forests

MDPI

Article

Dynamics in Stoichiometric Traits and Carbon, Nitrogen, and Phosphorus Pools across Three Different-Aged *Picea asperata* Mast. Plantations on the Eastern Tibet Plateau

Jixin Cao, Hong Pan, Zhan Chen and He Shang *

Key Laboratory of Forest Ecology and Environment of National Forestry and Grassland Administration, Research Institute of Forest Ecology, Environment and Protection, Chinese Academy of Forestry, Beijing 100091, China; caojx@caf.ac.cn (J.C.); 15176369309@163.com (H.P.); chenzhan0508@126.com (Z.C.)

* Correspondence: shanghechina@caf.ac.cn; Tel.: +86-10-628-886-32

Received: 28 October 2020; Accepted: 8 December 2020; Published: 17 December 2020

check for updates

Abstract: Understanding the variations in soil and plants with stand aging is important for improving management measures to promote the sustainable development of plantations. However, few studies have been conducted on the dynamics of stoichiometric traits and carbon (C), nitrogen (N), and phosphorus (P) pools across *Picea asperata* Mast plantations of different ages in subalpine regions. In the present study, we examined the stoichiometric traits and C, N, and P stocks in different components of three different aged (22-, 32-, and 42-year-old) *P. asperata* plantations by plot-level inventories. We hypothesized that the stoichiometric traits in mineral soil could shape the corresponding stoichiometric traits in soil microbes, tree roots and foliage, and the C, N, and P stocks of the total *P. asperata* plantation ecosystem would increase with increasing stand age. Our results show that the N:P ratio in mineral soil was significantly correlated with that in tree foliage and herbs. Additionally, the C:N ratio and C:P ratio in mineral soil only correlated with the corresponding stoichiometric traits in soil microbes and forest floor, respectively. Both the fractions of microbial biomass C in soil organic C and microbial biomass N in soil total N decreased with increasing stand age. The C, N, and P stocks of the total ecosystem did not continuously increase across stand development. In particular, the P stock of the total ecosystem exhibited a trend of increasing first and then decreasing. The aboveground tree biomass C accounted for more than 55% of the total ecosystem C stock regardless of stand age. In contrast, mineral soil and forest floor were the major contributors to the total ecosystem N and P stocks in all stands. This study suggested that all three different stands were N limited, and the stoichiometric homeostasis in the roots of *P. asperata* was more stable than that in the foliage. In addition, the soil microbial community assembly may change with increasing stand age for *P. asperata* plantations in the subalpine region.

Keywords: stoichiometric traits; elemental stocks and allocation; *Picea asperata* Mast. plantations; cutovers; subalpine areas

---

1. Introduction

The relative concentrations of carbon (C), nitrogen (N), and phosphorus (P), termed stoichiometry, are crucial traits of individual components in terrestrial ecosystems [1–3]. C is the main component of organisms and contributes to approximately 50% of plant dry matter [4]; additionally, N and P are two of the essential and most limiting nutrients in organisms [5]. N is a fundamental nutrient for synthesizing a range of molecules with vital structural and functional roles, and N in plant leaves is involved in photosynthetic processes [6,7]. P is a key component of many important compounds in cells, such as phospholipids, nucleic acids, and adenosine triphosphate (ATP) [8]. The ratios of C:N and C:P represent the ability of organisms to assimilate C when simultaneously consuming N and P [9]. The ratio of N:P has been widely used as an indicator of nutrient limitation in terrestrial net primary production [10]. Previous studies showed that stoichiometric traits were not constant and varied with the differences in species, individual organs of the same species, growth period, and environmental conditions [11–14]. In terrestrial ecosystems, plants provide substrates for soil microbes, and soil microbes are associated with soil nutrient turnover and supply mineralized nutrients to plants [15]. Additionally, soil, as a key basis of the terrestrial ecosystems, plays a pivotal role in the nutrients cycle in the ecosystem [16–18]. Thus, stoichiometric traits of chemical elements would reflect the interactions between plants, soil, and microbes [19,20], and C:N, C:P, and N:P ratios in soil may have a regulating impact on the corresponding stoichiometric traits in other ecosystem components, especially for foliage, root, and soil microbes.

During recent decades, China has launched large-scale afforestation programs, and the achievements have attracted worldwide attention [21]. Many studies have suggested that afforestation would favor environmental development, biodiversity conservation, and climate change mitigation [22–25]. However, the deterioration of ecological functions may occur during plantation development [26]. Stoichiometric traits are critical indicators of ecological processes [6,11], and a better understanding of the stoichiometric traits will be conducive to improving the management and promoting sustainable development of the plantations. Moreover, a clear evaluation of C, N, and P stocks in soil and plants is essential for understanding the ecological effects of afforestation.

The subalpine forest area of western Sichuan is on the eastern edge of the Tibetan Plateau and is a vital ecological barrier in the upper reaches of the Yangtze River, as it plays a crucial role in reducing soil erosion, regulating runoff, conserving water sources, and biodiversity [27]. Unfortunately, the primitive subalpine coniferous forests in these areas have been clear-cut since the middle of the last century [28], and it was not until 1998 that logging activities were completely banned [29]. After clear-cutting, spruce (*Picea* spp.) was commonly used to implement reforestation in these areas. After thousands of square kilometers of spruce plantations have been established on the cutovers, managing these plantations is very challenging [30,31]. Thus, it is essential to comprehensively realize the variations in these plantations with stand aging to draw up rational management measures. However, information on the dynamics of stoichiometric traits and their relationships between mineral soil and other components of spruce plantation ecosystems is still inadequate. Furthermore, the variations in the elemental stocks of the spruce plantation ecosystem, along with stand development, have also been rarely reported. We hypothesized that the stoichiometric traits in mineral soil could shape the corresponding stoichiometric traits in soil microbes, tree roots and foliage, and the C, N, and P stocks of the total *P. asperata* plantation ecosystem would increase with increasing stand age. The objectives of the present study are to (1) determine the stoichiometric traits of plants, forest floor, mineral soil, and soil microbes in different-aged *P. asperata* plantations, (2) analyze the relationships between the stoichiometric traits in mineral soil and other ecosystem components, and (3) evaluate the dynamics in ecosystem C, N and P stocks and the contributions of above- and belowground components.

2. Materials and Methods

2.1. Study Sites

The present study was conducted in Miyaluo (102°41′–102°44′ E, 31°42′–31°51′ N), which is in Lixian County, western Sichuan Province, China. This area belongs to the transition zone from the Tibetan Plateau to the Sichuan Basin and is an important part of the southwestern national forest region of China. In this area, the elevations of forestland range from 2400–3900 m. It is characterized by a montane monsoon climate with annual precipitation of 600–1100 mm and an annual mean temperature of 6–10 °C [32]. Soils are classified as mountain brown soil series in Chinese soil taxonomy.

2.2. Field Sampling and Measurements

The maximum age of existing *Picea asperata* Mast. plantation is approximately 50 years old in this subalpine area, and the age classes of *P. asperata* plantations are divided into young age (<20 years old), middle age (21 to 40 years old), near maturity (41 to 60 years old), respectively. Thus, three *P. asperata* plantations of different ages, namely, 22-, 32-, and 42-year-old, all with pure overstories, were chosen to represent the development process from middle age to near maturity. All the stands are on the eastern slope of the subalpine area (Table 1) and are located within a 5 km radius of each other. The field measurements and sampling were carried out in July 2018. Three 20 × 20 m plots were randomly selected in each stand. The diameter at breast height (DBH) and height (H) were recorded for all trees within each plot. Above- and belowground tree biomass in each plot was calculated using allometric equations with DBH and H [31]. In each plot, five trees were randomly selected and sampled for foliage, branch, trunk, and root. The foliage and branch samples were obtained from the middle canopy. The trunk samples were obtained by using an increment borer. The root samples were taken at five random points under the canopy for a soil depth of 0–40 cm. The soil was washed out by water to the roots. In each plot, foliage, branch, trunk, and root samples were mixed as one sample for each tree component. Three 1 × 1 m micro plots were distributed along a diagonal with each plot for herb investigation. In each micro plot, all herbs were excavated. Herb samples were separated into above- and belowground components after species had been identified. The forest floor was sampled by collecting the entire organic matter within three 0.2 × 0.2 m quadrates that were randomly placed in each plot. The samples of herbs and forest floor were oven-dried at 65 °C to constant weight. Soil samples of 0–20 cm and 20–40 cm were collected at five random sites in each plot. Five soil samples of the same layer were combined as one composite soil sample. Then, parts of the soil sample were kept on ice in an incubator for soil microbial C, N, and P measurements, and the other part of the soil samples was air-dried and sieved through a 2 mm mesh and further used to determine the organic C, total N, and P concentrations. Soil samples for bulk density of each correspondence depth were also collected using a 100 cm^3 soil ring and oven-dried at 105 °C to constant weight. After measuring the bulk density, these soil samples were sieved through a 2 mm mesh, and then, the volume of the fragments over 2 mm was determined using a graduated cylinder.

Table 1. Basic characteristics of the three *P. asperata* plantations.

Stand Age	Elevation (m a.s.l.)	Slope Degree (°)	Slope Aspect	Density (Trees hm^{-2})	Mean DBH (cm)
22	3308	15–20	East	1733	11.93
32	3330	18–25	East	1558	18.34
42	3360	11–24	East	792	23.32

DBH, Diameter at breast height.

Soil organic C concentration was determined by the potassium dichromate oxidation method [33]. Soil total N concentration was determined by the semi-micro-Kjeldahl method [33]. Soil total P and plant biomass P concentrations were determined by the ascorbic acid method and vanadium (V)-molybdenum (Mo) yellow colorimetry, respectively [34]. The soil samples were fumigated with chloroform, and soil microbial C and N concentrations were determined by a Vario TOC/TNb analyzer (Elementar, Langenselbold, Hesse, Germany). Plant biomass C and N concentrations were also determined by this method. Moreover, soil microbial P was determined by Mo blue colorimetry. Plantation ecosystem element stock was defined as the sum of the tree, herb, forest floor, and soil correspondence element stock. Soil C, N, and P stocks were estimated according to the formula based on the corresponding elemental concentration, the soil horizon thickness, the bulk density, and the volumetric percentage of fragments >2 mm [4]. The elemental concentration of herb was calculated as the biomass weighted average of each species for each micro plot. The C, N, and P stocks of different plant components were calculated by multiplying the component's biomass and the corresponding elemental concentrations, respectively. There is almost no shrub in the understory for cutting bush management. Thus, shrub was not measured in the present study.

2.3. Statistical Analysis

Statistical analysis was performed using the SPSS 18.0 (SPSS, Chicago, IL, USA). Data were first checked for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene's test. As the data met these analyses of variance (ANOVA) assumptions, the difference among the means of different stands and variations of different components within the same stand were respectively examined via one-way ANOVA and a Tukey test ($p < 0.05$). Pearson correlation analysis was performed to examine the correlation of the same variable in the different ecosystem components.

3. Results

3.1. C, N, and P Concentrations and Stoichiometric Traits in Mineral Soil and Soil Microbial Biomass

The highest concentrations of soil organic C and total N were observed in the 42-year-old stand at both the 0–20 cm and 20–40 cm soil depths (Figure 1a,b). However, the soil total P concentration in the 32-year-old stand was significantly higher than that in the other two stands at these two depths (Figure 1c). For the soil microbial biomass C concentration, the mean value at the 0-20 cm soil depth increased from 343.16 mg kg^{-1} in the 22-year-old stand to 443.64 mg kg^{-1} in the 42-year-old stand (Figure 2a). At the 20–40 cm soil depth, the soil microbial biomass C concentrations in the 22- and 32-year -old stands were not much different, and both of them were significantly lower than the soil microbial biomass C concentration in the 42-year-old stand ($p < 0.05$) (Figure 2a). In contrast, there were no significant differences in the microbial biomass N concentration among the three stands at each soil depth (Figure 2b). At both the 0-20 cm and 20–40 cm soil depths, the microbial biomass P concentration also did not change obviously with stand age and ranged from 3.15 mg kg^{-1} and 3.35 mg kg^{-1} to 3.63 mg kg^{-1} and 3.80 mg kg^{-1}, respectively (Figure 2c). Both the fractions of microbial biomass C in soil organic C and microbial biomass N in soil total N showed a decreasing trend with stand age at both the 0–20 cm and 20–40 cm soil depths (Figure 3a,b). Though the mean fraction of microbial biomass P in soil total P in the 32-year-old stands was lower than that in the other two stands at both the 0–20 cm and 20–40 cm soil depths, the differences did not reach statistical significance (Figure 3c). The soil C:N, C:P, and N:P ratios in the 42-year-old stands were all significantly higher than those in the 22- and 32-year-old stands at both the 0–20 cm and 20–40 cm soil depths ($p < 0.05$) (Figure 1d–f). In contrast, only the C:N ratio presented a significant variation among the three different stands for the soil microbial biomass (Figure 2d–f).

3.2. C, N, and P Concentrations and Stoichiometric Traits in Plants and Forest Floor

The variation patterns in C, N, and P concentrations of tree foliage differed from each other with stand age (Figure 4a–c). The highest concentrations of C, N, and P in tree foliage were found in the 22-, 42- and 32-year-old stands, respectively (Figure 4a–c). The C concentration of the tree roots did not significantly change with increasing stand age (Figure 4a); however, both the N and P concentrations in the tree roots significantly increased as stand age increased from 22 years to 32 years (Figure 4b,c). The mean C and N concentrations of the forest floor continuously increased from 252.76 g kg^{-1} and 10.77 g kg^{-1} in the 22-year-old stand to 301.86 g kg^{-1} and 11.45 g kg^{-1} in the 42-year-old stand, respectively (Figure 4a,b). In contrast, the P concentration of the forest floor first increased from 1.27 g kg^{-1} in the 22-year-old stand to 1.45 g kg^{-1} in the 32-year-old stand and then decreased to 1.35 g kg^{-1} in the 42-year-old stand (Figure 4c). With increasing stand age, the herb C, N, and P concentrations did not significantly change except for the aboveground C concentration (Figure 5a,c,e).

For the tree foliage and roots, the highest values of C:N, C:P, and N:P ratios were all observed in the 22-year-old stand, and C:N, C:P, and N:P ratios in the 22-year-old stand were significantly higher than those in the 32-year-old stand ($p < 0.05$) (Figure 4b,d,f). However, significant differences between the 22- and 32-year-old stands were found only for N:P ratios in the forest floor and the belowground herb ($p < 0.05$) (Figures 4f and 5f).

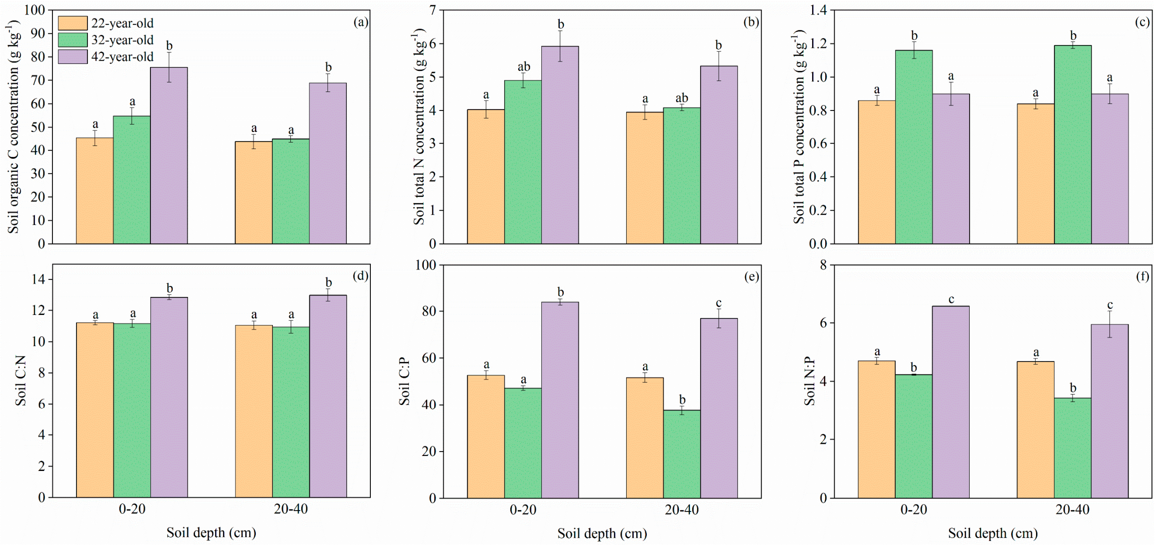

Figure 1. (**a**) C concentration, (**b**) N concentration, (**c**) P concentration, (**d**) C:N, (**e**) C:P and (**f**) N:P in the mineral soil of three different aged *P. asperata* plantations. Different lowercase letters indicate a significant difference between different aged stands at the same soil horizon ($p < 0.05$).

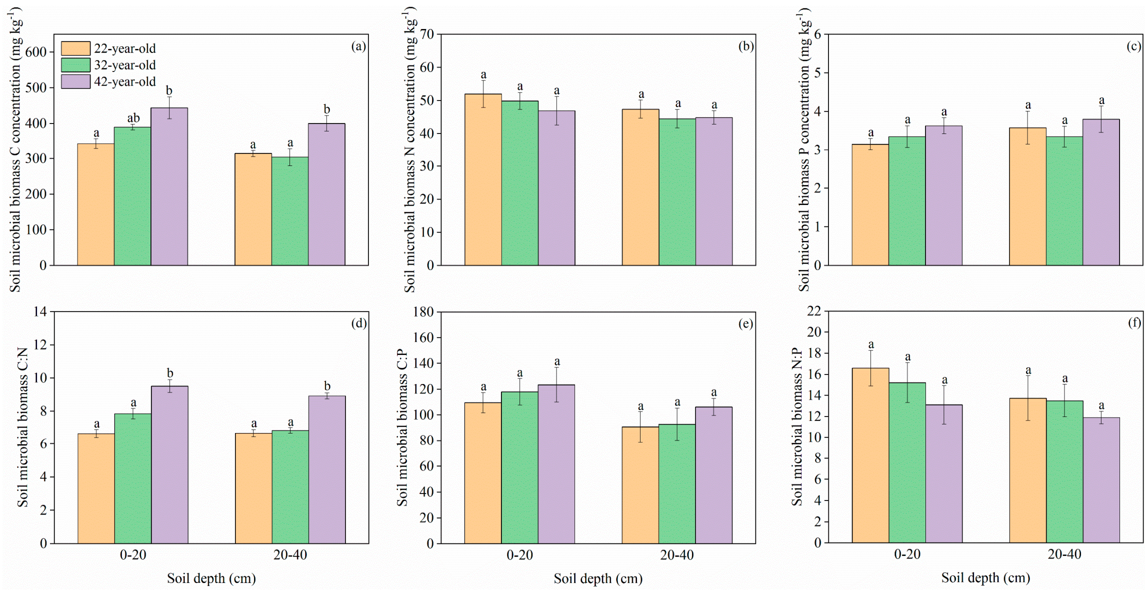

Figure 2. (**a**) C concentration), (**b**) N concentration, (**c**) P concentration, (**d**) C:N, (**e**) C:P, and (**f**) N:P in the soil microbes of three different aged *P. asperata* plantations. Different lowercase letters indicate a significant difference between different aged stands at the same soil horizon ($p < 0.05$).

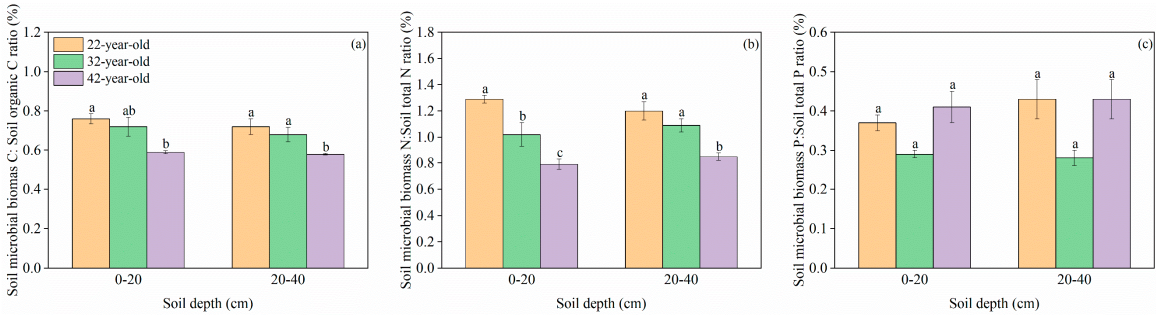

Figure 3. The fractions of (**a**) microbial biomass C in soil organic C, (**b**) microbial biomass N in soil total N, and (**c**) microbial biomass P in soil total P in three different aged *P. asperata* plantations. Different lowercase letters indicate a significant difference between different aged stands at the same soil horizon ($p < 0.05$).

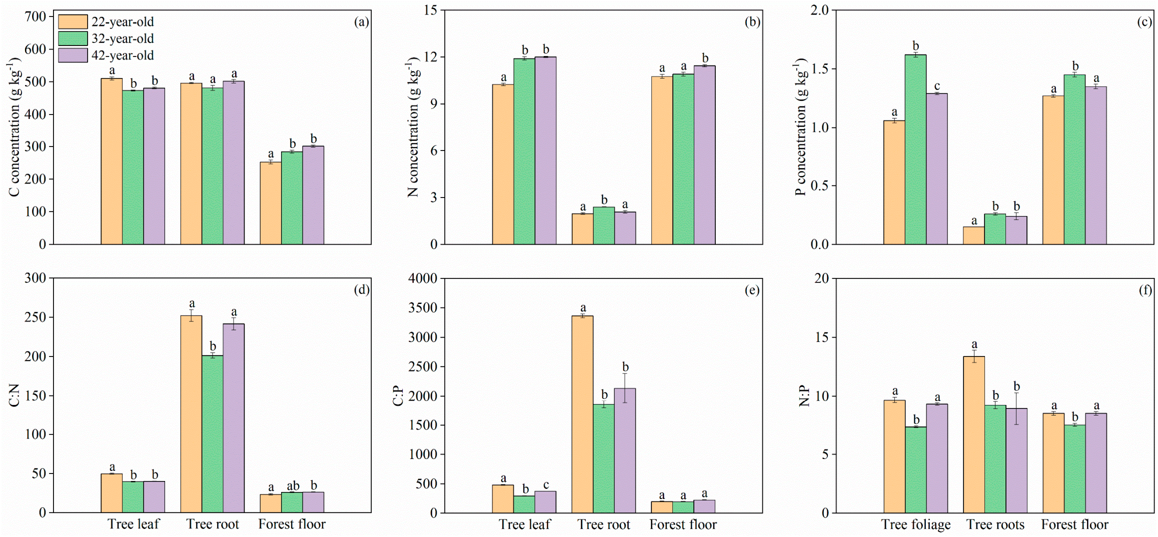

Figure 4. (**a**) C concentration, (**b**) N concentration, (**c**) P concentration, (**d**) C:N, (**e**) C:P and (**f**) N:P in the tree biomass and the forest floor in three different aged *P. asperata* plantations. Different lowercase letters indicate a significant difference between different aged stands for the same variable ($p < 0.05$).

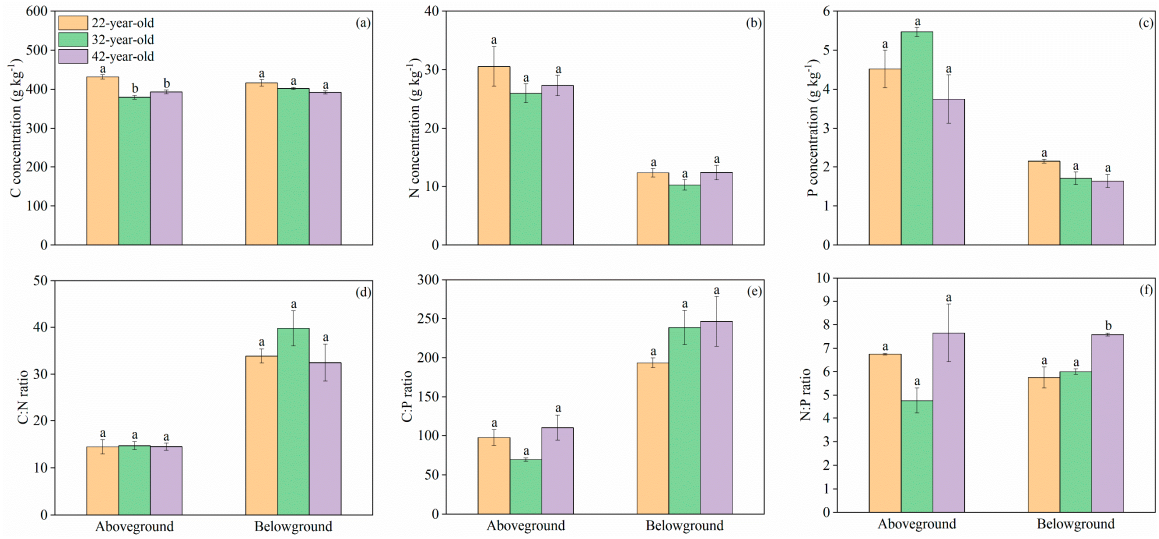

Figure 5. (**a**) C concentration, (**b**) N concentration, (**c**) P concentration, (**d**) C:N, (**e**) C:P and (**f**) N:P in the herb biomass of three different aged *P. asperata* plantations. Different lowercase letters indicate a significant difference between different aged stands for the same variable ($p < 0.05$).

3.3. Relationship between the Stoichiometric Traits of Mineral Soil and Other Ecosystem Components

The soil microbial biomass C:N ratio was significantly and positively correlated with the mineral soil C:N ratios at both the 0–20 cm and 20–40 cm soil depths ($p < 0.05$) (Table 2). The forest floor C:P ratio exhibited a significantly positive correlation with the mineral soil C:P ratio at both the 0–20 cm and 20–40 cm soil depths ($p < 0.05$) (Table 3). The N:P ratios in the belowground herb and the mineral soil were significantly and positively related at these two soil depths ($p < 0.05$) (Table 4). Furthermore, the N:P ratios in both tree foliage and the aboveground herb were also significantly and positively correlated with the mineral soil N:P ratio at a depth of 20–40 cm ($p < 0.05$) (Table 4). In contrast, the stoichiometric traits of the tree roots were not significantly related to those of the mineral soil (Tables 2–4).

Table 2. The relationship among the different components C:N in *P. asperata* plantations.

	Tree Leaf	Tree Root	Aboveground Herb	Belowground Herb	Forest Floor	Soil Microbes (0–20 cm)	Soil Microbes (20–40 cm)
Mineral Soil (0–20 cm)	−0.405	0.324	−0.189	−0.353	−0.340	0.734 *	0.919 **
Mineral Soil (20–40 cm)	−0.383	0.425	0.362	−0.274	0.514	0.743 *	0.840 **

* Significances are indicated at $p < 0.05$. ** Significances are indicated at $p < 0.01$.

Table 3. The relationship among the different components C:P in *P. asperata* plantations.

	Tree Leaf	Tree Root	Aboveground Herb	Belowground Herb	Forest Floor	Soil Microbes (0–20 cm)	Soil Microbes (20–40 cm)
Mineral Soil (0–20 cm)	0.046	−0.218	0.546	0.298	0.787 *	0.300	0.370
Mineral Soil (20–40 cm)	0.267	0.069	0.749 *	0.154	0.852 **	0.039	0.385

* Significances are indicated at $p < 0.05$. ** Significances are indicated at $p < 0.01$.

Table 4. The relationship among the different components N:P in *P. asperata* plantations.

	Tree Leaf	Tree Root	Aboveground Herb	Belowground Herb	Forest Floor	Soil Microbes (0–20 cm)	Soil Microbes (20–40 cm)
Mineral Soil (0–20 cm)	0.504	−0.307	0.652	0.834 **	0.596	−0.380	−0.388
Mineral Soil (20–40 cm)	0.717 *	0.083	0.803 **	0.711 *	0.799 *	−0.421	−0.278

* Significances are indicated at $p < 0.05$. ** Significances are indicated at $p < 0.01$.

3.4. Ecosystem C, N, and P Pools

With increasing stand age, the C and P stocks in the aboveground tree, tree roots, forest floor, and total ecosystem all increased first and then decreased (Tables 5–7). The N stock in the aboveground tree and tree roots and the P stock of mineral soil also followed the same variation pattern with increasing stand age (Tables 6 and 7). However, the mean C stock in mineral soil and the mean N stock in mineral soil and total ecosystem continuously increased with increasing stand age (Tables 5 and 6). Moreover, the C, N, and P stocks in aboveground herb and herb roots did not differ significantly among the three stands (Tables 5–7). The aboveground tree biomass was the dominant C pool, representing more than 55% of the total ecosystem C stock regardless of stand age (Table 5). In contrast, the mineral soil and the forest floor contributed much more than aboveground tree biomass to the total ecosystem N and P pool (Tables 6 and 7).

Table 5. The C Stocks of different components and their contributions to the total ecosystem C pool in three different aged stands.

	22-Year-Old Stand		32-Year-Old Stand		42-Year-Old Stand	
	C Stock (kg hm^{-2})	Ratio (%)	C Stock (kg hm^{-2})	Ratio (%)	C Stock (kg hm^{-2})	Ratio (%)
Aboveground tree	64,325.75 (12,202.95) a	57.87 (2.56) A	114,847.90 (10,212.70) b	62.55 (0.73) A	90,866.62 (9007.32) ab	56.32 (2.10) A
Aboveground herb	414.04 (119.37) a	0.37 (0.08) B	117.22 (8.84) a	0.06 (0.00) B	262.08 (96.10) a	0.16 (0.05) B
Forest floor	16,575.12 (1111.11) a	15.46 (1.26) C	24,780.46 (1232.39) b	13.61 (0.82) C	19,474.49 (1734.13) a	12.35 (1.79) C
Tree roots	10,871.25 (2209.40) a	9.75 (0.55) C	25,638.54 (2690.03) b	13.94 (0.48) C	22,484.83 (2123.27) b	13.94 (0.45) C
Herb roots	420.96 (112.14) a	0.38 (0.06) B	156.36 (12.04) a	0.09 (0.01) B	243.45 (80.67) a	0.15 (0.04) B
Mineral soil	17,140.35 (727.440) a	16.17 (1.91) C	17,731.46 (667.12) a	9.75 (0.54) D	27,295.93 (936) b	17.08 (0.80) C
Total	109,747.47 (16,062.27) a		183,271.83 (14,054.88) b		160,627.41 (10,247.59) ab	

Data are presented as the mean value with the standard error (SE) given in parenthesis. Mean values within a row followed by different lowercase letters are significantly different at $p < 0.05$, Mean values within a column followed by different Uppercase letters are significantly different at $p < 0.05$.

Table 6. The N Stocks of different components and their contributions to the total ecosystem N pool in three different aged stands.

	22-Year-Old Stand		32-Year-Old Stand		42-Year-Old Stand	
	N Stock (kg hm^{-2})	Ratio (%)	N Stock (kg hm^{-2})	Ratio (%)	N Stock (kg hm^{-2})	Ratio (%)
Aboveground tree	189.14 (38.62) a	7.36 (1.02) A	591.22 (51.90) b	17.97 (0.95) A	430. 45 (62.17) b	12.54 (1.52) A
Aboveground herb	30.87 (11.42) a	1.21 (0.39) B	7.95 (0.18) a	0.24 (0.01) B	18.79 (7.74) a	0.54 (0.22) B
Forest floor	710.50 (69.23) a	28.05 (0.83) C	950.67 (42.07) a	29.05 (1.49) C	739.66 (69.60) a	21.80 (2.55) C
Tree roots	43.72 (10.22) a	1.70 (0.29) B	127.11 (12.23) b	3.86 (0.23) B	93.36 (11.97) ab	2.72 (0.28) B
Herb roots	12.24 (2.87) a	0.48 (0.09) B	3.94 (0.08) a	0.12 (0.01) B	8.11 (3.26) a	0.24 (0.09) B
Mineral soil	1537.36 (69.52) a	61.21 (2.50) D	1597.77 (52.65) a	48.76 (1.15) D	2124.10 (101.40) b	62.16 (0.64) D
Total	2523.82 (178.50) a		3278.67 (111.71) b		3414.49 (129.34) b	

Data are presented as the mean value with the standard error (SE) given in parenthesis. Mean values within a row followed by different lowercase letters are significantly different at $p < 0.05$, Mean values within a column followed by different Uppercase letters are significantly different at $p < 0.05$.

Table 7. The P Stocks of different components and their contributions to the total ecosystem P pool in three different aged stands.

	22-Year-Old Stand		32-Year-Old Stand		42-Year-Old Stand	
	P Stock (kg hm^{-2})	Ratio (%)	P Stock (kg hm^{-2})	Ratio (%)	P Stock (kg hm^{-2})	Ratio (%)
Aboveground tree	17.82 (4.32) a	4.00 (0.81) A	54.57 (3.91) b	8.86 (0.35) A	33.33 (3.03) a	7.04 (0.70) A
Aboveground herb	4.56 (1.66) a	1.03 (0.35) A	1.69 (0.14) a	0.28 (0.02) BD	2.36 (0.67) a	0.50 (0.15) B
Forest floor	83.51 (7.46) a	18.97 (0.92) B	126.29 (3.61) b	20.60 (0.86) C	87.39 (9.53) a	18.35 (1.66) C
Tree roots	3.22 (0.62) a	0.72 (0.11) A	13.76 (1.01) b	2.24 (0.10) B	10.51 (1.92) b	2.19 (0.31) B
Herb roots	2.19 (0.60) a	0.50 (0.12) A	0.66 (0.01) a	0.11 (0.00) D	1.08 (0.44) a	0.23 (0.10) B
Mineral soil	327.54 (12.09) a	74.78 (2.22) C	417.27 (15.60) a	67.92 (0.41) E	340.12 (11.48) a	71.68 (0.92) D
Total	438.84 (21.32) a		614.24 (20.47) b		474.78 (19.22) a	

Data are presented as the mean value with the standard error (SE) given in parenthesis. Mean values within a row followed by different lowercase letters are significantly different at $p < 0.05$, Mean values within a column followed by different Uppercase letters are significantly different at $p < 0.05$.

4. Discussion

In the present study, the mineral soil C and N concentrations showed an increasing trend with increasing stand age, which is inconsistent with many previous studies [6,35,36]. These variations could be explained by the continuous input of litter and roots exudates along with stand development. However, there still exists some controversy regarding the effects of stand age on mineral soil C and N [37–39]. These controversial results may be caused by other influencing factors, such as previous land use, climate, and tree species, all of which may overshadow the impacts of stand age [4]. Different from the changing trend in mineral soil C and N concentrations, the highest value of mineral soil P concentration was observed in the 32-year-old stand and then decreased in the 42-year-old stand. With stand aging, the variation in soil total P concentration is more complex than that in soil C and N. Trees mobilize P from mineral soil into the biological P cycle, leading to a decrease in the soil total P concentration during the early growth process of the plantation; however, with increasing stand age, the litter input and decomposition gradually increase, which returns P to the soil and increases the total P concentration [40]. In addition, the decrease in soil pH with stand aging would reduce soil P availability because low pH drives phosphate to bind to Fe and Al precipitates [41]. Thus, the decrease in soil pH would also contribute to increasing soil total P concentration with increasing stand age. For the decrease in the total P concentration of mineral soil from the 32-year-old stand to the 42-year-old stand, this variation may be related to the plunge in tree density. During stand development, management by removing deadwood from plantations would not only decrease the elemental stock but also reduce the complement to soil P. Thus, we suggest that the total soil P concentration of mineral soil may exhibit a wave-changing pattern with increasing stand age.

In terms of soil microbes, we found that both the fraction of microbial C in soil organic C and the fraction of microbial N in soil organic N decreased with increasing stand age. The decreases in these two ratios indicate that the microbial biomass supported per unit C and N resources declined with stand aging [20]. Previous studies suggested that the fraction of microbial C in soil organic C was significantly and negatively correlated with the soil C:N ratio [42,43]. This negative relationship could be explained by soil microbes being suppressed by the lack of N resources under the condition of a high soil C:N ratio and then decreasing the C demand [44]. Moreover, the variation in the availability of soil C and N resources would further shape the assembly of the soil microbial community because bacteria are generally considered to require more N per unit biomass C accumulation than fungi [18,45]. Additionally, the accumulation of litter and decrease in pH is adverse for bacteria with stand development, which would also contribute to increasing the fungal abundance [18,46].

The C concentration of tree foliage decreased from the 22-year-old stand to the 32- and 42-year-old stands in the present study. However, the amplitude of variation is less than 10%, which was similar to the previous description for *Sonneratia apetala* plantations [47]. However, the N and P concentrations of tree foliage in the 32-and 42-year-old stands were much higher than those in the 22-year-old stand, resulting in a significant reduction in the C:N and C:P ratios in the two older *P. asperata* stands. Biomass is composed of different biochemical components that vary according to tree organ, tree species, geographical location, climate, and soil conditions [48]. Previous studies argued that increasing the C-rich metabolite concentration, such as tannins, could enhance the defense ability against diseases and insect pests [49], while the biomass N concentration and the N utilization efficiency could be changed by the variation in N supply [50,51]. Moreover, the growth rate hypothesis suggested that fast-growing organisms need relatively more P-rich RNA to support the rapid synthesis of large amounts of protein, resulting in a low biomass N:P ratio [52,53]. Hence, changes in the elemental concentrations and stoichiometric traits would reflect differences in the plant survival environment and adaptation strategies. In the present study, we suggest that both variations in nutrient demand and soil nutrient levels lead to increases in the N and P concentrations of tree foliage in the two older stands. It has been demonstrated that plant growth is limited by N if the N:P ratio < 14, constrained by P if the N:P ratio > 16, and co-limited by both N and P if the N:P ratio is between the two threshold values [54]. The present study found that all the N:P ratios in tree foliage, tree roots, and herbs were less than 14, and most of them were less than 10, indicating that all three different stands were severely scarce in terms of N nutrition. A deficiency in the N supply would reduce the production of cytokinins and accelerate leaf senescence because N nutrients are translocated from senescent leaves to young leaves [55]. The present study found that the N:P ratios in tree foliage, aboveground herbs, and belowground herbs were all significantly and positively correlated with the N:P ratio at a depth of 20–40 cm in mineral soil. In contrast, the N:P ratio in tree roots was not significantly correlated with the soil N:P ratio in any depth. These results may indicate that the stoichiometric trait in roots was less sensitive than that in tree foliage to the soil nutrient availability. These findings are consistent with the previous study that demonstrated the stoichiometric homeostasis in roots is more stable than that in foliage for both *Symplocos ramosissima* and *Machilus gamblei* seedlings [56]. However, some studies concerning other plant species suggested that stoichiometric homeostasis was stronger in foliage [17,57,58]. These contradictory results may be caused by the differences in tree species, growth stage, and external environment [59]. The present study suggests that *P. asperata* would prioritize meeting the requirements of N and P nutrient balance in the roots compared with foliage, which would be beneficial to sustainable growth under the environment of the thin soil layer in this subalpine area.

The total ecosystem C, N, and P stocks increased by more than 1.2-fold when increasing stand age from the 22-year-old stand to the 32-year-old stand. In contrast, the N stock was not significantly different between the 32-year-old and 42-year-old stands, and the mean C and P stocks even decreased in the 42-year-old stand, which is similar to the previous findings [60]. Our results did not in line with the hypothesis. The present study indicates that *P. asperata* plantations would not reach a stable state after 30 years of establishment, C and nutrient losses could occur during later stand development, and *P. asperata* plantations have been facing N limitation. Thus, the regular use of N fertilizers is suggested as an essential management measure for these different aged *P. asperata* plantations. In addition, other managements, such as adding organic matter and adjustment of tree density [30,61], may also contribute to improving the soil fertility of these *P. asperata* plantations, thereby achieving the goals of making land degradation neutrality and sustainable development [62,63].

5. Conclusions

The soil organic C and total N concentrations increased with increasing stand age; however, the soil total P concentration showed a changing process of increasing first and then descending. Additionally, all the stands of the *P. asperata* plantation were limited by the scarcity of N nutrition. The C:N, C:P, and N:P ratios of the mineral soil were only correlated with the corresponding stoichiometric traits in specific ecosystem components during stand development. The relative changes in soil C and N concentrations affected the microbial C:N ratio and may further shape the assembly of the soil microbial community. Compared with foliage, *P. asperata* would prioritize meeting the N requirement of the root. The C, N, and P stocks in the total ecosystem did not continue to increase significantly with increasing stand age due to the density of the *P. asperata* plantation plunging in the later growth stage. The proportion of aboveground tree biomass C to the C stocks of the total ecosystem was the highest regardless of stand age. In contrast, mineral soil and forest floor were the major contributors to the total ecosystem N and P stocks for all stands. The information provided by our study will provide a better understanding of the details of dynamics in *P. asperata* plantations established on cutovers of subalpine areas. Due to the limitation in the number of replicated stands in our study, the results do not represent the whole variation pattern of stand development. Thus, further evidence for the effects of stand age on stoichiometric traits and element stocks in *P. asperata* plantations will be required.

Author Contributions: Formal analysis, J.C.; Funding acquisition, H.S.; Investigation, J.C. and H.P.; Methodology, J.C., H.P., Z.C., and H.S.; Writing–original draft, J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Key R&D Program of China (No. 2017YFC0505001).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Sun, L.; Zhang, B.; Wang, B.; Zhang, G.; Zhang, W.; Zhang, B.; Chang, S.; Chen, T.; Liu, G. Leaf elemental stoichiometry of *Tamarix* Lour. species in relation to geographic, climatic, soil, and genetic components in China. *Ecol. Eng.* **2017**, *106*, 448–457. [CrossRef]
2. Elser, J.J. Ecological stoichiometry: From sea to lake to land. *Trends Ecol. Evol.* **2000**, *15*, 393–394. [CrossRef]
3. Elser, J.J.; Dobberfuhl, D.R.; Mackay, N.A.; Schampel, J.H. Organism size, life history and N:P stoichiometry. *Bioscience* **1996**, *46*, 674–684. [CrossRef]
4. Cao, J.; Wang, X.; Tian, Y.; Wen, Z.; Zha, T. Pattern of carbon allocation across three different stages of stand development of a Chinese pine (*Pinus tabulaeformis*) forest. *Ecol. Res.* **2012**, *27*, 883–892. [CrossRef]
5. Larsen, M.L.; Wihlelm, S.W.; Lennon, J.T. Nutrient stoichiometry shapes microbial coevolution. *Ecol. Lett.* **2019**, *22*, 1009–1018. [CrossRef]
6. Chen, L.; Deng, Q.; Yuan, Z.; Mu, X.; Kallenbach, R.L. Age-related C:N:P stoichiometry in two plantation forests in the Loess Plateau. *Ecol. Eng.* **2018**, *120*, 14–22. [CrossRef]
7. Peng, Y.; Li, F.; Zhou, G.; Fang, K.; Zhang, D.; Li, C.; Yang, G.; Wang, G.; Wang, J.; Yang, Y. Linkages of plant stoichiometry to ecosystem production and carbon fluxes with increasing nitrogen inputs in an alpine steppe. *Glob. Chang. Biol.* **2016**, *23*, 5249–5259. [CrossRef]
8. Bielski, R.L. Phosphate pools, phosphate transport, and phosphate availability. *Ann. Rev. Plant. Physiol.* **1973**, *24*, 225–252. [CrossRef]
9. Rong, Q.; Liu, J.; Cai, Y.; Lu, Z.; Zhao, Z.; Yue, W.; Xia, J. Leaf carbon, nitrogen and phosphorus stoichiometry of *Tamarix chinensis* Lour. in the Laizhou Bay coastal wetland, China. *Ecol. Eng.* **2015**, *76*, 57–65. [CrossRef]
10. Li, P.; Yang, Y.; Han, W.; Fang, J. Global patterns of soil microbial nitrogen and phosphorus stoichiometry in forest ecosystems. *Glob. Ecol. Biogeogr.* **2014**, *23*, 979–987. [CrossRef]
11. Leal, M.C.; Seehausen, O.; Matthews, B. The ecology and evolution of stoichiometric phenotypes. *Trends Ecol. Evol.* **2017**, *32*, 108–117. [CrossRef] [PubMed]
12. Yu, H.; Fan, J.; Li, Y. Foliar carbon, nitrogen, and phosphorus stoichiometry in a grassland ecosystem along the Chinese Grassland Transect. *Acta Ecol. Sin.* **2017**, *37*, 133–139. [CrossRef]
13. Cao, J.; Shang, H.; Chen, Z.; Tian, Y.; Yu, H. Effects of elevated ozone on stoichiometry and nutrient pools of *Phoebe bourneri* (Hemsl.) Yang and *Phoebe Zhennan* S. Lee et F. N. Wei seedlings in subtropical China. *Forests* **2016**, *7*, 78. [CrossRef]
14. Heuck, C.; Weig, A.; Spohn, M. Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. *Soil Biol. Biochem.* **2015**, *85*, 119–129. [CrossRef]

15. Fan, H.; Wu, J.; Liu, W.; Yuan, Y.; Hu, L.; Cai, Q. Linkages of plant and soil C:N:P stoichiometry and their relationships to forest growth in subtropical plantations. *Plant. Soil* **2015**, *392*, 127–138. [CrossRef]
16. Visser, S.; Keesstra, S.; Maas, G.; de Cleen, M.; Molenaar, C. Soil as a basis to create enabling conditions for transitions towards sustainable land management as a key to achieve the SDGs by 2030. *Sustainability* **2019**, *11*, 6792. [CrossRef]
17. Minden, V.; Kleyer, M. Internal and external regulation of plant organ stoichiometry. *Plant. Biol.* **2014**, *16*, 897–907. [CrossRef]
18. Fierer, N.; Strickland, D.L.; Bradford, M.A.; Cleveland, C.C. Global patterns in belowground communities. *Ecol. Lett.* **2009**, *12*, 1238–1249. [CrossRef]
19. Zhao, F.Z.; Ren, C.J.; Han, X.H.; Yang, G.H.; Doughty, R. Changes of soil microbial and enzyme activities are linked to soil C, N and P stoichiometry in afforested ecosystems. *For. Ecol. Manag.* **2018**, *427*, 289–295. [CrossRef]
20. Zhou, Z.-H.; Wang, C.-K. Changes of the relationships between soil and microbes in carbon, nitrogen and phosphorus stoichiometry during ecosystem succession. *Chin. J. Plant. Ecol.* **2016**, *40*, 1257–1266. [CrossRef]
21. Chen, C.; Park, T.; Wang, X.; Piao, S.; Xu, B.; Chaturvedi, R.K.; Fuchs, R.; Brovkin, V.; Ciais, P.; Fensholt, R.; et al. China and India lead in greening of the world through land-use management. *Nat. Sustain.* **2019**, *2*, 122–129. [CrossRef] [PubMed]
22. Peng, S.-S.; Piao, S.; Zeng, Z.; Ciais, P.; Zhou, L.; Li, L.Z.X.; Myneni, R.B.; Yin, Y.; Zeng, H. Afforestation in China cools local land surface temperature. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 2915–2919. [CrossRef] [PubMed]
23. Yang, X.; Wang, X.; Li, N. Combating climate change: What will China's Forestry do? *Ambio* **2010**, *39*, 340–343. [CrossRef]
24. Brockerhoff, E.G.; Jactel, H.; Parrotta, J.A.; Quine, C.P.; Sayer, J. Plantation forests and biodiversity: Oxymoron or opportunity? *Biodivers. Conserv.* **2008**, *17*, 925–951. [CrossRef]
25. Huang, M.; Zhang, L.; Gallichand, J. Runoff responses to afforestation in watershed of the Loess Plateau, China. *Hydrol. Process.* **2003**, *17*, 2599–2609. [CrossRef]
26. Li, C.; Zhou, X. Status and future trends in plantation silviculture in China. *Ambio* **2000**, *29*, 354–355. [CrossRef]
27. Miao, N.; Liu, S.; Shi, Z.; Yu, H.; Liu, X. Spatial patterns of dominant tree species in sub-alpine *Betula-abies* forest in West Sichuan of China. *Chin. J. Appl. Ecol.* **2009**, *20*, 1263–1270.
28. Liu, Y.; Chen, J.; Liu, Q.; Wu, Y. Nitrification and denitrification in subalpine coniferous forests of different restoration stages in western Sichuan, China. *Front. For. China* **2007**, *2*, 260–265. [CrossRef]
29. Zhang, Y.; Zhao, C.; Liu, S. The influence factors of sub-alpine forest restoration in Miyaluo, West Sichuan. *Sci. Silvae Sin.* **2005**, *41*, 189–193.
30. Pang, X.-Y.; Liu, Q.; Liu, S.-Q.; Wu, Y.; Lin, B.; He, H.; Zhang, Z.-J. Changes of soil fertility quality properties under subalpine spruce plantation in Western Sichuan. *Acta Ecol. Sin.* **2004**, *24*, 261–267.
31. Pang, X.; Huang, J.; Zhao, Q.; Feng, D.; Bao, W.; Tian, G. Ecosystem carbon stock across a chronosequence of spruce plantations established on cutovers of a high-elevation region. *J. Soil Sediments* **2016**, *17*, 2239–2249. [CrossRef]
32. Xu, Z.; Yin, H.; Xiong, P.; Wan, C.; Liu, Q. Short-term responses of Picea asperata seedlings of different ages grown in two contrasting forest ecosystems to experimental warming. *Environ. Exp. Bot.* **2012**, *77*, 1–11. [CrossRef]
33. Liu, G.S. *Soil Physical and Chemical Analysis and Description of Soil Profiles*, 1st ed.; Standards Press of China: Beijing, China, 1996; pp. 123–167.
34. Bao, S.D. *Agricultural Soil Analysis*, 3rd ed.; China Agriculture Press: Beijing, China, 1999; pp. 74–270.
35. Cao, J.; Yan, W.; Xiang, W.; Chen, X.; Lei, P. Stoichiometry characterization of soil C, N, and P of Chinese fir plantations of three different ages in Huitong, Hunan province, China. *Sci. Silvae Sin.* **2015**, *51*, 1–8.
36. Cui, N.; Liu, X.; Zhang, D.; Zhang, J.; Liu, Y.; Deng, C.; Ji, T.; Chen, Y. The distribution pattern of carbon, nitrogen and phosphorus and the stoichiometry characteristics of *Pinus massoniana* plantation in different ages. *Ecol. Environ. Sci.* **2014**, *23*, 188–195.
37. Li, Q.; Mo, Q.; Wang, F.; Li, Y.; Xu, X.; Zou, B.; Li, X.; Chen, Y.; Li, Z. Nutrient utilization by Casuarina equisetifolia plantation of different ages in the tropical coastal area of South China. *Chin. J. Appl. Environ. Biol.* **2015**, *21*, 139–146.

38. Mao, R.; Zeng, D.-H.; Hu, Y.-L.; Li, L.-J.; Yang, D. Soil organic carbon and nitrogen stocks in an age-sequence pf poplar stands planted on marginal agricultural land in Northeast China. *Plant. Soil* **2010**, *332*, 277–287. [CrossRef]
39. Noh, N.-J.; Son, Y.; Lee, S.-K.; Seo, K.-W.; Heo, S.-J.; Yi, M.-J.; Park, P.-S.; Kim, R.-H.; Son, Y.-M.; Lee, K.-H. Carbon and nitrogen storage in an age-sequence of *Pinus densiflora* stands in Korea. *Sci. China Life Sci.* **2010**, *53*, 822–830. [CrossRef]
40. Wu, H.; Xiang, W.; Chen, L.; Ouyang, S.; Xiao, W.; Li, S.; Forrester, D.I.; Lei, P.; Zeng, Y.; Deng, X.; et al. Soil phosphorus bioavailability and recycling increased with stand age in Chinese fir plantations. *Ecosystems* **2020**, *23*, 973–988. [CrossRef]
41. Wu, H.; Xiang, W.; Ouyang, S.; Xiao, W.; Li, S.; Chen, L.; Lei, P.; Deng, X.; Zeng, Y.; Zeng, L.; et al. Tree growth rate and soil nutrient status determine the shift in nutrient-use strategy of Chinese fir plantations along a chronosequence. *For. Ecol. Manag.* **2020**, *476*, 117896. [CrossRef]
42. Zhou, Z.H.; Wang, C.K. Reviews and syntheses: Soil resources and climate jointly drive variations in microbial biomass carbon and nitrogen in China's forest ecosystems. *Biogeosciences* **2015**, 6751–6760. [CrossRef]
43. Xu, X.; Schimel, J.P.; Thornton, P.E.; Song, X.; Yuan, F.; Goswami, S. Substrate and environmental controls on microbial assimilation of soil organic carbon: A framework for earth system models. *Ecol. Lett.* **2014**, *17*, 547–555. [CrossRef] [PubMed]
44. Zhou, Z.-H.; Wang, C.-K. Responses and regulation mechanisms of microbial decomposers to substrate carbon, nitrogen, and phosphorus stoichiometry. *Chin. J. Plant. Ecol.* **2016**, *40*, 620–630. [CrossRef]
45. Fanin, N.; Fromin, N.; Buatois, B.; Hättenschwiller, S. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. *Ecol. Lett.* **2013**, *16*, 764–772. [CrossRef] [PubMed]
46. Mao, R.; Cui, Q.; Zhao, Q.; Ai, G.; Li, L.; Zeng, D. Soil microbial biomass and activity in relation to stand age of poplar shelterbelts. *Chin. J. Appl. Ecol.* **2009**, *20*, 2079–2084.
47. Ren, H.; Chen, H.; Li, Z.; Han, W. Biomass accumulation and carbon storage of four different aged Sonneratia apetala plantations in Southern China. *Plant. Soil* **2010**, *327*, 279–291. [CrossRef]
48. Bert, D.; Danjon, F. Carbon concentration variations in the roots, stem and crown of mature Pinus Pinaster (Ait.). *For. Ecol. Manag.* **2006**, *222*, 279–295. [CrossRef]
49. Li, M.; Yu, L.; Du, M.; Huang, Z.; Shi, J. C, N, and P stoichiometry and their interaction with plants, litter, and soil in a *Cunninghamia lanceolata* plantation with different ages. *Acta Ecol. Sin.* **2018**, *38*, 7772–7781.
50. Nehe, A.S.; Misra, S.; Misra, S.; Murchie, E.H.; Chinnathambi, K.; Foulkes, M.J. Genetic variation in N-use efficiency and associated traits in Indian wheat cultivars. *Field Crops Res.* **2018**, *225*, 152–162. [CrossRef]
51. Ainsworth, E.A.; Rogers, A. The response of photosynthesis and stomatal conductance to rising [CO_2]: Mechanisms and environmental interactions. *Plant. Cell Environ.* **2007**, *30*, 258–270. [CrossRef]
52. Elser, J.J.; Sterner, R.W.; Gorokhova, E.; Fagan, W.F.; Markow, T.A.; Cotner, J.B.; Harrison, J.F.; Hobbie, S.E.; Odell, G.M.; Weider, L.J. Biological stoichiometry from genes to ecosystems. *Ecol. Lett.* **2000**, *3*, 540–550. [CrossRef]
53. Matzek, V.; Vitousek, P.M. N:P stoichiometry and protein: RNA ratios in vascular plants: An evaluation of the growth-rate hypothesis. *Ecol. Lett.* **2009**, *12*, 765–771. [CrossRef]
54. Koerselman, W.; Meuleman, A.F.M. The vegetation N:P ratio: A new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.* **1996**, *33*, 1441–1450. [CrossRef]
55. Güsewell, S. N:P ratios in terrestrial plants: Variation and functional significance. *New Phytol.* **2004**, *164*, 243–266. [CrossRef]
56. Shi, X.-M.; Qi, J.-H.; Song, L.; Liu, W.-Y.; Huang, J.-B.; Li, S.; Lu, H.-Z.; Chen, X. C, N and P stoichiometry of two dominant seedlings and their responses to nitrogen additions in the montane moist evergreen broad-leaved forest in Ailao Mountains, Yunnan. *Chin. J. Plant. Ecol.* **2015**, *39*, 962–970.
57. Schreeg, L.A.; Santiago, L.S.; Wright, S.J.; Turner, B.L. Stem, root, and old leaf N:P ratios are more responsive indicators of soil nutrient availability than new foliage. *Ecology* **2014**, *95*, 2062–2068. [CrossRef] [PubMed]
58. Garrish, V.; Cernusak, L.; Winter, K.; Turner, B.L. Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, *Ficus insipida*. *J. Exp. Bot.* **2010**, *61*, 3735–3748. [CrossRef] [PubMed]
59. Zhang, T.; Liu, W.; Huang, J.; Hu, T.; Tang, D.; Chen, Q. Characteristics of plant ecological stoichiometry homeostasis. *Guihaia* **2019**, *39*, 701–712. [CrossRef]

60. Alcañiz, M.; Úbeda, X.; Cerdà, A. A 13-Year Approach to Understand the Effect of Prescribed Fires and Livestock Grazing on Soil Chemical Properties in Tivissa, NE Iberian Peninsula. *Forests* **2020**, *11*, 1013. [CrossRef]
61. Novara, A.; Pulido, M.; Rodrigo-Comino, J.; Di Prima, S.; Smith, P.; Gristina, L.; Giménez-Morera, A.; Terol, E.; Salesa, D.; Keesstra, S. Long-term organic farming on a citrus plantation results in soil organic carbon recovery. *Geogr. Res. Lett.* **2019**, *45*, 271–286. [CrossRef]
62. Keesstra, S.; Mol, G.; de Leeuw, J.; Okx, J.; Molenaar, C.; de Cleen, M.; Visser, S. Soil-related sustainable development goals: Four concepts to make land degradation neutrality and restoration work. *Land* **2018**, *7*, 133. [CrossRef]
63. Keesstra, S.D.; Bouma, J.; Wallinga, J.; Tittonell, P.; Smith, P.; Cerdà, A.; Montanarella, L.; Quinton, J.N.; Pachepsky, Y.; van der Putten, W.H.; et al. The significance of soils and soil science towards realization of the United Nations sustainable development goals. *Soil* **2016**, *2*, 111–128. [CrossRef]

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.