


Short Communication

Effects of long-term phosphorus addition on soil ratios of phosphomonoesterase to phosphodiesterase in three tropical forests

Taiki Mori^{1,2,†}, Senhao Wang^{1,3,†}, Cong Wang⁴, Ji Chen^{5,6,7}, Cheng Peng^{1,3,8}, Mianhai Zheng^{1,3}, Juan Huang^{1,3}, Faming Wang^{1,3} , Zhanfeng Liu^{1,3}, Jiangming Mo^{1,3} and Wei Zhang^{1,3,*}

¹Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China, ²Kyushu Research Center, Forestry and Forest Products Research Institute, FFPRI, Kurokami 4-11-16, Kumamoto 860-0862, Japan, ³Center for Plant Ecology, Core Botanical Gardens, Chinese Academy of Sciences, Guangzhou 510650, China, ⁴State Key laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China, ⁵Department of Agroecology, Aarhus University, 8830 Tjele, Denmark, ⁶Aarhus University Centre for Circular Bioeconomy, Aarhus University, 8830 Tjele, Denmark, ⁷iCLIMATE Interdisciplinary Centre for Climate Change, Aarhus University, 4000 Roskilde, Denmark, ⁸University of Chinese Academy of Sciences, Beijing 100049, China

*Corresponding author. E-mail: zhangwei@scbg.ac.cn

†These authors contributed equally to this work.

Handling Editor: Zhiqun Huang

Received: 11 February 2022, **First Decision:** 17 April 2022, **Accepted:** 17 September 2022, **Online Publication:** 30 September 2022

Abstract

Soil microorganisms in tropical forests can adapt to phosphorus (P)-poor conditions by changing the activity ratios of different types of phosphatases. We tested whether microorganisms in P-poor tropical forest soils increased the phosphomonoesterase (PME) to phosphodiesterase (PDE) activity ratio, because a one-step enzymatic reaction of monoester P degradation might be more adaptive for microbial P acquisition than a two-step reaction of diester P degradation. A continuous 10-year P addition experiment was performed in three tropical forests. The activities of PME and PDE, and their ratio in soil, were determined under the hypothesis that the P-fertilized plots where P shortage is relieved would have lower PME:PDE ratios than the unfertilized controls. We demonstrated that long-term P addition in tropical forest soil did not alter the PME:PDE ratio in primary and secondary forests, whereas P fertilization elevated the PME:PDE ratio in planted forest. These results were in contrast to previous results. The long-term, large-scale P fertilization in our study may have reduced litter- and/or throughfall-derived PDE, which negated the lowered PME:PDE ratio via exogenous P inputs.

Keywords soil extracellular enzymes, phosphomonoesterase, phosphodiesterase, phosphorus fertilization, tropical forest

长期磷添加对热带森林土壤磷酸单酯酶与磷酸二酯酶活性比值的影响

摘要：热带森林土壤微生物可以通过改变不同类型的磷酸酶活性来适应低磷的环境。以往的研究认为，低磷环境中土壤微生物更倾向于分解有机磷单酯键而非二酯键。因此，磷酸单酯酶(PME)的活

性变化对生态系统磷输入响应可能更敏感，其与磷酸二酯酶(PDE)活性的比值在磷输入后将显著降低。为了检验土壤微生物在低磷的热带森林中对不同磷酸酶的“投资”策略，我们在鼎湖山3种热带森林进行了长达10年的磷添加实验。与以往的研究不同，本研究发现长期磷添加没有改变原生林与次生林的PME:PDE比值，但显著提高了人工林土壤PME:PDE比值。结果表明，长期磷添加可能减少了凋落物或穿透雨来源的PDE，从而使其土壤PDE活性下降较PME降低更快，因而PME:PDE比值未能降低。

关键词：土壤胞外酶，磷酸单酯酶，磷酸二酯酶，施磷，热带森林

INTRODUCTION

Phosphorus (P) is an important nutrient in natural ecosystems because it is essential for living organisms (Jelinčić *et al.* 2022; Guo *et al.* 2021). Tropical forests are P-poor ecosystems. P plays an important role in net primary production (Crews *et al.* 1995; Kitayama and Aiba 2002; Magadla *et al.* 2021; Vitousek and Sanford 1986) and soil microbial activity (Cleveland *et al.* 2002; Liu *et al.* 2012), although soil microbial activity is not necessarily limited by P (Mori *et al.* 2018, 2019). This is because that tropical forest soils are deeply weathered, and a large portion of the remaining P is present in occluded forms (Cross and Schlesinger 1995). In such ecosystems, P acquisition by plants and/or microbes is largely dependent on turnover and recycling of organic P compounds (Hidaka and Kitayama 2013; Turner and Engelbrecht 2011) via the production of extracellular phosphatases involved in the breakdown of organic P (Chen *et al.* 2020; Nannipieri *et al.* 2011; Turner 2008). Indeed, compared with other ecosystems, microbial investment in phosphatase in tropical forests is much larger relative to other types of enzymes due to lower soil P availability (Sinsabaugh *et al.* 2009; Turner and Wright 2014; Waring *et al.* 2014).

Organic P occurs in a variety of forms—including as diester P such as DNA, RNA and phospholipids—but many previous studies only focused on the role of phosphomonoesterase (PME) and neglected other enzymes participating in P decomposition (Turner and Engelbrecht 2011; Yokoyama *et al.* 2017, 2018). Diester P is degraded to orthophosphate by a two-step enzymatic reaction: initial hydrolysis from diester to monoester P by phosphodiesterase (PDE), and successive hydrolysis of monoester P into orthophosphate by PME (Turner and Haygarth 2005; Yokoyama *et al.* 2017). Although organisms may adapt to P-poor conditions by altering the activity ratios of the two phosphatases, few studies have investigated differences in PME and PDE activities between P-poor and P rich conditions (Yokoyama *et al.* 2017).

Yokoyama *et al.* (2017) tested the impact of P fertilization on four different types of phosphatases in tropical lowland forests in Malaysian Borneo, and found that P addition substantially reduced PME activity in soils, although its impact on PDE activity was unclear. Based on this phenomenon, they proposed the following novel hypothesis: in P-poor tropical forests, soil microbes (and/or plants) increase P allocation for PME production relative to PDE, because P acquisition via a one-step enzymatic reaction with lower energy cost might be more adaptive in P-poor soils (Yokoyama *et al.* 2017). However, since their report, no studies have tested this hypothesis. In the present study, we used data from a long-term (10-year) continuous P addition experiment to test the hypothesis of Yokoyama *et al.* (2017). Based on their hypothesis, we predicted that P addition would decrease PME:PDE ratios, because P acquisition via one-step PME reactions might be more adaptive in P-poor tropical forest soils. The present study will increase our understanding of the adaptation of soil microorganisms to P-poor conditions in tropical forests.

MATERIALS AND METHODS

The experiment was conducted in the Dinghushan Biosphere Reserve (DHSBR) in Guangdong Province, southern China (112°10' E, 23°10' N). The DHSBR experiences a monsoon climate. The annual mean temperature is 21.9 °C and the annual precipitation measured from February 2015 to January 2016 was 2431 mm (Zhou *et al.* 2018). There are three major forest types in the reserve: primary forest, secondary forest and planted forest (Supplementary Table S1). Soil in the reserve is lateritic red earth (Oxisols) formed from highly weathered sandstone (Mo *et al.* 2003). The soil properties of the three forests are shown in Supplementary Table S2. In February 2007, control and P-fertilized plots (5 m × 5 m) were established with five replications (Liu *et al.* 2012).

P (150 kg P ha⁻¹ yr⁻¹) in the form of NaH₂PO₄ was added bimonthly. In December 2017, soil cores were taken from the surface soils (0–10 cm) and sieved through a 2-mm mesh. The samples were frozen at –20 °C until use. PDE activity assays were conducted as described by Mori *et al.* (2020). Briefly, 1.0 g fresh soils and 50 mM acetate (100 mL, pH 5.0) were homogenized, and suspensions (500 µL) were then incubated with substrates labeled with 4-methylumbelliferone (4 h at 20 °C in the dark). Fluorescence was determined by a microplate spectrophotometer (365 nm excitation and 450 nm emission filters). PME activities determined using the Michaelis–Menten equation were reported by Mori *et al.* (2022); we calculated PME:PDE ratios based on their results. The impact of P addition on PME:PDE ratios was tested by a one-way ANOVA. PME:PDE ratios were natural log-transformed before statistical analyses, which were performed using R (version 4.1.1; R Core Team 2021).

RESULTS AND DISCUSSION

Long-term P addition in the three tropical forests significantly suppressed PDE activity (Table 1), which was consistent with previous studies. In primary and secondary forests, the decrease in PDE activity was similar to that in PME activity (Table 1), resulting in unaltered PME:PDE ratios (Fig. 1a and b). On the other hand, PME:PDE ratio was elevated by P fertilization in planted forest (Fig. 1c). These results were contrary to our initial hypothesis based on the prediction by Yokoyama *et al.* (2017) that P fertilization would result in lower PME:PDE ratios compared with unfertilized controls. The unchanged PME:PDE ratios also disagreed with several previous studies. According to Zhang *et al.* (2015), PME activity

decreased as P availability increased, but PDE activity did not, which supports the hypothesis of Yokoyama *et al.* (2017). Lang *et al.* (2017) also observed the highest PME:PDE ratio in the most P-poor soil among five different forests.

The inconsistent response of PME:PDE ratios to P addition in this study might be attributed to the activities of PME and PDE derived from leaf litters and throughfall to P fertilization. The large amount (150 kg P ha⁻¹ yr⁻¹) of P added in our study over a long period (10 years) should have increased the leaf P content (Zhu *et al.* 2015), and thus potentially reduced PDE activity in litter and/or PDE production by phyllosphere microbes providing coenzymes to soils, which could mask any decrease in the soil PME:PDE ratio (note that phyllosphere could be an important source of soil enzymes (Mori *et al.* 2019, 2021)) in primary and secondary forests (Fig. 1a and b) or even result in higher PME:PDE ratio in planted forest (Fig. 1c). This potential underlying mechanism requires the following assumptions: phosphatases from canopy leaves and litter make a large contribution to soil phosphatase activity; the decrease in PDE in leaves or the phyllosphere is larger than that in PME; and the responses of senescence-derived and phyllosphere microbe-derived PDE production to P fertilization depend on the duration and amount of P fertilization. Long-term P addition (9 years) in a Panamanian lowland tropical forest had similar results to those in our study: the ratio of PME to PDE was largely unchanged by P fertilization (Turner and Wright 2014), consistent with our proposed underlying mechanism. Experiments targeting enzymes in litters or throughfall (Mori *et al.* 2019, 2021) are necessary to test this idea. More data and P amendment experiments are needed to clarify the effects of P fertilization on PME and PDE

Table 1: Activities of PME and PDE in soils of the study forests

	Primary forest		Secondary forest		Planted forest	
	Control	P-added	Control	P-added	Control	P-added
PME activity ^{a,*} (µg g soil ⁻¹ hr ⁻¹)	5279.9 (840.2)	1103.7 (124.9)	2540.4 (332.6)	553.0 (63.9)	933.4 (171.9)	522.0 (62.3)
PDE activity [*] (µg g soil ⁻¹ hr ⁻¹)	270.4 (53.6)	59.0 (4.3)	108.5 (10.2)	27.5 (2.8)	84.0 (11.9)	29.8 (5.1)
PME:PDE	19.5	18.7	23.4	20.1	11.1	17.5

Values are means with standard errors in parentheses ($n = 5$). ^aData from Mori *et al.* (2022). ^{*}Significant differences in a linear mixed-effect model with forest type as a random effect ($P < 0.001$).

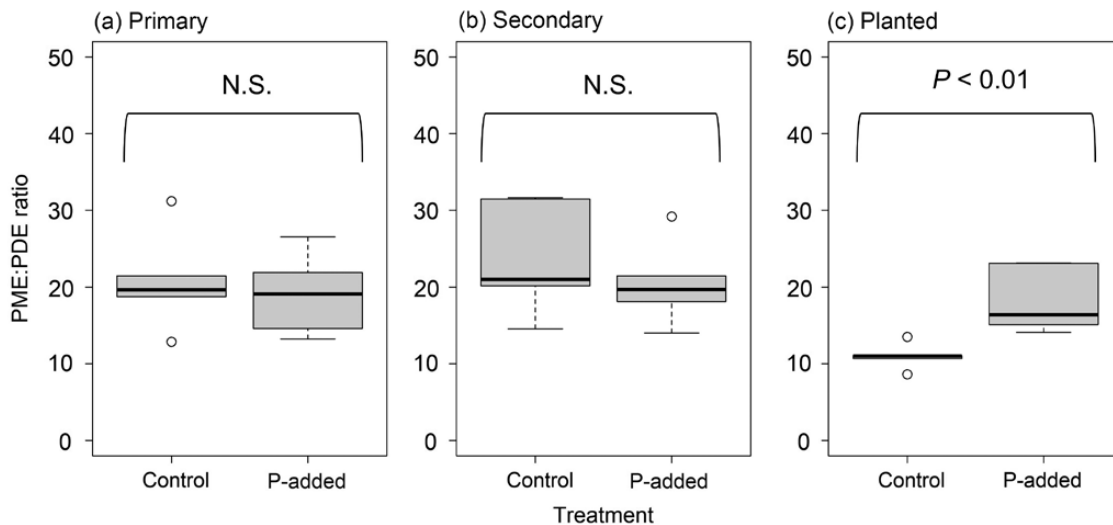


Figure 1: Effects of P addition on the ratio of PME and PDE activities (PME:PDE ratio) in (a) primary forest, (b) secondary forest and (c) planted forest. Data are illustrated using box plots ($n = 5$). Statistically significant differences were detected by one-way ANOVA. N.S., not statistically significant. PME:PDE ratios were natural log-transformed before statistical analyses. Means and standard errors are shown in Table 1.

activities produced by soil microorganisms, plants and phyllosphere microorganisms.

Supplementary Material

Supplementary material is available at *Journal of Plant Ecology* online.

Table S1: Indices of tree structure in three tropical forests.

Table S2: Soil properties in three tropical forests.

Funding

This study was supported by the National Natural Science Foundation of China (42077311 and 41731176), Grant-in-Aid for JSPS Postdoctoral Fellowships for Research Abroad (28.601), JSPS KAKENHI (JP19K15879) and a grant from the Sumitomo Foundation (153082).

Acknowledgements

We are grateful to the handling editor and anonymous reviewers for their insightful and constructive comments.

Conflict of interest statement. The authors declare that they have no conflict of interest.

REFERENCES

Chen J, van Groenigen KJ, Hungate BA, *et al.* (2020) Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems. *Glob Change Biol* **26**:5077–5086.

Cleveland C, Townsend A, Schmidt S (2002) Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. *Ecosystems* **5**:680–691.

Crews TE, Kitayama K, Fownes JH, *et al.* (1995) Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology* **76**:1407–1424.

Cross AE, Schlesinger WH (1995) A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* **64**:197–214.

Guo YP, Yan ZB, Zhang YW, *et al.* (2021) Environmental constraints on the inter-genus variation in the scaling relationship between leaf nitrogen and phosphorus concentrations. *J Plant Ecol* **14**:616–627.

Hidaka A, Kitayama K (2013) Relationship between photosynthetic phosphorus-use efficiency and foliar phosphorus fractions in tropical tree species. *Ecol Evol* **3**:4872–4880.

Jelinčić A, Šajna N, Zgorelec Ž, *et al.* (2022) Bracken-induced increase in soil P availability, along with its high P acquisition efficiency, enables it to invade P-deficient meadows. *J Plant Ecol* **15**:783–794.

Kitayama K, Aiba SI (2002) Ecosystem structure and productivity of tropical rain forests along altitudinal gradients with contrasting soil phosphorus pools on Mount Kinabalu, Borneo. *J Ecol* **90**:37–51.

Lang F, Krüger J, Amelung W, *et al.* (2017) Soil phosphorus supply controls P nutrition strategies of beech forest ecosystems in Central Europe. *Biogeochemistry* **136**:5–29.

Liu L, Gundersen P, Zhang T, *et al.* (2012) Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China. *Soil Biol Biochem* **44**:31–38.

Magadella A, Makhaye N, Perez-Fernandez M (2021) Symbionts in *Mucuna pruriens* stimulate plant performance through nitrogen fixation and improved phosphorus acquisition. *J Plant Ecol* **14**:310–322.

- Mo J, Brown S, Peng S, *et al.* (2003) Nitrogen availability in disturbed, rehabilitated and mature forests of tropical China. *For Ecol Manage* **175**:573–583.
- Mori T, Lu X, Aoyagi R, *et al.* (2018) Reconsidering the phosphorus limitation of soil microbial activity in tropical forests. *Funct Ecol* **32**:1145–1154.
- Mori T, Wang S, Zhang W, *et al.* (2019) A potential source of soil coenzymes: from the phyllosphere to soil via throughfall. *Appl Soil Ecol* **139**:25–28.
- Mori T, Wang S, Zhang W, *et al.* (2022) Microbial assembly adapted to low-P soils in three subtropical forests by increasing the maximum rate of substrate conversion of acid phosphatases but not by decreasing the half-saturation constant. *Eur J Soil Biol* **108**:103377.
- Mori T, Wang S, Zhou K, *et al.* (2021) Ratios of phosphatase activity to activities of carbon and nitrogen-acquiring enzymes in throughfall were larger in tropical forests than a temperate forest. *Tropics* **30**:25–29.
- Mori T, Zhou K, Wang C, *et al.* (2020) Effects of 14-year continuous nitrogen addition on soil arylsulfatase and phosphodiesterase activities in a mature tropical forest. *Glob Ecol Conserv* **22**:e00934.
- Nannipieri P, Giagnoni L, Landi L, *et al.* (2011) Role of phosphatase enzymes in soil. In Bunemann E, Oberson A, Frossard E (eds). *Phosphorus in Action: Biological Processes in Soil Phosphorus Cycling*. Springer, Berlin, 215–243.
- R Core Team (2021) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>.
- Sinsabaugh RL, Hill BH, Follstad Shah JJ (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* **462**:795–798.
- Turner BL (2008) Resource partitioning for soil phosphorus: a hypothesis. *J Ecol* **96**:698–702.
- Turner BL, Engelbrecht BMJ (2011) Soil organic phosphorus in lowland tropical rain forests. *Biogeochemistry* **103**:297–315.
- Turner BL, Haygarth PM (2005) Phosphatase activity in temperate pasture soils: potential regulation of labile organic phosphorus turnover by phosphodiesterase activity. *Sci Total Environ* **344**:27–36.
- Turner BL, Wright SJ (2014) The response of microbial biomass and hydrolytic enzymes to a decade of nitrogen, phosphorus, and potassium addition in a lowland tropical rain forest. *Biogeochemistry* **117**:115–130.
- Vitousek PM, Sanford RL (1986) Nutrient cycling in moist tropical forest. *Annu Rev Ecol Syst* **17**:137–167.
- Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. *Biogeochemistry* **117**:101–113.
- Yokoyama D, Imai N, Kitayama K (2017) Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant Soil*. **416**:463–476.
- Yokoyama D, Mori T, Wagai R, *et al.* (2018) Characteristics of phosphorus fractions in the soils derived from sedimentary and serpentinite rocks in lowland tropical rain forests, Borneo. *Soil Sci Plant Nutr*. **64**:218–221.
- Zhang X, Dong W, Dai X, *et al.* (2015) Responses of absolute and specific soil enzyme activities to long term additions of organic and mineral fertilizer. *Sci Total Environ* **536**:59–67.
- Zhou K, Lu X, Mori T, *et al.* (2018) Effects of long-term nitrogen deposition on phosphorus leaching dynamics in a mature tropical forest. *Biogeochemistry*. **138**:215–224.
- Zhu F, Lu X, Liu L, *et al.* (2015) Phosphate addition enhanced soil inorganic nutrients to a large extent in three tropical forests. *Sci Rep*. **5**:7923.