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

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

absorption and enhance Mn<sup>2+</sup> uptake by tea plants (as indicated by the increase in soil Fe/Mn
ratio). Overall, this study improved the understanding of soil OC and nutrient dynamics in tea
plantation ecosystems.

### 30 KEYWORDS

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

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# 33 **1. Introduction**

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

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

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

Over the past decade, soil nutrient stoichiometry (mainly C-N-P, rather than Ca-Mg or 64 Fe-Mn) has been broadly studied across the world (Tian et al., 2010; Yang et al., 2013; Zhang et 65 al., 2016; Yue et al., 2017; Yu et al., 2018; Qiao et al., 2020). A wide agreement exists amongst 66 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) contents as the soil depth increased (Yue et al., 2017; Yu et al., 2018; Qiao et al., 2020), whereas 69 conflicting vertical patterns were discovered for soil C/N, C/P, and N/P ratios. For instance, 70 decreasing trend of the C/P and N/P ratios was observed as the soil depth increased in the data of 71 the 2<sup>nd</sup> soil investigation in China (Tian et al., 2010). Nevertheless, larger C/N ratio in the deeper 72 soil layer, not the surface soil layer, was identified in a mollisol plain in the northeast China 73 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., 2013). As shown above, inconsistent vertical patterns have been reported for the C-N-P 76 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 primary particles as well as organic matter (OM) (Tisdall and Oades, 1982). According to the 80 differences of binding agents, soil aggregates can be classified into microaggregates (< 0.25 mm) 81 82 and macroaggregates (> 0.25 mm) (Tisdall and Oades, 1982). In general, persistent binding agents (like humified OM and polyvalent metal cation complexes) contribute to the binding of 83 primary particles into microaggregates (Six et al., 2004). Differently, temporary binding agents 84 (like fungal hyphae, plant roots, and polysaccharides) aggregating with microaggregates 85 conduces to the formation of macroaggregates (Six et al., 2004). As shown above, soil 86 aggregates with various sizes exert different abilities in the supply and reserve of soil OC and 87 nutrients. Thus, to improve the comprehension about the structure and function of soil 88 ecosystems, more efforts should be made to observe the soil nutrient stoichiometry within 89 aggregates (Xu et al., 2019; Cui et al., 2021). In recent period, lots of studies have reported the 90 OC, TN, and TP distribution in different sizes of aggregates, but these studies are ended with 91 different results. To be specific, some studies revealed the significant increases in the OC, TN, 92 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., 2021b). These show that the changes of soil OC, TN, and TP within aggregates have received 95 great attention, whereas soil exchangeable alkali cations (i.e.,  $Ca^{2+}$  and  $Mg^{2+}$ ) and available 96 micronutrients (i.e., Fe<sup>2+</sup> and Mn<sup>2+</sup>) are rarely investigated. 97

Our past studies indicated that the landuse shift from farmlands to tea plantations could ameliorate soil fertility level (Zheng et al., 2011). Nevertheless, during the process of tea growth, the variation in soil nutrient stoichiometry is still unclear. Meanwhile, since tea serves as a deep root plant, it is vital to reveal how nutrient stoichiometry changes with increasing soil depth in tea plantation ecosystems. Thus, the present study was carried out to investigate how soil OC and nutrient contents as well as their stoichiometric ratios vary with tea plantation age (8, 17, 25, and 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

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

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

An annual fertilizer regime in tea plantations is shown below. Both 0.65 Mg ha<sup>-1</sup> complex fertilizer (granule, N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O: 18%-6%-6%) and 12 Mg ha<sup>-1</sup> swine manure (slurry, 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 at the surrounding region vertically below tree crown. Subsequently, the top-dressing, applied to the site treated with replenished basal fertilizer, was replenished 3 times per year. Both 1.2 Mg ha<sup>-1</sup> complex fertilizer and 0.5 Mg ha<sup>-1</sup> 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

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

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

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

149 2.3. Litter and soil sampling

For every plot, the 5 surface litter (a stock) specimens had been acquired from the surface of soil in the 5 randomly chosen subplots (S = 1 m × 1 m), and afterwards were integrated into a composite litter specimen. An overall the 20 (4 tea plantation ages × 5 replicates) composite litter specimens were desiccated at the 80 °C until steady weight. Then, the weights of these desiccated litter specimens were measured, and the litter C (Nelson and Sommers, 1996) and N (Bremner, 1996) contents were detected. The amount of litter was 821, 974, 786, and 648 g m<sup>-2</sup> in the 8, 17, 25, and 43 years of tea plantations, respectively, and the C/N ratio of litter was
14.23, 12.68, 17.32, and 21.37, respectively.

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

# 168 2.4. Soil aggregate separation

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

177 2.5. Soil property analyses

Prior to the analyses of soil physical-chemical properties, soil specimens were subjected to atmospheric drying under indoor temperature condition. According to the cutting ring method (Lu, 2000), soil specimens were oven-dried at 105 °C to the stable weight in order to measure the 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 182 (mass : volume) as 1 : 2.5 (Lu, 2000). Soil OC and TN were identified via the acid dichromate 183 wet oxidation method (Nelson and Sommers, 1996) and the micro-Kjeldahl method (Bremner, 184 185 1996), separately. Soil TP was identified via the molybdate blue colorimetry method (Bray and Kurtz, 1945). Soil exchangeable alkali cations (i.e.,  $Ca^{2+}$  and  $Mg^{2+}$ ) were determined by the 186 ammonium acetate (CH<sub>3</sub>COONH<sub>4</sub>) (Thomas, 1982). In short, 2.5 g of every aggregate fraction 187 was weighted into Erlenmeyer flask to blend with 50 mL 1 M  $CH_3COONH_4$  (pH = 7.0). The 188 extract liquid was agitated for 30 min under 150 rpm, and afterwards sieved via Whatman No. 2 189 V filtration paper (quantitative and ashfree). Soil available micronutrients (i.e.,  $Fe^{2+}$  and  $Mn^{2+}$ ) 190 were determined by the diethylenetriamine pentaacetic acid (DTPA) (Lindsay and Norvell, 191 1978). In short, 10 g of every aggregate fraction was weighted into Erlenmeyer flask to blend 192 with 20 mL 0.005 M DTPA + 0.01 M CaCl<sub>2</sub> + 0.1 M TEA (triethanolamine) (pH = 7.0). The 193 extract liquid was agitated for 2 h under 180 rpm, and afterwards sieved. Entire extractable 194 metallic cations were detected by the atomic absorption spectrometer (AAS, Shimadzu, Japan). 195 196 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 197 between parallel specimen and experimental specimen was controlled in 5%. 198

199 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 MWD = 
$$\sum_{i=1}^{4} (\mathbf{X}_i \times \mathbf{M}_i)$$
,

in the formula,  $X_i$  indicates the *i*<sup>th</sup> size aggregates' mean diameter (mm) and  $M_i$  indicates the *i*<sup>th</sup> size aggregates' proportion (% in weight).

206 SPSS 22.0 was used for statistic analysis (Table 2). Means were tested by the Tukey's HSD 207 and significance was used at P < 0.05. Two-way analysis of variance (ANOVA) was taken for exploring the effects of soil depth, tea plantation age, and their interactions on the physico-chemical properties of bulk soil. Three-way ANOVA was taken for exploring the effects of soil depth, tea plantation age, aggregate size, and their interactions on the physico-chemical properties of soil aggregates. Besides that, Pearson correlation analysis was utilized to test the relationships between pH and stoichiometric ratios (i.e., Ca/Mg and Fe/Mn ratios) in bulk soil during the process of tea growth.

214 **3. Results** 

215 3.1. Composition and stability of soil aggregates

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

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

At the aggregate scales, soil OC (Figure 2) and TN (Figure 3) contents significantly increased with increasing aggregate size, but the distribution of soil TP (Figure 4) was even in different sizes of aggregates. From 8 to 43 years of tea plantations, the OC and TN contents in soil aggregates were significantly elevated by 22%-35% and 14%-24%, 11%-22% and 9%-17%, and 8%-18% and 9%-13% at the 0-10, 10-20, and 20-40 cm soil depths, respectively. Nevertheless, no significant variation existed in the aggregate-related TP content. Furthermore, at the 40-60 cm soil depth, the aggregate-related OC, TN, and TP contents did not show significant variations over time. Regardless of the tea plantation age, significant decreases in the 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

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

254 3.4. Contents of soil alkali cations and micronutrients

At the aggregate scales, soil exchangeable alkali cations (i.e.,  $Ca^{2+}$  and  $Mg^{2+}$ ) were more concentrated in the microaggregates (Figures 5 and 6). However, soil available micronutrients (i.e.,  $Fe^{2+}$  and  $Mn^{2+}$ ) were mainly existed in the coarse macroaggregates (Figures 7 and 8). From 8 to 43 years of tea plantations, the  $Ca^{2+}$  and  $Mg^{2+}$  contents in soil aggregates were significantly reduced by 31%-38% and 10%-24%, 23%-27% and 9%-18%, and 10%-16% and 5%-8% at the 0-10, 10-20, and 20-40 cm soil depths, respectively. However, the Fe<sup>2+</sup> and Mn<sup>2+</sup> contents in soil aggregates were significantly elevated by 16%-27% and 6%-9%, 11%-15% and 4%-7%, and 7%-12% and 3%-5%, respectively. In addition, at the 40-60 cm soil depth, the contents of aggregate-related exchangeable alkali cations and available micronutrients did not show significant variations over time. Irrespective of the tea plantation age, significant increases in the aggregate-related Ca<sup>2+</sup> and Mg<sup>2+</sup> contents were observed with increasing soil depth, but the aggregate-related Fe<sup>2+</sup> and Mn<sup>2+</sup> contents showed an opposite trend.

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

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

### 279 **4. Discussion**

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

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

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

Regardless of the tea plantation age, coarse macroaggregates were dominant in the topsoil 304 (0-10 cm) while microaggregates were dominant in the subsoil (10-60 cm), indicating 305 transformation of aggregate composition from coarse macroaggregate-prevailing 306 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 southwest Sichuan of China. In the present study, coarse macroaggregates were the prevailing 309 fractions in the topsoil, not the subsoil, which was attributed to the surface cumulation of soil OC 310 311 (Figure 2). As an essential cementing agent, soil OC could foster the formation of coarse

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

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

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

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

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

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

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

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

388

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

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

Regardless of the tea plantation age, soil C/N ratio decreased with increasing soil depth (Table S1), which coincided with the majority of studies (Cao et al., 2015; Feng and Bao, 2017, Yu et al., 2019). Batjes (1996) suggested that the decrease in soil C/N ratio as the soil depth increased was triggered by the stratification of humic substance in the soil profile. Moreover, in this study, the lower soil C/P and N/P ratios in the subsoil (Tables S2 and S3) backed the outcomes of past studies in terrestrial ecosystems of China, which were on the foundation of the 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).

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

# 427 4.4. Contents of soil alkali cations and micronutrients

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

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

At the 0-40 cm soil depth, the contents of soil Ca<sup>2+</sup> and Mg<sup>2+</sup> significantly decreased over 448 time (Figures 5 and 6), which might be due to the applications of urea and NH4<sup>+</sup>-N fertilizer in 449 the tea-planting course for increasing tea leaf outputs. Urea hydrolysis can promote the 450 production of ammonium ions which are readily nitrified into nitrate, and the excessive proton 451 produced by the nitrification can compete for the adsorption sites with  $Ca^{2+}$  and  $Mg^{2+}$  (Wang et 452 al., 2017a). As a result, these cations were easy to lose from soil in the manner of leaching. 453 Except at the 40-60 cm soil depth, continuous planting of tea led to the significant increases in 454 soil Fe<sup>2+</sup> and Mn<sup>2+</sup> contents (Figures 7 and 8), which were elevated by 7%-27% and 3%-9% from 455 8 to 43 years of tea planting, separately. This phenomenon was possibly caused by the soil 456 acidification (Table 1), which stimulates the release of soil Fe<sup>2+</sup> and Mn<sup>2+</sup> by mineralization and 457 desorption from soil OM and minerals (Wang et al., 2017a). Tea, as an aluminium (Al) 458 cumulating crop, is able to cumulate Al in leaves (Li et al., 2016). Soil acidification in the 459 tea-planting course was due to the substantial tea litter into the soil annually via trimmed 460 branches and leaves (Li et al., 2016). At the same time, the rhizosphere deposition of massive 461 organic acids (i.e., malate, lemon acid, and oxalate acid) around the tea roots could provoke 462 463 localized acidification (Xue et al., 2006). In addition, for increasing the output of tea, tea plantations needed to apply N fertilizers (i.e., urea and NH4+-N), thus leading to soil acidification 464 465 by the  $NH_4^+$  nitration (Yang et al., 2018).

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

## 472 4.5. Stoichiometric ratios of soil alkali cations and micronutrients

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

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

### 495 **5. Conclusions**

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

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.
- 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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Soil depth	Tea plantation age	Bulk density (g cm <sup>-3</sup> )	Clay (%)	pH
0-10 cm	8 years	$1.28\pm0.02~b$	34.69 ± 3.21 a	$4.57\pm0.02~a$
	17 years	$1.20\pm0.02~\text{c}$	$35.91 \pm 2.77$ a	$4.49\pm0.01\ ab$
	25 years	$1.26\pm0.01\ bc$	$33.12 \pm 2.46 \text{ a}$	$4.31\pm0.03\ b$
	43 years	$1.31\pm0.04\ b$	$35.08 \pm 2.41$ a	$4.15\pm0.02\;c$
10-20 cm	8 years	$1.30\pm0.03\ b$	$34.88\pm2.08\ a$	$4.55\pm0.03\ a$
	17 years	$1.22\pm0.03~\text{c}$	$32.59\pm3.02~a$	$4.50\pm0.01\ a$
	25 years	$1.30\pm0.03\ b$	$34.92 \pm 3.67 \ a$	$4.33\pm0.02\ b$
	43 years	$1.29\pm0.02\;b$	$32.35 \pm 2.68$ a	$4.17\pm0.02\;c$
20-40 cm	8 years	$1.32\pm0.04\ ab$	$35.26 \pm 1.45$ a	$4.60\pm0.04\ a$
	17 years	$1.31\pm0.01\ b$	$34.57 \pm 4.12$ a	$4.53\pm0.02\ a$
	25 years	$1.34\pm0.01\ ab$	34.51 ± 3.21 a	$4.34\pm0.04\ b$
	43 years	$1.33\pm0.04\ ab$	$34.29\pm3.54~a$	$4.19\pm0.03\ c$
40-60 cm	8 years	$1.36\pm0.01\ a$	$34.78 \pm 3.66 \ a$	$4.58\pm0.02\;a$
	17 years	$1.37\pm0.02\ a$	$36.89 \pm 2.98 \text{ a}$	$4.54\pm0.03\ a$
	25 years	$1.39\pm0.02\ a$	$33.68 \pm 1.91 \text{ a}$	$4.32\pm0.01\ b$
	43 years	$1.38 \pm 0.03$ a	35.81 ± 3.69 a	$4.21\pm0.01~bc$

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

Data represent the mean of 5 replicates  $\pm$  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.

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 Т  $\mathbf{S}\times\mathbf{T}$ S Т  $\mathbf{S}\times \mathbf{T}$ А  $\mathbf{S}\times\mathbf{A}$  $T \times A$  $S \times T \times A$  $\sqrt{}$  $\sqrt{}$ Bulk density  $\sqrt{}$ Clay × × Х  $\sqrt{}$ pН × Х  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ MWD  $\sqrt{}$  $\sqrt{\sqrt{}}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ Aggregate proportion  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$ Organic C  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$  $\sqrt{\sqrt{}}$ Total N  $\sqrt{1}$  $\sqrt{}$ Total P  $\sqrt{}$ × × × × х × х х  $\sqrt{}$ Exchangeable Ca<sup>2+</sup>  $\sqrt{\sqrt{}}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{\sqrt{}}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ Exchangeable Mg<sup>2+</sup>  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ Available Fe<sup>2+</sup>  $\sqrt{}$  $\sqrt{\sqrt{}}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{\sqrt{}}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ Available Mn2+  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ C/N ratio  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ × × × × × ×  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$  $\sqrt{}$ C/P ratio  $\sqrt{}$  $\sqrt{}$ N/P ratio

**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.

S: soil depth; T: tea plantation age; A: aggregate size.  $\sqrt{\sqrt{}}$ ,  $\sqrt{}$ , and  $\times$  indicate significant differences at P < 0.01, P < 0.05, and P > 0.05, respectively.

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Ca/Mg ratio

Fe/Mn ratio

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Soil	Tea plantation	MWD	Aggregate composition (%)			
depth	age	(mm)	> 2 mm	1-2 mm	0.25-1 mm	< 0.25 mm
0-10 cm	8 years	$1.88\pm0.03~b$	$44.26\pm3.24~bA$	$16.23 \pm 2.45 \text{ abC}$	$8.46 \pm 1.37 \; abD$	$31.05\pm5.78~bcB$
	17 years	$2.20\pm0.04\;a$	$53.18\pm2.78\;aA$	$18.02\pm1.63\ aB$	$6.69\pm0.98\ bC$	$22.11\pm4.01\ cB$
	25 years	$1.78\pm0.01\;b$	$40.29\pm4.01\;bA$	$17.97\pm2.03\ aC$	$8.81\pm0.88\ abD$	$32.93\pm3.58\ bcB$
	43 years	$1.53\pm0.03\ c$	$32.60\pm3.61~\text{cB}$	$19.61\pm2.04\ aC$	$7.64 \pm 1.57 \ bD$	$40.15\pm4.27\ abA$
10-20 cm	8 years	$1.62\pm0.02\ c$	$37.31\pm2.47~\text{cA}$	$13.58\pm1.56\ bB$	$9.24\pm2.04\ abC$	$39.87\pm2.69\ abA$
	17 years	$1.82\pm0.04\ b$	$43.02\pm2.69\ bA$	$14.31\pm1.38\ abC$	$8.87 \pm 1.14 \; abD$	$33.80\pm4.58\ bB$
	25 years	$1.56\pm0.03\ c$	$34.87 \pm 1.45 \ cB$	$15.03\pm2.47\ abC$	$9.36\pm1.09\ abD$	$40.74\pm3.94\ abA$
	43 years	$1.34\pm0.02\;d$	$29.24\pm3.28\ dB$	$13.97\pm1.65\ bC$	$7.28\pm0.82\ bD$	$49.51\pm2.56\;aA$
20-40 cm	8 years	$1.43\pm0.01\;cd$	$31.25\pm1.68\ cdB$	$15.47\pm2.49\ abC$	$7.62\pm0.47~bD$	$45.66\pm4.77~aA$
	17 years	$1.48\pm0.03\ cd$	$32.08\pm3.60\ cdB$	$16.89\pm2.51\ abC$	$8.91\pm2.14\ abD$	$42.12\pm2.05\ abA$
	25 years	$1.39\pm0.02\;d$	$30.72\pm3.25\;dB$	$14.23\pm0.58\ abC$	$6.81\pm1.36~bD$	$48.24\pm3.59\;aA$
	43 years	$1.48\pm0.03\ \text{cd}$	$32.49\pm2.98\ cdB$	$15.40\pm2.11\ abC$	$9.05\pm0.91\ abD$	$43.06\pm4.32~aA$
40-60 cm	8 years	$1.30\pm0.01\ d$	$28.48\pm2.57\;dB$	$12.02\pm3.08\ bC$	$10.05\pm0.58\ aC$	$49.45\pm3.68\ aA$
	17 years	$1.36\pm0.02\ d$	$29.68\pm2.61\ dB$	$13.78\pm1.14\ bC$	$9.47 \pm 1.03 \; abC$	$47.07\pm3.47~aA$
	25 years	$1.36\pm0.01~\text{d}$	$30.09\pm1.47\;dB$	$11.98\pm0.98\ bC$	$10.64\pm0.45~aC$	$47.29\pm4.01\;aA$
	43 years	$1.34\pm0.03\ d$	$28.42\pm3.02\;dB$	$14.33 \pm 1.57 \; abC$	$12.45\pm2.13~aC$	$44.80\pm2.99\;aA$

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

Data represent the mean of 5 replicates  $\pm$  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  $Ca^{2+}$  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 Mg<sup>2+</sup> 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  $Fe^{2+}$  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  $Mn^{2+}$  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 5



Figure 6



Figure 7

