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Changes in extracellular enzyme activity and microbial community structure with soil depth at the Luquillo Critical Zone Observatory

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ABSTRACT

Extracellular enzymes in soils mediate the decomposition of organic matter and catalyze key transformations in carbon, nitrogen and phosphorus cycling. However, most studies of extracellular enzyme activity have focused exclusively on relatively carbon and nutrient-rich surface soils. In tropical forests, several centimeters of nutrient-rich surface soil can overlay meters of resource-poor subsoil, of which the microbial ecology is poorly characterized. The goal of this study was to determine how extracellular enzyme activity changes as a function of depth across two soil orders (Oxisols and Inceptisols) and two forest types that occur at different elevations (Tabonuco, lower elevation; Colorado, higher elevation) at the Luquillo Critical Zone Observatory in northeast Puerto Rico. We excavated three soil pits to 140 cm at four different sites representing the four soil × forest combinations, and measured potential activities of four carbon-acquiring enzymes (α-glucosidase, β-glucosidase, β-xylosidase, cellobiohydrolase), one nitrogen-acquiring enzyme (N-acetyl glucosaminidase) and one organic phosphorus-acquiring enzyme (acid phosphatase) at six discrete depth intervals. We used phospholipid fatty acid (PLFA) analysis to assess viable microbial biomass and community structure. Overall, microbial biomass, specific enzyme activities and community structure were similar across the two soil and forest types, in spite of higher carbon concentrations and C:N ratios in the Colorado forest soil. Soil nutrients, microbial biomass and potential enzyme activities all declined exponentially with depth. However, when normalized to microbial biomass, specific enzyme activities either did not change with depth (β-glucosidase, β-xylosidase, cellobiohydrolase and N-acetyl glucosaminidase) or increased significantly with depth (α-glucosidase and acid phosphatase, $P < 0.05$). Principal components analysis of PLFA biomarkers revealed shifts in community structure with depth ($P < 0.01$), driven largely by a decline in fungal:bacterial ratios, an increase in gram positive and actinobacteria biomarkers, and a decrease in gram negative biomarkers. Shifts in community structure, upregulation of enzyme production in response to resource scarcity and decreased enzyme turnover rates may all contribute to high specific enzyme activities in subsoils. Our study indicates that low-carbon tropical subsoils contain small but metabolically active microbial communities, and that specific enzyme activities can be used to examine changes in microbial physiology across orders of magnitude gradients in soil carbon concentrations.

1. Introduction

Microbial communities in soils produce extracellular enzymes to acquire energy and resources from complex biomolecules in the soil environment (Burns, 1982). These enzymes are of interest on an ecosystem scale because they catalyze important transformations in the carbon (C), nitrogen (N) and phosphorus (P) cycles (Wallenstein and Burns, 2011). However, most studies of microbial communities and their associated enzymes have been restricted to the upper 15 cm of the soil, despite the fact that soil microbes influence biogeochemical processes throughout soil profiles (Blume et al., 2002; Fierer et al., 2003a; Buss et al., 2005). Tropical forest soils are often many meters deep and tropical subsoils store large amounts of C, contributing approximately 50% of the C stored at depths >1 m (Jobbágy and Jackson, 2000). It is thus reasonable to predict microbial communities may contribute to nutrient cycling

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in deeper parts of tropical soil profiles than have historically been measured.

Numerous soil properties can influence microbial communities, including carbon availability and composition (Griffiths et al., 1999; Bending et al., 2002), pH (Rousk et al., 2010), temperature (Zogg et al., 1997), redox status (DeAngelis et al., 2010), texture (Sessitsch et al., 2001) and mineralogy (Heckman et al., 2009). All of these properties can change with depth, some by orders of magnitude. The environmental gradient represented by depth profiles influences the abundance, composition and functions of soil microbial communities. For example, microbial biomass and substrate pools generally decline with soil depth (Blume et al., 2002; Fierer et al., 2003a; Fang and Moncrieff, 2005; Kramer et al., 2013). Furthermore, microbial community structure often changes with depth (Ekelund et al., 2001; Hansel et al., 2008; Hartmann et al., 2009; Eilers et al., 2012), possibly reflecting an increasing dominance of organisms that can maintain basal metabolism under conditions of low energy availability (Hoehler and Jorgensen, 2013). Several studies have found that potential enzyme activities decrease with depth (Taylor et al., 2002; Venkatesan and Senthurpandian, 2006; Snajdr et al., 2008; Gelsomino and Azzellino, 2011; Steinweg et al., 2013). However, specific enzyme activities (activity normalized to biomass or substrate availability) have not been measured in most cases, and it is therefore difficult to separate physiologically adaptive changes in enzyme activities from changes due solely to differences in biomass and substrate quantities (Trasar-Cepeda et al., 2008). The few studies that have normalized microbial assimilation or mineralization activities to the size of the microbial biomass show either similar values throughout the soil profile or an increase in specific activity with depth (Tate, 1979; Blume et al., 2002; Gelsomino and Azzellino, 2011; Kramer et al., 2013). Additionally, increased mineral association in subsoils can lead to greater stabilization of organic materials (Eusterhues et al., 2003; Rasse et al., 2005). Changes in the fraction of enzymes that are sorbed to clay minerals or organo–mineral complexes with depth can influence both potential enzyme activities and enzyme turnover rates (Taylor et al., 2002; Allison, 2006).

The primary objective of this study was to assess changes in the potential and specific activity of hydrolytic enzymes involved in C, N and P acquisition with increasing soil depth across the Luquillo Critical Zone Observatory (LCZO), a montane tropical forest in northeastern Puerto Rico. We hypothesized that energy (C) availability would be the major driver of the vertical distribution of enzyme activities. Specifically, we hypothesized that total enzyme activity would decline with depth; tracking declines in C availability and microbial biomass. We further hypothesized that enzyme activity per unit microbial biomass (i.e., specific activity) would increase with depth, reflecting greater microbial allocation to enzyme production in response to decreased resource (C and nutrient) availability (Sinsabaugh and Moorhead, 1994; Allison et al., 2011). Our second objective was to investigate changes in microbial community structure with depth at the LCZO. By doing so, we hoped to evaluate whether changes in enzyme activities were functional, or attributable to changes in community structure. Lastly, our experimental design allowed us to examine how landscape-scale gradients in geologic parent material and vegetation at the LCZO mediate changes in soil enzyme activities with depth. We predicted that in surface soils, enzyme activity would be principally driven by forest type, due to differences in leaf litter chemistry (and therefore substrate chemistry) among forest types (Fonte and Schowalter, 2004; Cusack et al., 2011). We predicted the influence of vegetation would decline with depth and that in subsoils, enzyme activity patterns would be influenced primarily by parent material.

2. Methods

2.1. Study site and sample collection

This study was conducted using soils from the Luquillo Critical Zone Observatory (LCZO) in northeastern Puerto Rico (18°18′N, 65°50′W). The LCZO offers a natural experiment for studying changes in microbial community characteristics with depth in the context of landscape-scale gradients in geology, vegetation and climate. The LCZO is composed of two dominant parent materials of differing age and mineralogy: 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 mountainous region is characterized by steep terrain and is highly dissected by slopes >30°. The mean annual temperature decreases from approximately 24 °C at 300 masl to 21 °C at 800 masl and precipitation increases from 3000 mm y^{-1} to 4000 m y^{-1} across the same elevation gradient (Brown et al., 1983). Most of the vegetation falls into four climate-designated life zones *sensu* Holdridge (1967): subtropical wet forest, subtropical rainforest, lower montane wet forest, and lower montane rainforest. The LCZO is covered primarily by mature Tabonuco (*Dacroydes excesla* Vahl) forest at low elevations (<600 masl) and Palo Colorado (*Cyrilla racemiflora* L.) forest at intermediate elevations (600–800 masl). Sierra palm forest is found across all elevations and is dominant at the highest elevations (800–1000 masl) (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 (Simon et al., 1990; Buss et al., 2010). The VC parent material weathers to produce Oxisols, which are classified as Humaquoxes on flat ridges, and Aquic and Inceptic Hapludoxes on slopes and in valleys (Table 1). The QD parent material weathers to produce Inceptisols, which are classified as Histic Humaquepts on ridges, and Aquic or Aquic Humic Dystrudepts on slopes and in valleys (Table 1, Soil Survey Staff, 2013). All soils are moderately to strongly acidic and contain mostly kaolinitic minerals, with iron and aluminum oxyhydroxides in the clay fraction. The Oxisols are strongly weathered, fine-textured soils that are high in iron and aluminum and contain <10% primary minerals, while the Inceptisols are coarser-textured and contain up to 40% primary minerals in surface soils (Scatena, 1989; Silver et al., 1994; Johnson and Hao, 2013).

To capture as much edaphic variability as possible in our study, we collected soils from four sub-watersheds (5–20 ha in size) throughout the LCZO that spanned 360–790 masl in elevation and represented Tabonuco and Colorado forest on VC and QD parent material (Table 1). Previous work has shown there are significant differences in soil properties associated with topographic position at the sub-watershed scale (Silver, 1994; Scatena and Lugo, 1995; Johnson et al., 2011); we therefore deliberately incorporated topographic variation into our sampling design. Within each sub-watershed 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. Five soil pits were excavated along each catena; three on slope terrain, one on a ridgetop and one in a valley. Soil profiles were excavated to 140 cm or bedrock using a bucket augur. Samples were collected from the wall of the pit at discrete 10 cm intervals beginning at the surface of the mineral soil. Samples were immediately refrigerated at 4 °C following collection, and shipped on ice to the University of Pennsylvania where they were field-fresh sieved to 5 mm to homogenize. Samples from one slope pit, the ridge pit and the valley pit were used for enzyme and PLFA analysis. Soil chemical analyses (see below) were performed on samples from all five pits. One subsample was frozen at −20 °C