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Changes in phosphatase kinetics with soil depth across a variable tropical landscape

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ABSTRACT

Phosphatase enzymes play a key role cycling phosphorus from organic to plant-available pools, particularly in tropical soils where inorganic phosphorus is often limited. However, most studies of phosphatase activity have focused only on surface soils, despite the large quantities of carbon and nutrients stored in tropical subsoils. The goal of this study was to determine how acid phosphatase kinetic parameters change with depth across two parent materials (represented by Oxisols and an Inceptisols) and two distinct forests (lower and upper montane) at the Luquillo Critical Zone Observatory in northeast Puerto Rico. We collected samples from five soil pits at each of four soil × forest types, and measured apparent phosphatase kinetic parameters ($^{App}V_{max}$ and $^{App}K_m$) and soil nutrients at 0, 20, 50, 80, 110 and 140 cm depths. Across all sites, $^{App}V_{max}$ declined 97% and $^{App}K_m$ declined 85% from the surface to 140 cm depth. The ratio of $^{App}V_{max}$ to $^{App}K_m$ (i.e., K_a) did not change through the first meter of soil profiles but was significantly reduced by 50% 140 cm. Total carbon, nitrogen and extractable phosphorus all declined exponentially with depth. Carbon concentrations and $^{App}V_{max}$ were both significantly greater in Oxisols compared with Inceptisols, and in the higher elevation montane forest compared to the lower elevation forest. The scaling relationship we observe between $^{App}V_{max}$ and $^{App}K_m$ is common for environmental systems, although the degree of correlation in our study ($R^2 = 0.48$) is unusually high, suggesting these parameters are both driven by changes in energy and nutrient availability along depth profiles. However, the consistency of K_a with depth indicates that overall catalytic capacity of phosphatase is maintained across a range of substrate concentrations. The larger variability in $^{App}V_{max}$ compared with $^{App}K_m$ suggests microorganisms exert more control over phosphatase production than substrate availability. Our findings indicate that subsoil microbial communities are not metabolically dormant, but rather contribute to P-cycling at rates comparable to their surface counterparts. Further research on ecology of microorganisms in resource-limited tropical subsoils is warranted to better understand microbial contributions to biogeochemical cycles throughout tropical soil profiles.

1. Introduction

Phosphorus (P) availability can limit net primary production (NPP) in tropical forests where rock-derived inorganic phosphate is depleted or geochemically bound (Cleveland et al., 2011; Davidson et al., 2007; Vitousek et al., 2010). In such ecosystems, plant-available P may be largely controlled by microbial mineralization of organic P (Harrison, 1987; Johnson et al., 2003; Turner and Engelbrecht, 2011). Recent research indicates that organic P in tropical soils occurs mainly as phosphate monoesters and phosphate diesters (Turner and Engelbrecht, 2011), which can be mineralized by extracellular phosphatase enzymes (Acosta-Martinez and Tabatabai, 2011). The activity of these phosphatases therefore plays a key role in regulating plant and microbial P availability in tropical soils.

Numerous researchers have measured soil phosphatase activity (Hui et al., 2013), and an inverse relationship with inorganic P availability is well established (Clarholm, 1993; Olander and Vitousek, 2000; Sinsabaugh et al., 2008; Treseder and Vitousek, 2001). However, most studies have focused exclusively on surface soils where organic P, carbon (C) and other nutrients are relatively abundant. A wide range of biotic and abiotic factors can influence the activity and kinetic behavior of extracellular enzymes, including soil resource (C and nutrient) availability (Allison and Vitousek, 2005; DeForest et al., 2012; Sinsabaugh et al., 2008),

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microbial biomass (Trasar-Cepeda et al., 2008), soil texture (Allison and Jastrow, 2006; Marx et al., 2005), mineral association (Allison, 2006), moisture (Baldrian et al., 2010; Steinweg et al., 2012), pH (Rao et al., 2000) and O_2 availability (Hall and Silver, 2013). All of these soil properties can change with soil depth (Fierer et al., 2003; Rumpel and Kogel-Knabner, 2011; Silver et al., 1999; Spielvogel et al., 2008). Thus, microbial contributions to P cycling may also vary widely with depth in tropical soil profiles.

Kinetic parameters provide a more complete description of an enzyme's behavior than potential activities, which are typically measured at a single saturating substrate concentration (German et al., 2011). These kinetic parameters can be used to improve uncertainty analysis and integrate microbial processes into biogeochemical models (Allison et al., 2010; Wieder et al., 2013). The kinetics of simple enzymes consisting of a single active site and substrate are described as a hyperbolic function by the Michaelis–Menten equation:

$$V = [V_{max} \times S]/[K_m + S] \quad (1)$$

where V is the reaction rate, S is the substrate concentration, V_{max} is the maximum rate of substrate conversion, and K_m, the half-saturation constant, is defined as the substrate concentration at which the rate of substrate conversion is $1/2V_{max}$ (Michaelis and Menten, 1913; Tabatabai and Bremner, 1971). K_m is also an indicator of enzyme-substrate binding affinity. In soils, these parameters are estimated for a pool of enzymes produced by many different organisms (Glazer and Nikaido, 1995; Zimmerman et al., 2013), which may vary considerably in their individual kinetic properties (Marx et al., 2005). Parameters estimated in soils are thus considered apparent V_{max} ($^{App}V_{max}$) and apparent K_m ($^{App}K_m$), with $^{App}V_{max}$ being a measure of enzyme abundance and $^{App}K_m$ being a measure of environmental substrate concentration (Chrost, 1991; Sinsabaugh and Follstad Shah, 2010). Ratios of $^{App}V_{max}$ to $^{App}K_m$ combine enzyme pool size and substrate concentration into a single metric. This metric has been defined as either the specificity constant K_a (Esti et al., 2011; Moscatelli et al., 2012) or the substrate turnover rate S_t (Crottereau and Delmas, 1998; Sinsabaugh and Follstad Shah, 2010). Larger values of K_a or S_t indicate superior catalytic performance.

Tropical subsoils store substantial quantities of C and nutrients (Jobbágy and Jackson, 2000). Extracellular phosphatases in tropical subsoils may therefore make an important contribution to P cycling, but we lack a basic understanding of their activity and kinetic behavior. The objective of this study was to evaluate how the kinetic parameters ($^{App}V_{max}$ and $^{App}K_m$) of acid phosphatase change with depth in the context of landscape-scale gradients in parent material and forest type present at the Luquillo Critical Zone Observatory. We predicted that $^{App}V_{max}$ and $^{App}K_m$ would decline with depth in accordance with declines in resource (C and nutrients) availability. Finally, we sought to determine whether the specificity constant (K_a) would shift (indicating an increase or decrease in overall catalytic performance) or remain constant with depth.

2. Methods

2.1. Site description and sample collection

This study was conducted using soils collected from the Luquillo Critical Zone Observatory (LCZO) in northeastern Puerto Rico (18°18′ N, 65°50′ W). The LCZO is composed of lower-Cretaceous volcaniclastic (VC) sediments of andesitic composition and an early-Tertiary age quartz-diorite (QD) pluton known as the Rio Blanco stock (Seiders, 1971a,b). The region is montane, wet tropical forest characterized by steep, highly dissected terrain. The mean annual temperature decreases from approximately 24 °C at 300 m to 21 °C at 800 m, and precipitation increases from 3000 mm y^{-1} to 4000 mm y^{-1} across the same elevation gradient (Brown et al., 1983). The LCZO is covered primarily by mature Tabonuco (*Dacroydes excesla* Vahl) forest at low elevations (<600 m), Palo Colorado (*Cyrilla racemiflora* L.) forest at intermediate elevations (600–800 m) and sierra palm forest at the highest elevations (800–1000 m (Brown et al., 1983; Weaver, 1991)).

Soils in the LCZO are 0.5–1.5 m thick, underlain by saprolite that ranges in thickness from ~2 m on steep hillslopes to up to 23 m on ridgetops (Buss et al., 2010; Simon et al., 1990). Soils from the VC parent material are fine textured, Fe- and Al-oxide rich, highly weathered Hapludoxes of the Zarzal and Cristal series. Soils from the QD parent material are coarse-textured, sandy Dystrudepts of the Picacho and Utuado series (Soil Survey Staff, 2013).

We collected soils from four sub-watersheds throughout the LCZO that represented Tabonuco and Colorado forest on VC and QD parent material (Table 1). These sub-watersheds spanned 360–780 m in elevation, and within each we sought pronounced local ridges (slope < 10°) that do not receive significant deposition from above. From each ridge, we sampled along catenas that descended into local ephemeral streams. We excavated five soil pits along each catena: three on slope terrain, one on a ridge top and one in a valley. These five pits were considered field replicates for each soil × forest type. Soil profiles were excavated to 140 cm or bedrock using a bucket augur. Samples were collected every 10 cm from the wall of the pit beginning at the surface of the mineral soil (considered 0 cm depth). We chose six depth intervals for enzyme assays: 0, 20, 50, 80, 110 and 140 cm, resulting in a total of 30 samples per soil × forest combination. Samples were field-fresh sieved to 5 mm to homogenize, and kept frozen at −20 °C until enzyme analysis. We acknowledge that freezing may have an effect on enzyme activities as has been observed in other studies (DeForest, 2009; German et al., 2011; Lee et al., 2007) but as it was not possible to assay this number of samples under field conditions in a timely manner, we focused on treating all samples identically to minimize sample storage effects on experimental outcomes. Repeated measurements of the same samples indicated that activity from frozen samples varied <10% over the duration of the experiment.

2.2. Soil characteristics

Total C and total N concentrations of soils were determined by dry combustion analysis using a Carlo-Erba NA 1500 elemental analyzer (Fisons Instruments, Beverly MA). In cases where carbon concentrations were <0.1% as measured by combustion analysis, samples were re-run on a stable isotope mass-spectrometer (Delta Plus, Thermo-Finnigan MAT, Bremen, Germany) to improve the precision of our measurements. With all soil pH values less than 6, and many less than 5 (Table 2), we assumed measured total C concentrations to be equivalent to organic C concentrations due to the absence of carbonates under acidic conditions. Available P was determined using a partial Hedley sequential fractionation based on the protocol of Tiessen and Moir (1993), with several modifications. First, 1–5 g of sample was extracted by shaking soil in 30 mL of 0.25 M $NaHCO_3$ solution for 16 h. Extracts were centrifuged and then filtered using a 0.45 μm nitrocellulose filter before running on ICP spectrometer (Genesis ICP-OES, Spectro Analytical Instruments Gmbh, Kleve, Germany) to determine total P in the extracts. The soil was then extracted again with 0.1 M NaOH following the same procedure. Total extractable P is reported as the sum of $NaHCO_3$ and NaOH-extractable P. Soil pH was determined in a 1:10 soil : distilled water slurry.