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# Influence of thinning on carbon storage mediated by soil physicochemical properties and microbial community composition in large Chinese fir timber plantation

Lei Huang<sup>1,2,3</sup> and Yunchao Zhou<sup>1,3\*</sup>

## Abstract

**Background** Thinning practices are useful measures in forest management and play an essential role in maintaining ecological stability. However, the effects of thinning on the soil properties and microbial community in large Chinese fir timber plantations remain unknown. The purpose of this study was to investigate the changes in soil physicochemical properties and microbial community composition in topsoil (0–20 cm) under six different intensities (i.e., 300 (R300), 450 (R450), 600 (R600), 750 (R750) and 900 (R900) trees per hectare and 1650 (R1650) as a control) in a large Chinese fir timber plantation.

**Results** Compared with the CK treatment, thinning significantly altered the contents of soil organic carbon (SOC) and its fractions but not in a linear fashion; these indicators were highest in R900. In addition, thinning did not significantly affect the soil microbial community diversity indices but significantly affected the relative abundance of the core microbial community. Proteobacteria, Acidobacteria, and Actinobacteria were the dominant bacterial phyla; the relative abundances of Proteobacteria and Acidobacteria were highest in R900, and that of Actinobacteria was lowest in R900. The dominant fungal phyla were Ascomycota, Basidiomycota and Mucoromycota; the relative abundance of Ascomycota was lowest in R900, and that of Mucoromycota was highest in R900. The fungal microbial community composition was more sensitive than the bacterial community composition. The activity of the carbon-cycling genes was not linearly correlated with thinning, and the abundance of C-cycle genes was highest in R900.

**Conclusions** These findings are important because they show that SOC and its fractions and the abundance of the soil microorganism community in large Chinese fir timber plantations can be significantly altered by thinning, thus affecting the capacity for carbon storage. These results may advance our understanding of how the density of large timber plantations could be modified to promote soil carbon storage.

**Keywords** Chinese fir, Carbon cycling, Thinning, Soil characteristics, Microbial community

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## Background

Soil carbon (C) storage is considered to be the greatest form of carbon storage in terrestrial ecosystems and has received much interest in recent years because of its potential for carbon storage and its important role in the global carbon cycle [1]. Soil organic carbon (SOC) is an important component of carbon storage. Cotrufo and Lavallee [2] reported that the C pool contained



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fast-cycling SOC and slow-cycling SOC; fast-cycling SOC mainly comprises plant derivatives, and slow-cycling SOC primarily comprises microbial necromass. Nevertheless, SOC is very sensitive to environmental variations, such as management measures and climate variability [1, 3]. Thinning is a primary measure of forest management that changes stand density, soil properties and understory vegetation, which ultimately affect soil nutrients and microbial activity and thus affect carbon storage in forest ecosystems [1, 4, 5]. Thinning can significantly affect plant communities and then influence litter, roots and plant exudates, which directly affect SOC and its fractions, thus affecting the structure of the soil microbial community in forest ecosystems [6]. In contrast, soil microorganisms release mineral elements near the rhizosphere to affect the growth of plants [7]. Owing to the complex interactions among thinning, soil physicochemical and microbial communities, the relationships among them are still largely unknown; thus, determining the effects of thinning practices on the soil carbon storage capacity is necessary.

Soil microorganisms are involved in most soil ecological processes, such as atmospheric CO<sub>2</sub> fixation, energy flow, soil fertility and carbon cycling [8–10]. Studies have reported that the effects of thinning on microbial diversity are based on different forest types, thinning times, and thinning intensities [11, 12]. Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) is an excellent fast-growing species that is widely used in southern China as a commercial wood source, with the largest planting area among artificial forests in China [13]. Owing to its special quality, it is considered an essential factor in the timber supply for human society, environmental protection, and maintaining stability in the global ecosystem [14]. During the past few decades, many Chinese fir trees have been planted, and some plantations, including large timber tree plantations, have been preserved. However, after decades of growth, high stand density, a low-diversity plant community and poor regeneration capacity are the main problems for maintaining forest ecosystems. Therefore, proper management measures should be taken to achieve sustainable development of these ecosystems. Previous studies have reported that forest thinning changes microbial activity, fine root production, and biomass, resulting in effects on the carbon inputs and outputs of Chinese fir [15, 16]. On the basis of these findings, we hypothesized that (1) thinning may influence the soil properties and microbial community of large Chinese fir timber plantations and that (2) the function of carbon cycling in Chinese fir ecosystems can be affected by thinning. However, the influence of forest thinning on soil microbes in large Chinese fir timber plantations

has not yet been investigated. Furthermore, how soil characteristics vary with stand density and the main drivers of carbon storage remain unknown.

In this study, eighteen experimental plots were subjected to six intensities of thinning in a 28-year-old large Chinese fir timber plantation, that is, 300 (R300), 450 (R450), 600 (R600), 750 (R750), 900 (R900) and 1650 (R1650, control treatment) trees per hectare. Five years after thinning, the soils under the different thinning intensities were studied to investigate (1) the variations in SOC and its fractions, the microbial community structure, and soil C storage under different the intensities; (2) the association between soil carbon storage and microorganism communities; and (3) the functional genes involved in C-cycling under the different intensities.

## Materials and methods

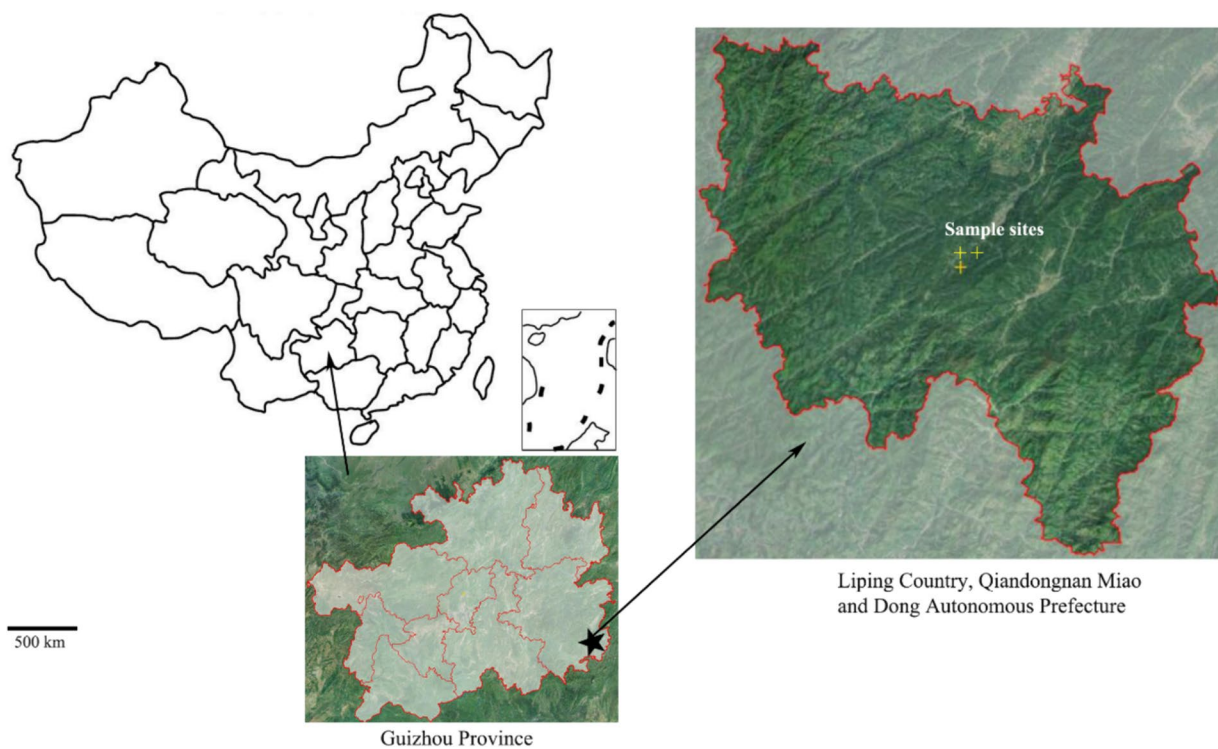
### Experimental site and thinning treatments

The study was performed in a 28-year-old Chinese fir plantation at Shijingshan State-owned Forest Farm (with 2797.17 hm<sup>2</sup> of pure Chinese fir forest) (109° 7′ 14″ E, 26° 11′ 17″ N) in Liping County, Southeast Guizhou Province, China (Fig. 1). The plantation has rich biodiversity, including *Taxus chinensis*, *Eurya nitida* Korthals, *Smilax china* L., *Michelia figo*, *Acer oblongum*, *Kalopanax septemlobus* and several other plant species with crucial scientific value and ecological status in China. The soil type is characterized by yellow or red-yellow soil. The area has a central subtropical monsoon humid climate with a mean temperature of 15.6 °C and annual average precipitation of 1325.9 mm, and the elevation is between 650 and 750 m.

Eighteen experimental plots (20 m × 30 m) were subjected to six thinning treatments in a completely randomized design with three replicates of each treatment. Each plot has a 15-m-wide perimeter buffer zone in the middle. The eighteen experimental plots had the same initial tree number before thinning. The thinning treatments were conducted in October 2017 after all small trees (with a breast diameter < 15 cm) and trees in close proximity (distance < 50 cm) were removed, with the exception of those showing strong growth. The residual stand densities after thinning were 300 (R300), 450 (R450), 600 (R600), 750 (R750), and 900 (R900) Chinese fir trees per hectare. The remaining three plots without thinning were used as controls (1650 trees per hectare).

### Soil sample collection

Forest soil sampling was conducted in September 2022. Ten sampling points were randomly chosen in each treatment. The topsoil (0–20 cm soil depth) was collected with a cutting ring (5 cm in diameter, 10 cm deep) to obtain soil samples. The 10 soil samples from each plot



**Fig. 1** Map showing sampling location of the study. The “+” indicates the sample sites

were thoroughly mixed to obtain one composite soil sample for each stand via a soil auger, resulting in 18 soil samples from the six different thinning densities. Roots were carefully removed from the soil samples, insects and gravels were removed, and the samples were then sieved through a 2 mm mesh. Each soil sample was mixed and divided into three portions: one portion was air-dried at room temperature and stored for soil physicochemical detection, one portion was stored at 4 °C for the analysis of microbial carbon (MBC) and organic carbon contents, and the third portion was stored at –80 °C for microbial DNA extraction and high-throughput sequencing [17–19].

#### Determination of soil physicochemical factors

SOC was detected via potassium dichromate oxidation with external heating. The concentration of easily oxidizable carbon (EOC) was determined in accordance with the process of Carter [20]. In brief, dissolved organic carbon (DOC) was determined from fresh soil with deionized water at a 1:5 ratio (soil:water) and detected with a multi-N/C 3100 TOC analyzer [21]. The concentration of particulate organic carbon (POC) was analyzed according to the methods of Elliott and Cabbardella [22]. Heavy fraction organic carbon (HFOC) and light fraction organic carbon (LFOC) analyses were performed

according to the methods of Wu et al. [23]. In addition, soil microbial biomass carbon (MBC) was extracted from fresh soil samples via chloroform fumigation—extraction and measured via a total organic carbon (TOC) analyzer [24, 25].

#### Microbial necromass C

Microbial necromass C is a component of SOC, and its content (fungal and bacterial necromass C) was measured via the method of Zhang and Amelung [26]. Amino sugar compounds were detected via a chromatographic column (250 mm\* 4.6 mm, 5 μm) on a gas chromatograph. We tested glucosamine (GlcN) and muramic acid (MurA) in the microbial cell wall, and the concentrations of microbial necromass carbon were calculated via the following equations:

$$\text{Bacterial necromass C} = 45 \times \text{MurA} \times 10^3$$

$$\begin{aligned} \text{Fungal necromass C} \\ &= (\text{GlcN}/179.12 - 2 \times \text{MurA}/251.23) \\ &\times 179.17 \times 9 \times 10^3 \end{aligned}$$

where 45 is the conversion factor from MurN to the bacterial necromass C, and 251.23 and 179.17 are the

molecular weights of MurA and GlcN, respectively. In addition, 9 represents the conversion value of fungal GlcN to fungal necromass. The microbial necromass C was calculated as the sum of the bacterial necromass C and fungal necromass C. The GlcN-to-MurA ratio represents the proportion of fungal and bacterial residue contributions to the SOC content.

#### DNA extraction and sequencing

Total DNA from each soil sample was extracted via a FastDNA Spin Kit according to the manufacturer's instructions. The quality and quantity were analyzed via 1% agarose gel electrophoresis and TBS-380, respectively, and the DNA purity was tested via a NanoDrop 2000 (Nanodrop Technologies Inc., CA, USA). And the qualified samples were sent to Shanghai Majorbio Biopharm Technology Co., Ltd. (Shanghai, China) to obtain metagenomic sequence data via Illumina HiSeq 4000 instruments.

#### De novo metagenomic assembly and functional annotations

The adaptor sequences and poor-quality regions of the raw data were removed via Trimmomatic software (version 0.35). A total of 18 separate assemblies were obtained via de novo assembly. Contigs and scaffold sequences were constructed via De Bruijn graph, and a comprehensive summary of assembly statistics was generated for the different metagenomes. CD-HIT-EST software was used to remove the duplicated and suboptimal contigs, resulting in the final assembly of each soil sample. Furthermore, the complete metagenomic sequences (length  $\geq 300$  bp) were submitted to the National Center for Biotechnology Information (NCBI) database (No. PRJNA994818).

The BLAST analyses of the unigenes were performed via the nonredundant protein sequence database (NR) and the Kyoto Encyclopedia of Genes and Genomes

(KEGG) repositories via Diamond software [27]. The annotated genes were subsequently assigned to different functional hierarchies via the KEGG Orthology (KO) database. Furthermore, the results of BLAST analyses of the unigenes were analyzed via the carbohydrate-active enzymes database (CAZy) by Hmmscan (<http://hmmer.janelia.org/search/hmmscan>).

#### Statistical analyses

The differences in the physiochemical properties of the soil and the relative abundances of functional genes were analyzed via one-way ANOVA and the Kruskal–Wallis test. The differences among the different thinning intensities were compared via Tukey's test.  $P < 0.05$  was considered significant. Constrained redundancy analysis (RDA) software was used to analyze the correlations between environmental changes and microbial communities. Nonmetric multidimensional scaling (NMDS) software was used to analyze the soil microbial communities based on Bray–Curtis distance metrics. A heatmap was constructed with TBtools.

## Results

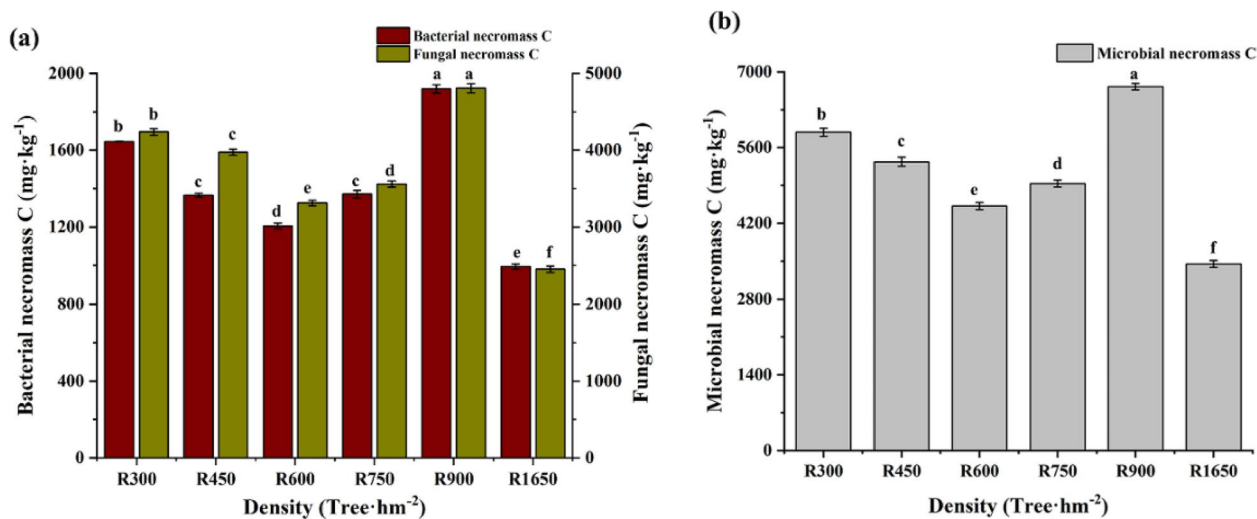
#### Soil physiochemical analyses under the different thinning densities

The soil characteristics significantly differed under the different thinning intensities (Table 1), and they did not display a linear relationship. Interestingly, all the tested soil physiochemical indicators were highest in R900, i.e., 35.71 g kg<sup>-1</sup> (SOC), 13.61 g kg<sup>-1</sup> (POC), 2.62% (EOC), 66.17 mg kg<sup>-1</sup> (DOC), 25.87 g kg<sup>-1</sup> (HFOC), 9.69 g kg<sup>-1</sup> (LFOC) and 6508.9 mg kg<sup>-1</sup> (MBC). Compared with those in R1650, the SOC content in R900 was 85.89% higher, the POC content was 86.69% higher, the DOC content was 36.63% higher, the LFOC content was 26% higher, the HFOC content was 134.54% higher, and the MBC content was 11.71% higher. These results may

**Table 1** Content of soil carbon fractions of large Chinese fir timber plantations under the different thinning densities

	R300	R450	R600	R750	R900	R1650
SOC (g kg <sup>-1</sup> )	27.75 ± 1.94b	24.33 ± 1.63c	20.40 ± 0.87d	23.54 ± 0.89e	35.71 ± 2.77a	19.21 ± 0.92f
POC (g kg <sup>-1</sup> )	8.95 ± 0.14c	12.20 ± 0.98b	4.96 ± 0.14f	6.73 ± 0.32e	13.61 ± 0.34a	7.29 ± 0.21d
EOC (%)	1.56 ± 0.11b	1.33 ± 0.14d	1.49 ± 0.16c	1.51 ± 0.10c	2.62 ± 0.18a	1.56 ± 0.08b
DOC (mg kg <sup>-1</sup> )	39.18 ± 0.36e	40.98 ± 1.85d	29.97 ± 0.96f	63.22 ± 2.50b	66.17 ± 1.55a	48.43 ± 0.79c
HFOC (g kg <sup>-1</sup> )	20.61 ± 0.50b	19.58 ± 0.78c	14.54 ± 0.35e	17.54 ± 0.35d	25.87 ± 0.94a	11.49 ± 0.46f
LFOC (g kg <sup>-1</sup> )	7.16 ± 0.30c	4.82 ± 0.14f	5.93 ± 0.40e	6.02 ± 0.25d	9.69 ± 0.20a	7.68 ± 0.21b
MBC (mg kg <sup>-1</sup> )	5938.5 ± 50.7b	5271.5 ± 136.9e	5011.5 ± 89.3f	5910.0 ± 99.4c	6508.9 ± 110.8a	5826.9 ± 101.1d

Soil organic carbon-SOC. Particulate organic carbon-POC. Easily oxidizable carbon-EOC. Dissolved organic carbon-DOC. Heavy fraction organic carbon-HFOC. Light fraction organic carbon-LFOC. Microbial biomass carbon-MBC. R300, R450, R600, R750, R900 and R1650 indicates trees of Chinese fir are 300, 450, 600, 750, 900 and 1650 per hectare, respectively. Treatments with different letters are significantly different at  $P < 0.05$



**Fig. 2** Changes in microbial necromass C under the different thinning intensities. Treatments with different letters are significantly different at  $P < 0.05$

indicate that R900 is more beneficial for SOC fractions than the other intensities are.

#### Contents of microbial necromass C under the different thinning intensities

The microbial necromass C varied significantly under the six different thinning intensities ( $P < 0.05$ , Fig. 2b) and was highest in R900, which contributed to an essential portion of the SOC. The content of the microbial necromass C was lowest in R1650, which may indicate that high-density trees were not beneficial for microorganism processes. However, the change trends of fungal necromass C were similar to those of microbial necromass C (Fig. 2a), whereas the change trends of bacterial necromass C differed. In addition, there was no significant difference in the bacterial necromass C among R750, R900 and R1650 ( $P > 0.05$ , Fig. 2a).

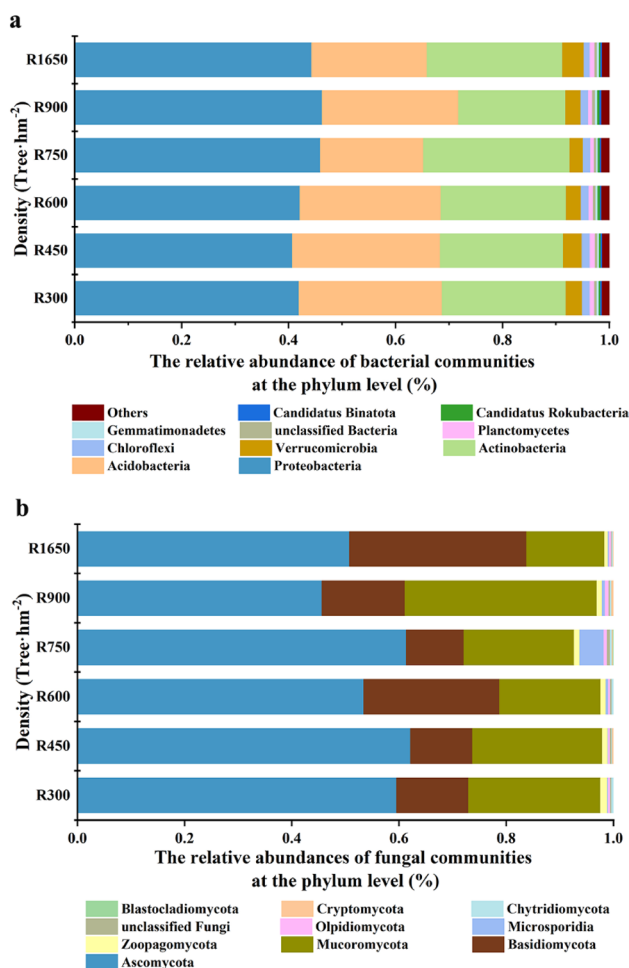
#### Genomic sequencing results

A total of 820.4 million clean reads were obtained for the eighteen soil samples, with an average of 45.6 million reads per sample obtained through metagenomic sequencing after the low-quality data were filtered out. The clean data were assembled via MEGAHIT. An average of 280,956, 271,815, 240,138, 301,979, 258,264 and 297,749 contigs were generated in the R300, R450, R600, R750, R900, and R1650 metagenomes, respectively. A total of 5,511,966 nonredundant open reading frames (ORFs) were obtained. Taxonomy-based annotations were collected for microbial community analysis from all unigenes.

#### Soil microbial community characteristics under the different thinning densities

A total of 223 phyla, 416 classes and 4440 genera were identified via whole-metagenome sequencing from all the soil samples. The results indicated that the microbial communities were not linearly correlated but rather sharply differed under the different thinning density levels. At the phylum level, the bacterial communities with a relative abundance  $> 1\%$  were Proteobacteria (40.71% to 46.28%), Acidobacteria (19.30% to 27.67%), Actinobacteria (20.03% to 27.35%), Verrucomicrobia (2.48% to 3.99%) and Chloroflexi (1.14% to 1.53%), which are the core phyla accounting for more than 80.0% of the total bacterial abundance (Fig. 3a). However, the relative abundance of Acidobacteria was highest in R900, and that of Actinobacteria was lowest in R900. Planctomycetes, unclassified bacteria, Gemmatimonadetes, Candidatus Rokubacteria and Candidatus Binatota had relative abundances of  $< 1\%$ . In addition, *Acidobacteria*, *Alphaproteobacteria*, *Actinomycetia* and *Hyphomicrobiales* were the four most abundant genera in all the samples (Fig. 4a).

Within the fungal community, Ascomycota, Basidiomycota and Mucoromycota were the dominant phyla, with relative abundances ranging from 45.63% to 62.16%, 10.75% to 33.03% and 1.835% to 3.81%, respectively (Fig. 3b). The relative abundance of Ascomycota was lowest in R900, and that of Mucoromycota was highest in R900. Moreover, *Rhizopus arrhizus*, *Rhizophagus irregularis*, and *Lipomyces starkeyi* were the most abundant species in all the samples (Fig. 4b).



**Fig. 3** The relative abundances of microbial communities at the phylum level. **a** Bacterial community. **b** Fungal community

NMDS analysis indicated that the phylogenetic distances of the soil microorganisms varied among the different thinning densities. The microbes associated with the different Chinese fir densities formed separate aggregations that segregated across the first and second coordinate axes (Fig. 5). RDA reflected the impacts of thinning and soil physicochemical properties on the soil microbial phyla and species community structure under the six different thinning densities (Fig. 6). The first two axes explained 74.78% of the microbial phyla composition (Fig. 6a). Pearson correlation analysis revealed that among the tested environmental factors, DOC, MBC, LFOC, and EOC were positively correlated with Proteobacteria and Candidatus Binatota ( $P < 0.05$ ) (Fig. 6c). Actinobacteria was negatively correlated with all the tested environmental factors. At the species level, the first two axes explained 63.69% of the microbial species composition (Fig. 6b). Pearson correlation analysis revealed that SOC and EOC were positively correlated

with *Hyphomicrobiales* and *Gammaproteobacteria* ( $P < 0.05$ ). Additionally, pH was negatively correlated with *Verrucomicrobia* and *Deltaproteobacteria* (Fig. 6d).

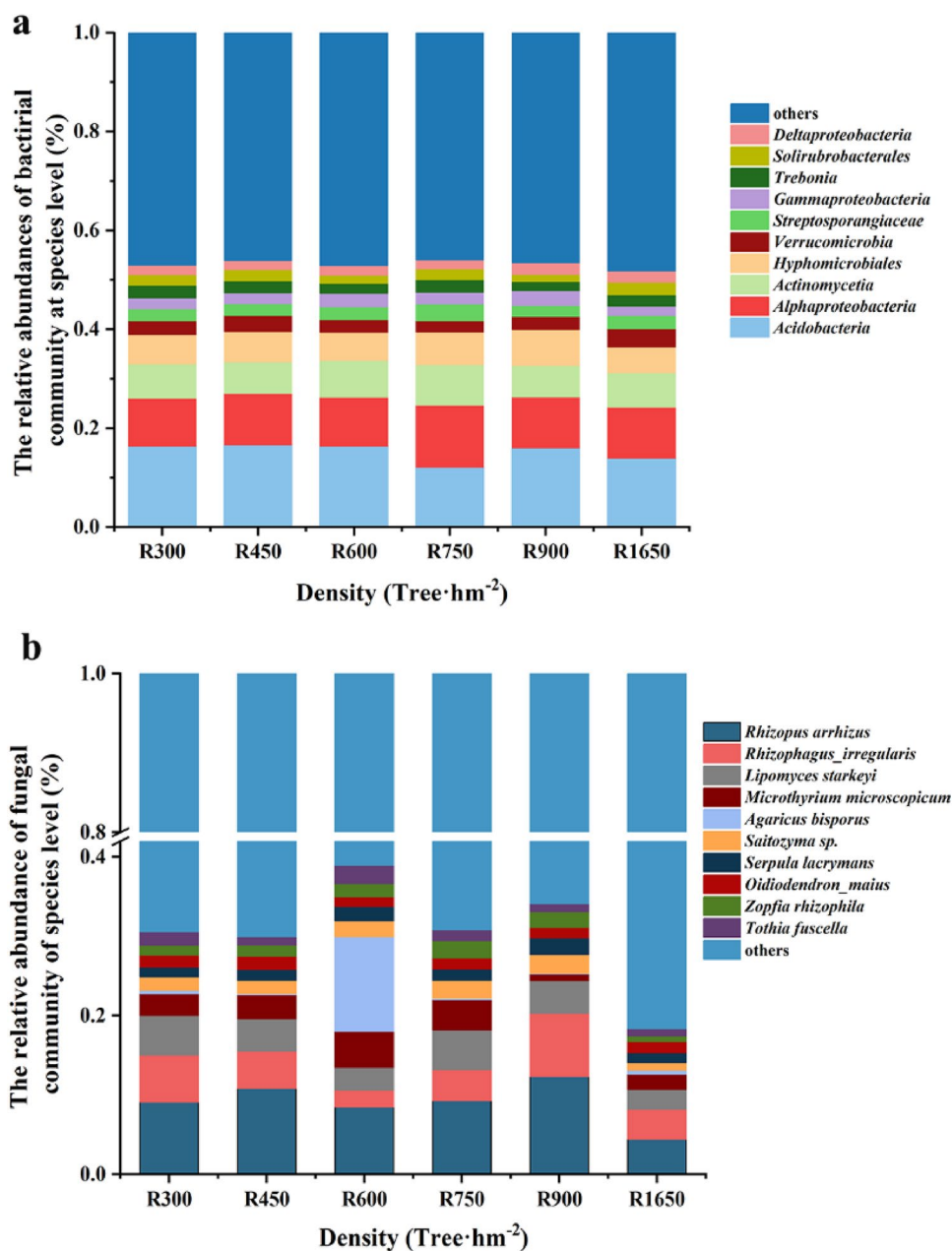
### Carbon cycling functional characteristics of the soil microbial communities

In this study, we analyzed the relative abundances of C-cycling functional genes under the different Chinese fir densities using the KEGG database. A total of 7203 KO genes were obtained that were distributed into the different KEGG level-two pathways, including carbohydrate metabolism, energy metabolism, cellular community-prokaryotes, signal transduction, xenobiotic biodegradation, and metabolism (Fig. 7a).

PCoA results indicated that the level 2 KEGG pathways formed distinct clusters under the different thinning densities, indicating that the thinning of Chinese fir trees resulted in different functional traits under the different thinning densities (Fig. 7b). Thinning and soil microorganisms play pivotal roles in tree growth and ecosystem balance. In addition, the practice of forest thinning may considerably affect the composition of the microbial community.

The results of the analysis of soil characteristics revealed that the carbon content in the soil varied widely. We focused mostly on the genes associated with carbon fixation and carbon metabolism with the purpose of studying potential microbial variations in soil C cycling. In particular, the number of KO genes associated with carbon metabolism related to the pathways of glycolysis/gluconeogenesis, the citrate cycle, the pentose phosphate pathway, pyruvate metabolism and glyoxylate and dicarboxylate metabolism significantly differed among the different thinning densities (Fig. 7c), along with genes associated with the Calvin cycle (*PRK*, *gapA*, *rbcS*, *rbcL*, and *rpiA*), the reductive TCA cycle (*porB*, *porD*, *porG*, and *korD*), the 3-hydroxypropionic acid cycle (*accA*), and reducing acetyl-CoA pathway circulation (*fdhB*) (Fig. 7d). The heatmap results revealed that the abundance of most of the genes was markedly higher in R900, which may indicate that pathways related to C-cycling were active in R900.

With the aim of identifying the effects of soil microorganisms on organic matter in the environment, the distribution of soil microbial carbohydrate-active enzymes was analyzed at different thinning densities via the CAZy database. The results revealed that the distribution of carbohydrate-active enzyme-encoding genes varied among the different thinning densities and represented 294 CAZy enzyme-encoding gene families. These families are distributed in major protein functional modules, including glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide



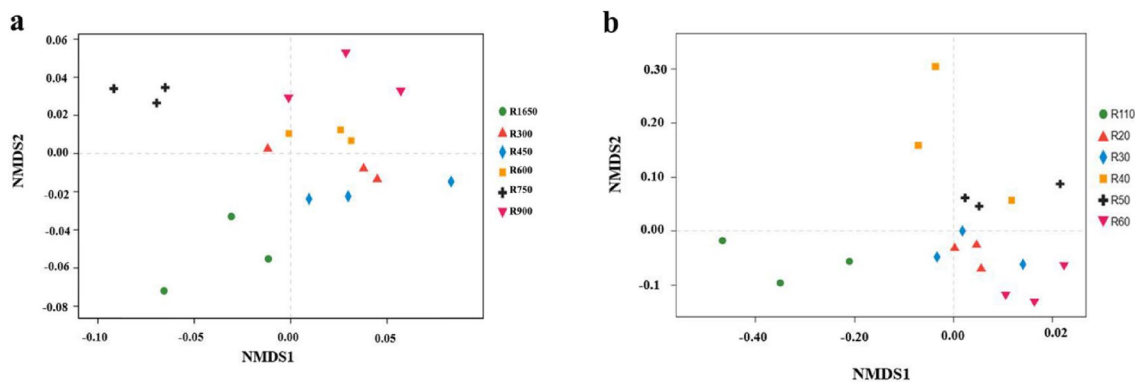
**Fig. 4** Composition and diversity of microbial communities at the species level. **a** Bacterial community. **b** Fungal community

lyases (PLs), carbohydrate-binding modules (CBMs), carbohydrate esterases (CEs) and auxiliary activities (AAs). Furthermore, there were 165 enzyme-encoding genes belonged to GHs, 107 enzyme-encoding genes belong to GTs, 49 enzyme-encoding genes belong to CBMs, and 36 genes belong to PLs. We analyzed the relative abundances of the 50 highest-ranking CAZy gene families under the different Chinese fir densities. The abundance of genes in each family noticeably differed under the different thinning densities. The number of

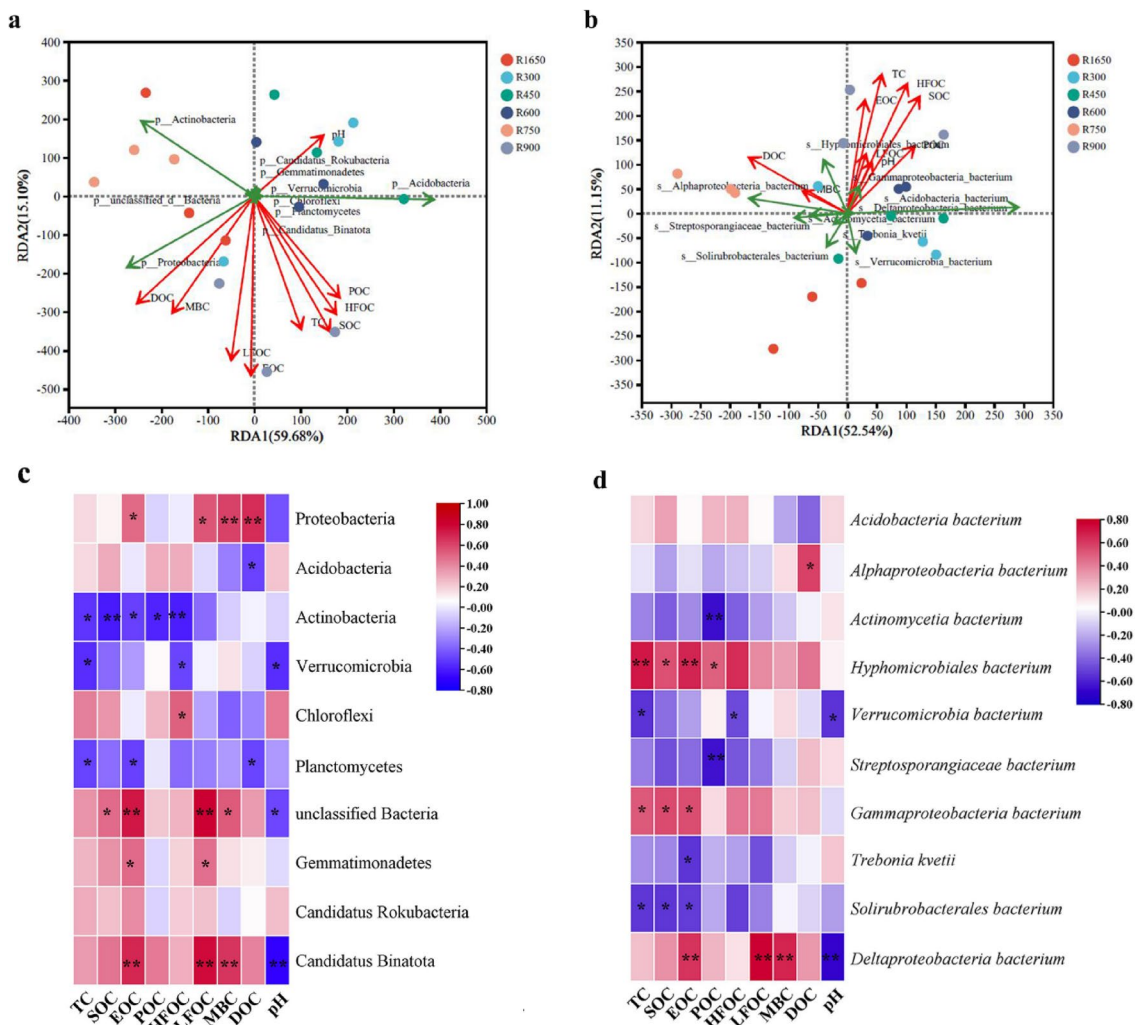
enzyme-encoding genes under R900 was significantly greater than that under the other densities, indicating that the microorganisms in this region participated in carbon cycle activities more actively (Fig. 8). This finding is similar to the results of the KEGG database analysis.

**Association analysis between functional genes and soil properties**

The correlations between the carbon-cycling functional genes and soil characteristics were analyzed via RDA

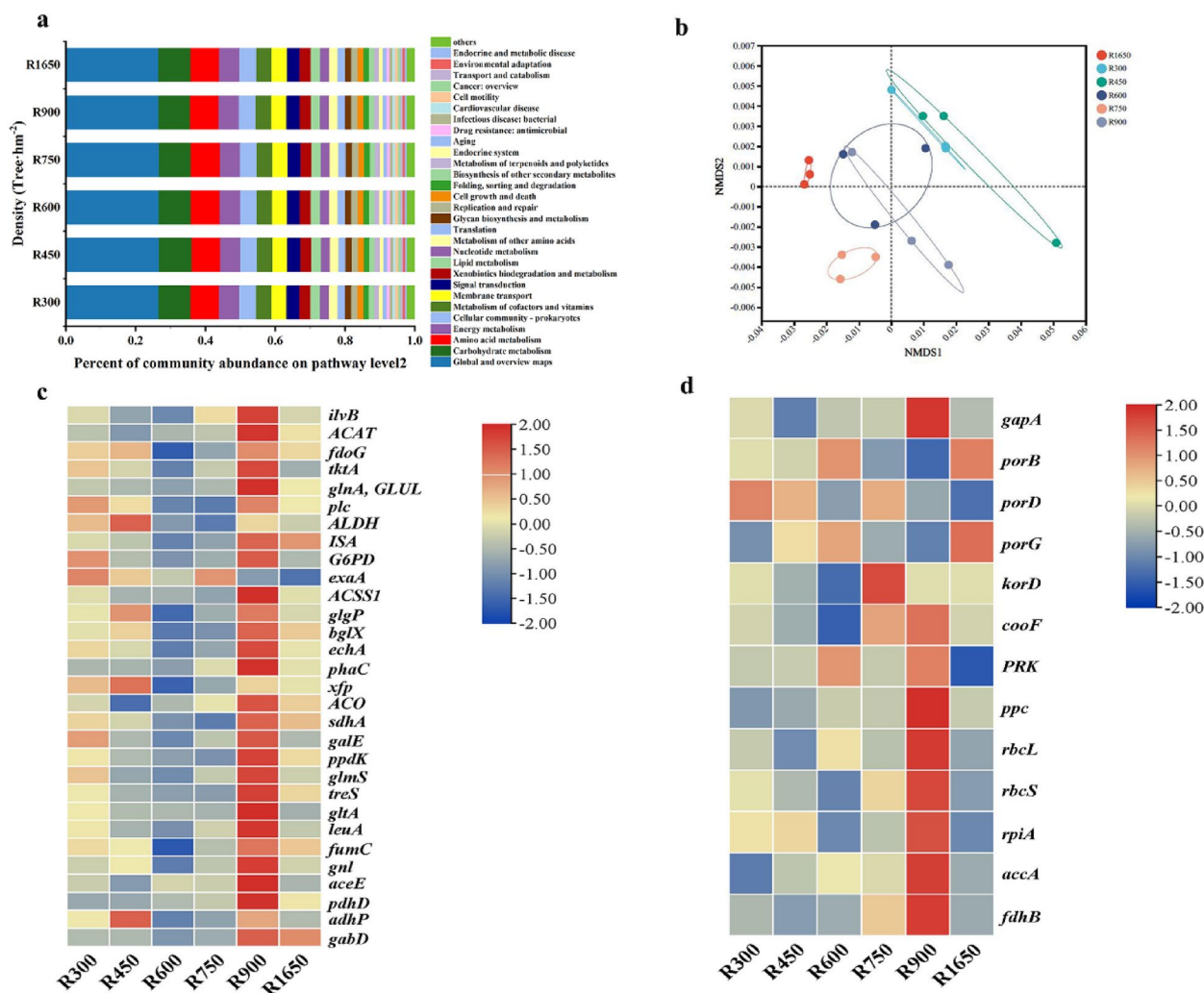


**Fig. 5** NMDS analysis based on Bray-distance under the different thinning densities. **a** Bacterial community. **b** Fungal community



**Fig. 6** RDA analysis and Pearson correlation analysis between soil physiochemical and microbial communities. **a, c** Phylum level. **b, d** Species level. Blue indicates negative correlation, red indicates positive correlation. \* indicates  $P < 0.05$ , and \*\* indicates  $P < 0.01$





**Fig. 7** Comparative analysis of functional pathways (a), NMDS analysis of functional gene families based on KEGG database (b), gene heatmap of carbon metabolism (c) and carbon fixation (d) under the different thinning densities

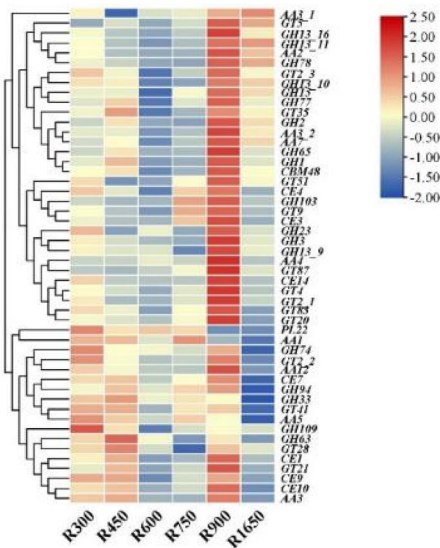
(Fig. 9). RDA1 and RDA2 constituted 26.50% and 11.39% of the carbon metabolism pathways, respectively. Among the soil environmental factors, TC and HFOC significantly influenced carbon metabolism (Fig. 9a). In terms of carbon fixation, RDA1 and RDA2 constituted 35.32% and 8.34%, respectively. pH, TC, HFOC, and POC significantly influenced carbon fixation (Fig. 9b).

## Discussion

### Response of soil characteristics to the different thinning densities

Appropriate thinning operations can maintain the structural and functional features of plantation ecosystems [28]. Compared with those in the CK treatment, the SOC content and its fractions did not display a linear relationship under the different thinning densities in the large Chinese fir plantation,

the SOC content in the R900 treatment was sharply (85.89%) higher, and the other fractions were differently elevated with thinning (Table 1). These results indicated that proper thinning had a positive effect on soil physiochemical properties in the large Chinese fir timber plantation, supporting our first hypothesis. The variation in SOC was related mainly to the change in litter carbon input caused by thinning [29]. In this study, the SOC concentration was highest in R900; however, the litterfall was not highest in R900 (Fig. S1). On the basis of these findings, we suggest that the shrubs and herbs had higher decomposition rates than did the other treatments in this study. Forest age can be an important factor influencing SOC. Zhou et al. [30] reported that SOC increased from the young to the overmature stage in Chinese fir plantations; however, Song et al. [31] reported that there was no significant correlation between SOC and stand



**Fig. 8** Heatmap of the top 50 CAZy gene families under the different thinning densities

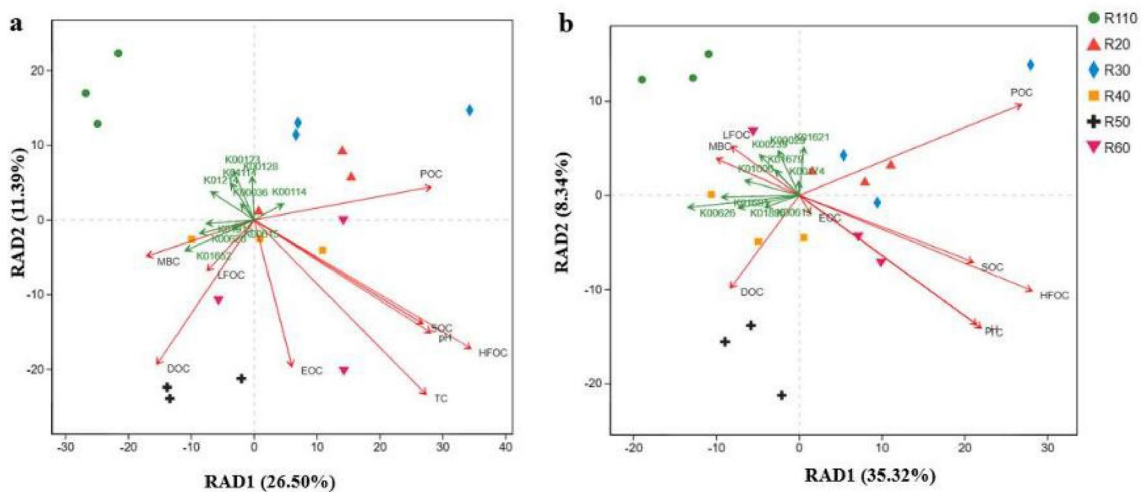
age in Chinese fir. Therefore, measuring SOC in Chinese fir forests of different ages is important for obtaining more information about its ecosystem value. POC is a portion of the SOC fraction and decomposes relatively easily during soil C storage. In this study, the POC contents significantly differed under the six different thinning intensities and were highest in R900 (Table 1).

MBC is an important fraction of SOC that is sensitive to short-term changes in soil ecosystems, and increasing MBC is beneficial to the stability of SOC [1]. In this study, the results revealed that under the different

thinning intensities, the MBC content and ratio to SOC obviously changed in large Chinese fir timber plantation, which may reveal that proper thinning practices are helpful for the carbon input and fixation of microbial necromass C. The proportion of MBC in this study was greater than that reported by Zhang et al. [1], indicating that the ratio of MBC to SOC is related to forest type. MBC mainly consists of microbial necromass C, and cell wall compounds contribute to at least 50% of SOC sequestration during microbial death in soil [1, 32]. The results of this study revealed that both fungal and bacterial necromass C were highest in R900, which contributed greatly to the SOC. Guizhou is a special place with karst landforms, most mountains are steep cliffs, and proper management may significantly affect the microclimate of the forest. In this study, the results revealed that thinning intensity strongly affected the SOC content and its fractions (Table S1). This finding indicates that thinning is an effective measure for managing large Chinese fir plantations.

**Taxonomic changes in the soil-based microbiome**

A previous study suggested that the structure of the microbial community is highly sensitive to environmental changes, including the practice of thinning [33]. In this study, clear patterns emerged in the soil microbial community structure, confirming that the diversity and richness did not differ significantly among the treatments, whereas the relative abundance of the soil microbial community of large Chinese fir timber plantations can be altered by thinning (Figs. 3, 4), supporting our first hypothesis. The results revealed that five bacterial phyla (Proteobacteria, Acidobacteria, Actinobacteria,



**Fig. 9** RDA analysis of gene abundance of the carbon fixation enzymes and environmental factors families under the different thinning densities. **a** Carbon metabolism. **b** Carbon fixation

Verrucomicrobia and Chloroflexi) contributed more than 80.0% of the total bacterial abundance (Fig. 3a). The dominant phyla in the fungal community were Ascomycota, Basidiomycota and Mucoromycota, and their relative abundances varied significantly across the different thinning intensities. These results suggested that thinning affected the relative abundance of key phyla, particularly in the fungal community. These findings may indicate that the fungal community structure in large Chinese fir timber plantations is more sensitive to thinning than the bacterial community structure is; this result is similar to that reported for mature Chinese pine plantations [11].

Soil microbes are essential for nutrient element cycling, organic matter degradation, mineral dissolution and other biogeochemical processes [34, 35]. Thinning altered the microbial community structure by changing soil nutrients and understory vegetation in mature Chinese pine plantations [11]. Zhang et al. [7] revealed that the net rate of carbon mineralization in the soil can be predicted by changes in the abundances of Proteobacteria and Acidobacteria. In our study, the relative abundance of Acidobacteria was greater in R900 than in the other treatments, and Acidobacteria are commonly considered oligotrophic bacteria [11]. This result indicated that Acidobacteria had opposing effects in the large Chinese fir timber plantation.

Fungi survive on carbon from the surrounding plants and soil, and tree density and understory plants may affect the dominant fungal composition. In our study, the abundance of Ascomycota in R900 was lower than that in the other treatments in the large Chinese fir timber plantation, and that of Mucoromycota was highest in R900. These findings indicated that thinning decreased the amount of litter and Chinese fir root and increased those of other understory plants, which may slightly increase the abundance of Ascomycotain low densities. This result was similar to that reported for mature Chinese pine plantations [11].

Additionally, microbial necromass C has a positive effect on soil C pool stability [1, 36]. Our results are similar to previously reported findings; the practice of thinning continually increased the microbial necromass C among total SOC. We suggest that microorganisms are essential drivers of SOC among large Chinese fir timber plantations under different thinning densities. However, the mechanism by which bacterial and fungal communities mediate SOC fractions under thinning is still unknown and needs further research.

### Responses of soil microbial functional genes involved in carbon cycling under the different thinning densities

Microbial biomass plays an essential role in carbon degradation, and the decomposition process cannot be predicted based on microbial biomass alone [37, 38]. Our study revealed that the microbial community and gene abundance related to carbon cycling clearly differed under the different thinning densities (Figs. 7 and 8), supporting our second hypothesis. Additionally, KO genes related to carbon metabolism (*ilvB*, *ACAT*, *fdoG*, *echA*, *ACO*, and *sdhA*) and carbon fixation (e.g., *ppc*, *PRK*, *gapA*, *porB*, and *porD*) were enriched under the different intensities, and most of the genes presented high C cycling activity in R900, indicating that R900 was a suitable intensity for carbon cycling in the large Chinese fir timber plantation. This finding may suggest that microorganisms were more active at this density, which promoted the degradation of litterfall. This result was consistent with the soil physiochemical properties. In contrast, the functional genes were significantly related to the soil properties, such as total C, DOC, and HFOC contents, which was similar to the findings of other studies [39]. This correlation may be because microorganisms degrade litterfall and plant communities, and the soil provides substrate for the growth of microorganisms and plants [40].

### Conclusions

Chinese fir is an important tree species in China. It is a fast-growing tree species that produces wood and sequesters carbon, and large timber trees have been preserved. To this end, it is important to understand the effects of thinning on soil properties and microorganism communities in large Chinese fir timber plantations. The results obtained from this research revealed that (1) thinning has a significant effect on the soil C balance of large Chinese fir timber plantations and may alter the SOC content and its fractions; furthermore, the R900 thinning intensity can be considered for carbon storage. (2) The abundances of the dominant microorganisms significantly differed under thinning; however, the fungal community was more sensitive than the bacterial community. The SOC, POC and HFOC contents were positively correlated with Acidobacteria. (3) Additionally, the relative abundance of C-cycling genes corresponds to different soil characteristics under the different thinning densities, and most of the genes in R900 were significantly more abundant than those in the other densities. Our findings contribute to a better understanding of the effects of thinning on large Chinese fir timber plantations, which are a resource for carbon storage in the environment.

## Abbreviations

R300, R450, R600, R750, R900 and R1650	Indicates trees of Chinese fir are 300, 450, 600, 750, 900 and 1650 per hectare, respectively
C	Carbon
SOC	Soil organic carbon
POC	Particulate organic carbon
EOC	Easily oxidizable carbon
DOC	Dissolved organic carbon
HFOC	Heavy fraction organic carbon
LFOC	Light fraction organic carbon
MBC	Microbial biomass carbon
NCBI	National center for biotechnology information
NR	Non-redundant protein sequence database
KEGG	Kyoto encyclopedia of genes and genomes
KO	KEGG Orthology
CAZY	Carbohydrate-active enzymes database
RDA	Redundancy analysis
NMDS	Nonmetric multidimensional scaling

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13021-024-00269-x>.

Supplementary Material 1.

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## Author contributions

Lei Huang methodology, designed, analyzed and wrote the manuscript. Yunchao Zhou designed the research work and provided critical suggestions for revising the manuscript. All authors reviewed the manuscript.

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## Availability of data and materials

The metagenomic assembly data has been deposited in the Sequence Read Archive (SRA) at the National Center for Biotechnology Information (NCBI). The accession number is PRJNA994818.

## Declarations

### Competing interests

The authors declare no competing interests.

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