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

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Outline

- 1 Introduction
- 2 Methods
- 3 Results
- 4 Conclusions

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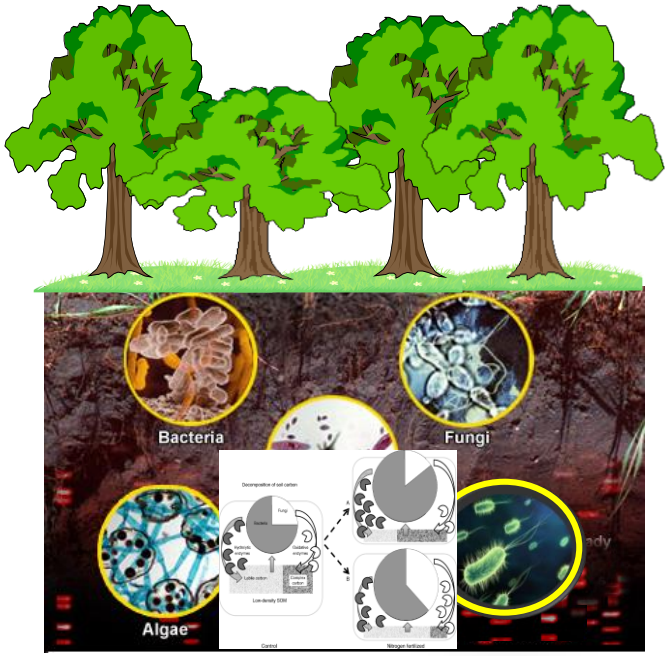
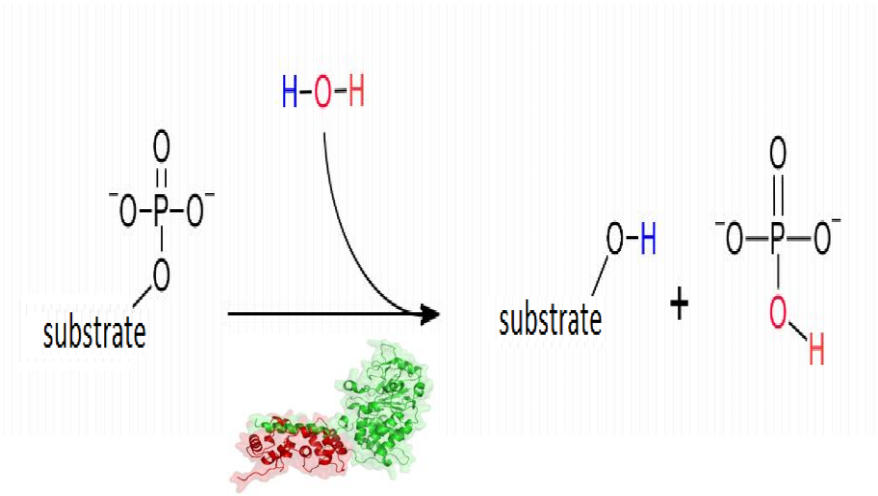
Applications of enzymes in ecology

- **Enzyme production and activities can affect ecosystem processes directly, can reflect the effects of climate change, land-use, and pollution…**
- **A phosphatase is an enzyme that uses water to cleave a phosphoric acid monoester into a phosphate ion and an alcohol .**
- **Soil phosphatases transform about 20%–80% of soil organic P to available P for plants and microbes uptake.**



Enzyme activity as an index



The general reaction catalyzed by a phosphatase enzyme

Michaelis-Menten kinetic parameters

◆**Enzymes mainly operate under non-saturated conditions in soils, hydrolytic enzyme activity is substrate (S) dependent and therefore follows the Michaelis–Menten equation.**

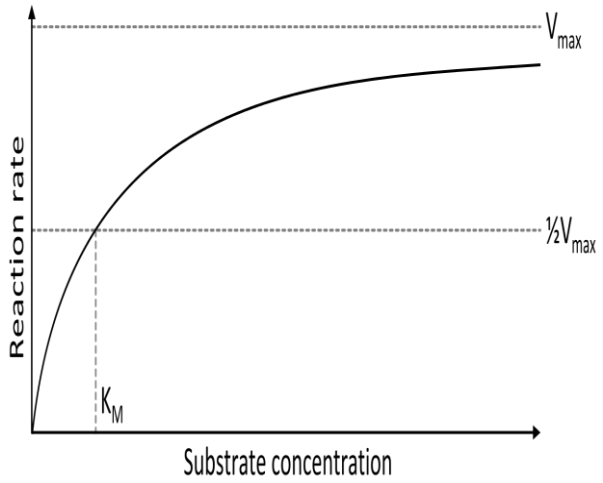
◆***K_m* and the catalytic efficiency are important parameters that can together represent microbial processes in biogeochemical models.**

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

***Vmax :* Maximal reaction rate** when all enzymes are substrate-saturated

***K_m :* Michaelis–Menten constant**, *reflects* the apparent binding affinity of the enzyme to the substrate ***,K_m↓ affinity*↑**

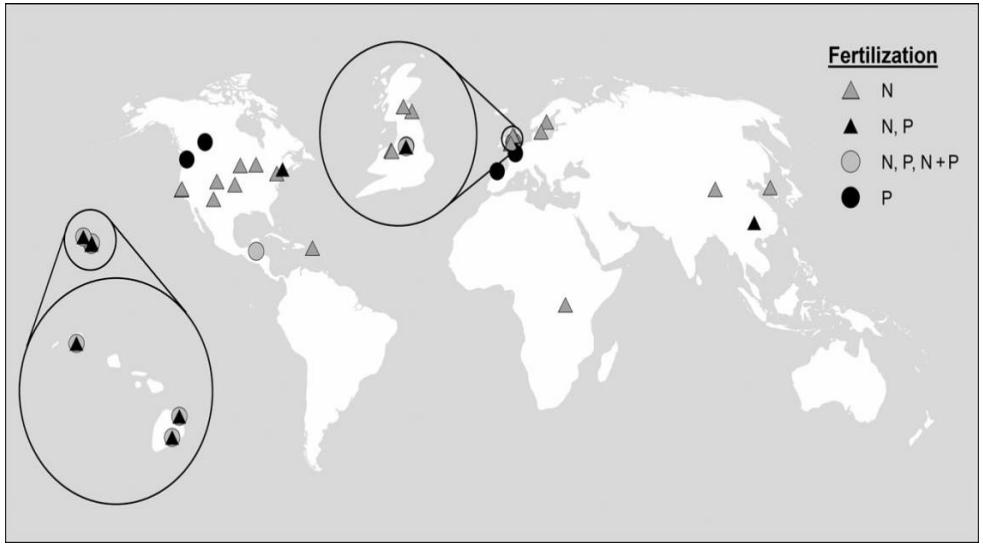
V_{max}/K_m: catalytic efficiency *reflects the enzyme catalytic* process relative to both the enzyme-substrate complex dissociation(*V_{max}*) *and the rate of enzyme-substrate* complex formation (*K_m*)



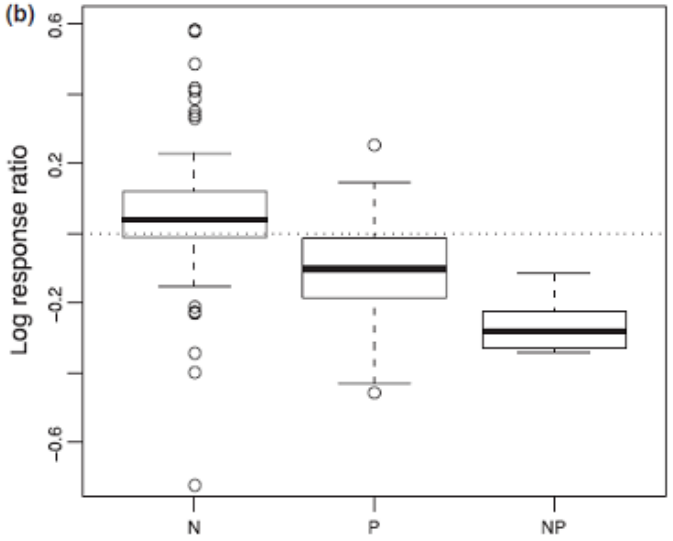
Michaelis–Menten kinetic

Phosphatase follows an economic principle

- **Micro-organisms will preferentially allocate energy toward the acquisition of whatever resource is most limiting .**
- **Results from meta-analysis indicated that, as N availability increased, microorganisms would allocate more resources towards the production of enzymes used to acquire P; P additions inhibited phosphatase under P only or combined N and P additions .**
- **However, only one study of N and P additions in a forest was included in the meta-analysis.**



Map of sites of phosphatase activity



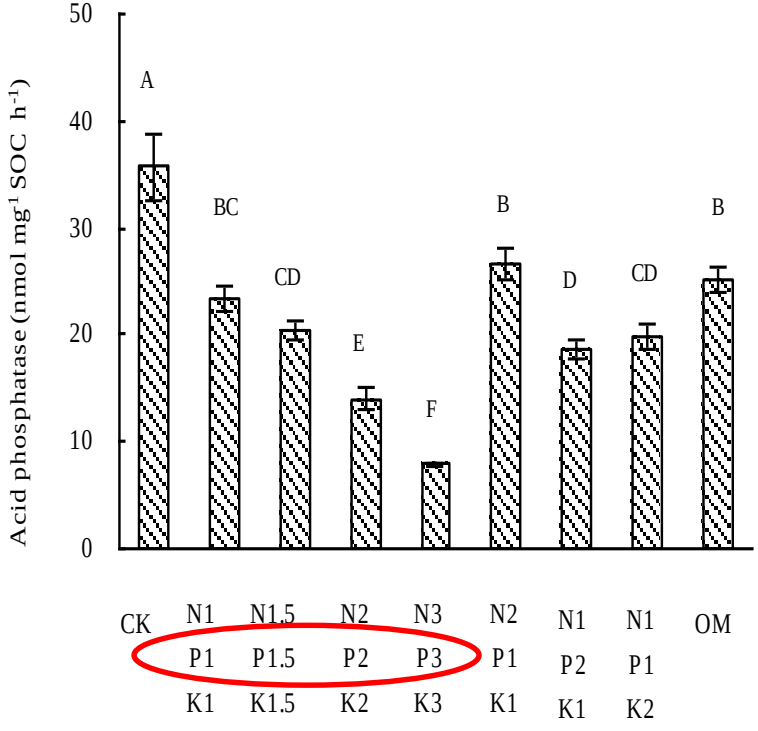
soil phosphatase responses to fertilization

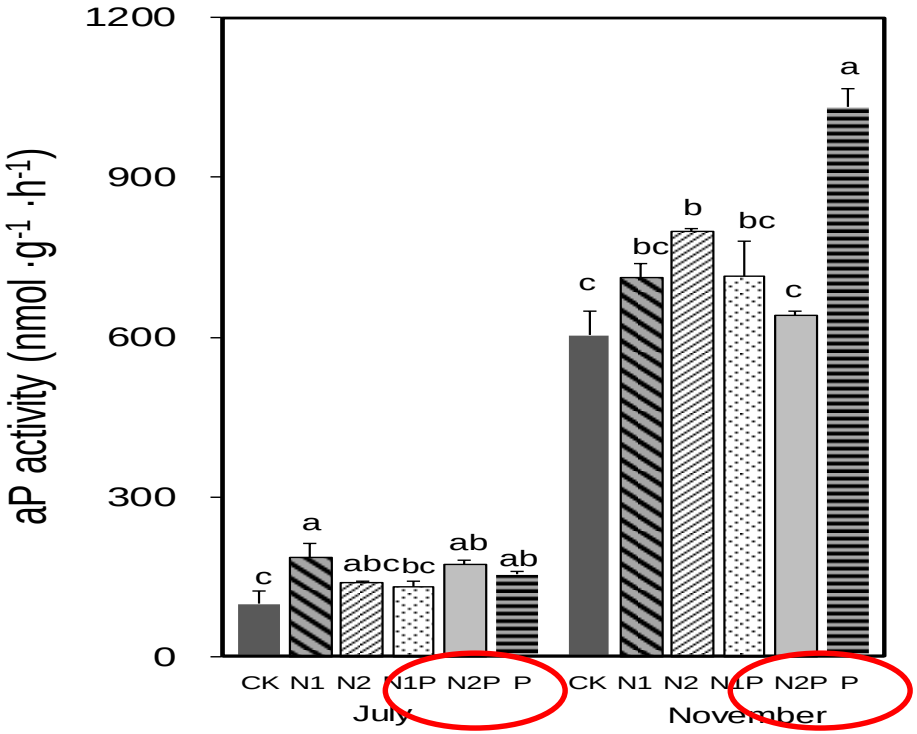
Marklein AR & Houlton BZ. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems , *New Phytologist*, 2012, 193(3): 696-704

Some studies did not follow the economic principle

◆**Our previous research showed that additions of N and P together could decrease soil phosphatase activity in subtropical agricultural soils.**

◆**But additions of P only or N and P together could increase soil phosphatase activity in subtropical forest soils .**





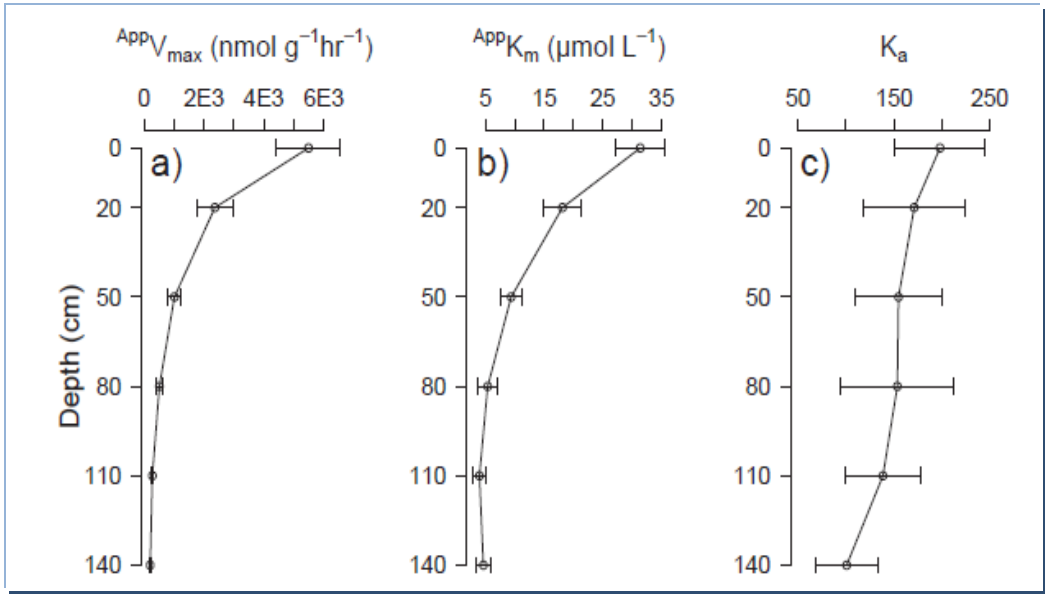
Dong WY & Zhang XY. Responses of soil microbial communities and enzyme activities to nitrogen and phosphorus additions in Chinese fir plantations of subtropical China .Biogeosciences, 2015,12:5537–5546

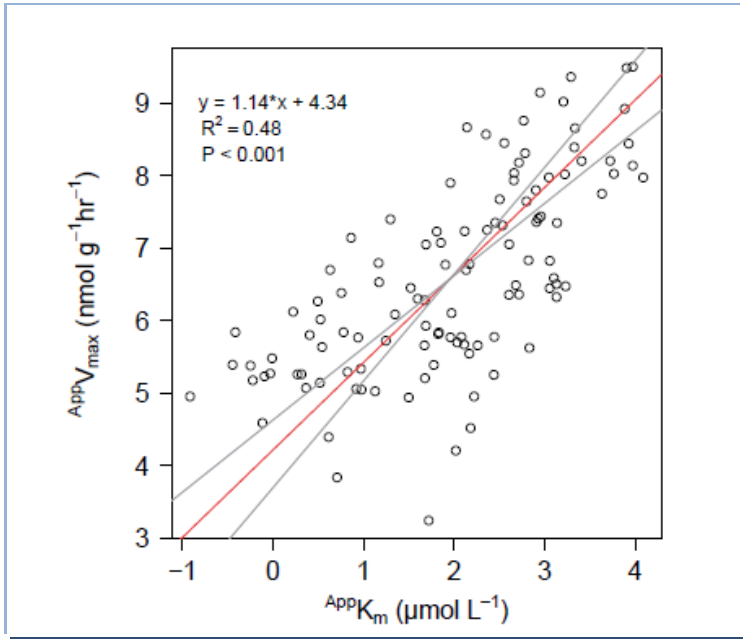
Zhang XY et al., Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. Science of the Total Environment, 2015,536:59–67

Soil phosphatase kinetic parameters variation?

◆**Soil 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.**

◆**How combined additions of N and P might affect the phosphatase kinetic parameters in forest soils are unclear.**





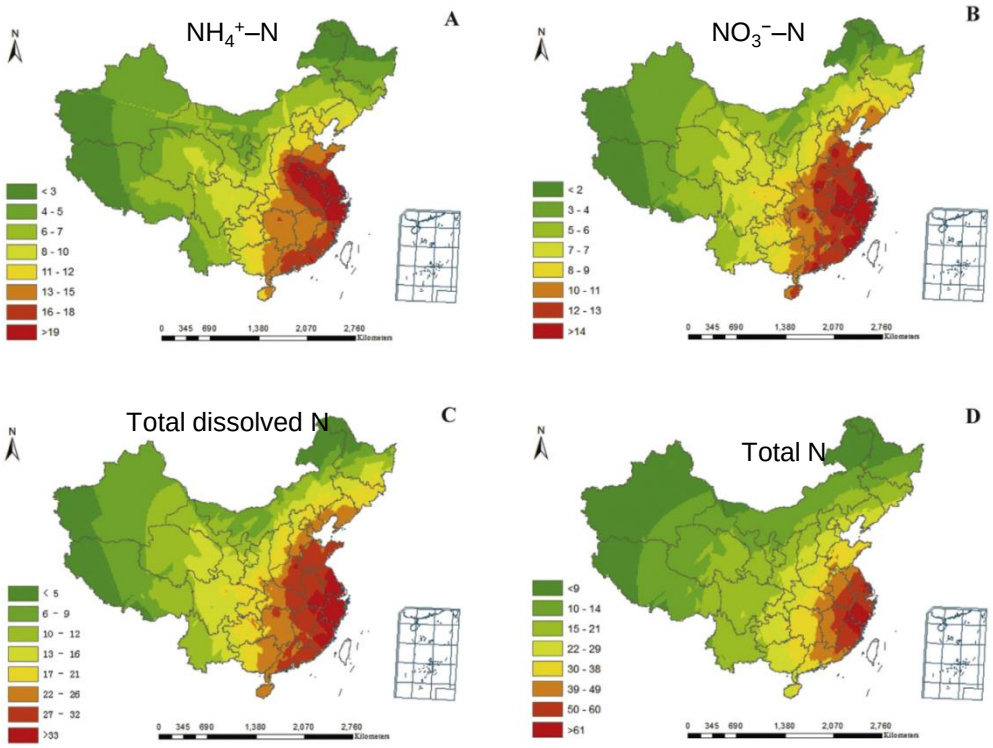
Stone MM & Plant AF. Changes in phosphatase kinetics with soil depth across a variable tropical landscape. Soil Biology & Biochemistry. 2014,71:61-67

Atmospheric nitrogen deposition is increasing

◆**Large quantities of N are deposited on subtropical and temperate forests throughout eastern China.**

◆**In subtropical forests, increasing N deposition may exacerbate existing P limitations; in temperate forests, increasing N deposition has been reported to shift nutrient limitation from N to P.**

◆**However, the complex linkages between N and P have not yet been resolved at an enzyme kinetic level.**



Zhu, J., et al. The composition, spatial patterns, and influencing factors of atmospheric wet nitrogen deposition in Chinese terrestrial ecosystems. Science of the Total Environment. 2015, 511: 777-785.

Objectives and hypotheses

Objectives:

Assessed the responses of phosphatase kinetic parameters to increased N and P availability

Hypotheses:

I. **the V_{max} and the catalytic efficiency would increase after N additions;**

II. **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**

III. **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.**

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Experimental treatments

◆The long term N and P addition experiments started in 2012 at Qianyanzhou and in 2013 on Changbai and Dinghu Mts.

◆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.

◆We sampled the 0-10 cm soils after 4-year nutrient additions.

Native conifer mixed broad-leaved forest

Chinese fir plantation

Secondary conifer mixed broad-leaved forest

◆control (CK);
◆ N (10 g N $m^{-2}yr^{-1}$（NH_4NO_3）;
◆ P（10 g P $m^{-2}yr^{-1}$ （NaH_2PO_4）;
◆NP（10 g N $m^{-2}yr^{-1}$（NH_4NO_3）
+10 g P $m^{-2}yr^{-1}NaH_2PO_4$）
（+5 g P m^{-2} yr^{-1} for Qianyanzhou）

CK	N
P	NP

NP	CK
N	P

NP	P
CK	N

P	N
NP	CK

Site description

	Changbai Mt.(CBM)	Qianyanzhou (QYZ)	Dinghu Mt. (DHM)
Climate zone	**temperate**	**subtropical**	**subtropical**
MAT (ºC)	2.8	16.8	22.0
MAP (mm)	731	1629	1733
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(*Pinuskoraiensis*), mongolian oak (*Quercusmongolica*)	Chinese fir (*Cunninghamialanceolata*)	schima root-bark (Schima superb), Chinese red pine (Pinusmassoniana Lamb)
pH	5.51±0.08 A	4.71±0.03 B	3.80±0.06 C
SOC (g kg^{-1})	87.5±1.18 A	16.5 ±0.71 B	11.1±1.21 C
TN (g kg^{-1})	5.6 ±0.05 A	1.2 ±0.07 B	0.9 ±0.05 C
TP (mg kg^{-1})	**1022±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**

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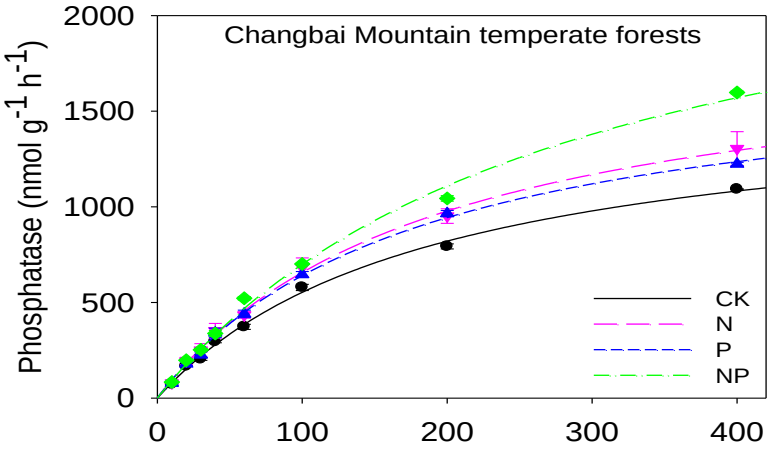
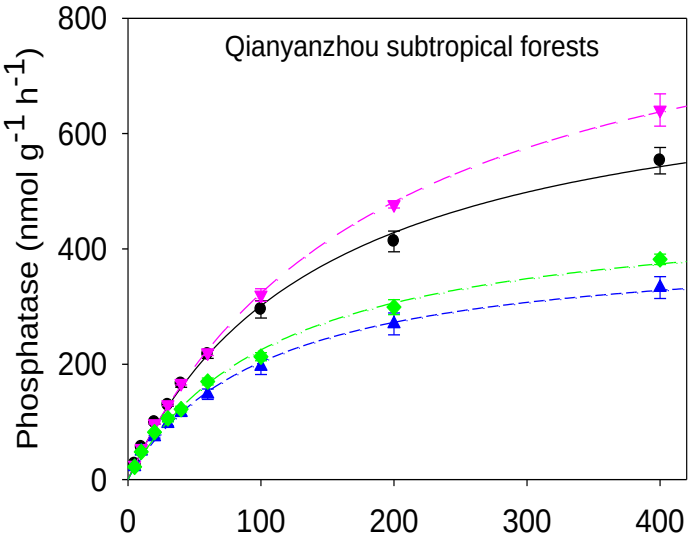
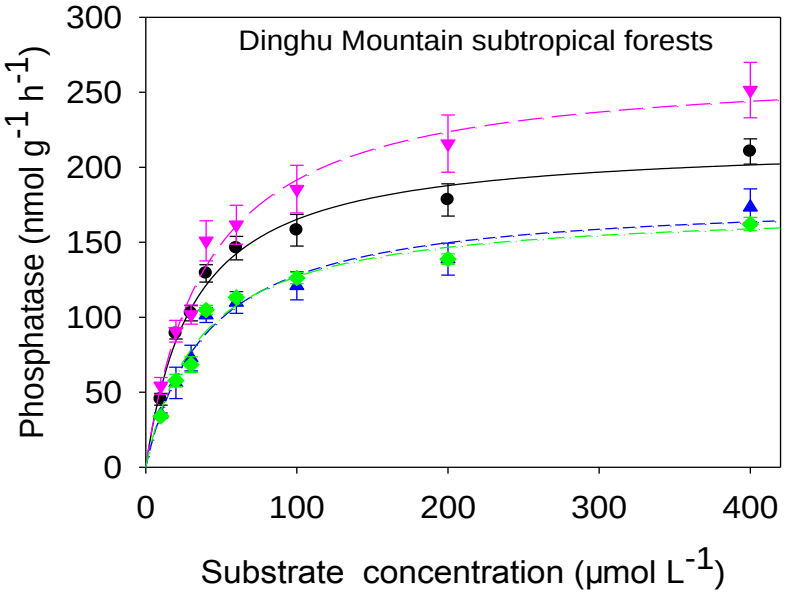
Michaelis-Menten plots of soil phosphatase

◆**The relationships between the substrate content and the phosphatase activities fitted well to the Michaelis–Menten equation.**

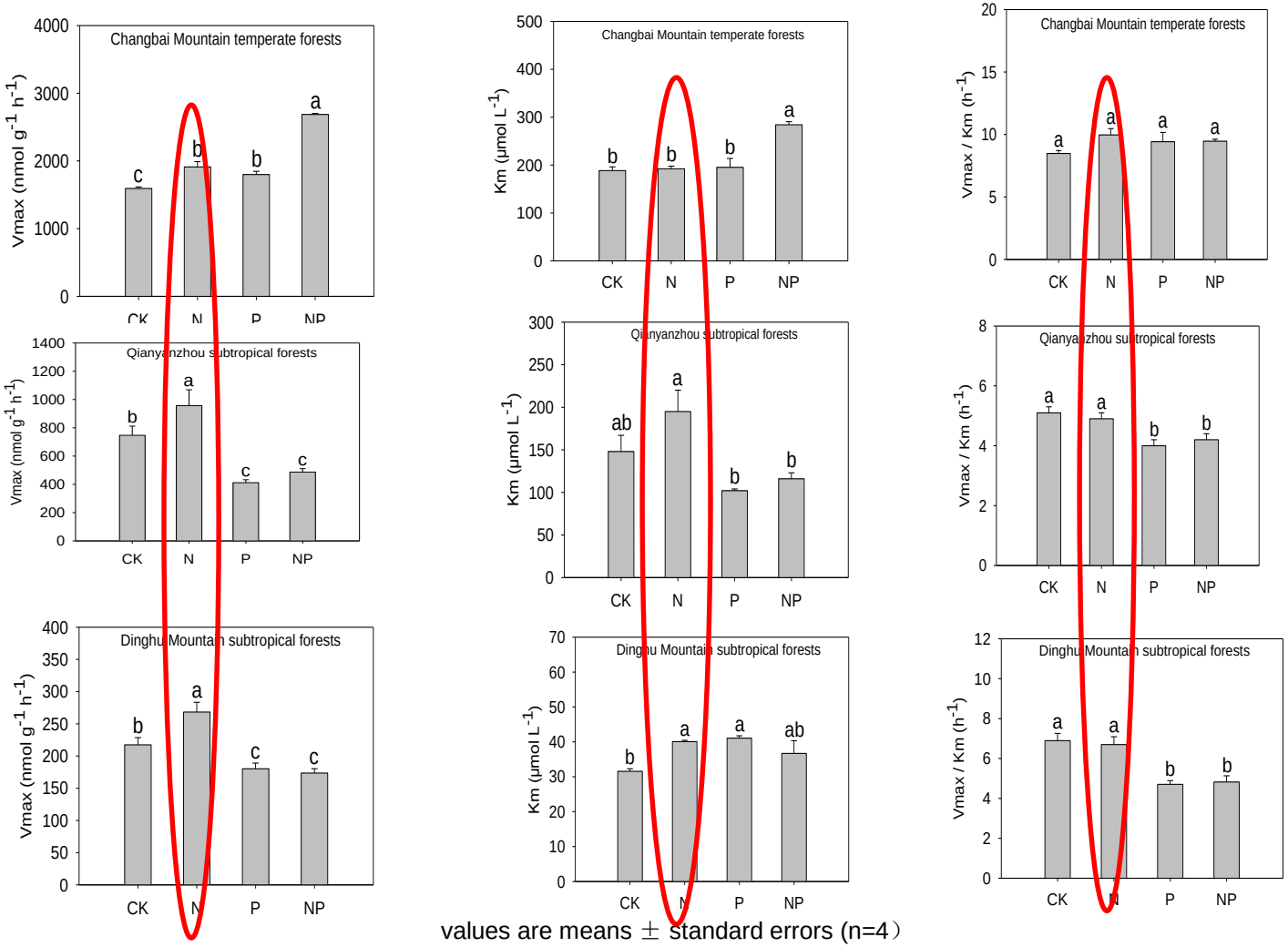






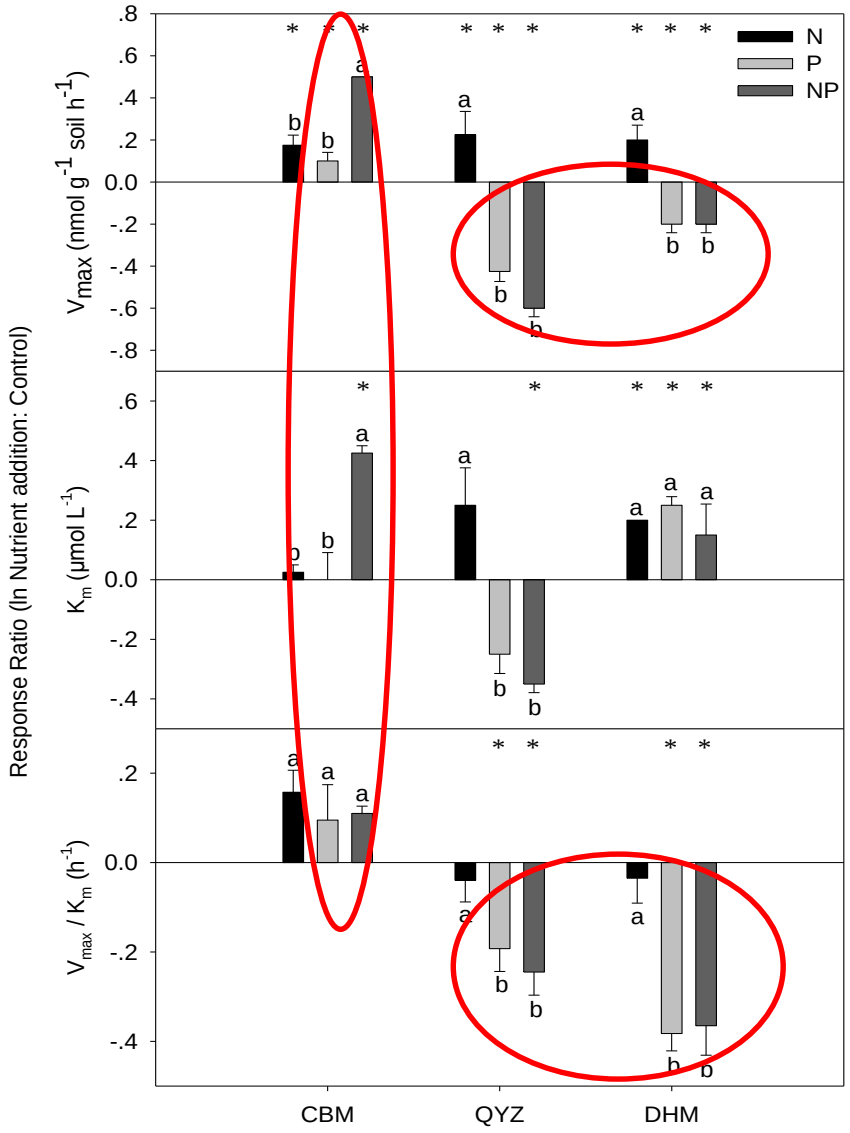
Responses of soil phosphatase kinetic parameters to N

◆**Our first 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.**



values are means ± standard errors (n=4）

Response ratios of soil phosphatase kinetic parameters to P and NP



◆The second 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.

◆In contrast to the second hypothesis in the temperate forest:

- ➢the V_{max} and catalytic efficiencies were increased after P additions.
- ➢the V_{max} and K_m increased about 70% 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.

Relationships between phosphatase kinetic parameters and soil nutrients

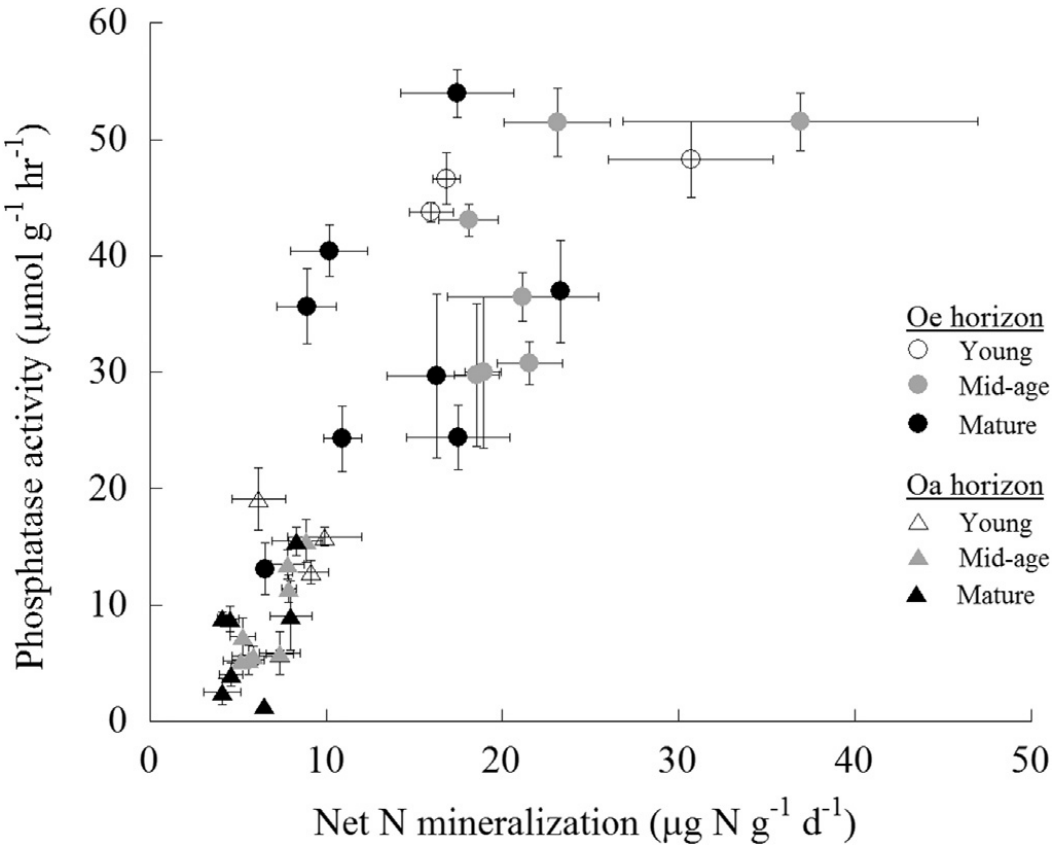
V_{max} and the K_m were positively related to NO_3^--N in the P-rich forest soils

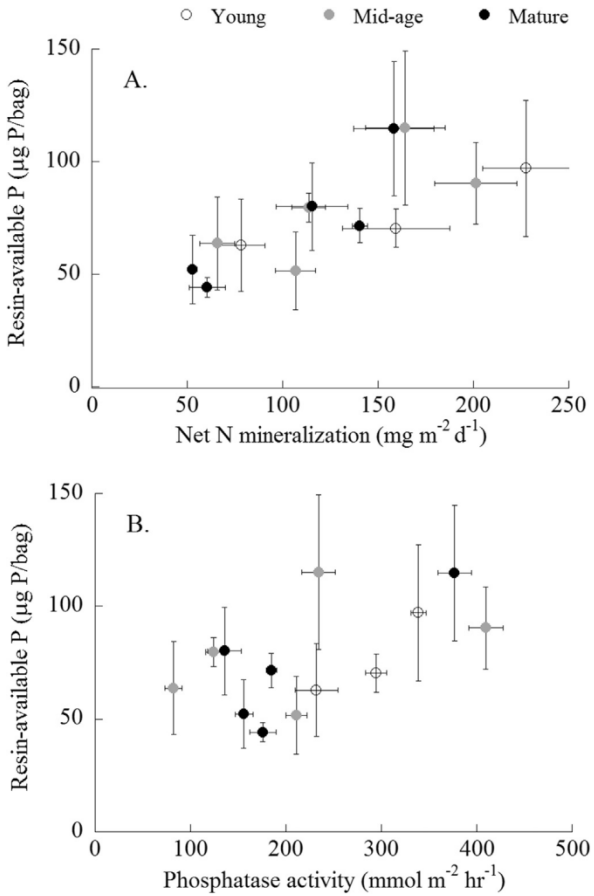
	Changbai Mountain			Qianyanzhou			Dinghu Mountain		
	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m	V_{max}	K_m	V_{max}/K_m
TP	**0.55***	NS	NS	**-0. 68****	**-0.59***	**-0.60***	**-0.60***	NS	**-0.80****
AvailableP	**0.58***	**0.56***	NS	**-0.72****	**-0.58***	**-0.74****	**-0.75****	NS	**-0.80****
AN/AP	**-0.53***	**-0.53***	NS	**0.76****	**0.63****	**0.71****	**0.79****	NS	**0.93****
TN/TP	NS	NS	NS	**0.80****	**0.70****	**0.69****	**0.66****	NS	**0.83****
SOC/TN	NS	NS	NS	**0.50***	**0.62***	NS	NS	NS	NS
SOC	**0.61***	**0.51***	NS	NS	NS	NS	NS	NS	NS
NO_3^--N	**0.68****	**0.62***	NS	NS	NS	NS	NS	NS	NS

* represents P<0.05, ** represents P<0.01, NS representP > 0.05, n=16.

Phosphatase activity is related to N availability

◆In hardwood forests in the northeastern United States, phosphatase activity is related to N availability but not P availability.



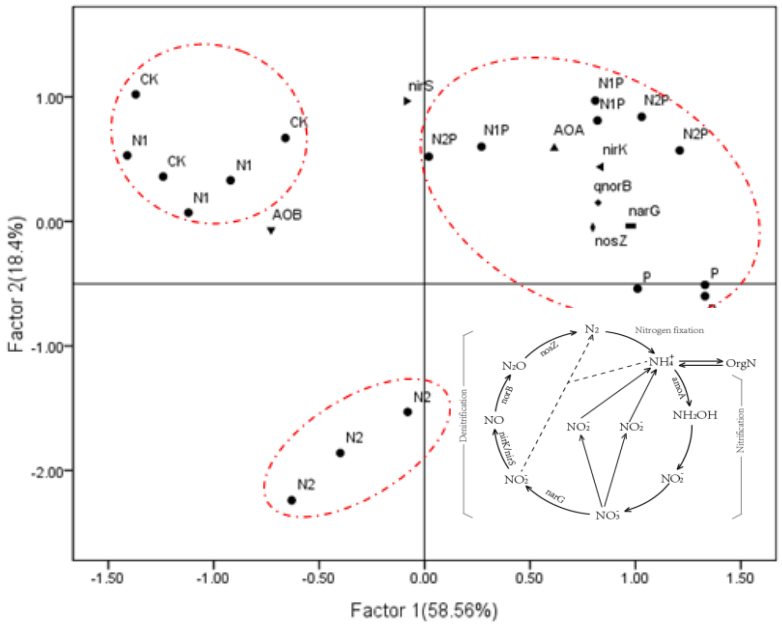


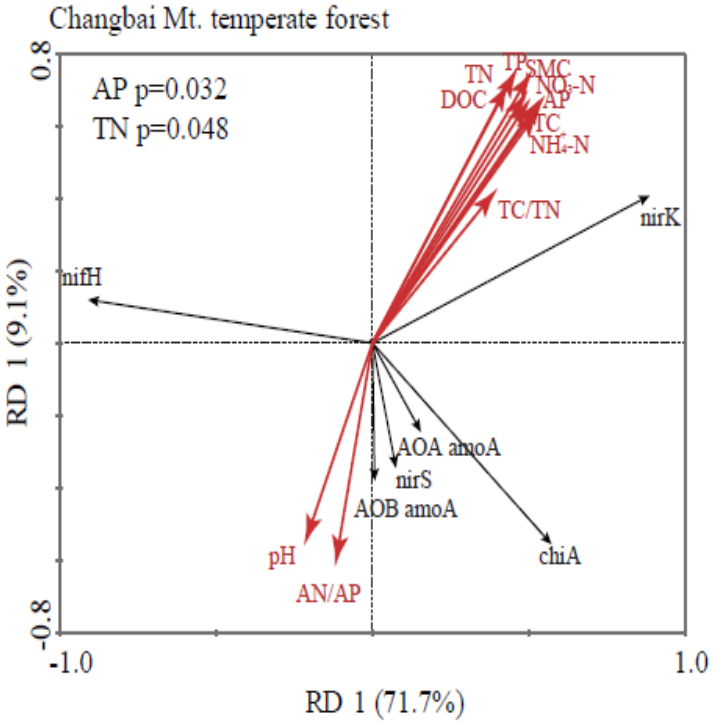
Ratliff TJ & Fisk MC . Phosphatase activity is related to N availability but not P availability across hardwood forests in the northeastern United States. Soil Biology and Biochemistry ,2016,**4: 61-69.**

P availability promoted the abundances of NFGs

◆The abundances of nitrogen functional genes (NFGs) were positively correlated with the concentrations of soil available P.

◆P and NP applications caused the soil nitrification rate and NO_3^--N concentrations to increase.





Tang YQ & Zhang XY. Impacts of nitrogen and phosphorus additions on the abundance and community structure of ammonia oxidizers and denitrifying bacteria in Chinese fir plantations. Soil Biology & Biochemistry, 2016,103: 284-293

Tang YQ & Zhang XY, Changes in nitrogen-cycling microbial communities with depth in temperate and subtropical forest soils. Applied Soil Ecology, 218, 124: 218–228

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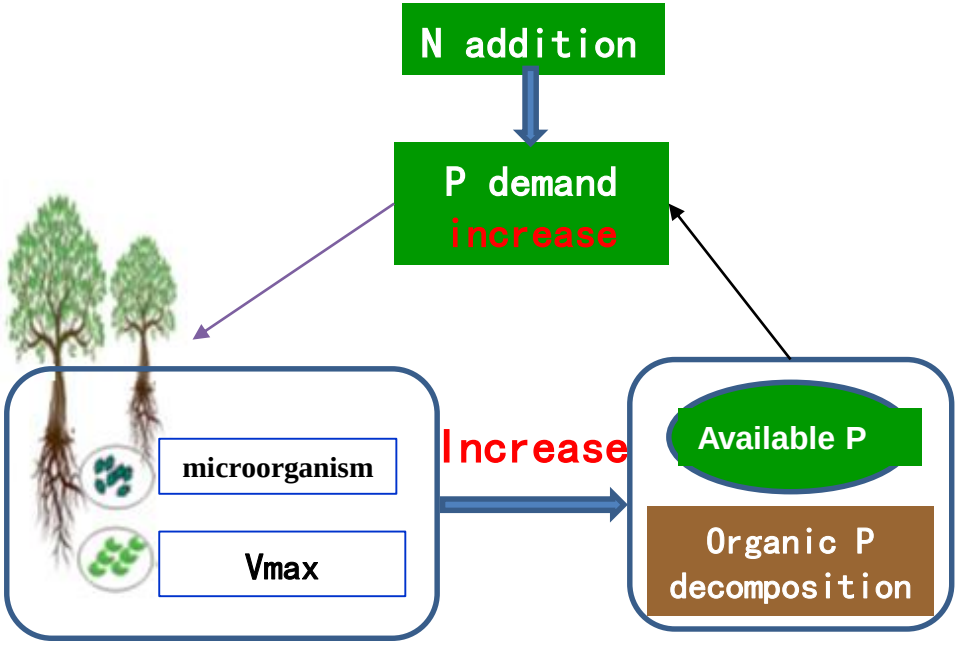
Conclusions

- N additions increased the V_{max} and K_m, but had no overall effect on the catalytic efficiency;
- while long-term N deposition might mitigate P limitation by increasing phosphatase secretion.

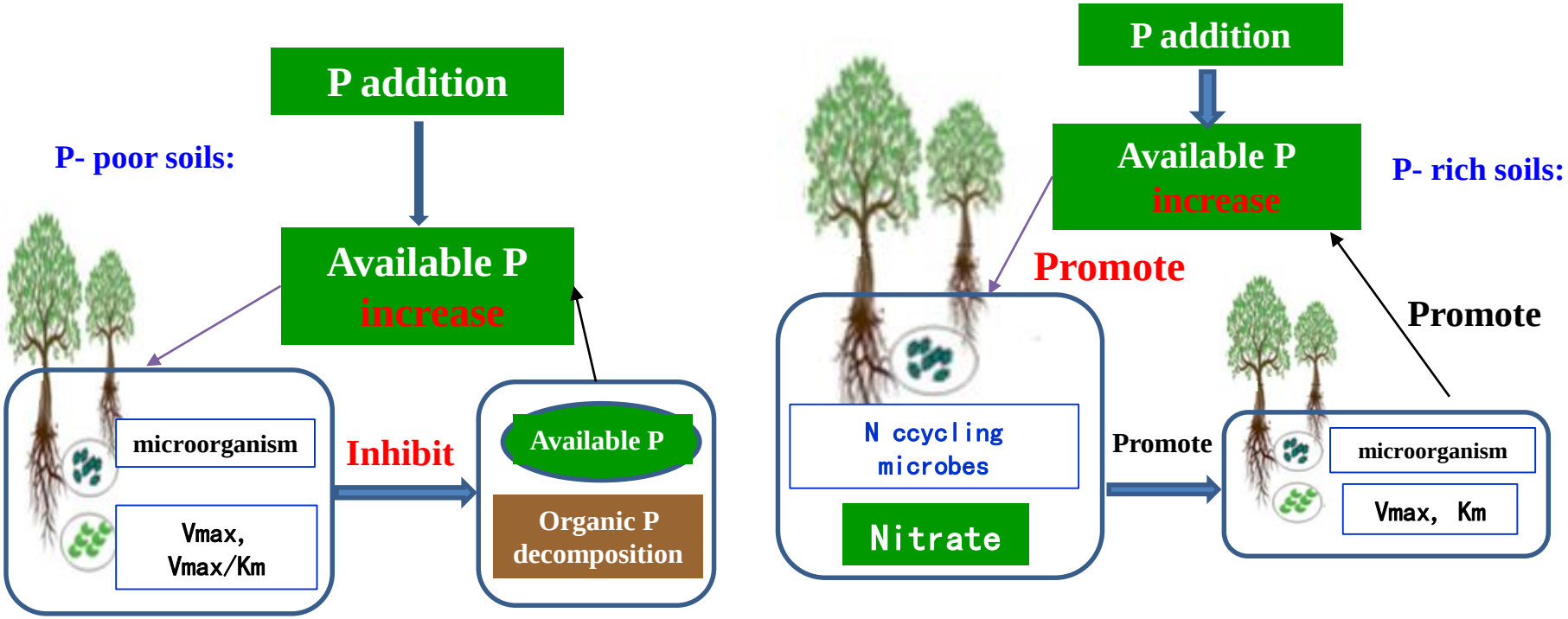


Conceptual scheme of N addition effects on phosphatase

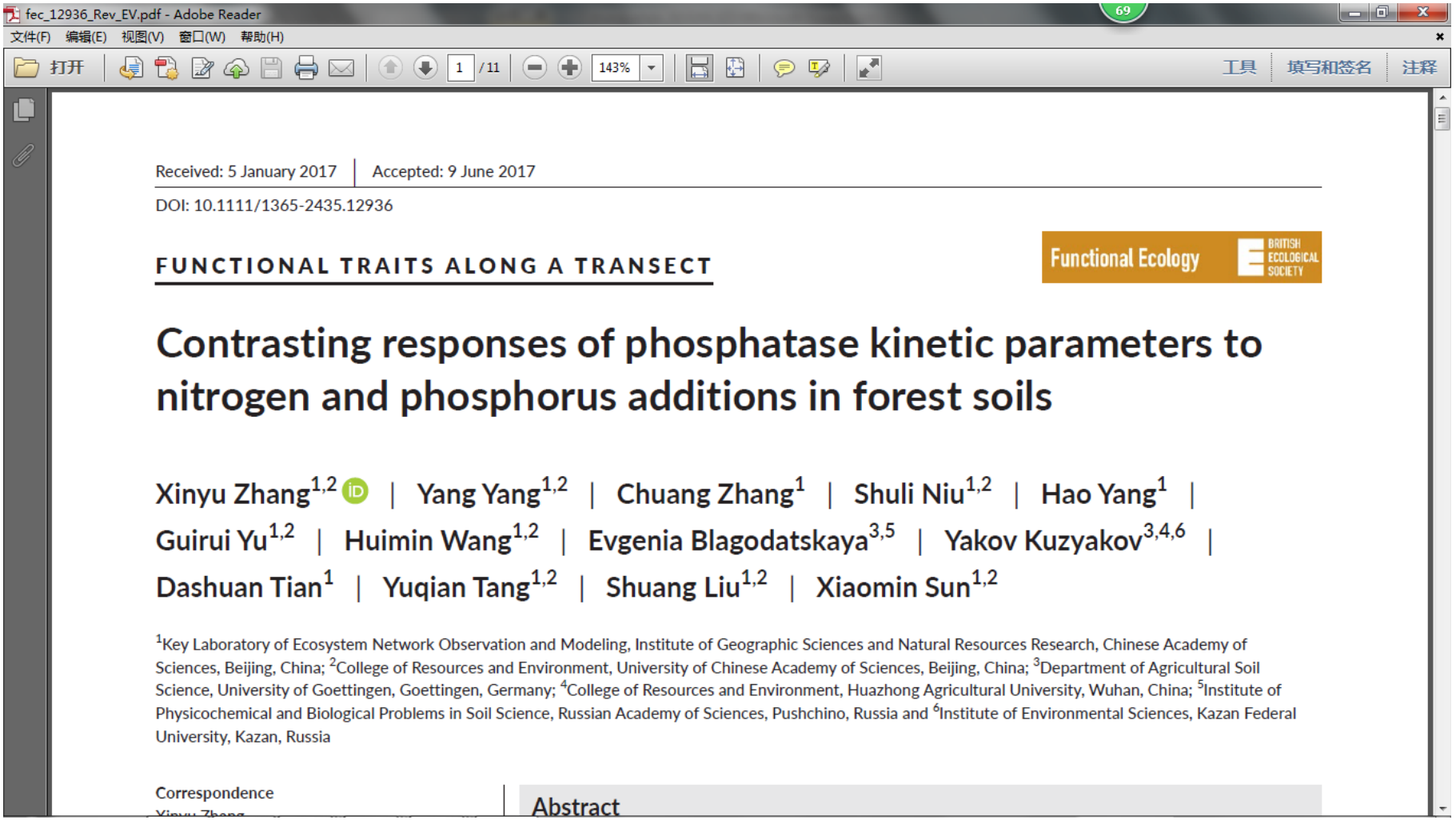
◆the responses of phosphatase kinetics to P fertilization should be considered differently in P-rich and P-poor soils:

I. in the P-poor forest soils, the combined NP and the P additions similarly decreased the V_{max} and the catalytic efficiency of phosphatase;

II. In contrast, in the P-rich forest soils, the combined NP additions increased the V_{max} and the K_m more than under the N or P additions.



Conceptual scheme of P addition effects on phosphatase



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FUNCTIONAL TRAITS ALONG A TRANSECT

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

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Abstract

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Thank you for your attention!