

## Article

# Variation Characteristics of Soil Organic Carbon Storage and Fractions with Stand Age in North Subtropical *Quercus acutissima* Carruth. Forest in China

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**Abstract:** Soil labile organic carbon sensitively reflects subtle changes in the soil carbon pool and is an important aspect of forest soil carbon pool research. However, little is known regarding soil labile organic carbon storage and its dynamic changes during the development of *Quercus acutissima* Carruth. forests. Consequently, we investigated the dynamic changes in soil organic carbon and its labile organic carbon fraction stocks at soil depths of 0–10 cm, 10–20 cm, and 20–40 cm along a 17-year-old, 26-year-old, and 65-year-old chronosequence in *Quercus acutissima* forests. We found that stand age significantly impacted particulate organic carbon (POC), light fraction organic carbon (LFOC), and soil organic carbon (SOC). The POC, LFOC, and SOC contents at different soil depths exhibited an increasing trend with stand age, which could be described by simple linear regression. However, there was no noteworthy difference in the soil water-soluble organic carbon (WSOC) content between different stand ages. Moreover, the 17-year-old, stand had higher POC, LFOC, and WSOC to SOC ratios. Soil nutrients significantly affected organic carbon and fractions, which revealed that POC, LFOC, WSOC, and SOC were remarkably positively correlated with alkaline hydrolysis nitrogen (AN) and available phosphorus (AP) ( $p < 0.05$ ). Furthermore, WSOC, POC, LFOC, and SOC were significantly positively correlated with available potassium (AK) ( $p < 0.05$ ). POC, LFOC, and SOC storage in the 0–40 cm soil layer increased with stand development, while WSOC storage decreased at 65a. In addition, LFOC stocks accounted for the highest proportion of organic carbon stocks. Our results indicated that the development of *Quercus acutissima* forests was a process of carbon sink; however, the soil organic carbon activity was high, and the soil structure was unstable during the early development stage.

**Keywords:** soil labile organic carbon; soil carbon pool; *Quercus acutissima* forests; chronosequence



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## 1. Introduction

Since the Industrial Revolution, the concentration of CO<sub>2</sub> in the ambient atmosphere has cumulatively increased by 50%, from 278 ppm before the Industrial Revolution to 417 ppm in June 2022 [1]. Global warming due to increased atmospheric CO<sub>2</sub> concentrations, leads to ocean warming, glacial retreat, rising sea levels, and extreme weather events that alter natural cycles worldwide [2]. As the largest carbon pool in terrestrial ecosystems, the continuous exchange of carbon between the soil carbon pool and ambient atmosphere significantly affects the global carbon cycle and climate [3,4]. Approximately 30.7% of Earth's land area is covered by forests (2016) [5], and more than two-thirds of the carbon in forest ecosystems is sequestered in soils and associated peat deposits [6]. Therefore,

small modifications in the organic carbon pools of forest soils can translate to changes in atmospheric CO<sub>2</sub> concentrations; thus, forests play a unique role in the reduction in atmospheric carbon dioxide concentrations [7,8] and play a critical role in regulating the global carbon cycle and mitigating global warming.

Forest-based warming mitigation strategies will play a key role toward achieving the rapid and deep net-emissions reductions required to prevent catastrophic climate change [9]. Differences in the canopy densities, stand structures, and vegetation compositions of forests across age sequences can lead to variations in the soil microclimate, as well as the quantity and quality of aboveground and belowground litter inputs [10–12]. Consequently, this can alter soil nutrient cycling and carbon flows due to complex plant-soil interactions, which leads to considerable uncertainties in the relationship between the ages of trees and carbon stocks for individual stands [13,14]. Although the variable characteristics of soil carbon storage have been investigated in independent studies of carbon storage for stands of different ages (e.g., 0 to 8-year-old *Eucalyptus* plantations [15]; 29 to 46-year-old Dragon spruce forests [7]; 0 to 110-year-old Scots pine [16]; and 40 to 120-year-old secondary tropical forests [17]), the dynamic changes related to stand age were complex. Consequently, detailed data are required to elucidate changes in the carbon stocks of soil at different developmental stages in forest ecosystems of specific regions. This, to provide a theoretical basis for the improved assessment of the balance between terrestrial and atmospheric carbon.

Studies have indicated that the stability of soil organic carbon depends on interactions between soil aggregates and soil resident minerals [18] and is positively correlated with the labile organic carbon input rate [19]. Soil labile organic carbon (LOC) is a mixture of organic matter that has high availability, and is easily decomposed and utilized by soil microorganisms, which can also directly supply nutrients to plants [20]. As soil labile organic carbon is extremely sensitive to changes in the soil environment, even minor shifts in soil ecosystems will alter its content; thus, it is often employed as an indicator for the evaluation of soil quality and environmental changes [21]. Labile fractions, such as water-soluble organic carbon (WSOC), light fraction organic carbon (LFOC), and particulate organic carbon (POC), are characterized by their easy decomposition and a brief turnover period [22]. Thus, they are typically considered as sensitive indices for changes in soil organic carbon that result from the dynamic responses of vegetation. Soil WSOC is comprised of various soluble organic residues, is the primary available material and energy source for soil microorganisms, and a potential source of carbon sequestration in soils [23], which is generally regarded as the most unstable fraction of soil organic carbon [24]. Therefore, soil WSOC plays an essential role in the accumulation of soil nutrients [25]. Soil POC consists of plant residues, seeds, and microbial debris, which are not enclosed within soil microaggregates [26]. Soil LOC fractions possess the attributes of easy mineralization, easy decomposition, and a short turnover period [22]. Since labile organic carbon can directly participate in the transformation of soil biochemistry and quickly respond to changes in soil organic matter, it is often utilized as an indicator to measure changes in soil carbon pools [27].

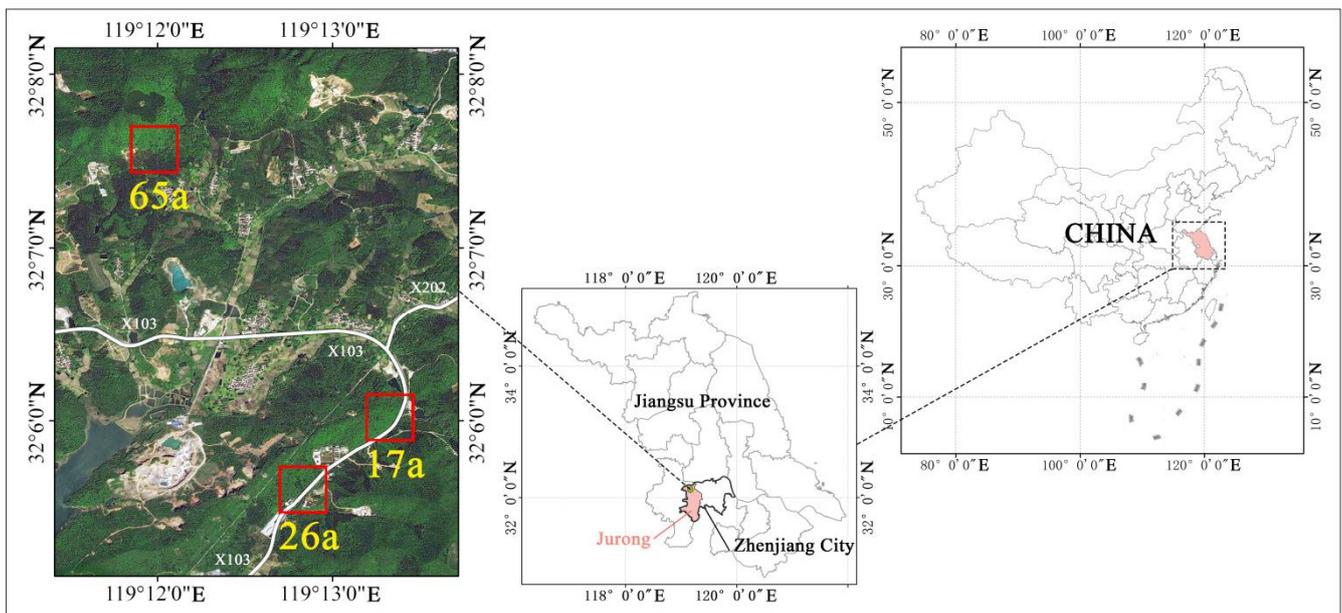
As a major contributor to ecosystem functionality, *Quercus acutissima* Carruth. is regarded as an indicator species for the localized assessment of forest health [28]. It is not only one of the most abundant *Quercus* species in East Asia; it can also adapt to an extensive range of ecological conditions and is widely distributed across China (18° N to 41° N, 91° E to 123° E) [29], spanning an estimated 7451.5 km<sup>2</sup> [30]. *Quercus acutissima* is a pioneer tree species of barren mountains and hills, as well as a pioneer tree species for soil and water conservation, which, in addition to having high ecological, economic, and landscape value [31], is used as timber, fuelwood, charcoal, and acorn production [32]. In the context of global climate change and multifunctional forest management, much research has been conducted on the cultivation technology, development and utilization, physiological and ecological characteristics, and genetic diversity of *Quercus acutissima* [33–36]. However, during the development of *Quercus acutissima* forests the dynamic changes in soil organic

carbon storage and organic carbon fractions, particularly those in labile organic carbon, remain poorly understood. Thus, the purpose of this study was to reveal the influences of stand age on changes in soil labile organic carbon and organic carbon storage and determine the key factors that affect soil labile organic carbon during *Quercus acutissima* development. Furthermore, this study assumed that the growth of *Quercus acutissima* stands was conducive to the accumulation of soil organic carbon.

## 2. Materials and Methods

### 2.1. Study Area

The study area is in the Xiashu and Jurong Forest Farms in Jurong City (32°07' N, 119°12' E), Jiangsu Province, China (Figure 1). The climate here is a subtropical humid monsoon with an average annual temperature of 15.5 °C and annual precipitation of 1126.4 mm (1969–2018). The average annual relative humidity is 79%, whereas the frost-free period is 233 days. The parent soil material is Quaternary Xiashu Formation (Q<sub>3x</sub>) loess-like sediments, which is primarily a yellow-brown soil and mountain yellow-brown soil. The thickness of the soil layer is generally 40–60 cm, and the soil texture is medium to heavy, with a pH of between 4.5 and 5.0. The forest vegetation is derived from the subtropical evergreen broad-leaved forest area of China, and the zonal vegetation is a deciduous broadleaved mixed forest. The experimental site is a natural secondary forest dominated by *Quercus acutissima*, with several *Quercus aliena* var. *acutiserrata*, and *Quercus variabilis* scattered sporadically. The main shrubs include *Rosa multiflora*, *Fortunearia sinensis*, *Callicarpa cathayana*, *Ilex cornuta*, and *Symplocos paniculata*, etc., whereas the herbs mainly include *Parthenocissus tricuspidata*, *Adiantum capillus-veneris*, and so on.



**Figure 1.** Location of the study site. 17a, 17-year-old stand; 26a, 26-year-old stand; 65a, 65-year-old stand.

### 2.2. Experimental Design and Sampling

Three differently aged *Quercus acutissima* forest stands were selected (young forest (17-years-old, 17a), half-mature forest (26-years-old, 26a), and mature forest (65-years-old, 65a)). Three experimental plots (20 m × 20 m) were selected according to their slope positions (e.g., upper, middle, and lower) in each forest stand, for a total of nine sample plots that were established, with an inter-plot distance of >20 m. Trees in the *Quercus acutissima* forest sample plots were measured; the diameters at breast height (DBH) and tree heights were recorded; and the overall stand topographies and characteristics were investigated (Table 1). We collected three soil sub-samples with a “diagonal” type distribution at each depth (i.e.,

0–10 cm, 10–20 cm, 20–40 cm) in each plot and mixed the three sub-samples of each plot to a composite soil sample and sifted through a 2 mm sieve to remove plant tissue, roots and gravel. Following natural air-drying and grinding, the physicochemical properties, organic carbon, and labile organic carbon fractions of the soil were analyzed.

**Table 1.** Basic characteristics of sampling plots studied.

Sample Plot Number	Stand Age/(a)	Stand Density/(Tree·m <sup>-2</sup> )	Slope Gradient/(°)	Slope Aspect	Diameter at Breast Height/(cm)	Height/(m)	Canopy Density/(%)
1	17	975	30	Southeast	10.2	8.60	48
2	17	700	34	Southeast	10.7	8.85	45
3	17	300	40	Southeast	14.2	10.31	35
4	26	775	8	North	13.4	9.94	80
5	26	825	8	North	15.2	10.63	80
6	26	600	8	North	14.2	10.31	75
7	65	225	28	Southeast	28.6	15.25	55
8	65	325	31	Southeast	26.9	14.73	45
9	65	350	28	Southeast	25.3	14.20	47

### 2.3. Soil Physical and Chemical Analyses

The soil texture was analyzed using a Laser Particle Analyzer (Microtrac Inc., North Largo, FL, USA), and the gravel volume percentage was measured via the Archimedes method. The soil bulk density (BD) was determined using the cutting ring (Ø50.46 mm × 50 mm high) method. The soil pH was quantified using the glass electrode technique, and the air-dried soil-to-water ratio was 1:2.5 (*m:v*). The alkaline hydrolysis nitrogen (AN), available potassium (AK), and available phosphorus (AP) contents of soil were measured using the alkali-diffusion, ammonium acetate extraction-flame photometer [37], and hydrochloric acid-sulfuric acid extraction and molybdenum-antimony anti-colorimetric methods, respectively.

### 2.4. Soil Organic Carbon and Labile Organic Carbon Analyses

The soil organic carbon (SOC) was determined using the potassium dichromate external heating oxidation-ferrous sulfate titration technique [38]. A 5 mL volume of 0.8 M K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> and 5 mL of H<sub>2</sub>SO<sub>4</sub> were added to 0.1 g of air-dried soil and passed through a 0.149 mm sieve, which was then boiled in an oil bath at 170–180 °C for five min and cooled, after which an indicator was added and titrated with 0.2 M FeSO<sub>4</sub>.

The soil particulate organic carbon (POC) was determined using the method of [39]. A 20 g volume of air-dried soil was dispersed by shaking overnight in 100 mL solution of sodium hexametaphosphate (5 g/L). The soil mixture was then passed through a 0.053 mm sieve and rinsed repeatedly with distilled water. The sieve residue (i.e., the particulate organic matter portion, >0.053 mm) was dried and weighed. Subsequently, the organic carbon content of the particulate organic matter was determined.

The soil water-soluble organic carbon (WSOC) was determined by modifying the technique described by Yang et al. [40], through extraction with deionized water (the mass ratio of water to soil was 2:1), after which the suspension was shaken at 210 rpm for 1 h, and then centrifuged at 3000 rpm for 15 min. The supernatant was filtered through a 0.45-µm polycarbonate membrane, and the filtrate was assessed using an automatic TOC analyzer (TOC-L CPH CN200, Shimadzu, Kyoto, Japan).

The soil light fraction organic carbon (LFOC) was fractionated using a method modified from McLauchlan and Hobbie [41]. A 10 g volume of air-dried soil was sifted through a 2 mm sieve and placed in 50 mL of a NaI solution with a specific gravity of 1.7 g/cm<sup>3</sup>. The soil was ultrasonically dispersed and following centrifugation the upper layer of the suspension was carefully passed through a 0.45-µm polycarbonate membrane. The NaI solution of a single sample was recovered via suction filtration, washed with a CaCl<sub>2</sub> solution to separate the light portion, repeatedly rinsed with deionized water, and then dried in a 60 °C oven for 48 h to a constant weight and weighed. The above process was

typically repeated two to three times, where the sum of the light groups separated multiple times was the number of light groups. The light soil group samples were passed through a 0.149 mm sieve and their organic carbon content was determined using the acid dichromate wet oxidation technique.

### 2.5. Stock Calculation of SOC and Fractions

The SOC and fraction storage ( $t \cdot \text{hm}^{-2}$ ) was calculated using the following equation:

$$S(t \cdot \text{hm}^{-2}) = \sum_i^n H_i \times \text{BD}_i \times C_i \times (100 - G_i) \times 10^{-3} \quad (1)$$

where  $S$  is the storage of SOC or fraction ( $t \cdot \text{hm}^{-2}$ ),  $H_i$  is the thickness of the  $i$  layer (cm),  $\text{BD}_i$  is the soil bulk density of the  $i$  layer ( $\text{g} \cdot \text{cm}^{-3}$ ),  $C_i$  is the organic carbon or fraction content of the  $i$  layer ( $\text{g} \cdot \text{kg}^{-1}$ ),  $G_i$  is the volume percentage (%) of gravel with diameters of  $\geq 2$  mm in the  $i$  layer.

### 2.6. Statistical Analyses

SPSS 22.0 statistical software was employed for the statistical analysis of all data, where the data of each variable were tested by normality and homogeneity. ANOVA was utilized to estimate the impacts of stand age on soil properties and organic carbon components. The LSD test revealed significant differences under  $p < 0.05$ . Redundancy analysis was used to study the relationships between organic carbon components and soil physicochemical properties for differently aged *Quercus acutissima*. Canoco5.0 and GraphPad Prism9.0 were used for plotting.

## 3. Results

### 3.1. Soil Properties

The soil sand, and silt contents of the three stand ages revealed significant differences. The clay content of the 17a stands was significantly lower than that of the 26a and 65a stands, and no significant differences in pH were measured between the three stand ages ( $p > 0.05$ ). The soil BD of the 26a stands was remarkably higher ( $p < 0.05$ ) than the other stand ages. The AN, AP, and AK contents of the 65a stands were higher than those in the 17a and 26a stands and the differences between soil layers were significant, which generally decreased with soil depth. Furthermore, there were no significant differences in the soil mechanical composition, BD, and pH between the soil layers (Table 2).

### 3.2. Soil Labile Organic Carbon

Soil depth had a significant influence on both soil organic carbon and labile organic carbon. In addition to WSOC, stand age significantly affected POC, LFOC, and SOC, while POC, WSOC, LFOC, and SOC were insensitive to the interactive impacts of stand age and soil depth (Table 3).

The POC content at different soil depths increased with stand age, which could be described by simple linear regression (Figure 2A). In the 17 to 65-years-old stands, the POC content within the 0–10 cm soil layer increased from  $4.74 \text{ g} \cdot \text{kg}^{-1}$  to  $9.68 \text{ g} \cdot \text{kg}^{-1}$ ; the 10–20 cm soil layer increased from  $2.34 \text{ g} \cdot \text{kg}^{-1}$  to  $6.53 \text{ g} \cdot \text{kg}^{-1}$ ; and the 20–40 cm soil layer increased from  $0.59 \text{ g} \cdot \text{kg}^{-1}$  to  $2.81 \text{ g} \cdot \text{kg}^{-1}$  (Figure 2A). The POC/SOC ratio within the 20–40 cm soil layer increased linearly by 6.40%, while the 0–10 cm and 10–20 cm soil layers did not exhibit an obvious trend with stand age (Figure 3A). Moreover, the ratio was higher in 17a and 65a, albeit the difference was not significant.

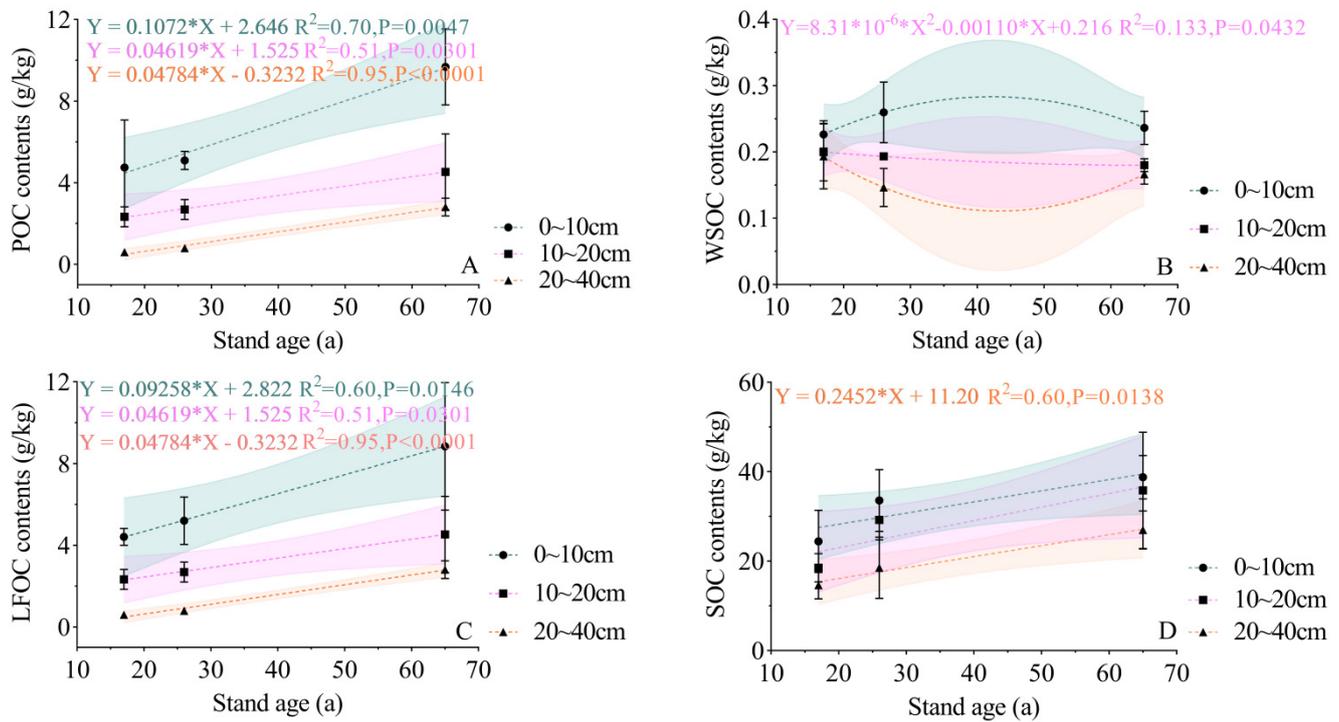
**Table 2.** Soil physical and chemistry properties of different stand ages. When  $p < 0.05$ , different capital letters represent differences between forest ages in the same soil layer, whereas different lowercase letters represent differences between soil layers at the same forest age. BD, bulk density; AN, alkaline hydrolysis nitrogen; AP, available phosphorus; AK, available potassium (mean  $\pm$  SE,  $n = 3$ ).

Stand Age (a)	17			26			65		
	0–10	10–20	20–40	0–10	10–20	20–40	0–10	10–20	20–40
Soil depth (cm)									
Sand (%)	63.75 $\pm$ 16.35 Aa	77.35 $\pm$ 8.15 Aa	59.40 $\pm$ 18.65 Aa	23.65 $\pm$ 19.78 Ba	27.23 $\pm$ 14.59 Ba	22.94 $\pm$ 7.18 Ba	40.81 $\pm$ 4.60 Ca	43.05 $\pm$ 2.87 Ca	38.89 $\pm$ 6.72 Ca
Silt (%)	34.15 $\pm$ 15.29 Aa	21.02 $\pm$ 7.23 Aa	36.72 $\pm$ 16.71 Aa	67.13 $\pm$ 15.72 Ba	64.52 $\pm$ 11.95 Ba	69.26 $\pm$ 6.41 Ba	50.31 $\pm$ 4.11 Ca	45.45 $\pm$ 3.65 Ca	52.91 $\pm$ 5.51 Ca
Clay (%)	2.11 $\pm$ 2.13 Aa	1.64 $\pm$ 0.92 Aa	3.88 $\pm$ 2.32 Aa	9.19 $\pm$ 4.16 Ba	8.18 $\pm$ 3.38 Ba	7.79 $\pm$ 1.36 Ba	8.87 $\pm$ 2.79 Ba	11.50 $\pm$ 3.46 Ba	8.20 $\pm$ 4.65 Ba
BD (g·cm <sup>-3</sup> )	1.35 $\pm$ 0.03 Aa	1.44 $\pm$ 0.18 Aa	1.38 $\pm$ 0.10 Aa	1.47 $\pm$ 0.02 Ba	1.70 $\pm$ 0.05 Ba	1.71 $\pm$ 0.05 Ba	1.32 $\pm$ 0.09 Aa	1.20 $\pm$ 0.33 Aa	1.28 $\pm$ 0.28 Aa
pH	4.68 $\pm$ 0.48 Aa	4.72 $\pm$ 0.16 Aa	4.47 $\pm$ 0.12 Aa	4.72 $\pm$ 0.05 Aa	4.60 $\pm$ 0.29 Aa	4.60 $\pm$ 0.03 Aa	4.48 $\pm$ 0.12 Aa	4.44 $\pm$ 0.10 Aa	4.52 $\pm$ 0.13 Aa
AN (mg·kg <sup>-1</sup> )	105.00 $\pm$ 32.08 Aa	72.33 $\pm$ 10.69 Ab	42.00 $\pm$ 7.00 Ac	119.00 $\pm$ 14.00 Aa	70.00 $\pm$ 7.00 Ab	53.67 $\pm$ 8.08 Ac	212.33 $\pm$ 32.33 Ba	135.33 $\pm$ 21.39 Bb	100.33 $\pm$ 10.69 Bc
AP (g·kg <sup>-1</sup> )	6.86 $\pm$ 1.76 Aa	8.48 $\pm$ 0.98 Ab	6.43 $\pm$ 2.15 Ac	10.27 $\pm$ 1.49 Aa	4.72 $\pm$ 0.71 Ab	2.71 $\pm$ 0.26 Ac	15.76 $\pm$ 4.22 Ba	7.82 $\pm$ 2.00 Bb	4.68 $\pm$ 2.20 Bc
AK (mg·kg <sup>-1</sup> )	155.42 $\pm$ 58.04 Aa	168.17 $\pm$ 23.01 Aa	133.56 $\pm$ 19.10 Aa	218.4 $\pm$ 13.34 Bb	193.75 $\pm$ 15.45 Bb	185.42 $\pm$ 15.62 Bb	226.73 $\pm$ 23.11 Bc	189.35 $\pm$ 5.32 Bc	201.74 $\pm$ 33.32 Bc

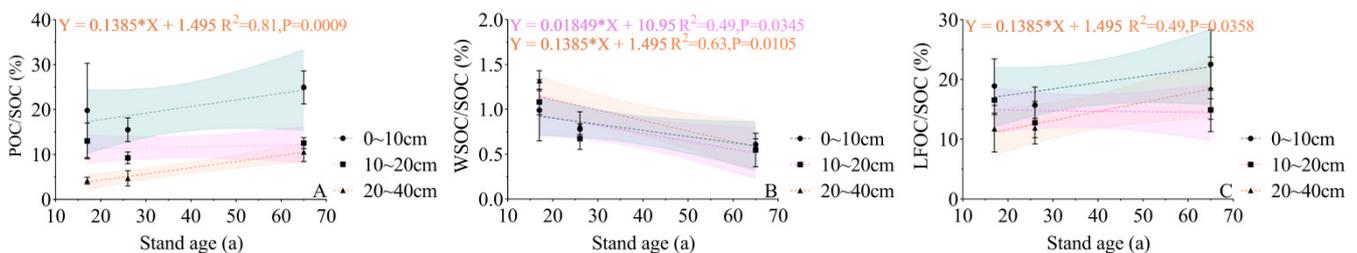
Note: International system, Stand 0.02–2 mm, Silt 0.002–0.02 mm, Clay <0.002 mm.

**Table 3.** Effects (F-ratio,  $p$ -values) of stand age, soil depth, and their interactions on the concentration of soil carbon fractions. SOC, soil organic carbon; POC, particulate organic carbon; WSOC, soil water-soluble organic carbon; LFOC, light fraction organic carbon. \* represents statistical significance, in which \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

Index	Stand Age		Soil Depth		Stand Age $\times$ Soil Depth	
	F Value	$p$ Value	F Value	$p$ Value	F Value	$p$ Value
POC	18.006	<0.001 ***	40.953	<0.001 ***	1.643	0.207
WSOC	0.349	0.710	12.739	<0.001 ***	1.291	0.311
LFOC	10.046	0.001 **	7.968	0.003 **	0.336	0.850
SOC	11.098	0.001 **	7.863	0.004 **	0.245	0.909



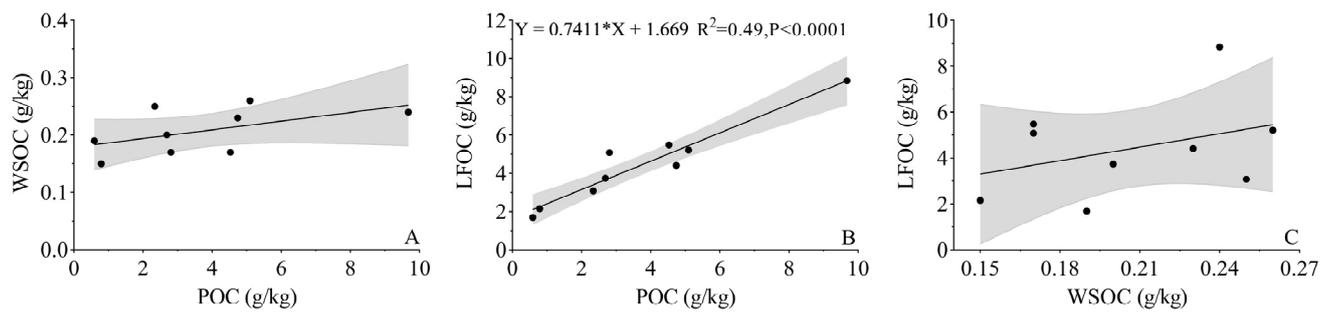
**Figure 2.** Variations in soil labile organic carbon with stand age. particulate organic fraction (POC, (A)); soil water-soluble organic carbon (WSOC, (B)); light fraction organic carbon (LFOC, (C)); soil organic carbon (SOC, (D)) (mean  $\pm$  SD, n = 3).



**Figure 3.** Ratio of soil labile organic carbon fractions and organic carbon in differently aged *Quercus acutissima* forests. Ratio of particulate organic carbon and organic carbon (POC/SOC, (A)); Ratio of soil water-soluble organic carbon and organic carbon (WSOC/SOC, (B)); Ratio of light fraction organic carbon and organic carbon (LFOC/SOC, (C)) (mean  $\pm$  SD, n = 3).

There was no significant difference in the soil WSOC content between stand ages (Table 3). The WSOC content within the 10–20 cm and 20–40 cm soil layers generally decreased with stand development, and the change trend within the 0–10 cm soil layer was not obvious (Figure 2B). Contrary to POC, the proportion of WSOC to SOC had a significantly negative correlation with stand age, and the proportion tended to increase with the greater soil depth (Figure 3B).

The tendency of the soil LFOC changes was akin to that of the POC, which increased with the changing chronosequence (Figure 2C). Moreover, the LFOC and POC had a good linear relationship (Figure 4B). The LFOC concentration within the 0–10 cm, 10–20 cm, and 20–40 cm soil layers of the 65a stands was 2.0, 1.8, and 3.0 times that of the 17a stands, respectively (Figure 2C). The ratio of LFOC to SOC within the upper soil layer (0–20 cm) was lowest in the 26a stands (12.75%), while the ratio of LFOC to SOC in the deeper soil layer (20–40 cm) increased during stand development (Figure 3C).

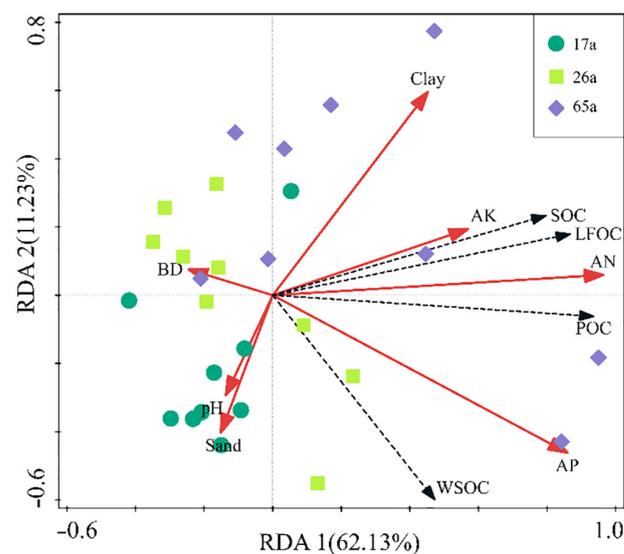


**Figure 4.** Relationships between labile organic carbon fractions in *Quercus acutissima* forest. Relationships between soil water-soluble organic carbon (WSOC) and particulate organic carbon (POC) (A); Relationships between light fraction organic carbon (LFOC) and particulate organic carbon (POC) (B); Relationships between light fraction organic carbon (LFOC) and soil water-soluble organic carbon (WSOC) (C).

During stand development, the SOC content increased significantly; 65a stands were roughly 1.6–1.9 fold and 1.2–1.5 fold higher than the 17a and 26a stands, respectively. Furthermore, compared with the 0–10 cm soil layer, the SOC concentration was higher than those in the 10–20 cm and 20–40 cm soil layers (Figure 2D).

### 3.3. Factors Affecting Composition of Soil Labile Organic Carbon

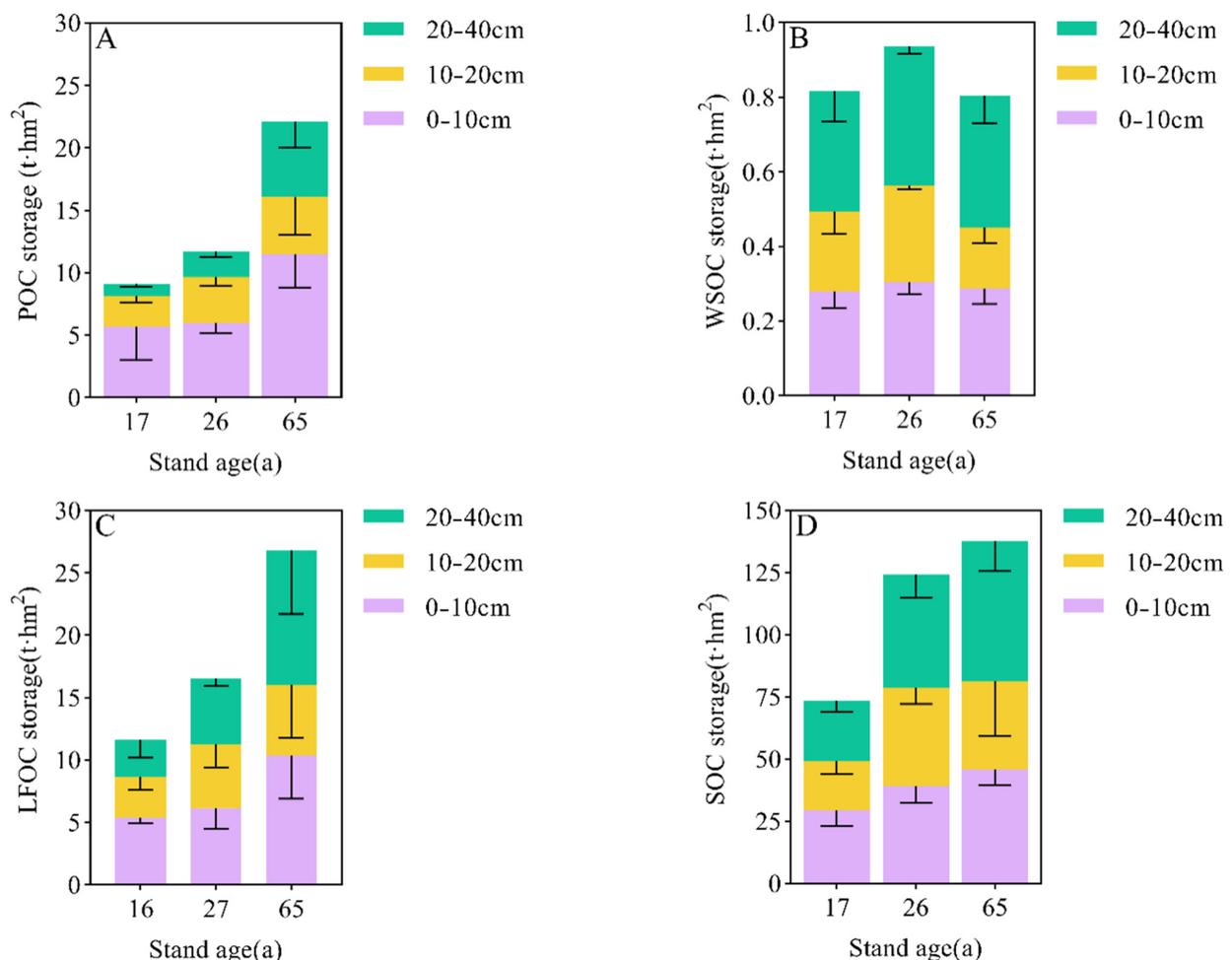
The redundancy analysis revealed the relationships between the physicochemical soil properties and organic carbon and labile organic carbon in differently aged *Quercus acutissima* forests (Figure 5). Axis 1 and 2 accounted for 62.13% and 11.2% of the total variation in soil organic carbon and its labile fractions, respectively. The POC, WSOC, LFOC, and SOC exhibited a notably positive correlation with AN and AP ( $p < 0.05$ ). The POC, LFOC, and SOC were positively correlated with AK, whereas WSOC was not. Only clay had a significant relationship with LFOC and SOC ( $p < 0.05$ ). Additionally, there were no significant relationships between the POC, WSOC, LFOC, SOC and sand, silt, BD, and pH.



**Figure 5.** Redundancy analysis screening of soil physicochemical properties (red arrows) affecting the soil carbon fraction (black arrows). SOC, soil organic carbon; POC, particulate organic carbon; WSOC, soil water-soluble organic carbon; LFOC, light fraction organic carbon; BD, bulk density; AN, alkaline hydrolysis nitrogen; AP, available phosphorus; AK, available potassium; 17a, 17-year-old stand; 26a, 26-year-old stand; 65a, 65-year-old stand.

### 3.4. Soil Carbon Storage

As revealed from the results, POC, LFOC, and SOC storage within the 0–40 cm soil layer increased with stand development, while WSOC storage decreased in the 65a stand (Figure 6). The SOC and labile organic carbon fraction content was significantly reduced with depth. However, this was not reflected in the SOC and labile organic carbon fraction stocks due to changes in the soil gravel volume percentage and bulk density, and carbon stocks did not change markedly with soil depth. The POC storage values of the 0–40 cm soil layer in the 26a and 65a stands were 1.28 and 2.42 times higher, respectively, than that in the 17a stands (Figure 6A). The total WSOC storage ranged from 0.80 to 0.93 t·hm<sup>2</sup> across the chronosequence (Figure 6B). The soil LFOC storage in the 0–40 cm soil depth of the 26a and 65a stands was 42.60% and 130.86% higher, respectively, than those in the 17a stands (Figure 6C). The SOC storage within the 0–40 cm soil layer in the 17a, 26a, and 65a stands was 73.37, 124.33, and 137.60 t·hm<sup>2</sup>, respectively (Figure 6D). The proportion of POC, WSOC, and LFOC fraction storage to SOC storage was 9.46%–16.26%, 0.60%–1.12%, and 13.38%–18.81%, respectively.



**Figure 6.** Soil carbon storage of three age groups. particulate organic fraction (POC, A); soil water-soluble organic carbon (WSOC, B); light fraction organic carbon (LFOC, C); soil organic carbon (SOC, D) (mean  $\pm$  SD, n = 3).

## 4. Discussion

### 4.1. Variations in SOC Content and Labile Organic Carbon Fractions across Forest Ages

This study found that stand age and soil depth notably affected changes in soil carbon (Table 3). Since numerous studies found that soil depth significantly impacted the SOC content and labile organic carbon fractions, which decreased with greater soil depth [12,42],

it does not appear in the discussion of this study. Stand age is related to the structure of forests, which can regulate the soil microenvironment and quantity of plant litter [10]; thus, the influence of the distribution of soil carbon fractions during stand development remains uncertain.

The primary sources of POC include microbial biomass debris, residual roots, stubble biomass, and animal manure [43]. LFOC is typically comprised of soil organic matter, which includes mainly animal and plant residues, mycelium, spores, monosaccharides, polysaccharides, and hemilignin [44,45]. For one thing, plant litter inputs are an important source of soil carbon, and aboveground litter tends to be enriched during stand development [46]. For another, as stands develop, soil microbial communities gradually become highly efficient and parsimonious, while the quantity, activities, and related intermediates of soil microbes increase [47]. Furthermore, in this study, the soil AP, AN, and AK generally increased with stand development (Table 2), where relationships between the soil AP, AN, AK, POC, WSOC, LFOC, and SOC concentrations were significantly positively correlated (Figure 5). This indicated that soil nutrients provided an adequate material guarantee for the metabolic activities of soil microorganisms during stand development. Therefore, the LFOC and POC contents tended to increase during the stand development stage, which could be well fitted by a linear function, while the contents of WSOC decreased with the stand development; thus, the correlation with stand age was not obvious (Figure 2). On one hand, litter residue inputs and root exudates were the main sources of soil WSOC [48,49], and the root biomass of *Quercus acutissima* forest decreased with stand development [50], which was consistent with the changing WSOC content with stand age (Figure 2B). Conversely, WSOC is a type of organic carbon in the soil that can be dissolved in water by extraction, which includes simple organic molecules such as carbohydrates, amino acids, and small proteins [51]. Moreover, WSOC is more mobile and robust during rainy summers [52]; therefore, it is less impacted by stand age.

LOC/SOC are generally considered as indicators of changes in soil nutrient status [53,54] and organic carbon dynamics [55], which can indirectly reflect the stability and resilience of carbon pools in forest ecosystems [11]. The ratio of soil labile organic carbon to total organic carbon can better reflect the status of the soil labile organic carbon pool. The higher the proportion of labile organic carbon in the total soil carbon, the greater the activity of soil carbon, and the lower its stability [56]. During stand development, except for WSOC, the proportions of POC and LFOC were higher in the 17a and 65a stands; however, the difference was not obvious (Figure 3A,C), which indicated that their soil organic carbon activities were high and carbon pool stability was low. While the WSOC:SOC ratio showed a decreasing trend, it was also found to be larger in the 17a stand (Figure 3B), which may have been due to the relatively high WSOC content. This meant that the soil structure in the early stage of *Quercus acutissima* forest development was unstable, and SOC was more easily depleted.

#### 4.2. Effects of Stand Age on Soil Carbon Storage

The average organic carbon stock of  $111.17 \text{ Mg}\cdot\text{hm}^{-2}$  reported in this study was within the average range of from 96 to  $344 \text{ Mg}\cdot\text{hm}^{-2}$ , which was reported from tropical, temperate, and boreal forest ecosystems [57]. Furthermore, the increase in soil carbon stocks with stand age in this study was similar to that reported for the first few decades following Pine, Masson pine, and Chinese fir afforestation [14,58,59]. This also validated our hypothesis that the development of *Quercus acutissima* stands was beneficial for the accumulation of soil organic carbon. Several studies revealed that soil carbon storage decreased with a greater stand age than half-mature forests [14,59]. Although the WSOC storage of our study illustrated this rule, carbon stocks of nearly-mature (40–50a) and overly-mature forests (>70a) were not investigated. Therefore, it remains unknown whether total organic carbon stocks and other labile organic carbon stocks continue to increase after middle-age in *Quercus acutissima* forests. Changes observed in the SOC pool with stand age indicated that stand age controlled biological processes in this ecosystem. The insignificant differences in carbon storage between the half-mature and mature forests in this research suggested that

the SOC pool was approaching the carrying capacity of forest soil C [59]. It may have been that the carbon inputs from litter and the forest floor can be balanced by increased carbon outputs driven by microbial decomposition, which results in relatively stable soil carbon pools over a chronosequence [60].

Changes in the SOC storage: length of period ratio may be employed as indicators to estimate the rates of increase or decrease in SOC storage [14,61]. There are significant variations in the rate and temporal duration required for carbon accumulation in soil, which are related to the productivity of restored vegetation, physical and biological conditions in the soil, and the history of SOC inputs and physical disturbances [62]. In this study, for *Quercus acutissima* forests from 17 to 26 years and 26 to 65 years, the increasing rates of SOC, POC, and LFOC storage within the 0–40 cm soil layer decreased from 566.16 to 34.04  $\text{g}\cdot\text{m}^{-2}\cdot\text{a}^{-1}$ , 28.23 to 26.79  $\text{g}\cdot\text{m}^{-2}\cdot\text{a}^{-1}$ , and 54.99 to 26.30  $\text{g}\cdot\text{m}^{-2}\cdot\text{a}^{-1}$ , respectively (Figure 6). These results showed that the increased rate of carbon storage in the later stage development of *Quercus acutissima* forests was lower than that during early-stage development. This may have been due to a considerable increase in the biomass of litter during the early stage of *Quercus acutissima* forest development, which promoted nutrient cycling in the young *Quercus acutissima* forest soil [63]. Moreover, related studies indicated that stand age may have the greatest impact on soil carbon storage during the first 10–30 years, and there were other factors that could not be included in our analysis [19,64,65]. Most of the reported rates of change in SOC stocks were typically in orders of magnitude, where the rate of increase in soil organic carbon stocks in the 17–26a *Quercus acutissima* forest of this study was higher than that for the 18–28a *Pinus massoniana* forest soil 0–40 cm (124  $\text{g}\cdot\text{m}^{-2}\cdot\text{a}^{-1}$ ) [66]. Furthermore, the rate of increase for soil LFOC storage was significantly higher than that of other soil organic carbon fractions, which signified that soil LFOC sensitively reflected the dynamic changes in soil organic carbon storage during *Quercus acutissima* forest development. He et al. [14] found that the soil carbon storage within the 0–20 cm soil layer in a Chinese fir forest increased from 0 to 17 years, and then decreased from 17 to 26 years; thus, the soil carbon storage changed from carbon sink to carbon source in 17 years. In this study, due to the lack of carbon storage data for near-mature forests (40–50a) and over-mature forests (>70a), it was not explored whether the soil carbon pool would transition from a carbon sink to a carbon source during the growth and development of the *Quercus acutissima* forest; thus, further investigations are required.

## 5. Conclusions

This study elucidated that the LFOC and POC contents showed an increasing trend during the development stage of forest stands, which could be well fitted to a linear function, while WSOC showed an opposite trend. During the stand development stage, the proportion of POC, WSOC, and LFOC in SOC in the 17a stand was relatively high, which indicated that the soil organic carbon activity was high, but the carbon pool stability was low during the early stage of *Quercus acutissima* forest stand development. With stand development, the POC, LFOC, and SOC carbon storage were increased; however, the increased rate of carbon storage during the latter stage of *Quercus acutissima* forest development was lower than that during the early stage. This indicated that the development of the *Quercus acutissima* forest involved a carbon sink process. Due to the lack of carbon storage data for near-mature forests (40–50a) and over-mature forests (>70a) in this paper, further research is required. Simultaneously, the soil LFOC storage capacity rate was significantly higher than that of other soil organic carbon fractions, which indicated that soil LFOC could sensitively reflect dynamic changes in soil organic carbon storage during the development of *Quercus acutissima* forests.

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