Article

How Elemental Stoichiometric Ratios in Microorganisms Respond to Thinning Management in *Larix principis-rupprechtii* Mayr. Plantations of the Warm Temperate Zone in China

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Abstract: The stoichiometric ratios of elements in microorganisms play an important role in biogeochemical cycling and evaluating the nutritional limits of microbial growth, but the effects of thinning treatment on the stoichiometric ratio of carbon, nitrogen, and phosphorus in microorganisms remain unclear. We conducted research in a *Larix principis-rupprechtii* Mayr. plantation to determine the main factors driving microbial carbon (C): nitrogen (N): phosphorus (P) stoichiometry following thinning and the underlying mechanisms of these effects. The plantation study varied in thinning intensity from 0% tree removal (control), 15% tree reduction (high density plantation, HDP), 35% tree reduction (medium density plantation, MDP), and 50% tree reduction (low density plantation, LDP). Our results indicated that medium density plantation significantly increased litter layer biomass, soil temperature, and other soil properties (e.g., soil moisture and nutrient contents). Understory vegetation diversity (i.e., shrub layer and herb layer) was highest in the medium density plantation. Meanwhile, thinning had a great influence on the biomass of microbial communities. For example, the concentration of phospholipid fatty acids (PLFA) for bacteria and fungi in the medium density plantation (MDP) was significantly higher than in other thinning treatments. Combining Pearson correlation analysis, regression modeling, and stepwise regression demonstrated that the alteration of the microbial biomass carbon: nitrogen was primarily related to gram-positive bacteria, gram-negative bacteria, soil temperature, and soil available phosphorus. Variation in bacteria, actinomycetes, gram-positive bacteria, gram-negative bacteria, and soil total phosphorus was primarily associated with shifts in microbial biomass carbon: phosphorus. Moreover, changes in microbial biomass nitrogen: phosphorus were regulated by actinomycetes, gram-negative bacteria, and soil temperature. In conclusion, our research indicates that the stoichiometric ratios of elements in microorganisms could be influenced by thinning management, and emphasizes the importance of soil factors and microbial communities in driving soil microbial stoichiometry.

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1. Introduction

Ecological stoichiometry is an effective tool to reveal the nutrient balance in forest ecosystems and the efficiency of microbial decomposition [1,2]. Exploring the microbial stoichiometric ratios of different forest types and their driving factors could also improve our understanding of the nutrient limitation in the process of microbial growth [3]. Previous research suggests that soil microbial biomass and its stoichiometric ratios might be sensitive to changes in ecological factors, such as soil hydrothermal condition, nutrient contents, and enzyme activity; soil microbe stoichiometry has been used to predict changes in soil microbial community structure and function after human management [4,5]. Therefore,

the responses of microbial carbon, nitrogen, and phosphorus stoichiometry characteristics to human management have received increased attention in forestry researchers.

Forest thinning is a common management technique that may increase soil nutrient availability and tree growth rates by altering tree densities in forests [6,7]. Previous research on thinning in plantation ecosystems has revealed several key insights. First, prior work illuminated that forest thinning may influence the growth of plantation trees by affecting light transmittance, throughfall, or other understory micro-environmental factors [8,9]. Second, manipulation of forest density directly affects the gap size and structure of the arborous layer, and indirectly alters the species diversity of the understory plant community [10,11]. Finally, thinning treatments could alter the decomposition rates of litter by adjusting understory micro-climate temperature, humidity, and microbial activity [12,13]. However, the impacts of different thinning intensities on the ratios of C, N, and P in soil microorganisms remain unknown.

Because of the intimate links between the plant communities and soil microbial communities [14], their responses to forest thinning practices may be coupled. For example, thinning directly affects the diversity and structure of understory plants, and the quality and composition of litter may respond significantly to changes in aboveground vegetation characteristics. Changes in litter quantity and quality are closely related to the activity of the soil microbial community and soil microbial biomass [15,16]. As a result, soil microbial communities may respond significantly to the changes in plant and litter characteristics after forest thinning management. In addition, some previous results also indicated that changes in stoichiometric ratios of forest soil microorganism communities are important for understanding microbial nutrients limitations and nutrient transformations in soil, and may also be tightly related to human management [17,18]. For example, Li et al. found that landscape and land use had a significant impact on soil microbial elemental stoichiometry and suggested that the stoichiometric ratios of microbial biomass were related to soil micro-environmental factors [17]. At the same time, elemental composition normally varies among different microbial groups (including bacteria, fungi, actinomycetes, and others) [19,20]. Changes to soil microbial groups may lead to differences in soil microbial biomass stoichiometric ratios [21]. Chen et al. suggested that observed changes in microbe stoichiometry were due to alterations to soil microorganism groups and abiotic variables under complex natural environmental conditions on the Tibetan Plateau [22]. Therefore, human management can greatly influence the biomass and stoichiometric characteristics of soil microorganisms by regulating changes in various ecological factors and microbial groups. However, at present, research on dynamic changes and mechanisms of regulating microbial stoichiometry in artificial forest ecosystems after human management remains controversial. In particular, after long-term field experimentation, it remains unclear whether microbial biomass C, N, and P ratios could be influenced by the variations in soil microbial groups and soil properties following thinning. Therefore, further study is critical for advancing our understanding of the regulatory mechanisms and dynamic processes governing microbial stoichiometry.

L. principis-rupprechtii is the primary planted tree species in Northern China, and is mainly used for timber production and environmental restoration [23,24]. However, due to inappropriate planting densities and low efficiency governance, soil productivity in *L. principis-rupprechtii* plantations is seriously degraded. Such forests have become unhealthy, leading to lower-efficiency forests, generally poor soil conditions, and *Larix* diseases. Therefore, these degraded plantations are a suitable ecosystem for understanding the ecological regulatory mechanisms of variation in microbial stoichiometry after human management. Previous study on thinning treatments has concentrated on improving plant biodiversity and soil fertility [25]. Past research has yet to elucidate whether density adjustments may alter the stoichiometric ratios in microorganisms by regulating soil properties and microbe groups. In this paper, we hypothesize that the elemental contents (including carbon, nitrogen, and phosphorus) and their ratios in microbial biomass likely respond to density adjustment as do vegetation communities, because of the close coupling

between above and belowground communities. Simultaneously, we predict that soil microbial biomass stoichiometry may not scale with the intensity of density regulation but instead peak at a suitable intensity, as rational thinning has been found to improve soil nutrient availability and increase forest productivity. In addition, we also predict that the soil factors and microbe groups may be altered by the adjustment of tree density, resulting in changes in microbial stoichiometry in the plantation ecosystem. Taken together, we expect that this research will indicate that the stoichiometric ratios of soil microbial biomass could be affected by density adjustment and are closely related to soil properties and microbial community structure.

2. Materials and Methods

2.1. Sampling Site Description and Thinning Experiment

Our research was carried out in *Larix principis-rupprechtii* Mayr. plantations located in Northern China. The plantations are located in the Tai Yue Mountain area of Shanxi Province (111°59′–112°05′ E, 36°40′–36°47′ N). The study area has a continental monsoon climate and the mean annual temperature (MAT) of the region is 6.2 °C, with mean lows of −10.3 °C in January and mean highs of 17.5 °C in August. Mean annual precipitation (MAP) is between 1700 and 2450 mm. The soils in the region are classified as Haplic luvisols, which developed from limestone. *Rhododendron micranthum* Turcz., *Hippophae rhamnoides* Linn., *Phlomis umbrosa* Turcz., and *Thalictrum aquilegifolium* var. *sibiricum* Linnaeus are the dominant understory species.

The experimental area was located in the *L. principis-rupprechtii* plantation, with an original density of 2600 trees ha^{−1}, which were planted in 1981. In 2007, thinning practice was carried out in experimental areas, and the density of plantations was maintained at 2095 trees ha^{−1}. In the spring of 2012, after topography and vegetation surveys, twelve 25 × 25 m plots (plots were at least 10 m apart) were built for a long-term field thinning experiment. Four thinning treatments were performed (each measure was repeated three times) to establish four plantation densities: 2095 trees ha^{−1} (control, unthinned), 1785 trees ha^{−1} (15% of trees removed, high density plantation), 1376 trees ha^{−1} (35% of trees removed, medium density plantation), and 1060 trees ha^{−1} (50% of trees removed, low density thinning) (Table S1) [23].

In 2018, we re-measured elevation, slope, diameter at breast height (DBH), and other plantation characteristics in 12 plots. To measure changes in understory vegetation diversity after thinning, five 1 m² and five 25 m² sub-quadrats were established in each plot (Table 1). Shannon indices, richness indices, and evenness indices were used to analyze the diversity of understory vegetation (including shrubs and herbs).

The equation of the Shannon index used for herb and shrub layers:

$$H = - \sum_{i=1}^n P_i \ln P_i \quad (1)$$

The equation of the richness index used for herb and shrub layers:

$$S = n \quad (2)$$

Evenness index of herb and shrub layers:

$$J = \left(- \sum P_i \ln P_i \right) / \ln S \quad (3)$$

n and S : number of species, P_i : species proportional to the total measure of all species.

Table 1. Soil properties under different density adjustment in *L. principis-rupprechtii* plantations.

Thinning Treatments	STC (g kg ⁻¹)	STN (g kg ⁻¹)	STP (g kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	NH ₄ ⁺ -N (mg kg ⁻¹)	SAP (mg kg ⁻¹)	pH Value	SWC (%)
Control	37.2 ± 1.0 a	3.0 ± 0.4 a	0.5 ± 0.1 a	7.1 ± 2.2 a	4.7 ± 0.6 a	4.0 ± 0.1 a	6.2 ± 0.1 a	32.4 ± 1.3 a
HDP	40.5 ± 1.3 b	3.4 ± 0.6 b	0.4 ± 0.1 a	9.1 ± 2.9 b	4.9 ± 0.6 a	4.3 ± 0.2 a	6.2 ± 0.1 a	45.5 ± 2.7 b
MDP	50.0 ± 1.4 c	3.9 ± 0.6 c	0.5 ± 0.1 a	10.0 ± 2.5 b	5.9 ± 0.5 b	4.9 ± 0.2 a	6.1 ± 0.1 a	50.7 ± 2.5 b
LDP	43.2 ± 1.3 b	3.6 ± 0.4 b	0.5 ± 0.0 a	8.6 ± 1.7 ab	5.1 ± 0.6 a	4.3 ± 0.1 a	6.2 ± 0.1 a	44.0 ± 2.8 b
df	3	3	3	3	3	3	3	3
F	10.19	9.86	2.43	7.34	6.41	1.36	0.95	8.91
p	<0.01	<0.01	0.37	0.02	0.03	0.43	0.56	<0.01

Values: mean ± standard error. Different lowercase letters: $p < 0.05$, significant difference between treatments. Control: control site; HDP: high density plantation; MDP: medium density plantation; LDP: high density plantation. F: the F value of the corresponding factor.

2.2. Soil and Litter Sampling and Laboratory Analysis

At each plot, nine replicate topsoil samples (0–10 cm depth) were collected with a tailor soil auger. Soil was sampled in August 2017 and August 2018. Concurrently, we used a temperature recorder (Spectrum Technologies, Inc., Chicago, IL, USA) to determine soil temperature at each of the 12 plots. All samples were passed through a 2 mm sieve and the plant debris and rocks were removed. After determining the soil water content (SWC), soil samples from the same plot were divided into three parts and immediately transported to the laboratory on ice. One part of the sample was used to measure the concentrations of soil available nutrients and microbial biomass (stored at 0 °C). The other portion of the soil sample was stored at −20 °C and analyzed for soil microbial community characteristics. The third portion was air-dried and prepared to measure pH value and nutrient indices of the soil. Litter samples were collected from surface soil in 12 plots and then sent to the laboratory. Before measuring the carbon, nitrogen, and phosphorus contents of the litter, all samples were over-dried for at least 72 h (at 60 °C).

Soil pH was measured in suspension with water (soil:water was 1:5) using a pH meter [26]. We measured soil total carbon (STC) and nitrogen (STN) using an elemental analyzer (FLASH 2000 CHNS/O, Thermo, Third Avenue, Waltham, MA, USA). Soil total phosphorus (STP) was measured with the HClO₄-H₂SO₄ oxidation digestion method. Soil ammonium nitrogen (NH₄⁺-N) and soil nitrate nitrogen (NO₃⁻-N) were extracted with the KCl extraction method and soil available phosphorus (SAP) was measured using the NaHCO₃ extraction method, then determined using an AA3 continuous flow analytical system (AA3, SEAL, Norderstedt, Germany). Soil water content (SWC) was measured by oven-drying to constant mass at 105 °C [26]. The carbon concentration in litter was analyzed with the dichromate oxidation method, the nitrogen concentration in litter was determined using the Kjeldahl method, and the litter phosphorus concentration was measured with spectrophotometry (using the colorimetric method) [25,26].

Microbial biomass C (MBC) and N (MBN) were analyzed through the fumigation–extraction method. Half of the fresh samples were needed to fumigate with chloroform in a vacuum (at least 24 h), and then after extraction with 0.5 M K₂SO₄, we measured all soil samples with a Total Organic Nitrogen/Carbon analyzer (Multi N/C 3000, Analytik Jena AG, Konrad-Zuse-Straße, Berlin, Germany) [26]. The method of Wu et al. [27] and Hedley & Steward [28] was used for the determination of microbial biomass P (MBP). The fumigation process also required chloroform. However, the nonfumigated and fumigation test samples were all extracted with 0.5 M NaHCO₃ and we then used the colorimetric method to measure the concentration of biomass phosphorus.

Previous studies suggested that phospholipid fatty acids (PLFA) could be used to analyze the variation in soil microbial community structure and composition. We extracted lipids from 8 g of soil (dry weight) with a mixture (including chloroform, methanol, and phosphate buffer). Subsequently, fatty acid methyl esters were recovered via mild alkaline methanolysis. Finally, we categorized soil microbial groups based on known content fatty acid methyl esters (19:0) as the internal standard. The PLFAs i13:0, i14:0, a14:0, a15:0, i15:0,

i16:0, a17:0, i17:0, and i18:0 (indicators of gram-positive bacteria; G⁺) [29,30] and 10:0 2OH, 15:1 w4c, 15:1 w6c, 16:0 2OH, 16:1 w6c, 16:1 w7c, 16:1 w9c, cy17:0, 17:1 w8c, 18:1 w5c, 18:1 w7c, and cy19:0 (indicators of gram-negative bacteria; G⁻) [29,31–33] and 14:0, 15:0, 17:0, and 18:0 were used as an indicator of bacteria biomass [33,34]. The fatty acids 16:1 w5c, 18:1 w9c, 18:2 w6c, and 18:2 w9c were used to represent the fungi [33,34]. The PLFAs 10 Me 16:0, 10 Me 17:0, 10 Me 18:0 were chosen to represent actinomycetes [35] (Table S2).

2.3. Statistical Analyses

The alteration of soil properties, understory vegetation, litter element contents, stoichiometric ratios in soil microorganisms, and microbe group biomass (e.g., bacterial biomass, fungal biomass) after thinning were tested using one-way analysis of variance (ANOVA) and least significant difference (LSD) multiple comparison. Pearson's correlation analysis was performed to reveal the correlations between the ratio of elements in microbial biomass and pH, moisture, temperature, and nutrient concentrations. We used regression analyses to evaluate relationships between soil microbe element ratios and microbe community biomass. To improve the variance distribution of soil microbial biomass stoichiometry, all data were transformed by log 10. Moreover, multiple linear stepwise regression was used to analyze whether variations in microbe biomass stoichiometric ratios were related to physicochemical properties and microbial communities in soil. Analyses were performed using SPSS 19.0 software (IBM, Corporation, Armonk, NY, USA).

3. Results

3.1. Soil Properties

As demonstrated in Table 1, thinning had a significant impact on the concentration of total carbon (STC), nitrogen (STN), phosphorus (STP), and available nutrients (NO₃⁻-N, NH₄⁺-N, and SAP) in soil. In particular, the concentrations of STC, STN, STP, NO₃⁻-N, NH₄⁺-N, and SAP were highest in the medium density plantation (Table 1). Meanwhile, soil moisture and pH value ranged from 31.6% to 53.3% and from 6.07 to 6.3, respectively (Table 1). Maximum soil temperature was 18.3 °C (LDP plantation) and minimum soil temperature was 9.9 °C (HDP plantation) (Figure S1).

3.2. Understory Vegetation Characteristics and Litter Properties

The Herb Shannon and evenness indices were significantly affected by density adjustment ($p < 0.05$) and peaked in the MDP plantation (5.19 ± 0.76 and 1.40 ± 0.20 , respectively). On the contrary, density adjustment had little effect on the Shannon, richness, and evenness indices of shrub plants. However, the highest Shannon index values were found in the HDP plantation (Table 2).

Table 2. Characteristics of understory vegetation after thinning practice.

Thinning Treatment	Shrub Biodiversity			Herb Biodiversity			Litter Layer Biomass (t ha ⁻¹)
	Shannon Index	Richness Index	Evenness Index	Shannon Index	Richness Index	Evenness Index	
Control	0.37 ± 0.63 a	1 ± 1 a	0.12 ± 0.21 a	4.18 ± 0.01 a	18 ± 3 a	1.13 ± 0.01 a	55.71 ± 9.57 a
HDP	0.46 ± 0.40 a	2 ± 1 a	0.23 ± 0.20 a	4.69 ± 0.47 ab	22 ± 5 a	1.26 ± 0.13 ab	60.44 ± 18.09 ab
MDP	0.44 ± 0.77 a	2 ± 2 a	0.32 ± 0.55 a	5.19 ± 0.76 b	25 ± 6 a	1.40 ± 0.20 b	69.15 ± 11.61 b
LDP	0.23 ± 0.40 a	1 ± 1 a	0.12 ± 0.20 a	4.51 ± 0.43 ab	23 ± 1 a	1.21 ± 0.12 ab	58.01 ± 14.27 a
df	3	3	3	3	3	3	3
F	0.76	0.61	0.53	7.23	1.56	7.53	7.25
p	0.64	0.50	0.46	0.02	0.27	0.02	0.02

Values: mean ± standard error. Different lowercase letters: $p < 0.05$, significant difference between treatments. F: the F value of the corresponding factor.

In addition, HDP and MDP treatment substantially increased the biomass in litter layer (Table 2). The carbon content of litter was higher in MDP and LDP than that of

the high density plantation and control site (Figure 1). The contents of nitrogen and phosphorus content in litter of high and medium density plantations was higher than in control and LDP (Figure 1). Overall, the litter properties were significantly affected by density adjustment during the sampling time in 2017 and 2018.

Figure 1. Characteristics of litter layer in *L. principis-rupprechtii* plantations with different density. Error bars indicate standard error ($n = 3$). Different lowercase letters: $p < 0.05$, significant difference between treatments.

3.3. The Stoichiometric Ratio of Soil Microbial Community

In 2017, soil microbial biomass carbon, nitrogen, and phosphorus in MDP and LDP were significantly higher than in the control site (Table 3). In August 2018, compared to the control, the elements of microbial biomass in soil (i.e., C, N, and P) were all higher in the thinning management. Compared to soil microbial biomass, MBN:MBP was less variable across forest thinning treatments. However, a similar difference was found in the stoichiometric characteristics of microbial biomass. In August 2017 and August 2018, MBC:MBN and MBC:MBP ratios in medium density plantation tended to increase significantly compared with the control site (Table 3).

Table 3. Comparison of soil microbial biomass and its stoichiometric ratios within 10 cm soil layers in various scale.

Treatment Methods		Soil Microbial Biomass			Soil Microbial Biomass Stoichiometry			References
		Microbial Biomass C (mg kg ⁻¹)	Microbial Biomass N (mg kg ⁻¹)	Microbial Biomass P (mg kg ⁻¹)	MBC:MBN	MBC:MBP	MBN:MBP	
2017	Control	433 ± 2 a	98 ± 2 a	26 ± 6 a	4.4 ± 0.4 ab	16.9 ± 3.0 ab	3.9 ± 0.8 a	In this paper
	HDP	512 ± 2 b	124 ± 10 b	32 ± 2 b	4.1 ± 0.2 a	16.0 ± 0.9 a	3.9 ± 0.3 a	
	MDP	737 ± 4 c	154 ± 9 c	37 ± 2 c	4.8 ± 0.5 b	19.8 ± 3.6 c	4.1 ± 0.0 a	
	LDP	547 ± 5 b	122 ± 2 b	40 ± 3 c	4.5 ± 0.1 ab	18.5 ± 1.7 b	4.1 ± 0.4 a	
	df	3	3	3	3	3	3	
	F	13.35	12.98	11.83	7.90	9.88	2.29	
	p	<0.01	<0.01	<0.01	<0.05	<0.01	>0.05	
2018	Control	578 ± 5 a	146 ± 2 a	34 ± 2 a	4.0 ± 0.1 a	17.1 ± 0.9 a	4.3 ± 0.3 a	
	HDP	617 ± 6 ab	152 ± 2 a	34 ± 1 a	4.0 ± 0.1 a	18.0 ± 0.8 ab	4.4 ± 0.2 a	
	MDP	726 ± 3 c	172 ± 1 b	38 ± 1 b	4.2 ± 0.0 b	19.1 ± 0.4 c	4.5 ± 0.1 a	
	LDP	653 ± 9 b	160 ± 2 b	36 ± 1 b	4.1 ± 0.0 ab	18.1 ± 0.8 b	4.4 ± 0.2 a	
	df	3	3	3	3	3	3	
	F	13.01 **	12.67 **	11.34 **	7.60 *	9.34 **	2.02	
	p	<0.01	<0.01	<0.01	<0.05	<0.01	>0.05	
Global average		680	105	161	7.6	42.4	5.6	Xu et al., (2013)
Temperate coniferous forest		508	80	86	6.3	70.6	7.4	Xu et al., (2013)

Different lowercase letters: $p < 0.05$, significant difference between treatments; MBC:MBN: microbial biomass C: microbial biomass N; MBC:MBP: microbial biomass C: microbial biomass P; MBN:MBP: microbial biomass N: microbial biomass P. F: the F value of the corresponding factor. * represents $p < 0.05$; ** represents $p < 0.01$.

Furthermore, soil microbial biomass C and N in the thinning treatments were near the average for temperate coniferous forests globally, whereas soil microbial biomass P was markedly lower than those in temperate coniferous forest globally. MBC:MBN and

MBN:MBP after thinning were slightly lower than in global temperate coniferous forests. Mean MBC:MBN in global temperate coniferous forests is 2.18–3.56 times higher than in the thinning treatments (Table 3).

3.4. Soil Microorganism Community Composition

As demonstrated in Figure 2, microbial abundance responded to thinning treatments. In August 2017, total PLFAs in the HDP ($25.34 \pm 5.97 \text{ nmol g}^{-1}$), MDP ($27.32 \pm 2.97 \text{ nmol g}^{-1}$), and LDP ($21.70 \pm 3.49 \text{ nmol g}^{-1}$) treatments were higher than in the control site ($20.36 \pm 4.30 \text{ nmol g}^{-1}$). Meanwhile, bacteria were significantly more abundant in MDP ($11.42 \pm 1.77 \text{ nmol g}^{-1}$) than in the control site ($8.24 \pm 1.60 \text{ nmol g}^{-1}$) and low density plantation ($8.29 \pm 1.75 \text{ nmol g}^{-1}$). Gram-positive bacteria, gram-negative bacteria, fungi, and actinomycetes were most abundant in the MDP plantation ($6.01 \pm 1.18 \text{ nmol g}^{-1}$, $5.34 \pm 1.84 \text{ nmol g}^{-1}$, $0.79 \pm 0.13 \text{ nmol g}^{-1}$, $1.95 \pm 0.26 \text{ nmol g}^{-1}$, respectively), and the biomass of gram-positive bacteria in the LDP ($4.38 \pm 0.88 \text{ nmol g}^{-1}$) plantation was lower than in control plots ($4.54 \pm 1.38 \text{ nmol g}^{-1}$). In August 2018, compared to the control, total PLFAs, gram-positive bacteria, gram-negative bacteria, and actinomycetes were all higher in the HDP, MDP, and LDP plantations. A similar pattern was found in mean fungi abundance, which was 0.06 nmol g^{-1} lower in the LDP plantation than in the control. Overall, the biomass of major microbial groups increased after thinning during the sampling time in 2017 and 2018 (Figure 2).

Figure 2. Soil microorganism PLFAs in *L. principis-rupprechtii* plantations with different density. Error bars indicate standard error ($n = 3$). Different lowercase letters: $p < 0.05$, significant difference between treatments.

3.5. The Influence of Soil Properties and Microbial Communities on Microbial Stoichiometric Ratios

MBC:MBN was positively correlated with soil temperature and available phosphorus contents ($r^2 = 0.51^*$, and $r^2 = -0.56^*$, respectively; Table 4), and MBC:MBP exhibited a positive association with total soil carbon, soil nitrogen, and soil phosphorus ($r^2 = 0.36^*$; $r^2 = 0.44^*$; $r^2 = 0.49^*$, respectively; Table 4). MBN:MBP was positively correlated with soil temperature ($r^2 = 0.49^*$; Table 4).

Table 4. Pearson correlation of microbial biomass C, N, and P ratios and pH value, moisture, temperature, and nutrient concentrations in soil across different thinning treatments.

Soil Microbial Biomass C, N, and P Ratios	Soil pH Value	SWC	Soil Temperature	STC	STN	STP	NO ₃ ⁻ -N	NH ₄ ⁺ -N	SAP
Soil microbial C	0.26	−0.16	0.46 *	0.61 **	0.16	0.24	0.14	0.57 **	0.35
Soil microbial N	0.26	−0.19	−0.23	0.58 **	0.30	0.16	0.18	0.45 *	0.39
Soil microbial P	0.25	−0.04	−0.28	0.27	0.11	0.08	0.04	0.20	0.42 *
MBC: MBN	−0.17	0.20	0.51 *	−0.27	0.18	−0.28	0.20	−0.21	−0.56 *
MBC: MBP	−0.24	0.09	0.24	0.36 *	0.44 *	0.49 *	0.07	−0.14	−0.12
MBN: MBP	−0.11	0.25	0.49 *	−0.13	−0.09	−0.18	−0.25	−0.15	−0.19

* represents $p < 0.05$; ** represents $p < 0.01$.

There was no significant correlation between the stoichiometric ratios of microorganism and the biomass of fungal (all $p > 0.05$; Figures 3–5). On the contrary, a significant correlation was found between MBC:MBN and gram-positive bacterial and gram-negative bacterial biomass ($r^2 = 0.281^*$, and $r^2 = 0.294^*$, respectively; Figure 3). MBC:MBP exhibited significant positive correlations with gram-positive bacterial biomass, gram-negative bacterial biomass, bacterial biomass, and actinomycetes biomass ($r^2 = 0.176^*$, $r^2 = 0.208^*$, $r^2 = 0.239^*$, and $r^2 = 0.242^*$, respectively; Figure 4), whereas a significant association existed between MBN:MBP and gram-negative bacterial biomass and actinomycetes biomass ($r^2 = 0.231^*$, and $r^2 = 0.227^*$, respectively; Figure 5).

Figure 3. Cont.

Figure 3. Relationships between MBC:MBN ratio and soil microbial groups in *L. principis-rupprechtii* plantations with different density.

Figure 4. Relationships between MBC:MBP ratio and soil microbial groups in *L. principis-rupprechtii* plantations with different density.

Figure 5. Relationships between MBN:MBP ratio and soil microbial groups in *L. principis-rupprechtii* plantations with different density.

The microbial biomass C, N, and P in soil were not significantly associated with soil pH, SWC, STN, STP, and NO_3^- -N (Table 4). However, soil microbial biomass C was positively associated with soil temperature ($r^2 = 0.46^*$; Table 4), and soil microbial biomass P was positively associated with soil available phosphorus content ($r^2 = 0.42^*$; Table 4). Moreover, soil STC and NH_4^+ -N content were significantly positively associated with the microbial biomass C ($r^2 = 0.61^{**}$, and $r^2 = 0.58^*$, respectively) and N ($r^2 = 0.57^{**}$, and $r^2 = 0.45^*$, respectively) in soil (Table 4).

Stepwise regression (Table 5) showed that the change in microbial biomass stoichiometric ratios was linked to soil microbial groups and soil properties following thinning. Specifically, changes in microorganism C:N were connected to shifts in soil temperature, G^+ bacterial abundance, and G^- bacterial abundance; changes in microorganism C:P were associated with variation in soil total P and bacterial, actinomycetes, G^+ bacterial abundance, and G^- bacterial abundance; and variation in microorganism N:P was linked to variations in actinomycetes abundance, G^- bacterial abundance, and soil temperature (Table 5).

Table 5. Stepwise regression analyses of microbial stoichiometry as predicted by soil nutrient variables and soil microbial groups in a *L. principis-rupprechtii* plantation.

Predicted Variables	R ²	Sig.	Contribution of the Individual Predictor (%)						
			Bacteria	Actinomycete	Gram-Positive Bacteria	Gram-Negative Bacteria	STP	Soil Temperature	SAP
Soil microbial C:N	0.274	<0.05			14.5	39.3		20.4	25.8
Soil microbial C:P	0.530	<0.01	34.1	16.9	13.7	11.8	23.5		
Soil microbial N:P	0.201	<0.05		65.1		17.6		17.3	

Soil microbial C:N: soil microbial biomass carbon: nitrogen; Soil microbial C:P: soil microbial biomass carbon: phosphorus; Soil microbial N:P: soil microbial biomass nitrogen: phosphorus.

4. Discussion

4.1. Shifts in Ecological Factors Following Thinning

It has previously been shown that the responses of soil nutrient concentrations, above-ground vegetation and litter properties to thinning may be positive, negative or neutral, depending on thinning method, time of application, deforestation intensity, and stand types [36–38]. A series of studies conducted in other forest ecosystems showed that the concentrations of organic matter, K, and Mg in unthinned plantation soil were lower than those in the thinned plantation soil, as was soil temperature, which may be the result of density adjustment [23,38]. This finding is similar to our results. In this study, we also found that thinning had a great influence on litter properties and the diversity of the shrub and herb layers. Selective reduction of forest density can increase gap size, thus increasing light transmittance, soil enzyme activity, and available nutrient content [39–41]. Changes in the micro-environment of the forest understory may influence vegetation growth and result in higher species diversity of the shrub and herb layers [15,42]. In addition, the elemental contents of litter also increased following thinning, as evidenced by higher content of carbon, nitrogen, and phosphorus contents in litter. Meanwhile, in the four thinning treatments, the change in litter layer biomass also reflected this pattern. This finding is similar to that of a study conducted in a Chinese fir plantation, which showed that carbon, nitrogen, and phosphorus contents in litter increased significantly in low density plantations [10].

4.2. Shifts in Microbial Community Characteristics Following Thinning

Our research also suggested that forest thinning had significant effects on the biomass of soil microorganisms (Table 3), particularly in the MDP plantation. Meanwhile, variation in soil temperature recorded and nutrient contents in our study may partly explain changes in soil microbial biomass after thinning (Table 4). Similarly, Kim et al. [43] and Ren et al. [8] found that soil microbial biomass was significantly related to soil moisture, temperature, texture, and other soil properties. Thinning may have a positive impact on soil moisture and temperature by improving forest density, providing a more appropriate hydrothermal condition for C and N mineralization in soil, and thus affecting microbial biomass in soil. Meanwhile, nutrient concentrations in soil determine the growth of vegetation, microbe groups, and enzyme activity. Previous studies have demonstrated that increases in microbe groups are frequently limited by the elemental contents of soil, and greater nutrient availability may contribute to carbon, nitrogen, and phosphorus accumulation in microbial biomass and higher microbial activity [13,18,36]. In this study, we found that soil nutrient contents in thinned plots were higher than those in the control site, which could contribute to greater carbon, nitrogen, and phosphorus storage in microbial biomass under different density adjustments.

Similarly, a series of studies have also found improvements in microbial community structure and composition following changes in management [44–46]. Compared with the control treatment, the medium density plantation had positive effects on the biomass of bacteria, fungi, actinomycetes, and other microbial groups. In general, the diversity of understory vegetation can be altered by different thinning methods [47,48], which

we also found in this study. Forest thinning can affect the composition and structure of forests, thus reducing competition among trees and understory plants, and leading to an increase in the species diversity of shrubs and herbs and the amount of leaf and root litter [49–51]. In addition to increases in decomposing material (e.g., leaf litter and root litter) after thinning, lower competition for nutrients may in turn promote the activity of microbial communities and lead to higher microbial biomass [16,52,53]. On the other hand, fungal biomass in LDP was lower than in the control plantation. This may be because the growth of fungi is closely related to soil moisture, root exudates, and soil carbon. High thinning may excessively reduce tree density and negatively affect soil moisture, thereby reducing the content of carbon substrates and root exudates, and affecting the activity and abundance of fungal biomass. [54,55].

4.3. Variation in Microbial Stoichiometry Linked to Microbe Groups and Soil Characteristics

In this study, the C:N, C:P, and N:P ratios of soil microbial biomass were higher in the medium density plantation than in the control site. Combined results of Pearson's correlation analysis, general linear models, and stepwise regression indicate that changes in soil nutrient concentrations, microbe group biomass, and soil temperature may be important factors determining changes in soil microbial stoichiometry following thinning [22]. Previous studies have provided evidence that thinning likely promotes the diversity of understory vegetation, soil properties (e.g., soil physical and chemical properties, enzyme activity, and microbial community composition) [56], increasing the amount of litter that can be decomposed rapidly (e.g., herb and shrub plant litter) and the decomposition rate of C-rich substrates [12,40,47], which is similar to our findings. Under these conditions, soil nutrients will accumulate, reducing competition for nutrients between microbial communities [19,20,57]. Under weak nutrient competition, soil microbial groups may continue to grow and increase their utilization rates of soil nutrients, leading to variations in cellular carbon, nitrogen, and phosphorus ratios of the microbial community [2,19,58]. Therefore, changes in plant diversity, soil nutrient conditions, and litter decomposition rates after thinning could lead to changes in soil microbial community activity, which may lead to the alteration of microbial biomass stoichiometric ratios [22,58].

In this study, we also found that bacteria, actinomycete and other microbe groups are significant indicators shaping microbial C:N:P stoichiometry. Previous results indicated that increases in soil element contents, enzyme activity, and plant diversity in the forest ecosystem had significant positive effects on microbial communities in soil [19]. Meanwhile, there may be a close relationship between microbe groups such as gram-positive bacteria, gram-negative bacteria and actinomycetes and the C:N:P ratios of microbial biomass [57,59]. For example, Qiu et al., indicated that the microbial biomass C:N ratio was affected by forest density and was related to variations in the abundance and diversity of microbial communities [57]. Therefore, changes in soil nutrient concentrations, understory vegetation, and other environmental variables after thinning may affect the abundance and structure of the soil microbe groups, resulting in changes to microbial stoichiometric ratios, as well observed in our study.

Previous studies have theorized that forest management practices have significant impacts on soil temperature, bulk density, silt content, and other soil physical properties, which may lead to variations in soil microbial C:N:P stoichiometry [59–61]. We observed a similar trend, which may be because increased soil temperature could increase the activity of soil microorganisms, thus promoting the accumulation of chemical elements in soil microbial biomass disproportionately. Furthermore, soil temperature was significantly associated with MBC:MBP and MBN:MBP (Tables 4 and 5). Generally, in areas with high soil temperature or high air temperature, the ability of soil microorganisms to fix effective elements is relatively significant [62,63]. Forest thinning significantly affected soil temperature and carbon and nitrogen concentrations, but had a relatively small impact on soil phosphorus content (mainly because phosphorus came from rock weathering and was relatively stable) (Figure S1 and Table 1). Under these conditions, the utilization efficiency of

carbon and nitrogen in microorganisms may be higher than that of phosphorus. Therefore, the effects of thinning on soil temperature may result in relatively more carbon and nitrogen stored in microbial biomass after thinning treatment, mainly influencing the MBC:MBN and MBC:MBP ratios. Meanwhile, these results may also further demonstrate that variation in temperature was closely related to the stoichiometric ratios of soil microorganisms (especially microbial C:P and N:P ratios).

After four forest thinning treatments, the ratios of soil microbial stoichiometry in *L. principis-rupprechtii* plantation were lower than the average values for global temperate coniferous forests [64]. One probable explanation for this is that the change in microbial stoichiometry could be attributed to variation in the concentration of soil elements, vegetation type, and litter properties. Specifically, STC, STN, and STP in *L. principis-rupprechtii* plantations were all lower than values reported by Xu et al. [64]. The relatively low rainfall and air temperature in the subalpine zone reduced the quantity and decomposition rate of litter, thus limiting the return of nutrients to soil, resulting in lower soil nutrient concentrations than the global average [64]. Generally, the decreases in soil nutrient pools could result in the decreases in the activity of microbe groups in soil, and because the nutrient contents in the soil of our study area are relatively low, the element contents accumulated in microbial biomass may be similarly low. On the other hand, the difference between the soil microbial stoichiometric ratios we reported and global averages could be due to the different utilization efficiency of different elements (especially C use efficiency) by microbial communities. In addition, previous studies have shown that higher soil silt content and relatively neutral pH could provide a more suitable soil microenvironment (e.g., moisture and temperature conditions), promote the decomposition of organic matter, and microbial mineralization, and thus increase the accumulation of nutrient elements in soil [8,13,22]. Therefore, differences in abiotic factors could also lead to the differences in the stoichiometric ratios of soil microbial biomass we observed.

5. Conclusions

Our results suggested that density adjustment had positive effects on the diversity of understory vegetation, litter properties, soil microbial community biomass, and soil properties. In particular, medium density plantation resulted in relatively higher soil microbial biomass, soil properties, and litter layer nutrient contents. Moreover, different thinning treatments did not significantly improve MBN:MBP ratio, but did affect the MBC:MBN and MBC:MBP ratios. Notably, changes in microbial biomass C:N, C:P, and N:P ratios were closely associated with soil microbial community, soil temperature, and soil nutrient contents. These findings demonstrate that soil properties and microbe group biomass played important roles in driving soil microbial biomass stoichiometry. Overall, our results emphasize the importance of alterations to soil properties and microbial communities through forest thinning in regulating microbial biomass stoichiometry, and further advancing our knowledge of nutrient cycling and dynamic balance of elements in warm temperate *L. principis-rupprechtii* plantations in China.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/f12060684/s1>, Table S1: Characteristics of different thinning management sites in *L. principis-rupprechtii* plantations, Table S2: PLFAs biomarkers selected to characterize microorganism community structure, Figure S1: Effect of forest thinning on soil temperature in *L. principis-rupprechtii* plantations. Error bars indicate standard error (n = 3). Different lowercase letters: $p < 0.05$, significant difference between treatments. Control: control site; LDP: low density plantation; MDP: medium density plantation; HDP: high density plantation.

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