

# Biochemical quality and accumulation of soil organic matter in an age sequence of *Cunninghamia lanceolata* plantations in southern China

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## Abstract

**Purpose** Biochemical protection is an important mechanism for maintaining the long-term stability of the soil carbon (C) pool. The labile and recalcitrant pools of soil organic matter (SOM) play different roles in regulating C and N dynamics; however, few studies have characterized the capacity of soil C sequestration while considering the biochemical quality of SOM. The aim of the present study was to assess the changes in the soil organic carbon (SOC) and nitrogen (N) pools during a traditional rotation period (25 years) of a Chinese fir (*Cunninghamia lanceolata*) plantation with an emphasis on SOM biochemical quality.

**Materials and methods** Three different forest stand development stages—young (6 years old), middle-aged (16 years old) and mature (25 years old)—were selected for soil sampling to a depth of 100 cm. Total C and total N of the soil was analysed to determine the changes in the SOC and N stocks among the three development stages using an equivalent soil mass (ESM) approach. Bulk soils were fractionated into labile and recalcitrant fractions using the acid hydrolysis method to identify the quality of SOM.

**Results and discussion** The mineral soil organic carbon pool at a 1-m depth slightly decreased from the young stand to the middle-aged stand and rapidly increased by 28 % to reach a maximum in the mature stand. SOC accumulation in the surface soil predominated the changes in total SOC stocks in all

three stands. The increased N was reflected in the entire depth, and the highest soil N accumulation was in the mature stand. The recalcitrant C concentration and SOC were positively correlated. The non-hydrolysable C proportion was lower in the middle-aged stand versus the young stand (8.69 % loss), while the labile C percentage was higher (13.89 % gain). In the mature stand, the recalcitrant C index increased to 39.84 %. The recalcitrant index of C decreased with an increasing soil depth, whereas the recalcitrant index of N dramatically increased.

**Conclusions** These results highlighted the significant effect of the stand age and the soil depth on the storage and biochemical availability of SOM in Chinese fir plantations of southern China. The recalcitrant index of C changed with the change in SOC concentration, indicating that biochemical protection mechanism plays an important role in soil C sequestration. In addition, more attention should be paid to subsoil C protection in the management of Chinese fir plantations because of low biochemical stability.

**Keywords** Acid hydrolysis method · Biochemical stability · Labile C · Recalcitrance index · Rotation period · Soil C stocks

## 1 Introduction

Plantations are expanding around the world at an annual rate of 2 % (van Dijk and Keenan 2007). Due to their rapid growth and high productivity, short-rotation plantations are considered to have a strong carbon (C) sequestration potential for mitigating rising atmospheric CO<sub>2</sub> (Houghton et al. 2009). A variety of studies have found that vegetation can capture C in biomass through photosynthesis over a rotation period (Thuille and Schulze 2006; Yang et al. 2011; Chen et al. 2013). However, it remains inconclusive whether the pools

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of soil organic carbon (SOC) serve as sinks or as sources for atmospheric CO<sub>2</sub>. In terrestrial ecosystems, the SOC pool likely represents the largest C sink (Schmidt et al. 2011), and the soil compartment holds two thirds of the forest ecosystem C (Lorenz et al. 2007). Therefore, even minor changes in the SOC pool have dominant effects on forest C dynamics.

The amount of carbon sequestered in soil is determined by a positive balance between input and output organic materials (Davidson and Janssens 2006). Reducing the turnover rate of soil organic matter (SOM) has been suggested as a more feasible strategy for enlarging the soil C pool, relative to increasing C input (Jastrow et al. 2007; Dungait et al. 2012). Carbon is sequestered in soil through three stabilization mechanisms: physical retention in soil aggregate, soil mineral association and biochemical protection; together, these mechanisms are responsible for prolonging residence time and promoting C accumulation. Humification alters small organic molecules to form new products that are more resistant to decomposition through biotic or abiotic condensation (Lorenz et al. 2007).

SOM is composed of diverse chemical compounds with varying turnover rates (Davidson and Janssens 2006). The recalcitrant SOM pool has mean residence times of 1500–3500 years and is generally 1000 years older than bulk soil C (Paul et al. 2001). Even some labile SOM has a mean turnover time of 22–125 years, which is longer than fresh plant materials (Schmidt et al. 2011; Dungait et al. 2012). In general, the long-lived recalcitrant SOM plays a critical role in regulating soil C stores, while short-term soil C or nitrogen (N) fluxes and nutrient cycles are more determined by the labile C pool as a result of its high sensitivity of quality (Belay-Tedla et al. 2009). Consequently, labile and recalcitrant C and N pool dynamics need to be better understood to effectively monitor and manage sequestration of C in soil.

Forest age may alter SOM quality (e.g., the relative distribution of labile and recalcitrant SOM) via changes to the input and decomposition of plant litters, which in coniferous forests is highly lignified and has low microbial availability. Stable organic compounds may become concentrated in the soil as a plantation matures if the input of plant tissues is continuous and if the labile fraction is decomposed rapidly in response to environmental change (Rovira and Vallejo 2002; Pérez-Cruzado et al. 2014). In addition, as forests age, there are changes in soil properties that also impact the soil C process. In the past decades, most studies have assessed changes in soil C stocks change over the cut rotation periods of plantations without considering SOM quality, whereas the relative composition of SOM is a crucial indicator for predicting C sequestration and plantation management (Pérez-Cruzado et al. 2014).

Despite their low C concentration, 46–63 % of the total soil C is stored below the first 30-cm depth worldwide (Batjes 1996). Most studies have sampled less than 30 cm of depth for carbon assessments, owing to field sampling challenges

(Liao et al. 2010; Mobley et al. 2015). Decreasing C/N ratio and increasing  $\delta^{13}$  and radiocarbon age throughout the entire soil profile (Rumpel and Kögel-Knabner 2011) suggest that subsoils could be expected to have higher biochemical stability compared to surface soils. However, studies of American and Mediterranean forest soils showed that the biochemical quality of SOM decreased with increasing soil depth (Tan et al. 2004; Rovira and Ramón Vallejo 2007). Even less information is available about how spatial distribution of SOM quality changes within the soil profile in subtropical forest soil.

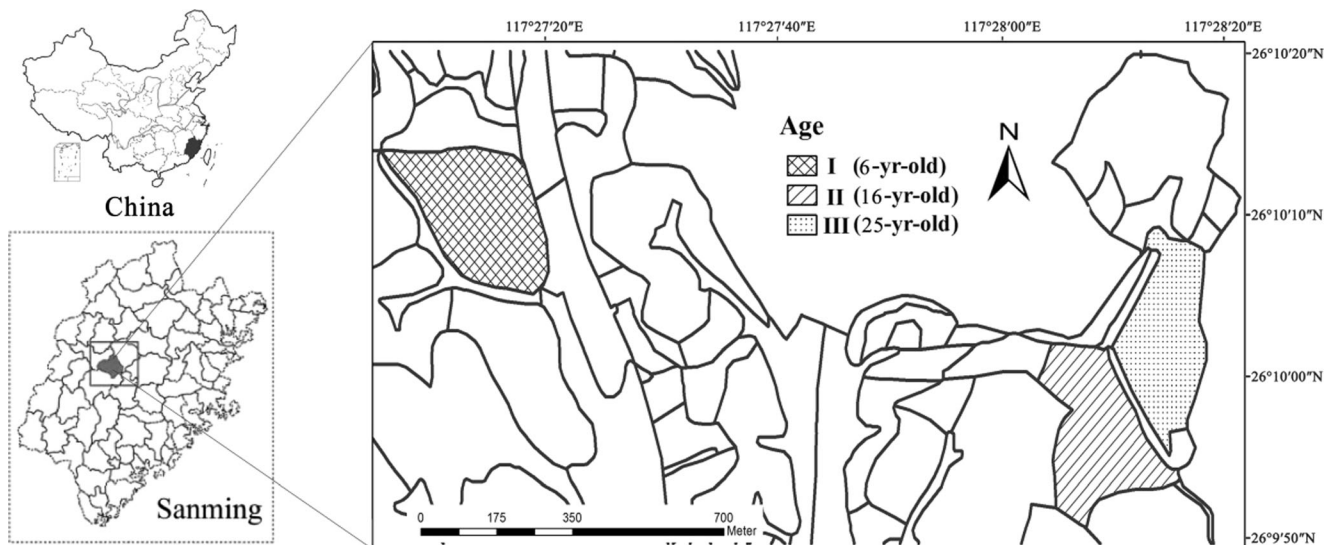
Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.) is an endemic species of south China that has a long cultivation history (>1000 years). Due to its rapid rate of growth, high yield and important economic value, these monospecific Chinese fir plantations cover approximately 12 million hm<sup>2</sup> and represent approximately 6.5 % of plantation forests around the world (FAO 2006). Hence, Chinese fir plantations have a significant effect on the global C balance. The majority of Chinese fir plantations are typically managed with successive rotations, and significant reductions in soil C storage over successive rotations have been observed (Zhang et al. 2004). Despite the close relationship between SOM quality and size of the soil C pool, there remains insufficient information about the dynamics of balance between the labile and recalcitrant pools of SOM as plantations age, particularly in Chinese fir plantations. An improved understanding of variations in the SOM quantity and quality with stand age over a traditional rotation period (~25 years) is crucial for developing adequate soil C management strategies.

In the present study, three different development stages (young, middle-aged and mature) were selected to investigate the volume as well as C quality in a set of soil profiles based on similar parent material, climate and slope aspect. We also analysed N in bulk soil and the N distribution in biochemical fractions because of the close interactions of C and N (Luo et al. 2006). Specially, findings from this study are expected to offer insights into (i) whether forest development stages lead to different amounts of SOM or different SOM quality; (ii) whether the biochemical quality of SOM is affected by soil depth (topsoil vs. subsoil) and (iii) what the role of biochemical mechanism is in sequestering soil C in Chinese fir plantations.

## 2 Materials and methods

### 2.1 Site description

The experimental sites were located at the Xiaohu work area of the Xinkou Experimental Forestry Centre of Fujian Agricultural and Forestry University, SanMing, Fujian (Fig. 1). The altitude ranges between 205 and 500 m above sea level. The experimental area is characterized as middle



**Fig. 1** Map of sampling region in China showing the location of the three Chinese fir (*Cunninghamia lanceolata*) plantations of varying ages

subtropical with a monsoonal climate. The mean annual temperature of the area is 19.1 °C, with maximum and minimum means of 28.2 °C (July) and 7.1 °C (January), respectively (Zhou et al. 2015). Mean annual rainfall is approximately 1749 mm, and most rainfall events are concentrated from March to August (Zhang et al. 2005). The mean relative humidity is approximately 81 %.

Three stands of second rotation 6-, 16- and 25-year-old Chinese fir plantations were selected for this study, representing archetypal growth stages (i.e., young, middle-aged, mature) according to the growing properties of Chinese fir. The maximum distance between any two stands was less than 2 km, which guarantees only minor differences in the climate and soil types to better attribute differences in soil organic matter quantity and quality to differences in stand age. The three Chinese fir stands were reforested after clear cutting and slash burning of the first rotation plantation, which was planted in evergreen broadleaf forests. The traditional rotation period is approximately 25 years for Chinese fir plantations (Ma et al. 2007). The middle-aged and mature plantations were thinned by the traditional practice (removal of alternate rows and cutting of the crowns of thinned trees) in 2011 and 2004, respectively.

Six subplots (20 m × 20 m) with southeastern slopes were established in each of the three sample stands, resulting in a total of 18 sample subplots. Buffer zones (4.0-m wide) were included in each direction to avoid edge effect. To minimize the effect of the slope position on soil characteristics, three subplots were selected on a downhill and three subplots were on an upslope position. Soils in all three stands were classified as ferralsol according to the Chinese soil classification, which corresponds to Ultisol in USDA taxonomy (Soil Survey Staff of USDA 1999); this type of soil is derived from siltstone and has a typical soil depth of 100 cm or more. The main

physiographical and textural characteristics of the selected environments are listed in Table 1.

Prior to soil collection, the understory plant communities were also surveyed. The Chinese fir plantation contained a small number of understory species and species richness. The predominant species of the understory were *Litsea cubeba* (Lour.) Pers., *Blechnum orientale* L., *Melastoma dodecandrum* Lour., *Ficus hirta* Vahl. and *Callicarpa kochiana* Makino.

## 2.2 Soil sampling

Soil samples were collected in January 2015. At each subplot, three soil profiles were excavated along a diagonal direction. Approximately 1 kg of soil samples were collected at an interval of 20 cm down to 100 cm (0–20, 20–40, 40–60, 60–80 and 80–100 cm). Soil samples were composited by the same soil depth and were homogenized well; then, approximately 1 kg of soil was placed in clean bags and was air dried at room

**Table 1** Stand characteristics of Chinese fir (*Cunninghamia lanceolata*) stands of varying age in Sanming, China

Stand characteristics	Young-aged	Middle-aged	Mature
Mean elevation(m)	213–246	207–250	215–236
Site index	20	23	23
Aspect	Southeast	Southeast	Southwest
Gradient (°)	33°–45°	25°–37°	28°–32°
Stems density ha <sup>-1</sup>	2267 (68)	1375 (46)	1225 (47)
Mean DBH	7.17 (0.16)	15.52 (0.46)	20.99 (0.34)
Mean height	5.57 (0.08)	12.52 (0.53)	20.62 (0.56)

Numbers in parenthesis indicate standard deviation of the mean DBH diameter at breast height (1.3 m above ground)

temperature for 5 days. In total, 90 composite samples were collected in each stand.

Two core soil samples were also collected to determine soil bulk density (BD) for every 20-cm sample interval using metal soil cores (200 cm<sup>3</sup>).

### 2.3 Basic soil characteristics analysis

Soil pH was determined using a glass electrode (PHSJ-4A, REX Instruments factory) at a soil/water ratio of 1:2.5. Gravimetric water content was determined by weighing core samples after collection; then, samples were dried at a constant weight at 105 °C in an oven. Stones (>2 mm) and large roots were subtracted from the core samples to correct for BD. Soil texture was obtained by the pipette method (Nanjing Institute of Soil Science 1978) after sodium hydroxide (NaOH) treatment to disperse the soil particles. The soil characteristics of the three stands are provided in Table 2.

### 2.4 SOC and N stocks calculations

Subsamples were ground through a 0.15-mm mesh prior to the determination of soil total C and total N, which were determined by combustion with a CN Elemental Analyser (ELEMENTAR VARIO EL III Analyser, Germany). No carbonates were in the soil samples tested by addition of 2 M HCl; so, the soil total C is equal to the organic carbon concentration.

SOC and N stocks were calculated based on equivalent soil mass (ESM) to avoid errors reflecting the different soil masses at a fixed soil depth (Wiesmeier et al. 2014). The mean soil mass of the middle-aged stand was calculated for each layer down to a depth of 1 m, representing the ESM. The difference between the soil mass of each layer of ESM and lighter soil (i.e., young and mature stands) was used to obtain depth corrections based on the following equation:

$$T_{\text{add}} \text{ (cm)} = \sum_i^{h_z} \frac{[M_{\text{EST}} \text{ (Mg ha}^{-1}) - M_i \text{ (Mg ha}^{-1})] \cdot 0.1 \text{ ha m}^{-2}}{BD_i \text{ (Mg m}^{-3})} \quad (1)$$

where  $T_{\text{add}}$  is the additional thickness of all soil horizons  $h_z$  down to a depth of 1 m, which was required for the soil depth corrections.  $M_{\text{EST}}$  is the equivalent soil mass of each layer in the middle-aged stand.  $M_i$  is the soil mass of horizon  $i$  and  $BD$  is the bulk density corresponding to horizon  $i$ , reflecting minor differences in the BD data at 80–100 cm among the three stand ages (Table 2); the 80–100-cm soil layer was not included in the calculations of the depth corrections. The additional thickness was performed at a horizon of 80–100 cm. The SOC or N stocks were calculated as the products of soil bulk density, SOC concentration and the depth correction as follows:

$$\text{Mg SOC ha}^{-1} = \sum_i^{h_z} [\text{SOC}_i \text{ (kg Mg}^{-1}) \cdot \text{BD}_i \text{ (Mg m}^{-3}) \cdot T_{i \text{ corr}} \text{ (m)} \cdot 10^{-3} \text{ Mg kg}^{-1} \cdot 10^4 \text{ ha}^{-1} \text{ m}^2] \quad (2)$$

where  $\text{SOC}_i$  is the concentration of SOC of the horizon  $i$ , and  $T_{i \text{ corr}}$  is the depth correction of horizon  $i$ , calculated as the fixed sample thickness (20 cm) plus the additional thickness

**Table 2** Soil characteristics from each Chinese fir (*Cunninghamia lanceolata*) stand age category to a depth of –1 m mineral soil in Sanming, China

Stand age	Soil depth (cm)	Gravimetric water content (%)	Bulk density (g cm <sup>-3</sup> )	pH (H <sub>2</sub> O)	Soil texture (%)		
					Sand	Silt	Clay
Young-aged	0–20	31.57 (4.01)	0.98 (0.12)	4.44 (0.20)	43.34 (3.78)	20.29 (2.41)	36.37 (5.39)
	20–40	29.03 (2.70)	1.11 (0.08)	4.37 (0.11)	41.76 (5.26)	20.14 (1.01)	38.10 (6.04)
	40–60	28.04 (3.66)	1.17 (0.08)	4.44 (0.08)	39.83 (3.55)	20.12 (2.34)	40.05 (5.60)
	60–80	27.79 (2.95)	1.22 (0.09)	4.41 (0.25)	40.04 (4.99)	19.74 (2.85)	40.22 (7.12)
	80–100	27.08 (2.53)	1.26 (0.07)	4.58 (0.12)	37.97 (3.34)	19.46 (0.99)	40.49 (4.25)
Middle-aged	0–20	29.58 (2.33)	1.21 (0.10)	4.20 (0.31)	31.39 (1.64)	37.73 (1.60)	30.89 (0.84)
	20–40	29.51 (2.53)	1.26 (0.09)	4.22 (0.23)	38.26 (7.70)	30.47 (10.24)	31.27 (5.33)
	40–60	30.26 (5.04)	1.24 (0.20)	4.20 (0.27)	32.23 (2.09)	35.44 (3.05)	32.33 (1.99)
	60–80	32.04 (5.74)	1.27 (0.15)	4.31 (0.17)	24.93 (9.86)	32.76 (2.21)	42.31 (9.02)
	80–100	31.94 (4.75)	1.23 (0.20)	4.24 (0.08)	35.14 (5.93)	31.29 (3.05)	33.57 (3.90)
Mature	0–20	33.55 (3.27)	1.14 (0.09)	4.22 (0.06)	18.84 (7.22)	35.04 (3.53)	46.12 (6.10)
	20–40	32.10 (2.46)	1.20 (0.06)	4.22 (0.07)	12.44 (4.92)	33.90 (2.89)	53.66 (6.78)
	40–60	32.77 (1.70)	1.23 (0.04)	4.24 (0.05)	11.43 (5.00)	30.97 (2.63)	57.59 (7.34)
	60–80	31.33 (2.34)	1.29 (0.04)	4.32 (0.06)	13.23 (5.76)	26.25 (6.69)	60.51 (6.38)
	80–100	31.70 (2.41)	1.28 (0.07)	4.43 (0.16)	13.26 (7.92)	24.95 (10.68)	61.79 (6.83)

Numbers in parenthesis indicate standard deviation of the mean

( $T_{\text{add}}$ ). The soil mass, depth corrections and SOC stocks corrections are shown in Table 3.

## 2.5 Labile and recalcitrant carbon and nitrogen pools

Stepwise chemical digestion with sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was used to evaluate the quality of the soil organic matter (Rovira et al. 2012; von Luetzow et al. 2007). In the present study, soil samples were subjected to a two-step sequence of acid hydrolysis with  $\text{H}_2\text{SO}_4$  to isolate and quantify labile and recalcitrant fractions of the soil organic matter (Belay-Tedla et al. 2009; Rovira and Ramón Vallejo 2007). Briefly, a 500-mg soil was reacted in sealed Pyrex tubes with 20 ml of 2.5 M  $\text{H}_2\text{SO}_4$  at 105 °C for 30 min, after which hydrolysate was recovered by centrifugation. The resultant residue was washed with 20-ml deionized water, and the washes were also recovered by centrifugation. The washes were added to the hydrolysate, which was used to represent the labile pool and was assigned the label 'labile pool I' (LPI). The solid residue, after drying at 60 °C, was hydrolysed for 12 h at room temperature with 2 ml of 13 M  $\text{H}_2\text{SO}_4$  on a reciprocating shaker (200 rpm). After cooling, deionized water was added to dilute the acid to 1 M, and the sample was hydrolysed for 3 h at 105 °C with occasional shaking. The hydrolysate was recovered as in the previous step and was labelled as 'labile pool II' (LP II). The remaining soil was used as the recalcitrant pool.

The organic carbon and total nitrogen in labile pools I and II (LP-C and LP-N, respectively) were measured with a TOC analyser (SHIMADZU TOC-L, Japan). Recalcitrant organic carbon (RC) and nitrogen (RN) pools were estimated as the difference between OC or TN in any given soil sample and the concentration of an element (i.e., SOC or TN) in labile pools (i.e., LPI + LPII). The recalcitrance indexes of C and N ( $RI_C$  and  $RI_N$ ) were calculated using Eq. (3) and Eq. (4) (Rovira and Vallejo 2002) to assess SOM biochemical stability:

$$RI_C = (\text{Recalcitrant C/SOC}) \times 100 \quad (3)$$

$$RI_N = (\text{Recalcitrant N/TN}) \times 100 \quad (4)$$

## 2.6 Statistical and data analysis

Two-way factorial ANOVA was performed to test for significant differences in soil carbon, total nitrogen, pH, bulk

density, labile and recalcitrant pools among stand ages and soil sampling depths. The data were analysed after testing for homogeneity of variance by using Levene's test. When constant variance was not satisfied, a log or square transformation was used. Multiple comparisons of the means were performed using Tukey's HSD test at a significance level of  $\alpha = 0.05$  if the main effect was significant. All statistical analyses were performed with SPSS 17.0. Simple linear fitting between the biochemical SOM fractions and the total carbon in the horizon were performed with Origin Pro 2015.

## 3 Results

### 3.1 SOC and total nitrogen TN concentration

In all three stands, SOC, total N and C/N was significantly different (Table 4;  $p < 0.001$ ), with decreasing mean values at greater soil depths (Fig. 2). The SOC concentration had a mean decrease of 41.41 % from the first (0–20 cm) to the second (20–40 cm) depth interval. Nitrogen concentration followed similar trends, with mean values ranging from  $1.41(\pm 0.22)$  g kg<sup>-1</sup> to  $1.15(\pm 0.20)$  g kg<sup>-1</sup> at a 20–40-cm soil depth in each stand.

The SOC concentration was lowest in the middle-aged stand at 0–20-cm soil depth and was highest in the mature stage. Although the SOC concentration was significantly different among the three stand ages (Table 4;  $p = 0.002$ ), there were no significant differences in the subsoil (below 20 cm) between the young, middle-aged and mature stands. The N concentrations were staggered according to stand age, irrespective of soil depth, with progressively higher total nitrogen from the young stand to the mature stand. The C/N ratio decreased with stand age from the young stand to the middle-aged stand and increased again for mature stand. The C/N ratio of the young stand at 0–20 and 60–80 cm was higher than that of the other two forest ecosystems (Fig. 2c).

### 3.2 SOC and N stocks

SOC stocks in surface soil (0–20 cm) occupied 35, 35 and 36 % of the entire soil carbon stock in the young, middle-aged and mature stands, respectively. Reflecting higher SOC

**Table 3** Soil masses, depth corrections and SOC stocks corrections within a depth of 1 m in young-aged and mature stands according to the equivalent soil masses (ESMs) of middle-aged stand

Stand age	Soil mass down to 1 m depth (Mg ha <sup>-1</sup> )	ESM middle-aged plantation at 1-m depth (Mg ha <sup>-1</sup> )	Depth corrections (cm)	SOC stocks correction (Mg ha <sup>-1</sup> )	N stocks correction (Mg ha <sup>-1</sup> )
Young-aged	112.8 (4.60)	124.22 (9.39)	11.72 (3.40)	13.49 (4.06)	1.55 (0.43)
Mature	121.89 (4.23)		2.86 (2.81)	4.10 (4.78)	0.53 (0.57)

Numbers in parenthesis indicate standard deviation of the mean

**Table 4** Results of two-way ANOVA on effects of stand age and soil depth on soil characteristics in Chinese fir (*Cunninghamia lanceolata*) stands of different ages in Sanming, China

Soil characteristic	Stand age		Soil depth		Stand age × soil depth	
	<i>F</i>	<i>P</i> value	<i>F</i>	<i>P</i> value	<i>F</i>	<i>P</i> value
SOC	<b>6.721</b>	<b>0.002</b>	<b>80.859</b>	<b>&lt;0.001</b>	0.594	0.780
TN	<b>51.607</b>	<b>&lt;0.001</b>	<b>42.258</b>	<b>&lt;0.001</b>	0.780	0.621
C:N	<b>30.458</b>	<b>&lt;0.001</b>	<b>61.004</b>	<b>&lt;0.001</b>	0.414	0.909
LPI-C	1.434	0.245	<b>34.540</b>	<b>&lt;0.001</b>	1.102	0.372
LPI-N	<b>12.367</b>	<b>&lt;0.001</b>	<b>20.157</b>	<b>&lt;0.001</b>	0.774	0.626
LPII-C	<b>9.549</b>	<b>&lt;0.001</b>	<b>12.311</b>	<b>&lt;0.001</b>	0.237	0.983
LPII-N	<b>8.176</b>	<b>0.001</b>	<b>10.525</b>	<b>&lt;0.001</b>	1.663	0.122
RC	<b>8.18</b>	<b>0.001</b>	<b>37.039</b>	<b>&lt;0.001</b>	0.585	0.787
RN	<b>64.031</b>	<b>&lt;0.001</b>	<b>2.738</b>	<b>0.035</b>	1.181	0.322
RI <sub>C</sub>	<b>4.881</b>	<b>0.01</b>	<b>3.317</b>	<b>0.015</b>	0.641	0.740
RI <sub>N</sub>	<b>33.272</b>	<b>&lt;0.001</b>	<b>12.574</b>	<b>&lt;0.001</b>	0.832	0.577

Bold numbers indicate significant differences among stands or soil depth ( $P = 0.05$ )

accumulation at the 0–20-cm depth in the mature compared with middle-aged stands, the SOC pool of the mineral soil (0–100 cm) in the mature stand was accordingly higher than that in the middle-aged stand (117.60 vs. 91.98 Mg SOC ha<sup>-1</sup>; Fig. 2a). Statistical analysis of the SOC at each soil depth category showed that at the 0–20-cm depth, the SOC was significantly different when averaged across ages ( $p < 0.05$ ). No other significant differences in SOC were detected for each soil depth below 20 cm.

N accumulation in surface soil is smaller than SOC accumulation with a mean distribution of 23 % (Fig. 2b). Total soil N stocks were significantly different by stand age ( $p < 0.001$ ). Total soil N stocks changed along the chronosequence, from a mean of  $14.39 \pm 1.48$  Mg ha<sup>-1</sup> in the young stand to a mean of  $17.55 \pm 1.66$  Mg ha<sup>-1</sup> in the middle-aged stand and a mean of  $19.18 \pm 1.02$  Mg ha<sup>-1</sup> in the mature stand (Fig. 2b).

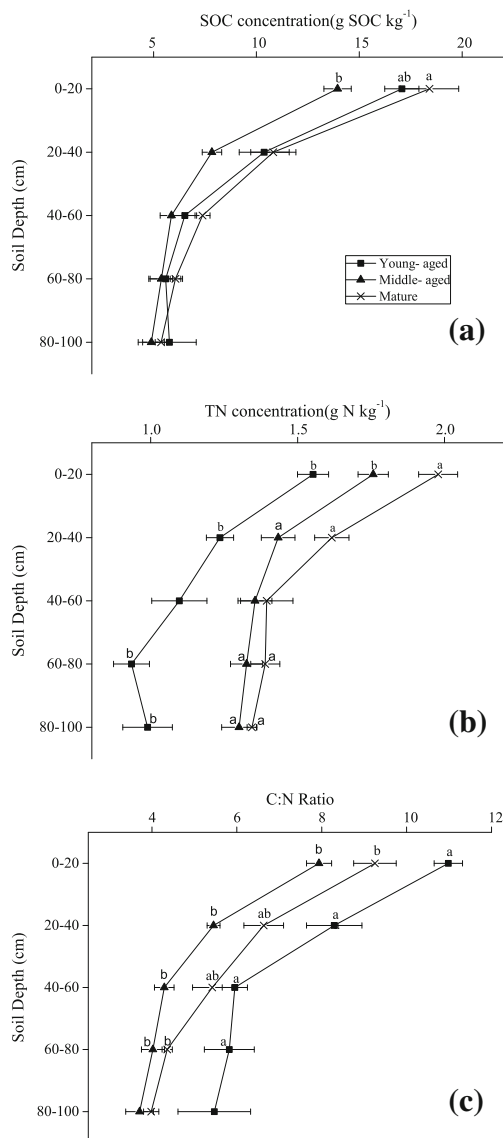
### 3.3 Labile and recalcitrant C and N pools

Recalcitrant indexes were used to determine the quality or stability of SOM. As mentioned previously, total C and N were affected by soil depth (Table 4), and the labile C and N also decreased progressively with the increasing depth (Fig. 4a–d). The change of recalcitrant C with depth was similar to the labile C pool in all three forest stands. The highest recalcitrant C concentration was in topsoil and then decreased through the soil horizons (Fig. 4e, g). The negative relationship between the recalcitrant N pool and the total N in the soil profile of all the stands showed that N stability increased sharply with soil depth (Fig. 4f, h).

Labile pool I C (LPI-C): the total organic carbon (OC) ratio was affected by stand age, accounting for 43.76 % of the total OC in the young stand, 57.65 % in the middle-aged stand and 41.33 % in the mature stand (Table 5). There was a clear

relationship between LPI-C and total OC in the horizons; however, LPI-C did not show significant differences between the stand ages (Table 5). The C in LPII contributed just 12–18 % of the total OC in the three stands. Data dispersion among the stands was larger than LPI-C, which means that measurable differences occurred among the forest ages for LPII-C. The mature Chinese fir stand had the highest C concentration in the LPII (Table 5). Based on multiple comparisons, the C concentration in LPII-C declined from the young stand to the middle-aged stand and then increased in the mature stand. A similar, non-significant trend was observed in recalcitrant C among the three stands. It is noteworthy that the recalcitrant C index (RI<sub>C</sub>) was different with respect to stand age ( $p = 0.01$ ). The recalcitrant C index in the middle-aged plantation was significantly lower than that in the young and mature stands, but it was similar in young and mature plantations.

As labile C was allocated more in the LPI, N was allocated in the LPI at an average of 37 % which was more than in LPII, but the two labile N pools appeared to be different depending on the growth stages of the Chinese fir (Fig. 4b, d). There was a constant decrease in the N concentration in LPI along with the chronosequence, which was not significant between the young and middle-aged stands (Table 5). The proportion of LPI-N to the total N pool appeared to decrease with progressive stand age. The N concentration of the LPII fraction was relatively consistent among the three stand ages. The degree of correlation between the total N proportion and the recalcitrant N concentration was very low as a result of high variability in the data (Fig. 4f), but on average, recalcitrant N concentration appeared to gradually increase along the different stages of Chinese fir plantation ages, with 0.36 mg g<sup>-1</sup> in the young stand to 0.72 mg g<sup>-1</sup> in the middle-aged stand and 0.95 mg g<sup>-1</sup> in the mature stand (Table 5). The non-hydrolysable N proportion showed a higher correlation with the total N in the



**Fig. 2** Soil organic carbon (SOC). **a** Total nitrogen (TN) **b** and carbon:nitrogen (C/N) ratio **c** profile distribution in Chinese fir (*C. lanceolata*) stands from three different age categories in SanMing, China. Error bars indicate standard deviation of the mean. Different lowercase letters show significant differences among stands in the same soil depth at  $p = 0.05$

horizon (Fig. 4h) and showed a consecutive increase in the recalcitrant index N ( $RI_N$ ) from the young to mature plantation ages but a non-significant difference between the middle-aged and mature stands (Table 5).

## 4 Discussion

### 4.1 SOC and N stocks

Wang et al. (2013) measured SOC storage of  $55.99 \text{ Mg ha}^{-1}$  at a depth of 0–40 cm, consistent with the results of the present study (uncorrected). However, the findings of the present

study indicated that the SOC storage (1 m depth) in the different stands was 29, 34 and 37 % higher, respectively, than those reported by Chen et al. (2013) for 7-, 16- and 21-year-old stands. For Chinese fir in limestone soil developed from dolomite, the SOC stocks at 1-m depths (Lan et al. 2016) were higher than those in the abovementioned studies. Thus, even for the same forest type, the capacity of SOC stocks is complicated to predict and depends on many factors, such as soil types, climate zones, management practices and stand origins (Paul et al. 2002). The average SOC storage of Chinese fir plantations within the upper 1 m as previously reported in published data ( $68.83\text{--}157.29 \text{ Mg ha}^{-1}$ ) was generally lower than the average value of  $193.55 \text{ Mg ha}^{-1}$  for the forest ecosystem in China (Zhou et al. 2000).

The magnitude of the SOC pool with plantation age did not show a consistent trend in short-rotation and intensely managed plantations (Sartori et al. 2007; Wellock et al. 2014; O'Neill et al. 2006; Peichl and Arain 2006). In this present study, we observed that the mean SOC storage at 1 m slightly decreased from the young to the middle-aged stands (up to ~8 % in 1-m soil depth) and a higher level was observed for the mature stage. This trend was consistent with the studies of differently aged Chinese fir plantations in two other provinces of China (Chen et al. 2013; Lan et al. 2016). We considered that low decomposition and reduced soil disturbance after the canopy closed and a high litter mass might contribute to the subsequent increase in SOC stocks in the mature stand (Mao et al. 2010; Cheng et al. 2015). The observed carbon loss from young to middle-aged stands might be attributed to the soil disturbance and erosion in intense management regimes for Chinese fir plantations (Zhang et al. 2004), such as site preparation, burning and clear cutting. In the early stage of Chinese fir plantations, the SOC protected through the soil aggregates and mineral becomes vulnerable to mineralization and transportation, and this effect can be enhanced in southern production regions, where steep slope, high temperature and high precipitation are common (Chen et al. 2013). The observed changes in soil C/N ratio among the three stand ages were consistent with the changes in SOC stocks, suggesting that the soil C/N ratio might be an indicator for changes in the SOC stocks (Mao et al. 2010). The steady increase in the C/N ratio in the soil profiles showed that the degradation of SOM would likely be inhibited after the stand reached middle age (Mujuru et al. 2014); the observed trend of increasing C sequestration after the middle-aged stage supports this (Fig. 3a).

Moreover, we observed significant SOC accumulation in surface soil, suggesting an effect of stand age on SOC stocks in the entire 1-m soil profile and negligible changes in the SOC pool below 20 cm across stand ages. Notably, a greater carbon loss (~20 % loss) was observed in the middle-aged stand compared with the young stands, mostly from the subsoil (Chen et al. 2013), in contrast to the results of the present

**Table 5** Biochemical fractions of C and N pools (mean ± 1SE): (a) C and N concentration in labile pool I and a proportion of total OC and N in labile pool I; (b) C and N concentration in labile pool II and a proportion of total OC and N in labile pool II; (c) C and N concentration and a proportion of total OC and N in recalcitrant pool

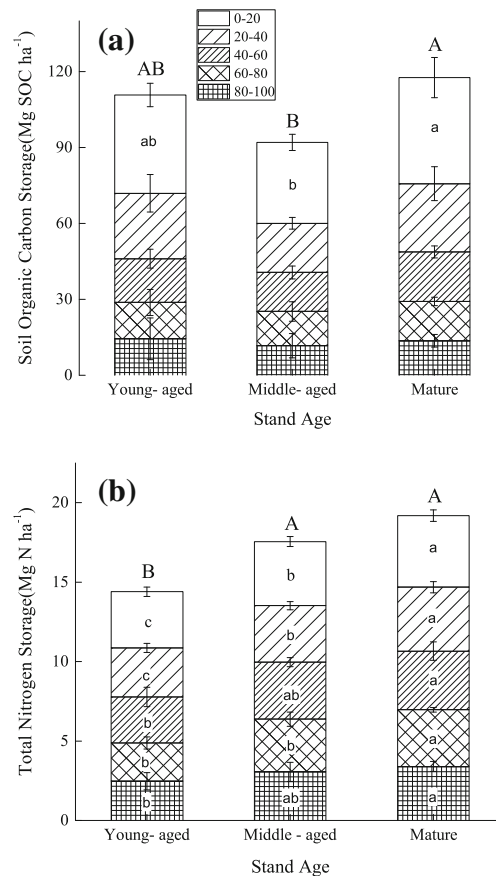
Fractions	Young	Middle-aged	Mature
<b>a. Labile pool I (LPI)</b>			
LPI-C (mg g <sup>-1</sup> )	3.78 (0.41)	4.07 (0.26)	3.80 (0.36)
LPI-N (mg g <sup>-1</sup> )	0.62 (0.05)a	0.51 (0.05)ab	0.37 (0.04)b
LPI-C (%)	43.76 (2.78)b	57.65 (2.67)a	41.33 (2.39)b
LPI-N (%)	52.17 (2.68)a	35.33 (3.37)b	23.56 (1.83)c
<b>b. Labile pool II (LPII)</b>			
LPII-C (mg g <sup>-1</sup> )	1.54 (0.22)ab	0.90 (0.10)b	1.72 (0.17)a
LPII-N (mg g <sup>-1</sup> )	0.17 (0.02)b	0.20 (0.03)ab	0.29 (0.03)a
LPII-C (%)	16.89 (2.12)ab	12.15 (1.28)b	18.93 (1.64)a
LPII-N (%)	14.53 (1.48)	14.11 (1.66)	18.34 (1.96)
<b>c. Recalcitrant pool</b>			
Recalcitrant C (mg g <sup>-1</sup> )	3.67 (0.45)	2.62 (0.41)	4.10 (0.57)
Recalcitrant N (mg g <sup>-1</sup> )	0.36 (0.03)c	0.72 (0.05)b	0.95 (0.04)a
Recalcitrant index of C (%)	38.89 (2.63)a	30.20 (2.51)b	39.84 (2.32)a
Recalcitrant index of N (%)	32.51 (3.08)b	50.57 (3.33)a	60.05 (2.48)a

Mean value of all five soil depths in each stand is shown in the table. Numbers in parenthesis indicate standard error of the mean. Different lowercase letters in the same row indicated significant difference among three stands by Tukey’s HSD test at *P* = 0.05 level. Only significant difference is shown by letters in the table

study. The higher soil carbon storage and low soil carbon loss observed in the present study might be ascribed to the relatively high clay and silt content, which created a stabilizing capacity for C with a high surface area (Wiseman and Püttmann 2006), particularly in deeper soil horizons (Paul et al. 2002). Study sites in Nanping has a soil clay content of approximately 36.08 % (Yang et al. 2001; Chen et al. 2013), which is much less than the soil contents in the plots of Sanming (Table 2). Unfortunately, there is little experimental evidence directly linking soil texture to differences in C stocks.

In this study, the size of the N pool follows the increased age grades, which is consistent with some of the previous studies (Dong et al. 2014; Uri et al. 2014). It has been suggested that over the short term, forest ecosystems have an intrinsic ability to maintain a growing demand for N via self-regulation of the C/N ratio in the soil or litter (Luo et al. 2006) and a long-term ability to accumulate substantial N in various ecosystem compartments (Yang et al. 2011). Some studies (Dijkstra et al. 2008; Phillips et al. 2011) have found significant interactions between the soil N capital and the soil C dynamics, acting through competition between plant roots and microbes. According to this, although long-term soil C sequestration can be maintained after an increase of the N input into an ecosystem, the limitation of N would be expected to accelerate soil C mineralization. The notable increase of soil N stocks observed in the young versus mature stands in this study suggests that N depletion was not an issue during stand development or that N input exceeded the demand of the existing Chinese fir biomass for growth.

The fixed depth method is widely employed in calculating carbon or nutrient storage in soil. However, this method generates systematic errors (Wendt and Hauser 2013) leading to



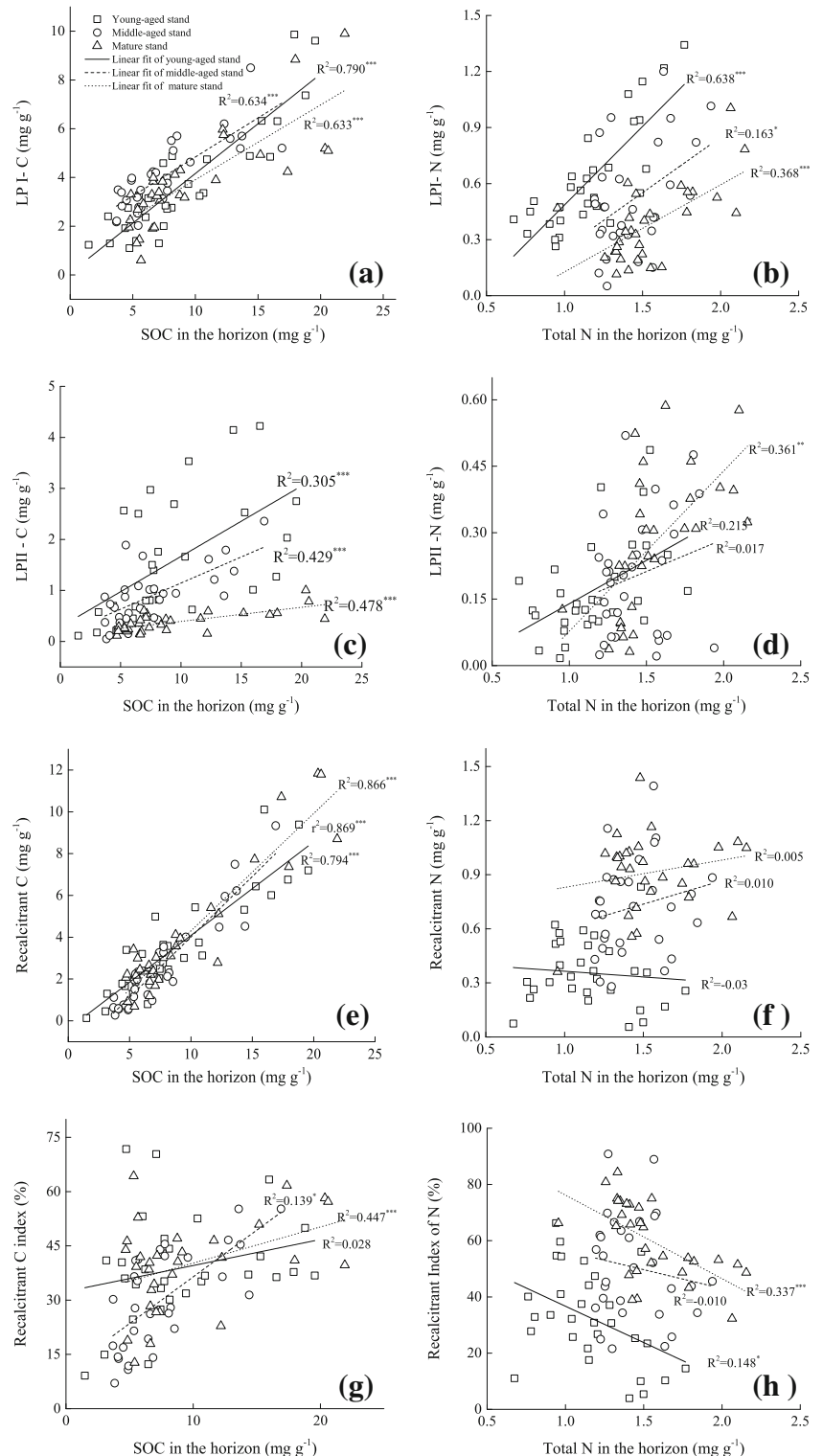
**Fig. 3** Soil organic carbon (a) and nitrogen (b) storage in each soil depth for three Chinese fir plantation development stages based on equivalent soil mass approach. Error bars indicate standard deviation of the mean. Different lowercase letters show significant differences among stands in the same soil depth at *p* = 0.05, different uppercase letters mark significant differences in the C or N profile totals (1 m mineral soil layer) among the three stands at *p* = 0.05. Only significant difference was shown by letters in figures



underestimations of the SOC stocks and false conclusions when comparing study sites with different soil bulk density (Mao et al. 2010; Wendt and Hauser 2013). Based on an ESM approach, the corrected amount of SOC and N stocks in the young and mature stands were 13.49, 4.10 and 1.55,

0.55 Mg ha<sup>-1</sup> (Table 3). Thus, to more accurately quantify the effects of stand age on SOC and N stocks and to evaluate the carbon budget of the forest, the worldwide use of a standard procedure (e.g., sampling method, measurement and calculation method) is needed.

**Fig. 4** Relationship between **a** total C concentration in labile pool I and SOC concentration in soil horizon; **b** N concentration in labile pool I and total N concentration in soil horizon; **c** total C concentration in labile pool II and SOC concentration in soil horizon; **d** N concentration in labile pool II and total N in soil horizon; **e** total C concentration in recalcitrant pool and SOC concentration in soil horizon; **f** N concentration in recalcitrant pool and total N in soil horizon; **g** recalcitrant index of carbon and SOC concentration in soil horizon; **h** recalcitrant index of nitrogen and N concentration in soil horizon. Each stand of a different age is fitted with linear regression. Soil depths were combined according to plantation age category. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$



## 4.2 Biochemical quality of SOM

The recalcitrant index of carbon and nitrogen was calculated to represent soil organic matter quality. At present, there is limited literature about the change in the biochemical quality of soil organic matter among plantations chronosequences. Close relationships between the SOC concentration and labile C and recalcitrant C (Fig. 4a, c, e) indicate that the biochemical fractions of SOC could well reflect the soil C concentration in each of the three stand ages. Gains in the C of the labile fractions (LPI + LPII) proportions and the downregulated recalcitrant index of C were observed over the entire soil profile in the middle-aged plantation; this denotes that C in the middle-aged stands is possibly more bioavailable, relative to the young or mature stands. Presumably, this might be explained by the low mineralization rate in the middle-aged stand as a consequence of a low microbial biomass carbon (Zhang et al. 2005). In general, low mineralization rates limit the degradation of labile C; hence, the relative distribution between labile C and recalcitrant C fractions would be changed (Shrestha et al. 2008). Moreover, artificial thinning in the middle-aged stands gave rise to an increased richness in understory species (Lin et al. 2001), which may induce the change of SOM composition through a large quantity of labile debris input to the soil (Ma et al. 2014). At the end of a plantation's rotation, in a mature stand, the recalcitrant index of C was significantly enhanced; in contrast to the middle-aged stand, the recalcitrant C fraction was similar to the levels in the young stand. Pérez-Cruzado et al. (2014) found that more stable soil organic substances accumulated in more advanced or mature stages of *Pinus radiata* plantations and that soil C recovered at the end of a rotation, compensating for the initial afforestation C losses.

For a long time, it was thought that subsoils have higher radiocarbon ages,  $^{13}\text{C}$  enrichment and microbial-derived carbon, which suggests that C in the surface soil is less recalcitrant than in deep soils. However, our findings show the decrease in the recalcitrant index of C for the depths in all three forest stands (Fig. 4g). Rumpel and Kögel-Knabner (2011) believe that the chemical composition of SOM depends on the specific soil type and that some environmental factors may limit deep SOM decomposition. Tan et al. (2004) and Rovira and Ramón Vallejo (2007) also found that  $\text{RI}_\text{C}$  was reduced from H- to B-horizons and revealed that SOM in the surface soil was biochemically resistant to microbial consumption. These phenomena may be related to fine root distribution patterns, labile C vertical mobility in the soil profile and non-hydrolysable lignin degradation in deep soils; these possible reasons have been detailed in Mediterranean forest soils (Rovira and Ramón Vallejo 2007). In addition, charcoals from slash burning that accumulated in superficial soils were observed in our plots, possibly related to increased recalcitrance (Rumpel and Kögel-Knabner 2011).

In our study, the recalcitrant index of N ( $\text{RI}_\text{N}$ ) tended to increase sharply with soil depth (Fig. 4f). A reasonable explanation for this trend may be the accumulated ammonium associated with clay particles at greater soil depths and the associated release of such complexes via acid hydrolysis that is difficult (Greenfield 1992).

## 5 Conclusions

The mean SOC storage at a 1-m depth slightly decreased from the young to middle-aged stands, with up to a maximum of  $117.60 \text{ Mg C ha}^{-1}$  for the mature stand; the SOC accumulation of surface soil predominated the changes in total SOC stocks with stand development. The N stocks increased with increasing stand age, and the increment of the N stocks was derived from the entire depth. The SOC that reaccumulated from the middle-aged stand to the mature stands might offset or at least mitigate the initial C loss resulting from the intense management practices on Chinese fir plantations.

At the end of the rotation, the proportion of C in the acid non-hydrolysable fraction recovered to the level of the young stage. Accumulated biochemical stable compounds from woody litter and the associated roots may lead to a reaccumulation of SOC at the late development stage of Chinese fir plantations. Differences in the recalcitrant index of C across the stand ages were consistent with the changes in soil C stocks. The closed linear relationship between the concentration of non-hydrolysable C and bulk soil C concentration indicated that the biochemical protection may be an important mechanism to sequester soil carbon in these stands. Finally, the decrease of recalcitrant C with increasing soil depth suggested that subsoil C was unstable relative to the topsoil, which implies that the protection of subsoil C should be considered in the management of Chinese fir plantations.

The results presented in this study showed a significant effect of the stand age on the storage and biochemical availability of SOM in *C. lanceolata* plantations of southern China. Nevertheless, to accurately assess the SOC sequestration of Chinese fir plantations, it is critical to further investigate the C dynamics (quantity and quality of C) in multiple age sequences, large-scale sites and successive generations over long-term periods.

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