BACTERIAL COMMUNITY SUCCESSION AND INFLUENCING FACTORS OF IMPERATA CYLINDRICA LITTER DECOMPOSITION IN A DEGRADED COPPER TAILINGS DAM OF CHINA

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Abstract

Litter decomposition is a critical component of the ecological nutritional transformation process. It is particularly important to investigate characteristics and interactions of bacterial communities in litter decomposition in heavy metal polluted degrade areas, which will help clarify driving mechanisms of organic matter and nutrient cycling in mining areas that harbor contaminated soil. Imperata cylindrical was the dominant plant species in the degrade area investigated; thus, we selected this species as research object. Here we explore bacterial community characteristics and key microbial groups as well as driving factors of litter decomposition using in-situ litter decomposition experiments. The nutrient content of I. cylindrica decreased, while the litter pH status increased as decomposition progressed in one of the three sub-dams investigated (i.e., S516). Proteobacteria and Actinobacteriota were the dominant bacterial phyla during the different litter decomposition stages. Moreover, the role of Friedmanniella was critical in sustaining both structure and function of the bacterial community during the early decomposition stage. Quadrisphaera became the dominant species as litter decomposition progressed. Litter properties and enzyme activities both had significant effects on litter bacterial community characteristics, whose driving factors varied during different restoration stages. The bacterial community dynamics of litter were affected primarily by litter properties during the decomposition process. Furthermore, the most crucial factors that impacted bacterial litter structure were pH and copper content. Findings will help to deepen our understanding of litter decomposition mechanisms in degraded ecosystems, while also providing a scientific basis for improving effectiveness of material circulation and nutrient transformation in degrade ecosystems.

INTRODUCTION

Within terrestrial ecosystems, litter acts as a pathway for plants to transfer nutrients to the soil (Naldini et al., 2021). Additionally, carbon (C) and nitrogen (N) cycling are governed by litter decomposition processes in terrestrial ecosystems, which determine soil organic carbon (SOC) and nutrient availability and subsequently the community composition and function of ecosystems, both aboveground and belowground (Berg and Rn, 2014). Studies have shown that litter also acts as an important global C pool, and 90% of terrestrial ecosystem biomass in a given year exists in the form of litter (Cebrian, 1999). Therefore, the exploration of litter decomposition characteristics and the main driving factors are critical for understanding the material circulation and nutrient flow of ecosystems.

Being the primary decomposer of litter, microorganisms influence both ecosystem material circulation and energy flow (He et al., 2016). Previous studies have found that bacterial communities simply colonize the litter surface, while relying on fungi to obtain energy through the decomposition of complex organic matter into small molecular products (Cline and Zak, 2015). In recent years, many studies have reported that bacteria play an important role in litter degradation. It has been reported that Acidobacteria can

degrade lignin (Bhatnagar et al., 2018), while α-proteobacteria and β-proteobacteria have been shown to significantly and positively correlate to most lignin and cellulolytic enzymes (Wang et al., 2019a; 2019b). *Pedobacter* secretes various carbohydrate-decomposing enzymes, which promotes the degradation of cellulose and hemicellulose. Additionally, Proteobacteria, Actinomycetes, and *Bacteroides* are the dominant bacterial groups during decomposition processes, while bacterial communities have shown to be involved in a series of succession processes alongside litter decomposition. During the initial decomposition stage, phyllosphere microbiology predominates but is rapidly replaced by bacterial groups that secrete protease and cellulase, such as *Frigobacterium* and*Sphingomonas* . *Bradyzhorium* , *Burkholderia* , and*Streptomyces* are the most common bacterial groups in litter during the latter decomposition stage (Tláskal et al., 2016). Therefore, exploring changes in bacterial community characteristics and the main driving factors of litter decomposition processes will help us better understand litter decomposition mechanisms.

Given their control over the litter decomposition process, extracellular enzymes produced by microorganisms have attracted broad attention (Zhang et al., 2019). Extracellular enzymes are subdivided into four categories, namely, cellulase, lignin-modifying enzymes, protease, and phosphatase, according to substrate properties. Cellulose can be hydrolyzed into glucose with the participation of cellulase (Wang et al., 2006). Lignin is the most difficult enzymatic component to degrade in litter, whose decomposition largely relies on ligninolytic enzymes, such as peroxidase, laccase, polyphenol oxidase, and catalase (Dong et al., 2019). Additionally, protease and urease are primarily associated with N cycling in litter decomposition (Yan et al., 2018). Therefore, it is also important to analyze characteristics of enzyme activities and their relationships with microbial communities during litter decomposition processes.

Based on the above information, we conducted an in-situ decomposition experiment that lasted for a total of 460 days.*Imperata cylindrica* litter was selected as the research object in a copper tailings area within the southeastern region of the Loess Plateau, China. We explored dynamic characteristics of litter properties and extracellular enzyme activities during different litter decomposition stages as well as the bacterial community structure and diversity in litter using high-throughput sequencing. The objectives of the study were to answer the following questions: (1) How did decomposition rates and nutrient content change during *I. cylindrica* litter decomposition in a copper tailings area? (2) In what way did bacterial communities dynamically change during the litter decomposition process and will this result in a relational change among microbial communities? (3) What were the key bacteria and main environmental factors that affected litter decomposition?

MATERIALS AND METHODS

Site description and experimental design

The Shibahe copper tailings dam (lat 35°15'˜35deg17' N, long 118deg38'˜111deg39' E) has been in existence since 1969, being an outgrowth of the Northern Copper Mine in southern Shanxi Province, China. Today, the dam consists of 16 sub-dams (Fig. 1). Continental monsoon constitutes the climate of the region. Additionally, average annual temperature and annual precipitation are 13.5degC and 631 mm, respectively (Jia et al., 2020). Our experiments were conducted at three of the 16 sub-dams (i.e., S516, S536, and S560, with a respective phytoremediation history of 50, 22, and 5 years) (Table S1).

In April 2019, we collected nine *I. cylindrical* litter samples from each of the three sub-dams. A portion of these samples was used for high-throughput sequencing (initial litter, D0), while other potions were airdried and placed in litter decomposition bags (8 g of litter per bag). The size of the nylon mesh bags was 20 cmx20 cm, and the aperture was 1 mmx1 mm. In May 2019, three sample plots were established in each of the three sub-dams, and three litter bags were placed in each plot. This provided a total of 27 litter bags for collection purposes (Fig. S1). Litter samples were collected after being allowed to decompose for 100 days (D100), 200 days (D200), and 460 days (D460). This provided a total of 36 litter samples. Following collection, litter samples were divided in two where one sample was stored (-20degC) to be used for high-throughput sequencing and the other was stored (4degC) with the remaining litter samples to ascertain physiochemical property and enzyme activity data.

Mass residual rate and chemical properties of litter

Litter bags were collected, and sediment was removed. Following this, the fresh litter weight (Wi) was measured. Sample portions were dried to a constant weight (65degC), after which water content (P) was measured. The mass residual rate of litter was then calculated (Zhang et al., 2019). The formula used to calculate the mass residual rate was as follows: (%)=W_i*(1-P)/W_0. Here, W_0 was the dry weight of the initial litter bag.

An elemental analyzer (vario EL/MACRO cube, Elementar Analysensysteme GmbH, Hanau, Germany) was used to measure total carbon (TC) and total nitrogen (TN) in litter samples. To measure the litter pH status, litter and water suspensions (1:20 mass/volume) were agitated for 30 min (Fioretto et al., 2000). Heavy metal (copper [Cu], zinc [Zn], lead [Pb], and cadmium [Cd]) litter concentrations were measured using atomic absorption spectrometry (AAS) (Agilent Technologies 200 Series AA, USA). Additionally, 3,5-Dinitrosalicylic acid (colorimetry) was used to measure litter sucrose and cellulase, respectively; phenol-sodium hypochlorite (colorimetry) was used to measure urease; potassium permanganate titration was used to measure catalase; the disodium phenyl phosphate colorimetric method was used to measure phosphatase (Jia et al., 2019).

DNA extraction, PCR amplification, and MiSeq sequencing techniques

A sterile phosphate buffer solution (PBS: NaCl, KCl, Na_2HPO_4, and KH_2PO_4) was used to wash litter samples three separate times before being filtered through a sterilized membrane filter (0.2 μm pore size) (Millipore, Jinteng, Tianjin, China). Sterile centrifuge tubes were used to seal filtered samples, which were then used to extract microbial DNA. This study used the E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek Inc, Norcross, GA, USA) to extract microbial DNA in litter following the manufacturer's directions. Additionally, we used the NanoDrop ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) for the purpose of quantifying extracted DNA. Primers 799F (5'-AACMGGATTAGATACCCKG-3') and 1193R (5'-ACGTCATCCCCACCTTCC-3') were used to amplify the V5–V7 hyper variable region of the 16S rRNA bacterial gene. DNA sequencing was conducted at Shanghai Majorbio Bio-pharm Technology (Shanghai, China), using the MiSeq platform (Illumina, Inc., USA).

Statistical analysis

QIIME software was selected for the purpose of integrating the original FASTQ format sequencing data (Caporaso et al., 2010). The USEARCH tool (version 7.0;*http://drive5.com/usearch/*) was used to vet and remove chimeric sequences. The operational taxonomic unit (OTU) partition threshold was identified at a 97% sequence similarity of classification results, which was subsequently used for the calculation of bacterial community diversity and relative abundance. To obtain species classification data corresponding to each OTU, the RDP Classifier (*http://rdp.cme.*msu.edu) was used to classify and analyze each OTU sequence. The Silva 138 database was used to compare bacterial sequences, with a 70% reliability threshold.

IBM SPSS version 24.0 was used to analyze physiochemical litter properties during the different decomposition stages, while the Duncan's Multiple Range test was used in conjunction with one-way ANOVA. A Student's t -test was used to compare the bacterial diversity and richness of litter at the different predetermined decomposition stages. In this study, we used non-metric multidimensional scaling (NMDS) analysis to determine bacterial community structure based on Bray–Curtis dissimilarity, while we used analysis of similarities (ANOSIM) to determine differences in intergroups. Additionally, we used variance inflation factor (VIF) analysis to remove high multicollinearity in environmental factors applying the "vegan package" in R3.5.3. We then used Canoco 5.0 (Microcomputer Power, USA) for redundancy analysis (RDA). Finally, the interactive platform Gephi was used to examine and visually perceive networks. Properties of the co-correlation network, such as the average degree, the network density, the average clustering coefficient, the network diameter, and the average path length, were then calculated (Jiao et al., 2020). The higher the average degree, the average clustering coefficient, and the network density, the closer the network connection was. Additionally, a lower path length and network diameter was indicative of a closer network connection (Ma et al., 2016).

RESULTS

Litter decomposition characteristics

In this study, we found a higher decomposition rate of species (*I. cylindrical*) litter during the early stage of decomposition, but this decomposition rate decreased after 100 days of decomposition (Fig. S1.). There was a significant difference in the residual rate of litter among the different years of recovery in the sub-dams ($P < 0.05$). During the latter stage of decomposition, the residual mass of*I. cylindrica* litter was from 27.05% to 67.14%, and the residual mass rate of *I. cylindrica* litter in the S536 sub-dam was the highest overall, followed in descending order by the S560 sub-dam and the S516 sub-dam.

Litter properties and enzyme activities during decomposition

The nutrient content of *I. cylindrica* litter decreased as litter decomposition progressed in the different sub-dams ($P < 0.05$). The C/N ratio of litter during the three restoration stages reached a peak after 100 days of decomposition, after which it decreased significantly. During the early litter decomposition stage, the C/N ratio of litter in the S536 sub-dam was highest when compared to the other sub-dams. Additionally, Cu, Zn, Pb, and Cd content gradually accumulated as litter decomposition progressed, and heavy metals also increased as litter decomposition progressed ($P < 0.05$), except for Zn, which peaked after 200 days of decomposition before decreasing. Litter pH increased as decomposition progressed in the S516 sub-dam ($P < 0.05$), where litter pH reached its maximum after 200 days of decomposition in the S536 and S560 sub-dams (Table 1).

We found no consistent variation trend for enzyme activities in litter as decomposition progressed in the different sub-dams (Table. 2). In S536 and S560 sub-dams, catalase activity in litter after 100 of decomposition was significantly higher compared to the initial decomposition stage ($P < 0.05$), but cellulase activity in litter during the D0 stage was higher compared to the other decomposition stages (Table 2). Urease reached its maximum value at the D200 stage (Table 2). Sucrase activity in litter from the S536 sub-dam increased as decomposition progressed, while it firstly increased before decreasing as decomposition progressed in the S516 and S560 sub-dams. Moreover, sucrase activity in the S560 sub-dam was significantly higher compared to corresponding values in the other sub-dams after 200 days of decomposition ($P < 0.05$).

Taxonomic distribution and bacterial diversity

This study obtained a total of 698,541 high-quality sequences from all samples. Based on 97% sequence similarity, we obtained a total of 1,271 bacterial OTUs in litter samples. Both diversity and richness indices (i.e., the Shannon index and the Ace and Chao1 indices, respectively) of bacterial community litter increased after 460 days of decomposition (D460) in the S516 sub-dam ($P < 0.05$; Fig. 2A). The Shannon index reached a peak after 200 days of decomposition (D200) in the S536 sub-dam (Fig. 2B). In the S560 sub-dam, the Shannon, Ace, and Chao1 indices of the bacterial litter community increased as litter decomposition progressed ($P < 0.05$; Fig. 2C). On the whole, litter bacterial community diversity and richness in this copper tailings area significantly increased as litter decomposition progressed, reaching a maximum value after 460 days (D460) of decomposition ($P < 0.05$; Fig. 2D). The Venn diagram revealed that common OTUs during the different decomposition stages accounted for 7.79%–10.02% of total OTUs. Additionally, shared OTUs became more common during the intermediate and latter decomposition stages, accounting for 29.76% (S516), 35.82% (S536), and 41.38% (S560) of total OTUs, respectively. This indicated that bacterial community composition during the latter litter decomposition stage was similar (Fig. 3).

Bacterial community composition among the different litter decomposition stages

Proteobacteria and Actinobacteria were the dominant phyla found in litter during the different decomposition stages, and their relative abundance compared to the bacterial community as a whole reached 91.658%. Actinobacteria was dominant during the initial litter decomposition stage in the S516 and S536 sub-dams with relative abundances of 42.26% and 45.19%, respectively. Actinomycetes decreased as litter decomposition progressed, gradually being replaced by Gammaproteobacteria ($P < 0.05$) (Fig. 4A, Fig. 4B, and Fig. 5A). Gammaproteobacteria was the dominant bacteria found during the initial (D0) stage in the S560

sub-dam, with a relative abundance of 55.88%, which decreased as litter decomposition progressed ($P<$ 0.05), while Alphaproteobacteria increased as litter decomposition progressed ($P < 0.05$) (Fig. 5C). Overall, Alphaproteobacteria remained stable during the litter decomposition process, while Gammaproteobacteria reached a peak of 38.81% after 100 days of decomposition (D100) (Fig. 5D).

This study found significant differences in how dominant bacteria changed in the sub-dams investigated (Fig. 4). *Sphingomonas* and*Massilia* gradually decreased as litter decomposition progressed, while *Burkholderia* and *Rhodanobacter* increased in the S516 sub-dam (Fig. 4E). *Sphingomonas* and *Burkholderia* exhibited significant differences among the different decomposition stages (Fig. 5E). *Sphingomonas* and *Modestobacter* were the dominant bacterial groups during the initial stage (D0), and their relative abundances decreased as decomposition progressed in the S536 sub-dam (Fig. 5F). In the S560 sub-dam, *Marseille* strains were the dominant bacteria during the initial stage (D0) with a relative abundance of 47.167%, while it differed significantly among the different decomposition stages (Fig. 4G; Fig. 5G).

Correlation among the different bacterial communities

We constructed co-correlation networks of bacteria communities during different decomposition stages to investigate the different types of interactions among bacterial communities during the decomposition of*I. cylindrica* litter (Fig. 6). Each node represents a bacterial genus, and each edge represents a correlation between different bacteria genera (Fig. 6). The bacterial network diagram contained 161 nodes and 1081 edges during the initial stage (D0). Node and edge numbers reached a maximum during the D200 stage but decreased after 460 days of decomposition (D460). Overall, the number of positive correlated edges was greater than 99%, which indicated the dominance of bacterial species that coexisted together during the litter decomposition process (Table 3).

Topological network properties reflected the connectivity and interaction between microbial communities. During the litter decomposition process, the average degree, the average clustering coefficient, and the network density gradually decreased as litter decomposition progressed, indicating that the connectivity among the bacterial networks gradually weakened as litter decomposition progressed (Table 3). Additionally, given that modularity can reflect the modular structure, in this study modularity increased as litter decomposition progressed. Moreover, Alphaproteobacteria, Actinobacteria, and Gammaproteobacteria contained greater than 50% of all nodes in the network.

According to the betweenness centrality (BC) value, which can reflect the degree of node connections, the top five groups in this study changed as litter decomposition progressed (Table 4). Actinomycetes was the key bacterial group, and *Friedmanniella* had the highest BC value at the initial stage (D0), which was indicative of the important role that these bacterial groups played in the structure and function of the bacterial community within *I. cylindrica* litter. These key bacterial groups were gradually replaced by *Azohydromonas* and*Hirschia* as litter decomposition progressed. *Quadrisphaera*played an important role in maintaining the structure and function of the bacterial community during the D460 stage, being at the core of the bacterial correlation network (Table 4).

Bacterial community structure and environmental variable correlations

Bray–Curtis dissimilarity was used for NMDS analysis of bacterial communities during different decomposition stages. Results showed that litter decomposition had a significant impact on the structure of the bacterial community within litter ($P < 0.05$) (Fig. 7). Canonical correlation analysis (CCA) showed that both litter properties and enzyme activities had significant impacts on the bacterial community ($P < 0.05$), while driving factors affecting the bacterial communities differed during different recovery years (Fig. 8). The dominant environmental factors which affected the bacterial community structure of litter in the S516 sub-dam were Zn ($R^2 = 0.844$; $P = 0.001$), TC ($R^2 = 0.634$; $P = 0.001$), and Pb ($R^2 = 0.565$; $P =$ 0.024). In the S536 sub-dam, Cu ($R^2 = 0.772$; $P = 0.001$), C/N ($R^2 = 0.583$; P = 0.012), Pb ($R^2 = 0.581$; $P = 0.012$), and Cd ($R^2 = 0.489$; $P = 0.049$) mainly affected the bacterial community structure of litter. Both Cu ($R^2 = 0.888$; $P = 0.002$) and TC ($R^2 = 0.632$; $P = 0.019$) significantly affected the bacterial community of the S560 sub-dam (Fig. 8). Variance partitioning analysis (VPA) showed that litter properties

and extracellular enzyme activities accounted for 17.96% and 11.13% of changes in the bacterial community, respectively. Overall, the bacterial community structure of litter was mainly affected by litter properties, especially litter pH ($R^2 = 0.844$; $P = 0.001$) and Cu content ($R^2 = 0.772$; $P = 0.001$).

There were significant differences in key bacterial groups that provided important contributions to litter decomposition among the different sub-dams (Fig. 9). Dominant bacterial genera (i.e., *Variovorax* , *Quadrisphaera* ,*Frigoribacterium* , *Kineococcus* , and*Actinomycetospora*) were significantly and positively correlated to urease activity in the S516 sub-dam ($P < 0.05$), which indicated that they were of great significance to N cycling in this mining area. *Promicromonospora* and *Afipia* positively correlated to sucrase activity ($P < 0.001$) in the S536 sub-dam (Fig. 9A), which indicated that these microorganisms may promote cellulose decomposition. *Pseudomonas* , *Burkholderia* ,*Bacilus* , *Mycobacterium* , and *Bradyrhizobium*positively correlated to catalase ($P < 0.01$) (Fig. 9B), which indicated that these bacteria may play important roles in lignin decomposition. *Bordetella* and *Steroidobacter* positively correlated to heavy metal content. Furthermore, *Massilia*positively correlated to cellulase ($P < 0.05$), while*Streptomyces* , *Kribbella* , *Caulobacter* ,*Chryseobacterium* , and *Pseudonocardia* positively correlated to sucrase and catalase activities, which indicated that they played key roles in litter decomposition in the S560 sub-dam. *Lechevalieria*positively correlated to heavy metal content, which indicated they possessed a certain level of heavy metal tolerance (Fig. 9C).

DISCUSSION

Characteristics of litter decomposition and nutrient release

In this study, litter decomposition rates closely correlated to the chemical properties of litter, while total organic carbon (TOC), TN, total phosphorus (TP), and ecological stoichiometry in litter affected litter decomposition rates (Sun et al., 2019). It has been reported that litter decomposition rates positively correlate to initial litter TN and TP content but negatively correlate to TOC, lignin, and cellulose (Cao et al., 2016). In this study, the initial TN content of *I. cylindrica* litter in the S516 and S560 sub-dams was significantly higher compared to the S536 sub-dam, which can potentially contribute greater N sources for microbial growth. Litter has also been reported to be a key factor affecting nutrient release (Santonja et al., 2017). Brady et al. (1960) found that the C/N ratio determined changes in litter N content. Additionally, N fixation occurred in litter when the C/N ratio was greater than 25, while N release occurred when the C/N ratio was less than 25. In this study, the initial (D0) C/N ratio in litter was greater than 25 in the different sub-dams, but litter TN content significantly decreased in the first 100 days of decomposition. This could have resulted from the rate of soluble substance decomposition in litter through eluviation processes during the early decomposition stage, which led to the release of a large amount of nutrients. Litter N in the different sub-dams increased after 200 days of decomposition (D200), which could have resulted from the litter C/N ratio reaching a peak after leaching and N fixation concurrently occurring in litter.

Bacterial community succession during litter decomposition

The main decomposers of material circulation in this mining area were the dominant litter microorganisms that effectually determined the litter decomposition rates (Gavito et al., 2021). Proteobacteria was the dominant bacteria during the early litter decomposition stage and was therefore considered the early decomposer (Zhang et al., 2017). In this study, Proteobacteria was consistently observed to be the dominant bacterial phyla in all samples. Moreover, as decomposition progressed, the dominant Proteobacteria (primarily Gammaproteobacteria) was gradually replaced by Actinobacteria, which is consistent with findings from other studies (Purahong et al., 2016; Lu et al., 2019). Proteobacteria can utilize nutrients produced by organic decomposition, such as ammonia (NH_3) and methane (CH_4), for growth requirements and metabolic processes (Lv et al., 2014). Sugar, soluble starch, and cellulose in litter gradually decomposed alongside litter decomposition. This resulted in extensive amounts of lignin to accumulate, which favor microbial colonization during the latter litter decomposition stage (Bonanomi et al., 2019).

Microbial community succession was more obvious at a lower classification level during litter decomposition. During the early litter decomposition stage, *Sphingomonas* , *Massilia* ,*Methylobacterium* , and *Modestobacter* were the dominant bacterial genera, which were gradually replaced by *Allorhizobium* ,*Burkholderia*

, and *Streptomyces* as litter decomposition progressed. It has been reported that *Sphingomonas* is a common bacterial group found during the early decomposition stage. Moreover,*Sphingomonas* is able to utilize various types of organic matter, including soluble organic matter and complex organic matter (Fredrickson et al., 1995). *Burkholderia* is a typical lignin decomposing bacteria that secretes laccase (Tian and Zhang, 2017).*Streptomyces* can effectively decompose humus and lignin, promoting litter decomposition (Purahong et al., 2016). *Rhizobium*is an important N-fixing bacteria, and its enrichment in litter may potentially be an important reason for the increase in N content observed during the latter litter decomposition stage (Sellstedt and Richau, 2013). Additionally, our results showed a significant correlation between *Burkholderia* , *Streptomyces* , and*Trichoderma* and various extracellular enzymes, such as sucrase and cellulase, as well as various types of heavy metals, indicating that these bacteria possess a certain tolerance to heavy metals and are consequently of great significance to litter decomposition in heavy metal polluted areas.

Microbial interactions during litter decomposition

Results showed a decrease in bacterial network connectivity after 100 days of litter decomposition (D100); however, after 200 days of litter decomposition (D200) bacterial network connectivity increased, at which point the number of nodes and edges reached their highest values. It has been reported that complex networks have higher resource and information transmission rates as well as higher functional diversity compared to relatively simple networks (Wagg et al., 2019). This increased network complexity also improved tolerance to environmental interference, helping to stabilize the microbial community (Shang et al., 2018). Results also showed an increase in material transfer efficiency among bacterial communities, while their tolerance to environmental disturbances increased during the latter decomposition stage. The complexity of the bacterial network decreased to a certain extent during the latter decomposition stage. This phenomenon could potentially be due to the fragmentation and fracturing of litter during the decomposition process, which reduces direct interactions between bacterial communities (Wang et al., 2013). Nutrients in litter are consumed gradually, and resource competition among microorganisms intensifies as the decomposition progresses, which in turn leads to niche differentiation and an increase in community functional diversity (Wagg et al., 2019). Accordingly, the modularity of the bacterial network increased during the latter decomposition stage. Additionally, the average patch length reflects the degree of environmental interference (Barranca et al., 2015). The average path length of the bacterial network increased in this study, and the connectivity between bacterial communities increased as litter decomposition progressed, indicating that the resistance of bacterial communities to external environmental interference increased during the latter decomposition stage.

Litter bacterial community and litter factor relationships

Numerous ecological factors, including the quality of litter, the pH status, temperature conditions, and water content, will have a significant effect on the microbial community structure during litter decomposition (Chen et al., 2021; Chen et al., 2020a). A previous study reported that TN and TP were the driving factors that effected the bacterial community structure of *Robinia pseudoacacia* litter (Xu et al., 2020). Lignin content and the C/N ratio of litter have also been shown to significantly correlate to bacterial community structure (Chen et al., 2021). Our study observed significant correlations among nutrient content, heavy metal content, and the pH status of litter with the litter bacterial community. Moreover, Cu and pH were the main factors that drove changes of bacterial structure. The pH status was also a key environmental factor affecting the soil microbial community, which significantly influenced microbial community structure and functional pathways (Lucas-Borja et al., 2019). It has been reported that the pH status as well as the TN and TN content of litter have significant effects on bacterial community structure during litter decomposition (Wang et al., 2019a; 2019b), indicating the important role that pH plays in litter microbial community assemblages.

At low concentrations, heavy metals (i.e., Cu, Zn, Fe, and Mg) are required for microbial growth processes, while also participating in numerous processes associated with biological metabolism (Chen et al., 2020b). However, heavy metals effect microbial composition and functional genes, diversity, and structure at higher concentrations (Chen et al., 2018). Results from this study showed a negative correlation between *Sphingomonas* and *Marseille* strains and Cu and Pb within the bacterial community structure of litter while being

correlated to invertase and cellulase in litter, indicating that heavy metals can potentially have an effect on extracellular enzymatic activities by changing the composition of the bacterial community that subsequently affects litter decomposition.

CONCLUSIONS

In this study, we found that significant differences were found in the litter decomposition of the different sub-dams, while litter decomposition in the S536 sub-dam was slowest overall, which is attributed to its lower litter quality. Litter decomposition significantly enriched heavy metals, such as Cu, Zn, Pb, and Cd. In litter, the microbial community composition underwent obvious successional changes during the litter decomposition process. Alphaproteobacteria and Gammaproteobacteria were the initial litter bacteria but were gradually replaced by Actinomycetes as litter decomposition progressed. Bacterial community diversity increased as litter decomposition progressed, and their resistance to environmental interference gradually increased. During the different decomposition stages, this study observed significant differences in bacterial community structure. Finally, litter properties and enzyme activities significantly correlated to bacterial community composition. The main influencing factors that drove changes in the bacterial structure of litter were Cu content and the pH status.

AUTHOR CONTRIBUTIONS

TJ conceived and designed the experiments. TG and XL performed the experiments. BC contributed new reagents. TJ wrote the manuscript. All authors read and approved the manuscript.

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Figure legends

Fig. 1 Sample plots were set up in copper tailings area, and litter samples were collected after being allowed to decompose for 100 days (D100), 200 days (D200), and 460 days (D460).

Fig. 2 The dynamics of litter bacterial community diversity indexes in S516 (A); S536 (B); S560 (C) and the whole copper mining area (D). Significance levels were denoted with $^{**}P < 0.01$ and $^{***}P < 0.001$.

Fig. 3 Venn diagrams of litter bacteria at OTU level in S516 (A), S536 (B), S560 (C) and the whole copper mining area (D).

Fig. 4 Litter bacterial community compositions with relative abundance greater than 1% and 4% at class (A,B,C,D) and genus(E,F,G,H)level respectively in S516 (A,E), S536 (B,F), S560 (C,G) and the whole copper mining area (D,H).

Fig. 5 Difference of dominant bacteria classes (A,B,C,D) and genera (E,F,G,H)at four litter decomposition stages in S516 (A,E), S536 (B,F), S560 (C,G) and the whole copper mining area (D,H). Significance levels were denoted with $^{**}P < 0.01$ and $^{***}P< 0.001$.

Fig. 6 Change of bacterial community co-correlation network at D0 (A), D100 (B), D200 (C) and D460 (D). Each node represented a bacterial genus, and the line represented a significant correlation between two genera ($P < 0.001$). The nodes in the left figure of each decomposition stage were colored by class level, and the nodes in the right figure were colored by module. The top 10 litter bacterial classes and modules were defined, and the other microbial were defined as others.

Fig. 7 NMDS of litter bacterial community at four decomposition stages in S516 (A), S536 (B), S560 (C) and the whole copper mining area (D)

Fig. 8 CCA analysis of the top 5 bacterial genera and litter properties (A,B,C,D)and enzyme activities (E,F,G,H)and bacterial community structure in S516 (A,E); S536 (B,F); S560 (C,G) and the whole copper mining area (D,H), as well as the VPA analysis (I) of litter properties and enzyme activities for differences in litter bacterial communities.

Fig. 9 Correlation analysis of litter properties and dominant bacteria genera in S516 (A), S536 (B), S560 (C) and the whole copper mining area (D).

Table 1 Properties of litters at the different decomposition stages.

Sub-dam	Decomposition stage	TC %	TN %	C/N	Cu mg·kg^{-1}	Zn mg·kg
S516	D0	43.578 ± 0.083a	1.261 ± 0.052a	34.597 ± 1.470b	11.181 ± 0.290c	26.722 ±
	D100	30.771 ± 9.193b	0.463 ± 0.055c	65.482 ± 12.658a	54.480 ± 36.994b	38.150 ±
	D200	26.648 ± 8.792c	0.769 ± 0.159b	37.653 ± 21.821b	64.477 ± 7.681b	76.929 ±
	D460	16.300 ± 0.191c	0.652 ± 0.014b	25.002 ± 0.227b	175.200 ± 2.221a	65.466 ±
S536	D0	43.158 ± 0.113a	0.583 ± 0.097a	75.378 ± 12.721b	13.762 ± 1.225c	18.818 ±
	D100	25.407 ± 8.658ab	0.287 ± 0.055b	86.677 ± 14.834a	182.013 ± 77.180b	44.908 ±
	D200	18.382 ± 6.677bc	0.412 ± 0.040b	44.096 ± 14.342b	269.613 ± 94.172ab	64.273 ±
	D460	12.300 ± 4.594c	0.484 ± 0.174b	25.265 ± 1.193b	317.790 ± 4.654a	43.652 ±

Sub-dam	Decomposition stage	TC %	TN %	C/N	Cu mg·kg^{-1}	Zn mg·kg
S560	D0	37.722 ± 0.142a	1.188 ± 0.036a	31.761 ± 0.962b	37.097 ± 1.828b	31.299 ±
	D100	20.231 ±8.310bc	0.506 ± 0.178c	39.255 ± 3.638a	254.773 ± 90.431a	48.535 ±
	D200	15.885 ± 2.999c	0.511 ± 0.068c	30.960 ± 1.659b	309.157 ± 18.502a	54.914 ±
	D460	27.991 ± 2.308b	0.916 ± 0.062b	30.610 ± 2.363b	345.247 ± 56.583a	36.373 ±

Data are means ± standard deviations. The different case letters indicate that the means are significantly different among vegetation restoration stages ($P < 0.05$) with Duncan test.

Table 2 The extracellular enzyme activities of litters during the different decomposition stages.

Sub-dam	Decomposition stage	Urease (mg·(g·24 h)$^{-1}$)	Sucrase (mg·(g·24 h)$^{-1}$)	Cellulase (mg·(g·72 h)$^{-1}$)	Cellulase (mg·(g·20 min)$^{-1}$)
S516	D0	3.369 ± 0.019a	3.310 ± 0.644c	0.811 ± 0.059b	5.626 ± 0.706ab
	D100	0.037 ± 0.005c	9.691 ± 0.383a	0.222 ± 0.025c	3.160 ± 1.766b
	D200	2.966 ± 0.042b	4.769 ± 0.063b	1.101 ± 0.038a	5.086 ± 2.312ab
	D460	3.229 ± 0.112ab	1.737 ± 0.012d	0.221 ± 0.050c	7.321 ± 0.133a
S536	D0	3.760 ± 0.308b	3.072 ± 0.716c	0.772 ± 0.054a	3.237 ± 0.231c
	D100	2.385 ± 0.063d	3.559 ± 0.027c	0.481 ± 0.031c	8.863 ± 1.413a
	D200	4.661 ± 0.064a	4.823 ± 0.119b	0.525 ± 0.026b	5.857 ±1.335b
	D460	2.934 ± 0.042c	6.967 ± 0.040a	0.551 ± 0.023b	5.626 ± 1.355b
S560	D0	2.959 ± 0.331c	2.299 ± 0.330c	0.623 ± 0.033a	0.848 ± 0.133b
	D100	1.160 ± 0.032d	4.216 ± 0.057c	0.383 ± 0.040b	7.707 ± 0.267a
	D200	4.810 ± 0.045a	18.450 ± 0.268a	0.310 ± 0.050c	7.784 ± 0.667a
	D460	4.157 ± 0.027b	12.796 ± 0.051b	0.585 ± 0.021a	8.169 ± 0.812a

Data are means ± standard deviations. The different case letters indicate that the means are significantly different among vegetation restoration stages ($P < 0.05$) with Duncan test.

Table 3 Topological properties of litter bacterial community correlation network as decomposition progressed.

Topological properties	D0	D100	D200	D460
Nodes	161	241	299	212
Edges	1081	1208	1740	649
Average degree	13.429	10.025	11.639	6.123
Network density	0.084	0.042	0.039	0.029
Modularity	0.353	0.662	0.794	0.714
Average clustering coefficient	0.908	0.912	0.894	0.697
Average path length	1.081	1.114	1.062	3.411
Positive correlation	100%	100%	100%	99.690%

Table 4 Key bacterial genera in correlation network during the litter decomposition process.

Decomposition stage	Genus	Class	Betweenness centrality (BC)
D0	*Friedmanniella*	Actinobacteria	14.6
	Conexibacter	Thermoleophilia	12.0
	Nocardioides	Actinobacteria	9.6
	Quadrisphaera	Actinobacteria	6.5
	Aureimonas	Alphaproteobacteria	6.0
D100	*Azohydromonas*	Gammaproteobacteria	24.0
	Modestobacter	Actinobacteria	21.0
	Paenibacillus	Bacilli	18.5
	Thiobacillus	Gammaproteobacteria	12.0
	Ellin6055	Alphaproteobacteria	11.5
D200	*Hirschia*	Alphaproteobacteria	11.5
	norank_f_Fimbriimonadaceae	Fimbriimonadia	11.0
	Ellin6055	Alphaproteobacteria	10.0
	Pedomicrobium	Alphaproteobacteria	9.5
	Sphingomonas	Alphaproteobacteria	8.0
D460	*Quadrisphaera*	Actinobacteria	1074.8
	MND1	Gammaproteobacteria	678.7
	Aureimonas	Alphaproteobacteria	656.9
	OM27_clade	Bdellovibrionia	550.0
	norank_f_Reyranellaceae	Alphaproteobacteria	550.0

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