

## RESEARCH ARTICLE

## Ecological stoichiometry of carbon, nitrogen, and phosphorus in soil layers and leaf biomass of large-leaf organic tea gardens

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This study was carried out in a certain area of organic tea garden in Pu'er, Yunnan, China by collecting soil and tea leaf samples from different soil layers (0 - 60 cm). The standard methods like combustion oxidation non-dispersive infrared method for organic carbon determination and automatic nitrogen determination method for total nitrogen measurement were used to explore the ecological stoichiometry of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) in large-leaf organic tea gardens to identify growth limiting factors and support nutrient management strategies. The results showed that, within the 0 - 60 cm soil layer, the pH was between 4.78 and 4.96. The content of soil alkali, hydrolysable nitrogen, ranged from 71.80 to 115.58 mg/kg, while available phosphorus was from 5.29 to 8.79 mg/kg. The total carbon (TC), total nitrogen, and total phosphorus contents varied from 18.04 to 30.65 g/kg, 1.25 to 1.96 g/kg, and 0.57 to 0.62 g/kg, respectively. As the soil depth increased, the levels of TC and TN gradually increased, while TP content first increased and then decreased. There were significant negative correlations between leaf TN and soil TC in the 0 - 10 cm layer, leaf TC and soil TP in the 10 - 20 cm layer, and leaf organic carbon with both soil TC and TN in the 20 - 40 cm layer. Conversely, leaf nitrogen showed significant positive correlations with soil TC and TN in the 20 - 40 cm layer. The soil carbon/nitrogen (C/N) ratio was greater than 11.90, carbon/phosphorus (C/P) ratio was less than 60.00, and nitrogen/phosphorus (N/P) ratio was less than 5.10, indicating that nitrogen might be a limiting factor for tea tree growth. Therefore, high nitrogen fertilizers may be needed in future tea garden management. This research provided a scientific basis for the sustainable nutrient management of large-leaf organic tea gardens, which is of great significance for promoting the healthy development of the tea industry.

**Keywords:** soil organic carbon; total nitrogen; total phosphorus; large-leaf organic tea gardens.

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### Introduction

Tea as a globally significant economic crop holds a prominent position, particularly in key

production countries such as China, India, and Kenya. Large-leaf tea plantations with their unique ecosystem structure and management practices present substantial research value in

the context of organic agriculture. Compared to conventional tea gardens, large-leaf tea plantations often exhibit distinct nutrient cycling and stoichiometric characteristics, which are attributed to their higher canopy coverage and more complex soil ecosystems. Investigating the dynamics of carbon (C), nitrogen (N), and phosphorus (P) in large-leaf tea plantations can provide valuable insights for the sustainable management of organic agriculture and tea plantations worldwide.

Ecological stoichiometry, as described by Sterner and Elser, focuses on the balance of energy and chemical elements, particularly carbon, nitrogen, and phosphorus within biological systems [1]. This scientific discipline offers a framework for understanding the complex web of ecological interactions [2, 3], which integrates biological theories across various levels from molecular to global scales, encompassing entire ecosystems [4]. On a global scale, extensive research has been conducted to understand the dynamics of energy and elemental balances in diverse biological systems. Studies have explored topics such as the role of stoichiometry in nutrient cycling, the impact of environmental changes on elemental ratios, and the consequences for ecosystem functioning [5, 6]. In China, research on ecological stoichiometry has surged in recent years [7, 8]. Researchers have concentrated on a broad range of fields, particularly on nutrient supply and demand in diverse ecosystems. Through analyzing the availability of essential elements like C, N, and P, researchers aim to determine the limiting factors that affect the growth and development of organisms [9, 10]. Furthermore, studies on stoichiometric metrics have provided valuable insights into the relationships between different elements and their influence on ecological processes [11]. Environmental factors such as topography and soil properties play a crucial role in shaping soil nutrient dynamics [12]. Topography can influence the distribution of water and nutrients, affecting plant growth and survival. Soil properties such as texture, pH, and organic matter content significantly impact nutrient

availability [13]. Understanding these dynamics is essential for effective fertilization management [14]. By considering the unique characteristics of the environment, farmers and land managers can make informed decisions regarding fertilization practices, optimizing crop growth while minimizing environmental impacts [15].

Ecological stoichiometry is a dynamic and evolving field that provides valuable insights into the complex interactions between energy and chemical elements in biological systems. The combination of global research and domestic studies in China has advanced the understanding of ecological processes and provided practical solutions for sustainable land management. By focusing on specific ecosystems, the soil nutrient dynamics can be explored continuously, and the strategies to ensure long-term sustainability of agricultural and natural ecosystems can be developed. This study aimed to identify growth-limiting factors and develop nutrient management strategies that could enhance the sustainability of tea plantations [16]. The research focused on an organic large-leaf tea plantation situated at an elevation of 1,600 meters. A comprehensive analysis of soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP) in various soil layers ranging from 0 to 60 centimeters were conducted to find distinct nutrient distribution patterns along the vertical soil profile to understand nutrient cycling and optimize nutrient applications, which would not only benefit tea production but also contribute to the overall health and stability of the ecosystem. The results of this study would help to the understanding of ecological stoichiometry in tea plantations as most existing research predominantly focused on surface layers and often overlooked the potential impacts of deeper soil layers on tea plant growth.

## Materials and methods

### Study region

The study site was selected in Maowei Mountain Organic Tea Tree Base located at 101.03°E,

23.07°N within a certain area of organic tea garden in Nanchun Town, Simao District, 12 kilometers from Pu'er city, Yunnan, China with the altitudes ranged from 1,230 to 1,600 meters. The site is characterized by high forest cover, providing an exceptional ecological environment conducive to organic agriculture and a subtropical climate with mild winters and cool summers and an annual average temperature of 21°C. The frost-free period lasts over 360 days [10]. The soil type in the study region is classified as red earth. The tea plants were grown in three plots with the average heights of 114.9 cm, 107 cm, 117.2 cm and average crown widths of 117 cm × 132 cm, 95 cm × 97 cm, and 116 cm × 128 cm in plots 1, 2, and 3, respectively. These growth characteristics reflected the suitability of the region for cultivating large-leaf tea species.

#### **Sample collection and processing**

The sampling location was selected at an altitude of 1,600 meters within a healthy tea plant area with a uniform slope. Three 10 m × 10 m plots were randomly chosen, and three quadrats were established along the diagonal of each plot. Five tea trees of average height and canopy width were sampled per plot. Leaves were collected from four cardinal directions including southeast, northeast, southwest, and northwest of each tree, labeled, and transported to the laboratory. Soil samples were collected from four distinct layers of 0 - 10 cm, 10 - 20 cm, 20 - 40 cm, and 40 - 60 cm within each quadrat. The samples were combined by layer. After removing impurities, approximately 1 kilogram soil per layer was air-dried and sieved. In the laboratory, tea leaves were washed using deionized water, dried at 105°C for 30 minutes, and then at 80°C until a constant weight was achieved. The leaves were ground and sieved through a 100-mesh sieve before stored for analysis [17].

#### **Determination of samples' nutrients**

For the determination of plant and soil organic carbons, a combustion oxidation non-dispersive infrared method was employed with an organic carbon analyzer in accordance with the National Environmental Protection Standard of the

People's Republic of China (HJ695-2014) [18, 19]. The TN of the plant was measured by an automatic nitrogen determination method after sulfuric acid and hydrogen peroxide digestion. Soil TN was similarly measured using the automatic nitrogen determination method [5]. The TPs of both plant and soil samples were determined using sulfuric acid and hydrogen peroxide digestion followed by analysis with a molybdenum-antimony anti-ultraviolet-visible spectrophotometer [1]. Soil pH was measured using a 2.5:1 water-to-soil ratio and the potentiometric method [13]. Alkaline hydrolysable nitrogen in soil was determined by the alkaline hydrolysis diffusion method, while available phosphorus was measured using the Olsen method [16].

#### **Data processing and analysis**

The data collected were processed using Microsoft Excel 2010 (Microsoft, Redmond, Washington, USA). SPSS 24.0 (IBM Corp., Armonk, NY, USA) was employed for statistical analysis. One-way ANOVA and multiple comparisons were performed with the least significant difference (LSD) test as the post-hoc analysis. Additionally, correlations between SOC, TN, TP in tea leaves and corresponding nutrient levels of total carbon (TC), TN, and TP in soil were assessed using the Pearson correlation method.

### **Results**

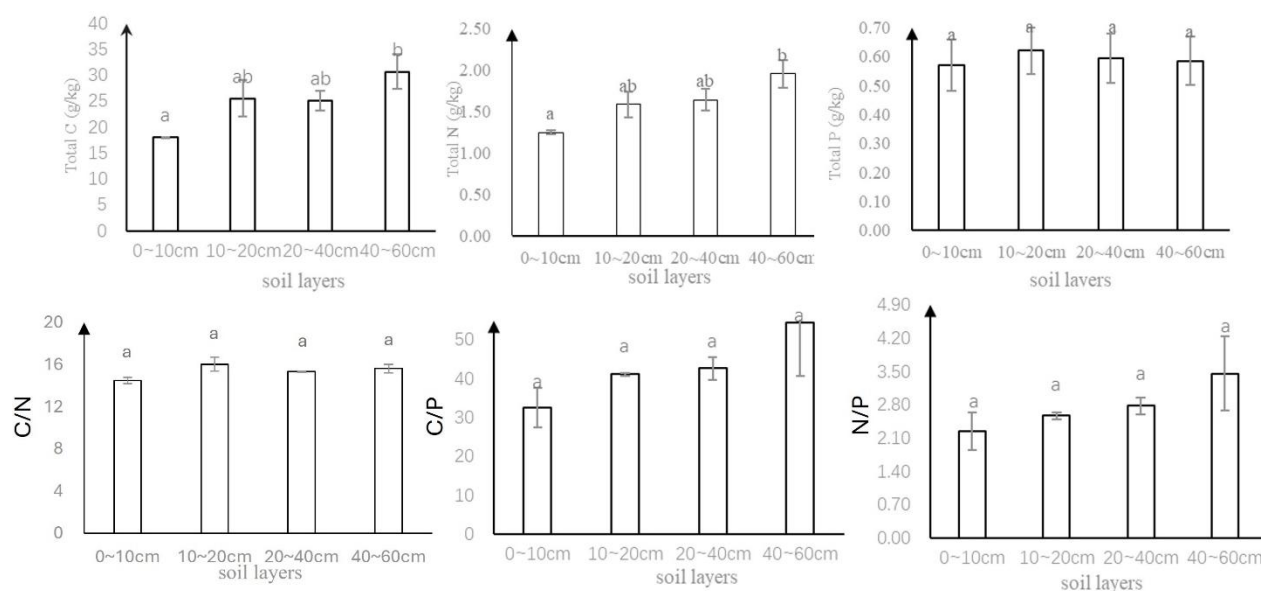
#### **Soil physicochemical properties in different soil layers of large-leaf tea plantations**

The results showed that, in the 0 - 60 cm soil layer, the pH values of the soil for large-leaf tea plants ranged from 4.78 to 4.96 (Table 1). While pH decreased with increasing soil depth, the differences were not significant [12]. The soil alkali-hydrolysable nitrogen content ranged between 71.80 and 115.58 mg/kg. An increasing trend in soil alkali-hydrolysable nitrogen was observed with soil depth. However, the differences between the 0 - 10 cm, 10 - 20 cm, and 20 - 40 cm layers were not significant, while the difference between these layers and the 40 -

**Table 1.** Basic physical and chemical properties of soils under different layers in big leaves tea gardens.

Soil layer (cm)	pH	Alkaline hydrolyzable nitrogen (mg/kg)	Available phosphorus (mg/kg)
0 - 10	4.96 ± 0.22 <sup>a</sup>	71.80 ± 0.75 <sup>b</sup>	5.29 ± 0.07 <sup>b</sup>
10 - 20	4.85 ± 0.33 <sup>a</sup>	94.45 ± 22.99 <sup>ab</sup>	5.59 ± 0.53 <sup>b</sup>
20 - 40	4.78 ± 0.15 <sup>a</sup>	89.49 ± 20.66 <sup>ab</sup>	8.79 ± 1.96 <sup>a</sup>
40 - 60	4.79 ± 0.16 <sup>a</sup>	115.58 ± 20.41 <sup>a</sup>	6.08 ± 0.15 <sup>b</sup>

Note: different letters in the same column indicated significant differences ( $P < 0.05$ ).



**Figure 1.** Soil TC, TN, and TP contents and stoichiometric ratios in different soil layers of large-leaved tea plantation. Different lowercase letters indicated significant differences between soil layers ( $P < 0.05$ ).

60 cm layer was significant, which might be attributed to long-term no-tillage practices in the organic tea garden soil, low rainfall during the year, and nutrient deposition following the rainy season. The soil available phosphorus content in large-leaf tea plants ranged from 5.29 to 8.79 mg/kg, which was considerably lower than that found in high-yield, high-quality tea plantations [14]. The available phosphorus content increased with soil depth up to a point, then decreased.

#### Soil TC, TN, and TP contents and stoichiometric ratios in different soil layers of large-leaf tea plantations

The contents of TC and TN in diverse soil layers progressively increased as the depth increased. The soil TC content ranged from 18.02 to 30.65 g/kg, and a significant difference was detected between the 0 - 10 cm layer and the 40 - 60 cm layer [1]. Likewise, the TN content spanned from 1.25 to 1.96 g/kg. There were significant differences between the 0 - 10 cm layer and the 40 - 60 cm layer, while no significant differences existed among other layers (Figure 1). The ratio of soil carbon (C) to nitrogen (N) demonstrated that the value of the C ratio was stably within the range of 14.5 to 15.64, which did not show significant differences among different soil layers. Similarly, the ratio of nitrogen to carbon was also maintained within a similar range, and no obvious changes were revealed by comparison among various soil layers. Such data performance not only highlighted the stability of soil characteristics on the vertical profile but also provided a strong basis to further understand the distribution of soil nutrients.

**Table 2.** SOC, TN, and TP contents and stoichiometric ratios of leaves of large-leaved tea plants.

Sample plot	SOC (mg/g)	TN (mg/g)	TP (mg/g)	C: N	C:P	N:P
Plot 1	664.50	26.90	2.50	24.70	265.80	10.76
Plot 2	686.90	24.30	2.40	28.27	286.21	10.13
Plot 3	672.00	23.20	2.20	28.97	305.45	10.55
Average value	674.47	24.80	2.37	27.31	285.82	10.48

**Table 3.** Correlations of C, N, P and stoichiometric ratio between leaves and soil.

Soil layer (cm)	Soil	Leaf					
		SOC	TN	TP	C:N	C:P	N:P
0 - 10	TC	-0.222	0.942	0.195	0.356	0.011	~0.943
	TN	0.991	-0.416	-0.009	0.539	0.214	-0.991
	TP	0.233	0.696	0.929	-0.589	-0.832	-0.235
	C:N	0.781	0.110	0.505	0.030	-0.317	-0.782
	C:P	0.565	-0.999*	-0.898	0.995	0.969	-0.564
	N:P	0.232	-0.946	-0.996	0.891	0.993	-0.231
10 - 20	TC	-0.970	0.723	0.379	-0.813	-0.561	-0.970
	TN	-0.966	0.736	0.396	-0.823	-0.576	0.965
	TP	-0.396	0.988	0.966	-0.956	-0.998*	0.394
	C:N	-0.995	0.618	0.244	-0.722	-0.438	0.995
	C:P	-0.959	0.276	-0.140	-0.407	-0.066	0.960
	N:P	-0.850	0.010	-0.399	-0.150	0.201	0.851
20 - 40	TC	-1.000**	0.545	0.156	-0.657	-0.355	1.000**
	TN	-0.999*	0.570	0.185	-0.679	-0.383	0.999*
	TP	-0.231	0.949	0.996	-0.891	-0.993	0.230
	C:N	-0.807	-0.067	-0.468	-0.073	0.276	0.808
	C:P	-0.805	-0.069	-0.470	-0.071	0.278	0.806
	N:P	-0.809	-0.064	-0.465	-0.076	0.273	0.810
40 - 60	TC	-0.722	-0.229	-0.606	0.091	0.429	0.701
	TN	-0.778	-0.145	-0.536	0.005	0.351	0.759
	TP	-0.200	0.946	0.996	-0.891	-0.993	0.230
	C:N	-0.096	-0.808	-0.978	0.718	0.914	0.066
	C:P	-0.449	-0.541	-0.837	0.418	0.707	0.422
	N:P	-0.478	-0.513	-0.819	0.388	0.683	0.451

Note: \* significantly correlated at the 0.05 level. \*\* significantly correlated at the 0.01 level.

### The contents and stoichiometric ratios of SOC, TN, and TP in the leaves of large-leaved tea plants

The contents of SOC, TN, and TP in the leaves of large-leaf tea plants were 674.47 mg/g, 24.8 mg/g, and 2.37 mg/g, respectively. Moreover, the average leaf C:N, C:P, and N:P ratios were 27.31, 285.82, and 10.48, respectively (Table 2) [8].

### Correlation analysis of leaf SOC, TN, and TP contents with soil TC, TN, and TP in large-leaf tea plants

The correlations between the leaf and soil contents of SOC, TN, TP and TC, TN, TP across various soil layers were analyzed according to the method of Zhou *et al.* [9]. The results showed that leaf TN had a negative correlation with soil TC in the 0 - 10 cm soil layer. Furthermore, leaf TC demonstrated a negative correlation with soil TP in the 10 - 20 cm soil layer, which was similar

to the results of Peñuelas *et al.* [20]. Additionally, a significant negative correlation was identified between leaf SOC and soil TC, as well as between leaf SOC and soil TN in the 20 - 40 cm soil layer, which were consistent with the findings of Gusewell [2]. Conversely, leaf TN showed a notable positive correlation with both soil TC and soil TN in the same 20 - 40 cm soil layer as mentioned by Dodds *et al.* (Table 3) [4].

## Discussion

### Soil TC, TN, and TP contents and chemical characteristics in different soil layers of large-leaf tea plantations

The concentrations of TC and TN increased with soil depth, while available phosphorus initially raised and then decreased [5]. This increase in TC content was likely due to nutrient fluxes from litter decomposition and root exudates, which contributed to organic carbon accumulation in deeper soil layers [13]. For the 0 - 60 cm soil layer, TP content ranged from 0.48 to 0.70 g/kg, exhibiting a relatively uniform distribution. This consistent vertical distribution suggested minimal cultivation disturbances in organic tea gardens, which effectively prevented nutrient loss [15, 21].

### SOC, TN, and TP contents and stoichiometric characteristics of large-leaf tea leaves

The average SOC content of tea leaves was 674.47 mg/g, surpassing both global averages for terrestrial plant leaves and the north-south transect of eastern China [8], which indicated a substantial carbon storage capacity in the tea plants of this region. Additionally, the average TN and TP contents of the tea leaves were 24.8 mg/g and 2.37 mg/g, respectively, which were higher than both global and Chinese averages [16]. These elevated nutrient contents suggested that the large-leaf tea plants thrived in favorable regional climatic conditions, which enhanced their nitrogen and phosphorus uptake. The leaf C:N, C:P, and N:P ratios were marginally lower than those in the north-south transect forest community of eastern China, pointing to faster

growth rates in the study area, likely due to less competition and optimized fertilization practices. These stoichiometric characteristics supported the notion that organic tea plantations were experiencing a favorable growth environment with efficient nutrient utilization.

### Correlation analysis of leaf SOC, TN, and TP with soil TC, TN, and TP and stoichiometric characteristics in different soil layers

Significant negative correlations were found between leaf TN and soil TC in the 0 - 10 cm layer, and between leaf C and soil TP in the 10 - 20 cm layer [9]. Leaf SOC showed a significant negative correlation with both soil TC and TN in the 20 - 40 cm layer [2]. In contrast, leaf N exhibited significant positive correlations with both soil TC and TN in the 20 - 40 cm layer [4]. These results suggested that soil nutrient content increased with depth, likely due to reduced cultivation cycles that helped form a protective mechanism against nutrient loss. A soil C:N ratio greater than 11.90 indicated nitrogen limitation in tea plant growth, signaling the need for high-nitrogen organic fertilizers to improve growth conditions. This study not only provided actionable nutrient management strategies for tea garden managers but also offered insights for similar economic crop systems globally. The findings on the dynamic changes in the C:N ratio could guide efforts to optimize nutrient use efficiency, enhancing tea quality and yield, and contributing to the sustainable development of organic agriculture. By integrating ecological stoichiometry with practical management practices, this study bridged the research gap on C:N balance in tea plantations and considered the effects of no-tillage practices on soil nutrient dynamics. These insights would serve as a scientific foundation for the sustainable management of tea plantations and similar crop systems.

### Impact of climate on soil nutrient distribution in tea plantations

Climatic factors play a crucial role in influencing the distribution of soil nutrients. In the study area, factors such as rainfall and temperature

likely impact the carbon, nitrogen, and phosphorus contents in both the soil and tea leaves. These effects are mediated through processes like organic matter decomposition, nutrient leaching, and variations in root nutrient uptake efficiency. During periods of higher rainfall, surface soil carbon and nitrogen may leach into deeper layers, resulting in nutrient accumulation in the lower soil horizons and depletion in the surface soil. Additionally, fluctuations in the average annual temperature affect tea plant growth rates and metabolic activity, which in turn influence the distribution of these essential nutrients. The observed trend of increasing nutrient concentrations with soil depth was likely linked to the specific climatic conditions of the region.

While this study provided valuable insights into the nutrient dynamics of large-leaf tea plantations, there were certain limitations. The study's scope was confined to a single region, and variations in climate conditions and soil types across different regions might affect the generalizability of the results. Furthermore, the relatively short duration of data collection called for more extended monitoring to capture long-term changes in soil nutrient levels, especially under changing climatic conditions. Future research should focus on long-term dynamic monitoring of soil and tea leaf nutrient levels, incorporating different fertilization strategies to optimize nutrient management. Additionally, molecular biology techniques could be employed to explore the response mechanisms of tea plants to fluctuations in soil nutrients. Expanding the study to include comparisons across different regions and planting systems will help to formulate more universally applicable strategies for sustainable tea plantation development. Furthermore, investigating how improvements in the ecological environment such as increasing vegetation diversity regulating soil nutrient cycling will help enhance tea quality, yield, and ecosystem stability, ultimately supporting the green development of the tea industry.

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