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Soil labile organic carbon and carbon-cycle enzyme activities under different thinning intensities in Chinese fir plantations

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ABSTRACT

Thinning is a silvicultural tool that is used to facilitate the growth of timber plantations worldwide. Plantations are important CO_2 sinks, but the mechanism by which thinning affects the quantity and stability of soil organic carbon (SOC) is poorly understood. In this study, we examined the effects of different thinning intensities (low-intensity thinning treatment with 30% of the trees removed; high-intensity thinning treatment with 70% of the trees removed; control treatment without tree removal) on the quantity and stability of SOC in Chinese fir (*Cunninghamia lanceolata* [Lamb.] Hook) plantations in southeastern China. The amounts of SOC, microbial biomass carbon (MBC), easily oxidizable carbon (EOC), cold-water- soluble organic carbon (CWSOC) and hot-water- extractable organic carbon (HWEOC) and the carbon-cycle-related enzyme activities (β-glucosidase, invertase and cellulose) were quantified. We found that thinning significantly decreased the amount of SOC compared with the control treatment, but the effect differed by sampling date. The MBC and EOC were significantly higher in the high-intensity thinning treatment than in the control and low-intensity thinning treatments, whereas the invertase and β-glucosidase activities were significantly higher in the control treatment. However, the amounts of CWSOC, HWEOC and cellulose activity did not differ among the treatments, which indicates that the MBC, EOC and the activities of invertase and β-glucosidase were better indicators of changes in SOC to thinning. In addition, the MBC, EOC, CWSOC and the β-glucosidase and cellulase activities peaked in the warmer months. Our results indicate that thinning treatments in Chinese fir plantations decreased the SOC quantity and enzyme activities and that high-intensity thinning may lead to an increase of labile SOC.

1. Introduction

Global forests store approximately 861 Pg of carbon (C), which represents nearly 44% of the carbon that is stored in the soil, and as a major C sink, even small impacts of the management of forest soils can strongly influence the global C cycle (Dixon et al., 1994; Pan et al., 2011). A major focus in forest management is to promote the increase of this sink (Jandl et al., 2007; Achat et al., 2015; Moreno-Fernandez et al., 2015). Soil organic carbon (SOC) can be divided into labile and recalcitrant fractions based on the mean residence times in the soil. Land management practices tend to have little influence on recalcitrant C because of its longer turnover time (Haynes, 2005). In contrast, labile SOC fractions, which have mean residence times that range from years to several decades, can respond rapidly to forest management and can be used as a sensitive indicator of changes in SOC because they are the main source of C that is released from soil to the atmosphere and have a potential to accelerate the decomposition of recalcitrant carbon by a priming effect through soil microorganisms and thus break soil carbon stability (Mao et al., 2012; Qiao et al., 2014; Shang et al., 2016). In addition, the activities of soil carbon cycle-related enzymes (e.g., β-glucosidase, invertase and cellulase) control the decomposition of organic carbon and reflect the metabolic

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requirements of the soil microbial community and the status of the available carbon resources; thus, these enzyme activities can aid in understanding the variations in SOC in response to forest management (Guan et al., 2014; Veres et al., 2015).

Thinning is an important forest management practice in both natural forests and plantations worldwide. This technique may increase forest nutrient availability, productivity and biodiversity, and it is an important tool for manipulating species composition (Verschuyl et al., 2011). Tree removal and the subsequent opening of the forest canopy by thinning change the microclimatic characteristics of the soil and affect the quantity and quality of potential organic inputs and the leaching of dissolved organic matter, thereby modulating SOC (Barg and Edmonds, 1999; Wic Baena et al., 2013). Thinning commonly decreases SOC by reducing litter inputs into the soil and possibly through the microclimate-driven acceleration of decomposition rates (Piene and Cleve, 1978; Covington, 1981; Jandl et al., 2007). However, long-term thinning practices have been reported to increase soil carbon by enhancing root system decomposition in thinned trees and understory growth after canopy removal (Selig et al., 2008). Despite an abundance of studies about the effects of thinning on the total SOC of the forest floor, few have examined how thinning influences labile carbon pools and carbon cycle-related enzymes in the mineral soil (Chiang et al., 2010; Geng et al., 2012; Chen et al., 2015), and the results are still controversial. For example, Bolat (2014) and Yuan et al. (2010) found thinning increased labile organic carbon, but decreases have been reported in other studies (Schilling et al., 1999; Hassett and Zak, 2005). In addition, because seasonal variations in soil temperature and aboveground vegetation might affect the ability or strategy of microbial decomposers to efficiently use SOC, the thinning effect on labile SOC and enzymatic activities may vary seasonally (Balser and Wixon, 2009; Liptzin and Silver, 2009; Keiblinger et al., 2010; Zhou et al., 2012).

Chinese fir (*Cunninghamia lanceolata* [Lamb.] Hook) is an important fast-growing, evergreen coniferous timber species that has been widely planted in southeastern China for more than 1000 years (Chen, 2003). Because of its high commercial value, Chinese fir plantations, which are typically high-density monocultures, have been widely established in previous natural broadleaf forests, and short rotation forestry has become common over the past century in an attempt to meet the rising demand for timber (Tian et al., 2011). These Chinese fir monoculture plantations are carbon sinks, but they are more susceptible to fires and pests than the forests that they have replaced. Furthermore, soil nutrient depletion and yield reduction become common problems after one or more rotations (Tian et al., 2011). Thus, silvicultural practices that recreate historic forest stand structures and thereby optimize the use of the soil, have been a focus of the sustainable management of Chinese fir plantations in recent years, particularly in terms of maintaining soil productivity and ecosystem sustainability.

This study aims to assess the response of SOC, its labile components and related enzyme activities to different thinning intensities. We investigated how the concentrations of SOC, microbial biomass carbon (MBC), easily oxidizable carbon (EOC), hot water-extractable organic carbon (HWEOC) and cold water-soluble organic carbon (CWSOC) as well as the activities of β-glucosidase, invertase and cellulase change following thinning. The specific objective was to assess (1) how different thinning intensities (0%, 30% and 70% stem-only thinning) influence these soil labile SOC fractions and carbon cycle-related enzyme activities and (2) whether the effects of thinning vary with the season. We hypothesized that with increasing thinning intensity, 1) the SOC and labile components will decrease because of the lower carbon inputs from trees after partial canopy removal; 2) the C-cycle enzyme activities will increase because the microclimate and light environment increase the SOC decomposition rate; and 3) the labile components and related enzyme activities peak in the summer months because of their high sensitivities to temperature and precipitation.

2. Materials and methods

2.1. Study area and thinning experiment

The study was conducted on a 26-year-old Chinese fir plantation at the Lishui Tree Farm Research Station (119°01′E, 31°36′N) of Nanjing Forestry University in Jiangsu, China. The site is located at an elevation of 100 m and has a slope of 15° with a southern orientation. The soil is a Haplic Luvisol (FAO, 1998) and is generally no more than 30 cm deep. The climate of the study area is subtropical with a mean annual temperature of 15.5 °C, a mean annual sunshine duration of 2146 h, a mean annual precipitation of 1005.7 mm, and a mean frost-free period of 220 days per year from 1995 to 2014. The monthly temperature and rainfall both peak in the summer months May–October (Supplementary Fig. S1).

Nine experimental plots (20 × 20 m) were established under an overstory that was dominated by Chinese fir in a completely randomized design with three replicates for each of the three treatments: no thinning (CK, control), low-intensity thinning (LIT, 30% of the trees removed) and high-intensity thinning (HIT, 70% of the trees removed). To reduce potential edge effects, each plot was surrounded by a 5 m-wide buffer zone. Whole-tree thinning was performed from February to April 2006. The trees in the thinning treatments were thinned from below (i.e., the subcanopy/suppressed trees were removed), and the distribution of the remaining trees was evened. All of the thinned stems were removed from the plots and used as commercial wood. The stand characteristics in the different treatments, including the plant species composition, mean tree height, diameter at breast height (DBH), stand density and biomass, were measured in October 2012 (Table 1). The fine root biomass was measured (Dong Wang and Xinli Chen, unpublished data) using 10 soil cores (5 cm diameter × 20 cm deep) that were collected from the site during the first weeks of January, March, May, July, September and November 2013. The fine roots were sorted into diameter ≤ 2 mm, dried at 85 °C for 48 h and weighed (Table 1).

2.2. Soil sampling

In each of the nine experimental plots, soil samples were taken at two mineral soil depths (S1, 0–10 cm; S2, 10–25 cm) using a 4.0-cm auger in the fall (October 3, 2012), winter (January 3, 2013), spring (April 3, 2013), and summer (July 3, 2013). Three sampling points were selected at random locations within each plot. The soil samples from different sites were pooled and mixed thoroughly, sieved (2-mm mesh), and visible roots and insects were removed. Each pooled soil sample was divided into two parts; one part was stored at 4 °C for the analysis of MBC, HWEOC, and CWSOC concentrations and soil β-glucosidase activity within 3 days, and the remaining part was air-dried to measure the SOC and EOC concentrations and both invertase and cellulose, whose activities can be maintained in air-dried at room temperature for 1 month (Schinner and Vonmersi, 1990). Soil temperatures at depths of 10 cm and 20 cm were recorded with temperature sensors (DS1921G-F5#, Maxim, USA) every 30 min from September 15, 2012 to July 3, 2013.

2.3. Chemical and biochemical analyses

The soil pH was determined using a glass electrode with a soil: solution ratio of 1:2.5. The moisture content was determined by oven drying the samples at 105 °C. The SOC was measured by wet

Table 1
Stand characteristics of the nine experimental plots. The values are the means ± SD for the plots.

Characteristic	CK		LIT		HIT	
Stand						
Stand density (trees ha^{-1})	3079 ± 53		2073 ± 34		1020 ± 22	
Stand basal area (m^2 ha^{-1})	27.6 ± 1.01		25.0 ± 0.85		17.4 ± 1.23	
DBH (cm)	10.69 ± 0.37		12.39 ± 0.26		14.73 ± 1.67	
Height (m)	11.83 ± 0.28		12.58 ± 1.22		15.42 ± 0.46	
Shrub biomass (t ha^{-1})	517.51 ± 23.37		956.25 ± 21.12		1307.53 ± 26.11	
Herb biomass (t ha^{-1})	362.48 ± 11.22		350.02 ± 15.33		529.98 ± 19.45	
Soil depth	0–10 cm	10–25 cm	0–10 cm	10–25 cm	0–10 cm	10–25 cm
Clay (100%)	1.13 ± 0.20	2.02 ± 0.19	0.88 ± 0.18	1.68 ± 0.20	1.34 ± 0.11	2.32 ± 0.21
Silt (100%)	32.81 ± 3.44	37.28 ± 2.12	32.94 ± 0.89	36.79 ± 2.44	34.22 ± 4.45	38.58 ± 2.68
Sand (100%)	66.06 ± 4.25	60.7 ± 3.12	66.18 ± 5.22	61.53 ± 4.21	64.44 ± 4.85	59.1 ± 5.11
Soil temperature (°C)	14.85 ± 7.48	14.46 ± 7.26	15.06 ± 7.87	15.17 ± 7.50	15.05 ± 7.79	15.29 ± 7.44
pH	4.62 ± 0.09	4.69 ± 0.13	4.69 ± 0.10	4.71 ± 0.06	4.63 ± 0.07	4.68 ± 0.07
Soil total N (g kg^{-1})	2.23 ± 0.48	1.61 ± 0.17	1.97 ± 0.16	1.52 ± 0.17	2.27 ± 0.32	1.48 ± 0.13
C/N	10.18 ± 0.85	8.74 ± 0.78	10.11 ± 1.06	8.28 ± 0.87	10.02 ± 0.96	8.20 ± 0.79
Soil total P (g kg^{-1})	0.45 ± 0.1	0.36 ± 0.06	0.39 ± 0.12	0.31 ± 0.07	0.46 ± 0.07	0.34 ± 0.07
	0–10 cm	10–20 cm	0–10 cm	10–20 cm	0–10 cm	10–20 cm
FRB(Chinese fir)	45.72 ± 2.04	33.53 ± 2.84	43.42 ± 3.74	33.40 ± 1.46	45.44 ± 5.36	33.68 ± 3.44
FRB (Other plants)	29.42 ± 2.63	13.27 ± 1.51	28.70 ± 2.34	20.53 ± 2.72	36.04 ± 3.08	21.46 ± 1.73
FRB (Dead)	18.52 ± 1.75	29.37 ± 4.09	14.77 ± 1.42	22.84 ± 2.80	20.38 ± 2.26	22.51 ± 2.81
FRB (Total)	93.66 ± 2.62	76.18 ± 6.36	86.90 ± 5.60	76.75 ± 5.15	101.87 ± 8.59	77.65 ± 6.67

CK: control; LIT: low-intensity thinning treatment; HIT: high-intensity thinning treatment, FRB: fine root biomass (similarly hereafter).

digestion with potassium dichromate (Snyder and Trofymow, 1984). Total nitrogen (N_{tot}) was measured with dry combustion and thermal conductivity detection using a C/N/S-analyzer (Vario EL III, Elementar, Germany).

The HWEOC and CWSOC were determined on fresh soil samples using the method of Bu et al. (2012). Each sample was extracted with distilled water (water: soil ratio of 2:1), shaken for 30 min at 80 °C and 20 °C for the HWEOC and CWSOC respectively and centrifuged for 20 min at 4000 rpm. All of the supernatants were filtered through 0.45 μm cellulose acetate membrane filters. The concentrations of HWEOC and CWSOC were immediately measured using a carbon analyzer (Shimadzu TOC-VCPN, Japan). The MBC was determined by the fumigation-extraction method (Vance et al., 1987) and was calculated as: $MBC = E_C/k_{EC}$, where E_C is the difference between the amount of organic carbon extracted from fumigated and non-fumigated soils and k_{EC} equals to 0.45. The EOC was determined by the method of 333 mmol L^{-1} $KMnO_4$ oxidation (Blair et al., 1995).

The soil β-glucosidase activity (NAG) was measured using a substrate of pNPG and expressed in μg *p*-nitrophenol (pNP) g^{-1} soil h^{-1} (Geng et al., 2012). The invertase and cellulase activities were determined using a substrate of carboxymethylcellulose and sucrose, and their activities were expressed in mg glucose g^{-1} soil (Schinner and Vonmersi, 1990; Deng and Tabatabai, 1994).

2.4. Data analysis

The repeated measures analysis of variance was used to determine the effect of the thinning treatments and sampling seasons on the SOC, MBC, CWSOC, HWEOC, and EOC concentrations and the activities of invertase, cellulase and β-glucosidase. When necessary, variables were $\log_{10}$ transformed to satisfy the analysis of variance assumptions of normality, sphericity, and homogeneous variances. In addition, the Pearson's correlation coefficients between the SOC, labile organic carbon fractions, soil enzyme activities and some soil physical-chemical variables were caculated. All of the statistical tests were conducted using SPSS 19.0 (SPSS, Chicago, IL, USA).

3. Results

3.1. Labile SOC fractions

Seven years after thinning, the SOC, EOC and MBC varied significantly with thinning treatment, with more pronounced effects in the upper (0–10 cm) than soil layer than the lower (10–25 cm) soil layer. In contrast, CWSOC and HWEOC did not differ with treatments (Table 2). In the upper soil, low-intensity and high-intensity thinning increased the SOC in October by 21.6% and 33.2%, respectively, compared with the control treatment, but decreased the SOC by 12.9% and 8.9% in April and 37.1% and 15.4% in July, respectively (Fig. 1). In the lower soil layer, both low-intensity

Table 2
Effects (F-ratio, p-values) of thinning intensity (T), sampling season (S) and their interaction on the quantity and stability of soil carbon as analyzed by repeated measures analysis of variance (n = 3).

Characteristic	Statistic	0–10 cm			10–25 cm		
		T	S	T × S	T	S	T × S
SOC	F	8.53	65.93	8.98	5.54	2.84	1.20
	p	0.018	<0.001	<0.001	0.043	0.169	0.381
MBC	F	26.46	23.72	2.12	6.992	11.24	2.05
	p	0.001	0.005	0.141	0.027	0.020	0.151
EOC	F	23.14	84.67	7.84	11.14	14.20	1.19
	p	0.001	<0.001	0.011	0.010	0.013	0.386
CWSOC	F	0.068	29.53	1.88	1.38	22.26	0.46
	p	0.935	0.003	0.180	0.322	0.006	0.824
HWEOC	F	0.97	22.59	0.61	2.00	26.77	2.09
	p	0.432	0.006	0.720	0.216	0.004	0.144
Glu	F	5.77	371.03	9.47	6.44	6.99	3.26
	p	0.040	<0.001	<0.001	0.032	0.045	0.048
Cellulase	F	1.96	26.21	1.73	2.40	0.49	2.14
	p	0.222	<0.001	0.171	0.172	0.696	0.098
Invertase	F	94.27	52.64	3.50	31.17	333.09	4.04
	p	<0.001	0.001	0.039	<0.001	<0.001	0.026

T: thinning treatments, S: season of the year, SOC: soil organic carbon, MBC: microbial biomass carbon, EOC: easily oxidizable carbon, CWSOC: cold-water soluble organic carbon, HWEOC: hot-water extractable organic carbon, Glu: β-glucosidase.

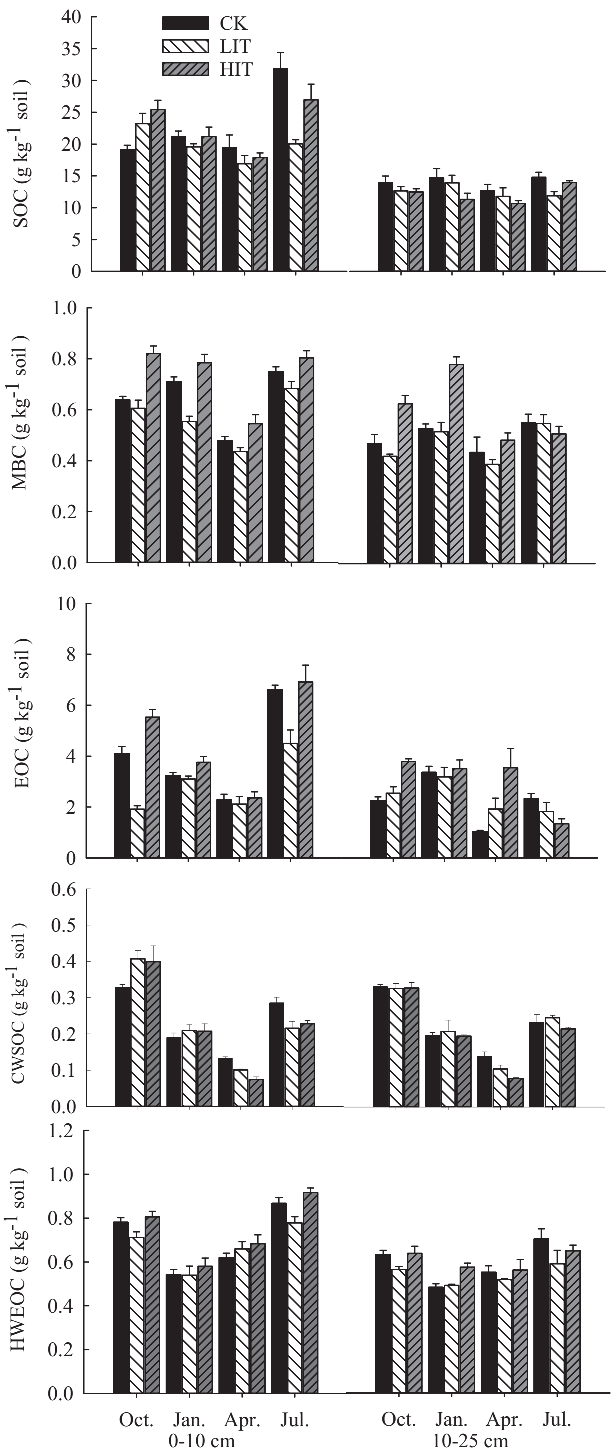

Fig. 1. Soil liable organic carbon fractions (mean values and standard error of mean) in the different thinning intensities (CK: control LIT, low intensity thinning; HIT, high intensity thinning) on four sampling dates.
SOC: soil organic carbon, MBC: microbial biomass carbon, EOC: easily oxidizable carbon, CWSOC: cold-water soluble organic carbon, HWEOC: hot-water extractable organic carbon.

and high-intensity thinning consistently decreased the SOC. The MBC was higher in the high-intensity thinning treatment than in both the control and low-intensity thinning treatments across all of sampling dates except for July, when the MBC in the lower soil was 7.8% lower in the thinned sites than in the control. The response of the EOC to thinning was similar to that of the MBC in the upper soil, but in the lower soil, the thinning treatments increased the EOC in October and January and decreased it in July (Table 2, Fig. 1).

Over the four sampling dates, the SOC and all of the labile SOC fractions, except for HWEOC, differed significantly, with more pronounced effects in the upper soil layer than in the lower soil layers (Table 2, Fig. 1). In the upper soil, the SOC was higher in the summer (July) than in the other seasons in both the control and high-intensity thinning sites; whereas the labile organic carbon fractions peaked in the warmer months (October and July). In the lower soil, no significant differences in SOC were observed between the seasons. Except for the HWEOC, which was lowest in January, the lowest values for the labile organic carbon fractions were observed in April (Fig. 1).

3.2. Soil β-glucosidase, cellulase and invertase activities

The soil invertase and β-glucosidase activities were also significantly affected by the thinning treatments and varied with sampling date and soil depth (Table 2). However, no difference in cellulase activity was observed among the treatments (Table 2). Thinning decreased the invertase activity across all of the sampling dates, with more pronounced effects in the upper soil than in the lower soil. In the upper soil, the thinning treatments decreased the β-glucosidase activity in October, April and July, but this general trend changed in January, when the β-glucosidase activity increased by 13.5% and 22.2% in the low- and high-intensity thinning treatments respectively. In the lower soil layer, the low-intensity thinning increased the β-glucosidase activity by 23.2% in January and decreased it by 32.4% and 18.3% in April and July respectively, and the high-intensity thinning decreased the β-glucosidase activity by 65.9% in April (Table 2, Fig. 2).

In all of the treatments, the activities of β-glucosidase and cellulase peaked in the warmer months (October and July) in the upper soil, whereas minimal or no significant differences were observed between the sampling dates in the lower soil. The highest invertase activity occurred in January in the upper soil, but the lowest invertase activity occurred in the lower soil (Table 2, Fig. 2).

3.3. Correlations

A correlation analysis showed that the SOC concentrations were positively correlated with the soil labile organic carbon fractions, enzymatic activities, soil temperature (T), water content (WC), total nitrogen concentration (TN) and SOC/TN. There were positive correlations between all of the labile organic carbon fractions and enzyme activities except for the invertase, whose activity was not correlated with MBC and WSOC. T was strongly positively correlated with EOC, HWEOC and the cellulase and β-glucosidase activities, whereas WC was positively correlated with MBC but negatively correlated with WSOC and the invertase activity. In addition, SOC/TN positively correlated with all of the labile organic carbon fractions and enzymatic activities. (Table 3).

4. Discussion

4.1. Soil organic carbon

Our study showed that the SOC concentration in the soil was significantly correlated with all of the labile carbon fractions and enzymatic activities, which has been observed in other studies (Xu et al., 2015; Li et al., 2016) and indicates that the labile organic carbon and C-cycle-related enzymatic activities were sensitive to SOC variations. Thinning reduced the SOC concentrations except for in October, which was partially consistent with our hypothesis,

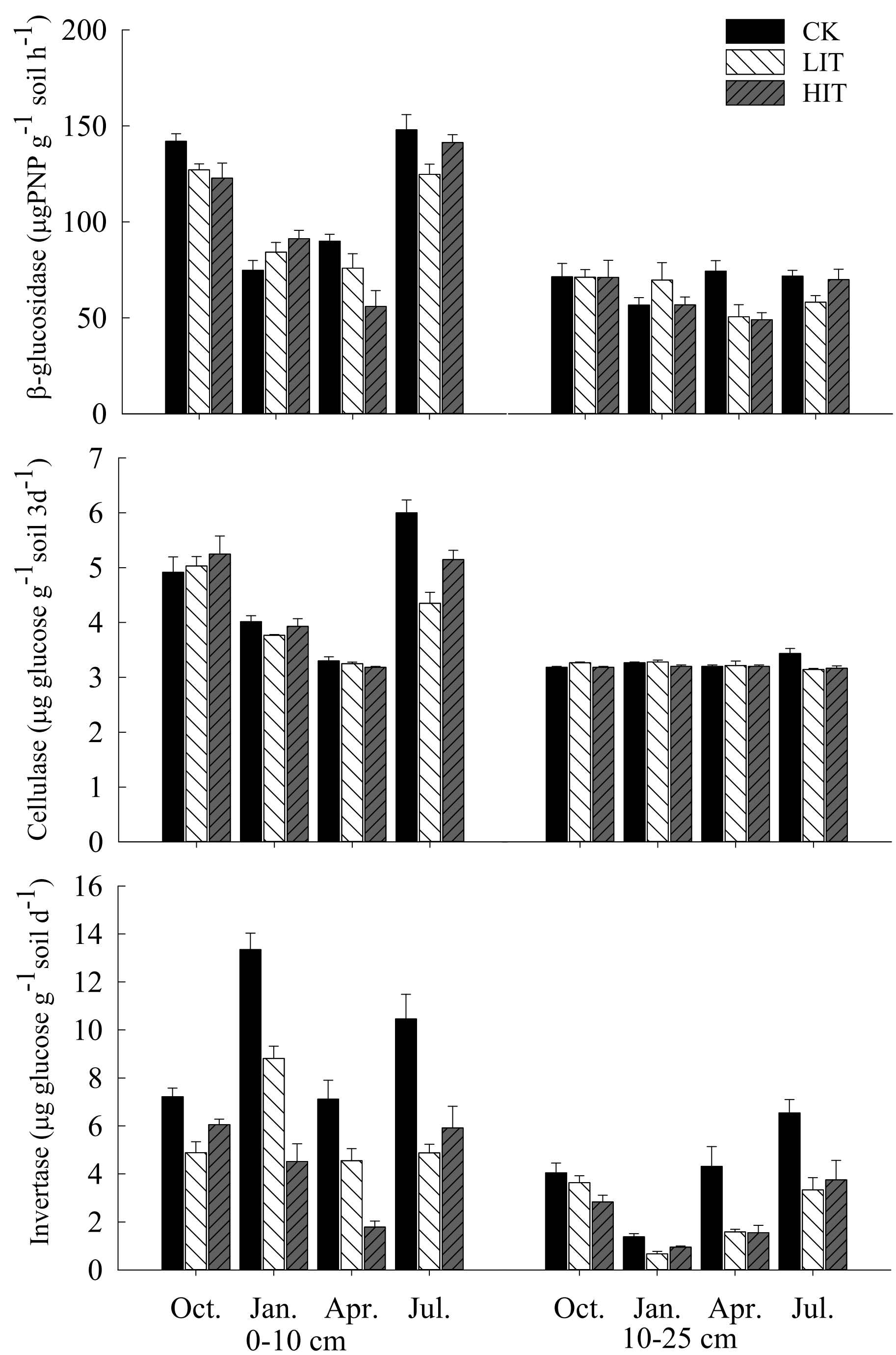

Fig. 2. Soil enzyme activities (mean values and standard error of mean) in the different thinning intensities (CK, LIT, HIT) on four sampling dates.

and the thinning effect was more pronounced in the upper soil layer than in the lower. An average SOC loss of 32.5% SOC in thinned sites was also found by Boerner et al. (2006), whereas Achat et al. (2015) found an SOC decrease of approximately 7% by tree removal in a meta-analysis. The decrease in SOC following the partial removal of canopy trees has been attributed to the reduction in the supply of substrate that enters the soil (Piene and Cleve, 1978; Jandl et al., 2007). The microclimate-driven acceleration of SOC decomposition rates due to decreased canopy closure also reduce the concentration of SOC (Piene and Cleve, 1978). Furthermore, rising groundwater level following thinning may increase SOC leaching losses (Laudon et al., 2009). However, the SOC concentrations were lower in the control than in the thinned sites in the autumn. Thinning may have allowed for the establishment of shrubs and graminoids with higher decomposition rates than the conifers in the summer (Table 1, S1), which allowed the thinned sites to compensate for the reduced total carbon inputs through an increased proportion of more rapidly decomposable leaf litter and root litter during the autumn (Chen and Shrestha, 2012; Brassard et al., 2013).

The SOC differed significantly only over the four sampling dates in the upper soil, and the warmer months (July and October) had higher SOC values than January and April. This seasonal variation likely reflects the rapid turnover of fine roots and associated mycorrhizae in subtropical climates (Yuan and Chen, 2010). However, this result will require further investigation. The response of SOC to thinning has been shown to differ significantly among different soil layers (Melero et al., 2009; Nave et al., 2010; Li

Table 3
Correlation matrix (r values) between the carbon fractions, enzymatic activities and environmental variables of the soils in the thinning and control sites.

	SOC	MBC	EOC	WSOC	HWEOC	Invertase	Cellulase	β-glucosidase
SOC	1							
MBC	0.34**	1						
EOC	0.59**	0.36**	1					
CWSOC	0.33**	0.30*	0.26*	1				
HWEOC	0.65**	0.68**	0.40**	0.32**	1			
Intervase	0.61**	ns	0.25*	ns	0.37**	1		
Cellulase	0.84**	0.34**	0.66**	0.47**	0.65**	0.50**	1	
Glu	0.81**	0.39**	0.62**	0.53**	0.75**	0.48**	0.84**	1
WC	0.39**	0.24*	ns	−0.27*	ns	−0.37**	ns	ns
T	0.45**	ns	0.54**	ns	0.88**	ns	0.51**	0.68**
TN	0.96**	0.34**	0.57**	ns	0.65**	0.60**	0.76**	0.74**
TOC/TN	0.82**	0.26*	0.46**	0.47**	0.48**	0.49**	0.71**	0.73**

NS: not significant.
* $P \le 0.05$.
** $P \le 0.01$.

et al., 2012). The SOC in the deeper soil is characterized by a longer mean residence time, and is therefore less sensitive to thinning (Fontaine et al., 2007). These differences can be attributed to the different plant species compositions among the various sites because different plants reach different soil depths with their roots, which control carbon sequestration and decomposition (Boone et al., 1998; Clemmensen et al., 2013).

4.2. Soil labile organic carbon fractions

We found that high-intensity thinning significantly increased the soil MBC and EOC compared with the control and low-intensity thinning treatments, and the effects were more pronounced in the upper mineral layer than in the lower mineral layer. Therefore, our hypothesis that thinning would reduce labile SOC was not supported. MBC and EOC are considered labile fractions of SOC and early indicators of soil quality because they control the dynamics of soil organic matter and nutrient availability and are highly sensitive to environmental changes and soil management (Blair et al., 1995; Rudrappa et al., 2006; Pignataro et al., 2012). Increases in MBC and EOC contents have also been previously observed following thinning treatments (Thibodeau et al., 2000; Yuan et al., 2010), and such increases have been attributed to soil temperature and the supply of organic carbon, which are dominant factors that control soil carbon stability (Fontaine et al., 2007; Karhu et al., 2014). In this study, the relatively high correlation between MBC, EOC, TN and TOC/TN indicate that the higher soil MBC and EOC concentrations in the high-intensity thinning treatment may have been caused by variations in the quantity and quality of the soil substrates. Compared with the control and low-intensity thinning sites, the high-intensity thinning treatment promoted non-tree vegetation and their fine roots would increase the input of labile carbon from root exudates, dead fine roots, and leaf and litterfall leachates, which would provide more available N, and thus increase the amount and activity level of the microbial biomass and provide additional sources of EOC (Purakayastha et al., 2008; Lou et al., 2011; Li et al., 2012).

We observed no differences in the CWSOC or HWEOC among the various treatments, which is consistent with the results of Chiang et al. (2010), who found that thinning did not have a significant effect on the water-soluble carbon concentration in the soil of *Cryptomeria japonica* plantations. Because rhizodeposition and root biomass are the main sources of CWSOC and HWEOC (Kalbitz et al., 2000), the loss of water-soluble organic carbons that were caused by the removal of Chinese fir trees could have been offset by the increase of non-tree vegetation, which produces easily decomposable litter. Furthermore, the higher positive correlation between HWEOC and soil temperature and the negative correlation between CWSOC and soil water content indicate that canopy removal enhanced sunlight penetration to the forest floor and may have changed the soil microclimate, potentially creating more favorable conditions to enhance CWSOC and HWEOC.

In terms of seasonal variations, all of the labile organic carbon fractions were higher in July and October than in the other months, which may be due to the high microbial activity that was driven by the higher temperatures and precipitation and the greater inputs from plant residues, roots, and exudates in the summer. Furthermore, all of the soil labile organic carbon fractions, except HWEOC were lowest in the spring, which coincided with the Nanjing plum-rain season (early April). The higher groundwater levels following precipitation events may have caused labile organic carbon leaching (Begueria et al., 2015).

4.3. Soil enzyme activities

Thinning significantly decreased the activities of invertase and β-glucosidase after 7 years of treatment and the cellulase activity also exhibited an insignificant decreasing trend during this period. Therefore, our hypothesis that the C-cycle enzyme activities will increase with thinning intensity was not supported. These results are consistent with those from a study in a Mediterranean pine forest, which showed decreases in β-glucosidase, urease, phosphatase and dehydrogenase activities after 8 years of thinning (Wic Baena et al., 2013). Similar results were also observed in the New Jersey Pine Barrens, where the cellulase and phenol oxidase activities decreased significantly after 1 year of thinning (Geng et al., 2012). Variations in the activities of the enzymes that degrade major soil organic matter components have been linked to shifts in decomposition rates and soil carbon storage, and the observed differences may indicate a reduction in the decomposition rates of soil organic matter and carbon cycling between the organic matter pools in the thinned sites (Xu et al., 2015; Li et al., 2016). One year fine root turnover experiments with litter bags at the same sites at the same time confirmed that the low and high intensity thinning significantly decreased fine root decomposition rates by 21% and 9%, respectively (Wang, 2013). These decreases in enzyme activities can be attributed to reductions in root activity. The mycorrhizae that are associated with the roots of Chinese firs produce extracellular enzymes, so thinning decreases enzyme activity in the surrounding soil (Kotroczo et al., 2014). Variations in the microbial composition may be another reason. Chen et al. (2015) found functional changes of soil organisms under different thinning treatments in the same sites and at the same time. The increase in the β-glucosidase activity in the thinning treatments in January might be attributable to variations in the microclimate.

Thinning changes the amount of light radiation and rainfall that reach the forest floor, which influences the activities of soil enzymes via altered soil temperature, moisture, pH and the composition and abundance of soil microorganisms (Kramer and Green, 2000; Baldrian et al., 2010).

Notably, the highly labile organic carbon content was not associated with an increase in the carbon cycle-related enzyme activities. However, the opposite relationship between the soil labile organic carbon and the activities of invertase and β-glucosidase was observed by Song et al. (2012), and this may be due to a decrease in the efficiency of soil microbial decomposition under high-intensity thinning. The final products of invertase and β-glucosidase are glucose and fructose, which are labile carbon and energy sources for microorganisms (Frankenberger and Johanson, 1983; Esen, 1993). The increase in the amount of labile organic matter supplied an adequate and easily decomposed substrate for microorganisms, which decreased their efficiencies in the high-intensity thinning treatment.

Our results showed that all three enzyme activities differed significantly between the four sampling seasons. Cellulase and β-glucosidase presented higher activity values in the summer and autumn and lower values in the winter and spring, which is consistent with the hypothesis that enzyme activities peak in the warmer months. This result can be attributed to seasonal variations in soil moisture and temperature that can significantly affect enzyme activities and microbiological variables in forest soils (Baldrian et al., 2010). The correlation analysis also indicated that an increase in soil temperature may increase enzyme activities. The negative relationship between WC and invertase activity indicated that the higher soil invertase activity in the upper soil layer in January may have been due to the low amount of precipitation. Invertase catalyzes the hydrolysis of disaccharides to monosaccharides, which are soluble soil carbohydrates that are strongly affected by the soil water content (Chendrayan et al., 1980; Li et al., 2010). The lower rainfall in the winter may have caused the soil to retain more of the soluble carbohydrates that were produced by the litter on the forest floor and thus increased the invertase activity in the upper soil.

5. Conclusion

In this study, we provide evidence for the effects of different thinning intensities on the labile SOC fractions and related enzyme activities in a Chinese fir plantation. We demonstrate that, compared with the control, the thinning treatments decreased the SOC content and enzyme activities and that high-intensity thinning increased the MBC and EOC. Shifts in the litter inputs associated with tree removal may result in more microbial biomass but lower efficiency. Furthermore, we found strong seasonal variations in the concentrations of the labile SOC fractions and C-cycle enzyme activities. The correlation analysis indicated that seasonal variations in the soil moisture and temperature control the observed seasonal variations in the labile organic carbon and soil enzyme activities. We concluded that the MBC, EOC and the activities of invertase and β-glucosidase were the most sensitive indicators to thinning to reflect SOC and that the seasonal dynamics of the labile SOC fractions and enzyme activities were likely driven by soil microclimatic changes and shifts in the availability and quality of the organic resources.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2016.05.016.

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