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Effects of thinning on soil saprotrophic and ectomycorrhizal fungi in a Korean larch plantation

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ABSTRACT

Thinning is an important silvicultural practice for improving the productivity and stability of plantations, but how thinning influences saprotrophic and mycorrhizal microbes and its ecosystem consequence are unclear. Here we conducted a thinning experiment with four treatments (quartic thinning with low-intensity, LT4; triple thinning with medium-intensity, MT3; twice thinning with high-intensity, HT2; and control) in a 60-year-old Korean larch (*Larix olgensis*) plantation in northeastern China. Our objective was to examine the thinning-induced changes in the community compositions of fungi, ectomycorrhizal (EcM) fungi, saprotrophic fungi, and bacteria using a high-throughput sequencing method. Thinning treatment explained 59%, 61%, and 45% variance of the total, EcM, and saprotrophic fungal community structures, respectively, but it had no effect on the bacterial community, suggesting that fungi are more sensitive to thinning than bacteria. LT4, MT3, and HT2 increased the saprotrophic fungal abundance by 67%, 67%, and 125%, respectively, but they reduced the EcM fungal abundance by 31%, 46%, and 84%, respectively. In addition, thinning enhanced the soil C accumulation and nutrients availability. Overall, these findings suggest that saprotrophic fungi may play a critical role in microbial-mediated ecosystem functions like EcM fungi in the thinned larch stands.

1. Introduction

China has the largest planted forests in the world, with an area up to 69 million ha (2009–2013; China Forestry Database). Larch (*Larix* spp.) is a dominant tree species of afforestation and reforestation in Northeast China, one of the three major forest regions in the country (Yu et al., 2011; Zhou et al., 2019). Thinning opens up the forest canopy and increases soil temperature and moisture (Wang et al., 2019). This silvicultural practice may lead to a positive effect on the microbial activity (Pang et al., 2013) because soil temperature and moisture influence the kinetics of microbial enzymes and shift the microbial community composition by altering microbial utilization of substrates, diffusion of soluble substrates, and extracellular enzyme activity (Hassett and Zak, 2005). However, thinning also changes the quality and quantity of microbial substrates. For example, long-term thinning has been suggested to significantly increase the soil recalcitrant C fraction via harvest residues that contain a large amount of lignin, wax, cutin, and suberin (Huang et al., 2011; Zhou et al., 2019). In addition, thinning-induced changes in fine root biomass and production further influence root exudation and root litter inputs, and thus regulate the soil microbial communities (Zwetsloot et al., 2018; Liu et al., 2018; Chen et al., 2019). Therefore, the potential positive effects of thinning on microbial activity due to the favorable microclimate may be enhanced or offset by the shifts in the substrate quantity and quality.

Compared to the microbial biomass production and activity, thinning has a more complex impact on microbial diversity and community composition at the genetic level, because the drivers of the community composition are inconsistent and thus less predictable (Hendershot et al., 2017). It remains inconclusive how thinning influences the relative abundance of mycorrhizal fungi *vs.* saprotrophic microbes. Mycorrhizal fungi play a critical role in the growth and survival of terrestrial plants because they can provide up to 80% of plant-required nitrogen (N) and phosphorus (P) (Van Der Heijden et al., 2015). Like most of the tree species in temperate and boreal forests, larch trees develop a symbiotic association with ectomycorrhizal (EcM) fungi (Yan et al., 2019) where the EcM fungi provide water and nutrients to the host trees by exploring the soil through their hyphal networks in exchange for carbohydrates from the trees (Smith and Read, 2010). The respiration of EcM fungi accounted for 41% of the rhizosphere respiration in larch plantations (Yan et al., 2019), indicating vigorous

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EcM fungal activity and emphasizing its important role in the C cycling of the forests. While thinning can rapidly inhibit the growth of mycorrhizal fungi by reducing fine root biomass (Lewandowski et al., 2015; Rasmussen et al., 2018), it creates forest gaps and increases the light availability and soil temperature, which likely decrease plant competition and enhance the growth of the remaining trees and their fine root production (López et al., 2003; Campbell et al., 2009; de Avila et al., 2017). For example, fine root biomass and production of the thinned stands can reach or even exceed those of the control stands three years after harvest (López et al., 2003; Campbell et al., 2009). Collectively, the net effects of thinning on mycorrhizal fungi is likely dependent upon thinning intensity, frequency, and recovery times after the thinning.

Several studies have suggested that mycorrhizal fungi can enhance nutrient uptake for plant growth, which will reduce C decomposition because of the increased nutrient limitation to the saprotrophic microbes (Orwin et al., 2011; Averill et al., 2014; Lindahl and Tunlid, 2015; Bödeker et al., 2016), i.e., “Gadgil effect” (Gadgil and Gadgil, 1971; Fernandez and Kennedy, 2016). Meanwhile, higher nutrient availability provided by the mycorrhizal symbiosis can stimulate plant C sequestration and subsequent C inputs to the soil (Orwin et al., 2011; Averill et al., 2014; Maaroufi et al., 2019). For example, Clemmensen et al. (2015) reported that the EcM fungi contributed significantly to the soil C accumulation in boreal forests through the production of recalcitrant fungal tissues. However, a recent study with a long-term multiple levels of N addition experiment in Northern Sweden reported that anthropogenic N deposition increased the soil C accumulation by altering saprotrophs rather than EcM fungi (Maaroufi et al., 2019), while Cheng et al. (2012) suggested that the CO_2 fertilization stimulated mycorrhizal fungal activity and then enhanced the soil C loss due to the increased decomposition. Therefore, the effect of changes in microbial composition on soil functions is still elusive.

In addition, our recent study had reported that long-term thinning treatments (quartic thinning with low-intensity, LT4; triple thinning with medium-intensity, MT3; twice thinning with high-intensity, HT2; and control) significantly increased the total and recalcitrant C in a 60-year-old Korean larch (*Larix olgensis*) plantation in Northeast China (Zhou et al., 2019). Therefore, increased soil recalcitrant C may be conducive to the growth of the *K*-strategy microbes, such as *Ascomycota* in fungi and *Acidobacteria* and *Actinobacteria* in bacteria (Zhou et al., 2017, 2018a, 2018b). On the contrary, our previous study also found that LT4, MT3 and HT2 treatments significantly increased the soil total and available N and P (Zhou et al., 2019). Proceeding from this angle, thinning may increase the abundance of *r*-strategy microbes, like *Proteobacteria* and *Bacteroidetes* within bacterial communities (Zhou et al., 2017, 2018a, 2018b). Taken together, the comprehensive effects of thinning on soil microbial communities at the genetic level are not clear and deserve further exploration. Our objective in the current study was to examine the thinning-induced changes in the community composition of fungi, EcM fungi, saprotrophic fungi, and bacteria in the Korean larch plantation. Specially, we addressed the following questions: (1) How do different thinning treatments affect the microbial community composition? (2) Do different microbial groups have different responses? And (3) how does thinning influence the microbial community composition through altering soil C fractions and nutrients?

2. Materials and methods

2.1. Study site and thinning treatments

The larch plantations were planted on a flat site located in the Mengjiagang National Forest Farm, Heilongjiang province, northeastern China (46.34–46.51° N, 130.55–130.88° E). The climate is characterized by a temperate continental climate, with mean annual air temperature and precipitation of 2.7 °C and 535 mm, respectively. In brief, our study included four thinning treatments on the larch stands planted in 1958, which were mechanically thinned with an intensity of ~45% in 1971. In specific, thinning treatments included: (1) quartic thinning with low-intensity (LT4): 17.1% in 1974, 5.8% in 1981, 13.5% in 1986, and 25.1% in 2000; (2) triple thinning with medium-intensity (MT3): 23.8% in 1974, 23.6% in 1981, and 15.3% in 2000; (3) twice thinning with high-intensity (HT2): 43.4% in 1974 and 35.6% in 2000; and (4) the control (only mechanically thinned in 1971). Three 20 × 20 m replicate plots were established in each treatment. Refer to Zhou et al. (2019) for the detailed information about the long-term thinning experiment.

2.2. Microbial sequence

The topsoil (0–10 cm depth without forest floor) samples were collected in July 2018 from seven random locations in each plot and homogenized as one composite sample. Composite sample did reduce the replicates, but one composite sample had seven random cores in each plot, which can represent the plot well. The fresh samples were sieved (< 2 mm) to remove roots and coarse objects (e.g., debris, rock). Soil pH was analyzed in 0.01 M $CaCl_2$ solution and the soil to solution ratio was 1:2.5. Soil carbon and nutrients fractions were measured synchronously and reported in our recent study (Zhou et al., 2019). Here, we consequently displayed the soil properties in the Table S1. The total genomic DNA from the soil samples was extracted using the Soil DNA Isolation Kit (Omega Bio-tek, Norcross, GA, U.S.) following the manufacturer's instructions. The 1% agarose gels were used to check the quality and size of the extracted DNAs. For bacteria, the 16S rRNA genes were amplified with the primers of 338F (ACTCCTACGGGAGG CAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). For Fungi, the ITS1 regions were amplified with the primers of ITS1F (CTTGGTCATT TAGAGGAAGTAA) and ITS2R (GCTGCGTTCTTCATCGATGC). The PCR was conducted using the TransStart Fastpfu DNA Polymerase (TransGen Biotech, Beijing, China) and performed in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, United States). The thermal cycling was based on an initial denaturation step at 95 °C for 3 min, followed by 35 cycles (fungi) or 27 cycles (bacteria) of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 45 s, and with a final extension at 72 °C for 10 min. The PCR products were extracted from 2% agarose gels and concentrated using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA). The PCR products were sent for the analysis on the Illumina’s MiSeq platform at the Majorbio Bio-Pharm Technology Co. Ltd (Shanghai, China). Reads were demultiplexed, quality-filtered, and processed using QIIME. Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE, and chimeric sequences were identified and removed using UCHIME. The taxonomic assignment of the 16S rRNA and ITS sequences was determined based on the bacterial SILVA reference database and fungal UNITE reference database using RDP Classifier. A total of 656,676 bacterial sequences and 787,238 fungal sequences were obtained with the 338F/806R and ITS1F/ITS2R primer sets for all the soil samples, respectively. The number of bacterial sequences varied from 47,495 to 63,354 per sample with an average length of 438, whereas the number of fungal sequences varied from 55,424 to 74,199 per sample with an average length of 265. The rarefaction curves for the observed OTUs for both bacteria and fungi reached saturation for each treatment (Fig. S1), suggesting that the analyzed reads were sufficient to detect most of sequence types. Finally, the sequences were deposited into the NCBI Sequence Read Archive under the accession number of PRJNA562993.

2.3. Statistical analysis

The saprotrophic and EcM fungi species were identified by FUNGuild through uploading fungal OTUs to FUNGuild (http://www.stbates.org/guilds/app.php) (Nguyen et al., 2016). Overall, a total of 1510 OTUs were found across all of the soils, but only half of those (839

OTUs) were assigned by FUNGuild. In addition, some fungi did not fall exclusively into a single guild because their presents are depending on life stage and environmental conditions (Nguyen et al., 2016). Therefore, the saprotrophic and EcM fungi in the following analysis were the species that absolutely belong to a single guild. We also calculated the percentage of EcM and saprotrophic fungal abundance and OTUs in the total assigned fungal guilds (Mushinski et al., 2018; Chen et al., 2019). One-way *ANOVA* analysis and the *Duncan's* multiple range test were used to examine the significant differences ($\alpha = 0.05$) in soil properties and microbial characteristics among the treatments.

The permutational multivariate analysis of variance (*PERMANOVA*) was performed to test the significance of separation among the four thinning treatments for fungal, bacterial, saprotrophic and EcM fungal communities. In addition, the non-metrical multidimensional scaling (NMDS) with the Bray–Curtis dissimilarity was conducted to assess the clustering of the soil microbial communities among the treatments with the R package of *vegan* (Oksanen et al., 2013).

Because of a strong collinearity among soil properties (Fig. S2), the distance-based redundancy analysis (db-RDA) was firstly used to identify the explanation (i.e., the adjusted R^2) of each soil property in the total variances of fungal, bacterial, and EcM fungal compositions, respectively; the stepwise analysis was then used to select the best model for changes in the microbial community composition against the ten soil properties investigated. All the statistical analyses were conducted using the R 3.6.0 software (R Core Team, 2013).

3. Results

The fungal community was dominated by *Ascomycota* and *Basidiomycota* phyla, which accounted for > 80% of the total fungal sequences (Fig. S3). The relative abundance of *Basidiomycota* decreased significantly ($F = 17.9$, $P < 0.001$) as the thinned intensity increased, i.e., LT4, MT3, and HT2 reduced the relative abundance of *Basidiomycota* by 47%, 49%, and 85%, respectively. However, LT4, MT3, and HT2 increased the relative abundance of *Ascomycota* by 1.7, 1.5, and 2.1 times, respectively ($F = 21.4$, $P < 0.001$). The first nine dominant bacterial phyla were *Proteobacteria*, *Actinobacteria*, *Verrucomicrobia*, *Acidobacteria*, *Chloroflexi*, *Rokubacteria*, *Bacteroidetes*, *Gemmatimonadetes*, and *Planctomycetes*, which did not differ significantly among the thinning treatments ($P > 0.05$) (Fig. S3).

Almost all of the assigned EcM fungi were from the fungal phylum of *Basidiomycota* (> 94%) (Fig. S3). The percentage of the EcM fungal abundance decreased significantly ($F = 10.9$, $P = 0.003$) as the thinning intensity increased (Fig. 1a). Specifically, LT4, MT3, and HT2 reduced the percentage of the EcM fungal abundance by 31%, 46%, and 84%, respectively (Fig. 1a). On the contrary, almost all of the assigned saprotrophic fungi were from the fungal phylum of *Ascomycota* (> 85%; Fig. S3). The percentage of the saprotrophic fungal abundance increased significantly ($F = 7.4$, $P = 0.010$) as the thinning intensity increased (Fig. 1b). Specifically, LT4, MT3, and HT2 increased the percentage of the saprotrophic fungal abundance by 67%, 67%, and 125%, respectively (Fig. 1b). However, the percentage of the assigned EcM ($F = 1.5$, $P = 0.299$) and saprotrophic ($F = 1.1$, $P = 0.411$) fungal OTUs was comparable among the treatments (Fig. 1).

The NMDS analysis based on the Bray-Curtis distance revealed clear separated samples for the total, EcM, and saprotrophic fungal communities among the treatments, but overlapped configuration of points for the bacterial community (Fig. 2). The *PERMANOVA* analysis further supported that thinning significantly altered the community compositions of fungi ($R^2 = 0.59$, $P = 0.001$), EcM fungi ($R^2 = 0.61$, $P = 0.001$), and saprotrophic fungal ($R^2 = 0.45$, $P = 0.011$), rather than that of bacteria ($P = 0.131$) (Fig. 2).

The soil properties contributed to the variance of community compositions of fungi and EcM fungi, among which the soil recalcitrant C explained the most variance (Fig. 3a, b). However, soil available P explained the most variance of the community compositions of saprotrophic fungi (Fig. 3c). For the bacterial community, soil pH, recalcitrant C, total C, N, and P had significant effects, among which soil pH explained the most variance (Fig. 3d). The stepwise analysis showed that the accepted predictors for the community compositions of the total fungi, EcM fungi, saprotrophic fungi, and bacteria were recalcitrant C ($R^2 = 0.23$, $P = 0.001$), recalcitrant C ($R^2 = 0.17$, $P = 0.003$), available P plus labile C pool I ($R^2 = 0.23$, $P = 0.002$), and soil pH ($R^2 = 0.17$, $P = 0.001$), respectively (Fig. 3d).

The linear regression analysis showed that soil recalcitrant C was positively correlated with the relative abundance of *Ascomycota* ($R^2 = 0.56$, $P = 0.003$), the relative abundance of saprotrophic fungi ($R^2 = 0.41$, $P = 0.015$), and the ratio of (*Acidobacteria* + *Actinobacteria*) to (*Proteobacteria* + *Bacteroidetes*) ($R^2 = 0.28$, $P = 0.045$), but negatively correlated with the relative abundance of EcM fungi ($R^2 = 0.38$, $P = 0.019$) (Fig. 4).

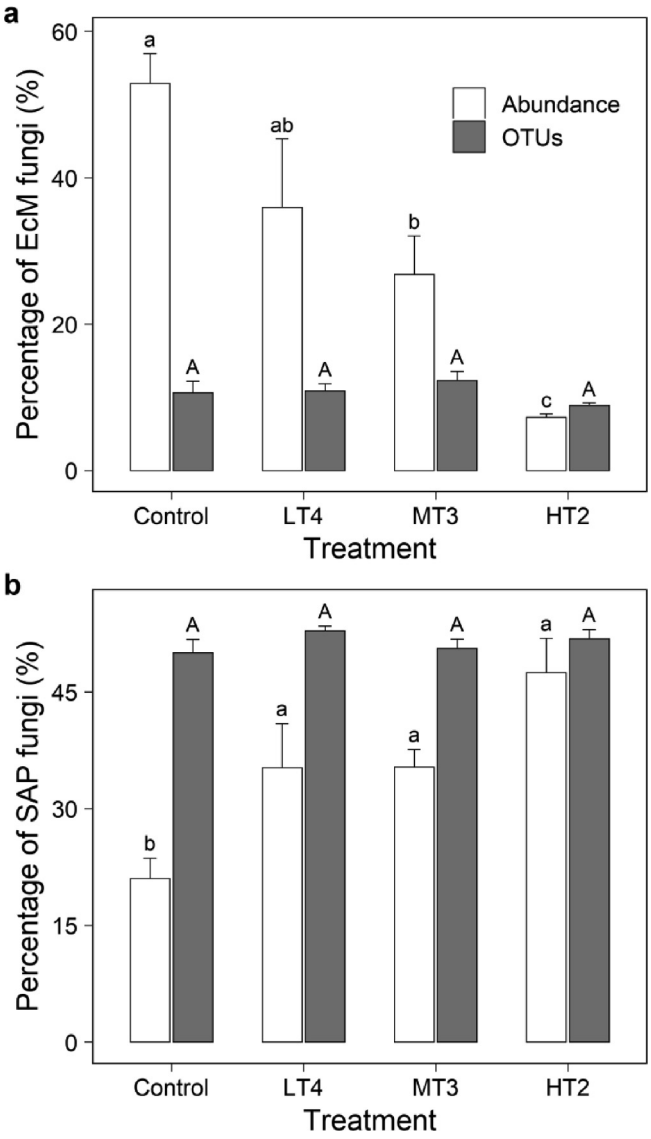


Fig. 1. The relative abundances and OTUs of ectomycorrhizal (EcM) and saprotrophic (SAP) fungi in the total assigned fungal guilds under the four thinning treatments. LT4, quartic thinning with low-intensity; MT3, triple thinning with medium-intensity; HT2, twice thinning with high-intensity.

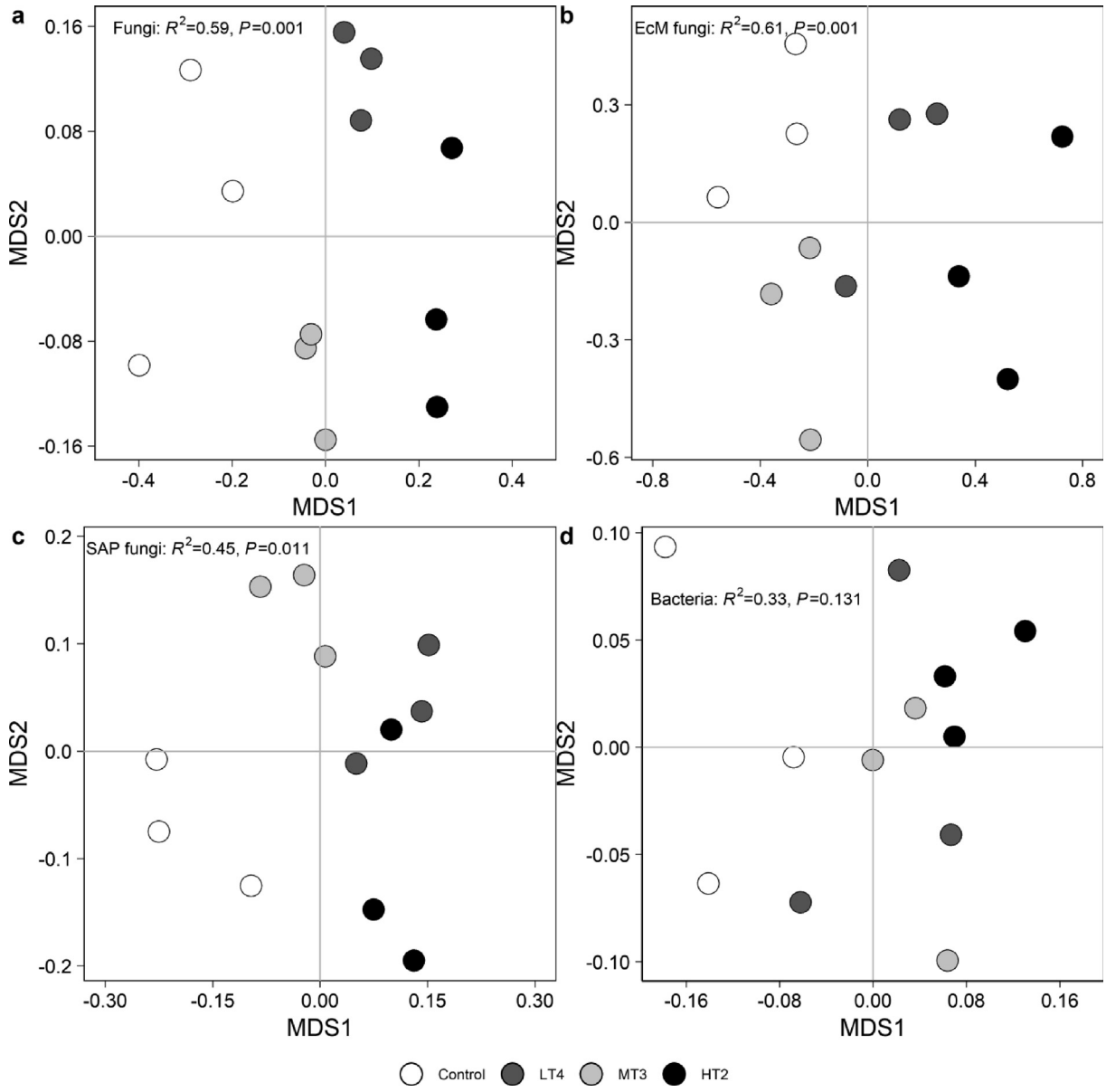


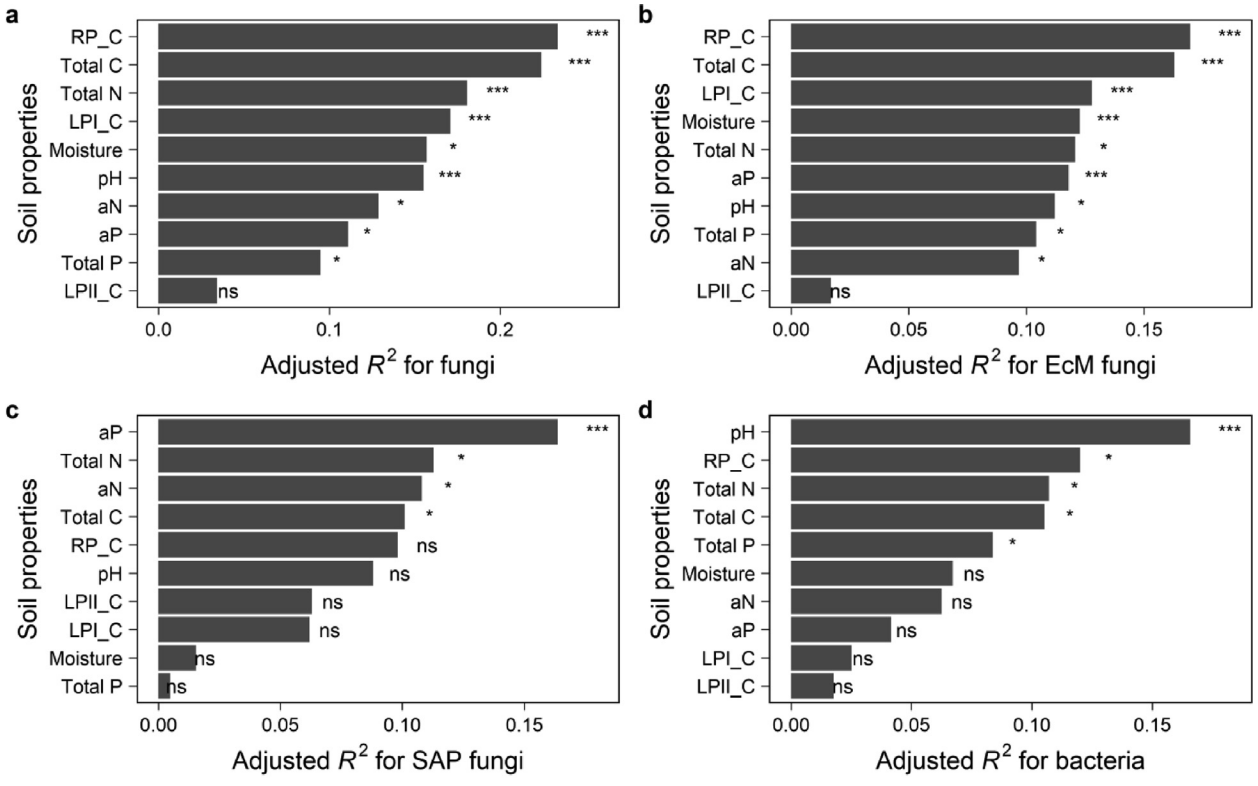
Fig. 2. Nonmetric multidimensional scaling (NMDS) to visualize the overall differences in the total fungal (a), ectomycorrhizal (EcM) fungal (b), saprotrophic (SAP) fungal (c), and bacterial (d) compositions for the four treatments.

4. Discussion

4.1. The community composition of fungi was more sensitive to thinning than that of bacteria

The *PERMANOVA* analysis revealed that thinning explained 59% of the variance of the fungal community composition, but the effect of thinning on the bacterial community composition was insignificant (Fig. 3). Additionally, thinning significantly altered the dominant phyla of fungi, but had no significant effect on any phylum of bacteria (Fig. S3); this is consistent with a long-term thinning experiment in the Chinese fir (*Cunninghamia lanceolata*) plantations in Eastern China (Cheng et al., 2018). Three possible mechanisms may contribute to the greater sensitivity to thinning of fungi than bacteria. First, fungal and bacterial community compositions have different drivers. Specifically, the dominant driver of fungi composition was soil recalcitrant C concentration (Fig. 4), which increased up to 71%, 69%, and 75% by LT4, MT3, and HT2, respectively (Table S1; Zhou et al., 2019). Consistent with previous findings (Fierer and Jackson, 2006), however, soil pH was the most important factor that regulated the bacterial community composition (Fig. 4), and LT4, MT3, and HT2 thinning increased pH by only 0.21, 0.17, and 0.30 units, respectively (Table S1; Zhou et al., 2019).

Second, fungi can produce various ligninocellulolytic enzymes necessary to degrade residual plant biopolymers and perforate plant cell walls; and they are assumed to be better decomposers of recalcitrant organic matter than bacteria in terrestrial ecosystems (De Boer et al., 2005). Thinning increases the inputs of recalcitrant C from woody residues with higher suberin and lignin concentrations (Huang et al., 2011; Zhou et al., 2019), which may stimulate the shifts in the fungal community composition (Cheng et al., 2018). Specifically, our thinning treatments increased the fungal phylum of *Ascomycota* by 70–110% (Fig. S3), which was closely related to the decomposition of recalcitrant C (Fig. 4a) (Zechmeister-Boltenstern et al., 2015). Within the bacteria, although we found a weak positive relationship between the ratio of (*Acidobacteria* + *Actinobacteria*) to (*Proteobacteria* + *Bacteroidetes*) and soil recalcitrant C (Fig. 4d), thinning had no effect on the relative abundances of *Acidobacteria*, *Actinobacteria*, *Proteobacteria*, and



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Microbes	Models	Adjusted R^2	F	P
Fungi	~RP_C	0.23	4.35	0.001
EcM Fungi	~RP_C	0.17	3.25	0.003
SAP Fungi	~aP+LPI_C	0.23	2.67	0.002
Bacteria	~pH	0.17	3.19	0.001

Fig. 3. Distance-based redundancy analysis (db-RDA) illustrating the effects of soil properties on the microbial community compositions. (a–d) The explanation (i.e., the adjusted R^2) of each soil property for the total fungi, ectomycorrhizal (EcM) fungi, saprotrophic (SAP) fungi, and bacteria, respectively. (e) The stepwise model built for the constrained ordination methods illustrating the most important predictors. LPI_C, labile C pool I; LPII_C, labile C pool II; RP_C, recalcitrant C pool; aN, available N; aP, available P.

Bacteroidetes (Fig. S3). In addition, such effect of recalcitrant C may be offset by the thinning-induced positive effects on the soil nutrients on overall bacterial community composition, because bacteria are characterized by high growth rate and demand for nutrients (Delgado-Baquerizo et al., 2017; Zhou et al., 2017, 2018a, 2018b).

Third, the changes in EcM and saprotrophic fungal community composition may also contribute to the changes in fungal community. In the present study, thinning decreased the relative abundance of *Basidiomycota*, the dominant EcM fungal species (Fig. S3), by 47–85% (Fig. 2d). This result was consistent with Hartmann et al. (2012) who also reported that timber harvesting negatively affected the EcM fungi at six forest sites in British Columbia, Canada. In addition, the thinning-induced significant increases in the relative abundance of *Ascomycota*, the dominant saprotrophic fungal species (Fig. S3), might also contribute to the changes in whole fungal community.

4.2. Thinning enhance saprotrophic fungi but suppress EcM fungi

Based on the FUNGuild (Nguyen et al., 2016), the present study found that thinning enhanced the saprotrophic fungi but suppressed the EcM fungi, which is in agreement with previous work in the Gulf Coastal Plain, USA (Mushinski et al., 2018). Timber harvesting decreases the fine root biomass and consequently reduces the fine root hosts for the EcM fungi, which is the potential interpretation for the short-term studies (Hartmann et al., 2012; Mushinski et al., 2018; Parladé et al., 2019). Nevertheless, fine root can be restored to the control level shortly after thinning (López et al., 2003; Campbell et al., 2009). Although we did not quantify the fine root biomass in this study, the soils were sampled 18 years after thinning and all the thinned stands had similar plant biomass currently (Zhou et al., 2019). Such clues suggest that fine root biomass in the thinned stands may reach to the control level. A recent long-term experiment found that timber harvesting increased the relative abundance of mycorrhizal fungi after 50-year recovery (Chen et al., 2019). These findings together suggest a delayed recovery of root-associated fungal communities compared with the fine roots. In addition, we found that soil recalcitrant C was positively correlated with the relative abundance of saprotrophic fungal guilds, but negatively correlated with the relative abundance of EcM

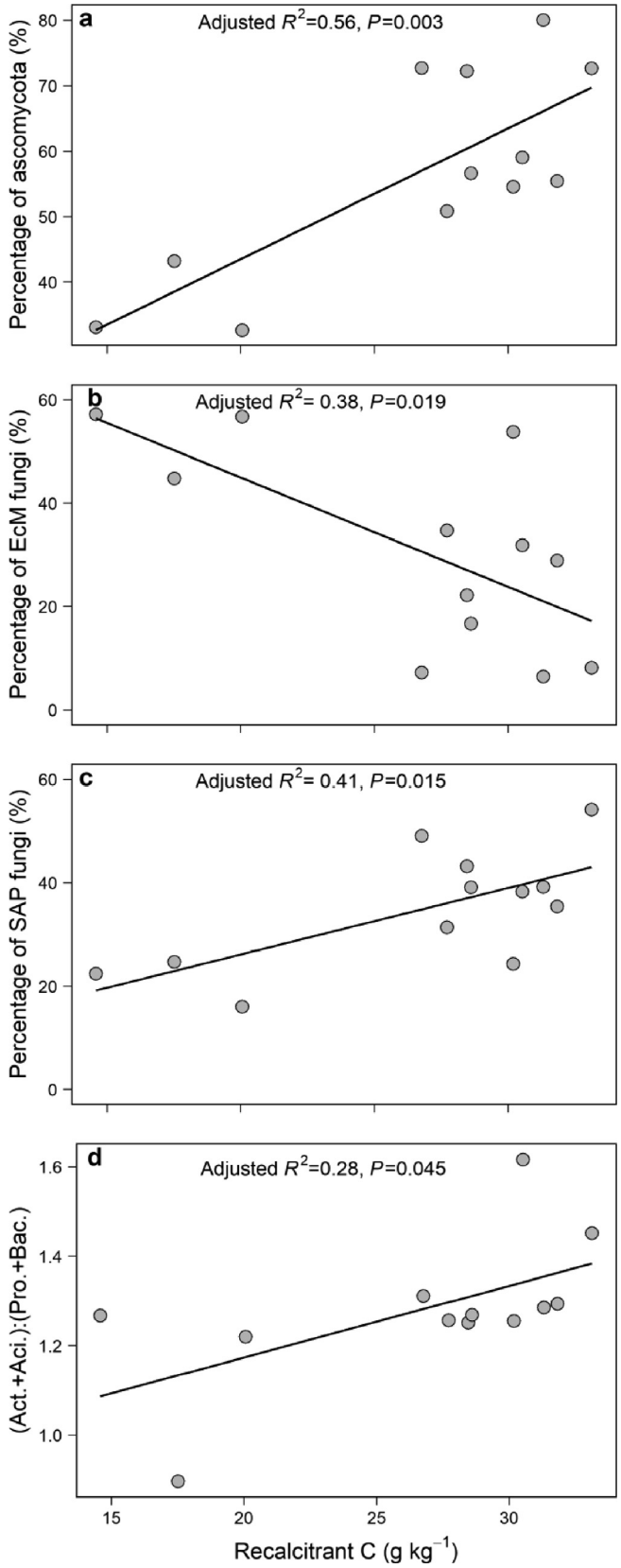

Fig. 4. Relationships between soil recalcitrant C and relative abundance of *Ascomycota* (a), relative abundance of ectomycorrhizal (EcM) fungi (b), relative abundance of saprotrophic (SAP) fungi (**c**), and the ratio of (*Acidobacteria* + *Actinobacteria*) to (*Proteobacteria* + *Bacteroidetes*) ((Aci. + Act.):(Pro. + Bac.), (d).

fungal guilds (Fig. 4). Therefore, thinning-induced increases in coarse-root woody residues and recalcitrant organic matter (Zhou et al., 2019; Table S1) would promote the growth of *K*-strategical phylum of *Ascomycota*. Increases in the relative abundance of saprotrophic fungal guilds would compete for the living space, which might also inhibit the growth of EcM fungi.

Mycorrhizal and saprotrophic fungal communities have partly overlapped niches (Bödeker et al., 2016), and saprotrophic fungi may also play a critical role in microbial-mediated ecosystem functions. EcM fungi are thought to inhibit the activities of saprotrophic microbes by nutrients limitation and then decrease the soil C decomposition (Orwin et al., 2011; Averill et al., 2014; Lindahl and Tunlid, 2015; Bödeker et al., 2016). However, a great activity of EcM fungi can also enhance the soil C loss (like a priming effect; Kuzyakov, 2010), because EcM fungi consume the nutrients in humus and litter, which can stimulate the N mining by saprotrophic microbes (Chen et al., 2014; Zechmeister-Boltenstern et al., 2015). In other words, high activity of EcM fungi may decrease the C use efficiency of saprotrophic microbes and negatively affect soil C pools. Moreover, LT4, MT3, and HT2 had positive effects on the soil total and available nutrients (Table S1), which illustrates an interesting connection between saprotrophic fungi and soil nutrients availability (Fig. 4). The enhanced soil N and P in turn can inhibit the growth and functions of mycorrhizal microbes evidenced by previous studies (Johnson et al., 2003; Sheldrake et al., 2017).

It should be mentioned that there are great challenges to regard the read abundance of high throughput amplicon sequences as a proxy for biological abundance. One the one hand, Palmer et al. (2018) had synthesized several limitations, including differences in DNA extraction efficiency, number of nuclei per cell, and the number of copies of the rRNA array for different taxa and/or cell types; a single isolate can have multiple ITS sequences; the ITS region is highly variable in length; ITS sequences vary in GC content; and there are a variable number of homopolymer repeats. PCR of mixed communities consequently introduces bias (Palmer et al., 2018). Several studies have pointed out that the read abundance from fungal high throughput amplicon sequences is not representative of relative biological abundance through the use of mock communities (Amend et al., 2010; De Filippis et al., 2017). The current findings involving the huge changes in EcM and saprotrophic fungal abundance possibly have such limitations. However, Taylor et al. (2016) suggested that the abundances were meaningful through the use of a fungal ITS mock community, and numerous studies continue to use abundance-based metrics to analyze high throughput amplicon sequences (Mushinski et al., 2018; Parladé et al., 2019; Chen et al., 2019).

In summary, we found that thinning significantly changed the community composition of fungi rather than bacteria due to different drivers and the decomposition of recalcitrant organic matter. Thinning treatments significantly enhanced saprotrophic fungi but suppressed EcM fungi, which together improved the soil C accumulation and nutrients availability. These findings suggest that EcM and saprotrophic fungal communities probably have overlapped niches, and saprotrophic fungi may play a critical role in microbial-mediated ecosystem functions in the thinned plantations.

CRediT authorship contribution statement

Zhenghu Zhou: Writing - original draft, Data curation, Software, Data curation. **Chuankuan Wang:** Conceptualization, Writing - review & editing. **Chengjie Ren:** Software, Writing - review & editing. **Zhihu Sun:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.117920.

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