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Contrasting responses of phosphatase kinetic parameters to nitrogen and phosphorus additions in forest soils

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Abstract

1. Global changes include increasing nitrogen (N) and phosphorus (P) deposition, which affect microbial nutrient demand and biogeochemical cycles. The responses of P-mineralizing enzymes to these global change components are poorly defined and are not specified in forest soils differing in P content.
2. We chose one site in a P-rich and two sites in P-poor forests and established sixteen 20 × 20 m plots at each site. Control, either N only, P only, or combined N and P, were randomly distributed through each forest site with four replicates. We investigated the effects of N and P additions over 4 years on the phosphomonoesterase potential activity (V_{max}), its half-saturation constant (K_m) and its catalytic efficiency (V_{max}/K_m).
3. Without N and P additions, the enzyme kinetic parameters V_{max}, K_m and V_{max}/K_m were higher in P-rich than in P-poor forest soils. These parameters increased with soil pH, SOC, TN and TP contents increased.
4. Remarkably, P additions caused the V_{max} and K_m to increase in P-rich soils, but had no effect on V_{max}/K_m. P additions to P-poor soils resulted in a decrease in the V_{max}/K_m via the inhibitory effects of inorganic P on the V_{max}. N additions had no effect on the V_{max}/K_m in P-rich and P-poor soils because of the similar increases in the V_{max} and K_m. The effects of combined N and P and P only additions to P-poor soils on the V_{max} and K_m were similar, but were stronger than the effects of N only or P only additions on the P-rich soils.
5. Phosphatase kinetic parameters were positively related to the availability of N and P in P-rich soils, but inorganic P inhibited phosphatase activity and caused a decrease in the catalytic efficiency in P-poor soils. More microbial community groups could contribute to the secretion of a broader spectrum of iso-enzymes under combined additions of N and P in P-rich soils. We conclude contrast responses of phosphatase kinetics to P and N inputs in P-rich and P-poor forest soils, while long-term N deposition might mitigate P limitation by increasing phosphatase secretion.

KEYWORDS
catalytic efficiency, kinetic parameters, nitrogen, phosphatase, phosphorus, subtropical forest, temperate forest

1 | INTRODUCTION

Global changes include increasing nitrogen (N) deposition and phosphorus (P) fertilization that affect plant and microbial nutrient demands (Marklein & Houlton, 2012; Yuan & Chen, 2015). Phosphorus (P) is an essential nutrient for plants and micro-organisms. Soil phosphatases transform about 20%–80% of soil organic P to available P for plants and microbes uptake (Houlton, Wang, Vitousek, & Field, 2008; Sinsabaugh & Follstad Shah, 2012). Hydrolytic enzyme activity is substrate (S) dependent and therefore follows the Michaelis–Menten equation, i.e. $v = V_{max}[S]/(K_m + [S])$, where V_{max} is the maximum rate of activity when all enzymes are substrate-saturated, and K_m is the Michaelis–Menten constant, which is the concentration of the substrate when the reaction velocity is equal to the half of V_{max} and reflects the apparent binding affinity of the enzyme to the substrate (Tabatabai & Bremner, 1971). The ratio of V_{max} to K_m reflects the enzyme catalytic process relative to both the enzyme-substrate complex dissociation (V_{max}) and the rate of enzyme-substrate complex formation (K_m), and is defined as the catalytic efficiency; higher catalytic efficiencies indicate superior catalytic performance of the enzyme (German, Marcelo, Stone, & Allison, 2012; Razavi, Blagodatskaya, & Kuzyakov, 2016; Stone & Plante, 2014). Enzymes mainly operate under non-saturated conditions in soils, so K_m and the catalytic efficiency are important parameters that can together represent microbial processes in biogeochemical models (Allison & Vitousek, 2005; Wieder, Bonan, & Allison, 2013).

Soil phosphatase follows an economic principle, i.e. micro-organisms will preferentially allocate energy toward the acquisition of whatever resource is most limiting their growth (Marklein & Houlton, 2012). It is thought that plants or micro-organisms need to invest about 15 g N g^{-1} P in phosphatase production (Houlton et al., 2008). Results from meta-analysis indicated that, as N availability increased, micro-organisms would allocate more resources towards the production of enzymes used to acquire P (Marklein & Houlton, 2012). In contrast, decadal N additions had no effect on soil phosphatase in either northern hardwood soils or in tropical rainforest soils (Turner & Joseph Wright, 2014; Weand, Arthur, Lovett, Sikora, & Weathers, 2010) .

In line with the economic principle, the meta-analysis indicated that P additions inhibited phosphatase under P only or combined N and P additions in grassland, wetland, or forest soils (Marklein & Houlton, 2012). However, only one study of N and P additions in a forest was included in the meta-analysis (Olander & Vitousek, 2000). Our previous research showed that additions of P only or N and P together could decrease soil phosphatase activity in agricultural soils (Zhang et al., 2015) and increase soil phosphatase activity in forest soils (Dong et al., 2015).

We therefore remain unsure as to how combined additions of N and P might affect the phosphatase kinetic parameters in forest soils, as, to date, few studies have examined these processes. Phosphatase kinetic parameters (V_{max} and K_m) declined from the surface downwards in tropical forest soil profiles, and the catalytic efficiencies remained constant at depths of up to 100 cm in soil profiles (Stone & Plante, 2014). Low substrate concentrations might inhibit a micro-organisms enzyme secretion, causing V_{max} to decrease (Sinsabaugh & Follstad Shah, 2012; Stone, DeForest, & Plante, 2014), while the K_m might decrease in the presence of different isoenzymes with high substrate affinity (Khalili, Nourbakhsh, Nili, Khademi, & Sharifnabi, 2011; Stone et al., 2014). In our previous study, the V_{max} and V_{max}/K_m for carbon-related hydrolases were higher in high altitude forest soils than in low altitude forest soils because they were positively correlated with the soil organic matter contents (Li et al., 2017). Stone et al. (2012) reported that the K_m decreased and the V_{max}/K_m of the carbon-related hydrolases increased in temperate forest soils after N additions for 20 years. The sparse findings from existing studies indicate that relationships between phosphatase kinetic parameters and soil properties under N and P additions are not well-defined for different forest soils.

Subtropical forest soils are highly weathered, strongly acidic, and P deficient, and their stoichiometric ratios of organic carbon (C), N and P are further evidence that they are P-limited (He, Hou, Liu, & Wen, 2016; Lu, Mao, Gilliam, Luo, & Mo, 2014; Ushio, Fujiki, Hidaka, Kitayama, & Poorter, 2015; Xu et al., 2017). Temperate forest soils have higher concentrations of organic C, N and P than subtropical forest soils, and are relatively rich in P compared with N (Xu et al., 2017). For example, soil total P content was lower in subtropical (Dinghu Mountain, 0.20 g/kg) than in temperate forests (Changbai Mountain, 1.67 g/kg), and the soil TN/TP ratio was higher in subtropical (Dinghu Mountain 8.9) than in temperate forests (Changbai Mountain, 3.6) (Xu et al., 2017). Atmospheric nitrogen (N) deposition is a key source of biologically available N for plants and micro-organisms. Large quantities of N are deposited on subtropical and temperate forests throughout eastern China (Zhu et al., 2015), which may exacerbate existing stoichiometric imbalances and P limitations. For example, in temperate forests, increasing deposition of atmospheric N has been reported to shift nutrient limitation from N to P (Crowley et al., 2012). To predict future P cycling and P release from soils, it is important to understand how phosphatase kinetic parameters respond to N and P additions. However, the complex linkages between N and P have not yet been resolved at an enzyme kinetic level.

In this study, we assessed the responses of phosphatase kinetic parameters to increased N and P availability and changes in the V_{max} and K_m related to soil nutrients in three ongoing N and P addition trials in two subtropical forests and one temperate forest. The subtropical forests were P-poor with high N atmospheric deposition and the temperate forest was P-rich with relatively low N atmospheric deposition. In line with the economic principle, i.e. soil P availability might inhibit the V_{max} and the catalytic efficiency of phosphatase, we hypothesized that (1) the V_{max} and the catalytic efficiency would be higher in P-rich temperate forests than in P-poor subtropical forests

TABLE 1 Characteristics and original properties of soils in the three experimental sites

	Changbai Mt. (CBM)	Qianyanzhou (QYZ)	Dinghu Mt. (DHM)
Climate zone	Temperate	Subtropical	Subtropical
MAT (°C)	2.8	16.8	22.0
MAP (mm)	731	1,629	1,733
Altitude (m)	758	102	240
Soil type	Albi-Boric Argosols	Ultisols	Latosol
Vegetation type	Native conifer mixed broad-leaved forest	Chinese fir plantation	Secondary conifer mixed broad-leaved forest
Main forest	Korean pine (*Pinus koraiensis*), Mongolian oak (*Quercus mongolica*)	Chinese fir (*Cunninghamia lanceolata*)	Schima root-bark (*Schima superb*), Chinese red pine (*Pinus massoniana* Lamb)
pH	5.51 ± 0.08^{A}	4.71 ± 0.03^{B}	3.80 ± 0.06^{C}
SOC (g/kg)	87.5 ± 1.18^{A}	16.5 ± 0.71^{B}	11.1 ± 1.21^{C}
TN (g/kg)	5.6 ± 0.05^{A}	1.2 ± 0.07^{B}	0.9 ± 0.05^{C}
TP (mg/kg)	1,022 ± 41^{A}	153 ± 8^{B}	131 ± 13^{B}
SOC/TN	15.5 ± 0.2^{A}	14.0 ± 0.6^{AB}	12.7 ± 0.8^{B}
TN/TP	5.6 ± 0.23^{B}	7.7 ± 0.11^{A}	6.8 ± 0.77^{AB}

MAT, mean annual temperature; MAP, mean annual temperature; pH, soil acidity; SOC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; SOC/TN, soil organic carbon to nitrogen ratios; TN/TP, soil total nitrogen to total phosphorus ratios. Values are *M* ± *SE* (*n* = 4). Different letters in each row represent significant differences between experimental sites ($p < .05$).

because of positive correlations between $V_{\max}$ and both the catalytic efficiency and substrate availabilities at the ecosystem scale; (2) the V_{max} and the catalytic efficiency would increase after N additions; (3) there would be a greater decrease in the $V_{\max}$ and the catalytic efficiency in P-rich soils than in P-poor soils after P additions, and (4) the $V_{\max}$ and the catalytic efficiency would either increase or decrease under combined N and P additions, depending on whether P was limited or abundant.

2 | MATERIALS AND METHODS

2.1 | Site description

The study sites were in the Changbai Mountain temperate native forest (hereafter called Changbai) (42°24′2″N, 128°05′42″E), Qianyanzhou subtropical plantation forest (hereafter called Qinayanzhou) (26°44′52″ N, 115°04′13″ E), and Dinghu Mountain subtropical secondary forests (hereafter called Dinghu) (23°9′41″N, 112°32′36″E) (Table 1). Changbai is a native conifer mixed broad-leaved forest dominated by Korean pine (*Pinus koraiensis*) and mixed mongolian oak (*Quercus mongolica*). The Qianyanzhou site comprises a Chinese fir (*Cunninghamia lanceolata*) plantation that was established in 2000. The secondary evergreen broad-leaved mixed pine forests on Dinghu are dominated by schima root-bark (*Schima superb*) and Chinese red pine (*Pinus massoniana* Lamb). Wet N deposition amounted to approximately 11, 33 and 36 kg N ha^{-1} yr^{-1} in the Changbai, Qianyanzhou and Dinghu forests respectively (Zhu et al., 2015).

The Changbai site is characterized by a continental temperate monsoon climate, with a mean annual temperature and precipitation of 2.8°C and 731 mm respectively (Table 1). The sites at Qianyanzhou and Dinghu are characterized by a subtropical monsoon climate, with mean annual temperatures of 16.8°C and 22.0°C respectively, and mean annual precipitation amounts of 1629 and 1733 mm respectively (Table 1). The soils at Changbai, Qianyanzhou, and Dinghu are classified as Argosols, Ultisols and Latosols respectively (Soil Survey Staff 2010). Before nutrient applications, the soils at the three sites were either P-rich or P-poor. Soil organic carbon (SOC), total N (TN), and total P (TP) contents, pH and C/N ratios were higher in the temperate forest than in the subtropical forests, while TN/TP ratios were higher in the subtropical forests than in the temperate forest (Table 1).

2.2 | Experimental treatments

The ongoing long term N and P addition experiments started in March 2012 at Qianyanzhou and in May 2013 on Changbai and Dinghu. Four treatments with four replicates were distributed randomly throughout each site, making a total of 48 plots. Each plot measured 20 × 20 m, and had an area of 400 m^2. A buffer zone of more than 20 m was established between each plot. There were four treatments, namely control (CK), N only, P only, and N and P combined (NP). For the N only treatment, 100 kg N ha^{-1} yr^{-1} were added as NH_4NO_3 to the three forest sites. For the P only treatment, 100 kg P ha^{-1} yr^{-1} were added to the Changbai and Dinghu sites, and 50 kg P ha^{-1} yr^{-1} were added at Qianyanzhou, as NaH_2PO_4. The combined treatments at the Changbai and Dinghu sites comprised 100 kg N ha^{-1} yr^{-1} and 100 kg P ha^{-1} yr^{-1} as NH_4NO_3 and NaH_2PO_4, and at the Qianyanzhou site, comprised 100 kg N ha^{-1} yr^{-1} and 50 kg P ha^{-1} yr^{-1}. At Qianyanzhou, the N and P fertilizers were mixed with sand and spread evenly with applications in March, June, September and December accounting for 30%, 30%, 20% and 20% of the total respectively. At the Dinghu and Changbai sites, N and P were dissolved in water and dispensed with a hand-operated water sprayer. The fertilizers were applied from May to October on Changbai, but were applied throughout the year on Dinghu. The approaches and frequencies of nutrient additions differed between sites so that they were compatible with the growing season at each site. Because of ongoing research trials at Qianyanzhou, the amount of P added at this site was half of that added at the Changbai and Dinghu sites.

2.3 | Soil sampling and analysis

We sampled the soil once at Qianyanzhou in November 2015 and at Dinghu and Changbai in August 2016, after nutrients had been added for 4 years. Soils in each plot were sampled to a depth of 10 cm at five randomly selected locations with an auger (diameter of 5 cm) and then mixed to form a composite sample. The fresh soil samples were sieved through a 2-mm mesh and stored at 4°C until analysis for phosphatase kinetic parameters, water extractable dissolved organic carbon (DOC), ammonium and nitrate N (NH_4^+-N, NO_3^--N), available P and pH. A subsample was air-dried, and then sieved through a 0.25-mm mesh before analysis for SOC, TN and TP.

Soil pH was measured in a soil-water suspension (1:2.5 v:v) by glass electrode (Bao, 2008). Soil NO_3^--N and NH_4^+-N were extracted with 2 M KCl (1:5 v:v) and measured by a continuous-flow auto-analyzer (Bran Lubbe, AA3, Germany) (Bao, 2008). Soil water extractable DOC was extracted with distilled water (1:5 v:v) and measured with a TOC analyzer (Liqui TOC *II*, Elementar, Germany). SOC and TN were determined with a CN analyzer (Vario Max, Elementar, Germany). Soil was digested with H_2SO_4-$HClO_4$ and TP was measured by the spectrophotometric method with a continuous flow automated analyzer (AA3, Bran+Luebbe, Germany) at 700 nm (Bao, 2008).

Since the original soil pH ranged from 3.8 to 5.5, acid phosphatase was assumed to provide a large part of the P that was available to plants or micro-organisms (Hui, Mayes, & Wang, 2013). We measured acid phosphatase (phosphomonoesterase) activity by acetate buffer at the ambient pH, following the method proposed by Saiya-Cork, Sinsabaugh, and Zak (2002) and modified by German et al. (2011). The microplates were incubated in the dark at 20°C for 4 hours, after which fluorescence was measured using a microplate fluorometer (Synergy^{H4}, BioTek) with excitation and emission filters of 365 and 450 nm respectively. Substrate (4-methylumbelliferone-phosphate: $C_{10}H_9O_6P$, Sigma M8883) concentration gradients of 5–400 umol/L were chosen to determine the phosphatase kinetic parameters. The phosphatase kinetic parameters, i.e. V_{max} and K_m, were estimated by fitting the Michaelis–Menten equation to the activities using hyperbola in SIGMAPLOT (SIGMAPLOT for Windows, Version 10.0). The phosphatase catalytic efficiency was calculated as the ratio of V_{max} to K_m. The V_{max} was expressed in units of nmol g^{-1} soil h^{-1}, K_m was expressed in units of μmol/L, and the catalytic efficiency was expressed in hr^{-1} (Stone et al., 2014).

2.4 | Statistics

All results were reported as means ± *SEs*. We calculated the natural log response ratios of the V_{max}, K_m and the V_{max}/K_m under N and P additions and compared them to the control to indicate the net effect of nutrient additions at the three sites. We used two-way ANOVA, multivariate general linear models, and Duncan's multiple comparisons to determine the effects of sites, nutrient additions, and their interactions on soil phosphatase kinetics parameters, and soil variables. One-way ANOVA and Duncan's multiple comparisons were used to determine the differences between the soil properties, soil phosphatase kinetic parameters and the natural log response ratios of the individual phosphatase kinetic parameters that resulted from the different N and P additions in each site. SPSS 17.0 was used for the analysis. The relationships between the V_{max}, K_m, V_{max}/K_m and the soil properties were determined by redundancy analysis using CANOCO 4.5. We used Monte Carlo permutations and automatic selection of means to test the significance of the variables. A significance level of $P < .05$ was applied.

3 | RESULTS

3.1 | Soil acidity and nutrient content under nitrogen and phosphorus additions

Apart from TN and SOC/TN, the soil acidity and nutrient contents were significantly different for different forest sites and under different nutrient additions (Table 2, Tables S1 and S2, $p < .05$). Additions of N, either alone or combined with P, resulted in decreases between 0.2 and 0.4 in the soil pH in the Changbai or Qianyanzhou forests, while P additions resulted in an increase of about 0.2 in soil pH in the Changbai forest (Table 3). Additions of P, either alone or combined with N, resulted in increases in both available P and total P and a decrease in the TN/TP and available N (ammonium and nitrate N) to available P (AN/AP) ratios in all three forest sites (Table 3, Tables S1 and S2). Additions of N alone or with P resulted in an increase in the NH_4^+-N content in the temperate forest (Table 3). Under the combined NP treatment, the NO_3^--N, SOC and DOC concentrations increased in Changbai and the NO_3^--N and SOC concentrations increased in the Qianyanzhou forest (Table 3, Table S1).

3.2 | Phosphatase kinetic parameters under nitrogen and phosphorus additions

The relationships between the substrate content and the phosphatase activities fitted well to the Michaelis–Menten equation (Figure 1). The phosphatase kinetic parameters and the catalytic efficiencies were significantly different both among the forest sites and nutrient additions (Tables 2, $p < .05$). The V_{max}, K_m and V_{max}/K_m were 2.1–8.5, 0.5–4.8 and 0.6–1.0 times higher in the temperate forest than in the subtropical forests (Figure 2).

When the natural log response ratio is greater than 0, N and P additions have a positive effect, while when the response ratio is less

TABLE 2 Summary statistics (*F* ratio, *p* value) from two-way ANOVA general linear models applied to phosphatase kinetic parameters and soil properties

	V_{max}	K_m	V_{max}/K_m	pH	AP	DOC	NH_4^+-N	NO_3^--N	SOC	TN	TP	SOC/TN	TN/TP	AN/AP
Site	**1,513, 0.00**	**253, 0.00**	**196, 0.00**	**873, 0.00**	**58, 0.00**	**237, 0.00**	**153, 0.00**	**182, 0.00**	**589, 0.00**	**347, 0.00**	**423, 0.00**	0, 0.79	**16, 0.00**	**21, 0.00**
Treatment	**30, 0.00**	**6, 0.00**	**7, 0.00**	**43, 0.00**	**49, 0.00**	**13, 0.00**	**8, 0.00**	**5, 0.01**	**7, 0.00**	2, 0.15	**10, 0.00**	2, 0.22	**49, 0.00**	**47, 0.00**
Site × Treatment	**49, 0.00**	**12, 0.00**	**5, 0.00**	**11, 0.00**	**13, 0.00**	**9, 0.00**	**5, 0.00**	**3, 0.02**	**5, 0.00**	2, 0.21	**3, 0.03**	1, 0.47	**7, 0.00**	**5, 0.00**

V_{max}, phosphatase potential activity; K_m, phosphatase half-saturation constant; V_{max}/K_m, phosphatase catalytic efficiency; AP, available P; DOC, dissolved organic carbon; AN/AP, ratio of ammonium and nitrate to available P; The other abbreviations are the same as Table 1. *p* values that are significant at .05 level and their corresponding *F* ratios are in bold.

TABLE 3 Soil acidity, NH_4^+-N and NO_3^--N, available P, and DOC contents after N and P additions

	pH			AvailableP (mg kg^{-1})			DOC (mg kg^{-1})			NH_4^+-N (mg kg^{-1})			NO_3^--N (mg kg^{-1})		
	CBM	QYZ	DHM	CBM	QYZ	DHM	CBM	QYZ	DHM	CBM	QYZ	DHM	CBM	QYZ	DHM
CK	5.30 ± 0.05^b	4.59 ± 0.08^a	3.87 ± 0.04^a	17 ± 2^b	3 ± 1^b	5 ± 1^b	73 ± 3.3^b	27 ± 1.6^{ab}	57 ± 3.5^a	20 ± 4.3^b	8 ± 0.5^a	6 ± 0.4^a	47 ± 7.6^b	4 ± 1.0^b	4 ± 0.2^a
N	**4.87 ± 0.03^d**	**4.25 ± 0.03^b**	3.85 ± 0.02^a	22 ± 2^b	3 ± 1^b	7 ± 1^b	91 ± 7.5^b	26 ± 2.7^b	53 ± 1.8^a	**39 ± 2.3^a**	14 ± 3.4^a	6 ± 0.7^a	65 ± 9.2^{ab}	5 ± 1.1^b	4 ± 0.9^a
P	**5.49 ± 0.02^a**	4.73 ± 0.06^a	3.87 ± 0.05^a	**176 ± 19^a**	**37 ± 4^a**	**56 ± 8^a**	134 ± 5.6^{ab}	29 ± 4^{ab}	57 ± 1.6^a	**43 ± 3.7^a**	11 ± 2.1^a	7 ± 0.7^a	55 ± 7.7^b	3 ± 0.7^b	5 ± 0.7^a
NP	**5.12 ± 0.07^c**	**4.21 ± 0.02^b**	3.85 ± 0.02^a	**192 ± 33^a**	**35 ± 8^a**	**50 ± 6^a**	**116 ± 10.4^a**	37 ± 4.5^a	59 ± 3.3^a	**33 ± 0.6^a**	15 ± 3.1^a	7 ± 0.4^a	**83 ± 9.4^a**	**9.5 ± 2.4^a**	4 ± 0.4^a
p value	**.00**	**.00**	.96	**.00**	**.00**	**.00**	**.00**	.18	.51	**.00**	.50	.33	**.05**	**.04**	.76

CBM, Changbai Mountain temperate forests; QYZ, Qianyanzhou subtropical forests; DHM, Dinghu Mountain subtropical forests; DOC, dissolved organic carbon. Different letters in each column represent significant differences between nitrogen and phosphorus additions ($p < .05$). Means ± *SE* ($n = 4$) and *p* values from Duncan's multiple comparisons (in bold).

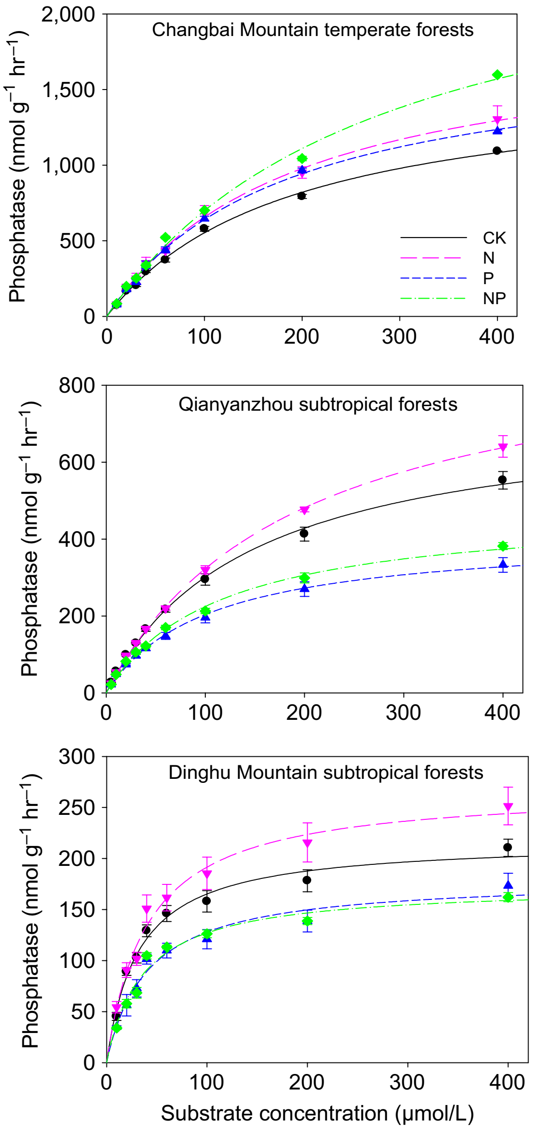


FIGURE 1 Michaelis–Menten plots of soil phosphatase under N and P additions, values are means ± *SEs* ($n = 4$)

than 0, N and P additions have a negative effect (Figure 3). The V_{max} increased but the K_m and the catalytic efficiencies did not change under the N treatments in the temperate forest (Figure 3). The V_{max} and the K_m increased in the N treatments in the subtropical forests, but the catalytic efficiencies were not affected (Figure 3). After P treatments in the two subtropical forests, the V_{max} and the catalytic efficiencies decreased but the K_m either increased or did not change (Figure 3). In contrast, the V_{max} increased about 13%, but the K_m and catalytic efficiencies were not affected, after P additions in the temperate forest (Figure 3). The responses of the V_{max}, K_m, and the catalytic efficiencies to additions of NP and P only were similar in the two subtropical forests. In the temperate forest, however, the V_{max} and K_m increased about 69% and 50% under NP additions than under CK, and increased more than under either N only or P only additions; the catalytic efficiencies remained steady under combined NP additions (Figure 3).

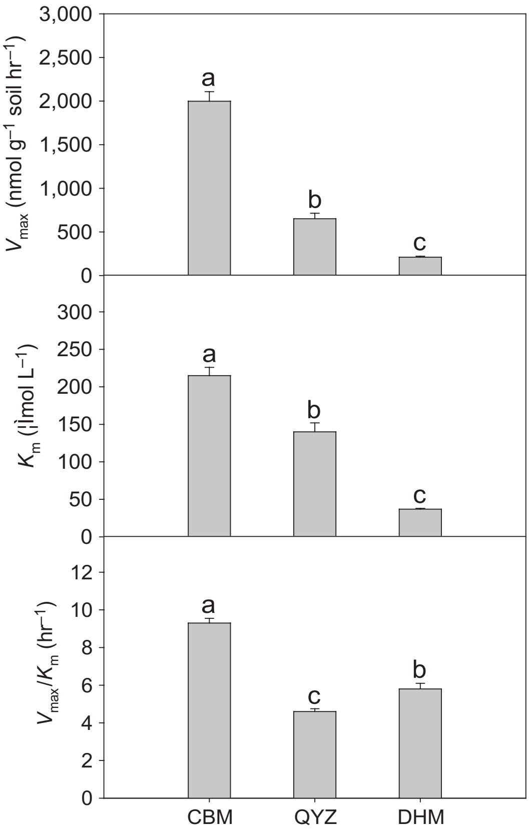


FIGURE 2 Kinetic parameters (V_{max} and K_m) and catalytic efficiencies (V_{max}/K_m) of phosphatase in the three forest soils (values are means ± *SEs*, $n = 16$). CBM, Changbai mountain temperate forests; QYZ, Qianyanzhou subtropical forests; DHM, Dinghu Mountain subtropical forests. The same below. Different letters for significant difference between each site at $p < .05$

3.3 | Relationships between phosphatase kinetic parameters and soil nutrients

Overall, the V_{max} and K_m were positively correlated ($R^2 = 0.84$, $p < .01$) (Figure 4). When examined by site, however, they were positively correlated in the Changbai ($R^2 = 0.80$, $p < .01$) and the Qianyanzhou ($R^2 = 0.91$, $p < .01$) sites, but not in the Dinghu ($R^2 = 0.01$, $p > .05$) site (Figure 4). In general, the correlations between the V_{max} and soil TP, available P and the TN/TP ratios were stronger than the correlations with K_m (Figure 5, Tables S3 and S4). Without N and P additions, the V_{max}, K_m, and the catalytic efficiencies were positively correlated with soil total (SOC, TN and TP) and available nutrient (NH_4^+-N, NO_3^--N and available P) contents in the temperate and subtropical forests, and the V_{max} was also positively correlated with soil pH but negatively correlated with the TN/TP ratios (Figure 5, Table S3).

Under nutrient additions, the V_{max} and the catalytic efficiencies were negatively correlated with TP and available P, and positively correlated with the N/P ratios (including TN/TP and AN/AP) in the subtropical forests (Figure 5, Table S4). The correlations between the K_m and the V_{max} and soil nutrients were similar, but were only significant for the Qianyanzhou site (Figure 5, Table S4). However, in the

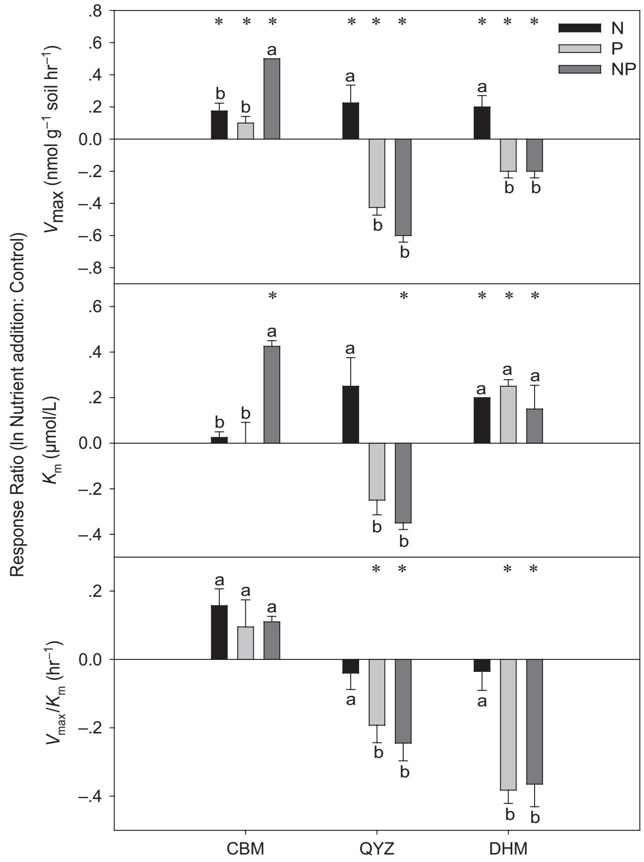


FIGURE 3 The natural log of the ratios of nutrient addition to control for kinetic parameters (V_{max} and K_m) and catalytic efficiencies (V_{max}/K_m) of phosphatase under N and P additions in each forest soil ($n = 4$). * and different lowercase for significant difference from control and between each nutrient additions at $p < .05$ respectively

temperate forest, we found that the V_{max} and K_m were positively correlated with NO_3^--N, SOC, total P and available P (Figure 5, Table S4).

4 | DISCUSSION

4.1 | Phosphatase kinetic parameters in phosphorus rich vs. poor forests

The first hypothesis was supported, and the V_{max} and the catalytic efficiency were higher in P-rich temperate forests than in P-poor subtropical forests (Figure 2). We found positive relationships between the V_{max} and available nutrients, in agreement with studies related enzyme activities with substrate availability (Loeppmann, Blagodatskaya, Pausch, & Kuzyakov, 2016; Tischer, Blagodatskaya, & Hamer, 2015). Furthermore, the kinetic parameters and the catalytic efficiency were positively related with soil pH, SOC, TN and TP contents. An enzyme's activity might increase when its target nutrient is present in a complex form and C and N are available (Allison & Vitousek, 2005). In subtropical forests, where soil microbial growth is expected to be more constrained by energy and nutrients than in temperate forests, natural selection might lead to a more efficient microbial community that produced enzymes with higher substrate affinities (lower K_m) so that, overall, fewer enzymes are produced (lower V_{max}) (Stone et al., 2014).

Substrates with differing availabilities of C and nutrients could change enzymatic systems in temperate or subtropical forests. Fast growing r-strategists were stimulated by easily available substrates with low substrate affinity, while slow growing K-strategists were more competitive under resource limitations with high substrate affinity (Loeppmann et al., 2016). This might

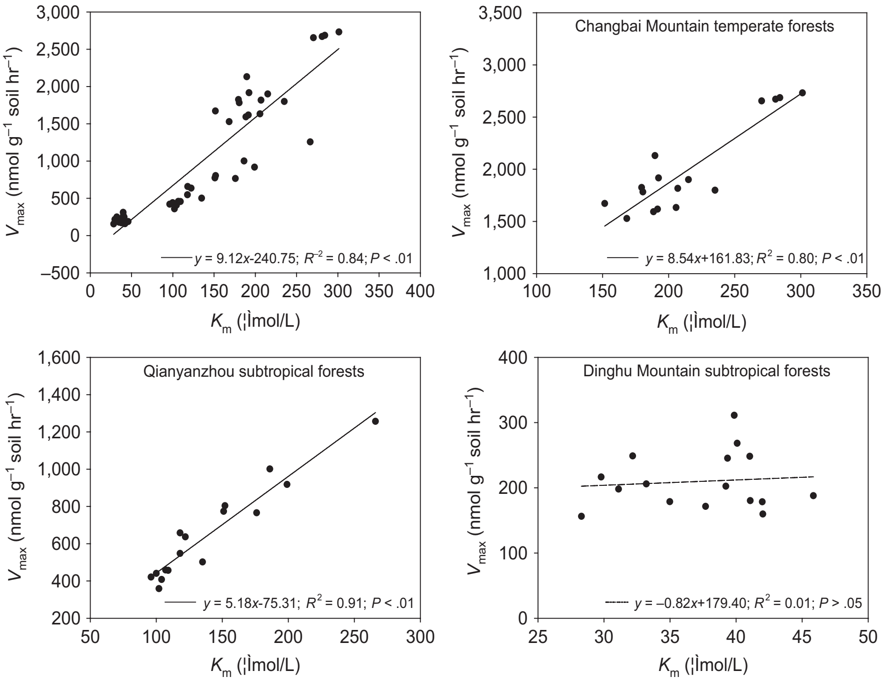
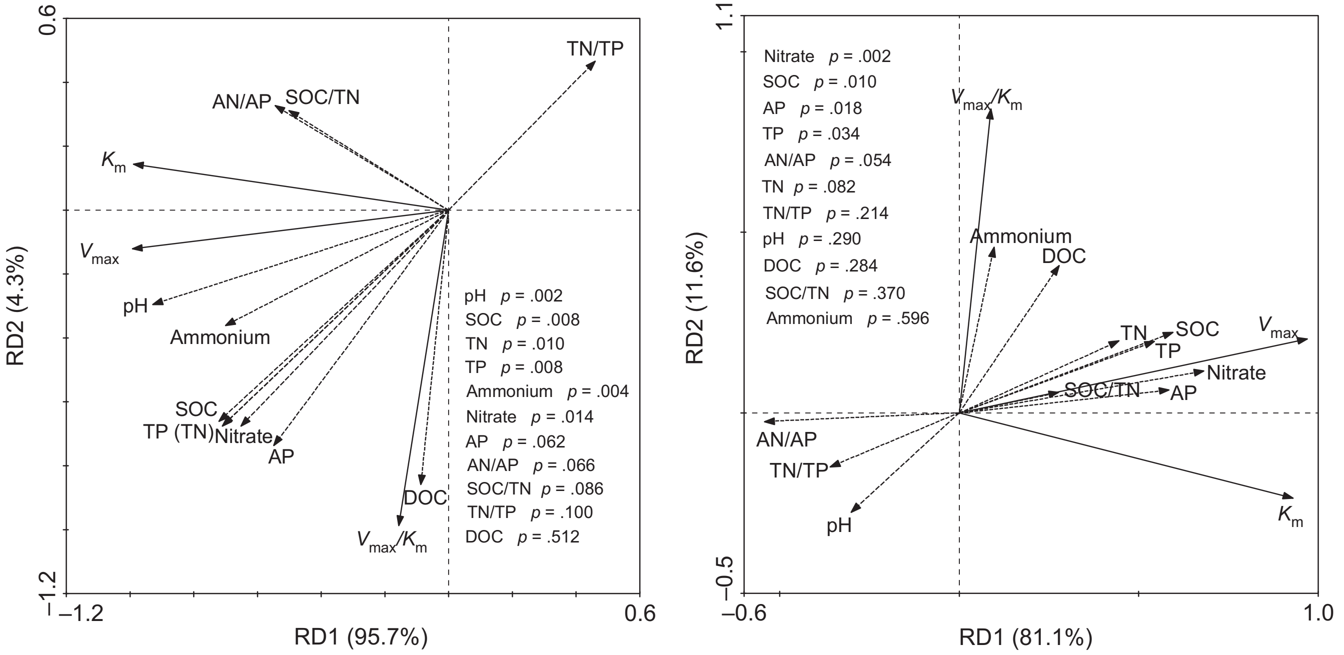
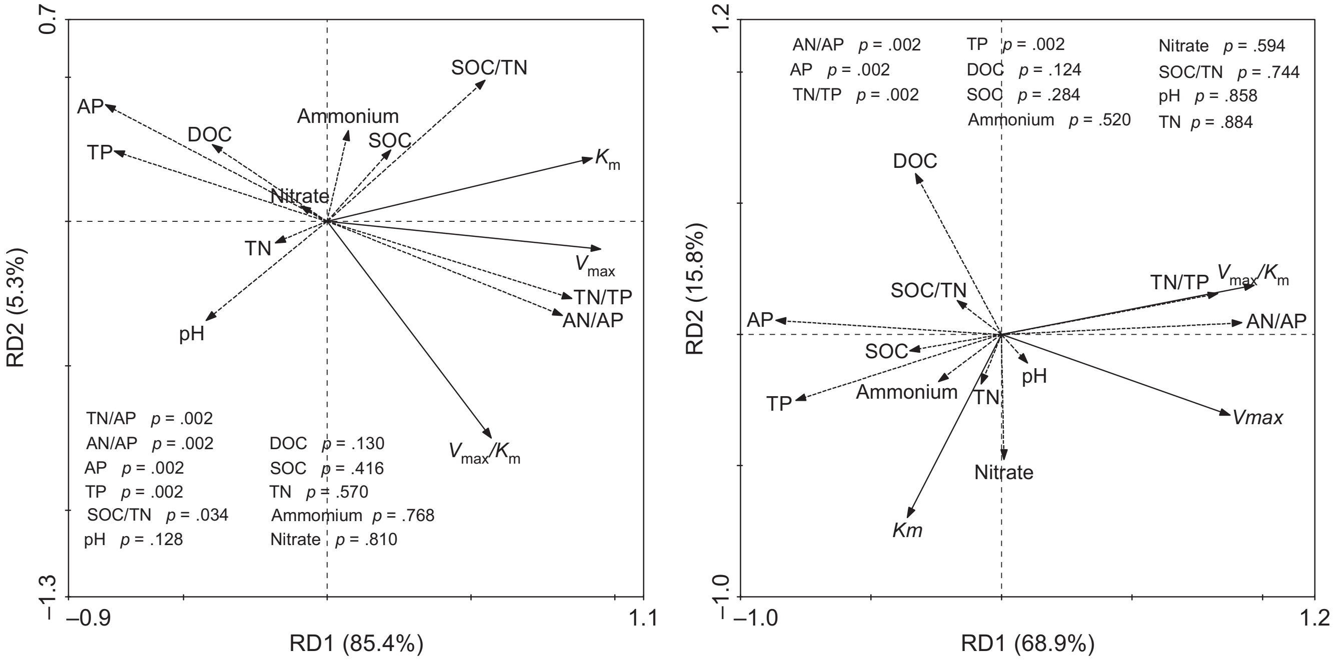


FIGURE 4 The relationships between the V_{max} and K_m of phosphatase in total ($n = 48$) and each forest site under N and P additions ($n = 16$)



Natural conditions

Changbai Mountain temperate forests



Qianyanzhou subtropical forests

Dinghu Mountain subtropical forests

FIGURE 5 Redundancy analyses (RDA) of the soil phosphatase kinetic parameters (V_{max} and K_m), catalytic efficiencies (V_{max}/K_m) and soil properties along natural forest sites ($n = 12$) and under N and P addtions in each forest site ($n = 16$). Soil properties are represented by dotted lines, and phosphatase kinetic parameters and catalytic efficiencies are indicated by solid lines. The abbreviations are the same as Tables 1 and 2

explain why the K_m was higher in the temperate soils than in the subtropical forest soils, and suggests a shift in dominating microbial population from r- to K-strategists from the P-rich to the P-poor forest soils. Microbial community structure may also affect the phosphatase affinity (Stone et al., 2014), e.g. the higher the bacterial community, the higher the substrate affinity (Tischer et al., 2015). Under substrate rich conditions, micro-organisms do not need a high substrate affinity to maintain high catalytic efficiency, but micro-organisms increase the substrate affinity of enzymes in substrate poor conditions. Because different microbial species might alter the soil organic matter decomposition rate (Tischer et al., 2015), microbial species, substrate affinities and catalytic efficiencies across different forest soils need to be linked in the future.

4.2 | Responses of phosphatase kinetic parameters to nitrogen and phosphorus additions

Our second hypothesis, that N additions would increase the V_{max} and the catalytic efficiency, was partly supported, as N additions resulted in consistent increases in the V_{max} and K_m but the catalytic efficiencies were relatively inert (Figure 3). The effect of N on the V_{max} was consistent with the results of the meta-analysis of Marklein and Houlton (2012); however, because of the positive relationships between K_m and V_{max} (Figure 4), the meta-analysis results cannot be extended from the V_{max} to the catalytic efficiency. While N deposition might impede organic matter decomposition in temperate forest soils where N did not limit microbial growth (Janssens et al., 2010), our results suggest that increasing the V_{max} and K_m simultaneously was a preferred microbial strategy for organic P mineralization. They also indicate that, if N deposition increased, the catalytic efficiency of the micro-organisms to mineralize organic P would not change in P-rich or P-poor soils.

The third hypothesis, that P additions caused the V_{max} and the catalytic efficiency to decrease, was only partly supported in the P-poor subtropical forests, but was not supported in the P-rich temperate forests. Additions of inorganic P resulted in a decrease in V_{max} in the subtropical forests, which was consistent with the conclusions of the meta-analysis (Marklein & Houlton, 2012), conceptual models (Cusack, Silver, Torn, Burton, & Firestone, 2011; Houlton et al., 2008), and experimental results (Wang, Wang, & Liu, 2008; Zheng, Huang, Chen, Wang, & Mo, 2015). Our results from correlation analyses confirmed that the V_{max} was inhibited by the end-product of soil available P in P-poor soils.

In contrast to our third hypothesis, the V_{max} was promoted by P additions to the P-rich forest soils (Figure 3). Previous studies have reported that the V_{max} in temperate forests was not influenced (Compton & Cole, 2001; DeForest, Smemo, Burke, Elliott, & Becker, 2012; Naples & Fisk, 2010; Yang & Zhu, 2015) or increased (Yang & Zhu, 2015) in response to P additions. Our correlation analysis showed that the V_{max} and the K_m were positively related to NO_3^--N in the P-rich forest soils. In a previous study, we found that soil P availability could promote the abundances of ammonia oxidizing archaea and that it caused the soil nitrification rate and NO_3^--N concentrations to increase (Tang et al., 2016). In hardwood forests in the northeastern United States, phosphatase activity was related to the net N mineralization rate but not P availability (Ratliff & Fisk, 2016). Allocation of excess N to phosphatase formation in the temperate forests might contribute to coupling of N and P cycling (Houlton et al., 2008).

The soil TN/TP ratios did not differ between the P and NP additions. The TN/TP ratios were higher in plants (28:1) and soil microbes (7:1) (Marklein & Houlton, 2012) than in soils under combined NP additions (3.8–4.8). The exogenous N might be more quickly assimilated by plants or microbes than P, such that the effects of P and combined NP additions on phosphatase kinetic parameters are similar. A portion of the exogenous N may be leached and may be unavailable to plants or microbes, which might weaken the effects of N on phosphatase.

When we considered the P-rich and P-poor forest soils together, we found that the V_{max} and K_m were closely related, which was consistent with the results of other studies of soil profiles in tropical forests (Stone & Plante, 2014) and in global aquatic and terrestrial ecosystems (Sinsabaugh et al., 2014). These positive relationships deserve recognition as the variations in K_m could weaken the effects of the V_{max} on the catalytic efficiency in large scale studies. The net effects of N and P additions on organic P decomposition rates might also be weaker than expected from the V_{max} response. However, the V_{max} and K_m in the Dinghu site were not correlated, and this decoupling might reflect the fact that the V_{max} and K_m were regulated by different factors (Tischer et al., 2015). For example, soil available and total P contents inhibited the V_{max} but not the K_m.

5 | CONCLUSIONS

The kinetic parameters and the catalytic efficiencies of phosphomonoesterase were higher in P-rich forest soils than in P-poor forest soils in eastern China. In natural forest ecosystems, the V_{max}, K_m and the V_{max}/K_m were positively correlated with soil pH and total and available P contents. The kinetic parameters and the catalytic efficiency responded differently to N and P additions: (1) N additions increased the V_{max} and $K_{m,}$ but had no overall effect on the catalytic efficiency; (2) P additions decreased the V_{max} and the catalytic efficiency in the P-poor soils, but caused about 13% increase in the V_{max} in the P-rich forest soils, and (3) in the P-poor forest soils, the combined NP and the P only additions had similar effects on the kinetic parameters and the catalytic efficiencies of phosphatase. In contrast, in the P-rich forest soils, the V_{max} and the K_m increased for about 69% and 50% under the combined NP additions compared to Control, and increased more than under the N only or P only additions. While inorganic P fertilization might inhibit or promote the secretion of phosphatase in P-poor or P-rich soils respectively, micro-organisms might allocate excessive N to phosphatase production to relieve P limitations under long-term N deposition. The positive relationships between V_{max} and K_m indicated that K_m could weaken the effects of N and P additions on the catalytic efficiency and should be included in future models of P cycling. In P-rich soils, the increases in V_{max} and K_m after the combined NP additions indicated that micro-organisms were sensitive to N and P, and that more microbial community groups secreted a broad spectrum of isoenzymes. Taken together, the responses of phosphatase kinetics to P fertilization should be considered differently in P-rich and P-poor soils, while long-term N deposition might mitigate P limitation by increasing phosphatase secretion.

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AUTHORS' CONTRIBUTIONS

X.Z., S.N., G.Y., H.W. conceived the ideas and designed the methodology; Y.Y., C.Z., H.Y., D.T., Y.T. and S.L. managed the field N and P additions, collected and analysed samples, and analysed the data; X.Z., Y.Y., E.B., Y.K. and X.S. discussed the results and led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

All data supporting the results in our manuscript have been archived in the Dryad Digital Repository https://doi.org/10.5061/dryad.8cq1s (Zhang et al., 2017).

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SUPPORTING INFORMATION

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