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Estimating decay dynamics for enzyme activities in soils from different ecosystems

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ABSTRACT

Extracellular enzymes in soil are central to the decomposition of plant and microbial detritus and they are increasingly incorporated into soil biogeochemical models as drivers of detritus breakdown. In enzyme-driven models, a critical parameter is the functional lifespan of the enzymes, yet this is poorly constrained by experimental data. We evaluated how long soil enzymes remain active in five soils spanning from arctic tussock tundra to a tropical forest/grassland soil. We incubated soils under continuous fumigation with $CHCl_3$ vapor to kill microbes and prevent the synthesis of new enzymes. We monitored the activities of six hydrolytic and two oxidative enzymes over a 12-week incubation. Initial activities of the various soil enzymes varied substantially across the ecosystems; they were generally highest (per gram soil) in the tussock tundra, followed by temperate hardwood forest, grassland, tropical forest/grassland, and then chaparral. In tussock tundra organic soil, activities of all enzymes decreased rapidly following first-order decay curves; the half-lives of enzyme activity were typically several weeks. In the mineral soils, the time course of loss of hydrolytic enzyme activities could always be described by a first-order decay curve, but in many cases, activity could equally be described by a zero-order, linear, decay function because the rate of loss was slow. Although α-glucosidase lost activity rapidly, for other enzymes substantial activity remained even after 12 weeks of incubation. This likely resulted from stabilization by mineral surfaces, stabilization that might constrain activity against native polymeric substrates. Measured turnover rate constants fell within the broad range that model have used, but that range remains exceedingly broad.

1. Introduction

Extracellular enzymes in soil are central to the decomposition of plant and microbial detritus. As such, measurements of their activities can describe how carbon and nutrient cycling are affected by environmental changes (Allison et al., 2007; Burns et al., 2013). Because of their importance, enzyme activities are increasingly incorporated into simulation models of soil processes as drivers of the rate of detritus breakdown (Sinsabaugh and Moorhead, 1994; Schimel and Weintraub, 2003; Lawrence et al., 2009; Wieder et al., 2013; Wang et al., 2013). In enzyme-driven models, a critical parameter is the functional lifespan of the enzymes because this regulates the size of the enzyme pool (Schimel and Weintraub, 2003; Allison, 2005; Manzoni et al., 2016). Enzyme-driven models must assign a value to this critical parameter, either by fitting the model to empirical data, or not uncommonly with little more than a wild guess (e.g. Schimel and Weintraub, 2003).

Unfortunately, the functional lifespans of soil enzymes are not well constrained (Burns, 1982; Allison, 2006; Wallenstein and Weintraub, 2008). Although enzyme decay dynamics and turnover times vary with their function and origin (Burns, 1982), it is unclear how they differ across enzymes or ecosystems; processes in soil may both degrade and stabilize enzyme activities. Enzymes may be broken down by proteolytic enzymes, by abiotic processes (including thermal denaturation and reacting with minerals), or by being taken up intact by microbes and metabolized intracellularly. They may be stabilized by interactions with minerals and organic matter (Nannipieri et al., 1996; Nannipieri and Gianfreda, 1998; Burns et al., 2013). When free in suspension, enzymes lose activity rapidly; for example the decay half-life for urease can be as low

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as 1.3 days (Pettit et al., 1976). However, in soil, enzymes are likely rarely free in solution. Stabilized enzymes may have their activity repressed (Allison, 2006) but lose activity more slowly, maintaining activity over weeks or even longer (Pettit et al., 1976; Marzadori et al., 1998). Components of the soil matrix (e.g. different minerals or organic matter) stabilize enzymes differently (Nannipieri et al., 1996; Quiquampoix et al., 1995; Servagent-Noinville et al., 2000; Burns et al., 2013). Thus, as we develop enzyme-driven models to be more than just theoretical constructs but rather field-applicable tools, we need to parameterize them with better estimates of enzyme turnover across soils and environmental conditions. Are values consistent enough across soils that we can use broadly applied parameters? Or must each soil be parameterized individually? How much do different enzymes vary in their stability?

In this study, we evaluated how long soil enzymes remain active in a range of soils and how activities and stabilities compare across ecosystems. We incubated five soils under continuous fumigation with $CHCl_3$ vapor to kill microbes and prevent the synthesis of new enzymes. Previous studies evaluating enzyme stability have generally used a single dose of gamma radiation to kill cells without degrading enzymes (e.g. Allison, 2006). However, gamma does not eliminate viable cells (Blankinship et al., 2014) and so may be unreliable for long-term incubations. We monitored the activities of six hydrolytic and two oxidative enzymes over a 12-week incubation. This enabled us to analyze the rate at which these enzymes lose activity due to natural abiotic and biochemical reactions over a period long enough to fit estimates of decay constants, even when decay may be slow due to stabilization by organic matter or minerals.

2. Materials and methods

2.1. Soil characterization

Soil samples were collected from a variety of ecosystems throughout the United States. Grassland samples were collected from the University of California Sedgwick Reserve near Santa Ynez, California. The grassland soil is a clay loam thermic Pachic haploxeroll in the Salinas series; the site experiences a mean annual precipitation of 38 cm. The vegetation is dominated by the non-native grasses *Bromus diandrus*, *Bromus hordaceous* and *Avena fatua*. Chaparral soil samples were collected 10 km northwest of Santa Barbara, California in the Santa Ynez mountains. The chaparral soil is a sandy loam Typic dystroxerept from the Maymen series; this area experiences annual average precipitation of approximately 60 cm. The chaparral site is dominated by manzanita (*Arctostaphylos tomentosa*) and chamise (*Adenostoma fasciculatum*). Both grassland and chaparral sites experience a Mediterranean-type climate with hot dry summers, cool wet winters, and wide variation in rainfall between wet and dry years. Temperate hardwood forest samples were collected from a mixed hardwood stand at the Harvard Forest in Petersham, Massachusetts. The forest is dominated by Oak (*Quercus velutina* and *Q. rubra*) with lesser amounts of *Betula lenta*, *Acer rubrum*, *Fagus grandifolia* and *Prunus serotine*. The soil is a sandy loam isotic, frigid Oxyaquic haplorthod from the Becket-Skerry association; average annual precipitation is ca. 120 cm. Tussock tundra soils were collected at the Toolik Field Station on the North Slope of the Brooks Range in Alaska. The soil is a coarse-loamy, mixed, acidic, gelic Typic aquiturbel. The site receives annual precipitation of ca. 30 cm. The dominant vegetation is *Eriophorum vaginatum* tussocks with mosses and *Betula nana* between the tussocks (Shaver et al., 2014) The tropical forest/grassland soils were collected in Hawaii from adjacent woodland and grassland sites at an elevation of 900 m above sea level. The soils are loamy sand Lithic haplustands; annual precipitation is between 100 and 200 cm. The woodland vegetation is *Metrosideros polymorpha* woodland with C3 shrub understory and scattered exotic C4 grasses, while the grassland is dominated by an African pasture grass (*Melinis minutiflora*) and scattered native shrubs (*Dodonaea viscosa*). Unfortunately, due to an error in sample handling, the samples from the two tropical vegetation types were composited into a single aggregated tropical forest/grassland soil sample.

Table 1 lists the soil characteristics of each site. Soil texture was determined by the hydrometer method of estimating particle size at Davis Analytics Lab at the University of California, Davis. Initial gravimetric water content (GWC) of each soil was determined by oven drying ~10 g subsamples at 60 °C for >48 h. The water holding capacity (WHC) was determined for each soil by saturating ~10 g subsamples with water in 1 μm pore size filter cups followed by passive draining for 24 h at room temperature (22 °C ± 1 °C) and 100% humidity. To measure soil pH, soil (10 g) was shaken with 10 mL of DI water for 4 h. Soil particles were allowed to settle before measuring the pH of the supernatant. To determine soil organic matter content, mineral soil (10 g) or organic soil (2 g) was first oven dried at 60 °C for 48 h then placed in a muffle furnace for 18 h at 500 °C. To determine the concentration of dissolved organic carbon, soil (10 g) was shaken with 50 mL of DI water for 3 h, then vacuum filtered with 1 μm pore sized glass fiber filters (Type A/E, Pall Co., Port Washington, NY, USA) until the eluent turned clear before diluting and measuring on a total organic carbon analyzer (Shimadzu Corporation, Series V Model CSN analyzer).

2.2. Soil sampling and processing

Samples from the top 10 cm (three to four samples pooled) of each site were collected between June and July 2013. All soils were shipped cold from field sites in plastic bags and immediately stored in the dark at 4 °C upon arrival. Chaparral, grassland, hardwood forest, and tropical forest/grassland soils were processed by sieving through a 4 mm screen and homogenizing. The tussock tundra soil is mostly composed of organic matter and decaying tussock. Therefore, to preserve the significantly different physical structure of the tussock tundra soil, the material was pulled apart into individual root strands rather than sieved.

Prior to fumigation, the water content of all soils was adjusted to normalize the initial conditions of the soil. The water content was set at 35% of water holding capacity to ensure that soil conditions not too dry (which would limit enzyme diffusion) or too wet (which could block $CHCl_3$ diffusion and potentially allow regrowth). Since the soils were effectively sterile, we didn't need to be excessively concerned with matching ambient moisture content. To adjust moisture content, sterile DI water was added to chaparral, grassland, and tropical forest/grassland soils, while tussock tundra soil had water removed by incubating the soil unsealed at 20 °C for 5 days. The temperate hardwood forest soil needed no adjustment. All five soils were then incubated for 72 h at room temperature and 100% humidity to equilibrate and remove any respiratory microbial CO_2 pulse created by the sudden change in water content. Adjusted soils were then stored airtight and in the dark at 4 °C the day before the start of fumigation.

Soil samples (~200 g each) were kept effectively sterile by continuously incubating with chloroform (vapor exposure at 22 °C) at 100% humidity in a 20 L heavy cast aluminum multilayered pressure cooker (30 cm diameter x 25 cm height) with an airtight lid and vacuum hose fitting. The entire set up was kept in a fume hood for the duration of the experiment. The pressure cooker was sealed, evacuated, and then returned to atmospheric pressure forcing chloroform vapor to reach soil micropores. Chloroform and water-soaked paper towels in the pressure cooker were restocked