Distinct storage mechanisms of soil organic carbon in coniferous forest and evergreen broadleaf forest in tropical China

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ABSTRACT

The impact of human activities on soil carbon (C) storage in tropical forests has aroused wide concern during the past decades because these ecosystems play a key role in ameliorating global climate change. However, there remain uncertainties about how land-use history alters soil organic carbon (SOC) stability and storage in different forests. In this study, we measured the C content and mass distributions of soil aggregates, density fractions, mineral-bound C and microbial biomass C in the organic horizon, 0-10 cm and 10-20 cm soil layers in coniferous forest and evergreen broadleaf forest at Dinghushan Biosphere Reserve in tropical China. The broadleaf forest had larger SOC stocks than the coniferous forest, but the proportion of SOC stored in different density fractions at 0-10 cm soils was similar between forest types, while a greater proportion of SOC was stored in microaggregates in the coniferous forest. Most of the SOC was held as light fraction C in the organic horizon in the coniferous forest, whereas the concentrations of mineral-bound C were higher in the broadleaf forest. These findings indicate clear differences in the protection of SOC between broadleaf and coniferous forests growing on the same soil type. We propose that historic conversion of broadleaf forest to coniferous forest has reduced soil C sequestration capacity by altering the diversity and quality of plant inputs to the soil, which in turn affected macroaggregate formation, soil chemical properties and microbial biomass. Our results thus demonstrate that changes in forest tree species composition could have long-lasting effects on soil structure and carbon storage, providing crucial evidence for policy decisions on forest carbon sink management.

1. Introduction

Soils play an important role as carbon (C) reservoir, as they contain around three times as much C as the vegetation at global scale (Scharlemann et al., 2014; Wiesmeier et al., 2019) and forest soils account for 16-26% of the global soil C pool (Pan et al., 2011; IPCC, 2013). Although much of the C stored in soils is thought to have long residence times (Trumbore, 2000; Carvalhais et al., 2014), human activities such as land-use change can cause substantial losses of soil organic C (Scharlemann et al., 2014). The impact of human activities on soil carbon storage is particularly concerning in (sub-)tropical forests because they contain *c.* 32% of all forest soil C (Pan et al., 2011). Widespread land-use change has resulted in substantial deforestation, especially in the tropics, and there remains only 34% of the world's forests as primary forests without clearly visible indications of human activity (FAO, 2020). Deforestation, forest regeneration, and the establishment of plantations have greatly affected the accumulation, distribution and stability of soil organic carbon (SOC) in tropical forests (Martin et al., 2013; Li et al., 2015; Powers et al., 2011). Although decades of research into the protection and storage of SOC in temperate soils, we still know very little about mechanisms of SOC stabilization in (sub-)tropical soils, especially during forest regeneration (Mobley et al., 2015; Wang et al., 2017; Coward et al., 2017; Sayer et al., 2019). Considering that the decomposition and turnover of unprotected organic matter is rapid under warm, humid tropical conditions, it is important to address the knowledge gaps on the protection mechanisms of SOC in (sub-)tropical forest soils under global climate change (Zech et al., 1997; Sayer et al., 2019; Sayer et al., 2020).

The stability of SOC depends on the extent of its protection from microbial mineralization via physical and chemical processes (Schmidt et al., 2011; Lehmann and Kleber, 2015). Physical protection of SOC occurs principally through the formation of soil aggregates, whereby microbial access to SOC generally declines with increasing aggregate size (van Veen and Kuikman, 1990). SOC can be additionally stabilized by microaggregate formation within macroaggregates in undisturbed soils (Kong et al.,2005; Six and Paustian,2014). Chemical protection of SOC often involves the formation of bonds between organic compounds and soil minerals, which also reduce the availability of SOC to microbial decomposers (Schmidt et al., 2011; Stockmann et al., 2013; Possinger et al., 2020). Approaches to measure the extent of SOC protection often focus on aggregate size distributions and the SOC associated with soil minerals (De Oliveira et al., 2018; Totsche et al., 2018). In particular, density fractionation of

SOC distinguishes a 'light' SOM fraction which is protected by occlusion within aggregates, and a 'heavy' SOC fraction that is also associated with soil minerals, affording a greater degree of protection from mineralization (Golchin et al., 1994). Thus, combining aggregate size and density fractionation can provide valuable information on the size and stability of SOC pools (Crow et al.,2007).

The formation and distributions of aggregate and density fractions are strongly influenced by the quantity and quality of plant inputs, as well as soil properties (Bouwman, 1990; Lal, 2004). The formation of water-stable soil aggregates of different sizes depends upon the form and quantity of the binding agents, whereby polysaccharides are primarily involved in the formation of the smallest microaggregates (<53 μm), fungal hyphae support the formation of intermediate aggregate sizes (53-250 μm and 250-2000 μm), and fine roots are principally involved in the formation of large macroaggregates (>2000 μm aggregate fraction; Six et al.,2002; Blanco-Canqui and Lal, 2004). Recent research on soil C storage suggests that 'high-quality' plant C inputs with low C/N ratios are more rapidly and strongly stabilized in organo-mineral complexes or occluded in soil microaggregates (Cotrufo et al., 2019; Lavallee et al., 2020). Therefore, high-quality litter inputs should promote the storage of SOC in microaggregates(Helfrich et al., 2008) and the formation of organo-mineral compounds, which make up a large part of the heavy SOC fraction (> 1.8 g cm^{-3}, Golchin et al., 1995a,b). By contrast, C derived from lower-quality litter with slow decomposition rates promotes the formation of macro-aggregates (Blanco-Canqui and Lal, 2004) and is more likely to be more retained in the light SOC fraction (< 1.8 g cm^{-3}; Golchin et al., 1994; Gregorich et al., 1996). However, soil properties also strongly influence organo-mineral interactions, for example via the presence of iron (Fe) and aluminium (Al) ions that can bind organic matter to form highly stable organo-mineral compounds (Stockmann et al., 2013). In addition, exchangeable calcium (Ca) plays a key role in SOC stabilization because it promotes aggregate formation and can also bind organic matter through ligand exchange or cation bridging (Rowley et al., 2018). Consequently, differences in the quantity and quality of litterfall, soil texture and soil minerals will result in distinct protection mechanisms of SOC in forest ecosystems, which could ultimately influence long-term SOC accumulation and storage (Cotrufo et al., 2019).

In subtropical China, coniferous forest and evergreen broadleaf forest are widely distributed and play critical roles in C storage (Xiang et al., 2013; Zhou et al., 2013, 2014). Coniferous forest is often assumed to represent the early stages of forest succession, whereas evergreen broadleaf forest

represents the late successional stages (Huang et al., 2013; Zhou et al., 2013). In addition, plantations of native Masson pine have been established in many areas due to their capacity to rapidly sequester C in aboveground biomass (Justine et al., 2017). The two forest types have distinct characteristics both above- and below-ground, including marked differences in plant diversity, aboveground biomass, litter quality, soil microbial communities, soil texture, as well as soil mineral elements and exchangeable cations, all of which have been extensively studied (Sun et al., 2013; Zhou et al., 2007; Yan et al., 2007; Li et al., 2013; Lu et al., 2015). The coniferous forest is generally characterized by lower annual litterfall and higher litter C/N ratio compared to evergreen broadleaf forest (Huang et al., 2011) and the coniferous forest soils have lower concentrations of Al and Fe, but similar concentrations of Ca, compared to evergreen broadleaf forest (Yan et al., 2007; Jiang et al., 2018). Such differences in the quantity and quality of plant litter inputs and soil characteristics between the two forest types are also associated with distinct patterns in C accumulation (Zhou et al., 2006; Tang et al., 2011), but there is lack of information about the protection mechanisms for SOC in the two forest types. Investigating the protection of SOC in these two forest types can therefore improve our understanding of SOC accumulation in subtropical forests. In addition, elucidating the protection mechanisms of SOC in forest types representing distinct successional stages will increase our knowledge of C storage by afforestation, as well as ecosystem C accumulation during natural forest succession (Zhou et al., 2006).

In this study, we aimed to determine differences in SOC protection and storage between coniferous forest and evergreen broadleaf forest by comparing surface SOC pools protected by distinct physical and chemical mechanisms, i.e. soil aggregates, density fractions, mineral-bound C, and SOC contained in microbial biomass. We tested the following hypotheses:

(H1) Given the greater annual litterfall and lower litter C/N ratios, the broadleaf forest will have a greater proportion of SOC stored in microaggregates and the heavy fraction compared to the coniferous forest;

(H2) As coniferous litter has lower decay rates and higher C/N ratios, the total SOC pool in the coniferous forest will be dominated by light fraction C;

(H3) As the broadleaf forest has higher concentrations of soil cations as well as lower litter C/N ratios, a greater proportion of SOC will be stabilized by organo-mineral-bonds in the broadleaf forest compared to the coniferous forest.

We then assessed how distinct protection mechanisms of SOC might influence total soil C storage by determining the relationships between total SOC content and the size of the microbial biomass, the mass of macro- or microaggregates, and the amount of SOC contained in the light vs. heavy fraction.

2. Materials and methods

2.1. Study sites and sample collection

The study site was located at the Dinghushan Biosphere Reserve in Guangdong province, southeast China (23°10'N, 112°10'E). The reserve has a typical humid monsoon climate, with a mean annual temperature of 21 °C and mean annual precipitation of 1927 mm (Huang & Fan, 1982). We chose two subtropical forests that are typical to the region: natural evergreen broadleaf forest (henceforth 'broadleaf forest') and plantations of coniferous Masson pine (henceforth 'coniferous forest'). The dominant tree species of the broadleaf forest are *Castanopsis chinensis* Hance, *Schima superba* Chardn. & Champ., *Cryptocarya chinensis* (Hance) Hemsl., and *Machilus chinensis* (Champ. Ex Benth.) Hemsl. In the 1930s, part of broadleaf forest was clear-cut and planted with the native pine *Pinus massoniana Lamb.*, which is the origin of the present coniferous forest. The understorey vegetation and litter in the coniferous forest were harvested frequently until the late 1990s. However, in contrast to the broadleaf forest, where highly decomposed organic material is incorporated into the surface mineral soil, the coniferous forest had a *c.* 5 cm thick Oe+Oa horizon at the time of sampling. Considering the topsoils are always sensitive to environmental changes and most fine roots were distributed there, we mainly focused on the top 0-10cm soils. The soil type in the region is lateritic red earth formed from sandstone, but the soil texture differs between forest types. The contents of sand, silt and clay are 19%, 61% and 18%, respectively, at 0-10 cm soils in the broadleaf forest, while they are 43% (sand), 43% (silt) and 13% (clay), respectively, in the coniferous forest (Table 1; Zhang et al.,2012).

In August 2019, we collected soil samples in sunny slope of broadleaf forest and coniferous forest (Fig.1). The slopes of the two forests are both approximately 30 degree and the distance of the two forests is approximately 2.5 kilometers. There were five random sampling plots in each forest, each plot was 10 × 20 m in size with at least 10-m distance between adjacent sampling plots. In the broadleaf forest, we collected three soil cores (3.5 cm in diameter) beneath the loose litter layer (Oi) at 0-10 cm (Oe+Oa + mineral soil mixed horizon; 'topsoil') and 10-20 cm (mineral soil horizon; 'subsoil') per

sampling plot. In the coniferous forest, we first collected samples of the Oe+Oa horizon from three 20 × 20 cm quadrats per plot and then we collected three cores from the mineral soil at 0-10 cm depth in the same quadrats. The soil samples within each soil layer were combined into one composite sample per plot, giving n = 5 samples per forest type and soil depth. Each composite sample was subsequently divided into three subsamples: one subsample was sieved to 8-mm mesh size to remove visible roots and stones before being air-dried for analysis of soil aggregates and density fractions. The other two subsamples were sieved to 2-mmmesh size after removing plant roots carefully by hand; one subsample was air-dried to measure soil pH and total C content and the other subsample was stored at 4 °C for microbial biomass C measurement within one week of collection. The C concentrations of the bulk soil and all fractions described below were measured on a C/N analyzer (IsoPrime100, Elementar Analysensysteme, Germany).

2.2. Measurement of soil properties

Gravimetric soil water content was determined by oven-drying the soils to constant weight at 105 ºC. Soil pH was measured by a glass electrode pH meter (FE28, Mettler Toledo, Shanghai, China) in a 1:2.5 soil/water suspension. Total soil organic carbon (SOC) and total nitrogen (TN) were measured after pretreatment of the samples with 10% hydrochloric acid to eliminate inorganic carbonate content (Matejovic, 1997). Soil microbial biomass carbon (MBC) was measured by the chloroform fumigation extraction method (Vance et al., 1987); MBC was determined from the difference in C content of paired fumigated and unfumigated extracts and a conversion factor (k_{ec} = 0.33; Mo et al., 2008) was applied to convert measured C to MBC. The bulk density (Zhang et al., 2012) was measured volumetrically as described by Fang et al. (2003).

2.3. Partitioning of soil aggregate and density fractions

We used the wet sieving method (Six et al., 1998) to partition water-stable aggregates in the mineral soil into size fractions using an aggregate analyzer (XY-100, Xiangyu Weiye Instrument equipment Co., LTD., Beijing, China) following the procedure described in Xu et al. (2018). In brief, 50 g pre-sieved (8-mm) soil was soaked in water for 10 minutes and then passed through a series of sieves (30 times per minute for 15 minutes) to divide the sample into four aggregate sizes, according to the apertures of the sieves:>2000 µm, 250-2000 µm, 53-250 µm and <53 µm. All aggregate fractions were oven-dried at 60 °C and their C concentration was measured. Here, the >2000 µm and 250-2000 µm size fractions were

collectively regarded as the macroaggregate fraction, whereas the 53-250 µm and <53 µm size fractions were regarded as the microaggregate fraction (Xu et al.,2018).

We determined two soil density fractions: the light fraction (< 1.8 g cm^{-3}) and the heavy fraction (> 1.8 g cm^{-3}) following Ye et al. (2018). In brief, 5 g of each pre-sieved (2-mm) soil sample was placed in 50 ml centrifuge tube with 30 ml NaI solution (1.8 g cm^{-3}). The samples were shaken at 300 rpm for 2 hours after dispersion by ultrasonication (300W and 40KHz; SB25-12DTDN, Ningbo, China), then centrifuged at 3000 g for 15 minutes and allowed to stand for 24 h; the supernatant (light fraction) was then removed by pipetting. The procedure was repeated three times to ensure complete separation of the light and heavy fractions. The light and heavy fractions were washed by deionized water, oven-dried at 60 °C and finely ground before determining the C concentration of each fraction.

2.4. Measurement of mineral-bound C

Mineral-bound C was determined by sequential chemical extraction of the heavy fraction. We firstly used sodium sulphate extraction to estimate calcium-bound C (henceforth: 'Ca-bound C'). Soil subsamples (0.5000 g) were extracted with a 1:50 soil/ 0.5 M Na$_2$SO$_4$ solution, the C content of the extract was interpreted as Ca-bound C (Xu and Yuan, 1993). The residue from the sodium sulphate extraction was used to determine iron and aluminum-bound C (henceforth: 'Fe/Al-bound C') using the dithionite-citrate-bicarbonate method (Lalonde et al., 2012). Briefly, the sample was placed in a 50 ml centrifuge tube with 2.5 ml 1 M sodium bicarbonate solution and 20 ml 0.3 M trisodium citrate solution. After heating the tube to 80 °C in a water bath, 0.50 g sodium dithionite was added to the tube and the suspension was stirred at 80 °C for 15 minutes. Finally, the tube was centrifuged at 3000 g for 10 minutes to separate supernatant and residue. The procedure was repeated three times to ensure complete extraction and the C concentration of the residue was determined. Fe/Al-bound C was calculated from the difference in the C content of the residue after sodium sulphate extraction and dithionite-citrate-bicarbonate extraction.

2.5. Data analysis

All SOC concentrations were expressed relative to total soil mass. The SOC stock (kg m^{-2}) in a given soil layer was calculated by multiplying SOC concentrations by bulk soil density (mineral soil layer) or the mass of the Oe+Oa horizon as follows:

SOC$_{soil}$ stock (kg m^{-2}) = [SOC concentration (gkg^{-1}) * bulk soil density (gcm^{-3}) * soil depth (cm)* c]/100

$$\text{(Eq.1)}$$

SOC$_{Oe+Oa}$ stock (kg m^{-2}) = [SOC concentration (gkg^{-1}) * Oe+Oa mass (kgm^{-2})]/1000   (Eq. 2)

where c (the proportion of sandy, silt and clay to total soil) is the correction factor for rock fragments.

    We used one-way Analysis of Variance (ANOVA) to examine differences between broadleaf and coniferous forest in soil pH, TN, SOC stocks and concentrations, and the C content or concentrations in soil aggregates and density fractions, as well as differences in the proportion of total SOC contained in MBC, density fractions, and aggregate classes. Given the distinct soil profiles in the two forest types, we compared only the 0-10 cm soil horizon between broadleaf and coniferous forest, but we also assessed pairwise differences between soil layers within forest types using Duncan's test at $P< 0.05$. We assessed the relationship between total SOC and density or aggregate size fractions using linear regressions. All analyses were performed with SAS v.8.1 (SAS Institute Inc., Cary, NC, USA) and we report significant results at $P< 0.05$.

3. Results

3.1. Comparison of mineral soil properties at 0-10 cm depth between forest types

    Overall, the broadleaf forest had significantly higher SOC concentration and stock at 0-10 cm depth compared to the coniferous forest (Table 1). Soil aggregate size distributions differed markedly between the two forest types: the broadleaf forest had a greater mass percentage of macroaggregates (73%) than the coniferous forest (54%), whereas the mass percentage of microaggregates was higher in the coniferous compared to the broadleaf forest (23% and 8%, respectively; Fig. 2a,b). Accordingly, macroaggregate-C concentrations in the broadleaf forest were2.3-fold higher than in the coniferous forest (Fig. 2c). However, as bulk density was much lower in the broadleaf forest than the coniferous forest, the C content in macroaggregates did not differ between forest types (Fig. 2e). By contrast, microaggregate-C concentrations did not differ between forest types, but microaggregate-C content in the coniferous forest was 2.3-fold higher than in the broadleaf forest (Fig. 2d,f). Finally, although the percentage of total SOC contained in macroaggregates did not differ between forest types (Fig. 2g), a lower percentage of SOC was contained in microaggregates in the broadleaf than in the coniferous forest (4% and 12%, respectively; Fig. 2h).

    The mass percentages of light and heavy fractions at 0-10 cm were similar in the two forest types

(Fig. 3a,b) and neither the C content (Fig. 3e,f) nor the percentage of total SOC (Fig. 3g,h) contained in light or heavy fractions differed between broadleaf and coniferous forest. However, the C concentrations of heavy and light fractions at 0-10 cm were two-fold and 2.5-fold higher in the broadleaf forest than in the coniferous forest, respectively (Fig. 3c,d). The broadleaf forest also had higher concentrations of Ca-bound and Fe/Al-bound soil Cat 0-10 cm than the coniferous forest (40% and 84% higher, respectively, Fig. 4a,b), as well as threefold greater MBC (Fig. 5a). However, the proportion of total SOC contained in MBC did not differ between forest types (Fig. 5b).

3.2. Soil aggregates, density fractions, and carbon pools in different soil layers

In the broadleaf forest, SOC concentrations were higher at 0-10 cm than 10-20 cm depth, but SOC stocks were similar between depths (Table 1, Fig. 2e). However, macroaggregate-C concentrations at 0-10 cm were 89% higher than at 10-20 cm (Fig. 2c) but there were no differences between soil depths in microaggregate-C concentrations or content (Fig. 2d,f) and a similar percentage of total SOC was contained in each aggregate size fraction at both depths (Fig. 2g,h). The two soil layers also had similar light fraction C concentration and content; whereas the C concentration in the heavy fraction was 52% higher at 0-10 cm than at 10-20 cm (Fig. 3c,d,e,f). Nonetheless, the percentage of the total SOC stock contained in the light fraction at 0-10 cm (15%) was lower than that at 10-20 cm (20%), whereas the percentage of the total SOC stock contained in the heavy fraction did not differ between soil depths (Fig. 3g). The concentrations and contents of Ca-bound C did not differ between soil depths (Fig. 4a,c) but Fe/Al-bound C concentrations were 56% higher at 0-10 cm than at 10-20 cm (Fig. 4b). Finally, neither MBC nor the proportion of MBC in SOC differed between soil depths (Fig. 5a,b).

In the coniferous forest, most of the SOC was concentrated in the Oe+Oa horizon (Table 1), hence, light fraction C concentrations, contents and the percentage of total SOC stock contained in the light fraction were179×, 1.6× and 6× greater, respectively, in the Oe+Oa horizon than in the mineral soil (0-10 cm depth; Fig. 3c,e,g). By contrast, although heavy fraction C concentrations did not differ between soil horizons, heavy fraction C content and the percentage of total SOC stocks contained in the heavy fraction were 80× and 26×greater, respectively, in the mineral soil than in the Oe+Oa horizon (Fig. 3f,h).The concentrations of Ca-bound and Fe/Al-bound C concentrations did not differ between the Oe+Oa horizon and the mineral soil (Fig. 4a,b). However, both Ca-bound and Fe/Al-bound C content were much higher in the mineral soil than in the Oe+Oa horizon (70× and 129× greater, respectively; Fig.

4c,d), as well as their proportions to total SOC (26× and 46×greater, respectively; Fig. 4e,f). Finally, MBC was14×higher in the Oe+Oa horizon than in the mineral soil (Fig. 5a), but the contribution of MBC to total SOC did not differ between soil horizons (Fig. 5b).

3.3. Relationships between total SOC and soil C fractions

Linear regressions showed that total SOC content decreased with the mass of microaggregates (R^2=0.66, p=0.0042, Fig. 6a) and the mass of the light fraction (R^2=0.56, P=0.020, Fig. 6c), but increased with the mass of macroaggregates (R^2=0.51, p=0.020, Fig. 6b) and MBC (R^2=0.79, p=0.0006, Fig. 6d).

4. Discussion

Our comparison of soil carbon stabilization in coniferous and broadleaf forest demonstrated clear differences between forest types in the overall proportion of SOC stored in aggregate size fractions and in the distribution of SOC among soil layers, aggregate size classes and density fractions. However, in contrast to our first hypothesis (H1), a greater proportion of SOC was stored in microaggregates in the coniferous forest, and the proportion of SOC stored in different density fractions in the mineral soil was similar among forest types. However, most of the SOC in the coniferous forest was held as light fraction C in the organic horizon (H2) and the concentrations of SOC stabilized by chemical bonds to soil minerals were higher in the broadleaf forest (H3). Here, we discuss the links between plant inputs to the soil, and the mechanisms of SOC stabilization in the two forests.

4.1. The mass distributions of soil aggregates and density fractions

The differences between our study forests in soil aggregate size distributions at 0-10 cm depth demonstrates the key role of plant inputs in shaping soil structure (Oades, 1993). Macroaggregates are formed by binding agents such as root hairs and fungal hyphae (Oades 1984; Tisdall, 1991) and the higher mass% of macroaggregates in the broadleaf forest (Fig. 2a) suggests there were more such binding agents in the surface soils (Yang et al., 2004; Lehmann et al., 2017; Chen et al., 2019), compared to the coniferous forest. Although we predicted a greater mass% of microaggregates in broadleaf forest (H1), the higher mass% of microaggregates in the coniferous forest (Fig. 2b) could be a result of the distinct soil texture, as low-clay soils often hold most of their organic matter in smaller microaggregates and the higher sand content could impede the formation of macroaggregates (Schweizer et al., 2019). In addition, as macroaggregates are more closely associated with fresh plant residues and more

susceptible to disturbance than microaggregates (Totsche et al.,2018), it is possible that the soil structure in the coniferous forest was strongly influenced during deforestation and replanting, and the subsequent low-quality plant litter inputs have limited the formation of new macroaggregates (Totsche et al., 2018). As the formation of soil aggregates is one of the key processes for increasing the mean residence time of SOC (Six et al., 2002), our results demonstrate that changes in forest tree species composition could have long-lasting effects on soil structure and C storage. Although the C in microaggregates is thought to be more stable than C stored in macroaggregates (Six et al., 2002; Blanco-Canqui and Lal, 2004), greater soil pore space and macrostructure with the higher mass% of macroaggregates in the broadleaf forest likely supports a greater diversity of soil organisms and higher water holding capacity (Horn et al., 1994; An et al., 2010).

The soil C associated with light and heavy density fractions is thought to be strongly related to soil aggregate size classes, whereby macroaggregate-C and light fraction-C represents the 'active' C pool, whereas C occluded in microaggregates and the heavy fraction represents the 'slow' C pool (Carter,2002; Torn et al.,2013; Hall et al.,2015). We therefore expected that the mass distributions of light- and heavy fractions in the two forest types would be similar to the mass distributions of macro-and microaggregates. However, the heavy fraction comprised *c*. 99% of soil C at 0-10 cm depth in both forests (Fig. 3a,b). Although our results seemingly contradict previous findings showing marked differences in the light and heavy fractions across numerous coniferous vs. broadleaf forests (Luo et al., 2020), the coniferous forest at our site was older and established on the same soil type as the broadleaf forest. Furthermore, we did not distinguish between 'free' and 'occluded' light and heavy fractions, which may reveal important differences in SOC stabilization between forests (Crow et al., 2007).

4.2. The distribution of C in soil aggregates and density fractions

The differences in the mass% of macroaggregates between sites was not reflected in macro-aggregate-C. Although the SOC concentration in macroaggregates was higher in the broadleaf than in the coniferous forest (Fig. 2c), the amount of C contained in the macroaggregate fraction and its contribution to total SOC stocks were similar (Fig. 2e,g). This lack of differences in macroaggregate-C between forests can be attributed to the minor contribution of macroaggregate-C to total SOC stocks, and the lower soil bulk density in the broadleaf forest (Table 1; Zhang et al., 2012), which is a key factor in calculating C stocks. By contrast, the patterns of microaggregate-C mirrored the pattern of mass%

distribution, reflecting the crucial importance of microaggregates for soil C sequestration (Six et al., 2002; Totsche et al., 2018). Despite similar C-concentrations within microaggregates (Fig. 2d), the amount of C associated with microaggregates was higher in the coniferous forest and the contribution of microaggregate-C to total SOC stocks at 0-10 cm was also greater than in the broadleaf forest (Fig. 2f,h). The greater proportion of SOC contained in microaggregates in the coniferous forest suggests that the SOC stock in the mineral soil is more stable than in the broadleaf forest, where the high concentrations of C in macroaggregates could indicate accumulation of more labile C in the relatively undisturbed broadleaf forest soil (Six et al.,2002).

Although SOC stocks at 0-10 cm in the mineral soil were lower in the coniferous forest than in the broadleaf forest, the total SOC stocks in Oe+Oa horizon and 0-10 cm depth in coniferous were similar with that in 0-10 cm depth in broadleaf forest (Table 1). As hypothesized (H2), the larger amount of light fraction C in the coniferous forest likely reflects the slower decay of lower-quality plant residues (Li et al., 2013). In addition, most of the light fraction C in the coniferous forest was contained in the organic horizons rather than incorporated into the mineral soil as in the broadleaf forest (Figs. 3a-d), which could indicate reduced abundance and activity of earthworms and other macrofauna (Verstraeten et al., 2018). However, our results suggest that much of the SOC in these forests is stabilized in heavy fractions (Fig. 3c,d,e,f), which is consistent with studies of other sites in the region(Luo et al., 2020). One possible explanation is that the acidic soils in Dinghushan forests have high concentrations of Fe^{2+}/Fe^{3+} and Al^{3+} ions (Jiang et al., 2018), which promote organo-mineral protection of SOC (Rasmussen et al., 2018).

4.3. Mineral-bound C and microbial biomass C in two forest types

Organo-mineral associations are of critical importance for protecting SOC both chemically and physically (Angst et al., 2017; Hemingway et al., 2019; Newcomb et al., 2017). In particular, Ca^{2+}, Fe^{2+} and Al^{3+}ions can bind C to form stable organo-mineral compounds (Stockmann et al., 2013; Yu et al., 2017). The highly weathered soils at our study site have also been subjected to a high rate of acid deposition (Lu et al., 2014, 2018) and have low soil pH (Table 1; Lu et al., 2015). Soils with low pH have higher concentrations of Fe^{2+}/Fe^{3+} and Al^{3+} (Jiang et al., 2018) and organo-mineral complexation becomes the dominant factor for SOC stabilization (Rasmussen et al.,2018). Accordingly, we measured greater content of Fe/Al-bound C than Ca-bound C in both forests in our study (Fig. 4a,b). Nonetheless, as hypothesized (H3), more heavy-fraction C was associated with Ca and Fe/Al in the broadleaf forest

than in the coniferous forest (Fig. 4a,b), which can be explained by the greater cation exchange capacity in soils with higher organic matter content (Jiang et al., 2018).Higher ion concentrations in the broadleaf forest might also be the result of root exudate inputs by a greater diversity of tree species (Weinhold et al., 2020) and minimal historic disturbance (Lu et al., 2014). Thus, soil C accumulation and stabilization in the forests at our study site are largely explained by interactions between soil minerals and the quality and diversity of plant inputs.

The higher MBC in the broadleaf forest compared to the coniferous forest (Fig. 5a) supports the view that greater plant diversity can promote SOC accumulation by supporting higher microbial biomass in soils (Prommer et al., 2020; Xu et al., 2020). However, the two forests had a similar MBC per unit SOC (Fig. 5b), and the size of SOC pool depends in part upon microbial activity (Liang et al., 2017; Sokol and Bradford, 2019). It is thus possible that differences in microbial activity or distinct microbial communities associated with coniferous vs. broadleaf trees play a greater role in SOC storage than microbial biomass.

4.4. Relationships between total SOC and different soil C fractions

Soil aggregates play an important role in protecting SOC via physical separation from microbial decomposers and up to 90% of the organic matter in topsoil is occluded in aggregates (Emerson, 1967). In our study, the relationships between SOC concentrations at 0-10 cm and aggregate mass, density fractions or MBC (Fig. 6) were strongly influenced by the differences in C stocks between forests (Fig. 6) as the higher SOC concentrations in the broadleaf forest were associated with higher mass% of macroaggregates and larger microbial biomass. However, most of the SOC in both forests was associated with the heavy fraction, which suggests that the lower C storage in the coniferous forest can be partly explained by the destruction of macroaggregates during plantation establishment, and reduced macroaggregate formation with lower-quality plant inputs (Totsche et al., 2018; Parwada and Tol, 2019). In the broadleaf forest, the high proportion of both macroaggregate and heavy fraction C suggest that occlusion of heavy fraction C within aggregates plays an important role in total SOC storage, possibly through the formation of microaggregates within macroaggregates, which can contribute to SOC stabilization in undisturbed soils (Six et al., 2002).Hence, our results suggest that the overall capacity of the coniferous forest soils to store C might be reduced by the destruction of macroaggregates and the change in tree species composition. Furthermore, soil parameters, such as soil pH and clay content, could also be key factors to affect SOC stocks in the two forests (Fierer et al., 2006; Sun et al., 2020).

As the percentage contribution of MBC to SOC was low (Fig. 5b), the strong relationship between MBC and SOC (Fig. 6d) indicates that SOC is an important source of C for soil microorganisms, and that higher SOC generally supports a greater microbial biomass C (Xu et al., 2018). Given that the forests in the study region are N-rich (Lu et al., 2018), the relationship between SOC and MBC could also indicate that the soil organic matter is an important source of other limiting nutrients, such as P (Stewart and Tiessen, 1987).

Conclusions

Our findings indicated clear differences in the protection of SOC between broadleaf and coniferous forest growing on the same soil type with different soil texture, which demonstrated that changes in forest tree species composition and soil texture could have long-lasting effects on C storage. The greater mass% of macroaggregates in the broadleaf forest can be attributed to the lack of soil disturbance. The majority of SOC in both forests was heavy fraction with physical protection or bounding to soil minerals, indicating that it is relatively stable. Importantly, our results indicate that the historic conversion of broadleaf forest to coniferous forest could have reduced soil C sequestration capacity in the long-term by altering the diversity and quality of plant inputs to the soil, which in turn affected macroaggregate formation, soil chemical properties and microbial biomass. Thus, our results highlight the importance of primary broadleaf forest in storing SOC and more attention should be taken on the protection of primary broadleaf forest. In addition, plant with higher fine root biomass or higher-quality litter may be a good choice during plantation establishment or afforestation projects.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

XKL and FLS designed this study. FLS, XKL, WBC collected soil samples and FLS did soil analysis. FLS and SX analyzed the data and drew the figures. XKL, FLS, SX, EJS, WBC and YD interpreted the results and wrote the paper.

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1 **Table 1** Comparison of soil properties in different soil layers in evergreen broadleaf forest (BF) and Masson pine coniferous forest (CF) in South China, showing

2 soil moisture, soil pH, total soil organic carbon (SOC), total soil nitrogen (N) and the soil C:N ratio, where Oe+Oa is the organic horizon; values shown are means

3 ± standard errors for *n* = 5. Different superscript letters represent significant differences among forests and soil horizons at *P*< 0.05.

Forest	Soil layer (cm)	Moisture (%)	pH	SOC (%)	Total N (%)	C:N ratio	SOC stock (kg/m^2)	Sand (%)	Silt (%)	Clay (%)	Bulk density (gcm^{-3})
CF	Oe+Oa	59±1.3[a]	3.43±0.05[d]	32.5±1.1[a]	1.35±0.07 [a]	24.2 ±0.7[a]	0.7±0.02[c]	-	-	-	-
	0-10	17±0.5[d]	4.01±0.04[a]	1.5±0.1[c]	0.10±0.00[d]	15.0±0.6[b]	1.9±0.09[b]	43.0*	42.5*	12.5*	1.52*
BF	0-10	26±0.6[b]	3.81±0.02[c]	3.1±0.4[b]	0.21±0.02 [b]	14.8±0.4[b]	2.6 ±0.34[a]	18.8*	61.3*	17.8*	0.94*
	10-20	23 ±0.4[c]	3.91±0.02[b]	2.0 ±0.2[c]	0.14±0.01[c]	13.9±0.5[b]	2.2±0.23ab	15.6*	63.0*	19.3*	1.28*

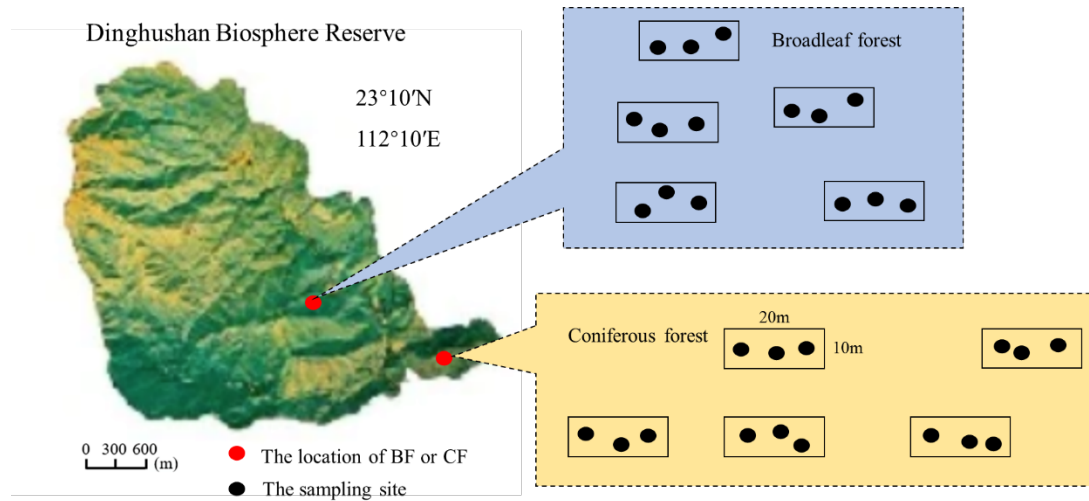4 * these data were reported by Zhang et al. (2012).

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Figure 1 Schematic map of the sampling location and the sampling scheme. BF, broadleaf forest; CF, coniferous forest. There are five random plots in each forest, with three sampling sites in each plot.
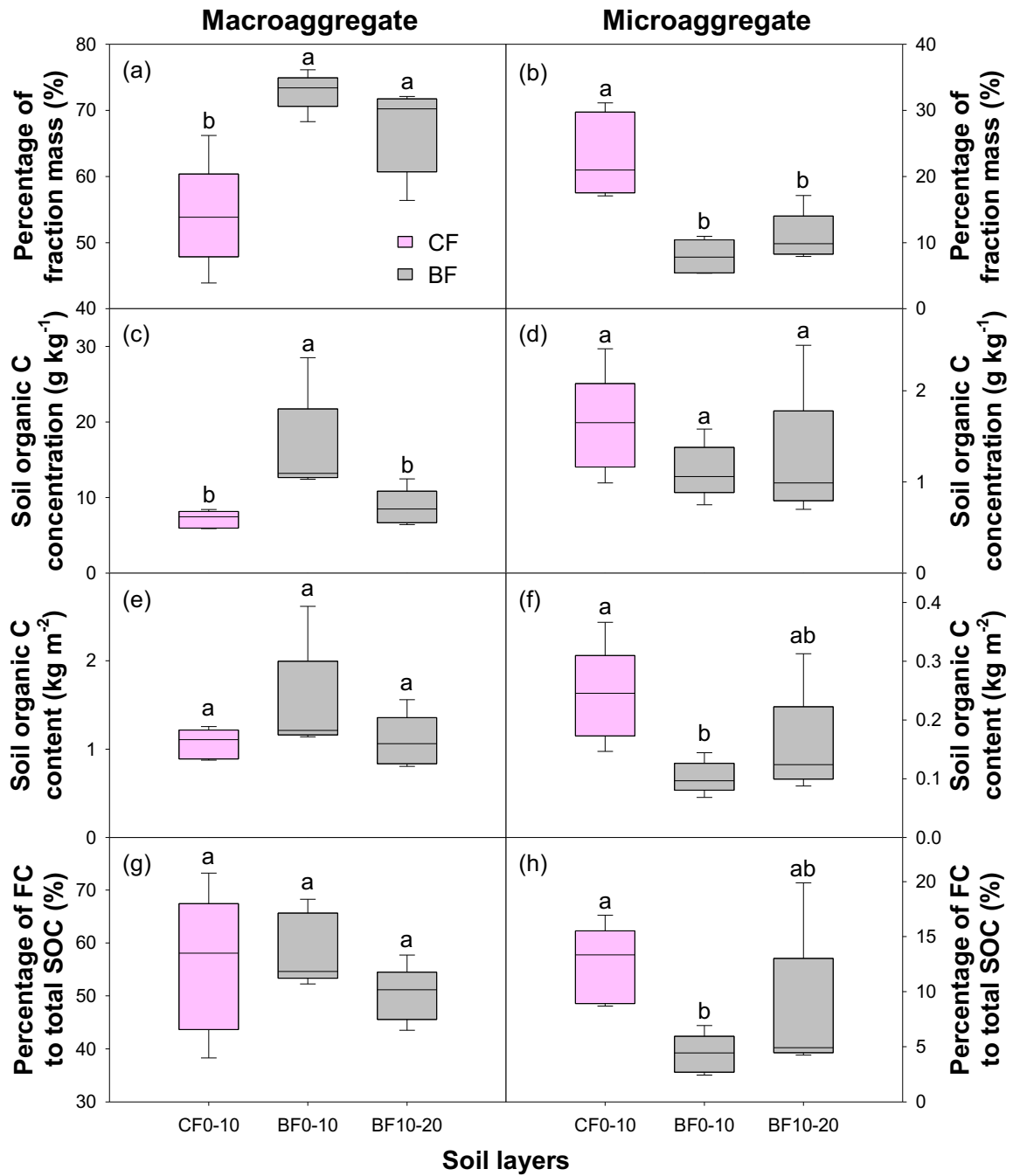
Figure 2 Differences in soil aggregates and associated soil organic carbon (C) at0-10 cm and 10-20 cm depth in coniferous forest (CF) and evergreen broadleaf forest(BF) in southeast China, showing (a,b) mass percentages, (c,d) soil organic carbon concentrations, (e,f) soil organic C stock, and (g,h) the percentage of total soil organic carbon (SOC)contained in macroaggregates (a,c,e,g) or microaggregates (b,d,f,h), where AC is aggregate-associated C. Box plots show the median, first and third quartiles, whiskers denote 1.5 × the upper and lower interquartile ranges, n = 5. Different lower-case letters represent significant differences between forests and soil depths at P< 0.05.
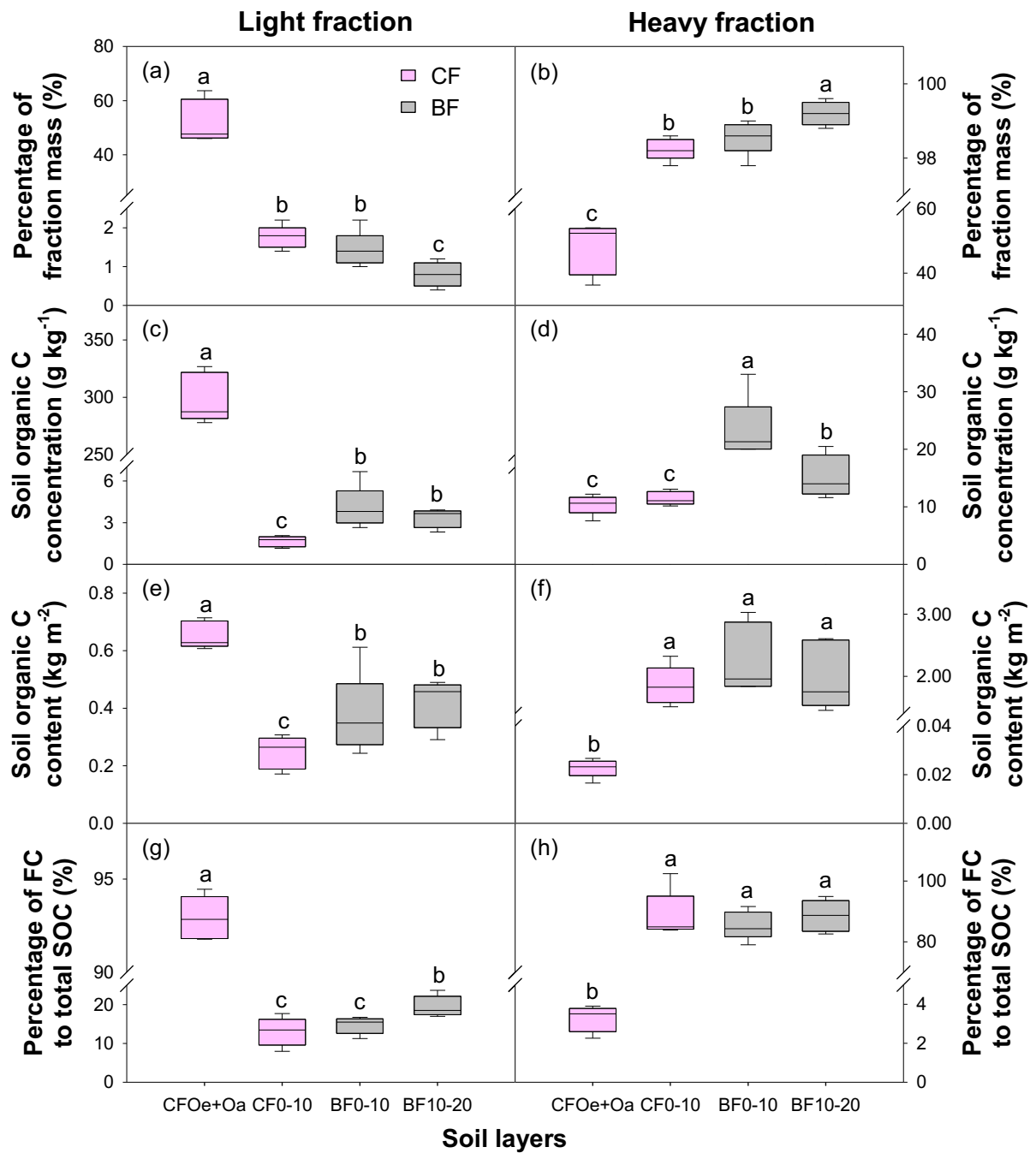
Figure 3 Differences in soil density fractions at 0-10 cm and 10-20 cm depth in coniferous forest (CF) and evergreen broadleaf forest(BF) in southeast China, showing (a,b) mass percentages, (c,d) soil carbon (C) concentrations, (e,f) soil C stocks, and (g,h) the percentage of total soil organic C (SOC) contained in the light fraction (a,c,e,g) or the heavy fraction (b,d,f,h), where FC is fraction-associated C. Box plots show the median, first and third quartiles, whiskers denote 1.5 × the upper and lower interquartile ranges, *n* = 5. Different lower-case letters represent significant differences between forests and soil depths at *P*< 0.05.
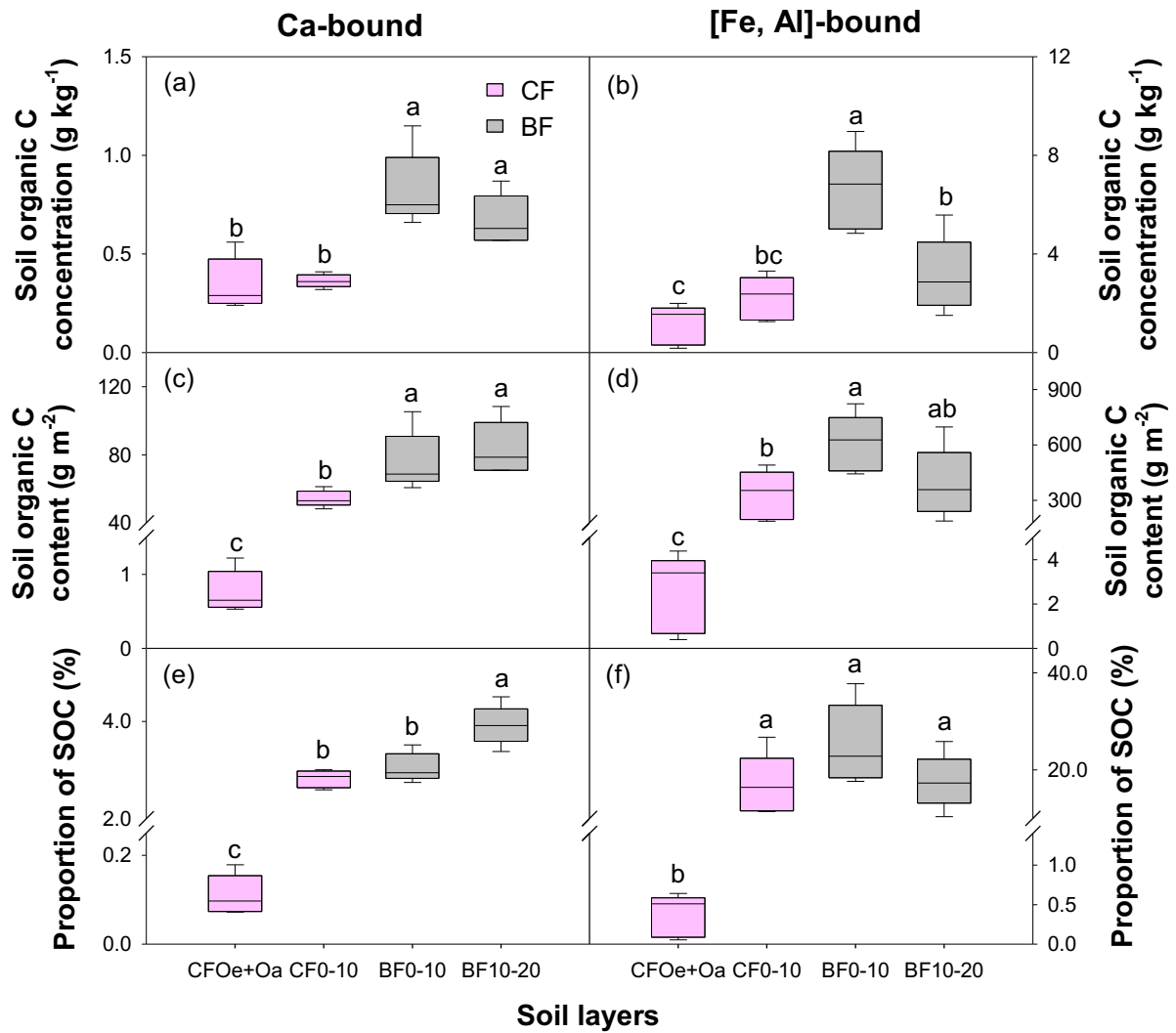
Figure 4 Concentrations of chemically bound soil organic carbon (C) in different soil layers (organic horizon, 0-10 cm and 10-20 cm) in coniferous forest (CF) and evergreen broadleaf forest(BF)in southeast China, showing concentrations of (a) calcium (Ca) bound (a) and iron or aluminum ([Fe, Al]) bound organic C, the stocks of (c) Ca-bound and (d) [Fe, Al]-bound (d) soil organic C and the percentage of total soil organic C (SOC) bound by (e) Ca and (d) [Fe, Al]. Box plots show the median, first and third quartiles, whiskers denote $1.5 \times$ the upper and lower interquartile ranges, $n = 5$. Different lower-case letters represent significant differences between forests and soil depths at $P < 0.05$.
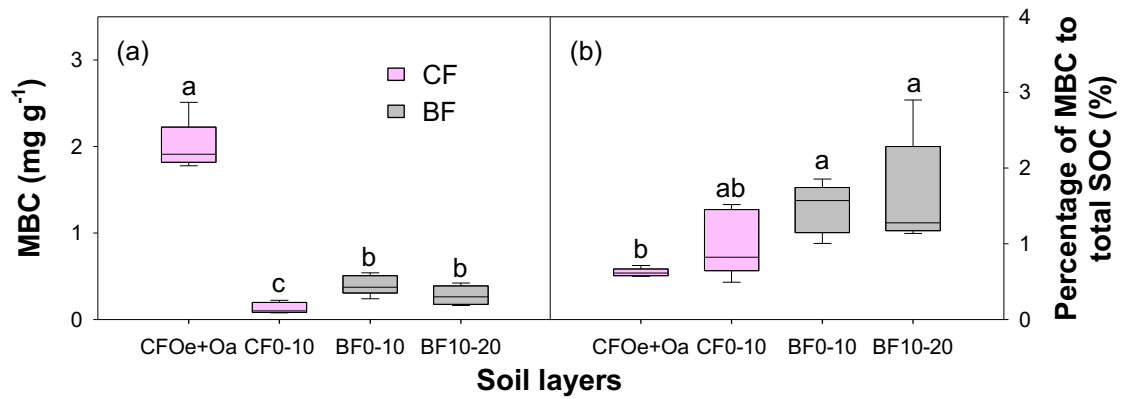
Figure 5 Microbial biomass carbon (MBC) in different soil layers (organic horizon, 0-10 cm and 10-20 cm) in coniferous forest (CF) and evergreen broadleaf forest (BF) in southeast China, showing, showing (a) MBC concentration and (b) the percentage contribution of MBC to total soil organic carbon (SOC). Box plots show the median, first and third quartiles, whiskers denote 1.5 × the upper and lower interquartile ranges. Different lower-case letters represent significant differences between forests and soil depths at *P*< 0.05.
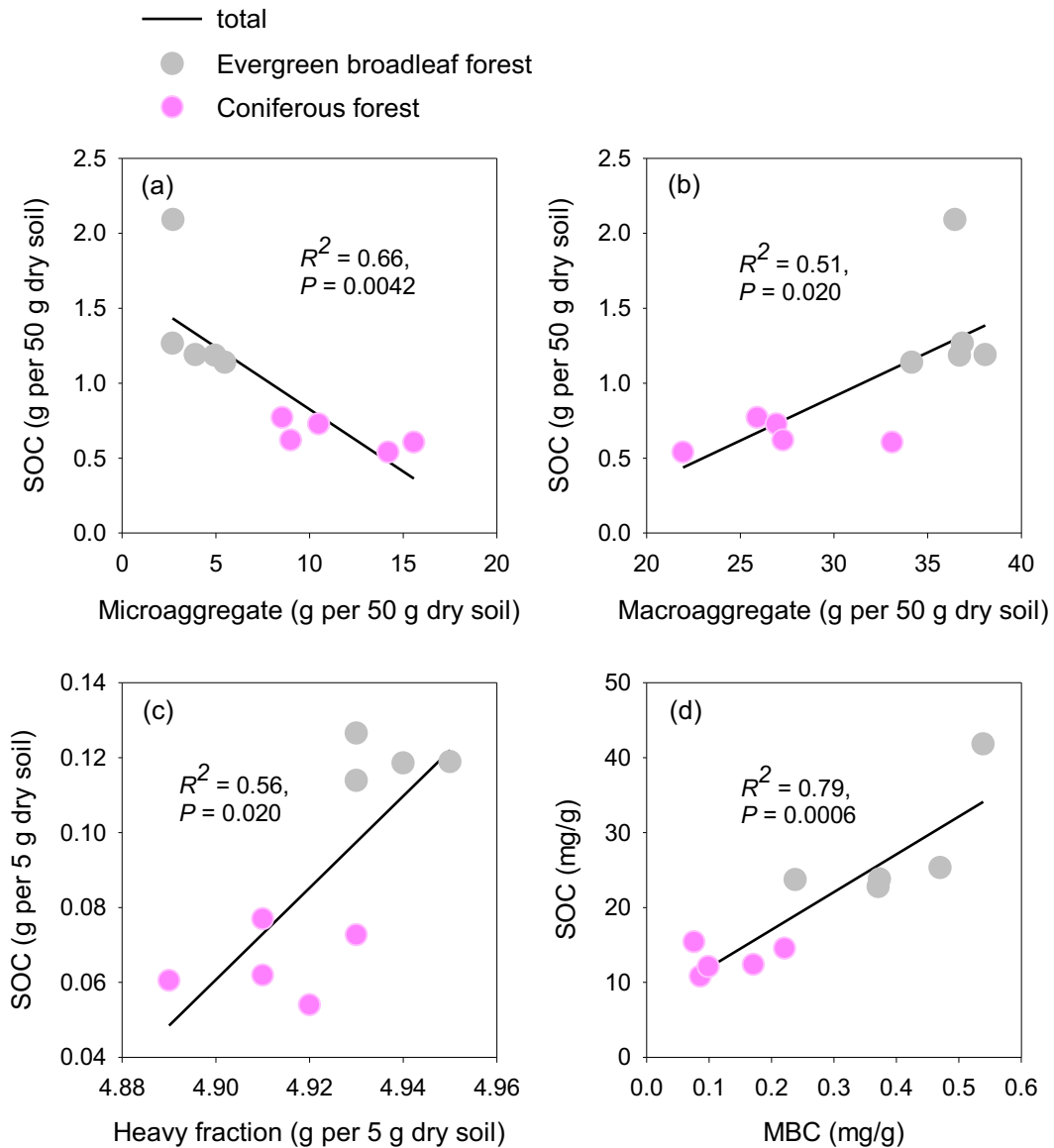
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Figure 6 Relationships between soil organic carbon (SOC) content and different soil fractions in coniferous forest (CF) and evergreen broadleaf forest (BF) in southeast China, showing the relationship between SOC and (a) microaggregate mass, (b) macroaggregates mass, (c) light fraction mass, and (d) microbial biomass carbon (MBC). The purple dots represent data from coniferous forest and the grey dots represent data from evergreen broadleaf forest. Regression lines are shown for significant relationships at $P<0.05$.