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The Effect of Forest Thinning on Soil Microbial Community Structure and Function

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Abstract: Microbial communities and their associated enzyme activities play key roles in carbon cycling in ecosystems. Forest thinning is likely to change the soil properties and feedbacks on the structure and function of microbial communities, consequently affecting microbial regulation on the soil carbon process. However, few studies have focused on the mechanism of how thinning affects the quantity and stability of soil carbon. To reveal the influence of thinning on soil carbon and to explore the regulated key factors, this study was conducted in a pure *Larix principis-rupprechtii* Mayr plantation with different thinning intensity (light, medium, and high) in Shanxi province, China. Soil properties (soil pH, soil water content, soil organic carbon, and soil microbial biomass carbon) were measured. Meanwhile, soil microbial communities were examined with the method of phospholipid fatty acid (PLFA), and soil enzyme activities were measured as indicators of soil microbial functions. The results showed that medium and high thinning has positive effects on soil organic carbon, microbial biomass carbon, soil microbial abundance, and soil enzyme activities. Actinomycetes and gram-negative bacteria were the major factors to affect soil microbial community function relating to carbon decomposition. Soil pH contributed to actinomycetes and gram-negative bacteria through direct influences on arbuscular mycorrhizal fungi. Moreover, there were strong correlations between soil pH and microbial community to control soil carbon turnover. The increasing of soil microbial abundance and the microbial regulation on soil carbon in forest thinning need to be considered for sustainable forest management practices in northern China.

Keywords: PLFA; soil microorganisms; soil enzyme activities; soil carbon properties; structural equation modeling

1. Introduction

In forest ecosystems, soil microorganisms play a critical role in carbon, nutrient cycling, and energy flow [1]. Microorganisms are also regarded as sensitive indicators of soil quality for their role in soil carbon processes, including soil organic matter decomposition and turnover [2]. Over the past two decades, soil microbial community and soil carbon process in forest management had been investigated by some studies [3,4], which theorized that soil carbon was affected by forest management through soil microbial regulations. Therefore, the effect of forest management on the soil microbial community structure and function have received increased research attention [5].

Forest thinning is one management strategy with strong potential. High stand densities of trees in pure forest may lead to poor growth and the reduction of forest productivity [6]. Parts of trees are selectively removed and form gaps to increase the availability of light, water, and nutrients to improve forest microclimatic conditions [7]. Soil microbial community structure and function are affected by thinning through the correlation between above- and below-ground processes [8]. Thinning directly affects carbon input (litter and roots), and then alters soil carbon process [9].

Forest thinning has shown positive or negative effects on soil microorganisms, while there remains no consensus on the influence on soil microbial community. Some previous studies theorized that thinning reduced soil microbial abundance, since large gaps promoted high soil and air temperatures and improved living conditions for bacteria- and fungi-feeding nematodes [10]. The positive effect of thinning on soil microbial diversity was observed in a Chinese fir plantation by a previous study, which theorized that thinning increased the availability of light, water, and nutrients to ameliorate forest microclimatic conditions [11]. However, the insignificant influence of thinning on soil microbial community was also supported by other literatures [12]. It is theorized that the decrease in soil microbial diversity is probably related to reductions in soil organic carbon and microbial abundance and that diversity can be improved by management practices [13].

Some previous works theorized that microbial communities are correlated with their function, and microbial community structure and function strongly influence soil carbon dynamics [14–16]. Soil microorganisms depolymerized soil organic matter by producing extracellular enzymes, which are involved in soil nutrient cycling [17]. The activities of soil enzymes are a direct expression of soil microbial community structure. The assay of soil enzymes, which can be divided into oxidases and hydrolases activities, gives an indication of functions that can be assumed by the microbial community [18]. Poor-quality and complex compounds such as lignin is degraded by oxidases which is produced primarily by fungi [19]. However, the hydrolase, which is produced primarily by bacteria, degrades cellulose and is related to soil carbon acquisition [20].

Many studies have investigated the effect of thinning on soil organic carbon [21,22], however, the regulation of soil microbial community on soil carbon with thinning treatment is still not well understood. After thinning treatment, soil organic carbon is altered following the variation in microbial community [23,24]. Therefore, studying the effect of microbial community structure on functions, as well as the microbial effect on soil carbon properties, is necessary for better understanding the belowground processes affecting carbon dynamics [25,26].

Thinning may also affect soil microbial communities by altering soil physicochemical factors in forest ecosystems [27]. The microclimates in different thinning intensity cause the variation in soil water content and pH [28]. It is known that soil pH has a significant effect on soil microbial communities and soil enzyme activities relating to carbon decomposition [29]. Meanwhile, soil microbes have the ability to maintain their intracellular pH [30]. There are many studies of the effect of soil water content on soil microbes [31,32]. Soil water maintains soil microbial activities and indirectly influences microbial substrate and oxygen availability. Therefore, it is necessary to note the effect of soil pH and soil water content on soil microbial community structure.

Larix principis-rupprechti Mayr is a prominent plantation tree species in the warm, temperate Taiyue Mountains of Shanxi province. In this paper, four thinning intensities were established to examine soil microbial community structure, function, and soil carbon with thinning in *L. principis-rupprechti* plantations. We measured the activities of hydrolases to assess soil microbial function relating to cellulose and chitin degrading capacity, and oxidases to assess microbial function relating to lignin degrading capability in driving soil carbon transformation.

Furthermore, we investigated the correlation between soil microbial community and function, studied how soil pH, soil water content, and shifts in microbial community drive the variation in soil carbon properties. We hypothesized that: (1) moderate thinning has positive effects on soil microbial structure and function; and (2) there were connections between soil microbial community structure and the function, which relates to soil organic carbon turnover; and (3) the variation in soil physicochemical properties influenced soil microbial function, which is regulated by soil microbial community structure, and finally affected soil carbon properties.

2. Material and Methods

2.1. Study Sites

The study was conducted in *L. principis-rupprechtii* plantations in the Taiyue Mountains (36°35′–36°53′ N, 111°91′–112°04′ E) of Shanxi province in northern China. This region is characterized as a warm temperate, continental monsoon climate with cold, dry winters and hot, wet summers. The mean annual temperature for the area is 8.6 °C. The annual average precipitation is about 600 mm and the rainy season is from June to August. The study site is located at an altitude of 2300 m (with study plots ranging from 2298–2358 m), with a slope of 23° (plots ranging 22–25°) and a northern aspect. The soil type is Haplic luvisols, *L. principis-rupprechtii* and *Betula platyphylla* Suk. are the dominant tree species on site. The understory is dominated by *Lonicera japonica* Thunb., *Corylus mandshurica* Maxim., *Rubus corchorifolius* L.f., *Rosa xanthina* Lindl., and *Lespedeza bicolor* Turcz.

2.2. Experimental Design and Treatments

In 1982, foveolate site preparation was carried out along contour lines in mountains, and 3-year-old *L. principis-rupprechtii* seedlings were planted with an initial density of 3000 trees hm^{-2}. In 2000, this plantation was thinned and maintained at 2100 trees hm^{-2}. In 2012, 12 25 × 25 m study plots were established in this area, and the plantation was again thinned, this time to four specified thinning densities: control (CK, 2100 trees hm^{-2}, thinning density: 0%), light (LT, 1850 trees hm^{-2}, thinning density: 15%), medium (MT, 1415 trees hm^{-2}, thinning density: 35%) and high (HT, 1100 trees hm^{-2}, thinning density: 50%). Each treatment was replicated three times (one of the control plots was destroyed by deforestation), and the plots were spaced at least 10 m apart in order to avoid edge effects. Detailed information for these four sites is shown in Table S1.

Five blocks of 2 × 2 m were distributed randomly in each study plot. Soil samples were collected on five different dates in August 2015, and April, June, August and October in 2016, with a depth of 0–10 cm at each block using a metal corer with an inner diameter of 5 cm. Soil samples of the same thinning treatment were mixed to create a composite sample. The soil samples were then sieved at 2 mm to remove roots and gravel. One part of the sample was stored at 4 °C for analyses of soil microbial community and soil enzyme activity. Another part of the sample was air-dried and passed through a 0.25 mm sieve for soil physicochemical analyses.

Soil Property Analyses

Soil water content was calculated from the mass loss by oven-drying samples at 105°C to a constant weight, for at least 48 h [33]. Soil pH was measured by Sartorius PB-10 with a soil solution ratio of 1:2.5. Soil organic carbon and soil total nitrogen was analyzed with an elemental analyzer (Thermo Fisher Scientific, FLASH 2000 CHNS/O, USA). Soil microbial biomass carbon was measured by the fumigation–extraction method [33]. Each sample was fumigated for 24 h at 25 °C with alcohol-free $CHCl_3$, using a 0.5M K_2SO_4 extracting agent and measuring with a TOC analyzer (Analytikjena, Multi N/C 3100 TOC, Germany).

2.3. Soil Microbial Community Structure and Function

Microbial community structure was determined using phospholipid fatty acid (PLFA). PLFA method is sensitive in detecting shifts in microbial community structure, with an inexpensive way of assessing the biomass and composition. PLFA provides the advantage of being an indicator of living organisms since it is rapidly hydrolyzed upon cell death [34]. The lipids in each freeze-dried soil sample were extracted in a single-phase mixture of chloroform, methanol, and phosphate buffer. The abundance of single PLFAs were calculated based on 19:0 internal standard content. After addition of an internal standard, the phospholipid fraction was subjected to a mild alkaline methanolysis, and the resulting fatty acid methyl esters were separated on a gas chromatograph [35,36]. The following soil microbial groups were classified using diagnostic fatty acids as the indicator: gram-positive

bacteria, gram-negative bacteria, saprotrophic fungi (Sap), arbuscular mycorrhizal fungi (AMF), and actinomycetes (Table S2). The abundance of soil microbial single PLFAs were used to analyze soil microbial diversity, which was calculated by the Shannon–Wiener index, species richness, and species evenness.

Shannon index of soil microbial community is

$$H = -\sum_{i=1}^{n} P_i \ln P_i$$

where n is the number of species and P_i is the measure of ith species proportional to the total measure of all species.

Species richness of soil microbial community is

$$S = n$$

where n is the number of species.

Species evenness of soil microbial community is

$$J = \left(-\sum P_i ln P_i\right)/lnS$$

where P_i is the measure of ith species proportional to the total measure of all species, and S is the number of species.

The activities of phenol oxidase and peroxidase were determined by using DOPA (3,4-Dihydroxy-L-phenylalamine) as the substrate. Soil suspension (1 g fresh soil with 1.5 mL 50 mmol L^{-1} sodium acetate buffer) and 2 mL 5 mmol L^{-1} L-DOPA were mixed for phenol oxidase assay. The same suspension was used with an addition of 0.2mL 0.3% H_2O_2 for peroxidase analyses. The activities of β-glucosidase (BG), *N*-acetyl-β-glucosaminidase (NAG) and cellobiohydrolase (CBH) were measured with p-nitrophenol assays [33].

2.4. Statistics

Analysis of variance (ANOVA) was used to determine the effect of thinning treatments on soil microbial community structure and function. Meanwhile, Fisher's least significant difference (LSD) multiple comparison test ($p < 0.05$) was used to compare soil microbial community structure and soil enzyme activities between different thinning intensities. Soil microbial composition and enzyme activities were also tested by repeated measures analysis of variance (RMANOVA) for the effects of thinning treatments across five sampling dates. These analyses were performed using SPSS 19.0 [3].

Redundancy analysis (RDA) was used to assess the correlation between soil microbial composition and soil enzyme activities, as well as the correlation between soil microbial community, soil pH, and soil water content. Based on Monte Carlo permutation with 499 iteration, the RDA was used with forward selection to filter the relative importance of explanatory variables of the microbial composition and soil enzyme activities. Meanwhile, the significant variables were used in the final analyses. Based on the RDA, partial redundancy analysis (pRDA) was used to partition variance of variables. Marginal effects indicate when the variable is used as the only factor. Conditional effects showed the additional variance, and each variable is indicated when it is included in the model. These analyses were completed in CANOCO 4.5 software (Wageningen University and Research Centre, Wageningen, The Netherlands) for Windows.

Structural equation modeling (SEM) was used to test the hypothetical connections between soil physicochemical variables and soil microbial communities, as well as the correlation between microbial community structure and the function, which is relating to soil organic carbon turnover in different thinning treatments. In the path model depicting the hypothesis on the regulatory pathway of soil microbial community structure and function, soil pH and soil water content were considered as the important indicators of changes in soil microbial composition; the abundance of gram-negative bacteria,

saprotrophic fungi, and actinomycetes were the key factors to represent the structural attributes of microbial community. We estimated the model parameters by maximum likelihood estimation using Amos 22.0. The adequacy of model fitting was assessed by a χ^2 test ($p > 0.05$, CMIN/df < 2), the comparative fit index (CFI > 0.9) and the root square mean error of approximation (RMSEA < 0.05) [32]. Numbers on arrows are standardized direct path coefficients. R^2 value represents the proportion of total variance explained for the specific dependent variable.

3. Results

3.1. Soil Parameters

Soil water content increased with the increasing of thinning intensity in August 2015 and June and October in 2016. However, soil water content increased in the LT treatment in August 2016. In August 2015 and April 2016, soil pH significantly increased in the HT treatment. Meanwhile, soil pH increased gradually during growing season in 2016. Soil organic carbon and total nitrogen increased significantly in the MT treatment, however, soil microbial biomass carbon and the ratio of organic carbon to total nitrogen had no consistent trend. In August 2015, and June, August, and October in 2016, soil microbial biomass carbon increased in the MT and HT treatment (Table S3).

3.2. Soil Microbial Community Structure

After thinning treatment, the trend of microbial community structure diversity varied among sampling dates (Table 1). In August 2015 and June 2016, soil microbial Shannon–Wiener index and microbial species richness increased in the MT and HT treatments. In April and October 2016, microbial Shannon–Wiener index and microbial species richness decreased in LT and MT. In the sampling date of August 2016, these indexes decreased in the HT treatment.

Table 1. Soil microbial community structure diversity in each sampling date after thinning.

Months	Treatments	Shannon–Wiener index	Species Richness	Species Evenness
August, 2015	CK	3.38 ± 0.01b	78.50 ± 0.71a	0.78 ± 0.00a
	LT	3.40 ± 0.01b	78.33 ± 1.15a	0.78 ± 0.01a
	MT	3.47 ± 0.03a	79.00 ± 5.57a	0.79 ± 0.02a
	HT	3.42 ± 0.02b	80.00 ± 2.65a	0.78 ± 0.01a
April, 2016	CK	3.22 ± 0.00a	54.00 ± 2.83a	0.81 ± 0.01a
	LT	3.12 ± 0.07a	50.00 ± 2.65a	0.80 ± 0.01a
	MT	3.14 ± 0.09a	52.00 ± 2.65a	0.79 ± 0.01a
	HT	3.21 ± 0.01a	54.33 ± 0.58a	0.80 ± 0.00a
June, 2016	CK	3.27 ± 0.08b	55.00 ± 4.24c	0.82 ± 0.01a
	LT	3.29 ± 0.06b	54.67 ± 1.53c	0.82 ± 0.01a
	MT	3.38 ± 0.03ab	62.67 ± 3.21b	0.82 ± 0.00a
	HT	3.43 ± 0.02a	69.33 ± 2.31a	0.81 ± 0.00a
August, 2016	CK	3.40 ± 0.02a	60.50 ± 0.71a	0.83 ± 0.01a
	LT	3.41 ± 0.12a	64.33 ± 7.37a	0.82 ± 0.01a
	MT	3.37 ± 0.12a	65.67 ± 7.09a	0.81 ± 0.01a
	HT	3.33 ± 0.04a	59.33 ± 6.03a	0.82 ± 0.03a
October, 2016	CK	3.46 ± 0.01a	70.50 ± 3.54ab	0.81 ± 0.01a
	LT	3.39 ± 0.04b	63.67 ± 2.52b	0.82 ± 0.01a
	MT	3.40 ± 0.04b	68.00 ± 9.00ab	0.81 ± 0.02a
	HT	3.47 ± 0.00a	78.00 ± 2.00a	0.80 ± 0.00a

The values are mean ± standard error. Different letters indicate significant differences (p <0.05) among the different thinning treatments. CK: control; LT: light thinning; MT: medium thinning; HT: high thinning.

The RMANOVA results showed that soil microbial abundance was significantly affected by thinning treatment across the sampling times ($p < 0.05$) (Table 2). In the thinning treatments, gram-positive, gram-negative bacteria, AMF, Sap, actinomycetes, and total PLFAs were changed after

different thinning intensities (Table 3). In August 2015 and August 2016, microbial abundance increased in the MT treatment, and then decreased in the HT treatment. However, in April, June, and October in 2016, the abundance of microbial composition in HT was higher than those in other thinning intensities. Moreover, during the growing season in 2016, the microbial abundance increased gradually from April to October.

Table 2. F-values and *p*-values (in parentheses) for significance tests of the effects in different thinning treatments on soil microbial variables.

Microbial Composition	Time of Sampling					Repeated Measurement
	August, 2015	April, 2016	June, 2016	August, 2016	October, 2016	
Total PLFAs	1.63 (0.27)	2.36 (0.16)	63.83 (0.00) ***	1.19 (0.38)	11.19 (0.01) **	116.27 (0.00) ***
Gram-positive bacteria	1.77 (0.24)	1.70 (0.25)	36.60 (0.00) ***	1.00 (0.45)	9.72 (0.01) **	302.55 (0.00) ***
Gram-negative bacteria	0.72 (0.57)	0.60 (0.64)	45.70 (0.00) ***	0.93 (0.48)	8.57 (0.01) **	142.09 (0.00) ***
AMF	1.08 (0.42)	1.27 (0.38)	74.39 (0.00) ***	0.70 (0.58)	8.77 (0.01) **	91.20 (0.00) ***
Sap	1.07 (0.42)	0.682 (0.59)	2.16 (0.18)	0.67 (0.60)	2.08 (0.19)	17.96 (0.01) **
Actinomycetes	0.85 (0.51)	0.94 (0.47)	97.27 (0.00) ***	0.75 (0.56)	11.94 (0.00) **	242.71 (0.00) ***

AMF: arbuscular mycorrhizal fungi; Sap: saprotrophic fungi. **, $p < 0.01$, ***, $p < 0.001$.

Table 3. Soil microbial PLFAs in different thinning treatments.

Microbial Composition	Thinning	Time of Sampling				
		August, 2015	April, 2016	June, 2016	August, 2016	October, 2016
Total PLFAs	CK	83.21 ± 1.82a	16.21 ± 1.72ab	17.08 ± 1.26a	19.23 ± 3.34a	34.29 ± 3.33a
	LT	81.84 ± 7.41a	15.00 ± 1.34a	18.57 ± 1.31a	23.96 ± 5.73a	24.15 ± 2.81b
	MT	91.23 ± 12.81a	15.79 ± 2.65ab	27.64 ± 1.97b	25.97 ± 6.17a	28.03 ± 5.16b
	HT	75.46 ± 7.12a	18.91 ± 1.59b	36.05 ± 2.28c	19.58 ± 3.34a	40.98 ± 3.22c
Gram-positive bacteria	CK	22.02 ± 0.49a	3.00 ± 0.50a	3.41 ± 0.39a	4.41 ± 1.56a	8.31 ± 0.10a
	LT	20.99 ± 1.96a	2.85 ± 0.46a	3.81 ± 0.35a	4.91 ± 1.61a	5.50 ± 0.79b
	MT	24.45 ± 4.16a	2.86 ± 0.76a	6.31 ± 0.65b	6.06 ± 1.64a	6.59 ± 1.59b
	HT	19.51 ± 2.05a	3.71 ± 0.33a	8.80 ± 1.00c	4.15 ± 0.81a	10.58 ± 1.27c
Gram-negative bacteria	CK	23.27 ± 1.70a	2.94 ± 0.81a	3.11 ± 0.36a	3.20 ± 0.99a	7.71 ± 1.07a
	LT	23.57 ± 2.57a	2.75 ± 0.47a	3.54 ± 0.49a	3.94 ± 1.45a	4.69 ± 1.25b
	MT	25.00 ± 5.71a	2.70 ± 0.86a	5.49 ± 0.61b	4.81 ± 1.60a	6.27 ± 1.27b
	HT	20.77 ± 2.12a	3.33 ± 0.39a	8.02 ± 0.61c	3.32 ± 0.72a	9.03 ± 0.66c
AMF	CK	2.10 ± 0.11a	0.26 ± 0.04a	0.28 ± 0.04a	0.28 ± 0.09a	0.76 ± 0.14ab
	LT	2.39 ± 0.40a	0.25 ± 0.05a	0.31 ± 0.01a	0.36 ± 0.14a	0.50 ± 0.08a
	MT	2.78 ± 0.73a	0.27 ± 0.09a	0.56 ± 0.06b	0.44 ± 0.17a	0.70 ± 0.19a
	HT	2.23 ± 0.20a	0.34 ± 0.03a	0.76 ± 0.05c	0.31 ± 0.10a	1.02 ± 0.07b
Sap	CK	1.37 ± 0.18a	0.17 ± 0.06a	0.17 ± 0.01a	0.39 ± 0.16a	0.43 ± 0.06ab
	LT	1.26 ± 0.08a	0.16 ± 0.06a	0.26 ± 0.11ab	0.37 ± 0.13a	0.37 ± 0.12ab
	MT	1.54 ± 0.83a	0.12 ± 0.04a	0.31 ± 0.10ab	0.28 ± 0.04a	0.31 ± 0.03a
	HT	0.88 ± 0.18a	0.17 ± 0.04a	0.38 ± 0.10b	0.30 ± 0.01a	0.50 ± 0.13b
Actinmycete	CK	8.15 ± 0.58a	1.08 ± 0.16a	1.25 ± 0.16a	1.42 ± 0.38a	3.00 ± 0.23a
	LT	7.84 ± 0.43a	1.01 ± 0.09a	1.40 ± 0.12a	1.59 ± 0.55a	1.95 ± 0.28b
	MT	7.89 ± 1.07a	1.06 ± 0.28a	2.31 ± 0.18b	1.91 ± 0.47a	2.33 ± 0.50b
	HT	7.15 ± 0.74a	1.24 ± 0.09a	3.00 ± 0.07c	1.42 ± 0.33a	3.42 ±0.12a

The values are mean ± standard error. Different letters indicate significant differences ($p < 0.05$) among the four different thinning treatments. AMF: arbuscular mycorrhizal fungi; Sap: saprotrophic fungi; CK: control; LT: light thinning; MT: medium thinning; HT: high thinning.

3.3. Soil Enzyme Activities

The soil enzyme activities were significantly affected by thinning treatment across the sampling times ($p < 0.05$) (Table 4). The LT treatment had positive effects on the activities of BG and NAG (Figure 1). In August 2015, thinning treatments decreased the activity of phenol oxidase, while increased the activity of peroxidase. Meanwhile, the activity of BG declined but the activities of NAG and CBH increased in the MT treatment. In April 2016, the MT treatment increased the activities of phenol oxidase and peroxidase, while decreased the activities of NAG and CBH. In the sampling dates of June, August, and October in 2016, the MT treatment increased the activities of phenol oxidase, BG, NAG, and CBH.

Table 4. F-values and p-values (in parentheses) for significance tests of the effects in different thinning treatments on soil enzyme variables

Soil Enzyme	Time of Sampling					Repeated Measurement
	August, 2015	April, 2016	June, 2016	August, 2016	October, 2016	
Phenol oxidase	9.53 (0.01) **	21.98 (0.00) **	4.52 (0.05) *	14.93 (0.00) **	6.17 (0.02) *	96.82 (0.00) ***
Peroxidase	3.42 (0.08)	14.10 (0.00) **	6.05 (0.02) *	0.94 (0.47)	5.66 (0.03) *	56.41 (0.00) ***
BG	1.85 (0.23)	2.23 (0.17)	31.99 (0.00) ***	5.01 (0.04) *	0.69 (0.59)	37.09 (0.00) **
NAG	7.99 (0.01) *	4.81 (0.04) *	34.76 (0.00) ***	16.56 (0.00) **	2.58 (0.14)	26.72 (0.00) ***
CBH	7.23 (0.02) *	6.89 (0.02) *	71.10 (0.00) ***	28.32 (0.00) ***	23.78 (0.00) ***	4.88 (0.00) **

BG: β-glucosidase, NAG: *N*-acetyl-β-glucosidase, CBH: cellobiohydrolase. *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

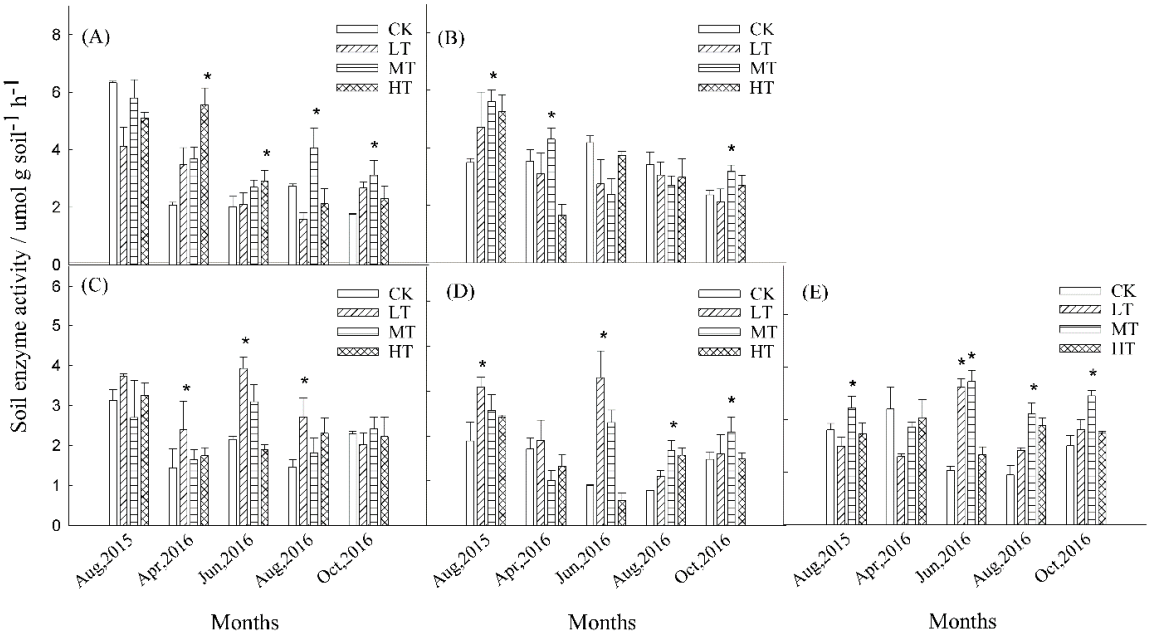

Figure 1. Variation in the activities of phenol oxidase (**A**), peroxidase (**B**), BG (**C**), NAG (**D**), and CBH (**E**) after different thinning treatments. BG: β-glucosidase; NAG: *N*-acetyl-β-glucosidase; CBH: cellobiohydrolase; LT: light thinning; MT: medium thinning; HT: high thinning. * indicate significant differences ($p < 0.05$) compare with the CK treatments.

3.4. Correlation between Soil Microbial Community Structure and Soil Enzyme Activities

We investigated the correlation among gram-positive, gram-negative bacteria, AMF, Sap, and actinomycetes, with enzyme activities. For the RDA ordination, the Monte Carlo permutation test showed that four factors significantly correlated with soil enzyme activities, including actinomycete, gram-positive, gram-negative bacteria, and AMF. The first two RDA axes explained 97.4% of the variance in the relationship between soil microbial community structure and soil enzyme activities. The RDA ordination biplot showed that actinomycetes were positively associated with BG and CBH, while gram-positive, gram-negative bacteria, and AMF were associated with NAG (Figure 2). Together, these microbial compositions explained 40.2% of the variance in soil enzyme activities. Forward selection of the four variables in the RDA ordinations showed that actinomycetes and gram-negative bacteria as the key factors had primarily affected soil enzyme activities (Table 5). AMF had a relatively high marginal effect, however, the conditional effect of AMF was negligible. The result of variance partitioning showed that gram-negative bacteria and actinomycetes explained 36% of the variance in soil enzyme activities. Compared with actinomycetes, the variation explained by gram-negative bacteria alone had a relatively high predictive power (19.7%) and an interactive effect between them explained 8.5%.

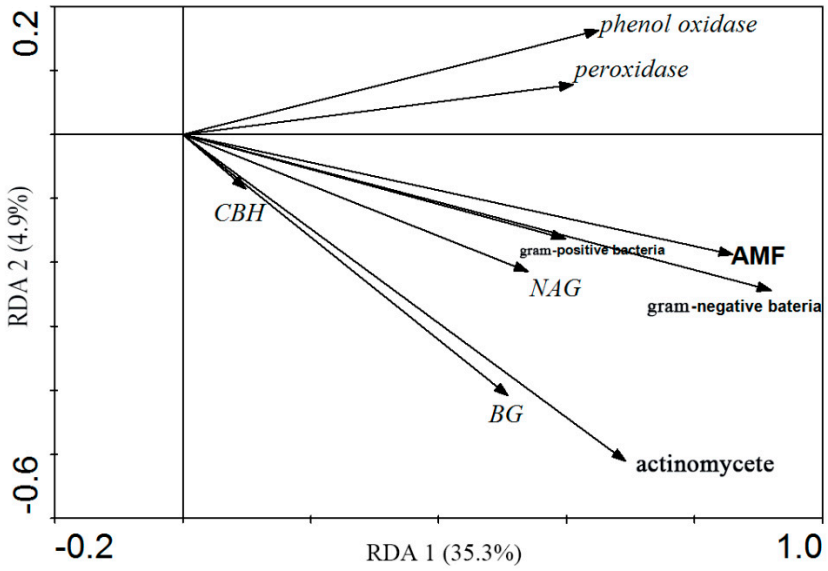

Figure 2. Redundancy analysis (RDA) ordination biplot of soil microbial community and soil enzyme activities. AMF: arbuscular mycorrhizal fungi; BG: β-glucosidase; NAG: *N*-acetyl-β-glucosidase; CBH: cellobiohydrolase.

Table 5. Marginal and conditional effects of microbial composition on soil enzyme activities obtained from forward selection in redundancy analysis (RDA).

Variables	Lambda-A [1]	Lambda-B [2]	*P* [3]	F-Ratio [4]
Actinomycetes	0.18	0.18	0.01	4.03
Gram-negative bacteria	0.30	0.20	0.00	5.41
Gram-negative bacteria	0.13	0.02	0.61	0.66
AMF	0.26	0.01	0.92	0.20

[1] Describe marginal effects, which show the variance explained when the variable is used as the only factor. [2] Describe conditional effects, which show the additional variance each variable explains when it is included in the model. [3] The level of significance corresponding to Lambda-B when performing Monte Carlo test at the 0.05 significance level. [4] The Monte Carlo test statistics corresponding to Lambda-B at the 0.05 significance level. AMF: arbuscular mycorrhizal fungi.

3.5. Effects of Soil PH and Soil Water Content on Soil Microbial Community

The path of the soil oxidase-related variables passed the statistical test for adequacy (p = 0.33, CNMI/df = 1.13, CFI = 1.00, RMSEA < 0.05) and the non-significant pathways were deleted. The model explained 39% and 47% of the variance in the activities of phenol oxidase and peroxidase respectively. The path analysis suggested that soil water content was negatively related with pH, and directly affected actinomycetes. In addition, soil water content exerted indirect effects on gram-negative bacteria, and then affected the activity of phenol oxidase. Soil pH had a directly negative effect on the activity of peroxidase. Meanwhile, pH exerted positive and indirect effects on the activities of phenol oxidase and peroxidase by influencing AMF and gram-negative bacteria. Compared with soil water content, soil pH had a relatively stronger effect on soil microbial community in this path model. AMF had a strongly positive effect on the activity of peroxidase (Figure 3).

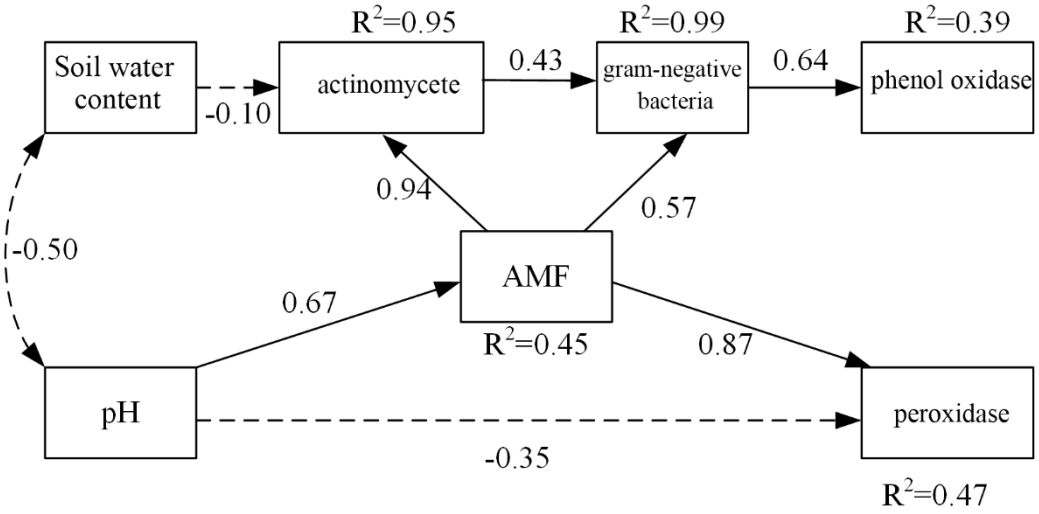

Figure 3. Path of soil oxidase controlled by soil pH, soil water content, and microbial community. AMF: arbuscular mycorrhizal fungi; dash-line arrows indicate negative effects.

The path of the soil hydrolase-related variables passed the statistical test for adequacy (p = 0.43, CNMI/df = 1.02, CFI = 1.00, RMSEA = 0.02) and the non-significant pathways were deleted. The model explained 58, 22, and 42% of the variance in the activities of BG, NAG, and CBH, respectively. Soil pH directly affected AMF, and then indirectly influenced soil hydrolase by affecting gram-negative bacteria and actinomycetes. Soil water content had a direct effect on the activity of CBH. Compared with soil water content, soil pH had a relatively stronger effect on soil microbial community in this path model. AMF had a direct and strongly positive effect on the actinomycetes, which directly influenced the activity of NAG (Figure 4).

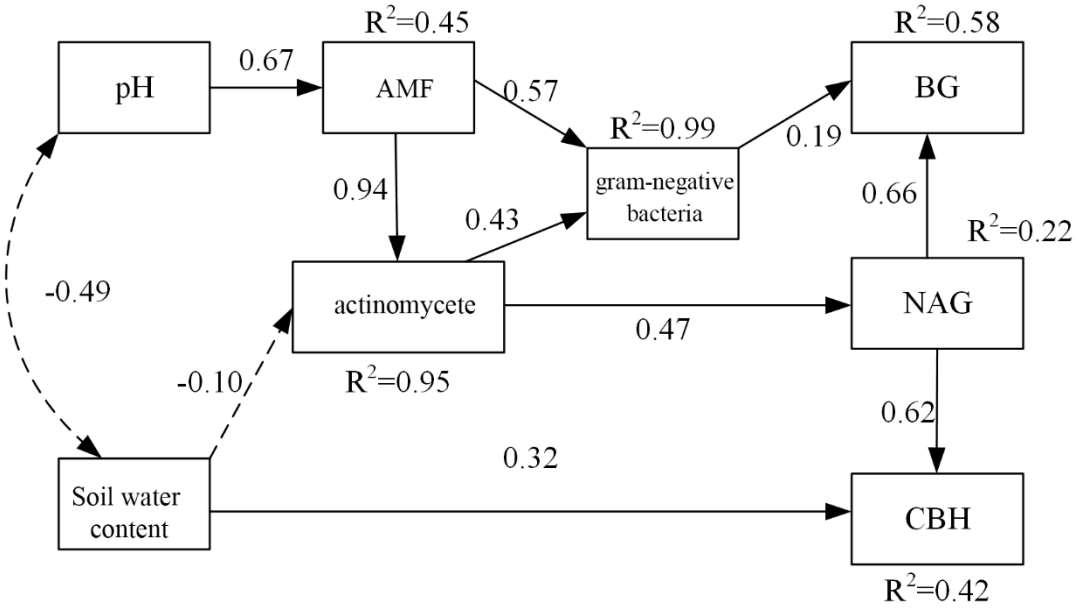

Figure 4. Path of soil hydrolase controlled by soil pH, soil water content, and microbial community. AMF: arbuscular mycorrhizal fungi; BG: β-glucosidase; NAG: *N*-acetyl-β-glucosidase; CBH: cellobiohydrolase. Dash-line arrows indicate negative effects.

3.6. Effects of Soil PH, Soil Water Content, and Microbial Factors on Soil Carbon Properties

Among these variables, pH, soil water content, Shannon–Wiener index, phenol oxidase, peroxidase, BG, and NAG were found to significantly affect soil carbon properties. For the RDA ordination, forward

selection of these variables and a Monte Carlo permutation test showed that soil pH, Shannon–Wiener of soil microorganisms, and the activities of phenol oxidase, peroxidase, and NAG were the main factors influencing soil organic carbon and microbial biomass carbon (Table 6). Soil water content and BG were removed from variance partitioning. Even though there was a notable effect of soil water content and BG, the conditional effect was negligible. The result of variance partitioning showed that soil pH and microbial variables explained 73.7% of the variance in soil carbon. Soil microbial factors and soil pH explained 20.3% and 7.6% of the variance, respectively, while the interactive effect between the two explained 45.8%.

Table 6. Marginal and conditional effects of soil pH, soil water content, and microbial community on soil carbon obtained from forward selection in redundancy analysis (RDA).

Variables	Lambda-A [1]	Lambda-B [2]	*p* [3]	F-Ratio [4]
Soil pH	0.53	0.53	0.00	42.83
NAG	0.23	0.08	0.00	10.37
peroxidase	0.19	0.06	0.00	9.23
Shannon–Wiener index	0.26	0.04	0.00	6.94
Phenol oxidase	0.25	0.03	0.02	4.81
Soil water content	0.22	0.00	0.79	0.22
BG	0.16	0.00	0.93	0.07

[1] Describe marginal effects, which show the variance explained when the variable is used as the only factor. [2] Describe conditional effects, which show the additional variance each variable explains when it is included in the model. [3] The level of significance corresponding to Lambda-B when performing Monte Carlo test at the 0.05 significance level. [4] Monte Carlo test statistics correspond to Lambda-B at the 0.05 significance level. BG: β-glucosidase, NAG: *N*-acetyl-β-glucosidase.

4. Discussion

4.1. Soil Microbial Community Structure and Soil Enzyme in Thinning Treatments

One finding of our study was that soil microbial abundance was significantly affected by forest thinning. The consistent results on the effects of thinning on soil microbial communities were reported by some previous studies [12,37]. It is theorized that differential responses of microbial communities to thinning might be related to the sensitivity of microbes to environmental change and the variation in microbes to carbon source utilization [38]. However, some studies have shown that thinning had little influence on soil microbial community structure because of different thinning intensities and methods [39,40].

Our results suggested that medium and high thinning increased the abundance and diversity within the microbial community. Gap formation resulting from thinning increased light transmittance to understory plants, and improved forest microclimate and created more favorable conditions for microbial decomposers [41,42]. Our results were consistent with those from a study in Chinese fir plantations, which showed increases in soil microbial biomass after seven years of thinning with high thinning treatment [43]. Similar results were also observed in *Pinus densiflora Sieb. et Zucc.* forests, where the abundance of soil microbes increased significantly after seven years of thinning [44]. This is because thinning had increased carbon inputs from litter, root exudates, and dead fine root [43,44]. However, decreased soil microbial abundance in forest thinning had also been reported [11]. In 2016, soil microbial abundance increased gradually from the beginning to the end of the growing season. One reason for this result was that soil temperature and moisture conditions were suitable for microbial growth and reproduction in the summer. Meanwhile, leaf litter in autumn led to an increase below-ground carbon inputs and nutrient utilization. In this study, we found that medium thinning increased microbial diversity in summer (August 2015 and June 2016). However, microbial diversity decreased in the medium thinning in spring and autumn (April and October 2016). There is precedence in the literature for seasonal shifts in microbial diversity [11], with soil effective nutritive elements, litter quality, and seasonal carbon inputs varying by season and influencing microbial composition [45]. In addition,

increased understory plant diversity following thinning can also stimulate soil microbial community diversity [36,46], although we did not test this directly in our study. However, some research found that plant community better predicts the variation in soil microbial community structure following the changes in the quality and quantity of litter and soil physicochemical properties [47]. Future studies should assess the relationship between soil microbial diversity and understory plant diversity.

Our finding showed that soil enzyme activities were significantly affected by forest thinning. The light and medium thinning treatments had elevated hydrolase activities, however, there was no obvious regularity in oxidase activities. The increasing of the efficiency of litter decomposition due to thinning promoted the activities of enzyme relating to carbon decomposition [48]. Our finding was inconsistent with previous results that thinning decreased the activities of BG and cellulose, possibly due to reduced turnover of extraradical hyphae of mycorrhizae [43]. An investigation indicated that thinning decreased the activities of phenol oxidase and cellulose, but increased peroxidase activity. Our results also showed that medium thinning increased the activities of phenol oxidase, CBH, and NAG, suggesting that moderate thinning had positive effects on soil enzyme activities. With light and medium thinning, the variation of hydrolase activities peaked in June, and then declined in August and October. This finding was not consistent with previous studies. Previously, it was found that the activities of oxidase were higher in spring than in summer and autumn, however, the seasonal variation in hydrolase activities was irregular [49].

4.2. Correlation between Soil Microbial Community Structure and Soil Enzyme Activities, Controlled by Soil PH and Soil Water Content

Soil enzyme activities were used to assess the function of carbon transformation, which was related to microbial community structure [50]. Specific soil microbial composition was involved in regulating a specific process. It is theorized that the variation in hydrolases activities were related to bacterial abundance, and oxidases activities were affected by the abundance of fungi [51]. In this paper, we found that thinning can modulate the microbial structure–function relationship. After thinning treatment, the abundance of bacteria and AMF were correlated with the activity of NAG, while actinomycetes were positively associated with the activities of BG and CBH. These results were consistent with the finding of previous study [33], who reported that gram-negative bacteria, AMF, and actinomycetes correspond with the activities of hydrolases. It is suggested that thinning weakens the effects of oxidase on complex decomposed carbon sources, while it enhances the effects of hydrolase on easily decomposed carbon sources. Actinomycetes and gram-negative bacteria were the major factors to affect soil microbial function, and gram-negative bacteria had relatively higher predictive power. Recent studies conformed that bacteria had strong correlations with the capacity of soil carbon decomposition [52], and soil microbial diversity contributed to key soil microbial functions, regulating soil carbon dynamics [53]. These results are consistent with our finding. These microbial compositions explained 40.2% of the variance in soil enzyme activities. This relatively low predictive power suggests that there are other missing factors affecting soil enzyme activities.

Soil water content and soil pH were the key factors to regulate soil microbial community structure, function, and soil carbon process [54]. The result showed that thinning treatment increased soil water content. One reason is that thinning altered canopy density and increased the amount of water reaching the soil surface via throughfall in forest. Soil pH increased in the HT treatment in some sampling dates. The variation of soil pH and soil water content altered soil microbial structure, exerting direct and indirect impacts on soil microbial function. In the SEM model, soil pH directly affected AMF, and then exerted indirect and positive effects on oxidase and hydrolase. Soil water content had a stronger influence on oxidase and hydrolase through influences on gram-negative bacteria and actinomycetes. This phenomenon was inconsistent with prior study that soil pH was not correlated with soil microbial community structure [55]. However, the study theorized that soil water content and pH were the main factors regulating soil microbial community, as soil water content changed plant nutrient availability, which was strongly correlated with soil pH. The importance of soil pH and soil water content for

microbial community structure was widely proved [56]. Previous literatures suggested that soil pH, as a driver of soil bacterial community, significantly affected soil microbial function [57].

4.3. Effects of Soil PH, Soil Water Content, and Microbial Factors on Soil Carbon Properties

Soil carbon properties were controlled by individual biotic and abiotic factors and their interactive effects, and the structural shifts in soil microbial communities were related to alterations of their functioning, such as in mediating soil organic carbon dynamics [36]. Our result showed that the MT treatment significantly increased soil organic carbon. The MT or HT treatment stimulated soil microbial growth and increased microbial biomass carbon in different sampling dates, which had been observed in other studies [58]. As a detector of soil organic matter availability, soil microbial biomass carbon was sensitive to environmental variation. The increasing of microbial biomass carbon was related to the variation of soil water and soil pH. Previous studies theorized that the increasing of soil organic carbon and microbial biomass carbon in moderate thinning treatment may be caused by the changes of the quantity and quality of soil substrates [43]. In this paper, soil pH, soil water content, microbial community diversity and functional properties were used to assess the contribution on soil carbon. As far as the conditional effects be concerned, there were strong effects of soil pH, oxidase, NAG, and microbial diversity on soil carbon. Soil pH was the key factor that affected the variation of soil carbon properties. Some studies had consistent results, which showed that moderate soil pH stimulated soil microbial growth and accelerated microbial decomposition [59]. Oxidase is involved in the degradation of lignin, the most abundant component of litter. In this study, gram-negative bacteria and AMF significantly and directly contributed to oxidase activities. Compared with oxidase activities, there was no notable influence of hydrolase on the soil carbon process. This phenomenon was inconsistent with the findings of previous studies, in which oxidase was produced directly by fungi [60]. Oxidase enzymes may result from a positive interaction between AMF and other fungal groups [61].

Our finding suggested that soil pH and microbial community had a strong influence on soil carbon properties, whereas the effect of soil water content was negligible. The marginal effects of soil pH and microbial community were 7.6% and 20.3%, respectively. However, the conditional effects of them had a significantly strong power. It was suggested that there were strong correlations between soil pH and microbial community to control soil carbon turnover. The previous study theorized that soil physicochemical properties and microbiological diversity contributed to carbon turnover and transformation [54]. After thinning treatment, the variation in soil pH and soil water content influenced soil microbial function relating to carbon decomposition, which was regulated by soil microbial community structure, and finally affected soil carbon properties.

5. Conclusions

Our study revealed that medium or high forest thinning has positive effects on microbial abundance and microbial community function. After forest thinning, variation in soil pH and soil water content altered soil microbial structure, and actinomycetes and gram-negative bacteria were the major factors to affect soil microbial function relating to carbon decomposition. Soil pH contributed to actinomycetes and gram-negative bacteria through direct influences on AMF. Meanwhile, there were strong correlations between soil pH and microbial community to control soil carbon turnover. This study showed that thinning can alter soil physicochemical properties and further affect soil carbon via microbial regulations. These findings have important implications for forest management. Soil microbial abundance, which is improved by thinning, is probably related to the increases in soil organic carbon. Therefore, the increasing of soil microbial abundance and microbial regulation on soil carbon needs to be considered for sustainable forest management practices in northern China.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/10/4/352/s1, Table S1: Basic sample plot information.

Author Contributions: R.W., H.H., and X.C. conceived and designed the experiments; R.W. performed the experiments; R.W. analyzed the data; X.C. and H.H. contributed materials and analysis tools; R.W. wrote the paper.

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