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# Distribution and eco-stoichiometry of carbon and nitrogen of the plant-litter-soil continuum in evergreen broad-leaved forest

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## ABSTRACT

In order to understand the nutrient distribution characteristics and eco-stoichiometry of the evergreen broad-leaved forest in Dagang Mountain of Jiangxi Province, China, we investigated the distribution and stoichiometry of carbon and nitrogen along the plant-litter-soil continuum in evergreen broad-leaved forest. The total N concentration of the fine roots and litter had a high coefficient of variation of 16.22% and 9.17%, with a high spatial heterogeneity, respectively. The relationship between the carbon and nitrogen concentration and the functional traits of leaves and roots of constructive species was analyzed. The total C and N concentrations in leaves and roots were positively related. C concentration in the leaves and fine roots of *S. discolor* was relatively high, as 418.16 g·kg<sup>-1</sup> and 381.82 g·kg<sup>-1</sup>, leading to the strong carbon storage capacity in the whole plant. *A. fortunei* had low leaf and fine root C: N ratios (14.94 and 19.96), mainly causing its high growth rate. Compared to evergreen tree species, deciduous tree species had higher nitrogen concentration in leaves and roots, larger specific leaf area, smaller root surface area, and root length. With the increase of soil depth, the concentration of soil SOC, ROC, total N, alkali nitrogen, NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N decreased, but the variation of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N was minor. Among the components, alkaline N had a strong relation with soil C and N, and ROC was highly related with soil ammonium and nitrate nitrogen. The result in this study reveals the different survival strategies and relative adaptive mechanism of different species.

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## Introduction

The earth's ecosystems are changing on a global scale. Much of this change is caused by human activities. These changes have been exacerbated by increased technological and economic developments since the industrial revolution, and by rapid population growth since the Second World War. The structure of terrestrial ecosystems, and patterns in vegetation distribution, in particular, were the result of long-term climatic effects, and as such are at risk of change due to human activities. In-depth understanding of ecological processes and dynamics of nutrient cycling, in particular, the carbon cycle and the nitrogen cycle in terrestrial ecosystems, can provide a scientific basis from which to solve major global problems such as climate change and biodiversity loss (Guirui, Xuanran, and Ning 2014). These two cycles are closely linked through a series of material and energy transfer processes in the soil-plant-atmosphere system (Jiahui, Ning, and Congcong 2018).

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They are coupled together by a series of biochemical oxidation and reduction reactions. Affected not only by the stability of the quantitative balance between resource supply and demand but also by biological factors related to resource utilization and transformation efficiency (Shijie, Zhiyou, and Yunxi 2016).

Plants store C through photosynthesis and gradually replenish soil C and nutrients by depositing leaf litter (Ladanai, Ågren, and Olsson 2010). The distribution of elemental C and N among plant organs is restricted by the availability of soil nutrients, as well as by plant growth strategies, physiological characteristics, and life history. The distribution of C and N is therefore the result of interactions between environmental factors and species development (Gao, Li, and Xu et al. 2007). Litter is the starting point of the detrital food chain in forest ecosystems (Kang et al. 2010), an intrinsic part of the nutrient cycle and the main source of soil organic matter (Sayer 2006), which plays an important role in maintaining soil fertility and increasing forest productivity. As a link between plants and soil, litter plays a decisive role in nutrient cycling and energy flow in forest ecosystems (Domke, Perry, and Walters 2016). The return of nutrients, soil nutrient supply, plant nutrient requirements, and self-regulation of plants during litter decomposition all increase the complexities of researching plant-litter-soil system nutrient concentration (Ladanai, Ågren, and Olsson 2010).

Ecological chemometrics is a discipline that studies the relationship between multiple chemical elements during ecological processes (Elser et al. 2000). By combining the basic principles of biology, chemistry and physics, measurement of stoichiometric relationships serves as a means to integrate the different scales of the micro- and macro-worlds, providing links between individuals, populations, communities, and ecosystems. This strategy has been successfully used to explore the balance between nutrients, energy, and multiple chemical elements within an ecosystem (Bin, Yongjun, and Wenguang 2010; Dehui and Guangsheng 2005). Eco-chemochemistry unifies the differently scaled components of ecological entities at the elemental level by measuring carbon, nitrogen and phosphorus concentration and calculating their ratios. By doing this, we can better clarify the interactions among ecosystem components and the dynamic balance of chemicals in the process. An ecosystem's composition, function, and response to environmental changes can also be assessed using stoichiometric relationships. The relative abundance of different chemical components of the ecosystem can control the rate of nutrient cycling and energy flow; thus, we can simply use the ratio of elements to reveal the relationship between carbon and nitrogen cycles in an ecosystem, as well as the constraints and regulations of carbon and nitrogen in the process of mass transfer (Elser et al. 2000; Hessen, Elser, and Sterner 2013; Shaoqiang and Guirui 2008). The carbon and nitrogen cycles both transfer elements between plants, litter, and soil. Using plant-litter-soil as a complete continuum, the carbon and nitrogen concentration and ratios of leaves, litter, and soil in the biome can reveal the interaction between elements and the relationship between material balance and constraints in the chemical process (Shijie, Zhiyou, and Yunxi 2016).

The subtropical southern region of China is the most concentrated area of evergreen broad-leaved forests in the world. These are complex, multi-species broad-leaved forests composed mainly of evergreen trees from families such as Fagaceae, Lauraceae, and Theaceae. This type of forest has a complex functional structure, large biomass, and is rich in biodiversity, and thus is a valuable concentration of biological resources (Ying 1986). C and N are essential nutrients for plant production. In addition, their stoichiometry is indicative, of plant growth and development, and therefore of life history and survival strategies (Yongjing, Mengjuan, and Tongping 2018). Most of the study on stoichiometry is about the concentration of chemical elements and the characteristics change of their stoichiometric ratio, but there were few studies analyzing the relationship between stoichiometry and the functional traits of roots and leaves. In this study, the measurement of the carbon and nitrogen concentration of leaves, roots, litters and in the soil of six dominant tree species were applied to explore the different survival strategies of different species and their relative adaptive mechanism.

## Materials and methods

### Study site

The study site is located in the Dagangshan National Forest Ecological Station (114°30'~114°45'E, 27°30'~27°50'N) in Xinyu City of Jiangxi Province (Figure 1). The soil type is Kandiodults, whose main clay mineral is kaolinite. The vertical zonal change in altitude is very slight, and the soil remains very moist, loose, and has a thick humus layer. Other soils in the area include zonal low hilly red soil, yellow soil, and their sub-categories. The yellow soil is widely distributed, mainly at an altitude of 300–700 m; the red soil is mostly distributed in low hills (200 m above sea level). The pH value of the soil ranges from 4.0 ~ 5.0. The maximum elevation is 1091.8 m above sea level.

The climate is a subtropical monsoon humid climate. The average annual temperature is 16.8°C. Annual average solar radiation is 486.6 KJ·cm<sup>-2</sup>, annual average relative humidity is 81%, annual average evaporation is 1503 mm, and annual precipitation is 1593.7 mm with rainfall mostly concentrated in the period from April to June. The average annual frost-free period is 268 days. The percentage of forest cover of this area is 76.4%, and the vegetation is mainly natural secondary evergreen broad-leaved forest, deciduous broad-leaved forest, various coniferous and broad-leaved mixed forest, bamboo forest, and natural evergreen broadleaf forest.

### Methods and the collection of samples

#### Collection of soil, litter and plant samples

##### (1) Soil samples

Three sampling plots (20 m × 20 m) in the evergreen broad-leaved forest were randomly selected. Soils, litters, and plant samples were collected in November, 2017. In each plot, five subsampling plots (1 m × 1 m) were randomly selected, and samples (50 cm in diameter) from two layers of soil (0–10 cm and 10–20 cm) were taken using a soil drill, and then mixed evenly. Before measuring the NH<sub>4</sub><sup>+</sup>-N and

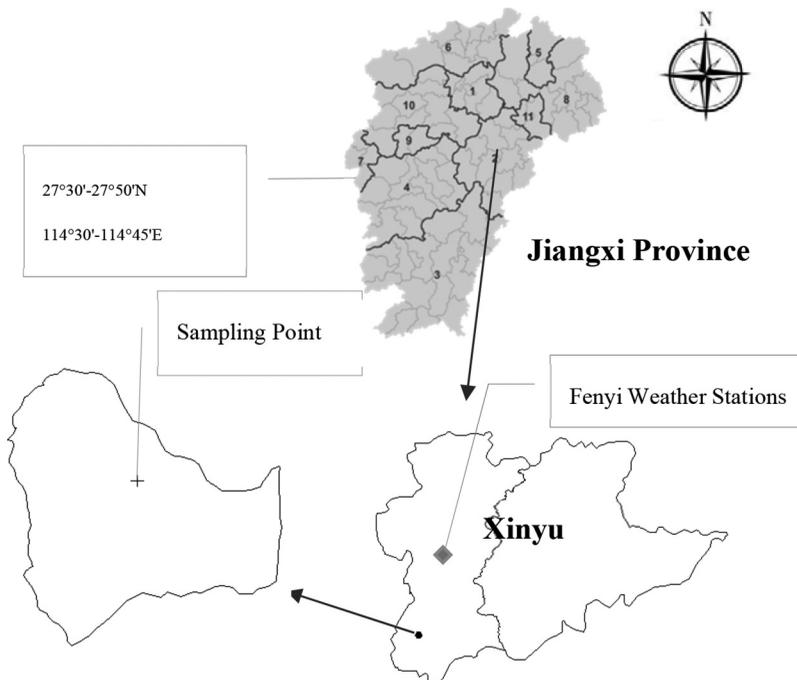


Figure 1. The location of the study area.

$\text{NO}_3^-$ -N concentration, the soil sample was passed through a 2 mm sieve. We then air-dried other soil samples, and they were then filtered through a 0.25 mm sieve to determine TN (total nitrogen), through a 2 mm sieve to determine Alkaline N, SOC, and ROC.

### (2) Litter samples

Five brackets made from nylon gauze (1 m × 1 m) were used for the collection of litters (dry weight 1175.85 g) in each plot. These samples were then mixed together in a Kraft paper bag, and dried in the oven at 80°C to a constant weight. The sample was then ground into a powder of 0.10 mm by a pulverizer for elemental analysis.

### (3) Plant samples

According to the community survey data, the constructive species of evergreen broad-leaved forest were *Sapium discolor* (Champ. ex Benth.) Muell., *Castanopsis fargesii* Franch., *Cyclobalanopsis myrsinifolia* (Blume) Oersted, *Machilus pauhoi* Kanehira, *Alniphyllum fortunei* (Hemsl.) Makino, and *Vernicia fordii* (Hemsl.) Airy Shaw (Table 1). For each species, we randomly selected three plants without obvious symptoms from which to collect the mature leaves in the central and upper parts of the plant facing four directions (i.e., north, east, south, and west). The samples were brought back to the laboratory to test the carbon and nitrogen concentration and to measure leaf functional traits. At the same time, in order to avoid inference, select a position 0.5 m from the trunk and less shrubs and herbs, and collect a 40 cm-deep fine root sample using a 50 mm diameter soil drill. Digging root segments that can clearly distinguish 1–2 grade of roots without breakage or breakage were used to determine root functional traits. We dug three cores, randomly placed, of the roots of each plant. These soil samples were rinsed with running water using a sieve with a pore size of 0.5 mm to leave the fine roots. The fine root samples were then placed in a 65°C oven, dried to a constant weight, and ground into a 0.10 mm powder by a pulverizer for measuring the total carbon and total nitrogen concentration.

We measured specific leaf area, leaf lifespan, specific root length, and area. Leaf area was scanned with Epson-v37 scanner for 6 to 10 leaves for each tree, then killed in the oven for a constant weight and weighed. Specific leaf area is equal to the leaf area to dry weight. Specific root length: Scanned all roots of each tree with the scanner Epson-v37 and calculated the length and surface area of the roots with WinRHIZOTRON, then dried at 60°C in an oven for 48–72 h and weigh with an electronic balance. Specific root length is equal to the root length to the dry weight.

According to the scaly marks on the branches, the growth age of the branches was identified, and the number of leaves on the branches of different ages was calculated. At the age of one year, the leaves that were developed in the year were 1 year old leaves, and the leaves that grew last year were 2 years old leaves, and so on. According to this, the leaf life was compiled and the expected life of the blade was calculated by the formula. The formula is as follows:

$$L_x = \frac{N_x + N_{x+1}}{2} \quad (1)$$

$$T_x = \sum_x^{\infty} L_x \quad (2)$$

$$E_x = \frac{T_x}{N_x} \quad (3)$$

**Table 1.** Average tree height and DBH of the sampling tree.

Number	SD	CF	CM	MP	AF	VF
Height (m)	22.4	18.2	17.4	21.5	15.3	24.8
DBH (cm)	29.4	39.1	28.7	31.0	25.9	39.3

In the formula:  $X$  is the leaf age level;  $L_x$  is the average number of surviving leaves from  $x$  to  $x + 1$  age;  $N_x$  is the number of leaves initially surviving at the age of  $x$ ;  $T_x$  is the total numbers of leaves remaining in the  $x$ -age and above ages;  $E_x$  is the average life expectancy at the beginning of the  $x$  period.

### Stoichiometric element determination

The concentration of organic carbon in plant leaves, litter, fine roots, and soil was determined using the Walkley-Black chromic acid wet oxidation method. To determine the total nitrogen concentration, plant leaves, litter, and fine root samples were digested by  $H_2SO_4-H_2O_2$ , and the resulting solution was analyzed using a Kjeldahl nitrogen analyzer (KDY-9380). To determine the total soil nitrogen concentration, a sulfuric acid-mixed catalyst was first used, and then the nitrogen concentration in the digested solution was determined by a Kjeldahl nitrogen analyzer (KDY-9380). Soil ammonium nitrogen ( $NH_4^+$ -N) and nitrate nitrogen ( $NO_3^-$ -N) concentration were measured using a continuous flow analyzer (SKALAR San++ continuous flow analyzer). ROC was measured with  $333 \text{ mmol}\cdot\text{l}^{-1}$ . Soil alkaline nitrogen was measured using the diffusion method (Rukun 1999). The total number of leaves, roots, litter, and soil samples were measured 207 times (leaves:  $6 \times 3 \times 3$ , roots:  $6 \times 3 \times 3$ , litter:  $3 \times 5 \times 3$ , soil:  $3 \times 2 \times 5 \times 3$ ).

### Data analysis

The C and N concentrations of the leaves, fine roots, and collected litter of the constructive species were all presented as mass percentage, and C:N ratios were presented as elemental mass ratios. We calculated Pearson's correlation coefficient to assess the correlation between C:N ratios in leaves, fine roots, litter, and soil from different layers and different trees, and we used One-way ANOVA (Tukey) to assess significance in differences between each category. Statistical analysis was carried out using SPSS 20.0 software ( $\alpha = 0.05$ ).

## Results

### C and N stoichiometry in the leaf-litter-soil continuum

Carbon, nitrogen, and C:N ratio concentration of leaves were  $401.87 \pm 20.56 \text{ g}\cdot\text{kg}^{-1}$ ,  $21.41 \pm 1.75 \text{ g}\cdot\text{kg}^{-1}$  and  $19.27 \pm 2.58 \text{ g}\cdot\text{kg}^{-1}$ , as Table 2. The coefficient of variation (CV) of the average concentration of C and N in the leaves was 5.12% and 8.17%. Carbon, nitrogen, and C:N ratio concentration of fine root were  $348.64 \pm 11.64 \text{ g}\cdot\text{kg}^{-1}$ ,  $15.73 \pm 1.40 \text{ g}\cdot\text{kg}^{-1}$  and  $23.97 \pm 1.12$ . The coefficient of variation (CV) of the average concentration of C and N in the litter was 3.34% and 8.90%. Carbon, nitrogen, and C:N ratio concentration of litter were  $323.06 \pm 20.35 \text{ g}\cdot\text{kg}^{-1}$ ,  $12.76 \pm 2.07 \text{ g}\cdot\text{kg}^{-1}$  and  $25.58 \pm 2.52$ . The coefficient of variation (CV) of the average concentration of C and N in the litter was 6.3% and 16.22%. Carbon, nitrogen, and C:N ratio concentration of soil were  $16.40 \pm 1.02 \text{ g}\cdot\text{kg}^{-1}$ ,  $1.09 \pm 0.10 \text{ g}\cdot\text{kg}^{-1}$  and  $16.27 \pm 1.22$ . The coefficient of variation (CV) of the average concentration of C and N in the litter was 6.22% and 9.17%.

**Table 2.** Average, maximum, and minimum values of leaf, litter, fine root and soil C and N concentration of six constructive species in Dagangshan, Jiangxi Province.

	C(g·kg <sup>-1</sup> )	N(g·kg <sup>-1</sup> )	C:N
Leaf	401.87(20.56)	21.41(1.75)	19.27(2.58)
	349.71–489.78	16.72–27.00	14.97–23.33
Fine root	348.64(11.64)	15.73(1.40)	23.97(1.12)
	318.55–384.92	10.06–22.50	17.11–34.24
Litter	323.06(20.35)	12.76(2.07)	25.58(2.52)
Soil	16.40(1.02)	1.09(0.10)	16.27(1.22)
	15.32–17.34	0.98–1.16	15.44–17.67

Note: Numbers in the parentheses are standard deviation.

### The C&N stoichiometry and functional traits of leaves and fine roots of different species

The descending orders of C concentration in leaves were *C. Myrsinifolia*, *S. discolor*, *V. fordii*, *C. fargesii*, *A. fortune*, and *M. pauhoi*, as Figure 2. N concentration descending orders were *V. fordii*, *C. myrsinifolia*, *A. fortune*, *M. pauhoi*, *S. discolor*, and *C. fargesii*. The C:N ratios descending orders were *S. Discolor*, *C. fargesii*, *C. myrsinifolia*, *M. pauhoi*, *V. fordii*, and *A. fortune*.

C concentration in fine roots descending orders were *M. pauhoi*, *S. Discolor*, *C. fargesii*, *C. myrsinifolia*, *A. fortune*, and *V. fordii* as Figure 3. N concentration in fine roots descending orders were *M. pauhoi*, *S. Discolor*, *A. fortune*, *V. fordii*, *C. Myrsinifolia*, and *C. fargesii*. The C:N ratios in fine roots descending orders were *C. fargesii*, *C. Myrsinifolia*, *V. fordii*, *A. fortune*, *S. Discolor* and *M. pauhoi*.

*S. discolor*, *A. fortunei* and *V. fordii* are all deciduous species, while *C. fargesii*, *C. myrsinifolia*, and *M. pauhoi* are evergreen as Table 3. Leaf lifespan is longer for evergreen trees, with the longest of all being found in *S. discolor* and the shortest in *V. fordii*. Specific leaves area of deciduous tree species have on average a larger area than those of evergreen trees, the largest was *V. fordii* and the smallest was *C. fargesii*. The root surface area of evergreen tree species was larger than deciduous tree species: the largest was *C. myrsinifolia*, and the smallest was *S. discolor*. Specific root length was larger in deciduous species than in evergreen tree species, with the largest being found in *A. fortunei*, and the smallest in *C. fargesii*.

### Distribution of C and N in different soil layers and their ecological stoichiometry

With the increase of soil depth, the concentration of soil SOC, ROC, total N, alkali nitrogen,  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  decreased, the variation of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were minor as Table 4. At 0–10 cm soil depth, the total N, alkali nitrogen,  $\text{NO}_3^-\text{-N}$ , and C:N ratio had a significant CV; at 10–20 cm soil depth, the CV of ROC, alkaline nitrogen, and  $\text{NO}_3^-\text{-N}$  were significant. In general, the horizontal spatial heterogeneity of soil C and  $\text{NH}_4^+\text{-N}$  levels in the study area was not obvious, whereas the horizontal spatial distribution of soil  $\text{NO}_3^-\text{-N}$  concentration was significantly heterogeneous.

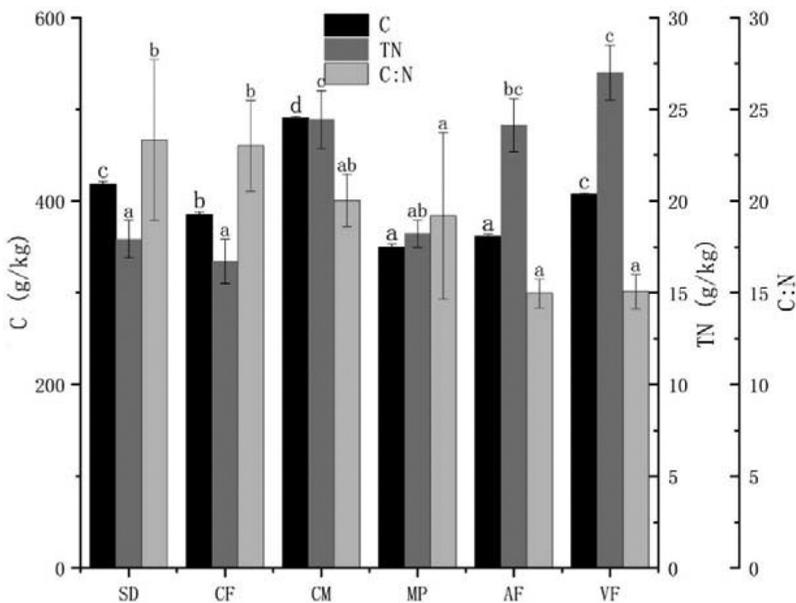
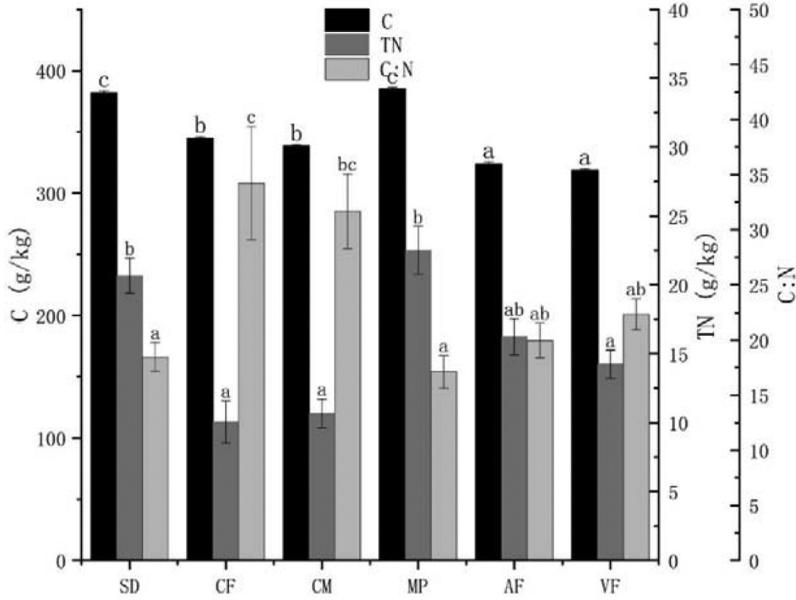


Figure 2. Concentration of carbon and nitrogen in leaves of six species of evergreen broad-leaved forest.



**Figure 3.** Concentration of carbon and nitrogen in fine roots of six species of evergreen broad-leaved forest.

**Table 3.** Characteristics of leaves and roots of six species of evergreen broad-leaved forest.

	leaves		fine roots	
	Lifespan(a)	SLA(cm <sup>2</sup> ·g <sup>-1</sup> )	Surface area(cm <sup>2</sup> )	SRL(m·g <sup>-1</sup> )
SD	0.67(0.11)	135.81(4.62)	0.17(0.005)	95.63(4.83)
CF	2.36(0.27)	104.88(3.78)	0.338(0.005)	73.45(3.21)
CM	1.46(0.41)	153.37(3.56)	0.357(0.006)	85.15(3.45)
MP	1.76(0.74)	106.32(4.89)	0.302(0.014)	83.42(3.89)
AF	0.60(0.07)	285.86(5.31)	0.219(0.008)	118.16(5.08)
VF	0.62(0.41)	1286.88(9.83)	0.235(0.007)	79.37(4.81)

Note: Same as Table 3. SLA is specific leaf area, and SRL is specific root length.

**Table 4.** Distribution and stoichiometry of soil carbon and nitrogen at different depths.

Depths	SOC	ROC	N	Alkaline N	NH <sub>4</sub> <sup>+</sup> - N	NO <sub>3</sub> <sup>-</sup> - N	C:N
	g·kg <sup>-1</sup>	g·kg <sup>-1</sup>	g·kg <sup>-1</sup>	mg·kg <sup>-1</sup>	mg·kg <sup>-1</sup>	mg·kg <sup>-1</sup>	
0-10	20.0 (1.38)	15.24 (0.43)	1.46 (0.28)	70.73 (25.67)	1.72 (0.11)	3.19 (2.01)	14.21 (3.70)
10-20	12.73 (0.89)	6.13 (1.14)	0.72 (0.08)	36.47 (6.47)	1.57 (0.11)	3.07 (1.80)	17.75 (0.72)

Note: SOC is soil organic carbon. ROC is soil oxidizable carbon. N is soil nitrogen concentration. \*\* is significantly correlated at the 0.01 level, and \* is significantly correlated at the 0.05 level.

### **Correlation between C and N distribution and ecological stoichiometry**

As shown in Table 5, at 0-10 cm soil depth, alkaline nitrogen in the study area was positively correlated with soil SOC. On the other hand, at 10-20 cm soil depth, soil alkaline nitrogen was positively correlated with total N; ROC in the study area was positively negatively with NH<sub>4</sub><sup>+</sup>-N at 0-10 cm soil depth. On the other hand, at 10-20 cm soil depth, ROC was positively correlated with NO<sub>3</sub><sup>-</sup>-N .

**Table 5.** Correlation between different carbon and nitrogen components.

		SOC	N	NH <sub>4</sub> <sup>+</sup> – N	NO <sub>3</sub> <sup>-</sup> – N
0–10 cm	Alkaline N	0.724*	-	-	-
	ROC	-	-	-0.637*	-
10–20 cm	Alkaline N	-	0.787**	-	-
	ROC	-	-	-	0.591*

Note: \*\* is significantly correlated at the 0.01 level, \* significantly correlated at the 0.05 level. – is not significant for correlation.

## Discussion

### *C and N concentration and their ecological stoichiometry in different plant parts*

As shown in Table 2, the CV of the average N concentration in fine roots and litter was 8.90% and 16.22%, respectively, indicating that there is a large degree of the spatial distribution of total N concentration in the fine roots of different tree species in the study area. Since the major pathways for C assimilation, photosynthesis, and nutrient absorption are different pathways, C is usually not a limiting element of plant growth, and thus C concentration shows littler variation in most plants (Zhaoxia, Kelin, and Xiaoli 2015).

Studies have shown that the total C and N concentration of the leaves of 102 dominant plants in southeastern China ranges from 374.1 to 646.5 g·kg<sup>-1</sup> and from 8.4 to 30.5 g·kg<sup>-1</sup>, respectively (Shujie, Guirui, and Chunming 2013). In this study, we measured the leaf N concentration of six representative species of evergreen broad-leaved forest in the study area and found the average values were consistent with the values of the dominant plant leaves in the eastern and southern transects. The average carbon concentration was 401.87 g·kg<sup>-1</sup>, which was lower than that of 492 terrestrial plants worldwide (464 g·kg<sup>-1</sup>) (Elser, O'Brien, and Dobberfuhl 2000). This may be explained by the fact that our sample collection was done in autumn, a season when plants are not metabolically active and the turnover rate is not as high as that during other seasons. The average concentration of leaf N in the six representative trees was 21.41 g·kg<sup>-1</sup>, which was slightly higher than the global average concentration of N in plant leaves (20.09 g·kg<sup>-1</sup>) (Shujie, Guirui, and Chunming 2013). This value was higher than one in 753 Chinese terrestrial plants (18.6 g·kg<sup>-1</sup>) (Wenxuan et al. 2005). This may be related to the high resorption rate of leaf N, thus promoting nitrogen retention and reuse of N and enlarging the leaf N pool. Previous studies have shown the average concentration of C and N in fine roots of Chinese terrestrial plants were 473.9 g·kg<sup>-1</sup> and 9.16 g·kg<sup>-1</sup>, respectively. The litter value is within the range of global average root N concentration (9.90 ~ 11.2 g·kg<sup>-1</sup>). The worldwide data also showed that fine root N concentration and soil nutrients are positively correlated (Yuzhu, Quanlin, and Bingjie 2015). The average concentration of fine root N in our six species was 15.73 g·kg<sup>-1</sup>, which was higher than the fine root N concentration of terrestrial plants both in China and globally.

### *Soil C and N concentration and their ecological stoichiometry*

Using soil samples collected from different depths, we found that the soil SOC, ROC, total N, and alkali nitrogen concentration in our study area decreased with an increase of soil depth. A possible reason for this is that the decomposition of litter occurs on the surface layer of soil, and constantly moves downward (Qingni, Qingpei, and Dingkun 2013; Rad 2017). The deposition, leaching, ammoniation, and denitrification of atmospheric nitrogen have a great impact on the changes of soil nitrogen concentration (Baorong, Quanchao, and Qishan 2017; Guohong, Guangsheng, and Li 2006). In our study area, soil NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N did not display a significant difference between 0–10 cm soil samples and 10–20 cm soil samples. This may be because the effects of ammoniation and nitrification on the concentration of NH<sub>4</sub><sup>+</sup>-N and NO<sub>3</sub><sup>-</sup>-N in soil were greater than those of atmospheric nitrogen deposition and leaching. The soil NO<sub>3</sub><sup>-</sup>-N concentration in the study area was higher

than that of  $\text{NH}_4^+$ -N, which is consistent with the results of Song et al. (Jones, Leafe, and Stiles 1978). In their study, the authors revealed that the soil  $\text{NH}_4^+$ -N pool is larger in winter and spring, whereas in summer and autumn, the soil  $\text{NO}_3^-$ -N pool is larger.

Alkaline N is an important indicator of the level of total N, and it can accurately reflect soil N dynamics and N supply levels in an organism (Shuiqing, Shan, and Doudou 2017). At 0–10 cm soil depth, the Alkaline N in the study area was positively correlated with soil SOC, and at 10–20 cm soil depth, the alkaline N was significantly positively correlated total N, indicating that the nitrogen supply capacity is high when the soil carbon concentration is abundant. ROC is an important part of soil active organic carbon, which can sensitively reflect changes in soil quality (Xue, Shiji, and Shuqi 2016). It may have certain effects on soil microbes, thus affecting the positive correlation between ammonification rate and soil  $\text{NH}_4^+$ -N concentration. The soil ROC was negatively correlated with  $\text{NH}_4^+$ -N at 0–10 cm soil depth. On the other hand, at 10–20 cm soil depth, ROC was positively correlated with  $\text{NO}_3^-$ -N. At the same time, the absorption of roots and the soil moisture status affect the migration and distribution of nutrients in the soil, and the effect on nitrate nitrogen is greater than that of ammonium nitrogen (Barber 1984). Xue Ming's research results show that different nitrogen form ratios are beneficial to the consumption of ROC by microorganisms (Xue, Y X, and Ma et al. 2018). When ammonium nitrogen is used as the main nitrogen supply form, microorganisms consume the most ROC. However, the ratio of ammonia nitrate to nitrate and nitrate-based worksheets is more favorable for ROC content in SOC.

### ***Relationship between plant functional traits and C and N concentration and their eco-stoichiometry***

C and N concentration and eco-stoichiometry characterize plant growth strategies. Carbon and nitrogen are indispensable elements for plant growth and development: carbon is the most important element in plant dry matter, while nitrogen is a limiting element in plant growth (Wenhua, Zhihong, and Wende 2006; Ying, Shuying, and Ping 2014). The average C: N ratio of leaves and fine roots of deciduous species was smaller than that evergreen trees. The evergreen tree species are generally distributed in the lower layer, and the deciduous tree species are relatively distributed in the upper layer (Jones, Leafe, and Stiles 1978). The evergreen tree species have a slow growth strategy. This survival strategy is the result of long-term adaptation of the organism to the environment, and the plant regulates the concentration of the elements in the body to achieve a better living state. The concentration of C and N in the leaves and the leaf lifespan determine the concentration of C and N in the litter and the input amount, which affects soil carbon concentration and nutrient supply. The leaf lifespan of deciduous tree species was shorter than that of evergreen tree species, which means that the vegetative cycle rate of deciduous tree species is higher than that of evergreen tree species. Compared with evergreen tree species, there are more nutrients in the environment for deciduous tree species to absorb and utilize. The functional traits of leaves and roots of deciduous tree species tend to grow rapidly, such as short leaf life, large leaf area, small surface, and large roots. The functional traits of leaves and roots of evergreen trees tend to grow slowly, and absorb and store more nutrients. Then, the strategies for reabsorption of nitrogen by the leaves are also different, the difference of the litter input amount and frequency of results in the differences of the soil carbon and nitrogen concentration. It also explains why the N concentration of leaves and roots in deciduous tree species is greater than that of evergreen tree species. Because their rate of litter nutrient cycling is fast, the specific root length of deciduous trees is greater than that of evergreen trees. In order to adapt to the slower rate of nutrient cycling, the evergreen tree species showed a larger root surface area to facilitate nutrient absorption. Because the nutrient cycling rate interacts between different components of the plant-litter-soil continuum, the coefficient of variation of the N concentration of soil and litter is high.

Among the six representative species we studied, *S. discolor* contained a higher amount of C in leaves and fine roots, indicating that its carbon storage capacities are remarkable. As a main element in chlorophyll, N affects plant chlorophyll activity, photosynthetic rate, enzymatic activities during dark

reactions, and photorespiration rate (Xuefeng, Wei, and Zhijun 2013). The rate of respiration is related to the amount of N in plant tissues (McGroddy, Daufresne, and Hedin 2004). In plant cells, 90% of N is present in proteins, and these proteins need energy to self-repair (Elser, Acharya, and Kyle 2003). The N concentration and its ratio to C in plants are strong indicators of the plant's internal stability. The concentration of C and N in leaves and fine roots of our six different species was generally consistent, showing a positively correlating relationship (i.e., if the concentration of C is abundant, then the concentration of N is also abundant and vice versa). By comparing the C:N ratios of the leaves and fine roots of different tree species, we found that when the C:N ratio is higher, N is less abundant. Among the six species we studied, *C. fargesii*, *C. myrsinifolia*, *A. fortunei* and *V. fordii* contained higher leaf N concentration than root N concentration, and the ratio of root N to C is higher than that of leaf N to C, suggesting these species have a high nitrogen transfer rate. However, *S. discolor* and *M. pauhoi* showed that the root N concentration was greater than the leaf N concentration. The difference is that *S. discolor* grows slower in deciduous tree species and *M. pauhoi* faster in evergreen tree species. The distribution of elements in plants is due to the changes in competitive strategies. Among the deciduous species, the surface area of fine roots of *S. discolor* was the smallest, and the surface of fine roots of *M. pauhoi* was the smallest in the evergreen tree species, indicating that the surface of fine roots may be related to nitrogen absorption and transfer. The different C: N of plant organs is the result of different processes of plant absorption, transportation, distribution, utilization, and release of chemical elements, which makes the growth rate of plants different. For most organisms, the high growth rate often corresponds to a high N:C ratio (Wenxia 2016). The high N concentration of both leaves and roots implicated a fast growth rate of the plant. In our study, we found that consistent with the growth rate hypothesis, that *A. fortunei* had high leaf and fine root N:C ratios, and the growth rate of this species was high. This species is a fast-growing species, and at the same time a pioneer species in secondary bare land.

The previous analysis showed that the C and N contents and functional traits of different organs of the plant were the results of the life history strategies of the plant. The concentration of C and N in various plant organs has a high spatio-temporal heterogeneity. At the same time, the plant's reabsorption of N in leaf litter is the N concentration being used and transported in the plant, this mechanism should pay more attention in the future study.

Besides, there are also differences in the turnover rates of C and N of different species due to the different leaf lifespan by measuring the input of leaf litter C and N concentration on soil C and N concentration is helpful to recognize the nutrient carrying capacity and the plant functionality. The concentration of phosphorus is also important in the stoichiometry which should be considered with C and N together in the future.

## Conclusion

According to the investigation of the C and N stoichiometric characteristics of leaves, litter, fine roots, and soil of evergreen broad-leaved forests, the following main conclusions can be drawn:

(1) Carbon and nitrogen distribution characteristics of leaves, litters, and fine roots in evergreen broad-leaved forests

The average C and N concentration in leaves, fine roots, and litter was significantly different from the average concentration of soil. The spatial distribution of total N concentration is greater than that of C in fine roots of the different tree species in the study area. The C:N ratio of litter is greater than the average leaf C:N ratio, which is probably due to the reabsorption of N nutrients by plant leaves.

(2) Carbon and nitrogen stoichiometry characteristics of dominant tree species in leaves and fine roots

Of the six plants, the descending orders of C concentration in leaves were *C. Myrsinifolia*, *S. discolor*, *V. fordii*, *C. fargesii*, *A. fortune*, and *M. Pauhoi*. The average N concentration descending orders were *V. fordii*, *C. myrsinifolia*, *A. fortune*, *M. pauhoi*, *S. discolor*, and *C. fargesii*. The C:N ratios descending orders were *S. Discolor*, *C. fargesii*, *C. myrsinifolia*, *M. pauhoi*, *V. fordii*, and *A. fortune*.

C concentration in fine roots descending orders were *M. pauhoi*, *S. Discolor*, *C. fargesii*, *C. myrsinifolia*, *A. fortune* and *V. fordii*. N concentration in fine roots descending orders were *M. pauhoi*, *S. Discolor*, *A. fortune*, *V. fordii*, *C. Myrsinifolia*, and *C. fargesii*. The C:N ratios in fine roots descending orders were *C. fargesii*, *C. Myrsinifolia*, *V. fordii*, *A. fortune*, *S. Discolor* and *M. pauhoi*. The C concentration in the leaves and fine roots of *S. discolor* were relatively high, and so its carbon storage capacity is strong. *A. fortunei* had low leaf and fine root C: N ratios, and the growth rate of this species was high. This species is a fast-growing species, and at the same time a pioneer species in secondary bare land. The nitrogen concentration of leaves and roots showed that deciduous trees were larger than evergreen trees. The other traits were characterized in that the specific leaf area of the deciduous tree species was larger than that of the evergreen tree species, the root surface area of the deciduous tree species was smaller than that of the evergreen tree species, and the specific root length of the deciduous tree species is greater than that of the evergreen tree species.

### (3) Correlation of carbon and nitrogen components in different soil layers

With the increase of soil depth, the concentration of soil SOC, ROC, total N, alkali nitrogen,  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N decreased, the change of  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N were minor. At 0–10 cm soil depth, the alkaline nitrogen was positively correlated with SOC, and ROC was negatively correlated with  $\text{NH}_4^+$ -N. And at 10–20 cm soil depth, alkaline nitrogen was positively correlated with N, and ROC was positively correlated with  $\text{NO}_3^-$ -N. It indicated that the nitrogen supply capacity is also high when soil carbon is abundant. The difference in the relationship between carbon and nitrogen components was the result of leaching and microbial differences in active carbon and nitrogen utilization.

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