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Differential Responses of Soil Extracellular Enzyme Activity and Stoichiometric Ratios under Different Slope Aspects and Slope Positions in *Larix olgensis* Plantations

Mingwei Wang, Li Ji, Fangyuan Shen, Jun Meng, Junlu Wang, Chengfeng Shan and Lixue Yang *

Key Laboratory of Sustainable Forest Ecosystem Management-Ministry of Education, School of Forestry, Northeast Forestry University, Harbin 150040, China; wmwnefu2022@163.com (M.W.); jlnefu@hotmail.com (L.J.); shuke9197@126.com (F.S.); mengjun035@163.com (J.M.); wangjunlu522@163.com (J.W.); shanchengfeng@hotmail.com (C.S.)

* Correspondence: ylx_0813@163.com; Tel.: +86-0451-82191122

Abstract: Soil enzymes play an important role in nutrient biogeochemical cycling in terrestrial ecosystems. Previous studies have emphasized the variability of soil enzyme activities and stoichiometric ratios in forest ecosystems in northern China. However, much less is known about soil enzyme activity, enzymatic stoichiometry ratios and microbial nutrient limitations in *Larix olgensis* plantations under different microsites. In this study, four specific extracellular enzyme activities (β-glucosidase, β-1,4-N-acetylglucosaminidase, L-leucine aminopeptidase, Acid phosphatase), and soil physicochemical properties were measured in the 0–20 cm soil layer. The results showed that slope aspect and slope position had a significant effect on soil moisture, soil bulk density, soil porosity, soil organic matter, ammonium nitrogen and nitrate-nitrogen. Meanwhile, slope aspect and slope position had a significant effect on β-glucosidase, β-1,4-N-acetylglucosaminidase, L-leucine aminopeptidase and Acid phosphatase activities while the highest activity of β-glucosidase (or β-1,4-N-acetylglucosaminidase), L-leucine aminopeptidase, and Acid phosphatase was observed in the upper slope of the east, the upper slope of the south, and the upper slope of the north; soil porosity, pH and soil organic matter were the main factors affecting soil extracellular enzyme activities. The log-transformed ratios of soil C-, N-, and P-acquiring enzyme activities were 1.00:1.06:1.17, indicating that soil microbial growth in this region was limited by N and P. Therefore, these findings highlight that N and P inputs should be considered in the management of *L. olgensis* plantations to improve soil microbial enzyme activity, alleviating N and P limitations.

Keywords: enzyme activity; enzymatic stoichiometry; slope aspect; slope position; *Larix olgensis* plantations

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1. Introduction

Ecological stability is one of the goals of sustainable forest management [1–3]. Topography(slope aspect, slope position) [4] is an important environmental condition affecting forest ecological resources [5]. Microclimate, hydrological and ecological conditions [6] are shaped by slope aspect and slope position which can significantly alter the subsurface organic matter decomposition processes [7] and determine how soil nutrients are stored and cycled [8]. Previous studies have shown that slope aspect and slope position have an important impact on tree growth [9], species diversity [10] and soil physicochemical properties [11]. Currently, few studies have explored the effects of soil extracellular enzymes on tree growth which limits our understanding of the role of soil enzymology in forest sustainable management.

As a protein with a specific catalytic capacity [12] secreted by soil microorganisms [13] as well as other organic tissues (e.g., plant roots and residues and soil fauna), soil extracellular enzymes play a crucial role in biogeochemical processes [14–16], driving nutrient cycling [17], and they are sensitive indicators of subtle changes in soil habitats [18].

Sinsabaugh et al. [19] speculated that hydrolytic enzyme activity could represent the microbial nutrient requirements. In recent years, β-1,4-glucosidase (BG), N-acetyl-β-aminoglucosidase (NAG), leucine aminopeptidase (LAP), acid phosphatase (AP) have been extensively studied [20]. In the forest ecosystem, soil extracellular enzyme activity (EEA) is varied in response to environmental variabilities [21]. Many studies suggest that soil moisture leads to higher EEA until the soil becomes anaerobic and substrate diffusion or oxygen content limits EEA [22]. Xu et al. [23] found a negative correlation between BG and NAG and annual precipitation in forest ecosystems of northeastern China. Gomez E J et al. [24] expressed that EEA showed the maximum rates of wet conditions (high water availability). It is well known that pH is one of the vital soil characteristics affecting soil microbial diversity and EEA [25]. The optimum pH for hydrolytic exoenzymes varies significantly and can be a limiting factor for microbiological decomposition [26], such as EEA was mainly influenced by climate and pH [23]. Many studies have focused on large regional scales. Nevertheless, the topography also modifies the climatic conditions in a small area [27], but we still know little about the patterns of soil enzyme changes in micro-site environments. As a result, conducting research on soil enzyme activity in micro-site environments is beneficial to our more comprehensive understanding of the variation pattern of soil enzyme activity.

Enzymatic stoichiometry is an important indicator to reveal the growth and metabolic processes of microorganisms [28] and to evaluate the limitation status of soil nutrient resources [29]. On a global scale, Sinsabaugh et al. [20] found that the stoichiometric ratios of C, N and P metabolic enzymes in soil followed the relationship of 1:1:1. However, the growth of soil microorganisms is affected by environmental stress and nutrient limitation, and the stoichiometric ratio of enzymes may not follow the relationship of 1:1:1 [29]. Previous studies have shown that available substrates, abiotic and biotic factors influence enzymatic stoichiometry [30]. For example, Xu et al. [23] reported that $C:N_{eea}$ decreased as the pH increased in different regions of forest soils in China. Peng et al. [31] found a remarkable positive correlation between total phosphorus and soil enzyme stoichiometry ratio of ln(NAG + LAP):ln(AP) in temperate grasslands of China. To further elucidate the characteristics of microbial metabolism, Moorhead et al. [32] proposed to calculate microbial metabolic limitations by converting stoichiometric ratios related to C, N, and P metabolism enzymes into vector lengths and angles to account for microbial nutritional requirements. [33]. Enzymatic stoichiometry connects microbial decomposition to nutrient mineralization and improves our understanding of nutrient cycling in terrestrial ecosystems [34]. Therefore, the study of enzymatic stoichiometry is beneficial to the management of *L. olgensis* plantations.

L. olgensis is characterized by a fast growth rate, good wood material, straight stem, drought and flood resistance. It is commonly used for fiber and construction timber and plays a major role in local water-soil conservation and ecosystem protection. In recent years, there has been an increasing number of studies on soil enzymology. However, studies on the effect of slope position and slope aspect on soil extracellular enzymes in *L. olgensis* plantations are limited. Our aim is to promote the sustainable use of soil resources and enhance the management of *L. olgensis* plantations. In this study, we focused on the changes and characteristics of soil extracellular enzyme activity and enzyme stoichiometry under topographic factors (slope aspect, slope position). Thus, we propose the following hypothesis: (1) The EEA in the upper and middle slopes will be higher than those in the lower slopes, and EEA in the eastern and southern slopes will be higher than those in the northern slopes. Soil porosity and pH will be the main factors affecting soil EEA. (2) Given the increasingly recognized P-limit for microorganisms in the soil [35], we project microorganisms will be limited by P in the study region.

2. Materials and Methods

2.1. Study Site

The region of study is located in the Greater Khingan Mountains, Heilongjiang province, China (124°5′35.58″ E, 50°15′35.58″ N) (Figure 1), at an altitude of 400–700 m above sea level (a.s.l.). The region is a cold temperate continental monsoon climate characterized by a frost-free period of 85–130 days, a large temperature difference between day and night, adequate light, abundant rainfall, and frequent cold air activity. The average annual temperature is −1.2 °C. The extreme maximum temperature reached 37.3 °C and the extreme minimum temperature was −45.4 °C. The average annual precipitation is 494.8 mm, the average annual sunshine is 2493 h. The soils are mainly brown coniferous forest soils with the largest distribution region, followed by chernozems soil, grey forest soil, meadow soils, swamp soils and stony soils (classification according to FAO (IUSS and Working Group WRB, 2006)). The species composition in this area is dominated by *Pinus sylvestris var. mongolica, Betula platyphylla, Populus davidiana, Vaccinium uliginosum, Fabaceae Cyperaceae*. In 1992, *L. olgensis* plantations were established with 2 year old seedlings and the planting densities were all 2500 trees/ha. There was no artificial tending after afforestation.

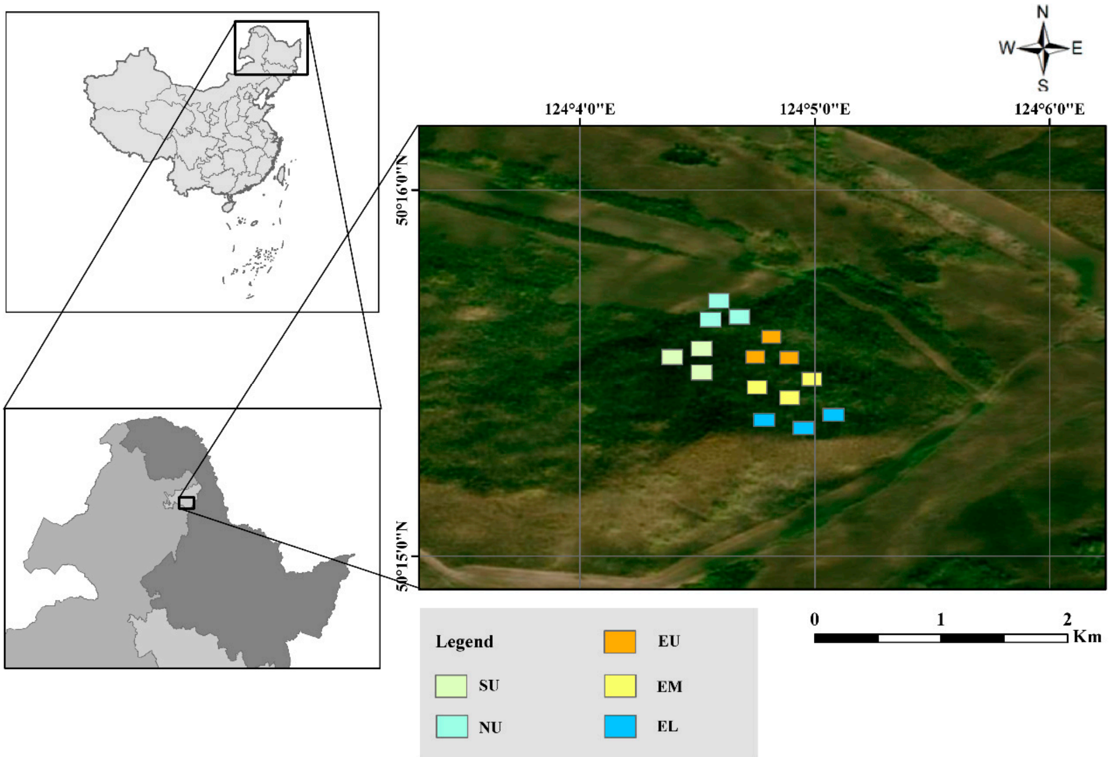

Figure 1. Location and basic information of the study area and sampling sites. The abbreviations of sampling sites are as follows: SU, the upper slope of the south; NU, the upper slope of the north; EU, the upper slope of the east; EM, the middle slope of the east; EL, lower slope of the east.

2.2. Experimental Design

In July 2021, 15 standard plots (20 × 30 m) were set up in *L. olgensis* plantations, including three randomly replicated plots on the upper, middle and lower slopes of the east slope, three randomly replicated plots on the upper slope of the south slope and three plots on the upper slope of the north slope (Figure 1). The detailed information was recorded in Table 1. Soil samples were collected at the 0–20 cm depth with a 10 cm diameter auger after removing the surface litter, roots and gravel. These samples were pooled as a composite sample. The fresh soil samples were stored in a portable refrigerator at −20 °C and brought back to the laboratory and stored in a −20 °C refrigerator. Some of the fresh soil samples were passed through a 2-mm sieve (10 mesh) for the measurement of soil extracellular enzymes, soil ammonium nitrogen (NH_4^+-N) and nitrate nitrogen (NO_3^--N), the others were air-dried and used for the measurement of soil physical and chemical properties.

Table 1. Site characteristics of different topography of *L. olgensis* plantations.

Sample Site	Altitude (m)	Crown Density	Soil Thickness (cm)	Diameter (cm)	Height (m)	Basal Area $(cm)^2$
EL	417	0.50	70	16.80 ± 0.52	11.56 ± 0.16	221.56 ± 0.62
EL	418	0.50	70	15.82 ± 0.92	11.85 ± 0.24	196.46 ± 1.02
EL	420	0.50	75	16.28 ± 0.44	13.25 ± 0.18	208.06 ± 0.54
EM	425	0.60	47	16.62 ± 1.17	14.27 ± 1.10	216.84 ± 1.27
EM	423	0.65	48	15.48 ± 0.37	12.07 ± 0.20	188.11 ± 0.47
EM	422	0.60	49	14.97 ± 0.38	12.54 ± 0.17	175.92 ± 0.48
EU	428	0.60	36	14.90 ± 0.41	12.48 ± 0.17	174.28 ± 0.51
EU	429	0.55	34	13.43 ± 0.54	12.45 ± 1.25	141.59 ± 0.64
EU	428	0.60	33	13.67 ± 0.49	13.18 ± 1.16	146.69 ± 0.59
NU	418	0.60	32	12.70 ± 0.36	11.77 ± 0.19	123.84 ± 0.69
NU	418	0.60	34	14.67 ± 0.37	13.70 ± 0.90	163.68 ± 0.68
NU	418	0.60	32	12.49 ± 0.34	11.96 ± 0.18	121.87 ± 0.59
SU	429	0.70	42	15.14 ± 0.59	12.31 ± 0.24	177.57 ± 0.46
SU	430	0.70	41	15.99 ± 0.58	12.10 ± 0.22	200.71 ± 0.47
SU	430	0.70	43	14.97 ± 0.49	12.27 ± 0.21	175.92 ± 0.44

Note: SU, the upper slope of the south; NU, the upper slope of the north; EU, the upper slope of the east; EM, the middle slope of the east; EL, lower slope of the east.

2.3. Soil Physicochemical Properties and Soil Enzyme Activity

The soil samples were collected in aluminum boxes and weighed, at 105 °C to a constant weight immediately to calculate the soil moisture. Soil bulk density and soil porosity were measured using the ring knife method [36]. Soil total carbon (TC) and soil total nitrogen (TN) were determined using a carbon and nitrogen analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Langenselbold, Germany), and soil total phosphorus (TP) was determined by spectrophotometry after wet digestion with $HClO_4$-H_2SO_4 and extracted with 0.5 M $NaHCO_3$ and measured with a spectrophotometer (Mapada Corporation, Shanghai, China). pH was determined using a pH meter (MT-5000, Shanghai, China). Soil ammonium nitrogen (NH_4^+-N) and soil nitrate nitrogen (NO_3^--N) were determined by a continuous flow analyzer (Auto Analyzer 3, SEAL Analytical GmbH, Norderstedt, Germany). Soil organic matter was determined by the potassium permanganate titration method [37].

Four hydrolytic enzymes were determined according to the method of German et al. [38] (Table 2). The fluorescence values were measured using a multifunctional enzyme standard (Spectrum-Max M5, Molecular Devices, San Jose, CA, USA) with the fluorescence excitation and detection light at 365 and 450 nm, respectively, the enzyme activity was expressed as moles of substrate produced per gram of dry matter per hour (nmol·g^{-1}·h^{-1}). All experiments conducted with these soils maintained three independent replicates (*n* = 3).

Table 2. Types, substrates and functions of soil extracellular enzymes.

Enzyme	EC	Substrate Proxy	Substrate Concentration	Function
L-leucine aminopeptidase (LAP)	EC 3.4.11.1	L–Leucine–7-amino–4–methylcoumarin	200 μmol·L^{-1}	Catalyze the cleavage of amino acids from proteins or other peptide substrates.
β-glucosidase (BG)	EC 3.2.1.21	4–MUB–b–D–glucoside	200 μmol·L^{-1}	Decomposition of readily degradable carbon and release of glucose from cellulose.
Acid phosphatase (AP)	EC 3.1.3.2	4–MUB–phosphate	200 μmol·L^{-1}	Release of phosphate from Hydrolyzed phosphoglycans and phospholipids.
β-1,4-N-acetylglucosaminidase (NAG)	EC 3.2.1.61	4–MUB–N-acetyl–β–D–glucosaminide	200 μmol·L^{-1}	Hydrolyze N-acetylglucosamine (an amino sugar) in chitin.

2.4. Data Analysis

One-way ANOVA was used to test the significance of differences between treatments (LSD, α = 0.05), and Spearman correlation analysis was performed between soil factors and soil extracellular enzyme activity using SPSS 19.0 (IBM Corp., Armonk, NY, USA). Redundancy analysis (RDA) was used to determine the interrelationships between soil enzyme activities and environmental conditions. The forward selection procedure in RDA, based on a Monte Carlo permutation with 499 iterations, was performed to determine the most significant discriminating variables of the specific soil extracellular enzyme activities, and the significant variables (p < 0.05) were used in the final analyses. Significant tests for RDA were carried out using CANOCO v4.56 software (Biometris-Plant Research International Wageningen, The Netherlands). Standardized major axis (SMA) analysis of enzyme stoichiometric ratios was performed using the 'smart' package in R. The slope of the regression represents the relative degree of limitation by C, N or P. A greater deviation from 1 indicates a greater relative degree of restriction. The enzyme vector angle and length were calculated, and the relatively long vector length indicated the degree of limitation by C nutrients, the vector angle >45° indicated the degree of limitation by P nutrients, and the vector angle <45° indicated the degree of limitation by N nutrients. Graphs were created by using Origin 2018 software (OriginLab, Northampton, MA, USA). All results were reported as means $\pm$ standard errors (SE).

Vector analysis (vector length [L, unit less] and vector angle [A, $\circ$]) of soil EEAs was conducted to evaluate microbial nutrient limitations. Vector length and angle were calculated as follows:

$$\text{Vector length (L)} = \sqrt{[\text{Ln}(BG)/\text{Ln}(NAG + LAP)]^2 + [\text{Ln}(BG)/\text{Ln}(AP)]^2}$$

$$\text{Vector angle (A) = Degress } \{ATAN2[(\text{Ln }(BG)/\text{Ln }(AP), (\text{Ln }(BG)/\text{Ln }(NAG{+}LAP)]\}$$

3. Results

3.1. Soil Physicochemical Properties at Different Slope Aspects and Slope Positions

Slope aspects and slope positions had significant effects on SM, DB, SP, SOM, $NH_4{}^+$-N and $NO_3{}^-$-N, but did not have significant effects on ST, pH, TC, TN and TP (Table 3). Compared to other sites, SP increased by 1.95%, 47.51%, 16.41, 13.38 on the EM, SOM increased by 38.49%, 15.11%, 9.35%, 40.48% on the NU, SM increased by 53.94%, 38.04%, 97.58%, 22.12% on the NU, respectively.

Table 3. Soil physicochemical properties in *L. olgensis* plantations at different slope aspects and slope positions.

Site	SM %	BD g·cm^3	SP %	ST °C	pH	TC g·kg^{-1}	TN g·kg^{-1}	TP g·kg^{-1}	SOM g·kg^{-1}	$NH_4{}^+$-N mg·kg^{-1}	$NO_3{}^-$-N mg·kg^{-1}
EU	55.23 ± 3.84 ab	0.87 ± 0.09 b	170.52 ± 15.33 a	15.43 ± 0.52 a	5.69 ± 0.11 a	86.61 ± 5.59 b	3.84 ± 0.32 a	0.90 ± 0.19 a	50.49 ± 4.46 a	211.80 ± 9.88 a	0.83 ± 0.06 b
EM	61.95 ± 5.33 ab	0.84 ± 0.08 b	173.84 ± 15.87 a	13.80 ± 0.36 ab	5.73 ± 0.19 a	104.20 ± 6.37 a	4.85 ± 0.62 a	0.75 ± 0.04 a	67.81 ± 8.82 a	157.45 ± 1.76 b	1.96 ± 0.29 a
EL	43.03 ± 3.57 b	1.20 ± 0.05 a	117.85 ± 6.75 b	14.87 ± 0.87 ab	5.76 ± 0.10 a	109.69 ± 5.32 a	5.20 ± 0.77 a	0.75 ± 0.18 a	66.93 ± 14.64 a	155.3 ± 21.15 b	1.89 ± 0.22 a
SU	69.62 ± 11.89 ab	1.00 ± 0.05 ab	148.61 ± 9.34 ab	15.13 ± 0.58 ab	5.35 ± 0.14 a	85.38 ± 6.68 b	5.09 ± 0.71 a	0.96 ± 0.06 a	71.76 ± 9.82 a	208.50 ± 15.32 a	0.67 ± 0.05 b
NU	85.02 ± 16.49 a	1.18 ± 0.06 a	153.32 ± 14.7 ab	13.50 ± 0.30 b	5.41 ± 0.14 a	119.95 ± 6.46 a	3.81 ± 0.74 a	0.73 ± 0.16 a	55.67 ± 13.04 a	193.12 ± 11.66 ab	0.78 ± 0.08 b
F	2.63 *	6.05 **	2.99 *	2.28 ns	1.88 ns	0.70 ns	1.08 ns	0.54 ns	6.01 **	4.05 **	15.20 **

Note: SU, the upper slope of the south; NU, the upper slope of the north; EU, the upper slope of the east; EM, the middle slope of the east; EL, lower slope of the east. SM, soil moisture; BD, bulk density; SP, soil porosity; ST, soil temperature; SOM, soil organic matter; TC, soil total carbon; TN, soil total nitrogen; TP, soil total phosphorus. Values presented are means $\pm$ SE. Different lower-case letters indicate significant differences among different treatments ns: not significant; * p < 0.05, ** p < 0.01.

3.2. Characteristics of Soil Extracellular Enzyme Activity Changes at Different Slope Aspects and Slope Positions

Slope aspects and slope positions had significant effects on BG, NAG, LAP and AP activities (Figure 2), while the highest BG activities were observed in the EM and insignificant differences in the activities of the other four sites; the highest NAG activities were observed in the EM, the least active was observed in the EB, and the difference in activity among the other three sites was not significant; the highest LAP activities were observed in the SU, the least active was observed in the NU, and the difference in activity among the other three sites was not significant; the highest AP was observed in the NU, and the least active was observed in the EU, and the difference in activity among the other three sites was not significant.

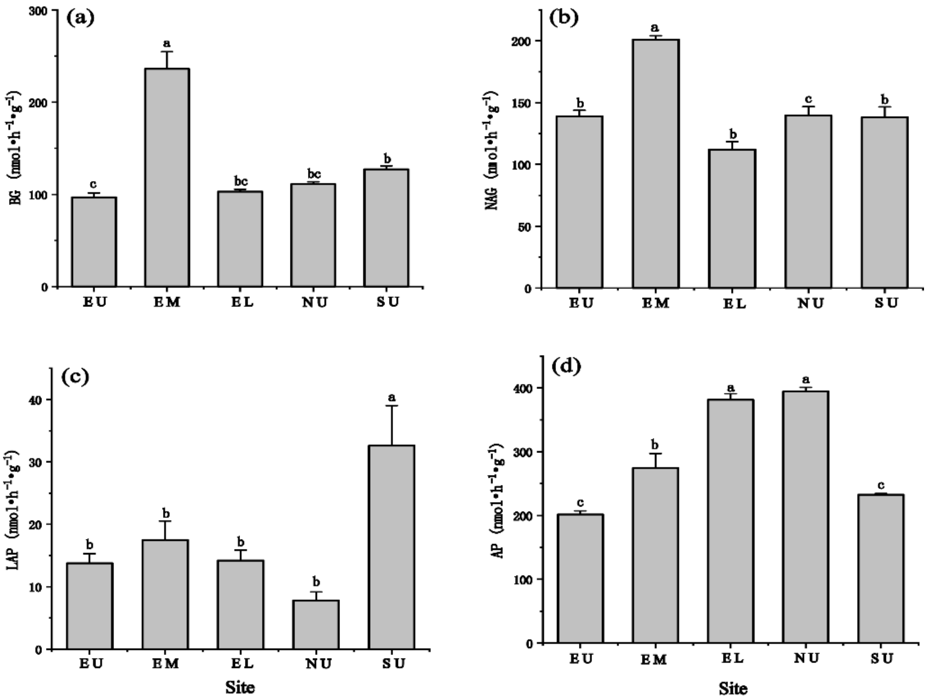

Figure 2. Soil extracellular enzyme activity under different site conditions. SU, the upper slope of the south; NU, the upper slope of the north; EU, the upper slope of the east; EM, the middle slope of the east; EL, lower slope of the east. BG, β–glucosidase (**a**); NAG, β–1,4–N–acetylglucosaminidase (**b**). LAP, leucine aminopeptidase (**c**); AP, acid phosphatase (**d**); Significant differences are marked by lowercase letters ($p < 0.05$).

3.3. Soil Extracellular Enzyme Stoichiometry Ratios at Different Slope Aspects and Slope Positions

Slope aspects and slope positions had significant effects on ln (BG): ln (NAG + LAP), ln (BG): ln (AP), and ln (NAG + LAP): ln (AP) (Table 4). The range of variation of $C:N_{eea}$, $C:P_{eea}$, and $N:P_{eea}$ was 0.78–1.01, 0.78–0.89, and 0.81–0.97 (Figure 3). Overall, $C:N_{eea}$, $C:P_{eea}$, and $N:P_{eea}$ all had the highest ratios in the EM. The standardized major axis analysis (SMA) indicated that the soil C:N and N:P enzyme activity ratios showed a significant linear relationship and the C:P enzyme activity ratio showed a non-significant linear relationship; the slopes of the soil C:N, N:P and C:P enzyme activity ratios were 0.87, 1.13 and 1.29 (Figure 4), and the enzyme vector lengths of the different stand conditions were the highest in the EM and vector angles were highest in the EL, that was 1.41 and 50.91°, respectively (Table 4).

Table 4. Length and angle of extracellular enzyme vectors in different subsurface soils.

Site	VL	VA
EU	1.23 ± 0.02 c	45.25 ± 1.24 b
EM	1.41 ± 0.03 a	45.97 ± 0.67 b
EL	1.24 ± 0.01 c	50.91 ± 0.36 a
SU	1.23 ± 0.01 c	50.18 ± 0.35 a
NU	1.30 ± 0.01 b	46.76 ± 0.36 b

Note: SU, the upper slope of the south; NU, the upper slope of the north; EU, the upper slope of the east; EM, the middle slope of the east; EL, lower slope of the east. VL, Soil extracellular enzyme vector length; VA, Soil extracellular enzyme vector angle. Lower-case letters in different columns indicate significant differences ($p < 0.05$).

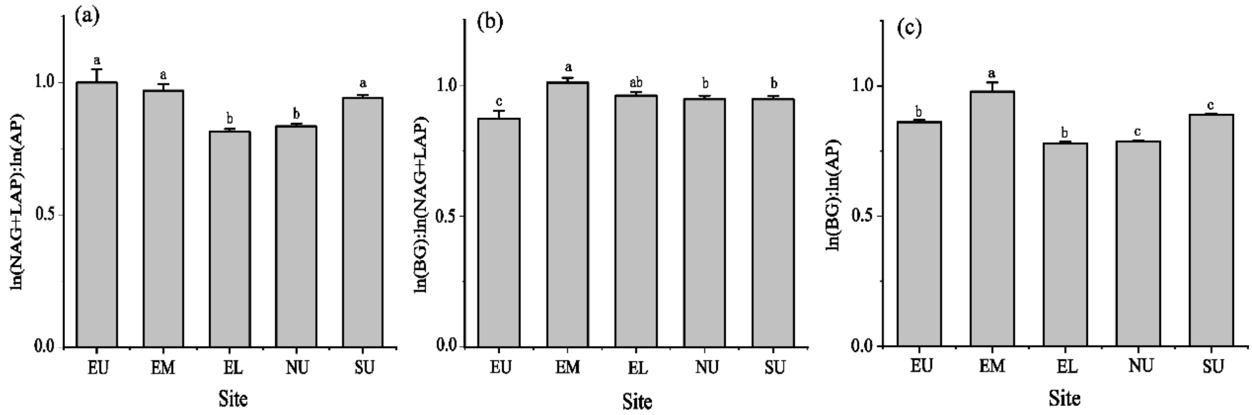

Figure 3. Soil enzyme stoichiometric ratios under different site conditions. C/N_{eea} (**a**), C/P_{eea} (**b**), N/P_{eea} (**c**). Letters above error bars indicate significant differences at p < 0.05.

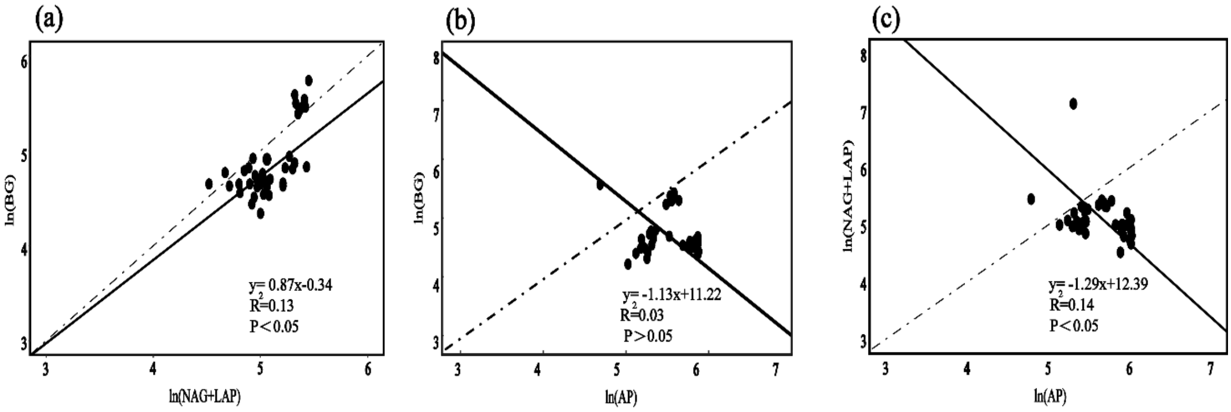

Figure 4. Standardized major axis regressions of the log-transformed soil C–, N–, and P– acquiring enzyme activities in different sites. C–acquiring enzyme activities (**a**), N–acquiring enzyme activities (**b**), and P–acquiring enzyme activities (**c**).

3.4. Relationship between Soil Extracellular Enzyme Activity and Stoichiometric Ratio and Soil Factors

Soil extracellular enzyme activity was used as the response variable and soil factors as explanatory variables for redundancy analysis and the Monte Carlo test. The first two components of the RDA axes explained 79.7% of the variance in the relationship between soil enzyme activity and environmental variables (Figure 5). More than 79% of the spatial variations in soil enzyme activities were explained by the combination of the ten factors. Forward selection of the ten factors in the RDA ordinations showed that three factors were significantly related to spatial variations in soil enzyme activity, including SP, pH, and SOM. However, the effect of other environmental factors on soil enzyme activities was completely negligible.

Spearman correlation analysis (Table 5) showed that there was a significant positive correlation between TC, TN, and SOM with AP; a significant positive correlation between SM and BG, and a significant negative correlation between pH and BG. There were significant positive correlations of stoichiometric ratio C:N_{eea} with SM and NH_4^+-N, and significant negative correlations with NO_3^--N and pH; there were significant positive correlations of C:P_{eea} with SM and significant negative correlations with pH.

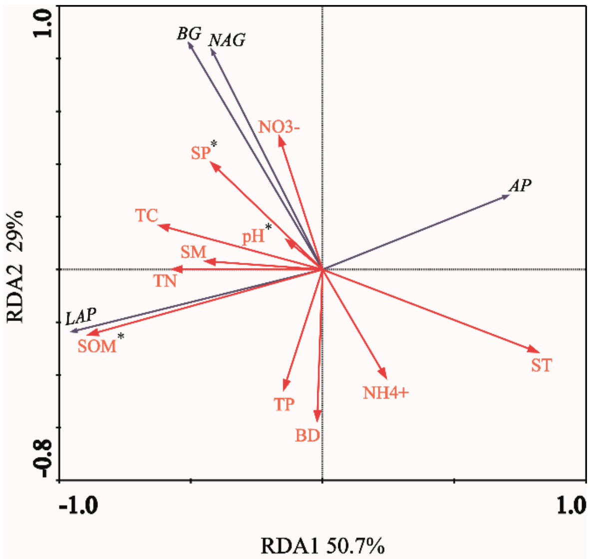

Figure 5. Redundancy analysis (RDA) of soil extracellular enzyme activities and soil factors. SM, soil moisture; BD, soil bulk density; SP, soil porosity; ST, Soil temperature; SOM, soil organic matter; TC, soil total carbon; TN, soil total nitrogen; TP, soil total phosphorus. BG, β–glucosidase; LAP, leucine aminopeptidase; AP, acid phosphatase; NAG, β–1,4–N–acetylglucosaminidase. * $p < 0.05$.

Table 5. Spearman correlation coefficients between soil extracellular enzyme activities and soil factors.

Soil Factor	BG	AP	NAG	LAP	$C:N_{eea}$	$C:P_{eea}$	$N:P_{eea}$
Total nitrogen	0.04	0.54 *	0.02	−0.04	0.05	−0.22	−0.22
Total carbon	0.08	0.513 *	0.06	−0.08	0.03	−0.15	−0.13
Total phosphorus	0.19	0.35	−0.13	0.07	0.23	0.06	−0.20
$NH_4{}^+$-N	0.44	0.49	−0.28	0.08	0.52 *	0.21	−0.37
$NO_3{}^-$-N	−0.44	−0.31	0.50	0.01	−0.60 *	−0.30	0.43
Soil organic matter	0.18	0.63 **	−0.08	−0.06	0.22	−0.12	−0.32
Soil temperature	−0.23	−0.21	0.01	−0.22	−0.18	−0.22	−0.01
Soil moisture	0.55*	0.09	−0.18	0.19	0.51 *	0.53 *	−0.06
Soil bulk density	0.20	0.41	0.41	−0.05	−0.12	−0.01	0.21
Soil porosity	0.01	−0.2	−0.38	−0.17	0.27	0.09	−0.27
pH	−0.69 **	−0.3	0.14	0.07	−0.62**	−0.57 *	0.12

Note: BG, β–glucosidase; LAP, leucine aminopeptidase; AP, acid phosphatase; NAG, β–1,4–N–acetylglucosaminidase. $C:N_{eea}$, ln (BG): ln (NAG + LAP); $C:P_{eea}$, ln (BG): ln (AP); $N:P_{eea}$, ln (NAG + LAP): ln (AP). * $p < 0.05$; ** $p < 0.01$.

4. Discussion

4.1. Response of Soil Extracellular Enzymes to Slope Aspects and Slope Positions in L. olgensis Plantation Forests

It had been noted that soil extracellular enzyme activity had a collaborative effect with soil nutrients [39]. In this study, we found the highest BG activity in EM, the highest LAP activity in NU, the highest NAG activity in EM, and the highest AP activity in SU (Figure 2). This study also discovered (Figure 5) that SOM, pH, and SP were the main factors affecting soil extracellular enzyme activity which was consistent with our first hypothesis. Topography is the main factor affecting soil formation and plays a key role in organic carbon and nutrient storage, cycling, and retention [8]. Slope position and slope aspect adjust the hydrological processes and the level of solar radiation reaching the ground, influencing microclimate on a micro-scale [4]. Different climate conditions influence soil enzyme activities by affecting the decomposition of litter, root exudate content and microbial diversity and number [15]. Salehi et al. [40] showed that the slope aspect had a significant effect on soil bulk, soil porosity, soil organic carbon and total nitrogen storage. Liu et al. [41] demonstrated higher organic carbon and total nitrogen content in soils on southern slopes.

Microclimates (solar radiation, soil temperature and moisture) influence the developmental processes of the soil [11]. Generally, the topography is higher at the top and southern slopes due to the transport of soil moisture and sediments along the slopes [42]. Soil microorganisms can rapidly adapt to environmental changes [43] and are drivers of soil nutrient cycling [34]. Soil microorganisms promote the conversion and fixation of C in the soil through both microbial in vivo and in vitro pathways using catabolism and anabolism, thereby promoting SOC accumulation where the in vitro pathway is mainly through extracellular enzyme action and the in vivo pathway is mainly through microbial cellular transport [44].

At the same time, our results showed that C and N had a significant positive correlation with AP activity, which may be a microbial strategy to stimulate AP enzyme activity by increasing C and N and thus avoiding P limitation. Soil pH exhibited significant negative correlations on BG activity, $C:N_{eea}$ and $C:P_{eea}$. With increasing pH, BG activity, $C:N_{eea}$ and $C:P_{eea}$ decreased, which is consistent with the findings of Deforest J L et al. [45] on the effect of pH on soil C, N and P enzymes. Soil enzyme activity significantly decreased with increasing pH, due to the acidic soils in the study region, which may increase or fix C content [46]. Some studies found that soil temperature had a significant effect on soil enzyme activity [47], but this study found no significant effect of soil temperature on soil extracellular enzyme activity, which is due to the small difference in soil temperature under each stand in the study region and the diurnal temperature fluctuations at high altitudes, resulting in the possibility of soil extracellular enzymes adapting to a wide range of temperatures [48]. SM produced a significant positive correlation in BG activity, $C:N_{eea}$ and $C:P_{eea}$, with a significant increase in BG activity, $C:N_{eea}$ and $C:P_{eea}$ with increasing SM, probably because SM is related to microbial and plant root secretions, which increase the range of root activity with increasing water in a certain soil moisture range [49], promoting microbial activity, exudation of root exudates, and thus stimulating BG activity [24]. Therefore, soil factors have a very important role in soil enzyme activity and more attention should be paid to micro-site conditions when conducting research.

4.2. Soil Enzyme Stoichiometric Ratios Reveal Nutrient Limitation of Microorganisms in L. olgensis Plantation Forests

Soil extracellular enzyme stoichiometric ratios are controlled by the effectiveness of soil C, N, and P nutrient resources [50] and reflect the status of microbial nutrient requirements [16]. Our study discovered that the study region is limited by N, P which is consistent with the second hypothesis. The results show that slope positions and slope aspects had a significant effect on the stoichiometric ratio that was the largest in the middle slope of the eastern slope (Figure 3). In addition, the stoichiometric ratio was 1.00:1.06:1.17, which deviated from the global stoichiometric ratio of soil C, N, and P enzymes that follows 1:1:1 [19], indicating that the study region was limited by N and P. The enzyme vector length and angle [33] further illustrated the limitation of microbial nutrients, with the enzyme vector angle >45° under each stand (Table 4). The reasons for this situation may be the following: (1) *Larix olgensis* is more inclined to P uptake [51] and slower decomposition and release when the nutrients, mainly in the form of litter, are returned to the soil. (2) Because the microorganisms have a higher demand for P, reflecting the deficiency of elemental P in the study region. This is in agreement with the findings of Cui et al. [15] who concluded that phosphorus is a limiting factor for ecosystem productivity. According to the principle of "nutrient balance" in microorganisms [52], they will release more N- and P-acquiring enzymes to meet their nutrient requirements. Therefore, more attention should be paid to the input of N and P elements in the management of *L. olgensis.*

5. Conclusions

In this study, we aimed to reveal the changes in soil extracellular enzyme activities and the nutrient-limiting elements under different slope aspects and slope positions in *Larix olgensis* plantations. Our results showed that the highest activity of BG (or NAG), LAP, AP was observed in the EM, SU, NU. The main features affecting the EEA are SP, pH and SOM. In addition, microbiological growth is limited by N and P. Thus, N and P inputs should be considered in the long-term management of *L. olgensis* plantations. The results of this study provided important insights into the sustainable management of *L. olgensis* plantations. The effect of slope aspects and slope positions on soil extracellular enzyme activity and stoichiometric ratios was focused on in the study; we still need to understand the effects of litter substrate addition, nitrogen deposition and warming on soil extracellular enzyme activity. In addition, the mechanism of soil enzymology in sustainable forest management needs to be further elucidated.

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