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ORIGINAL PAPER

Changes in soil organic carbon and aggregate stability following a chronosequence of *Liriodendron chinense* plantations

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Abstract The objectives for this study were to determine changes in soil organic carbon (SOC) components and water-stable aggregates for soil profiles from different ages of plantations of *Liriodendron chinense* and to clarify which organic carbon component is more closely associated with the formation and stability of soil aggregates. Three layers of soil (depths 0–20 cm, 20–40 cm, 40–60 cm) were collected from young, half-mature and mature stages of *L. chinense*. SOC, readily oxidizable organic carbon, chemically stable organic carbon and aggregate composition were determined. Intermediate stable organic carbon, the microbial quotient and aggregate stability (mean weight diameter) were calculated. SOC and aggregate stability in the *L. chinense* plantation did not increase linearly with an increase in *L. chinense* age; rather, they first decreased, then increased with increasing age of *L. chinense*. The microbial quotient had a negative effect on the level of organic carbon and the stability of aggregates, while chemically stable organic carbon had a positive effect, which explained 55.0% and 19.3% of the total variation, respectively ($P < 0.01$). Therefore, more attention should be paid of these two indicators in the future.

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Introduction

In the natural ecosystem, *Liriodendron chinense* (Magnoliaceae) is an endangered deciduous tree that grows naturally in small populations scattered among montane, broad-leaved forests of southern China and northern Vietnam (Li et al. 2016; Yang et al. 2014; Yao et al. 2008). Due to its fast growth, large biomass and high wood quality, it is a high-grade, valuable decorative wood (Zhang et al. 2011). It also contains a variety of biologically active ingredients that can be developed into value-added medicines and spices (Zhang et al. 2011). Because it can remove particulate matter and significantly improve air quality and is aesthetic pleasing, it is often used for urban greening (Wu et al. 2018b). Thus, its market demand is very large, and many forest farms in China have been planting *L. chinense* on a large scale since the 1980s.

Long-term plantation of a single tree species, however, may affect soil fertility and quality (Lemenih et al. 2004). The stability of soil aggregates, the basic units of soil structure, is a key property for many soil ecological processes and functions such as water-holding capacity, root penetration, organic carbon turnover and soil respiration (Wang et al. 2019; Winstone et al. 2019; Yang et al. 2019). Moreover, as biogeochemical reactors, soil aggregates are the key factors in soil fertility and quality (Mueller et al. 2013; Wang et al. 2018). Soil organic carbon (SOC) is also critical for regulating various physical, chemical, and biological processes in soils. Soil aggregation processes in turn play a crucial role in protecting SOC to sustain soil fertility and quality (Qian et al. 2018). In addition, SOC is the major binding agent on aggregate hierarchy (Six et al. 2000). Therefore, understanding the reaction between organic carbon and aggregates and their interrelationships is critical to defining soil fertility and quality. However, in previous studies, scientists have paid more attention to the relationship between aggregate stability and unstable carbon components, such as fulvic acid and labile organic carbon and so on (Li et al. 2015; Pollakova et al. 2018). The stability of aggregates is more strongly correlated with labile carbon (Wang et al. 2018), and soil aggregation is mainly affected by labile organic carbon (Dai et al. 2019). Less attention was paid to the relationship between stable organic carbon components and aggregate stability. However, unstable carbon pools are more sensitive to environmental changes and have larger fluctuations and variations than stable carbon pools do (Kuzyakov et al. 2019; Mi et al. 2016), which may not be as good as stable organic carbon at showing the temporal variation of plantations over a long period of time. Therefore, we predicted that the stable organic carbon components have a stronger correlation with the aggregate stability than the unstable organic carbon components.

In addition, SOC is influenced by a range of biotic and abiotic factors including climate, topography, soil properties and vegetation management, and other anthropogenic conditions (Tan et al. 2004), that often interact and regulate carbon inputs to and losses from the soil (Boca et al. 2014). The input of SOC mainly depends on the input of organic residues such as forest litter and roots, and the input of root exudates. Root biomass and litter increase with the increase of forest age (Chen et al. 2013). Therefore, the organic carbon content is expected to increase with the increase of plantation age. Due to the interdependence between organic carbon and aggregates, the aggregates will change correspondingly.

The aboveground litter and root system of a forest are distributed differently in different soil depths. The topsoil contains litter such as fallen leaves, which are more easily degraded. In general, the fine root biomass and root exudation decreases with increasing depth (Pei et al. 2018; Tuckmantel et al. 2017). In addition, as the soil depth increases, the oxygen content decreases, which is more conducive to the accumulation of stable organic carbon (less easily oxidized). Therefore, we predicted that as the soil depth increases, the organic carbon content will decline, the easily oxidized organic carbon may decrease, and the stable organic carbon may increase.

In addition, soil microbial activity differs at different soil depths and different forest ages, which thus the level of soil respiration differs, which in turn affects carbon output processes in the soil (Yu et al. 2014), and thus the formation and stability of SOC and soil aggregates (Heimann and Reichstein 2008; Luo et al. 2017; Tamura et al. 2017). Therefore, soil microbial activity may be an important index to study the changes in SOC and soil aggregates in different soil depths of *L. chinense* cultivated for a long time.

The objectives of this study were (1) to examine whether SOC and aggregate stability increase with the increase of *L. chinense* ages; (2) to determine whether SOC and aggregate stability decrease with increasing soil depths; and (3) to confirm whether there is a strong positive correlation between stable organic carbon and aggregate stability.

Materials and methods

Study site and experimental design

This study was conducted at Yangkou forest farm in Nanping City, Fujian Province, China (26°49′04″ N, 117°54′21″ E). The area has a typical subtropical monsoon climate with a mean annual temperature of 18.5 °C and relative humidity of 82%. The mean annual rainfall is 1669 mm (mainly occurred from March to August). The soil is classified as mountain red soil developed from weathering of the granite parent material according to the Chinese soil classification and as a Ferralsol by the FAO (Food and Agriculture Organization) classification (IUSS Working Group WRB 2015). The basic physical and chemical properties of the surface soil (0–20 cm) as tested using the methods of Lu (2000) were bulk density 1.16 g cm^{-3}, soil organic matter 26.12 g kg^{-1}, total nitrogen 1.62 g kg^{-1}, hydrolytic nitrogen 65.15 mg kg^{-1}, available phosphorus 1.03 mg kg^{-1}, available potassium 109.23 mg kg^{-1}, and pH 4.85.

Since the 1980s, natural forests have been continuously felled and planted with *L. chinense*, gradually forming an age gradient. In 2016, we selected a chronosequence of three stages of forest development: 5-year-old stands (young forest), 13-year-old stands (half-mature forest), and 31-year-old stands (mature forest). Three 400 m^2 plots (20 × 20 m) were established at each stand in December 2016, and thus nine sample areas were established for this study (Chen et al. 2013; Wei et al. 2014). The stand characteristics of the different age groups of *L. chinense* plantations are shown in Table 1.

Soil sampling and analysis

The soil profile was excavated in a typical section of the sample area. At depths of 0–20 cm, 20–40 cm and 40–60 cm, about 1 kg of undisturbed soil blocks were taken for aggregate analysis on December 28, 2016. In addition, about 200 g fresh soil samples were taken and stored in ice bags for transport back to the laboratory for microbial biomass carbon (MBC) analysis. The aggregates samples were gently broken apart along natural break points and put it into a hard plastic lunch box to avoid extrusion deformation and brought

Table 1 Stand characteristics of the different age groups of *Liriodendron chinense* plantations

Age group	Age (years)	Tree height (m)	Diameter at breast height (cm)	Treedensity (stem ha^{-1})	Canopy cover (%)	Slope (°)
Young forest	5	6	7	1111	70	27
Half-mature forest	13	12	15	278	85	28
Mature forest	31	27	38	123	85	26

back to the laboratory. In the laboratory, the samples were properly air-dried and gently smashed into small pieces of 1–2 cm along natural sections and passed through an 8-mm sieve. Stone, roots and other debris in the soil samples were carefully removed by hand. After thorough mixing, 100 g was taken for aggregate samples sieving, and the other part was used to analyze the soil properties (Wu et al. 2018a; Yu et al. 2012a).

Aggregate distribution and stability were determined using wet-sieving methods (Elliott 1986; Yu et al. 2012b; Zhang et al. 2017): 2 mm, 0.25 mm and 0.053 mm mesh sieves from top to bottom; 100 g of the soil sample was placed on the top sieve and immersed in deionized water for 5 min, then the sieves were mechanically moved up and down 50 times for 2 min, with a moving distance was 3 cm. Thus, the soil samples were divided into four classes of aggregates: (1) large macroaggregates (> 2 mm), (2) small macroaggregates (2–0.25 mm). (3) microaggregates (0.25–0.053 mm), (4) silt + clay particles (< 0.053 mm). After separation, the fractions of the aggregates were dried at 50 °C and then weighed.

Soil water stability was determined by the mean weight diameter of aggregates (*MWD*) which was calculated using the following equation (Karami et al. 2012; Zhang et al. 2014):

$$MWD = \sum_{i=1}^{n} \bar{X}_i W_i \quad (1)$$

where $\bar{X}_i$ is the mean diameter of aggregates for each sieve size, W_i is the weight of the aggregates in that size range as a fraction of total wet sieve weight of the sample analyzed, and n is the number of sieves.

The SOC concentration was determined by chemical oxidation using $K_2Cr_2O_7$ solution (Lu 2000; Walkley and Black 1934; Wu et al. 2018a). MBC was measured using the fumigation-extraction method (Li et al. 2013; Mi et al. 2018; Vance et al. 1987). The soil readily oxidizable organic carbon (ROC) was analyzed using 333 mmol L^{-1} potassium permanganate ($KMnO_4$) oxidation (Chen et al. 2017). Chemically stable organic carbon (CSOC) refers to organic carbon that has not been removed by $Na_2S_2O_8$, based on methodology adapted and modified from Eusterhues et al. (2003). Briefly, after the soil passed the 0.25 mm sieve, 0.5 g soil samples and 20 g $Na_2S_2O_8$ were weighed and dissolved in 250 mL deionized water, buffered with 22 g $NaHCO_3$ for 48 h at 80 °C. Using the suction filter to make the filtrate pass through a 0.45 μm membrane, repeatedly washing to remove excess $Na_2S_2O_8$, freeze-dried and analyzed for the remaining organic carbon content. Intermediate stable organic carbon (ISOC) is defined as the portion of organic carbon that is more stable than ROC and more active than CSOC. It was calculated as ISOC = SOC – ROC – CSOC. Note in this equation that we do not consider the influence of other relatively small carbon pools, such as MBC or dissolved organic carbon, but only focus on ROC and CSOC and the third carbon pool, which is neither of them. Moreover, the microbial quotient (MQ) was used as an indicator of soil microbial activity, which was calculated as the ratio of MBC to SOC (Haynes 1999; Kaschuk et al. 2010).

Statistical analysis

IBM SPSS Statistics 20 (IBM, Armonk, NY, USA) was used for all analyses except the redundancy analysis (RDA). One-way analysis of variance (ANOVA) was used to analyze the effects of different forest ages on different soil depths, and a least significant difference test (LSD) was used to compare means at $P < 0.05$. RDA of SOC components and aggregate size classes was done using CANOCO software 5.0 (Biometrics, Netherlands).

Results

The distribution of water-stable aggregates

The composition of soil aggregates in the half-mature forest was significantly different from that in the young and the mature forests (Fig. 1). Small aggregates (2–0.25 mm) and microaggregates (0.25–0.053 mm) of half-mature forest accounted for the largest two proportions, accounting for 47.33%–56.03% and 23.94%–34.53% of the total soil mass, respectively. However, small aggregates (2–0.25 mm) and large aggregates (> 2 mm) accounted for the two largest proportions of young and mature forests. With increasing age of the trees (from 5 to 31 years), the content of large aggregates (> 2 mm) in the soil decreased significantly and then

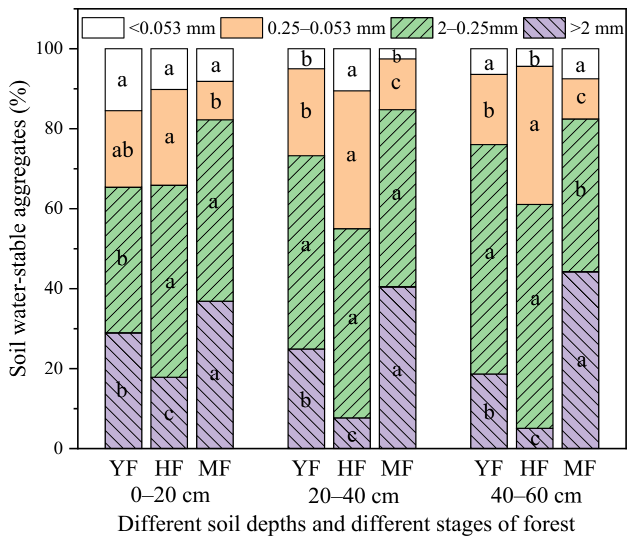

Fig. 1 Distribution of soil water-stable aggregates in different soil depths of different ages of *Liriodendron chinense* plantations. For the same soil depth and aggregate fraction, different lowercase letters indicate significant differences between different forest ages at $P<0.05$. *YF* young forest, *HF* half-mature forest, *MF* mature forest, >2 mm: large macroaggregates, 2–0.25 mm: small macroaggregates, 0.25–0.053 mm: microaggregates; <0.053 mm: silt+clay particles

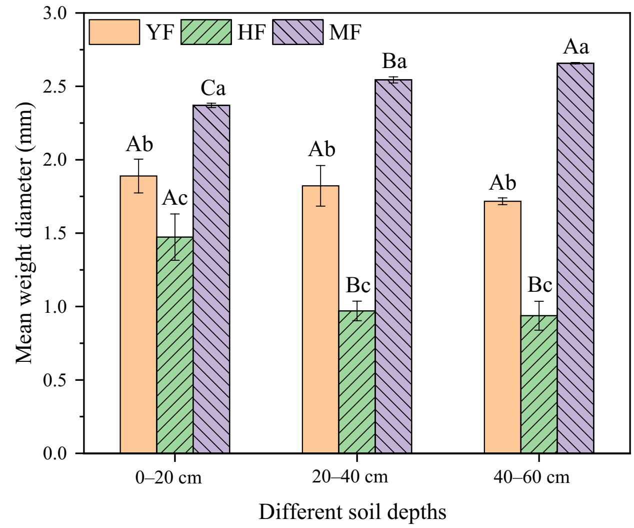

Fig. 2 Mean weight diameter (MWD) in different soil depths of different ages of *Liriodendron chinense* plantations. Different uppercase letters indicate significant differences in MWD between different soil depths in the same forest age at $P<0.05$. Different lowercase letters indicate significant differences between different forest ages in the same soil depth at $P<0.05$. *YF* young forest; *HF* half-mature forest; *MF* mature forest

increased significantly. In young and half-mature forests, the content of large aggregates (>2 mm) in the soil tended to decrease with increasing soil depth. In contrast, the content of large aggregates (>2 mm) in the mature forest soil tended to increase with increasing soil depth.

Stability of water-stable aggregates

The MWD is used to characterize the water stability of soil aggregates. The greater the value of MWD, the stronger the stability of the soil aggregates. Regardless of the soil depth, MWD always decreased significantly first and then increased significantly with increasing tree age (Fig. 2). In young forests, there was no significant difference in MWD among the three soil depths. In half-mature forests, the MWD of 0–20 cm was the largest and was significantly higher than in the 20–40 cm and 40–60 cm layers. In mature forests, MWD significantly increased with increasing soil depth.

SOC components and microbial quotient

The SOC content in soils of different tree ages decreased with increasing soil depth (Fig. 3) and first decreased significantly and then increased significantly with increasing tree ages. Among the three different carbon components of SOC, ISOC accounted for the largest proportion of SOC, followed by ROC, and CSOC accounted for the smallest proportion. At the 0–20 cm soil depth, there was no significant difference in CSOC among the three forest ages. ISOC of the half-mature forest was significantly lower (about 25.12%) than in the young forest at 0–20 cm. Moreover, ISOC of

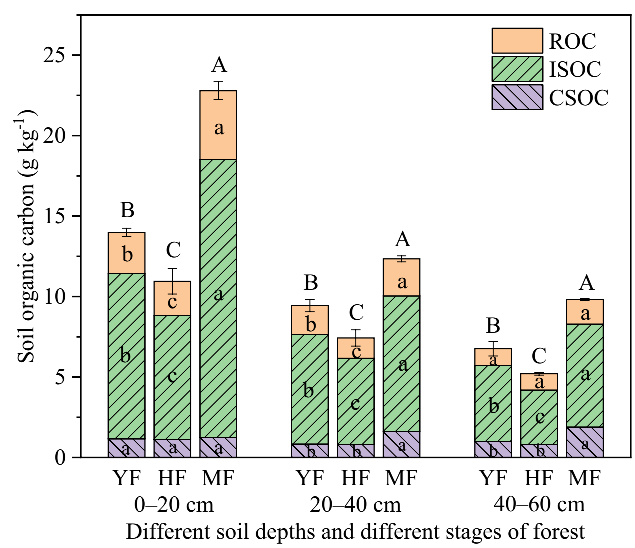

Fig. 3 Soil organic carbon (SOC) components and contents in different soil depths of different ages of *Liriodendron chinense* plantations. Different uppercase letters indicate significant differences in SOC between different forest ages in the same soil depth at $P<0.05$. For the same soil depth and SOC fraction, different lowercase letters indicate significant differences between different forest ages at $P<0.05$. *YF* young forest, *HF* half-mature forest, *MF* mature forest, *ROC* readily oxidizable organic carbon, *ISOC* intermediate stable organic carbon, *CSOC* chemically stable organic carbon

the mature forest was significantly higher (124.29%) than in the half-mature forest at 0–20 cm. Compared with the ROC in the young and the half-mature forest, the ROC of the 0–20 cm soil layer increased by 68.11% and 101.42%, respectively. At 20–40 cm and 40–60 cm, the CSOC of the mature forest was significantly higher than in the other two forest groups. Moreover, the CSOC of the mature forest increased significantly with the increasing soil depths. At all soil depths, MQ was the highest in the half-mature forest, significantly higher than in the young forest and mature forest (Fig. 4).

Correlations between SOC components and water-stable aggregates

The RDA plot shows the contribution of each SOC component to the total variance among the distribution of aggregates and the relationships among the SOC components, samples, and aggregate composition (Fig. 5). The eigenvalues of axis 1 and axis 2 in the RDA biplot were 0.73 and 0.012, respectively, which jointly explained 74.70% of the total variation in the distribution of aggregates. Moreover, the two largest explanatory variables were MQ and CSOC, which explained 55.0% and 19.3%, respectively ($P < 0.01$). CSOC was positively correlated with the large macroaggregates (> 2 mm) and MWD along the negative axis of axis 1. In contrast, MQ was negatively correlated with the large macroaggregates (> 2 mm) and MWD along the positive axis of axis 1. In addition, the MWD and large

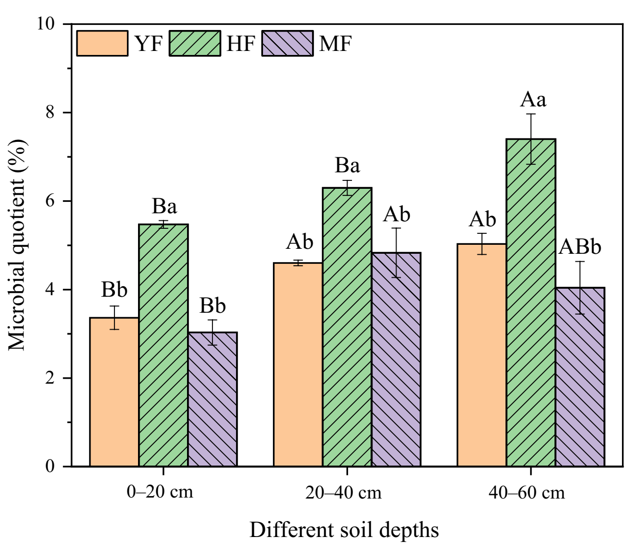

Fig. 4 Microbial quotient (MQ) in different soil depths of different *Liriodendron chinense* ages. Different uppercase letters indicate significant differences in MQ between different soil depths in the same forest age at $P < 0.05$. Different lowercase letters indicate significant differences between different forest ages in the same soil depth at $P < 0.05$. *YF* young forest, *HF* half-mature forest, *MF* mature forest

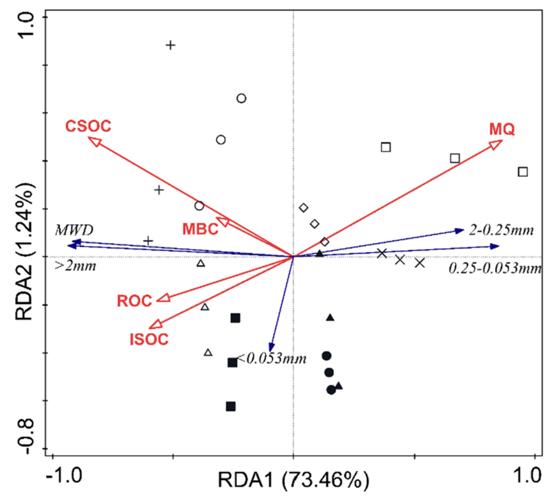

Fig. 5 Redundancy analysis (RDA) of the distribution of water-stable aggregates (> 2 mm, 2–0.25 mm, 0.25–0.053 mm and < 0.053 mm) and MWD constrained by the soil organic carbon components and microbial quotient (ROC, ISOC, CSOC, MBC and MQ) in different ages of *Liriodendron chinense* plantations. Different symbols indicated: filled square—young forest (0–20 cm); open diamond—half-mature forest (0–20 cm); open triangle—mature forest (0–20 cm); filled circle—young forest (20–40 cm); multi sign—half-mature forest (20–40 cm); open circle—mature forest (20–40 cm); filled triangle—young forest (40–60 cm); open square—half-mature forest (40–60 cm); plus sign—mature forest (40–60 cm)

macroaggregates (> 2 mm) were negatively correlated with the small macroaggregates (2–0.25 mm) and microaggregates (0.25–0.053 mm) along the positive axis of axis 1. Remarkably, the MWD was positively correlated with the large macroaggregates (> 2 mm). CSOC had a positive impact on mature forest (0–20 cm, 20–40 cm and 40–60 cm) and had a negative impact on half-mature forest (0–20 cm, 20–40 cm and 40–60 cm). MQ had a positive impact on half-mature forest (0–20 cm, 20–40 cm and 40–60 cm) and had a negative impact on mature forest (0–20 cm, 20–40 cm and 40–60 cm).

Discussion

Changes in SOC components and aggregate stability in *L. chinense* plantation of different ages

SOC was hypothesized to increase with forest age, but our results showed an initial downward trend followed by an upward trend. There were two potential reasons for the change in SOC pools with forest age. First of all, in the maturation from young forest to half-mature forest (from 5 to 13 years), *L. chinense* grew rapidly and required more nutrients. The MQ, which characterizes soil microbial activity, increased significantly (Fig. 4), increasing soil respiration, prompting the release of organic carbon pool nutrients and reducing the organic carbon content. Previous studies on Chinese fir [*Cunninghamia lanceolata* (Lamb.) Hook.] have shown similar results that soil carbon reserves are significantly lower in the medium-age forest than in the young (Chen et al. 2013). Second, during growth from the half-mature forest to the mature forest (from 13 to 31 years), we found that MQ significantly decreased, indicating a decrease in microbial activity and a decrease in organic carbon output. At this time, the *L. chinense* continue to grow, and the organic matter of root exudates and litter increases the carbon input of the soil, so the carbon balance tends to be in the direction of carbon accumulation (Luo et al. 2017). During the transition from half-mature forest to mature forest, carbon input in soil was greater than the carbon output, thus SOC was cumulative (Heimann and Reichstein 2008).

Our results showed that the stability of aggregates is consistent with the direction of the change in organic carbon, as shown previously (Das et al. 2014), mainly because the aggregates can physically protect against loss of organic carbon, and the organic carbon in turn acts as a cementing substance that promotes the formation of large aggregates and improves the stability of aggregates (Mao et al. 2014; Six et al. 2004). Therefore, when SOC is affected by microbial activity and tends to accumulate carbon, the aggregates are relatively stable, while in the case of carbon loss, stability of the aggregates decreases.

Changes in SOC components and aggregate stability with different soil layers in *L. chinense* plantations

As expected, the total SOC and chemically unstable components (ROC and ISOC) decreased as the depth of the soil increased in the three forest ages of *L. chinense*. During the maturity of *L. chinense*, CSOC increased with the increase of soil depth. We speculate that one reason may be that the test area has red soil. Due to the presence of leaching and sedimentation, deeper soil accumulates more iron-aluminum oxide than the topsoil (Ma and Xu 2010). In addition, Fe oxides and Al oxides have been suggested to be important stabilizing agents and have help stabilize aggregates (Demenois et al. 2017; Liang and Balser 2008). The second reason may be that as the depth of the soil increased, the oxygen content of the soil decreased, resulting in anaerobic conditions. The SOC that remained at deeper soil depths was slower in cycling and not easily decomposed; biochemical protection by recalcitrant alkyl C may be the primary mechanisms for SOC preservation and aggregate stability (Li et al. 2017). Therefore, more CSOC will be accumulated and the soil aggregates are more stable in the deeper soil of long-term plantations of *L. chinense*.

Relationship between SOC components and aggregate stability in *L. chinense* plantations

As expected, there was a stronger positive correlation between CSOC and aggregate stability which was shown in our RDA results (Fig. 5). We speculate that, as a relatively stable carbon component, CSOC may be the "carbon core" of the aggregate interior and protected by the aggregates. Some studies suggested that CSOC could be an aromatic carbon like charcoal or black carbon (Eusterhues et al. 2003). Therefore, CSOC is difficult to oxidize and highly stable, and levels were strongly positively correlated with aggregate stability. The unstable carbon pools, however, are more sensitive to environmental changes and have greater fluctuations and variations (Mi et al. 2016). Therefore, CSOC should be receive more attention in the future and may be a better indicator to explain and characterize changes in soil aggregate stability during long-term forest growth.

Conclusions

This study confirms that SOC and aggregate stability did not increase with increasing stand age, but declined significantly until the half-mature age, then increased significantly as the plantations of *L. chinense* aged. MQ seems to have a negative impact on the formation of organic carbon and the stability of aggregates, leading to such results. The evidence presented here indicates that CSOC increased with soil depth of the mature *L. chinense* plantation and was highly correlated with the MWD. Future study should focus on the accumulation of CSOC in deep soil and the participation of microorganisms.

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