

The stability of soil organic carbon across topographies in a tropical rainforest

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Mechanisms of soil organic carbon (SOC) stability are still unclear in forests ecosystems. In order to unveil the influences of topography on the SOC stability, the 60ha dynamic plot of a tropical montane rainforest were selected in Jianfengling, in Hainan Island, and soil was sampled from 60 quadrats. The chemical fractions of SOC were detected with ¹³C CPMAS/NMR and path analyses were used to explore the mechanisms of SOC stability in the two local topographies (flat areas and steep areas). The chemical fractions of SOC showed alkyl carbon > O-alkyl carbon > carboxyl carbon > aromatic carbon. The decomposition index (DI) values were greater than 1 in different topographies and the averaged DI value was 1.29, which indicated that the SOC in the study area were stable. The influencing factors of SOC stability varied in topographies, where SOC, soil moisture (SM), ammoniacal nitrogen (NH₄⁺-N, AN) were the main influencing factors in the relative flat areas (RF), as well as SM and AN were the main factors in the relative steep areas (RS). More SOC and AN strengthened the SOC stabilization, while higher soil moisture lowered SOC stability. Inertia index (II) also higher in RS than that in RF, indicating that local topography significantly affects SOC content and SOC stability through changing soil environment factors. Topography cannot be neglected in considering SOC stability and further C budgets.

1 **The stability of soil organic carbon across topographies in a tropical rainforest**

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12 **Abstract:** Mechanisms of soil organic carbon (SOC) stability are still unclear in forests
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27 budgets.

28 **Keywords:** soil organic carbon stability; topography; tropical forest; ^{13}C CPMAS/NMR; path
29 analysis

30

31 1. Introduction

32 Soil organic carbon (SOC) of forests plays an important role in the global carbon cycle (Pan
33 et al. 2011). Changes in SOC storage can significantly impact the global carbon cycle and climate
34 change (Stockmann et al. 2013). SOC stability is the ability of soil organic matter to resist
35 disturbance and restore original level under current conditions (Wiesmeier et al. 2014). This high
36 SOC stability would be beneficial to the accumulation of SOC (Schmidt et al. 2011). The SOC
37 stabilization mechanisms that are commonly accepted include chemical stabilization, physical
38 stabilization, and biochemical stabilization (Christensen 1996; Fontaine et al. 2007; Six et al.
39 2002). In terms of chemical stabilization, SOC consists of chemical fractions with different
40 stability and has long been considered as the decisive mechanism of SOC stability (Yang et al.
41 2020). Solid ^{13}C NMR technology is one of the methods to dissect SOC chemical fractions and

42 has been applied into tropical forest soil stability research. Previous studies illustrated that the
43 composition of SOC chemical fractions are affected by soil microorganisms, soil properties, and
44 carbon input (ElberlingBreuning-Madsen & Knicker 2013; González-Pérez et al. 2008; Ktigel-
45 Knabner 1997; LorenzLal & Jiménez 2010). Topographic factors, such as elevation, slope,
46 concavity and convexity, affect water flow and erosion, plants growth and litter decomposition,
47 and hence influence SOC content and quality (Fernández-RomeroLozano-García & Parras-
48 Alcántara 2014; SunZhu & Guo 2015). As such there is inherent heterogeneity in SOC stability
49 associated with the spatial variability of topographic factors. However, studies on SOC stability
50 influencing factors mainly focused on tree species, soil minerals protection or altitude (Angst et
51 al. 2018) rather than topography.

52 Topography itself is a comprehensive factor, thus, it should be taken into consideration in
53 SOC stability. Topography dominates the allocation of water and heat resources, which affects the
54 spatial allocation of vegetation and may thus the quantity and quality of SOC (SunZhu & Guo
55 2015). In addition, the movement of water and nutrients due to different topography types
56 contributes to soil properties heterogeneity (TsuiChen & Hsieh 2004). Studies found topography
57 influences nutrients availability in tropical forests, and the heterogeneity of nutrient cycling may
58 influence the dynamics of SOC across diverse tropical landscapes (Cleveland et al. 2011;
59 Randerson et al. 2009; Townsend et al. 2011). We hypothesized that nutrients would be the key
60 influencing factors of SOC stability.

61 The Jianfengling montane rainforest is one of the best preserved tropical primeval forest in
62 China (Li et al. 2012). Previous studies found terrain heterogeneity in this area had a strong effect
63 on the soil properties (Shi 2012) and pointed that SOC stability is significantly influenced by soil
64 properties (Liu et al. 2007). Soil properties on varying topographic positions also have distinctive
65 characteristics under micro-climate conditions (Zhu et al. 2014), which fundamentally influences
66 nutrients, hydrological processes, and hence the SOC stability across topographies. However, it
67 lacks systematic investigations to particularly address how topographies influence the SOC
68 stability via changing soil properties. The objectives of this study are to investigate the differences
69 in soil properties and SOC stability at four topographic positions, and to identify the mechanisms
70 of SOC stability in different topographies in tropical rainforests. To address this objective, we
71 collected soils from 60 quadrats in the 60ha dynamic plot in different topographical areas in the

72 Jiangueling tropical montane rainforest. The stability of SOC was demonstrated by SOC chemical
73 fractions which were analyzed by ^{13}C -NMR.

74 **2. Materials and Methods**

75 The study area, a 60ha dynamics plot in the tropical montane rain forest ($18.23^\circ - 18.50^\circ\text{N}$,
76 $108.36^\circ - 109.05^\circ\text{E}$) with an elevation being 800 – 1000 m, located in Jianfengling, in the
77 southwest region of Hainan Island, China(Li & Zhou 2002). The climate of studied area is tropical
78 rainy climate, with a mean annual precipitation of 1700 – 2600 mm, and a mean annual temperature
79 of 20 – 25°C (Yang et al. 2016). The main plant species are Lauraceae, Rubiaceae, Fagaceae,
80 Myrtaceae (Li & Zhou 2002) and the soil belongs to Ultisols order according to USDA Soil
81 Taxonomy.

82 Based on the survey technical specification of the Center for Tropical Forest Science,
83 Smithsonian Institute, the 60 ha dynamics plot was divided into 1500 quadrats, each 20 m \times 20 m
84 in size. Figure 1 shows the distribution of the 60 randomly selected soil sampling sites from the
85 1500 quadrats. In each sampling site, cutting ring method was used to obtain topsoil (0-10 cm)
86 samples (each sample including four replications). In total, 240 soil samples were collected and
87 brought back to the laboratory. A part of soil sample was stored under 4°C to measure acid
88 phosphatase activity (APA). The rest of soil sample were air-dried and ground into 1 mm to
89 analyze soil total nitrogen (TN), total phosphorus (TP), available phosphorus (AP), soil pH, and
90 SOC chemical fractions.

91 Soil samples were pretreated with 10% (v/v) HF solutions before solid-state ^{13}C -CPMAS
92 NMR analysis, to remove Fe^{3+} and Mn^{2+} in soil, improving the signal/noise ratio. In detail, 10 g
93 ground soil sample was vibrated for 2 hours after adding 50 ml HF solutions and then centrifuged
94 with a centrifuge (3,000 rpm) for 10 minutes to remove the suspension. The above steps were
95 repeated five times to get the precipitate (Mathers et al. 2002). The precipitate was washed 5 times
96 with 50 ml deionized water to remove residual hydrofluoric acid, then freeze-dried for further
97 analysis. The fractions of SOC were measured with ^{13}C CPMAS/NMR, 4 mm probe pulse
98 sequence. CPTOSS ^{13}C resonant frequency is 100.38 MHz, ^1H resonant frequency is 399.16
99 MHz, rotate speed is 5 kHz, and the contact time is 3 ms, a cycle time is 1 s, sampling frequency
100 of 4 k. According to previous study (Baldock et al. 1992), different peaks represent different C
101 compounds: 0 – 45 ppm is alkyl carbon, 45 – 110 ppm is O-alkylcarbon, 110 – 160 ppm is aromatic

102 carbon, and 160 – 220 ppm is carbonyl carbon. Total of 240 soil samples were detected in the
103 Analytical Center of Institute of Chemistry Chinese Academy of Sciences (Beijing City). MestRe-
104 C software was used to analyze chemical fractions of SOC. We used two indices, decomposition
105 index (DI) and inertia index (II), to represent SOC stability.

$$106 \quad DI = \frac{\text{alkyl carbon}}{\text{o-alkyl carbon}} \quad (1)$$

$$107 \quad II = \frac{\text{alkyl carbon} + \text{aromatic carbon}}{\text{o-alkyl carbon} + \text{carboxyl carbon}} \quad (2)$$

108 The DI can reflect microbial processing with higher ratios indicating losses of more labile C
109 relative to poorer-quality C compounds (Cusack et al. 2011), therefore, the higher ratio, the more
110 stable is the SOC (Chen 2012; Ostertag et al. 2008). The higher II value means more alkyl carbon
111 and aromatic carbon exist in the SOC, indicating higher SOC stability (Ostertag et al. 2008), which
112 is benefit to SOC accumulation.

113 Soil organic carbon and total N contents were determined by the $\text{K}_2\text{Cr}_2\text{O}_7\text{-H}_2\text{SO}_4$ oxidation
114 method and Kjeldahl procedure respectively (Liu et al. 2012). Soil total phosphorus (TP) were
115 extracted by semimicro kelvin method and determined by automatic flow analyzer (PROXIMA
116 1022/1/1, ALLIANCE instruments, France). Soil ammoniacal nitrogen ($\text{NH}_4^+\text{-N}$) contents were
117 extracted using 2 M KCl on an orbital shaker for 1 h under ambient temperature and then the
118 suspension was filtered. The extracts were analyzed by a continuous flow analytical system
119 (SKALAR San++, SKALAR Co., Netherlands). Soil available phosphorus (AP) was determined
120 by the ammonium chloride-hydrochloric acid extraction method (Bao 2000), and acid phosphatase
121 activity (APA) was determined by the phosphoric acid bisacid colorimetric method (Guan 1986).
122 Soil moisture (SM) was represented as the ratio of dry soil weight to soil water weight after fresh
123 soil samples were dried at 105°C in 24 h. Soil pH was detected by the potentiometric method,
124 where 10 g air-dried soil was put in 25 ml beaker and added with distilled water and kept for 30
125 minutes, and then the suspension's pH value was measured with corrected pH meter.

126 To investigate the effects of local topography on soil properties, we binned each data point
127 into a topography type based on elevations, slopes and convexities by fuzzy *C*-mean clustering
128 cluster analysis (Xu et al. 2015). Four topography types were identified, the flap area, the relative
129 steep area, the steep area and the mountaintop area. We then performed one-way ANOVAs to test
130 the differences of soil properties between topography groups. The soil property was significant
131 different if P value was ≤ 0.05 . All results are shown as mean \pm SE. Path analysis provides a way

132 to examine the multiple relationships between SOC stability and environmental factors. Thus, path
133 analytic framework was applied to examine the influencing factors of SOC stability in different
134 topographies. To get the eligible results of path analyses with enough data in each topography, we
135 classified the flat and the top area into the relative flat (RF) areas and the rest two types into the
136 relative steep (RS) areas.

137 All analyses were conducted using SPSS Version 20 and figures were generated by Origin
138 (version 92 E).

139 **3. Results**

140 **3.1. Soil properties in different topography types**

141 Soil properties varied in different topographies. To be specific, soil organic carbon increased from
142 flat to top, where the contents of SOC was 29.66 g/kg in flat areas and 41.97 g/kg in top areas. Soil
143 TN tended to increase in top area, in which the contents of TN were 1.47 g/kg in flat areas and
144 1.78 g/kg in top areas. However, soil C:N ratios were similar in different topographies. The
145 contents of AN, TP, SM were also the highest in the top areas, whereas the contents of pH were
146 the highest in the flat areas (Figure 2. Table 1).

147 **3.2. SOC components in different topography types**

148 The alkyl carbon, averaged 41.67% of the SOC, contributed the most to the SOC chemical
149 fractions in the four different topography types (Figure 3). The aromatic carbon which accounted
150 only 2.61%, contributed the lowest to SOC. The O-alkyl carbon and carboxyl carbon took up
151 33.08% and 22.80%, respectively. As illustrated in figure 4, the overall distributions of four
152 fractions of SOC were similar in different topography types. However, alkyl carbon and carboxyl
153 carbon proportions were significantly altered by topography. The content of alkyl carbon increased
154 from flat to top, where the content was 38.87% in flat areas and 43.34% in top areas, but the content
155 of carboxyl carbon decreased from flat to top, where the content was 24.78% in flat areas and
156 21.24% in top areas.

157 **3.3. SOC stability index in local topography types**

158 In the four topographies, decomposition index (DI) was all higher than 1, and inertia index
159 (II) were all higher than 0.7. The DI was not significantly different among topographies, while II

160 was significantly different in different topographies with the lowest II value in flat areas (0.72)
161 and the highest value in steep areas (0.91) (Figure 5).

162 3.4. Influencing factors of SOC stability in different topography types

163 Figure 6 showed the influencing factors and mechanisms of SOC stability were different in
164 different topography types. In the RF areas, SOC, AN and SM together explained 52% variability
165 of SOC stability. SOC and AN positively and directly affected SOC stability, high SOC and AN
166 could strengthen SOC stability. SM negatively and directly influenced SOC stability, however the
167 direct negative effect of SM was partly offset by the indirect positive effect of SM on AN and
168 SOC, thus, SM contributed a little in total effects of SOC stability.

169 In the RS areas, SM and AN also affected SOC stability. The effect direction was the same
170 as in the RF areas. The effect of SM was larger than that of AN on SOC stability. Increasing SM
171 would decrease SOC stability, this negative effect was partly offset by its indirect effect on AN.
172 AN positively and directly affected SOC stability.

173 Soil AN and SM were the same influencing factors of SOC stability in the RF areas and the
174 RS areas, however the effect forces were different. In detail, SM was the negligible limiting factor
175 in the RF areas, whereas SM was the most important limiting factor in the RS areas. The results
176 of path analyses illustrated that R square of the models was 0.53 in the RF areas, while it was 0.42
177 in the RS areas (Table S1).

178 4. Discussion

179 The chemical fractions of SOC in different topography areas are characterized by alkyl carbon
180 > O-alkyl carbon > carboxyl carbon > aromatic carbon. The SOC fractions ranking order is similar
181 to those of subtropical evergreen board-leaved forest and Masson pine in China (Shang et al. 2013),
182 subtropical natural forest, hoop pine plantation in Australia (ChenXu & Mathers 2004). The alkyl
183 carbon accounted for the most of the SOC fractions, indicating a higher percentage of passive
184 carbon in SOC. The DI (alkyl carbon/O-alkyl carbon) at plot scale was 1.29, which is higher than
185 moist semi-deciduous tropical forest soils in Ghana (1.25) (ElberlingBreuning-Madsen & Knicker
186 2013), old live oak forest (0.87) and old-growth dry tropical forests (0.70) in Costa Rica
187 (LorenzLal & Jiménez 2010), plantations in the subtropics of China (*Pinus massoniana*,
188 *Castanopsis hystrix*, *Michelia macclurei*, *Mytilaria laosensis*, all < 0.8)(Wang 2010), evergreen

189 broad-leaved forest (0.76), *Cunninghamia lanceolata* forest (0.89), *Cryptomeria fortune* forest
190 (0.78), and coniferous and broad-leaved mixed forest (0.53) in the subtropics of China (Zhang et
191 al. 2015). The DI in different topography types were all higher than 1, indicating a rather stable
192 SOC in the tropical montane rainforest of Jiangfengling, Hainan Island (González-Pérez et al.
193 2008)

194 Path analysis showed SOC contents only affected SOC stability in RF areas, while AN and
195 SM affected SOC stability in both topographies. Studies found the interaction between SOC and
196 mineral surfaces could stabilize SOC, whereby stable organic-mineral bonds were formed through
197 anion and inner-sphere ligand-exchange reaction (Wiesmeier et al. 2019). Tropical forest with
198 highly weathered soils contains high concentrations of iron (Sanchez 1976) that could protect C
199 from microbes and enhance SOC stability. The SOC only affected SOC stability in RF areas might
200 be due to the SOC content in RF areas was significantly higher than that in the RS areas ($37.21 >$
201 31.32 g/kg, $P < 0.01$).

202 SOC stability depends not only on its chemical characteristics, but also on soil
203 microenvironment (Yang et al. 2020). Soil biotic community and the soil microenvironment were
204 key factors affecting the SOC stability (Schmidt et al. 2011; Yang et al. 2018). Soil N, especially
205 in the bioavailable forms, such as ammoniacal nitrogen is an essential component of all living
206 organisms. Soil N dynamics are mainly driven by microbe (Levy-BoothPrescott & Grayston 2014)
207 and microbes mainly assimilate of AN (Cheng et al., 2015); however, others found nitrogen
208 addition negatively affects soil microbial growth, composition and function (Chen et al. 2018).
209 The soil AN content in the study area is rather high, which is about 40.4 mg/kg while the value in
210 Amazon pristine forest is 5.7 mg/kg (Hamaoui et al. 2016) and 2.12 mg/kg in a secondary tropical
211 forest in Southern China (Wang et al. 2014). Therefore, microorganism activities and functions
212 might be suppressed by the high AN content in this area, resulting in a slow decomposition rates
213 and consequently high SOC stability in this tropical forest.

214 Soil moisture is a key factor influencing SOC mineralization in terrestrial ecosystems
215 (LiuZhang & Wan 2009; MoyanoManzoni & Chenu 2013). Soil moisture could affect SOC
216 decomposition directly or indirectly. Topography influences soils by the transport of fine soil
217 particle, base of slopes and positions of lower slope which called depositional areas tend to have
218 high organic materials and water-holding capacity (Chapin III Matson & Vitousek 2012). The
219 above process consequently results in active carbon accounted more compared with the stubborn

220 carbon, indicating the low DI and II in RF. This explained why SM was the negative influencing
221 factor of SOC stability in RS and RF areas (Figure 6). Moreover, SOC in flap areas was the most
222 unstable carbon in the four topography types (Figure 4). In terms of indirectly ways, decomposition
223 of soil organic matter depends on factors such as soil mineralogy, redox potential and electron
224 acceptor availability, which are controlled by soil water regimes (RoJi & Lee 2018). Studies found
225 increase soil water content benefits dissolved organic carbon dissolution and nutrients transfer and
226 stimulate the activity of microorganisms which involved in organic carbon decomposition (Goebel
227 et al. 2007). The higher SM resulted in higher rates of substrate supply to microbes and higher
228 microbial growth. In addition, soil moisture availability could affect the vegetation distribution
229 and structure (D'Odorico et al. 2007; Ruiz-Sinoga et al. 2011), then, affect the amount and quality
230 of litter, which in turns influences the soil organic matter decomposition rate (Rodríguez-Iturbe &
231 Porporato 2007). On the other hand, AN is water soluble nitrogen which is vulnerably influenced
232 by soil water. Hence, SM could negatively affect SOC stability by positively influencing the soil
233 microbe and AN content.

234 **5. Conclusions**

235 Our synthesis on the effects of small-scale topography on SOC stability in a tropical mountain
236 rain forest showed that topographic heterogeneity altered SOC stability via changing soil nutrients
237 and soil moisture. Our findings indicated that SOC is rather stable in the tropical montane
238 rainforest topsoil, AN and SM were the main influencing factors of SOC stability both in RF and
239 RS areas. Further, SOC and AN are positive promoting factors while SM is limiting factors in
240 study areas. Accordingly, soil nutrients were the key influencing factors in RF areas but not the
241 RS areas. We hypothesized nutrients be the key influencing factor in study areas was not fully
242 confirmed. Considering the path analysis model explained less than 50% in the RS areas, thus
243 other factors remain to be further explored.

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247 **Conflict of interest**

248 The authors declare no competing financial interests.

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Figure 1

Distribution of soil sampling sites in the 60ha plot in tropical forests

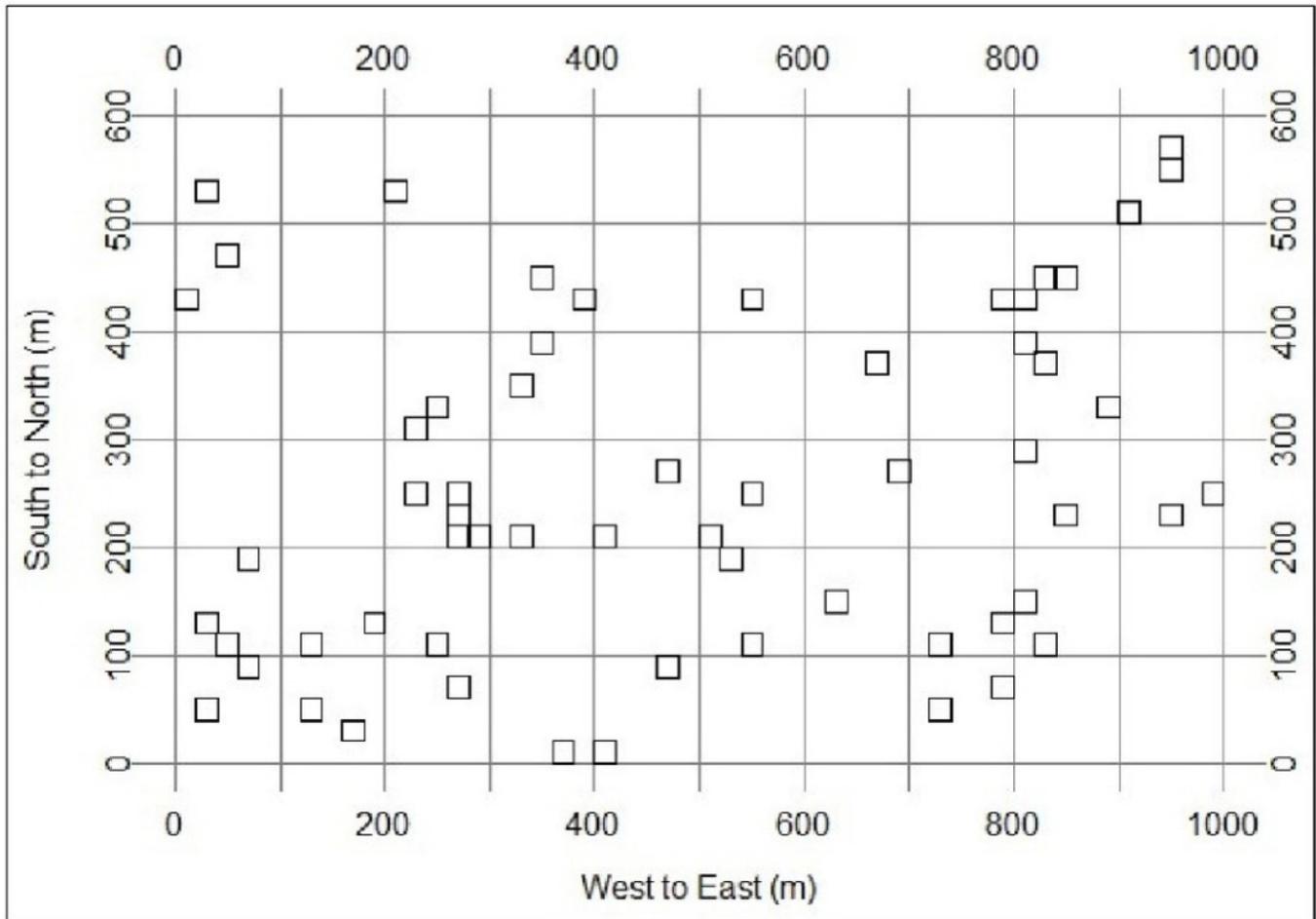


Figure 2

The changes of soil SOC (a), TN (b) and C:N ratio (c) among four topographies. The different lowercases mean significant differences among topographies ($P < 0.05$)

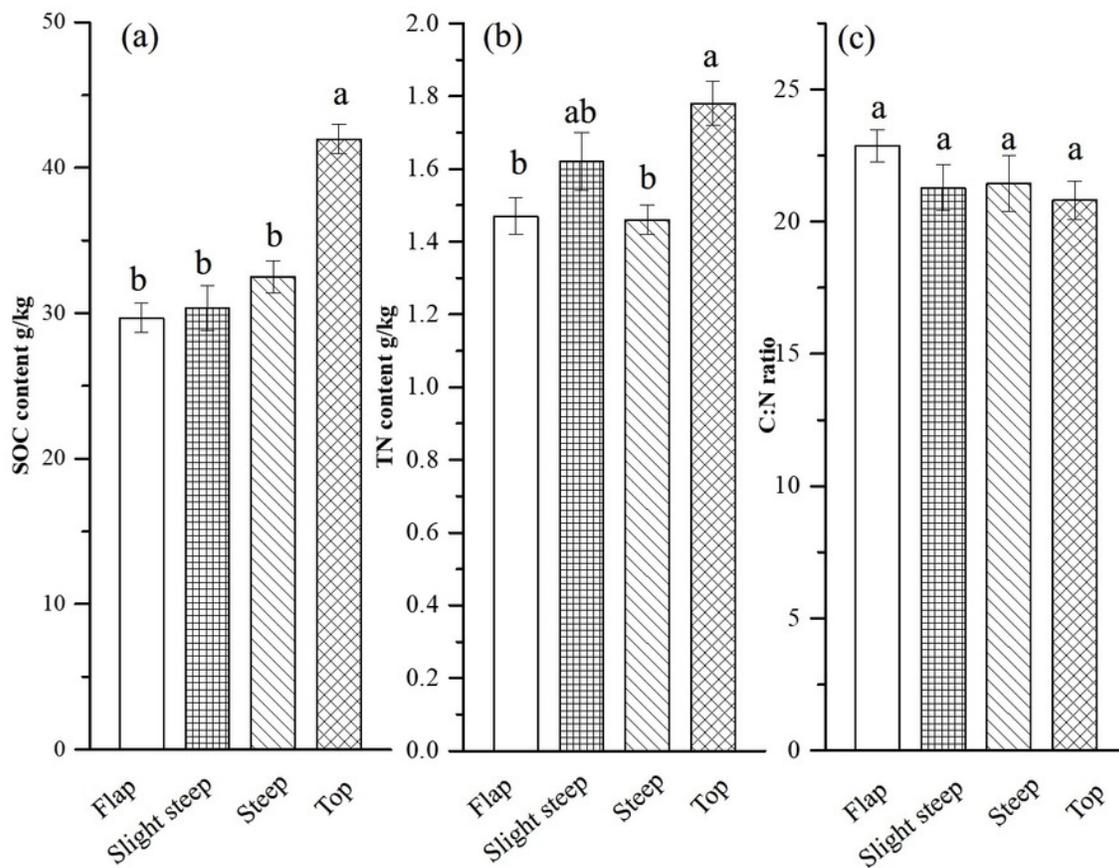


Figure 3

The distributions of SOC components in this tropical rainforest. Errors Bars represent the standard error (SE)

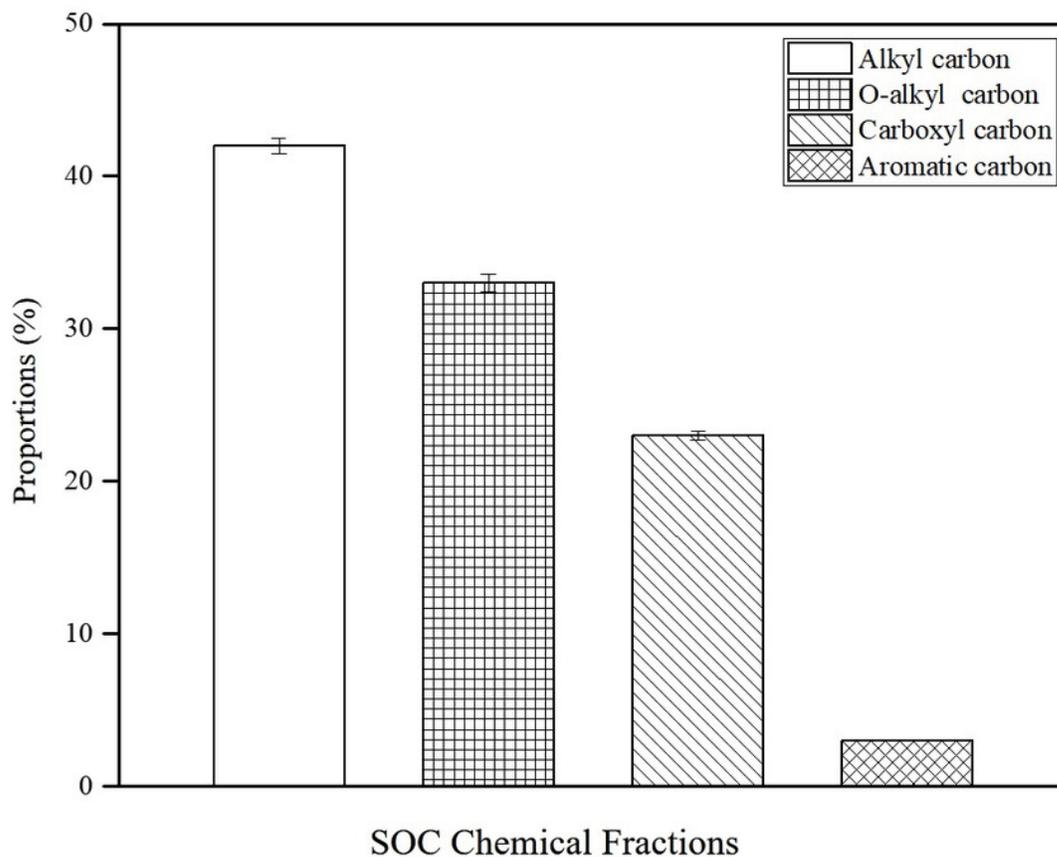


Figure 4

The comparisons of SOC fractions in the four topography types. (a) alkyl carbon, (b) O-alkyl carbon, (c) carboxyl carbon, and (d) aromatic carbon. Errors Bars represent the standard error. The different lowercases mean significant differences among topogra

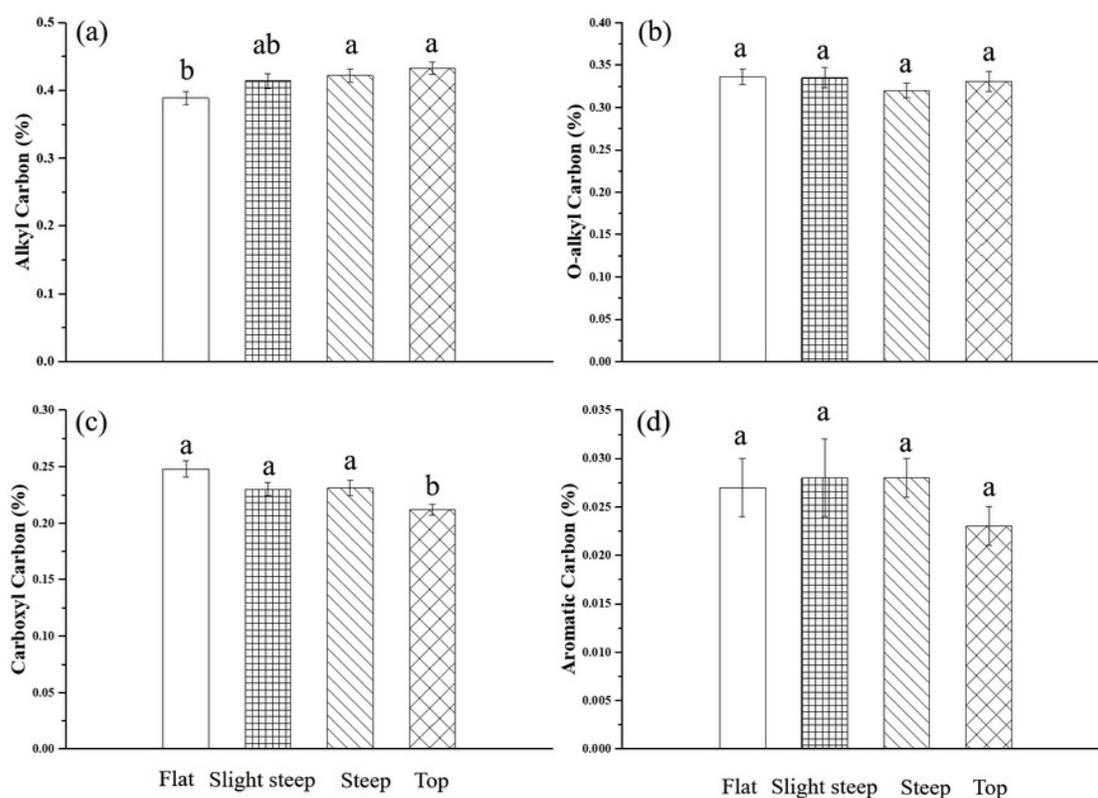


Figure 5

Difference of decomposition index (DI) and inertia index (II) among topographies. (a) decomposition index, (b) inertia index. Errors Bars represent the standard error. The same letter means variables in the different topography types are not significantly

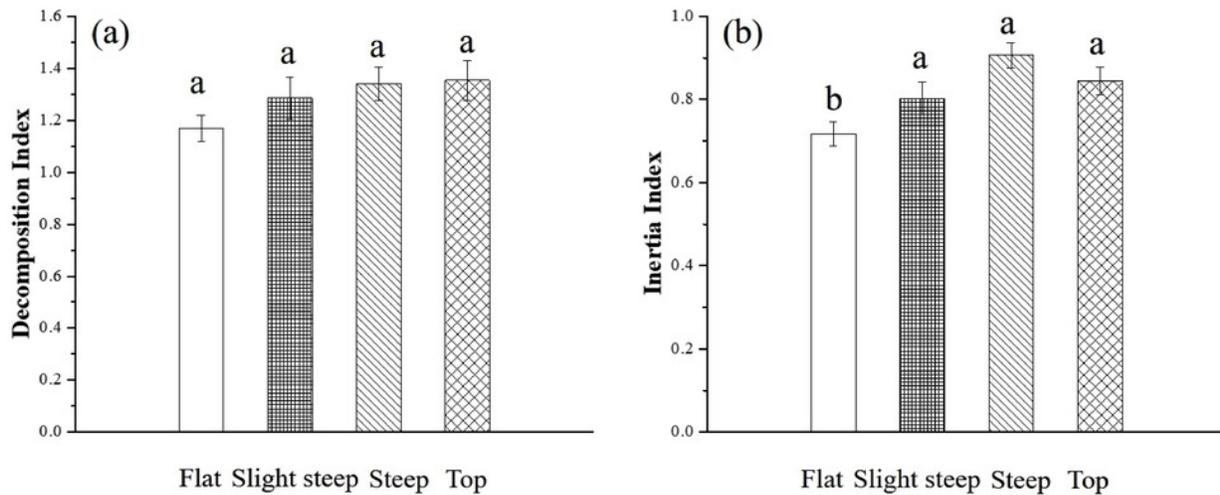


Figure 6

Influencing factors and mechanisms of SOC stability in two topographies. Values aside the straight line are direct path coefficient, and values aside the curve are indirect path coefficient. The direction of arrow is the effect direction.

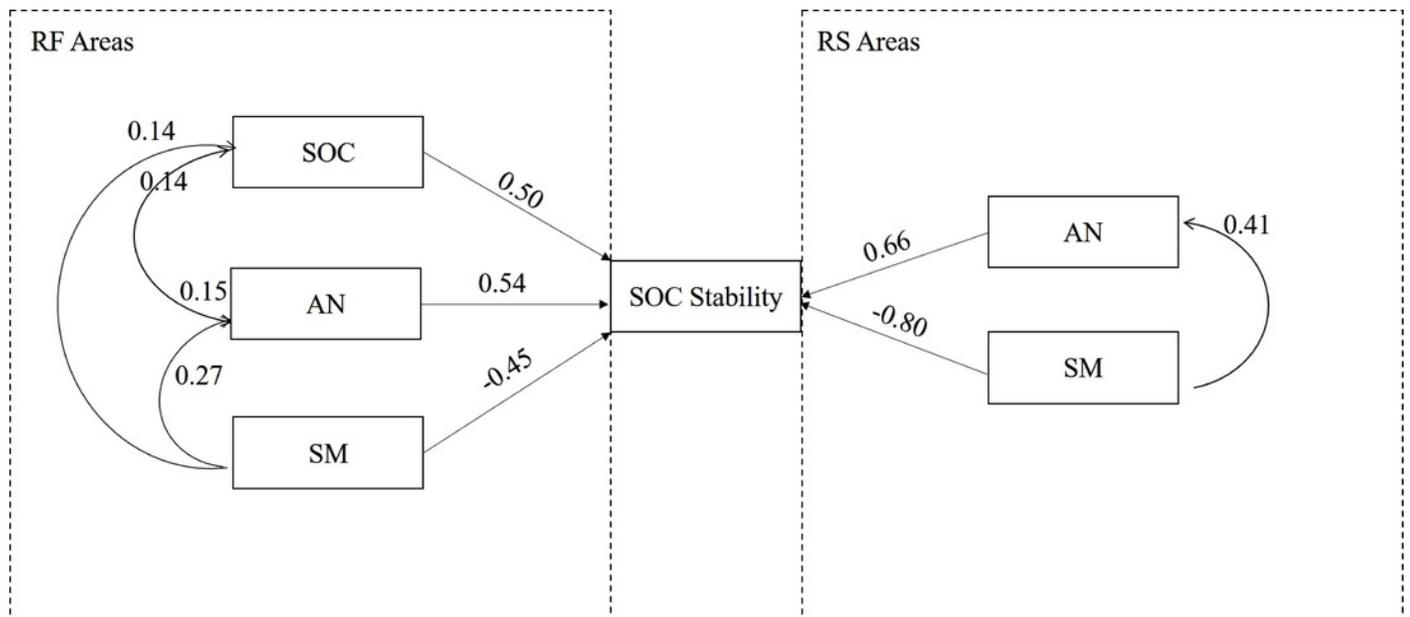


Table 1 (on next page)

Soil properties in different topographic areas (n=60)

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Table 1. Soil properties in different topographic areas (n=60)

Variables	Flat (n=12)	Slight steep (n=16)	Steep (n=13)	Top (n=19)	Average (n=60)
AN (mg/kg)	30.29±6.04 bc	42.16±4.32a b	29.21±5.69c	53.03±3.41a	40.40±2.62
TP (mg/kg)	74.12±5.64 b	82.50±5.62a b	77.66±5.07b	90.91±3.68a	82.40±2.54
AP (mg/kg)	2.71±0.18a	3.03±0.10a	2.92±0.08a	2.30±0.11a	2.93±0.06
C:P ratio	468.65±34.09a	433.61±25.56a	442.74±43.57a	407.08±24.19a	434.20±15.36
N:P ratio	20.56±1.42a	20.26±0.66a	20.39±1.46a	19.68±0.96a	20.16±0.54
SM	20.88±1.66c b	21.20±1.52 b	16.86±0.83dc	25.28±1.22a	21.49±0.77
pH	4.42±0.05a	4.32±0.05b	4.31±0.04ab	4.15±0.02c	4.28±0.02

4 Abbreviation: AN was ammoniacal nitrogen; TP was total phosphorus; AP was available
5 phosphorus; C:P ratio was calculated as SOC/ TP; N:P ratio was calculated as TN/TP; SM was
6 soil moisture, and n was sample size. All values were expressed as mean values ± SE. The different
7 lowercases mean significant differences among topographies (P < 0.05).

Table 2 (on next page)

Table S1 The parameters of path analysis for SOC stability in the two different topography types <!--[if !supportAnnotations]--> <!--[endif]--> <!--[if !supportAnnotations]--> <!--[endif]-->

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Table S1. The parameters of path analysis for SOC stability in the two different topography types

Topography type	Factors	SCC	DPC	SOC	AN	SM	Decision coefficient	Determination coefficient	R square of the model
Relative flat areas	SOC	0.52	0.50	-	0.15	-0.13	0.27	0.26	0.53
	AN	0.45	0.54	0.14	-	-0.23	0.19	0.24	
	SM	-0.05	-0.45	0.14	0.27	-	-0.16	0.02	
Relative Steep areas	SM	-0.40	-0.80		0.41		0.00	0.32	0.42
	AN	0.16	0.66			-0.50	-0.22	0.11	

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