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Effects of Post-Thinning Precipitation on Soil Acid Phosphomonoesterase Activity in *Larix principis-rupprechtii* Mayr. Plantations

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Abstract: Soil phosphorus (P) is one of the essential macronutrients for plant growth. Phosphatase-mediated P mineralization in particular is critical for the biogeochemical cycling of P, and its activity reflects the organic P (P_o) mineralization potential in soils. In recent years, global climate change has led to changes in precipitation, which inevitably has affected the P cycle as well. To study these effects of precipitation on soil acid phosphomonoesterase (AcPME) activity, the following combined thinning and precipitation treatments were conducted across *Larix principis-rupprechtii* Mayr. plantations in China: control (CK), light (LT), moderate (MT), and high thinning (HT). The precipitation treatments included natural precipitation (NP), 30% reduced precipitation (RP30), and 60% reduced precipitation (RP60). Soil moisture, microbial biomass carbon (MBC), and soil P fractions were also determined to link their effects on soil AcPME. The results show that soil AcPME activity was significantly higher in the rainy season, which is associated with higher microbial activity and increased P demand, than in the dry season. Generally, soil AcPME activity was found to increase with thinning intensity. In the dry season, the NP treatment was more conducive to improving soil AcPME activity. In the rainy season, the RP60 treatment inhibited soil AcPME activity under all thinning treatments. The RP30 treatment was only found to offer a significant boost for MT. These results indicate that the potential transformation rate of P_o may be more dependent on water in the dry season than in the rainy season. If drought occurs, the P_o mineralization rate would decrease for all *L. principis-rupprechtii* plantations, but excessive rainfall in the rainy season would also impact the turnover of P_o into MT adversely.

Keywords: precipitation; thinning; acid phosphomonoesterase; *Larix principis-rupprechtii* Mayr

1. Introduction

Soil phosphorus (P) occurs in both inorganic (P_i) and organic (P_o) forms, and consists of compounds with varying degrees of solubility and bioavailability [1]. Most soil P is bound in detritus as P_o and remains inaccessible to plants. Apart from the weathering of parent material in soils [2,3], P input depends on the mineralization of soil organic matter [4,5], soil-nesting insect pollinators [6], and soil-dwelling arthropods [7], who alter soil microbial communities and their function associated with P mineralization by altering frass and waste inputs, and by selectively consuming plant materials. In addition, P inputs depends on the plant chemistry and their chemical composition [8]. Phosphatases, which originate from fungi, bacteria, and root exudates, catalyze the hydrolysis of ester bonds between phosphate and carbon compounds in organic substrates to enhance P availability to ecosystems [9,10]. The production of phosphatase increases when the demand for P increases in forest ecosystems [11]. These enzymes therefore play an important role in maintaining and controlling the rate of P cycling in

forest ecosystems [12]. Of the phosphatases, phosphomonoesterases are the most widely studied [13]. Phosphomonoesterase activity provides useful information on the P_o mineralization potential and biological activity in soils [14]. Usually, acid phosphomonoesterase (AcPME) prevails in acidic soils whereas alkaline phosphomonoesterase prevails in alkaline soil [15].

Global climate change models have predicted that precipitation patterns will undergo major changes in certain geographic regions in the second half of the 21st Century. In particular, precipitation is expected to decrease in northern China [16]. These predicted changes will greatly alter the soil moisture dynamics [17], which may in turn affect the dynamics of the soil P cycle [18,19]. Soil moisture is a key factor controlling soil P availability via several processes including P_o mineralization [20], the demand of P for plant growth, and microbial activity [12,21]. For example, in the Mediterranean region, soil phosphatase activity was reduced during an increasingly strong drought and the P available for uptake by plants also decreased, which resulted in a significant limitation for plant growth [22,23]. Sardans and Peñuelas [24] found that a 21% reduction of soil moisture resulting from runoff and rainfall exclusion lowered AcPME activity by 31%–40%. Similarly, a six-year drought manipulation experiment where soil moisture was lowered by 13% to 29% showed that water stress results in reduced microbial activity; soil AcPME activity was lowered by 22% to 27%. At the same time, drought augmented the availability of organic extractable P [25]. Therefore, changes in precipitation are likely to affect the rate of P turnover and soil P availability, but droughts may offer benefits too. Because soil AcPME is associated with P cycling in forest ecosystems, it is a good measure of how changes in precipitation affect the P supply overall [26]. However, to our best knowledge, the effects of precipitation on soil AcPME activity have been unstudied in warm temperate forests. Understanding how soil phosphatase activity responds to drought events will help inform understanding of future soil dynamics and aid in developing proper silvicultural guidelines in this new global change context [27].

In the warm temperate zone, *Larix principis-rupprechtii* Mayr. is one of the typical afforestation species [28]. The high initial planting density and the management lag have resulted in incredibly dense canopies in existing stands, which reduces soil fertility and forest quality with low yield and efficiency [29]. Reasonable thinning can promote improve the light, water, temperature and soil nutrient availability conditions in forests, thereby improving forest productivity [30]. These *L. principis-rupprechtii* plantations provide an excellent opportunity to study soil P cycling at different thinning intensities. Previous studies have shown that soil P availability varies with the degree of thinning, with maximum P availability under moderately thinned (MT) plantations [31]. In this study, we aim to study how soil AcPME activity responds to precipitation in plantations having different P availabilities.

We conducted a field experiment to apply various precipitation treatments (natural precipitation (NP), 30% reduced precipitation (RP30) and 60% reduced precipitation (RP60)) across *L. principis-rupprechtii* plantations following different thinning regiments to test the hypotheses: (1) Regarding the influence of seasonal precipitation, soil AcPME activity will be greater during the rainy season than the dry season; (2) Plantations having different thinning regiments will differ in their response to the precipitation treatments, but soil AcPME activity will be greatest in MT plantations; (3) Precipitation treatments will have different effects on soil AcPME activity during the dry and rainy seasons.

2. Materials and Methods

2.1. Site Description and Experimental Design

The study was conducted at Mt. Taiyue in Shanxi, North China (36°31′–36°43′ N, 112°01′–112°15′ E; elevation 2273–2359 m.a.s.l.). The area has a semi-humid temperate monsoon climate, consisting of cold, dry winters and hot, rainy summers. The mean annual temperature is about 8.7 °C, with a minimum monthly average temperature of −10.4 °C in January and a maximum monthly average temperature of 17.4 °C in July. The average frost-free period lasts 125 days, with an early frost in October and a late frost in April. The mean annual precipitation ranges from 600 mm to 650 mm, of which

more than 60% falls from May to October (rainy season) and less than 40% falls between November and April (dry season). The soil type is Alfisol, according to the U.S. soil classification system [32]. The zonal vegetation is a temperate deciduous broad-leaved forest, and the dominating tree species are *Betula platyphylla* Suk., *Quercus wutaishanica* Mayr., *Pinus tabulaeformis* Carr. and *L. principis-rupprechtii*.

In spring 1982, several three-year-old *L. principis-rupprechtii* seedlings were planted at a density of 3000 trees ha⁻¹. The seedlings were planted along contour lines in the mountainous areas of the bush vegetation, following forestry guidelines used to meet timber demands. In April 2010, thinning management treatments were carried out for entire stands on *L. principis-rupprechtii* plantations to lower the density to 2160 trees ha⁻¹. After thinning, twelve 25 m × 25 m plots were set up at a distance of at least 10 m apart in order to avoid edge effects. In March 2012, the twelve plots were randomly assigned to four distinct treatments of secondary thinning: light thinning (LT, thinned to 1835 trees ha⁻¹), moderate thinning (MT, thinned to 1413 trees ha⁻¹), high thinning (HT, thinned to 1086 trees ha⁻¹), and the control (CK, unthinned). Thus, this study was conducted in a completely randomized design with three replications per treatment. Detailed information on these four thinning treatments can be found in Table S1 [31].

In April 2016, within each of the 12 plots, we randomly established three precipitation treatments, including natural precipitation (NP), 30% reduced precipitation (RP30), and 60% reduced precipitation (RP60) with three replicate subplots (3 m × 3 m) for each treatment. In the reduced precipitation (RP) subplots, a 3.5 m × 9 cm concave transparent polyvinyl chloride (PVC) plate was evenly installed over which a rain reduction frame was made in series with iron wire to intercept the precipitation. The gap between the transparent plates of the RP30 subplot was 21 cm, while the gap for the RP60 subplot was 6 cm. The rain reduction frame was placed ~80–100 cm above the ground to prevent rainwater from entering the subplots, and the corresponding area of the subplot was uniformly covered to form a rainproof surface. To prevent surface runoff and lateral movement of moisture in the surrounding soil, thick PVC plates were inserted into the ground to a depth of 15 cm around each RP subplot. The control subplots, receiving natural precipitation, were built beside these RP subplots. The litter that fell onto the rain reduction frames of RP subplots was removed manually and periodically sprinkled onto the soil below the plates.

2.2. Soil Sampling

We conducted soil sampling in April (the dry season) and August (the rainy season) of 2018. Nine soil cores (three random points per subplot from each three replicate subplots) at 0–10 cm (2.5 cm diameter) were collected for each precipitation treatment in each plot and mixed to form a composite soil sample. Soil samples transferred into sealed plastic bags and immediately taken to the laboratory. After removing roots and plant residues, the composite sample was sieved through a 2-mm mesh sieve and divided into two parts. One was immediately stored at 4 °C for measuring soil AcPME activity and microbial biomass carbon (MBC). The other was air-dried and further sieved to 0.25 mm for chemical property analysis of soil total carbon (TC), total nitrogen (TN) and P fractionation, and through a 2-mm sieve for soil pH analysis.

2.3. Soil Analysis

Soil moisture of the top 10 cm was measured twice a month within a subplot using a Theta Probe ML2X with an HH2 moisture meter (Delta-T Devices, Cambridge, England). Soil pH value was measured in an aqueous extract (1:2.5 soil:water) with a pH-Meter (pH-10, Sartorius, Göttingen, Germany). Soil TC and TN were determined by dry combustion with an elemental analyzer (FLASH2000 CHNS/O, Thermo, Third Avenue, Waltham, MA, USA). Soil MBC was determined using the chloroform fumigation extraction technique [33]: a 5-g quantity (dry weight equivalent) of soil was fumigated for 24 h at 25 °C. Organic C from fumigated and non-fumigated (control) soil samples extracted with 0.5 M K₂SO₄ and quantified with a total organic carbon analyzer (Multi N/C 3000, Analytik Jena AG, Konrad-Zuse-Straße, Berlin, Germany). Then non-fumigated values were subtracted from fumigated

values and MBC was calculated using a k_{EC} factor of 0.45 [34]. Soil AcPME activity was assayed via the standard method of Tabatabai and Bremner [35]. Briefly, 1 g of fresh soil was well mixed with modified universal buffer (MUB, pH 6.5) and substrate (*p*-nitrophenyl phosphate) solution and then incubated at a temperature of 37 °C for 1 h. Then the amount of *p*-nitrophenol release was measured spectrophotometrically.

The sequential extraction procedure developed by Hedley, et al. [36] and modified by Tiessen and Moir [37] was used to assess soil P fractions. In general, 0.5 g of air-dried soil was sequentially extracted with an anion exchange resin membrane (defined as R- P_i fraction), 0.5 M $NaHCO_3$ (Bic- P_i and Bic- P_o fractions), 0.1 M NaOH (OH- P_i and OH- P_o fractions), 1.0 M HCl (Dil.HCl- P_i fraction) and hot concentrated HCl (Conc.HCl- P_i and Conc.HCl- P_o fractions). Soil residue was digested with concentrated H_2SO_4 and H_2O_2 (Res- P_i fraction). All extraction solutions were measured using a modified phosphomolybdate blue method [38].

2.4. Statistical Analysis

Both main effects and interactive effects of thinning and season on soil properties were tested using repeated-measures analysis of variance (ANOVA). Differences were examined between the dry and rainy seasons for soil moisture, soil properties and soil AcPME activity using the *t*-test for independent samples. One-way ANOVA, followed by Tukey's multiple comparisons post hoc test at $p < 0.05$ level, was used to evaluate the differences in soil moisture, soil properties and soil AcPME activity among the NP treatments in types of thinned plantations; and the effects of precipitation intensity on dependent variables among the treatments separately for each season and thinning. Correlations between soil property and AcPME activity were assessed using Pearson's correlation coefficients. All data were checked for normality and homoscedasticity prior to performing the statistical analyses and were log-transformed when necessary to correct for deviations from these assumptions. P fractions were log-transferred to normalize data distribution. Analyses were performed using SPSS, version 21.0 (IBM, Chicago, IL, USA).

3. Results

3.1. Soil Moisture

In all plantations, the mean soil moisture was significantly greater in the rainy season (May to October) than in the dry season (November to April) (Figure 1). For the NP treatment, annual mean soil moisture was relatively higher in MT and HT than in LT and CK. Specifically, soil moisture increased in MT ($p = 0.029$) and HT ($p = 0.048$) in the dry season more than CK (Figure S1). The values of mean annual soil moisture across all thinning treatments were 26.1% for NP, 23.5% for RP30, and 20.5% for RP60. Compared to NP, the RP60 soil moisture decreased significantly (Figure S2).

Figure 1. Seasonal dynamics of soil moisture under different precipitation treatments from January to December 2018 in the control (a), lightly thinned (b), moderately thinned (c), and highly thinned (d) *L. principis-rupprechtii* plantations. NP, natural precipitation; RP30, 30% reduced precipitation; RP60, 60% reduced precipitation.

3.2. Soil Chemical and Microbial Properties

In the dry season, there were no significant differences in pH, TC, and TN among the three precipitation treatments. NP had greater TP ($p = 0.021$) and MBC ($p = 0.015$) than RP60 under CK and MT respectively (Table 1). The concentrations of Bic-P_o and Conc.HCl-P_o decreased as the amount of precipitation was reduced, and accordingly they were significantly higher under the NP treatment than the RP60 treatment, except for in the HT (Table 2). In the rainy season, the RP60 treatment significantly lowered soil pH in LT to a greater extent than the NP treatment did (Table 1). Concentrations of TP, Bic-P_o, OH-P_o, and Conc.HCl-P_o were almost always lower in the RP60 treatment than the NP treatment (Tables 1 and 2). The only exceptions to this trend were samples collected in MT (Bic-P_o) and HT (Conc.HCl-P_o). Most soil properties were significantly correlated with soil AcPME, except for TC ($p = 0.194$) and Bic-P_i ($p = 0.678$) (Table 3).

Table 1. Soil properties in the dry and rainy seasons and their responses to the various precipitation and thinning treatments in *L. principis-rupprechtii* plantations.

Season	Thinning	Precipitation	pH	TC(g kg ⁻¹)	TN(g kg ⁻¹)	TP(g kg ⁻¹)	MBC(mg kg ⁻¹)
Dry	CK	NP	6.4 ± 0.2	43.7 ± 3.7	3.7 ± 0.4	0.34 ± 0.01	345.0 ± 2.2 a
		RP30	6.0 ± 0.0	66.1 ± 11.7	5.2 ± 1.0	0.29 ± 0.00	273.4 ± 12.6 ab
		RP60	6.0 ± 0.1	44.1 ± 1.1	3.4 ± 0.1	0.32 ± 0.02	231.4 ± 31.3 b
		<i>p</i>	0.113	0.112	0.189	0.062	0.017
	LT	NP	6.3 ± 0.1	38.8 ± 2.2	3.1 ± 0.2	0.41 ± 0.03	343.2 ± 7.3
		RP30	5.7 ± 0.1	39.7 ± 5.2	3.7 ± 0.1	0.36 ± 0.01	349.2 ± 108.8
		RP60	6.2 ± 0.4	41.0 ± 1.5	3.7 ± 0.3	0.37 ± 0.00	331.7 ± 76.1
		<i>p</i>	0.290	0.898	0.196	0.130	0.987
	MT	NP	6.1 ± 0.2	53.4 ± 0.7	4.8 ± 0.2	0.63 ± 0.04 a	389.2 ± 13.5
		RP30	5.9 ± 0.1	63.3 ± 4.8	5.0 ± 0.4	0.53 ± 0.04 ab	355.1 ± 23.0
		RP60	5.8 ± 0.0	56.8 ± 3.3	4.6 ± 0.2	0.44 ± 0.02 b	348.1 ± 19.0
		<i>p</i>	0.207	0.188	0.565	0.025	0.326
	HT	NP	6.1 ± 0.0	54.8 ± 4.8	4.9 ± 0.5	0.46 ± 0.04	383.1 ± 14.5
		RP30	5.7 ± 0.1	58.0 ± 0.8	4.4 ± 0.1	0.43 ± 0.02	425.6 ± 37.6
		RP60	5.8 ± 0.1	59.5 ± 8.1	4.7 ± 0.5	0.42 ± 0.02	338.5 ± 37.2
		<i>p</i>	0.065	0.825	0.769	0.712	0.230
Rainy	CK	NP	6.1 ± 0.0	48.2 ± 4.0	2.0 ± 0.1	0.51 ± 0.00 a	649.9 ± 12.3
		RP30	6.2 ± 0.1	45.1 ± 5.0	2.2 ± 0.2	0.44 ± 0.01 b	659.2 ± 60.5
		RP60	6.2 ± 0.1	47.2 ± 4.1	2.0 ± 0.2	0.38 ± 0.01 c	624.8 ± 33.9
		<i>p</i>	0.760	0.878	0.736	< 0.001	0.831
	LT	NP	6.1 ± 0.1 a	39.4 ± 3.1	3.2 ± 0.4	0.57 ± 0.02 a	537.8 ± 5.9
		RP30	5.9 ± 0.0 ab	45.2 ± 1.3	3.6 ± 0.1	0.47 ± 0.02 b	561.7 ± 86
		RP60	5.8 ± 0.0 b	43.2 ± 0.4	3.4 ± 0.1	0.46 ± 0.02 b	650.7 ± 41.7
		<i>p</i>	0.045	0.174	0.601	0.007	0.376
	MT	NP	6.1 ± 0.0	58.0 ± 4.8	2.3 ± 0.3	0.65 ± 0.00 a	614.4 ± 16.3
		RP30	6.3 ± 0.1	60.5 ± 3.7	2.4 ± 0.2	0.53 ± 0.01 b	621.4 ± 44.5
		RP60	6.3 ± 0.1	57.2 ± 2.3	2.2 ± 0.2	0.53 ± 0.02 b	754.1 ± 72.8
		<i>p</i>	0.361	0.813	0.874	0.001	0.166
	HT	NP	6.2 ± 0.2	45.4 ± 2.8	2.6 ± 0.5	0.59 ± 0.02 a	694.3 ± 33.3
		RP30	6.1 ± 0.2	57.2 ± 3.7	2.9 ± 0.2	0.47 ± 0.02 b	616.2 ± 27.0
		RP60	6.0 ± 0.1	54.4 ± 2.1	2.7 ± 0.2	0.53 ± 0.02 ab	719.8 ± 10.9
		<i>p</i>	0.664	0.068	0.809	0.030	0.070

CK, control site; LT, light thinning; MT, moderate thinning; HT, high thinning. NP, natural precipitation; RP30, 30% reduced precipitation; RP60, 60% reduced precipitation. TC, total carbon; TN, total nitrogen; TP, total phosphorus; MBC, microbial biomass carbon. Data (mean ± standard error, *n* = 3) followed by different letters within a column differ significantly according to Tukey's test (*p* < 0.05). When the difference is not statistically significant, no letter notation is given.

Table 2. Soil phosphorus fractions in the dry and rainy seasons under different precipitation and thinning treatments in *L. principis-rupprechtii* plantations.

Season	Thinning	Precipitation	R-P _i	Bic-P _i	Bic-P _o	OH-P _i	OH-P _o	Dil.HCl-P _i	Conc.HCl-P _i	Conc.HCl-P _o	Res-P _i
mg kg ⁻¹											
Dry	CK	NP	6.5 ± 0.1	7.7 ± 0.5	5.5 ± 0.5 a	10.8 ± 1.6	76.8 ± 5.4	38.8 ± 2.2 a	46.7 ± 2.8 a	83.8 ± 1.0 a	61.0 ± 3.7
		RP30	7.1 ± 1.1	7.8 ± 0.7	3.1 ± 0.1 b	11.3 ± 0.2	65.7 ± 3.4	25.2 ± 1.1 b	29.9 ± 1.6 b	71.1 ± 1.1 b	64.2 ± 4
		RP60	6.9 ± 0.5	6.7 ± 0.0	3.0 ± 0.1 b	12.5 ± 1.1	87.7 ± 10.2	35.1 ± 1.1 a	54.5 ± 3.8 a	45.2 ± 3.1 c	64.2 ± 8
		<i>p</i>	0.850	0.323	0.002	0.577	0.163	0.002	0.002	< 0.001	0.900
	LT	NP	6.1 ± 1.0	7.0 ± 1.1	14.1 ± 0.2 a	11.8 ± 2.5	87.6 ± 7.4	40.0 ± 5.7	43.6 ± 3.1	114.8 ± 9.5 a	85.5 ± 21.2
		RP30	10.5 ± 0.6	7.5 ± 0.3	15.9 ± 1.5 a	11.6 ± 0.8	77.6 ± 4.3	34.8 ± 2.4	38.3 ± 1.6	66.2 ± 6.2 b	86.8 ± 0.5
		RP60	9.3 ± 1.7	7.7 ± 1.2	7.0 ± 1.4 b	12.8 ± 0.5	92.3 ± 2.2	37.4 ± 2.7	43.6 ± 1.7	59.3 ± 3.0 b	97.8 ± 7.4
		<i>p</i>	0.087	0.894	0.004	0.839	0.199	0.654	0.236	0.002	0.771
	MT	NP	13.4 ± 0.9	11.6 ± 2.7	21.7 ± 4.7 a	17.4 ± 2.1	167.1 ± 20.1	84.8 ± 8.9	30.9 ± 2.0	132.0 ± 8.9 a	155.2 ± 14.1 a
		RP30	11.7 ± 0.3	14.2 ± 1.3	17.0 ± 1.2 ab	17.3 ± 1.1	137.1 ± 26.2	73.6 ± 18.5	24.5 ± 2.1	115.7 ± 2.8 ab	115.0 ± 7.5 ab
		RP60	12.6 ± 1.3	11.6 ± 3.0	8.9 ± 0.9 b	16.3 ± 1.5	115.2 ± 4.3	62.4 ± 5.6	33.7 ± 3.7	95.4 ± 5.1 b	87.8 ± 11.1b
		<i>p</i>	0.509	0.702	0.049	0.872	0.240	0.483	0.125	0.016	0.015
	HT	NP	10.2 ± 2.8	10.0 ± 1.1	14.4 ± 4.6	10.8 ± 0.2	125.8 ± 8.1	66.7 ± 16.1	40.1 ± 2.2	76.7 ± 3.8	100.7 ± 15.3
		RP30	12.1 ± 0.9	12.3 ± 1.3	18.2 ± 1.9	12.3 ± 0.6	118.7 ± 4.3	58.3 ± 10.4	37.0 ± 1.3	66.7 ± 2.0	93.6 ± 2.9
		RP60	12.8 ± 1.0	9.6 ± 0.5	9.3 ± 0.8	14.4 ± 1.9	115.2 ± 17.0	75.6 ± 2.4	42.1 ± 1.0	65.6 ± 2.8	80.6 ± 2.0
		<i>p</i>	0.602	0.195	0.174	0.185	0.798	0.577	0.154	0.069	0.350
Rainy	CK	NP	10.3 ± 0.7	8.4 ± 0.2	10.1 ± 2.7 ab	12.3 ± 0.0 b	135.2 ± 0.8 a	51.8 ± 0.1	31.2 ± 0.1	96.7 ± 3.7 a	155.9 ± 8.5
		RP30	7.4 ± 0.9	9.4 ± 1.3	15.0 ± 0.2 a	14.1 ± 0.5 a	83.1 ± 9.2 b	42.7 ± 5.5	28.5 ± 2.7	88.8 ± 2.3 a	150.3 ± 12.5
		RP60	7.4 ± 0.5	7.7 ± 0.0	6.5 ± 0.5 b	10.3 ± 0.2 c	83.1 ± 2.3 b	39.1 ± 5.1	28.9 ± 1.2	50.0 ± 2.4 b	150.2 ± 5.6
		<i>p</i>	0.063	0.330	0.025	< 0.001	0.001	0.181	0.519	< 0.001	0.885
	LT	NP	10.6 ± 1.0	9.4 ± 0.7	10.5 ± 1.3	21.7 ± 0.1	129.7 ± 4.2 a	61.7 ± 2.4	32.8 ± 1.0	128.3 ± 5.9 a	162.6 ± 10.2
		RP30	7.5 ± 0.6	6.2 ± 0.7	15.0 ± 2.8	20.2 ± 0.1	92.0 ± 9.9 b	52.3 ± 5.5	39.6 ± 5.1	74.1 ± 6.2 b	164.8 ± 10.8
		RP60	8.7 ± 0.8	7.5 ± 0.8	10.8 ± 0.6	19.4 ± 1.3	75.1 ± 1.5 b	55.5 ± 7.4	44.6 ± 2.0	79.0 ± 2.1 b	163.1 ± 11.3
		<i>p</i>	0.090	0.066	0.226	0.173	0.002	0.505	0.100	< 0.001	0.989
	MT	NP	10.9 ± 1.2	8.5 ± 0.7	18.5 ± 1.7 a	20.4 ± 0.2	166.7 ± 7.8 a	105.9 ± 9.8	31.6 ± 0.3	150.5 ± 6.0 a	135.9 ± 4.4
		RP30	12.1 ± 2.7	8.7 ± 1.4	15.7 ± 0.6 a	24.9 ± 3.9	102.0 ± 1.5 b	100.7 ± 3.5	23.9 ± 3.5	108.7 ± 2.1 b	135.9 ± 7.5
		RP60	15.4 ± 1.8	7.9 ± 1.1	10.1 ± 0.4 b	18.8 ± 2.0	79.7 ± 2.1 c	105.3 ± 15.8	25.2 ± 2.2	121.9 ± 4.9 b	142.5 ± 8.4
		<i>p</i>	0.318	0.874	0.005	0.288	< 0.001	0.935	0.122	0.002	0.754
	HT	NP	13.1 ± 1.6	8.3 ± 0.8	19.0 ± 0.3 a	22.0 ± 0.2	123.2 ± 5.4 a	83.6 ± 8.9	31.2 ± 0.3	131.5 ± 18.9	159.7 ± 10.9
		RP30	11.3 ± 2.5	8.1 ± 1.4	13.3 ± 0.8 b	20.4 ± 1.8	98.5 ± 6.3 b	50.0 ± 9.3	32.3 ± 8.6	90.3 ± 6.3	149.5 ± 6.9
		RP60	9.5 ± 1.3	10.5 ± 2.5	8.8 ± 0.9 c	19.7 ± 0.9	100.7 ± 2.6 b	77.8 ± 10.6	39.8 ± 4.4	109.4 ± 8.7	155.5 ± 5.5
		<i>p</i>	0.442	0.580	< 0.001	0.424	0.024	0.098	0.533	0.146	0.684

R-P_i, inorganic phosphorus extracted by resin strip; Bic-P_i and Bic-P_o, extracted by NaHCO₃; OH-P_i and OH-P_o, extracted by NaOH; Dil.HCl-P_i, extracted by 1 M HCl; Conc.HCl-P_i and Conc.HCl-P_o, extracted by concentrated HCl; Res-P_i; digested by concentrated H₂SO₄ and H₂O₂. CK, control site; LT, light thinning; MT, moderate thinning; HT, high thinning. NP, natural precipitation; RP30, 30% reduced precipitation; RP60, 60% reduced precipitation. Data (mean ± standard error, *n* = 3) followed by different letters within a column differ significantly according to Tukey's test (*p* < 0.05). When the difference is not statistically significant, no letter notation is given.

Table 3. Coefficient (r) for the Pearson's correlation between soil acid phosphomonoesterase (AcPME) and soil properties in *L. principis-rupprechtii* plantations.

Soil Properties	pH	TC	TN	TP	MBC	R-P _i	Bic-P _i	Bic-P _o	OH-P _i	OH-P _o	Dil.HCl-P _i	Conc.HCl-P _i	Conc.HCl-P _o	ResP _i
AcPME	0.24 _*	0.15	−0.54 **	0.71 **	0.77 **	0.35 **	0.05	0.46 **	0.64 **	0.33 **	0.56 **	−0.48 **	0.58 **	0.70 *

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

3.3. Soil AcPME Activity

Soil AcPME activity was significantly higher in the rainy season than in the dry season ($p < 0.01$), almost 1.9 times more so (Figure 2). Throughout the two seasons, soil AcPME activity levels in the NP treatment were as follows, in decreasing order: HT ($447.2 \pm 50.8 \mu\text{g g}^{-1} \text{h}^{-1}$), MT ($441.2 \pm 49.6 \mu\text{g g}^{-1} \text{h}^{-1}$), CK ($390.9 \pm 64.1 \mu\text{g g}^{-1} \text{h}^{-1}$) and LT ($376.3 \pm 55.6 \mu\text{g g}^{-1} \text{h}^{-1}$).

The effect of precipitation on soil AcPME activity varied with season and thinning treatment (Figure 2). In the dry season, the NP treatment was most conducive to improving soil AcPME activity in all four thinning scenarios (Figure 2a). Specifically, for CK and LT, the NP treatment resulted in significantly greater activity than RP30 ($p = 0.004$ and 0.036) and RP60 ($p = 0.003$ and 0.012) respectively. As for MT and HT, although there was no significant difference in soil AcPME activity among precipitation treatments, they still displayed a downward trend with precipitation decline. The descending order of treatments for observed soil AcPME activity in MT was NP ($336.9 \pm 31.3 \mu\text{g g}^{-1} \text{h}^{-1}$) > RP30 ($323.6 \pm 6.8 \mu\text{g g}^{-1} \text{h}^{-1}$) > RP60 ($256.9 \pm 1.6 \mu\text{g g}^{-1} \text{h}^{-1}$). In HT, soil AcPME activity decreased by 11.1% and 29.3% in RP30 and RP60, respectively, compared to NP.

Figure 2. Soil acid phosphomonoesterase (AcPME) activity in the dry (A) and rainy (B) seasons under different precipitation and thinning treatments in *L. principis-rupprechtii* plantations. CK, control site; LT, light thinning; MT, moderate thinning; HT, high thinning. NP, natural precipitation; RP30, 30% reduced precipitation; RP60, 60% reduced precipitation. Error bars indicate standard error ($n = 3$). Different lowercase letters denote significant differences between precipitation treatments according to Tukey's test ($p < 0.05$).

In the rainy season, soil AcPME activity decreased significantly in RP60 compared with NP (Figure 2b). Specifically, soil AcPME activity in CK, LT, MT and HT were 40.6%, 31.3%, 14.4% and 14.6% lower, respectively, for the RP60 than the NP treatment. However, with the exception of MT, soil AcPME activity did not differ significantly between the RP30 and NP treatments. In contrast in MT, soil AcPME activity increased significantly in the RP30 treatment (Figure 2b). Among the four thinning scenarios, the responses of soil AcPME activity to precipitation were the most pronounced in MT. The activity in all three precipitation treatments differed significantly in MT.

4. Discussion

4.1. Soil AcPME Activity in the Dry and Rainy Seasons

Consistent with the hypothesis, our study found that soil AcPME activity was significantly higher in the rainy season than the dry season (Figure 2). Higher soil moisture (Figure 1) and MBC (Table 1) were observed in the rainy season. The higher AcPME activity during the rainy season could be partly explained by the relatively high MBC and soil moisture content, which together promoted P_o mineralization [39]. To some extent, this trend is in line with another study conducted in a desert

steppe of northwestern China [40] and the southern USA [20], where the authors found that soil AcPME activities were significantly stimulated by regimes of increasing precipitation. They proposed that precipitation significantly affects the soil microbial biomass, thereby changing the activity of soil extracellular enzymes involved in P (AcPME) metabolism. Huang, et al. [41] also found that soil phosphatase activity increased significantly during the rainy season, which might have been a response to the increasing P demand caused by the vigorous growth of plants and strong microbial activity. On the contrary, the dry season was not favorable for microbial growth and activity and was the low-point for biological activity [42]. This conclusion is in line with most experiments studying enzymatic production under different levels of water availability, which tend to show lowered phosphatase activity under drought conditions [43].

4.2. Effects of Thinning on Soil AcPME Activity under Natural Levels of Precipitation

Our results showed that MT and HT exhibited higher soil AcPME activities than CK and LT. Both soil microenvironment and nutrient status of the *L. principis-rupprechtii* plantations were recognized as the major contribution to soil AcPME activity, after thinning treatment [44,45]. In our study, soil chemical properties were closely related to soil AcPME activity (Table 3), and the soil nutrient conditions of MT and HT were superior to those of CK and LT (Supplementary Table S2 and Figure S3). This was also partly supported by other studies [12,46,47], which suggests that the better the soil nutrient condition, the higher the soil AcPME activity should be. In addition, MT and HT had a soil microenvironment (high soil moisture) that is more conducive to microbial growth [31], as well as higher soil microbial biomass (Table 1) [48] and microbial community function [49]. This also contributed to high soil AcPME activity because this enzyme is partly secreted from microbes [50,51]. Moreover, Renella et al. [52] indicates that microbial production of AcPME was higher in forest soil with a pH of 5.1–7.

4.3. Effects of Precipitation Treatments on Soil AcPME Activity

In the dry season, the NP treatment was more conducive to improving soil AcPME activity in all thinning scenarios, but precise effects differed among the different degrees of thinning (Figure 2a). In CK and LT, which had low soil moisture, soil AcPME activity was greatest in the NP treatment, indicating that water was an important regulator of soil P transformation in these two plantations [53]. Tian, et al. [31] reported that the seasonal precipitation in the spring is insufficient to meet the exuberant growth potential of plants. Thus, the RP treatments potentially inhibited plant growth, thereby reducing the P demand [23,24]. This could also be attributed to the decreased activity of microorganisms under low moisture conditions, which results in limited P_o mineralization [1,20]. Furthermore, in the dry season, concentrations of TP, MBC and $Bio-P_o$ were significantly higher in NP than RP treatments (Tables 1 and 2). A similar result was obtained by Hou, et al. [54], who believed that the increase in soil AcPME activity may be a combined result of the higher MBC and the easily mineralizable labile P_o . Bhandari, et al. [20] also found that the higher enzyme activities including P mineralization was associated with higher levels of SOC, TN, MBC, and MBN. In MT and HT, precipitation treatments had no significant effect on soil AcPME activity, probably due to their well-established self-regulation mechanism [55].

In the rainy season, soil AcPME activities showed different response patterns to precipitation treatments under different thinning scenarios. Soil AcPME activity and soil moisture were lower in RP60 than in NP treatments under all thinning scenarios (Figure 2b and Figure S2). Other studies have also found similar results reported, which indicate that soil moisture is a major driving factor of phosphatase activity [12,25]. Our results show that in the rainy season, RP60 treatments significantly reduced $Bio-P_o$, $OH-P_o$ and $Conc.HCl-P_o$ concentrations (Table 2), which could be one of the reasons for the decrease in soil AcPME activity. Our analysis is consistent with that of Turner and Haygarth [56], who proposed that high P_o in soil represent the most useful P fractions for predicting potential phosphatase activity. P_o fractions account for labile, moderately labile, and non-labile forms [57,58], but the fraction

overall is a suitable indicator of the ability of soil-plant system to obtain labile P (mineralization) because it is a natural potential substrate for phosphatase [12]. This reinforces the idea that all three forms are needed to better predict the P cycle capacity of forest ecosystems. In addition, in LT, the RP60 treatment led to further acidification of the soil (Table 1), which might also be a factor causing the decline of soil AcPME activity. When the soil pH value is relatively low, considerable aluminum toxicity occurs, which inhibits plant root growth and nutrient absorption [59,60]. These factors reduce soil AcPME activity, which is closely related to microbe and root growth [61,62]. The decrease of soil acid phosphatase activity caused by drought would lead to the decrease of P availability for plants, which would lead to long-term competition for P in regional forest ecosystems.

However, in the rainy season, the RP30 treatment did not inhibit soil AcPME activity, and even promoted it in MT. This is attributed to the fact that the abundant natural precipitation met the water demand of plant growth during the rainy season, though excessive precipitation is not advantageous for plants in a way that it may inhibit the mineralization potential of soil P_o [63]. Because soil moisture was already high in MT (Figure 1), excessive natural precipitation was not conducive to the diffusion of oxygen in the soil [64,65], which may have caused soil hypoxia, thus limiting plant root growth and soil microbial activity [66]. Therefore, when precipitation is appropriately reduced (i.e., RP30), the soil hypoxic environment is alleviated, thereby increasing soil AcPME activity, as in the RP30 treatment of MT. This would also improve the mineralization capacity of soil P_o .

5. Conclusions

Driven by the seasonality of precipitation, soil acid phosphomonoesterase (AcPME) activities showed an obvious seasonal pattern. That is, soil AcPME activity was significantly higher in the rainy season, which is associated with higher microbial activity and increased P demand, than in the dry season. Soil AcPME activity gradually increased with thinning intensity. That is, high thinning (HT) and moderate thinning (MT) were higher than light thinning (LT) and control (CK), indicating that HT and MT had good soil conditions. Our results also indicate that a reasonable precipitation distribution is an important factor in controlling the soil P cycle in a *L. principis-rupprechtii* plantation. In the dry season, the natural precipitation (NP) treatment was more conducive to improving soil AcPME activity for all thinning scenarios, which attribute to increasing microbial biomass carbon (MBC) and easily mineralizable labile P_o . In the rainy season, soil AcPME activity was significantly lower in the 60% reduced precipitation (RP60) treatment than in the NP treatment under all thinning scenarios. These influences of precipitation on soil AcPME are likely driven by RP60 effects on P_o fractions (including Bio- P_o , OH- P_o and Conc.HCl- P_o) and further acidification of the soil. The 30% reduced precipitation (RP30) treatment did not inhibit soil AcPME activity, and even promoted it in MT. In summary, our results indicate that a year-long drought would not be conducive to the transformation of soil P_o in the forest ecosystem, but an increase in rainfall during the rainy season would also be detrimental to the mineralization of soil P_o in MT *L. principis-rupprechtii* plantations of North China.

Supplementary Materials: The following are available online at <http://www.mdpi.com/1999-4907/10/9/734/s1>, Table S1: Information about the study sites under the four thinning treatments in 2014. Table S2: Repeated-measures ANOVA results for soil properties under the four thinning treatments in *L. principis-rupprechtii* plantations. Figure S1: Soil moisture in the dry season under natural precipitation (NP) treatments in *L. principis-rupprechtii* plantations following thinning. Figure S2: Soil moisture under different precipitation treatments in *L. principis-rupprechtii* plantations. Figure S3: Soil phosphorus fractions under natural precipitation (NP) treatments in *L. principis-rupprechtii* plantations following thinning.

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