

1   **Soil nutrient stoichiometry varied with tea plantation age and soil depth at an aggregate**  
2   **scale in the southern Guangxi of China**

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8   **ABSTRACT**

9   Soil ecological stoichiometry offers a tool to explore the distribution, cycling, limitation, and  
10   balance of chemical elements in tea plantation ecosystems. This study was aimed to explore how  
11   soil organic C (OC) and nutrient contents (total N (TN), total P (TP),  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$ )  
12   as well as their stoichiometric ratios (C/N, C/P, N/P, Ca/Mg, and Fe/Mn) vary with tea plantation  
13   age (8, 17, 25, and 43 years) and soil depth (0-10, 10-20, 20-40, and 40-60 cm) within aggregates  
14   in the southern Guangxi of China. Our results showed that tea plantation age and soil depth  
15   significantly influenced soil nutrient stoichiometry in different sizes of aggregates. Among  
16   different ages of tea plantations, soil OC, TN, and TP contents as well as C/N, C/P, and N/P  
17   ratios significantly decreased as the soil depth increased. In addition, soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents  
18   were significantly lower in the surface soil layer than the deeper soil layer, whereas soil  $\text{Fe}^{2+}$  and  
19    $\text{Mn}^{2+}$  contents showed totally opposite trends, and no significant differences were detected  
20   among different soil depths in Ca/Mg and Fe/Mn ratios. Tea plantation age could influence the  
21   variation in soil nutrient stoichiometry, but such effect was more obvious at the 0-40 cm soil  
22   depth in contrast to the 40-60 cm soil depth. At the 0-40 cm soil depth, continuous planting of  
23   tea was beneficial for the significant increases in soil OC, TN,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$  contents, whereas  
24   soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents significantly decreased over time. During the process of tea growth,  
25   the losses of soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , especially the  $\text{Ca}^{2+}$  (as indicated by the decrease in soil Ca/Mg  
26   ratio), could lead to the soil acidification. Meanwhile, soil acidification could reduce  $\text{Fe}^{2+}$

27 absorption and enhance  $Mn^{2+}$  uptake by tea plants (as indicated by the increase in soil Fe/Mn  
28 ratio). Overall, this study improved the understanding of soil OC and nutrient dynamics in tea  
29 plantation ecosystems.

30 **KEYWORDS**

31 Tea plantation age; Soil depth; Soil aggregate; Ecological stoichiometry

32

33 **1. Introduction**

34 In the past century, under the remarkable increase in population pressure, continuous tillage  
35 and overmuch deforestation resulted in the dramatic decrease in soil fertility level in the southern  
36 Guangxi of China (Jiang et al., 2018). For the purpose of tackling these challenges, the Chinese  
37 government has rolled out the Grain for Green program in the hope of alleviating land  
38 deterioration via converting farmlands to forest lands or grass lands (Zeng et al., 2020). Since the  
39 initiation of such program, the south part of Guangxi has initiated the mode of transforming  
40 farmlands into tea (*Camellia sinensis* L.) plantations as per the local geography and natural  
41 resources (Zhang et al., 2017). Tea, as a pivotal cash crop, is commonly cultivated in the  
42 developing nations, particularly in China, India, Kenya, and Sri Lanka. China is the first nation  
43 to plant tea across the globe, with the tea-planting area reaching 3.17 million hectares in 2020  
44 and presenting an elevating trend in the future (Chinese Tea Committee, 2020). Guangxi has the  
45 subtropic monsoon climate and marks the key tea-planting region in China. According to the  
46 statistics from Chinese Tea Committee (2020), more than 80% tea plantations of Guangxi are  
47 situated at impoverished counties, and tea-planting industry turns to be the staple industry on  
48 which poor counties depend to throw off poverty.

49 Ecological stoichiometry offers a tool to explore the distribution, cycling, restriction, and  
50 balance of nutrients in terrestrial ecosystems (Yu et al., 2019), and plays a critical role in  
51 recognizing the influence factors and drive mechanisms in ecological processes (Su et al., 2019).  
52 On the one hand, carbon (C) is the most commonly seen element in plants (Prescott et al., 2020),

53 and nitrogen (N) and phosphorus (P) are critical control factors for the growth of plants (Krouk  
54 and Kiba, 2020). The relationships amongst the three different elements are coupled (Elser et al.,  
55 2003). Soil C/N, C/P, and N/P ratios represent not only the equilibrium features of soil C, N, and  
56 P, but also the dynamics of fertility characteristics during the process of soil genesis (Bai et al.,  
57 2020). On the other hand, calcium (Ca), magnesium (Mg), iron (Fe), and manganese (Mn) are  
58 pivotal metallic nutritive elements for the development of plants (Liu et al., 2021a). Soil total Ca,  
59 Mg, Fe, and Mn may exceed the demand of a single plant by more than a thousand-fold and  
60 cannot sensitively reflect the needs of plants (Miner et al., 2018), but the available fractions of  
61 these nutrients may be insufficient or redundant, resulting in the deficiencies or abundances of  
62 plant nutrients (Otero et al., 2013). Thus, soil exchangeable Ca and Mg as well as available Fe  
63 and Mn generate significant effects on the development of plants.

64 Over the past decade, soil nutrient stoichiometry (mainly C-N-P, rather than Ca-Mg or  
65 Fe-Mn) has been broadly studied across the world (Tian et al., 2010; Yang et al., 2013; Zhang et  
66 al., 2016; Yue et al., 2017; Yu et al., 2018; Qiao et al., 2020). A wide agreement exists amongst  
67 these studies that soil depth is vital for the regulation of soil nutrient stoichiometry. Substantial  
68 studies have identified the decreasing trend of soil organic C (OC), total N (TN), and total P (TP)  
69 contents as the soil depth increased (Yue et al., 2017; Yu et al., 2018; Qiao et al., 2020), whereas  
70 conflicting vertical patterns were discovered for soil C/N, C/P, and N/P ratios. For instance,  
71 decreasing trend of the C/P and N/P ratios was observed as the soil depth increased in the data of  
72 the 2<sup>nd</sup> soil investigation in China (Tian et al., 2010). Nevertheless, larger C/N ratio in the deeper  
73 soil layer, not the surface soil layer, was identified in a mollisol plain in the northeast China  
74 (Zhang et al., 2016). Moreover, the C/N ratio displayed no remarkable change throughout  
75 different soil depths in an investigation of alpine grassland on the Qingzang Plateau (Yang et al.,  
76 2013). As shown above, inconsistent vertical patterns have been reported for the C-N-P  
77 stoichiometric ratios in different soil ecosystems. Meanwhile, these studies were mainly focused  
78 on the regional or global scales, rather than on the aggregate scales.

79 As the basic unites of soil structure, soil aggregates are complex ensembles composed of  
80 primary particles as well as organic matter (OM) (Tisdall and Oades, 1982). According to the  
81 differences of binding agents, soil aggregates can be classified into microaggregates (< 0.25 mm)  
82 and macroaggregates (> 0.25 mm) (Tisdall and Oades, 1982). In general, persistent binding  
83 agents (like humified OM and polyvalent metal cation complexes) contribute to the binding of  
84 primary particles into microaggregates (Six et al., 2004). Differently, temporary binding agents  
85 (like fungal hyphae, plant roots, and polysaccharides) aggregating with microaggregates  
86 conduces to the formation of macroaggregates (Six et al., 2004). As shown above, soil  
87 aggregates with various sizes exert different abilities in the supply and reserve of soil OC and  
88 nutrients. Thus, to improve the comprehension about the structure and function of soil  
89 ecosystems, more efforts should be made to observe the soil nutrient stoichiometry within  
90 aggregates (Xu et al., 2019; Cui et al., 2021). In recent period, lots of studies have reported the  
91 OC, TN, and TP distribution in different sizes of aggregates, but these studies are ended with  
92 different results. To be specific, some studies revealed the significant increases in the OC, TN,  
93 and TP contents as the aggregate size decreased (Sarker et al., 2018; Piazza et al., 2020).  
94 Nevertheless, some other studies drew the totally opposite trends (Lu et al., 2019; Liu et al.,  
95 2021b). These show that the changes of soil OC, TN, and TP within aggregates have received  
96 great attention, whereas soil exchangeable alkali cations (i.e.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) and available  
97 micronutrients (i.e.,  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) are rarely investigated.

98 Our past studies indicated that the landuse shift from farmlands to tea plantations could  
99 ameliorate soil fertility level (Zheng et al., 2011). Nevertheless, during the process of tea growth,  
100 the variation in soil nutrient stoichiometry is still unclear. Meanwhile, since tea serves as a deep  
101 root plant, it is vital to reveal how nutrient stoichiometry changes with increasing soil depth in  
102 tea plantation ecosystems. Thus, the present study was carried out to investigate how soil OC and  
103 nutrient contents as well as their stoichiometric ratios vary with tea plantation age (8, 17, 25, and  
104 43 years) and soil depth (0-10, 10-20, 20-40, and 40-60 cm) within aggregates (< 0.25, 0.25-1,

105 1-2, and > 2 mm). In addition, we hypothesized that the responses of soil OC and nutrient  
106 contents and their stoichiometric ratios to tea plantation age would be different amongst different  
107 soil depths.

108 **2. Materials and methods**

109 **2.1. Experiment site**

110 In January 2019, the present study was completed at the Hengxian Agriculture Experiment  
111 Center of Guangxi University (altitude of 557-563 m and slope degree of 13-15 °) (Figure 1).  
112 Subtropic monsoon climate is predominant. Yearly average rainfall and temperature register  
113 1304 mm and 21.6 °C, separately. Exposed soil horizon occurs early in the Mesozoic, which  
114 gradually formed the Ultisols agrotype (IUSS Working Group, 2014). As early as in 1960s, due  
115 to the high economic value of tea, massive hectares of farmlands were developed to tea  
116 plantations in such region.

117 The “*Baimao* tea” refers to a major cultivar in such area, and the ages of these tea  
118 plantations are distinct. Tea plantations were both experimental trials (Guangxi University) and  
119 commercial plantings, and were managed by different owners. In the tea-planting course, tillage  
120 method is no tillage and tea-planting density is almost  $6 \times 10^4$  plants  $\text{ha}^{-1}$ . Herbicides were not  
121 applied and yellow sticky boards were used to prohibit pests, because the color may attract pests  
122 and get them stuck on the boards. In addition, all tea plants were subjected to slight pruning in  
123 September each year.

124 An annual fertilizer regime in tea plantations is shown below. Both  $0.65 \text{ Mg ha}^{-1}$  complex  
125 fertilizer (granule, N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O: 18%-6%-6%) and  $12 \text{ Mg ha}^{-1}$  swine manure (slurry,  
126 N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O: 0.54%-0.48%-0.36%) were applied yearly in mid-November as the basal fertilizer  
127 at the surrounding region vertically below tree crown. Subsequently, the top-dressing, applied to  
128 the site treated with replenished basal fertilizer, was replenished 3 times per year. Both  $1.2 \text{ Mg}$   
129  $\text{ha}^{-1}$  complex fertilizer and  $0.5 \text{ Mg ha}^{-1}$  urea were applied onto soil surface in mid-March, while

130 0.65 Mg ha<sup>-1</sup> complex fertilizer and 0.3 Mg ha<sup>-1</sup> urea were applied in late-June and in  
131 early-September.

132 2.2. Experiment design

133 In general, examining the same location persistently has been considered a quite effective  
134 approach in the monitoring of the variations in soil with time (Sparling et al., 2003).  
135 Nevertheless, the challenges in long-period soil monitoring have made it urgent to develop  
136 substitutional approaches to research the changes of soil over time, amongst which the most  
137 common approach is the ‘space-for-time’ alternative (Zanella et al., 2018).

138 In this study, such approach was used to explore the variation in soil nutrient stoichiometry  
139 in a chronological sequence of tea plantations. In general, certain underlying mixture effects  
140 exist in the spatial variations of soil, hence the present study manages to mitigate such effects via  
141 choosing tea plantations, which were cultured with the same tea variety (“*Baimao* tea”) with  
142 different planting ages (8, 17, 25, and 43 years), and were located at the same unit associated  
143 with geomorphological status.

144 Each of the 4 tea plantation age groups was replicated in 5 locations for a total of 20  
145 experimental units. Separation amongst these units was completed with distances of > 800 m  
146 between each other, hence decreasing the spatial autocorrelation and avoiding the  
147 pseudo-replication. For every unit ( $S \approx 1 \times 10^4$  m<sup>2</sup>), a plot ( $S = 20$  m  $\times$  20 m) was randomly  
148 established with distance of > 50 m away from the unit margin.

149 2.3. Litter and soil sampling

150 For every plot, the 5 surface litter (a stock) specimens had been acquired from the surface of  
151 soil in the 5 randomly chosen subplots ( $S = 1$  m  $\times$  1 m), and afterwards were integrated into a  
152 composite litter specimen. An overall the 20 (4 tea plantation ages  $\times$  5 replicates) composite  
153 litter specimens were desiccated at the 80 °C until steady weight. Then, the weights of these  
154 desiccated litter specimens were measured, and the litter C (Nelson and Sommers, 1996) and N  
155 (Bremner, 1996) contents were detected. The amount of litter was 821, 974, 786, and 648 g m<sup>-2</sup>

156 in the 8, 17, 25, and 43 years of tea plantations, respectively, and the C/N ratio of litter was  
157 14.23, 12.68, 17.32, and 21.37, respectively.

158 Soil sampling was completed in the same sites of the litter sampling. For every plot, the 5  
159 soil specimens had been acquired by a spade from every soil layer (i.e., 0-10, 10-20, 20-40, and  
160 40-60 cm) in the 5 subplots ( $S = 1 \text{ m} \times 1 \text{ m}$ ), and afterwards were integrated into a composite  
161 soil specimen. An overall the 80 (4 tea plantation ages  $\times$  4 soil layers  $\times$  5 replicates) composite  
162 soil specimens were gently separated into naturally formed aggregates, which were sieved by a 5  
163 mm sifter to realize the removals of small stones, coarse roots, and macrofauna. After that, soil  
164 specimens were used for the aggregate separation. For every plot, moreover, extra 5 soil  
165 specimens were randomly chosen via cutting rings (volume =  $100 \text{ cm}^3$ , diameter = 50.46 mm,  
166 and depth = 50 mm) from every soil layer to assess the bulk density, clay ( $< 0.002 \text{ mm}$ ), pH, OC,  
167 and nutrients of bulk soil (Table 1).

168 2.4. Soil aggregate separation

169 As per the process of wet screening, 250 g of every composite soil specimen was sieved via  
170 the 2, 1, and 0.25 mm sieves in a successive way (Kemper and Chepil, 1965). To be specific, the  
171 composite soil specimens were soaked by the aqua destillata for 15 min, and afterwards were  
172 oscillated in the vertical direction for 15 min at the  $1 \text{ s}^{-1}$  oscillating rate and 5 cm amplitude.  
173 Consequently, we obtained 4 different sizes of aggregates, covering microaggregates ( $< 0.25$   
174 mm), fine (0.25-1 mm), medium (1-2 mm), and coarse ( $> 2 \text{ mm}$ ) macroaggregates. All of the  
175 aggregates were desiccated and weighted, and then aggregate-related OC and nutrients were  
176 detected.

177 2.5. Soil property analyses

178 Prior to the analyses of soil physical-chemical properties, soil specimens were subjected to  
179 atmospheric drying under indoor temperature condition. According to the cutting ring method  
180 (Lu, 2000), soil specimens were oven-dried at  $105^\circ\text{C}$  to the stable weight in order to measure the  
181 bulk density. Soil clay was detected by the hydrometer (TM-85, Veichi, China) (Lu, 2000). Soil

pH was detected by the glassy electrode (MT-5000, Ehsy, China), with the ratio of soil : water (mass : volume) as 1 : 2.5 (Lu, 2000). Soil OC and TN were identified via the acid dichromate wet oxidation method (Nelson and Sommers, 1996) and the micro-Kjeldahl method (Bremner, 1996), separately. Soil TP was identified via the molybdate blue colorimetry method (Bray and Kurtz, 1945). Soil exchangeable alkali cations (i.e.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) were determined by the ammonium acetate ( $\text{CH}_3\text{COONH}_4$ ) (Thomas, 1982). In short, 2.5 g of every aggregate fraction was weighted into Erlenmeyer flask to blend with 50 mL 1 M  $\text{CH}_3\text{COONH}_4$  (pH = 7.0). The extract liquid was agitated for 30 min under 150 rpm, and afterwards sieved via Whatman No. 2 V filtration paper (quantitative and ashfree). Soil available micronutrients (i.e.,  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) were determined by the diethylenetriamine pentaacetic acid (DTPA) (Lindsay and Norvell, 1978). In short, 10 g of every aggregate fraction was weighted into Erlenmeyer flask to blend with 20 mL 0.005 M DTPA + 0.01 M  $\text{CaCl}_2$  + 0.1 M TEA (triethanolamine) (pH = 7.0). The extract liquid was agitated for 2 h under 180 rpm, and afterwards sieved. Entire extractable metallic cations were detected by the atomic absorption spectrometer (AAS, Shimadzu, Japan). In this study, 5 standard specimens (GBW-07401), 5 blank specimens, and 80 parallel specimens (accounted for 20% of the total soil specimens) were used to control quality, and the error between parallel specimen and experimental specimen was controlled in 5%.

## 2.6. Calculations and statistics

The mean weight diameter (MWD, mm) was utilized to indicate the stability of soil aggregates. To be specific, if the MWD value is higher, the aggregate stability is stronger (Kemper and Chepil, 1965):

$$203 \quad \text{MWD} = \sum_{i=1}^4 (X_i \times M_i),$$

in the formula,  $X_i$  indicates the  $i^{\text{th}}$  size aggregates' mean diameter (mm) and  $M_i$  indicates the  $i^{\text{th}}$  size aggregates' proportion (% in weight).

SPSS 22.0 was used for statistic analysis (Table 2). Means were tested by the Tukey's HSD and significance was used at  $P < 0.05$ . Two-way analysis of variance (ANOVA) was taken for

208 exploring the effects of soil depth, tea plantation age, and their interactions on the  
209 physico-chemical properties of bulk soil. Three-way ANOVA was taken for exploring the effects  
210 of soil depth, tea plantation age, aggregate size, and their interactions on the physico-chemical  
211 properties of soil aggregates. Besides that, Pearson correlation analysis was utilized to test the  
212 relationships between pH and stoichiometric ratios (i.e., Ca/Mg and Fe/Mn ratios) in bulk soil  
213 during the process of tea growth.

214 **3. Results**

215 **3.1. Composition and stability of soil aggregates**

216 At the 0-10 and 10-20 cm soil depths, continuous planting of tea resulted in significant  
217 variations in the proportions of different sizes of aggregates, apart from the medium and fine  
218 macroaggregates (Table 3). To be specific, the proportions of coarse macroaggregates  
219 significantly rose within the first 17 years and afterwards significantly dropped, whereas the  
220 proportions of microaggregates displayed an opposite trend over time. Meanwhile, the greatest  
221 value of soil MWD was identified in the tea plantations of 17 years (Table 3). Notably, the role  
222 of tea plantation age in the aggregate composition and stability is limiting at the 20-40 and 40-60  
223 cm soil depths. Across the 4 tea plantation ages, the coarse macroaggregates were dominant at  
224 the 0-10 cm soil depth, which accounted for 32.60%-53.18% of bulk soil. However, at the 10-20,  
225 20-40, and 40-60 cm soil depths, the microaggregates were dominant, which accounted for  
226 33.80%-49.51%, 42.12%-48.24%, and 44.80%-49.45%, respectively. These results showed that  
227 the coarse macroaggregate proportions significantly reduced while the microaggregate  
228 proportions significantly elevated with increasing soil depth.

229 **3.2. Contents of soil C, N, and P**

230 At the aggregate scales, soil OC (Figure 2) and TN (Figure 3) contents significantly  
231 increased with increasing aggregate size, but the distribution of soil TP (Figure 4) was even in  
232 different sizes of aggregates. From 8 to 43 years of tea plantations, the OC and TN contents in  
233 soil aggregates were significantly elevated by 22%-35% and 14%-24%, 11%-22% and 9%-17%,

234 and 8%-18% and 9%-13% at the 0-10, 10-20, and 20-40 cm soil depths, respectively.  
235 Nevertheless, no significant variation existed in the aggregate-related TP content. Furthermore,  
236 at the 40-60 cm soil depth, the aggregate-related OC, TN, and TP contents did not show  
237 significant variations over time. Regardless of the tea plantation age, significant decreases in the  
238 aggregate-related OC, TN, and TP contents were observed as the soil depth increased.

### 239 3.3. Stoichiometric ratios of soil C, N, and P

240 A three-way ANOVA analysis showed that the lone and interactive effects of soil depth, tea  
241 plantation age, and aggregate size on the C/P and N/P ratios were significant, and the effects of  
242 soil depth, aggregate size, and their interactions on the C/N ratio were significant (Table 2). In  
243 this study, significant increases in aggregate-related C/N (Table S1), C/P (Table S2), and N/P  
244 (Table S3) ratios were accompanied by the increasing aggregate size. At the 0-10, 10-20, and  
245 20-40 cm soil depths, aggregate-related C/N ratio did not show significant variation while  
246 aggregate-related C/P and N/P ratios significantly increased with the increase in tea plantation  
247 age. Moreover, there was little role of tea plantation age in the aggregate-related C/N, C/P, and  
248 N/P ratios at the 40-60 cm soil depth. Among different ages of tea plantations, aggregate-related  
249 C/N, C/P, and N/P ratios significantly dropped as the soil depth increased. For example, at the  
250 0-10 cm soil depth, aggregate-related C/N, C/P, and N/P ratios across the 4 tea plantation ages  
251 fluctuated in 20.81-23.04, 28.81-37.07, and 1.31-1.67, respectively. Meanwhile, at the 40-60 cm  
252 soil depth, aggregate-related C/N, C/P, and N/P ratios fluctuated in 16.41-20.74, 13.44-22.88,  
253 and 0.84-1.08, respectively.

### 254 3.4. Contents of soil alkali cations and micronutrients

255 At the aggregate scales, soil exchangeable alkali cations (i.e.,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) were more  
256 concentrated in the microaggregates (Figures 5 and 6). However, soil available micronutrients  
257 (i.e.,  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) were mainly existed in the coarse macroaggregates (Figures 7 and 8). From  
258 8 to 43 years of tea plantations, the  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents in soil aggregates were significantly  
259 reduced by 31%-38% and 10%-24%, 23%-27% and 9%-18%, and 10%-16% and 5%-8% at the

260 0-10, 10-20, and 20-40 cm soil depths, respectively. However, the  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  contents in soil  
261 aggregates were significantly elevated by 16%-27% and 6%-9%, 11%-15% and 4%-7%, and  
262 7%-12% and 3%-5%, respectively. In addition, at the 40-60 cm soil depth, the contents of  
263 aggregate-related exchangeable alkali cations and available micronutrients did not show  
264 significant variations over time. Irrespective of the tea plantation age, significant increases in the  
265 aggregate-related  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents were observed with increasing soil depth, but the  
266 aggregate-related  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  contents showed an opposite trend.

267 **3.5. Stoichiometric ratios of soil alkali cations and micronutrients**

268 A three-way ANOVA analysis showed that the effect of tea plantation age on the  $\text{Ca}/\text{Mg}$   
269 and  $\text{Fe}/\text{Mn}$  ratios in soil aggregates was significant (Table 2). In this study, soil  $\text{Ca}/\text{Mg}$  (Table  
270 S4) and  $\text{Fe}/\text{Mn}$  (Table S5) ratios did not vary among different sizes of aggregates. At the 0-10,  
271 10-20, and 20-40 cm soil depths, aggregate-related  $\text{Ca}/\text{Mg}$  ratio significantly decreased while  
272 aggregate-related  $\text{Fe}/\text{Mn}$  ratio significantly increased in the tea-planting course. Moreover, there  
273 was little role of tea plantation age in the aggregate-related  $\text{Ca}/\text{Mg}$  and  $\text{Fe}/\text{Mn}$  ratios at the 40-60  
274 cm soil depth. In tea plantations, no significant variations were observed amongst different soil  
275 depths in aggregate-related  $\text{Ca}/\text{Mg}$  and  $\text{Fe}/\text{Mn}$  ratios. For example, at the 0-10 cm soil depth,  
276 aggregate-related  $\text{Ca}/\text{Mg}$  and  $\text{Fe}/\text{Mn}$  ratios across the 4 tea plantation ages ranged from 1.81 to  
277 1.96 and 0.76 to 0.85, respectively. Meanwhile, at the 40-60 cm soil depth, aggregate-related  
278  $\text{Ca}/\text{Mg}$  and  $\text{Fe}/\text{Mn}$  ratios ranged from 1.88 to 1.92 and 0.78 to 0.82, respectively.

279 **4. Discussion**

280 **4.1. Composition and stability of soil aggregates**

281 Tea plantation age significantly influenced the aggregate composition and stability at the  
282 0-10 and 10-20 cm soil depths, whereas the effect at the 20-40 and 40-60 cm soil depths was  
283 quite limited. In the early (8-17 years) period, tea planting was beneficial for the transition from  
284 microaggregates to coarse macroaggregates at the 0-10 and 10-20 cm soil depths (Table 3). By  
285 comparison, in the middle (17-25 years) and late (25-43 years) periods, tea planting induced

286 coarse macroaggregate destruction and microaggregate release (Table 3). According to the  
287 hierarchical concept of soil aggregates (Six et al., 2004), the quality of plant litter returning to the  
288 soil determines the distribution of decomposition products of litter in different sizes of  
289 aggregates, which ultimately impacts the aggregate composition. In the early period of tea  
290 planting, tea litter displayed greater availability (as indicated by the lower litter C/N ratio),  
291 revealing that the decomposition products of litter were easily combined into the coarse  
292 macroaggregates, hence fostering the formation of coarse macroaggregates (Tisdall and Oades,  
293 1982). Reversely, in the middle and late periods of tea planting, tea plants naturally encountered  
294 aging processes and litter was progressively subjected to humification, which induced the  
295 decomposition of coarse macroaggregates into microaggregates (Six and Paustian, 2014).  
296 Moreover, the reduced litter amount and covering area after 17 years of tea planting enhanced  
297 the rainfall eluviation and artificial interferences (i.e., pruning of tea plants and application of  
298 fertilizers), which also caused the destruction of coarse macroaggregates. In the tea-planting  
299 course, variation in aggregate stability was indicated via the change of MWD value (Table 3). At  
300 the 0-10 and 10-20 cm soil depths, the MWD value was the greatest in the 17 years of tea  
301 planting, which was associated with the highest proportions of coarse macroaggregates in the  
302 17-year tea plantations. These findings indicated that the 17-year tea plantations exhibited  
303 stronger aggregate stability in contrast to other plantations at the 0-10 and 10-20 cm soil depths.

304        Regardless of the tea plantation age, coarse macroaggregates were dominant in the topsoil  
305 (0-10 cm) while microaggregates were dominant in the subsoil (10-60 cm), indicating  
306 transformation of aggregate composition from coarse macroaggregate-prevailing to  
307 microaggregate-prevailing with the increase in soil depth (Table 3). Also, alike outcomes were  
308 corroborated by Li et al. (2015) and Zhu et al. (2017) from studies on tea plantations in the  
309 southwest Sichuan of China. In the present study, coarse macroaggregates were the prevailing  
310 fractions in the topsoil, not the subsoil, which was attributed to the surface cumulation of soil OC  
311 (Figure 2). As an essential cementing agent, soil OC could foster the formation of coarse

312 macroaggregates (Al-Kaisi et al., 2014). Moreover, the reduced proportions of coarse  
313 macroaggregates as the soil depth increased were also because of the elevated soil compactness  
314 (as indicated by the bulk density) (Table 1). Soil densification could prevent the growth of plant  
315 roots, hence causing the activities of soil microorganisms decreased, especially soil fungi (Kurmi  
316 et al., 2020). Reduced activities of soil fungi could diminish the production of polysaccharose  
317 and glomalin-related soil protein (GRSP) from the fungal hyphae, hence inducing the  
318 proportions of soil macroaggregates decreased (Ji et al., 2019). Likewise, as per our past studies  
319 (Wang et al., 2017b; Zhu et al., 2019), soil microbial activities and GRSP content served as the  
320 vital effects in the formation and stabilisation of soil macroaggregates, and presented the higher  
321 levels in the topsoil compared with the subsoil in tea plantation ecosystems. With increasing soil  
322 depth, the decrease in MWD value was mainly related to the change of soil aggregate  
323 composition (Table 3), especially for the decomposition of coarse macroaggregates into  
324 microaggregates, implying that the topsoil exhibited stronger aggregate stability in contrast to the  
325 subsoil.

#### 326 4.2. Contents of soil C, N, and P

327 In this study, more contents of soil OC and TN could be detected in coarse macroaggregates  
328 (Figures 2 and 3), which conformed to the findings of Six et al. (2004) that macroaggregates  
329 were comprised of microaggregates via temporary binding agents; meanwhile, macroaggregates  
330 could provide the protection for the OM, hence causing the cumulation of OC and TN in  
331 macroaggregates. Unlike soil OC and TN, soil TP was evenly distributed in different sizes of  
332 aggregates (Figure 4). Moreover, Bhatnagar and Miller (1985) also detected alike outcomes from  
333 soil specimens subjected to fresh poultry manure treatments, and promoted the mechanisms  
334 affecting the distribution of TP in soil aggregates. Specifically, (i) introduced P was firstly  
335 adsorbed by clay particulates in soil and clay particulates were discrepant in different sizes of  
336 aggregates, and (ii) introduced P had selective absorptive properties for the different sizes of

337 aggregates. According to our findings, stochasticity seems to be one probable mechanism that  
338 sheds light on the TP distribution in soil aggregates.

339 Tea plantation age could positively affect the cumulation of soil OC and TN, but such  
340 positive effects were more obvious at the 0-40 cm soil depth in contrast to the 40-60 cm soil  
341 depth. In this study, soil OC and TN contents exhibited a significant growing trend over time  
342 (Figures 2 and 3), which was possibly associated with the following mechanisms. First, many  
343 long-period tests had demonstrated the proactive roles of manure and chemical fertilizer  
344 applications in soil OM cumulation (Tong et al., 2009; Zhou et al., 2013). Similarly, in the  
345 tea-planting course, growing soil OC and TN contents were probably caused by the applications  
346 of substantial swine manure every year ( $12 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) in this tea-planting region (Wang and  
347 Ye, 2020). Second, plants serve as the prime OM sources in soil via root exudates and litter  
348 remains (Franklin et al., 2020). In the tea-planting course, soil OC and TN cumulation probably  
349 occurred as a result of the growing root systems and the increasing amounts of aboveground  
350 litter attained from trimmed branches and leaves. Third, no tillage could provide physical  
351 protection for the OM combined with soil aggregates, and then further improve soil OC and TN  
352 sequestration (Wulanningtyas et al., 2021). Notably, although the positive correlations of OC and  
353 TN contents with clay content in soil have been reported, the present study revealed that  
354 significant increases in the OC and TN contents were accompanied by no significant variation in  
355 the clay content during the process of tea growth (Table 1). Similarly, Li et al. (2015) and Wang  
356 et al. (2018) discovered as well that the changes of soil OC and TN contents were not influenced  
357 by the clay content over time in tea plantation ecosystems, mainly because soil OC and TN  
358 contents primarily depend on fertilization, tillage, root exudates, and litter remains, but soil clay  
359 content is mainly controlled by its parent material (Rakhsh et al., 2020). Unlike soil OC and TN,  
360 regardless of the soil depth, no significant difference existed in soil TP content amongst different  
361 aged tea plantations (Figure 4), which implied the resistance of soil TP content to the change of  
362 tea plantation age. Also, past studies verified that soil TP content was not related to the tea

363 plantation age (Wu et al., 2018; Yan et al., 2018), as soil P primarily derives from the weathering  
364 release of soil minerals, instead of the short-period biology cycle (Cui et al., 2019).

365 In tea plantation ecosystems, the decreasing OC, TN, and TP contents with increasing soil  
366 depth (Figures 2, 3, and 4)coincided with some past findings in other ecosystems, such as tropic  
367 forests, bushlands, and grasslands (Stone and Plante, 2014; Yu et al., 2018; Qiao et al., 2020). In  
368 the present study, the higher contents of OC, TN, and TP in the topsoil were associated with the  
369 higher OM input, in which the soil OM content in the topsoil was enriched by the input of  
370 surface tea litter, root debris and exudates, and swine manure.

371 4.3. Stoichiometric ratios of soil C, N, and P

372 Soil C/N, C/P, and N/P ratios act as vital indicators of soil health (Liu et al., 2018), which  
373 can be employed for exploring C circulation and guiding the equilibrium between N and P in soil  
374 ecosystems (Sardans et al., 2012). In this study, soil C/N ratio grew with growing aggregate size  
375 (Table S1), which indicated that the OM in macroaggregates was younger and more unstable in  
376 contrast to microaggregates (Six et al., 2004). Meanwhile, the OM associated with  
377 microaggregates experienced more degradation, resulting in the lower C/N ratio in the  
378 microaggregates (Xu et al., 2019). Among different ages of tea plantations, soil OC and TN were  
379 predominantly distributed in the coarse macroaggregates, but the TP was evenly distributed in  
380 different sizes of aggregates. As a result, the associations of C/P and N/P ratios to aggregate size  
381 primarily depended on the relationships of OC and TN contents with aggregate size (Tables S2  
382 and S3). As far as we know, the changes of soil C/P and N/P ratios within aggregates are rarely  
383 examined, although these kinds of knowledge are imperative because of the biogeochemical  
384 cycles of N and P being influenced by the dynamics of soil aggregates (Cui et al., 2021).  
385 Consequently, the impact generated by the aggregate size on the C/P and N/P ratios ought to be  
386 studied more for the accurate forecast of soil N and P cycling under natural or man-intervened  
387 ecosystems.

388 Irrespective of the soil depth, soil C/N ratio showed little significant variation in the

389 tea-planting course (Table S1). Meanwhile, tea plantation age significantly affected soil C/P and  
390 N/P ratios at the 0-40 cm soil depth, not the 40-60 cm soil depth (Tables S2 and S3). Soil C/N  
391 ratio is generally treated as the critical indicator which affects the formation and degradation of  
392 soil OM (Khan et al., 2016). Since response of soil TN content to soil environment change is  
393 almost the same as soil OC content (Wang et al., 2018), soil C/N ratio did not show significant  
394 difference amongst different aged tea plantations (Table S1). Likewise, Zhou et al. (2018)  
395 proved that no close correlation existed between soil C/N ratio and vegetation coverage, because  
396 C and N are structure elements and their cumulation and consumption in soil remain relative  
397 consistency. Soil C/P ratio is the indicator suggesting P effectiveness, and higher C/P ratio often  
398 denotes lower P effectiveness (Khan et al., 2016). In acidic soil (Table 1), available P was  
399 adsorbed on the surfaces of Fe/Al oxides and clay minerals in a preferential way, because Fe/Al  
400 oxides and clay minerals with greater surface areas could afford enough sites to available P  
401 adsorption (Wu et al., 2018). As the tea plantation age increased, therefore, soil acidification led  
402 to the decrease in P effectiveness (evidenced by the significant increase in soil C/P ratio) (Table  
403 S2). Soil N and P are the prohibiting factors mostly seen during the process of plant growth, and  
404 thus, N/P ratio can be utilized as one efficient indicator that shows nutrient restriction (Khan et  
405 al., 2016). In this study, soil N/P ratio significantly increased in the tea-planting course (Table  
406 S3), mainly because soil TN content experienced significant increase while no such significant  
407 change was found in TP content over time.

408        Regardless of the tea plantation age, soil C/N ratio decreased with increasing soil depth  
409 (Table S1), which coincided with the majority of studies (Cao et al., 2015; Feng and Bao, 2017,  
410 Yu et al., 2019). Batjes (1996) suggested that the decrease in soil C/N ratio as the soil depth  
411 increased was triggered by the stratification of humic substance in the soil profile. Moreover, in  
412 this study, the lower soil C/P and N/P ratios in the subsoil (Tables S2 and S3) backed the  
413 outcomes of past studies in terrestrial ecosystems of China, which were on the foundation of the  
414 data from both the 2<sup>nd</sup> soil investigation in China (Tian et al., 2010) and the Chinese Ecosystem

415 Research Network (CERN) (Chai et al., 2015).

416 Across the 4 tea plantation ages, the mean contents of OC and TN in bulk soil (0-20 cm)  
417 were 16.70 and 0.77 g kg<sup>-1</sup>, separately, which were below the mean contents of OC (21.30 g kg<sup>-1</sup>)  
418 and TN (2.17 g kg<sup>-1</sup>) in Chinese tea plantations (Sun et al., 2020; Xie et al., 2020). Moreover, in  
419 this tea-planting region, the mean content of TP in bulk soil (0-20 cm) was 0.57 g kg<sup>-1</sup>,  
420 corresponding to the moderate level in Chinese tea plantations, where TP content varied in the  
421 range of 0.35-1.20 g kg<sup>-1</sup> (Wu et al., 2018; Sun et al., 2020). Herein, soil C/N ratio is higher  
422 compared with other tea-planting regions in China, whereas soil C/P and N/P ratios are much  
423 lower (Sun et al., 2020). These findings are primarily associated with the lower contents of soil  
424 OC and TN, especially the TN. In general, N is the most limiting element in the net primary  
425 production of tea plantation ecosystems (Miner et al., 2018), and this phenomenon also appeared  
426 in the southern Guangxi of China.

427 4.4. Contents of soil alkali cations and micronutrients

428 According to the findings from Adesodun et al. (2007) and Emadi et al. (2009), the higher  
429 contents of exchangeable alkali cations (including Ca<sup>2+</sup> and Mg<sup>2+</sup>) were detected in both 2-4.76  
430 and < 0.25 mm aggregates in the non-tillage soil. In the tillage course, however, the contents of  
431 these two cations decreased in the 2-4.76 mm aggregates and increased in the < 0.25 mm  
432 aggregates, revealing that the tillage practice could cause soil Ca<sup>2+</sup> and Mg<sup>2+</sup> to redistribute in  
433 different sizes of aggregates. In comparison, the present study exhibited that the distribution of  
434 soil Ca<sup>2+</sup> and Mg<sup>2+</sup> in aggregates was similar among different ages of tea plantations (Figures 5  
435 and 6), implying that the distribution of these two cations in aggregates was seldom influenced  
436 by the tea plantation age. To be specific, coarse macroaggregates had the lowest contents of Ca<sup>2+</sup>  
437 and Mg<sup>2+</sup>, whereas microaggregates exhibited the highest contents. These findings could be  
438 ascribed to the larger specific surface areas of microaggregates (Adesodun et al., 2007), which  
439 increased microaggregates' adsorption to Ca<sup>2+</sup> and Mg<sup>2+</sup> derived from root exudates, litter  
440 remains, and manure (Emadi et al., 2009). Unlike exchangeable alkali cations, the contents of

441 soil available micronutrients (including  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$ ) usually correspond to the content of soil  
442 OM (Wang et al., 2017a), which are more abundant in macroaggregates (Six et al., 2004).  
443 Similarly, this study also found that the  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  had a similar distribution pattern with OC  
444 within aggregates (Figures 7 and 8). Since the decomposition products of litter can be easily  
445 integrated to the coarse macroaggregates (Six et al., 2004), the nutrient cycling of plant-soil  
446 systems might lead to the higher contents of soil  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  in the coarse macroaggregates  
447 (Wang et al., 2017a).

448 At the 0-40 cm soil depth, the contents of soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  significantly decreased over  
449 time (Figures 5 and 6), which might be due to the applications of urea and  $\text{NH}_4^+ \text{-N}$  fertilizer in  
450 the tea-planting course for increasing tea leaf outputs. Urea hydrolysis can promote the  
451 production of ammonium ions which are readily nitrified into nitrate, and the excessive proton  
452 produced by the nitrification can compete for the adsorption sites with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Wang et  
453 al., 2017a). As a result, these cations were easy to lose from soil in the manner of leaching.  
454 Except at the 40-60 cm soil depth, continuous planting of tea led to the significant increases in  
455 soil  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  contents (Figures 7 and 8), which were elevated by 7%-27% and 3%-9% from  
456 8 to 43 years of tea planting, separately. This phenomenon was possibly caused by the soil  
457 acidification (Table 1), which stimulates the release of soil  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  by mineralization and  
458 desorption from soil OM and minerals (Wang et al., 2017a). Tea, as an aluminium (Al)  
459 cumulating crop, is able to cumulate Al in leaves (Li et al., 2016). Soil acidification in the  
460 tea-planting course was due to the substantial tea litter into the soil annually via trimmed  
461 branches and leaves (Li et al., 2016). At the same time, the rhizosphere deposition of massive  
462 organic acids (i.e., malate, lemon acid, and oxalate acid) around the tea roots could provoke  
463 localized acidification (Xue et al., 2006). In addition, for increasing the output of tea, tea  
464 plantations needed to apply N fertilizers (i.e., urea and  $\text{NH}_4^+ \text{-N}$ ), thus leading to soil acidification  
465 by the  $\text{NH}_4^+$  nitration (Yang et al., 2018).

466 Across the 4 tea plantation ages, the contents of soil  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  were higher in the  
467 topsoil than the subsoil (Figures 7 and 8), primarily owing to the usage of swine manure and the  
468 inputs of tea litter and roots in the topsoil (Miner et al., 2018). Nevertheless, the contents of soil  
469  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  showed an opposite trend as the soil depth increased (Figures 5 and 6), because  
470 soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  were easy to move from topsoil to subsoil in the manner of leaching (Hansen  
471 et al., 2017).

472 4.5. Stoichiometric ratios of soil alkali cations and micronutrients

473 Tea plantation age exerted a significant influence on the  $\text{Ca}/\text{Mg}$  and  $\text{Fe}/\text{Mn}$  ratios at the  
474 0-40 cm soil depth, not the 40-60 cm soil depth (Tables S4 and S5). To be specific, a significant  
475 decline in the  $\text{Ca}/\text{Mg}$  ratio was found at the 0-40 cm soil depth over time. From 8 to 43 years of  
476 tea planting, the contents of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  at the 0-40 cm soil depth decreased by 10%-38% and  
477 5%-24%, separately, which revealed that the role of tea plantation age in the content of soil  $\text{Ca}^{2+}$   
478 was greater than that of soil  $\text{Mg}^{2+}$  (Figures 5 and 6). Lu et al. (2014) suggested that the selective  
479 losses of soil exchangeable alkali cations ( $\text{Ca}^{2+} > \text{Mg}^{2+}$ ) could lead to the disequilibrium of soil  
480 metal ions in forest ecosystems. Similarly, in this study, the preferential loss of soil  $\text{Ca}^{2+}$  relative  
481 to  $\text{Mg}^{2+}$  was the prime cause of the significant decline in the soil  $\text{Ca}/\text{Mg}$  ratio in the tea-planting  
482 course. The depletion of soil exchangeable alkali cations (especially the  $\text{Ca}^{2+}$ ) could lead to the  
483 decrease in soil buffering capacity and soil acidification (Hansen et al., 2017). Thus, the  $\text{Ca}/\text{Mg}$   
484 ratio at the 0-40 cm soil depth was positively related ( $P < 0.05$ ) to soil pH across the 4 tea  
485 plantation ages (Figure S1). Soil acidification accelerated the mineralization and desorption of  
486 soil available micronutrients from soil OM and minerals (Wang et al., 2017a), conducive to the  
487 significant increases in  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  contents at the 0-40 cm soil depth, especially the  $\text{Fe}^{2+}$   
488 (Figures 7 and 8). In a chronological sequence of tea plantations, the negative relationship ( $P <$   
489  $0.05$ ) of soil  $\text{Fe}/\text{Mn}$  ratio with soil pH in different soil depths indicated more cumulation of soil  
490  $\text{Fe}^{2+}$  relative to  $\text{Mn}^{2+}$  over time (Figure S1). Moreover, the change of soil  $\text{Fe}/\text{Mn}$  ratio was also  
491 triggered by the antagonistic relationship between soil  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  during the process of tea

492 plant uptake (Wang et al., 2017a). Tian et al. (2016) discovered that soil acidification could  
493 reduce  $\text{Fe}^{2+}$  absorption and enhance  $\text{Mn}^{2+}$  uptake by various plant species, thereby causing the  
494 increase in soil Fe/Mn ratio and threatening plant productivity.

495 **5. Conclusions**

496 Herein, soil OC, TN, and TP contents as well as C/N, C/P, and N/P ratios decreased as the  
497 soil depth increased. Moreover, soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents were lower in the topsoil than the  
498 subsoil, whereas soil  $\text{Fe}^{2+}$  and  $\text{Mn}^{2+}$  contents showed an opposite trend, and no differences were  
499 detected amongst different soil depths in soil Ca/Mg and Fe/Mn ratios. Tea plantation age could  
500 influence the variations in soil OC and nutrient contents and their stoichiometric ratios, but such  
501 effects were more obvious at the 0-40 cm soil depth in contrast to the 40-60 cm soil depth, thus  
502 supporting our hypothesis. At the 0-40 cm soil depth, continuous planting of tea was favorable to  
503 the increases in soil OC, TN,  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$  contents, whereas soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  contents  
504 decreased over time. Compared with other tea-planting regions in China, soil C/N ratio is higher  
505 in this tea-planting region, whereas soil C/P and N/P ratios are much lower, indicating that soil  
506 OC and TN contents in the present study were lower, especially the TN. Therefore, an  
507 appropriate increase in the amount of N fertilizer should be applied in this tea-planting region. In  
508 the tea-planting course, the losses of soil  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , especially the  $\text{Ca}^{2+}$  (as indicated by the  
509 decrease in soil Ca/Mg ratio), could lead to the soil acidification. Meanwhile, soil acidification  
510 could reduce  $\text{Fe}^{2+}$  absorption and enhance  $\text{Mn}^{2+}$  uptake by tea plants (as indicated by the increase  
511 in soil Fe/Mn ratio). Overall, the present study improved the understanding of soil OC and  
512 nutrient dynamics in tea plantation ecosystems.

513 **Data availability**

514 The data supporting the discovered information here can be presented by the relevant author  
515 based on reasonable requests.

516 **Author contribution**

517 S.W. and S.Y. designed the experiments; L.M. carried out the experiments; S.W. and L.M.  
518 analyzed the experimental results; L.M., S.W. and S.Y. wrote and edited the manuscript.

519 **Competing interests**

520 The authors declare no conflict of interest.

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**Table 1** Effects of soil depth and tea plantation age on the bulk density, clay, and pH in bulk soil.

Soil depth	Tea plantation age	Bulk density (g cm <sup>-3</sup> )	Clay (%)	pH
0-10 cm	8 years	1.28 ± 0.02 b	34.69 ± 3.21 a	4.57 ± 0.02 a
	17 years	1.20 ± 0.02 c	35.91 ± 2.77 a	4.49 ± 0.01 ab
	25 years	1.26 ± 0.01 bc	33.12 ± 2.46 a	4.31 ± 0.03 b
	43 years	1.31 ± 0.04 b	35.08 ± 2.41 a	4.15 ± 0.02 c
10-20 cm	8 years	1.30 ± 0.03 b	34.88 ± 2.08 a	4.55 ± 0.03 a
	17 years	1.22 ± 0.03 c	32.59 ± 3.02 a	4.50 ± 0.01 a
	25 years	1.30 ± 0.03 b	34.92 ± 3.67 a	4.33 ± 0.02 b
	43 years	1.29 ± 0.02 b	32.35 ± 2.68 a	4.17 ± 0.02 c
20-40 cm	8 years	1.32 ± 0.04 ab	35.26 ± 1.45 a	4.60 ± 0.04 a
	17 years	1.31 ± 0.01 b	34.57 ± 4.12 a	4.53 ± 0.02 a
	25 years	1.34 ± 0.01 ab	34.51 ± 3.21 a	4.34 ± 0.04 b
	43 years	1.33 ± 0.04 ab	34.29 ± 3.54 a	4.19 ± 0.03 c
40-60 cm	8 years	1.36 ± 0.01 a	34.78 ± 3.66 a	4.58 ± 0.02 a
	17 years	1.37 ± 0.02 a	36.89 ± 2.98 a	4.54 ± 0.03 a
	25 years	1.39 ± 0.02 a	33.68 ± 1.91 a	4.32 ± 0.01 b
	43 years	1.38 ± 0.03 a	35.81 ± 3.69 a	4.21 ± 0.01 bc

Data represent the mean of 5 replicates ± standard deviations. Means in the same column with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages.

**Table 2** Three-way ANOVA regarding the effects of soil depth, tea plantation age, aggregate size, and their interactions on the physico-chemical properties of soil aggregates, and Two-way ANOVA regarding the effects of soil depth, tea plantation age, and their interactions on the physico-chemical properties of bulk soil.

Soil properties	Three-way ANOVA							Two-way ANOVA		
	S	T	A	S × T	S × A	T × A	S × T × A	S	T	S × T
Bulk density								√	√	√
Clay								×	×	×
pH								×	√	×
MWD								√	√	√
Aggregate proportion	√	√	√/√	√	√	√	√			
Organic C	√/√	√/√	√/√	√/√	√/√	√/√	√/√	√/√	√/√	√/√
Total N	√/√	√/√	√/√	√/√	√/√	√/√	√/√	√/√	√/√	√/√
Total P	√	×	×	×	×	×	×	√	×	×
Exchangeable Ca <sup>2+</sup>	√	√/√	√	√	√	√	√	√	√/√	√
Exchangeable Mg <sup>2+</sup>	√	√	√	√	√	√	√	√	√	√
Available Fe <sup>2+</sup>	√	√/√	√	√	√	√	√	√	√/√	√
Available Mn <sup>2+</sup>	√	√	√	√	√	√	√	√	√	√
C/N ratio	√	×	√	×	√	×	×	√	×	×
C/P ratio	√	√	√	√	√	√	√	√	√	√
N/P ratio	√	√	√	√	√	√	√	√	√	√
Ca/Mg ratio	×	√	×	×	×	×	×	×	√	×
Fe/Mn ratio	×	√	×	×	×	×	×	×	√	×

S: soil depth; T: tea plantation age; A: aggregate size. √/√, √, and × indicate significant differences at  $P < 0.01$ ,  $P < 0.05$ , and  $P > 0.05$ , respectively.

**Table 3** Effects of soil depth and tea plantation age on the aggregate stability and composition.

Soil depth	Tea plantation age	MWD (mm)	Aggregate composition (%)			
			> 2 mm	1-2 mm	0.25-1 mm	< 0.25 mm
0-10 cm	8 years	1.88 ± 0.03 b	44.26 ± 3.24 bA	16.23 ± 2.45 abC	8.46 ± 1.37 abD	31.05 ± 5.78 bcB
	17 years	2.20 ± 0.04 a	53.18 ± 2.78 aA	18.02 ± 1.63 aB	6.69 ± 0.98 bC	22.11 ± 4.01 cB
	25 years	1.78 ± 0.01 b	40.29 ± 4.01 bA	17.97 ± 2.03 aC	8.81 ± 0.88 abD	32.93 ± 3.58 bcB
	43 years	1.53 ± 0.03 c	32.60 ± 3.61 cB	19.61 ± 2.04 aC	7.64 ± 1.57 bD	40.15 ± 4.27 abA
10-20 cm	8 years	1.62 ± 0.02 c	37.31 ± 2.47 cA	13.58 ± 1.56 bB	9.24 ± 2.04 abC	39.87 ± 2.69 abA
	17 years	1.82 ± 0.04 b	43.02 ± 2.69 bA	14.31 ± 1.38 abC	8.87 ± 1.14 abD	33.80 ± 4.58 bB
	25 years	1.56 ± 0.03 c	34.87 ± 1.45 cB	15.03 ± 2.47 abC	9.36 ± 1.09 abD	40.74 ± 3.94 abA
	43 years	1.34 ± 0.02 d	29.24 ± 3.28 dB	13.97 ± 1.65 bC	7.28 ± 0.82 bD	49.51 ± 2.56 aA
20-40 cm	8 years	1.43 ± 0.01 cd	31.25 ± 1.68 cdB	15.47 ± 2.49 abC	7.62 ± 0.47 bD	45.66 ± 4.77 aA
	17 years	1.48 ± 0.03 cd	32.08 ± 3.60 cdB	16.89 ± 2.51 abC	8.91 ± 2.14 abD	42.12 ± 2.05 abA
	25 years	1.39 ± 0.02 d	30.72 ± 3.25 dB	14.23 ± 0.58 abC	6.81 ± 1.36 bD	48.24 ± 3.59 aA
	43 years	1.48 ± 0.03 cd	32.49 ± 2.98 cdB	15.40 ± 2.11 abC	9.05 ± 0.91 abD	43.06 ± 4.32 aA
40-60 cm	8 years	1.30 ± 0.01 d	28.48 ± 2.57 dB	12.02 ± 3.08 bC	10.05 ± 0.58 aC	49.45 ± 3.68 aA
	17 years	1.36 ± 0.02 d	29.68 ± 2.61 dB	13.78 ± 1.14 bC	9.47 ± 1.03 abC	47.07 ± 3.47 aA
	25 years	1.36 ± 0.01 d	30.09 ± 1.47 dB	11.98 ± 0.98 bC	10.64 ± 0.45 aC	47.29 ± 4.01 aA
	43 years	1.34 ± 0.03 d	28.42 ± 3.02 dB	14.33 ± 1.57 abC	12.45 ± 2.13 aC	44.80 ± 2.99 aA

Data represent the mean of 5 replicates ± standard deviations. Means in the same column with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means in the same row with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 1** Location of the experiment site.

**Figure 2** Effects of soil depth and tea plantation age on the organic C content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 3** Effects of soil depth and tea plantation age on the total N content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 4** Effects of soil depth and tea plantation age on the total P content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 5** Effects of soil depth and tea plantation age on the exchangeable  $\text{Ca}^{2+}$  content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 6** Effects of soil depth and tea plantation age on the exchangeable  $\text{Mg}^{2+}$  content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 7** Effects of soil depth and tea plantation age on the available  $\text{Fe}^{2+}$  content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

**Figure 8** Effects of soil depth and tea plantation age on the available  $\text{Mn}^{2+}$  content in bulk soil and different sized aggregates. Data represent the mean of 5 replicates and error bars represent the standard deviations. Means with the same lower case letter are not significantly different ( $P > 0.05$ ) among different soil depths and tea plantation ages. Means with the same capital letter are not significantly different ( $P > 0.05$ ) among different sized aggregates.

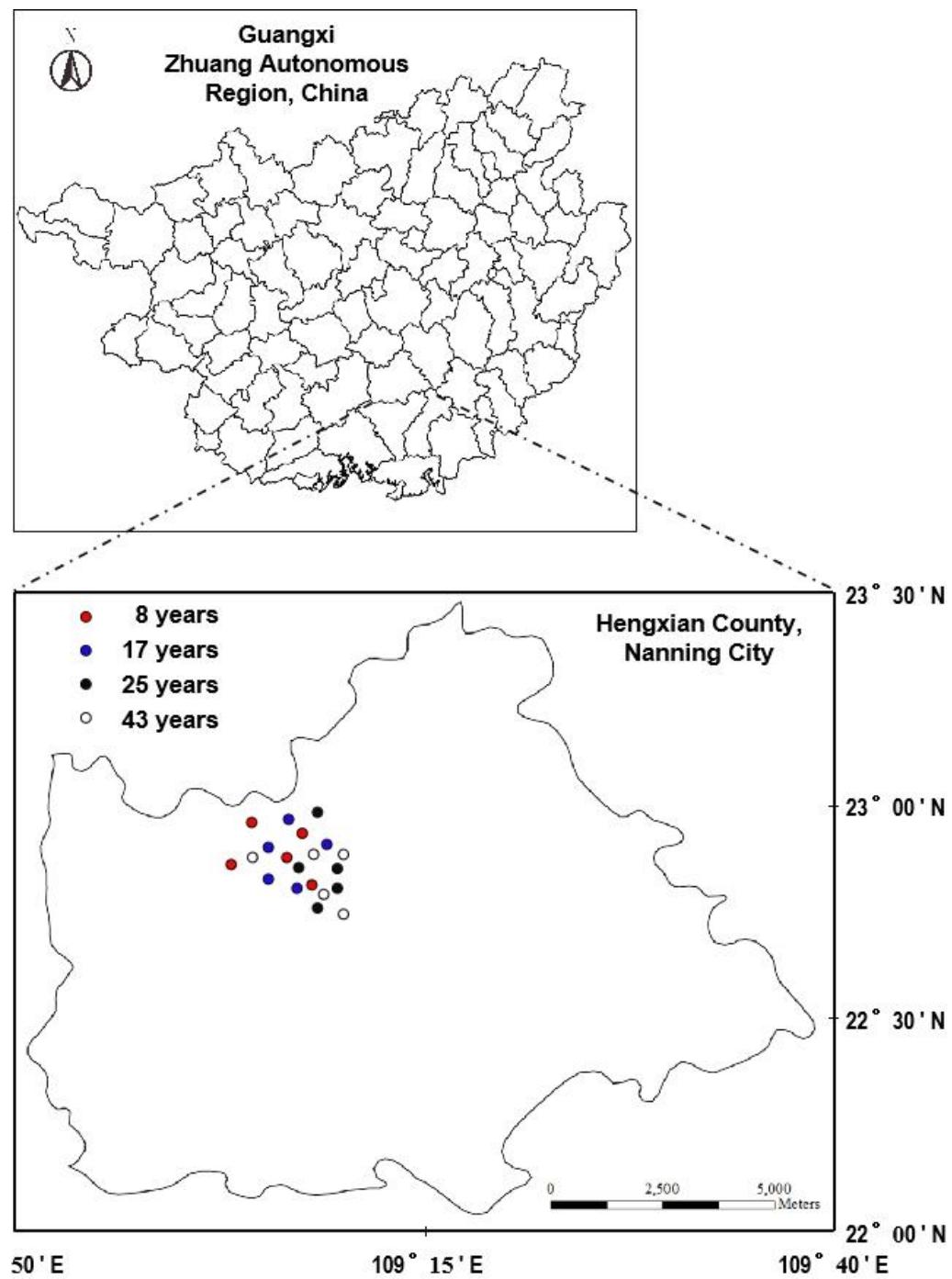


Figure 1

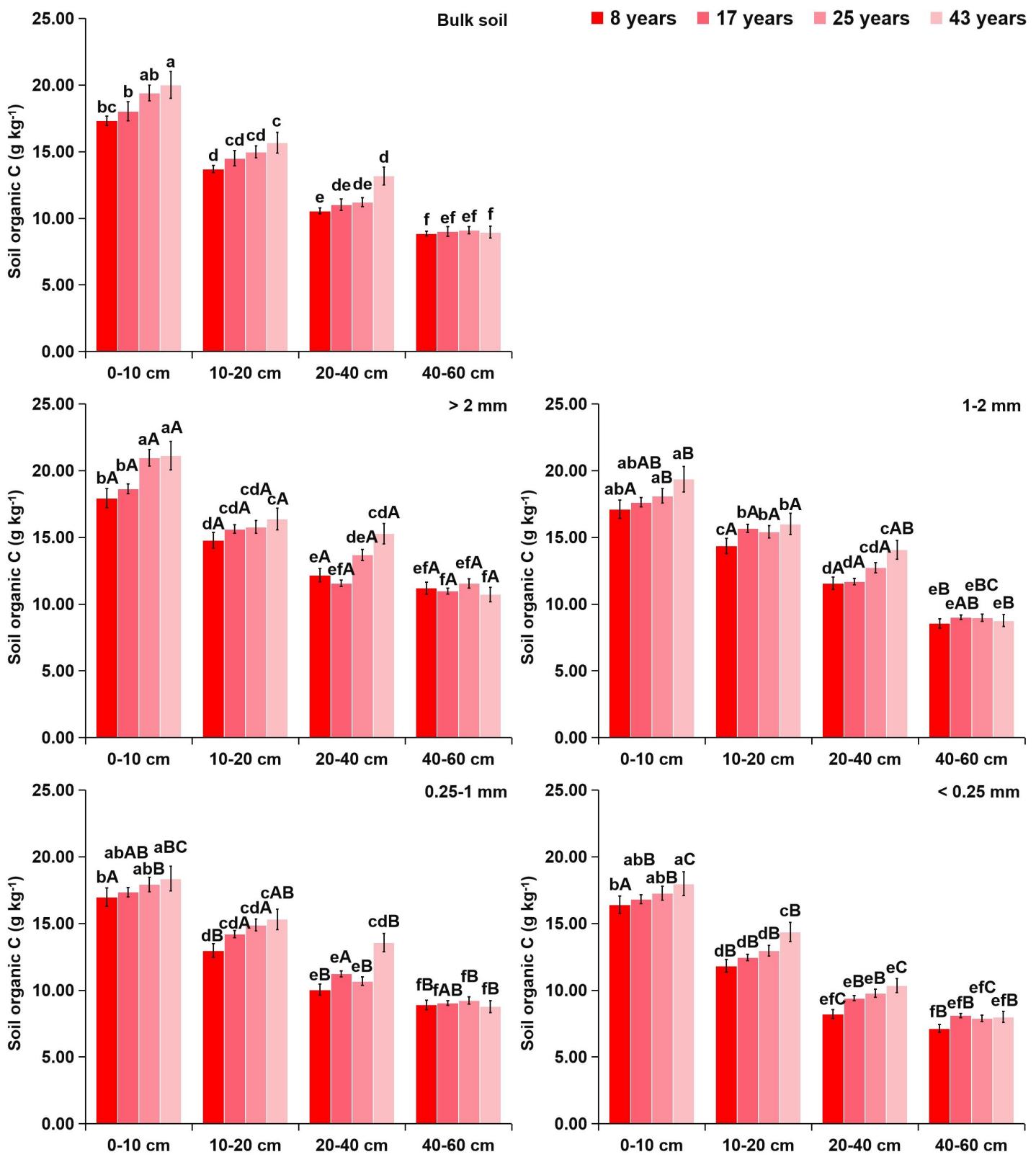


Figure 2

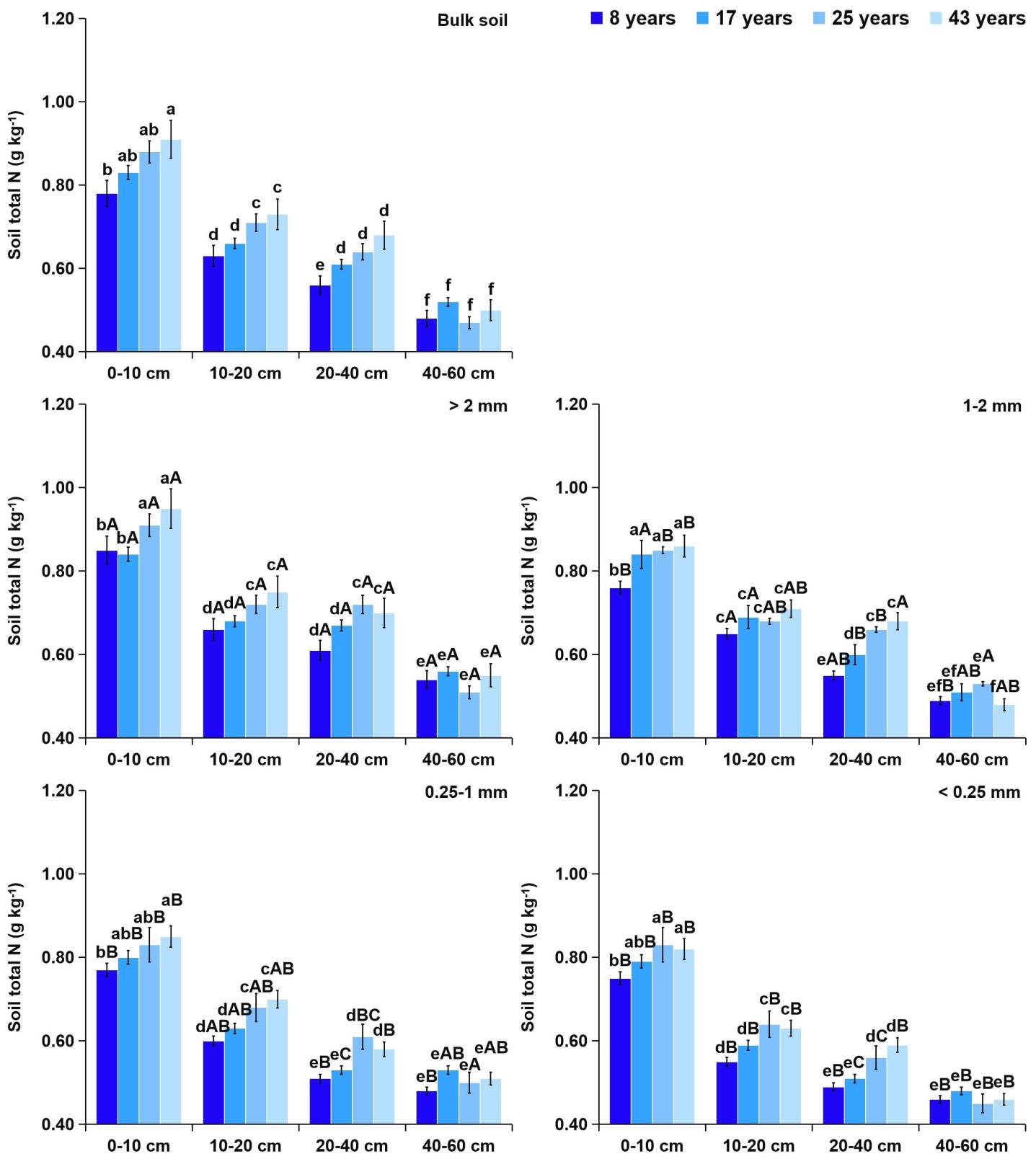


Figure 3

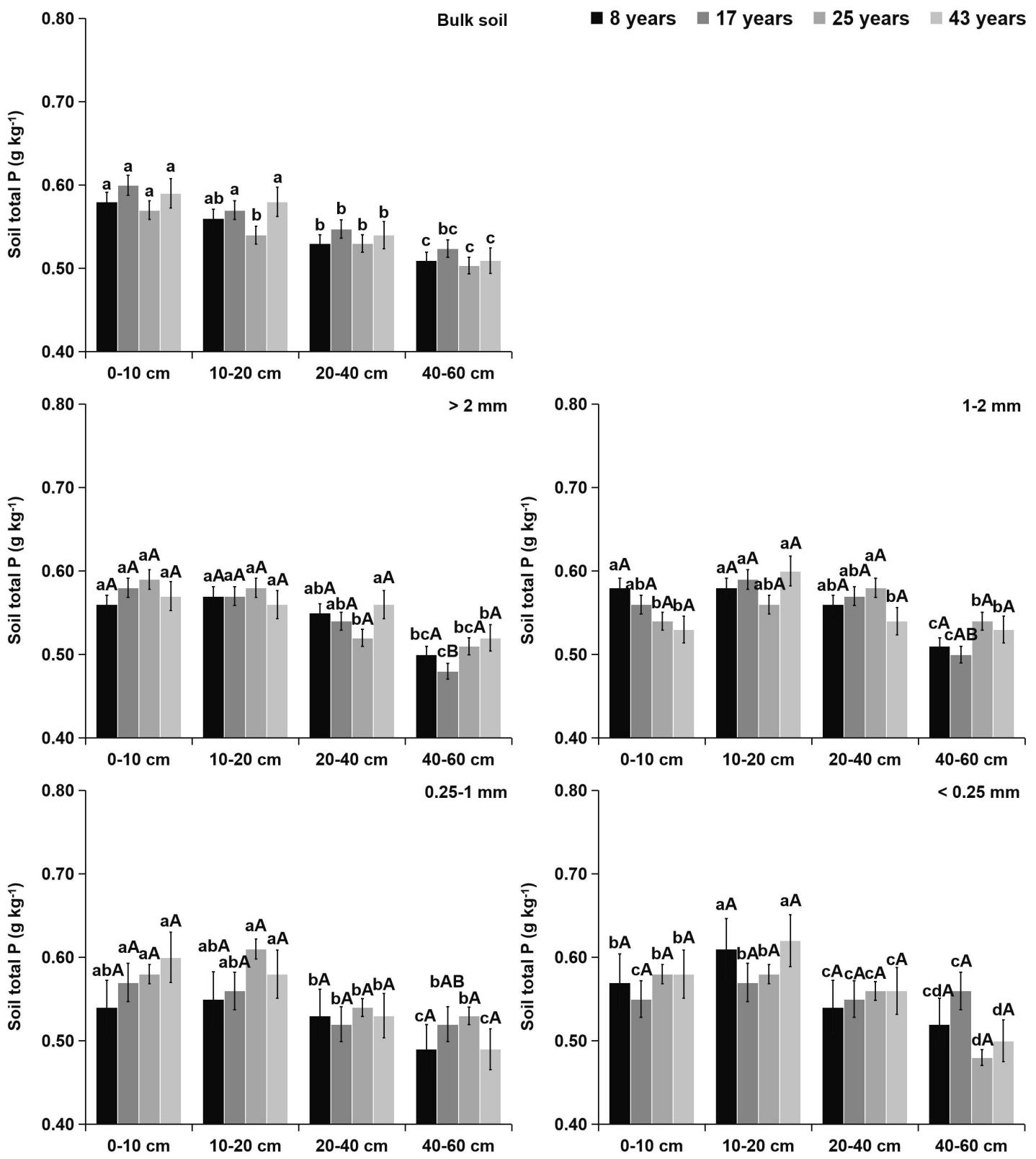


Figure 4

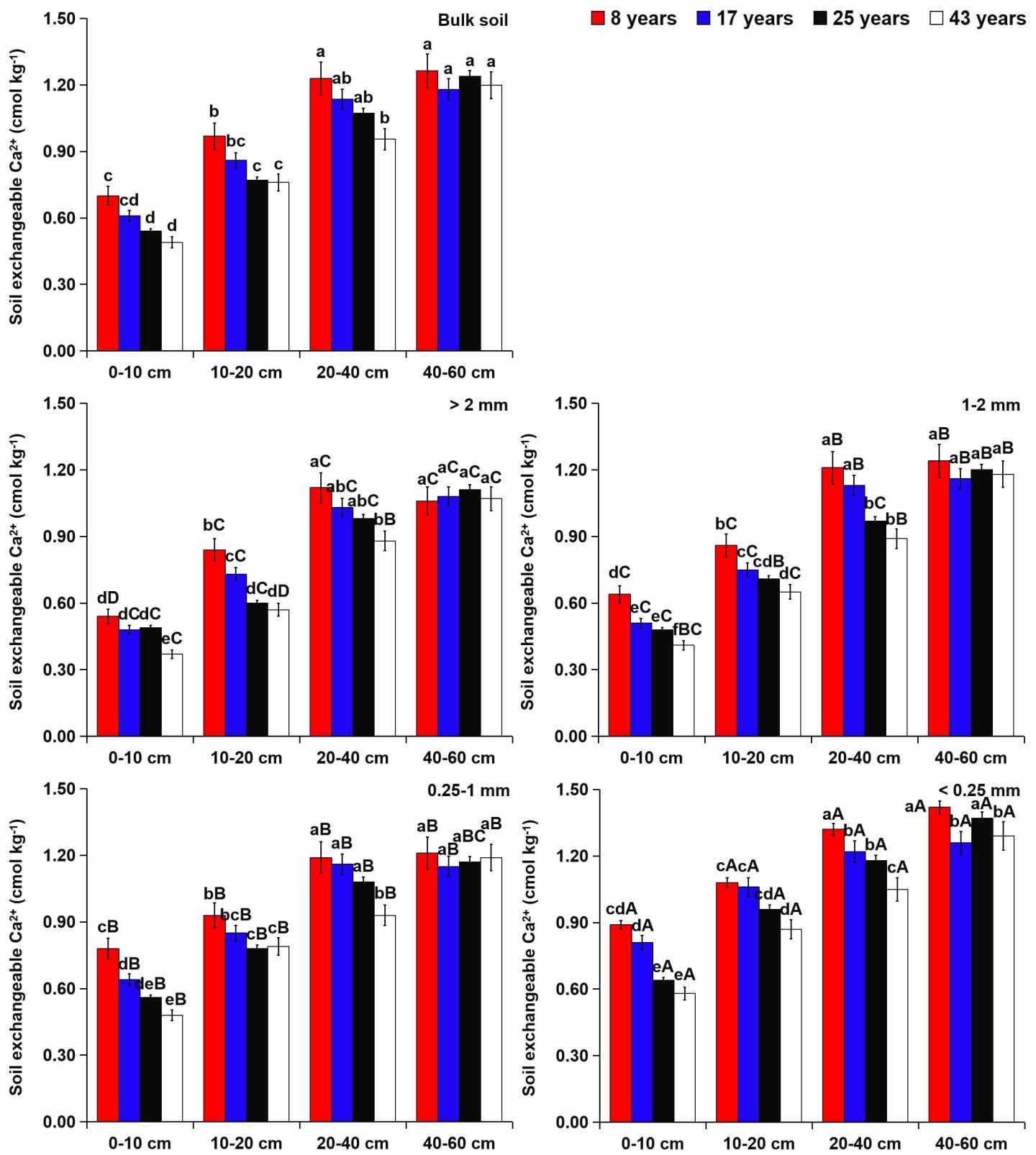


Figure 5

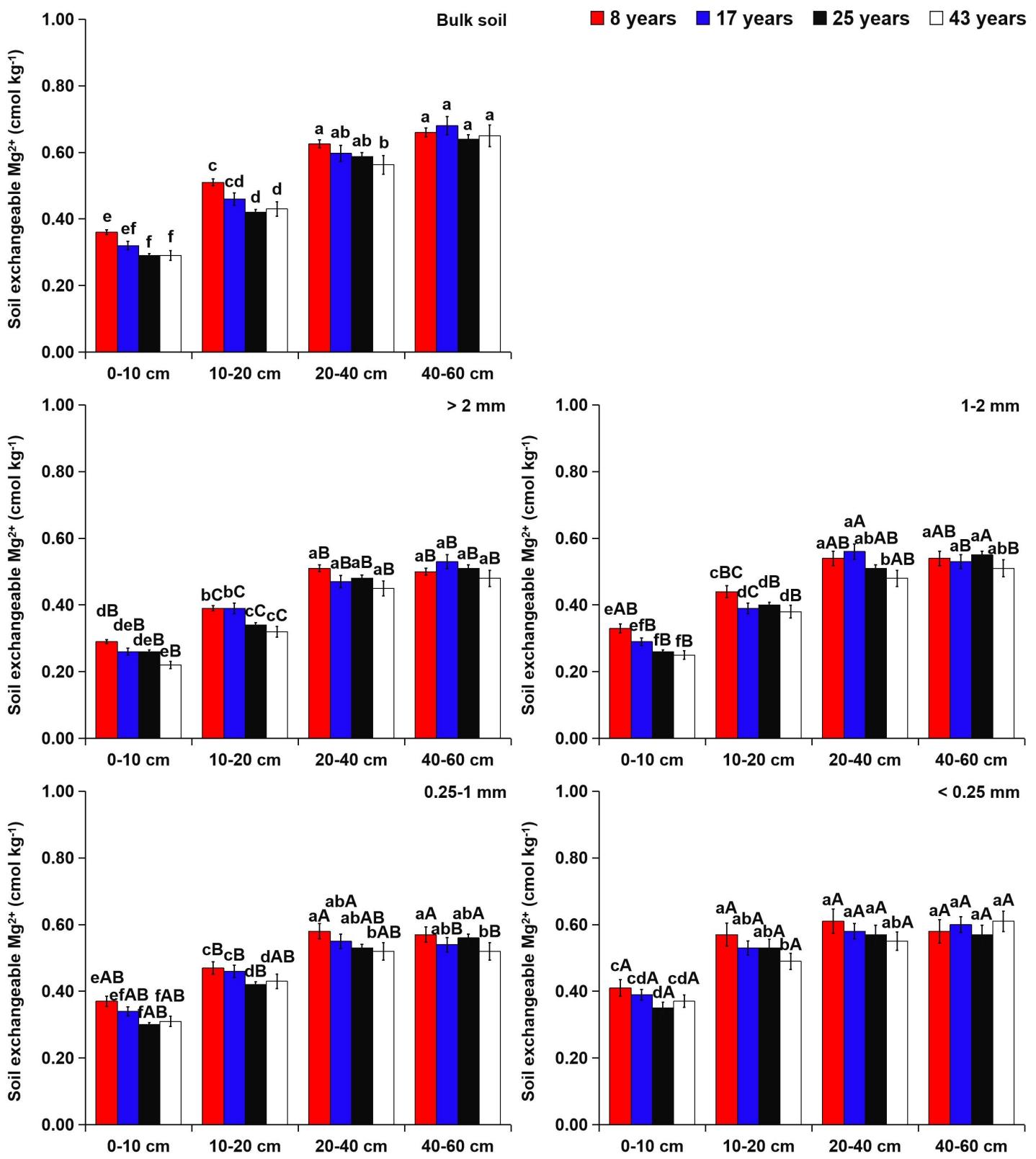


Figure 6

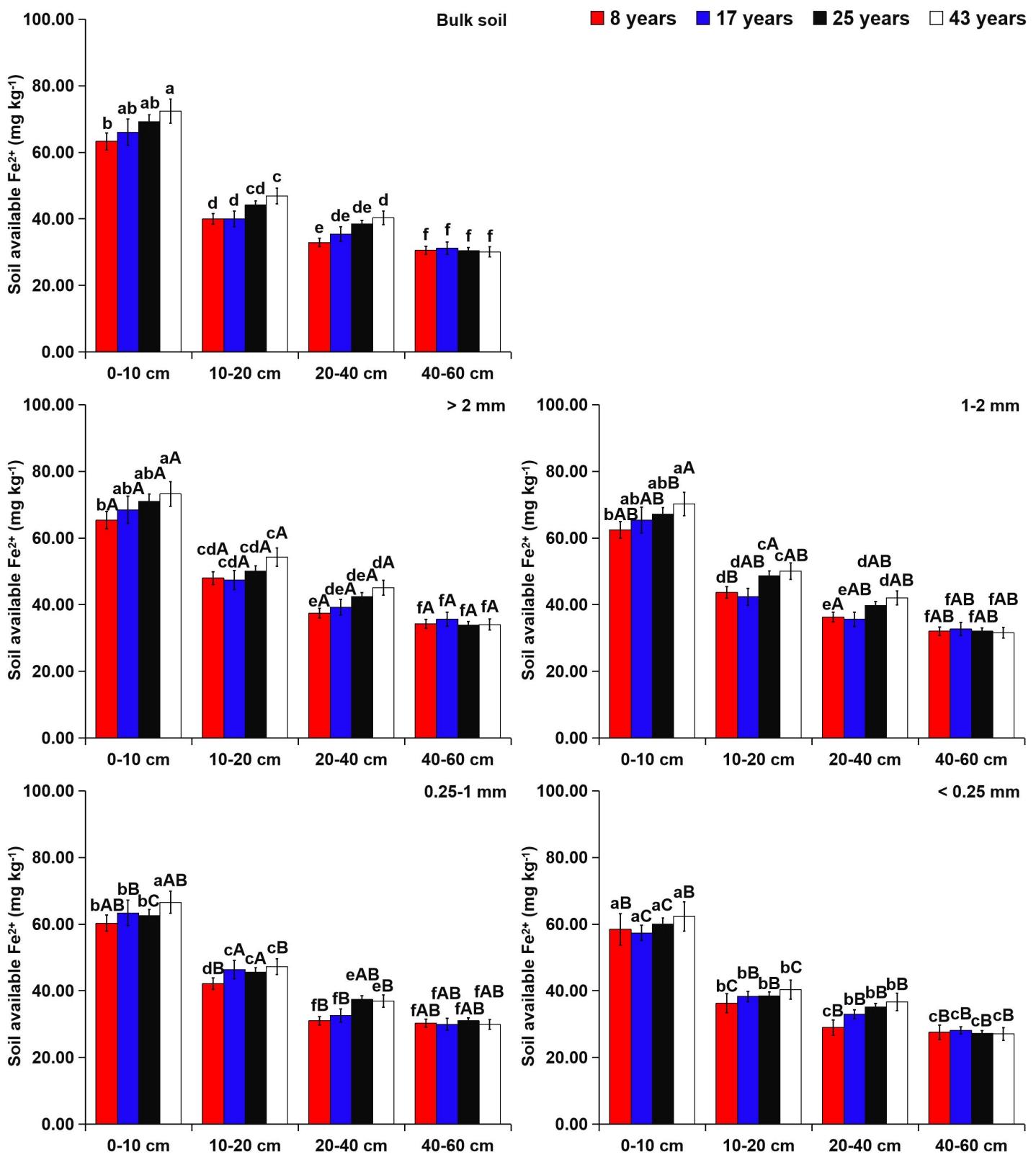


Figure 7

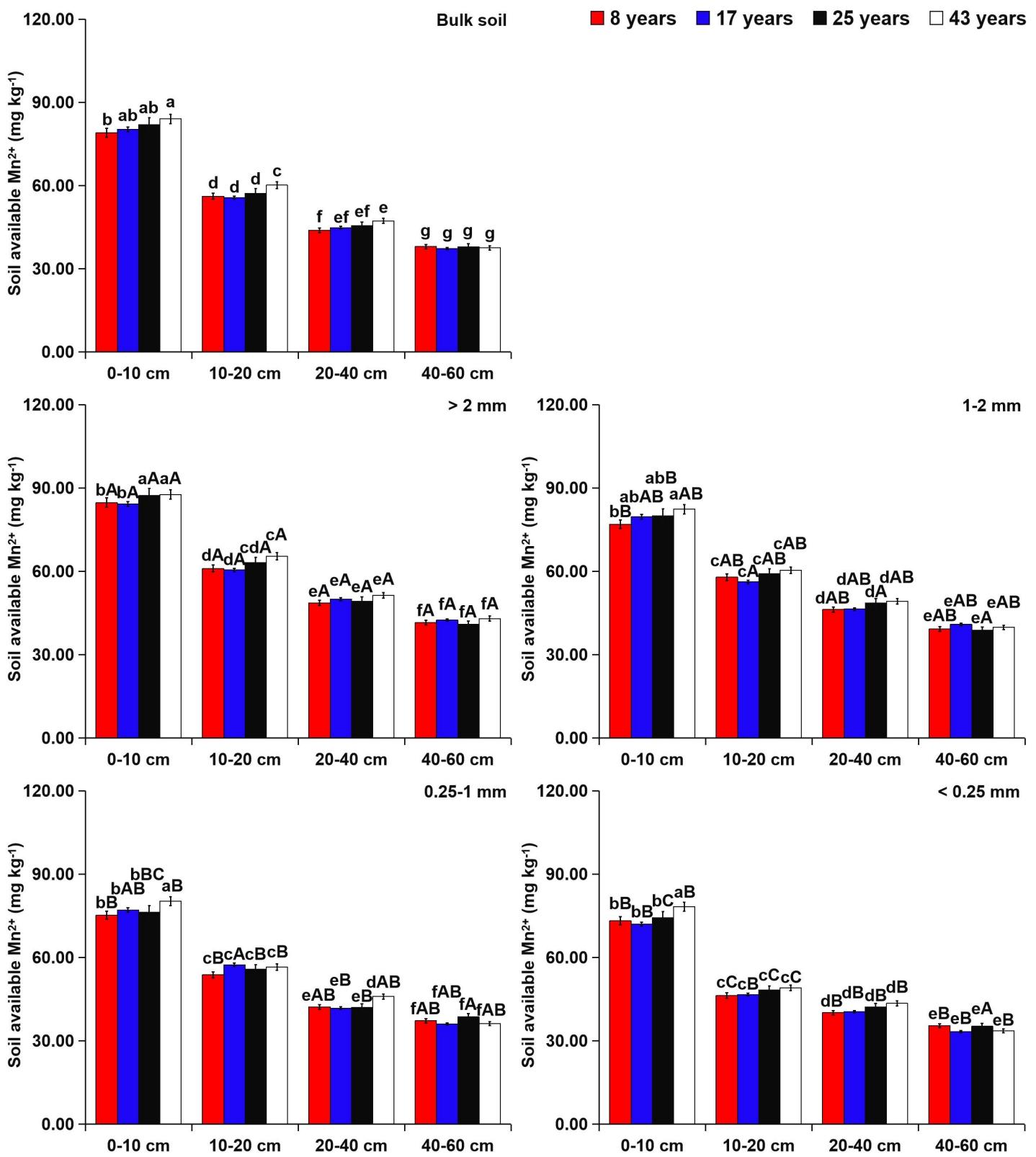


Figure 8