relative abundances of 75.2% and 57.3% in undecomposed and decomposed layers, respectively. Proteobacteria and Planctomycetes were dominant in the humus layer, with a relative abundance of 53% (Figure 3c). Overall, the relative abundances of Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes decreased with

increasing decomposition, while those of Planctomycetes, Acidobacteria, Verrucomicrobia, Patescibacteria, Gemmatimonadetes, and Chloroflexi increased (Figure 3c).

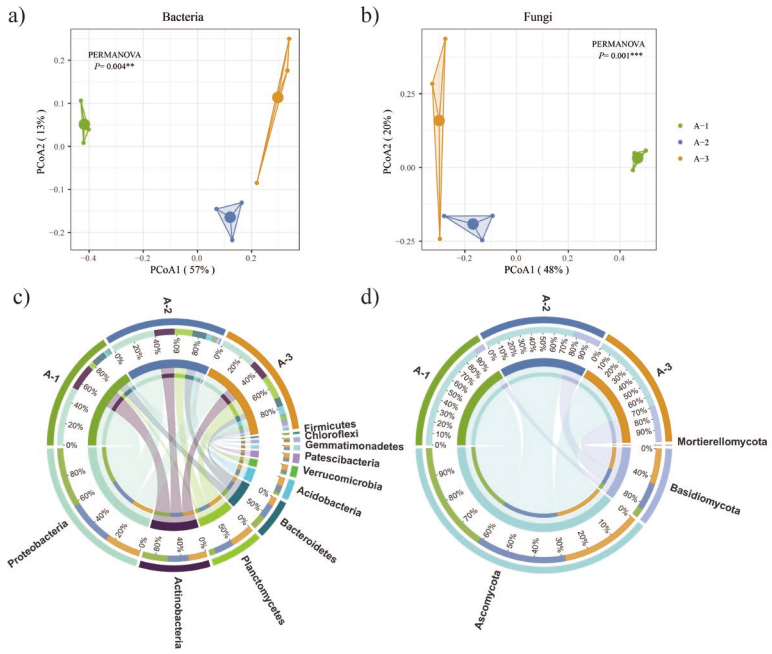

Figure 3. Composition of bacterial (**a**,**c**) and fungal (**b**,**d**) communities of different litter layers of the natural *Picea crassifolia* forest in the Qinghai–Tibet Plateau. (A-1, A-2, and A-3 represent the litter layers of undecomposed litter layer, decomposed litter layer, and humus layer, respectively. Note: $p \geq 0.05$ is not marked; $0.01 < p < 0.05$ is marked as *; $0.001 < p < 0.01$ is marked as **; $p \leq 0.001$ is marked as ***).

At the phylum level, fungi were dominated by Ascomycota, Basidiomycota, and Mortierellomycota (Figure 3d). Ascomycota was the most abundant in all litter layers, with average relative abundances of 92.1%, 76.6%, and 68.3% in different decomposition layers (Figure 3d). In general, the relative abundances of Ascomycota, Basidiomycota, and Mortierellomycota varied significantly between different decomposition layers. The relative abundance of Mortierellomycota was less than 0.1%, increasing from 0.009% in the undecomposed layer to 0.151% in the humus layer (Figure 3d). The relative abundance of Ascomycota decreased with increasing decomposition, while that of Basidiomycota and Mortierellomycota (a subphylum of Zygomycota) increased (Figure 3d).

LEfSe analysis showed that there were 250 bacterial branches as indicator species across 3 different litter decomposition layers, with 73 branches in the undecomposed litter, mainly indicating Proteobacteria species at the phylum level, and Bacteroidetes, Proteobacteria, Armatimonadetes, Actinobacteria, and Firmicutes at the order level. Additionally, 29 branches were enriched in the decomposed litter, primarily in the orders of Proteobacteria, Cyanobacteria, and Actinobacteria. In the humus layer, 148 branches were enriched, including WPS_2, Gemmatimonadetes, Hydrogenedente, Acidobacteria, Verrucomicrobia, Patescibacteria, and Nitrospirae (Supplementary Figures S2a and S3a). In the fungal community, 65 branches were indicator species for different litter layers, with 44, 4, and 148 branches enriched in the undecomposed, decomposed, and humus layers, respectively, mainly including species at the genus level of Basidiomycota and Ascomycota (Supplementary Figures S2b and S3b).

3.4. Bacterial and Fungal Networks in Different Litter Layers

Network construction was used to differentiate the co-occurrence patterns of bacterial and fungal communities in different litter layers. Network topology parameters, including

the number of nodes and edges, as well as the proportion of negative and positive correlations, were employed to assess the complexity of bacterial and fungal networks across different litter layers (Figures 4c and 5d). The number of nodes and edges, along with the proportion of positive correlations, indicated that the complexity of the soil bacterial community significantly increased with the progression of litter decomposition. Notably, there was no significant difference in the number of nodes between layers A-2 and A-3 ($p < 0.05$). Additionally, both positive and negative correlations in the bacterial network showed significant differences across different decomposition layers ($p < 0.05$). As decomposition progressed, the proportion of negative correlations in the network gradually decreased.

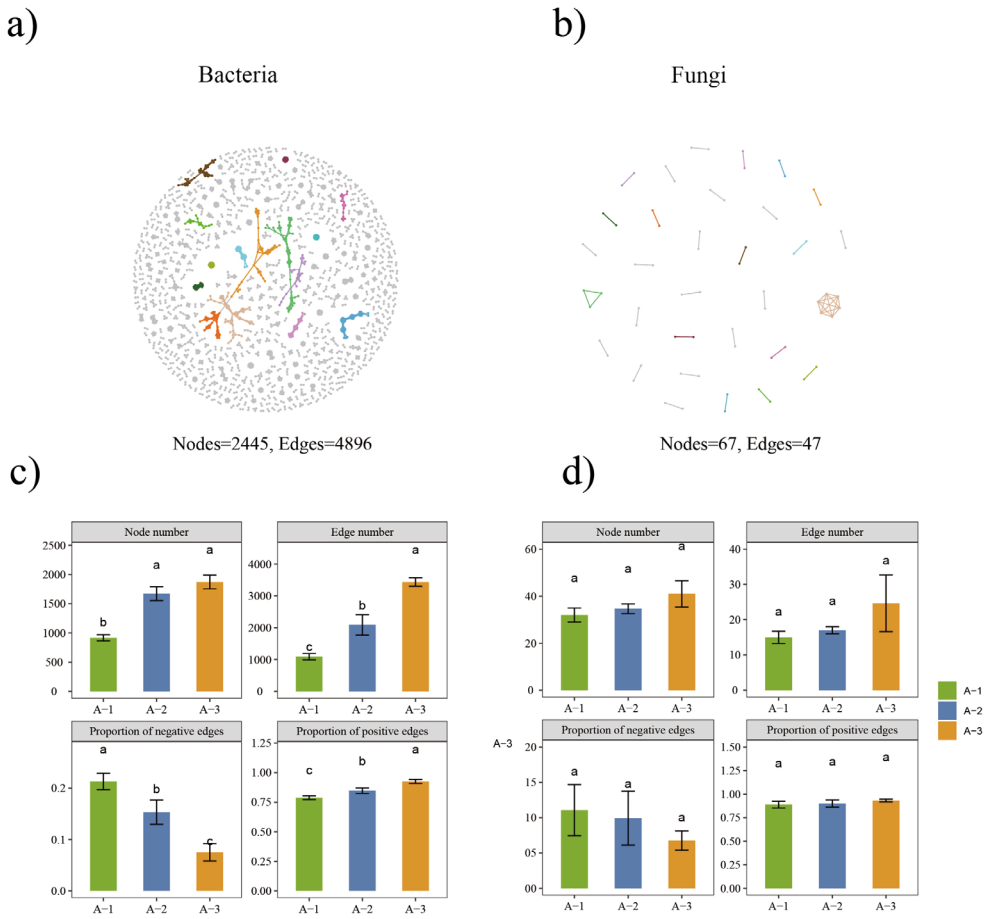

Figure 4. Ecological networks of bacteria (**a**) and fungi (**b**), and multiple indices were used to estimate the complexity of co-occurrence networks. (Data are means ± SD. Bars (n = 3) with different lowercase letters indicate significant differences as revealed by Tukey's HSD tests ($p < 0.05$). Nodes indicate litter biotic taxa (bacteria (**c**) and fungi (**d**) at OTU levels); Edges indicate the correlation between taxa; A-1, A-2, and A-3 represent the litter layers of undecomposed litter layer, decomposed litter layer, and humus layer, respectively. Note: different lowercase letters indicate differences between groups).

Regarding the fungal network, there were no significant changes in the number of nodes, edges, and the proportions of positive and negative correlations across different litter decomposition layers. Consequently, the increase in the complexity of the fungal network with the progression of litter decomposition was not significant.

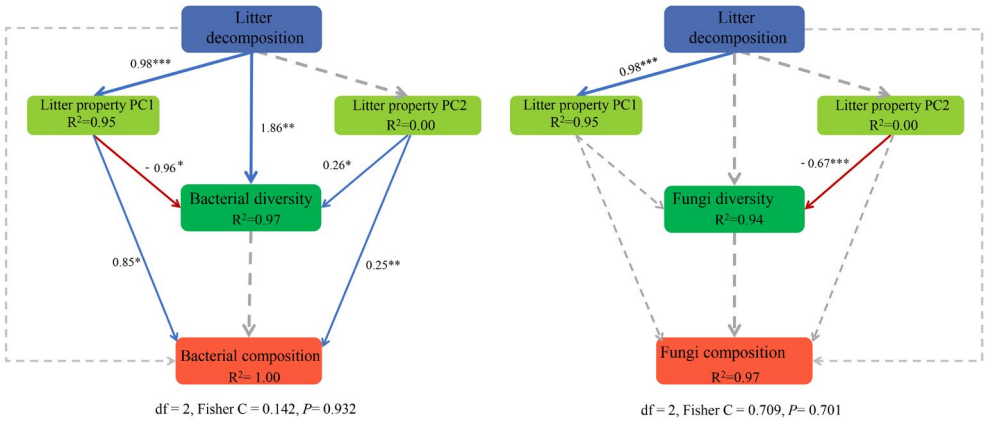

Figure 5. Using a fitted structural equation model (SEM) to identify the direct and indirect pathways through which litter property affects the litter bacterial and fungal community. (The bacterial and fungal composition is represented by NMDS1. Blue lines indicate positive relationships, red lines indicate negative relationships and numbers associated with lines indicate the correlation coefficient. Paths with non-significant coefficients are presented as grey lines. Note: $p \geq 0.05$ is not marked; $0.01 < p < 0.05$ is marked as *; $0.001 < p < 0.01$ is marked as **; $p \leq 0.001$ is marked as ***).

3.5. Factors Influencing Microbial Community Composition in Different Litter Layers

The influence of abiotic and biotic factors on soil microbial communities in different litter decomposition layers was investigated using piecewise structural equation modeling. In the two models, bacterial and fungal communities were explained by 100% and 97%, respectively. The structural equation model revealed that the first principal component (PC1) of litter properties was significantly negatively correlated with bacterial diversity ($p < 0.05$), with a path coefficient of −0.96. It was also significantly positively correlated with bacterial community composition ($p < 0.05$), with a path coefficient of 0.85. The second principal component (PC2) of litter properties showed a significant positive correlation with both bacterial diversity and composition ($p < 0.05$), with path coefficients of 0.26 and 0.25, respectively.

Interestingly, the PC1 of litter properties showed no significant relationship with either fungal diversity or community composition. However, the PC2 was significantly negatively correlated with fungal diversity ($p < 0.05$), with a path coefficient of −0.67, but it had no significant relationship with fungal community composition. Additionally, there were no significant relationships between bacterial and fungal diversity and composition (Figure 5).

4. Discussion

4.1. Changes in Litter Traits across Different Decomposition Layers

The decomposition process of litter exhibits both phase-specific and continuous characteristics. Changes in components and properties during the decomposition process lead to significant variations in litter traits at different stages. Significant differences were found in the traits of *Picea crassifolia* litter at various stages of decomposition. Specifically, the content of insoluble polysaccharides (ISPs), lignin, cellulose, hemicellulose, starch, total sugars, and total carbon gradually decreased with the increasing degree of litter decomposition, with significant differences observed in the contents of cellulose, hemicellulose, and starch. The most likely reason for this is that as decomposition progresses, substrates are increasingly broken down and consumed by microbes [42,43]. Small molecular compounds such as carbohydrates (starch) and sugars are preferentially decomposed, while more complex components like cellulose and lignin are less readily degraded [44]. The significant changes in the contents of cellulose, hemicellulose, and starch may be related to microbial nutrient preferences [27].

It is noteworthy that the pH of the litter increases with the degree of decomposition. This could be a result of both the intrinsic properties of the litter (such as surface properties,

tissue structure, nutrient elements, types, and amounts of organic compounds) and external environmental factors (such as temperature, moisture, and soil site quality) [9,45,46].

The chemical properties of litter, or substrate quality, are indicative of the litter's relative decomposability. Physicochemical properties such as the C/N ratio, lignin/N ratio, and cellulose/N ratio also influence the decomposition rate at different stages of decomposition [47–49], leading to differences in litter traits at various decomposition stages. The C/N ratio, lignin/N ratio, and cellulose/N ratio of *Picea crassifolia* litter decrease with increasing decomposition and are affected in the later stages by external environmental factors or the input of exogenous nutrients [50]. An increase in the number and activity of soil microbes leads to the gradual utilization of difficult-to-decompose substances like residual lignin, cellulose, and tannins in the decomposing residues, resulting in a reduction in substrate quality [50].

4.2. Changes in Microbial Communities across Different Litter Decomposition Layers

The number of species of fungi and bacteria is an important characteristic of the soil microbial community [51]. This study found that the α-diversity of bacteria differs significantly across different litter decomposition layers, increasing significantly with the degree of litter decomposition, and the diversity of bacteria is positively correlated with the decomposition process [52]. The α-diversity of fungi did not show significant differences across the litter decomposition layers. On one hand, this might be due to the increase in bacterial diversity as a result of nutrient release from the litter during different stages of decomposition, leading to increased competition among species within the microbial community [53]. On the other hand, compared to bacteria, fungi are more stable, and thus their trend of change is not significant in different litter layers (Supplementary Figure S5). Additionally, differences in growth strategies, competitiveness, and resource utilization between fungi and bacteria lead to different responses in diversity between fungi and bacteria [54,55].

Additionally, this study discovered that although there are significant differences in bacterial diversity among the litter layers at different decomposition stages, the types of unique bacterial taxa contained in the microbial communities of these layers are consistent. These taxa include Proteobacteria, Actinobacteria, and Bacteroidetes [56]. These three microbial groups constitute at least 10% of the bacteria in litter [56]. Due to their broad adaptability, they are commonly found in forest litter at various stages of decomposition and are effective in decomposing litter. In this study, the relative abundance of these three dominant phyla in different litter decomposition layers exceeded 50%, consistent with the conclusions of other researchers [57]. Further research indicates that among these, Proteobacteria and Bacteroidetes, which belong to the Gram-negative bacterial groups, are capable of decomposing cellulose [58]. This correlates with the findings in this study; that is, the cellulose content in the litter of natural *Picea crassifolia* forests at different decomposition stages decreased with the progression of decomposition, and correspondingly, the relative abundance of Proteobacteria and Bacteroidetes bacteria groups decreased [59]. Notably, Acidobacteria, as a major enriched bacterial group in the litter community [60], exhibits extensive phylogenetic diversity, spanning 26 branches [61]. Its genomic, physiological, and metabolic diversity allows it to be widely distributed in fluctuating environments. In this study, the relative abundance of Acidobacteria in different litter layers increased with the progression of decomposition. The decrease in the abundance of Actinobacteria in different litter layers with increased litter decomposition was unexpected, considering its involvement in the decomposition of organic matter and its significant role in organic matter turnover and carbon cycling [62]. Other studies also indicate an increase in Actinobacteria abundance during the later stages of litter decomposition [63,64]. However, overall, the relative abundance of Actinobacteria in the undecomposed layer is only slightly higher than in the litter decomposition and humus layers, but Actinobacteria remain a dominant phylum among the bacterial groups in different litter layers.

Compared to bacteria, fungi, which are capable of degrading complex compounds like lignin, are considered the primary decomposers in litter and play a dominant role in decomposing cellulose and hemicellulose [65,66]. Ascomycota and Basidiomycota are typically the dominant fungal phyla at the soil–litter interface [67]. In *Picea crassifolia* litter, Ascomycota is dominant, with smaller proportions of Basidiomycota and the subphylum Mortierellomycota. As litter decomposes, the relative abundance and diversity of Ascomycota decrease, while those of Basidiomycota and Mortierellomycota increase. Most Ascomycetes are saprophytic and early colonizers in fungal community succession, explaining their large proportion in all litter layers. Basidiomycetes can degrade lignin-rich recalcitrant litter [67,68], particularly in the later stages of decomposition. This aligns with findings that Basidiomycetes have the highest relative abundance in the humus layer of different *Picea crassifolia* litter layers [68,69].

4.3. Changes in Network Complexity across Different Litter Decomposition Layers

Network complexity can reflect the coexistence patterns of microbes in different litter layers and reveal complex interactions among microbes [70]. The increase in complexity of the soil bacterial community with the progression of litter decomposition is evident through the number of nodes and edges and the proportion of positive correlations. Notably, there is no significant difference in the number of nodes and edges between points A-2 and A-3, indicating that bacterial networks become more complex with the progression of litter decomposition, potentially strengthening cooperation among bacteria [71]. Furthermore, with the increase in decomposition, the proportion of negative correlations in the network gradually decreases, suggesting a potential reduction in bacterial competition [72]. This could be due, on the one hand, to the increase in microbial diversity as a result of nutrient release from the decomposing litter, making the bacterial network more complex and strengthening cooperation among microbes [72]. On the other hand, changes in environmental physicochemical factors and shifts in microbial preferences can alter microbial community interactions and drive changes in the microbial community [73], and the alleviation of resource limitations also contributes to reducing competition among microbes, explaining the decrease in the proportion of negative correlations in the network [74].

4.4. Microbial Community Response to Changes in Litter Traits

Changes in litter traits at different stages of decomposition drive variations in microbial communities. Structural equation modeling indicates that different litter decomposition layers indirectly influence microbial diversity and composition through their effects on litter traits. Specifically, the first principal component (PC1) of litter properties shows a significant negative correlation with bacterial diversity and a significant positive correlation with bacterial community composition. The second principal component (PC2) is significantly positively correlated with both bacterial diversity and composition. This is mainly because the positive direction of the PCA1 axis represents a decrease in resources such as total carbon, cellulose, and hemicellulose (Supplementary Figure S4). The consumption of litter resources implies an increase in microbial resource acquisition [72], leading to significant changes in diversity composition.

Furthermore, the PC1 of litter properties shows no significant relationship with fungal diversity or community composition. The PC2 is significantly negatively correlated with fungal diversity but has no significant relationship with fungal community composition. This suggests that bacteria are more sensitive to changes in litter properties than fungi [75]. On the one hand, this could be due to the more resistant cellular structures of fungi, allowing stable fungal community structures to withstand external stress and disturbances [76]. On the other hand, bacteria typically have smaller body sizes and reproduce faster than fungi, enabling them to preferentially access nutrients released from decomposing litter [77].

In summary, changes in litter traits can drive variations in microbial communities, particularly in relation to bacteria. This underscores the potential importance of bacteria in predicting litter decomposition and mineral dynamics.

5. Conclusions

This study investigated the changes in microbial communities and their response to litter trait variations in different decomposition layers of *Picea crassifolia* in the alpine hilly region of the Qinghai–Tibet Plateau. The study's results reveal significant changes in the microbial community structure and an increase in network complexity as litter decomposition progresses. Bacterial diversity shows an increase, while fungal diversity remains largely unchanged. Additionally, this research highlights that despite significant differences in bacterial community structure and diversity across varying degrees of decomposition, the dominant microbial groups remain consistent across different litter layers. For fungi, though differences in community structure and diversity are not significant, the dominant groups are consistent across decomposition layers. With more advanced decomposition, the microbial networks within *Picea crassifolia* litter layers become increasingly complex, notably in bacterial networks where complexity significantly rises and the proportion of negative correlations within the network decreases gradually. Additionally, the findings revealed a significant negative correlation between the PC1 of litter properties and bacterial diversity and a significant positive correlation with the composition of bacterial communities. No significant relationship was found with fungal diversity or community composition, indicating a tighter link between bacteria and litter traits compared to fungi.

This research provides new insights into the mechanisms by which litter traits influence microbial community changes. Future studies focusing on litter traits, especially chemical properties, are crucial for better understanding microbial community dynamics and enhancing forest ecosystem functions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15050797/s1, Supplementary Table S1: Statistical Table of the Number of OTUs and Tags of Bacteria in Different Litter Samples; Supplementary Table S2: Statistical Table of the Number of OTUs and Tags of Fungi in Different Litter Samples; Supplementary Table S3: Significant Litter Traits in the Redundancy Analysis (RDA) of the Bacterial Community; Supplementary Table S4: Significant Litter Traits in the Redundancy Analysis (RDA) of the Fungal Community; Supplementary Figure S1: The rarefaction curves of litter bacteria (a) and fungi (b); Supplementary Figure S2: Linear Discriminant Analysis (LDA) analysis of the abundance of (a) bacteria and (b) fungi at different in the different litter layers of *Picea crassifolia* forest; Supplementary Figure S3: LEfSe analysis of the abundance of (a) bacteria and (b) fungi at different in the different litter layers of *Picea crassifolia* forest; Supplementary Figure S4: Principal Component Analysis (PCA) Based on Litter Traits Significantly Affecting Bacterial and Fungal Communities; Supplementary Figure S5: Community assembly analysis is based on the Standardized Effect Size of Mean Nearest Taxon Distance (ST) for bacterial and fungal communities.

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