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Changing rainfall frequency affects soil organic carbon concentrations by altering non-labile soil organic carbon concentrations in a tropical monsoon forest

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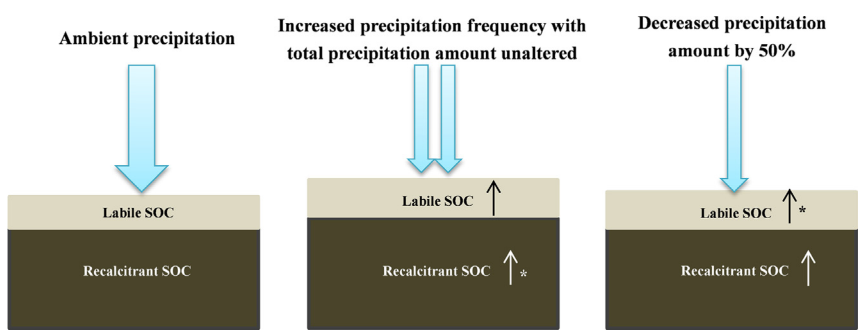
HIGHLIGHTS

- This study was conducted to clarify effects of precipitation changes on SOC fractions.
- Increased rainfall frequency and decreased rainfall amount were manipulated.
- Rainfall frequency increase with the amount unaltered increased the SOC concentration.
- The non-labile fraction contributed a substantial proportion to this increase.
- Rainfall amount decrease by 50% did not significantly change the SOC concentration.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil stores a substantial proportion of carbon (C), making it the greatest terrestrial C pool and pivotal to stabilizing the global climate system. Rainfall amounts and regimes have been changing in many places, but effects of precipitation changes on soil organic C (SOC) stabilization are not completely understood. Considerable attention has been focused on the consequences of changes in rainfall amounts, with rainfall regimes having been less studied. This study was conducted in a tropical climax forest to clarify the effects of rainfall changes on SOC fractions, with permanganate oxidation and density fractionations employed to divide the labile and non-labile SOC fractions. Two rainfall manipulation treatments, i.e., increased rainfall frequency with the total rainfall amount unchanged (IRF) and decreased rainfall amount by 50% with rainfall frequency unaltered (DRA), were conducted for two years, with ambient rainfall (AR) as the control. As a result, the IRF treatment increased the SOC concentration that mainly originated from increases in the non-labile SOC content. Relative to the AR control, the DRA treatment did not change the total SOC concentration although the labile SOC concentration increased. This typically is due to a small proportion of the labile fraction to the total SOC content. Our results suggest that this water-rich mature forest is resistant to rainfall amount changes to a great extent (e.g., decrease of 50% as in the present study) from the SOC stabilization perspective, while changes in rainfall frequency could exert more notable effects.

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1. Introduction

Soil is the greatest terrestrial carbon (C) pool that is more than the total of the vegetation and atmospheric C pools, making soil vital to stabilizing the climate system. Globally, the first 1 m of surface soil stores approximately 1500 Pg C (Jobbágy and Jackson, 2000; Scharlemann et al., 2014), with a substantial percentage sequestrated in low latitude forests (Jobbágy and Jackson, 2000). Thus, any small change in the soil organic C (SOC) pool may cause a drastic fluctuation in the atmospheric C concentration (Cox et al., 2000), consequently accelerating global climate changes such as warming and precipitation changes (IPCC, 2013). Although considerable attempts have been made for decades, drawing a firm conclusion on soil C dynamics under climate change scenarios remains difficult, due to many factors affecting soil C balances (Allison et al., 2010; Schimel et al., 2001; Schmidt et al., 2011). Moreover, available data are distributed unevenly across different regions and generate great uncertainties for soil C predictions (Scharlemann et al., 2014). Specifically, there have been few data of SOC content available in China that include diverse ecosystems (Scharlemann et al., 2014). Increasing research in these data-poor regions would help to improve the modelling precision of global and regional C dynamics.

SOC is a C continuum that consists of various classes of organic materials with different decomposability by soil microorganisms (Schmidt et al., 2011). Although an emerging view shows that turnover of soil organic compounds could be determined by a combination of the decomposer community and the energy needed for their activity, properties and abundance of soil minerals, and supply of numerous resources (Lehmann and Kleber, 2015), these C-containing materials are often regarded as a black box (i.e., the total SOC) or divided into different pools (such as labile and non-labile SOC pools) to simplify studies in practice (von Lützow et al., 2007). In previous literature, physical, chemical and biological methods have been proposed to quantify the labile and non-labile SOC fractions (Blair et al., 1995; McLauchlan and Hobbie, 2004; Six et al., 2001). Despite the diverse methodologies, labile C fractions typically constitute organic compounds that are active and sensitive to environmental changes, whereas non-labile fractions are often considered as containing the mineral-associated and stable organic compounds (von Lützow et al., 2007). Labile and non-labile SOC fractions have been widely used to study the response of SOC to environmental changes (Chen et al., 2012; Durigan et al., 2017; Schnecker et al., 2016), and they are likely to respond differently to environmental changes (Chen et al., 2012; McLauchlan and Hobbie, 2004). Previous studies suggest that labile SOC is more sensitive than non-labile SOC due to its relatively lower molecular recalcitrance and structural protection (Six et al., 2002; von Lützow et al., 2006).

Climate changes can influence hydrologic cycles and precipitation patterns (including precipitation amount, timing, intensity, and frequency), which have been altering in many places (Beier et al., 2012; IPCC, 2013; Knapp et al., 2015). Precipitation changes are expected to obviously influence soil water content (Deng et al., 2012; Harper et al., 2005; Thomey et al., 2011). The resulting changes in water supply may affect the growth and community composition of plants that provide C inputs to the soil via litterfall and root exudates and death (Kardol et al., 2010; Thomey et al., 2011; Zhao and Running, 2010), and soil C outputs by modifying gaseous C emissions (Harper et al., 2005; Huang et al., 2015; Knorr et al., 2008) and aqueous C loss via leaching of dissolved organic C and runoff (Deng et al., 2018; Ma et al., 2014). Finally, trade-offs between altered soil C inputs and outputs determines the direction and magnitude of SOC responses under precipitation changes. Moreover, soil moisture conditions could affect SOC stabilization by means of modifying the abundance of functional soil microbial groups (Canarini et al., 2016), and shifted soil microbial communities may consequently give rise to changes in SOC accumulation due to the varied microbial contribution (e.g., bacteria vs. fungi) (Shao et al., 2017). Although considerable attention has been given to precipitation in recent decades, most studies have focused on changes in precipitation amounts (e.g., Chen et al., 2015; Talmon et al., 2011), with other precipitation attributes having been less studied (Beier et al., 2012). Precipitation changes (such as timing and frequency), however, could even exhibit substantially greater effects than altered precipitation amounts in several ecosystems (Deng et al., 2018; Wu et al., 2012) and deserve further studied.

Moreover, a majority of precipitation manipulation has been conducted at the medium latitudes ranging from 30 to 60°, with only 4% of the manipulations occurring at latitudes < 30° and no one has taken into account changes in precipitation variability in forests (Beier et al., 2012; Vicca et al., 2014). Southern China has been experiencing precipitation changes since the 1980s; although total precipitation has been not altered, no rain and heavy rain days have increased, while light rain has decreased (Zhou et al., 2011). Associated with increased air temperature, soil moisture has been significantly declining in this region (Zhou et al., 2011). Chen et al. (2015) showed that in three forests of southern China, a four-year precipitation removal significantly decreased the SOC content (especially the non-labile fraction), which was accompanied with changed C inputs in terms of both quantity and quality, while a doubling of precipitation had negligible effects. Although a growing body of literature has reported changes in precipitation attributes (IPCC, 2013; Knapp et al., 2015; Zhou et al., 2011), the consequences, e.g., whether precipitation regime change affects SOC fractions in diverse ways, remain less studied (Beier et al., 2012). Limited results derived from studies manipulating precipitation frequency greatly increase the uncertainty related to quantifying soil C dynamics.

Forests contribute to 92% of the global biomass, and tropical forests account for two-thirds of the total forest biomass (262.1 Pg C; Pan et al., 2013). With substantially high gross and net primary productivity, this results in a great proportion of C stock in tropical forests (Pan et al., 2013), and therefore, it is critical to the global C balance. Old-growth monsoon forests in southern China can sequester C in the soil, and this region has been projected to be a significant C sink (Piao et al., 2009; Zhou et al., 2006). With ongoing precipitation changes in the region, however, whether SOC fractions and the soil C sink function would be altered has not been well addressed. Although precipitation change was reported to influence soil respiration (Deng et al., 2018; Moyano et al., 2013; Vicca et al., 2014) and soil C stocks in other ecosystems (Aanderud et al., 2010), 100% higher precipitation did not significantly alter soil respiration or SOC content (including both of the labile and non-labile fractions) in this regional climax forest relative to ambient precipitation (Chen et al., 2015; Deng et al., 2012). A recent study showed that precipitation seasonality greatly affected the dominant soil fungal taxa in a neighbouring evergreen forest (Zhao et al., 2016), and our parallel study demonstrated that an increase in precipitation frequency stimulated the total and dissolved organic carbon (DOC)-driven soil respiration rates (Deng et al., 2018), suggesting an altered microbial community structure and activity under precipitation changes. This scenario could further affect the SOC fractions because soil microbial communities drive soil C processes (Cotrufo et al., 2013).

This precipitation manipulation experiment was conducted in a tropical monsoon forest of southern China to observe how precipitation changes affect the contents of the total, labile and non-labile SOC fractions. Two precipitation treatments were included in the experiment: 1) increase rainfall frequency with the total rainfall amount remaining unchanged (IRF) and 2) decrease the total rainfall amount by 50% (DRA) relative to ambient rainfall (AR). The IRF treatment that reduces the water amount of each precipitation event could be beneficial to water retention for plant and microbial activities (Deng et al., 2018) rather than favouring runoff loss (Laporte et al., 2002). Higher plant productivity and microbial activities could transfer more C into the soil rapidly as microbial products. This scenario may favour soil C stabilization (Cotrufo et al., 2013; Schmidt et al., 2011) because microbial products have been observed to contribute substantially to stable soil organic matter possibly due to a high organo-mineral association and patchy fragment formation between soil aggregates and microbial products

(Kleber et al., 2011; Miltner et al., 2011). Therefore, we expected that the IRF treatment would increase the SOC concentration, especially that of the non-labile C fraction. Conversely, reduced precipitation frequency with the total amount unaltered could affect the synthesis of organic materials and soil CO_2 loss, resulting in lower SOC content (Knapp et al., 2002). The DRA treatment was expected to decrease the contents of the SOC and its fractions, based on previous observations in this region or elsewhere (Chen et al., 2015; Cuevas et al., 2013).

2. Materials and methods

2.1. Site description

This study was conducted in a tropical monsoon forest that locates in the Dinghushan Biosphere Reserve (DBR; 112°30′39″–112°33′41″E, 23°09′21″–23°11′30″N). A chain of undulating hills exist in the reserve, with a slope mostly ranging in 35° to 45°. Forests including the studied climax forest and other secondary coniferous and mixed forests are widely distributed in those parts of the DBR with an elevation being lower than 800 m (more details could refer to the website of DBR; http://www.dhs.scib.cas.cn/). Due to the substantial effects of the land-sea thermal difference and cold air in winter, the study site has a tropical monsoon climate with an annual air temperature of 21.4 °C; mean air temperature is 28.0 °C in the warmest month and 12.6 °C in the coolest month. The air temperature in the investigation period was consistent with the temporal dynamic of the annual air temperature with the warmest August on average at 29.4 °C and the coldest January being 15.2 °C (https://rp5.ru/). The annual precipitation reaches 1956 mm, of which approximately 80% occurs in the wet season from April to September, and the remaining precipitation occurs in the dry season including the remaining months of a calendar year. Precipitation manipulation treatments were conducted in an evergreen broadleaved forest that is the climax in this humid and warm region. With protection by Buddhist monks in a neighbouring temple, the forest has been developed with few anthropogenic activities for >400 years. The dominant tree species include *Castanopsis chinensis*, *Schima superba*, *Cryptocarya chinensis*, and *Machilus chinensis* (Deng et al., 2012). Soils are shallow ultisol overlying sandstone and shale bedrock, with a soil pH (KCl) of 3.9; bulk density of 0.90 g cm^{-3}; and sand, silt and clay proportions of 48.8%, 26.3% and 23.9%, respectively (Chen et al., 2015; Deng et al., 2012).

2.2. Experimental design

The experimental platform including three blocks was established in June 2013, following a randomized block design. In each block, three quadrats with a size of 5 × 5 m^2 were set up, and the two treatments and control were randomly assigned to the quadrats. Polyvinyl chloride (PVC) panels were inserted into the soil to prevent disturbances from surface runoff in and out of the quadrats. The buffering distance between each two quadrats was >5 m.

The rainfall manipulations included a control receiving ambient rainfall (AR) and two treatments, IRF by increasing rainfall frequency with the total rainfall amount unchanged and DRA by decreasing the total rainfall amount by 50% relative to the AR control. In the DRA plots, 50% of the total throughfall produced in each precipitation was intercepted using a semicircle and transparent PVC panels with a diameter of 10 cm because half of the plot areas were sheltered from the rain. For the IRF treatment, half of the throughfall was also intercepted as occurred in the DRA plots and then collected in two big buckets. After rain events, the collected throughfall was reapplied to the corresponding quadrat to simulate increased rainfall frequency without a change in total rainfall amount. Detailed illustrations and photos of the experimental manipulations are referenced to Deng et al. (2018). The water in the buckets was sprayed back evenly 4–5 days after the rain in the dry season or 1–2 days in the wet season. If the collected throughfall was >10 mm in the dry season or >20 mm in the wet season due to sustained rainy days or individual extreme rainfall event, it was reapplied in multiple events with 10-mm water per application event during the dry season and 20 mm per application event in the wet season. In the treated quadrats, litterfall intercepted by the PVC panels was returned under the panels accordingly to avoid changing litterfall inputs. Finally, the annual throughfall was 1854.8 and 1067.8 mm in the AR and DRA plots, respectively, while the IRF plots received 1767.7 mm of throughfall with 55 rainfall events (51%) more than the AR plots (Deng et al., 2018). Both precipitation manipulations did not significantly affect the soil temperature but significantly reduced the soil water content by 28% in the DRA plots (Deng et al., 2018).

2.3. Soil collections, preparations and analyses

Soil samples were collected in soil layers of 0–10, 10–20, and 20–40 cm in the typically dry and wet seasons (January and July, respectively), after two years of experimental manipulations. A round soil auger with an inner diameter of 5 cm was used for soil samplings. In each quadrat, five soil cores were collected randomly in each of the three soil layers after removing the litter and mixed into a composite soil sample. All the composite soil samples were passed through a 2-mm sieve and then air-dried to determine the content of SOC fractions.

The total SOC concentration was quantified by a modified Walkley-Black method, with a $K_2Cr_2O_7$ solution serving as the oxidant and $FeSO_4$ used to titrate the remaining $K_2Cr_2O_7$ (Wang et al., 2012). The readily oxidizable SOC (ROC) concentration was determined by a chemical oxidation method. Air-dried soil samples (<2 mm) were further ground to pass through a 149-μm sieve. For each sample, soils containing 15 mg C were then weighed into a centrifuge tube and 25 mL of 333 mM $KMnO_4$ solution was used as the oxidant to oxidate the labile C fractions (Blair et al., 1995). After shaking for labile C oxidation, these tubes were centrifuged for 5 min at a speed of 2000 r min^{-1}, and then, the supernatants were diluted 250 times with deionized water to read colour development at a wavelength of 565 nm on a UV spectrophotometer (Chen et al., 2012). Three controls with no soil added but with the same procedures (including shaking, centrifuge, and dilution) were also analysed for colour development. The difference in absorbance between the sample and the control was used to calculate the ROC concentration, assuming that 1 mmol MnO_4 was consumed to oxidize 9 mg of carbon (Blair et al., 1995). For each sample, the difference between SOC and ROC concentrations was regarded as the non-readily oxidizable SOC (NROC) content. The air-dried soil samples were also separated by a NaI solution with a density of 1.70 g cm^{-3}, filtered and oven-dried to obtain light fraction and heavy fraction soil aggregates (Janzen et al., 1992). Then, the light fraction and heavy fraction soil aggregates were used to determine the light fraction SOC (LFOC) and heavy fraction SOC (HFOC) concentrations, respectively, in a Vario EL elemental analyser (Elementar, Hanau, Germany). The difference between the SOC and LFOC in each sample was used to adjust the corresponding HFOC content, with a determinant coefficient being 0.88 between the estimated and observed HFOC contents. This high correlation ensures the validity of the density fractionation method. The soil pH value was measured in a 1:2.5 soil/water suspension and water content was determined by oven-drying the soil samples at 24 °C and weighing them. Soil microbial biomass C was tested using the CH_3Cl fumigation and extraction method as described by Vance et al. (1987).

2.4. Statistics

Three-way analyses of variance (ANOVAs) were conducted to detect significant effects on SOC fractions, with rainfall manipulation, soil layer and sampling season serving as the three fixed factors. Because seasonal effects and their interactions with the other two factors were mostly nonsignificant (Table 1), we pooled the data obtained in the wet and dry seasons to conduct further data analyses. Univariate ANOVA with the Tukey HSD method was used to test the significance of rainfall

Table 1
Summary of three-way ANOVAs on soil organic carbon (SOC) fractions, with rainfall manipulation treatment (RMT), soil layer (SL) and season (S) serving as three fixed factors. The statistical *F* and significance level *p* are presented in the table. ROC stands for readily oxidizable organic carbon, NROC stands for non-readily oxidizable organic carbon, LFOC stands for light fraction organic carbon, and HFOC stands for heavy fractions organic carbon.

		RMT	SL	S	RMT × SL	RMT × S	SL × S	RMT × SL × S
SOC	*F*	8.9	86.8	1.2	1.4	3.4	1.7	1.0
	p	0.001	<0.001	0.271	0.263	0.046	0.206	0.428
ROC	*F*	11.3	86.5	1.0	0.6	0.9	0.4	0.1
	p	<0.001	<0.001	0.331	0.672	0.428	0.681	0.976
NROC	*F*	10.2	90.4	2.8	1.7	3.1	2.0	1.3
	p	<0.001	<0.001	0.101	0.166	0.058	0.152	0.303
LFOC	*F*	7.3	43.8	0.1	0.6	1.2	1.3	0.3
	p	0.002	<0.001	0.705	0.671	0.299	0.276	0.877
HFOC	*F*	7.2	83.2	2.2	1.4	1.7	0.7	1.4
	p	0.002	<0.001	0.144	0.238	0.196	0.528	0.258

manipulations on SOC in each of the three soil layers and in the soil layer under each of the three precipitation manipulation treatments, with block as a random factor. When normality and variance homogeneity assumptions were not met, data were rank-transformed using the Rankit method to obtain normal scores for data analyses. Bivariate correlations were used to test the significance between each two of the SOC fractions and environmental factors including soil water content, pH and microbial biomass C. The significance level was set at $p < 0.05$. All the statistical analyses were conducted in IBM SPSS Statistics 22 (IBM Corp., New York, US), and graphs were created in Sigmaplot 10.0 (Systat Software Inc., California, US).

3. Results

3.1. Changes in SOC fractions

Three-way ANOVAs showed that the rainfall manipulation and soil layer significantly affected the SOC fractions ($p < 0.05$ for all), with no interaction detected for any of these parameters ($p > 0.05$ for all; Table 1). In the investigated soil layers, SOC fractions did not respond to the rainfall manipulations differently between the wet and dry seasons, as indicated by the nonsignificant seasonal effects ($p > 0.05$; Table 1). For the tested SOC fractions, there is no significant interactive effect among rainfall manipulation, soil layer and sampling season ($p > 0.05$), with the only exception being that SOC was interactively affected by rainfall manipulation and sampling season ($p = 0.046$; Table 1).

Relative to the AR control, the IRF treatment significantly increased the SOC concentration by 13.3 ± 4.2 g kg^{-1} in the first surface soil layer (i.e., 0–10 cm), while the DRA treatment did not significantly change the SOC concentration (Fig. 1). As soil depth increased (i.e., 10–20 and 20–40 cm), rainfall manipulation effects became nonsignificant, although the increasing trend was maintained ($p = 0.069$ and 0.103, respectively; Fig. 1). The IRF and DRA treatments tended to increase ROC and LFOC contents in the 0–10 cm soil layer ($p = 0.061$ and 0.062, respectively) and significantly increased ROC content in the 10–20 and 20–40 cm soil layers and LFOC content in the 10–20 cm soil layer ($p < 0.05$; Fig. 2a and b). For the two non-labile fractions of NROC and HFOC, the IRF treatment significantly increased the contents in the first soil layer ($p < 0.05$ for the both), but such a trend became nonsignificant in the 10–20 and 20–40 cm soil layers ($p > 0.05$; Fig. 2c and d). The DRA treatment, however, did not significantly change the NROC and HFOC contents in the three soil layers ($p > 0.05$; Fig. 2c and d).

In addition, we calculated the proportion of differences in the labile and non-labile SOC fractions that contributed to the total difference in SOC concentration under each precipitation treatment relative to that in the control. The results showed that most of the total SOC increased

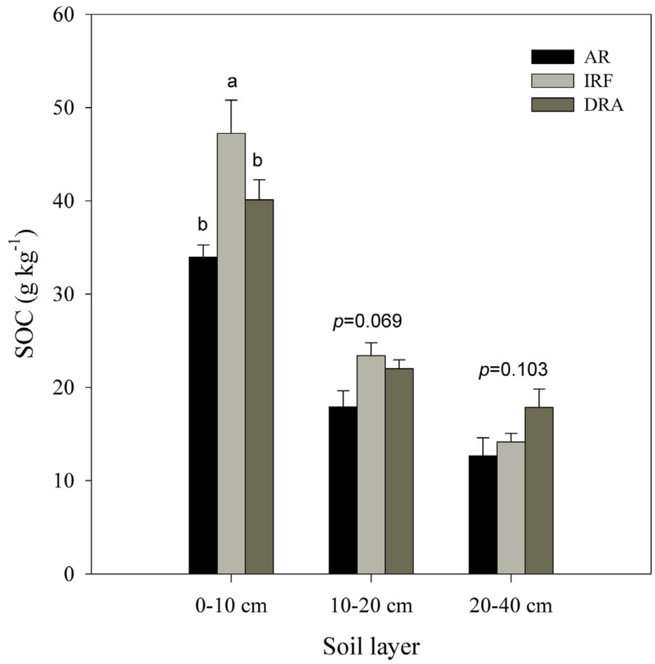

Fig. 1. Soil organic carbon (SOC) concentrations under the three rainfall manipulation treatments in the three soil layers. Error bars represent standard errors (n = 3). Different lowercase letters above bars indicate significant differences at $p < 0.05$. An exact *p* value is given when $p \geq 0.05$. In the figure, AR is ambient rainfall, IRF is increased rainfall frequency with the total rainfall amount unaltered, and DRA is decreased rainfall amount by 50% relative to ambient rainfall.

under the IRF and DRA treatments due to the accumulation of non-labile SOC fractions (NROC and HFOC) that contributed on average 75% to the total SOC increases (53%–91%; Fig. 3).

3.2. Bivariate correlations

There were significant correlations between the total SOC and SOC fractions, a pattern that consistently occurred in the three soil layers ($p < 0.05$ for all; Table 2). However, the SOC fractions did not show significant correlations with soil water content or microbial biomass C in most combinations ($p > 0.05$, Table 2). Soil pH generally had negative relationships with the SOC fractions, which were statistically significant in the 0–10 and 20–40 cm soil layers ($p < 0.05$; Table 2). When pooling all the data, these correlations between each of the two variables were maintained, and the statistical power was further increased, as indicated by the greater correlation coefficients (Table 2). Furthermore, the SOC fractions showed significant correlations with soil water content, pH and microbial biomass ($p < 0.05$; Table 2), implying that these variables contributed significantly to variations in the SOC fractions across soil layers. This implication was demonstrated by the significant correlations between the SOC fractions and environmental factors across the soil layers under each of the rainfall manipulations ($p < 0.05$).

4. Discussion

In the present study, the IRF treatment increased the SOC concentration relative to the AR control (Fig. 1), a result supporting our expectation. However, a direct correlation between soil water and C content was not observed in the two surface soil layers (Table 2), which could be attributable to the vertical hydraulic lifting by plant roots and fast evaporation in the surface soils (Caldwell et al., 1998). Previous studies suggest that increased rainfall frequency could stimulate plant and microbial activities and affect ecosystem processes (Knapp et al., 2008; Laporte et al., 2002). Under the IRF treatment in our study, 50% of the throughfall generated in each rainfall event was collected and then

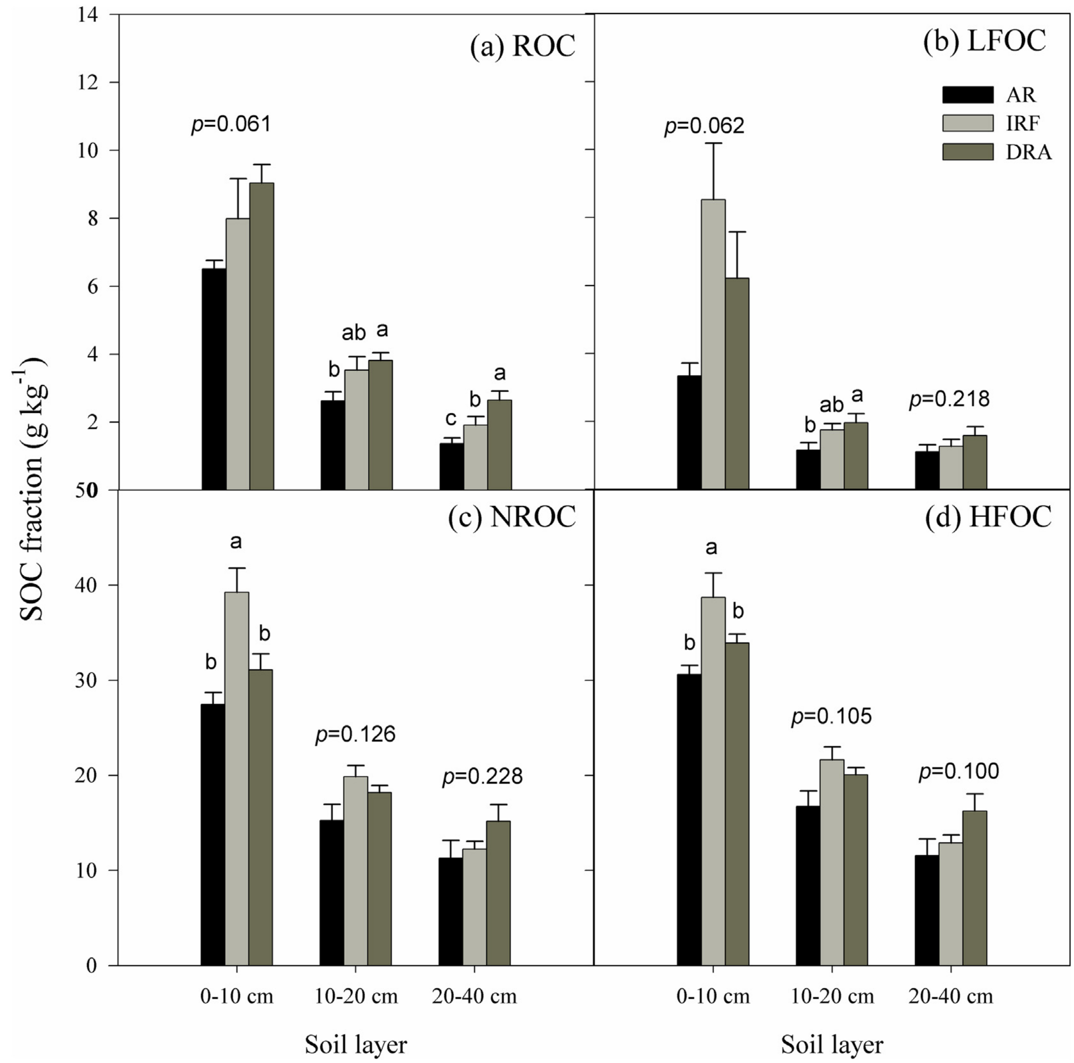

Fig. 2. Soil organic carbon (SOC) fractions under the three rainfall manipulation treatments in the three soil layers. Error bars represent standard errors (n = 3). Different lowercase letters above bars indicate significant differences at $p < 0.05$. An exact p value is given when $p \geq 0.05$. In the figure, AR is ambient rainfall, IRF is increased rainfall frequency with the total rainfall amount unaltered, and DRA is decreased rainfall amount by 50% relative to ambient rainfall. The abbreviations ROC and NROC represent readily and non-readily oxidizable organic carbon, respectively, while LFOC and HFOC represent light-fraction and heavy-fraction organic carbon, respectively.

reapplied in the corresponding quadrats several days after the rain. This treatment was able to reduce the rainfall intensity of each rainfall event (Deng et al., 2018) and may have decreased the water loss through runoff because a previous study suggested that rainfall events > 14 mm could generate runoff in this region (Zuo and Ma, 2005). Extra water retention could maintain higher activity of plant growth

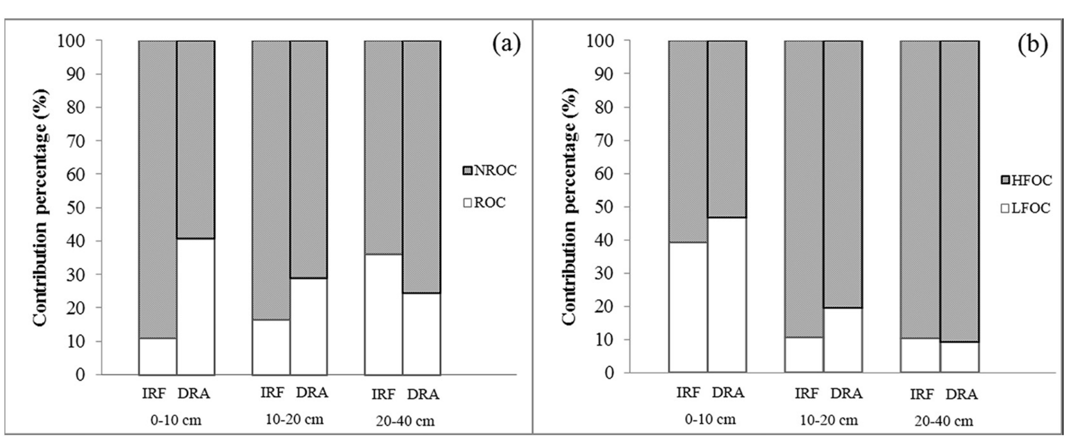

Fig. 3. Contribution of changes in the labile and non-labile SOC fractions to total change in the SOC concentration under the rainfall manipulation treatments. In the figure, IRF is increased rainfall frequency with the total rainfall amount unaltered and DRA is decreased rainfall amount by 50% relative to ambient rainfall. The abbreviations ROC and NROC represent readily and non-readily oxidizable organic carbon, respectively, while LFOC and HFOC represent light-fraction and heavy-fraction organic carbon, respectively.

Table 2
Bivariate correlations between SOC fractions and environmental factors in the three soil layers. Sample number (n) is 18 for each of the three soil layers, while n equals 54 when all the data were pooled. In the table, * indicates a significant correlation at $p < 0.05$ level and ** indicates a significant correlation at $p < 0.01$. Italics indicate marginally significant correlations at $0.05 < p < 0.10$ level. The four SOC fractions ROC, NROC, LFOC and HFOC stand for the readily oxidizable organic carbon, the non-readily oxidizable organic carbon, the light fraction organic carbon, and the heavy fractions organic carbon, respectively. SWC is soil water content and MBC is microbial biomass carbon.

		ROC	NROC	LFOC	HFOC	SWC	pH	MBC
0–10 cm	SOC	0.71**	0.98**	0.91**	0.94**	−0.10	−0.73**	*0.45*
	ROC		0.54*	0.51*	0.61**	−0.32	−0.34	0.25
	NROC			0.94**	0.94**	−0.02	−0.76**	*0.46*
	LFOC				0.75**	−0.26	*−0.46*	0.50*
	HFOC					0.05	−0.83**	0.54*
10–20 cm	SOC	0.67**	0.98**	0.60**	0.99**	0.20	−0.40	0.14
	ROC		0.51*	0.68**	0.61**	−0.32	−0.44	−0.22
	NROC			0.52*	0.99**	0.31	−0.42	0.21
	LFOC				0.48*	−0.11	−0.10	0.30
	HFOC					0.23	−0.42	0.10
20–40 cm	SOC	0.71**	0.99**	0.77**	1.00**	0.47*	−0.52*	0.08
	ROC		0.61**	0.39	0.72**	0.27	−0.64*	0.05
	NROC			0.79**	0.99**	0.48*	−0.54*	0.16
	LFOC				0.68**	*0.41*	−0.49*	0.18
	HFOC					0.50*	−0.54*	0.13
All	SOC	0.92**	1.00**	0.90**	1.00**	0.67**	−0.87**	0.60**
	ROC		0.89**	0.85**	0.92**	0.59**	−0.88**	0.53**
	NROC			0.89**	0.99**	0.69**	−0.85**	0.60**
	LFOC				0.87**	0.57**	−0.77**	0.57**
	HFOC					0.68**	−0.87**	0.59**

and promote plant productivity (Knapp et al., 2008; Laporte et al., 2002; Nijp et al., 2014), thus increasing soil C inputs.

In the studied forest, a recent study showed that relative to the AR plots, the IRF treatment significantly increased fine root biomass (Deng et al., 2018), which is thought to closely correlate with the aboveground biomass in primary forests (Sun et al., 2018). Although respired C loss was also enhanced by more frequent rainfall events (Deng et al., 2018; Laporte et al., 2002), increased plant activity could enhance soil C inputs by higher litterfall productivity and root exudates with altered C quantity and quality (Knapp et al., 2008). Moreover, higher microbial activities may transfer litter C more rapidly into the soil (e.g., litter-leached DOC; Deng et al., 2018). The microbial products are thought to contribute greatly to SOC stabilization (Cotrufo et al., 2013; Schmidt et al., 2011; von Lützow et al., 2006), possibly due to high organo-mineral association and patchy fragment formation between soil aggregates and microbial products (Kleber et al., 2011; Miltner et al., 2011). The balance between C inputs and outputs determines the direction and magnitude of SOC changes (Knapp et al., 2008). Therefore, the SOC increases under the IRF treatment could be due to the rainfall frequency increases the benefits to plant-derived C inputs more than microbial decomposition (i.e., increased C input > increased C output). Moreover, soil C loss through runoff could also be lower in the nutrient-rich upper soils (Polyakov and Lal, 2004). The two processes favour soil C accumulation.

The SOC increases under the IRF treatment were derived from increases in both of the labile and non-labile fractions (Figs. 2 and 3), as supported by consistent patterns resulting from the physical (density fractionation) and chemical (permanganate oxidation) SOC fractionations in this study. Therein, increases in the non-labile fraction contributed substantial proportions to the SOC pattern (Fig. 3). On the one hand, this result is likely due to increases in the plant-derived C inputs by aboveground litterfall that are more rapidly transferred into soils, as well as increases in belowground productivity (e.g., fine root) under the IRF treatment (Deng et al., 2018). This direct C transfer, however, could only account for a minor proportion of the increased SOC because an emerging view has been expressed that SOM stabilization is mainly derived from the interaction between the soil matrix and organic compounds that have been processed by microbial activities (Cotrufo et al., 2013; Lehmann and Kleber, 2015), although chemical recalcitrance has been highly recommended to determine SOC storage in several ecosystems such as seasonally dry tropical forests (Campo and Merino, 2016). On the other hand, this increase in the non-labile fraction, as indicated by both of the NROC and HFOC contents, could also be associated with more favourable nutrient availability and consequently higher microbial activity. A parallel study showed that the IRF treatment significantly enhanced litter-leached C, N and P concentrations and fluxes and increased fine root biomass (Deng et al., 2018), both of which could promote soil C inputs. Combined with the relatively higher labile SOC under the IRF treatment as observed in the present study (Fig. 2a and b), soil nutrient availability should have been improved to support higher soil microbial activities to process C into non-labile forms. Microbial products are important to ensuring stable SOC and account for a substantial proportion of the total SOC (Canarini et al., 2016; Cotrufo et al., 2013; Schmidt et al., 2011).

Reduced rainfall could induce extra SOC loss across different ecosystems (Dai et al., 2015; Li et al., 2015; Meier and Leuschner, 2010). However, decreasing the rainfall amount by 50% did not significantly affect the SOC concentration relative to ambient rainfall in the studied forest (Fig. 1). This result is opposite from our expected result that DRA would decrease the SOC concentration. In a recent study, 100% removal of rainfall for 4 years significantly decreased the SOC concentration, which was accompanied by reduced soil water content and microbial biomass C in this studied forest (Chen et al., 2015). In this forest, there could be a soil moisture threshold (15%) beneath which water deficit may occur and stress ecosystem functioning (such as soil respiration) (Deng et al., 2010). The studied forest is a climax forest in southern China with sufficient water supply and therefore does not have a water-deficit in most of the year (Deng et al., 2012; Deng et al., 2018). This scenario may make the forest resistant to precipitation decreases to some extent. Moreover, mature forests with few disturbances are generally less sensitive to climate change (Kroel-Dulay et al., 2015), although extreme events are very likely to change the SOC concentration (e.g., Chen et al., 2015). The treatment magnitude and duration could greatly affect the response of ecosystem functions and processes (Beier et al., 2012; Wilcox et al., 2017).

This is a pioneering study to explicitly conduct a manipulation of precipitation frequency in a tropical forest to clarify precipitation effects on SOC fractions. From the perspective of soil C fractions, we conclude that this tropical monsoon forest is vulnerable to changes in precipitation frequency even in a relatively short period of time. This pattern indicates that the current trend of fewer but more intensive precipitation events in this region (Zhou et al., 2011) may be adverse to SOC stabilization and could weaken the regional C sink function as identified in previous studies (Piao et al., 2009; Zhou et al., 2006). In contrast, this humid tropical forest is very resistant to changes in precipitation amounts, indicating that a change in the quantity of precipitation would not substantially affect SOC content. However, additional events such as a complete removal of precipitation for a period would result in a significant effect on soil C stabilization in this forest (Chen et al., 2015). As proposed in recent studies, the persistence of SOC in an ecosystem that is controlled by a combination of factors including climate conditions such as precipitation is prone to changes in these controlling factors (Lehmann and Kleber, 2015; Schmidt et al., 2011). Moreover, our study presents asymmetric responses of SOC fractions to precipitation changes along the soil layers. Despite the underlying mechanisms remaining unclear, this result highlights a necessity to explicitly represent soil C processes with altered process parameters as recommended by Schmidt et al. (2011).

However, methodological limitations remain in the present study, and therefore, the results should be interpreted with caution. First, a list of recent studies demonstrate that soil organic C is actually a continuum of organic compounds that have been processed to different decomposition stages by soil microbial communities (Lehmann and Kleber, 2015; Schmidt et al., 2011). Fractionating SOC into different pools is too robust to capture SOC change in detail, although some implications can be obtained at a relatively low resolution. Several high-resolution techniques such as nuclear magnetic resonance and mass spectrometry and fine-scale imaging techniques to address spatial arrangements of soil matrixes are highly recommended for studying SOC in response to environmental changes (Schmidt et al., 2011). Second, the SOC concentration was analysed using a modified Walkley-Black method (Wang et al., 2012). This wet chemical oxidation method could overestimate C content if the SOC concentration is extra low (e.g., <5 g kg^{-1}; Wang et al., 2012), and this method has limitations such as altered recovery proportion and potential risk of Cr pollution (Chatterjee et al., 2009; De Vos et al., 2007); however, it can provide reliable estimates for soil samples that contain relatively high C content (Wang et al., 2012). Moreover, despite being not presented in this study, combining observations of soil bulk density can shed a light on the SOC stock in response to precipitation changes and then provide further information on SOC dynamics under global changes.

5. Conclusions

In the studied forest, increased rainfall frequency for a relatively short period of time was able to increase the SOC concentration that was accompanied by a significant increase in the non-labile SOC content. Relative to the labile SOC, the non-labile SOC fraction contributed on average 75% to the SOC increases. A removal of 50% of the total rainfall amount increased the labile SOC concentration but did not significantly change the non-labile SOC content. Consequently, the total SOC concentration was not significantly altered by a decrease of 50% of the total precipitation amount because the labile SOC contributed a small proportion to the total SOC content. This scenario typically is associated with resistance of the studied climax forest, and the DRA treatment in the present study did not result in a water-deficit condition in such a water-rich forest. Our results suggest that the soil C concentration in this old-growth tropical forest could be more vulnerable to changes in rainfall frequency than changes in rainfall amount and that the current trend in precipitation changes in this region may be adverse to the SOC stabilization.

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