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Phosphatase activity in relation to key litter and soil properties in mature subtropical forests in China

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HIGHLIGHTS

- The potential activities of AcPME and PDE were positively correlated in all horizons.
- AcPME activity was significantly lower in the L than the F/H horizon while PDE activity was comparable between them.
- The relationships between phosphatase activities and key edaphic properties vary with horizon.

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ABSTRACT

Phosphatase-mediated phosphorus (P) mineralization is one of the critical processes in biogeochemical cycling of P and determines soil P availability in forest ecosystems; however, the regulation of soil phosphatase activity remains elusive. This study investigated the potential extracellular activities of acid phosphomonoesterase (AcPME) and phosphodiesterase (PDE) and how they were related to key edaphic properties in the L horizon (undecomposed litter) and F/H horizon (fermented and humified litter) and the underlying mineral soil at the 0–15 cm depth in eight mature subtropical forests in China. AcPME activity decreased significantly in the order of F/H horizon > L horizon > mineral soil horizon, while the order for PDE activity was L horizon = F/H horizon > mineral soil horizon. AcPME (*X* axis) and PDE (*Y* axis) activities were positively correlated in all horizons with significantly higher slope in the L and F/H horizons than in the mineral soil horizon. Both AcPME and PDE activities were positively related to microbial biomass C, moisture content and water-holding capacity in the L horizon, and were positively related to soil C:P, N:P and C:N ratios and fine root (diameter ≤ 2 mm) biomass in the mineral soil horizon. Both enzyme activities were also interactively affected by forest and horizon, partly due to the interactive effect of forest and horizon on microbial biomass. Our results suggest that modulator(s) of the potential extracellular activity of phosphatases vary with horizon, depending on the relative C, P and water availability of the horizon.

1. Introduction

Human-induced elevation of atmospheric CO_2 concentration and increases in nitrogen (N) deposition have led to a strong imbalance with phosphorus (P), conferring an increasingly important role of P availability in the function of natural ecosystems (Peñuelas et al., 2012). Phosphatase-mediated P mineralization is one of the critical processes in P biogeochemical cycling and determines P availability in forest ecosystems (Marklein and Houlton, 2012; Olander and Vitousek, 2000) as well as other natural ecosystems (Marklein and Houlton, 2012; Turner and Haygarth, 2005). Identification of the key environmental factors regulating phosphatase activity is critical for advancing our understanding of the dynamics and availability of P in forest ecosystems (Henry, 2012; Nannipieri et al., 2011).

Phosphatases (e.g. acid phosphomonoesterase) have been found in both plants and microbes, with an exception that alkaline phosphomonoesterase has only been found in microbes (Nannipieri et al., 2011). Of the phosphatases present in soil, phosphomonoesterases are the most widely studied; usually, acid phosphomonoesterase prevails in acidic soils whereas alkaline phosphomonoesterase prevails in alkaline soil (Nannipieri et al., 2011). Phosphomonoesterases act on a range of low molecular weight P compounds with monoester bonds (namely phosphomonoesters), including mononucleotides, sugar phosphatases, and polyphosphates, and can also catalyze the hydrolysis of low-order inositol phosphatases (Nannipieri et al., 2011; Turner and Haygarth, 2005). Phosphodiesterases are a group of enzymes

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involving the hydrolysis of P compounds with diester bonds (namely phosphodiesters) such as phospholipids and nucleic acids, which constitute the majority of the fresh organic P inputs to soil (Bunemann, 2008; Koukol et al., 2006; Turner and Haygarth, 2005). Hydrolysis of a phosphodiester is initiated by phosphodiesterase to release a phosphomonoester, which must then be hydrolyzed by phosphomonoesterase to release a free phosphate for biological uptake (Bunemann, 2008; Turner and Haygarth, 2005).

The production of phosphatase is mainly regulated by the demand for P by organisms and environmental P availability (Olander and Vitousek, 2000; Sinsabaugh and Follstad Shah, 2012), in addition to other edaphic factors (e.g. C and N availability) (Allison and Vitousek, 2005; Marklein and Houlton, 2012; Naples and Fisk, 2010). Soil phosphatase activity is usually inversely related to soil P availability as a result of negative feedback by soil available P to the production and activity of phosphatase (Olander and Vitousek, 2000), but may be positively related to the amount of soil extractable organic P due to the enhancement of phosphatase activity by the availability of hydrolyzable organic P sources (Tarafdar and Claassen, 1988). Soil phosphatase activity is usually reported to be positively related to soil C and N availability (Allison and Vitousek, 2005; Marklein and Houlton, 2012), mainly because the production and release of phosphatase are significant C- and N-consuming processes (Allison and Vitousek, 2005). After its release into soil, phosphatase is subject to degradation and stabilization (Nannipieri et al., 2011; Sinsabaugh and Follstad Shah, 2012) and is affected by a number of edaphic factors such as soil organic matter (Zornoza et al., 2007, 2007), contents of clay (Nannipieri et al., 2011) and moisture (Henry, 2012), and soil pH (Turner, 2010). Since extracellular phosphatase activity can be regulated by multiple environmental factors, the relationships between phosphatase activity and key litter and soil properties vary widely in different terrestrial ecosystems (Marklein and Houlton, 2012; Sinsabaugh et al., 2008) in relation to site background C, N, and P availability (Allison and Vitousek, 2005; Olander and Vitousek, 2000), variation in organic matter (or C) content with soil depth (Chen et al., 2000; Sinsabaugh et al., 2008), or organism composition, which affects nutrient assimilation and allocation (Naples and Fisk, 2010). Moreover, the extracellular activities of different phosphatases may be dissimilarly regulated by the same edaphic factors (e.g. soil total organic P) (Turner, 2010; Turner and Haygarth, 2005), probably as a result of their different functions in P mineralization (Turner and Haygarth, 2005) and/or their different vulnerabilities to degradation and stabilization (Turner, 2010).

Recent studies suggest that soil P availability is a significant limiting factor in the growth of both plant and soil microorganisms in natural subtropical forests in China (Hou et al., 2012; Huang et al., 2013). Phosphatase-mediated P mineralization is considered to be one of the significant processes governing soil P availability in these forest ecosystems (Huang et al., 2013). However, what drives soil phosphatase activity in these forests remains elusive.

This study sampled forest floor litters (separated into L and F/H horizons) and the underlying soil at the 0–15 cm depth from eight subtropical forests in China. The potential extracellular activities of acid phosphomonoesterase and phosphodiesterase and contents of total C, total N, total P, microbial biomass C, and moisture were determined. Soil extractable inorganic P and organic P fractions and fine root biomass in the 0–15 cm soil horizon were also determined. The main objective was to identify the edaphic properties that could significantly modulate AcPME and PDE activities in litter and soil in mature subtropical forests using a relationship analysis approach. In particular, we were interested in whether and how phosphatase activities might be related to C:N:P stoichiometry in litter and soil, as an increasing number of studies have suggested that soil C and N availability can have impacts on soil P availability by influencing the soil phosphatase activity (Allison and Vitousek, 2005; Marklein and Houlton, 2012).

2. Materials and methods

2.1. Site description

The study was conducted in the Dinghushan Biosphere Reserve, located in the middle of Guangdong Province in China (112°31′ E to 112°34′ E, 23°09′ N to 23°12′ N). The Reserve covers an area of 1155 ha and has a typical subtropical monsoon climate. More than 1800 plant species have been identified and documented across the Reserve. Mean annual temperature at the site is about 21 °C, and mean annual precipitation is around 1900 mm. Nearly 80% of the precipitation falls in the wet season (from April to September) and 20% in the dry season (from October to March). Elevation ranges from 10 to 1000 m above sea level. Surface forest soils have developed from Devonian sandstone and shale during the Holocene (Shen et al., 2001), and are classified as Ferralsols according to the FAO classification.

Table 1 shows the basic information of eight selected forests, which are within a straight-line distance of 4 km from each other in Dinghushan, China. They represent five major types of forests and cover a large range of topographic conditions in south China. All sites have been protected well since their establishment, except for the pine forest site which suffered frequent harvest of litter and understory by local residents between the 1930s and early 1990s (Mo et al., 1995). Harvest of the understory (mainly seedlings of broadleaved species and herbs) has impeded the succession of pine forest to pine and broadleaved mixed forest at this site (Mo et al., 1995).

2.2. Sampling and sample preparation

Litters and soils were sampled from eight selected forests in October 2010, at the start of dry season. At each site, four subplots (20×20 m^2) were randomly set up with a distance of at least 10 m between them. In each subplot, three sampling areas (20×20 cm^2) were randomly selected with the constraints that they were 1–2 m away from the nearest tree (diameter at breast height $\geq$ 5 cm) and at least 5 m from their nearest neighbor. Fine litter (senesced branches, bark, flowers and fruits with diameters $\leq$ 1 cm, and leaf litter) within the sampling area were fully collected and separated into the L horizon (undecomposed litter) and F/H horizon (mixture of partly decomposed litter and amorphous humus) in the field. After the litters were collected, a soil profile was excavated in the bare soil area. Soil at 0–15 cm depth was sampled by three successive cutting rings (height: 5 cm; volume: 100 cm^3) from top to bottom (5 cm depth per cutting ring). The three litter samples from the same layer of each subplot were bulked together as one composite sample (one composite L horizon sample and one composite F/H horizon sample per subplot). Nine soils (three cutting rings per area $\times$ three areas) from each subplot were bulked together to form one composite soil sample. Both litter and soil samples were stored at 4 °C in the refrigerator within 4 h after sampling.

For each litter sample, fresh weight was measured and then the sample was mixed well. A subsample was oven-dried at 65 °C to constant weight and weighed for the determination of moisture content, after which the oven-dried subsample was ground for the determination of total C, total N, and total P. The rest of the fresh litter was cut into small pieces and then stored at 4 °C prior to the determination of microbial biomass C, enzyme activities, and water-holding capacity.

For each soil sample, fresh weight was measured, and then the sample was mixed well and sieved to pass through a 4-mm mesh, with stones of diameter >4 mm picked out and weighed. A first subsample of the sieved soil was oven-dried at 105 °C to constant weight for the determination of moisture content. A second subsample was stored at 4 °C for the determination of microbial biomass C, enzyme activities, and water-holding capacity. A third subsample was weighed and air-dried for 2 weeks prior to grinding and determination of soil chemical properties. The remaining sieved fresh soil was weighed and used for the calculation of fine root biomass. In general, living fine roots