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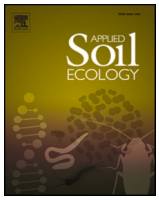

A less complex but more specialized microbial network resulted in faster fine-root decomposition in young stands of *Robinia pseudoacacia*

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ABSTRACT

Microorganisms play an important role in litter decomposition. Bacteria and fungi colonize litter during the decomposition process; therefore, understanding the interactions of bacterial and fungal communities can yield insight into litter decomposition dynamics. Most studies have focused on leaf litter decomposition, but thus far, there has been little information on the dynamic changes in soil microbial composition at the different stages of fine-root decomposition. Therefore, we conducted a 210-day incubation experiment on the fine roots of *Robinia pseudoacacia* to explore how the temporal dynamics of soil enzyme activities and microbial communities are related to the decomposition of *R. pseudoacacia* at four different stand ages (15, 25, 35 and 45 years) during vegetation restoration. The results showed that the fine-root decomposition rate was higher for young stands of *R. pseudoacacia*. We found that the oxidase activity was significantly higher in the young stands (15 and 25 years) than in the old stands (35 and 45 years), while the hydrolase activity showed the opposite trend. The main reason for the higher decomposition rate in young stands of *R. pseudoacacia* was the high oxidase enzyme activities and less complex but more specialized microbial network of younger stands. The occurrence network was used to identify the keystone taxa by statistical analysis, and the key taxa changed from Acidobacteria to Proteobacteria during vegetation restoration. These findings demonstrate that vegetation restoration alters the soil microorganism community and network structure during fine-root decomposition of *R. pseudoacacia*. This finding is of great significance for understanding the role of microorganisms in regulating fine-root decomposition dynamics.

1. Introduction

Plant litter decomposition is one of the key processes driving carbon and nutrient cycling in ecosystems (Guo et al., 2021; Krishna and Mohan, 2017). It is estimated that root litter accounts for 48 % of the annual plant litter input, which is larger than the proportion accounted for by leaf litter (Freschet et al., 2013). As an active part of roots, fine roots (diameter < 2 mm) have a high turnover rate and are the largest contributor to root litter (McCormack et al., 2015; Xia et al., 2015). However, our understanding of the role of fine-root decomposition on soil carbon and nutrient cycling is still inadequate relative to that of the decomposition of aboveground litter.

To date, many studies have been conducted to explore the factors driving fine-root decomposition. The classic theory suggests that climate, substrate quality, and soil microorganisms regulate litter decomposition (Beidler and Pritchard, 2017; Bradford et al., 2015). Climate is a dominant factor on a large scale (Guo et al., 2021), while the effects of substrate quality, enzyme activities, and the soil microbial community on fine-root decomposition occur at a local scale (Dong et al., 2020; Fu et al., 2022; Solly et al., 2014; Wang et al., 2019b). Roots are composed of a variety of complex organic compounds, such as lignin, cellulose, and hemicellulose (Poirier et al., 2018), and their decomposition requires the action of enzymes. Hydrolases such as β-glucosidase can be used to depolymerize cellulose, and oxidases such as phenol oxidase and peroxidase can be employed to degrade lignin (Margida et al., 2020). Bacterial and fungal communities differ in substrate preference and strategies for nutrient acquisition (Wang et al., 2019a). Proteobacteria are involved in plant decomposition at an early stage, while Actinobacteria and Firmicutes can decompose plant detritus in a continuous process. Some groups of Actinobacteria and Firmicutes are

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fast-growing bacteria that can secrete β-glucosidase and xylanase and participate in the degradation of plant polymers (Wang et al., 2020b). As the main decomposers of litter, fungi are the main group of microorganisms that secrete hydrolases and oxidases (Floudas et al., 2012). For example, cellobiohydrolase can remove monomers and dimers at the end of the cellulose chain, while β-glucosidase converts glucose cellobiose dimers to glucose via hydrolysis (Pérez et al., 2002). Phenol oxidase and peroxidase, produced by Basidiomycetes fungi, participate in lignin degradation (Zheng et al., 2021). Although the dynamics of extracellular enzyme activities and microbial succession have been studied for leaf litter, the mechanism of fine-root decomposition has received scant attention in recent years.

Because the microbial community plays a vital role in the decomposition of litter, the composition of the microbial community may be one of the factors affecting the decomposition rate of fine roots. Differences among the stages of vegetation restoration may determine the initial microbial composition and thus affect the enzyme activities related to fine-root decomposition (Zhang et al., 2022). Previous studies investigated the correlation of litter properties and enzyme activities with the microbial community (Liu et al., 2020; Xu et al., 2020); however, this relationship was obtained from one sampling. To date, there remains a paucity of evidence on the variations in the dynamics of soil extracellular enzyme activities and the microbial community with the dynamics of fine-root decomposition. In addition, whether microbial network structure and connectivity differ under different environmental conditions during fine-root decomposition remains unclear. Fine-root decomposition is a complex process that involves many interactions among microorganisms, and the results of decomposition can be influenced by these interactions. Network analysis is suitable for determining the combined abundance of microorganisms, obtaining a comprehensive understanding of microbial community structure and assembly patterns, and further identifying keystone taxa (Banerjee et al., 2018). Degree and centrality can be used to reveal the close groups in the network and to identify the keystone taxa (Banerjee et al., 2016; Banerjee et al., 2021). Keystone taxa play a special and important role in the microbial community, and removing them affects the structure and function of the microbial community (Romdhane et al., 2021). To date, however, the response of microbial networks to the dynamics of fine-root decomposition in *Robinia pseudoacacia* plantation restoration is still unclear.

High erodibility, steep terrain, and human activities cause serious water and soil erosion on the Loess Plateau (Wang et al., 2017). The Chinese government has implemented a series of measures to restore vegetation since 1950, and in 1999, the Grain for Green program was implemented, which effectively restrained the further deterioration of soil and water (Fu et al., 2017). *R. pseudoacacia* plays a significant role in vegetation restoration on the Loess Plateau because of its strong nitrogen fixation ability, high growth rate, and drought tolerance (Feng et al., 2017; Jiao et al., 2018). To date, most studies have focused on the leaf litter decomposition of *R. pseudoacacia*, while few reports have examined the dynamics of the underground components. The production and decomposition of aboveground and underground litter is a key process linking plants and soil (Zhang et al., 2013), and the soil net carbon balance depends on the balance of exogenous organic matter (litter and root exudates) input and decomposition. Understory biomass and litter biomass increased simultaneously under vegetation restoration (Zhang et al., 2019a; Zhang et al., 2019b), and fine root yield and biomass also increased with stand age (Chen et al., 2016; Zhang et al., 2013). Therefore, understanding the decomposition of fine roots during vegetation restoration is of great importance for understanding soil carbon sequestration on the Loess Plateau.

The objectives of this study were to assess the potential changes in soil enzyme activities and the microbial community during the recovery of *R. pseudoacacia* plantations and how these changes affect the decomposition of fine roots of *R. pseudoacacia* at different ages. To control the influence of other factors and focus on the changes in microorganisms, we conducted a 210-day (d) incubation experiment in a climate chamber. We hypothesized that (1) the fine-root decomposition rate would gradually decrease with *R. pseudoacacia* plantation restoration and that (2) the variation in the soil microbial community would be the major factor driving the changes in fine-root decomposition. To test the above hypotheses, we investigated the dynamics of fine-root decomposition and nutrient release, changes in lignocellulosic enzyme activity, and interactions between bacterial and fungal communities during *R. pseudoacacia* plantation restoration and further identified keystone groups involved in fine-root decomposition at different ages during vegetation restoration.

2. Materials and methods

2.1. Study site

Sampling was performed in Huaiping Forest Farm in Yongshou County, southern Shaanxi Province, Loess Plateau, in July 2020 (34°12′12″-34°50′50″N, 108°05′7″-108°05′11″E) (Table S1). Yongshou County lies in the hill and gully region of the Loess Plateau, which has a warm temperate continental climate. The average annual temperature in this area is 10.8 °C, and the average minimum and maximum temperatures are −2.9 °C and 23.7 °C, respectively. This area belongs to the temperature subhumid climate zone, with an average annual precipitation of 605 mm, and >60 % of the precipitation occurs from July to September (Liu et al., 2017). The altitude ranges from 1123 m to 1464 m, and the soil type is loessial (Liu et al., 2020). The Huaiping forest farm was founded in 1958, covering an area of 64.55 km^2. It is one of the main afforestation areas in Yongshou County. Vegetation restoration in Huaiping Forest Farm has experienced human interference. Currently, there are few natural secondary forest species in this area, such as *Quercus liaotungensis* and *Betula platyphylla*, and the major plantation species are *R. pseudoacacia* and *Pinus tabuliformis*. There was a relatively comprehensive chronosequence for *R. pseudoacacia*. After talking with the local elderly and looking up references, we chose stands with ages ranging from 15 years to 45 years old for the survey. The understory vegetation recovered well, and the dominant herb species were *Rubus parvifolius, Aster tataricus, Humulus scandens, Leonurus japonicus, Carpesium abrotanoides, Artemisia argyi, Chenopodium album* and *Artemisia selengensis*.

2.2. Sampling and processing of samples

We chose *R. pseudoacacia* stands with four different ages as the experimental sites, and three plots of 20 m × 20 m were established at each site. Table S1 shows detailed information on the topography, climate and vegetation of each site. Soil samples were taken from 12 plots from the four stands of different ages (three independent replicate plots for each stage). After removing the surface litter and humus in each plot, nine soil samples were randomly taken from a depth of 0–20 cm in each replicated plot. The subsoil samples from one plot were mixed to form one sample, yielding a total of 12 soil samples. We then removed roots and other debris through 2 mm sieves and mixed samples from the same vegetation restoration stages. Fresh soil samples were divided into two groups: one was stored at 4 °C and − 80 °C to measure soil enzyme activities and the soil microbial community, and the other was used to perform fine-root decomposition experiments. Air-dried soil was used to measure the basic physical and chemical properties (Table S2). At the same time, we sampled fine roots from the stands of four different ages. Because there is a high need for fine roots, we identified fine roots through color, smell and the presence of nodules and directly dug them out. Vernier calipers were used to measure root diameter, and when the diameters were >2 mm, the roots were removed. The remaining fine roots were taken back to the laboratory, cleaned with distilled water and dried to constant weight in a 60 °C oven. One part was used to measure the elemental content, and the others were stored at room temperature

for the decomposition incubation experiment.

2.3. Fine-root decomposition incubation

Fine-root decomposition was conducted in microcosms (10 cm in height and 9 cm in diameter) with small pots. Fine roots of each plantation age were incubated in the soil collected at the corresponding stand age. To prevent the loss of fine and small soil particles and maintain water infiltration, mesh nylon cloth was used to wrap the bottom of each pot. Fifteen pots were set for each stand age, and all the pots were filled with fresh soil equivalent to 300 g of oven-dried soil. Then, the corresponding fine roots (2 g) were evenly distributed into 150-μm nylon mesh bags (7.5 cm × 7.5 cm) and buried at a depth of 4–5 cm. This allowed microorganisms to pass while preventing the loss of fine roots via decomposition. Soil moisture was maintained at 60–70 % field capacity. Every two days, the pots were weighed, and water was added to maintain soil humidity. A dark, closed, and humid climate chamber was chosen to culture fine roots at 25 °C. The pots were removed from each treatment at sampling times of 0 d, 30 d, 110 d, 154 d and 210 d, and a total of 60 samples (4 plantation ages × 5 sampling times × 3 replicates = 60 samples) were obtained in our study. Then, the fine roots were dried in an oven to a constant weight at 60 °C to measure the fine-root elemental content, and the corresponding soil samples were collected to measure soil enzyme activities, soil microbial community composition and other basic physical and chemical properties.

2.4. Determination of the physical and chemical properties of fine roots and soil

We analyzed the carbon, nitrogen, and phosphorous contents of fine roots by soil agrochemical analysis (Bao, 2000). The cellulose, hemicellulose, and lignin components of the fine roots were extracted in a series of neutral and acid detergents and analyzed in accordance with the National Energy Laboratory Procedure (NREL) (Rowland and Roberts, 1994). The soil water content was determined by the drying method in an oven, and the soil bulk density was determined by the cutting-ring method. The soil pH was measured with a pH meter, and the soil organic carbon content was measured by a potassium dichromate oxidation process. The soil total nitrogen content was determined by the Kjeldahl method, and the soil phosphorus content was determined by the $HClO_4$-H_2SO_4 method. The soil soluble carbon and nitrogen levels were determined by a total organic carbon (TOC) analysis instrument, and the ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N) levels were measured by using a continuous flow analysis system (Autoanalyzer 3, Bran and Luebbe, Germany). Soil particles were analyzed by a laser particle size analyzer.

2.5. Determination of enzyme activities

Root litter consists of cellulose, hemicellulose, and lignin. Therefore, we measured the activities of two potential soil hydrolases and oxidases involved in the degradation of lignin and cellulose: β-1,4-glucosidase (BG), cellobiohydrolase (CBH), peroxidase (PERX), and phenol oxidase (PPO). The microplate fluorescence method was used to measure all hydrolase activities, and a microplate spectrophotometer was used for all oxidases (DeForest, 2009; Saiya-Cork et al., 2002). The detailed determination methods were as follows: the activities of the two hydrolases were measured with black 96-well plates. Samples, enzyme substrates, reference standards, and Tris-HCl buffer were placed in specific wells in the plate. One gram of fresh soil sample was dissolved in 50 ml of buffer. The sample well contained 150 μl of soil suspension and 50 μl of enzyme substrate. The blank well contained 150 μl of soil suspension and 50 μl of buffer. The negative control well contained 150 μl of buffer and 50 μl of enzyme substrate. The quenching well contained 150 μl of suspension and 50 μl of standard substance. The reference standard well contained 50 μl of standard substance and 150 μl of buffer. The standard substance for BG and CBH was 4-methylumbelliferone (MUB), and the substrates were 4-MUB-β-D-glucoside and 4-MUB-cellobioside, respectively; the incubation times were 2 h and 4 h, respectively. The reaction was stopped with 10 μl of 1.0 M NaOH solution, and then, the fluorescence was measured with excitation at 365 nm and emission at 450 nm (Spectra Max M2, Molecular Devices, California, USA). The unit of enzyme activity was nmol h^{-1} g^{-1}. The enzyme substrate for oxidase was L-DOPA, and oxidase activity measurement was similar to the method used for hydrolase activity. The absorbance value at 450 nm was determined by a spectrophotometer to quantify the activity.

2.6. MiSeq sequencing of soil fungal and bacterial communities

The fungal ITS1 region was amplified using ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′). Bacterial 16S rRNA genes were amplified using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR mixtures contained 5 × *TransStart* FastPfu buffer (4 μl), 2.5 mM dNTPs (2 μl), 5 μM forward primer (0.8 μl), 5 μM reverse primer (0.8 μl), *TransStart* FastPfu DNA Polymerase (0.4 μl), template DNA (10 ng), and ddH_2O (to a final volume of 20 μl). The PCR amplification protocol was as follows: initial denaturation at 95 °C for 3 min, followed by 27 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 45 s and a final extension at 72 °C for 10 min, ending at 4 °C. The PCRs were performed in triplicate. The PCR products were extracted from a 2 % agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, USA). The V4 region of 16S rRNA and ITS1 region of fungi were sequenced using the Illumina MiSeq PE300 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

The raw sequence reads were demultiplexed, quality-filtered by fastp (https://github.com/OpenGene/fastp, version 0.20.0) (Chen et al., 2018) and merged by FLASH (http://www.cbcb.umd.edu/software/flash, version 1.2.7) (Magoč and Salzberg, 2011). We used UPARSE (http://drive5.com/uparse/, version 7.1) to cluster operational taxonomic units (OTUs) with 97 % similarity and to identify and remove chimeric sequences (Edgar, 2013). The taxonomy of each representative OTU sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/, version 2.2) against the 16S rRNA database (v138, https://www.arb-silva.de/) and Unite database (Release 8.0 https://unite.ut.ee/) using a confidence threshold of 70 % (Wang et al., 2007).

2.7. Data analysis

2.7.1. Fine-root decomposition and soil enzyme activities

Fine-root decomposition (FRD) was calculated as the fine-root mass remaining relative to the initial fine-root mass in the nylon bags (Fu et al., 2021). We calculated the decomposition values of fine roots for different stand ages of *R. pseudoacacia* per day during the decomposition period. Soil enzyme activities were expressed as nmol g^{-1} h^{-1} during data processing. We used repeated-measures ANOVA to compare the differences in fine-root decomposition and soil enzyme activities for *R. pseudoacacia* stands of different ages.

2.7.2. Microbial community

We analyzed the OTU richness of fungi and bacteria. According to the Bray–Curtis dissimilarity in the normalized OTU data, we used principal coordinate analysis (PCoA) to assess microbial β-diversity, and the 'Adonis' function of the 'vegan' package was used for significance tests with R. 3.6.2. In addition, repeated-measures ANOVA was performed to compare the effect of different stand ages of *R. pseudoacacia* on microbial OTU richness at different decomposition times.

2.7.3. Network analysis and keystone taxa

Network analyses were performed to assess the complexity of the microbial community and to identify potential keystone taxa involved in fine-root decomposition at different ages. To avoid false correlations, we chose the top 300 dominant bacterial OTUs and the top 100 dominant fungal OTUs. Spearman rank correlation was used to assess the association of microbial OTUs at all sampling times. We used the *psych* package to calculate the correlation coefficients of the networks in R. A p value <0.01 and a coefficient of correlation >0.8 were considered statistically robust (Zheng et al., 2021). Then, we generated the networks. The visualization of the network was performed on the Gephi interactive platform (Bastian et al., 2009). We chose the undirected network (edges without direction) and *Fruchterman-Reingold* layout. Related network node scores and detailed parameters, such as degree, path length, diameter, and closeness centrality, were obtained from Gephi. We used high degree and high closeness centrality values to statistically identify keystone taxa. After Z score transformation of the sum of these two values, the top 20 OTUs with the highest Z score were used to identify keystone taxa. In addition, the network topological properties of each sample were obtained with the subgraph function via the 'igraph' package as described by Ma et al. (2016) and Qiu et al. (2021).

Pearson's correlation was used to quantify the relationships between fine root decomposition indicators and soil enzyme activities, microbial richness, community composition and network properties. In addition, we used partial least squares path modeling (PLSPM) to evaluate the direct and indirect relationships between stand age, decomposition time, hydrolase enzyme activities (BG and CBH), oxidase enzyme activities (PERX and PPO), microbial community composition (represented by the first principal components of PCoA), microbial network properties (node numbers and average path length), and fine root decomposition indicators (fine root decomposition, content of carbon, content of nitrogen and content of phosphorus) using the R package "PLSPM." The model fit was assessed based on the goodness of fit (GOF).

3. Results

3.1. Fine-root decomposition dynamics

Fine roots (collected from the same stands as soils for incubation) at different restoration times showed different decomposition parameters. The lowest decomposition rate was observed for 45-year-old (45Y) fine roots (Fig. 1 a), while the other three stands presented higher decay rates at 110 d ($F = 18.325$, $P < 0.001$) and 154 d ($F = 18.325$, $P < 0.001$). After 210 d of fine-root decomposition, the stands of different ages (15 years–45 years) showed decay rates of 47.5 %, 51.5 %, 52 % and 43.83 %. The fine-root decomposition rate constant for 45Y was significantly lower than that for the other three stand ages ($P = 0.019$, Fig. S1). Both different vegetation restoration years and decomposition times had

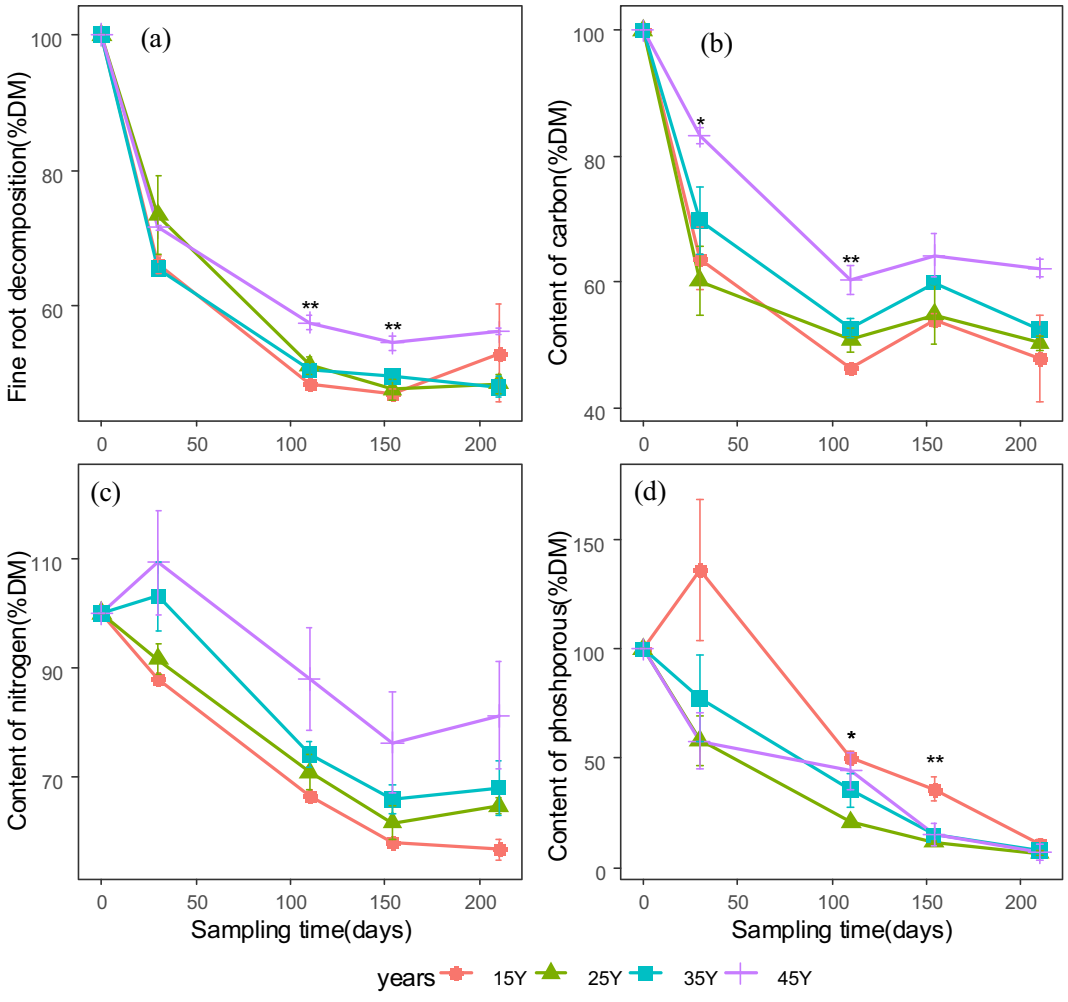

Fig. 1. Fine-root decomposition and changes in fine root quality in 15Y (red), 25Y (green), 35Y (blue) and 45Y (purple). Fine-root decomposition was calculated as the percentage of fine root mass remaining at each decay time (a). Figures b-d indicate changes in carbon (b), nitrogen (c), and phosphorus (d) content levels. * and ** indicate significant differences at $P < 0.05$ and $P < 0.01$, respectively. Error bars represent the standard error (n = 3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significant effects on fine-root decomposition (Table S4, $P < 0.05$).

The variation in fine root nutrients demonstrated differences among the different stand ages of *R. pseudoacacia* (Fig. 1 b-d). The levels of carbon, nitrogen, and phosphorus all showed a decreasing trend during fine-root decomposition. The carbon and nitrogen levels of the 45Y fine roots were higher than those of the other three stands, and the nitrogen content of 45Y fine roots showed higher values at 30 d ($P = 0.031$, F = 4.960) and 110 d ($P = 0.003$, F = 11.735) (Fig. 1b). Levels of phosphorous decreased rapidly during the early stage (110 d, $P = 0.038$, F = 4.550), and the results were significantly different at 110 days and 154 days for all four stand ages (Fig. 1d, 110 d: P = 0.038, F = 4.550; 154 d: $P = 0.008$, F = 8.077) (Fig. 1d). Both restoration years and decomposition time had significant effects on fine-root quality variation (Table S4).

3.2. Dynamics of enzyme activities

The activities of peroxidase, cellobiohydrolase, phenol oxidase, and β-1,4-glucosidase differed significantly across the four stand ages (Fig. 2a-d). The activities of peroxidase and phenol oxidase were significantly higher in the young stands (15Y and 25Y) than in the old stands (35Y and 45Y) (Fig. 2a, c). The hydrolase activities of cellobiohydrolase and β-1,4-glucosidase showed the opposite trend: they were higher in old stands (35Y and 45Y) than in young stands (15Y and 25Y), and they were significantly different at the later stages. All enzyme activities showed the lowest values at a sampling time of 110 d.

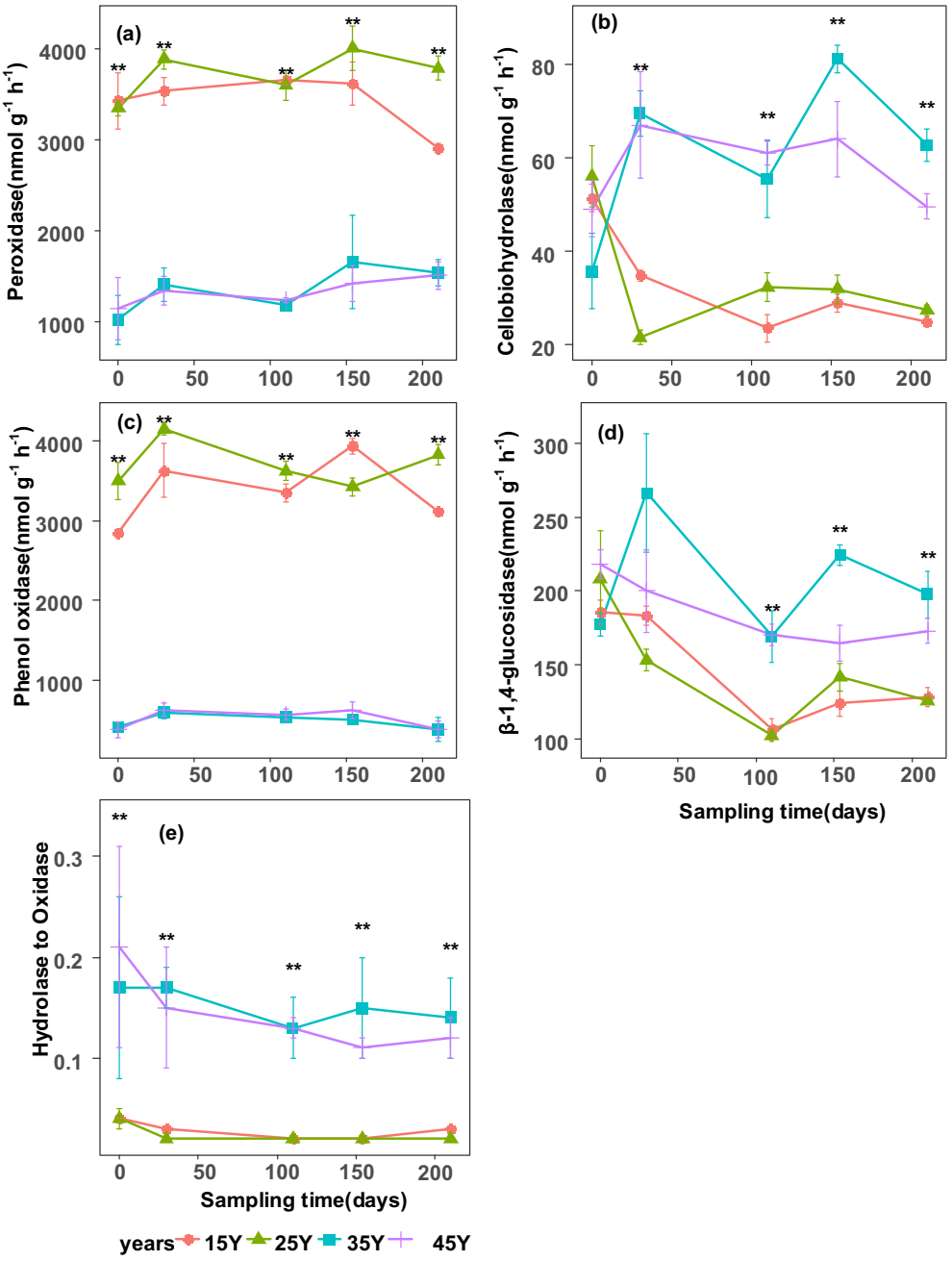

Fig. 2. Changes in peroxidase (a), cellobiohydrolase (b), phenol oxidase (c), β-1,4-glucosidase (d) activities, and hydrolase to oxidase ratio (e) in *R. pseudoacacia* at different stand ages. The unit of enzyme activities was nmol g^{-1} h^{-1}. ** represents significant difference at $P < 0.01$ level. The error bars represent the standard error ($n = 3$).

3.3. Microbial community succession during fine-root decomposition

Both bacterial and fungal OTU richness changed significantly during fine-root decomposition (Table S5). The initial sample represents the field soil from *R. pseudoacacia* stands of different ages, while the other samples were obtained during the decay process (30–210 d). The bacterial OTU richness decreased immediately after fine-root decomposition (Fig. 3a). After 30 d of decomposition, the richness of bacterial OTUs of 15Y—35Y increased with time and was significantly higher than the richness of OTUs of 45Y at 110–154 d, peaking at 110 d. However, the richness of bacterial OTUs of 45Y decreased with time (Fig. 3a). The bacterial community of *R. pseudoacacia* varied significantly among different stand ages ($P < 0.001$, Fig. 3c), and these differences were noticeable at each sampling time point (Fig. S2 and Table S6). Actinobacteria, Proteobacteria, Acidobacteria and Chloroflexi were the dominant bacterial phyla in all samples (Fig. 3e), which showed different trends at different stand ages. In summary, these results showed that different soil bacterial groups vary with stand age at specific stages during fine-root decomposition.

Vegetation restoration time had a significant effect on the bacterial and fungal OTU richness (Table S5). Similar to that of the bacterial community, the OTU richness of fungi decreased immediately after decomposition. The OTU richness of fungi in 15-45Y decreased with time after 30 d, and the OTU richness in 45Y was significantly lower than that in 15-35Y at 0 d (Fig. 3b). The fungal community of *R. pseudoacacia* varied significantly among different stand ages ($P < 0.001$, Fig. 3d), and these differences were noticeable at each sampling time point (Fig. S3 and Table S6). Ascomycota, Mortierellomycota, and Basidiomycota were the dominant fungal phyla in all samples (Fig. 3e), showing different trends among different stand ages.

3.4. Co-occurrence networks and keystone taxa

There were significant differences in the complexity of the microbial networks of the four different *R. pseudoacacia* plantations. The networks for the first three stand ages (15-35Y) had lower edges (514–684), lower average degrees (4.16–4.80) and lower average cluster coefficients (0.19–0.31) (Fig. 4 and Table 1). Compared with the 15-35Y stands, 45Y had a more complex microbial network with more edges (3366), a higher average degree (20.52), and a higher average cluster coefficient (0.51) (Fig. 4 and Table 1). The main bacterial taxa belonged to Acidobacteria, Actinobacteria, Chloroflexi, Proteobacteria, Firmicutes, and Gemmatimonadota, while the fungal taxa belonged to Ascomycota and Basidiomycota (Table S7). To assess the effect of removing key OTUs on the microbial network, we removed 20 key OTUs and reconstructed the microbial network (Fig. S4 and Table S8). The results showed that the microbial network complexity decreased without these key OTUs, and removing 20 key OTUs made the important parameters (such as nodes, edges, average degree values and average cluster coefficients) simpler, having an especially strong effect on the microbial network of 45Y *R. pseudoacacia*.

3.5. Relationship of fine root decomposition, enzyme activities, microbial community composition and microbial network properties

The correlation analysis showed that fine root decomposition (represented by fine root mass remaining) was significantly positively correlated with soil β-1,4-glucosidase activities, fungal richness and fungal community composition but negatively correlated with microbial network properties (such as node number) (Fig. 5, $P < 0.05$). In addition, there were significant positive correlations between fine root qualities (C and N) and soil hydrolase activities, fungal richness and microbial community composition (Fig. 5 and Fig. 6, $P < 0.05$). However, fine root qualities were negatively correlated with oxidase activities, node numbers, and average path length.

The PLS-PM results showed that stand age significantly negatively affected oxidase enzyme activities, microbial network properties and fine root decomposition indicators. Time had a negative influence on fine root decomposition indicators. Soil enzyme activities and microbial community composition significantly positively affected fine root decomposition indicators, while soil microbial network parameters significantly negatively affected fine root decomposition indicators. Stand age and decomposition time jointly regulated fine root decomposition by influencing soil enzyme activities and microbial traits (Fig. 6).

4. Discussion

Soil microorganisms play an important role in fine-root decomposition. Our previous studies demonstrated that the litter decomposition rate decreased with vegetation restoration (Zhong et al., 2017; Zhong et al., 2018). However, it is unclear whether fine-root decomposition has a similar trend. Our study showed that stand age had an effect on fine-root decomposition, and the fine roots of young stands tended to decompose more quickly than those of older stands. Analysis showed that oxidase activity was significantly higher in the young stands (15Y and 25Y) than in the old stands (35Y and 45Y), while hydrolase activity showed the opposite trend. The higher decomposition rate of fine roots in young stands was mainly because of higher hydrolase enzyme activities and a less complex but more specialized microbial network.

The oxidative enzyme activities were much higher in younger stands, the hydrolase activity was higher in older stands, and the hydrolase: oxidase ratio was much higher in older stands throughout the incubation period (Fig. 2e). These patterns may be attributed to two reasons: (1) differences in pH values among the four stands of different ages may have led to differences in enzyme activities. A previous study demonstrated that there is a positive relationship between pH and peroxidase and phenol oxidase activities (Sinsabaugh, 2010). In our study, the pH values were 7.97, 8.03, 6.57, and 6.68, respectively, from 15Y to 45Y (Table S2); therefore, the higher soil pH in the young stands may have led to higher oxidase activities. (2) Young stands have much higher oxidase activities, while older stands have much higher hydrolase activities. The higher oxidase activities of young stands indicate that microorganisms in young stands are more limited by carbon and need more energy supply (Lashermes et al., 2016). Microbial communities were colimited by carbon and phosphorous in all vegetation restoration types (Cui et al., 2019), and P limitation increased with the restoration time of *R. pseudoacacia* (Zhang et al., 2019b). Therefore, the microorganisms secreted more hydrolases to mine soil organic matter for nitrogen and phosphorus nutrients, leading to much higher hydrolase activities in older stands than in young stands. The difference in oxidase and hydrolase activities among different stand ages may be attributed to different microbial resource utilization strategies and the trade-offs of microorganisms acquiring nutrients and energy at the different stages.

Soil enzymes are the main drivers of plant residue decomposition in most ecosystems (Allison and Vitousek, 2004; Shen et al., 2021). However, the relationship between fine-root decomposition and enzyme activities in stands of different ages is unclear. A previous study demonstrated that the litter decomposition rate increased with vegetation restoration (Zhang et al., 2022). Improvement in litter quality drives the variation in fungal community composition and then accelerates litter decomposition. Moreover, studies have shown that the litter decay rate gradually decreases when vegetation succession climaxes. The findings indicated that the difference in the litter decomposition rate may be attributed to different litter qualities (C:P ratio), soil nutrient availability, and soil enzyme activities (Liu et al., 2021; Zhang et al., 2022; Zhong et al., 2017). Our study found that fine root decomposition rates decreased with vegetation restoration, which may be attributed to the following three reasons. First, stand age influenced fine root decomposition by regulating soil enzymes. Oxidase activities decreased and hydrolase activities increased with vegetation restoration (Fig. 2). Both oxidase activities and hydrolase activities were directly

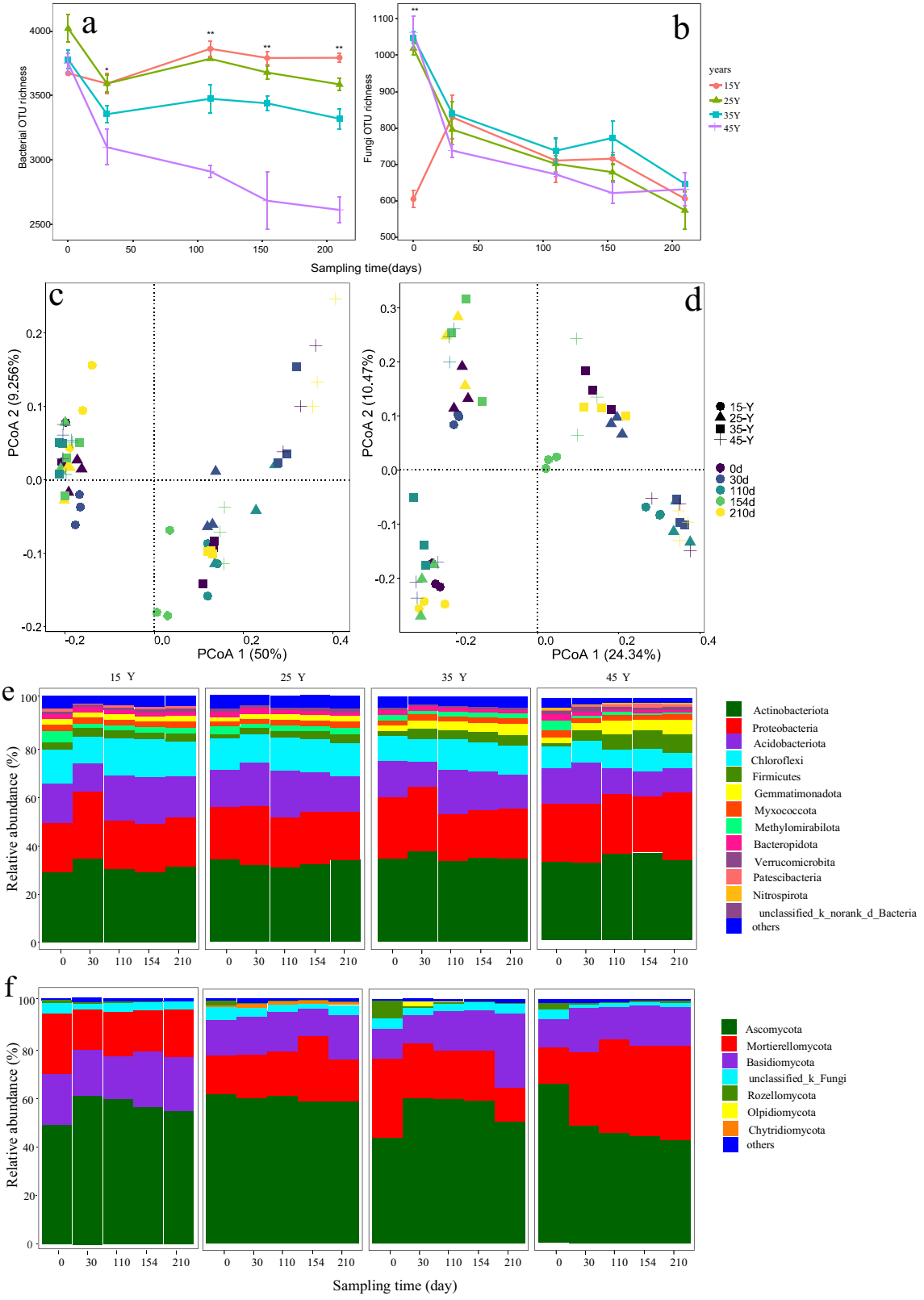

Fig. 3. Variations in bacterial and fungal OTU richness, community structure, and taxonomic composition during the 210 d fine-root decomposition experiment at different stand ages. Bacterial (a) and fungal (b) richness as shown by OTU richness. Changes in bacterial (c) and fungal (d) community structure (at the phylum level) were assessed by principal coordinate analysis (PCoA) of the UniFrac distance matrix. Changes in the relative abundance of the most dominant bacterial (e) and fungal (f) phyla. * and ** represent significant differences at $P < 0.05$ and $P < 0.01$ levels, respectively (Duncan's test). Error bars indicate the standard error (n = 3).

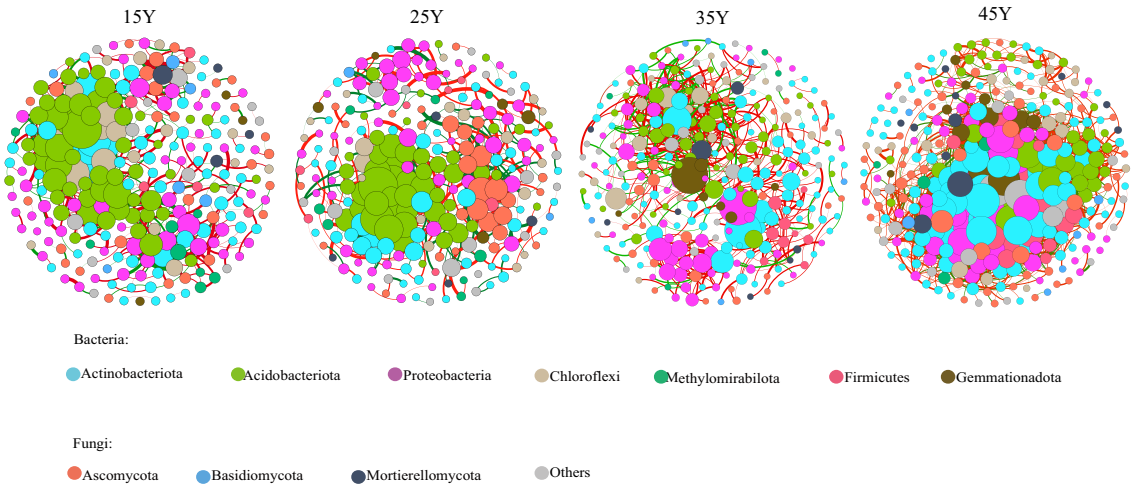

Fig. 4. Bacterial and fungal OTU co-occurrence networks during vegetation restoration. The Spearman correlation coefficients ($r > 0.8$ or $r < -0.8$ significant at $P < 0.01$) are shown. Nodes are colored according to phylum; red edges represent positive a correlation, and green edges represent a negative correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Network parameters and keystone taxa at four different succession ages of *R. pseudoacacia.*

Network	Nodes/edges	Avg. degree	Char. path length	Diameter	Clust. coeff.	Positive links (%)	Negative links (%)	Keystone taxa
15Y	252/605	4.80	5.37	15	0.31	67.77	32.23	Acidobacteria
25Y	247/514	4.16	5.36	14	0.28	64.98	35.02	Acidobacteria
35Y	294/684	4.65	5.01	13	0.19	75.88	24.12	Acidobacteria
45Y	328/3366	20.52	3.52	11	0.51	59.57	40.43	Proteobacteria

positively regulated fine root decomposition indicators (Fig. 6), but oxidase activities (0.5538) had a higher direct positive influence on fine root decomposition than hydrolase activities (0.1848). Previous studies have demonstrated that higher enzyme activities result in faster litter decomposition (Fan et al., 2019; Lin et al., 2019). Therefore, higher oxidase enzyme activities at a younger age may lead to higher fine root decomposition. Second, stand age influenced fine root decomposition by regulating microbial network complexity. Network topological properties (such as node numbers, edges, average degree) increased with vegetation restoration (Table 1), and the topological properties were negatively related to fine root decomposition indicators (Fig. 5), which showed that a less complex but more specialized microbial network resulted in faster fine-root decomposition in young stands of *R. pseudoacacia*. In addition, stand age also affected fine root decomposition by influencing microbial community composition (Fig. 6), which is consistent with results reported by Lin et al. (2021). Microbial species redundancy may exist in fine root decomposition; therefore, changes in microbial richness may not be as significant as changes in community composition during fine root decomposition (Martínez et al., 2020). Hence, stand age not only directly regulated fine root decomposition indicators but also significantly affected the soil enzyme activities, microbial community composition, and microbial network properties, all of which jointly regulate fine root decomposition in vegetation restoration.

During the different stages of litter decomposition, the dynamic variations in the soil bacterial and fungal communities depend on the mutual interactions of oligotrophs and copiotrophs (Lian et al., 2019; Rezgui et al., 2021; Sauvadet et al., 2019). The copiotroph groups, such as Firmicutes and Ascomycota, were enriched (except for Ascomycota, which was depleted in 45Y), while oligotroph groups, such as Acidobacteria and Chloroflexi, were depleted after fine-root addition, which indicated that microbial patterns changed based on the obtained resource and nutrient status. A previous study demonstrated that Actinobacteria and fungal communities play different roles in fine-root decomposition (Fu et al., 2021). We found that Actinobacteria dominated the bacterial community and Ascomycota dominated the fungal community. A previous study demonstrated that Actinobacteria and Alphaproteobacteria are the largest contributors to genes encoding lignocellulose-degrading enzymes (Wang et al., 2020a). As the main participators in early litter decomposition, Ascomycota species play a vital role in fine-root decomposition (Zhan et al., 2021). The abundance of Ascomycota decreased while the abundance of Basidiomycota increased during the decomposition process, which may be because of the reduction in the levels of easily available compounds and the remaining complex compounds, and Basidiomycota species are better at using complex compounds than Ascomycota species (Wang et al., 2019c). In summary, these findings demonstrate that differences in microbial groups resulted in variations in fine-root decomposition.

Microbial community composition influences litter decomposition, and litter composition influences the microbiota. Generally, high-quality litter (with higher nitrogen content and lower lignin/N) is susceptible to degradation (Eastman et al., 2022; Guo et al., 2021). In our study, the chemical composition of fine roots with increasing restoration years showed significant differences in the TN and cellulose content and lignin/N ratio (Table S3, $P < 0.05$). As a low-quality litter with a lower N content and higher lignin/N ratio, the fine roots of 15-year-old stands were more difficult to decompose. Bacteria and fungi have different substrate preferences (Wang et al., 2019a). Basidiomycota can form large mycelia and secrete various enzymes to degrade recalcitrant substrates (Purahong et al., 2016). Therefore, the relative abundance of Basidiomycota was higher in the young stands (Fig. 3f). In addition, with litter C and N rapidly released in the early stages, more recalcitrant substrate occurred in the late stages (210 days). Microorganisms with low abundance gradually become dominant, and these changes may reflect the selection of populations under carbon/energy or environmental pressure (Moitinho et al., 2018). This indicated the role of the substrate composition in microbial selection to some extent.

Network analysis can be used to study why some species appear

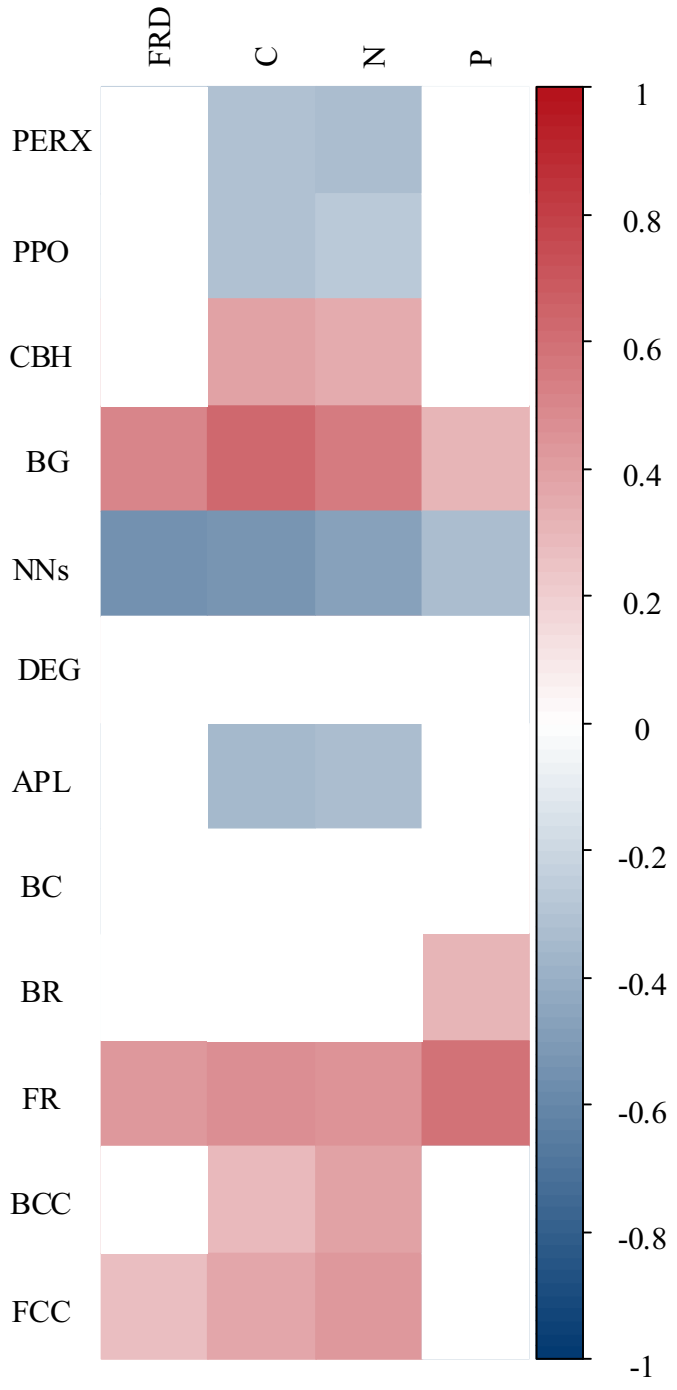

Fig. 5. Correlation analysis of fine root decomposition indicators (FDR, C, N and P) with enzyme activities (PERX, PO, CBH, and BG), microbial network properties (node numbers, degree, average path length, and betweenness centralization), microbial richness (fungal OTU richness and bacterial OTU richness), and microbial community composition (bacterial and fungal community composition). Red indicates a positive correlation, blue indicates a negative correlation, and the color depth indicates Pearson coefficients $p < 0.05$, and the blank colour indicates $p > 0.05$. FRD: fine root decomposition, C: content of carbon; N: content of nitrogen; P: content of phosphorus. PERX: peroxidase, CBH: cellobiohydrolase, PPO: phenol oxidase, BG: β-1,4-glucosidase. NNs: node numbers; DEG: degree; APL: average path length; BC: betweenness centralization; BR: bacterial OTU richness; FR: fungal OTU richness; BCC: bacterial community composition; FCC: fungal community composition. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

together in an ecological niche and to statistically identify the high-connectivity taxa in a community (Ge et al., 2021; Wu et al., 2022). In our study, we explored the variations in the soil microbial network structure at different stand ages during fine-root decomposition, and the results demonstrated that there were differences in the topology of the cooccurrence network. Specifically, the nodes, edges, diameters, and cluster coefficients of the microbial network were higher in the young stands (15-35Y) than in the old stands (45Y). The number of edges and nodes increased in the process of vegetation restoration, which indicated that more links and complex networks were formed in the older stands than in the young stands (Moitinho et al., 2018; Zheng et al., 2021). In other words, stand age influenced fine root decomposition by regulating microbial network properties. It is possible that a less complex but more specialized microbial network causes faster fine-root decomposition in young stands of *R. pseudoacacia*. A previous study demonstrated that oligotrophic groups such as Acidobacteria might prefer nutrient-poor environments (Zhong et al., 2020), while many Proteobacteria are thought to be copiotrophic and well adapted to nutrient-rich conditions (Jiang et al., 2021; Zhong et al., 2020). The changes in keystone groups from 15Y to 45Y indicated that vegetation restoration improved the previously poor soil environment and made the structure of the soil bacterial community develop in a direction conducive to soil quality (Lu et al., 2022; Zhong et al., 2020). In addition, Wang et al. (2020a) reported that Betaproteobacteria was the major class involved in the decomposition of mixed litter. Our study also identified some keystone taxa involved in fine root decomposition, and much work needs to be conducted to further confirm their abilities to decompose litter via subsequent culturing.

In addition, network analysis can be used to study how potential ecological networks are affected by the environment (Banerjee et al., 2016). For example, a network with a short path length can transmit environmental fluctuations to the whole network in a short time, thus rapidly changing the structure and function of the whole network (Barranca et al., 2015). This is especially true for networks of older stands. We found that the path length and modularity were lower in the older stands than in the younger stands (Table 1 and Table S8), and the average links and cluster coefficient were relatively high in the older stands (Table 1), indicating that the network easily responded to environmental variations in the older stands (Zhan et al., 2021).

The interaction in the network comes from a large microbial modularity (Banerjee et al., 2016), which can be seen as a functional unit of the microbial community and potential niche (Chaffron et al., 2010). Network interactions have many positive and negative correlations within and between bacterial and fungal modules. In our decomposition process, positive correlations decreased and negative correlations increased from 15Y to 45Y (Table 1). Niche differentiation may lead to much antagonism in the microbial network during the late stage of decomposition, which shows that there is stronger resource competition between microorganisms. These links and complex networks demonstrated that there was much higher interaction among soil bacteria and fungi in older stands (Pan et al., 2021). In short, network analysis revealed changes in the microbial niche (Purahong et al., 2016), and these variations may affect the process of fine root decomposition.

Overall, our study showed that the microbial community of *R. pseudoacacia* plantations has complex characteristics. Different decay stages have different decomposition patterns due to differences in microbial taxa. This can be seen as an ecological network, representing a transformation from a simple network to a complex network. However, it should be noted that the keystone taxa identified from network analysis are based on statistical data, and it is necessary to further complement these data with experiments to identify keystone taxa and verify the ability of the microbial community to decompose fine roots.

5. Conclusion

Our results demonstrated that enzyme activities associated with

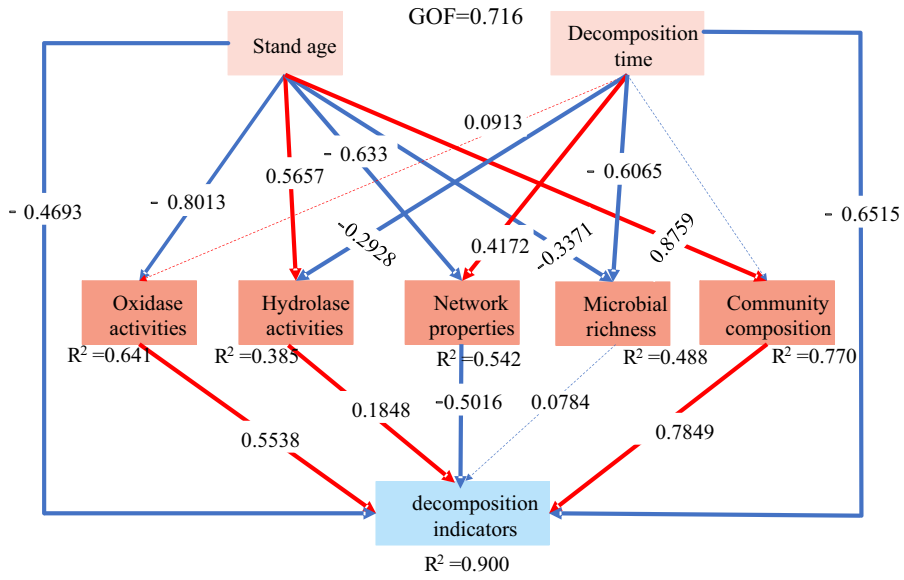

Fig. 6. A Partial Least Squares Path Modeling (PLSPM) of stand age, decomposition time, enzyme activities, microbial community composition, microbial network properties and fine root decomposition indicators. Solid thick arrows indicate a significant difference ($P < 0.05$). Red indicates a positive correlation, blue indicates a negative correlation. The model was evaluated using goodness of fit (GOF). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

lignocellulose degradation, the microbial community and network patterns varied during the fine-root decomposition process and were driven by vegetation restoration age. The higher decomposition rate of fine roots in young stands was mainly attributed to the higher oxidase enzyme activities and less complex but more specialized microbial network in younger stands. In addition, we identified the keystone taxa in the microbial cooccurrence network, with Acidobacteria replaced by Proteobacteria along the vegetation restoration chronosequence. Consequently, our findings revealed that soil microbial succession mediated by vegetation restoration determines fine-root decomposition.

Declaration of competing interest

This article has no conflict of interest and has not been published in other journals.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2022.104735.

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