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Seasonal Variations in Carbon, Nitrogen and Phosphorus Concentrations and C:N:P Stoichiometry in the Leaves of Differently Aged *Larix principis-rupprechtii* Mayr. Plantations

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Abstract: The concentrations and stoichiometry of certain elements (carbon, nitrogen and phosphorus) are critical to the maintenance of plant functional and environmental adaptation during plant growth. We explore how the concentrations of C, N and P and the ratios of C:N, C:P, and N:P in the leaves of differently aged *Larix principis-rupprechtii* Mayr. plantations changed with growing season and stand age from 2012 to 2015 in the Qinling Mountains, China. The results showed that the element concentration and stoichiometric ratios in leaves were significantly affected by sampling month, stand age and sampling year; and multiple correlations with stand age were observed in different growing seasons. Compared to global element concentrations and stoichiometry in plants, the leaves of larch stands in the study region had higher C and P concentrations and C:N and C:P ratios but lower N concentrations and N:P ratios than global levels. The leaf N:P ratios of all of the larch stands were generally less than 14, suggesting that the growth of larch stands was limited by N in the study region. Our study facilitates the management and restoration of forest plantation and provides a valuable contribution to the global pool of leaf nutrition and stoichiometry data.

Keywords: *Larix principis-rupprechtii* Mayr.; carbon; nitrogen and phosphorus; stoichiometry; Qinling Mountains; stand age

1. Introduction

Chemical elements are essential components of plants and are the basic components of ecology and stoichiometry [1]. Carbon (C), nitrogen (N) and phosphorus (P) are essential for plant growth and are vital in nutrient cycling, forest succession and the sustainable management of forest ecosystems. Carbon forms the structural basis of plants and constitutes one-half of the dry mass of plants [2–5].

Most plants are completely dependent on soil N because they lack symbiotic bacteria that fix N. Higher concentrations of N are associated with higher leaf area index values, extended photosynthesis duration and greater nutrient uptake [6–8]. Phosphorus influences photosynthetic assimilation and biomass production in plants [9] and represents the main component of RNA, DNA and ATP. In natural ecosystems, plant growth is typically limited by the lack of a particular nutrient element [5]. Generally, N and P are the primary limiting nutrients for forest plants [10], but C can also act as a limiting element [11].

The concentrations of nutritional elements (C, N and P) in plants reflect nutrient uptake, utilization efficiency and adaptation to the environment during different growth stages [12,13]. In forest plantations, changes in N and P concentrations with plant growth stage have attracted particular attention due to their importance for the development of nutrient management practices [14,15]. Thus, the relationships between elemental concentrations and growing season based changes in plant tissues represent key information for the management and fertilization of forest plantation. Ecological stoichiometry is an integrative discipline that involves the study of multiple nutritional elements in ecological interactions and involves determining the elemental compositions of living organisms. Numerous studies have indicated that the C:N:P stoichiometry of plants can affect litter decomposition, species composition and grazing in forest ecosystems [16,17], and that stoichiometry is affected by environmental conditions (temperature, elevation and drought), plant age (stand age) and growing season (sampling time) [15,18]. The examination of C, N and P dynamics during stand development facilitates the quantification of the temporal pattern of C:N:P stoichiometry over the age sequence in the forest ecosystem. Agren (2008) suggested that ecological patterns occurring through time might differ from those observed across space [11]. However, our knowledge of the temporal variability of C, N and P concentrations and C:N:P stoichiometric ratios is much less developed than that of their spatial patterns. Furthermore, it remains unknown whether the C, N and P concentrations and C:N:P stoichiometric ratios of plants are constrained through time. Thus, it is vital to determine how changes over the growing season and plant age are related to the adaptation of perennial plant to various environments.

The Qinling Mountains have historically been home to warm temperate evergreen broadleaf forests with high biodiversity. However, over the past century, the natural forests of the Qinling Mountains have been logged and replaced by plantation forests [19]. At middle elevations within the Qinling Mountains, forests of conifers, such as *Prince Rupprecht's* Larch. (*Larix principis-rupprechtii* Mayr.) are present. This species is native to the mountainous regions of Shaanxi, Henan and Hebei Provinces in northern China. Furthermore, *L. principis-rupprechtii* Mayr. is a more appropriate tree species for planting in forest plantations in northern China [20].

In this study, we assessed the temporal patterns of C, N and P concentrations and C:N:P stoichiometric ratios in larch leaves by conducting a long-term field experiment in a larch plantation ecosystem. The specific objectives of this study are (1) to assess how growing season and stand age affect leaf C, N and P concentrations and C:N:P ratios and (2) to determine the relationships between stand age and leaf C, N and P concentrations and C:N:P ratios.

2. Materials and Methods

2.1. Study Site

This study was conducted in an undisturbed larch (*L. principis-rupprechtii* Mayr.) plantation in Taibai County of Baoji City, Shaanxi Province, China (34°02'18.1"N, 107°20'51.1"E), from 2012 to 2015. The site is characterized by a mountain climate within the overall context of a typical continental monsoon climate. The average annual temperature is 7.7 °C and the annual mean precipitation is 1000 mm. The average elevation ranges from 1620 to 1780 m. Brown soil (Luvisol of FAO classification) is present within the study area, and the basic characteristics of the forest floor (0–20 cm) are shown in Table 1. Larch plantations are widespread in this area, and larch is the primary species used for afforestation. Larch plantations cover large portions of the study area, and their total area is 196.27 ha.

Table 1. Properties of the forest floor (0–20 cm) in the study region.

Chemical Characteristics	Site	Nutrient Content				Nutrient Classification	Description
		2012	2013	2014	2015		
Organic matter (SOM) (g kg ⁻¹)	A	18.68	19.72	16.22	11.45	III (SOM content: 20–30 g kg ⁻¹) V (SOM content: 10–20 g kg ⁻¹)	Normal (III) Very poor (V)
	B	20.36	20.96	15.58	10.22		
	C	25.89	24.93	17.17	13.77		
	D	39.55	39.67	19.30	24.85		
	E	57.14	37.43	28.53	23.98		
Total N (TN) (g kg ⁻¹)	A	1.18	1.16	0.97	1.1	III (TN content: 1–1.5 g kg ⁻¹) IV (TN content: 0.75–1 g kg ⁻¹)	Normal (III) Poor (IV)
	B	1.21	1.3	0.92	0.95		
	C	1.44	1.2	0.95	1.16		
	D	1.62	1.73	1.56	1.50		
	E	1.81	1.67	2.40	2.34		
Total P (TP) (g kg ⁻¹)	A	1.51	1.48	1.42	1.05	I (TP content: >1 g kg ⁻¹) III (TP content: 0.6–0.8 g kg ⁻¹)	Very high (I) Normal (III)
	B	1.57	1.52	1.51	1.03		
	C	1.53	1.51	1.52	0.67		
	D	1.59	1.51	1.56	1.16		
	E	1.58	1.33	1.40	1.34		

Soil nutrient classification based on the national standard classification for soil nutrient content in 1982. Roman numerals represent different soil nutrition classifications. The nutrient classification standard is based on data from the Second National Soil Survey of China. **A:** sapling forest; **B:** young forest; **C:** half-mature forest; **D:** near-mature forest and **E:** mature forest.

2.2. Experimental Design, Plant Sampling and Soil Sampling

The areas containing the larch plantations are separated from each other by 3 km in the study region (except for the areas of sapling forest and young forest, which are separated by 1.5 km). In 2011, after topography and vegetation surveys, we preliminarily selected experimental sites with similar topography and vegetation types within each area. All the experimental sites have been fenced since 2011 to prevent human activities from influencing these areas. In 2012, the ages of the larch plantations were 5 years (sapling forest), 10 years (young forest), 20 years (half-mature forest), 30 years (near-mature forest) and 40 years (mature forest), and the mean forest densities were 2750, 2750, 2500, 2510 and 2580 individual trees per hectare, respectively. In the study area, larch plantation trees typically bud (i.e., begin their physiological activities and growth) in late April; grow rapidly throughout July, August and early September; grow slowly in late September and early October; and fully abscise their leaves in early November. Thus, sampling times were scheduled in the middle of May, June, July, August, September, and October from 2012 to 2015. The growth stages of the larch plantations were divided into an early growing season (May–June), a fast growing season (July–September) and a late growing season (October).

In April 2012, we selected an experimental site within each area. Three plots (20 × 20 m) were established randomly within each experimental site (the location of these plots remained fixed from 2012 to 2015, and the topography and composition of the undergrowth were nearly identical among the plots). Two of the three plots were adjacent to each other, and an isolation strip containing two rows of trees was established. A distance of at least 5 m was maintained between the adjacent plots. The third plot was separated from the others by 0.5 km. When sampling in each month, three quadrats (5 × 5 m) were established randomly at each plot. When possible, samples were collected from trees of similar size (similar diameter and height) in each plot. To standardize the sample collection, sun-exposed and fully expanded leaves were collected from five individuals in each quadrat. Leaves were collected at mid-height using a pole pruner, and each sample consisting of leaves from five trees was placed in a separate bag (5-g samples (fresh mass) were obtained from each tree and placed in a paper envelope). We applied different markers to the trees after sampling to avoid resampling and secondary resampling from the same trees. All of the samples were oven dried at 105 °C for 15 min, then oven dried at 75 °C for 24 h. The dry weights were determined, and the dried samples were ground into a uniform powder fine enough to pass through a 1.0-mm sieve for analysis. Additionally, soil samples were randomly collected in triplicate from depths of 0–20 cm within the same quadrats in mid-April from 2012 to 2015. All of the soil samples were air dried and passed through a 0.25-mm sieve to remove gravel and plant remnants. The soil samples were then ground to a fine powder for analysis.

2.3. Measurements of Plant and Soil Samples

The organic C (OC) concentrations in the leaves and soil samples were measured using the potassium dichromate/sulphuric acid mixture titration method [21]. The total N (TN) concentrations in the leaves and soil samples were measured using the semimicro-Kjeldahl method [22] with a Kjeldahl Auto-analyser (K9840 Kjeldahl Analyser, Hanon, Jinan, China). The total P (TP) concentrations in the leaves and soil samples were analysed colourimetrically with an ultraviolet spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) using the molybdenum blue method following digestion in concentrated H₂SO₄ and H₂O₂ [23]. All of the leaf and soil data are expressed as mass concentrations (mg·g⁻¹ and g·kg⁻¹).

2.4. Statistical Analysis

Before statistical analysis, a normality test was applied to the data, and the homogeneity of the variance was examined using Levene's test. One-way analysis of variance (ANOVA) and Tukey's post hoc multiple comparison tests were used to test for significant differences in C, N and P concentrations and C:N:P stoichiometric ratios among growing seasons and stand ages. Multivariate

ANOVA tests were used to assess the effects of sampling year, sampling month and stand age on the leaf C, N and P concentrations and C:N:P stoichiometric ratios. Variance component estimation (VCE) was used to assess the contribution percentage of sampling year, sampling month, stand age and residual components to the leaf C, N and P concentrations and C:N:P stoichiometric ratios, and the total variance was partitioned by using the residual maximum likelihood (REML) method. All of the statistical analyses were performed with SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). The linear correlations of the C, N and P concentrations and the C:N:P stoichiometric ratios with stand age were assessed using ordinary least squares regression (OLS) with the model $y = ax + b$, and these statistical analyses were performed with Sigma-Plot Suite V13 (Systat Software Inc., San Jose, CA, USA).

3. Results

3.1. Seasonal Variations in the C, N and P Concentrations in the Leaves of Differently Aged Stands

Large seasonal variations in leaf N and P concentrations were observed in all larch stands from 2012 to 2015 (Table 2), whereas no significant variation in leaf C concentrations were observed in the different growing seasons (i.e., sampling month). Leaf C concentrations across stands increased slightly with growth and peaked in the fast growing season in each sampling year, but the differences among growing seasons were not significant. In each sampling year, large differences in the leaf N and P concentrations of all stands were observed among the different growing seasons. The leaf N concentrations were highest in the fast growing season and lowest in the late growing season. Additionally, a second peak in leaf N concentration occurred in the early growing season. The leaf P concentrations generally decreased with growth in each sampling year. The maximum leaf P concentrations were observed in the early growing season, and the minimum values were observed in the late growing season. No significant differences in leaf C concentration were observed among the different larch stands within the same sampling month. Generally, the sapling forest had higher leaf N and P concentrations than the other larch stands in the same sampling month from 2012 to 2015.

Sampling month, sampling year, stand age and the interaction of sampling year \times sampling month significantly affected the leaf N and P concentrations in all larch stands ($p < 0.05$), but they did not significantly affect leaf C concentrations (Table 3). Based on the VCE analysis, sampling month determined the greatest amount of variation in leaf N concentration (44.38%), whereas sampling year determined the greatest amount of variation in leaf P concentration (26.76%), followed by sampling month (20.43%). Interestingly, the residual variance was the largest source of variation (53.43%) in leaf C concentration (Table 4).

Table 2. C, N and P concentrations ($\text{mg}\cdot\text{g}^{-1}$) in the leaves of differently aged stands in different growing seasons from 2012 to 2015.

Element	Year	Stand Age	Leaf C, N and P Concentrations ($\text{mg}\cdot\text{g}^{-1}$) in the Different Sampling Months					
			May	Jun	Jul	Aug	Sept	Oct
C	2012	A	486.59 ± 2.92 C	498.83 ± 17.68 AB	506.04 ± 13.01 AB	515.42 ± 24.07	504.88 ± 54.08	497.74 ± 37.58
		B	536.80 ± 14.51 A	512.16 ± 61.30 A	543.79 ± 4.29 A	490.41 ± 43.46	506.70 ± 5.07	512.92 ± 27.82
		C	485.76 ± 25.00 C	484.07 ± 7.40 B	498.07 ± 37.17 AB	522.31 ± 4.52	488.22 ± 25.00	501.77 ± 38.67
		D	545.40 ± 9.50 a A	519.76 ± 3.31 b A	509.78 ± 6.78 b AB	524.73 ± 13.64 b	516.36 ± 11.15 b	513.85 ± 6.92 b
		E	511.94 ± 31.04 AB	489.06 ± 13.97 B	487.51 ± 14.44 B	503.43 ± 11.18	527.73 ± 2.71	496.76 ± 29.26
	2013	A	480.84 ± 25.74	513.59 ± 8.25 AB	504.99 ± 15.75	517.12 ± 23.06	530.69 ± 6.11	526.82 ± 18.58
		B	487.41 ± 16.05	510.53 ± 13.59 AB	516.27 ± 14.18	512.69 ± 20.20	528.06 ± 7.45	497.93 ± 43.43
		C	508.67 ± 7.88 ab	523.67 ± 10.66 ab A	497.76 ± 16.25 b	513.38 ± 4.43 ab	534.64 ± 18.51 a	504.76 ± 3.25 ab
		D	495.04 ± 0.57 c	485.06 ± 3.08 c B	501.20 ± 14.19 bc	522.18 ± 5.00 ab	539.89 ± 6.77 a	523.05 ± 14.77 ab
		E	491.39 ± 3.71 bc	483.92 ± 17.96 c B	499.58 ± 1.77 bc	516.36 ± 4.25 ab	537.46 ± 9.74 a	503.62 ± 6.94 bc
	2014	A	499.75 ± 13.78	496.76 ± 4.27	505.40 ± 15.87	504.56 ± 5.32	492.52 ± 12.91	495.54 ± 10.75
		B	512.58 ± 8.82	507.65 ± 19.65	506.90 ± 12.75	502.11 ± 21.40	492.90 ± 13.99	501.11 ± 7.51
		C	505.41 ± 3.56	498.52 ± 16.60	495.54 ± 11.45	516.83 ± 12.76	503.12 ± 2.78	489.62 ± 21.58
		D	533.36 ± 22.29	504.66 ± 5.86	512.02 ± 6.61	527.62 ± 32.04	495.65 ± 6.35	521.72 ± 24.30
		E	529.36 ± 11.50 a	504.99 ± 5.78 ab	448.38 ± 26.35 c	511.74 ± 6.64 ab	482.42 ± 12.42 bc	510.29 ± 9.70 ab
	2015	A	513.09 ± 8.42 AB	493.82 ± 1.09	504.72 ± 34.94	510.91 ± 8.39 B	538.48 ± 25.28 AB	506.15 ± 4.40 B
		B	528.58 ± 5.88 ab A	522.70 ± 5.97 bc	510.43 ± 10.70 bc	519.50 ± 2.15 bc B	540.52 ± 5.33 a AB	505.24 ± 7.28 c B
		C	529.18 ± 5.28 bc A	485.20 ± 36.39 c	523.41 ± 0.27 bc	599.95 ± 13.37 a A	592.74 ± 24.98 a A	556.67 ± 9.20 ab A
		D	515.82 ± 5.37 AB	524.80 ± 7.11	560.48 ± 49.44	582.74 ± 12.08 A	527.26 ± 32.75 AB	542.62 ± 29.43 AB
		E	502.93 ± 11.85 B	512.35 ± 3.56	546.29 ± 8.09	509.59 ± 19.04 B	513.61 ± 32.20 B	526.96 ± 28.70 AB
N	2012	A	20.75 ± 1.57 a	15.43 ± 0.23 c B	17.41 ± 1.28 bc C	21.51 ± 1.10 a A	19.71 ± 1.04 ab B	9.41 ± 0.28 d A
		B	22.16 ± 1.50 b	17.01 ± 0.11 d AB	15.63 ± 0.60 d C	19.84 ± 0.96 c AB	26.60 ± 0.63 a A	9.26 ± 0.22 e A
		C	20.74 ± 0.16 a	17.35 ± 0.51 b AB	20.85 ± 1.95 a AB	19.30 ± 0.21 ab B	18.13 ± 0.55 b B	5.98 ± 0.15 c C
		D	20.07 ± 0.79 a	18.62 ± 0.46 a A	18.55 ± 0.73 a BC	18.35 ± 0.73 a B	18.79 ± 0.50 a B	7.89 ± 0.66 b B
		E	21.26 ± 0.30 b	18.46 ± 1.53 c A	23.83 ± 0.25 a A	18.69 ± 0.61 c B	18.66 ± 1.24 c B	6.63 ± 0.79 d BC
	2013	A	19.62 ± 0.21 bc A	18.52 ± 0.09 c A	20.57 ± 0.06 ab AB	22.33 ± 0.49 a A	21.12 ± 1.43 ab A	12.01 ± 0.48 d B
		B	17.06 ± 0.52 ab BC	14.16 ± 0.678 c C	17.74 ± 0.67 a B	17.41 ± 0.71 ab B	17.79 ± 0.87 a BC	15.73 ± 0.79 bc A
		C	18.07 ± 0.67 bc B	17.68 ± 0.53 bc AB	21.50 ± 0.97 a A	16.86 ± 0.57 bc BC	18.84 ± 1.61 b BC	15.69 ± 0.27 c A
		D	16.44 ± 0.64 b C	16.57 ± 0.71 b B	20.39 ± 0.85 a AB	16.87 ± 0.25 b BC	20.12 ± 0.80 a AB	12.33 ± 0.76 c B
		E	16.75 ± 0.43 ab BC	16.90 ± 0.15 ab B	19.09 ± 1.91 a AB	15.92 ± 0.71 b C	16.91 ± 1.01 ab C	5.94 ± 0.04 c C
	2014	A	26.20 ± 0.80 a AB	22.77 ± 0.96 b AB	15.95 ± 0.96 c BC	16.96 ± 0.24 c B	15.48 ± 0.91 c A	5.35 ± 0.27 d B
		B	23.70 ± 0.76 a BC	20.87 ± 1.32 b BC	14.27 ± 1.51 c C	21.98 ± 0.70 ab A	16.45 ± 0.90 c A	5.73 ± 0.55 d AB
		C	26.81 ± 1.37 a A	24.85 ± 1.02 a A	18.19 ± 1.15 c AB	21.88 ± 1.48 b A	14.82 ± 0.61 d AB	5.54 ± 0.25 e AB
		D	24.23 ± 1.09 a BC	19.88 ± 1.53 a B	16.09 ± 1.00 c BC	20.71 ± 1.21 b A	12.90 ± 0.54 d B	6.38 ± 0.19 e A
		E	22.79 ± 0.72 a C	22.78 ± 0.80 a AB	19.65 ± 0.99 b A	17.05 ± 1.80 b B	8.35 ± 0.53 c C	5.32 ± 0.33 d B

Table 2. Cont.

Element	Year	Stand Age	Leaf C, N and P Concentrations (mg·g ⁻¹) in the Different Sampling Months					
			May	Jun	Jul	Aug	Sept	Oct
P	2015	A	13.15 ± 0.04 b C	11.16 ± 1.02 b D	11.50 ± 2.33 b BC	21.47 ± 1.18 a B	18.38 ± 1.26 b B	6.00 ± 0.44 c D
		B	12.31 ± 0.63 ab CD	8.76 ± 0.11 c D	10.87 ± 0.69 b C	11.62 ± 0.32 ab C	13.20 ± 1.22 a B	11.46 ± 0.63 ab B
		C	26.87 ± 0.32 b A	24.85 ± 1.46 b A	16.70 ± 1.32 c A	29.37 ± 0.81 a A	16.53 ± 0.34 c A	13.23 ± 0.25 d A
		D	18.90 ± 0.54 b B	18.68 ± 1.28 b B	14.77 ± 1.02 bc AB	24.82 ± 4.50 a AB	16.25 ± 1.52 b A	9.77 ± 0.51 c C
		E	11.22 ± 0.36 b D	13.89 ± 0.55 a C	13.51 ± 1.20 a AB	12.08 ± 0.69 ab C	12.26 ± 0.48 ab B	5.81 ± 0.92 c D
	2012	A	2.85 ± 0.02 a A	1.95 ± 0.03 c BC	2.20 ± 0.16 b A	1.35 d	1.35 d	1.33 d AB
		B	2.89 ± 0.04 a A	2.15 ± 0.02 c A	2.38 ± 0.04 b A	1.35 d	1.35 d	1.35 d A
		C	2.22 ± 0.01 a C	1.70 ± 0.02 c D	1.87 ± 0.01 b B	1.35 d	1.35 d	1.31 e B
		D	2.88 a A	2.10 ± 0.04 c AB	2.32 ± 0.02 b A	1.35 d	1.35 d	1.33 d AB
		E	263 ± 0.04 a B	1.84 ± 0.10 b C	1.91 ± 0.09 b B	1.35 c	1.35 c	1.31 ± 0.03 c B
	2013	A	2.50 ± 0.04 b AB	2.06 ± 0.05 c B	2.13 ± 0.04 c B	2.76 ± 0.19 a A	2.43 ± 0.05 b A	1.70 ± 0.02 d AB
		B	2.72 ± 0.20 a A	2.24 ± 0.09 b B	1.84 ± 0.08 c CD	1.89 ± 0.15 c B	2.06 ± 0.04 bc B	1.83 ± 0.05 c A
		C	2.27 ± 0.12 a BC	2.44 ± 0.09 a A	1.75 ± 0.07 b D	1.54 ± 0.05 b B	1.75 ± 0.07 b C	1.58 ± 0.06 b B
		D	1.96 ± 0.14 ab C	1.83 ± 0.03 bc C	2.10 ± 0.09 a BC	1.80 ± 0.12 bc B	1.58 ± 0.11 c C	1.58 ± 0.06 c B
		E	2.41 ± 0.18 a AB	1.84 ± 0.03 b C	2.42 ± 0.19 a A	1.67 ± 0.39 bc B	2.17 ± 0.04 ab B	1.22 ± 0.09 c C
	2014	A	2.42 ± 0.10 a D	2.36 ± 0.06 ab	1.72 ± 0.15 d B	1.86 ± 0.06 cd	2.11 ± 0.09 bc AB	0.84 ± 0.07 e A
		B	2.92 ± 0.03 a B	1.97 ± 0.31 c	2.15 ± 0.07 b A	1.94 ± 0.06 b	2.27 ± 0.19 b A	0.78 ± 0.04 c A
		C	2.73 ± 0.06 a C	2.31 ± 0.11 b	2.13 ± 0.17 bc A	2.10 ± 0.17 c	1.86 ± 0.04 c B	0.72 ± 0.02 d A
		D	2.91 ± 0.04 a BC	1.96 ± 0.25 bc	1.80 ± 0.22 c AB	1.88 ± 0.06 c	2.30 ± 0.02 b A	0.79 ± 0.07 d A
		E	3.16 ± 0.09 a A	2.12 ± 0.19 b	1.88 ± 0.04 c AB	1.96 ± 0.04 bc	1.29 ± 0.01 d C	0.58 ± 0.02 e B
	2015	A	2.43 ± 0.06 d D	4.19 ± 0.06 b A	4.09 ± 0.11 b B	3.80 ± 0.10 b A	5.57 ± 0.27 a A	2.90 ± 0.18 c A
		B	3.07 ± 0.03 c A	4.74 ± 0.64 a A	4.70 ± 0.15 ab A	3.92 ± 0.12 b A	4.15 ± 0.07 ab B	2.49 ± 0.24 c A
		C	2.72 ± 0.01 a BC	2.40 ± 0.04 b B	2.28 ± 0.06 c D	2.15 ± 0.04 d B	2.30 ± 0.03 bc C	1.73 ± 0.05 e B
		D	2.78 ± 0.01 a B	2.51 ± 0.45 a B	2.33 ± 0.01 a CD	2.51 ± 0.09 a B	2.11 ± 0.45 a C	1.21 ± 0.13 b C
		E	2.66 ± 0.02 a C	2.77 ± 0.07 a B	2.55 ± 0.09 a C	2.36 ± 0.50 a B	2.49 ± 0.11 a C	1.10 ± 0.18 b C

Values are the mean ± standard deviation of the mean ($n = 3$). Lowercase letters represent significant differences among different months at $p < 0.05$. Capital letters represent significant differences among the differently aged stands at $p < 0.05$. The same letters indicate no significant difference at $p < 0.05$. A: sapling forest; B: young forest; C: half-mature forest; D: near-mature forest and E: mature forest.

3.2. Seasonal Variations in the C:N:P Stoichiometric Ratios in the Leaves of Differently Aged Stands

Large seasonal variations in the leaf C:N, C:P and N:P ratios were observed in all larch stands from 2012 to 2015 (Table 5). Generally, the leaf C:N and C:P ratios increased with growth and peaked in the fast growing season. In each sampling year, the maximum leaf C:N and C:P ratios were observed in the late growing season, and the minimum values were observed in the early growing season. However, a different pattern of seasonal variation was observed in the leaf N:P ratios. Generally, the leaf N:P ratio began to increase in the early growing season, peaked in the fast growing season and decreased in the late growing season. The maximum leaf N:P ratios were observed in the fast growing season, and the minimum values were observed in the late growing season. No clear differences in stoichiometric ratios were observed among the different larch stands in the same sampling month from 2012 to 2015.

Sampling month, sampling year, stand age and their interactions (sampling year \times sampling month, sampling year \times stand age, sampling month \times stand age and sampling year \times sampling month \times stand age) significantly affected the leaf C:N:P stoichiometric ratios of all larch stands ($p < 0.05$) (Table 3). Based on the VCE analysis, sampling month determined the greatest amount of variation in the leaf C:N ratio (57.35%), whereas the interaction of sampling year \times sampling month determined the greatest amount of variation in the leaf C:P and N:P ratios (38.19% and 31.78%, respectively) (Table 4).

Table 3. Multivariate ANOVA results for the effects of sampling year (SY), sampling month (SM) and stand age (SA) on the C, N and P concentrations and C:N, C:P, N:P ratios in leaves.

Element	SY	SM	SA	SY \times SM	SY \times SA	SM \times SA	SY \times SM \times SA
	d.f. = 3	d.f. = 5	d.f. = 4	d.f. = 15	d.f. = 12	d.f. = 19	d.f. = 57
C	F = 5.09	F = 1.45	F = 1.94	F = 0.97	F = 0.93	F = 0.52	F = 0.43
	$p = 0.075$	$p = 0.370$	$p = 0.268$	$p = 0.576$	$p = 0.591$	$p = 0.855$	$p = 0.933$
N	F = 7.18	F = 59.25	F = 9.65	F = 7.21	F = 4.97	F = 2.13	F = 1.00
	$p = 0.044$	$p = 0.001$	$p = 0.025$	$p = 0.035$	$p = 0.067$	$p = 0.243$	$p = 0.587$
P	F = 133.16	F = 11.17	F = 31.94	F = 11.41	F = 16.87	F = 2.31	F = 2.29
	$p \leq 0.001$	$p = 0.001$	$p = 0.003$	$p = 0.015$	$p = 0.007$	$p = 0.217$	$p = 0.219$
C:N	F = 73.91	F = 635.25	F = 52.11	F = 49.33	F = 18.42	F = 19.53	F = 6.93
	$p = 0.001$	$p \leq 0.001$	$p = 0.001$	$p = 0.001$	$p = 0.006$	$p = 0.005$	$p = 0.035$
C:P	F = 134.62	F = 270.46	F = 34.57	F = 59.87	F = 8.73	F = 6.03	F = 3.02
	$p \leq 0.001$	$p \leq 0.001$	$p = 0.002$	$p = 0.001$	$p = 0.025$	$p = 0.047$	$p = 0.144$
N:P	F = 101.26	F = 34.10	F = 20.67	F = 18.49	F = 10.12	F = 2.28	F = 2.00
	$p \leq 0.001$	$p = 0.002$	$p = 0.006$	$p = 0.006$	$p = 0.019$	$p = 0.221$	$p = 0.264$

d.f.: degree of freedom.

Table 4. Partitioning of total variance (%) in C, N and P concentrations and C:N, C:P and N:P ratios into sampling year (SY), sampling month (SM), stand age (SA) and residual components.

Variation Source	Partitioning of Total Variance (%)							
	SY	SM	SA	SY \times SM	SY \times SA	SM \times SA	SY \times SM \times SA	Residual
C	17.37	2.75	5.20	11.59	7.49	2.17	0	53.43
N	0	44.38	2.47	20.69	11.05	4.27	3.07	14.06
P	26.76	20.43	4.63	13.44	18.20	0.06	7.56	8.93
C:N	0.84	57.35	1.83	17.16	3.78	5.41	11.59	2.04
C:P	7.92	35.17	3.27	38.19	3.22	2.40	6.47	3.37
N:P	24.43	7.31	4.00	31.78	13.21	0.44	10.64	8.19

The variance in each factor was converted to percentages at each level. Analyses were performed on all data from 2012 to 2015.

Table 5. C:N, C:P and N:P ratios in leaves of differently aged stands in different growing seasons from 2012 to 2015.

Ratio	Year	Stand Age	Leaf C:N, C:P and N:P Ratios (by Mass) in the Different Sampling Months					
			May	Jun	Jul	Aug	Sept	Oct
C:N	2012	A	23.53 ± 1.61 c B	32.34 ± 1.38 b A	29.16 ± 2.10 bc AB	24.00 ± 1.73 c	25.61 ± 2.39 c A	52.88 ± 3.74 a C
		B	24.29 ± 1.59 c AB	30.11 ± 0.79 b AB	34.83 ± 1.57 b A	24.81 ± 3.18 c	19.06 ± 0.63 d B	55.40 ± 2.20 a C
		C	23.43 ± 1.35 b B	27.91 ± 0.65 b BC	24.13 ± 3.80 b BC	27.06 ± 0.25 b	26.93 ± 1.21 b A	83.87 ± 6.08 a A
		D	27.20 ± 0.69 b A	27.92 ± 0.66 b BC	27.52 ± 1.45 b B	28.62 ± 0.83 b	27.49 ± 1.01 b A	65.48 ± 5.67 a BC
		E	24.07 ± 1.14 bc AB	26.59 ± 1.99 bc C	20.45 ± 0.50 c C	26.97 ± 1.43 bc	28.36 ± 1.84 b A	75.31 ± 5.28 a AB
	2013	A	24.52 ± 1.52 c B	27.73 ± 0.41 b B	24.55 ± 0.74 c B	23.17 ± 1.18 c B	25.20 ± 1.59 bc C	43.88 ± 0.24 a B
		B	28.60 ± 1.53 b A	36.09 ± 1.55 a A	29.11 ± 0.30 b A	29.50 ± 2.03 b A	29.72 ± 1.24 b AB	31.68 ± 2.57 ab C
		C	28.18 ± 1.39 b A	29.64 ± 1.13 ab B	23.18 ± 1.37 c B	30.47 ± 1.23 ab A	28.49 ± 2.11 ab AB	32.47 ± 0.70 a C
		D	30.14 ± 1.14 b A	29.32 ± 1.34 bc B	24.59 ± 0.39 d B	30.96 ± 0.17 b A	26.86 ± 1.11 cd BC	42.50 ± 2.02 a B
		E	29.34 ± 0.72 bc A	28.63 ± 0.82 bc B	26.33 ± 2.43 c AB	32.48 ± 1.29 b A	31.85 ± 1.60 b A	84.83 ± 1.68 a A
	2014	A	19.08 ± 0.42 c C	21.84 ± 0.96 c BC	31.79 ± 2.49 b AB	29.75 ± 0.43 b A	31.92 ± 2.63 b C	92.68 ± 4.20 a
		B	21.64 ± 0.67 c B	24.40 ± 2.02 bc A	35.74 ± 3.08 b A	22.88 ± 1.70 c B	30.02 ± 1.44 bc C	88.01 ± 9.54 a
		C	18.88 ± 0.88 c C	20.09 ± 1.30 c B	27.33 ± 1.98 bc BC	23.67 ± 0.98 c B	33.99 ± 1.30 b BC	88.53 ± 7.22 a
		D	22.02 ± 0.51 d AB	25.50 ± 2.19 d A	31.92 ± 2.28 c AB	25.55 ± 2.37 d AB	38.44 ± 1.17 b B	81.70 ± 2.14 a
		E	23.23 ± 0.29 cd A	22.18 ± 0.67 d BC	22.89 ± 2.26 cd C	30.23 ± 3.05 c A	57.91 ± 3.23 b A	96.09 ± 5.00 a
2015	A	39.02 ± 0.55 bc B	44.50 ± 3.91 b B	45.37 ± 11.25 b	22.83 ± 0.90 d B	29.40 ± 2.74 cd B	84.57 ± 5.44 a A	
	B	43.02 ± 1.98 b A	59.67 ± 1.16 a A	47.05 ± 3.09 b	44.72 ± 1.06 b A	41.23 ± 4.32 b A	44.18 ± 2.13 b B	
	C	19.70 ± 0.29 c D	19.63 ± 2.69 c E	31.46 ± 2.39 b	20.44 ± 0.91 c B	35.89 ± 2.25 b AB	42.08 ± 1.40 a B	
	D	27.30 ± 0.90 bc C	28.21 ± 2.35 bc D	38.22 ± 6.17 b	23.94 ± 3.83 c B	32.72 ± 4.54 bc AB	55.67 ± 4.29 a B	
	E	44.88 ± 1.92 b A	36.92 ± 1.57 b C	40.64 ± 3.21 b	42.24 ± 2.22 b A	41.99 ± 3.81 b A	92.48 ± 17.60 a A	
C:P	2012	A	170.53 ± 2.07 c C	255.49 ± 12.62 b AB	231.45 ± 20.12 bc AB	380.65 ± 17.70 a	372.62 ± 40.00 a	372.96 ± 28.33 a
		B	185.72 ± 2.60 c BC	238.50 ± 7.27 b B	228.52 ± 4.88 bc AB	363.38 ± 32.27 a	374.06 ± 3.71 a	380.73 ± 20.55 a
		C	219.28 ± 11.06 c A	285.44 ± 7.92 b A	266.88 ± 21.56 bc A	387.44 ± 3.34 a	361.60 ± 18.45 a	382.38 ± 29.73 a
		D	189.38 ± 3.35 d BC	248.12 ± 6.85 b B	219.50 ± 4.92 c B	389.30 ± 10.10 a	382.51 ± 8.48 a	385.62 ± 6.28 a
		E	194.59 ± 13.06 c B	267.13 ± 19.14 b AB	256.41 ± 19.47 b AB	372.03 ± 8.19 a	389.62 ± 1.96 a	378.05 ± 14.63 a
	2013	A	192.06 ± 7.28 de BC	249.09 ± 9.11 b AB	236.83 ± 11.91 bc B	188.31 ± 18.70 e B	218.71 ± 2.60 cd D	309.30 ± 7.04 a B
		B	180.37 ± 19.34 c C	228.55 ± 10.22 b BC	280.44 ± 5.60 a A	272.46 ± 25.55 ab AB	256.92 ± 8.68 ab C	272.28 ± 21.58 ab B
		C	224.26 ± 12.27 d AB	214.48 ± 8.87 d C	284.22 ± 14.56 c A	334.41 ± 7.62 a A	304.83 ± 4.16 bc B	320.65 ± 9.76 ab B
		D	253.72 ± 18.04 b A	264.56 ± 2.22 b A	239.14 ± 15.53 b B	290.23 ± 20.89 ab AB	343.30 ± 28.68 a A	331.24 ± 21.03 a B
		E	204.66 ± 13.24 c BC	263.30 ± 12.70 bc A	207.44 ± 15.91 c B	323.07 ± 86.58 ab A	247.22 ± 5.64 bc CD	413.66 ± 38.11 a A
	2014	A	206.82 ± 4.62 c A	210.57 ± 6.81 c	295.72 ± 23.90 b A	271.65 ± 8.16 bc	234.24 ± 14.90 bc C	593.94 ± 57.34 a B
		B	175.56 ± 1.49 c BC	263.49 ± 56.62 b	236.33 ± 12.66 bc B	259.11 ± 12.54 b	217.91 ± 14.95 bc C	645.31 ± 30.66 a B
		C	185.01 ± 3.60 d B	215.93 ± 14.81 cd	233.82 ± 21.22 bc B	247.60 ± 19.65 bc	270.53 ± 5.50 b B	678.75 ± 18.39 a B
		D	183.32 ± 9.79 d B	261.30 ± 37.46 bc	287.32 ± 37.64 b A	281.05 ± 10.43 bc	215.61 ± 4.42 cd C	658.90 ± 31.37 a B
		E	167.41 ± 1.88 d C	239.14 ± 17.70 c	239.06 ± 18.54 c B	260.60 ± 7.87 c	375.38 ± 12.49 b A	882.05 ± 44.12 a A

Table 5. Cont.

Ratio	Year	Stand Age	Leaf C:N, C:P and N:P Ratios (by Mass) in the Different Sampling Months					
			May	Jun	Jul	Aug	Sept	Oct
N:P	2015	A	211.51 ± 3.48 a A	117.80 ± 1.53 C B	123.30 ± 6.44 c B	134.41 ± 5.37 c B	96.87 ± 9.02 d C	175.16 ± 10.75 b C
		B	172.44 ± 2.41 b D	111.45 ± 16.42 c B	108.64 ± 5.02 c B	132.61 ± 3.59 c B	130.34 ± 2.37 c BC	203.78 ± 16.88 a C
		C	194.43 ± 1.96 d B	202.32 ± 12.08 d A	229.73 ± 6.08 c A	278.97 ± 10.56 b A	257.68 ± 8.43 b A	322.59 ± 8.42 a B
		D	185.26 ± 2.11 b C	213.78 ± 38.97 b A	240.40 ± 20.05 b A	232.34 ± 5.23 b A	260.90 ± 76.37 b A	450.75 ± 27.96 a A
		E	189.20 ± 2.89 b BC	184.84 ± 5.23 b A	214.43 ± 10.72 b A	222.75 ± 46.82 b A	205.77 ± 3.84 b AB	489.28 ± 91.90 a A
	2012	A	7.27 ± 0.59 b BC	7.90 ± 0.06 b C	7.98 ± 1.13 b B	15.89 ± 0.81 a A	14.55 ± 0.77 a B	7.05 ± 0.22 b A
		B	7.67 ± 0.48 cd BC	7.92 ± 0.03 c C	6.57 ± 0.24 d B	14.70 ± 0.71 b AB	19.63 ± 0.47 a A	6.87 ± 0.16 cd A
		C	9.36 ± 0.08 c A	10.23 ± 0.37 bc A	11.16 ± 0.96 bc A	14.32 ± 0.15 a B	13.43 ± 0.40 a B	4.56 ± 0.12 d C
		D	6.97 ± 0.28 cd C	8.89 ± 0.33 b B	7.99 ± 0.25 bc B	13.61 ± 0.54 a B	13.92 ± 0.37 a B	5.92 ± 0.49 d B
		E	8.08 ± 0.16 c B	10.06 ± 0.54 b A	12.53 ± 0.69 a A	13.81 ± 0.46 a B	13.78 ± 0.91 a B	5.04 ± 0.53 d BC
	2013	A	7.84 ± 0.21 cd AB	8.98 ± 0.24 ab A	9.64 ± 0.19 a B	8.13 ± 0.69 bc	8.70 ± 0.43 ab C	7.05 ± 0.19 d C
		B	6.30 ± 0.40 b C	6.35 ± 0.52 b B	9.63 ± 0.15 a B	9.23 ± 0.44 a	8.66 ± 0.61 a C	8.60 ± 0.20 a B
		C	7.96 ± 0.37 c AB	7.24 ± 0.06 c B	12.26 ± 0.10 a A	10.99 ± 0.70 ab	10.73 ± 0.63 b B	9.97 ± 0.51 b A
		D	8.41 ± 0.39 bc A	9.04 ± 0.46 bc A	9.73 ± 0.72 b B	9.38 ± 0.71 bc	12.79 ± 1.01 a A	7.81 ± 0.67 c BC
		E	6.98 ± 0.47 ab BC	9.19 ± 0.19 a A	7.91 ± 0.70 a C	9.93 ± 2.51 a	7.77 ± 0.35 ab C	4.87 ± 0.35 b D
	2014	A	10.84 ± 0.14 a A	9.65 ± 0.40 b	9.32 ± 0.63 b A	9.14 ± 0.40 b BC	7.35 ± 0.29 c A	6.40 ± 0.39 d B
		B	8.12 ± 0.28 b CD	10.75 ± 1.68 a	6.66 ± 0.88 b B	11.35 ± .59 a A	7.26 ± 0.21 b AB	7.40 ± 1.03 b B
		C	9.81 ± 0.58 ab B	10.75 ± 0.12 a	8.55 ± 0.16 bc AB	10.47 ± 0.94 a AB	7.96 ± 0.16 c A	7.69 ± 0.54 c AB
		D	8.33 ± 0.49 b C	10.21 ± 0.57 ab	9.06 ± 1.62 ab AB	11.05 ± 0.90 a AB	5.61 ± 0.25 c C	8.07 ± 0.43 b AB
		E	7.21 ± 0.13 cd D	10.78 ± 0.79 a	10.47 ± 0.54 ab A	8.68 ± 0.98 bc C	6.50 ± 0.48 d B	9.20 ± 0.73 ab A
2015	A	5.42 ± 0.11 a C	2.66 ± 0.21 bc D	2.82 ± 0.65 bc C	5.65 ± 0.42 a C	3.30 ± 0.14 b C	2.08 ± 0.15 c C	
	B	4.02 ± 0.23 b D	1.87 ± 0.24 d D	2.31 ± 0.13 d C	2.97 ± 0.03 c D	3.18 ± 0.28 c C	4.61 ± 0.24 a B	
	C	9.87 ± 0.15 b A	10.38 ± 0.78 b A	7.34 ± 0.78 c A	13.65 ± 0.39 a A	7.19 ± 0.22 c AB	7.67 ± 0.34 c A	
	D	6.79 ± 0.16 b B	7.64 ± 1.67 ab B	6.34 ± 0.46 b AB	9.90 ± 1.82 a B	7.97 ± 1.90 ab A	8.16 ± 1.18 ab A	
	E	4.22 ± 0.14 D	5.01 ± 0.29 C	5.31 ± 0.60 B	5.25 ± 0.86 CD	4.92 ± 0.36 BC	5.34 ± 0.75 B	

Values are the mean ± standard deviation of the mean ($n = 3$). Lowercase letters represent significant differences among different months at $p < 0.05$. Capital letters represent significant differences among the differently aged stands at $p < 0.05$. The same letters indicate no significant difference at $p < 0.05$. A: sapling forest; B: young forest; C: half-mature forest; D: near-mature forest and E: mature forest.

3.3. Relationships of the C, N and P Concentrations and Their Stoichiometric Ratios in Leaves with Stand Age

Large variations in leaf C, N and P concentrations and their stoichiometric ratios over age sequences were observed from 2012 to 2015. However, no unidirectional linear correlation between stand age and any of leaf C, N and P concentrations or C:N:P stoichiometric ratios were observed, and variable linear correlations were observed in the different sampling months from 2012 to 2015 (Table 6). For 2012–2014 (but not 2015), positive correlations between the leaf C concentrations and stand age were observed in May, August and September, whereas significant negative correlations were observed in July. Furthermore, variable linear correlations were observed in June and October. For leaf N concentrations, variable linear correlations with stand age were observed in the different sampling months. Interestingly, no significant correlation between leaf P concentration and stand age was observed in any of the different growing seasons from 2012 to 2015.

Table 6. Correlations between stand age and the C, N, and P concentrations and C:N, C:P, and N:P ratios in leaves in different sampling months from 2012 to 2015.

Element	Year	Sampling Month					
		May	Jun	Jul	Aug	Sept	Oct
C	2012	1.00 ***	−0.99 ***	−1.00 ***	0.60 ns	1.00 ***	−0.97 **
N		0.28 ns	0.90 *	0.98 **	0.85 ns	0.74 ns	−0.96 **
P		0.33 ns	0.29 ns	0.50 ns	0.10 ns	0.31 ns	0.63 ns
C:N		0.41 ns	−0.97 **	−0.99 **	0.99 **	0.97 **	1.00 **
C:P		0.99 **	0.99 ***	1.00 ***	−0.99 **	0.99 ***	0.97 **
N:P		0.54 ns	0.94 *	0.97 **	0.86 ns	0.64 ns	−0.95 *
C		2013	0.99 **	−1.00 ***	−0.98 **	0.96 *	0.98 **
N	−0.93 *		0.87 ns	0.70 ns	0.87 ns	−0.96 **	−0.95 *
P	0.54 ns		0.63 ns	0.62 ns	0.59 ns	0.46 ns	−0.89 *
C:N	0.67 ns		0.65 ns	0.22 ns	0.96 **	0.99 **	0.99 **
C:P	1.00 ***		1.00 ***	−1.00 ***	1.00 ***	1.00 ***	1.00 ***
N:P	0.48 ns		0.95 **	0.16 ns	0.81 ns	0.97 **	0.57 ns
C	2014		1.00 ***	0.99 **	−0.99 ***	1.00 ***	0.96 **
N		−0.95 *	0.04 ns	0.96 *	0.78 ns	−0.97 **	0.07 ns
P		0.84 ns	0.39 ns	0.17 ns	0.20 ns	0.78 ns	0.80 ns
C:N		0.97 **	0.26 ns	−0.99 ***	0.93 *	0.99 ***	0.40 ns
C:P		−1.00 ***	1.00 ***	0.91 *	0.77 ns	1.00 ***	1.00 ***
N:P		−0.97 **	0.35 ns	0.96 **	−0.93 *	0.76 ns	0.97 **
C		2015	−1.00 ***	1.00 ***	1.00 ***	0.99 **	−1.00 ***
N	0.98 *		0.99 **	0.94 *	0.94 *	−0.98 **	0.83 ns
P	0.03 ns		0.85 ns	0.86 ns	−0.92 *	−0.92 *	−0.97 **
C:N	−0.99 ***		−1.00 ***	−0.99 **	1.00 ***	1.00 ***	1.00 ***
C:P	−0.97 **		1.00 ***	1.00 ***	1.00 ***	1.00 ***	1.00 ***
N:P	0.74 ns		0.97 **	0.95 *	0.96 **	0.88 ns	0.96 *

Asterisks (*) show significant correlations at $p < 0.05$, double asterisks (**) show significant correlations at $p < 0.01$, and triple asterisks (***) show significant correlations at $p < 0.001$. ns indicates no significance at the 5% level.

A negative correlation between the leaf C:N ratio and stand age was observed in July in each sampling year (except 2015). However, a positive correlation was observed from the mid-late stage of the fast growing season to the late growing season (August, September and October). In May and June of each sampling year, variable linear correlations were observed. In each sampling year, a positive correlation between the leaf N:P ratio and stand age was observed in June and July, whereas in the early growing season (May), the mid-late fast growing season (August and September) and the late growing season (October), variable linear correlations were observed. Interestingly, a unidirectional linear correlation was observed between the leaf C:P ratio and stand age throughout the growing season in each sampling year, and the leaf C:P ratio was positively correlated with stand age.

4. Discussion

4.1. Patterns of the Leaf C, N and P Concentrations across All Differently Aged Stands

Across all of the larch stands, the leaf C concentrations (leaves sampled in July) and the mean leaf C concentrations (leaves sampled in July–September) were higher than those of the arid saline biomes of China ($396.7 \pm 45.4 \text{ mg}\cdot\text{g}^{-1}$) [24] and the global value ($461.3 \pm 72.2 \text{ mg}\cdot\text{g}^{-1}$) [25,26], but the leaf N concentrations (leaves sampled in the same month) were generally lower than those of Chinese flora ($20.2 \pm 8.4 \text{ mg}\cdot\text{g}^{-1}$) [18], the arid saline biomes of China ($28.1 \pm 9.4 \text{ mg}\cdot\text{g}^{-1}$) [24], the global value ($20.1 \pm 8.7 \text{ mg}\cdot\text{g}^{-1}$) [25,26] and the desert regions of northern China ($24.5 \pm 8.1 \text{ mg}\cdot\text{g}^{-1}$) [27]. However, the leaf P concentrations of all larch stands (leaves sampled in the same month) were generally higher than those of Chinese flora ($1.46 \pm 0.99 \text{ mg}\cdot\text{g}^{-1}$) [18], the global flora ($1.77 \pm 1.1 \text{ mg}\cdot\text{g}^{-1}$) [25,26] and the desert flora of northern China ($1.74 \pm 0.88 \text{ mg}\cdot\text{g}^{-1}$) [27] (Tables 2 and S3). These differences in the leaf C, N and P concentrations among different studies are likely due to differences in sampling seasons, vegetation types and soil nutrients. Previous studies have reported both organ-specific and site-specific differences (e.g., temperature and precipitation differences), which may account for much of the variability in plant nutrient concentrations [28]. In conclusion, the larch plantations in the Qinling Mountains had higher C and P concentrations but lower N concentrations than those reported in other areas. A complete description of element allocation in different plant tissues is critical for explaining plant functional diversity [28]. Seasonal variations in nutrient elements in plant tissue also represent a dynamic growth response. Such variations may be related to the migration of nutrient elements caused by changes in the balance between the uptake and utilization efficiency of these elements and differences in C fixation ability and N and P-use efficiency among the different growing seasons. These phenomena may lead to differences in the C, N and P concentrations of plant leaves [3,4,29].

In this study, the leaf C, N and P concentrations of all larch stands showed significant variations among the different growing seasons from 2012 to 2015, and the leaf concentrations of different nutrient elements were significantly affected by growing season, sampling year, and stand age. Across all of the larch stands, the leaf C and N concentrations were generally higher in the fast growing season than in the other growing seasons. This phenomenon was likely due to the higher growth rate during the fast growing season, as rapid growth promotes greater accumulation of C and N via enhanced photosynthesis [30,31]. Moreover, rapidly growing organs require abundant N-rich proteins to maintain their rapid growth rates [32,33]. In addition, plants require P-rich ribosomal RNA to initiate growth [31], which may explain why the highest leaf P concentrations were observed in the early growing season. In the late growing season, the leaf N and P concentrations were significantly lower than they were in the other growing seasons, which may reflect a response to the ontogenetic development of the plant [34]. Generally, nutrient elements are allocated to specific organs for plant survival, including the transfer of elements from leaves to winterized or perennial organs [35–37]. Thus, decreases in leaf N and P concentrations with growth may be caused by the re-allocation of nutrient elements to other organs in the late growing season. The results of this study indicate that leaf N and P concentrations were more easily influenced than was C concentration by growing season, sampling year and stand age, which is consistent with the results of other reports [14,15,25]. Across all of the larch stands, large variations in leaf C, N and P concentrations were observed, and the leaves of saplings displayed greater demand for N and P than did the other stands. This result may explain why the demand for nutrients in young plants is higher than that in old plants, as these elements are critical to plant growth and play a key role in enzyme production [11]. However, in consideration of the un-repeatability in this study (due to age factors), the variations in leaf C, N and P concentrations among all of the larch stands might be due to age but could be due to differences in soil nutrients or other factors. Yang et al. (2011b) indicated that increasing N and P concentrations in the soil increased leaf N and P concentrations [4]. Although soil nutrients were highly related to plant tissue nutrients, this finding does not suggest that a stable relationship existed between the soil nutritional status

and nutrient concentrations or stoichiometric ratios in plants. The relationships between soil nutrient and plant nutrient concentrations should be investigated in the future.

Previous studies have indicated that nutrient elements in plants are affected by a dilution effect caused by increases in plant size and biomass [16,38,39]; moreover, the element concentrations in leaves decrease with plant ontogenetic development [3,4,8,40]. In our study, the dilution effect affected P concentrations more than it did C and N concentrations. The leaf C and N concentrations did not show a dilution effect; in particular, the leaf C concentrations increased slightly with growth, which is consistent with the results of other studies [38]. This pattern can be explained in terms of an association between the assimilative capacity of C and plant growth [41].

4.2. Patterns of Leaf C:N:P Stoichiometric Ratios across All Differently Aged Stands

Comparison of our results with those of other studies in which samples were collected in July or July–August revealed that the leaf C:N ratios of all larch stands were higher than those of the arid saline biomes of China (15.7 ± 5.6) [24] and the global value (23.8 ± 17.3) [25,26]. Furthermore, the leaf C:P ratios were higher than those of the arid saline biomes of China (229.4 ± 63.7) [24] but lower than the global value (300.9 ± 236.8) [25,26]. In addition, the leaf N:P ratios were lower than those of Chinese flora (16.3 ± 9.3) [18], the arid saline flora of China (15.4 ± 3.7) [24], the desert flora of northern China (15.77 ± 7.5) [27] and the global value (13.8 ± 9.5) [25,26] (Table 5 and Table S3). The differences in the leaf C:N, C:P and N:P ratios among different studies are likely due to differences in ecosystem type, sampling time, abiotic factors (such as temperature, elevation, precipitation, and drought) [25] and biological factors [26]. In conclusion, the leaves of larch plantations in the Qinling Mountains had higher C:N and C:P ratios but lower N:P ratios than the results from other regions.

Elser et al. [31] found that the leaf C:N, C:P and N:P ratios of all larch stands were significantly co-affected by sampling year, sampling month and stand age; in our study, the leaf C, N and P stoichiometric ratios showed significant variation among the different growing seasons from 2012 to 2015. The capacities of plants to fix C varies by growing season, and differences in the allocation patterns of N and P can lead to different C:N, C:P, and N:P ratios [42,43]. In general, both the leaf C:N and leaf C:P ratios increased in the later sampling months during the growing season in this study. This pattern is explained by plant accumulation of structural material that is enriched in C as the plant grows. Moreover, N and P become diluted as the leaf area increases, leading to higher C:N and C:P ratios [11,13].

C:N stoichiometry is used to estimate the N-use efficiency and plant responses to N assimilation, as the C:N ratio is positively correlated with plant N-use efficiency and negatively correlated with plant growth rate [44,45]. Variations in C fixation ability and N-use efficiency with growing season were observed in the leaves of all of the larch stands. In each larch stand, the late growing season was associated with both the highest N-use efficiency and the lowest growth rate. C:P stoichiometry is used to estimate P-use efficiency and plant responses to P assimilation, as the C:P ratio is positively correlated with plant P-use efficiency and negatively correlated with plant growth rate [43,44]. The leaf C:P ratios of all of the larch stands peaked in the late growing season in our study, indicating that the highest P-use efficiency in the leaves and the lowest growth rate occur at that time. Variation in the leaf C:N and C:P ratios with growth represent a dynamic response to changes in N- and P-use efficiency in plants in different growth stages and corresponds to patterns of plant ontogeny. Thus, the leaf C:N and C:P ratios increased in the later sampling months, reflecting the accumulation of compounds with higher ratios of C to N or P [11]. The growth rate hypothesis (GRH) suggests that the N:P ratio is negatively correlated with growth rate [37]. Generally, the leaf N:P ratios initially increased (from the early growing season to the fast growing season) and then decreased (from the fast growing season to the late growing season). This pattern is similar to that reported by Orgeas et al., (2003) [46]. However, unlike Agren (2008), who concluded that the leaf N:P ratio decreased with plant growth in terrestrial ecosystems [11], Elser et al., (1996) found that the leaf N:P ratio was affected by sampling

time (growing month) [31]. We found that the leaf N:P ratio was also affected by stand age, sampling year and their interactions.

The leaf N:P ratio can be used to determine potential N or P limitations in plants [40,47,48]. A ratio of <14 indicates N limitation, and a ratio of >16 indicates P limitation. In our study, the leaf N:P ratios of all of the larch stands were generally <14 during the entire growing season from 2012 to 2015. Considering the background soil nutrient concentrations (Table 1), the growth of larch plantations was limited much more strongly by N than by P in our study region. However, in some of the sampling months from 2012 to 2015, the leaf N:P ratios were between 14 and 16, indicating an equal limitation of N and P in the growth of larch trees [31]. These results indicate that the type and intensity of nutrient limitation in plants may vary with growing season, plant age and degree of plant development. Additionally, in terrestrial ecosystems, the N and P concentrations in different plant tissues are affected by the N and P concentrations in the soil [49]. The N and P in plants are derived from the soil pool, and the C, N and P concentrations in the soil depend mainly on the release of C, N and P through litter decomposition by microbes. Therefore, changes in N and P concentrations in soil can affect C:N:P stoichiometric ratios in leaves [4]. However, litter is difficult to decompose by microbial action in soil with a C:N ratio >25 [50]. In our study, the leaf C:N ratios of all the larch stands were significantly greater than 25 in the late growing season, limiting the cycling of C, N and P in the soil in the study region. This phenomenon likely explains the N-limited growth pattern of the larch plantations. Across all of the larch stands, large differences in the C:N, C:P and N:P ratios were observed in the same sampling month, but no clear tendencies were found. This result is explained by the N and P concentrations in the plant tissue being more easily affected by various factors in this study.

4.3. Correlation Study

Analysing the variations in C, N and P concentrations and their stoichiometric ratios over age sequences provides important information for forest management [51]. In our study, the C, N and P concentrations and their stoichiometric ratios in the leaves of all larch stands were significantly affected by stand age, and large variations over the age sequence were observed among the different growing seasons from 2012 to 2015.

In our study, in the early growth stage and the fast growing stage, leaf C concentration increased significantly with stand age. This pattern can be explained by the fact that the most active growth occurs in young plants; thus, C fixation in the leaves of young plants is stronger than that in old plants. Markovic et al., (2009) reported that N concentrations decreased and P concentrations increased with stand age in some or all tissues of lucerne [52]. Chen et al., (2004) found that leaf P concentrations of *Pinus sylvestris* var. decreased with stand age, and that the leaf N concentrations of a *Pinus* spp. plantation did not show consistent changes with stand age [53]. However, in the present study, the leaf N and P concentrations did not display any significant relationship with stand age in the different sampling months. This result can be attributed to the limited responses of N and P activity in plants to sampling year, sampling time, plant age and their interaction, compared with their responses to other environmental factors [16].

Previous studies have indicated that increases in woody biomass and the lignification of plants in forest ecosystems may lead to increases in the C:N and C:P ratios in different tissues with increasing stand age [13,54]. In this study, no unidirectional linear correlations between stand age and leaf C:N, C:P and N:P ratios were observed in the different growing seasons from 2012 to 2015. Overall, the leaf C:N and C:P ratios increased with stand age during the fast growing season; in particular, the leaf C:P ratio increased with stand age throughout the entire growing season. This pattern is inconsistent with the findings of Yang and Luo (2011), who reported that leaf C:N and C:P ratios increased with stand age [13]. A previous study indicated that the leaf N:P ratios in lucerne decreased and then increased with stand age [14]; consistent with our findings. However, the correlation between leaf N:P ratio and stand age varied by sampling month and sampling year. The results indicate that in

perennials, the C, N and P concentrations and C:N:P stoichiometric ratios in tissues may change with stand age because of differences in the utilization efficiency of nutrient elements among differently aged plants. Although the effects of stand age on C, N and P concentrations and their stoichiometric ratios are considerable, they are not completely understood. Further investigations of the relationships of nutrient element concentrations and stoichiometric ratios with stand age should be conducted. Moreover, the GRH suggests that the C:N, C:P and N:P ratios are negatively correlated with plant growth rate [37,43,44]. In this study, the leaf C:N and C:P ratios generally increased with stand age, indicating that the young larch plantation trees had a higher growth rates than did the old stands. In addition, the results indicated that the leaves of the young larch plantation trees had a greater photosynthetic capacity than did the leaves of the old stands. This result is similar to that reported by Reich et al. (2009) [55].

5. Conclusions

In contrast to nutrient cycling in natural forests, nutrient cycling within plantation forests may depend on artificial management practices. Seasonal variations in C, N and P and their stoichiometric ratios in different growth stages can mirror specific geographic environments and may reflect the physiological and nutritional requirements of plants. However, short-term data can limit our ability to predict nutrient limitations in forest ecosystems. In our study, we attempted to elucidate the variations in C, N and P concentrations and their stoichiometric ratios through a long-term field experiments in a larch plantation ecosystem. Our study suggests that the variations in leaf C, N and P concentrations and C:N:P stoichiometric ratios may be caused by variation in the nutrient allocation patterns or physiological demands of leaves among different growing seasons. The evidence presented here reinforces the view that nutrient limitation in forest ecosystems is a complex phenomenon.

Our study indicates that caution should be taken when considering C, N and P concentrations or C:N, C:P and N:P ratios as functional traits because sampling month and plant age strongly influence the concentrations of nutritional element and their stoichiometric ratios in leaves. However, these patterns could be used as a new methodology to understand plant growth and formation in plant communities. Our study also provides a valuable contribution to the global pool of leaf stoichiometric data. Collectively, our results should be helpful for improving the sustainability of larch plantation production by providing guidance on the optimal levels of N fertilization that should be applied in the early growing season in this region. Further investigations of the relationship between the stoichiometric ratios in plants and the growing season or stand age should be conducted to provide insights into the conservation and management of plantation forests.

Supplementary Materials: The following are available online at www.mdpi.com/1999-4907/8/10/373/s1, Table S1: Analysis of variance of the C, N and P concentrations in leaves at various growth stages from 2012 to 2015 (DOC). Table S2: Analysis of variance of the C:N, C:P and N:P ratios in leaves at various growth stages from 2012 to 2015 (DOC). Table S3: The mean C, N, and P concentrations and C:N, C:P, and N:P ratios (mass ratio) in leaves from July to September (DOC).

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