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Environmental controls on density-based soil organic carbon fractionations in global terrestrial ecosystems

Xiaolu Sun[1,2,3] | Michael G. Ryan[3,4] | Zuoxin Tang[2,5] | Bisheng Wang[1] | Quanxiao Fang[1] | Osbert Jianxin Sun[2]

[1]Agronomy College, Qingdao Agricultural University, Qingdao, Shandong, PR China

[2]School of Ecology and Nature Conservation, Beijing Forestry University, Beijing, PR China

[3]Department of Ecosystem Science and Sustainability, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, Colorado, USA

[4]Rocky Mountain Research Station, USDA Forest Service, Fort Collins, Colorado, USA

[5]College of Agriculture and Life Sciences, Kunming University, Kunming, PR China

Correspondence
Osbert Jianxin Sun, Institute of Forestry and Climate Change Research, Beijing Forestry University, Beijing 100083, PR China.
Email: sunjianx@bjfu.edu.cn

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Abstract

Categorization of soil organic carbon (SOC) into different functional subpools according to their recalcitrance and protective mechanisms helps better understand ecosystems organic carbon (OC) dynamics, and various attempts have been made to explore the suitable experimental fractionation method for such purpose. However, most previous studies neglected the influences of environmental factors on the effectiveness of varying fractionation methods. Density fractionation has shown great promise in elucidating SOC immobilization mechanisms. Here, we compared three varying types of density fractionation methods (density, density + dispersion, and density + other procedures) for categorizing the SOC into three functional pools, that is, active OC (OC_{active}), moderately stable OC ($OC_{m\text{-}stable}$), and stable OC, (OC_{stable}) using global data compiled for 95 sites in 31 published studies, and examined the influences of climate (mean annual temperature [MAT] and annual precipitation), vegetation type, and soil properties (soil depth, clay content, and soil type) on SOC fractions determined by the three density fractionation methods. The percentage of $OC_{m\text{-}stable}$ fraction was found to be highest using the density method and lowest using the density + dispersion method, due to differential density ranges between the two methods. At a global scale, the contents of total SOC and its OC fractions decreased with temperature. Precipitation had no apparent influences on the subdivided SOC fractions using either the density + dispersion method or the method of density + other procedures, whereas soil type constrained the effect of precipitation on SOC fractions using the density method. The percentage of $OC_{m\text{-}stable}$ determined by the density + dispersion method was more responsive to MAT and vegetation type than that by the other two methods. The percentage of OC_{stable} determined by the method of density + other procedures was significantly and positively related to the clay content as the OC_{stable} based on this method included small particles. For all the three methods of fractionation, soil type had a greater influence than the clay content on the SOC fractions, especially the $OC_{m\text{-}stable}$ and the OC_{stable}. For soil type characterized by rich metal oxides, both the density method and the method of density + other procedures could be used for SOC fractionation. For soil type rich in nutrients, the density + dispersion method would have higher sensitivity for distinguishing the $OC_{m\text{-}stable}$.

KEYWORDS
density fractionation method, mean annual temperature, soil organic carbon, soil type, vegetation type

1 | INTRODUCTION

Soil stores about three-quarters of the terrestrial organic carbon (OC) (Smith et al., 2008; Stockmann et al., 2013). Determinations of soil organic carbon (SOC) dynamic is very important for evaluating and predicting the influence of global climate change on terrestrial ecosystem carbon cycling. However, the underlying mechanisms in the responses of SOC to environmental changes are still unclear because of the intrinsic complexity in SOC turnover. Previous studies have indicated that instead of the constraints by variable chemical composition and recalcitrance, this complexity is resulted more from the physicochemical protections of SOC (Kleber, 2010; Schmidt et al., 2011). Based on theoretical understanding of different SOC protection mechanisms, previous studies generally fractionated SOC into three functional subpools: the mineral-associated OC fraction, the physically protected OC fraction, and the free OC fraction (Sollins et al., 1996; von Lützow et al., 2006). The mineral-associated OC fraction is the most stable component of SOC tightly bonding with mineral particles, making them inaccessible to reductions by microbes and enzymes (Dungait et al., 2012; Six, Conant, et al., 2002). Physically protected OC represents moderately stable SOC component spatially inaccessibility to soil microbial decomposers and was isolated by aggregation or other processes (e.g., phyllosilicates intercalation and organic macromolecules encapsulation) (Gupta & Germida, 2015; Six et al., 2004; Six, Feller, et al., 2002; von Lützow et al., 2006). The free OC fraction is the active SOC component that is not biologically or physically protected.

Various attempts have been made to experimentally explore the fractionation methods that could differentiate SOC pool into different subpools according to those physicochemical protection mechanisms. von Lützow et al. (2007) reviewed most physical and chemical fractionation methods and concluded that soil microbial biomass determined by the chloroform fumigation-incubation method and the light density fraction determined by the density fractionation method may best represent the active SOC pool. Poeplau et al. (2018) compared 20 different SOC fractionation methods using agricultural soils from three experimental sites and found that the coarse light SOC fraction determined by the density fractionation method better represented the newly derived SOC component. There are also studies showing the great promise of using the density fractionation method to elucidate SOC decomposition progression and mineral association status (Baisden et al., 2002; Chenu & Plante, 2006; Golchin et al., 1997; Jones & Singh, 2014; Wagai et al., 2009).

The density fractionation, accomplished with heavy liquids such as sodium iodide (NaI) and sodium polytungstate (SPT, $Na_6[H_2W_{12}O_{40}]$), usually separates soil organic matter (SOM) into heavy fraction (HF) and light faction (LF). The heavy fraction, with density over 1.6–2.0 g cm^{-3}, mainly consists of organo-mineral associated complexes, which often has a low C/N ratio and a higher degree of decomposition. In contrast, light fraction mainly consists of pieces of plant residues, often representing the active SOC component (Christensen, 1992; von Lützow et al., 2007; Wagai et al., 2009).

However, in order to better represent functional SOC subpools in simulation modeling, many methods are developed to further fractionate SOC into three or more subdivided fractions of varying stability or recalcitrance. The sequential density fractionation method can separate SOC more intensively according to their chemical bonds with mineral particles. Fraction with a density of 1.8–2.2 g cm^{-3} is dominated by the ligand exchange reactions and H-bonding between phyllosilicate and oxidized OC species (C–O, C=O, O=C–O) or protonated amide (Jones & Singh, 2014; Kögel-Knabner et al., 2008; von Lützow et al., 2006). However, in the ferrosol, fraction with density >2.0 g cm^{-3} is dominated by monodentate or bidentate covalent bond between unsatisfied hydroxyl group of Fe or Al oxide and negatively charged organic domain (such as hydrolyzed carboxylic acids or phenolic compounds) (Kögel-Knabner et al., 2008; Mikutta et al., 2006; Sollins et al., 1996). Fraction with density > 2.6 g cm^{-3} is dominated by H-bonding and ligand bonding between quartz and feldspar and SOM enriched in aliphatic C and protonated amide (Jones & Singh, 2014). As a result, heavier density SOC is likely be more stable, as the bond between SOC with the metal oxide mineral particle associated by ligand exchange is much stronger than the bond between SOC with phyllosilicate by polyvalent cation bridge.

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 & Vallejo, 2003; Swanston et al., 2005). Other researchers have also used glass beads or other methods to disrupt aggregates (Gaudinski et al., 2000; Jastrow, 1996; Karhu et al., 2010; Lima et al., 2006; Schwendenmann & Pendall, 2006; Six et al., 1998). The third fraction type isolated by these methods, known as "occluded" low density fraction, has commonly been designated as an intermediary SOM fraction formed during the progressive decay of plant detritus accompanied by mineral association (Golchin et al., 1997; Wagai et al., 2009). However, a review by Wagai et al. (2009) shows that the chemical composition of this fraction varies greatly among different studies and has variable mean residence times, indicating that the role of occluded LF in SOM dynamics can change depending on various factors, such as soil type, mineralogy, vegetation, climate and the disturbance regime.

There are other physical or chemical methods combined with the density fractionation method to isolate the physically protected OC fraction. One common approach has been the use of chemical extractions following density fractionation. After removing LF (mainly containing fresh plant materials), the remaining HF materials are further

characterized chemically by acid hydrolysis or oxidation. Several chemical fractionation methods, such as hydrolysis by hydrochloric acid (HCl) (Oades, 1988; Paul et al., 1997; Trumbore & Zheng, 1996) or sulfuric acid (H_2SO_4) (Balesdent, 1996), and oxidation by hydrogen peroxide (H_2O_2) (Eusterhues et al., 2005; Helfrich et al., 2007; Leifeld & Kögel-Knabner, 2001; Mikutta et al., 2006), hydrofluoric acid (HFA) (Trumbore et al., 1989) or sodium hypochlorite (NaOCl) (Helfrich et al., 2007), have been used for isolating stable SOC. Another method combines density fractionation with particle size fractionation (John et al., 2005; Six, Callewaert, et al., 2002) based on the fact that OC 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).

Many factors, such as climate (Lugato et al., 2021; Smith et al., 2008), vegetation (Lugato et al., 2021; Schrumpf et al., 2013; Stockmann et al., 2013), and soil characteristics (Jones & Singh, 2014; Kögel-Knabner et al., 2008; Rasmussen et al., 2018; Schrumpf et al., 2013), can affect SOC fractions. Studying their influences on SOC fractions would help effectively evaluate the adequacy of the specific SOC fractionation methods in categorizing functional SOC pools and improve our understanding of the SOC dynamics in the global terrestrial ecosystems.

It has been widely proven that labile SOC decompose more rapidly as temperature increases, resulting in an overall more recalcitrant SOC pool (Du et al., 2014; Fissore et al., 2008; Garten, 2011; Garten & Hanson, 2006; Lugato et al., 2021; Pisani et al., 2014; Tian et al., 2016). However, there are studies demonstrating a lack of difference in or a less temperature sensitivity of labile OC compared with more recalcitrant OC (Hakkenberg et al., 2008; Percival et al., 2000; Sun et al., 2019). This conflict might be caused by either the influences of the fractionation methods or the constraints of study sites. Some existing studies have shown that precipitation could affect SOC by changing soil microbial and enzyme activities (Kang & Freeman, 1999; Nielsen & Ball, 2015; Turner, 2010). A study by Lugato et al. (2021) on the physical SOM fractionation with European-wide soils indicated that the sensitivity of particulate and mineral-associated SOM fractions to precipitation could be different owning to differences in land covers.

Vegetation affects SOC fractions by modification of soil microbial community composition through differential quantity and quality of organic matter inputs and by altering the micro-environment and soil characteristics (Quideau et al., 2001; Stockmann et al., 2013; Wang et al., 2023; You et al., 2014; Zhang et al., 2023). Especially, different plant species could strongly regulate SOC aggregation formation by affecting hypha and root hair (Gao et al., 2022; Gupta & Germida, 2015). In contrast, there are also studies finding no difference in SOC fractions among different forest types (Fissore et al., 2009; Sun et al., 2019; Zimmermann et al., 2010).

Soil mineralogy can alter the chemical protection of SOC by controlling the number and type of chemical bonds formed with SOM. Mathieu et al. (2015) reviewed studies on soil radiocarbon profiles and noted that SOC dynamics in deep soil are driven more by soil type than by climate. Compared with larger soil particles, SOC (usually the alky-C) interactions with clay particle often have a longer mean residence time (Paul, 2016; von Lützow et al., 2006); whereas some researches have indicated that aluminum- or iron complexes is more important in SOC stability than clay especially in the acid soil (Percival et al., 2000; Rasmussen et al., 2018).

Despite the existing experimental studies on evaluation of different SOC fractionation methods in search for optimized fractionation procedures (e.g., Helfrich et al., 2007; Marzaioli et al., 2010; Poeplau et al., 2018; Poeplau & Don, 2014; Trumbore & Zheng, 1996), most of these works have been done without consideration of the impacts of environmental factors. Thus, in this study, we compared SOC fractions determined by different density fractionation methods in 31 published studies by taking into account of the influences of selective environmental factors. We anticipated that the performance of varying SOC fractionation methods would differ globally owing to differences in climatic conditions, vegetation types, and soil properties.

2 | MATERIALS AND METHODS

2.1 | Data collection

We collected data on SOC fractions from the studies of density fractionation categorizing SOC into three functional subpools: the mineral-associated stable OC fraction (OC_{stable}), the physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the free OC fraction (OC_{active}). The SOC fractionation results were compiled from 31 published studies at 95 sites globally, consisting of a total of 255 datum records (see Supporting information Appendix S1). The types of data and associated information include fractionation methods, OC contents of the bulk soil and in each of categorical fractions, site location, climate conditions (mean annual temperature [MAT] and annual precipitation), soil type, vegetation type, soil sample depth, and clay content.

2.1.1 | SOC fractionation data

All the SOC data we collected from each study was exclusively determined by specific density fractionation methods. We grouped the fractionation methods into three categories: (1) by density, with the SOC fractions separated by sequencing density of heavy liquid (e.g., Baisden et al., 2002); (2) by density + dispersion, with the SOC fractions separated by density before or after soil physical dispersion using ultrasound and glass beads (e.g., Golchin et al., 1994; Jastrow, 1996); and (3) by density + other procedures, with the SOC fractions separated by density combined with other physical and chemical methods like acid hydrolyzation, oxidization and size fractionation (Helfrich et al., 2007).

To better compare and mechanistically interpret the results across studies, instead of using the original data on SOC fractions, we merged some of the categorical fractions when they were too finely distinguished and standardized the SOC fractions into the three

subpools by considering the SOC physicochemical protection mechanism. For studies adopting the density method, we assigned the heaviest fraction OC with density >2.0 or 2.2 g cm^{-3} as the OC_{stable}, the lightest fraction OC with density <1.6 g cm^{-3} as the OC_{active}, and the intermediate density fraction OC with a density range of 1.6–2.0 or 2.2 g cm^{-3} as the $OC_{m\text{-}stable}$. For those adopting the density + dispersion method, we classified the free light OC fractions with density less than the threshold values (the threshold values was ranged from 1.6 to 1.85 g cm^{-3} among different studies, see Supporting information Appendix S1) before physical dispersion as the OC_{active}, the occluded light OC fractions with density less than the threshold values after dispersion as the $OC_{m\text{-}stable}$, and the heavy OC fractions with density more than the threshold values after dispersion as the OC_{stable}. For studies combining the density fractionation with other procedures, we classified the light OC fractions with density less than the threshold values as the OC_{active}, the chemically resistant heavy fractions or OC bond to fine soil particles (>63 or 53 μm) as the OC_{stable} and the rest unresistant heavy OC fraction as the $OC_{m\text{-}stable}$. The details on how to reassign SOC fractions from the literature are described in the Supporting information Appendix S1. Soil recovery rate was calculated by dividing the sum of each OC fraction by the total SOC content of the original soil sample.

For convenience of comparisons, when OC was reported as the stock (C_t Mg ha^{-1}), we converted those values into carbon content (C_c g kg^{-1}) as follows:

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

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

To account for the soil depth effect, the soil data from literature were rearranged into two generic layers: the topsoil and the subsoil. The topsoil included data for 0–30 cm or the A horizon. If the topsoil layer was greater than 30 cm, we also included this into topsoil. The subsoil included data for soils below 30 cm (including those non-topsoil layers that the upper limit was less than 30 cm, but lower limit was more than 30 cm) or the B and/or C horizons.

2.1.2 | Other data

Location

Latitude and longitude were primarily collected from the literature sources and their references (of the same study site). If these were not reported, the latitude and longitude were determined by using Google Earth with information on study site description.

Climate

We compiled data on MAT and annual precipitation (P) for each study site. They were primarily derived from the literature. When MAT and P were not reported, the location coordinates were used to extract climatic values from the CRU database for the year(s) of research (http://www.cru.uea.ac.uk; 0.5° × 0.5° or finer grid).

Soil properties

The soil variables included sample depth, soil type, and clay content. The FAO classification system (FAO/UNESCO, 1974) was used to describe soil types. When soil type was reported according to the USDA soil taxonomy or local classifications, an equivalent FAO classification was added to the database according to other studies for the same sites. If soil type was not described in the study, we completed our database by cross referencing information with other better referenced studies for the same or nearby sites, and ultimately, missing data were filled using information extracted from the Harmonized World Soil Database (V 1.2, the spatial resolution of the SMUs varies by region depending on the source data).

Vegetation type

As a strong human intervention (e.g., organic fertilizer addition, harvest, and straw return) on agricultural soil would greatly affect the SOC density fractionation results (Dignac et al., 2017), we selected literature studies on ecosystems with little or no apparent anthropogenic influences such as natural forests and grassland. Vegetation type for each study site was divided into six broad categories: conifer forest, mixed forest, broadleaf forest, rainforest, grass, and forest and grass (including savanna and other ecosystems with both woods and grass).

2.2 | Statistical analysis

The total SOC and each of the OC fractions were tested by Kruskal–Wallis test to determine the effects of soil depth and soil type, and the relationships of clay content with total SOC and each of the OC fractions were tested by Pearson correlation analysis. Based on the priori knowledge that the influences of climate and vegetation on SOC were affected by soil depth (Jobbágy & Jackson, 2000; Mathieu et al., 2015), and the fact that soil depth significantly influenced on SOC and its fractions in our study (Figure 1a), and to eliminate the effect of soil depth, the relationships of MAT, annual precipitation, and vegetation types with SOC and each of the OC fractions were tested by mixed linear models with studyID as random effect and soil depth as a fixed effect. All data were analyzed using R 3.6.1 (www.r-project.com) with significance level defined as $\alpha < 0.05$, and the analyses with mixed linear model were performed using "lmerTest" package of R 3.6.1. All figures were drawn in the R 3.6.1 environment with "ggplot2," "grid," "ggrepel," and "corrplot" packages.

3 | RESULTS

3.1 | Variations of OC fractions by varying density fraction methods

The value of total SOC content significant differed among the three fractionation methods ($\chi^2 = 14.52$, $p < 0.001$). The mean SOC content was highest by the density method (43.15 ± 9.64 g kg^{-1} soil), and lowest by the density + other procedures (26.99 ± 2.92 g kg^{-1} soil;

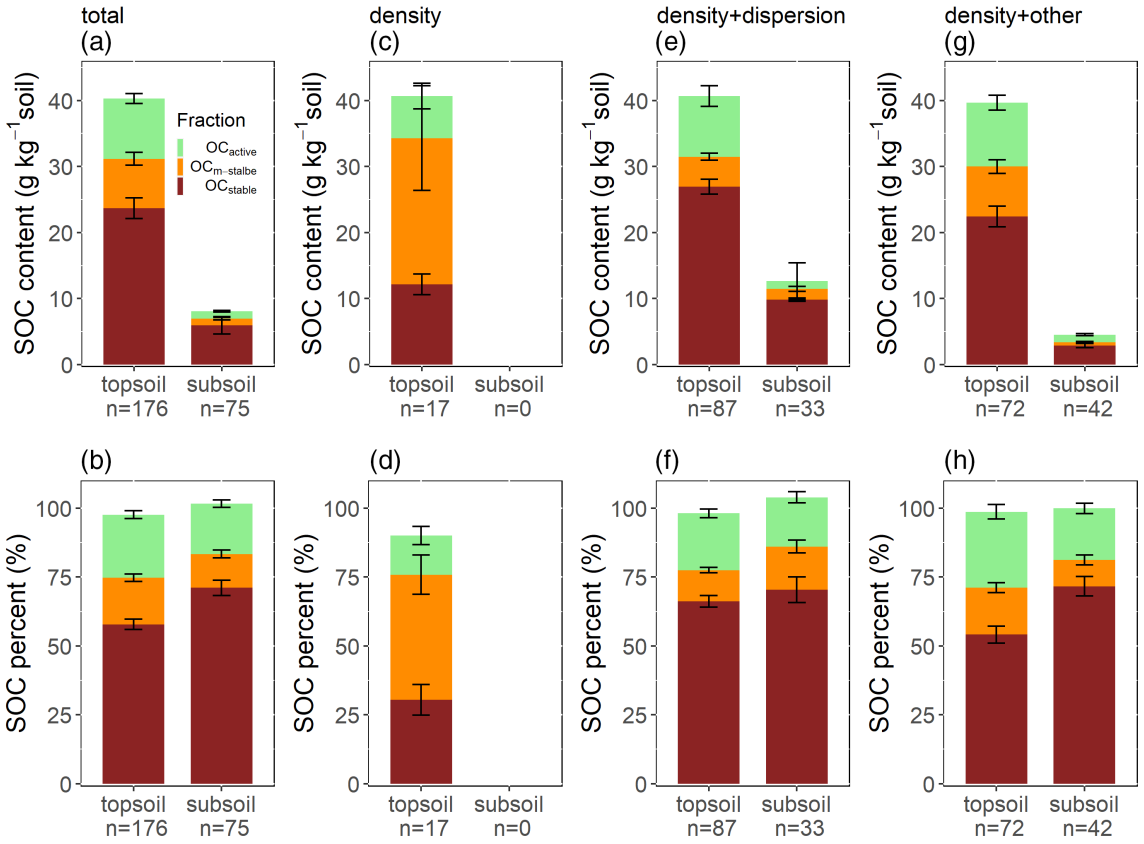

FIGURE 1 The different soil carbon fractions content (a,c,e,g) and percentage (b,d,f,h) in topsoil and subsoil of three different methods. The three experimental methods were density (SOC fractionation by sequential density), density + dispersion (SOC fractionation by density before and after soil physical dispersion, and density + other (SOC fractionation by density combined with other physical and chemical methods). The three fractions were the mineral-associated stable OC fraction (OC_{stable}), the physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the free OC fraction (OC_{active}). OC, organic carbon; SOC, soil organic carbon. [Colour figure can be viewed at wileyonlinelibrary.com]

Figure 2a). The SOC recovery rate was significantly lower by the density method (77.90 ± 7.35%) than by other two methods ($\chi^2 = 11.35$, $p = 0.003$; 93.67 ± 1.05% for the density + dispersion and 96.12 ± 0.98% for the density + other procedures; Figure 2b). The percentage and content of OC_{active} (averaged 21.73 ± 1.06% and 6.84 ± 0.57 g kg^{-1}soil, respectively) did not significantly vary with the fractionation methods. However, the contents and percentages of $OC_{m\text{-}stable}$ and OC_{stable} significantly differed among the three methods (for content: $\chi^2 = 19.24$ and 22.05, p all <0.001. For percentage: $\chi^2 = 29.68$ and 26.78, p all <0.001). The content and percentage of $OC_{m\text{-}stable}$ were lowest by the density + dispersion method (3.74 ± 0.42 g kg^{-1} soil and 12.54 ± 0.98%), and highest by the density method (20.74 ± 6.48 g kg^{-1} soil and 43.76 ± 5.77%); the content and percentage of OC_{stable} were lowest by the density method (10.99 ± 3.19 g kg^{-1} soil and 27.49 ± 4.76%, Figure 2b).

3.2 | The effect of climate

With soil depth as a fixed effect, the mixed linear model showed that the contents of total SOC and the three OC fractions were all negatively correlated to MAT (Figure 3a), and the slopes of the mixed linear model indicated that they averagely decreased by 1.16 g kg^{-1} (total SOC), 0.26 g kg^{-1} (OC_{active}), 0.31 g kg^{-1} ($OC_{m\text{-}stable}$) and 0.63 g kg^{-1} (OC_{stable}), respectively, with an 1°C increase in MAT. No significant correlations were found between the percentage of soil OC fractions and MAT and between the content and percentage of soil OC fractions and annual precipitation. However, the relationships of the OC content with MAT and annual precipitation differed with the type of OC fractions and the fractionation methods. When determined by the density method, only the total SOC content was negatively correlated to MAT (Figure 3b); it decreased by 1.27 g kg^{-1} with a 1°C increase in MAT, as inferred by the slope of the linear model. The contents of total SOC, OC_{active}, and OC_{stable} and the percentage of OC_{stable} were positively correlated to annual precipitation (Figure 3b); on average, they increased by 3.17, 0.75, and 4.05 g kg^{-1} and 4.56%, respectively, with 100 mm increases in annual precipitation. For the density + dispersion method, the total SOC content and the content and percentage of $OC_{m\text{-}stable}$ were significantly and negatively correlated to MAT (Figure 3c); on average, they decreased by 1.19 g kg^{-1}, 0.44 g kg^{-1}, and 0.82%, respectively, with a 1°C increase in MAT increase. The contents of total SOC, $OC_{m\text{-}stable}$, and

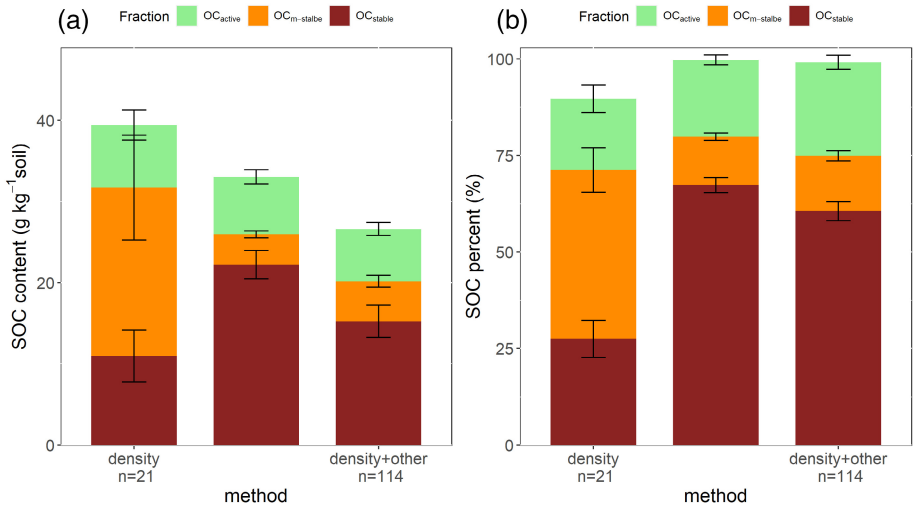

FIGURE 2 The content (a) and percentage (b) of different soil carbon fractionations by three different experimental methods. The three experimental methods were density (SOC fractionation by sequential density), density + dispersion (SOC fractionation by density before and after soil physical dispersion, and density + other (SOC fractionation by density combined with other physical and chemical methods). The three fractions were the mineral-associated stable OC fraction (OC_{stable}), the physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the free OC fraction (OC_{active}). OC, organic carbon; SOC, soil organic carbon. [Colour figure can be viewed at wileyonlinelibrary.com]

OC_{stable} determined by the density + dispersion method were positively correlated to annual precipitation (Figure 3c); on average, they increased by 1.56, 0.27, and 1.46 g kg^{-1}, respectively, with 100 mm increases in annual precipitation. The percentage of OC_{active} was negatively correlated to annual precipitation (Figure 3c), and it decreased by 1.17% with 100 mm increases in annual precipitation. For the method of density + other procedures, the contents of total SOC and the three OC fractions were all significantly and negatively correlated to MAT (Figure 3d), and they decreased by 1.33, 0.29, 0.24, and 0.93 g kg^{-1}, respectively, with a 1°C increase in MAT; the contents of total SOC and OC_{stable} were significantly and negatively correlated to annual precipitation (Figure 3d), and they decreased by 1.11 and 0.89 g kg^{-1}, respectively, with 100 mm increases in annual precipitation.

3.3 | The effect of vegetation type

With soil depth as a fixed effect, the mixed linear model did not detect significant overall effect of vegetation type on the total SOC and its OC fractions. However, the percentages of all three OC fractions determined by the density + dispersion method were significantly affected by vegetation type ($F = 2.64$, 3.07 and 3.11, respectively; $p = 0.037$, 0.021, and 0.018, respectively). The rainforest in the hot and humid environment (MAT of 18.5 ± 1.3°C, and annual precipitation of 2143 ± 101 mm; Figure 4c) had the lowest percentage of OC_{active} (8.62 ± 3.06%; Figure 4c); whereas the broadleaf, mixed, and conifer forests with temperate climate (MAT of 9.9 ± 0.4, 11.4 ± 2.6, and 8.4 ± 0.6°C, respectively; annual precipitation of 686 ± 28, 1280 ± 78, and 975 ± 60 mm, respectively) had higher percentage of OC_{active} (22.41 ± 2.13, 20.80 ± 3.89, and 24.21 ± 2.26%, respectively). The grass and forest-grass vegetation types (annual precipitation of 824 ± 30 and 699 ± 138 mm and MAT of 9.6 ± 0.5 and 16.5 ± 1.4°C, respectively) were associated with lower percentage of OC_{active} (9.41 ± 2.53 and 13.05 ± 2.30%, respectively) and higher percentage of OC_{stable} (77.35 ± 3.58 and 70.10 ± 1.40%, respectively; Figure 4c). Furthermore, the percentage of $OC_{m\text{-}stable}$ was highest in rainforest (16.36 ± 4.56%, Figure 4c). There was no significant effect of vegetation type on SOC fractions determined by either the density method or the method of density + other procedures.

3.4 | The effect of soil properties

The contents of total SOC and OC in all three fractions were significantly lower in the subsoil than in the topsoil (χ^2 all >14, p all <0.001; Figure 1a). Furthermore, the percentages of $OC_{m\text{-}stable}$ and OC_{stable} significantly differed between the topsoil and the subsoil ($\chi^2 = 7.07$ and 19.31, $p = 0.008$ and <0.001); the topsoil had higher percentage of $OC_{m\text{-}stable}$ and lower percentage of OC_{stable} than the subsoil. No significant difference was found in the percentage of OC_{active} between the topsoil and the subsoil (Figure 1b). Unfortunately, a lack of sufficient datum points for the subsoil did not allow for examination of the influences of soil layer on SOC fractions determined by the density method (Figure 1c,d). For the density + dispersion method, the contents of total SOC and all the three OC fractions were also significantly lower in the subsoil than in the topsoil (χ^2 all >14, p all <0.001; Figure 1e), but the percentage of OC in all fractions did not significant differ between the topsoil and the subsoil (Figure 1f). And as for the method of density + other procedures, the

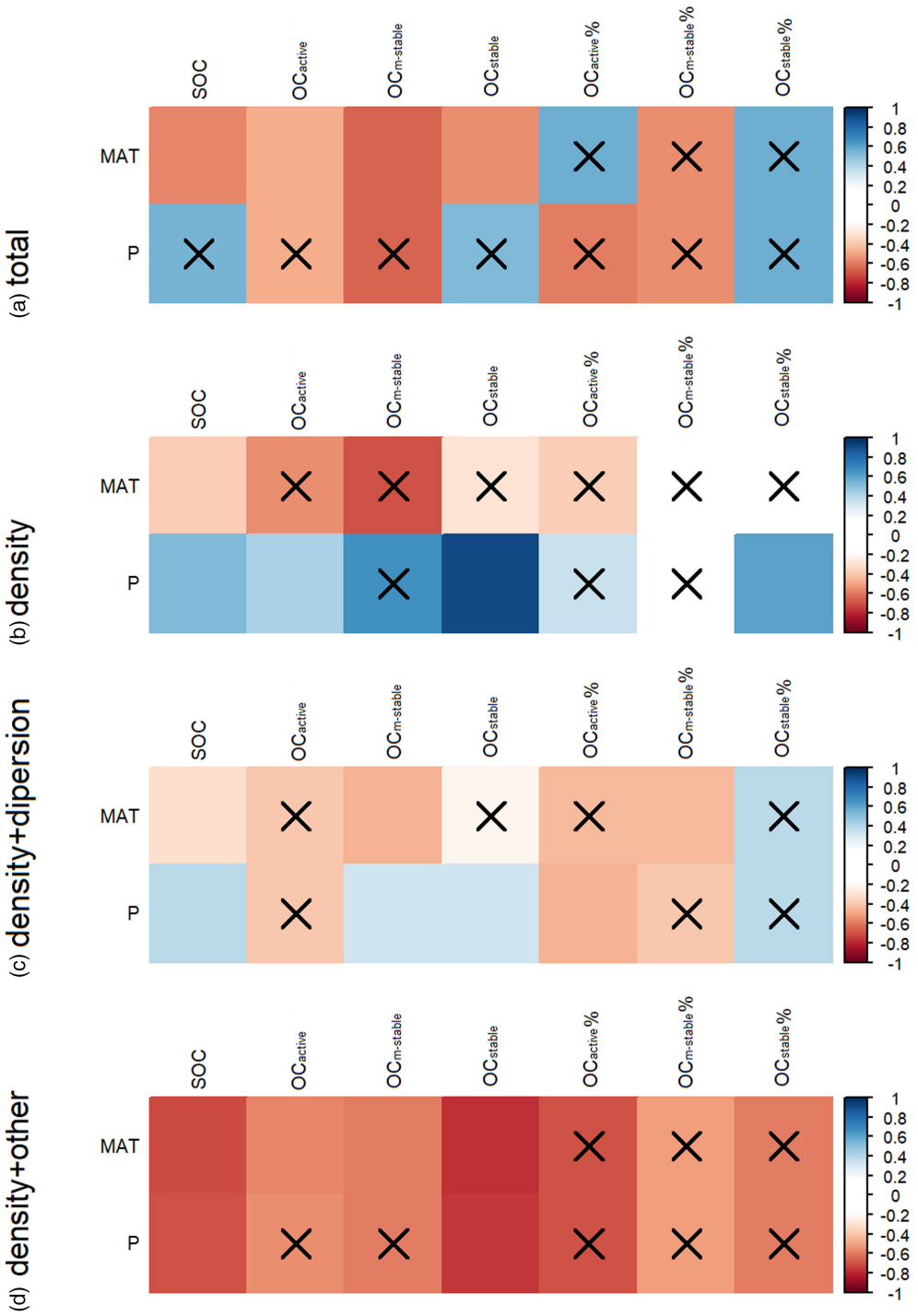

FIGURE 3 The relationships of soil organic carbon content (SOC) and different soil carbon fractionations with mean annual temperature (MAT) and annual precipitation (P) determined by a mixed regression model using study ID as a random factor and soil depth as a fixed effect. The R^2 were scaled from white to dark blue for positive correlations and dark red for negative correlations. Those correlations with $p < 0.05$ were considered not significant (×). The three experimental methods were density (SOC fractionation by sequential density), density + dispersion (SOC fractionation by density before and after soil physical dispersion, and density + other (SOC fractionation by density combined with other physical and chemical methods). The three fractions were the mineral-associated stable OC fraction (OC_{stable}), the physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the free OC fraction (OC_{active}). And the percent sign (%) after the fraction names represented the percentage of this fractions. OC, organic carbon. [Colour figure can be viewed at wileyonlinelibrary.com]

contents of total SOC and all OC fractions were also significantly lower in the subsoil than in the topsoil (χ^2 all >40, p all <0.001; Figure 1g); the percentages of OC_{active} and $OC_{m\text{-}stable}$ were significantly higher in the topsoil than in the subsoil ($\chi^2 = 4.00$ and 20.41, $p = 0.045$ and <0.001), with the reverse trend for the percentage of OC_{stable} ($\chi^2 = 22.89$, $p < 0.001$; Figure 1h).

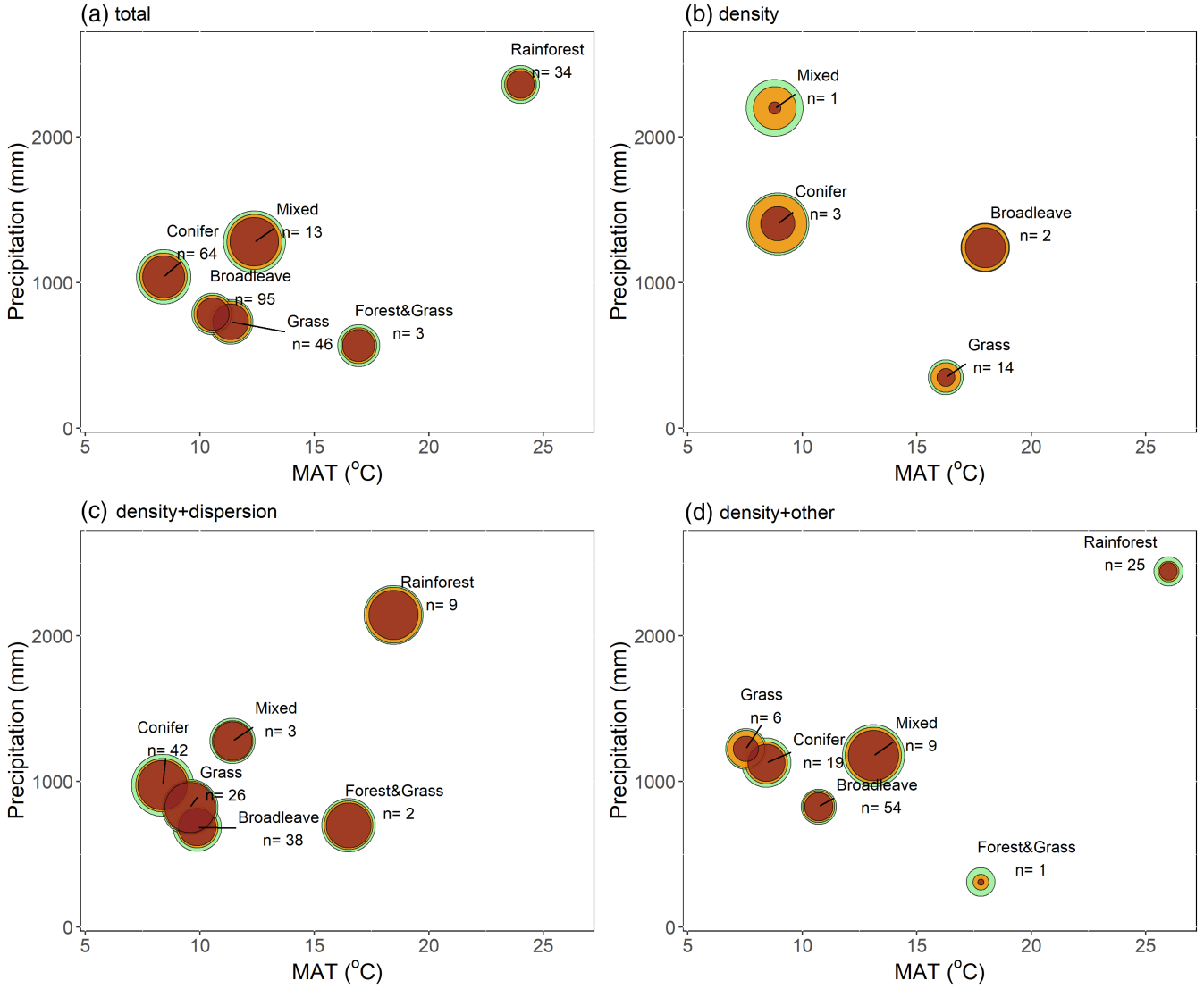

FIGURE 4 Soil organic carbon (SOC) and its fractionation contents of top soil with different vegetation types of three different experimental methods. The three experimental methods were density (SOC fractionation by sequential density), density + dispersion (SOC fractionation by density before and after soil physical dispersion, and density + other (SOC fractionation by density combined with other physical and chemical methods). The area of the light green rings indicated the content of free OC fraction (OC_{active}), the area of the orange rings indicated the content of physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the area of the dark brown circles indicated the content of mineral-associated stable OC fraction (OC_{stable}). 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 annual precipitation of the corresponding vegetation type site. [Colour figure can be viewed at wileyonlinelibrary.com]

The Pearson correlation analysis showed that the contents of total SOC, $OC_{m\text{-}stable}$, and OC_{stable} were negatively related to soil clay content ($p = 0.007$, 0.019, and 0.021, respectively; Figure 5). The contents of total SOC and $OC_{m\text{-}stable}$ as well as the percentage of $OC_{m\text{-}stable}$ determined by the method of density + other procedures were negatively related to soil clay content ($p = 0.031$, 0.007, and 0.001, respectively), but the percentage of OC_{stable} was positively related to soil clay content ($p = 0.037$).

The $OC_{m\text{-}stable}$ content was significantly affected by soil type ($\chi^2 = 23.89$, $p = 0.004$). Among the soil types, the andosol had significantly highest $OC_{m\text{-}stable}$ content (63.08 ± 28.37 g kg^{-1}; Figure 6a) as well as the highest contents of SOC and OC_{active} (92.73 ± 57.42 g kg^{-1} and 11.22 ± 5.16 g kg^{-1}, respectively), but the lowest percentage of OC_{stable} (10.44 ± 3.53%). The ferralsol had the lowest SOC content (19.53 ± 5.17 g kg^{-1}; Figure 6a) and the $OC_{m\text{-}stable}$ content (1.33 ± 0.53 g kg^{-1}); whilst the SOC content was second to the lowest (24.71 ± 6.53 g kg^{-1}) and the contents of OC_{active} and OC_{stable} were the lowest (3.91 ± 1.58 and 5.37 ± 1.74 g kg^{-1}, respectively) in the fluvisol. For the density method, the $OC_{m\text{-}stable}$ content was significantly affected by soil type ($\chi^2 = 11.29$, $p = 0.046$), being significantly largest in the andosol (135.79 g kg^{-1}; Figure 6b); whereas the ferralsol was highest in both the content and percentage of OC_{stable} (51.63 g kg^{-1} and 84.49%, respectively). For the density + dispersion method, the contents of $OC_{m\text{-}stable}$ and OC_{stable} as well as the

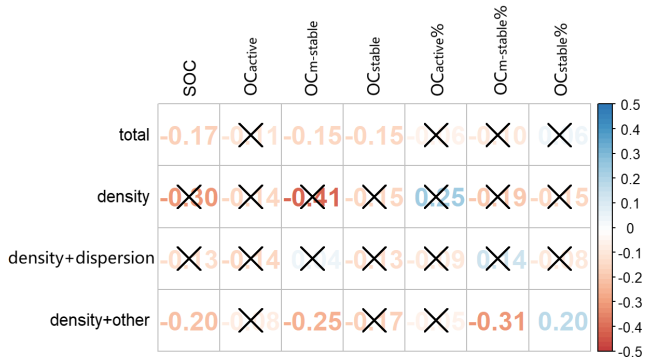

FIGURE 5 The relationships of total soil organic content (SOC) and different soil carbon fractionations with soil clay content determined by Pearson correlation analysis. The number in the box was Pearson correlations, which scaled from dark blue for positive correlations to dark red for negative correlations. Those correlations with $p < 0.05$ are considered not significant (×). The three experimental methods were density (SOC fractionation by sequential density), density + dispersion (SOC fractionation by density before and after soil physical dispersion, and density + other (SOC fractionation by density combined with other physical and chemical methods). The three fractions were the mineral-associated stable OC fraction (OC_{stable}), the physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the free OC fraction (OC_{active}). And the percent sign (%) after the fraction names represented the percentage of this fractions. [Colour figure can be viewed at wileyonlinelibrary.com]

percentages of all three OC fractions were significantly affected by soil type ($\chi^2 = 14.07$, 21.59, 17.35, 18.78, and 20.70, respectively, and $p = 0.029$, 0.003, 0.008, 0.005, and 0.004, respectively); both the content and percentage of $OC_{m\text{-}stable}$ were highest in the acrisol (7.95 ± 1.71 g kg^{-1} and 22.74 ± 4.10%, respecitvely; Figure 6c), and lowest in the ferralsol (1.73 ± 0.90 g kg^{-1} and 4.65 ± 1.56%, respectively); the leptosol had the lowest content and percentage of OC_{stable} (13.94 ± 2.38 g kg^{-1} and 44.96 ± 7.67%) and highest percentage of OC_{active} (35.80 ± 1.87%); the luvisol had the highest percentage of OC_{stable} (78.29 ± 2.71%) and lowest percentage of OC_{active} (11.81 ± 1.61%); and the podsol had the highest OC_{stable} content (33.37 ± 4.62 g kg^{-1}). For the method of density + other procedures, there were significant effects of soil type on the content and percentage of $OC_{m\text{-}stable}$ and on the percentage of OC_{stable} ($\chi^2 = 15.33$, 26.47, and 17.74, respectively; $p = 0.018$, <0.001, and =0.007, respectively); the andosols had the highest content and percentage of $OC_{m\text{-}stable}$ (26.72 ± 7.79 g kg^{-1} and 72.92 ± 1.33%) and the lowest percentage of OC_{active} and OC_{stable} (10.21 ± 5.46% and 12.58 ± 4.36%); the ferralsol had the lowest content and percentage of $OC_{m\text{-}stable}$ (0.57 ± 0.14 g kg^{-1} and 6.95 ± 0.78%); and the podsol had the highest OC_{stable} content (33.37 ± 4.62 g kg^{-1}).

4 | DISCUSSION

In this study, we found that uses of different density fractionation methods led to varying distributions of OC fractions in soils (Figure 2). In general, the percentage and content of OC_{active} (LF or free LF) did not vary with the density fractionation methods, which might be because that all the three fractionation methods used the similar density threshold values (1.6–1.85 g cm^{-3}) and procedures to determine this component. However, due to the greater density range (1.6–2.0 or 2.2 g cm^{-3}) adopted, the medium density fraction ($OC_{m\text{-}stable}$) determined by the sequential density fractionations was greater than that by other two methods, which resulted in greatly reduced heavy density fraction (OC_{stable}) by the sequential density fractionation method (Figure 2). The density method also had the lowest recovery rate compared to the other two methods, which might be because that the density fractionation procedure was repeated multiple times (usually 4–6 times), and each time the suspension and filtering operation would loss some DOC and OC bonding to tiny mineral particles (Baisden et al., 2002; Poeplau et al., 2018). Whereas for the density + dispersion method, due to the relatively narrower density range (less than the density threshold values ranged from 1.6 to 1.85 g cm^{-3}) and uncertainty disruptive power (Poeplau & Don, 2014), the percentage of occluded LF ($OC_{m\text{-}stable}$) fraction was lower compared to by the other two methods; this resulted in higher percentage of the stable OC fraction by the density + dispersion method than by other two methods. As marked variations in the SOC content, environmental factors, and soil types existed in our database, one very important controversy to the above explanations is that the differences in the OC content and percentage among different fractionation methods might actually be due to the natural variability of these soils as they were sampled from variable environments with contrasting soil types. In a study comparing different SOC fractionation methods with the same soil samples, Poeplau et al. (2018) found that the recovery rate of the density method similar to the one in our study was lower than the equivalent density + dispersion method and the method of density + other procedures, and that the percentage of OC in the medium density fraction by the density + dispersion method was higher than that of the occluded LF by the method of density + other procedures. These findings help prove the authenticity of our results.

4.1 | The effect of climate on SOC fractions based on density methods

Our results indicated that the contents of total SOC and its fractions decreased with increasing temperature (Figure 3). And the percentage of occluded LF ($OC_{m\text{-}stable}$) by the density + dispersion method had a negative relationship with MAT, indicating that the occluded LF OC fraction was more susceptible to temperature rises compared to the free LF and HF OC. The occluded LF OC was mostly released from transient and temporary aggregation, which formed by partially decomposed organic residues, smaller primary organic–mineral complexes, and mineral particles though the adhesive effect of organic stabilizing agent like microbial- and plant-derived polysaccharides, roots, and hypha (particularly vesicular arbuscular mycorrhizal hyphae); this adhesive effect is usually less strong than the chemical bonds inside the primary organic-mineral complexes (Dexter

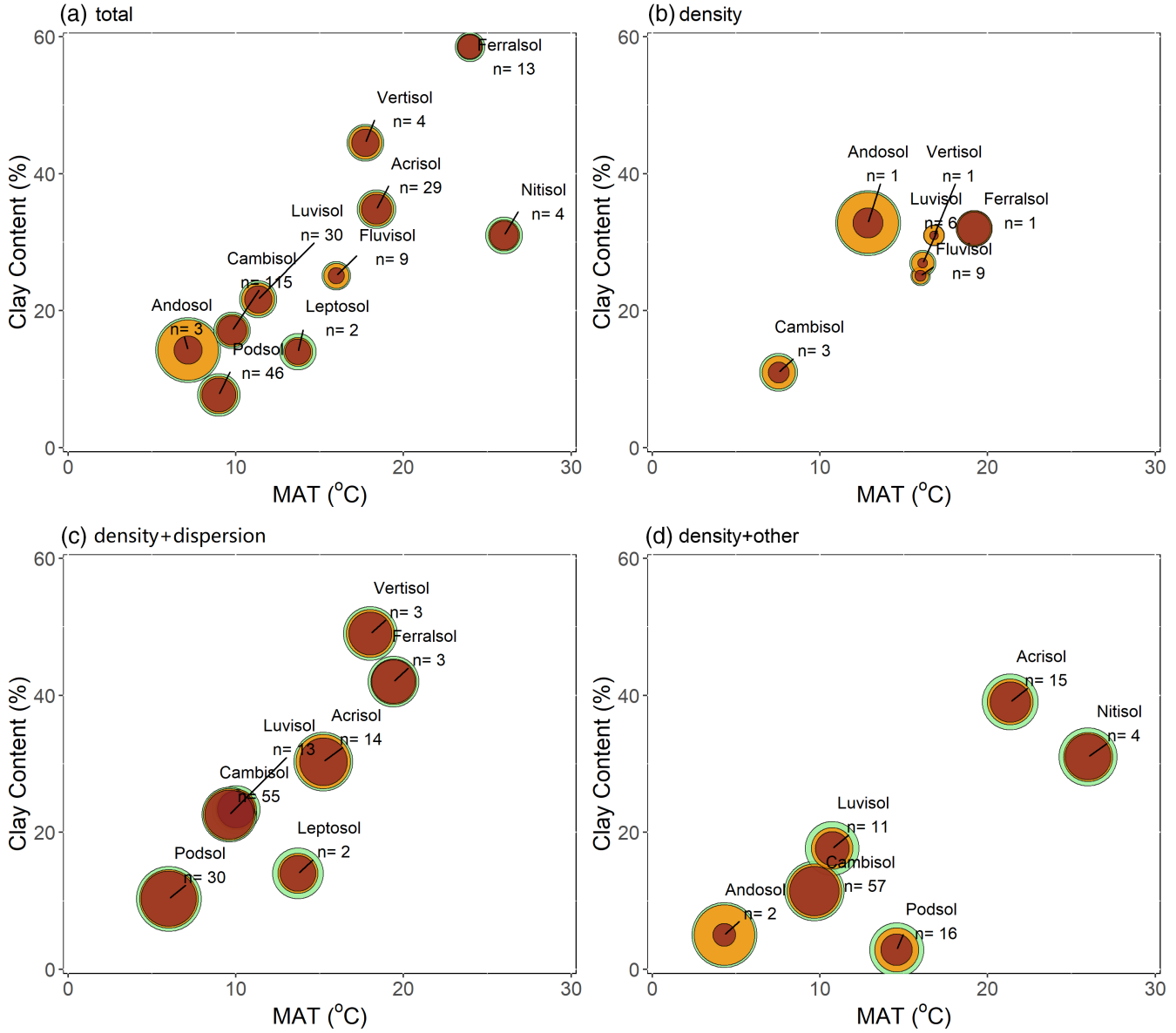

FIGURE 6 Soil organic carbon (SOC) and its fractionation contents for different soil types of three different experimental methods. The three experimental methods were density (SOC fractionation by sequential density), density + dispersion (SOC fractionation by density before and after soil physical dispersion, and density + other (SOC fractionation by density combined with other physical and chemical methods). The area of the light green rings indicated the content of free OC fraction (OC_{active}), the area of the orange rings indicated the content of physically protected and moderately stable OC fraction ($OC_{m\text{-}stable}$), and the area of the dark brown circles indicated the content of mineral-associated stable OC fraction (OC_{stable}). 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. [Colour figure can be viewed at wileyonlinelibrary.com]

et al., 2008). Thus, in the tropical or subtropical region with high MAT, drastic variations in soil water content affected by monsoon activity (in our study the data of Freixo et al., 2002; and Wagai et al., 2008) can disrupt the aggregation structure and release the partially decomposed occluded OC (Edwards, 2013); these OC fractions would then be exposed to microbes and thus degraded more rapidly compared to those biochemically recalcitrant free OC fraction in the humid and hot environments.

The relationships of SOC and its variable OC fractions with annual precipitation differed among the assessments by different fractionation methods (Figure 3), suggesting the complexity of the influences by precipitation on SOC dynamics. We found that both the content and percentage of OC_{stable} by the density method increased with increasing precipitation. It needs to note that the purpose of sequential density fractionation method is to separate OC fractions of contrasting stability according to their differential chemical bonds with mineral particles; the heavy fraction with density >2.0 or 2.2 g cm^{-3} by this method more represents the OC band to phyllosilicate clay particle, aluminum and iron oxides, quartz, and feldspar (Jones & Singh, 2014; Kögel-Knabner et al., 2008; Mikutta

et al., 2006; Sollins et al., 1996; von Lützow et al., 2006). And in our study, for the density method, the soil types with lower participation were mainly luvisol and fluvisol, which lack clay and iron-bearing minerals in the upper layer and would lead to a lower OC_{stable} percentage, while the soil types with higher participation were mainly cambisol and ferrosol with accumulation of fine texture and metal oxides, which would led to a higher OC_{stable} percentage (Farmer, 1982; Jordanova, 2017). Thus, an increasing content and percentage of the heavy density OC_{stable} by the density method with participation might actually be constrained by differences of soil type.

4.2 | The effect of vegetation on SOC fractions based on density methods

Our analysis showed that vegetation type only significantly influenced the percentage of the three OC fractions by the density + dispersion method. Broadleaf, mixed, and conifer forests with temperate climate had higher percentage of active OC, while rainforest and forest and grass with higher MAT having lower percentage OC_{active}. These patterns might be caused by variations in MAT among vegetation types as increasing MAT can lead to a lower decomposition activation energy (as its labile chemical composition) and higher availability to micro-organisms and enzymes (due to lack of physical and chemical protection) of labile or active OC fraction (Conant et al., 2011). The findings that grassland had the highest percentage of OC_{stable} and the lowest percentage of $OC_{m\text{-}stable}$ by the density + dispersion method, which might be explained by less structural litter input (e.g., less lignin and aliphatic OC), led to less free and occluded OC fraction and occurrence of mainly partially decomposed residues from those structural litter in the topsoil (Kleber, 2010; von Lützow et al., 2006). These results demonstrated that the occluded light OC by the density + dispersion method was more responsive to vegetation type.

4.3 | The effect of soil properties on SOC fractions based on density methods

The contents of total SOC and each of the OC fractions were found to be significantly lower in the subsoil than in the topsoil regardless of the density fractionation methods. This is supported by findings from other studies, occurring as a result that the soil carbon input processes (from plant root or litter) are mostly taken place in the topsoil rather than in the subsoil (Jobbágy & Jackson, 2000).

Most previous studies had demonstrated that clay content was positively related to the stable OC (Dexter et al., 2008; Malamoud et al., 2009; Mathieu et al., 2015). But in our study, the contents of SOC, $OC_{m\text{-}stable}$, and OC_{stable} were all negatively related to clay content. This might be because, in our data, most forests occurred on acid soil sites; in the acid soil, the aluminum- or iron complexes are usually more important in determining the SOC stability than does the clay (Percival et al., 2000; Rasmussen et al., 2018). We also found that the percentage of OC_{stable} by the method of density + other procedures was significantly and positively related to clay content, which can be explained by the fact that some small particle HF OC (<53 or 63 μm) was included in the OC_{stable} fractions as a result of combining the density method with particle size separation.

For all the three density fractionation methods, soil type mainly affected the content of $OC_{m\text{-}stable}$. Because the high organic content and aggregation of soil materials (Kögel-Knabner & Amelung, 2014), the andosols with desirable water-holding and nutrient supply capacity had distinctively highest SOC content as well as the content and percentage of $OC_{m\text{-}stable}$. Furthermore, we found that both the content and percentage of OC_{stable} were higher in the acrisol, ferralsol, and podsol by all the three fractionation methods, as the pedogenic processes in acrisol, ferralsol, and podsol favor the formation of secondary iron oxyhydroxides (e.g., ferrihydrite, goethite, and lepidocrocite) or hydroxyaluminium silicate (e.g., allophane and kaolinite) (Farmer, 1982; Jordanova, 2017), which could bond with negatively charged organic domains through ligand exchange and form a stable organo-mineral complex (Jones & Singh, 2014). However, with the same soil type, the separation of the OC fractions could differ by the fractionation methods; we found that the percentage of OC_{stable} in the luvisol was higher by the density + dispersion method, but lower by the density method. The lower percentage of OC_{stable} fraction (the heaviest density) determined by the density method could be because that the luvisol is devoid of clay and iron-bearing minerals; whereas the higher percentage of OC_{stable} by the density + dispersion method (HF < 1.6 to 1.85 g cm^{-3}) might be caused by loss of reactive OC (OC_{active} and $OC_{m\text{-}stable}$) in leaching (The Editors of Encyclopaedia Britannica, 2016b). These results show that soil type could affect the OC_{stable} or $OC_{m\text{-}stable}$ fractions through both the soil mineralogy and the pedologic formation process; these processes could also interactively co-vary with climate and vegetation factors from a macro perspective. For example, the luvisol is formed mainly under climatic regimes ranging from cool temperate to warm Mediterranean (The Editors of Encyclopaedia Britannica, 2016b); whereas the acrisol is mainly formed under humid tropical climate with a natural vegetation of woodland (The Editors of Encyclopaedia Britannica, 2016a), with generally higher MAT than that experienced by the luvisol.

4.4 | Suggestion for SOC density fractionation method selection

Synthesizing the effects of all factors considered in this study, soil type appeared to be more influential than climate and vegetation as inferred by the results from all the three density fractionation methods. This is because, on one hand, soil type had a greater influence on SOC fractions, especially the $OC_{m\text{-}stable}$ and OC_{stable} fractions; and on the other hand, the influences of climate and vegetation can be reflected by the variations in soil type. Thus, we recommend the preferential consideration of soil type in selection of the SOC density fractionation methods.

For soil types characterized by an accumulation of metal oxides, particularly iron and aluminum oxides, like acrisol, ferralsol, and

podsol, because of their higher percentages of OC_{stable}, we recommend the use of the density method (e.g., multiple HF with sequencing density fractionation) and the method of density + other procedures (e.g., combining density and particle fractionations), which better characterize mineral properties. Although the low recovery rate and operation complexity might result in a narrower scope of its application (Baisden et al., 2002; Poeplau et al., 2018), the density method could better reflect the OC stability because of its favorable representation of chemical bonds between mineral particles and SOM (Jones & Singh, 2014; Mikutta et al., 2006; Sollins et al., 1996). Researchers may opt to either of the two methods according to their own experimental conditions and research objectives.

For soil types with higher nutrient capacity and higher SOC content like andosol, the density + dispersion method is recommended for its high sensitivity of separating the occluded LF-C ($OC_{m\text{-}stable}$) fraction. In studies of SOC with different climate conditions and/or vegetation types, the density + dispersion method could be preferable because of the higher responsiveness of the occluded LF C to changes in MAT and variable vegetation types. However, the higher sensitivity and the greater variations in chemical composition of the occluded LF-C fraction (Wagai et al., 2009), in combination with the uncertainty in the disruptive power by the density + dispersion method (Poeplau & Don, 2014), might lend to an inconsistent fractionation result. For example, the disruption of aggregation, like freeze–thaw circle (Edwards, 2013) and tillage (Amézketa, 1999), frequently occur in soil, and the fractionation results on free LF-C and the occluded LF-C might vary a great deal with such events.

Clearly, there is a need for more comparative studies on the techniques and application of fractionation methods with systematic experimental designs and better controls across a wide range of climate, soil types, and vegetation cover. The work of Poeplau et al. (2018) has set a good example with the uses of agricultural soils from three experimental sites for such a purpose.

5 | CONCLUSIONS

The physically protected $OC_{m\text{-}stable}$ fraction by the sequential density method had the highest percentage and the $OC_{m\text{-}stable}$ by the density + dispersion method had the lowest percentage. The contents of total SOC and its OC fractions decreased with increasing temperature. Precipitation had no influence on the SOC fractions either by the density + dispersion method or the method of density + other procedures, whereas soil type constrained the effect of precipitation on the SOC fractions by the density method. The percentage of occluded light OC fraction by the density + dispersion method was more responsive to MAT and vegetation type. Only the percentage of OC_{stable} by the method of density + other procedures was found to be significantly and positively related to clay content as the OC_{stable} fraction determined by this method included small particles. Regardless of the density fractionation methods, soil type had a greater influence than the clay content on the SOC fractions, especially $OC_{m\text{-}stable}$ and OC_{stable}. For soil types characterized by an accumulation of metal oxides, the density method and the method of density + other procedures could either be chosen in consideration of the environmental conditions and research objectives. For soil types rich in nutrients, the density + dispersion method would be a better choice because of its high sensitivity for separating the occluded LF OC.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supplementary material of this article.

ORCID

Xiaolu Sun https://orcid.org/0000-0003-1317-9196

Osbert Jianxin Sun https://orcid.org/0000-0002-8815-5984

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SUPPORTING INFORMATION

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