Density-Based Soil Organic Carbon Fractionation: Experimental Method Comparison and Influential Factors

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Research

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Abstract

Background: Because soil organic carbon (SOC) variation is a result of its physicochemical protection, fractionating SOC into different functional subpools according to its protection mechanism and studying the mechanism of different SOC fractions' responses to environmental change will help guide the study of SOC dynamics. Therefore, we conducted an analysis of density-based SOC fractionation of 107 study sites from 35 literature sources to answer the following questions: (1) Will different fractionation methods yield different amounts in the three organic carbon pools: free organic carbon (FOC), occluded organic carbon (OOC) and mineral associated organic carbon (MOC)? (2) Does the distribution of these three SOC fractions differ with climate (mean annual temperature, MAT; mean annual precipitation, MAP), soil characteristics (e.g., soil layer, soil type, clay content) or vegetation type when controlling for any method differences?

Results: Experimental method significantly affected OOC and MOC but not FOC results, and OOC separated by density and soil physical dispersion (density+disperse) was underestimated, thus a suitable SOC fractionation method should be carefully selected. SOC and MOC contents were negatively related to MAT; and highest SOC content appeared at moderate MAP, and when MAP increased or decreased, SOC decreased. SOC, FOC, and MOC were significantly affected by vegetation type; presumably due to anthropogenic disturbance or precipitation, plantations, grass and rainforest had the lower SOC contents and higher OOC and MOC percentages; and conifer, broadleaf, and mixed forests had similar FOC, OOC and MOC percentages, indicating less effect of tree species on SOC variation. The contents of both SOC and each fraction decreased in deeper sol layer; SOC, FOC and OOC contents were significantly affected by soil type; and SOC and MOC contents were negatively related to soil clay content, but the influences of soil characters on SOC and its fractions were less than experimental method and climate condition.

Conclusion: Experimental methods for fractionation of SOC significantly affected fraction results. Climate, vegetation type and soil character also significantly influenced SOC and its factions, but the influences of soil characters on SOC and its fractions were not as strong as experimental method and climate condition.

1. Introduction

Soil organic carbon (SOC) stores about three-quarters of the terrestrial organic carbon (Smith et al., 2008; Stockmann et al., 2013). However, because of the complexity of soil organic matter (SOM), the mechanisms of SOC responses to environmental changes are still unclear. Recent studies have indicated that this complexity resulted more from SOM physicochemical protection than from its chemical composition (Sollins et al., 1996; Mayer, 2004; von Lützow et al., 2006; Kleber, 2010; Schmidt et al., 2011). Fractionating the SOC pool into different subpools according to their different physicochemical protection mechanisms would help detect and predict SOC dynamics more exactly. However, discussion continues regarding how to effectively separate SOC into functional subpools which would better represent organic carbon dynamics.

Generally, according to the different SOC protection mechanisms, most previous research has fractionated SOC into three functional subpools: the mineral-associated SOC fraction (also known as micro-aggregation or primary organo-mineral interaction SOC), the physically isolated SOC fraction (also known as macro-aggregation or secondary organo-mineral interaction SOC), and the free SOC fraction (Sollins et al., 1996; Mayer, 2004; von Lützow et al., 2006; Schmidt et al., 2011). The mineral-associated SOC fraction is the most stable part of the SOC pool, characterized by tight bonding with mineral particles making SOC inaccessible to microbes and their enzymes (Jastrow and Miller, 1997; Six et al., 2002a; Eusterhues et al., 2003; von Lützow et al., 2006; Dungait et al., 2012). Physically isolated SOC is formed by aggregation or other processes such as intercalation with phyllosilicates or encapsulation in organic macromolecules (von Lützow et al., 2006). This SOC fraction is often thought to be moderately stable because of spatial inaccessibility to soil microbial decomposers (Christensen, 2001; Six et al., 2002b, 2004; von Lützow et al., 2006; Gupta and Germida, 2015). The free SOC fraction refers to SOC that is not protected by any of the mechanisms mentioned above (Sollins et al., 1996; Mayer, 2004; von Lützow et al., 2006; Schmidt et al., 2011).

1.1. Fractionation method based on density

Modeling SOC variation would be improved by using fitted appropriate methods to fractionate SOC into functional subpools according to physiochemical protection mechanisms. Density fractionation, accomplished with heavy liquids such as sodium iodide (NaI) and sodium polytungstate (SPT, $Na_6(H_2W_{12}O_{40})$), has shown great promise in elucidating SOM decomposition progression and mineral association status (Golchin et al., 1997; Baisden et al., 2002; Chenu and Plante 2006; Wagai et al., 2009; Jones and Singh, 2014). Usually, this method separates SOM into heavy fraction (HF) and light faction (LF). Heavy fraction, with density over 1.6-2.0 g cm^{-3}, mostly consists of organo-mineral associated complexes, and often has low C/N ratio and a higher degree of decomposition. In contrast, light fraction, either free or occluded, mostly consists of pieces of plant residues, often representing the active SOC pool (Christensen, 1992; von Lützow et al., 2007; Wagai et al., 2009). In order to better represent functional SOC subpools, many methods have been used to further fractionate SOC into three or more fractions.

The sequential density fractionation method can separate SOC according to the chemical bonds with mineral particles. Phyllosilicate dominates the fraction with a density of 1.8 to 2.2 g cm^{-3} and can be associated with oxidized OC species (C-O, C = O, O = C-O) or protonated amide forms by ligand exchange reactions and H-bonding (Jones and Singh, 2014; von Lützow et al., 2006; Kögel-Knabner et al., 2008). Iron oxides dominated fractions with density > 2.0 g cm^{-3} in the ferrosol and were abundant in monodentate and bidentate covalent bonds formed through ligand exchange between negatively charged organic domains (such as hydrolyzed carboxylic acids or phenolic compounds) and unsatisfied hydroxyl groups of Fe or Al oxides (Jones and Singh, 2014; Sollins et al., 1996; Mikutta *et al.*, 2007; Kögel-Knabner et al., 2008). Quartz and feldspar dominated fractions with density > 2.6 g cm^{-3} and were enriched in aliphatic C and protonated amide forms (Jones and Singh, 2014). Also, heavier density SOC would likely be more stable, as the bond between SOC with metal oxide mineral particles associated by ligand exchange is much stronger than the bond between SOC with phyllosilicates by polyvalent cation bridges (von Lützow et al., 2006; Mikutta et al., 2006; Kögel-knabner *et al.*, 2008).

To further separate the light density fraction from the mineral dominant fraction, Golchin et al. (1994) developed a technique using ultrasound energy (sonication) to disrupt aggregates and to break the bonds between detritus and mineral particles (Golchin et al., 1994; Rovira and Vallejo, 2003; Swanston et al., 2005). Other researchers have used glass beads or other methods to disrupt aggregates (Jastrow *et al.*, 1996; Six et al., 1998; Gaudinski et al., 2000; Lima et al., 2006; Schwendenmann *et al.*, 2006; Karhu et al., 2010). The third fraction isolated by these methods, named as "occluded", "aggregate-protected" or "mineral-associated low density" fraction, is commonly designated as an intermediary SOM that forms during the progressive decay of plant detritus accompanied by mineral association (Golchin et al., 1994; Golchin et al., 1997; von Lützow et al., 2006; Wagai et al., 2009); Yet the review of Wagai et al. (2009) showed that the chemical composition of this fraction varied greatly among different studies and had variable mean residence times.

Some researchers have used other physical or chemical methods combined with the density method to isolate a third SOC fraction. One common approach used chemical extractions after density fractionation. After removing LF (mainly containing rather fresh plant materials), the remaining HF was further characterized chemically by acid hydrolysis or oxidation (Trumbore and Zheng, 1996; Trumbore et al., 1989; Echeverría et al., 2004; Helfrich et al., 2007). Several chemical fractionation methods, such as hydrolysis by hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) and oxidation by hydrogen peroxide (H_2O_2) or hydrofluoric acid (HF), have been used in isolating stable SOC (von Lützow et al., 2007; Paul, 2016). Using both ^{13}C and ^{14}C analysis, wet oxidation with H_2O_2 can remove younger SOC (Eusterhues et al., 2005; Helfrich et al., 2007). Residues after H_2O_2 treatment were predominantly made of polymethylenic type OM (e.g., waxes, vascular plant residues), N-containing compounds (derived from mineral surface interaction), and pyrogenic materials (e.g., lignite, charcoal, and ash) (Eusterhues et al., 2005; Leifeld and Kögel-Knabner, 2001; Mikutta et al., 2006). Acid hydrolysis using 6M HCl could effectively remove carbohydrate and protein materials by disrupting hydrolytic bonds, and the residues were mostly biologically recalcitrant alkyl and aryl materials (e.g., lignin, suberin, cutin, and waxes) (von Lützow et al., 2007). However, polyvalent cations involved in clay flocculation or microaggregation can also be removed by acid treatments, which results in increasing solubility of occluded and complexed SOM (Oaodes, 1988; von Lützow et al., 2007). Although Paul et al. (1997) indicated that the HCl-hydrolyzable pool generally was about 1500 years BP younger than the non-hydrolyzable C pool in surface soils, Balesdent (1996) suggested that acid hydrolysis was not able to separate functional OM pools. HF was used to separate mineral-associated OM from non-mineral associated OM by dissolving the hydrated silicate minerals and Fe/Al oxide-type organo-mineral complexes (von Lützow et al., 2007), and the remaining non-hydrolyzable SOM in HF fractions of temperate soils represents an extremely resistant SOM pool with a minimum estimate of ^{14}C content (Trumbore and Zheng, 1996). However, this method was ineffective in isolating old C from tropical soils (Trumbore and Zheng, 1996). Additionally, this removal procedure might not completely remove the mineral-associated OM fraction, has unclear stabilization mechanisms, and the removing effect also strongly depends on pedogenesis (Eusterhues et al., 2007). Another method combines density fractions with particle size fractionation (Six et al., 2002c; John et al., 2005). This fractionation is based on the concept that sorption is an important stabilization mechanism. SOM absorbed by smaller particles often has a higher degree of decomposition (more alkyl-C, lower C/N ratio) and a longer turnover time (von Lützow et al., 2007).

The wide variety of SOC fractionation methods makes the results of different studies difficult to compare. An evaluation of these fractionation methods is needed to assist in choosing suitable SOM fractionation methods or to further develop appropriate new methods.

1.2. Factors affecting SOC fractionation dynamics

Many factors, such as climate, vegetation, and soil characteristics can affect SOC fractionations. Evaluating those effects can provide a better understanding of their influential mechanisms, and potentially improve predictions of SOC responses to future environmental changes. Our previous study (Sun et al., 2019) showed that total SOC content decreased along an increasing mean annual temperature (MAT) gradient in eastern China, but was not affected by vegetation type or soil characteristics. However, we additionally wanted to know if this result was universal, or just an isolated phenomenon.

Although our previous study showed no significant trend of different SOC fractions with MAT, many other studies have indicated that labile SOC is decomposed more easily as temperature increases, which leads to an overall more resistant SOC pool (Garten and Hanson, 2006; Fissore et al., 2008; Garten, 2011; Du et al., 2014; Pisani et al., 2014; Tian et al., 2016). More data from different studies would help to resolve this contradiction. How precipitation affects SOC processes and fractionation is much more complex (Cotrufo et al., 2013), and few studies have indicated how precipitation could affect SOC by changing soil microbial and enzyme activities (Kang and Freeman, 1999; Turner, 2010).

Our previous result showed no difference in SOC fractions among different forest types, as also reported in some other studies (Fissore et al., 2009; Zimmermann *et al.*, 2010). However, some studies indicated that vegetation could affect SOC pool size by changing soil microbial community composition through differing quantity and quality of organic matter inputs and by altering the micro-environment and soil characteristics (Quideau et al., 2001; Stockmann et al., 2013; You et al., 2014). For example, aggregate formation was strongly affected by hypha and root hair amount in the rhizosphere, and as a result, different species would indirectly affect SOC protection (Amézketa, 1999; Gupta and Germida, 2015).

Soil type can also affect the size of the SOC pool due to differences in soil mineralogy, soil texture, and other soil physiochemical characteristics. Mathieu et al. (2015) reviewed studies of soil radiocarbon profiles and indicated that SOC dynamics at deeper soil depths are driven more by soil type than by climate. For example, soil mineralogy can alter the chemical protection of SOC by controlling the number and type of chemical bonds formed with organic carbon (von Lützow et al., 2006; Jones and Singh, 2014; Paul, 2016). And compared with larger soil particles, SOC (usually the alky-C) interactions with clay particles often has a longer mean residence time (von Lützow et al., 2006; Paul, 2016). However, soil effects often interacted with climate and vegetation, making it hard to determine their true effect.

To evaluate different SOC fractionation methods based on density and to determine how environmental factors affect SOC fractions, we compared SOC and its fractions at 107 sites reported in 35 published studies, and attempted to answer these questions:

1. Will different methods of SOC fractionation yield different amounts of different functional SOC subpools (free SOC, occluded SOC, and mineral-associated SOC)?
2. Does the distribution of these three SOC fractions differ with climate (temperature, precipitation), soil characteristics (soil layer, soil type, clay content) or vegetation type when controlling for any method differences?

2. Materials And Methods

2.1. Data collection

Because density fractionation is widely applied and provides a useful explanation mechanism for SOC fractionation, we only chose those studies based on density fractionation methods among all the available soil fractionation studies. We extracted SOC fractionation results at 107 study sites from 35 previsously published studies throughout the world (226 data recordings) (Fig. 1). All data regarding fractionation methods, organic carbon content of soil and each fraction, study location, climate characteristics (MAT and mean annual precipitation, MAP), soil type, vegetation type, soil layer, and other soil properties were also collected.

2.1.1. SOC fractionation data

Fractionation method: All the SOC fractionation data we collected from each study was based on the density fractionation method. Because the fractionation methods differed somewhat among the studies, after comparing all studies, we divided the fractionation methods into three categories: 1. density: includes SOC fractionation separated by multiple density; 2.density + disperse: includes SOC fractionation separated by density before and after soil physical dispersion; 3. density + other: includes SOC fractionation separated by density and other physical and chemical methods.

SOC Fraction: To better compare the results of each study and to explain the results mechanically, instead of using the original fractions, we categorized the fractions of some studies (which fractionated SOC into more than three fractions) into only three fractions: free organic carbon (FOC), occluded organic carbon (OOC), and mineral associated organic carbon (MOC). Usually, by considering the SOC physicochemical protection mechanism, we were able to combine two or more fractions in those studies to create the three fractions that we used for our analysis. For the studies using the multiple density method, we assigned the heavy fractions with density > 2.0 or 2.2 g cm^{-3} to MOC, the light fractions with density < 1.6 g cm^{-3} to FOC, and the medium density fractions to OOC. For the studies using the density + disperse method, we classified light fractions with density < 1.6 to 1.85 g cm^{-3} before ultrasonic or physical dispersion as FOC, classified light fractions with the same density after dispersion as OOC, and classified heavy fractions with density > 1.6 to 1.85 g cm^{-3} after dispersion as MOC. For the literature sources using density + other methods, we classified the light fraction without dispersion as FOC, classified the resistant heavy fractions or OC bond to fine soil particles (e.g., slit or clay) as MOC, and classified the rest as OOC. The details of how to reassign SOC fractions for each literature source are provided in the appendix.

Soil layer: To easily compare results from previously published literature with soil divided into more than one layer, we divided soil data into only two different layer groups: an upper layer and a deeper layer. The upper layer included soil data from 0–30 cm soil layer or the A horizon. The deeper layer included soil data from the soil layer deeper than 30 cm or the B or C horizons. If the top soil layer was greater than 30 cm, we also included this layer in upper layer.

2.1.2. Other data

Location: Latitude, longitude, and altitude were primarily collected from the literature sources and their references (of the same study site). If those data were not reported, the latitude, longitude, and altitude were determined from Google Earth by using the study site description provided in the reference.

Climate data: We collected MAT and MAP data for each study site. Those data were also primarily collected from the literature and their references. When climate data were not provided, the location coordinates were used to extract climatic values from the CRU database (http://www.cru.uea.ac.uk; New *et al.*, 2000).

Soil characteristics: In addition to SOC data, we also collected soil clay content, soil clay CEC values, and soil type for each study site. For all study locations, those data were also primarily collected from the literature and their references. For soil type, we use the FAO soil type. When soil type was reported according to the USDA soil taxonomy or to a local classification, an equivalent FAO classification was added to the database according to other studies on the same sites. If those data were not mentioned, we completed our database by cross referencing information with other better referenced studies, and ultimately, default data was extracted from the Harmonized World Soil Database (V 1.2).

Vegetation type: Vegetation type for each study site was divided into seven broad categories: conifer forest, mixed forest, broadleaf forest, rainforest, grass, plantation, and forest and grass (including savanna and other ecosystems with both forest and grass).

2.2. Statistical analysis

For easier comparisons, when organic carbon was reported as carbon stock (C_t t ha^{-1}), we converted those values into carbon content (C_c g kg^{-1}) as follow:

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$$C_c = \frac{C_t}{BD \times D \times 10}$$

where BD is soil bulk density (g cm^{-3}), and D is soil sampling depth (cm).

When calculating the weighted mean and error of each SOC fraction, sample size was regarded as the sample number reported in the literature. If no sample number was reported, the sample size was defined as 1.

The effect of study method, soil layers, vegetation type, and soil type on different OC fractions was tested by ANOVA. To eliminate the effect of fractionation method, the effect of MAT, MAP, vegetation type, and soil type on different OC fractions was tested again by ANCOVA by using fractionation method as a covariant. The effects of MAT, MAP and clay content on each SOC fraction was tested by mixed linear model. To eliminate the effect of fractionation method, it was defined as a fixed factor in the mixed linear model. All data were analyzed using R 3.6.1 (www.r-project.com) with significance defined as $\alpha < 0.05$, and all figures were made by R 3.6.1 with “ggplot2” package.

3. Results

3.1. The effect of study methods

The percentage and content of FOC showed no significant difference among the different study methods, and the average of FOC content and percentage was 8.74 ± 0.46 g kg^{-1}soil and 21.14 ± 0.61 % (Fig. 2). However, the percentages and contents of MOC and OOC fractions had significant differences among the three different methods (for content: df = 2, F = 26.71 and 3.53, $p < 0.001$ and = 0.031; for percentage: df = 2, F = 28.33 and 16.25, p all < 0.001). The OOC percentage of density + disperse method was lowest (10.55 ± 0.69 %), the OOC percentage of density method was highest (47.54 ± 5.03 %), and the MOC percentage of density method was lowest (28.36 ± 4.13 %).

3.2. The effect of climate

The total SOC and MOC contents were negatively related to MAT (adjusted R^2 = 0.017 and 0.017, p = 0.029 and 0.027) (Fig. 3a, c), SOC and MOC decreased 0.71 and 0.39 g kg^{-1} with a 1 °C MAT increase, and the mixed liner model showed these patterns were not affected by fractionation method. The total SOC and MAP was second order related (adjusted R^2 = 0.086, $p < 0.001$) (Fig. 3b), according to their fitting formula ($SOC = -2.51 \times 10^{-5} MAP^2 + 0.073 MAP - 4.01$), the highest SOC content appeared at 1450 mm annual precipitation. However, different SOC factions have different variation pattern with MAT and MAP under different method; the FOC content determined by the density+disperse method was significantly negatively related to MAP (p = 0.012, adjusted R^2 = 0.069, Fig. 3d), the OOC content determined by the density + disperse method was significantly positively related to MAT (p = 0.017, adjusted R^2 = 0.061, Fig. 3e), and the MOC content determined by the density method was significantly negatively related to MAT (p = 0.027, adjusted R^2 = 0.017, Fig. 3f).

3.3. The effect of vegetation type

Total SOC, FOC, and MOC contents were significantly affected by vegetation type (F = 5.97, 3.58, and 5.10, p < 0.001, = 0.002, and < 0.001), and after controlling the effect of fractionation methods, the ANCOVA results also showed that SOC, FOC, and MOC contents were significantly different among different vegetation types (F = 6.09, 3.54 and 4.38, p < 0.001, = 0.002 and < 0.001). The plantations in our database were mostly located in warm areas (mean MAT = 18.9 ± 0.5 ^{o}C) and had the lowest SOC content (17.38 ± 1.42 g kg^{-1}), highest MOC percentage (71.82 ± 6.74 % and 12.55 ± 1.55 g kg^{-1} for content), highest OOC percentage (36.49 ± 7.94 % and 5.92 ± 1.31g kg^{-1} for content) and lowest FOC content (3.57 ± 0.37 g kg^{-1} and 20.96 ± 1.88 % for percentage) (Fig. 4). Grass, with the lowest precipitation (873 ± 62 mm) had the second lowest SOC content (27.14 ± 3.82 g kg^{-1}) and lowest FOC percentage (11.09 ± 1.92 % and 3.73 ± 0.80 g kg^{-1} for content), and rainforest with the highest precipitation (2298 ± 30 mm) in the warm area (mean MAT = 18.3 ± 0.7 ^{o}C) also had lower SOC content (31.23 ± 2.98 g kg^{-1}). Broadleaf, mixed, and conifer forests had moderate SOC content (32.87 ± 2.41, 51.44 ± 4.12, and 53.90 ± 2.32 g kg^{-1}) and similar FOC, OOC and MOC contents and percentages. The forest and grass vegetation type had the highest SOC content (72.89 ± 13.38 g kg^{-1}), highest FOC content (19.92 ± 3.68 g kg^{-1}) and percentage (29.83 ± 5.78 %), and highest OOC content (27.96 ± 6.65 g $kg^{-1}$and 36.49 ± 7.94 % for percentage).

3.4. The effect of soil characteristics

Total SOC and all fractions contents were significantly lower in the deeper soil layer than in the upper soil layer (F = 58.02, 36.11, 11.10and 32.36, p all $\leq$ 0.001, Fig. 5a). Also, the percentages of FOC, and OOC were significantly greater in the upper soil than in the deeper soil layer (F = 6.65 and 8.42, p = 0.011 and 0.004), but MOC percentage was significantly lower in the upper soil than in the deeper soil layer (F = 24.34, p < 0.001, Fig. 5b). After controlling for the effect of fractionation methods, the ANCOVA test result showed that SOC content, FOC, OOC and MOC contents and percentages were also significantly different between the upper and deeper soil layers.

Total SOC, FOC and OOC contents (F = 4.73, 1.88, and 39.09, p < 0.001, = 0.034, and < 0.001, Fig. 6) and FOC, OOC and MOC percentages were significantly affected by soil type (F = 3.50, 5.56 and 7.53, p all < 0.001, Fig. 6). And the ANCOVA test result showed similar result after controlling for the effect of fractionation methods. The SOC content of andosols was largest (82.43 ± 22.04 g kg^{-1}) with the highest OOC content and percentage (61.92 ± 18.55 g kg^{-1} and 73.36 ± 7.94 %) and lowest FOC and MOC percentages (11.08 ± 2.00 and 10.33 ± 2.00 %). The SOC contents of lithosols and podsols were mederately high (55.25 ± 8.94 and 47.31 ± 2.40 g kg^{-1}) with highest FOC content and percentage for

 her MOC content for podsols (33.21 ± 1.41 g

kg^{-1} and 70.05 ± 3.04 % for percentage), but the MOC percentage of ferralsols was highest (77.65 ± 96.74 % and 21.57 ± 1.87 g kg^{-1} for content). The SOC of fluvisols (17.1 ± 6.35 g kg^{-1}) was lowest with lowest FOC and MOC content (3.91 ± 1.58 and 5.37 ± 1.74 g kg^{-1}). Luvisols, vertisols, leptosols and nitisols also have mederately low SOC contents (25.78 ± 1.94, 28.70 ± 4.63, 31.00 and 33.05 ± 4.68 g kg^{-1}, respectively), and the OOC content and percentage of nitisols were lowest (2.48 ± 0.94 g kg^{-1} and 5.58 ± 1.53 %).

The mixed liner model showed that after controlling for the effect of fractionation methods, the total SOC and MOC contents were negatively related to soil clay content (p = 0.023 and 0.029) (Fig. 7a, b), SOC and MOC decreased 0.27 and 0.15 g kg^{-1} with a 1% soil clay content increase. And the MOC percentages determined by different methods have different relationship with soil clay content (Fig. 7c), MOC percentage determined by density method negatively related to soil clay content (adjusted R^2 = 0.013, p = 0.050), but MOC percentage determined by density + other method positively related to soil clay content (adjusted R^2 = 0.028, p = 0.040).

4. Discussion

4.1. Effect of fractionation methods

Our results indicated fractionation method did not significantly affect the percentage and content of FOC (Fig. 2). This was because no matter which experimental method was used, all studies determined FOC as the LF fraction using with similar density (< 1.6 to 1.85 g cm^{-3}).

The percentage and content of MOC and OOC fractions were significantly different among the three different fractionation methods, with the highest OOC and lowest MOC percentages determined by the density method, and highest the MOC and lowest OOC percentages determined by the density + disperse method (Fig. 2). These differences were caused by the different experimental procedures used by those methods. With the density method, the fraction with medium densities of 1.6 to 2.0 or 2.2 g cm^{-3} was categorized as OOC. In contrast, the density + disperse method categorized light fractions (density < 1.6 or 1.8 g cm^{-3}) after dispersion as OOC, resulting in the density method including a greater proportion of OC as OOC due to its larger density range, and thus leading to a lower MOC percentage for the density method. SOC fractionations separated by multiple density methods could partly represent SOC stability due to their different dominant chemical bonds. However, this needs to be combined with mineral analyses by soil pedogenesis, thus limiting the application of this method. Also, to better understand the role that SOC mineral associated protection plays in SOC turnover processes, research regarding residue time or age of different SOC fractions by the multiple density method are needed. The density + disperse method was the most popular method due to its easy operation and clear mechanism. However, the underestimation of the OOC fraction made it difficult to ideally represent the intermediate SOC pool (Jones and Singh, 2014; von Lützow et al., 2006; Kögel-Knabner et al., 2008; Wagai et al., 2009). The

 methods based on density (density + other) varied in this study, and the MOC and OOC percentages of these methods were moderate compare to the other two methods (density and density + disperse). Some research has already been done to compare these methods with same soil samples, and those studies indicated that the results of density combined with particle fractionation or H_2O_2 oxidation might better represent the functional SOC pool (von Lützow et al., 2007; Eusterhues et al., 2005; Helfrich et al., 2007). Thus, careful consideration of SOC protection mechanisms and other affected factors (e.g., soil pedogenesis and mineral characteristics) should be conducted before a suitable SOC fractionation method can be chosen.

4.2. The effect of climate, soil characteristics, or vegetation type

4.2.1. Climate

The total SOC content and MOC content were both negatively related to MAT, which indicated a decreasing quantity of SOC with increasing temperature. Although most previous studies indicated a SOC decrease with increasing temperature, yet a relative more decrease of labile or active OC fraction were often reported in other researches (Garten and Hanson, 2006; Fissore et al., 2008; Garten, 2011; Du et al., 2014; Pisani et al., 2014; Tian et al., 2016); as the decomposition rate of labile SOC increased more rapidly with increasing temperature caused by both a lower decomposition activation energy for FOC (as its labile chemical composition) and the higher availability of free SOC to micro-organisms and enzymes (due to lack of physical and chemical protection) (Davidson and Janssens, 2006; Conant et al., 2011; Paul, 2016). The great variation of soil OC content as in our studies might weaken the effect of temperature and making the influence of MAT on FOC and OOC fractions not significant. Also, as our results showed no significant variation pattern of MAT on any fractions percentage, the decreasing MOC content with MAT might merely be caused by the decreasing total SOC with MAT.

Our result also indicated that the highest SOC content appeared at moderate MAP (about 1450 mm annual), and when MAP increased or decreased, SOC content decreased. This result was similar to other studies which indicated that in wetland regions, reducing precipitation increased soil respiration and reduced SOC storage by relieving an oxygen limitation, while in arid and semi-arid climates, increasing precipitation increased microbial metabolism and thus stimulated SOC decomposition (Davidson and Janssens, 2006; Bradford et al., 2016; Nielsen and Ball, 2015).

What's more, in our study, different SOC factions have different variation pattern with MAT and MAP under different method; so, when the SOC fractions of different research studies needed to be compared, the fractionation experimental method should be same, or the effects of experimental method should be carefully excluded previously.

4.2.2. Vegetation

Our analysis showed that vegetation type significantly influenced total SOC, FOC and MOC contents after eliminating experimental method effects. Plantations had the lower SOC content, presumably due to the less SOC input by intense anthropogenic disturbance. Grass and rainforest with lowest and highest MAP also had lower SOC with lower percentage of FOC, which was consistent to our previous result that SOC content was highest in the moderate MAP. In grass, less SOC input as lower plant productivity and less litter quantity lend to a lower SOC content and lowest FOC percentage (Guo and Gifford, 2002); however, the OOC percentage of grass was high, this might because that the proportion of underground biomass of grass was much higher than forest, which help formatting aggregated occluded OC (Amézketa, 1999; Gupta and Germida, 2015). In rainforest, hot and humid environment might accelerate the decomposition rate of SOC, especially labile SOC, and lend to a lower SOC content and FOC percentage (Davidson and Janssens, 2006; Smith et al., 2008; Conant et al., 2011). The high SOC content for the forest and grass vegetation type might be caused by the higher OOC content in the research of Baisden et al., (2002). As most forest and grass SOC data from this research had a higher OOC percentage when using the multiple density method, the OOC fraction of forest and grass might have been overestimated. Conifer, broadleaf, and mixed forests had moderate SOC content and similar FOC, OOC and MOC contents and percentages, indicating less effect of tree species on SOC variation over the large scale of our study.

4.2.3. Soil characteristics

Both total SOC and each fractions contents were significantly lower in the deeper sol layer than in the upper layer, and this relationship was not affected by experimental method, this result was similar to other study (Jobbágy and Jackson, 2000).

Total SOC, FOC and OOC were significantly affected by soil type. The largest SOC content of andosols with the highest OOC content and percentage was caused by the data extracted from Golchin et al. (1997). This study was conducted in a forest and grass that experienced annual burning. Fire can create a large abundance of complex, highly condensed, aromatic chemicals known as black carbon or charred OM. This type of SOC, with usually long residence time (500–10,000 years), was categorized as OOC according to the density + disperse method that they used (Skjemstad et al., 1998; Schmidt et al., 2002). Yet there is growing evidence that charred OM is not inert in soils and can be degraded by adding an easily available C source (the priming effect) (Bird *et al.*,1999; Hamer et al., 2004). The high OOC percentage for the fluvisols might because that all fluvisols data was extracted from the research of Baisden et al., (2002) which using the multiple density method. MOC contents and percentages of acrisols, ferralsols, podsols and cambisols were higher, and the pedogenic processes for acrisols, ferralsols, cambisols and podsols prefer to form secondary iron oxyhydroxides (e.g., ferrihydrite, goethite, and lepidocrocite) or hydroxyaluminium silicate (e.g., allophane and kaolinite) (Farmer, 1982; Jordanova, 2017), which bond with negatively charged organic domains through ligand exchange to form a stable organo-mineral complex (Jones and Singh, 2014). Considering there were only few recordings (1 to 4 sites) for vertisols, lithosols, leptosols and nitisols in our database, it would be too arbitrary to discuss the influences of soil characters for these soil types.

Differ from common thoughts that high clay amount would supply more surface area to interact with SOM, thereby leading to more MOC (Dexter et al., 2008; Malamoud et al., 2009; Mathieu et al., 2015), our

 ely related to soil clay content, this might because that the highly positive relationship of clay content and MAT (adjusted $R^2 = 0.321$, $p < 0.001$), which negatively related with SOC and MOC, weaken the impacts of clay itself. Also, the result that MOC percentages determined by different methods have different relationship with soil clay content, this indicated that the influences of soil characters on SOC and its fractions were not as strong as experimental method and climate condition.

5. Conclusions

In conclusion, experimental methods for fractionation of SOC significantly affected fraction results. Careful consideration of SOC protection mechanisms and other affected factors should be conducted before a suitable SOC fractionation method is selected. SOC and MOC contents were both negatively related to MAT, and the highest SOC content appeared at moderate MAP, when MAP increased or decreased, SOC content decreased. Vegetation type significantly influenced total SOC, FOC and MOC contents after eliminating experimental method effects. Both total SOC and each fractions contents were significantly lower in the deeper sol layer than in the upper layer, total SOC, FOC and OOC were significantly affected by soil type, and SOC and MOC contents were negatively related to soil clay content, but the influences of soil characters on SOC and its fractions were not as strong as experimental method and climate condition.

Declarations

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Availability of data and materials

Available on request.

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Authors’ contributions

MGR, OJS, and XS designed the research. Data were collected and analyzed by XS and ZT. Manuscript was prepared by MGR, OJS, and XS. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not Applicable.

Consent for publication

Not Applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

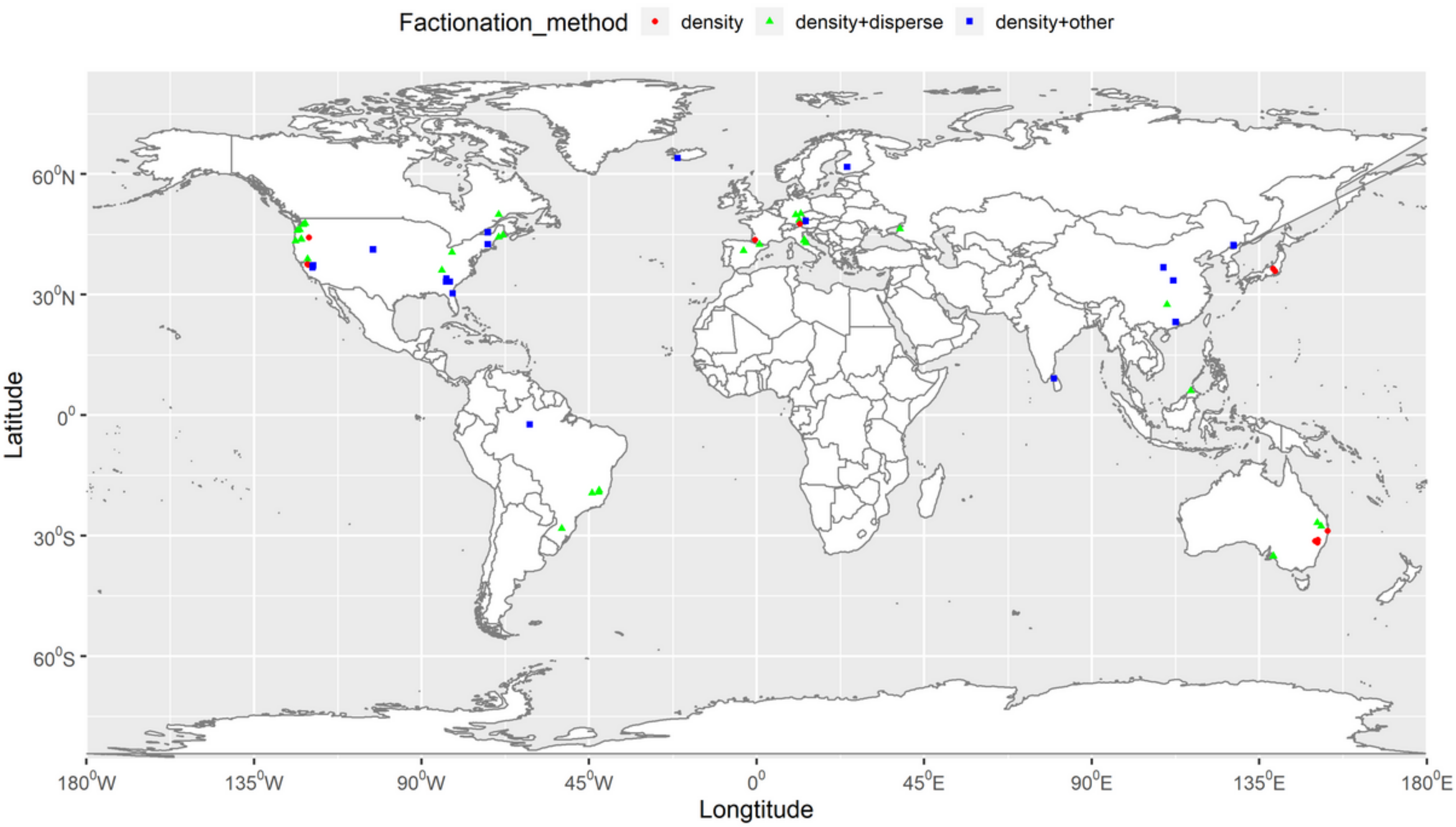

Figure 1

Distribution of 107 study sites from 35 published studies.

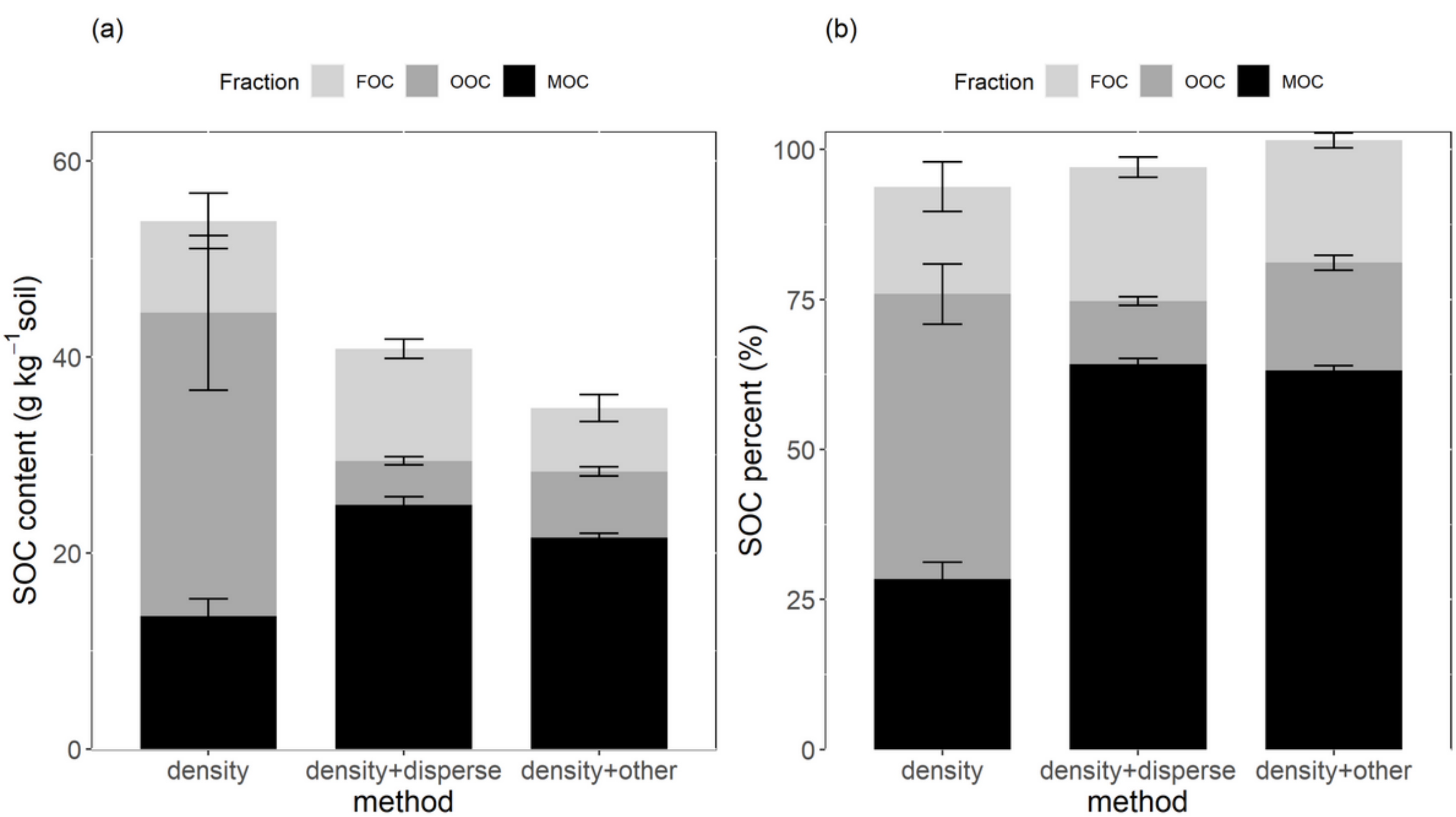

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Figure 2

The content (a) and percentage (b) of different soil carbon fractionations by three different experimental methods. The three SOC fractionations were free organic carbon (FOC), occluded organic carbon (OOC), mineral associated organic carbon (MOC). The three experimental methods were density (SOC fractionation by multiple density), density+disperse (SOC fractionation by density before and after soil physical dispersion, and density+other (SOC fractionation by density and other physical and chemical methods)

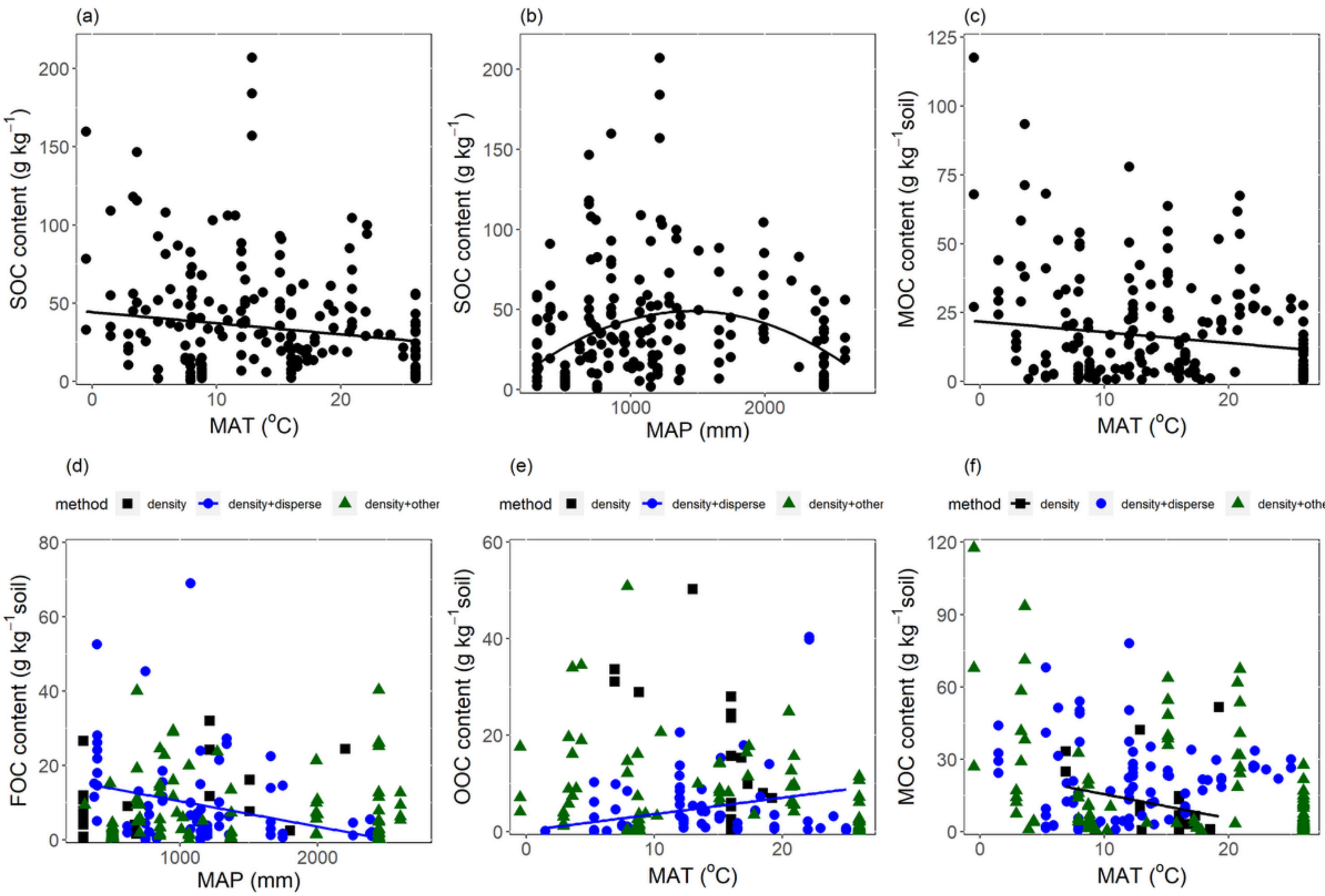

Figure 3

The relationship of soil organic content (SOC) and different soil carbon fractionations with mean annual temperature (MAT) and mean annual precipitation (MAP) under three different experimental methods. The three SOC fractionations were free organic carbon (FOC), occluded organic carbon (OOC), mineral associated organic carbon (MOC). The three experimental methods were density (SOC fractionation by multiple density), density+disperse (SOC fractionation by density before and after soil physical dispersion, and density+other (SOC fractionation by density and other physical and chemical methods). (a) The relationship of total SOC content with MAT; (b) The relationship of total SOC content with MAP ; (c) The relationship of MOC content with MAT; (d) The relationship of FOC content with MAP under different experimental methods; (e) The relationship of OOC content with MAT under different experimental methods; (f) The relationship of MOC content with MAT under different experimental methods.

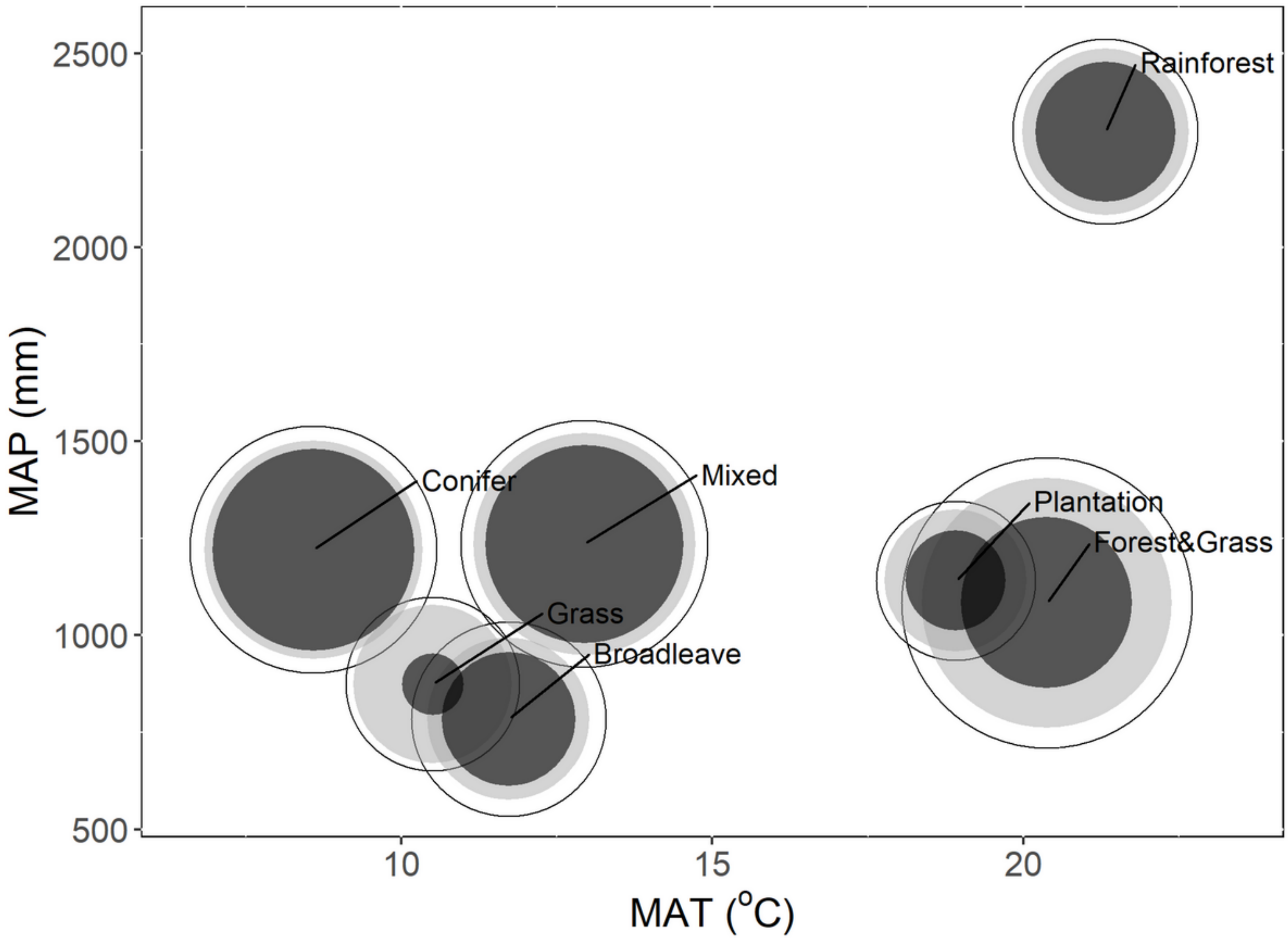

Figure 4

Soil organic carbon (SOC) and its fractionation contents with different vegetation types. The area of the light gray ring indicates the free organic carbon (FOC) content; the area of the dark gray ring indicates the occluded organic carbon (OOC) content; the area of the black circle indicates the mineral associated organic carbon (MOC) content; and the total area of the bubble indicates the total SOC content. The X-axis value of each circle center indicates the average mean annual temperature (MAT) of the corresponding vegetation type site, and the Y-axis value of circle center indicates the average MAP (mean annual precipitation) of the corresponding vegetation type site.

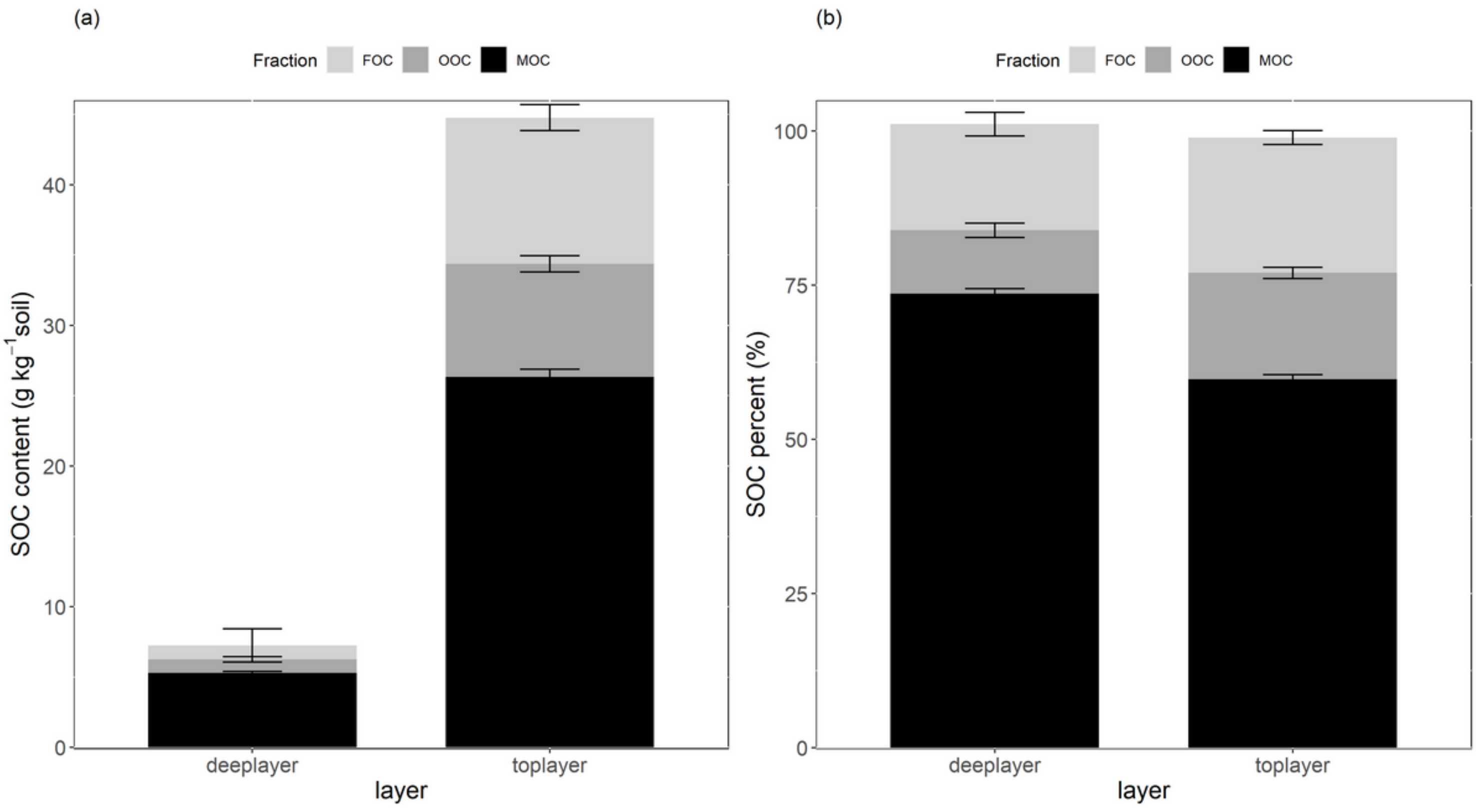

Figure 5

The content (a) and percentage (b) of different soil carbon fractions in different soil layers. (SOC, soil organic carbon; MOC, mineral associated organic carbon; OOC, aggregate occluded organic carbon; FOC, free organic carbon).

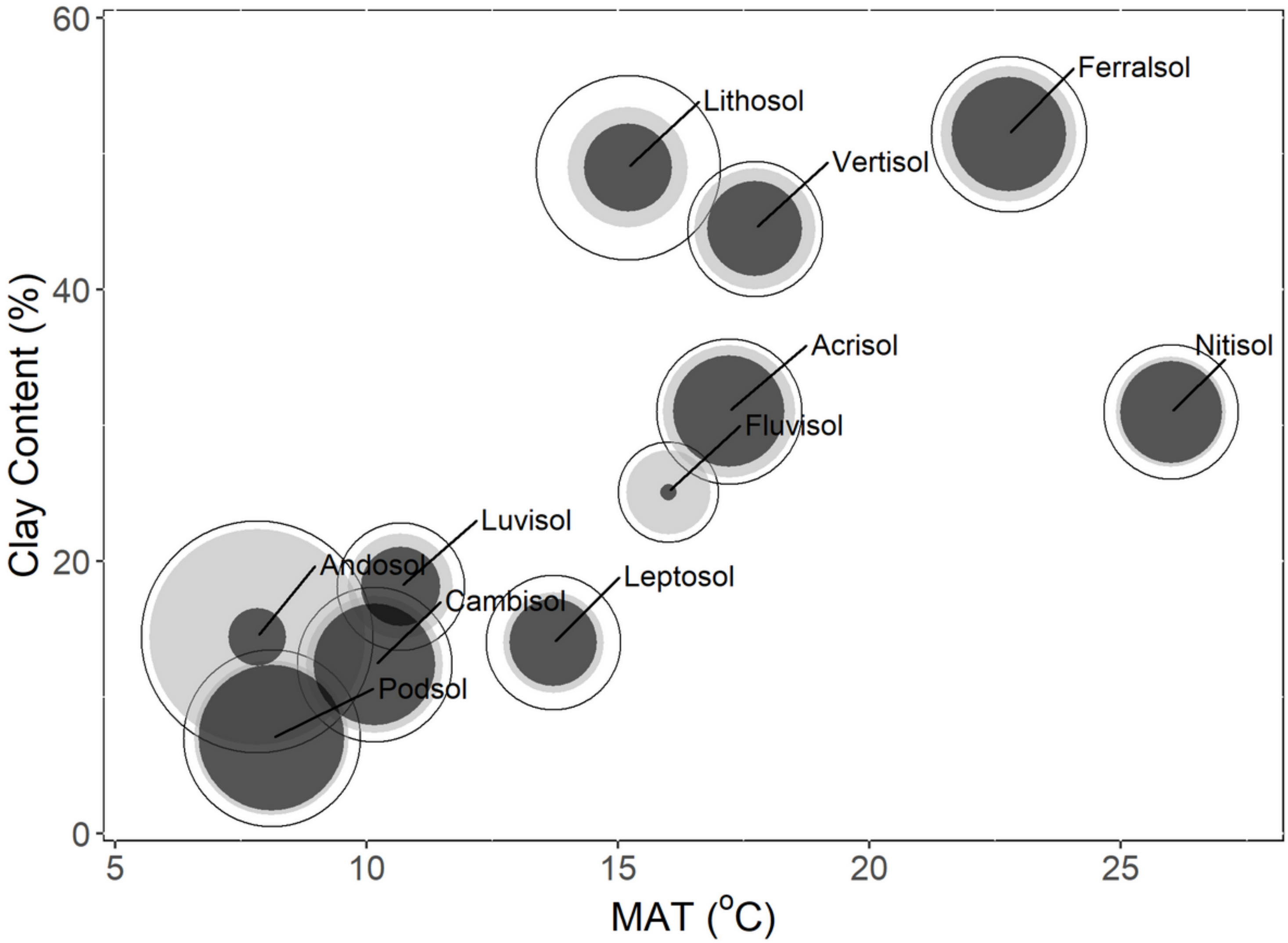

Figure 6

Soil organic carbon (SOC) and its fractionation contents for different soil types. The area of the light gray ring indicates the free organic carbon (FOC) content; the area of the dark gray ring indicates the occluded organic carbon (OOC) content; the area of the black circle indicates the mineral associated organic carbon (MOC) content; and the total area of the bubble indicates the total SOC content. The X-axis value of the circle center indicates the average mean annual temperature (MAT) of the corresponding soil type site, and the Y-axis value of the circle center indicates the average clay content of the corresponding soil type.

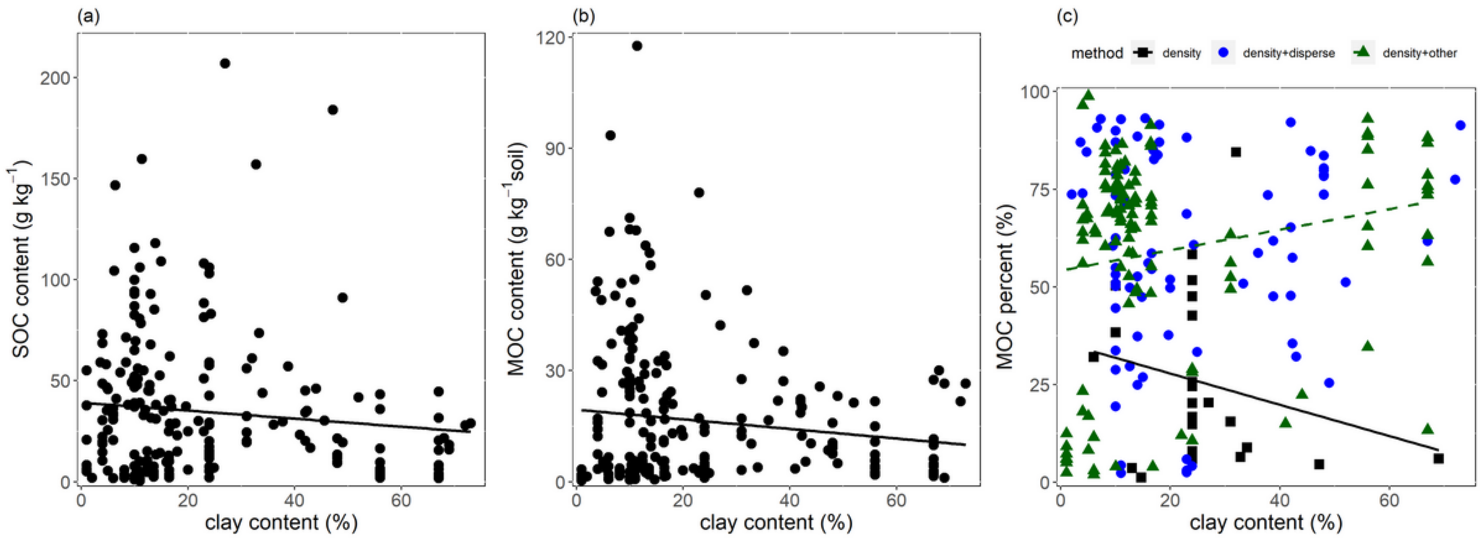

Figure 7

The relationship of total soil organic content (SOC) and mineral associated organic (MOC) content and percentage with soil clay content. (a) The relationship of total SOC content with clay content; (b) The relationship of MOC content with clay content; (c) The relationship of MOC percentage with clay content under different experimental methods.

Supplementary Files

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