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Soil C:N:P stoichiometry in tropical forests on Hainan Island of China: Spatial and vertical variations

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Abstract

Soil carbon (C), nitrogen (N), and phosphorus (P) are three important elements. The study of stoichiometric relationships of soil C, N, and P in tropical forests on Hainan Island, China could improve our understanding of nutrient cycling and provide valuable information for forest management. Soil samples were collected at five different depths from 0-100 cm at 100 sites among four different forest types on Hainan Island, and total C, N, and P concentrations were measured. Soil C and N concentrations and soil C:P and N:P ratios declined from the surface soil layer to the deeper soil layers and soil P and C:N ratio had relatively small variations among different depths, due to that soil C and N were mostly controlled by biological processes such as photosynthesis and N_2-fixation, while P was more influenced by bedrock. Large spatial variations were found for soil C, N, P concentrations and their ratios. Soil C and N concentrations were significantly influenced by longitude and vegetation cover, while soil P concentration and C:P and N:P ratios were significantly controlled by latitude. This study produced a comprehensive data set of soil C, N, and P stoichiometry, and their variation patterns and controls in the tropical forests. The information generated here could help improve ecosystem models for better understanding of forest element stoichiometry, ecosystem productivity, and plant-environment relationships.

Keywords C:N:P stoichiometry; Nutrient limitation; Soil depth; Tropical forests

1. Introduction

Carbon (C), nitrogen (N) and phosphorus (P) are three fundamental elements of plants and ecosystems. Carbon is a basic structural element that constitutes about half of plant dry biomass (Mooney 1972). Nitrogen is an important component of enzymes and chlorophyll (Olson et al. 1982; Santiago 2015). Phosphorous is a key component of nucleic acid, phospholipids, ATP, and NADP (Elser et al. 2007; Deng et al. 2015). While the source of C for plant growth is from the atmosphere through photosynthesis, the uptakes of P primarily come from bedrock. Sources of N mainly come from N_2-fixation and soil mineral N decomposed from litter, with more N from the atmosphere in the tropical forests. Soil C, N, and P and ratios in terrestrial ecosystems have been central to our understanding of plant physiology and growth, C sequestration, nutrient cycles, and nutrient limitations to ecosystem productivity (McGroddy et al. 2004; Aponte et al. 2010; Hui and Luo 2014; Deng et al. 2015; Xu et al. 2015; Bing et al. 2016). Quantifying the patterns and detecting the controls of the soil C, N, P stoichiometry in different ecosystems has become an important task.

The C:N ratio in soil or litter has long been recognized as a quality indicator of organic matter (Swift et al. 1979; Batjes 1996; Zhang et al. 2008; Ostrowska and Porębska 2015). For example, Batjes (1996) found that different soil types may have different C decomposition rates and reported that mean soil C:N ratio range from 9.9

for Yermosols to 25.8 for Histosols. The C:P ratio is another useful quality indicator of organic matter and its decomposition rate (Paul et al. 2007). The ratio of N:P is related to nutrient constraints in ecosystems (Gusewell and Gessner 2009; Peňuelas et al. 2012; Bui and Henderson 2013). These ratios have been built into processed-based ecosystem models to regulate nutrient limitations on ecosystem C dynamics and to predict ecosystem C sequestration in a changing environment (Parton et al. 1988; Deng et al. 2015).

While soil often exhibits a higher degree of stoichiometric homeostasis in terms of the major nutrients (i.e., C, N, and P), previous studies have shown that many factors may influence soil C:N, C:P and N:P ratios, such as management practices (e.g., fertilization), disturbances (e.g., land use change and fire), climate, topography, and biotic factors (e.g., plant type) (McGroddy et al. 2004; Cleveland and Liptzin 2007; Bui and Henderson 2013; Bing et al. 2015; Yuan et al. 2017; Tang et al. 2018a). For example, Li et al. (2012) evaluated the effect of land use change on soil C:N:P ratios in subtropical China and found that land use plays an important role in influencing soil stoichiometry. A large-scale study on the C:P and N:P ratios in Chinese soils found that climate, soil order, soil depth, and weathering stage all regulate their variations (Tian et al. 2010). Soil N and P concentrations vary dramatically across different vegetation types and ages. Soil N tends to be poor in temperate forests, but rich in tropical forests. In contrast, P is often considered as a limiting factor for plant productivity in tropical forests (Vitousek and Farrington 1997; Hedin et al. 2003). Plants in different forests may have different nutrient use efficiencies and different adaptations to the local growth conditions. As a result, soil N and P concentrations could be influenced.

Variations of soil C, N, and P concentrations in terrestrial ecosystems and the mechanisms influencing soil C:N:P stoichiometry at different spatial scales have been investigated in recent years (Aponte et al. 2010; Kirby et al. 2011; Li et al. 2012; Mooshammer et al. 2012; Beermann et al. 2015; Bing et al. 2016). For examples, Aponte et al. (2010) investigated the stoichiometry of C, N, and P in the soil of Mediterranean forests and found that season, vegetation type, and soil depth regulate C:N:P stoichiometry (Bui and Henderson 2013). Compared to C:N ratio, the variations of C:P and N:P ratios are larger. Fan et al. (2015) studied plant and soil C:N:P stoichiometry in subtropical plantations in Fujian, China and found that soil C and P decrease with the age of Eucalyptus trees, and plant N:P ratio is strongly related to soil N:P ratio. But up today, the study on the stoichiometry of soil C, N, and P in tropical forests such as those on Hainan Island is still relatively limited (Kirkby et al. 2011; Li et al. 2012; Yu et al. 2018).

Tropical forests only occupy 6% of land area in the world but contain about 40% of the stored C in the terrestrial biosphere (Ashton et al. 2012; Ren et al. 2014). Hainan is the largest tropical island in China. It serves as an ideal place for tropical soil C, N, and P study for two reasons: 1) High temperature and precipitation in this region result in fast biogeochemical cycles of C, N, and P, high rate of organic matter decomposition, and high primary productivity (Conant et al. 2011); and 2) Many different forest types and soil types exist on the island (Ren et al. 2014). The influences of vegetation type and soil type on soil C:N:P stoichiometry could be investigated. Revealing the patterns and mechanisms of soil C, N, and P stoichiometry in the tropical forests on Hainan Island could improve our understanding and prediction of the biogeochemical cycling in tropical forests (Zechmeister-Boltenstern et al. 2015).

In this study, we investigated soil C, N, P concentrations and their ratios from 100 sites in the tropical forests on Hainan Island, China. The primary goal of this study was to examine the spatial and vertical variations of stoichiometric relationships of soil C, N, and P concentrations and their influencing factors. We hypothesized that: 1) Soil C, N, P concentrations and their ratios would be influenced by habitat factors, as latitude, longitude, and elevation could influence climatic factors, plant nutrient uptakes and growth, and litter decomposition, and further soil C, N, and P; 2) Vegetation variables such as vegetation cover and tree growth would have different impacts on nutrient uptakes and litter decomposition, and soil C, N, P concentrations and their ratios. Soil C would increase with vegetation cover and growth, but soil N and P concentrations could be decreased with these factors. The specific objectives were 1) to quantify the spatial and vertical variation of soil C, N, P concentrations, and the ratios of C:N, C:P, and N:P across forest sites on Hainan Island; 2) to detect whether and how soil C, N, P concentrations and their ratios vary with habitat (i.e., latitude, longitude, and elevation), environmental factors (i.e., temperature and precipitation) and vegetation (i.e., vegetation cover and tree height) variables.

2. Materials and methods

2.1. Site description

Hainan Island is located at the northern edge of the tropics (latitude 18°10'-20°10'N, longitude 108°37'-111°03'E) with a land area of 33920 km^2 (Ren et al. 2014). The climate in the region is tropical monsoon climate. There are distinct dry and wet seasons, with average annual rainfall of 1500-2500 mm and average annual temperature of 22-26°C. Soil is mainly laterite. The main forest types are tropical rain forest, with more than 4200 plant species including about 2000 tropical species (Zhou 1995).

2.2. Experimental design and site selection

We used a stratified sampling approach for soil collections based on vegetation classification, forest area, and tree age on Hainan Island (Ren et al. 2014). Vegetation classification was based on remote sensing and image processing (Ren et al. 2014). Six major vegetation types are distributed on the island including tropical natural rain forest, *Eucalyptus* plantation, rubber plantation, *Casuarina* plantation, coniferous plantation, and orchard. Based on forest type, spatial distribution, forest area, stand volume, and age class, 100 field sampling plots on the island were established in 2012 (Fig. S1). Those samples represented 91% of vegetation types on the island. The number of plots for each forest type was as follows: 50 for natural forest (mostly tropical rain forest), 8 for *Eucalyptus* plantation, 24 for rubber plantation, 2 for *Casuarina* plantation, 3 for *Acacia* plantation, 3 for *Pinus* plantation, 1 for mixed coniferous and broad-leaved species forest, and 9 for orchard (including 3 for mango orchard, 3 for betel nut orchard, 2 for lychee orchard, and 1 for longan orchard).

There were three replicate quadrats in each plot. The area per quadrat was 3600 m^2 for natural forest, 800 m^2 for plantation, and 400 m^2 for orchard. At each sampling site, plot specific data were collected in 2012, including tree information, management practices, and plot properties, such as plot number, latitude, longitude, topography, soil type, vegetation type, name of dominant species, successional stage (young, medium, and mature forests for both planation and natural forests), management practices (i.e., fertilization, grazing, thinning, fire, and others), human interference (no, medium, and severe), vegetation cover, age of trees, and height of trees. Topography included mountain, hill, and plain. Mountain is a geographic feature rising higher than 500 m, and often includes steep slopes and a defined summit or peak (Zhang et al. 2015). Plain is a flat landmass that generally does not change much in elevation, and the elevation is less than 200 m. Hill has a lower elevation than a mountain, usually higher than 200 m but lower than 500 m, and has a rounded top with no well-defined summit. Soil type included Latosolic red soil, Red soil, Mountain yellow soil, Latosols red soil, Yellow soil, Sandy loam soil, Yellow sandy soil, Red sandy soil, Podzol soil, and Sandy soil (Liang 1988). The corresponding soil orders in soil taxonomy for Latosolic red soil included Inceptosols, Oxisols, and Ultisols; for Red soils included Inceptisols and Ultisols; and for Yellow soil included Inceptisols and Ultisols. Forest type was regrouped into tropical rain forest, evergreen broadleaf forest, tropical conifers forest, and evergreen deciduous broadleaf mixed forest. For management practices, if fertilization was applied, we labeled fertilization. Fertilization rate or type of fertilization were not separated for these sites. Human interference was ranked based on the influences of human management practices on forest ecosystems. Mean annual temperature and total precipitation at each site were collected from the nearest meteorological stations using the geographical coordinates (National Meteorological Information Center, 2020).

2.3 Soil sampling and soil C, N, P measurements

For determination of C, N, and P in the forest soil, we collected three soil cores 100 cm deep for each of the three quadrats with a soil auger (4 cm diameter) in 2014. We separated into five depths (0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, and 50-100 cm). For soil bulk density measurement, soil was collected for every 1 m soil profile at each sampling plot using a soil auger. Soil at the five soil depths along two diagonal lines was collected and brought to the laboratory for measurement (Tang et al. 2018a, 2018b).

The soil samples were processed by the potassium dichromate oxidation method for determination of soil C concentration (% of dry mass) (Liu et al. 1996). Total N concentration was measured using the micro-Kjeldahl method (Bremner 1996). Total P concentration was quantified using the ammonium molybdate method after persulfate oxidation with soil samples digested with $HClO_4$-H_2SO_4 mixture (Kuo 1996).

2.4 Data analysis

One-way ANOVA was conducted to identify the significant differences in soil C, N, and P, and their ratios of the whole 0-100 cm soil profile and among different soil depths caused by topography, soil type, forest type, successional stage, and human interference. Logarithm transformation was performed on data before ANOVA when soil C, N, P concentrations and their ratios were not normally distributed. Kolmogorov-Smirnov test was conducted for normality test. Least significant difference (LSD) method was used for multiple comparison among means when a significant effect was detected. Results presented in multiple comparisons were back-transformed. To test whether the concentrations and ratios of soil C, N, and P were influenced by habitat variables (latitude, longitude, and elevation), vegetation variables (vegetation cover, tree height, and age), temperature, and precipitation, scatter plots were constructed and linear, power function, or quadratic regression analyses were developed. Multiple regression was further conducted to develop the optimal regression models of soil C, N, P concentrations and their ratios with habitat and vegetation variables for the while soil profile and different soil depths. Since simple regression showed quadratic relationships with latitude, $latitude^2$ was included in the multiple regression model. Stepwise method was used for variable selection with $p<0.10$ for variable to be entered into the model and $p<0.05$ for variable to remain in the model. All statistical analysis in this study was performed using the SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA; Hui and Jiang 1996).

3. Results

3.1 Distributions, means, and variations of soil C, N, P concentrations and their ratios at different depths in tropical forests on Hainan Island

Soil C concentration varied greatly from 1.17 to 69.81 mg g^{-1} from different depths across all sampling sites (Fig. 1a; Table 1). Soil C concentration followed a normal distribution (Table S1). For the 0-10 cm depth, soil C concentration showed a distribution with the highest frequency appeared around 30 mg g^{-1} with a mean soil C concentration of 23.87 mg g^{-1}. Moving towards deeper soil depths, the distribution shifted towards the lower concentration. For example, the mean value of soil C concentration for the 50-100 cm depth was only 7.29 mg g^{-1}. Soil N and P concentrations did not follow normal distribution. Soil N concentration varied largely from 0.18 to 3.92 mg g^{-1} across all sites. Compared to soil C concentration, the distribution of soil N concentration was less skewed towards left (Fig. 1b). Mean soil N concentration decreased from 1.65 mg g^{-1} at the 0-10 cm depth to 0.60 mg g^{-1} at the 50-100 depth. The distribution of soil P concentration was similar to soil N concentration, but the concentration was much smaller, ranging from 0.07 to 1.69 mg g^{-1} for all depths and the relative variation (CV) was larger (Fig. 1c; Table 1). The mean soil P concentration was 0.41 mg g^{-1} at the 0-10 cm depth and decreased to 0.29 mg g^{-1} at the 50-100 cm depth.

Soil C:N ratio was mostly normally distributed, with the most sites having a value of 15 and a range from 2.07 to 80.75 with majority of values falling between 10 and 20 (Fig. 1d). The mean values at different depths did not change significantly with an overall mean of 15.43 (range of 14.29 to 16.28). The distribution of soil C:P ratio was slightly left-skewed, with a range from 5.94 to 223.24 for all depths (Fig. 1e). The mean value of soil N concentration declined from 79.73 mg g^{-1} at 0-10 depth to 37.46 mg g^{-1} at the 50-100 cm depth (Table 1). Soil N:P ratio showed a similar distribution pattern with soil C:P, with a range of 0.30 to 13.83 (Fig. 1f). The mean value of soil N:P ratio declined from 5.41 at 0-10 cm depth to 2.97 at the 50-100 cm depth (Table 1).

3.2 Influences of forest type, soil type and other variables on soil C, N, P concentrations and their ratios in tropical forests on Hainan Island

Soil C concentration at the whole soil profile (0-100 cm depth) was significantly influenced by the topography, soil type, forest type, successional stage, management practice, and human interference (Table 2). Soil N concentration was only influenced by soil type while soil P concentration was only influenced by human interference. Similar results were found for soil layers from 0-10 cm to 50-100 cm (Table S2). Management practice significantly influenced soil P concentration in the top 0-10 cm soil layer. For soil C:N ratio in the whole soil profile, topography, soil type and management practice had significant influences. All factors significantly influenced soil C:P ratio except soil type. Only forest type and human influence had significant effects on soil N:P ratio (Table 2). For the top 0-10 cm soil, all factors investigated here significantly influenced soil C:N and C:P ratios, but not soil N:P ratio (Table S2). MANOVA also showed that soil C, N, P concentrations and their ratios, as a whole, were significantly influenced by the topography, soil type, forest type, successional stage, management practice, and human interference for the complete soil profile and different soil layers (Table S3).

For different soil types, mountain yellow sandy, red sandy and podzol soils had higher soil C concentration than sandy loam and sandy soils (Fig. 2). No significant differences in soil C concentration were found among other soil types. Sandy loam soil had higher soil N concentration but lower C:N and C:P ratios. Sandy soil also had lower soil C:N ratio compared to some other soil types (Fig. 2). Regarding the soil topography, sites in the mountain area had significantly higher soil C concentration, soil C:N ratio and soil C:P ratio than sites in the plain area (Fig. S2).

Forest type had significant influences on soil C concentration, soil C:N, C:P, and N:P ratios (Fig. 3). Soil C concentration and soil C:N ratio in the tropical rainforest were significantly higher than that in the tropical coniferous forest, but insignificantly differed from other two forest types (Fig. 3). Soil C:P ratio was the lowest in the evergreen broad-leaved forest. Soil N:P ratio was higher in the tropical coniferous forest than other three forests.

Successional stage significantly influenced soil C, P concentrations and soil C:N, C:P, and N:P ratios (Table 2; Fig. 4). Soil C concentration was the highest in the middle mature forest, and the lowest in the middle plantation. Soil P concentration was higher in the young and old plantations than young natural forest. Soil C:N ratio did not change much among different successional stages, but soil C:P and N:P ratios were significantly higher in the young natural forest than others. Management practices significantly influenced soil C concentration, and soil C:N and C:P ratios

(Fig. S3). Grazing had the lowest soil C, N, and P concentrations, and fire tended to increase soil C concentration and soil C:N ratio. No disturbance had higher soil P concentration, and lower soil C:P and N:P ratios. Human interference had significant impacts on soil C and P concentrations, and soil C:N, C:P, and C:P ratios (Fig. S4). Non-disturbed soils had the highest soil C concentration, C:N and C:P ratios, while the medium disturbed soils had higher soil P concentration and lower C:N and C:P ratios (Fig. S4).

3.3. Relationships of soil C:N, C:P, and N:P ratios with soil C, N, and P concentrations across sampling sites

Across all sites, soil C:N ratio had a strong significant relationship with soil C concentration than soil N concentration, but had no significant relationship with soil P concentration (Fig. S5a, d, g). Soil C:P ratio showed a significant power functional relationship with soil P concentration, and a linear relationship with soil C concentration (Fig. S5b, e, h). Soil N:P ratio, like soil C:P ratio, showed a significant power functional relationship with soil P concentration, and a weak yet significant linear relationship with soil N concentration (Fig. S5c, f, i).

3.4. Relationships of soil C, N, P concentrations and soil C:N, C:P, and N:P ratios with habitat and vegetation variables

For simple regression, soil C concentration was significantly influenced by latitude, elevation, and vegetation cover (Fig. 5). Longitude and vegetation height had no influences on soil C, N, P and their stoichiometry. Soil C concentration had a quadratic relationship with latitude, initially increased with latitude, reached the highest value and declined with latitude (Fig. 5a). Soil C concentration increased linearly with elevation and vegetation cover. Soil N concentration was not correlated with habitat and vegetation variables while soil P concentration only increased with latitude (Fig. 5). Soil C:N ratio was significantly influenced by latitude, elevation, and vegetation cover, similarly to soil C concentration (Fig. 6). Soil C:P ratio was also influenced by the latitude, elevation, and vegetation cover, but the relationships with latitude and elevation were a quadratic relationship. Soil N:P ratio was significantly influenced by latitude and elevation.

Multiple regression showed that soil C and N concentrations were regulated by both habitat variables (longitude and elevation) and vegetation variable (vegetation cover) for the whole soil profile, but soil P concentration was only related to habitat variable (latitude). Soil C:N ratio was regulated by latitude, vegetation cover, and precipitation while soil C:P and N:P ratios were only regulated by latitude. In the top 0-10 cm soil layer, soil C concentration was significantly influenced by vegetation cover, soil N concentration was only regulated by longitude, and soil P concentration was influenced by latitude (Table S2). Soil C:N and C:P ratios were related to elevation, vegetation cover, and precipitation, and soil N:P ratio was only influenced by latitude. Soil C, N, and P concentrations in the deep soils were mostly regulated by habitat factors such elevation and latitude.

4. Discussion

By measuring soil C, N, and P concentrations from 100 sites in forests on Hainan Island, China, we quantified the spatial and vertical variations of soil C, N, P concentrations and their stoichiometric ratios. Our results showed that mean soil C:N:P ratio decreased from surface soil to deep soil as expected, and variations of soil C and N concentrations, soil C:P ratio, and N:P ratio were larger than those of soil P concentration and C:N ratio. Soil C concentration, C:N ratio, and C:P ratio varied among topographies, soil types, forest types, successional stages, and human interferences. While soil C and N concentrations were regulated by habitat (longitude and elevation) and vegetation (vegetation cover) variables, soil P was only regulated by habitat variable (i.e., latitude). These findings broadened our understanding of the biogeochemical cycling of soil C, N, and P in tropical forests and provided a comprehensive dataset for parameterization and validation of biogeochemical models in the region (Wang et al. 2011).

4.1. Variations and causes of soil C, N, P concentrations and their ratios in tropical forests on Hainan Island

Spatial distributions and variations of soil C, N, P concentrations and their stoichiometric ratios have not come to a definitive conclusion, but a decline of soil C:N:P ratio from surface to the deep layers has been often reported. Our results showed similar results of declining C:N:P ratios with soil depth, which are consistent to some previous reports such as Bing et al. (2015) that reported C:N:P ratio varies among different depths from 343:16:1 in the A horizon to 63:3:1 in the C soil layer.

Fanin et al. (2015) also found that soil C:N:P ratio is 151:10:1 in an undisturbed Amazonian rainforest. The lower C:N:P ratio in our study might be due to the fact that, on the Hainan Island, annual precipitation was high (~2500 mm) which modulated the nutrient availability and leaching of nitrogen. In addition, species diversity on the island was relatively high. More nutrients would be used by plants and soil nutrients could be reduced, resulting in a low C:N:P ratio (Long et al. 2012).

While Cleveland and Liptzin (2007) reported a constant stoichiometric ratios of soil C, N, and P (212:15:1) in mostly the surface soils across a wide range of global forest soils, some recent studies showed great variations among different ecosystems. Our results showed that C:N, C:P, N:P ratios varied dramatically across the sites (2.1 to 80.8, 5.9 to 223.2, and 0.3 to 13.8, respectively). Xu et al. (2013) found that soil C:N:P ratio varied from 64:5:1 to 1347:72:1 with an average of 287:17:1 using a global data set of 3422 measurements. For Chinese soils, Tian et al. (2010) reported a ratio of 60:5:1, and Li et al. (2012) reported 80:7.9:1 for top soils (0-20 cm) in subtropical China. Our results were within the ranges of these reported values.

The spatial heterogeneity in soil C, N, P distributions and their ratios may be caused by many factors such as habitat (latitude, longitude or elevation), soil types, topography, and plant productivity (height, biomass) (McGroddy et al. 2004). On Hainan Island, there was a decreasing trend of precipitation concertation from west to east, based on precipitation datasets from 1967 to 2012 (Chen et al. 2015). The highest precipitation occurred in the interior of the island where latitude was also higher (Li et al. 2015). High precipitation might stimulate plant growth and C inputs into the soil. As a result, we observed that soil C concentration showed a quadratic response to latitude (Fig. 5). In a comprehensive study, Bing et al (2016) showed that the ratios of C:P, C:N and N:P varied in different ecosystems. Soils in alpine ecosystems have much higher C:P in the O and A horizons, and N:P ratio is comparable with global forest soils and grassland soils. They attribute the difference to the complex conditions in alpine ecosystems, which are currently experiencing strong climatic warming, more precipitation, and anthropogenic impacts (Bing et al. 2016). In this study, the tropical rainforest had higher C:N and C:P ratio, compared to C:N ratio for the tropical coniferous forest and C:P ratio for the evergreen broad-leaved forest. The highest N:P ratio appeared in the tropical coniferous forest where relatedly low P was observed. The lower P concentration was also reported in broad-leaved forest, broadleaf-coniferous forest, and coniferous forest soils by Bing et al. (2016).

As plants will take up nutrients from the soil and return the nutrients back to soil through litterfall, conduct photosynthesis and possible nitrogen fixation, different plants will influence changes of C, N, and P in soils (McGroddy et al. 2004; Bing et al. 2015). It has been shown that the savanna and grassland ecosystems have relatively consistent stoichiometry, but rainforests and tall open eucalypt forests have variable C:N:P ratios (Bing et al. 2016). Agreeing with the findings from previous studies (e.g., Hedin 2004) and partially supporting our hypothesis one, our data showed that soil P tended to increase and N:P ratio tended to decrease with latitude. Elevation seemed to have more influences on soil C, N, and P concentrations and their ratios. He et al. (2016) studied soil nutrient stoichiometry in mountain areas of subtropical China and found that soil C and N concentrations increased linearly with elevation, which was similar to our results. Soil P concentration was not significantly related to elevation but showed a similar trend of quadratic response revealed in He et al. (2016). Similar response patterns for soil C:N, C:P, and N:P ratios were found between our study and He et al. (2016). Soil C concentration linearly increased with vegetation cover, partially supporting our hypothesis two. Soil N concentration was also significantly regulated by habitat variable (longitude) and vegetation variable (vegetation cover). Furthermore, soil N concentration increased with vegetation cover, perhaps due to increased litterfall and decomposition, and nutrients returning to the soil. As soil C concentration increased more with vegetation cover, soil C:N ratio was increased with vegetation cover due to enhanced C input to soil. Soil C:P and N:P ratios were only regulated by habitat variable.

4.2. Implication for nutrient limitation in tropical forests of southern China

Soil C:N, C:P and N:P ratios could be indicators of soil quality and limitation of certain nutrients in soils in terrestrial ecosystems (Tian et al. 2010; Izquierdo et al. 2013). In tropical forests, the role of soil nutrients, especially P, in the distribution and growth of tropic vegetation have been a controversial issue over years (Tanner et al. 1998; Cleveland et al. 2002; Feller et al. 2003; Vitousek et al. 2010; Townsend et al. 2011; Bing et al. 2015). Soil P, particularly the ratio of N:P, may be a key variable associated with the delimitation between rainforest and open eucalypt forests. Our results showed that soil N:P ratio decreased with soil depth, and coniferous and young forests had higher soil N:P ratio. The N:P ratio was 5.4 in the top soil (0-10 cm), suggesting that N might be a limiting nutrient for ecosystems and could influence plant N:P ratio (Tessier and Raynal 2003; Bui and Henderson 2013; Fan et al. 2015). For soil C:P ratio, high (>300) C:P ratio indicates net immobilization of nutrients. Based on the above criteria, vegetation in the tropical forest on Hainan Island was mostly limited by the soil N (He et al. 2016), not P. Previous studies in the tropical forests mostly show that nutrient deficiency can eventually limit net primary production (Schuur and Maston 2001; Wardle et al. 2004; Silk et al. 2013). In Bornean tropical forests, Fujii (2014) found that pH, more than P, might be a key factor influencing vegetation distribution. Soil pH was low on Hainan Island, mostly due to high precipitation and N deposition (about 2.5-4.0 g N m^{-2} y^{-1}) in southern China, particularly surrounding urban areas (Du et al. 2015; Li et al. 2017; Tian et al. 2018). Reducing air pollutions and adequate fertilization to plantation forests are needed to improve forest productivity on the island.

It is worth noting that while we found that soil C, N, P concentrations and their rations were significantly regulated by certain habitat and vegetation variables, their variations could be explained by these variables were mostly very low (Table 3). This indicated that some other variables, such as geologic parent materials and geological factors such as soil age, and soil erosion could significantly influence soil elements and their stoichiometry (Torn et al. 1997; Hugget 1998; Porder and Chadwick 2009). For example, soil P content can be strongly influenced by soil age and weathering intensity of the parent material. Further studies should also consider these variables.

5. Conclusion

Soil C and N concentrations and C:P and N:P ratios in tropical forests on Hainan Island exhibited large vertical heterogeneity, but the vertical variations of soil P concentration and C:N ratio among different depths were relatively small. These vertical variations were caused by biological controls and physical limitations. Soil C and N were mostly controlled by biological processes such as photosynthesis and N_2-fixation, while P was more influenced by bedrock. Spatially, variations of soil C and N concentrations were larger than those of soil P concentration. Latitude, vegetation type, soil type, and altitude played certain roles in the soil element stoichiometry. Our study has provided at least two new insights into soil stoichiometry. 1) Although the soil C and P concentrations and stoichiometry showed no clear geographic patterns along latitude and longitude, they exhibited distinct patterns along altitude. Perhaps this result is reflecting a relationship between plant stoichiometry and vegetation types across different altitudes. 2) Topography, soil type, forest type and management practice seemed to have more profound effects on soil C concentration than on soil N and P concentrations. Soil element stoichiometry is influenced more by the environmental factors than vegetation cover and tree height. Our results provide useful information for the stoichiometry of soil C, N, and P in tropical forests. Furthermore, this study provides additional benefits to modeling in tropical forests and for better management of forests in the region.

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Compliance with Ethical Standards

The authors declare that they have no conflict of interest.

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Table 1. The concentrations of total C, N, P and the ratio of C:N, C:P, and N:P of soil at different depths in tropical forests on Hainan Island, China

		Total C (mg g^{-1})	Total N (mg g^{-1})	Total P (mg g^{-1})	C:N ratio	C:P ratio	N:P ratio
0-10 cm[a]	Mean	23.87	1.65	0.41	16.28	79.73	5.41
	SE[b]	1.07	0.06	0.03	0.70	4.93	0.31
	CV[c]	44.74	37.87	60.47	42.68	61.54	57.60
10-20 cm	Mean	16.89	1.27	0.39	14.89	64.24	4.79
	SE	0.74	0.05	0.03	0.67	4.18	0.27
	CV	44.02	39.14	72.10	44.71	64.70	56.00
20-30 cm	Mean	12.37	1.00	0.36	14.82	49.40	3.91
	SE	0.51	0.04	0.03	0.88	3.17	0.23
	CV	41.33	38.48	71.04	58.81	63.82	57.29
30-50 cm	Mean	9.70	0.82	0.35	14.29	42.36	3.49
	SE	0.40	0.04	0.03	0.69	2.84	0.21
	CV	41.31	43.20	84.42	48.19	66.71	60.63
50-100 cm	Mean	7.29	0.60	0.29	15.94	37.46	2.97
	SE	0.33	0.03	0.02	1.18	2.81	0.18
	CV	45.50	49.22	75.10	73.51	74.67	60.30
0-100 cm	Mean	10.90	0.86	0.33	15.43	46.54	3.59
	SE	0.41	0.03	0.02	0.78	2.85	0.19
	CV	37.48	37.79	70.77	50.04	60.99	53.71

[a] Sample size n=100 for 0-10 cm, and n=99 for other depths. [b] SE is standard error, [c] CV is coefficient of variance.

Table 2. Results of ANOVA on the effects of topography, sol type, forest type, sessional stage, management practice, and human influence on soil C, N, P, and their ratios across all depths in tropical forests in Southern China. Data of soil N, P concentrations and C:P, N:P ratios were log-transformed before ANOVA.

	Soil C		Soil N		Soil P		Soil C:N		Soil C:P		Soil N:P	
Factor[b]	F	*P*	F	*P*	F	*P*	F	*p*	F	*p*	F	*p*
Topography	**18.78**[a]	**<0.01**[a]	0.89	0.41	1.18	0.31	**10.6**	**<0.01**	**12.24**	**<0.01**	2.17	0.12
Soil type	**4.09**	**<0.01**	**5.18**	**<0.01**	1.11	0.36	**2.71**	**0.01**	1.07	0.40	0.21	0.99
Forest type	**13.58**	**<0.01**	1.47	0.23	1.66	0.18	2.4	0.10	**4.62**	**<0.01**	**2.98**	**0.04**
Successional stage	**8.29**	**<0.01**	1.53	0.19	1.76	0.13	2.02	0.10	**5.57**	**<0.01**	2.29	0.05
Management practice	**13.34**	**<0.01**	1.21	0.31	1.93	0.10	**3.62**	**0.03**	**4.08**	**<0.01**	1.78	0.12
Human Influence	**14.2**	**<0.01**	2.30	0.10	**3.86**	**0.02**	3.04	0.05	**20.77**	**<0.01**	**4.85**	**0.01**

[a] Bold fonts mean significant at alpha=0.05 or 0.01 level. [b] Topography includes mountain, hill and plain; Soil type includes latosolic red soil, red soil, mountain red soil, latosols red soil, yellow soil, sandy loam soil, yellow sandy soil, red sandy soil, podzol soil, and sandy soil; Forest type

includes mixed forest, evergreen broad-leaved forest, tropical coniferous forest, and tropical rainforest; Management practice includes no disturbance, thinning, fire, grazing, fertilization and other; Human influence include no influence, middle and severe influences.

Table 3. Multiple regression of soil C, N, P concentrations and their ratios with habitat and vegetation variables

Model [a]	R^2
C=-289.818-2.695Long+0.003Ele+0.064Cover	0.39
N=-25.054+0.234Long+0.003Cover	0.10
P=-1.529+0.0051Lat^2	0.11
CN=-1902+201.534Lat-5.334Lat^2+0.0570Cover+0.005Prep	0.32
CP=-16527+1759.419Lat-46.674Lat^2	0.23
NP=13.756-0.028Lat^2	0.06

[a] Lat: latitude; Long, longitude; Ele, elevation; Cover: vegetation cover; Prep: Precipitation. R^2, coefficient of determination.

Figure Legends

Fig. 1 Histograms of soil total C, N, P, C:N ratio, C:P ratio, and N:P ratio at different depths of soils in tropical forests on Hainan Island, China. All ratios are calculated on a weight basis. Sample size is 100.

Fig. 2 Comparisons of soil total C and N concentrations and C:N, C:P, and N:P ratios among different soil types. Sample size is 100.

Fig. 3 Comparisons of soil total C concentration and C:N, C:P, and N:P ratios among different forest types. Sample size is 100.

Fig. 4 Comparisons of soil total C, N, P concentrations and C:N, C:P, and N:P ratios among different forest successions. Sample size is 100.

Fig. 5 Relationships between soil C, N, P concentrations and latitude, longitude, elevation, vegetation cover, and tree height. Sample size is 100.

Fig. 6 Relationships between soil C:N, C:P, N:P ratios and latitude, longitude, elevation, vegetation cover, and tree height. Sample size is 100.

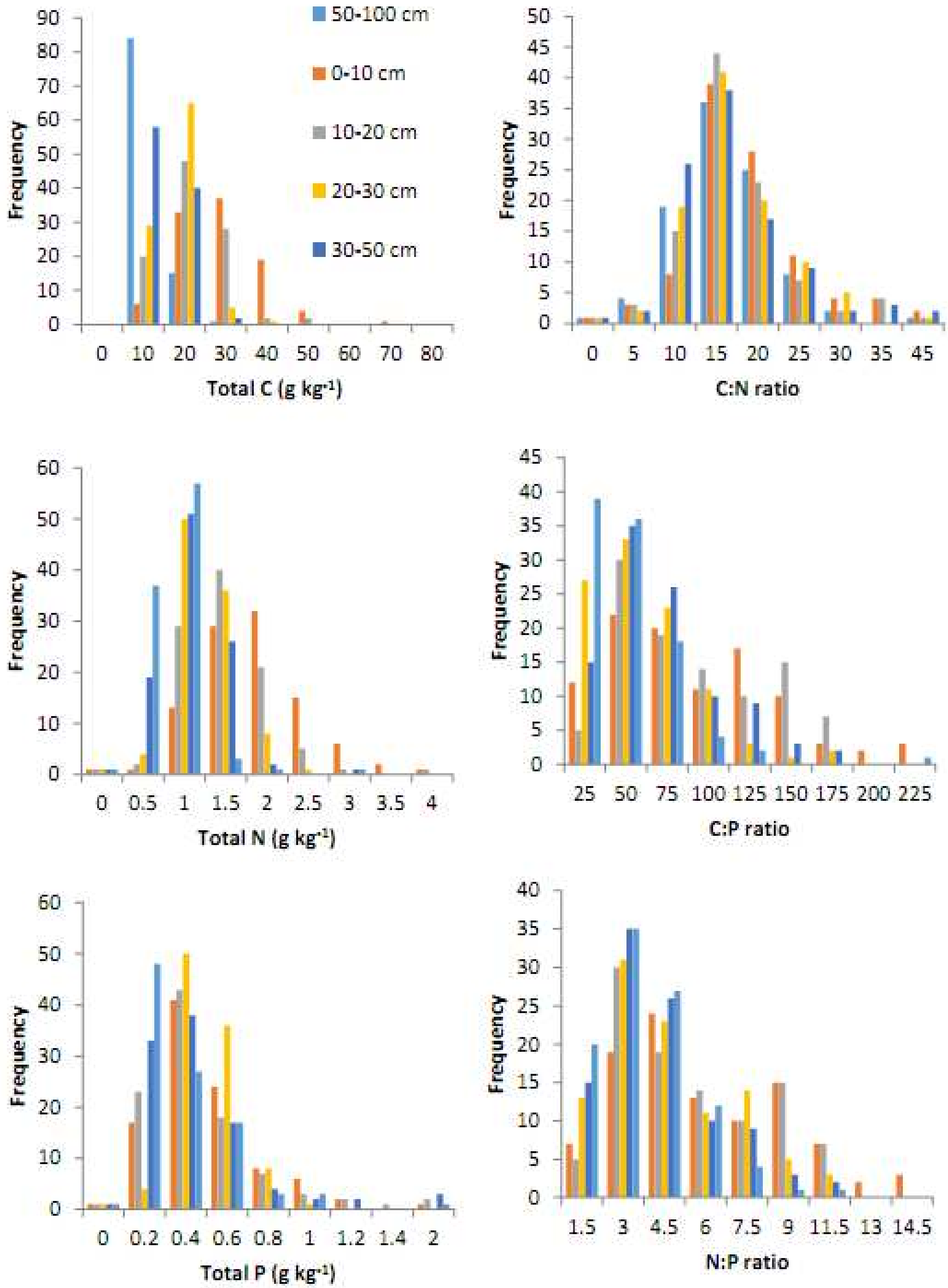

Fig. 1 Histograms of soil total C, N, P, C:N ratio, C:P ratio, and N:P ratio at different depths of soils in tropical forests on Hainan Island, China. All ratios are calculated on a weight (not molecular) basis. Sample size is 100.

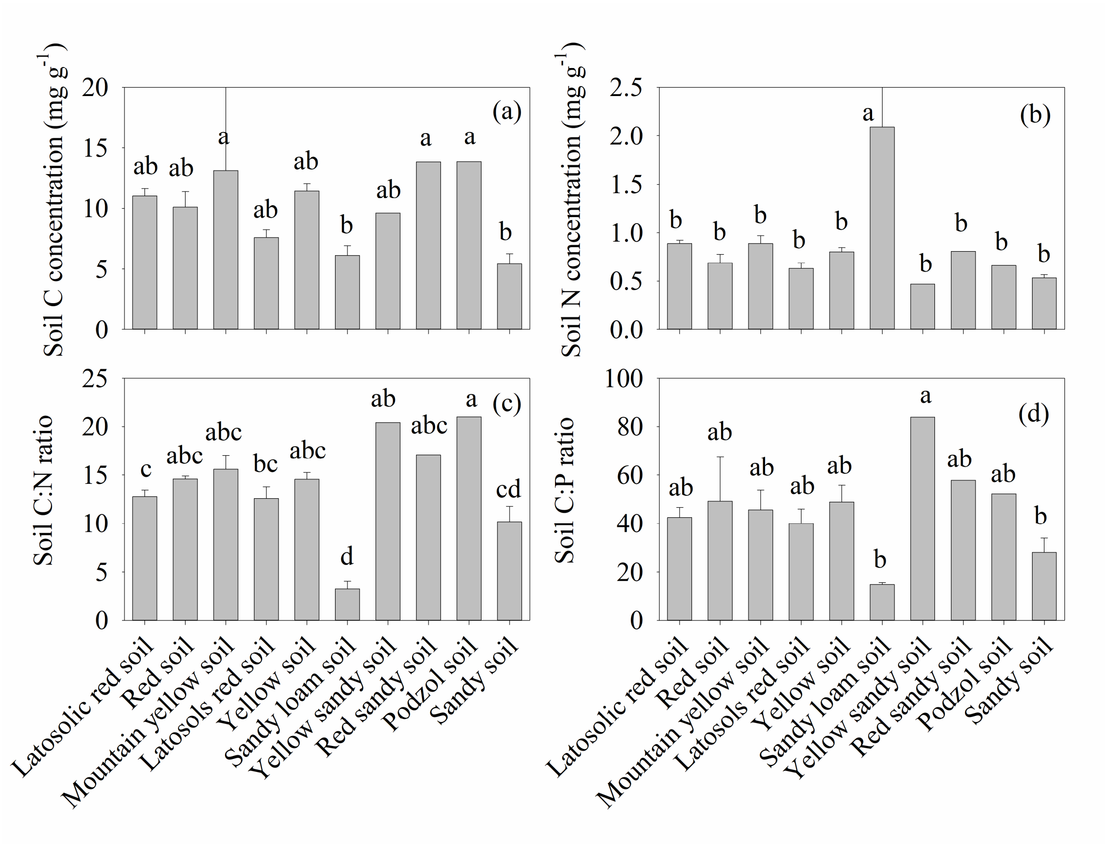

Fig. 2 Comparisons of soil total C and N concentrations and C:N and C:P ratios among different soil types. Sample size is 100.

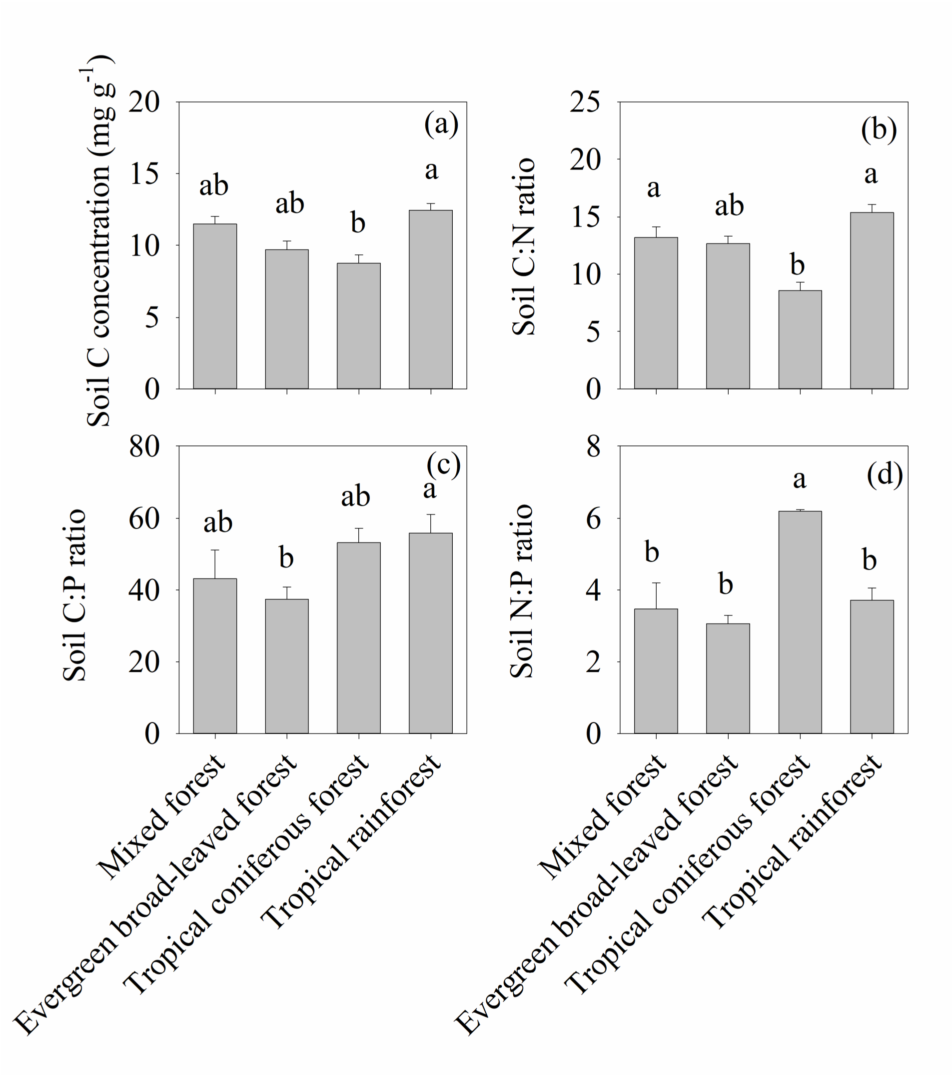

Fig. 3 Comparisons of soil total C concentration and C:N, C:P, and N:P ratios among different forest types. Sample size is 100.

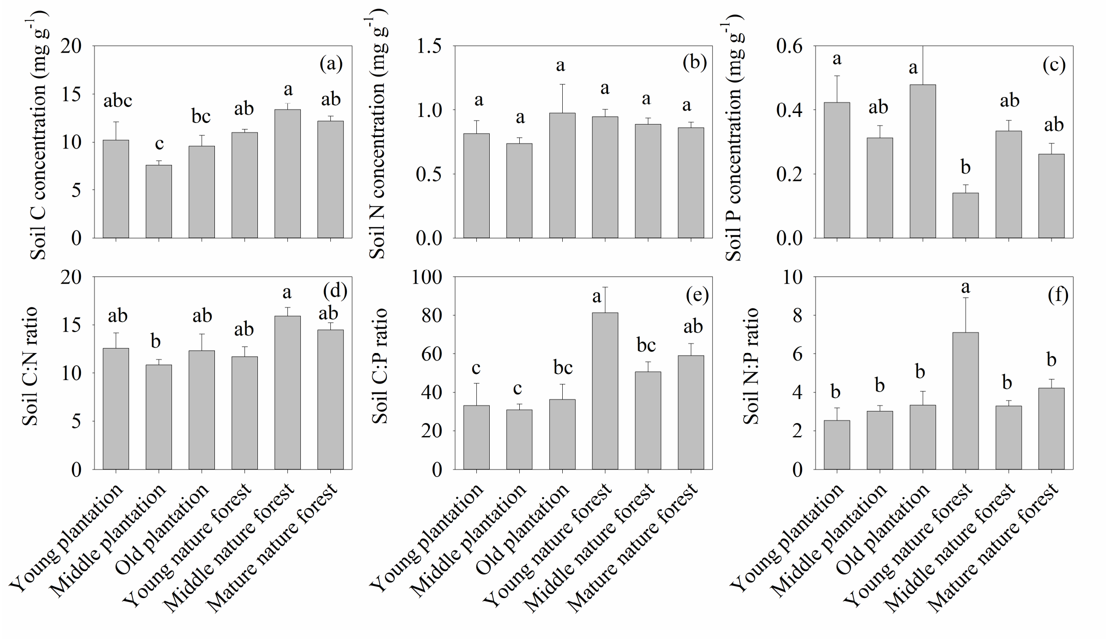

Fig. 4 Comparisons of soil total C, N, P concentrations and C:N, C:P, and N:P ratios among different forest successions. Sample size is 100.

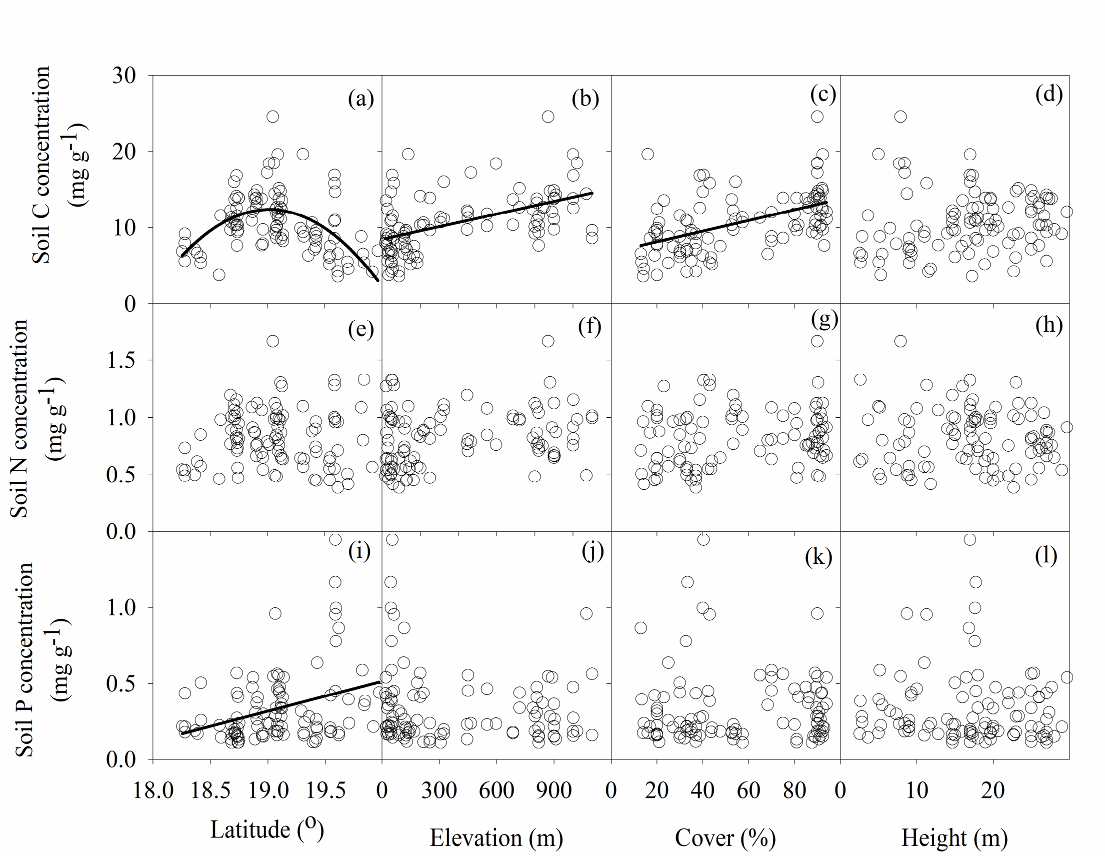

Fig. 5 Relationships between soil C, N, P concentrations and latitude, elevation, vegetation cover, and tree height. Sample size is 100.

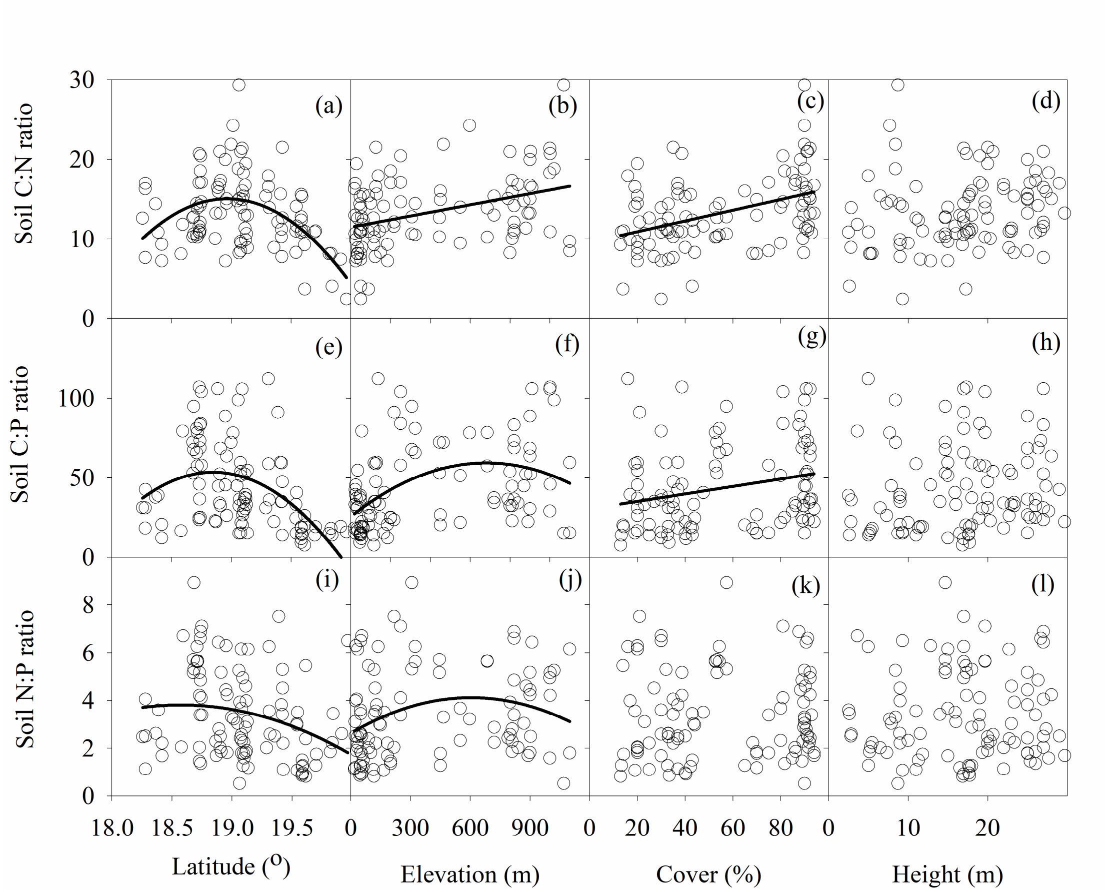

Fig. 6 Relationships between soil C:N, C:P, N:P ratios and latitude, elevation, vegetation cover, and tree height. Sample size is 100.

Supplemental Materials

Soil C:N:P Stoichiometry in Tropical Forests on Hainan Island of China. Plant and Soil. Dafeng Hui, Xitian Yang, Qi Deng, Qiang Liu, Xu Wang, Huai Yang, Hai Ren.

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Fig. S1 Map of soil sampling sites on Hainan Island, China.

Fig. S2 Comparisons of soil total C concentration and C:N and C:P ratios among different topographies. Sample size is 100.

Fig. S3 Comparisons of soil total C, N, P concentrations and C:N, C:P, and N:P ratios among different management practices. Sample size is 100.

Fig. S4 Comparisons of soil total C, N, P concentrations and C:N, C:P, and N:P ratios among different human impacts. Sample size is 100.

Fig. S5 Relationships between soil C:N, C:P, N:P ratios and soil C, N, P concentrations. Sample size is 100.

Table S1 Kolmogorov-Smirnov normality test for soil total C, N, and P concentrations and C:N, C:P, and N:P ratios with original data and logarithm transformed data.

Table S2 Results of ANOVA on the effects of topography, sol type, forest type, sessional stage, management practice, and human influence on soil C, N, P, and their ratios at different soil depths (0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, and 50-100 cm) in tropical forests in Southern China. Data of soil N, P concentrations and C:P, N:P ratios were log-transformed before ANOVA.

Table S3 Results of MANOVA of soil total C,N,P concentrations and C:N, C:P, and N:P concentrations under different topographies, soil types, forest types, forest succession, management practices, and human impacts.

Table S4 Multiple regression of soil C, N, P concentrations and their ratios with habitat and vegetation variables at different soil depths.

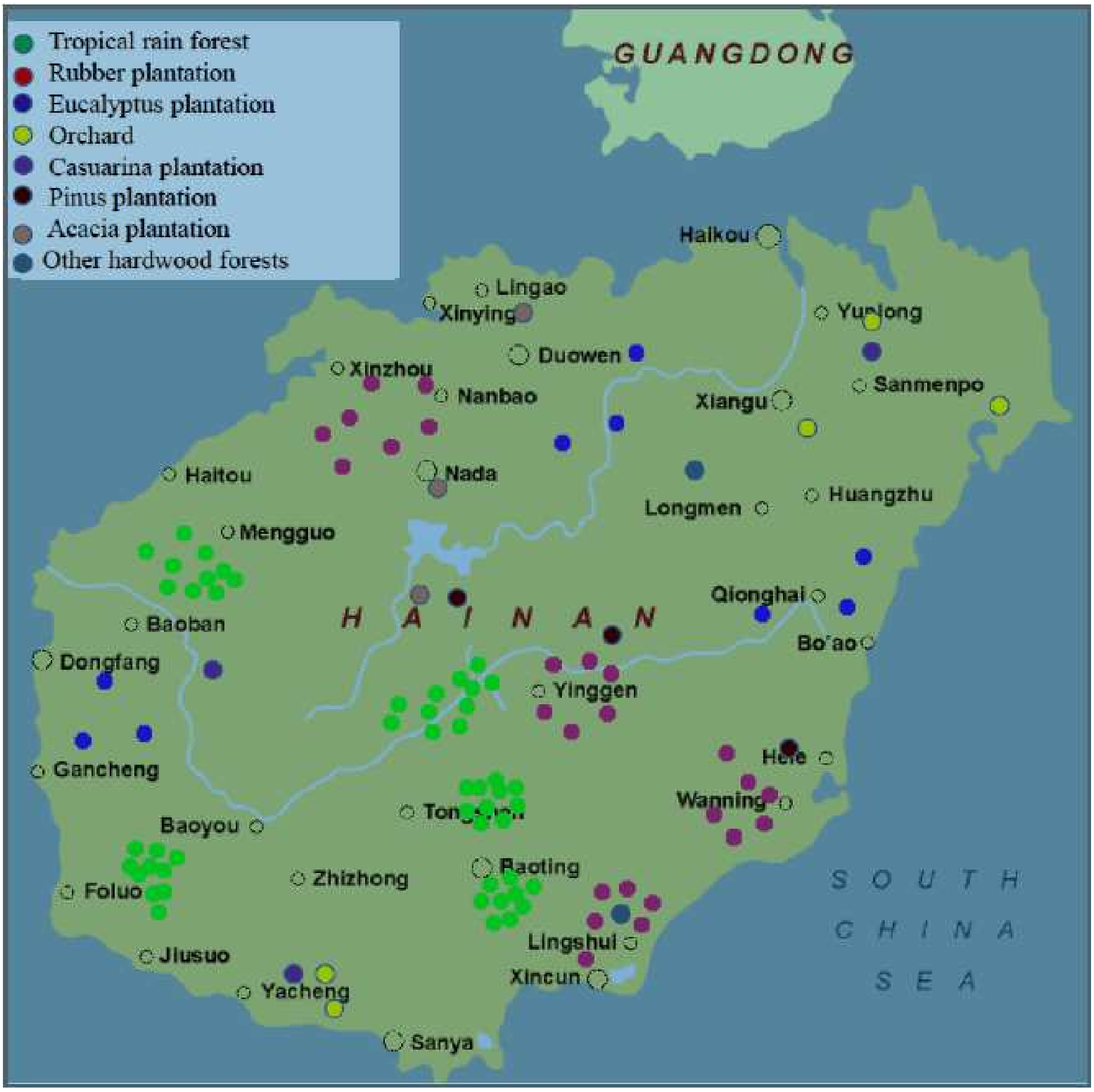

Fig. S1 Map of soil sampling sites on Hainan Island, China.

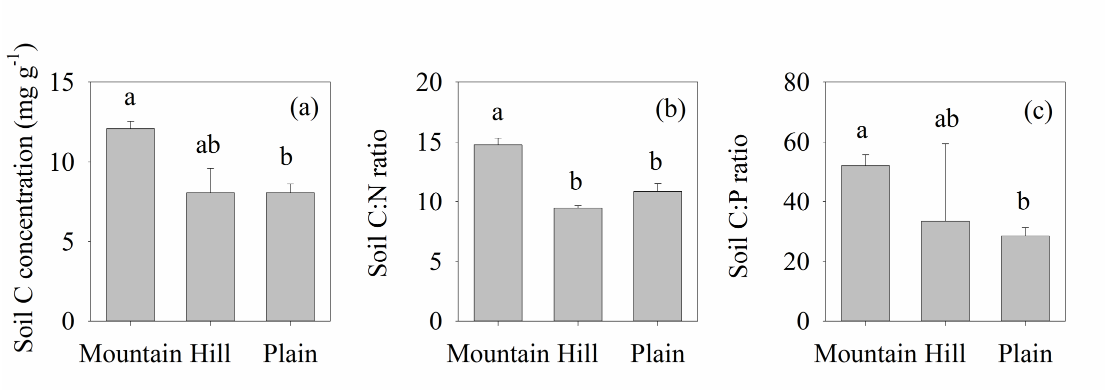

Fig. S2 Comparisons of soil total C concentration and C:N and C:P ratios among different topographies. Sample size is 100.

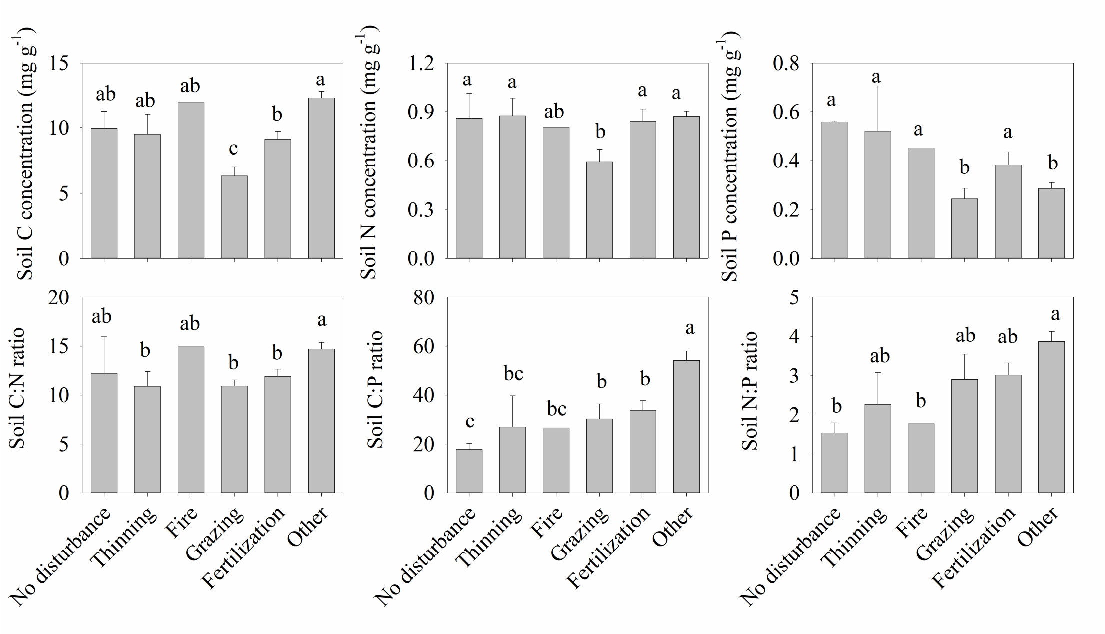

Fig. S3 Comparisons of soil total C, N, P concentrations and C:N, C:P, and N:P ratios among different management practices. Sample size is 100.

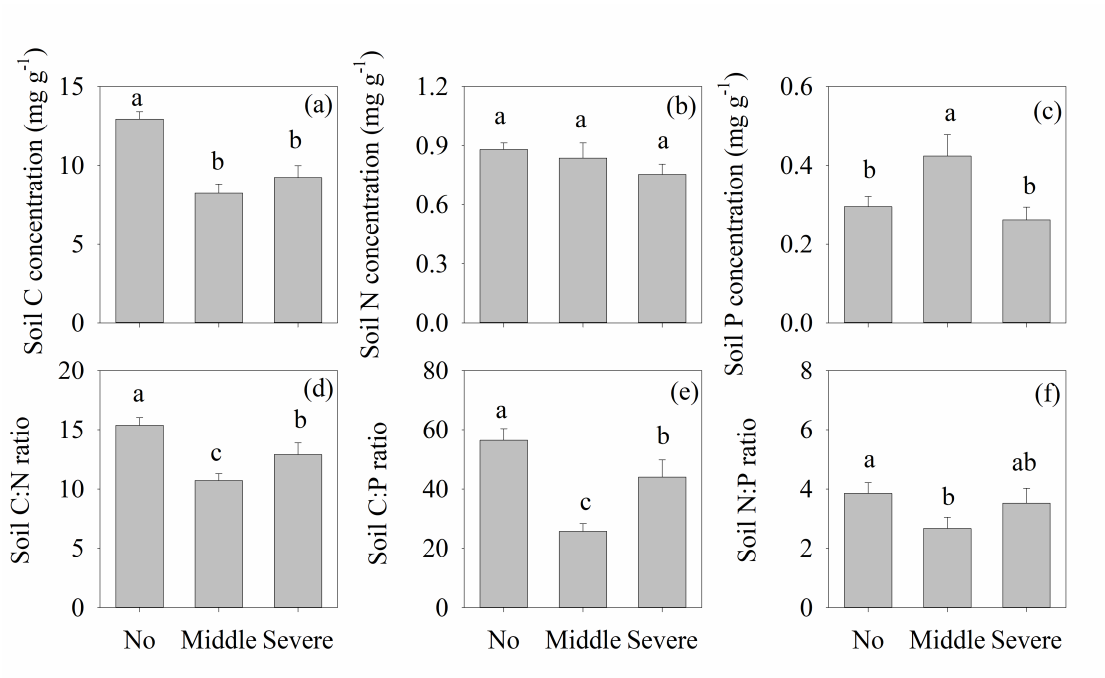

Fig. S4 Comparisons of soil total C, N, P concentrations and C:N, C:P, and N:P ratios among different human impacts. Sample size is 100.

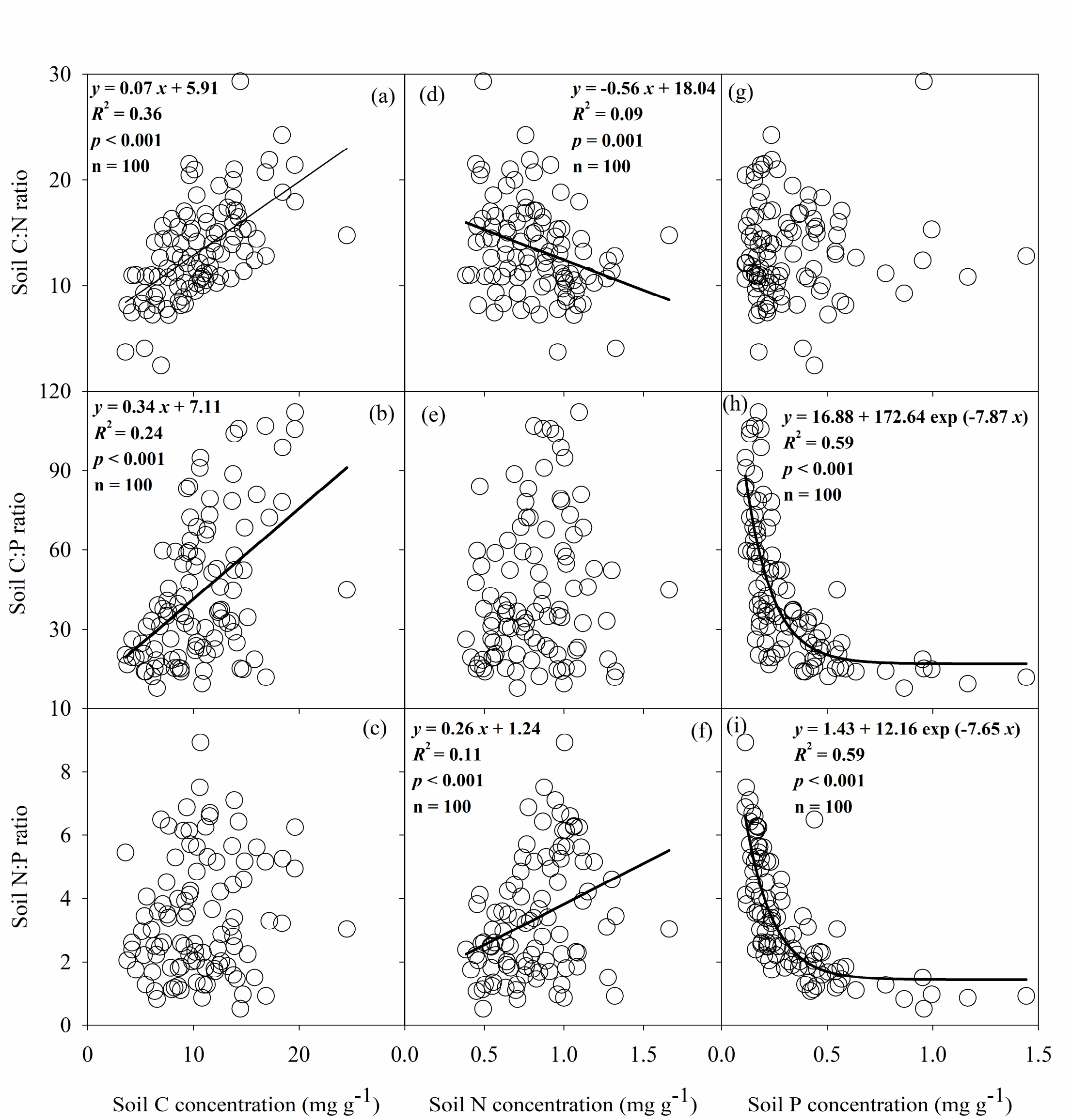

Fig. S5 Relationships between soil C:N, C:P, N:P ratios and soil C, N, P concentrations. Sample size is 100.

Table S1 Kolmogorov-Smirnov normality test for soil total C, N, and P concentrations and C:N, C:P, and N:P ratios with original data and logarithm transformed data.

	Original data		Logarithm transformed data	
Variable	D	p	D	p
C	0.06	>0.15	-	-
N	0.11	<0.01	0.06	>0.15
P	0.17	<0.01	0.12	<0.01
C:N ratio	0.07	>0.15	-	-
C:P ratio	0.14	<0.01	0.06	>0.15
N:P ratio	0.1327	<0.01	0.0756	>0.15

Table S2 Results of ANOVA on the effects of topography, sol type, forest type, sessional stage, management practice, and human influence on soil C, N, P, and their ratios at different soil depths (0-10 cm, 10-20 cm, 20-30 cm, 30-50 cm, and 50-100 cm) in tropical forests in Southern China. Data of soil N, P concentrations and C:P, N:P ratios were log-transformed before ANOVA.

	Soil C (0-10 cm)		Soil N (0-10 cm)		Soil P (0-10 cm)		Soil C:N (0-10 cm)		Soil C:P (0-10 cm)		Soil N:P (0-10 cm)	
Factor[b]	F	*P*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Topography	**15.01**	**<0.01**	0.37	0.70	1.87	0.16	**23.21**	**<0.01**	**4.17**	**0.02**	0.12	0.89
Soil type	**2.62**	**0.01**	**4.53**	**<0.01**	1.80	0.08	**2.98**	**<0.01**	**3.80**	**<0.01**	1.36	0.22
Forest type	**4.93**	**<0.01**	0.82	0.48	2.13	0.10	**8.42**	**<0.01**	**6.97**	**<0.01**	1.40	0.24
Successional stage	**11.94**	**<0.01**	1.03	0.40	2.36	0.06	**22.11**	**<0.01**	**6.97**	**<0.01**	1.40	0.24
Management practice	**5.21**	**<0.01**	0.97	0.44	**2.57**	**0.03**	**8.61**	**<0.01**	**3.93**	**<0.01**	2.29	0.05
Human Influence	**15.52**	**<0.01**	1.79	0.15	**4.26**	**<0.01**	**27.45**	**<0.01**	**9.33**	**<0.01**	2.56	0.06
	Soil C (10-20 cm)		Soil N (10-20 cm)		Soil P (10-20 cm)		Soil C:N		Soil C:P		Soil N:P	

							(10-20 cm)		(10-20 cm)		(10-20 cm)	
Factor[b]	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Topography	**18.74**	**<0.01**	1.33	0.27	2.79	0.07	**24.29**	**<0.01**	2.74	0.07	1.33	0.27
Soil type	**5.40**	**<0.01**	**5.90**	**<0.01**	1.84	0.07	**4.84**	**<0.01**	**4.02**	**<0.01**	1.36	0.22
Forest type	**7.05**	**<0.01**	**2.95**	**0.04**	1.57	0.20	**8.80**	**<0.01**	**3.82**	**0.01**	**1.54**	**0.21**
Successional stage	**15.03**	**<0.01**	1.15	0.33	1.29	0.28	**21.35**	**<0.01**	**3.24**	**0.02**	**1.08**	**0.37**
Management practice	**2.83**	**0.02**	0.71	0.62	1.54	0.19	**4.45**	**<0.01**	1.96	0.09	1.37	0.25
Human Influence	**16.90**	**<0.01**	1.50	0.22	**3.00**	**0.03**	**22.60**	**<0.01**	**3.89**	**0.01**	2.52	0.06

	Soil C (20-30 cm)		Soil N (0-10 cm)		Soil P (0-10 cm)		Soil C:N (0-10 cm)		Soil C:P (0-10 cm)		Soil N:P (0-10 cm)	
Factor[b]	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Topography	**17.81**	**<0.01**	1.63	0.20	0.96	0.38	**18.60**	**<0.01**	**3.10**	**0.05**	0.63	0.53
Soil type	**7.40**	**<0.01**	**4.98**	**<0.01**	1.16	0.33	**5.41**	**<0.01**	**4.17**	**<0.01**	1.03	0.42
Forest type	**7.16**	**<0.01**	1.09	0.36	**2.90**	**0.04**	**7.10**	**<0.01**	**2.71**	**0.05**	1.73	0.17

Successional stage	**13.30**	**<0.01**	2.47	0.05	1.65	0.17	**14.20**	**<0.01**	**4.00**	**<0.01**	1.13	0.34
Management practice	2.09	0.07	0.71	0.62	1.71	0.14	**3.18**	**0.01**	1.54	0.19	1.65	0.15
Human Influence	**14.23**	**<0.01**	1.45	0.23	1.47	0.23	**13.58**	**<0.01**	**2.70**	**0.05**	**2.78**	**0.04**

	Soil C (30-50 cm)		Soil N (30-50 cm)		Soil P (30-50 cm)		Soil C:N (30-50 cm)		Soil C:P (30-50 cm)		Soil N:P (30-50 cm)	
Factor[b]	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Topography	**8.10**	**<0.01**	0.32	0.73	2.59	0.08	**13.37**	**<0.01**	0.11	0.90	1.41	0.25
Soil type	**3.93**	**<0.01**	**3.06**	**<0.01**	1.18	0.32	**3.61**	**<0.01**	**2.89**	**<0.01**	1.15	0.34
Forest type	**3.71**	**0.01**	0.19	0.90	**3.39**	**0.02**	**3.95**	**0.01**	1.58	0.20	2.03	0.12
Successional stage	**5.52**	**<0.01**	0.32	0.86	1.85	0.12	**9.53**	**<0.01**	0.61	0.66	1.68	0.16
Management practice	2.28	0.05	0.13	0.99	1.49	0.20	**2.38**	**0.04**	0.11	0.99	1.93	0.10
Human Influence	**6.46**	**<0.01**	0.36	0.79	**2.78**	**0.04**	**10.10**	**<0.01**	0.28	0.84	2.14	0.10

	Soil C (50-100 cm)		Soil N (50-100 cm)		Soil P (50-100 cm)		Soil C:N (50-100 cm)		Soil C:P (50-100 cm)		Soil N:P (50-100 cm)	
Factor[b]	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*P*
Topography	**7.72**	**<0.01**	0.29	0.75	1.04	0.36	**8.19**	**<0.01**	0.04	0.96	0.59	0.56
Soil type	**4.21**	**<0.01**	**5.50**	**<0.01**	1.44	0.18	**2.84**	**<0.01**	**4.35**	**<0.01**	1.22	0.29
Forest type	2.39	0.07	0.15	0.93	**3.79**	**0.01**	1.98	0.12	0.34	0.80	**2.95**	**0.04**
Successional stage	**6.82**	**<0.01**	0.39	0.81	1.18	0.32	**7.83**	**<0.01**	0.80	0.53	1.00	0.41
Management practice	**1.89**	**0.10**	0.73	0.60	1.31	0.26	1.90	0.10	1.33	0.26	1.58	0.18
Human Influence	**8.41**	**<0.01**	0.48	0.69	2.68	0.05	**8.33**	**<0.01**	0.50	0.68	2.68	0.05

[a] Bold fonts mean significant at alpha=0.05 or 0.01 level. [b] Topography includes mountain, hill and plain; Soil type includes latosolic red soil, red soil, mountain red soil, latosols red soil, yellow soil, sandy loam soil, yellow sandy soil, red sandy soil, podzol soil, and sandy soil; Forest type includes mixed forest, evergreen broad-leaved forest, tropical coniferous forest, and tropical rainforest; Management practice includes no disturbance, thinning, fire, grazing, fertilization and other; Human influence include no influence, middle and severe influences.

Table S3 Results of MANOVA of soil total C,N,P concentrations and C:N, C:P, and N:P concentrations under different topographies, soil types, forest types, forest succession, management practices, and human impacts

	0-100 cm		0-10 cm		10-20 cm		20-30 cm		30-50 cm		50-100 cm	
Variable	Wilk's Lambda	p	Wilk's Lambda	p	Wilk's Lambda	p	Wilk's Lambda	p	Wilk's Lambda	p	Wilk's Lambda	p
Topography	0.63	<0.001	0.63	<0.001	0.53	<0.001	0.64	<0.001	0.69	0.001	0.75	0.012
Soil type	0.30	<0.001	0.32	<0.001	0.30	<0.001	0.24	<0.001	0.40	0.001	0.26	<0.001
Forest type	0.68	0.007	0.68	0.010	0.54	<0.001	0.63	0.001	0.62	0.001	0.68	0.011
Forest succession	0.46	<0.001	0.42	<0.001	0.44	<0.001	0.46	<0.001	0.58	0.002	0.61	0.007
Management practice	0.54	0.002	0.54	0.004	0.62	0.066	0.61	0.041	0.69	0.305	0.63	0.085

Human impact	0.50	<0.001	0.38	<0.001	0.42	<0.001	0.46	<0.001	0.56	<0.001	0.64	0.002

Table S4 Multiple regression of soil C, N, P concentrations and their ratios with habitat and vegetation variables at different soil depths

Model [a]	R^2
0-10 cm soil layer	
C=1.913+0.014Cover	0.15
N=-3.487+0.034Long	0.03
P=-0.253+0.0008Lat^2	0.20
CN=-104.46+0.009Ele+0.189Cover+3.714Temp+0.014Prep	0.43
CP=-51.592+0.501Long+0.0004Ele+0.009Cover-0.0289Height-0.001Prep	0.34
NP=-1.359+0.005Lat^2	0.09
10-20 cm soil layer	
C=12.756+0.010Ele	0.27
N=1.174+0.0003Ele	0.04
P=-1.865+0.006Lat^2	0.12
CN=10.774+0.074Cover	0.10
CP=-25314+2693.8Lat-71.44Lat^2	0.23
NP=20.773-0.028Lat2	0.06
20-30 cm soil layer	0.39
C=-30.859+0.291Long+0.002Ele-0.000002Ele^2	0.23
N=no significant variable	0.00
P=-0.227+0.0008Lat^2	0.14
CN=-344.06+3.202Long+0.004Ele+0.067Cover	0.27
CP=-25.27+0.245Long+0.0003Ele-0.0004Prep	0.14
NP=-1.490+0.005Lat^2	0.09

30-50 cm soil layer	
C=2.241+0.0009Ele	0.08
N=-0.828+0.003Lat^2	0.09
P=-0.622+0.002Lat^2	0.14
CN=-296.367+2.753Long+0.003Ele+0.050Cover	0.29
CP=-18.704+0.178Long	0.06
NP=-1.871+0.006Lat^2	0.10
50-100 cm soil layer	
C=-100.266+0.899Long+0.042Cover+0.002Prep	0.26
N=154.974-16.397Lat+0.434Lat^2+0.0002Ele	0.23
P=-0.961+0.0032Lat^2	0.24
CN=-221.864+1.977Long+0.078Cover+0.004Prep	0.34
CP=-13.213+0.126Long	0.40
NP=-1.355+0.004Lat^2	0.05

[a] Lat: latitude; Long, longitude; Ele, elevation; Cover: vegetation cover; Temp: temperature; Prep: Precipitation. R^2, coefficient of determination.