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

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Abstract

Soil microorganisms in tropical forests can adapt to 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 compared to 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
Introduction

Phosphorus (P) is an important nutrient in natural ecosystems because it is essential for living organisms. Tropical forests are P-poor ecosystems. P plays an important role in net primary production (Crevis et al., 1995; Kitayama and Aiba, 2002; Vitousek et al., 2010) 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., 2019a, 2018). This is because 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; Mori, 2022; 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 to 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.

Material 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 2,431 mm (Zhou et al., 2018). There are three major forest types in the reserve: primary forest, secondary forest, and planted forest (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 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 twice
monthly. 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, 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 to 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 ecoenzymes 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, 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 activities produced by soil microorganisms, plants, and phyllosphere microorganisms.
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|                  | Primary forest | Secondary forest | Planted forest |
|------------------|----------------|------------------|----------------|
|                  | Control        | P-added          | Control        | P-added          | Control        | P-added          |
| PME activity $^\S$,$^*$ | 5279.9 (840.2) | 1103.7 (124.9)  | 2540.4 (332.6) | 553.0 (63.9)    | 933.4 (171.9)  | 522.0 (62.3)    |
| (µg g soil$^{-1}$hr$^{-1}$) |                |                  |                |                 |                |                 |
| PDE activity $^*$  | 270.4 (53.6)   | 59.0 (4.3)       | 108.5 (10.2)   | 27.5 (2.8)      | 84.0 (11.9)    | 29.8 (5.1)      |
| (µg g soil$^{-1}$hr$^{-1}$) |                |                  |                |                 |                |                 |
| PME:PDE           | 19.5           | 18.7             | 23.4           | 20.1            | 11.1           | 17.5            |

Values are means with standard errors in parentheses (n = 5). $^\S$ Data from Mori et al. (2022).

$^*$ Significant differences in a linear mixed-effect model with forest type as a random effect ($p < 0.001$).
**Figure legends**

**Figure 1:** Effects of P addition on the ratio of phosphomonoesterase (PME) and phosphodiesterase (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.
