agronomy

Article

Stoichiometry of Soil, Microorganisms, and Extracellular Enzymes of *Zanthoxylum planispinum* var. *dintanensis* Plantations for Different Allocations

Yitong Li, **Yanghua Yu** * and **Yanping Song**

School of Karst Science, State Engineering Technology Institute for Karst Decertification Control,
Guizhou Normal University, Guiyang 550001, China; liyitong0711@163.com (Y.L.); syp_2022@163.com (Y.S.)
* Correspondence: yuyanghua2003@163.com; Tel.: +86-138-8505-4791

Abstract: Plantations with different allocation patterns significantly affect soil elements, microorganisms, extracellular enzymes, and their stoichiometric characteristics. Rather than studying them as a continuum, this study used four common allocations of plantations: *Zanthoxylum planispinum* var. *dintanensis* (hereafter *Z. planispinum*) + *Prunus salicina*, *Z. planispinum* + *Sophora tonkinensis*, *Z. planispinum* + *Arachis hypogaea*, and *Z. planispinum* + *Lonicera japonica* plantations, as well as a single-stand *Z. planispinum* plantation as a control. Soil samples from depths of 0–10 and 10–20 cm at the five plantations were used to analyze the element stoichiometry, microorganisms and extracellular enzymes. (1) One-way analysis of variance (ANOVA) showed that the contents of soil organic carbon (C), nitrogen (N), phosphorus (P), and potassium (K) of *Z. planispinum* + *L. japonica* plantation were high, while those of calcium (Ca) and magnesium (Mg) were low compared to the *Z. planispinum* pure plantation; soil microbial and enzyme activities were also relatively high. Stoichiometric analysis showed that soil quality was good and nutrient contents were high compared to the other plantations, indicating that this was the optimal plantation. (2) Two-way ANOVA showed that stoichiometry was more influenced by plantation type than soil depth and their interaction, suggesting that plantation type significantly affected the ecosystem nutrient cycle; soil microbial biomass (MB) C:MBN:MBP was not sensitive to changes in planting, indicating that MBC:MBN:MBP was more stable than soil C:N:P, which can be used to diagnose ecosystem nutrient constraints. (3) Pearson's correlation and standardized major axis analyses showed that there was no significant correlation between soil C:N:P and MBC:MBN:MBP ratios in this study; moreover, MBN:MBP had significant and extremely significant correlations with MBC:MBN and MBC:MBP. Fitting the internal stability model equation of soil nutrient elements and soil MBC, MBN, and MBP failed ($p > 0.05$), and the MBC, MBN, and MBP and their stoichiometric ratios showed an absolute steady state. This showed that, in karst areas with relative nutrient deficiency, soil microorganisms resisted environmental stress and showed a more stable stoichiometric ratio. Overall stoichiometric characteristics indicated that the *Z. planispinum* + *L. japonica* plantation performed best.

Keywords: *Zanthoxylum planispinum* var. *dintanensis*; allocations; plantations; stoichiometry; soil microorganisms; soil extracellular enzymes; internal stability

Citation: Li, Y.; Yu, Y.; Song, Y. Stoichiometry of Soil, Microorganisms, and Extracellular Enzymes of *Zanthoxylum planispinum* var. *dintanensis* Plantations for Different Allocations. *Agronomy* **2022**, *12*, 1709. https://doi.org/10.3390/agronomy12071709

Academic Editor: Yanyou Wu

Received: 18 May 2022
Accepted: 16 July 2022
Published: 19 July 2022

1. Introduction

Soil, as a substrate for plant growth, and a source and sink of water–fertilizer, serves as a medium of continuous material and energy exchanges. The relationship between soil and plant growth can be characterized by the elements, microorganisms, extracellular enzymes of soil, and their interactions [1]. Among them, soil carbon (C), nitrogen (N), phosphorus (P), and other elements are important components of soil fertility, which directly affect plant growth, soil microbial dynamics, litter decomposition, and the accumulation and circulation of soil nutrients [2]. Soil microorganisms mineralize organic matter mainly by secreting extracellular enzymes, which helps absorption of soil nutrients, so as to balance element acquisition and investment [3,4]. Ecological stoichiometry is a discipline that studies ecology from multiple perspectives [5], including the balance of energy and various chemical elements in ecosystems [6], in order to analyze and predict ecosystem structure and dynamics. Soil nutrient stoichiometry can reveal the coupling relationship and effectiveness of soil nutrients. Soil microbial biomass stoichiometry can be used as an important C, N, and P ecosystem flux to reflect soil microbial mineralization or fixation of soil elements [7]. Soil extracellular enzyme stoichiometry is an important indicator to reveal microbial nutrient status and relative resource constraints [8]. Therefore, the study of soil ecological stoichiometry helps to clarify the mechanism of soil nutrient availability, circulation, and balance, and explain soil microbial metabolic activities [9,10]. Studying the continuum of soil, microorganisms, and extracellular enzymes can help to integrate and analyze the circulation rule of soil nutrients in forest ecosystems and explore the balance among different soil components.

Researchers have carried out many studies on soil ecological stoichiometry. Some scholars [11,12] analyzed the soil C:N:P ratio in the Loess Plateau in China and other places, and found that C, N, and P showed significant correlation in varying degrees, which indicates that element stoichiometry is sensitive to variation in contents. Previous studies found that soil element stoichiometry has a close relationship with microbial biomass stoichiometry, and soil microorganisms have strong steady-state behavior, indicating that soil microorganisms adaptively respond to nutrient levels [13–15]. Other scholars [16,17] proposed that the theory of extracellular enzyme stoichiometry can clarify the balance between microbial biomass stoichiometry and soil element composition. Wu et al. [18] clarified that there is a strong covariant relationship between soil, microorganisms, and extracellular enzyme stoichiometry. In conclusion, forest soil stoichiometry emphasizes the correlation between soil elements, microorganisms, and extracellular enzymes. Rather than studying them as a continuum [18,19], most studies focus on elements C, N, and P, and pay less attention to potassium (K), calcium (Ca), and magnesium (Mg). In addition, different plantation types produce different litter and root exudates, resulting in different nutrient transport to the soil, further affecting soil element stoichiometry, microbial dynamics, and nutrient cycles [20,21]. Yet, there have been few studies on the soil stoichiometry of plantations.

Zanthoxylum planispinum var. *dintanensis* (hereafter *Z. planispinum*) has the characteristics of Ca preference and tolerance to drought and rocky soils. It is the most suitable tree species for ecological restoration in many typical rocky desertification areas. It has a balanced variety and rich content of amino acids, proteins, vitamins, mineral elements, and other metabolic substances with outstanding quality advantages. Due to its unique fragrance and numb taste [22], it has become a major seasoning. However, in recent years, some problems, including soil quality degradation, fruit yield, quality reduction, and stand instability, have arisen, probably due to the single-stand structure of plantations. Therefore, clarifying the soil stoichiometry of *Z. planispinum* plantations in different allocations is critical for the improvement of stand stability and delay of any decline. In view of this, this study took four common allocations of plantations in a rocky desertification area of central Guizhou Province, China: *Z. planispinum* + *Prunus salicina*, *Z. planispinum* + *Sophora tonkinensis*, *Z. planispinum* + *Arachis hypogaea*, and *Z. planispinum* + *Lonicera japonica* plantations, as well as a single-stand plantation as a control to study the stoichiometry of soil, microorganisms, and extracellular enzymes. The following issues were addressed: (1) the changing rules of soil ecological stoichiometry characteristics with plantation type; (2) the sensitivity comparison of microbial ecological stoichiometry and soil element stoichiometry indicating nutrient limitation, and (3) the internal relationships among the stoichiometry of elements, microorganisms, and enzymes. The purpose is to understand the interaction rule between soil nutrients and microorganisms in *Z. planispinum* plantations, and to provide a theoretical foundation for optimal stand plantations and soil nutrient regulation.

2. Materials and Methods

2.1. Overview of the Research Site

The study area is located in Beipanjiang Town, Zhenfeng County, Guizhou, and is part of the Beipan River Basin. The habitat is uniquely characterized as (1) a subtropical humid monsoon climate with a dry and hot climate, and an average annual rainfall of about 1100 mm. It is rich in heat resources, with an annual total accumulated temperature of 6542.9 °C, annual average temperature of 18.4 °C, and annual extreme maximum and minimum temperatures of 32.4 and 6.6 °C, respectively; (2) the valley terrain is in the altitude range of 530–1473 m asl and has typical climate characteristics of the region; (3) the habitat has experienced rocky desertification, with low forest coverage. Exposed bedrock is high, with carbonate rocks accounting for 78.45%, and soil is mainly lime soil [23]; and (4) cultivated vegetation includes *Z. planispinum*, *Juglans regia,* and *Tectona grandis*; agricultural crops are mainly *Hylocereus polyrhizus* and maize (*Zea mays*), and shrubs are mainly *L. japonica*.

2.2. Sample Plot Setting and Sample Collection

One sample plot was set up, with basically the same environmental conditions, for each of the five selected stand types to measure geographical factors such as longitude, latitude, altitude, and slope, as well as the community structure indexes of *Z. planispinum*, such as tree age, density, plant height, crown width, and coverage (Table 1). The *Z. planispinum* were planted in five sample plots in 2012. From the end of 2015 to 2016, *Prunus salicina*, *Sophora tonkinensis*, *Arachis hypogaea*, and *Lonicera japonica* were allocated around the *Z. planispinum*, and the allocation time was determined according to plant growth habits. The allocation density was 600–750, 1500–1800, 2500, and 450–600 plants ha^{-1}, respectively. In implementing the policies of returning farmland to forest and reducing maize planting, the ground was already cultivated, which ensured that the soil fertility level of all sample plots was similar before planting *Z. planispinum*. The same fertilization measures were adopted in all sample plots. The specific fertilization measures were to use compound fertilizers based on N, P, and K ($N:P_2O_5:K_2O = 15:15:15$, total nutrients $\geq 45\%$), fertilizing once in the first 10 days of September and once in the first half of March, both of which used about 0.2 kg of fertilizer per plant, spreading the fertilizer in a ring 20–30 cm away from the main stem of *Z. planispinum* (because *Z. planispinum* fine roots are densely distributed in this area), and covering with 1–2 cm of soil after fertilization to avoid fertilizer loss. After planting *Z. planispinum* for 3 years, fruits were harvested once a year during July–August, producing about 3–4 kg per plant, without other special treatment measures. After allocating *P. salicina* for 3 years, fruits were harvested once a year (a total of twice by the sampling time), producing about 5–6 kg per plant each time. The whole plants of *S. tonkinensis* were harvested once in the third year of allocation. After *A. hypogaea* allocation, the whole plants were harvested once a year, and were harvested a total of four times by the sampling time. *Lonicera japonica* was harvested following the second year of allocation, making a total of three harvested by the sampling time. The soil was mainly sandy and loam, with pH 6.23–8.16; the soil layer was shallow, and the thickness was mainly about 10–18 cm. Soil was mainly yellow and red, plasticity was low, and aggregate structure was poor. The content of gravel in soil was high, and the mass ratio was about 30%.

Table 1. General information of plots.

Plantation Type	Longitude	Latitude	Altitude (m asl)	Slope (°)	Tree Age (a)	Density (m)	Height (m)	Crown Width (m)	Coverage (%)
YD1	105°40′28.33″ E	25°37′57.41″ N	764	10	8	3 × 3	3.5	2 × 2.3	70
YD2	105°40′19.79″ E	25°39′25.75″ N	728	10	8	2 × 2	2.0	1.2 × 1.8	60
TD3	105°38′36.32″ E	25°39′23.64″ N	791	10	8	2 × 2	2.5	2.5 × 2.8	85
YD4	105°38′36.35″ E	25°39′22.29″ N	814	10	8	3.5 × 3	2.5	1.5 × 2.5	70
YD5	105°38′35.64″ E	25°39′23.35″ N	788	10	8	3 × 4	2.2	2.5 × 2.3	65

YD1: *Z. planispinum* + *P. salicina*; YD2: *Z. planispinum* + *S. tonkinensis*; YD3: *Z. planispinum* + *A. hypogaea*; YD4: *Z. planispinum* + *L. japonica*; YD5: *Z. planispinum* pure plantation.

Soil samples were collected in mid-November 2020. The plants were dormant at this time, with weak soil material exchange and low soil microbial activity; thus, soil material composition was more stable. It had been sunny for more than 15 consecutive days before sampling, resulting in relatively low soil variation degree. Due to the high levels of heterogeneous and discontinuous soil in this karst area, three 10 m × 10 m repeated quadrats were set for each sample plot with a sufficient buffer zone between them. Three to five points were selected along an "S" curve in each sample plot. The soil was divided into 0–10 and 10–20 cm sublayers (actual depth was used if it was less than 20 cm). When sampling, we avoided the fertilization area, 10–30 cm around the trunk of *Z. planispinum*, to reduce effects from fertilization, weeding, and other human interference. A total of 30 soil samples were collected from the five sample plots. The fresh samples were divided into two parts after removing the root system, gravel, and animal and plant remains. One part was screened through 2 mm sieves and stored at 4 °C for timely determination of microbial and extracellular enzyme-related indexes; the other part was dried naturally, ground, and screened through a 0.15 mm sieve for soil nutrient analyses.

2.3. Index Analysis Methods

2.3.1. Soil Chemical Properties Analysis

Soil organic C (SOC) was determined by the $K_2Cr_2O_7$-external heating method, total N was determined by the semi-micro Kjeldahl method, total P was determined by $HClO_4$–H_2SO_4 digestion–molybdenum antimony anticolorimetry–ultraviolet spectrophotometry, total K was determined by sodium hydroxide melting-flame photometry, and total Ca and total Mg were determined by atomic absorption spectroscopy [24].

2.3.2. Soil Biological Properties Analysis

Soil fungi, bacteria, and actinomycete concentrations were determined by the plate counting method with beef peptone medium, potato glucose agar medium, and Gao's No. 1 medium [25], respectively. Soil microbial biomass C, N, and P were determined by chloroform fumigation extraction [26]. Soil extracellular enzyme activity (EEA) was measured by fluorescence spectrophotometry [27]. The activity of four hydrolases was determined: β-1,4-glucosidase (BG) for C, β-1,4-n-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP) for N and acid phosphatase (AP) for P. These four enzymes mainly participate in the terminal catalytic reaction, which reflects the metabolic levels of soil C, N, and P. On this basis, the soil extracellular enzyme stoichiometric ratios can be used to characterize the limitations of soil energy and nutrients [28].

2.4. Data Processing and Analysis

Soil microbial biomass C, N, and P were calculated according to Wang [29]. Soil EEAs were calculated according to Bell [27], and their stoichiometry calculated according to Sinsabaugh et al. [30]. Data calculation and sorting were carried out by Microsoft Excel 2013 (version 2013, Microsoft, Redmond, WA, USA). All data were analyzed using SPSS 20.0 (version 20.0, IBM SPSS, New York, NY, USA). One-way analysis of variance (ANOVA) with least significant difference was used, taking the planting or soil depth as an independent variable, to test the differences in soil elements, microorganisms, and extracellular enzyme stoichiometry between different plantations of the same depth or between different depths of the same plantation. Two-way ANOVA was used to clarify the effects of planting, soil depth, and their interactions on stoichiometry of soil elements, microorganisms, and extracellular enzymes. Pearson's correlation was used to verify relationships among soil elements, microbes, and extracellular enzyme C:N:P. The relationship between soil elements and microbial biomass was examined using standardized major axis (SMA) estimation and was evaluated using the software SMATR 2.0 (version 2.0, Daniel Falster, Sydney, Australia). Before data analysis, log conversion was performed to improve the distribution and uniformity of variance. Figures were created in Origin 8.6 (version 8.6, OriginLab

Corporation, Northampton, MA, USA), and data in the map and table are presented in the form of mean ± standard deviation.

3. Results

3.1. Soil Elements and Stoichiometry of Z. planispinum Plantations

The SOC and total N contents in the 0–10 cm soil layer were the highest in plot 4 (59.55 and 5.07 g kg^{-1}, respectively), and significantly higher than for plots 2, 3, and 5. Total P was highest in plot 4 (1.73 g kg^{-1}) and lowest in plot 2 (0.99 g kg^{-1}); there was a significant difference between these two plots, but not the other plots. Total K was 5.58–13.00 g kg^{-1}, and significantly lower in plots 1 and 2 than in plots 3–5. Total Ca and total Mg were highest in plot 5, and significantly higher than for the other four plots. Trends for all elements were also similar for the 10–20 cm soil layer. Overall, *Z. planispinum* + *L. japonica* plantation was conducive to accumulation of SOC, total N, total P, and total K on the surface, and the pure plantation was conducive to accumulation of characteristic elements, such as Ca and Mg, in karst areas (Figure 1).

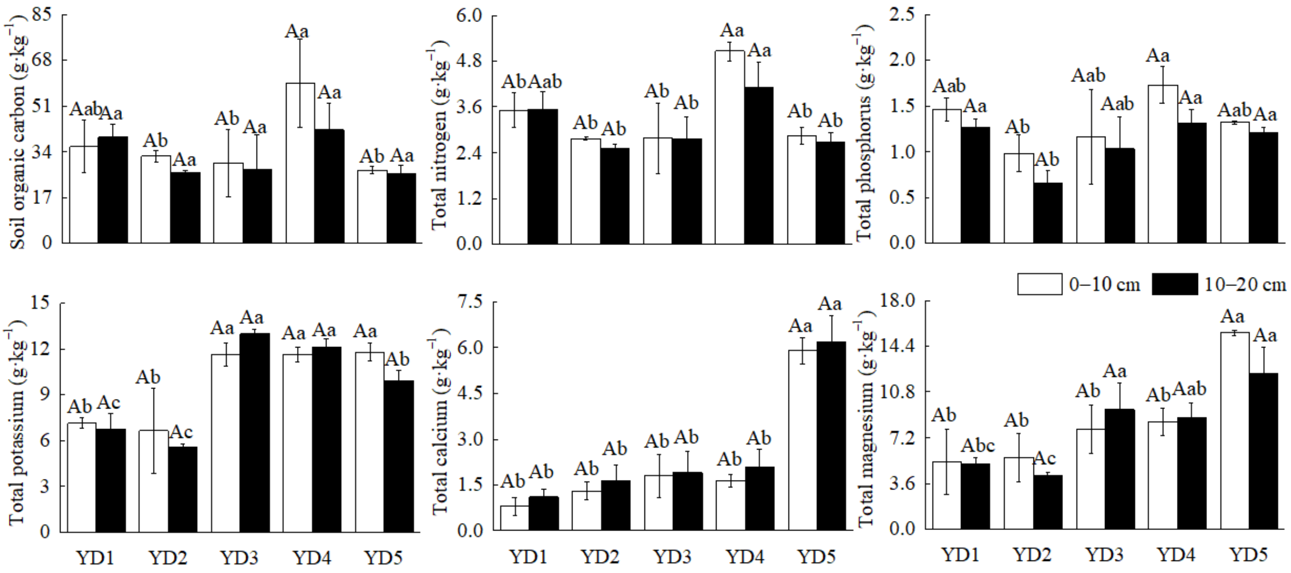

Figure 1. Soil element contents of *Z. planispinum* plantations for different allocations. YD1: *Z. planispinum* + *P. salicina*; YD2: *Z. planispinum* + *S. tonkinensis*; YD3: *Z. planispinum* + *A. hypogaea*; YD4: *Z. planispinum* + *L. japonica*; YD5: *Z. planispinum* pure plantation. The same notation is used in the other figures. Lower case letters, significant differences between different plantation types of the same depth at $p < 0.05$; upper case letters, significant differences between different depths of the same plantation types at $p < 0.05$.

The C:N values were in the range of 9.54–11.7, with no significant differences among the five plots; the values of C:P and N:P were in the order of plot 2 > plot 4 > plot 1 > plot 3 > plot 5, and were significantly greater in plots 2 and 4 than in plot 5, indicating that there was a stable equilibrium relationship between C and N. The values of C:K, N:K, and P:K were in the order of plot 1 > plot 2 > plot 4 > plot 5 > plot 3, indicating that the change trends of C, N, and P contents were basically the same. The C:Ca was highest in plots 1 and 4, and significantly higher than in plot 5; Ca:Mg in plot 5 (0.45) was significantly higher than in other plots. In general, although the variation range of soil element stoichiometry was greater in the 0–10 than the 10–20 cm soil layer, the trends were similar; the effects of plantation type on K, Ca, and Mg were greater than on C, N, and P (Figure 2).

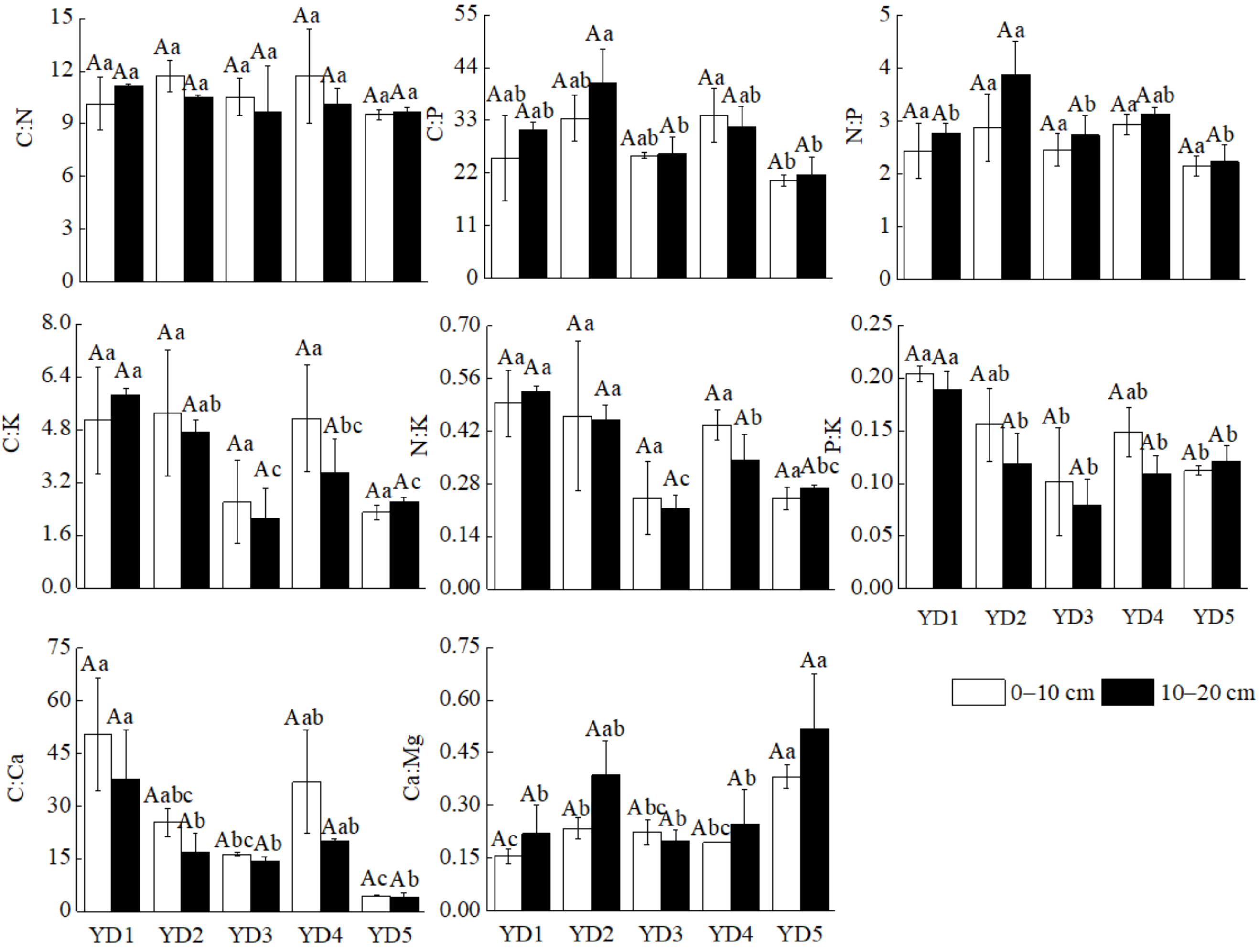

Figure 2. Stoichiometry of soil elements of *Z. planispinum* plantations. C:N, soil C:N ratio; C:P, soil C:P ratio; N:P, soil N:P ratio; C:K, soil C:K ratio; N:K, soil N:K ratio; P:K, soil P:K ratio; C:Ca, soil C:Ca ratio; Ca:Mg, soil Ca:Mg ratio. Lower case letters, significant differences between different plantation types of the same depth at $p < 0.05$; upper case letters, significant differences between different depths of the same plantation types at $p < 0.05$.

3.2. Soil Biological Properties and Stoichiometry of *Z. planispinum* Plantations

There were no significant differences in the fungi and actinomycete concentrations in both soil layers among the five plots. In the 0–10 cm soil layer, the highest and lowest values of bacterial concentration were in plots 2 and 5, respectively, with no significant differences among other plots. In the 10–20 cm soil layer, the bacteria concentration was highest in plots 1 and 4 and lowest in plot 5. The concentration of soil microorganisms was lowest in plot 5, indicating a stressful soil environment. The effect of plantation type on fungi and actinomycetes was less than on bacteria, and there was no clear effect of soil depth on concentration of soil microorganisms (Figure 3).

In the 0–10 cm soil layer, microbial biomass P (MBP) in plot 4 was significantly higher than in plots 1 and 5, and there was no significant difference in microbial biomass C (MBC) and microbial biomass N (MBN) among the five plots. In the 10–20 cm soil layer, MBC was significantly higher in plot 4 than in plot 1, and there were no significant differences in MBN and MBP among the five plots. The results showed that MBN was more stable than MBC and MBP, and that plantation type had little effect on MBC and MBP. There were no significant differences in MBC:MBN and MBN:MBP between the two soil layers; in the 0–10 cm layer, MBC:MBP in plot 1 (1.95) was significantly higher than in plots 2–4 (1.77, 1.71, and 1.72, respectively), and there were no significant differences in the 10–20 cm layer (Figure 4).

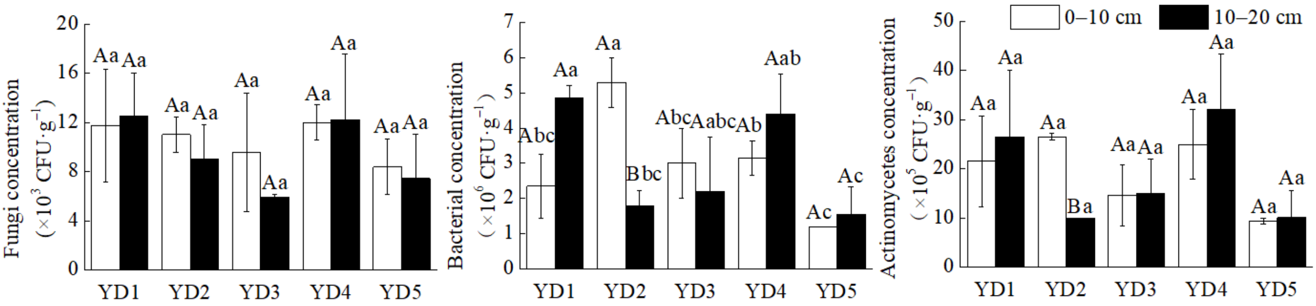

Figure 3. Concentration of soil microorganisms of *Z. planispinum* plantations. Lower case letters, significant differences between different plantation types of the same depth at $p < 0.05$; upper case letters, significant differences between different depths of the same plantation types at $p < 0.05$.

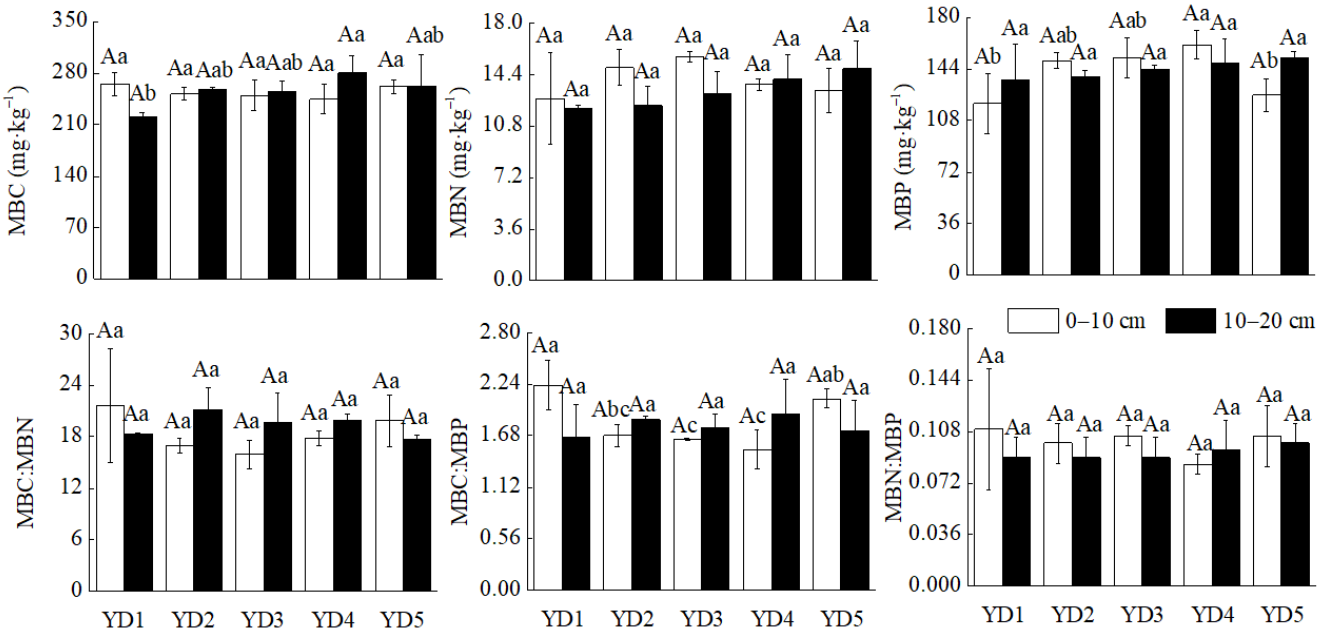

Figure 4. Soil microbial biomass and stoichiometry of *Z. planispinum* plantations. MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen; MBP, soil microbial biomass phosphorus; MBC:MBN, soil microbial biomass carbon to microbial biomass nitrogen ratio; MBC:MBP, soil microbial biomass carbon to microbial biomass phosphorus ratio; MBN:MBP, soil microbial biomass nitrogen to microbial biomass phosphorus ratio. Lower case letters, significant differences between different plantation types of the same depth at $p < 0.05$; upper case letters, significant differences between different depths of the same plantation types at $p < 0.05$.

In the 0–10 cm soil layer, the four EEAs showed no significant differences among the five plots; in the 10–20 cm soil layer, all EEAs were highest in plot 4. Except for plot 1, the EEA decreased significantly with increasing soil depth, with no significant differences among the other plots. The differences in (NAG + LAP):AP were reflected in the 10–20 cm soil layer, being significantly higher for plot 4 than for plots 1–3; there were no significant differences for BG:(NAG + LAP) and BG:AP in the same soil layer in different plots, as well as in different soil layers in the same plot, indicating that soil extracellular enzymes followed a strict proportional relationship (Figure 5).

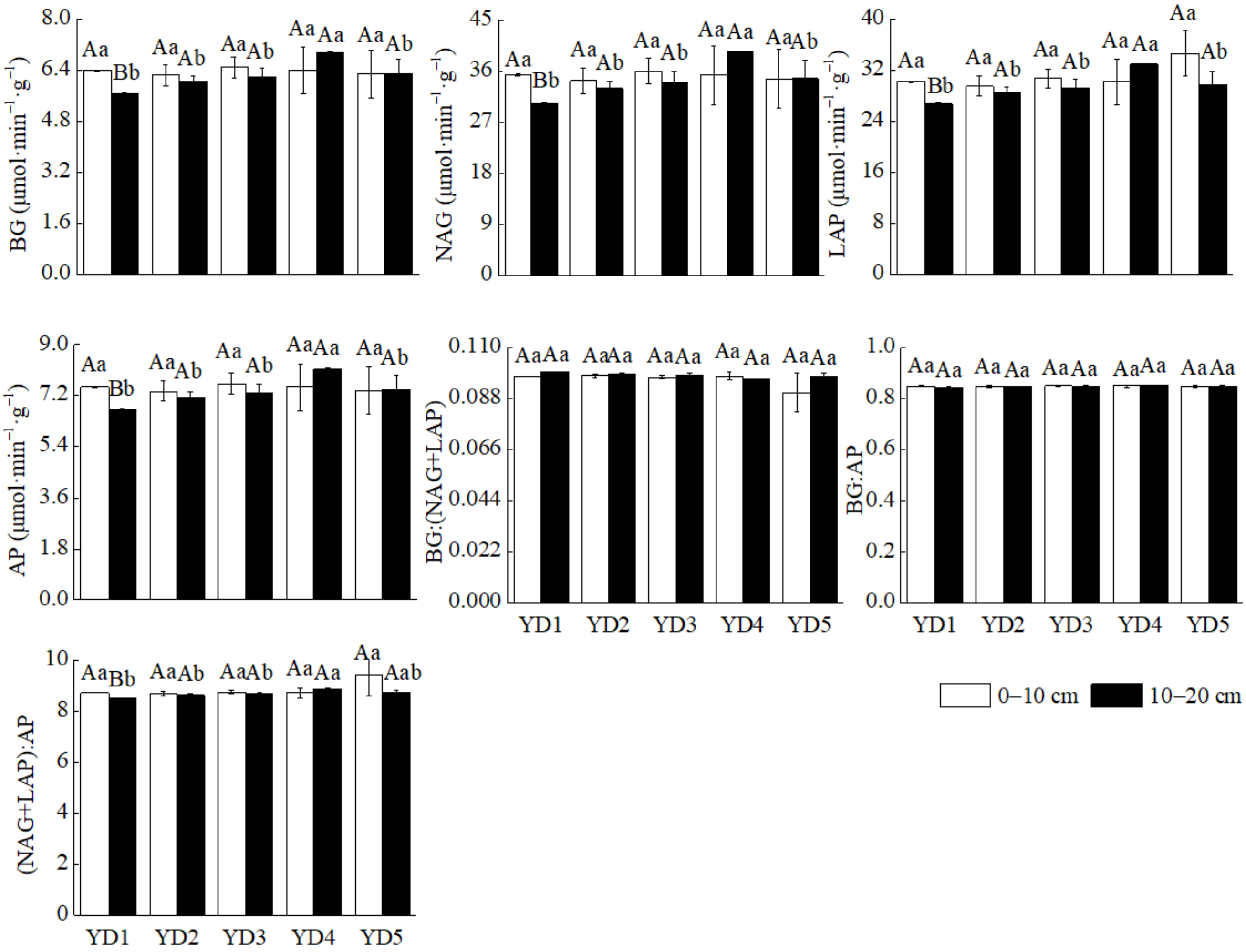

Figure 5. Soil extracellular enzymes activities (EEAs) and their stoichiometry of *Z. planispinum* plantations. BG, β-1,4-glucosidase; NAG, β-1,4-n-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase; BG:(NAG + LAP), ratio of β-1,4-glucosidase to the sum of β-1,4-n-acetylglucosaminidase and leucine aminopeptidase; BG:AP, β-1,4-glucosidase to acid phosphatase ratio; (NAG + LAP):AP, ratio of the sum of β-1,4-n-acetylglucosaminidase and leucine aminopeptidase to acid phosphatase. Lower case letters, significant differences between different plantation types of the same depth at $p < 0.05$; upper case letters, significant differences between different depths of the same plantation types at $p < 0.05$.

3.3. *Effects of Plantation Types and Soil Depth on Soil Properties*

Plantation type had significant effects on soil elements and stoichiometry to varying degrees except for C:N. Plantation type had no significant effects on soil biological properties except for bacterial concentration. Soil depth had no significant effect on soil elements and stoichiometry except for total P and Ca:Mg. The interaction between plantation type and soil depth only had significant effects on bacterial concentration. These comprehensive results showed that stoichiometry was more influenced by plantation type than soil depth and their interaction. In terms of stoichiometry, soil microorganisms and extracellular enzymes were more stable than soil elements (Table 2).

3.4. *Correlation of Soil Element, Microorganism, and Extracellular Enzyme C:N:P of Z. planispinum Plantations*

Soil C had high and significant positive correlations with N and P. The absolute value of the negative correlation coefficient between MBN:MBP and MBC:MBN was less than the positive correlation coefficient between MBN:MBP and MBC:MBP, indicating that MBC was more sensitive to the change of MBN than MBP. The correlations among BG:(NAG + LAP), BG:AP, and (NAG + LAP):AP were all significant ($p < 0.01$), indicating a close relationship among EEAs (Figure 6).

Table 2. Two-way ANOVA for the effects of plantation type and soil depth on soil, microorganisms, and extracellular enzymes and stoichiometry (value in the table are F ratios).

Factors	**SOC**	**TN**	**TP**	**TK**	**TCa**	**TMg**			
A	4.831 *	12.521 ***	5.389 *	30.478 ***	61.275 ***	20.602 ***			
B	1.326	1.678	5.200 *	0.413	1.636	0.669			
A × B	0.750	0.696	0.322	1.423	0.059	1.312			
	C:N	**C:P**	**N:P**	**C:K**	**N:K**	**P:K**	**C:Ca**	**Ca:Mg**	
A	0.804	6.671 **	5.434 *	6.616 **	9.099 **	9.224 **	6.411 **	8.538 **	
B	0.641	1.336	4.734	0.409	0.157	3.254	2.418	5.177 *	
A × B	0.576	0.697	0.848	0.676	0.383	0.595	0.357	0.950	
	fungi	**bacteria**	**actinomycetes**	**MBC**	**MBN**	**MBP**	**MBC:MBN**	**MBC:MBP**	**MBN:MBP**
A	1.570	5.432 *	3.474	0.682	0.925	2.120	0.312	0.890	0.198
B	0.551	0.022	0.033	0.003	1.235	0.106	0.510	0.215	0.798
A × B	0.269	7.235 **	1.369	2.087	1.332	1.676	1.529	2.952	0.319
	BG	**NAG**	**LAP**	**AP**	**BG: (NAG + LAP)**	**BG:AP**	**(NAG + LAP):AP**		
A	1.473	1.472	2.805	1.472	1.478	1.325	1.574		
B	0.470	0.477	3.444	0.485	2.049	0.274	2.147		
A × B	1.393	1.390	2.298	1.406	1.325	1.156	1.383		

A, plantation type; B, soil depth; A × B, the interaction between plantation type and soil depth. SOC, soil organic carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; TCa, total calcium; TMg, total magnesium; C:N, soil C:N ratio; C:P, soil C:P ratio; N:P, soil N:P ratio; C:K, soil C:K ratio; N:K, soil N:K ratio; P:K, soil P:K ratio; C:Ca, soil C:Ca ratio; Ca:Mg, soil Ca:Mg ratio; fungi, soil fungi concentration; bacteria, soil bacteria concentration; actinomycetes, soil actinomycetes concentration; MBC, soil microbial biomass carbon; MBN, soil microbial biomass nitrogen; MBP, soil microbial biomass phosphorus; MBC:MBN, soil microbial biomass carbon to microbial biomass nitrogen ratio; MBC:MBP, soil microbial biomass carbon to microbial biomass phosphorus ratio; MBN:MBP, soil microbial biomass nitrogen to microbial biomass phosphorus ratio; BG, β-1,4-glucosidase; NAG, β-1,4-n-acetylglucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase; BG:(NAG + LAP), ratio of β-1,4-glucosidase to the sum of β-1,4-n-acetylglucosaminidase and leucine aminopeptidase; BG:AP, β-1,4-glucosidase to acid phosphatase ratio; (NAG + LAP):AP, ratio of the sum of β-1,4-n-acetylglucosaminidase and leucine aminopeptidase to acid phosphatase. *, **, *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

3.5. Internal Stability Analysis of Soil Elements and Microbial Biomass of Z. planispinum Plantations

The ecological stoichiometry internal stability model proposed by Sterner and Elser [31] is expressed in the following equation: $Y = CX^{1/H}$. In the equation, Y represents soil microbial biomass C, N, and P and their stoichiometric ratio; X represents the corresponding soil C, N, and P and their stoichiometric ratio; C is the fitting constant; H is the internal stability index. When the equation fitting result is successful ($p < 0.05$), $H > 4$ shows that Y is in a steady state; $2 < H < 4$ indicates that Y is in a weak steady state; $1.33 < H < 2$ means that Y is in a weakly sensitive state; and $H < 1.33$ indicates that Y is in a sensitive state. When the fitting result of the equation is unsuccessful ($p > 0.05$), Y is in an absolute steady state. Fitting the internal stability model equation of soil nutrient elements and soil microbial biomass C, N, and P was not successful ($p > 0.05$), and the dependent variable showed an absolute steady state [32]. This indicated that soil microorganisms had strong internal stability (Table 3).

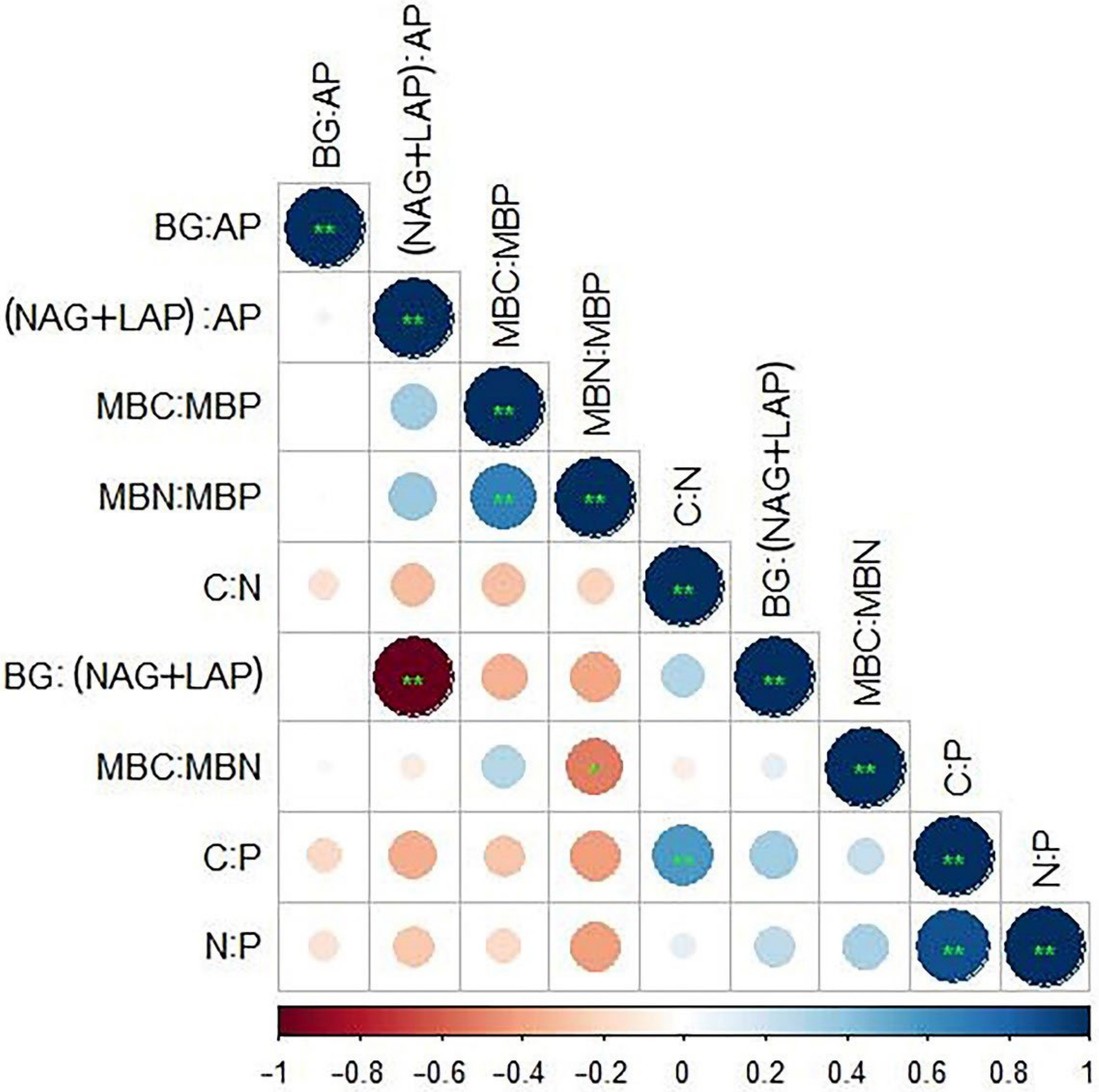

Figure 6. Correlations of soil element, microorganism, and extracellular enzyme C:N:P of *Z. planispinum* plantations. C:N, soil C:N ratio; C:P, soil C:P ratio; N:P, soil N:P ratio; MBC:MBN, soil microbial biomass carbon to microbial biomass nitrogen ratio; MBC:MBP, soil microbial biomass carbon to microbial biomass phosphorus ratio; MBN:MBP, soil microbial biomass nitrogen to microbial biomass phosphorus ratio; BG:(NAG + LAP), ratio of β-1,4-glucosidase to the sum of β-1,4-n-acetylglucosaminidase and leucine aminopeptidase; BG:AP, β-1,4-glucosidase to acid phosphatase ratio; (NAG + LAP):AP, ratio of the sum of β-1,4-n-acetylglucosaminidase and leucine aminopeptidase to acid phosphatase. *, ** indicates significant differences at $p < 0.05$ and $p < 0.01$, respectively.

Table 3. Standardized major axis analysis of C, N, and P concentrations in soil and soil microbial biomass.

Variable		*n*	r^2	*p*	**Intercept**	**Slope**
X	**Y**					
SOC	MBC	30	0.003	0.828	2.792	−0.2547
TN	MBN	30	0.005	0.768	1.380	−0.4954
TP	MBP	30	0.001	0.924	2.126	0.3874
C:N	MBC:MBN	30	0.009	0.693	2.405	−1.114
C:P	MBC:MBP	30	0.099	0.176	1.129	−0.6038
N:P	MBN:MBP	30	0.189	0.055	−0.6543	−0.8425

n: sample size.

4. Discussion

4.1. Soil Element Contents and Stoichiometry of *Z. planispinum* Plantations

The C, N, and P contents were higher for *Z. planispinum* + *L. japonica*/*P. salicina* plantations, indicating that these two plantations were conducive to nutrient accumulation. This is because the roots of *L. japonica* and *P. salicina* are generally well-developed, frequently exchanging materials with soil, where large amounts of nutrients accumulate due to dead root decomposition or root exudates; these things considered, abundant and easily decomposed litter returns nutrients back into soil as fertilizer. In addition, *P. salicina*, a tree with deep roots, can transport nutrients from the deep soil to the surface [33]. The total N and total P contents of the *Z. planispinum* + *S. tonkinensis* plantation were the lowest (2.64 and 0.82 g kg^{-1}, respectively), and because *S. tonkinensis* is a N-fixing plant, this is inconsistent with the conclusion that N-fixing plants help the accumulation of total N [34]. Possible reasons include that (1) the litter of *S. tonkinensis* was less, and the whole plants were harvested, which led to the reduction of nutrients back into soil; and (2) legume plantations had a high absorption of soil P [20], resulting in a relative lack of soil P under the same fertilization measures. Simultaneously, because P deficiency inhibited the secretion of N-fixing enzymes by N-fixing bacteria, it reduced the amount of N fixation [35]. Future management measures will be taken to supplement soil P to improve soil conditions. In this study, total P decreased with deeper soil. Compared with other elements, P was significantly affected by soil depth (Table 2), consistent with the results of Yan et al. [36]. The reason is that P is easily fixed under neutral or alkaline soil conditions, which is altered in the surface soil when there is an abundance of microorganisms and organic matter [37]. In this study, the soil Ca and Mg were in the range of 0.95–6.05 and 4.95–13.85 g kg^{-1}, respectively, which were relatively enriched compared with other subtropical areas. This is because the weathering of carbonate rocks generated this neutral or slightly alkaline soil environment, with Ca and Mg enriched. At the same time, *Z. planispinum* is a calciphile with a strong ability to enrich Ca, which increases the Ca concentration in soil; the soil Ca and Mg contents of *Z. planispinum* plantations for the four allocations were significantly lower than for the pure plantation, indicating that the compound planting mode increased Ca and Mg consumption compared with the pure plantation. Therefore, it is necessary to supplement the corresponding mineral element fertilizers appropriately.

The C:N in the soil tillage layer was inversely proportional to the decomposition rate of organic matter [38]. The soil C:N (10.47) in this area was lower than the Chinese average (14.4), indicating that N was sufficient for microbial growth, and the excess was released into the soil. The lowest value was for the *Z. planispinum* pure plantation (9.59), indicating that soil mineralization rate was highest for the pure plantation. This implies that compound management can reduce mineralization, increase organic matter accumulation, and improve soil C sequestration capacity. The soil C:P is an index of P availability, which reflects the metabolic trend of soil-available P by microorganisms. In this study, the soil C:P (28.9) was less than 200 and lower than the Chinese average (136), indicating that soil microorganisms mineralized SOC to supply the soil-effective P pool, and the soil was mainly limited by C. The soil N:P is an index indicating that the soil is limited by N or P (less than 10 indicates soil N deficiency). In this study, the N:P (2.76) was lower than the Chinese average (9.3), indicating that the soil N limitation was greater than P. The C:P and N:P of *Z. planispinum* + *S. tonkinensis* and *Z. planispinum* + *L. japonica* were significantly higher than for the others, and soil C and N of the *Z. planispinum* + *S. tonkinensis* plantation were low, suggesting that *Z. planispinum* + *S. tonkinensis* and *Z. planispinum* + *L. japonica* promoted the balance of soil C, N, and P; the *Z. planispinum* + *L. japonica* plantation may effectively alleviate the relative lack of C and N. Our results of P saturation are inconsistent with the conclusion that karst areas are generally limited by P [39]. Possible reasons include: (1) intense karstification promoted rock weathering, and with special hydrothermal conditions, the dissolution rate of soil microorganisms was increased, so that more P was dissolved and extracted into soil and (2) weak soil microorganism activity somehow strengthened soil P fixation [37].

4.2. Soil Microbial Properties and Stoichiometry of Z. planispinum Plantations

In this study, the concentrations of soil groups in the five sample plots were in the following order: bacteria > actinomycetes > fungi. This is because the soil was alkaline. This environment was suitable for the growth and reproduction of bacteria and actinomycetes, but unfavorable to the survival of fungi. Therefore, the concentration of bacteria was somewhat higher than that of fungi. The concentrations of bacteria, fungi, and actinomycetes were highest when *Z. planispinum* was mixed with *P. salicina*, *S. tonkinensis*, and *L. japonica*, respectively, followed by that of *Z. planispinum* + *Arachis hypogaea*, and that of the pure plantation was the lowest (Figure 3), indicating that plant species restricted the composition of the soil microbial community. This is because, compared with pure plantations, mixed plantations can increase productivity, improve soil nutrients, and increase soil microbial community abundance and EEAs by increasing the chemical composition of C substrates such as root exudates and litter [40,41]. In this study, MBC:MBN was greater than 10, indicating that the dominant microbial community was fungi, suggesting that local soil nutrients were relatively poor [42].

The MBC:MBN (18.8) was higher for the *Z. planispinum* + *L. japonica* plantation than that of Chinese soil, generally [43] (7.6), and the mean values of MBC:MBP (1.81) and MBN:MBP (0.1) were much lower than that for Chinese soil (70.2 and 6, respectively). This showed that the biological availability of soil N was low; the soil microbial P release supplemented the P pool, ensuring rich P in soil (Figure 1). The relatively poor N in soil limited the MBN fixation. Meanwhile, plants increased investment to P-rich ribosomal RNA in P-rich soil environments [18], resulting in the low N:P ratio (Figure 2).

In this study, the soil enzymatic C:N ratio (0.1) was lower than the mean value of major terrestrial ecosystems (1.41), and the enzymatic C:P (0.85) and N:P (8.8) were higher than the mean of global terrestrial ecosystems (0.62 and 0.44, respectively), indicating that NAG and LAP activities were high and AP activity was low. This is because soil microorganisms secrete more N-decomposing enzymes to relieve N restriction [30] and less P-related enzymes to deal with the P saturation environment. A soil enzymatic C:N ratio of 1:1 indicates that C and N are mineralized at the same rate. We conclude that the mineralization rate of soil N and P was greater than that of soil C in this area. The four enzymes showed higher activities in the *Z. planispinum* + *L. japonica* plantation than in the other modes (Figure 5), and the soil C, N, and P contents in this mode were all the highest (Figure 1). This showed that the higher the nutrient contents, the stronger the EEA, consistent with the conclusions of Zuo et al. [44]. It is speculated that *L. japonica* may persist by regulating soil microorganisms to secrete extracellular enzymes to mineralize more organic matter [45]. In addition, SOC and total N were energy sources for soil enzyme production and secretion: the total N can increase biomass of underground fine roots, promote growth of rhizosphere microorganisms, and finally increase EEAs. In contrast, enzymes are also proteins, and high soil EEA can provide more N to maintain enzyme production. Concerning the effect of soil depth on extracellular enzymes, except for *Z. planispinum* + *L. japonica*, the soil EEA of plantations decreased with increasing soil depth. The reason is that the C input and rhizosphere effect of bottom soil are lower than that of the surface [46]. In contrast, the soil EEA of *Z. planispinum* + *L. japonica* was high in the bottom layer. It may be that the fine roots were deeply distributed, and the root system secreted more enzymes, thus, assisting nutrient absorption. The specific mechanism needs to be further explored.

4.3. Correlation and Internal Stability Analysis of Soil Stoichiometry of Z. planispinum Plantations

The ratio of BG:(NAG + LAP):AP had no significant correlation with soil C:N:P and MBC:MBN:MBP (Figure 6). The reason is that extracellular enzymes are complexes affected by soil properties, vegetation type, and other factors, as well as the fact that EEA is affected by some unmeasured soil biological or non-biological factors [47]. There was no significant correlation between soil C:N:P and MBC:MBN:MBP ratios in this study;

moreover, MBN:MBP had significant and extremely significant correlations with MBC:MBN and MBC:MBP (Figure 6), indicating that soil microorganisms were not affected by soil elements. This is consistent with the results of Cleveland et al. [48] and Hartman et al. [49]. The reason is that soil microorganisms have self-balancing mechanisms which can change their community composition by adjusting their own structure composition and population dynamics to maintain the relative stability of C:N:P [50]. This also indicated that soil microorganisms resist environmental stresses through adjusting their activities, resulting in a stricter C:N:P ratio [13]. This study also showed that soil microbial biomass C:N:P was not sensitive to changes in plantation type (Table 2), indicating that soil microbial biomass C:N:P was more stable than soil C:N:P, which can be used to diagnose ecosystem nutrient constraints.

5. Conclusions

In this study, the soil C:N:P was low, indicating that C and N contents in soil were relatively low, while P content was relatively high, which reflected the low vegetation productivity in this area; moreover, the area was mainly limited by C and N. At the same time, soil microorganisms mineralize soil organic matter to supplement the soil-available P pool. The soil MBC:MBN in this area was high, while MBC:MBP and MBN:MBP were low, indicating that biological availability of soil N was low, and the P released by soil microorganisms supplemented the available P pool. In this study, the low soil enzymatic C:N ratio and the high soil enzymatic C:P and N:P ratios showed that NAG and LAP activities were high and AP activity was low. This is because soil microorganisms secrete more N-decomposing enzymes to relieve N restriction and less P-related enzymes to deal with the P saturation environment. The impact of plantation type on ecological stoichiometry was greater than that of soil depth and their interaction, suggesting that stand type had a more significant impact on ecosystem nutrient cycling. The *Z. planispinum* + *L. japonica* plantation had a large amount of nutrients returned, which improved soil fertility and promoted the growth of soil microorganisms. This study indicated that application of *L. japonica* slowed the nutrient deficit and improved the soil environment. The *Z. planispinum* + *L. japonica* was the best plantation type. Fitting of the internal stability model equation of soil nutrient elements and soil microbial biomass C, N, and P was unsuccessful ($p > 0.05$), and the dependent variable showed an absolute steady state. This indicated that soil microorganisms resisted environmental stress through adjustment of their activities, resulting in a stricter C:N:P ratio. The soil MBC:MBN:MBP was more stable internally than soil C:N:P, which could be applied to diagnose ecosystem nutrient constraints.

Author Contributions: Conceptualization, Y.Y.; formal analysis, Y.L. and Y.Y.; software, Y.L. and Y.S.; investigation, Y.L. and Y.S.; writing—original draft preparation, Y.L.; writing—review and editing, Y.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Guizhou Province Science and Technology Support Plan Project (Qian-ke-he Zhicheng [2022] Yiban 103).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are not publicly available due to privacy.

Acknowledgments: We thank International Science Editing (http://www.internationalscienceediting.com) for editing this manuscript (accessed on 3 May 2022).

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

References

1. Wang, L.J.; Wang, P.; Sheng, M.Y.; Tian, J. Ecological stoichiometry and environmental influencing factors of soil nutrients in the karst rocky desertification ecosystem, southwest China. *Glob. Ecol. Conserv.* **2018**, *16*, e00449. [CrossRef]
2. Wang, M.M.; Chen, H.S.; Zhang, W.; Wang, K.L. Soil nutrients and stoichiometric ratios as affected by land use and lithology at county scale in a karst area, southwest China. *Sci. Total Environ.* **2018**, *619*, 1299–1307. [CrossRef] [PubMed]

3. Yuan, J.H.; Wang, L.; Chen, H.; Chen, G.L.; Wang, S.Q.; Zhao, X.; Wang, Y. Responses of soil phosphorus pools accompanied with carbon composition and microorganism changes to phosphorus-input reduction in paddy soils. *Pedosphere* **2021**, *31*, 83–93. [CrossRef]
4. Zhang, H.Z.; Shi, L.L.; Wen, D.Z.; Yu, K.L. Soil potential labile but not occluded phosphorus forms increase with forest succession. *Biol. Fertil. Soils* **2016**, *52*, 41–51. [CrossRef]
5. Anderson, T.R.; Boersma, M.; Raubenheimer, D. Stoichiometry: Linking elements to biochemical. *Ecology* **2004**, *85*, 1193–1202. [CrossRef]
6. Elser, J.J.; Fagan, W.F.; Denno, R.F.; Dobberfuhl, D.R.; Folarin, A.; Huberty, A.; Interlandi, S.; Kilham, S.S.; McCauley, E.; Schulz, K.L.; et al. Nutritional constraints in terrestrial and freshwater food webs. *Nature* **2000**, *408*, 578–580. [CrossRef]
7. Ren, C.J.; Zhao, F.Z.; Kang, D.; Yang, G.H.; Han, X.H.; Tong, X.G.; Feng, Y.Z.; Ren, G.X. Linkages of C:N:P stoichiometry and bacterial community in soil following afforestation of former farmland. *For. Ecol. Manag.* **2016**, *376*, 59–66. [CrossRef]
8. Wang, J.P.; Wu, Y.H.; Li, J.J.; He, Q.Q.; Bing, H.J. Soil enzyme stoichiometry is tightly linked to microbial community composition in successional ecosystems after glacier retreat. *Soil Biol. Biochem.* **2021**, *162*, 108429. [CrossRef]
9. Gai, X.; Zhong, Z.K.; Zhang, X.P.; Bian, F.Y.; Yang, C.B. Effects of chicken farming on soil organic carbon fractions and fungal communities in a Lei bamboo (Phyllostachys praecox) forest in subtropical China. *For. Ecol. Manag.* **2021**, *479*, 118603. [CrossRef]
10. Zhong, Z.K.; Li, W.J.; Lu, X.Q.; Gu, Y.Q.; Wu, S.J.; Shen, Z.Y.; Han, X.H.; Yang, G.H.; Ren, C.J. Adaptive pathways of soil microorganisms to stoichiometric imbalances regulate microbial respiration following afforestation in the Loess Plateau, China. *Soil Biol. Biochem.* **2020**, *151*, 108048. [CrossRef]
11. Huang, R.; Lan, T.; Song, X.; Li, J.; Ling, J.; Deng, O.P.; Wang, C.Q.; Gao, X.S.; Li, Q.Q.; Tang, X.Y.; et al. Soil labile organic carbon impacts C:N:P stoichiometry in urban park green spaces depending on vegetation types and time after planting. *Appl. Soil Ecol.* **2021**, *163*, 103926. [CrossRef]
12. Fang, Z.; Li, D.D.; Jiao, F.; Yao, J.; Du, H.T. The Latitudinal Patterns of Leaf and Soil C:N:P Stoichiometry in the Loess Plateau of China. *Front. Plant Sci.* **2019**, *10*, 85. [CrossRef]
13. Hu, N.; Li, H.; Tang, Z.; Li, Z.F.; Li, G.C.; Jiang, Y.; Hu, X.M.; Lou, Y.L. Community size, activity and C:N stoichiometry of soil microorganisms following reforestation in a karst region. *Eur. J. Soil Biol.* **2016**, *73*, 77–83. [CrossRef]
14. Ravindran, A.; Yang, S.S. Effects of vegetation type on microbial biomass carbon and nitrogen in subalpine mountain forest soils. *J. Microbiol. Immunol. Infect.* **2015**, *48*, 362–369. [CrossRef]
15. Wang, Y.; Ren, Z.; Ma, P.P.; Wang, Z.M.; Niu, D.C.; Fu, H.; Elser, J.J. Effects of grassland degradation on ecological stoichiometry of soil ecosystems on the Qinghai-Tibet Plateau. *Sci. Total Environ.* **2020**, *722*, 137910. [CrossRef]
16. Dai, X.L.; Zhou, W.; Liu, G.R.; Liang, G.Q.; He, P.; Liu, Z.B. Soil C/N and pH together as a comprehensive indicator for evaluating the effects of organic substitution management in subtropical paddy fields after application of high-quality amendments. *Geoderma* **2019**, *337*, 1116–1125. [CrossRef]
17. Zechmeister-Boltenstern, S.; Keiblinger, K.M.; Mooshammer, M.; Penuelas, J.; Richter, A.; Sardans, J.; Wanek, W. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. *Ecol. Monogr.* **2015**, *85*, 133–155. [CrossRef]
18. Wu, X.Z.; Yan, X.; Wang, B.; Liu, R.T.; An, H. Effects of desertification on the C:N:P stoichiometry of soil, microbes, and extracellular enzymes in a desert grassland. *Chin. J. Plant Ecol.* **2018**, *42*, 1022–1032. (In Chinese) [CrossRef]
19. Yin, S.; Wang, C.K.; Jin, Y.; Zhou, Z.H. Changes in soil-microbe-exoenzyme C:N:P stoichiometry along an altitudinal gradient in Mt. Datudingzi, Northeast China. *Chin. J. Plant Ecol.* **2019**, *43*, 999–1009. (In Chinese) [CrossRef]
20. Chen, H.; Chen, M.L.; Li, D.J.; Mao, Q.G.; Zhang, W.; Mo, J.M. Responses of soil phosphorus availability to nitrogen addition in a legume and a non-legume plantation. *Geoderma* **2018**, *322*, 12–18. [CrossRef]
21. Hernández, J.; del Pino, A.; Vance, E.D.; Califra, Á.; Del Giorgio, F.; Martínez, L.; González-Barrios, P. Eucalyptus and Pinus stand density effects on soil carbon sequestration. *For. Ecol. Manag.* **2016**, *368*, 28–38. [CrossRef]
22. Yu, Y.H.; Wang, Z.Y.; Ying, B.; Yang, D.L. *Management Principle and Technology of Ecological Industry in Karst Plateau Canyon*, 1st ed.; China Environmental Science Press: Beijing, China, 2021; pp. 28–30. (In Chinese)
23. Yu, Y.; Song, Y.P.; Zhong, X.P.; Li, Y.T.; Ying, B. Growth decline mechanism of *Zanthoxylum planispinum* var. *dintanensis* in the canyon area of Guizhou karst plateau. *Agron. J.* **2021**, *113*, 852–862. [CrossRef]
24. Bao, S.D. *Soil Agrochemical Analysis*, 3rd ed.; China Agriculture Press: Beijing, China, 2000; pp. 22–173. (In Chinese)
25. Shen, P.; Fan, X.R.; Li, G.H. *Microbiology Experiment*, 3rd ed.; Higher Education Press: Beijing, China, 1999; pp. 92–95. (In Chinese)
26. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
27. Bell, C.W.; Fricks, B.E.; Rocca, J.D.; Steinweg, J.M.; McMahon, S.K.; Wallenstein, M.D. High-throughput Fluorometric Measurement of Potential Soil Extracellular Enzyme Activities. *J. Vis. Exp.* **2013**, *81*, e50961. Available online: https://www.jove.com/video/50961 (accessed on 26 October 2021). [CrossRef]
28. Hu, Z.Y.; Li, J.T.; Shi, K.W.; Ren, G.Q.; Dai, Z.C.; Sun, J.F.; Zheng, X.J.; Zhou, Y.W.; Zhang, J.Q.; Li, G.L.; et al. Effects of Canada Goldenrod Invasion on Soil Extracellular Enzyme Activities and Ecoenzymatic Stoichiometry. *Sustainability* **2021**, *13*, 3768. [CrossRef]
29. Wang, L.D.; Yao, T.; Wang, F.L.; Wei, L.Y.; Guo, C.X. Soil microbial and soil enzyme activity in a discontinued farmland by the Lower Shiyang River. *Acta Ecol. Sin.* **2016**, *36*, 4769–4779. [CrossRef]

30. Sinsabaugh, R.L.; Hill, B.H.; Shah, J.J.F. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* **2009**, *462*, 795–798. [CrossRef]
31. Sterner, R.W.; Elser, J.J. *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*, 1st ed.; Princeton University Press: Princeton, NJ, USA, 2002; pp. 117–795.
32. Persson, J.; Fink, P.; Goto, A.; Hood, J.M.; Jonas, J.; Kato, S. To be or not to be what you eat: Regulation of stoichiometric homeostasis among autotrophs and heterotrophs. *Oikos* **2010**, *119*, 741–751. [CrossRef]
33. Ding, Y.; Huang, X.; Li, Y.; Liu, H.Y.; Zhang, Q.C.; Liu, X.M.; Xu, J.M.; Di, H.J. Nitrate leaching losses mitigated with intercropping of deep-rooted and shallow-rooted plants. *J. Soils Sediments* **2021**, *21*, 364–375. [CrossRef]
34. Stinca, A.; Chirico, G.B.; Incerti, G.; Bonanomi, G. Regime Shift by an Exotic Nitrogen-Fixing Shrub Mediates Plant Facilitation in Primary Succession. *PLoS ONE* **2015**, *10*, e0123128. [CrossRef] [PubMed]
35. Png, G.K.; Turner, B.L.; Albornoz, F.E.; Hayes, P.E.; Lambers, H.; Laliberte, E. Greater root phosphatase activity in nitrogen-fixing rhizobial but not actinorhizal plants with declining phosphorus availability. *J. Ecol.* **2017**, *105*, 1246–1255. [CrossRef]
36. Yan, Y.J.; Dai, Q.H.; Jin, L.; Wang, X.D. Geometric morphology and soil properties of shallow Karst fissures in an area of karst rocky desertification in SW China. *Catena* **2019**, *174*, 48–58. [CrossRef]
37. Cen, L.P.; Yan, Y.J.; Dai, Q.H.; Jiao, Q.; Hu, G.; Gao, R.X.; Fu, W.B. Occurrence characteristics of organic carbon and phosphorus in fissured soil under different land use types in Karst area. *Acta Ecol. Sin.* **2020**, *40*, 7567–7575. (In Chinese) [CrossRef]
38. Li, Y.Q.; Zhao, X.Y.; Zhang, F.X.; Awada, T.; Wang, S.K.; Zhao, H.L.; Zhang, T.H.; Li, Y.L. Accumulation of soil organic carbon during natural restoration of desertified grassland in China's Horqin Sandy Land. *J. Arid Land* **2015**, *7*, 328–340. [CrossRef]
39. Chen, H.; Li, D.J.; Mao, Q.G.; Xiao, K.C.; Wang, K.L. Resource limitation of soil microbes in karst ecosystems. *Sci. Total Environ.* **2019**, *650*, 241–248. [CrossRef]
40. Wolfe, B.E.; Klironomos, J.N. Breaking new ground: Soil communities and exotic plant invasion. *Bioscience* **2005**, *55*, 477–487. [CrossRef]
41. Rousk, J.; Brookes, P.C.; Baath, E. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Appl. Environ. Microbiol.* **2009**, *75*, 1589–1596. [CrossRef]
42. Sun, J.; Lang, J.X.; Kong, D.J.; Guo, X.N.; Wei, Y.D.; Zhou, T. Effects of biochar and straw on the C:N:P stoichiometry of soil, microbes, and extracellular enzymes in an aeolian sandy soil. *Acta Pratacult. Sin.* **2021**, *30*, 29–39. (In Chinese) [CrossRef]
43. Xu, X.F.; Thornton, P.E.; Post, W.M. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* **2013**, *22*, 737–749. [CrossRef]
44. Zuo, Y.P.; Zhang, X.Y.; Zeng, H.; Wang, W. Spatiotemporal Dynamics of Soil Extracellular Enzyme Activity and Its Influence on Potential Mineralization Rate of Soil Organic Carbon in Forests of Daxing'an Mountain Range. *Acta Sci. Nat. Univ. Pekin.* **2018**, *54*, 1311–1324. (In Chinese) [CrossRef]
45. Xu, Z.W.; Yu, G.R.; Zhang, X.Y.; He, N.P.; Wang, Q.F.; Wang, S.Z.; Wang, R.L.; Zhao, N.; Jia, Y.L.; Wang, C.Y. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). *Soil Biol. Biochem.* **2017**, *104*, 152–163. [CrossRef]
46. Peng, X.Q.; Wang, W. Stoichiometry of soil extracellular enzyme activity along a climatic transect in temperate grasslands of northern China. *Soil Biol. Biochem.* **2016**, *98*, 74–84. [CrossRef]
47. Xie, M.Y.; Feng, X.X.; Ma, H.F.; Hu, H.; Wang, J.Y.; Guo, Y.X.; Ren, C.J.; Wang, J.; Zhao, F.Z. Characteristics of soil enzyme activities and stoichiometry and its influencing factors in Quercus aliena var. acuteserrata forests in the Qinling Mountains. *Chin. J. Plant Ecol.* **2020**, *44*, 885–894. (In Chinese) [CrossRef]
48. Cleveland, C.C.; Liptzin, D. C:N:P stoichiometry in soil: Is there a "Redfield ratio" for the microbial biomass? *Biogeochemistry* **2007**, *85*, 235–252. [CrossRef]
49. Hartman, W.H.; Richardson, C.J. Differential Nutrient Limitation of Soil Microbial Biomass and Metabolic Quotients (qCO2): Is There a Biological Stoichiometry of Soil Microbes? *PLoS ONE* **2013**, *8*, e57127. [CrossRef]
50. Khan, K.S.; Joergensen, R.G. Stoichiometry of the soil microbial biomass in response to amendments with varying C/N/P/S ratios. *Biol. Fertil. Soils* **2019**, *55*, 265–274. [CrossRef]