forests

MDPI

Article

Soil Microbial Community and Soil Abiotic Factors Are Linked to Microorganisms' C:N:P Stoichiometry in *Larix* Plantations

Mengke Cai, Xiaoqin Cheng, Li Liu, Xinhao Peng, Tianxiong Shang and Hairong Han *

School of Ecology and Nature Conservation, Beijing Forestry University, Beijing 100083, China; cmkwhjxnyzh@163.com (M.C.); cloud2014@bjfu.edu.cn (X.C.)
* Correspondence: hanhr6015@bjfu.edu.cn; Tel.: +86-10-6233-6015

Abstract: Ecological stoichiometry is an essential tool to understand carbon (C), nitrogen (N), and phosphorus (P) cycles and nutrient limitations. Plantations are usually managed to maintain specific age structures, but the impact of such management on microbial biomass and stoichiometric ratios remains unclear. We compared the stand ages of four *Larix principis-rupprechtti* Mayr. Plantations that were 15 years old, (young plantation, Lar15), 24 years old, (middle aged plantation, Lar24), 40 years old, (near-mature plantation, Lar40), and 50 years old, (mature plantation, Lar50), respectively, to determine the main factors that drive differences in the C:N:P stoichiometry of microorganisms. We demonstrated that the temperature, moisture, and nutrient concentrations in surface soil increased significantly with forest age. The stoichiometric ratios of elements in soil and microorganisms reached their maxima in the Lar40 and Lar50 plantations. Additionally, forest stand ages had a great influence on the biomass of microbial communities. Moreover, soil microbial community and soil abiotic factors are closely related to soil microorganisms' C:N:P stoichiometric ratios. Specifically, changes in the microbial biomass C:N (MBC:MBN) were primarily correlated with bacteria, Gram-positive bacteria (G^+), temperature, NH_4^+-N, and moisture in soil. Shifts in G^+, actinobacteria, soil temperature, and total phosphorus were primarily associated with variation in microbial biomass C:P (MBC:MBP). Alterations in microbial biomass N:P (MBN:MBP) were correlated with bacteria, NH_4^+-N, water content, Gram-negative bacteria, and soil temperature. Overall, these results suggest that microbial elemental stoichiometric ratios could be affected by stand age and emphasize the importance of microbial communities and soil abiotic factors in shifting this dynamic change process.

Keywords: soil and microorganism stoichiometry; forest age; abiotic factor; microbial community; environmental variables

Citation: Cai, M.; Cheng, X.; Liu, L.; Peng, X.; Shang, T.; Han, H. Soil Microbial Community and Soil Abiotic Factors Are Linked to Microorganisms' C:N:P Stoichiometry in *Larix* Plantations. *Forests* **2023**, *14*, 1914. https://doi.org/10.3390/f14091914

Academic Editor: Wenjie Liu

Received: 21 August 2023
Revised: 13 September 2023
Accepted: 16 September 2023
Published: 20 September 2023

1. Introduction

Ecological stoichiometry has been used to understand nutrient cycling in terrestrial ecosystems [1,2]. Soil stoichiometric ratios also reflect the rates of nutrient element (e.g., C, N, and P) mineralization in soil following human management. Generally, the elemental ratios of forest soil and microorganisms might vary across successional gradients and have positive impacts on ecosystem nutrient cycling through their effects on biological communities and soil micro-environmental factors [3–5]. Therefore, the responses of soil and microbe element stoichiometry to plantation management models have attracted the attention of ecological researchers.

Understanding the influences of forest management methods (e.g., afforestation) on element cycling and microbial activity is undoubtedly necessary for increasing the yield of forest tree products and maintaining ecosystem functioning [6,7]. Many plantations are managed according to specific age structures. Emerging evidence indicates that plantation age may affect the vertical structure of forest by influencing the gap size, soil hydrothermal conditions, or other understory micro-climate conditions [8,9]. Moreover, manipulation of the forest age directly affects the net ecosystem productivity of the tree layer and indirectly

changes the elemental content in plant organs and woody debris [10,11]. Furthermore, forest age could influence the decomposition efficiency of plant residue by adjusting understory micro-environmental conditions, microbial characteristics, and soil properties [12,13]. To date, the effects of stand ages on stoichiometric ratios in soil and microorganisms remain unclear.

Owing to the close relationships between aboveground vegetation and belowground microorganisms [14], their responses to plantation age gradients may be coupled. Recent studies reveal that age structure could alter the net primary productivity and composition of plantations, and the characteristics of plant residue may respond strongly to changes in aboveground vegetation characteristics. The variations in litter or root residues have been proven to be intimately related to soil element concentrations and microbial community compositions [15]. Liu et al. (2020) [16] demonstrated that the stoichiometric ratio of soil can be very sensitive to changes in elemental concentrations and ratios in leaves, litter, and roots after afforestation. Moreover, forest age could alter soil nutrient elemental contents by altering soil moisture conditions and light intensity [17,18]. These results indicate that the concentration and ratios of soil elements may depend strongly on forest age and changes in aboveground vegetation characteristics.

Other previous results also indicated that soil microbial biomass is a relatively labile nutrient component in soil and that changes in forest soil microbial stoichiometric ratios may also be tightly correlated with plantation age. An increasing body of evidence shows that elemental composition normally varies among bacteria, fungi, actinobacteria, or other microbial groups [19,20]. Therefore, stand age can greatly influence microbial stoichiometry characteristics via driving soil temperature, water content, nutrient content, microbial groups, and other ecological factors [21,22]. Evaluating the dynamic characteristics and driving mechanisms of soil and microorganisms' stoichiometric ratios across stand ages would benefit the management and conservation of plantation ecosystems. However, whether microbial biomass C:N:P ratios could be affected by soil abiotic factors and the microbial community after afforestation remains unclear.

Larix principis-rupprechtii Mayr, the primary conifer tree species planted in Shanxi Province, northern China, has been predominantly utilized for timber production and soil erosion prevention since the late 1960s [23,24], and the elemental contents and ratios in the soil may vary with forest age in *Larix* plantations [23]. Liu et al. (2020) [16] observed a significant decrease in the ratios of C:P and N:P in microorganisms along an age gradient in a *Robinia pseudoacacia* plantation. Conversely, the soil microbial C:N ratio remained stable in the surface soil. Additionally, variations in stoichiometric ratios of elements in different plant tissues during plantation development have been reported. Zeng et al. (2017) [24] and Liang et al. (2017) [25] indicated notable changes in C:N:P ratios across different plant organs, including leaves, fine roots, and branches, with plantation age. Hence, plantations of varying stand ages provide a chronological sequence for comprehending the ecological regulatory mechanisms underlying the variation in microbial C:N:P stoichiometry within managed ecosystems. While earlier studies on plantation management methods focused on improving stand structure, soil nutrient levels, and enhancing plantation biodiversity [25], recent research remains inconclusive regarding whether plantation age can modify stoichiometric ratios in microorganisms and soil by regulating soil properties, microbial communities, and other ecological factors.

We posit that elemental C, N, and P contents, as well as their ratios, in both soil and microbial biomass, are likely to exhibit correlations with stand age. These correlations are expected to be influenced by shifts in vegetation communities, stemming from the intricate interconnections between above- and below-ground ecosystems. Concurrently, we anticipate that environmental variables, including litter characteristics, soil physical and chemical attributes, and microbial biomass, may not exhibit a linear relationship with stand age. Instead, they might peak at an optimal age structure, as certain age configurations are more apt to enhance soil nutrient levels and augment forest product yield. Furthermore, we hypothesize a significant influence of stand age on soil parameters

and microbial composition, consequently prompting shifts in the stoichiometry of microbial C:N:P within plantations.

2. Materials and Methods

2.1. Site Description and Experimental Design

Our research was conducted on Taiyue Mountain, situated in the subalpine region of Shanxi Province, northern China. This area features a warm continental mountainous climate (111°59′45″–112°02′23″ E, 36°38′00″–36°42′23″ N). The average annual temperature of the region is 6.2 °C, and the mean annual precipitation varies from 1700 to 2450 mm, with 60%–70% occuring between May and October. The soil type prevalent in this area is Haplic luvisol (IUSS Working Group WRB, 2015) originating from limestone parent material [20,26,27]. Its bulk density varies from 1.18 g cm^{-3} to 1.41 g cm^{-3} (Table S1). This soil type exhibits a brown coloration and generally maintains a thickness exceeding 30 cm before reaching the bedrock [26]. The predominant vegetation within the study area is temperate deciduous broad-leaved forest, dominated by *L. principis-rupprechtii* Mayr. and *Pinus tabuliformis* Carr. trees. *Rosa xanthine* Lindl and *Dendranthema chanetii* Stapf are the dominant understory species. The main moss species are *Barbula constricta* Mitt, *Marchantia polymorpha* L., and *Thuidium kanedae* Sak. The main animals in this study area were *Otis tarda*, *Sus scrofa*, Siberian roe deer, and *Strigiformes*.

Since the late 1960s, *L. principis-rupprechtii* plantations have been one of the main species used in afforestation in the study area. The local forestry management department has also taken measures to protect natural forests. Following topographic and vegetation surveys, we established twelve plots (i.e., three replicates per stand age) to study four growth stages of Larix plantations: 15 years old (young plantation, Lar15), 24 years old (middle-aged plantation, Lar24), 40 years old (near mature plantation, Lar40), 50 years old (mature plantation, Lar50). These were established in July 2014, except for the middle-aged plantation, which was established in August 2016. Each age treatment is at least 2 km apart from the next closest and has similar topography, climate, and understory plant species compositions (Tables S1 and S2). We included three replicate plots for each plantation age, for a total of twelve sampled plots. We kept each 20 × 30 m plot separated from its nearest neighbor by at least 8 m to avoid potential edge effects. The detailed site information of each forest age treatment is listed in Table S1.

2.2. Sampling and Laboratory Analysis

Soil temperature at a depth of 0–10 cm was measured in each of the twelve plots using temperature loggers. Additionally, the water content (SWC) of the soil at the same depth was determined using a moisture meter. Concurrently, nine replicate samples were collected from the surface soil using a tailor soil auger. Following the removal of plant residue through a soil sieve with a 2 mm mesh width, samples were divided into three parts. One part was utilized to assess the concentrations of available nutrients and microbial biomass in the soil and was stored at 0 °C. Another portion was stored at −20 °C for the analysis of soil microbial community characteristics. The third part underwent an air-drying process and was prepared for measuring the pH value and nutrient indices of the soil.

To sample fresh, undecomposed litter and live fine roots, we collected samples from the twelve plots according to methods described by Zeng et al. (2017) [24]. We selected seven to ten healthy *Larix* trees in each plot and collected fine root samples with a tailor fine root auger (10 cm diameter). We also collected undecomposed leaf litter from all plots. The fine root and leaf litter samples underwent a drying process in which they were placed in an oven at 105 °C for 15 min, followed by further drying at a minimum temperature of 60 °C for 48 h. Once completely dry, the root and litter samples were crushed and prepared for laboratory analysis.

Soil pH was assessed by preparing a water suspension with a soil-to-water ratio of 1:5, and measurements were taken using a pH meter [28]. Total soil carbon (STC) and nitrogen

(STN) were determined using an elemental analyzer (Thermo Fisher Scientific, FLASH 2000 CHNS/O, Thermo, Third Avenue, Waltham, MA, USA) [20]. For total soil phosphorus (STP), the $HClO_4$-H_2SO_4 oxidation digestion method was employed, and samples were then measured on an AA3 continuous flow analytical system (AA3, SEAL, Norderstedt, Germany) [28]. Soil ammonium nitrogen (NH_4^+-N) and soil nitrate nitrogen (NO_3^--N) concentrations were measured using the KCl extraction method. Additionally, the soil available phosphorus (SAP) concentration was determined using the $NaHCO_3$ extraction method. These analyses were also conducted using an AA3 continuous flow analytical system. For the analysis of litter and root samples, we determined the carbon content through a process involving H_2SO_4-dichromate oxidation [29,30]. To ascertain the nitrogen concentration within these samples, we utilized the Kjeldahl method [31]. Additionally, the phosphorus contents of litter and root samples were measured using the AA3 system after acid-H_2O_2 digestion [28].

2.3. The Characteristics of Microbial Communities Analysis

Microbial biomass C (MBC) and N (MBN) were assessed using the fumigation-extraction method. To accomplish this, half of the fresh samples underwent fumigation with chloroform under vacuum conditions for at least 24 h. Following the fumigation process, the samples were subjected to extraction and treated with 0.5 M K_2SO_4. Subsequently, measurements were conducted on all soil samples using a total organic nitrogen/carbon analyzer (Multi N/C 3100, Analytik Jena AG, Konrad-Zuse-Straße, Berlin, Germany) [28]. The methodology outlined by Wu et al. (2006) [32] and Hedley and Steward (1982) [33] was employed for the determination of microbial biomass P (MBP). In this procedure, the fumigation process also necessitated the use of chloroform. However, for the test samples, an extraction step with 0.5 M $NaHCO_3$ was conducted prior to applying the colorimetric method for the quantification of their biomass.

We examined the diversity of soil microbial community structure and composition using phospholipid fatty acid (PLFA) analysis. The process involved the extraction of lipids from each sample (which had been dried and stored at low temperatures) using a mixture containing chloroform, methanol, and phosphate. Following this, fatty acid methyl esters were obtained through mild alkaline methanolysis. Further details can be found in Table S3, which provides comprehensive information regarding the analysis.

2.4. Statistical Analyses

We employed the least significant difference (LSD) multiple comparison method to analyze changes in soil properties, root and litter characteristics, stoichiometric ratios in soil and microorganisms, as well as bacterial and fungal biomass across stands of varying ages. Additionally, we utilized repeated measures analysis of variance (ANOVA) to examine the impact of plantation ages and sampling years on soil physical and chemical properties, stoichiometric ratios in both soil and microorganisms, and the biomass of microbe groups. For investigating the variation in the ratios of elements in the soil and microbial biomass, as well as their connections with pH, moisture, temperature, and nutrient concentrations, we conducted Pearson's correlation analysis. To evaluate the associations between soil microbial element ratios and microbial community biomass, regression analyses were applied. In order to enhance the variance distribution of soil microbial biomass stoichiometry, all data were subjected to a log-10 transformation. Furthermore, we utilized multiple linear stepwise regression to explore whether variations in microbial carbon, nitrogen, and phosphorus ratios were linked to nutrient factors and microbial groups within the soil.

3. Results

3.1. Variation in Environmental Parameters with Plantation Age

The age of the plantation significantly influenced the concentrations of various chemical elements, including carbon (C), nitrogen (N), and phosphorus (P), within leaf litter and fine roots. Specifically, the carbon and nitrogen contents in leaf litter were notably

higher in Lar50 and Lar15 plantations compared to Lar24 and Lar40 plantations, whereas the phosphorus concentration in litter followed the order Lar24 < Lar15 < Lar40 < Lar50 (as shown in Table 1). Furthermore, fine roots from Lar24 plantations exhibited a significantly lower carbon content when compared to those from Lar40 and Lar50 plantations. The concentrations of nitrogen and phosphorus in roots also exhibited significant variation across different plantation ages, particularly noticeable in Lar40 and Lar50 plantations. Regarding the litter layer biomass, it was found to be 16% to 31% higher in Lar24, Lar40, and Lar50 plantations in comparison to the Lar15 plantation (as indicated in Table 1). Interestingly, the impact of plantation age on the Shannon diversity, richness, and evenness indices of shrub and herbaceous vegetation appeared to be limited. However, the highest diversity of understory species was observed in mature plantations (as detailed in Table S2).

Table 1. The characteristics of litter and roots in *Larix* plantations.

Treatments	Litter Characteristics				Root Characteristics		
	Biomass (t ha^{-1})	Element Content (g kg^{-1})			Element Content (g kg^{-1})		
		C	N	P	C	N	P
Lar15	50.68 ± 6.78 ^{a}	70.71 ± 1.48 ^{ab}	6.57 ± 0.65 ^{ab}	0.59 ± 0.04 ^{a}	52.07 ± 2.11 ^{ab}	3.05 ± 0.24 ^{a}	0.33 ± 0.02 ^{a}
Lar24	58.74 ± 11.26 ^{b}	66.92 ± 1.14 ^{a}	5.88 ± 0.34 ^{a}	0.51 ± 0.02 ^{b}	48.91 ± 1.04 ^{a}	3.48 ± 0.19 ^{a}	0.42 ± 0.01 ^{b}
Lar40	56.93 ± 8.10 ^{b}	68.34 ± 1.31 ^{ab}	6.21 ± 1.03 ^{ab}	0.62 ± 0.04 ^{a}	57.46 ± 3.72 ^{b}	4.11 ± 0.43 ^{b}	0.51 ± 0.03 ^{c}
Lar50	66.21 ± 13.79 ^{c}	72.48 ± 1.64 ^{b}	6.92 ± 0.71 ^{b}	0.66 ± 0.01 ^{a}	63.77 ± 2.34 ^{c}	4.27 ± 0.56 ^{b}	0.56 ± 0.02 ^{c}

The values are presented as mean ± standard error. Distinct lowercase letters are used to indicate significant differences between treatments ($p < 0.05$). Lar15: young plantation; Lar24: middle-aged plantation; Lar40: near-mature plantation; Lar50: mature plantation.

During August of both 2016 and 2017, the age of the plantation exhibited a pronounced influence on the levels of total and available soil nutrients, as detailed in Table S4. Notably, the concentrations of total carbon (STC), total nitrogen (STN), total phosphorus (STP), nitrate nitrogen (NO_3^--N), and ammonium nitrogen (NH_4^+-N) (with the exception of available phosphorus (SAP)) had a significant increase in the near-mature and mature plantations. This trend was evident in the age-based sequence (Table 2). Harvest year affected nutrient concentrations (except the STC content in the mature plantation) in four different plantation ages (Table S4). Soil moisture and temperature ranged from 30.2% to 47.7% and from 9.4 °C to 16.1 °C, respectively (Figure 1). The maximum soil pH was 6.47 (mature plantation) and the minimum soil pH was 5.95 (young plantation; Figure 1).

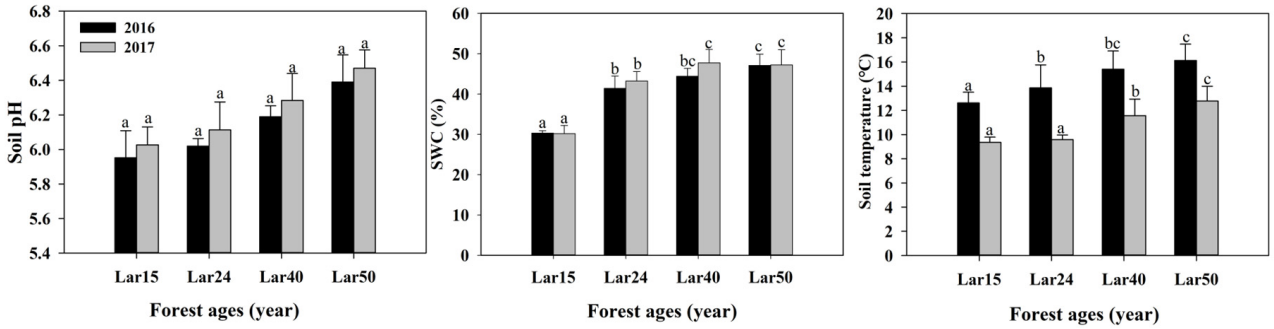

Figure 1. Soil pH, water content and temperature in four plantation age classes. SWC: soil water content. Error bars indicate standard error ($n = 3$). Distinct lowercase letters are used to indicate significant differences between treatments within the measured soil physical properties ($p < 0.05$).

Table 2. Soil nutrient concentrations and microbial biomass for each sampling period across four plantation ages classes.

Sampling Time	Thinning Treatments	STC (g kg^{-1})	STN (g kg^{-1})	STP (g kg^{-1})	MBC (mg kg^{-1})	MBN (mg kg^{-1})	MBP (mg kg^{-1})	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	SAP (mg kg^{-1})
August 2016	Lar15	16.41 ± 0.42 ^{Aa}	1.42 ± 0.26 ^{Aa}	0.28 ± 0.02 ^{Aa}	371.37 ± 16.46 ^{Aa}	92.49 ± 3.74 ^{Aa}	20.64 ± 2.40 ^{Aa}	6.77 ± 1.32 ^{Aa}	3.00 ± 0.47 ^{Aa}	2.74 ± 0.17 ^{Ac}
	Lar24	33.04 ± 1.51 ^{Ab}	2.84 ± 0.25 ^{Ab}	0.39 ± 0.01 ^{Ab}	460.62 ± 17.63 ^{Ab}	106.93 ± 5.48 ^{Ab}	23.41 ± 1.14 ^{Aa}	9.37 ± 0.65 ^{Ab}	4.31 ± 0.03 ^{Ab}	2.62 ± 0.21 ^{Ab}
	Lar40	45.65 ± 0.89 ^{Ac}	3.66 ± 0.08 ^{Ac}	0.49 ± 0.00 ^{Ac}	626.00 ± 6.55 ^{Ac}	143.61 ± 10.07 ^{Ac}	32.45 ± 0.64 ^{Ab}	12.43 ± 4.13 ^{Ac}	5.49 ± 0.50 ^{Ac}	2.25 ± 0.57 ^{Aab}
	Lar50	52.71 ± 2.85 ^{Ac}	4.35 ± 0.11 ^{Ad}	0.57 ± 0.02 ^{Ac}	783.37 ± 9.36 ^{Ad}	175.53 ± 4.22 ^{Ad}	37.24 ± 0.93 ^{Ac}	11.89 ± 6.19 ^{Ac}	7.30 ± 0.15 ^{Ad}	1.84 ± 0.76 ^{Aa}
August 2017	Lar15	12.23 ± 1.12 ^{Ba}	1.13 ± 0.10 ^{Ba}	0.19 ± 0.02 ^{Ba}	226.04 ± 67.86 ^{Ba}	64.49 ± 11.17 ^{Ba}	19.31 ± 2.77 ^{Aa}	3.20 ± 1.14 ^{Ba}	2.08 ± 0.27 ^{Ba}	5.48 ± 0.50 ^{Bb}
	Lar24	26.24 ± 1.36 ^{Bb}	2.10 ± 0.18 ^{Bb}	0.28 ± 0.03 ^{Bb}	355.28 ± 12.85 ^{Bb}	92.26 ± 10.43 ^{Ab}	22.76 ± 1.08 ^{Aa}	6.46 ± 3.72 ^{Bb}	3.33 ± 0.28 ^{Bb}	5.19 ± 0.21 ^{Bb}
	Lar40	37.76 ± 2.40 ^{Bc}	2.77 ± 0.09 ^{Bb}	0.39 ± 0.01 ^{Bc}	620.67 ± 42.46 ^{Ac}	142.27 ± 7.20 ^{Ac}	32.07 ± 1.76 ^{Ab}	6.36 ± 2.21 ^{Bb}	4.78 ± 0.37 ^{Bc}	3.87 ± 0.11 ^{Ba}
	Lar50	51.09 ± 3.98 ^{Ad}	3.79 ± 0.09 ^{Bc}	0.48 ± 0.02 ^{Bd}	756.70 ± 21.82 ^{Ac}	170.86 ± 11.15 ^{Ad}	36.31 ± 1.02 ^{Ab}	8.50 ± 3.13 ^{Bc}	6.32 ± 0.34 ^{Bd}	3.35 ± 0.06 ^{Ba}

Different capital letters signify significant differences in soil indicators between 2016 and 2017; distinct lowercase letters are used to denote significant differences between treatments within the measured nutrient parameters ($p < 0.05$).

3.2. The Characteristics of Soil and Microorganisms

The contents of microbial C, N, and P within the topsoil increased along the age gradient in August 2016 and August 2017 (Table S5). The soil microbial biomass C in Lar24 and Lar40 decreased significantly compared with that of the mature-age plantation and had higher concentrations than that of the youngest age plantation. The microbial biomass N in soil followed a similar pattern, increasing with stand age and reaching its peak in the Lar50 plantation. In addition, the microbial biomass P in surface soil showed few differences between Lar15 and Lar24, which may indicate that growing *Larix* for 15–24 years has little influence on the soil microbial biomass phosphorus (Table 3).

Table 3. Effect of different plantation ages on microbial biomass and its stoichiometric ratios and soil stoichiometric ratios.

Treatment Methods		Soil Stoichiometry			Microbial Biomass			Microbial Biomass Stoichiometry		
		STC:STN	STC:STP	STN:STP	Microbial Biomass C (mg kg^{-1})	Microbial Biomass N (mg kg^{-1})	Microbial Biomass P (mg kg^{-1})	MBC:MBN	MBC:MBP	MBN:MBP
2016	Lar15	10.60 ± 0.51 ^{a}	60.98 ± 0.61 ^{a}	5.77 ± 0.34 ^{a}	371.37 ± 16.46 ^{a}	92.49 ± 3.74 ^{a}	20.64 ± 2.40 ^{a}	4.01 ± 0.02 ^{a}	18.21 ± 2.81 ^{a}	4.53 ± 0.67 ^{a}
	Lar24	11.71 ± 1.51 ^{b}	84.44 ± 5.68 ^{b}	7.25 ± 0.48 ^{b}	460.62 ± 17.63 ^{b}	106.93 ± 5.48 ^{b}	23.41 ± 1.14 ^{a}	4.32 ± 0.29 ^{b}	19.69 ± 0.65 ^{b}	4.57 ± 0.19 ^{a}
	Lar40	12.47 ± 0.50 ^{c}	93.87 ± 1.61 ^{c}	7.53 ± 0.22 ^{c}	626.00 ± 6.55 ^{c}	143.61 ± 10.07 ^{c}	32.45 ± 0.64 ^{b}	4.37 ± 0.32 ^{b}	19.29 ± 0.39 ^{b}	4.43 ± 0.39 ^{a}
	Lar50	12.12 ± 0.85 ^{c}	92.43 ± 2.26 ^{c}	7.64 ± 0.36 ^{c}	783.37 ± 9.36 ^{d}	175.53 ± 4.22 ^{d}	37.24 ± 0.93 ^{c}	4.47 ± 0.16 ^{c}	21.05 ± 0.77 ^{c}	4.71 ± 0.03 ^{a}
2017	Lar15	11.84 ± 0.25 ^{a}	69.13 ± 6.74 ^{a}	5.85 ± 0.69 ^{a}	226.04 ± 67.86 ^{a}	64.49 ± 11.17 ^{a}	19.31 ± 2.77 ^{a}	3.51 ± 1.01 ^{a}	11.65 ± 3.06 ^{a}	3.33 ± 0.12 ^{a}
	Lar24	12.53 ± 0.61 ^{b}	93.40 ± 3.79 ^{b}	7.46 ± 0.31 ^{b}	355.28 ± 12.85 ^{b}	92.26 ± 10.43 ^{b}	22.76 ± 1.08 ^{a}	3.87 ± 0.29 ^{a}	15.62 ± 0.30 ^{b}	4.05 ± 0.26 ^{b}
	Lar40	13.61 ± 0.68 ^{c}	97.06 ± 6.18 ^{b}	7.13 ± 0.36 ^{b}	620.67 ± 42.46 ^{c}	142.27 ± 7.20 ^{c}	32.07 ± 1.76 ^{b}	4.36 ± 0.14 ^{b}	19.42 ± 2.07 ^{c}	4.44 ± 0.06 ^{bc}
	Lar50	13.48 ± 0.98 ^{c}	107.00 ± 9.02 ^{c}	7.94 ± 0.16 ^{c}	756.70 ± 21.82 ^{d}	170.86 ± 11.15 ^{d}	36.31 ± 1.02 ^{c}	4.44 ± 0.36 ^{b}	20.85 ± 0.71 ^{c}	4.70 ± 0.24 ^{c}

Distinct lowercase letters are used to signify significant differences between treatments ($p < 0.05$).

The stoichiometry characteristics of microbial biomass exhibited a similar trend (Table 3). Plantation age had a considerable impact on the ratios of MBC:MBN and MBC:MBP. However, the N:P ratio within the microbial biomass did not exhibit significant differences among the various plantation ages in August 2016.

The abundance of microorganisms demonstrated a correlation with the stand age (Figure 2, Table S6). In August 2016 and August 2017, compared with the Lar15 plantation, total PLFAs were higher in the Lar24, Lar40, and Lar50 plantations. This pattern was consistent across the average abundance of Gram-positive bacteria, Gram-negative bacteria, fungi, and actinobacteria. These were all 1.23–2.75 times higher in the Lar24, Lar40, and Lar50 plantations than in the Lar15 plantation. Additionally, the variation in bacteria and Gram-positive bacteria between Lar24 and Lar40 did not demonstrate significant differences, similar to the variation in fungi PLFA concentrations between Lar50 and Lar40. In general, throughout the development of Larix plantations, the biomass of major microbial groups experienced an increase (Figure 2).

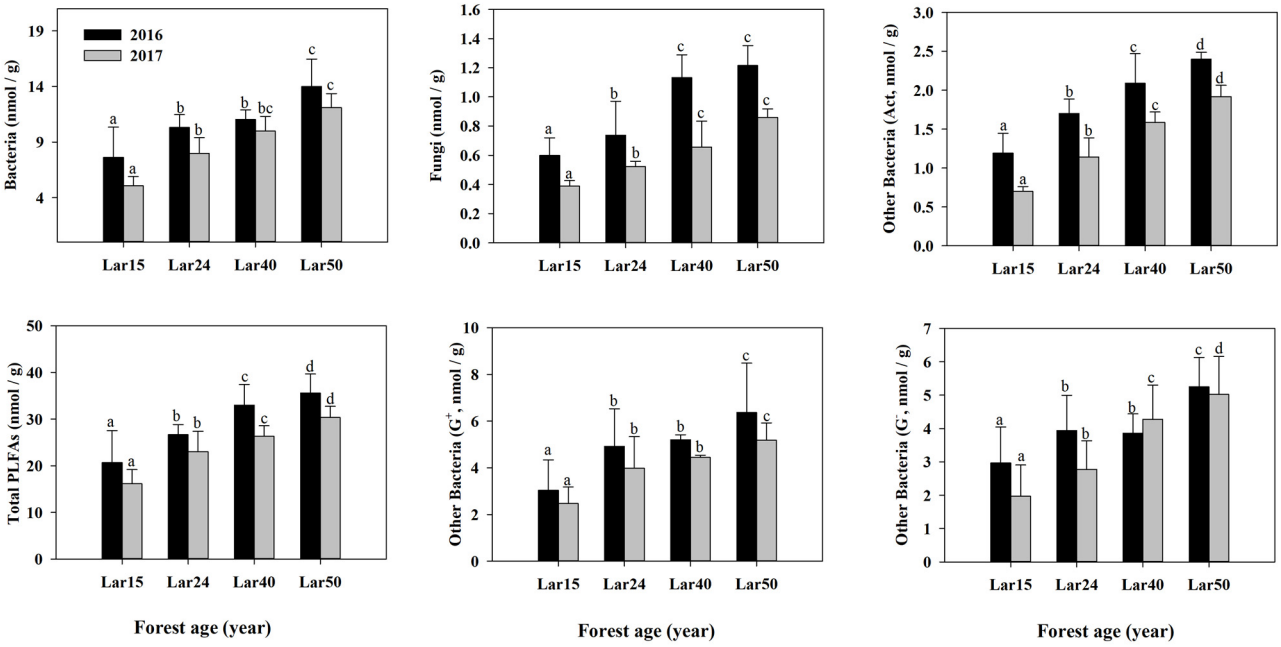

Figure 2. The biomass of different soil microbial PLFAs in August 2016 and August 2017. Error bars in the figures represent the standard error (n = 3). Different lowercase letters are used to denote significant differences between treatments ($p < 0.05$). G^+: Gram-positive bacteria; G^-: Gram-negative bacteria; Act: actinobacteria.

3.3. The Stoichiometric Ratios of Elements in Soil and Microbial Biomass

In 2016, soil C:N (10.60 ± 0.51), C:P (60.98 ± 0.61), and N:P (5.77 ± 0.34) ratios in Lar15 were significantly lower compared to the middle-aged plantation, near-mature plantation, and mature plantation (Table 3). The soil C:N ratio (12.47 ± 0.50) and C:P (93.87 ± 1.61) ratios in the near-mature plantation were higher than in Lar50 (12.12 ± 0.85, 92.43 ± 2.26, respectively). Soil C:N (13.61 ± 0.68) ratios in the near mature plantation were higher than in Lar50 (13.48 ± 0.98) in 2017. Notably, the carbon, nitrogen, and phosphorus ratios within the young plantation remained the lowest among the plantations (Table 3).

In comparison to the soil stoichiometric ratios, the alteration in microbial stoichiometric ratios with varying plantation ages appeared relatively modest. During both August 2016 and August 2017, the ratios of MBC:MBN, MBC:MBP, and MBN:MBP within the near-mature and mature plantations demonstrated a notable tendency to increase significantly, distinguishing them from the other two plantation types (Table 3).

3.4. Combined Effects of Soil Properties and Microbial Communities on Microbial Stoichiometric Ratios

The C:N, C:P, and N:P ratios in soil were not significantly correlated with soil temperature (Table S7). However, soil C:N exhibited positive associations with soil pH, soil water content, and soil available phosphorus content (R^2 = 0.485 *, R^2 = 0.586 **, and R2 = −0.629 **, respectively; Table S7). Similarly, the soil N:P showed positive correlations with pH value, soil water content, NH_4^+-N content, and soil available phosphorus (R^2 = 0.680 **, R^2 = 0.869 **, R^2 = 0.693 **, and R^2 = 0.671 **, respectively; Table S7). Additionally, significant positive associations were observed between soil pH value, soil moisture, NO_3^--N content, NH_4^+-N content, and the C:N ratio (R^2 = 0.624 **, R^2 = 0.844 **, R^2 = 0.455 *, and R^2 = 0.736 **, respectively) within the soil (Table S7).

Pearson correlations further revealed that the microbial C:N ratio showed positive correlations with soil properties except for soil NO_3^--N content and soil available phosphorus content (R^2 = 0.378 and R^2 = −0.233; Table S8), and the microbial C:P ratio had positive associations with all soil properties, except for soil available phosphorus content (R^2 = −0.240; Table S8). The microbial N:P ratio demonstrated positive correlations with all

soil properties except soil pH value and soil available phosphorus content (R^2 = 0.393 and R^2 = −0.154; Table S8).

Regarding correlations between microbial stoichiometric ratios and microbial biomass, there were no significant associations with the biomass of fungi (all $p > 0.05$, Figures 3–5). In contrast, significant correlations were identified between microbial C:N and the biomass of Gram-positive bacteria (R^2 = 0.176 *), Gram-negative bacteria (R^2 = 0.326 *), and total bacteria (R^2 = 0.348 *; Figure 3). Microbial C:P showed significant positive correlations with the biomass of bacteria, Gram-positive bacteria, Gram-negative bacteria, and actinobacteria (R^2 = 0.426 *, R^2 = 0.531 *, R^2 = 0.664 *, and R^2 = 0.482 *, respectively; Figure 4). Moreover, the microbial N:P ratio demonstrated a significant association with bacterial biomass and Gram-negative bacterial biomass (R^2 = 0.412 * and R^2 = 0.525 *, Figure 5).

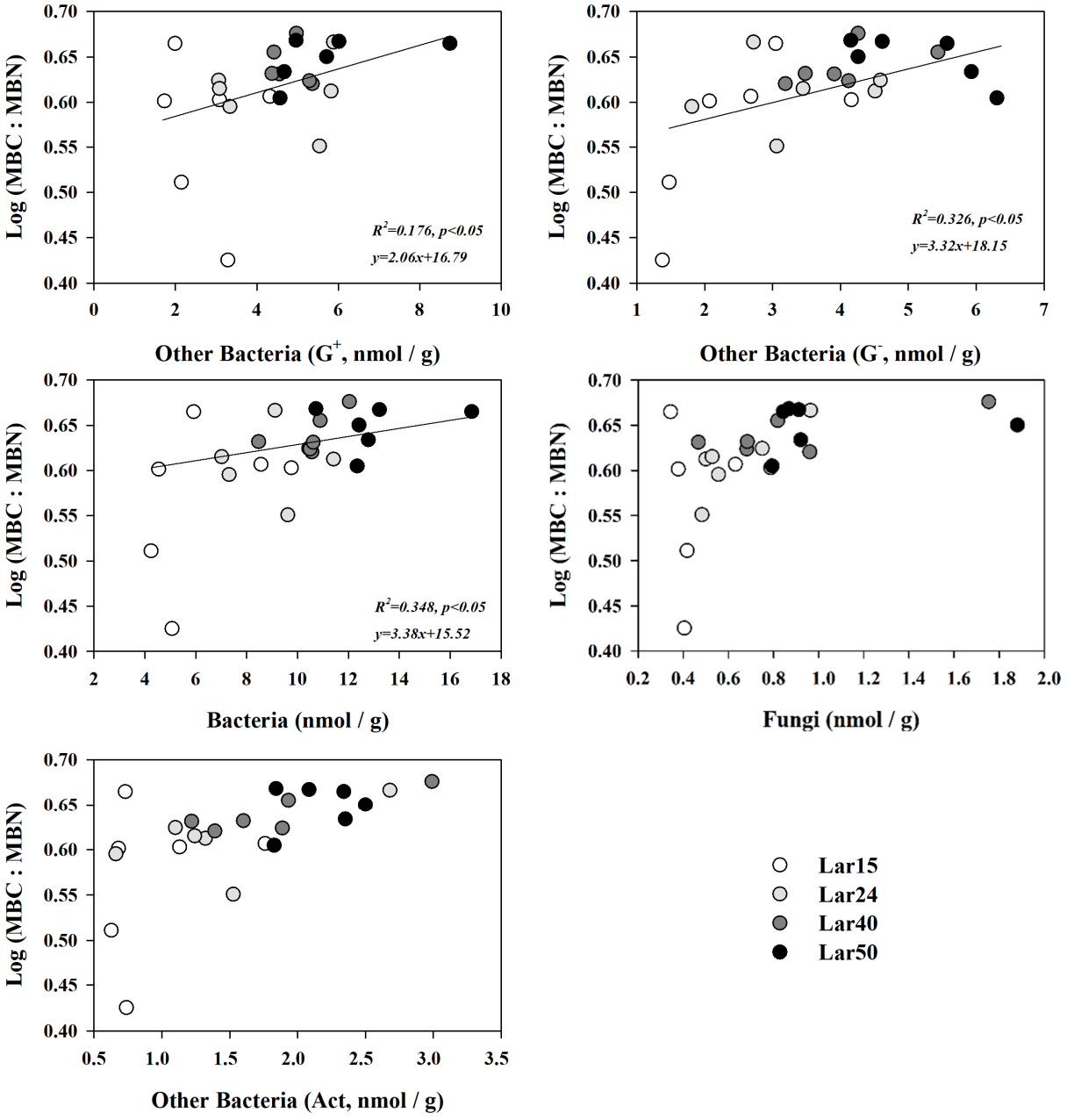

Figure 3. The connections between the MBC:MBN ratio and microbial groups in soil across four plantation age classes. G+: Gram-positive bacteria; G−: Gram-negative bacteria; Act: actinobacteria.

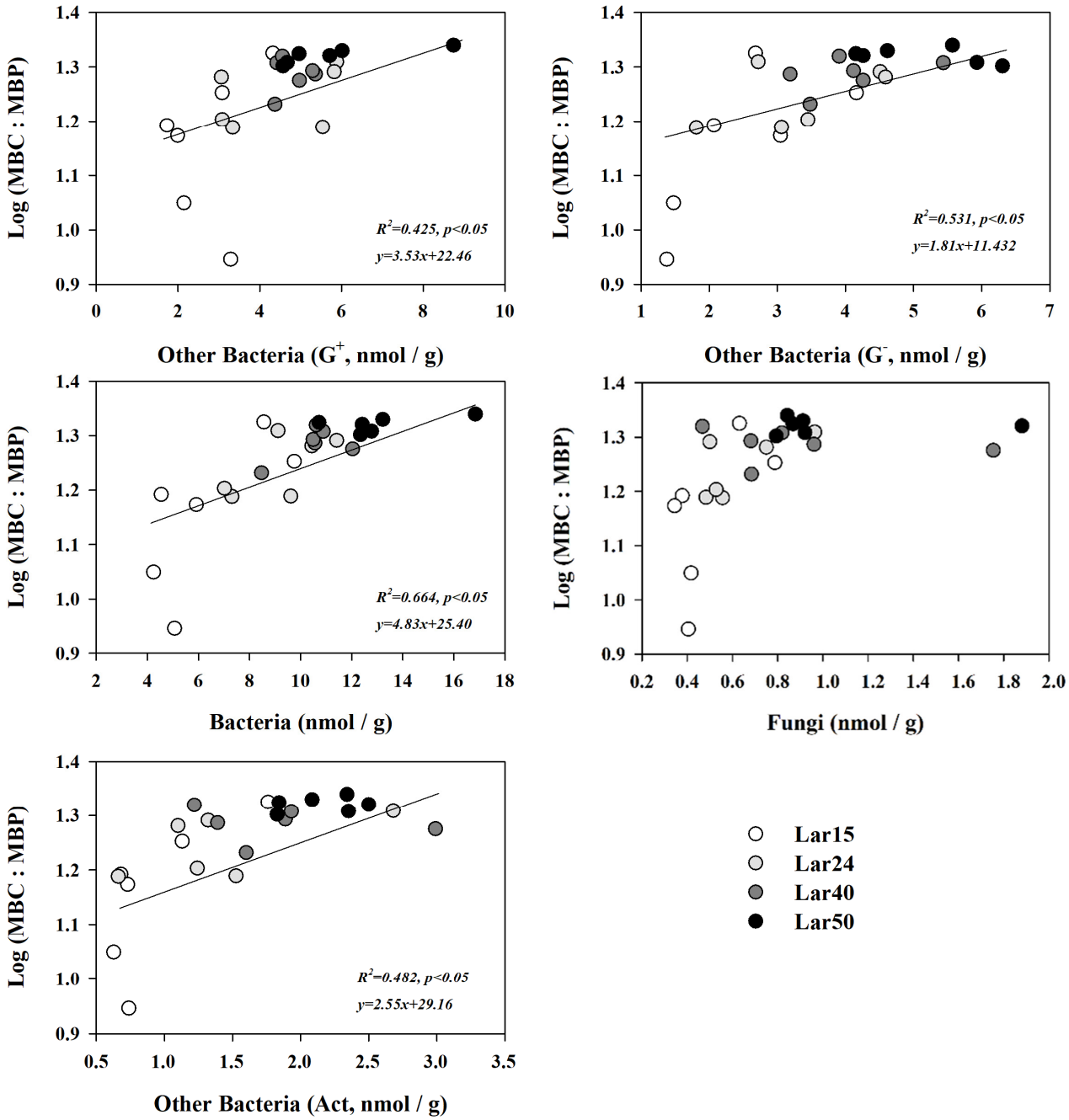

Figure 4. The connections between the MBC:MBP ratio and microbial groups in soil across four plantation age classes. G+: Gram-positive bacteria; G−: Gram-negative bacteria; Act: actinobacteria.

In addition, the changes in microbial biomass stoichiometric ratios were associated with soil microbial groups and soil properties (Table 4). Specifically, shifts in the microbial C:N ratio were associated with variations in soil temperature soil moisture, NH_4^+-N content, bacteria, and Gram-positive bacterial abundance. Variations in the microbial C:P ratio were related to changes in soil temperature, soil total P, and actinobacteria and Gram-positive bacterial abundance. Furthermore, variations in the microbial N:P ratio were linked to fluctuations in bacterial abundance, actinobacteria abundance, soil temperature, and soil moisture (Table 4).

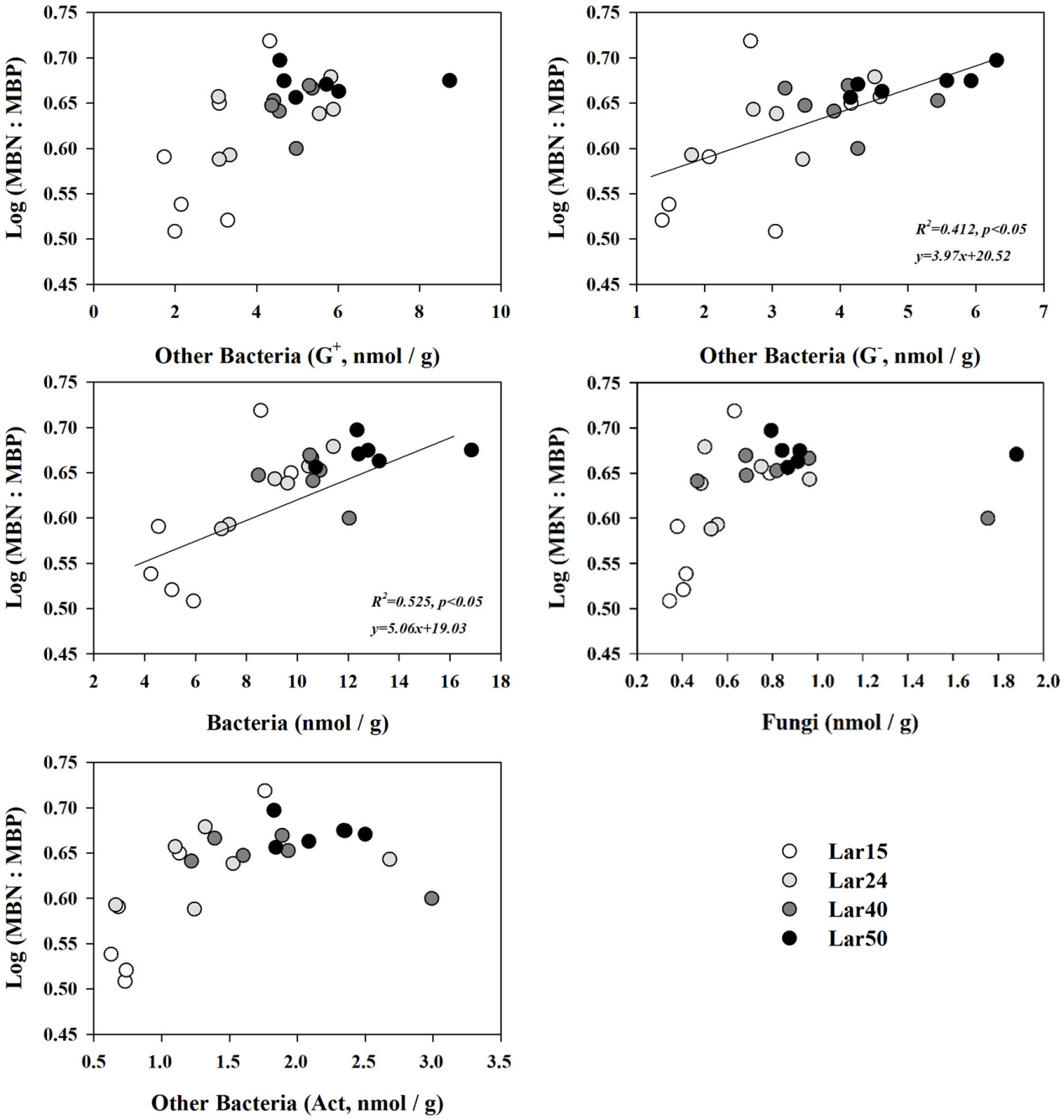

Figure 5. The connections between the MBN:MBP ratio and microbial groups in soil across four plantation age classes. G+: Gram-positive bacteria; G−: Gram-negative bacteria; Act: actinobacteria.

Table 4. The results of stepwise regression analysis, revealing the relationship between microbial C:N:P stoichiometry, soil nutrient factors, and soil microbial communities within *Larix* plantations.

Predicted Variables	R^2	Sig.	Contribution of the Individual Predictor (%)							
			Bacteria	Actinobacteria	Gram-Positive Bacteria	Gram-Negative Bacteria	Soil Temperature	SWC	STP	NH_4^+-N
Microbial C:N	0.27	<0.05	29.1		23.8		22.4	10.4		14.3
Microbial C:P	0.53	<0.01		26.9	33.7		23.5		15.9	
Microbial N:P	0.20	<0.05	34.6			13.9	14.9	17.3		19.3

4. Discussion

4.1. Shifts in Basic Environmental Variables under Different Plantation Ages

Our findings revealed a clear increase in fine root nutrient concentrations as plantation ages advanced, reaching its peak in mature plantations. Previous research has suggested that the age of forests could influence the efficiency of nutrient absorption by fine roots, the contents of available nutrients in soil, and microbial abundance, ultimately leading to significant shifts in carbon, nitrogen, and phosphorus concentrations within roots [34]. Yuan et al. (2011) [34] indicated that the content of nitrogen in soil was closely associated with elemental concentrations in roots. Chen et al. (2016) [35] also reported that the carbon and nitrogen concentrations in fine roots of *Pinus tabulaeformis* plantations are related to the concentration of soil elements, a result similar to our own.

We found that the concentrations of litter nutrients in Lar15 were significantly lower than in other plantation age classes. McGroddy et al. (2004) [36] found that the soil nutrient concentration could affect the nutrient adsorption capacity of leaves. Aber et al. (1989) [37] found that in nutrient poor conditions, plants have a relatively poor ability to resorb nutrient elements. Therefore, the reduction in the soil nutrient pool might lead to a decrease in nutrient concentrations in leaf litter. Moreover, in August 2016 and August 2017, young and middle-aged plantations showed lower soil temperatures and water content compared to Lar40 and Lar50 plantations. This phenomenon could be attributed to the relatively greater mean tree height, diameter at breast height, and litter layer biomass in Lar40 and Lar50 plantations, which might contribute to elevated surface soil temperatures and humidity. A thick litter layer may prevent soil water content from drying out and increase soil temperature.

4.2. The Influence of Forest Age on the Soil Elemental Stoichiometric Ratios and Microbial Characteristics

Our research indicates that plantation age had significant effects on soil stoichiometric ratios (Tables 3 and S5), particularly in Lar40 and Lar50 plantations. In line with the findings of Tian et al. (2018) [38], soil elemental ratios showed significant relationships with soil properties including moisture, temperature, and texture. For instance, the age of the forest could be positively linked to improvements in soil moisture and temperature conditions, stemming from enhanced stem growth in plants. This, in turn, could create a more favorable hydric and thermal environment for the mineralization of carbon (C) and nitrogen (N) within the soil, thereby influencing nutrient concentrations and stoichiometric ratios. Therefore, age sequence affects the stoichiometric ratios of soil elements by changing hydrothermal conditions and available nutrients in soil. Moreover, increases in forest age can increase litter and root biomass, thus improving available substrate supply, soil enzyme activity, and understory vegetation diversity [34–36]. These changes in the forest understory's microenvironment could potentially impact microbial metabolic efficiency and nutrient turnover rate, ultimately leading to the accumulation of soil nutrients [15,37].

Over time, there was a discernible increase in soil nutrient availability, as indicated by higher contents of NO_3^--N, NH_4^+-N, and SAP in the near-mature and mature plantations. However, contrary to this trend, soil pH did not exhibit the same pattern across the four plantation ages. A similar observation was reported in a study conducted in a *Pinus massoniana* plantation, where available nutrient contents (such as NH_4^+-N and SAP) in soil exhibited significant increases in mature-age plantations. In parallel, Chen et al. (2016) [35] reported notable rises in soil available nitrogen and available phosphorus concentrations in *Pinus tabulaeformis* and *Robinia pseudoacacia* plantations as they matured. Such disparities could potentially stem from variations in element concentrations, plant organ biomass, and the promotion of litter decomposition (including leaves and roots), consequently contributing to increased available nutrients within the soil. Moreover, the soil N:P ratio has been utilized as an indicator of nutritional limitations during the growth phase. Ren et al. (2018) [39] and Fan et al. (2015) [40] suggested that soil phosphorus becomes increasingly limited compared to nitrogen and potassium in forest soils. Although total soil phosphorus

content might exhibit an increase as plantation age advances, available phosphorus concentrations could decline as stand age increases. In alignment with this hypothesis, our study found that Lar40 and Lar50 plantations showed higher total nitrogen and total phosphorus contents in soil, while SAP decreased significantly with plantation age. These findings suggest that phosphorus could be the limiting nutrient for the growth of *Larix* plantations.

Our study also revealed a significant influence of plantation age on soil microbial community characteristics. This finding is in line with a range of previous studies that have observed an increase in microbial community diversity and abundance with progressing age gradients [39–41]. In comparison to young and middle-aged plantations, near-mature and mature plantations exhibited higher relative abundances of microbial groups, including bacteria, fungi, and actinobacteria. In general, forest age can induce shifts in the vertical structure, litter characteristics, and understory micro-environment [42,43], which were also apparent in our study. The maturation of forests can modify their composition and structure, resulting in reduced competition among trees and understory plants. This, in turn, enhances nutrient utilization efficiency among plants, leading to increased leaf and root litter production [44–46]. As forest age advances, the quantity of decomposing material, such as leaf litter and root litter, tends to increase. This shift in resource availability and competition dynamics may improve microbial community activity and elevate microbial biomass [16,47,48]. Additionally, the activity of microbial communities is intricately linked with factors such as soil moisture, root exudates, and soil carbon content. Younger forests might exhibit smaller crown areas and lower heights of the lowest branches, potentially impacting soil moisture and subsequently reducing carbon substrate content and root exudates. These factors, in turn, could influence microbial abundance and activity [49,50].

Soil microbial biomass is regarded as an indicator of the growth status of soil microorganisms and can be influenced by various factors, including vegetation growth, soil moisture and temperature conditions, and the availability of labile carbon compounds in the soil. Typically, as forest stands age, the growth of microbial communities tends to be promoted. This phenomenon can be attributed to older stands providing a greater abundance of decomposable carbon and nitrogen substrates, thereby facilitating microbial anabolism and metabolic activities. Chen et al. (2018) [41] also observed that the microbial carbon content in young forest is much lower than that in mature forest. In our study, we found that the content of microbial biomass elements in Lar50 plantations was significantly higher than in other plantation age classes, a pattern that echoes the findings of previous research. This observation reinforces the notion that as forests mature, they can offer a more conducive environment for microbial communities to thrive in due to the increased availability of organic substrates that fuel their growth and activity.

4.3. Strong Linkages between Microbial C:N:P Ratios, Soil Properties, and Microbial Communities

Our results suggest that alterations in soil temperature, soil moisture, soil nutrient concentrations, and the biomass of various microbial groups are likely crucial determinants of shifts in soil microbial C:N:P stoichiometry across varying forest ages [22]. Previous research has indicated that forest management practices can exert a significant impact on soil pH, water content, and soil properties, leading to alterations in soil microbial biomass [51–53]. Our results also reflect this trend. Furthermore, soil water content and nutrient concentrations showed significant associations with microbial biomass C, N, and P ratios (Tables 4 and S8). In areas characterized by high soil moisture or atmospheric humidity, soil microorganisms tend to exhibit enhanced capabilities to sequester C and N elements [54,55]. In this context, the utilization efficiency of C and N by microorganisms may surpass that of P (due to phosphorus being derived from rock weathering). Consequently, the effect of plantation age on soil water content and nutrient concentrations could contribute to a relatively higher accumulation of carbon and nitrogen in microbial biomass, thereby influencing the ratios of MBC:MBN and MBC:MBP.

Moreover, it is theorized that microorganisms commonly have specific elemental stoichiometries (e.g., the C:N ratio of bacteria is four and that of fungi is ten) [19]. Additionally,

clear differences in metabolic rate and nutrient requirements have been observed among microbial groups [56]. For example, the metabolic rates and P requirements of fungi are lower than those of bacteria. Compared with bacterial groups, fungi groups tend to utilize substrates with higher C:N ratios. Thus, alterations in soil nutrient concentrations, substrate availability, and physicochemical properties as forests age can influence the composition and abundance of soil microbial groups. This, in turn, may bring about shifts in microbial stoichiometric ratios. Our observations were close to those of previous studies.

Previous research has provided evidence that forest age is related to variation in the vertical structure, litter properties, root properties, hydric and thermal conditions, and other micro-environment factors which influence soil nutrient concentrations. These intricate relationships have a direct influence on the stoichiometric ratios of microbial C:N:P in soil [57], which aligns with our findings. As forests mature, there is a likelihood of enhanced stem growth, improved soil moisture, and elevated soil temperature, leading to accelerated decomposition rates and an increase in C-rich substrates such as litter, fine roots, and branches [35,42,58]. These conditions foster the accumulation of soil nutrients, thus reducing the competition among microbial communities for these essential elements [19,20]. In an environment with reduced nutrient competition, microbial groups are likely to experience growth and expansion along with heightened rates of nutrient utilization. Consequently, this dynamic leads to changes in the carbon, nitrogen, and phosphorus content within microbial cells, altering the stoichiometric ratios of the microbial community as a whole [2,19,59]. Across the span of the four distinct stand ages we studied, the transformations in vertical plant structure, soil hydrothermal conditions, and litter quantity and decomposition rates could collectively drive variations in soil microbial community activity. This, in turn, could influence shifts in microbial biomass stoichiometric ratios, particularly the microbial C:N and N:P ratios.

Furthermore, previous studies indicated that the PLFA method may miss some other microbes (such as prokaryotes, fungi, and other eukaryotes). In contrast, high-throughput sequencing technologies, such as next-generation sequencing, offer the advantage of analyzing a wide range of genetic material from environmental samples. This approach provides a more comprehensive view of microbial diversity, allowing researchers to identify a broader spectrum of microorganisms, including those that may have been missed by PLFA analysis. It enables the identification of specific microbial taxa at various taxonomic levels and can reveal more nuanced insights into community dynamics and response processes to environmental changes. Thus, combining multiple methods, such as PLFA analysis and high-throughput sequencing, can provide a more comprehensive understanding of microbial community dynamics and ultimately contribute to a more holistic comprehension of ecosystem functioning and responses to management practices.

5. Conclusions

Our findings demonstrated that the increase in chemical element contents of fine roots and litter, coupled with higher soil nutrient concentrations and microbial biomass in mature plantations, indicates the positive influence of plantation age on ecosystem nutrient availability and microbial activity. The observation of an increasing soil N:P ratio with plantation age suggests that phosphorus might become a limiting factor for *Larix* plantations as they mature. This insight has implications for forest management practices, as it suggests that phosphorus supplementation or management strategies to enhance phosphorus availability might be beneficial in maintaining healthy and productive plantation ecosystems. Additionally, the close relationship between changes in microbial biomass stoichiometric ratios and soil microbial group biomass, along with the influence of soil properties, emphasizes the complex interplay between abiotic and biotic factors in shaping microbial elemental stoichiometry. This finding underscores the importance of considering both soil characteristics and microbial community structure when analyzing ecosystem nutrient cycling processes. Overall, our research contributes valuable insights into ecological stoichiometry and its relationship to forest age and microbial communities.

The results offer practical guidance for forest management strategies, especially in terms of nutrient management and understanding the intricate connections between soil properties and microbial processes in forest ecosystems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f14091914/s1. Table S1: Characteristics of sampling sites in Larix plantations. Table S2: Species diversity of understory vegetation characteristics in Larix plantations. Table S3: PLFAs biomarkers used to characterize microbial community structure. Table S4: Repeated-measures ANOVA for soil physical and chemical properties in *Larix* plantations of different ages. Table S5: Repeated-measures ANOVA for soil microbial biomass and the stoichiometric ratios in soil and microorganisms in *Larix* plantations of different ages. Table S6: Repeated-measures ANOVA for microbe group biomass in *Larix* plantations of different ages. Table S7: Linkage of C:N, C:P and N:P ratios and pH value, moisture, temperature and nutrient concentrations in topsoil across 4 different plantation ages. Table S8: Linkage of microbial C:N, C:P and N:P ratios and pH value, moisture, temperature and nutrient concentrations in topsoil under 4 different plantation ages. Refs [60–67] are cited in Supplementary Materials.

Author Contributions: M.C., H.H. and X.C. designed field experiments; Laboratory analytical work was performed by M.C., L.L., X.P. and T.S. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Key Research and Development Program of China (No. 2019YFA0607304) and the Tibet Science and Technology Plan project (XZ202201ZY002N).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We are very grateful to Prof. Zhiyong Zhou for his support in the analysis of soil samples in the laboratory.

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

References

1. Peñuelas, J.; Sardans, J. Elementary factors. *Nature* **2009**, *460*, 803–804. [CrossRef]
2. Vicca, S.; Stocker, B.D.; Reed, S.; Wieder, W.R.; Bahn, M.; Fay, P.A.; Janssens, I.A.; Lambers, H.; Peñuelas, J.; Piao, S. Using research networks to create the comprehensive datasets needed to assess nutrient availability as a key determinant of terrestrial carbon cycling. *Environ. Res. Lett.* **2018**, *13*, 125006. [CrossRef]
3. Sterner, R.W.; Elser, J.J. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*; Princeton University Press: Princeton, NY, USA, 2002.
4. Heyburn, J.; McKenzie, P.; Crawley, M.J.; Fornara, D.A. Effects of grassland management on plant C:N:P stoichiometry: Implications for soil element cycling and storage. *Ecosphere* **2017**, *8*, e01963. [CrossRef]
5. Fanin, N.; Fromin, N.; Buatois, B.; Hättenschwiler, 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]
6. Magruder, M.; Chhin, S.; Palik, J.B.B. Thinning increases climatic resilience of red pine. *Can. J. For. Res.* **2013**, *43*, 878–889. [CrossRef]
7. Hooper, D.U.; Bignell, D.E.; Brown, V.K.; Brussaard, L.; Dangerfield, J.M.; Wall, D.H.; Wardle, D.A.; Coleman, D.C.; Giller, K.E.; Lavelle, P.; et al. Interactions between aboveground and belowground biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. *BioScience* **2000**, *50*, 1049–1061. [CrossRef]
8. Bao, Y.; Qiuliang, Z.; Liming, W. Comprehensive characteristics of the vertical structure of middle young over cutting forest of *Larix* gmelinii. *Sci. Silvae Sin.* **2015**, *51*, 132–139.
9. Lunstrum, A.; Chen, L. Soil carbon stocks and accumulation in young mangrove forests. *Soil Biol. Biochem.* **2014**, *75*, 223–232. [CrossRef]
10. Pregitzer, K.S.; Euskirchen, E.S. Carbon cycling and storage in world forests: Biome patterns related to forest age. *Glob. Chang. Biol. Bioenergy* **2010**, *10*, 2052–2077. [CrossRef]
11. Hember, R.A.; Kurz, W.A.; Metsaranta, J.M.; Black, T.A.; Guy, R.D.; Coops, N.C. Accelerating regrowth of temperate-maritime forests due to environmental change. *Glob. Chang. Biol. Bioenergy* **2012**, *18*, 2026–2040. [CrossRef]
12. Wang, Q.; Wang, Y.; Wang, S.; He, T.; Liu, L. Fresh carbon and nitrogen inputs alter organic carbon mineralization and microbial community in forest deep soil layers. *Soil Biol. Biochem.* **2014**, *72*, 145–151. [CrossRef]
13. Wang, Z.; Yao, X.; Wang, W. Variation of soil carbon pools in *Pinus* sylvestris, plantations of different ages in north China. *Acta Ecol. Sin.* **2018**, *38*, 248–254. [CrossRef]

14. Ren, C.; Zhao, F.; Kang, D.; Yang, G.; Han, X.; Tong, X.; Feng, Y.; Ren, G. Linkages of C:N:P stoichiometry and bacterial community in soil following afforestation of former farmland. *For. Ecol. Manag.* **2016**, *376*, 59–66. [CrossRef]
15. Chen, X.-L.; Wang, D.; Chen, X.; Wang, J.; Diao, J.-J.; Zhang, J.-Y.; Guan, Q.-W. Soil microbial functional diversity and biomass as affected by different thinning intensities in a Chinese fir plantation. *Appl. Soil Ecol.* **2015**, *92*, 35–44. [CrossRef]
16. Liu, Y.; Fang, Y.; An, S.S. How C:N:P stoichiometry in soils and plants responds to succession in *Robinia pseudoacacia* forests on the Loess Plateau, China. *For. Ecol. Manag.* **2020**, *475*, 118394. [CrossRef]
17. Dang, P.; Gao, Y.; Liu, J.; Yu, S.; Zhao, Z. Effects of thinning intensity on understory vegetation and soil microbial communities of a mature Chinese pine plantation in the Loess Plateau. *Sci. Total Environ.* **2018**, *630*, 171–180. [CrossRef] [PubMed]
18. Yang, Y.; Geng, Y.; Zhou, H.; Zhao, G.; Wang, L. Effects of gaps inthe forest canopy on soil microbial communities and enzyme activity in a Chinese pine forest. *Pedobiologia* **2017**, *61*, 51–60. [CrossRef]
19. Chen, X.; Han, Y.H.; Chen, X.; Wang, J.; Chen, B.; Wang, D.; Guan, Q. Soil labile organic carbon and carbon-cycle enzyme activities under different thinning intensities in Chinese fir plantations. *Appl. Soil Ecol.* **2016**, *107*, 162–169. [CrossRef]
20. Ma, J.Y.; Han, H.R.; Cheng, X.Q. Soil temperatures and active carbon components as key drivers of C stock dynamics between two different stand ages of *Larix principis-rupprechtii* plantation. *Peerj* **2020**, *8*, e8384. [CrossRef]
21. Chen, Y.L.; Chen, L.Y.; Peng, Y.F.; Ding, J.Z.; Li, F.; Yang, G.B.; Kou, D.; Liu, L.; Fang, K.; Zhang, B.B.; et al. Linking microbial C:N:P stoichiometry to microbial community and abiotic factors along a 3500-km grassland transect on the Tibetan Plateau. *Glob. Ecol. Biogeogr.* **2016**, *25*, 1416–1427. [CrossRef]
22. Cheng, C.; Wang, Y.; Fu, X.; Xu, M.; Dai, X.; Wang, H. Thinning effect on understory community and photosynthetic characteristics in a subtropical *Pinus massoniana* plantation. *Can. J. For. Res.* **2017**, *47*, 1104–1115. [CrossRef]
23. Li, H.L.; Crabbe, M.J.C.; Xu, F.L.; Wang, W.L.; Niu, R.L.; Gao, X.; Zhang, P.; Chen, H.K. Seasonal variations in carbon, nitrogen and phosphorus concentrations and C:N:P stoichiometry in the leaves of differently aged *Larix principis-rupprechtii Mayr*. Plantations. *Forests* **2017**, *8*, 373. [CrossRef]
24. Zeng, Q.; Lal, R.; Chen, Y.; An, S. Leaf and root ecological stoichiometry of Caragana korshinskii on the Loess Plateau of China in relation to plantation age. *PLoS ONE* **2017**, *12*, e0168890. [CrossRef]
25. Liang, X.; Liu, S.; Wang, H.; Wang, J. Variation of carbon and nitrogen stoichiometry along a chronosequence of natural temperate forest in northeastern China. *J. Plant Ecol.* **2017**, *11*, 339–350. [CrossRef]
26. Sun, X.L.; Zhao, J.; You, Y.M.; Sun, O.J.X. Soil microbial responses to forest floor litter manipulation and nitrogen addition in a mixed-wood forest of northern China. *Sci. Rep.* **2015**, *6*, 19536. [CrossRef] [PubMed]
27. FAO. *World Reference Base for Soil Resources 2014, Update 2015 International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Reports No. 106*; The Food and Agriculture Organization: Rome, Italy, 2015.
28. Bao, S. *Soil and Agricultural Chemistry Analysis*; China Agriculture Press: Beijing, China, 2000. (In Chinese)
29. Schumacher, B.A. *Methods for the Determination of Total Organic Carbon (TOC) in Soils and Sediments*; US Environmental Protection Agency, Office of Research and Development, Ecological Risk Assessment Support Center: Washington, DC, USA, 2002.
30. Zeng, Q.; Li, X.; Dong, Y.; An, S.; Darboux, F. Soil and plant components ecological stoichiometry in four steppe communities in the Loess Plateau of China. *Catena* **2016**, *147*, 481–488. [CrossRef]
31. Bremner, J.M. Determination of nitrogen in soil by the Kjeldahl method. *J. Agric. Sci.* **1960**, *55*, 11–33. [CrossRef]
32. Wu, J.S.; Lin, Q.M.; Huang, Q.Y.; Xiao, H.A. *Soil Microbial Biomass–Methods and Applications*; China Meteorological Press: Beijing, China, 2006.
33. Hedley, M.J.; Stewart, J.W.B. Method to measure microbial phosphate in soils. *Soil Biol. Biochem.* **1982**, *14*, 377–385. [CrossRef]
34. Yuan, Z.Y.; Chen, H.Y.H.; Reich, P.B. Global-scale latitudinal patterns of plant fine root nitrogen and phosphorus. *Nat. Commun.* **2011**, *2*, 55–73. [CrossRef] [PubMed]
35. Chen, L.; Mu, X.; Yuan, Z.; Deng, Q.; Chen, Y.; Yuan, L.Y.; Ryan, L.T.; Kallenbach, R.L. Soil nutrients and water affect the age-related fine root biomass but not production in two plantation forests on the Loess Plateau, China. *J. Arid Environ.* **2016**, *135*, 173–180. [CrossRef]
36. McGroddy, M.E.; Daufresne, T.; Hedin, L.O. Scaling of C:N:P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. *Ecology* **2004**, *85*, 2390–2401. [CrossRef]
37. Aber, J.D.; Nadelhoffer, K.J.; Steudler, P.; Melillo, J.M. Nitrogen saturation in northern forest ecosystems. *BioScience* **1989**, *39*, 378–386. [CrossRef]
38. Tian, L.M.; Zhao, L.; Wu, X.D.; Fang, H.B.; Zhao, Y.H.; Hu, G.J.; Yue, G.Y.; Sheng, Y.; Wu, J.C.; Chen, J.; et al. Soil moisture and texture primarily control the soil nutrient stoichiometry across the Tibetan grassland. *Sci. Total Environ.* **2018**, *622–623*, 192–202. [CrossRef] [PubMed]
39. Ren, H.; Kang, J.; Yuan, Z.; Xu, Z.; Han, G. Responses of nutrient resorption to warming and nitrogen fertilization in contrasting wet and dry years in a desert grassland. *Plant Soil* **2018**, *432*, 65–73. [CrossRef]
40. 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]
41. Chen, L.L.; Deng, Q.; Yuan, Z.Y.; Mu, X.M.; Kallenbach, R.L. Age-related C:N:P stoichiometry in two plantation forests in the Loess Plateau of China. *Ecol. Eng.* **2018**, *120*, 14–22. [CrossRef]
42. Yang, Y.H.; Luo, Y.Q. Carbon: Nitrogen stoichiometry in forest ecosystems during stand development. *Glob. Ecol. Biogeogr.* **2011**, *20*, 354–361. [CrossRef]

43. Zhang, L.; Wang, J.; Bai, Z.; Lv, C. Effects of vegetation on runoff and soil erosion on reclaimed land in an opencast coal-mine dump in a loess area. *Catena* **2015**, *128*, 44–53. [CrossRef]
44. Rice, S.K.; Westerman, B.; Federici, R. Impacts of the exotic, nitrogen-fixing black locust (*Robinia pseudoacacia*) on nitrogen-cycling in a pine-oak ecosystem. *Plant Ecol.* **2004**, *174*, 97–107. [CrossRef]
45. Maassen, S.; Wirth, S. Soil microbiological monitoring of a pine forest after partial thinning for stand regeneration with beech seedlings. *Soil Sci. Plant Nutr.* **2004**, *50*, 815–819. [CrossRef]
46. Van der Heijden, M.G.A.; Bardgett, R.D.; van Straalen, N.M. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.* **2008**, *11*, 296–310. [CrossRef] [PubMed]
47. Lado-Monserrat, L.; Lidón, A.; Bautista, I. Litterfall, litter decomposition and associated nutrient fluxes in *Pinus halepensis*: Influence of tree removal intensity in a Mediterranean forest. *Eur. J. For. Res.* **2015**, *5*, 833–844. [CrossRef]
48. Thibodeau, L.; Raymond, P.; Camiré, C.; Munson, A.D. Impact of precommercial thinning in balsam fir stands on soil nitrogen dynamics, microbial biomass, decomposition, and foliar nutrition. *Can. J. For. Res.* **2000**, *30*, 229–238. [CrossRef]
49. Bauhus, J.; Paré, D.; Coté, L. Effects of tree species, stand age and soil type on soil microbial biomass and its activity in a southern boreal forest. *Soil Biol. Biochem.* **1998**, *30*, 1077–1089. [CrossRef]
50. Lemma, B.; Nilsson, I.; Kleja, D.B.; Olsson, M.; Knicker, H. Decomposition and substrate quality of leaf litters and fine roots from three exotic plantations and a native forest in the southwestern highlands of Ethiopia. *Soil Biol. Biochem.* **2007**, *39*, 2317–2328. [CrossRef]
51. Zhao, F.Z.; Ren, C.J.; Han, X.H.; Yang, G.H.; Wang, J.; 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]
52. Liu, J.; Yang, Z.; Peng, D.; Zhu, H.; Yang, G.; Ha, V.N.; Zhong, Z. Response of soil microbial community dynamics to *Robinia pseudoacacia* L. afforestation in the loess plateau: A chronosequence approach. *Plant Soil* **2018**, *423*, 327–338. [CrossRef]
53. Wang, Y.; Zhang, X.; Huang, C. Spatial variability of soil total nitrogen and soil total phosphorus under different land uses in a small watershed on the Loess Plateau, China. *Geoderma* **2009**, *150*, 141–149. [CrossRef]
54. Lazzaro, L.; Mazza, G.; d'Errico, G.; Fabiani, A.; Giuliani, C.; Inghilesi, A.F.; Lagomarsino, A.; Landi, S.; Lastrucci, L.; Pastorelli, R.; et al. How ecosystems change following invasion by Robinia pseudoacacia: Insights from soil chemical properties and soil microbial, nematode, microarthropod and plant communities. *Sci. Total Environ.* **2018**, *622–623*, 1509–1518. [CrossRef] [PubMed]
55. Delgado-Baquerizo, M.; Maestre, F.T.; Reich, P.B.; Jeffries, T.C.; Gaitan, J.J.; Encinar, D.; Berdugo, M.; Campbell, C.D.; Singh, B.K. Microbial diversity drives multifunctionality in terrestrial ecosystems. *Nat. Commun.* **2016**, *7*, 10541. [CrossRef] [PubMed]
56. Mooshammer, M.; Wanek, W.; Hammerle, I.; Fuchslueger, L.; Hofhansl, F.; Knoltsch, A.; Schnecker, J.; Takriti, M.; Watzka, M.; Wild, B.; et al. Adjustment of microbial nitrogen use efficiency to carbon: Nitrogen imbalances regulates soil nitrogen cycling. *Nat. Commun.* **2014**, *5*, 3694. [CrossRef] [PubMed]
57. Yang, B.; Pang, X.Y.; Hu, B.; Bao, W.K.; Tian, G.L. Does thinning- induced gap size result in altered soil microbial community in pine plantation in eastern Tibetan Plateau? *Ecol. Evol.* **2017**, *7*, 2986–2993. [CrossRef] [PubMed]
58. Zhang, W.; Liu, W.; Xu, M.; Deng, J.; Han, X.; Yang, G.; Feng, Y.; Ren, G. Response of forest growth to C:N:P stoichiometry in plants and soils during *Robinia pseudoacacia* afforestation on the Loess Plateau, China. *Geoderma* **2019**, *337*, 280–289. [CrossRef]
59. Radtke, A.; Ambraß, S.; Zerbe, S.; Tonon, G.; Fontana, V.; Ammer, C. Traditional coppice forest management drives the invasion of *Ailanthus altissima* and *Robinia pseudoacacia* into deciduous forests. *For. Ecol. Manag.* **2013**, *291*, 308–317. [CrossRef]
60. Fichtner, A.; von Oheimb, G.; Härdtle, W.; Wilken, C.; Gutknecht, J.L.M. Effects of anthropogenic disturbances on soil microbial communities in oak forests persist for more than 100 years. *Soil Biol. Biochem.* **2014**, *70*, 79–87. [CrossRef]
61. Kourtev, P.S.; Ehrenfeld, J.G.; Häggblom, M. Exotic plant species alter the microbial community structure and function in the soil. *Ecology* **2002**, *83*, 3152–3166. [CrossRef]
62. Bossio, D.A.; Fleck, J.A.; Scow, K.M.; Fujii, R. Alteration of soil microbial communities and water quality in restored wetlands. *Soil Biol. Biochem.* **2006**, *38*, 1223–1233. [CrossRef]
63. Gavazov, K.; Ingrisch, J.; Hasibeder, R.; Mills, R.T.; Buttler, A.; Gleixner, G.; Pumpanen, J.; Bahn, M. Winter ecology of a subalpine grassland: Effects of snow removal on soil respiration, microbial structure and function. *Sci. Total Environ.* **2017**, *590–591*, 316–324. [CrossRef] [PubMed]
64. Myers, R.T.; Zak, D.R.; White, D.C.; Peacock, A. Landscape-level patterns of microbial community composition and substrate use in upland forest ecosystems. *Soil Sci. Soc. Am. J.* **2001**, *65*, 359–367. [CrossRef]
65. Zhang, B.; Li, Y.; Ren, T.; Tian, Z.; Wang, G.; He, X.; Tian, C. Short-term effect of tillage and crop rotation on microbial community structure and enzyme activities of a clay loam soil. *Biol. Fertil. Soils* **2014**, *50*, 1077–1085. [CrossRef]
66. Brockett, B.F.T.; Prescott, C.E.; Grayston, S.J. Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. *Soil Biol. Biochem.* **2012**, *44*, 9–20. [CrossRef]
67. Frostergård, A.; Bååth, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol. Fertil. Soils* **1996**, *22*, 59–65. [CrossRef]