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Distribution of organic carbon fractions in soil aggregates in Chinese fir plantations with different stand ages



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Abstract

Background: Revealing the variations in soil aggregate-related organic carbon (OC) and labile organic carbon (LOC) fractions in a chronosequence of Chinese fir plantations plays an important role in better understanding the impact of soil carbon sink or source on the Chinese fir plantation ecosystem. In this study, soil samples in a depth of 0–20 cm were collected from Chinese fir plantations at different stand ages (0, 9, 17, and 26 years old) in Guangxi, China. With the optimal moisture sieving method adopted, the soil aggregates of 4 different sizes were obtained, including > 2-mm, 2–1-mm, 1–0.25-mm, and < 0.25-mm aggregates. Soil OC and LOC fractions were measured in the aggregates of different sizes. The LOC fractions included readily oxidizable carbon (ROC), particulate organic carbon (POC), microbial biomass carbon (MBC), water-soluble organic carbon (WOC), and mineralized organic carbon (MOC).

Results: Soil aggregate stability, as indicated by the mean weight diameter (MWD), was the highest in the 17-year-old Chinese fir plantations and was significantly positively related (p < 0.05) to the concentrations of OC and LOC fractions (except for the ROC and MOC), with the POC in particular. As for all stand ages of Chinese fir plantations, the concentrations of soil OC and LOC fractions were significantly increased as the aggregate size decreased. Consequently, there were more OC and LOC fractions distributed in the < 0.25-mm aggregates. During the stand development, the concentrations of soil OC and LOC fractions first increased and then decreased, with the highest levels detected in the 17-year-old Chinese fir plantations, indicating that the 17-year-old Chinese fir plantations were conducive to the accumulation of soil OC and LOC fractions.

Conclusion: After 17 years of planting, promoted soil carbon (especially for the POC) accumulation contributes significantly to enhancing soil aggregate stability for the Chinese fir plantations in Guangxi, China.

Keywords: Chinese fir plantation, Soil aggregates, Organic carbon, Labile organic carbon fractions

Introduction

Over the last century, extensive deforestation and longterm tillage have resulted in land degradation given the sharp rise in population pressure across China. In order to address these issues, the Chinese government has implemented the Grain for Green Program, with the aim to reverse land degradation (soil erosion) by restoring cultivated lands to forestlands (Zeng et al. 2020). Since this program was launched, the cultivated lands distributed in the north of Guangxi have been restored to Chinese fir (*Cunninghamia lanceolata*) plantations (Huang et al. 2020). At present, afforestation is recognized as an effective solution to land restoration through soil organic carbon (OC) sequestration (Zhang et al. 2017). However, to increase soil OC concentration and storage by afforestation needs to take a long time and the total OC is ineffective in revealing the effect of soil carbon source or sink accurately (Deng et al. 2014).

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As the active parts of soil OC, labile organic carbon (LOC) fractions, including readily oxidizable carbon (ROC), particulate organic carbon (POC), microbial biomass carbon (MBC), water-soluble organic carbon (WOC), and mineralized organic carbon (MOC), are possibly more sensitive to the conversion of land use than soil OC (Sheng et al. 2015). To be specific, ROC is oxidized by 333 mmol L⁻¹ KMnO₄ to serve as a free and less stable fraction of soil OC (Blair et al. 1995). POC consists of plant residues, seeds, and microbial debris and is not occluded with soil micro-aggregates (Cadisch et al. 1996). MBC is the living fraction of soil OC and has been suggested as an effective indicator of variation in the status of organic matters (OMs) (Powlson et al. 1987). WOC consists of various soluble organic residues and is regarded as the least stable fraction of soil OC (Schnabel et al. 2002). MOC comprises small molecular weight compounds resulting from microbial processing and is prone to the decomposition by soil microorganisms (Zhao et al. 2008). Thus, soil LOC fractions are characterized by easy mineralization, easy decomposition, and short turnover period (Li et al. 2018).

Soil aggregates serve as the fundamental units of soil structure; their composition can regulate the interactions between minerals and organic substances in numerous soil physical, chemical, and biological processes (Six and Paustian 2012); and their stability can evaluate the soil structure dynamic (Castro et al. 2002). According to the hierarchy concept, soil aggregates are categorized into micro- (< 0.25 mm) and macro-aggregates (> 0.25 mm) (Six et al. 2004). During their formation processes, primary particles get integrated into micro-aggregates under the action of persistent binding agents (e.g., humified OMs and polyvalent metal cation complexes) (Voltolini et al. 2017). Meanwhile, macro-aggregates are constituted by the micro-aggregates under the effect of temporary binding agents (e.g., fungal hyphae, plant roots, microbial- and plant-originated polysaccharides) (Li et al. 2019). As a result, the concentrations of soil OC and LOC fractions vary among the aggregates of different sizes. Therefore, it is essential to determine the distribution of OC and LOC fractions in soil aggregates for better understanding the dynamic of soil carbon in the forest ecosystem (Bai et al. 2020).

Chinese fir plantations, which are considered as one of the most broadly distributed forest types in China (Tong et al. 2021), have significantly contributed to the development of the forest economy and the improvement of net primary productivity (Yang et al. 2021). In 2019, the area of Chinese fir plantations in China reached 10.96 million ha and this figure has been on the increase continuously over time (Yang et al. 2019). Due to the advantageous geographical and climatic conditions, Guangxi has established itself as one of the major provinces

planted Chinese fir in China (Chen and Cen 2016). According to the previous studies, the conversion of land use from cultivated lands into Chinese fir plantations could be effective in improving soil structure and promoting soil microbial development, which might be attributed to the accumulation of soil OC derived from the tree roots and leaves in the fir plantations (Huang et al. 2020). During the development of Chinese fir plantations, however, the dynamics of soil OC and LOC fractions, especially at aggregate scales, remained poorly understood. Thus, this study is aimed to reveal the effect of stand age on (i) the composition and stability of soil aggregates, (ii) OC and LOC fraction concentrations, and (iii) their storage in soil aggregates. Moreover, it is hypothesized in this study that the stand development of Chinese fir would be favorable to the accumulation of soil OC and LOC fractions.

Materials and methods

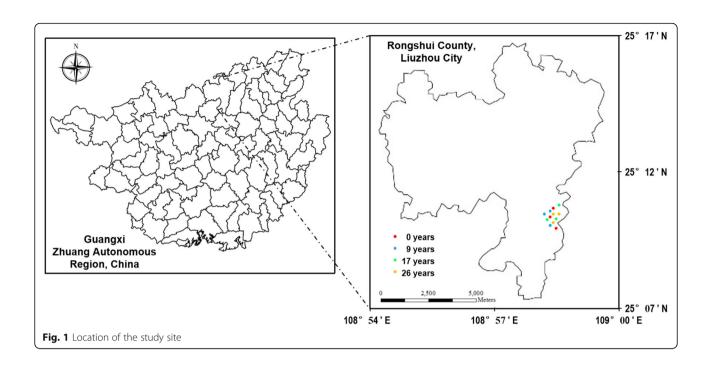
Study site

This study was carried out in June 2019 at the Beijiang River Forest Farm in Rongshui County, Liuzhou City, Guangxi Zhuang Autonomous Region (Fig. 1). Since the 1950s, for the economic value of Chinese fir, numerous hectares of cultivated lands were converted into Chinese fir plantations. Thus, the Beijiang River Forest Farm serves as the central production area of Chinese fir in Guangxi. In this area, the subtropical monsoon climate is prevailing, with the year-round average temperature of 18.8 °C and the year-round average precipitation of 1824.8 mm. The low mountains and hills make up the majority of landform, with an altitude of 500-900 m and a gradient of 18-23°. Soil parent materials include limestone, granite, and argillaceous sandstone. Soil types include krasnozem and latosol (IUSS Working Group 2014), with the loamy clay texture. The density of Chinese fir planting was set to nearly 1000 plants ha⁻¹, with the planting spacing nearly $3 \text{ m} \times 3 \text{ m}$. The fir plantations adopt near nature management, so that the undergrowth vegetation evolves naturally and reduces human disturbance. The undergrowth vegetation species mainly include Allantodia hachijoensi, Cibotium barometz, and Allantodia metteniana.

Experimental design

In this study, the variations in soil aggregate-related OC and LOC fractions during the development of Chinese fir plantations were examined by the space-for-time substitution method (Sparling et al. 2003). In general, some potential confounding effects might exist in soil spatial variations; thus, we tried to minimize these effects through selecting representative Chinese fir plantations, which were located at the similar geomorphology-related units. Based on the site survey, we selected 4 different stand ages of Chinese fir plantations, including 0 years

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(cultivated lands), 9 years (young forests), 17 years (middle-aged forests), and 26 years (mature forests). Each stand age was replicated in triplicate and then produced 12 Chinese fir plantations. Separation among these plantations was made with a distance of $> 800\,\mathrm{m}$ between each other to reduce the spatial autocorrelation and to prohibit the pseudoreplication. In each Chinese fir plantation, one plot $(20\,\mathrm{m}\times20\,\mathrm{m})$ was randomly constructed at a distance of $> 50\,\mathrm{m}$ away from the edge of the plantation.

Litter and soil sampling

In each plot, based on 3 randomized sub-plots (1 m \times 1 m), 3 litter samples were collected on the soil

surface into the plastic sacks prior to soil sampling, and then the 3 litter samples were combined to a composite litter sample. A total of 12 composite litter samples were dried using the oven on the condition of 80 °C till the weight of these litter samples remained unchanged, and then the dried litter samples were weighed (Table 1).

Soil samples were collected at the sites of litter sampling. In each plot, 3 soil samples were collected by a spade from 0- to 20-cm depth into the plastic boxes, and then the 3 soil samples were combined to a composite soil sample. A total of 12 composite soil samples were carefully separated along natural planes of weakness into natural aggregates. These natural aggregates were filtered by

Table 1 Litter and soil properties in the Chinese fir plantations with different stand ages (0 years, 9, 17, and 26 years old)

Sample	ltem	Stand age			
		0 years	9 years	17 years	26 years
Litter	Amount (g m ⁻²)	372 ± 21 c	492 ± 32 b	643 ± 27 a	516 ± 23 b
Soil	рН	5.82 ± 0.13 a	$5.61 \pm 0.08 \text{ b}$	5.43 ± 0.22 c	$5.12 \pm 0.16 d$
	Bulk density (g cm ⁻³)	1.23 ± 0.02 a	1.20 ± 0.04 a	1.18 ± 0.02 a	1.24 ± 0.03 a
	OC concentration (g kg^{-1})	$9.34 \pm 0.01 d$	13.51 ± 0.34 b	20.28 ± 0.18 a	10.83 ± 0.10 c
	ROC concentration (g kg ⁻¹)	2.51 ± 0.14 a	2.87 ± 0.14 a	2.95 ± 0.27 a	2.49 ± 0.03 a
	POC concentration (g kg^{-1})	$2.89 \pm 0.11 \text{ b}$	3.00 ± 0.01 b	4.41 ± 0.14 a	1.99 ± 0.01 c
	MBC concentration (mg kg ⁻¹)	125.00 ± 7.02 b	127.00 ± 3.79 b	174.00 ± 6.66 a	122.33 ± 5.36 b
	WOC concentration (mg kg^{-1})	77.61 ± 4.42 ab	90.13 ± 4.04 a	87.08 ± 4.08 a	67.94 ± 1.53 b
	MOC concentration (mg kg ⁻¹)	75.96 ± 0.94 c	87.65 ± 2.88 b	97.37 ± 3.08 a	93.73 ± 1.22 ab

Data represent the average of 3 replicates ± standard errors. Different lowercase letters indicate significant differences among the different stand ages. OC, organic carbon; ROC, readily oxidizable carbon; POC, particulate organic carbon; MBC, microbial biomass carbon; WOC, water-soluble organic carbon; MOC, mineralized organic carbon

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a 5-mm sieve to screen out macro-fauna, large roots, litter residues, and small stones. At the same time, another 3 soil samples in each plot were randomly collected by the cutting ring ($V = 100 \, \mathrm{cm}^{-3}$, depth = 50 mm, $\emptyset = 50.46 \, \mathrm{mm}$) to measure pH, bulk density, and concentrations of OC and LOC fractions in bulk soil (Table 1).

Soil aggregate separation

Soil aggregates were separated by the optimal moisture sieving method as described by Bach and Hofmockel (2014). In brief, field-moist clods (< 5 mm) were cooldried at 4°C until reaching a gravimetric water concentration of $\sim 60 \,\mathrm{g\,kg^{-1}}$, at which point they were able to be sieved into different sized aggregates at room temperature. The cool-dried clods (300 g) were vertically oscillated for 15 min with an amplitude of 50 mm and a rate of 1 oscillation s⁻¹; then, they were filtered with the sieves at 2-mm, 1-mm, and 0.25-mm continuous diameters. Through the above procedures, soil aggregates with different sizes were obtained, including > 2-mm, 2-1mm, 1–0.25-mm, and < 0.25-mm fractions. Afterwards, and LOC fraction the aggregate-related OC concentrations were measured.

Soil analyses

According to the cutting ring method (Lu 2000), soil samples were oven-dried at 105 °C to the stable weight in order to measure the bulk density. Soil pH was measured using the glass electrode method and the soil-towater ratio was l:2.5 (m:v) (Lu 2000). Soil OC was measured using the acid dichromate wet oxidation method (Nelson and Sommers 1996). Five milliliters of 0.8 M $K_2Cr_2O_7$ and 5 mL of H_2SO_4 were added into the soil and then boiled at 170–180 °C for 5 min. The remaining $K_2Cr_2O_7$ was titrated with 0.2 M FeSO₄.

Soil ROC was determined following the method of Liu et al. (2008). 0.5 g of air-dried soil was placed into a test tube and 10 mL of 0.2 M mixed liquor (1:6 $K_2Cr_2O_7$ and 1:3 H_2SO_4) was added. Next, the test tube was boiled at 130–140 °C for 5 min. Once the test tube was cooling down, we transferred all of the solution into a 250-mL Erlenmeyer flask. The remaining $K_2Cr_2O_7$ was titrated with 0.2 M FeSO₄.

Soil POC was measured using the method of Cambardella and Elliott (1993). Twenty grams of air-dried soil was dispersed in 100 mL of sodium hexametaphosphate by shaking for 18 h. The resulting soil suspension was poured over a 53- μ m screen under a flow of distilled water to ensure separation. All of the materials remaining on the screen were washed into a dry dish and were oven-dried at 60 °C for 12 h.

Soil MBC was determined following the chloroform fumigation extraction method (Vance et al. 1987). Both the fumigated and non-fumigated soil was extracted in 0.5 M $\rm K_2SO_4$ with a soil-to-extraction ratio of 1:2.5 (m:v). The MBC was calculated by subtracting the $\rm K_2SO_4$ -extracted carbon of non-fumigated soil from that of the fumigated soil using an extraction efficiency factor ($\rm K_C$) of 2.64.

Soil WOC was measured using the method of Yang et al. (2006). Twenty grams of air-dried soil was placed into a 50-mL polypropylene centrifuge tube and then 50 mL of distilled water was added. The centrifuge tube was shaken at 120 rpm for 1 h at room temperature and was centrifuged at 1000 rpm for 15 min. Next, each sample was filtered through a 0.45-µm polycarbonate membrane and the WOC in the filtrate was measured.

Soil MOC was determined following the method of Shen et al. (2012). Twenty-five grams of soil was incubated in 1-L mason jars and went through 28 days of incubation at 25 °C and \sim 60% of water holding capacity. Meanwhile, the control (without soil) was also incubated. Fifty-milliliter vials containing 10 mL of 1 M NaOH were placed in the jars all at once and we removed them after different amounts of time (1, 4, 7, 14, 21, and 28 days) to trap respired CO_2 .

Calculations and statistical analyses

Soil mean weight diameter (MWD, mm) serves as a key indicator to evaluate the soil aggregate stability. To be specific, if the MWD value is higher, the aggregate stability is higher. The MWD value was calculated as the following formula (Castro et al. 2002)

$$MWD = \sum_{i=1}^{4} X_i W_i$$

in which X_i is the mean diameter of aggregate fraction i (mm) and W_i is the proportion of the ith aggregate fraction in bulk soil (% in weight).

Soil OC storage (g m⁻²) was calculated as the following formula (Eynard et al. 2005):

$$OCstorage = \sum_{i=1}^{4} (W_i \times OC_i) \times B_d \times H \times 10$$

in which W_i is the proportion of the ith aggregate fraction in bulk soil (% in weight), OC_i is the concentration of OC in the ith aggregate fraction (g kg $^{-1}$), $B_{\rm d}$ is the soil bulk density (g cm $^{-3}$), and H is the soil depth (cm). Similarly, soil LOC fraction storages were also calculated.

The SPSS 22.0 software was adopted for all statistical analyses and the results were expressed as the mean from 3 replicates. The one-way analysis of variance (ANOVA) was used for estimating how stand age affects the litter and bulk soil characteristics. Comparisons were made among 4 aggregate sizes and 4 stand ages for the composition of soil aggregates and the concentrations of

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soil OC and LOC fractions with a variation of a split-plot design. In the model, aggregate size was considered the main factor and stand age was considered the subplot factor. Aggregate size, stand age, and the aggregate size × stand age interaction were treated as the fixed effects and replicate was treated as the random effect. Comparison of the means for aggregate sizes and stand ages were made using Tukey's honestly significant difference. The relationships of soil aggregate stability with OC and LOC fraction concentrations were determined by Pearson's correlation analysis.

Results

Soil aggregate composition and stability

Across the 4 stand ages, the main fractions of soil aggregates were the > 2-mm aggregates (with a mean value of 51.90%), followed by the < 0.25-mm (24.85%) and 2-1mm (16.79%) aggregates, and the 1-0.25-mm aggregates (6.88%) were related with the lowest proportions (Table 2). The proportions of > 2-mm aggregates were significantly (p < 0.05) higher in the 17-year-old stand compared with stands of all other ages. However, the proportions of < 0.25-mm aggregates presented an inverse tendency during the development of Chinese fir plantations. Among the 4 stand ages, there were no significant (p > 0.05) differences in the proportions of 2-1-mm and 1-0.25-mm aggregates. Moreover, the value of soil MWD was first increased and then decreased over time, and the highest value was observed in the 17-year-old fir plantations (Fig. 2).

Soil carbon concentration in aggregates Organic carbon

Regardless of the stand age, the OC concentration in < 0.25-mm (12.83–31.30 g kg $^{-1}$) and 1–0.25-mm (10.58–27.85 g kg $^{-1}$) aggregates was significantly (p < 0.05) higher than that in > 2-mm aggregates (6.80–16.13 g kg $^{-1}$) (Table 3). In the course of stand development, the OC concentration in soil aggregates was first increased

and then decreased, and the OC concentration was significantly (p < 0.05) higher in the 17-year-old fir plantations in comparison to other fir plantations.

Readily oxidizable carbon

Irrespective of the stand age, the ROC concentration in < 0.25-mm aggregates (3.03–3.77 g kg⁻¹) was significantly (p < 0.05) higher than that in > 2-mm aggregates (2.02–2.60 g kg⁻¹) (Table 4). In all aggregates (except for the 2–1-mm aggregates), there was no significant (p > 0.05) difference in the ROC concentration among the different stand ages.

Particulate organic carbon

The POC concentration in < 0.25-mm (1.98–5.95 g kg⁻¹) and 1–0.25-mm (2.47–5.59 g kg⁻¹) aggregates was significantly (p < 0.05) higher than that in > 2-mm aggregates (1.67–3.81 g kg⁻¹) (Table 5). With the increase of plantation age, the POC concentration in soil aggregates was first increased and then decreased, and the POC concentration was significantly (p < 0.05) higher in the 17-year-old fir plantations than other fir plantations.

Microbial biomass carbon

Regardless of the stand age, the MBC concentration in < 0.25-mm aggregates (147.24–202.76 mg kg $^{-1}$) was significantly (p < 0.05) higher than that in 2–1-mm (116.28–181.46 mg kg $^{-1}$) and > 2-mm (96.66–150.49 mg kg $^{-1}$) aggregates (Table 6). With the stand development, the MOC concentration in soil aggregates was first increased and then decreased, and the MOC concentration was significantly (p < 0.05) higher in the 17-year-old fir plantations in comparison to other fir plantations.

Water-soluble organic carbon

Irrespective of the stand age, the WOC concentration in < 0.25-mm aggregates (90.24–110.25 mg kg $^{-1}$) was significantly (p < 0.05) higher than that in 1–0.25-mm (74.96–103.54 mg kg $^{-1}$), 2–1-mm (65.33–94.09 mg kg $^{-1}$),

Table 2 Effects of aggregate sizes (> 2, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0, 9, 17, and 26 years old) on the soil aggregate composition in a chronosequence of Chinese fir plantations

Stand age (years)	Soil aggregate	composition (%	6)		Effects						
	> 2 mm	2–1 mm	1-0.25 mm	< 0.25 mm	Aggregate size (A), df = 3		Stand age (S), $df = 3$		A × S, df= 9		
					F	P	F	P	F	Р	
0	47.28 ± 1.11 c	17.33 ± 0.82 a	7.34 ± 0.57 a	28.05 ± 0.64 b	1249.508	< 0.0001	40.037	< 0.0001	37.026	< 0.0001	
9	52.31 ± 1.51 b	18.47 ± 0.84 a	5.24 ± 0.52 a	23.98 ± 1.94 c							
17	61.02 ± 1.15 a	16.02 ± 0.88 a	6.89 ± 1.06 a	16.07 ± 1.73 d							
26	42.28 ± 1.17 d	17.33 ± 0.41 a	7.41 ± 1.07 a	32.98 ± 0.72 a							
Mean	51.90 ± 2.22 A	16.79 ± 0.40 C	6.88 ± 0.45 D	24.85 ± 2.15 B							

Data represent the average of 3 replicates \pm standard errors. Different lowercase letters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

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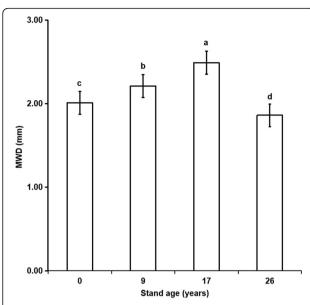


Fig. 2 Soil aggregate stability (as indicated by the MWD) in the Chinese fir plantations with different stand ages (0, 9, 17, and 26 years old). Data represent the average of 3 replicates and error bars represent the standard errors. Different lowercase letters indicate significant differences among the different stand ages. MWD, mean weight diameter

and > 2 mm (49.76–83.18 mg kg $^{-1}$) aggregates (Table 7). With the stand development, the WOC concentration in soil aggregates was first increased and then decreased. The WOC concentration in > 2-mm, 2-1-mm, and 1-0.25-mm aggregates was the highest in the 9-year-old fir plantations, and the WOC concentration in < 0.25-mm aggregates was the highest in the 17-year-old fir plantations.

Mineralized organic carbon

As shown in Table 8, the MOC concentration in < 0.25-mm aggregates $(60.87-145.02\,\mathrm{mg\,kg^{-1}})$ was

significantly (p < 0.05) higher than that in 2–1-mm (66.55–103.77 mg kg⁻¹) and > 2-mm (73.88–97.72 mg kg⁻¹) aggregates. With the increase of fir plantation age, the MOC concentration in soil aggregates was first increased and then decreased. The MOC concentration in > 2-mm and < 0.25-mm aggregates was the highest in the 17-year fir plantations, and the MOC concentration in 2–1-mm and 1–0.25-mm aggregates was the highest in the 9-year fir plantations.

Relationship of soil aggregate stability with carbon concentration

Pearson's correlation analysis explained the relationships of soil aggregate stability (as indicated by the MWD value) with the concentrations of soil OC and LOC fractions (including ROC, POC, MBC, WOC, and MOC) (Fig. 3). As revealed from these results, the MWD value was positively correlated with the concentrations of OC ($R^2 = 0.808$, p < 0.01), POC ($R^2 = 0.912$, p < 0.01), MBC ($R^2 = 0.401$, p < 0.05), and WOC ($R^2 = 0.485$, p < 0.05) in bulk soil during the fir-planting process.

Soil carbon storage in aggregates

At the 0–20-cm depth, soil OC and LOC fraction storage was higher in the >2-mm and <0.25-mm aggregates compared to other aggregates among the 4 stand ages of fir plantations (Fig. 4). For example, soil OC storage across the 4 stand ages was $0.79-2.32 \, \mathrm{kg}$ m $^{-2}$ and $0.93-1.28 \, \mathrm{kg} \, \mathrm{m}^{-2}$ in the >2-mm and <0.25-mm aggregates, respectively, which contributed to 24.5-40.7% and 31.9-48.0% of the OC storage in bulk soil, respectively. Also, soil LOC fraction storage werewas mainly distributed in the >2-mm and <0.25-mm aggregates. With the stand development, the storage of soil OC and some LOC fractions (including ROC, POC, and MBC) increased until the 17 years old and then decreased. In addition, the highest storage of soil WOC was found in the 9-year-old fir

Table 3 Effects of aggregate sizes (> 2 mm, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0, 9, 17, and 26 years old) on the soil organic carbon concentration in a chronosequence of Chinese fir plantations

Stand age (years)	Soil organic ca	arbon concentrati	ion (g⋅kg ^{−1})		Effects						
	> 2 mm	2–1 mm	1–0.25 mm	< 0.25 mm	Aggregate size (A), df = 3		Stand age (S), $df = 3$		$A \times S$, d.f. $df = 9$		
					F	P	F	P	F	Р	
0	6.80 ± 0.70 b	8.84 ± 0.45 c	10.58 ± 0.72 c	13.50 ± 0.89 c	78.217	< 0.0001	192.986	< 0.0001	6.450	< 0.0001	
9	$8.29 \pm 0.60 \text{ b}$	15.86 ± 1.41 b	18.39 ± 0.77 b	22.30 ± 1.01 b							
17	16.13 ± 1.12 a	23.21 ± 1.62 a	27.85 ± 1.46 a	31.30 ± 1.34 a							
26	8.82 ± 0.95 b	10.84 ± 0.67 c	12.12 ± 1.12 c	12.83 ± 0.30 c							
Mean	10.01 ± 1.15 B	14.69 ± 1.72 AB	17.23 ± 2.10 A	20.16 ± 2.38 A							

Data represent the average of 3 replicates \pm standard errors. Different lowercase letters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

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Table 4 Effects of aggregate sizes (> 2, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0, 9, 17, and 26 years old) on the soil readily oxidizable carbon concentration in a chronosequence of Chinese fir plantations

Stand age (years)	Soil readily o	Soil readily oxidizable carbon concentration (g kg ⁻¹)					Effects						
	> 2 mm	2–1 mm	1–0.25 mm	< 0.25 mm	Aggregate size (A), $df = 3$		Stand age (S), $df = 3$		$A \times S$, df = 9				
					F	P	F	P	F	Р			
0	2.02 ± 0.01 a	2.57 ± 0.29 b	2.74 ± 0.45 a	3.25 ± 0.32 a	7.093	0.009	0.554	0.511	0.150	0.997			
9	2.48 ± 0.29 a	3.28 ± 0.06 a	3.25 ± 0.39 a	3.35 ± 0.11 a									
17	2.60 ± 0.65 a	3.31 ± 0.72 a	3.40 ± 0.02 a	3.77 ± 0.25 a									
26	2.07 ± 0.01 a	$2.44 \pm 0.08 b$	2.59 ± 0.17 a	3.03 ± 0.11 a									
Mean	2.29 ± 0.11 B	2.90 ± 0.14 AB	$3.00 \pm 0.23 \text{ AB}$	$3.34 \pm 0.18 \text{ A}$									

Data represent the average of 3 replicates ± standard errors. Different lowercase letters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

plantations and soil MOC storage was successively increased over time.

Discussion

Soil aggregate composition and stability

In this study, the > 2-mm aggregates were predominant in bulk soil, irrespective of the stand age (Table 2). At the early phase (initial 17 years), fir planting contributed to the increase of > 2-mm aggregates, while at the late phase (after 17 years), fir planting led to the decrease in these aggregates (Table 2). These findings are consistent with the previous studies suggesting that soil aggregation is a dynamic process in nature and that the composition of soil aggregates is affected by the stand age to a significant extent (Xiao et al. 2019; Bai et al., 2020). The change of soil aggregate stability was readily reflected by the variation in soil MWD, which plays a vital role in the assessment of aggregate stability (Six and Paustian 2012). After 17 years of fir planting, the decrease of soil MWD was caused by the conversion of > 2-mm aggregates into < 0.25-mm aggregates (Fig. 2), indicating that the soil aggregate stability was higher in the 17-year-old fir plantations than in other plantations. The peak of soil aggregate stability appeared in the 17-year-old fir plantations, which is suspected to result from the accumulation of soil OC and LOC fractions in these fir plantations.

It has now been demonstrated in some studies that soil aggregate stability (as presented by the MWD) is significantly positively related to OC concentration in the forest ecosystem (Su et al. 2009; Ayoubi et al. 2012). Similarly, it was also found out that there was a significantly positive correlation between soil aggregate stability and OC concentration (Fig. 3a). This is because soil OMs served as the most important binding agents for aggregate formation and soil OC concentration was closely related to the level of aggregate stability (Six et al., 2004). Meanwhile, the correlation between soil aggregate stability and LOC fraction concentrations was tested, and the results showed that aggregate stability was positively correlated with POC, MBC, and WOC concentrations in bulk soil to a significant extent (Fig. 3c-e), implying that soil aggregate stability was largely affected by

Table 5 Effects of aggregate sizes (> 2, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0, 9, 17, and 26 years) on the soil particulate organic carbon concentration in a chronosequence of Chinese fir plantations

Stand age (years)	Soil particulate	e organic carbon	concentration	(g kg ⁻¹)	Effects						
	> 2 mm	2–1 mm	1-0.25 mm	< 0.25 mm	Aggregate size (A), $df = 3$		Stand age (S), $df = 3$		$A \times S$, df = 9		
					F	P	F	Р	F	Р	
0	2.02 ± 0.17 bc	2.69 ± 0.20 bc	3.06 ± 0.19 c	3.66 ± 0.11 c	65.524	< 0.0001	267.583	< 0.0001	4.718	< 0.0001	
9	$2.41 \pm 0.10 \text{ b}$	$3.25 \pm 0.17 \text{ b}$	$3.76 \pm 0.29 \text{ b}$	$4.09 \pm 0.17 \text{ b}$							
17	3.81 ± 0.14 a	5.09 ± 0.22 a	5.59 ± 0.11 a	5.95 ± 0.08 a							
26	1.67 ± 0.08 c	2.08 ± 0.06 c	2.47 ± 0.16 c	1.98 ± 0.05 d							
Mean	2.48 ± 0.26 B	3.28 ± 0.35 AB	$3.72 \pm 0.38 \text{ A}$	3.92 ± 0.44 A							

Data represent the average of 3 replicates ± standard errors. Different lower caseletters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

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Table 6 Effects of aggregate sizes (> 2, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0, 9, 17, and 26 years old) on the soil microbial biomass carbon in a chronosequence of Chinese fir plantations

Stand age (years)	Soil microbial b	Soil microbial biomass carbon (mg kg ⁻¹)					Effects						
	> 2 mm	2–1 mm	1–0.25 mm	< 0.25 mm	Aggregate size (A), $df = 3$		Stand age (S), <i>df</i> = 3		$A \times S$, $df = 9$				
					F	P	F	P	F	Р			
0	96.66 ± 5.49 b	126.48 ± 4.18 b	141.51 ± 3.25 b	160.22 ± 2.73 b	80.063	< 0.0001	92.674	< 0.0001	0.829	0.594			
9	105.84 ± 4.06 b	125.61 ± 2.46 b	151.63 ± 5.57 b	162.73 ± 8.04 b									
17	150.49 ± 5.95 a	181.46 ± 2.39 a	192.61 ± 7.16 a	202.76 ± 6.19 a									
26	102.44 ± 8.07 b	116.28 ± 5.60 b	138.50 ± 4.03 b	147.24 ± 3.60 b									
Mean	113.86 ± 7.32 C	137.46 ± 7.93 B	156.56 ± 6.87 AB	168.24 ± 6.70 A									

Data represent the average of 3 replicates ± standard errors. Different lowercase letters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

the LOC fraction concentrations, especially for the POC, in the fir plantations.

Soil carbon concentration as affected by aggregate size

According to the hierarchy concept, soil aggregaterelated OC has a significant impact on the dynamics of soil OMs, which are the main agents for soil aggregation (Six et al. 2004). Therefore, the determination of OC concentration in different sized aggregates plays an important role in evaluating the levels of soil fertility and health (Wang et al. 2020). In some studies, it has been indicated that the macro-aggregates (> 0.25 mm) were comprised of the micro-aggregates (< 0.25 mm) under the action of temporary binding agents in the soil ecosystem (Bucka et al. 2019; Cooper et al. 2020). In the meantime, the protection of soil OMs can be provided by macro-aggregates, thus resulting in the accumulation of soil OC in the macro-aggregates (Egan et al. 2018; Li et al. 2019). However, our findings suggested the opposite. In all stand ages of fir plantations, there was higher soil OC concentration observed in the micro-aggregates (Table 3), which is consistent with the results obtained by Six et al. (2004), who suggested that the larger specific surface area of soil micro-aggregates could promote

the adsorption of OMs from root exudates and litter residues. Moreover, soil micro-aggregates were derived from the combination of primary particles and persistent binding agents, and their structure was relatively stable. Soil OMs associated with the micro-aggregates were difficult to be decomposed and utilized by soil microorganisms (Xu et al. 2021). Therefore, the level of OC concentration in the micro-aggregates is shown to be relatively high in this study.

As the temporary binding agents, soil LOC fractions play a vital role in the formation and stabilization of soil aggregates (Li et al. 2015). Revealing the distribution of soil LOC fractions in different sized aggregates may help shed light on the dynamics of soil aggregates (Li et al. 2018). In the following parts, our findings about the distribution of each LOC fraction in soil aggregates will be detailed. Firstly, the < 0.25-mm aggregates had the highest concentration of soil ROC (Table 4), which is consistent with the results of Li et al. (2008) and Hua et al. (2009). According to their results, soil ROC in the < 0.25-mm aggregates exhibited the lowest sensitivity to environmental changes and microbial decomposition. Therefore, < 0.25-mm aggregates are deemed suitable for soil ROC accumulation. Secondly, soil POC was

Table 7 Effects of aggregate sizes (> 2, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0 years, 9, 17, and 26 years) on the soil water-soluble organic carbon concentration in a chronosequence of Chinese fir plantations

Stand age (years)	Soil water-solu	Soil water-soluble organic carbon concentration (mg kg ⁻¹)					Effects						
	> 2 mm	2–1 mm	1-0.25 mm	< 0.25 mm	Aggregate size (A), $df = 3$		Stand age (S), $df = 3$		$A \times S$, df = 9				
					F	P	F	P	F	P			
0	61.12 ± 1.97 c	65.33 ± 1.29 b	88.14 ± 4.53 b	98.99 ± 2.96 b	86.733	< 0.0001	45.693	< 0.0001	4.271	0.001			
9	83.18 ± 2.24 a	94.09 ± 6.89 a	103.54 ± 1.11 a	99.28 ± 2.50 b									
17	71.54 ± 2.70 b	77.72 ± 4.53 b	89.95 ± 3.24 b	110.25 ± 2.67 a									
26	49.76 ± 1.77 d	66.85 ± 1.96 b	74.96 ± 3.25 c	90.24 ± 1.87 c									
Mean	66.40 ± 3.85 C	76.00 ± 3.92 C	89.15 ± 3.36 B	99.69 ± 2.40 A									

Data represent the average of 3 replicates ± standard errors. Different lowercase letters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

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Table 8 Effects of aggregate sizes (> 2, 2–1, 1–0.25, and < 0.25 mm) and stand ages (0, 9, 17, and 26 years) on the soil mineralized organic carbon concentration in a chronosequence of Chinese fir plantations

Stand age (years)	Soil mineralize	d organic carbor	concentration (mg kg ⁻¹)	Effects						
	> 2 mm	2–1 mm	1–0.25 mm	< 0.25 mm	Aggregate size (A), $df = 3$		Stand age (S), <i>df</i> = 3		$A \times S$, df = 9		
					F	P	F	P	F	P	
0	73.88 ± 1.96 b	69.25 ± 4.63 c	123.13 ± 1.21 b	68.80 ± 2.80 c	7.524	0.001	35.533	< 0.0001	156.552	< 0.0001	
9	90.68 ± 5.21 a	103.77 ± 2.51 a	132.55 ± 2.08 a	60.87 ± 1.37 d							
17	97.72 ± 3.77 a	66.55 ± 2.48 c	54.82 ± 4.23 d	145.02 ± 0.91 a							
26	87.30 ± 2.14 a	80.18 ± 3.64 b	71.62 ± 1.09 c	127.40 ± 3.54 b							
Mean	97.19 ± 10.38 B	95.53 ± 10.01 B	79.94 ± 4.62 AB	87.40 ± 3.01 A							

Data represent the average of 3 replicates ± standard errors. Different lowercase letters indicate significant differences among the different stand ages. Different capital letters indicate significant differences among the different aggregate sizes

concentrated in the micro-aggregates (Table 5), which may be attributed to the micro-aggregates having smaller pores and lower O₂ concentration, thus inhibiting the decomposition of soil POC (Wang et al. 2012). Thirdly, the concentration of soil MBC was the highest in the < 0.25-mm aggregates but the lowest in the > 2mm aggregates (Table 6), which is consistent with the findings of Wang et al. (2021b), who proposed that the more soil ROC in the < 0.25-mm aggregates could make more carbon sources available for microbial growth. Fourthly, soil WOC concentration in soil aggregates increased as the decreasing aggregate size (Table 7), because micro-aggregates have a larger specific surface area than macro-aggregates, which makes microaggregates capable of adsorbing more soil WOC (Gao et al. 2014). Lastly, the distribution of soil MOC concentration in aggregates was found to show the same trends as POC (Table 8), since POC can be rapidly decomposed by the microorganisms in soil, thus converting to MOC (Jastrow 1996).

As discussed above, regardless of the stand age, soil OC and LOC fractions were concentrated in the microaggregates (< 0.25 mm). According to the micro-aggregate theory (Edwards and Bremner 1967), the formation of soil micro-aggregates was envisioned as a solid-phase reaction occurring among the OMs, polyvalent metals, and electrically neutral clays. The OMs complexed into the micro-aggregates would be physically protected and made inaccessible to soil microorganisms, thus leading to the accumulation of soil OC and LOC fractions in the microaggregates. Similarly, the results obtained in this study suggest that the formation of soil micro-aggregates plays a significant role in the sequestration of soil carbon during the stand development, which supports the microaggregate theory.

Soil carbon concentration as affected by stand age

With the stand development, the concentrations of soil OC and LOC fractions (including ROC, POC, MBC,

WOC, and MOC) first increased and then decreased, with the highest levels found in the 17-year-old fir plantations, thus rejecting our hypothesis. In the forest ecosystem, trees themselves act as the primary source of OMs into soil by root exudates and litter residues (Zeng et al. 2010). At the early phase (initial 17 years) of fir planting, soil OC and LOC fractions were accumulated due to the progressively growing root systems (Luan et al. 2010) as well as the enhanced quantity of above-ground tree litter (Table 1). At the late phase (after 17 years) of fir planting, however, the quantity of tree litter declined gradually due to the fir plantations undergoing the natural aging process (Table 1), which led to the decreases in soil OC and LOC fraction concentrations (Luan et al. 2010). In addition, the 17-year-old fir plantations had relatively stable soil aggregates (Fig. 2), which is conducive to protecting the OMs (Six et al. 2004). As a result, soil OC and LOC fractions accumulated in the 17-year-old fir plantations. Meanwhile, as revealed by Pearson's correlation analysis (Fig. 3), the concentrations of OC and LOC fractions (except for the ROC and MOC) were positively correlated (p < 0.05) with MWD in bulk soil during the stand developmentfirplanting process, which confirms that the variations in soil OC and LOC fraction concentrations were largely affected by the stability of soil aggregates. The ratios of LOC fractions to OC can reflect the quality and turnover of soil OC (Huo et al. 2013). According to Wang et al. (2021a), an increase in the LOC/OC ratios was accompanied by a decrease in the OC availability in forest soil. In this study, soil LOC/OC ratios (including ROC/OC, POC/OC, MBC/ OC, WOC/OC, and MOC/OC ratios) first decreased and then increased over time, with the lowest levels observed in the 17-year-old fir plantations (Table 9), suggesting that the soil OC in the 17-year-old fir plantations experienced greater degradation.

Soil carbon storage in aggregates

Across the 4 different stand ages, the storage of soil OC and LOC fractions in the >2-mm and <0.25-mm

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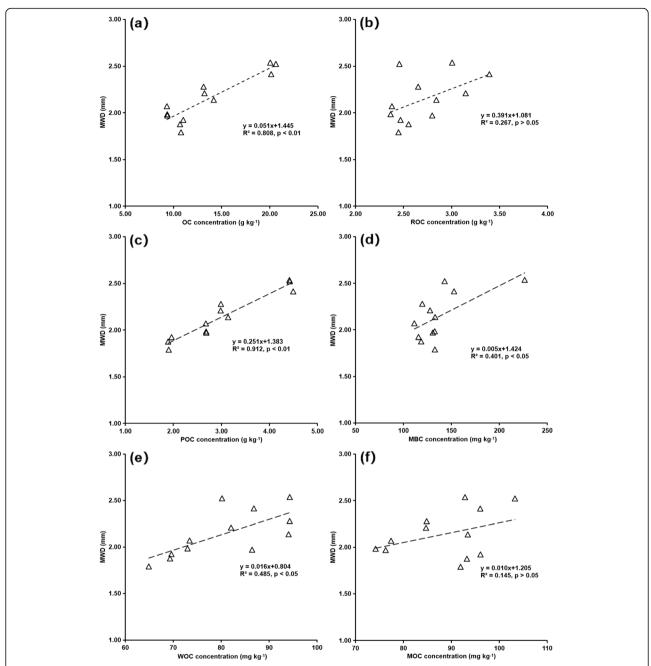


Fig. 3 Relationships of soil aggregate stability (as indicated by the MWD) with the carbon concentration in a chronosequence of Chinese fir plantations. MWD, mean weight diameter; OC, organic carbon; ROC, readily oxidizable carbon; POC, particulate organic carbon; MBC, microbial biomass carbon; WOC, water-soluble organic carbon; MOC, mineralized organic carbon

aggregates made greater contribution to that in bulk soil at the depth of 0–20 cm (Fig. 4), implying that both > 2-mm and < 0.25-mm aggregates were the dominant carriers of soil OC and LOC fraction storages in the fir plantations. Notably, although > 2-mm aggregates had the lowest concentrations of soil OC and LOC fractions at the depth of 0–20 cm, the contribution of OC and LOC fraction storage in > 2 mm aggregates to thosethat in bulk soil was greater, because > 2-mm aggregates

accounted for a higher proportion in all aggregates (Table 2). These results indicated that the storage of soil aggregate-related OC and LOC fractions in this study was mainly determined by the proportion of different sized aggregates rather than the concentrations of soil aggregate-related OC and LOC fractions, which contradicts the findings of Egan et al. (2018) and Bai et al. (2020). As for the soil ecosystem, Egan et al. (2018) and Bai et al. (2020) revealed that only when the proportion

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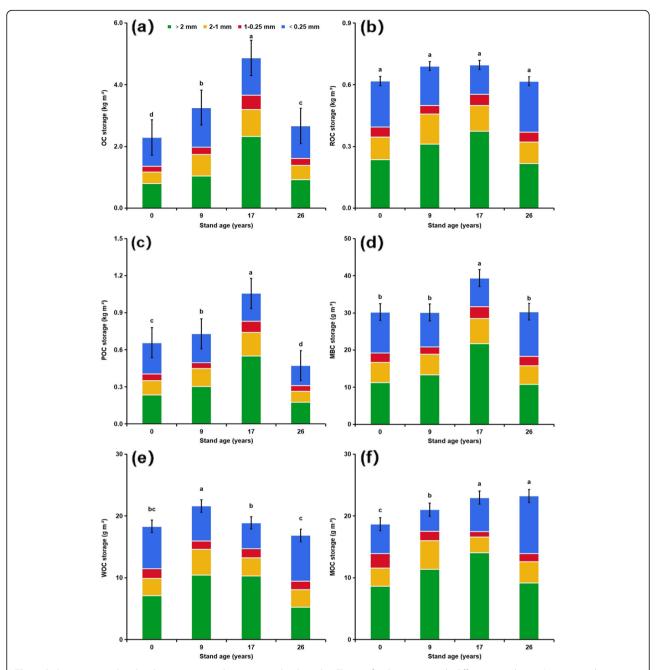


Fig. 4 Soil aggregate-related carbon storage at the 0–20-cm depth in the Chinese fir plantations with different stand ages (0, 9, 17, and 26 years old). Data represent the average of 3 replicates and error bars represent the standard errors. Different lowercase letters indicate significant differences among the different stand ages. OC, organic carbon; ROC, readily oxidizable carbon; POC, particulate organic carbon; MBC, microbial biomass carbon; WOC, water-soluble organic carbon; MOC, mineralized organic carbon

of soil aggregates was not significantly different among the different sized aggregates, the carbon concentration at aggregate scales would influence the carbon storage.

The change in soil OC storage in a period of planting divided by the length of this period is utilized as an index for estimating the increased or decreased rate of soil OC storage (Su et al. 2009). Most of the

reported change rate of soil OC storage is in an order of magnitude, with the average of 33 g OC m⁻² year⁻¹ for forest and agricultural chronosequences (Post and Kwon 2000). In this study, as the fir-plantingstand age increased from 0 to 17 years, the increased rates of soil OC, ROC, POC, and MBC storages were 49.99 g OC m⁻² year⁻¹, 4.60 g ROC m⁻² year⁻¹, 13.54 g POC m⁻²

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Table 9 The proportions of labile organic carbon fractions (ROC, POC, MBC, WOC, and MOC) accounting for organic carbon in the Chinese fir plantations with different stand ages (0, 9, 17, and 26 years old)

Stand age (years)	ROC/OC (%)	POC/OC (%)	MBC/OC (%)	WOC/OC (%)	MOC/OC (%)
0	26.96	31.04	1.34	0.83	0.82
9	21.13	22.09	0.94	0.66	0.65
17	14.38	21.50	0.85	0.42	0.47
26	23.18	18.53	1.14	0.63	0.87

OC, organic carbon; ROC, readily oxidizable carbon; POC, particulate organic carbon; MBC, microbial biomass carbon; WOC, water-soluble organic carbon; MOC, mineralized organic carbon

year⁻¹, and 0.61 g MBC m⁻² year⁻¹, respectively (Fig. 4). In contrast, from 17 to 26 years of stand age, the decreased rates of soil OC, ROC, POC, and MBC storage were 41.94 g OC m⁻² year⁻¹, 8.81 g ROC m⁻² year⁻¹, $20.91 \text{ g POC m}^{-2} \text{ year}^{-1}$, and $1.08 \text{ g MBC m}^{-2} \text{ year}^{-1}$, respectively (Fig. 4). As shown above, the change rates (including increased and decreased rates) of soil OC storage in this area were above average (33 g OC m⁻² year⁻¹) and the conversion of carbon sink into carbon source in soil was found in the 17-year-old fir plantations. Moreover, both increased rate (initial 17 years) and decreased rate (after 17 years) of soil POC storage were evidently greater than those of other LOC fraction storages, indicating that soil POC can sensitively reflect the dynamic of soil OC storage during the development of Chinese fir -plantationsing process.

Conclusions

In the present study, the highest stability of soil aggregates was observed in the 17-year-old Chinese fir plantations, and soil aggregate stability was closely associated with the concentrations of OC and LOC fractions (except for the ROC and MOC), with the POC in particular. During the development of Chinese fir plantationsing process, the concentrations of soil OC and LOC fractions increased until the 17 years old and then decreased. Therefore, after 17 years of fir planting, promoteding soil carbon (especially for the POC) accumulation is crucial for enhancing soil aggregate stability in the Chinese fir plantations of Guangxi, China.

Abbreviations

MWD: Mean weight diameter; OMs: Organic matters; OC: Organic carbon; LOC: Labile organic carbon; ROC: Readily oxidizable carbon; POC: Particulate organic carbon; MBC: Microbial biomass carbon; WOC: Water-soluble organic carbon; MOC: Mineralized organic carbon

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Authors' contributions

SW and SY conceived and designed the experiments. XH, YH, and QZ performed the experiments, analyzed the data, and wrote the manuscript. All of the authors read and approved the final manuscript.

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