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# Ecoenzymatic Stoichiometry in the Rhizosphere and Bulk Soil of a *Larix principis-rupprechtii* Plantation in North China

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**Abstract:** Soil extracellular enzymes play an important role in ecosystem energy conversion and material cycling. Ecoenzymatic stoichiometry can reflect the relationship between the soil's microbial nutrient cycle and nutrient limitation. However, there have been few studies on the differences in ecoenzymatic stoichiometry and nutrient limitation between rhizosphere soil and bulk soil. This study examined soil nutrients and enzyme activities in rhizosphere soil and bulk soil in a *Larix principis-rupprechtii* plantation in north China. The results showed that the levels of soil organic carbon (C), total nitrogen (N), and available nutrients in the rhizosphere soil were significantly higher than those in the bulk soil, whereas the total potassium (TK) level was significantly lower. The soil C:N, C:P, and N:P ratios of the rhizosphere soil also exceeded those of the bulk soil. The acid phosphatase (ACP), urease (UE), and β-glucosidase (β-GC) activities in the rhizosphere soil exceeded those in the bulk soil, whereas the activities of N-acetyl-β-D-glucosidase (NAG), aminopeptidase (LAP), and nitrogenase (NA) were lower. The ratios of C, N, and P acquisition activities changed from 1:1.7:1 in the rhizosphere soil to 1:2:1 in the bulk soil. Redundancy analysis showed that the available K and soil water content in the rhizosphere soil were the most important soil factors affecting soil enzyme activities and ecoenzymatic stoichiometry; those in the bulk soil were soil N:P and soil water content. These results suggest that not all soil enzyme activities present rhizosphere effects and that bulk soil is more susceptible to N limitation in *Larix principis-rupprechtii* plantations. Plant roots play an important role in regulating soil nutrients and soil activities, and future studies should examine the underlying mechanisms in more detail.

**Keywords:** soil enzyme activity; rhizosphere soil; bulk soil; ecoenzymatic stoichiometry



**Citation:** Yang, L.; Jia, Y.; Li, Q.; Cui, H.; Lu, J.; Ma, J.; Xu, Z. Ecoenzymatic Stoichiometry in the Rhizosphere and Bulk Soil of a *Larix principis-rupprechtii* Plantation in North China. *Forests* **2023**, *14*, 1315. https://doi.org/10.3390/f14071315

Academic Editor: Benjamin L. Turner

Received: 9 May 2023
Revised: 19 June 2023
Accepted: 20 June 2023
Published: 27 June 2023



## 1. Introduction

Soil extracellular enzymes are an important part of the soil ecosystem [1], and play vital roles in soil biochemical processes, energy conversion [2], and the ecosystem nutrient cycle [1,3,4]. Given their abundance and rapid reproduction, soil microorganisms are the main source of soil enzymes [5,6]. Plant root exudates, such as sugar, amino acids, and organic acids, represent an additional source of soil enzymes [5,7]. These exudates mainly affect the rhizosphere microenvironment [8,9] and play an important role in regulating forest rhizosphere ecological processes [10]. Recent studies have looked at the ecological stoichiometry of soil enzymes within the examination of the soil nutrient cycle and nutrient limitation [11–14].

β-glucosidase (β-GC) is an indicator of carbon (C) demand, whereas N-acetyl-β-D-glucosidase (NAG) and aminopeptidase (LAP) are indicators of nitrogen (N) demand, and acid phosphatase (ACP) is an indicator of phosphorus (P) demand. The ecological stoichiometry of soil enzymes can reflect the ability of microorganisms to allocate nutrients and can restrict elements, indicating microbial growth [11,12,15], such as a high BG:(NAG + LAP) ratio, suggesting C limitation relative to N [16–18]. These indicators can

be used to represent the release of soil extracellular enzymes by microorganisms during the assimilation of major nutrients [19–22]. A previous study found the convergence of ratios of specific C, N, and P acquisition activities at 1:1:1 on a global scale, indicating the relative stability of soil C:N:P [11]. However, the localized stoichiometric ratio of ecological enzymes is influenced by a wide variety of biological and abiotic factors, leading to significant differences between different ecosystems [21,23,24].

The rhizosphere is a narrow soil region encompassing plant roots [25], and provides an environment suitable for microorganisms [26]. The rhizosphere also provides an interface for interactions between soil, plants, and microorganisms [27,28]. Bulk soil generally has a lower nutrient transformation rate, microbial quantity, and activity than rhizosphere soil due to a lack of root regulation [22,29,30]. The rhizosphere effect is induced by the rhizodeposition transfer of C, N, and other nutrients from plants' fine roots to the soil [27,31,32], leading to differences in physical, chemical, and biological characteristics between rhizosphere soil and bulk soil [28,33–35]. The influences of root morphology, metabolism, and soil characteristics result in differences in the mineral nutrients and enzyme activities in rhizosphere soil among different habitats [27,28,36,37]. The most recent studies on soil enzyme activities have focused on either bulk soil or rhizosphere soil [15,24,38,39]. For example, Xu et al. (2017), compared the bulk soil enzyme activities in nine forest ecosystems in different temperature zones in eastern China [40] and Cui et al. (2018) measured the enzyme activities in rhizosphere soil with different vegetation and soil types in the arid area of the northern Chinese Loess Plateau [41]. However, these studies did not simultaneously consider rhizosphere soil and bulk soil, thereby ignoring the important effects of roots on soil enzyme activity and ecological stoichiometry. Consequently, the differences and relationships between forest rhizosphere soil and bulk soil remain unclear [26,42].

The aim of the present study was to compare the enzyme activities and ecological stoichiometry of bulk soil with those of rhizosphere soil in a *Larix principis-rupprechtii* plantation in the temperate zone of northern China. The hypotheses of the present study are as follows: (1) rhizosphere effects exist in soil C, N, and P; however, soil enzyme activities do not show rhizosphere effects due to abundant nutrients in rhizosphere soil; and (2) N limitation in bulk soil exceeds that in rhizosphere soil because of the wide-ranging N limitation in boreal forests. The present paper reveals the main factors influencing the enzyme activities and stoichiometric characteristics of rhizosphere soil and bulk soil. The results of the present study can improve knowledge regarding the role of plant roots in regulating soil enzyme activities.

## 2. Methods

### 2.1. Study Sites

The study area of the present study was in the Mulanweichang National Forestry Administration, Hebei Province, China (116°32′ E–118°14′ E, 41°35′ N–41°40′ N). The study area falls within an elongated branch of the Greater Khingan Range, Inner Mongolia Plateau, Yanshan Mountains. The terrain of the study area decreases from northwest to southeast, with an altitude of between 750 and 2050 m above sea level (a.s.l.). The soil type in this study area is Burozem from the classification of China, which is called Colorful Luvisol and Braunerde from the classifications of the United Nations and Russia, respectively. The study area falls in a temperate semihumid and semiarid continental monsoon mountain climate zone with maximum, minimum, and average annual temperatures of 29.8 °C, −42.9 °C, and 3.3 °C, respectively. The study area has an annual average precipitation of 445 mm, with precipitation occurring in summer from June to August.

A field sampling program was implemented in a 30-year-old *Larix principis-rupprechtii* plantation on a hill with a slope of 15°. The average diameter at breast height (DBH), tree height, and stand density of each sample plot were 12.37 cm, 9.2 m, and 1575 plants/hm$^2$, respectively. The dominant herbs of the understory of the plot were identified to include *Moehringia lateriflora*, *Carex rigescens*, *Galium boreale*, and *Carex siderosticta*. The present study accounted for the large spatial heterogeneity in the soil nutrients and enzyme activities in

the study area by establishing 16 replicate sample plots. Each sample plot had an area of 20 m × 20 m, with a 10 m buffer zone between each sample plot along the contour line. The sample plots were enclosed using railings from August 2018, to eliminate livestock and human interference. Field investigation and sampling were conducted in August 2019, after one year of enclosure.

### 2.2. Soil Sampling

Three quadrats, each with an area of 0.5 m × 0.5 m, were established along a diagonal line in each plot. In each quadrat, all plant roots were extracted and rhizosphere soil within 4 mm of the root surfaces was collected [43]. The rhizosphere soil samples collected from three quadrats in the same plot were amalgamated into a single sample, resulting in the collection of 16 rhizosphere soil samples. Five samples of surface soil taken from a depth of 0–10 cm, representing bulk soil, were randomly collected from each plot, and amalgamated into a single sample. The collected soil samples were refrigerated and analyzed within a week. They were filtered through a 2 mm sieve to remove the roots, litter, stones, and other debris. Each soil sample was divided into two parts; one part was air-dried for the determination of soil total nutrients, whereas the other was stored in a refrigerator at 4 °C before laboratory measurements of soil enzyme activity, soil microbial biomass carbon, nitrate nitrogen ($NO_3^-$-N), and ammonium nitrogen ($NH_4^+$-N) were taken.

### 2.3. Measurements of Soil Properties

The present study used the potassium dichromate external heating method to determine the soil contents of organic carbon (SOC), total nitrogen (TN), total phosphorus (TP), total potassium (TK), available phosphorus (AP), available potassium (AK), ammonium nitrogen ($NH_4^+$-N), nitrate nitrogen ($NO_3^-$-N), microbial biomass carbon (MBC), and pH. Soil TN was determined using Kjeldahl nitrogen analyzer (Kjeltec 2300, Foss Tecator AB, Sweden, Sweden). TP was determined via potassium dichromate–sulfuric acid digestion (UV-1800, Shanghai, China). TK was analyzed via flame atomic absorption spectrophotometry (M360, Sherwood, WV, USA); AP was analyzed using Mo-Sb anti-spectrophotometry method and extracted with sodium bicarbonate (UV-1800, Shanghai, China) [44]; AK was analyzed via $NH_4OAc$ extraction flame spectrophotometry (M360, Sherwood, WV, USA); $NO_3$-N and $NH_4^+$-N were analyzed using a continuous flow analyzer (Skalar san++, Skalar, the Netherlands); soil pH (soil/water, 1:2.5) was analyzed using a pH meter (HI2211, Hanna, Italy); and MBC was analyzed utilizing the chloroform fumigation–potassium sulfate leaching method (multi 2100 s C/N, Analytik Jena AG, Jena, Germany). These methods can be seen in the work of Bao, 2000 [45].

The present study determined the activities of various soil enzymes, including β-glucosidase (β-GC), leucine aminopeptidase (LAP), N-acetyl-β-D-glucosidase (NAG), acid phosphatase (ACP), urease (UE), and nitrogenase (NT). Table 1 summarizes the specific functions of the enzyme activities. β-GC, LAP, NAG, ACP, and UE were determined using microplate fluorometry [46]. Following the method instructions, reagents were added to soil samples on a 96-well cell culture plate, following which an enzyme-labeling instrument was used to identify the enzyme activity. The activity potential of soil nitrogenase was determined via the acetylene reduction method [47] according to the following sequence: (1) a 10 g subsample of fresh soil from each soil sample was placed into a 150 mL sterilized serum bottle, following which a 4 mL solution of glucose (1 mg C $g^{-1}$ dry soil) and disodium malate (1 mg C $g^{-1}$ dry soil) was added to ensure nonlimiting C availability; (2) a total of 10% of the air in the bottle (10 mL) was replaced with pure $C_2H_2$ (99.99%), following which the bottle was incubated at 26 °C for 24 h; (3) after incubation was completed, 1 mL of the reaction gas was extracted and injected into a gas chromatograph (gc7890b, Agilent, CA, USA).

### 2.4. Data Processing and Analysis

The soil enzyme C:N, C:P, and N:P stoichiometric characteristics were characterized by EEAC:N, EEAC:P, and EEAC:N, respectively, using the following formulae [48]:

$$EEA_{C:N} = \frac{ln(BG)}{ln(LAP + NAG)}, \quad (1)$$

$$EEA_{C:P} = \frac{ln(BG)}{ln(ACP)}, \quad (2)$$

$$EEA_{N:P} = \frac{ln(LAP + NAG)}{ln(ACP)}. \quad (3)$$

**Table 1.** Description of the extracellular enzymes included in this study.

| Enzyme Name | EC Number | Abbreviation | Function |
|---|---|---|---|
| β-glucosidase | 3.2.1.2.1 | β-GC | Hydrolyzes glucose from cellobiose |
| Leucine aminopeptidase | 3.4.11.1 | LAP | Hydrolyzes leucine and other hydrophobic amino acids from the N terminus of polypeptides |
| N-acetyl-β-D-glucosidase | 3.4.11.1 | NAG | Degrades chitin and other β-1,4 glucosamine polymers |
| Acid phosphatase | 3.1.3.2 | ACP | Hydrolyzes phosphosaccarides and phospholipids to release phosphate |
| Urease | 3.5.1.5 | UE | Hydrolyzes $NH_3$-N in urea |
| Nitrogenase | — | NT | Reduces nitrogen molecules to ammonia |

The vector length and vector angle were used for the vector analysis of soil enzyme activity, as follows [17,49]:

$$Length = \sqrt{\left(EEA_{C:N}\right)^2 + \left(EEA_{C:P}\right)^2}, \quad (4)$$

$$Angle(o) = Degress[Atan2(EEA_{C:P}), (EEA_{C:N})]. \quad (5)$$

The present study applied analysis of variance (ANOVA) in SPSS 20 (SPSS Inc., Chicago, IL, USA) to determine significant differences in enzyme activity and the enzyme stoichiometry ratio between rhizosphere and bulk soil. Redundancy analysis (RDA) was performed using Canoco 5.0 (Cobe Information Technology Co., Ltd.; Shanghai, China) to determine the relationship between soil nutrients and enzyme activities in rhizosphere soil and bulk soil. Origin 2022 software (OriginLab, 8.0, Northampton, MA, USA) was used for producing maps.

## 3. Results

### *3.1. Chemical Properties of Rhizosphere and Bulk Soil*

Table 2 summarizes the differences in chemical properties between rhizosphere soil and bulk soil in the studied *Larix principis-rupprechtii* plantation. The SOC, TN, $NH_4^+$-N, $NO_3^-$-N, AP, AK, and MBC of the rhizosphere soil all significantly exceeded those of the bulk soil ($p < 0.01$), by factors of 1.42, 3.21, 2.49, 1.95, 1.68, 1.61, 2.28, and 1.48, respectively, indicating a positive rhizosphere effect (R/S > 1). The TK and pH of the bulk soil significantly exceeded those of the rhizosphere soil ($p < 0.01$) by factors of 1.23 and 1.03, respectively, representing a negative rhizosphere effect (R/S < 1). While the TP of the rhizosphere soil exceeded that of the bulk soil, the difference was not statistically significant. In addition, the soil C:N, C:P, and N:P ratios of the rhizosphere soil significantly exceeded those of the bulk soil ($p < 0.01$) by factors of 1.25, 2.13, and 1.7, respectively.

**Table 2.** Chemical properties of rhizosphere and bulk soil in the studied *Larix principis-rupprechtii* plantation.

| Soil Type | TN (g/kg) | TP (g/kg) | TK (g/kg) | AP (mg/kg) | AK (mg/kg) | SOC (g/kg) | $NH_4^+$-N (mg/kg) | $NO_3^-$-N (mg/kg) | MBC (mg/L) | SWC (%) | pH | C:N | C:P | N:P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rhizosphere soil | 10.49 ± 1.04 [a] | 1.02 ± 0.15 [a] | 7.02 ± 0.33 [a] | 26.82 ± 3.82 [a] | 496.98 ± 68.63 [a] | 172.99 ± 21.07 [a] | 13.96 ± 6.61 [a] | 87.48 ± 21.80 [a] | 1591.11 ± 249.10 [a] | 58.21 ± 13.49 [a] | 5.79 ± 0.27 [a] | 16.38 ± 1.53 [a] | 172.22 ± 14.51 [a] | 10.65 ± 1.14 [a] |
| Bulksoil | 5.38 ± 0.52 [b] | 0.89 ± 0.20 [a] | 8.66 ± 0.28 [b] | 16.01 ± 2.49 [b] | 308.12 ± 23.36 [b] | 69.61 ± 5.17 [b] | 9.15 ± 3.05 [b] | 27.23 ± 7.24 [b] | 697.04 ± 28.47 [b] | 39.4 ± 4.91 [b] | 6.01 ± 0.12 [b] | 13.06 ± 1.23 [b] | 81.03 ± 10.02 [b] | 6.26 ± 0.87 [b] |

Abbreviations: TN—total nitrogen; TP—total phosphorus; TK—total potassium; AP—available phosphorus; AK—available potassium; SOC—soil organic carbon; MBC—microbial biomass carbon; SWC—soil water content; [a] and [b] indicate the significance of rhizosphere and bulk soil. ($p < 0.01$).

### 3.2. Enzyme Activities in Rhizosphere and Bulk Soils

Figure 1 shows the differences in enzyme activities between rhizosphere and bulk soils in the studied *Larix principis-rupprechtii* plantation. The average activities of ACP, β-GC, UE, LAP, NAG, and NT in the rhizosphere soil were 1580.83 ± 36.26 nmol $g^{-1}$ $h^{-1}$, 3410.78 ± 246.92 nmol $g^{-1}$ $h^{-1}$, 1.13 ± 0.09 μ$g^{-1}$ $d^{-1}$, 482.5 ± 50.05 nmol $g^{-1}$ $h^{-1}$, 1946.02 ± 147.65 nmol $g^{-1}$ $h^{-1}$, and 0.21 ± 0.03 nmol $C_2H_4$ $g^{-1}$ $h^{-1}$, respectively; those in the bulk soil were 1520.40 ± 68.94 nmol $g^{-1}$ $h^{-1}$, 3020.77 ± 545.72 nmol $g^{-1}$ $h^{-1}$, 0.97 ± 0.05 μ $g^{-1}$ $d^{-1}$, 609.33 ± 72.06 nmol $g^{-1}$ $h^{-1}$, 4223.90 ± 240.26 nmol $g^{-1}$ $h^{-1}$, and 0.36 ± 0.13 nmol $C_2H_4$ $g^{-1}$ $h^{-1}$, respectively. The activities of ACP, β-GC, and UE in the rhizosphere soil exceeded those in the bulk soil by factors of 1.04, 1.13, and 1.17, respectively; the activities of LAP, NAG, and NT in the bulk soil exceeded those in the rhizosphere soil by factors of 1.26, 2.17, and 1.69, respectively. There were significant differences in the ACP, NAG, LAP, UE, and NT activities between the rhizosphere soil and the bulk soil ($p < 0.01$), whereas there was no significant difference regarding β-GC activities ($p > 0.05$).

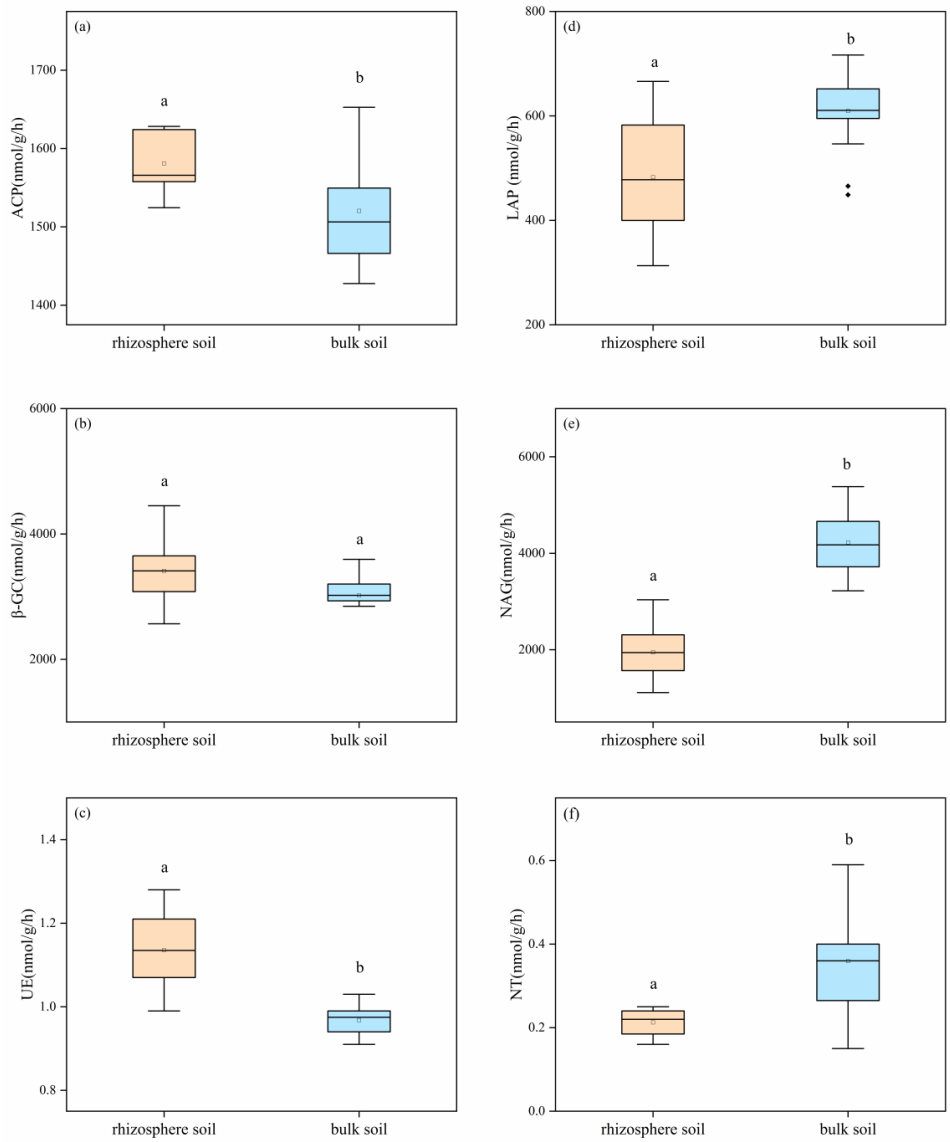


**Figure 1.** Enzyme activities in rhizosphere and bulk soils in a *Larix principis-rupprechtii* plantation. (**a**) Acid phosphatase (ACP); (**b**) β-glucosidase (β-GC); (**c**) N-acetyl-β-D-glucosidase (NAG); (**d**) aminopeptidase (LAP); (**e**) urease (UE); (**f**) nitrogenase activity (NT). Different letters represent significant differences between rhizosphere and bulk soil enzyme activities.

### *3.3. Stoichiometric Ratio and Vector Characteristics of Soil Enzymes*

Figure 2 shows the ratios of enzyme activities between the rhizosphere soil and bulk soil. The average of (NAG + LAP): ACP in the rhizosphere soil (1.54 ± 0.37) was significantly lower than that in the bulk soil (3.19 ± 0.47) ($p < 0.01$). β-GC: ACP (2.16 ± 0.47) and β-GC: (NAG + LAP) (1.48 ± 0.44) in the rhizosphere soil significantly exceeded those in the bulk soil (1.98 ± 0.33 and 0.63 ± 0.13, respectively) ($p < 0.01$).

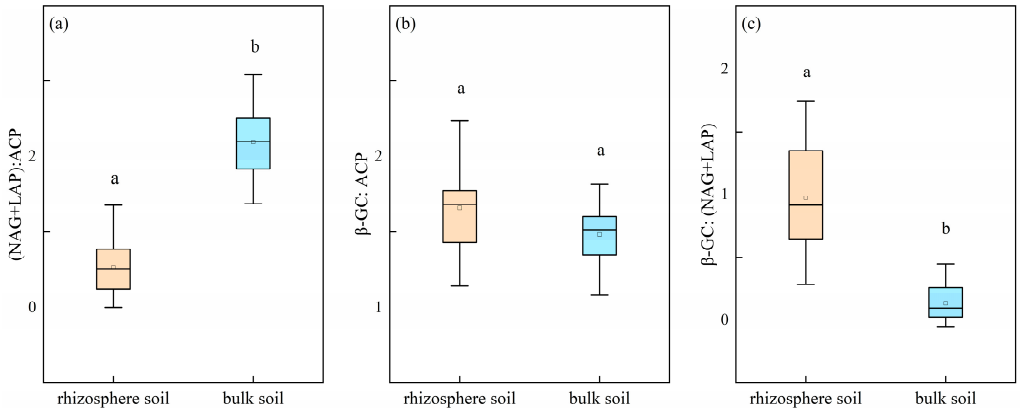


**Figure 2.** Enzyme activity ratios in the rhizosphere and bulk soils in a *Larix principis-rupprechtii* plantation. (**a**) (NAG + LAP): ACP represents the comparison of N and P acquisition activities; (**b**): β-GC: ACP represents the comparison of C and P acquisition activities; (**c**) β-GC: (NAG + LAP) represents the comparison of C and N acquisition activities. Different letters represent significant differences between rhizosphere and bulk soil enzyme activities. Abbreviations: NAG—N-acetyl-β-D-glucosidase; LAP—aminopeptidase; ACP—acid phosphatase; β-GC—β-glucosidase.

The natural logarithms of $EEA_{C:N}$, $EEA_{C:P}$, and $EEA_{N:P}$ in the rhizosphere soil were 0.59, 1.1, and 1.86, respectively (Figure 3a), whereas the mean ecoenzymatic C:N:P activity ratio was approximately 1:1.7:1; those of the bulk soil were 0.54, 1.09, and 2.01 (Figure 3b), respectively, with a mean ecoenzymatic C:N:P activity ratio of approximately 1:2:1.

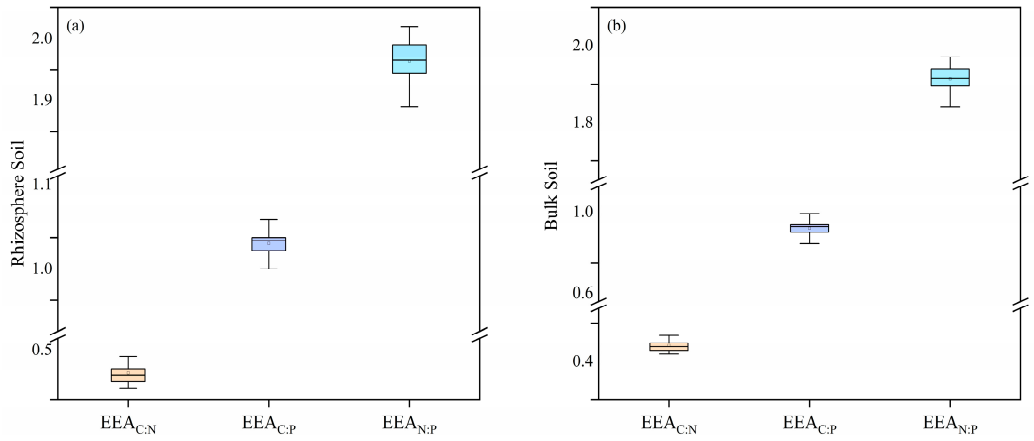


**Figure 3.** Natural logarithms of enzyme stoichiometric ratios in rhizosphere and bulk soils in a *Larix principis-rupprechtii* plantation. $EEA_{C:N}$ is the ratio between the logarithm of β-GC and the logarithm of (NAG + LAP); $EEA_{C:P}$ is the ratio between the logarithm of β-GC and the logarithm of ACP; and $EEA_{N:P}$ is the ratio between the logarithm of NAG + LAP and the logarithm of ACP. (**a**) for rhizosphere soil; (**b**) for bulk soil.

The average vector length of the rhizosphere soil exceeded that of the bulk soil ($p > 0.05$), indicating a greater C limitation in the former. The vector angles of the rhizosphere and bulk soils were both <45°, indicating the occurrence of N limitation in both soils, although to a greater degree in the bulk soil (Figure 4).

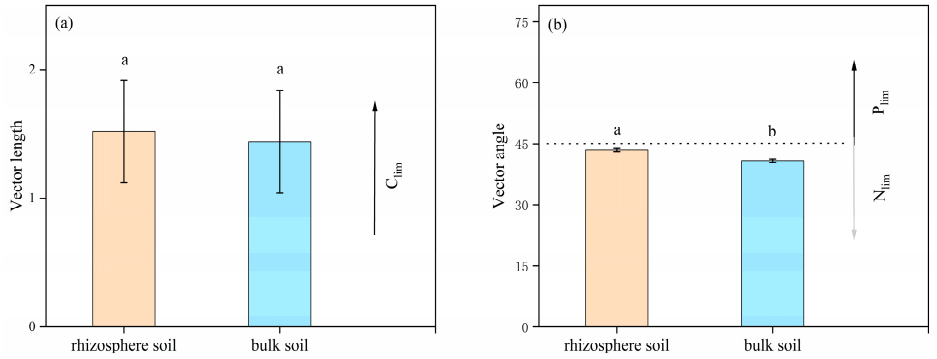


**Figure 4.** Vector characterization of enzymes in rhizosphere and bulk soils of a *Larix principis-rupprechtii* plantation. (**a**) for soil vector length, vector length represents the C limitation, C limitation increases with the vector length; (**b**) for soil vector angle, vector angle represents relative P and N limitations, A vector angle of >45° represents microbial P limitation, and that of <45° represents N limitation. Different letters represent significant differences between rhizosphere and bulk soil vector characterization of enzymes.

### *3.4. Factors Influencing Soil Enzyme Activity and Ecological Enzyme Stoichiometry*

The present study involved conducting an RDA analysis, in which soil enzyme activity and the stoichiometric ratio were set as response variables and rhizosphere soil nutrients and bulk soil nutrients were set as explanatory variables (Figure 5).

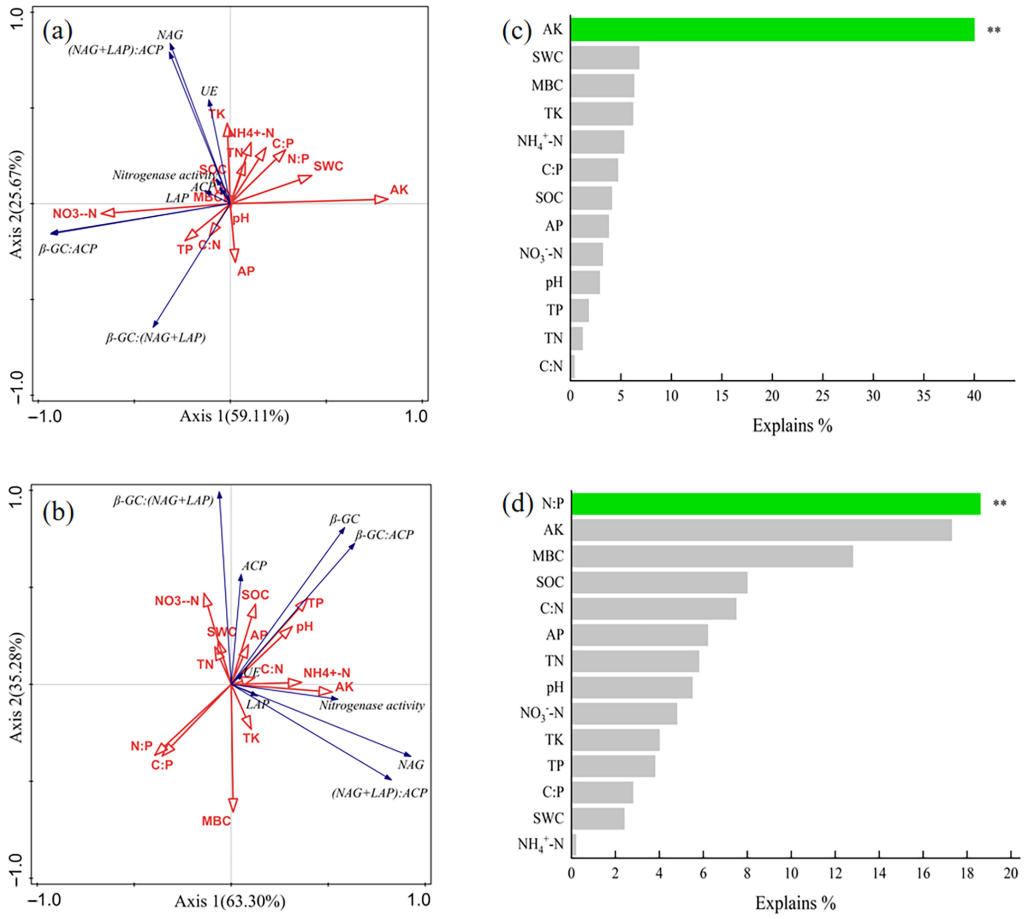


**Figure 5.** Redundant analysis diagram of the influence of soil nutrients on soil enzyme activity and stoichiometric ratios in a *Larix principis-rupprechtii* plantation. (**a**) for rhizosphere soil; (**b**) for bulk soil; (**c**) explanation of rhizosphere soil; (**d**) explanation of bulk soil. ** $p < 0.01$.

The results showed that environmental variables explained 84.78% of the observed variation in the soil enzyme activities in the rhizosphere soil, with the first and second axes explaining 59.11% and 25.67% of the observed variation, respectively. AK was the most explanatory variable, which explained 46.5% of the observed variation in the soil enzyme activities and enzyme stoichiometry in the rhizosphere soil ($p < 0.01$, Figure 5c).

Environmental variables explained 98.58% of the soil enzyme activities in the bulk soil, with the first and second axes explaining 63.30% and 35.28% of the observed variation, respectively. N:P was the most explanatory variable, which explained 18.7% of the observed variation in the soil enzyme activities and enzyme stoichiometry in the bulk soil ($p < 0.01$, Figure 5d).

## 4. Discussion

### *4.1. Characteristics of Nutrients and Enzyme Activities in Rhizosphere and Bulk Soils*

Most of the previous related studies used soil indicators, such as total C, TN, and TP, to show that the nutrients in rhizosphere soil exceeded those in bulk soil [50]. The results of the present study showed that, besides TK and pH, the nutrient indices of the rhizosphere soil exceeded those of the bulk soil (Table 2), demonstrating a clear positive rhizosphere effect (R/S > 1). Forest plants store the products of photosynthesis in the roots [32,36]; meanwhile, rhizosphere soils provide large quantities of organic carbon readily utilized by microorganisms, thereby increasing microbial biomass and activity in the rhizosphere microzone and accelerating microbial turnover. The results of the present study show that the MBC of the rhizosphere soil significantly exceeded that of the bulk soil. Plants distribute C to rhizosphere soil via the roots, and the presence of C in the rhizosphere promotes the microbial mineralization of organic N and P [51]. Consequently, the nutrients and microbial biomass of the rhizosphere soil exceed those of the bulk soil. The growth and mineralization of soil microorganisms are generally limited by C. Although the results of the present study showed that the SOC content of the rhizosphere soil exceeded that of the bulk soil, C limitation occurred in the rhizosphere soil, as was also confirmed by the results of the vector length analysis (Figure 4a). The results of the present study indicate an overall positive rhizosphere effect of soil nutrients in the studied *Larix principis-rupprechtii* plantation.

Xu et al. (2017), identified average soil β-GC, NAG, LAP, and ACP activities in nine different forest ecosystems to be 4460 ± 370 nmol $g^{-1}$ $h^{-1}$, 1930 ± 220 nmol $g^{-1}$ $h^{-1}$, 3590 ± 450 nmol $g^{-1}$ $h^{-1}$, and 1626 ± 177 nmol $g^{-1}$ $h^{-1}$ [40], respectively, consistent with the results of the present study (Figure 1). A study of a temperate coniferous and broad-leaved mixed natural secondary forest in northeast China by Chen et al. (2018b) found that the activities of β-GC, ACP, and NAG in rhizosphere soil exceeded those in bulk soil [39]. Brzostek et al. (2013) similarly determined that the activities of NAG and ACP in rhizosphere soil exceeded those in bulk soil [38]. However, inconsistent with previous studies, the results of the present study showed that the activities of LAP, NAG, and NT in bulk soil exceeded those in rhizosphere soil, whereas the opposite pattern was observed for ACP, β-GC, and UE. These results indicate that bulk soil and rhizosphere soil are limited by N and P, respectively. Under sufficient available nutrients (such as N), rhizosphere microorganisms prefer to obtain nitrogen via the decomposition of root exudates rather than via the decomposition of soil organic matter, since the former can be more easily decomposed [31]. This preference in turn reduces the need for the secretion of extracellular enzymes required for decomposing organic nitrogen, and represents one possible reason for the activities of NAG and LAP in bulk soil exceeding those in rhizosphere soil. However, the rhizosphere soil contained higher concentrations of TP and AP compared with the bulk soil, whereas the opposite pattern was observed for the soil C:P and N:P (Table 2). The global mean ratio of microbial biomass nitrogen (MBN) and microbial biomass phosphorus (MBP) was 6.7 in the topsoil [52]. However, in our study, the rhizosphere soil N:P was 10.65 (Table 2), which was higher than the soil MBN/MBP. This indicated that rhizosphere soil microorganisms were limited by soil P relative to N. Therefore, the rhizosphere soil microorganisms secrete more extracellular enzymes to obtain P (Figure 1). These results confirm that rhizosphere soil contains available C and N due to root exudation, whereas P

is limited within it. The present study hypothesized that root exudation in a *Larix principis-rupprechtii* plantation provided sufficient C and N to support rhizosphere microorganisms, but provided insufficient P, resulting in P limitation in the rhizosphere soil. Future studies should examine this hypothesis in greater detail.

### 4.2. Ecoenzymatic Stoichiometry of Enzymes in Rhizosphere and Bulk Soils

The stoichiometric ratio of soil enzymes on a global scale is relatively conservative at ±1:1:1 [11]. This result can principally be attributed to conservative microbial biomass C:N:P [53], which fluctuates in a relatively stable range, even if affected [53,54]. The present study determined the natural logarithms of $EEA_{C:N:P}$ in the rhizosphere and bulk soil of the *Larix principis-rupprechtii* plantation to be approximately 1:1.7:1 and 1:2.01:1, respectively, differing from the mean ratio of soil ecological enzymes on a global scale. Previous related studies have also demonstrated significant differences in enzyme activity between different localized areas due to the environment, biology, and other factors [19]. The results of the present study can be used to rank the soil enzymes of rhizosphere soil and bulk soil according to activity: (NAG + LAP) > β-GC > ACP. These results suggest relatively high enzyme activity related to N acquirement, indicating that the soil of the *Larix principis-rupprechtii* plantation was mainly limited by N. This result is consistent with the results of the vector angle analysis (Figure 4b) in the present study and with the results of most previous related studies, which have shown that nutrient cycling in temperate forests is mostly restricted by N [40,55]. Moreover, the N limitation in the bulk soil in the present study exceeded that in the rhizosphere soil, possibly because rhizosphere exudation plays a role in regulating the circulation and utilization of N in the rhizosphere microzone [37]. This is consistent with the results of soil total nitrogen and the soil N:P ratio in this study, in that they are greater in rhizosphere soil than in bulk soil, as seen in Table 2. This is because the exudates from plant roots tend to enhance N cycling by stimulating the microbial growth process, which can induce microbes to release extracellular enzymes that depolymerize SOM via priming effects [56,57]. The rhizosphere soil showed higher enzyme activity related to the N cycle compared with that in the bulk soil, leading to a significantly higher availability of $NH_4^+$ and $NO_3^-$ in the rhizosphere soil (Table 2). This result is consistent with the theory of microorganism resource allocation, which states that microorganisms prefer to increase the input of enzyme resources related to elements with low availability [58]. This result is also consistent with the "optimal allocation" model of ecological economics [59]. Certainly, *Larix principis-rupprechtii* seasonally drop needles, which may affect soil enzyme activity. Previous studies have suggested that litter has effects on some soil enzyme activities but no effects on other enzyme activities [60–63]. Therefore, the effects of fallen needles from *Larix principis-rupprechtii* should be studied in further work.

### 4.3. Key Factors Affecting Enzyme Activity and Their Stoichiometric Ratio in Rhizosphere and Bulk Soils

The results of the present study suggest that there are significant differences in the enzyme activity and stoichiometric ratio between rhizosphere soil and bulk soil in the studied *Larix principis-rupprechtii* plantation, and that different environmental factors affect the two soil types. Redundancy analysis showed that the main factors influencing soil enzyme activity in the bulk soil were soil N:P and SWC (Figure 5b). N:P was significantly negatively correlated with bulk soil enzyme activity and the enzyme stoichiometric ratio ($p < 0.01$), whereas SWC showed a significantly positive correlation ($p = 0.052$). A study of bulk soil enzyme activities in nine forests along the North–South Transect in eastern China by Xu et al. (2017) found that β-GC and NAG were significantly negatively correlated with soil N:P, consistent with the results of the present study. This result indicates that the regulation of soil N:P regarding soil enzyme activities occurs on different spatial scales. Under low available soil N and P, soil microorganisms increase the secretion of N- and P-related enzymes to meet their nutrient requirements [40], reflecting a trade-off between

soil nutrient conditions and microbial demand. Soil moisture has been shown to be an additional factor having an important effect on soil enzyme activity [64]. Similarly, the results of the present study show a significant positive correlation between SWC and soil enzyme activity and between SWC and the stoichiometric ratio in bulk soil. This is also consistent with the results of a study of a central subtropical forest in China [65]. Collectively, these results indicate that an increase in soil moisture can improve soil enzyme activity.

In general, rhizosphere soil had higher wetness than bulk soil. Roots have a huge absorption surface, increasing the moisture of rhizosphere soil [66,67]. Moreover, roots absorb more water to decompose SOM [68]. The results of the present study have also shown weak negative correlations between SWC and enzyme activity and between SWC and the stoichiometric ratio in rhizosphere soil (Figure 5a). Previous related studies have indicated the presence of a threshold in soil moisture, below which soil moisture shows a positive relationship with microbial enzyme activity [30]. However, an increase in soil moisture above this threshold can gradually lead to the formation of an anaerobic environment, resulting in a decline in enzyme activity. The results of the present study indicate an average SWC of the rhizosphere soil of 58.2%, which significantly exceeds that of the bulk soil, which was 39.2%. This higher SWC of the rhizosphere soil may have exceeded the soil moisture threshold, demonstrating its relationship with microbial activity. The results of the present study also show that soil AK had important influences on rhizosphere enzyme activity and the stoichiometric ratio. Interestingly, while the AK of the rhizosphere soil exceeded that of the bulk soil, the reverse relationship was found for TK. It can be hypothesized that plant roots and rhizosphere microorganisms are highly dependent on K. Future studies should investigate this dependency in greater detail. The differences in enzyme activities and influencing factors between rhizosphere soil and bulk soil indicate that roots play an important role in the relationship between microorganisms and soil.

## 5. Conclusions

The present study investigated the differences in nutrients, enzyme activities, and stoichiometric ratios between the bulk and rhizosphere soils of a *Larix principis-rupprechtii* plantation in the northern Yanshan Mountain, China. Consistent with our hypotheses, the C, N, and P in the rhizosphere soil were higher than in the bulk soil, indicating the significant effects of rhizosphere soil. However, not all of the soil enzyme activities were higher in the rhizosphere soil than in the bulk soil. The enzyme activities related to C and P acquisition in the rhizosphere soil exceeded those in the bulk soil, whereas the enzyme activities related to N acquisition in the bulk soil exceeded those in the rhizosphere soil. The natural logarithm of $EEA_{C:N:P}$ and the vector angles both indicated that the soil of the *Larix principis-rupprechtii* plantation was mainly limited by N, with that in the bulk soil exceeding that in the rhizosphere soil. The factors affecting enzyme activity and the stoichiometric ratio of the rhizosphere soil were shown to be different to those affecting the bulk soil, indicating that roots play an important role in the relationship between microorganisms and soil.

**Author Contributions:** Y.J. and Z.X. contributed to the design of this study. L.Y., J.M. and J.L. performed the experiment. L.Y., Q.L. and H.C. performed the statistical analysis. L.Y. wrote the first draft of the manuscript with contributions from all authors. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by the National Natural Science Foundation of China (31700377) and the Natural Science Foundation of Hebei (C2018204096).

**Data Availability Statement:** The original contributions presented in this study are included in this article; further inquiries can be directed to the corresponding author.

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

## References


1. Wu, X.D.; Zhao, L.; Fang, H.B. Soil Enzyme Activities in Permafrost Regions of the Western Qinghai-Tibetan Plateau. *Soil Boil. Biochem.* **2012**, *76*, 1280–1289. [CrossRef]
2. Burns, R.G. Enzyme activity in soil: Location and A Possible Role in Microbial Ecology. *Soil Boil. Biochem.* **1982**, *14*, 423–427. [CrossRef]
3. Huang, H.L.; Zong, N.; He, N.P.; Tian, J. Soil Enzyme Stoichiometric Characteristics at Different Altitudes in Alpine Meadow of Qinghai Tibet Plateau. *J. Appl. Ecol.* **2019**, *30*, 3689–3696.
4. Liu, C.H.; Ma, J.Y.; Qu, T.T.; Xue, Z.J.; Li, X.Y.; Chen, Q.; Wang, N.; Zhou, Z.C.; An, S.S. Extracellular Enzyme Activity and Stoichiometry Reveal Nutrient Dynamics during Microbially-Mediated Plant Residue Transformation. *Forests* **2022**, *14*, 34. [CrossRef]
5. Guan, S.Y. *Soil Enzymes and Their Research Methods*; Beijing Agricultural Press: Beijing, China, 1986; pp. 14–19.
6. Ladd, J.N. Origin and Range of Enzymes in Soil. In *Soil Enzymes*; Burns, R.G., Ed.; Academic Press: London, UK, 1978; pp. 51–96.
7. Tabatabai, M.A. Enzymes. In *Methods of Soil Analysis*; Weaver, R.W., Augle, S., Bottomly, P.J., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A., Eds.; Soil Science Society of America: Madison, WI, USA, 1994; pp. 775–833.
8. Jones, D.L.; Hodge, A.; Kuzyakov, Y. Plant and Mycorrhizal Regulation of Rhizodeposition. *New Phytol.* **2004**, *163*, 459–480. [CrossRef] [PubMed]
9. Bais, H.P.; Weir, T.L.; Perry, L.G.; Simon, G.; Jorge, M.V. The Role of Root Exudates in Rhizosphere Interactions with Plants and other Organisms. *Annu. Rev. Plant Biol.* **2006**, *57*, 233–266. [CrossRef]
10. Drake, J.E.; Darby, B.A.; Giasson, M.A.; Kramer, M.A.; Phillips, R.P.; Finzi, A.C. Stoichiometry Constrains Microbial response to Root Exudation-insights from a Model and a field Experiment in a Temperate Forest. *Biogeosciences* **2013**, *10*, 821–838. [CrossRef]
11. Sinsabaugh, R.L.; Lauber, C.L.; Weintraub, M.N.; Ahmed, W.B.; Allison, S.D.; Crenshaw, C.; Contosta, A.R.; Marcy, D.S.; Gallo, T.B.; Gartner, S.E.; et al. Stoichiometry of Soil Enzyme Activity at Global Scale. *Ecol. Lett.* **2008**, *11*, 1252–1264. [CrossRef]
12. Waring, B.G.; Weintraub, S.R.; Sinsabaugh, R.L. Ecoenzymatic Stoichiometry of Microbial Nutrient Acquisition in Tropical Soils. *Biogeochemistry* **2014**, *117*, 101–113. [CrossRef]
13. Tapia-Torres, Y.; Elser, J.J.; Souza, V.; García-Oliva, F. Ecoenzymatic Stoichiometry at the Extremes: How Microbes Cope in an Ultra-oligotrophic Desert Soil. *Soil Boil. Biochem.* **2015**, *87*, 34–42. [CrossRef]
14. He, Q.Q.; Wu, Y.H.; Bing, H.J.; Zhou, J.; Wang, J.P. Vegetation Type rather than Climate Modulates the variation in Soil Enzyme Activities and Stoichiometry in Subalpine Forests in the Eastern Tibetan Plateau Geoderma. *Geoderma* **2020**, *374*, 114424. [CrossRef]
15. Xiao, L.; Liu, G.B.; Xue, S.; Li, Q.; Xue, S. Ecoenzymatic Stoichiometry and Microbial Nutrient Limitation during Secondary Succession of Natural Grassland on the Loess Plateau, China. *Soil Till Res.* **2020**, *200*, 104605. [CrossRef]
16. Chen, H.; Li, D.; Zhao, J.; Zhang, W.; Xiao, K.; Wang, K. Nitrogen addition aggravates microbial carbon limitation: Ev-idence from ecoenzymatic stoichiometry. *Geoderma* **2018**, *329*, 61–64. [CrossRef]
17. Moorhead, D.L.; Sinsabaugh, R.L.; Hill, B.H.; Weintraub, M.N. Vector analysis of ecoenzyme activities reveal constraints on coupled C, N and P dynamics. *Soil Boil. Biochem.* **2016**, *93*, 1–7. [CrossRef]
18. Mori, T. Does ecoenzymatic stoichiometry really determine microbial nutrient limitations? *Soil Boil. Biochem.* **2020**, *146*, 107816. [CrossRef]
19. Sinsabaugh, R.L.; Hill, B.H.; Follstad, S.; Jennifer, J. Ecoenzymatic Stoichiometry of Microbial Organic Nutrient Acquisition in Soil and Sediment. *Nature* **2009**, *462*, 795–798. [CrossRef]
20. Sinsabaugh, R.L.; Follstad, S.; Jennifer, J. Ecoenzymatic Stoichiometry and Ecological Theory. *Annu. Rev. Ecol. Evol. Syst.* **2012**, *43*, 313–343. [CrossRef]
21. Peng, X.Q.; Wang, W. Stoichiometry of Soil Extracellular Enzyme Activity along a Climatic Transect in Temperate Grasslands of Northern China. *Soil Boil. Biochem.* **2016**, *98*, 74–84. [CrossRef]
22. Cui, Y.X.; Bing, H.; Fang, L.; Mao, J.; Shen, G.T.; Yu, J.L.; Wang, X.; Zhu, H.; Wu, Y.H.; Zhang, X.C. Extracellular Enzyme Stoichiometry reveals the Carbon and Phosphorus Limitations of Microbial Metabolisms in the Rhizosphere and Bulk Soils in Alpine Ecosystems. *Plant Soil* **2019**, *468*, 7–20. [CrossRef]
23. Zhang, W.; Xu, Y.D.; Gao, D.X.; Wang, X.; Liu, W.C.; Deng, J.; Han, X.H.; Yang, G.H.; Feng, Y.A.; Ren, G.X. Ecoenzymatic Stoichiometry and Nutrient Dynamics along a Revegetation Chronosequence in the Soils of abandoned Land and Robinia Pseudoacacia Plantation on the Loess Plateau, China. *Soil Boil. Biochem.* **2019**, *134*, 1–14. [CrossRef]
24. Yang, Y.; Liang, C.; An, S.S.; Cheng, H.; An, S.S.; Chang, S.X. Soil Extracellular Enzyme Stoichiometry reflects the Shift from P-to N-limitation of Microorganisms with Grassland Restoration. *Soil Boil. Biochem.* **2020**, *149*, 107928. [CrossRef]
25. Hartmann, A.; Rothballer, M.; Schmid, M. Lorenz Hiltner, A Pioneer in Rhizosphere Microbial Ecology and Soil Bacteriology Research. *Plant Soil* **2008**, *312*, 7–14. [CrossRef]
26. Gartner, T.B.; Treseder, K.K.; Malcolm, G.M.; Sinsabaugh, R.L. Extracellular Enzyme Activity in the Mycorrhizospheres of a Boreal Fire Chronosequence. *Pedobiologia* **2012**, *55*, 121–127. [CrossRef]
27. Neumann, G.; Römheld, V. Rhizosphere Chemistry in Relation to Plant Nutrition. In *Marschner's Mineral Nutrition of Higher Plants*; Academic Press: Cambridge, MA, USA, 2012; pp. 347–368.
28. Marschner, P. Rhizosphere biology. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Academic Press: Colac, Australia, 2012; pp. 369–388.

29. Ai, C.; Liang, G.; Sun, J.; Wang, X.; Zhou, W. Responses of Extracellular Enzyme Activities and Microbial Community in Both the Rhizosphere and Bulk Soil to Long-term Fertilization Practices in a Fluvo-aquic Soil. *Geoderma* **2012**, *173*, 330–338. [CrossRef]
30. Zhu, X.; Liu, M.; Kou, Y.; Liu, D.Y.; Liu, Q.; Zhang, Z.L.; Jiang, Z.; Yin, H.J. Differential Effects of N addition on the Stoichiometry of Microbes and Extracellular Enzymes in the Rhizosphere and Bulk Soils of an Alpine Shrubland. *Plant Soil* **2020**, *449*, 285–301. [CrossRef]
31. Cheng, W.; Kuzyakov, Y. Root Effects on Soil Organic Matter Decomposition. *Roots Soil Manag. Interact. Roots Soil* **2005**, *48*, 119–143.
32. Finzi, A.C.; Abramoff, R.Z.; Spiller, S.K.; Brzostek, E.R.; Darby, B.A.; Kramer, M.A.; Phillips, R.P. Rhizosphere Processes are Quantitatively Important Components of Terrestrial Carbon and Nutrient Cycles. *Gcb Bioenergy* **2015**, *21*, 2082–2094. [CrossRef]
33. Hinsinger, P.; Bengough, A.G.; Vetterlein, D.; Young, I.M. Rhizosphere: Biophysics, biogeochemistry and ecological relevance. *Plant Soil* **2009**, *321*, 117–152. [CrossRef]
34. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; Putten, V.D.; Wim, H. Going back to the Roots: The microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **2013**, *11*, 789–799. [CrossRef]
35. Zhao, Y.; Li, T.; Shao, P.; Sun, J.; Xu, W.; Zhang, Z. Variation in bacterial community structure in rhizosphere and bulk soils of different halophytes in the yellow River Delta. *Front. Ecol. Evol.* **2022**, *9*, 816918. [CrossRef]
36. Phillips, R.P.; Finzi, A.C.; Bernhardt, E.S. Enhanced Root Exudation induces Microbial Feedbacks to N Cycling in a Pine Forest Under long-term $CO_2$ Fumigation. *Ecol. Lett.* **2011**, *14*, 187–194. [CrossRef]
37. Avazpoor, Z.; Moradi, M.; Basiri, R.; Mirzaei, J.; Taghizadeh-Mehrjardi, R.; Kerry, R. Soil Enzyme Activity variations in Riparian Forests in relation to Plant Species and Soil Depth. *Arab. J. Geosci.* **2019**, *12*, 708. [CrossRef]
38. Brzostek, E.R.; Greco, A.; Drake, J.E.; Finzi, A.C. Root Carbon inputs to the Rhizosphere stimulate Extracellular Enzyme Activity and increase Nitrogen Availability in Temperate Forest Soils. *Biogeochemistry* **2013**, *115*, 65–76. [CrossRef]
39. Chen, X.; Ding, Z.J.; Tang, M.; Zhou, B. Greater Variations of Rhizosphere Effects within Mycorrhizal Group than between Mycorrhizal Group in a Temperate Forest. *Soil Boil. Biochem.* **2018**, *126*, 237–246. [CrossRef]
40. 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. *Soil Boil. Biochem.* **2017**, *104*, 152–163. [CrossRef]
41. Cui, Y.X.; Fang, L.C.; Guo, X.B.; Wang, X.; Zhang, Y.J.; Lia, P.F.; Zhang, X.C. Ecoenzymatic Stoichiometry and Microbial Nutrient Limitation in Rhizosphere Soil in the Arid Area of the Northern Loess Plateau, China. *Soil Boil. Biochem.* **2018**, *116*, 11–21. [CrossRef]
42. Sun, X.; Ye, Y.; Liao, J.; Soromotin, A.V.; Smirnov, P.V.; Kuzyakov, Y. Organic Mulching Increase Microbial Activity in Urban Forest Soil. *Forests* **2022**, *13*, 1352. [CrossRef]
43. Phillips, R.P.; Fahey, T.J. The Influence of Soil Fertility on Rhizosphere Effects in Northern Hardwood Forest Soils. *Soil Sci. Soc. Am. J.* **2008**, *72*, 453–461. [CrossRef]
44. Qiu, X.; Peng, D.; Tian, H.; Wang, H.; Liu, X.; Cao, L.; Li, Z.; Cheng, S. Soil ecoenzymatic stoichiometry and microbial resource limitation driven by thinning practices and season types in *Larix principis-rupprechtii* plantations in North China. *For. Ecol. Manag.* **2021**, *482*, 118880. [CrossRef]
45. Bao, S.D. *Soil and Agricultural Chemistry Analysis*; China Agricultural Press: Beijing, China, 2000; pp. 26–114.
46. Chen, H.; Li, D.J.; Xiao, K.C.; Wang, K.L. Soil microbial processes and resource limitation in karst and non-karst forests. *Funct. Ecol.* **2018**, *32*, 1400–1409. [CrossRef]
47. Patra, A.K.; Abbadie, L.; Clays-Josserand, A.; Degrange, V.; Grayston, S.J.; Guillaumaud, N.; Loiseau, P.; Louault, F.; Mahmood, E.; Nazaret, S.; et al. Effects of Management Regime and Plant Species on the Enzyme Activity and Genetic Structure of N-fixing, Denitrifying and Nitrifying Bacterial Communities in Grassland Soguils. *Environ. Microbiol.* **2006**, *8*, 1005–1016. [CrossRef] [PubMed]
48. Keeler, B.L.; Hobbie, S.E.; Kellogg, L.E. Effects of long-term nitrogen addition on microbial enzyme activity in eight forested and grassland sites: Implications for litter and soil organic matter decomposition. *Ecosystems* **2009**, *12*, 1–15. [CrossRef]
49. Fanin, N.; Moorhead, D.; Bertrand, I. Eco-enzymatic stoichiometry and enzymatic vectors reveal differential C, N, P dynamics in decaying litter along a land-use gradient. *Biogeochemistry* **2016**, *129*, 21–36. [CrossRef]
50. Dai, X.Q.; Fu, X.L.; Kou, L.; Wang, H.M.; Shock, C.C. C:N:P Stoichiometry of Rhizosphere Soils Differed Significantly among Overstory Trees and Understory Shrubs in Plantations in Subtropical China. *Can. J. For. Res.* **2018**, *48*, 1398–1405. [CrossRef]
51. Meier, I.C.; Pritchard, S.G.; Brzostek, E.R.; McCormack, M.L.; Phillips, R.P. The Rhizosphere and Hyphosphere Differ in their Impacts on Carbon and Nitrogen Cycling in Forests exposed to elevated $CO_2$. *New Phytol.* **2015**, *205*, 1164–1174. [CrossRef]
52. Gao, D.; Bai, E.; Wang, S.; Zong, S.; Liu, Z.; Fan, X.; Zhao, C.; Hagedorn, F. Three-dimensional mapping of carbon, nitrogen, and phosphorus in soil microbial biomass and their stoichiometry at the global scale. *Glob. Change Biol.* **2022**, *28*, 6728–6740. [CrossRef]
53. 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]
54. Wojciech, P.; Ewa, B.; Jaroslaw, L.; Lukac, M. A Comparison of C:N:P Stoichiometry in Soil and Deadwood at An advanced Decomposition Stage. *Catena* **2019**, *179*, 1–5.
55. Wardle, D.A.; Walker, L.R.; Bardgett, R.D. Ecosystem Properties and Forest decline in Contrasting long-term Chronosequences. *Science* **2004**, *305*, 509–513. [CrossRef]

56. Cheng, W.X.; Parton, W.J.; Gonzalez-Meler, M.A.; Phillips, R.P.; Asao, S.; McNickle, G.G.; Brzostek, E.; Jastrow, J.D. Synthesis and modeling perspectives of rhizosphere priming. *New Phys.* **2014**, *201*, 31–44. [CrossRef]
57. Meier, I.C.; Finzi, A.C.; Phillips, R.P. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. *Soil Boil. Biochem.* **2017**, *106*, 119–128. [CrossRef]
58. Sinsabaugh, R.L.; Moorhead, D.L. Resource Allocation to Extracellular Enzyme Production: A Model for Nitrogen and Phosphorus Control of Litter Decomposition. *Soil Boil. Biochem.* **1994**, *26*, 1305–1311. [CrossRef]
59. Treseder, K.K.; Vitousek, P.M. Effects of Soil Nutrient Availability on Investment in Acquisition of N and P in Hawaiian rain Forests. *Ecology* **2001**, *82*, 946–954. [CrossRef]
60. Zhao, Q.; Classen, A.T.; Wang, W.W.; Zhao, X.R.; Mao, B.; Zeng, D.H. Asymmetric effects of litter removal and litter addition on the structure and function of soil microbial communities in a managed pine forest. *Plant Soil* **2017**, *414*, 81–93. [CrossRef]
61. Liu, R.; Zhang, Y.; Hu, X.F.; Wan, S.; Wang, H.; Liang, C.; Chen, F.S. Litter manipulation effects on microbial communities and enzymatic activities vary with soil depth in a subtropical Chinese fir plantation. *For. Ecol. Manag.* **2021**, *480*, 118641. [CrossRef]
62. Wang, L.; Deng, D.; Feng, Q.; Xu, Z.; Pan, H.; Li, H. Changes in litter input exert divergent effects on the soil microbial community and function in stands of different densities. *Sci. Total Environ.* **2022**, *845*, 157297. [CrossRef]
63. Ai, L.; Wu, F.; Fan, X.; Yang, Y.; Zhang, Y.; Zheng, X.; Zhu, J.; Ni, X. Different effects of litter and root inputs on soil enzyme activities in terrestrial ecosystems. *Appl. Soil Ecol.* **2023**, *183*, 104764. [CrossRef]
64. Li, X.; Zhang, Y.; Ding, C.; Liu, Y.; Wu, K.; Jiang, F.; Su, D. Water addition promotes vegetation recovery of degraded alpine meadows by regulating soil enzyme activity and nutrients in the Qinghai–Tibetan Plateau. *Ecol. Eng.* **2020**, *158*, 106047. [CrossRef]
65. Shi, L.J.; Wang, H.M.; Fu, X.L.; Kou, L.; Meng, S.W.; Dai, X.Q. Soil Enzyme Activities and their Stoichiometric Characteristics of Typical Plantations in the Middle Subtropical Zone. *J. Appl. Ecol.* **2020**, *31*, 1980–1988.
66. Montagnoli, A.; Di Iorio, A.; Terzaghi, M.; Trupiano, D.; Scippa, G.S.; Chiatante, D. Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. *Eur. J. For. Res.* **2014**, *133*, 957–968. [CrossRef]
67. Coners, H.; Leuschner, C. In situ measurement of fine root water absorption in three temperate tree species-temporal variability and control by soil and atmospheric factors. *Basic Appl. Ecol.* **2005**, *6*, 395–405. [CrossRef]
68. Kuzyakov, Y. Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* **2002**, *165*, 382–396. [CrossRef]