

Original Research

# Variations in Carbon, Nitrogen and Phosphorus Stoichiometry during a Growth Season Within a *Platycladus orientalis* Plantation

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## Abstract

Knowledge of seasonal variations of carbon (C), nitrogen (N), and phosphorus (P) concentrations plus C:N:P ratios can provide insight into the dynamics of biological processes, nutrient cycling, resource acquisition, and mass partitioning in plants and soils. *Platycladus orientalis* (Cupressaceae) is an important tree species distributed throughout northern China. We studied the seasonal dynamics of C, N, and P concentrations and their stoichiometry in leaves, branches, roots, and soil in a *P. orientalis* plantation. Plant and soil C, N, and P concentrations, and their stoichiometry varied significantly among ecosystem components and growth season sampling times, indicating nutrient allocation patterns and mobility in the *P. orientalis* plantation. Accurately quantifying and comparing C:N:P stoichiometry in the plant and soil of *P. orientalis* requires a study of plant organs, soil depth, and sampling times. The mean N concentration in leaves was 14.06 g kg<sup>-1</sup> and the mean N:P ratio was 11.82, indicating that the growth of *P. orientalis* was N limited during the growing season. N and P concentrations and stoichiometry in plants were correlated with those in soil, suggesting coupled nutrient control between plant and soil. The C:N:P ratio was regulated in different components of the ecosystem.

**Keywords:** *Platycladus orientalis*, C:N:P stoichiometry, plant-soil, nutrient limitation

## Introduction

Carbon (C), nitrogen (N), and phosphorus (P) are primary nutrients that regulate important ecological functions, structures, and processes [1]. Ecological stoichiometry involves the balance of multiple

chemical elements in an organism and how this shapes ecological interactions [2, 3]. Ecological stoichiometry is important for understanding the adaptive strategies of plants. It provides insight into the relationships between organism and ecosystem structure and the relationship between the C:N:P stoichiometry of a species and its environment [4-6].

Nutrient concentrations in plants reflect nutrient uptake and utilization efficiency during growth. Nutrient concentrations are important in the maintenance

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of plant function and in adaptation to a changing environment [5]. Fast-growing species or fast-growing stages require more N and P compared to C, and more P compared to N [2, 7]. The leaf N:P ratio is an important index that reflects the shift between N- and P-limitations [8, 9]. Soil is the main nutrient source for plants. Extractable nutrients in soil regulate plant growth. Studies on ecological stoichiometry and seasonal dynamics of soil C, N, and P concentrations reveal the effectiveness and limitations of soil nutrients [10]. The plant-soil feedback mechanism involves the nutrient relationship between plant and soil. It is therefore necessary to study ecological stoichiometry on the whole plant-soil system [11, 12].

Nutrient concentrations of plants can vary within and between growing seasons. The resorption of nutrients is essential in supporting spring growth in deciduous species and forbs, which show large seasonal variations in nutrient concentrations. In contrast, evergreen species can retain leaf nutrients and resorption can occur gradually over the growing season [13, 14]. Some stoichiometry studies have been conducted on plants and soils over a particular season or time period [15-17], but seasonal variations of stoichiometry remain poorly understood. Studying the seasonal dynamics of C, N, and P concentrations and their stoichiometry could increase our understanding of nutrient allocation and re-use; further study could also improve forest management, particularly for tree species that grow in ecologically fragile and nutrient-limited areas.

*Platycladus orientalis* (Cupressaceae) is an evergreen tree species that is highly resistant to cold, drought, and saline-alkaline soil. It is long-lived and well adapted to many different environments. Due to the ability to thrive under a wide range of climate and soil conditions, *P. orientalis* is an important afforestation species. It is widespread throughout China, particularly in ecologically fragile sites and mining areas, and is used for sand stabilization and soil erosion control. Its wood is strong and decay-resistant, making it valuable for building, ship construction, and furniture [18-21]. In this study, we hypothesized that *P. orientalis* growth in the Jiulong Mountains is limited by N or P in the entire growing season. We also hypothesized that *P. orientalis* and soils would show seasonal variation in their nutrient concentrations and stoichiometric ratios. We determined the C, N, and P concentrations as well as the C:N, C:P and N:P ratios of trees and soils in a *P. orientalis* plantation among different stages of a growing season. The specific objectives were to: (1) compare the differences of C:N:P stoichiometry for tree organs, soil, and growth season stage; (2) determine the relationships between stoichiometry between plant organs and soil, and (3) detect the nutrient limitations of *P. orientalis* plantations.

## Materials and Methods

### Study Area

The study area was a *P. orientalis* plantation the Jiulong Mountains (100-997 m altitude, 115°59'-116°07'E, 39°54'-39°59'N). This region has a temperate continental monsoon climate. The average annual temperature is 11.8°C, and the mean annual precipitation is 623 mm. The soil is a brown, rocky mountain forest soil with a high stone content, and the average soil layer thickness ranges from 20 to 50 cm. The forest was planted in 1981 at a density of 3000 trees per hectare. The dominant tree species are *P. orientalis*, *Pinus tabulaeformis*, and *Quercus variabilis* [22, 23].

### Sample Sampling

This study was conducted during April 2016. Three plots of 20 m × 20 m were established in a typical stand that could represent the average state (roughly estimated DBH, tree height, slope) of the *P. orientalis* plantation stand in the study area. Mean diameter at breast height (DBH) was 1.3 m and height in the inventory plots was 6.6 cm and 7.3 m. Buds of *P. orientalis* start to burst, and tree growth begins, in mid-April. Trees grow rapidly during July, August, and early September; growth slows in late October. The growth stages of *P. orientalis* were divided into an early growing season (April-June), a fast growing season (July-September), and a late growing season (October).

Plant samples (leaves, branches, and roots) were collected in each plot in the middle of April, June, August, and October 2016. Five standard trees, representing average DBH range, medium size, growth, and crown width, were selected based on the average of the site index of *P. orientalis*. Leaves and branches were sampled from 12 directions of each standard tree using a pole pruner (the directions were the upper, middle, and lower parts of the eastern, southern, western, and northern tree crowns). The sampled leaves and branches were mixed for each plot. Roots were sampled 50 cm from the base of each standard tree at depths of 0-10 cm and 10-20 cm using 10 cm diameter soil cores. All visible roots were collected. Leaves, branches, and roots sampled were washed with tap water and oven-dried at 65°C to a constant mass. These plant samples were then ground (CT 193 Cyclotec, FOSS, Denmark) and passed through a 10-mesh sieve for C, N, and P content analysis [24].

Soil samples were collected at the same times as plant sampling. Soil samples were collected at depths of 0-10 cm and 10-20 cm with an 8 cm diameter hand core after removing mulch from the plot. In each plot, five soil cores of each soil layer were randomly collected and mixed. All soil samples were air-dried and ground (CT 193 Cyclotec, FOSS, Denmark) to a fine powder

Table 1. Descriptive statistics of C, N, and P concentrations and their stoichiometric ratios for leaves, branches, roots, and soil.

Element	Items	Mean	Range	Minimum	Maximum	SD	CV
C (g kg <sup>-1</sup> )	Leaves	446.22	32.27	431.27	463.54	10.32	2.31%
	Branches	462.10	29.73	446.21	475.94	8.38	1.81%
	Roots (0-10 cm)	419.19	100.65	368.69	469.34	25.48	6.08%
	Roots (10-20 cm)	417.98	96.01	369.94	465.95	30.01	7.18%
	Soil (0-10 cm)	32.50	26.73	22.50	49.23	9.53	29.31%
	Soil (10-20 cm)	21.00	12.04	14.25	26.29	4.05	19.30%
N (g kg <sup>-1</sup> )	Leaves	14.06	5.51	10.85	16.36	1.82	12.95%
	Branches	6.57	2.29	5.14	7.43	0.68	10.32%
	Roots (0-10 cm)	9.38	3.91	7.65	11.56	1.16	12.40%
	Roots (10-20 cm)	8.05	4.10	6.50	10.60	1.38	17.14%
	Soil (0-10 cm)	2.09	1.54	1.48	3.02	0.56	26.68%
	Soil (10-20 cm)	1.78	2.01	1.01	3.02	0.53	29.88%
P (g kg <sup>-1</sup> )	Leaves	1.23	1.05	0.80	1.85	0.32	26.37%
	Branches	0.73	0.33	0.55	0.88	0.11	15.61%
	Roots (0-10 cm)	0.40	0.33	0.22	0.55	0.09	22.31%
	Roots (10-20 cm)	0.39	0.22	0.28	0.50	0.06	16.24%
	Soil (0-10 cm)	0.63	0.75	0.30	1.05	0.22	35.26%
	Soil (10-20 cm)	0.59	0.54	0.26	0.80	0.18	30.51%
C:N	Leaves	32.30	15.03	26.36	41.39	4.78	14.81%
	Branches	71.12	28.85	62.76	91.61	8.53	11.99%
	Roots (0-10 cm)	45.38	21.10	34.75	55.85	6.55	14.43%
	Roots (10-20 cm)	53.40	28.69	36.33	65.02	10.20	19.11%
	Soil (0-10 cm)	15.52	2.98	14.10	17.08	0.98	6.29%
	Soil (10-20 cm)	14.22	1.69	13.48	15.17	0.44	3.12%
C:P	Leaves	386.83	320.42	250.75	571.17	101.25	26.17%
	Branches	644.85	286.26	542.19	828.45	106.42	16.50%
	Roots (0-10 cm)	1111.90	1294.30	674.48	1968.78	328.73	29.56%
	Roots (10-20 cm)	1086.80	860.11	776.95	1637.06	213.01	19.60%
	Soil (0-10 cm)	55.65	61.71	35.87	97.58	18.32	32.91%
	Soil (10-20 cm)	39.32	46.77	19.26	66.03	13.80	35.10%
N:P	Leaves	11.82	6.03	8.81	14.84	1.64	13.88%
	Branches	9.06	3.45	7.63	11.08	1.00	10.98%
	Roots (0-10 cm)	24.37	18.29	19.22	37.51	5.26	21.58%
	Roots (10-20 cm)	20.78	14.70	16.39	31.09	4.44	21.38%
	Soil (0-10 cm)	3.62	3.76	2.23	5.99	1.25	34.47%
	Soil (10-20 cm)	2.77	3.27	1.37	4.64	0.99	35.64%

Notes - SD: standard deviation, CV: coefficient of variation

(100 mesh sieve) in order to remove gravel and plant remnants for C, N, and P content analysis.

### Measuring C, N, and P Concentrations in Plants and Soil

The organic carbon (OC) in the plant and the soil samples was analyzed using the  $H_2SO_4$ - $K_2Cr_2O_7$  wet oxidation method [25]. Total nitrogen (TN) in the plant and the soil was determined using the Kjeldahl method [24]. Total phosphorus (TP) in the plant and the soil was determined using the  $HClO_4$ - $H_2SO_4$  colorimetric method [24]. All data were expressed as mass ( $g\ kg^{-1}$ ). C, N, and P stoichiometric ratios of the different samples were calculated as OC vs. TN (C:N), OC vs. TP (C:P), and TN vs. TP (N:P).

### Data Analysis

All data were tested for homogeneity of variance and, if necessary, log-transformed. Data were then analyzed by the following three steps. First, one-way analysis of variance (ANOVA) and Tukey's post-hoc multiple comparison tests were used to test the significant differences in C, N, and P concentrations and C:N:P stoichiometric ratios among plant organs and sampling season and among soil depths and sampling season. Second, a generalized linear model (GLM) was used to assess the effects of plant organs (or soil depths), sampling seasons, and their interactions on plant and soil C, N, and P concentrations and C:N:P stoichiometric ratios. The coefficient of variation (CV) was used to describe the degree of variation. Third, Pearson correlation analysis was used to analyze the correlation of C:N:P stoichiometry among plant organs and soil depth. All of the statistical analyses were performed using SPSS (v 19.0, SPSS Inc., Chicago, IL, USA).

## Results

### Descriptive Statistics

Carbon concentrations varied from  $14.25\ g\ kg^{-1}$  (in soil at 10-20 cm) to  $475.94\ g\ kg^{-1}$  (in branches) (Table 1). N concentrations varied from  $1.01\ g\ kg^{-1}$  (in soil at 10-20 cm) to  $16.36\ g\ kg^{-1}$  (in leaves), and P concentrations varied from  $0.26\ g\ kg^{-1}$  (in soil at 10-20 cm) to  $1.85\ g\ kg^{-1}$  (in leaves). Mean C concentrations were in the order (high to low) of branches>leaves>roots (0-10 cm)>roots (10-20 cm). The N concentration order (high to low) was leaves>roots (0-10 cm)>roots (10-20 cm)>branches. P concentrations were the highest in leaves, followed by branches, and P concentrations in 10-20 cm roots were the lowest.

Among plant organs, branches had the highest C concentration ( $462.10\ g\ kg^{-1}$ ) and leaves contained the highest N and P concentrations. Leaves had the

greatest variation of N and P contents, whereas roots at 0-10 cm had the greatest variation of C contents. CVs of C contents were low in roots at 10-20 cm (<7.18%) and 29.31% in soil at 0-10 cm. CVs of N and P contents varied from 10.32% to 35.26%.

For stoichiometry, low P contents and high C contents led to the highest C:P ratios in both plant organs and in soil, ranging from 19.26 (soil at 10-20 cm) to 1968.78 (roots at 0-10 cm). C:N and N:P remained at a low level compared to C:P, changing from 1.37 (soil at 10-20 cm) to 91.61 (branches). CVs of these ratios also varied greatly, with a low of 3.12% and a high of 35.64%.

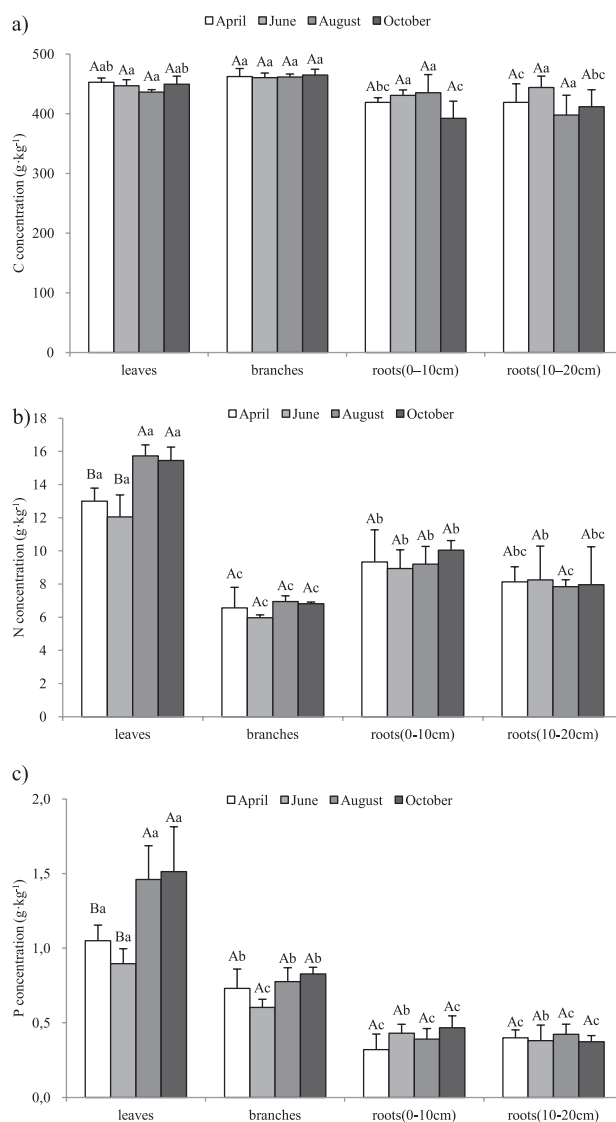


Fig. 1. Seasonal variations of the C, N, and P concentrations (mean + standard error) in leaves, branches, and roots: a) seasonal variations of the C concentration in leaves, branches, and roots; b) Seasonal variations of the N concentration in leaves, branches, and roots; and c) seasonal variations of the P concentration in leaves, branches, and roots; different uppercase letters represent significant differences among different sampling seasons at  $p < 0.05$ ; different lowercase letters represented significant differences among different organs at  $p < 0.05$

Table 2. GLM analysis of effects of plant organ (PO), sampling season (SS), and soil depth (SD) on plant and soil C:N:P stoichiometric characteristics.

Components	Factors	d.f.	Sum of squares ( <i>p</i> )					
			C	N	P	C:N	C:P	N:P
Plant	PO	3	16713.01 (0.000**)	377.48 (0.000**)	5.57 (0.000**)	9492.70 (0.000**)	4488578.92 (0.000**)	1889.02 (0.000**)
	SS	3	1755.16 (0.212)	12.63 (0.042*)	0.40 (0.000**)	385.20 (0.136)	300702.41 (0.057)	47.90 (0.263)
	PO×SS	9	5434.86 (0.146)	20.91 (0.131)	0.56 (0.001**)	216.23 (0.940)	470633.37 (0.209)	146.70 (0.221)
Soil	SD	1	1889.02 (0.000**)	2.22 (0.002**)	0.01 (0.563)	10.22 (0.000**)	1601.32 (0.008**)	4.31 (0.040*)
	SS	3	47.90 (0.038*)	0.88 (0.173)	0.45 (0.008**)	4.50 (0.025*)	2600.61 (0.014*)	13.16 (0.011*)
	SD×SS	3	146.70 (0.057)	0.93 (0.157)	0.02 (0.008**)	2.29 (0.142)	336.59 (0.606)	0.88 (0.795)

Notes – d.f.: degree of freedom, *p*: *p* value (\*, *p*<0.05; \*\*, *p*<0.01)

Seasonal Variations of C, N, and P Stoichiometry in Leaves, Branches, and Roots

C, N, and P concentrations varied significantly among leaves, branches, and roots (*p*<0.05). Concentrations in branches were significantly greater than concentrations in roots in April and October, while both N and P concentrations of leaves were significantly higher than those of branches and roots. C, N, and P concentrations did not vary significantly among seasons in branches and roots, while N and P concentrations in leaves differed significantly among growth seasons (*p*<0.05). Both N and P concentrations in leaves were higher in the fast-growing season and lower in the early growing season. Plant organ and

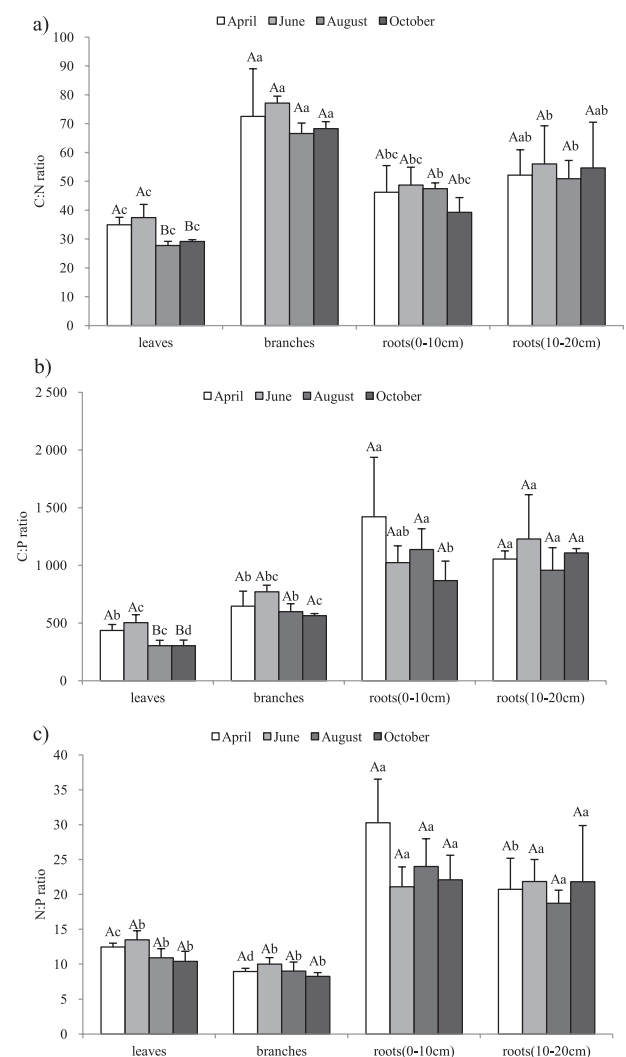


Fig. 2. Seasonal variations of the C:N, C:P and N:P ratios (mean + standard error) in leaves, branches and roots: a) seasonal variations of the C:N ratio in leaves, branches and roots; b) Seasonal variations of the C:P ratio concentrations in leaves, branches and roots; and c) seasonal variations of the N:P ratio in leaves, branches and roots; different uppercase letters represented significant differences among different sampling seasons at *p*<0.05; different lowercase letters represent significant differences among different organs at *p*<0.05

Table 3. Seasonal variations in the C, N, and P concentrations and their stoichiometric ratios in different soil depths of *P. orientalis* stands.

Element	Soil depth (cm)	Sampling seasons			
		April	June	August	October
C (g kg <sup>-1</sup> )	0-10	25.45±3.20Ca	27.54±2.1BCa	44.23±8.07Aa	32.77±10.07ABCa
	10-20	20.99±4.74Aa	21.92±3.24Aa	22.13±3.64Ab	18.97±5.90Aa
N (g kg <sup>-1</sup> )	0-10	1.75±0.16Aa	1.76±0.24Aa	2.68±0.44Aa	2.15±0.75Aa
	10-20	1.47±0.29Aa	1.56±0.23Aa	1.52±0.30Ab	1.35±0.43Aa
P (g kg <sup>-1</sup> )	0-10	0.41±0.14Aa	0.57±0.11Aa	0.72±0.29Aa	0.80±0.14Aa
	10-20	0.39±0.16Aa	0.60±0.15Aa	0.61±0.17Aa	0.75±0.04Aa
C:N	0-10	14.50±0.55Aa	15.80±1.04Aa	16.47±0.55Aa	15.32±0.68Aa
	10-20	14.18±0.67Aa	14.02±0.19Ab	14.59±0.54Ab	14.07±0.08Ab
C:P	0-10	65.67±12.77Aa	49.61±13.40Aa	66.6±27.25Aa	40.73±6.41Aa
	10-20	56.56±9.87Aa	37.55±5.11Ba	37.88±11.42Ba	25.27±6.62Bb
N:P	0-10	4.56±1.03Aa	3.17±1.00Aa	4.06±1.72Aa	2.67±0.53Aa
	10-20	4.01±0.78Aa	2.67±0.33Ba	2.60±0.79Ba	1.80±0.48Ba

sampling season interactions significantly affected the P concentrations. GLM analysis showed that organ type was the largest contributor to the variation in the C, N, and P concentrations (Fig. 1, Table 2).

C, N, and P stoichiometry varied among leaves, branches, and roots (Fig. 2). For example, C:N ratios of branches were significantly higher than those of leaves and roots (0-10 cm) ( $p<0.05$ ), while C:P and N:P ratios of roots (0-10 and 10-20 cm) differed significantly from leaves and branches. Leaves C:N and C:P significantly differed among seasons ( $p<0.05$ ). However, the interaction between organ type and season did not affect C:N, C:P and N:P ratios (Table 2).

#### Seasonal Variations of C, N, and P Stoichiometry in Soil

Soil C, N, and P concentrations and their stoichiometric ratios decreased along with soil depth, except for the P concentrations in June (Table 3). Soil C and N concentrations reached a peak in August (both 0-10 cm and 10-20 cm), while soil P concentrations reached a peak in October. Similarly, soil C:N and C:P ratios reached a peak in August (0-10 cm), while soil N:P reached a peak in April (both 0-10 cm and 10-20 cm). GLM analysis showed that soil depth was the greatest contributor to the variation in the C and N concentrations, while the sampling season determined the greatest amount of variation in the P concentration (Table 2).

Soil C, N, and P concentrations and their stoichiometric ratios were significantly affected by soil depth and sampling season individually. However, they were not significantly affected by the interaction effect of soil depth and sampling season, except for P concentrations. GLM analysis showed that soil depth

was the greatest contributor to the variation in the C:N ratio, while the sampling season determined the greatest amount of variation in the C:P and N:P ratios (Table 2).

#### Correlation between Stoichiometry in Plant and Soil

Most C, N, and P stoichiometry among leaves, branches, and roots showed no significant correlations. Only P ( $p<0.05$ ) concentrations and C:P ( $p<0.01$ ) and N:P ( $p<0.05$ ) ratios between leaves and branches were significantly correlated (Table 4).

N concentrations in leaves were positively correlated ( $p<0.05$ ) with N concentrations in the 0-10 cm depth soil, and the P concentration in leaves was positively correlated with the P concentration in the 0-10 cm and 10–20 cm depth soil. Soil N and P contents were significantly correlated ( $p<0.05$ ) with leaves' N and P contents, respectively. However, most stoichiometry between plant organs and soil showed no significant correlations. Only the N:P ratio between roots (10-20 cm) and soil (0-10 cm) were significantly correlated (Table 4).

## Discussion

### C, N, and P Concentrations in Leaves, Branches, and Roots

C compounds provide the sugars that are the basis for growth, reproduction, and structure, constituting roughly 50% of plant dry mass [1]. As a supporting and organizing organ, branches are mainly composed of polysaccharides, such as lignin and cellulose, which are rich in carbon [26]. This structural property

Table 4. Correlation between plant and soil stoichiometry.

Element	Items	Leaves	Branches	Roots (0-10 cm)	Roots (10-20 cm)	Soil (0-10 cm)
C (g kg <sup>-1</sup> )	Branches	-0.308				
	Roots (0-10 cm)	-0.087	-0.326			
	Roots (10-20 cm)	0.470	-0.512	0.135		
	Soil (0-10 cm)	-0.318	0.090	0.374	-0.151	
	Soil (10-20 cm)	-0.143	0.042	0.564	0.411	0.533
N (g kg <sup>-1</sup> )	Branches	0.521				
	Roots (0-10 cm)	0.101	-0.528			
	Roots (10-20 cm)	-0.286	-0.398	0.428		
	Soil (0-10 cm)	<b>0.732*</b>	0.406	-0.141	-0.224	
	Soil (10-20 cm)	0.160	0.237	-0.561	-0.535	0.556
P (g kg <sup>-1</sup> )	Branches	<b>0.668*</b>				
	Roots (0-10 cm)	0.291	-0.133			
	Roots (10-20 cm)	0.128	-0.030	-0.008		
	Soil (0-10 cm)	<b>0.747**</b>	0.460	0.488	-0.035	
	Soil (10-20 cm)	<b>0.628*</b>	0.511	0.427	-0.038	<b>0.771**</b>
C:N	Branches	0.442				
	Roots (0-10 cm)	0.094	-0.412			
	Roots (10-20 cm)	-0.066	-0.409	0.508		
	Soil (0-10 cm)	-0.321	-0.216	0.017	-0.007	
	Soil (10-20 cm)	-0.401	-0.220	0.057	-0.073	0.319
C:P	Branches	<b>0.734**</b>				
	Roots (0-10 cm)	0.290	-0.210			
	Roots (10-20 cm)	0.439	0.299	0.010		
	Soil (0-10 cm)	0.033	0.018	0.446	-0.132	
	Soil (10-20 cm)	0.464	0.357	0.568	0.108	<b>0.708**</b>
N:P	Branches	<b>0.653*</b>				
	Roots (0-10 cm)	0.269	-0.016			
	Roots (10-20 cm)	0.204	-0.199	-0.231		
	Soil (0-10 cm)	0.193	0.007	0.439	-0.219	
	Soil (10-20 cm)	0.453	0.260	<b>0.663*</b>	-0.041	<b>0.764**</b>

Notes – \*,  $p < 0.05$ ; \*\*,  $p < 0.01$

determines the highest C concentration in the branches (462.10 g kg<sup>-1</sup>) among plant organs (Table 1, Fig. 1). N is an important nutritional element and a fundamental component of all enzymes and chlorophyll in plants [27]. P influences photosynthetic assimilation and biomass production and is a key component of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and adenosine triphosphate (ATP). P is vital in the storage and translation of genetic information, energy storage, and cell structure [28, 29]. During the growth

stage, plants transfer a large amount of nutrients to leaves in order to meet the growth requirements [30], Branches and roots serve as nutrient absorption and transport channels, storing fewer nutrients [31]. Thus, N (14.06 g kg<sup>-1</sup>) and P (1.23 g kg<sup>-1</sup>) concentrations were higher in leaves than in branches and roots.

Seasonal variations of nutrients in plant tissues represent a dynamic growth response. These variations may be related to the migration of nutrients caused by changes in the balance between nutrient uptake and

utilization efficiency [32-35]. N and P concentrations in leaves differed significantly with the growing season, showing an initial rise leading to a peak and finally reaching a stable level. Being the main photosynthetic organs, leaves were capable of synthesizing a large number of proteins and nucleic acids [36]. In the early growing season, related to slow leaf growth, cell division was generally slow, requiring fewer proteins and nucleic acids. In the fast-growing season, a large amount of proteins and nucleic acids were needed for the fast growth rate of plants, requiring more N and P. At the end of the growing season, the leaf growth almost stopped, leading to low N and P demands. This result was consistent with previous reports showing that the demand for N and P in plants is higher in the fast-growing season than in the slow-growing season [37, 38]. Compared to the rapid increase in leaf biomass, the absorption of nutrients by plants cannot keep up with the rate of cell swelling. Therefore, the nutrients were diluted in various organs, showing a decline in N and P [2, 39]. However, compared to herbs [40-42], arborvitae trees are a slow-growing species. Thus, N and P concentrations were not reduced by the 'dilution effect' of cell division. This phenomenon has also been confirmed in shrubs [30, 43] and other coniferous tree species such as larch [44, 45].

#### Stoichiometry Patterns of C:N:P in Leaves, Branches and Roots

The stoichiometric characteristics in plant organs at the same developmental stage differed and this was related to the selective absorption and distribution patterns of C, N, and P. In this study, C:N and C:P ratios in the leaves were the lowest compared to branches and roots, N:P ratios in both leaves and branches were less than 14, and the N:P ratios in roots (0-10 and 10-20 cm) were greater than 20 (Table 1). These results were similar to previous studies finding that structural (rough roots and stems) and metabotropic (leaves, reproductive structure, and fine roots) organs have different functions, resulting in different nutrient contents and stoichiometry [46]. We also found that leaves do not represent the overall nutrient content of the tree. C:N:P stoichiometry was not well-constrained by organs in *P. orientalis*. Therefore, future studies on *P. orientalis* stoichiometry should extend to several different organs such as fine roots.

CVs indicated that the C:N ratios were more stable than C:P and N:P ratios during the growing season (Table 1, Fig. 2). The C:N, C:P and N:P ratios in leaves increased first and then decreased along with further growth. The highest C:N, C:P and N:P ratios in leaves occurred in the early growing season, and the lowest ratios in the fast-growing and the late-growing seasons. This may be related to soil N and P concentrations, which were higher in August and October and lower in April and June (Table 3). Generally, both C:N and

C:P stoichiometry can be used to estimate N-use efficiency and P-use efficiency. High C:N and C:P ratios represented high utilization rates of N and P, but low plant growth rates [36, 47]. In the case of a shortage of nutrient elements, plants often have a higher nutrient use efficiency and resorption efficiency. This is a survival strategy for plants in barren environment conditions [16, 35, 48]. In this study, *P. orientalis* was more prone to nutrient limitation and shortage in the early growing season. Therefore, it was possible for plants to satisfy nutrient needs by increasing the efficiency of N and P utilization.

The leaf N:P ratio was an indicator of the relative limitation of N vs. P. plant growth, limited by concurrent N concentrations <20 g kg<sup>-1</sup> and N:P ratios <14, whereas P concentration <1 g kg<sup>-1</sup> and N:P ratio >16 often signify P limitation [8, 49]. In this study, the average leaf N:P ratio was 11.82, and the average N concentration was 14.06 g kg<sup>-1</sup>. This indicated that *P. orientalis* in the rocky mountainous area of northern China is more restricted by N elements during the whole growing season. The N limitation threshold varies in interspecific ecosystems and growing sites [29, 50]. A more robust evaluation of nutrient limitations within an ecosystem involves nutrient inputs and fertilization experiments [51].

#### Soil C, N, and P Concentrations

Soil depth significantly affected the C and N concentrations in *P. orientalis* soil. C and N concentrations in 0-10 cm-deep soil were higher than those in 10-20 cm soil. The results of this study were similar to those of previously published information [41]. Leaves, branches, and harvest residues were deposited on the surface layer of soil and then the humus formed after decomposition accumulated in the upper soil [52, 53]. P concentration was mainly affected by the weathering of soil parent materials. Rock weathering is a long process [54], and P concentrations in soil showed no significant difference between soil layers.

Sampling season significantly affected soil C and P concentrations in *P. orientalis*. This is consistent with the results of other studies [55, 56]. Soil C and P concentrations of *P. orientalis* peaked in August because the increased microbial activity at higher temperatures favored the capacity of the system to utilize nutrients or organic materials. As a result, nutrients and organic materials may enter the soil [57].

#### Soil C:N:P Stoichiometry

Soil stoichiometry showed significant differences between soil depths. C:N, C:P, and N:P ratios of the 0-10 cm soil layer were higher than those in 10-20 cm soil layer. This result was similar to results of a previous study [58] showing that the C:N, C:P, and N:P ratios of the organic-rich soil layer (0-10 cm) were significantly higher than those in total soil depths. This was the result of relatively stable soil P content throughout the



soil profile compared to the rapid decline in C and N along with soil depth.

Sampling seasons had a significant effect on soil stoichiometry. The C:N ratio was more stable than C:P and N:P ratios during the whole growing season. Plants are the major source of total soil C and N in terrestrial systems, and the biogeochemical cycles of C and N are tightly coupled in terrestrial ecosystems, requiring a relative fixation ratio of carbon [59, 60]. Soil C:P and N:P ratios of 10-20 cm peaked in April, which was due to the low soil P concentration in April.

The soil N:P ratio can be used as a diagnostic indicator of N saturation and to determine the threshold for nutrient limitation [52]. The average soil N:P ratio in the study was 2.77-3.62, which is lower than the average of China (9.3) and the global average (13.1) [61]. The low soil N:P ratio in our study area indicates a high possibility of soil N deficiency.

#### Correlation between Plant and Soil Stoichiometry

P concentration, C:P, and N:P ratios in leaves and branches were significantly positively correlated (Table 4), indicating that nutrient use efficiency was similar between leaves and branches. This study was similar to an earlier study [62] that found that significant correlations of C, N, and P concentrations among organs mainly occurred in the above-ground parts of *Vitex rotundifolia*, especially between twigs and creeping stems. This study also confirmed previous results [38] showing that the C:P ratio in leaves and branches had a significant positive correlation. The branches and leaves collected in this study were located on the same branch. Since the branches were the most adjacent nutrient transport channels to leaves, the extraction and absorption of nutrients in branches and leaves were synchronized. This could explain the significantly positive correlation between P concentration, C:P, and N:P ratios in leaves and branches.

N and P concentrations in leaves were significantly positively correlated with soil N and P concentrations, respectively. Similarly, N:P in 10-20 cm roots was significantly positively correlated with the N:P in 0-10 cm soil (Table 4). These data confirmed the results of previous studies. For example, N and P concentrations in plants were positively correlated with soil N and P concentrations in an evergreen broad-leaved forest in Southwest China [63]. Leaf P concentrations and the N:P ratio of plant leaves were significantly correlated with soil P levels in a P-enriched area of the Lake Dianchi watershed in southwestern China [64]. These results also indicate that soil nutrient concentrations are coupled with plant nutrient concentrations [63, 64]. In this study, leaf nutrient concentrations were highly related to soil nutrient concentrations, but this did not indicate that a stable relationship exists in the stoichiometric ratios between the leaves and soil (Table 4). This result confirmed previous studies [32]. Plants

absorb nutrients from the soil and have a significant allometric growth relationship between the absorption of N and P in leaves [65]. However, the restriction of N or P elements in soil may alter this allometric relationship [38]. In fact, we found that *P. orientalis* was limited by N elements during the entire growing season.

Soil is the main nutrient source for plant photosynthesis and growth and plants return some nutrients back to the soil in the form of litter and its decomposition products [66, 67]. Therefore, C, N, and P concentrations of the vegetation and soil are significantly correlated [68]. The C:N:P ratio was regulated in different components of the ecosystem [69], and our study mainly focused on the system transfer processes of how C:N:P varied from low C:N and C:P (soil) to high C:N and C:P (leaves, branches, and roots). In the karst area of northwestern Guangxi [70], the Loess Plateau [71], and subtropical China [72], the C:N, C:P, and N:P values of the forest soil were lower than those in plants, which is consistent with the present study.

#### Conclusions

Seasonal variations in C, N, and P concentrations and their stoichiometric ratios were studied in a *P. orientalis* plantation. The results reflect the physiological and nutritional demands of these trees and mirror the specific geographic environment. We found that:

(1) Plant organs, soil depths, and sampling seasons need to be included when quantifying and comparing C:N:P stoichiometry in both plants and soil in *P. orientalis* plantations. Organ type was the largest contributor to the variations in the C, N, and P concentrations and C:N:P stoichiometry of trees. Soil depth was the largest contributor to the variations in the C, N concentrations and C:N ratio. Sampling time contributed the greatest amount of variation in the P concentration, C:P and N:P ratios. In both trees and soil, the C:N ratios were more stable than C:P and N:P ratios during the growing season.

(2) Significant variations among C, N, and P concentrations or C:N, C:P and N:P ratios were observed among leaves, branches, and roots during the stages of the growing season, indicating patterns of nutrient allocation and mobility.

(3) N concentration in leaves was 14.06 g kg<sup>-1</sup>, and the N:P ratio was 11.82, indicating a N limitation in *P. orientalis* during stages of the growing season – especially in the early growing season stage. This offers insight into the fertilizer requirements of forested ecosystems.

(4) Significant correlation between the N and P concentrations of leaves and soil N and P concentrations suggested the coupled mobility or control between plant and soil, and the C:N:P ratio was regulated in different components of the ecosystem.

In summary, ecological chemometric studies in *P. orientalis* can refine population stoichiometry. These results are applicable to the sustainable management of *P. orientalis* plantations and provide a scientific procedure for soil nutrient diagnosis and data for enabling of large-scale ecosystem chemometrics study and transformation.

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### Conflicts of Interest

The authors declare no conflict of interest.

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