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Extracellular enzyme kinetics and thermodynamics along a climate gradient in southern California

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ABSTRACT

Microbial decomposers produce extracellular enzymes to degrade complex plant polymers, making plant C available for metabolism and eventual respiration back to the atmosphere as CO_2. Knowledge of how extracellular enzyme kinetics and microbial activity vary with climate is therefore valuable for predicting how future carbon cycling may be affected by climate change, but studies investigating such dynamics in more xeric ecosystems are underrepresented in the literature. We investigated how microbial biomass, litter chemistry, and extracellular enzymes (V_{max} and K_m) and their temperature sensitivities varied along a Mediterranean climate gradient in southern California. Total microbial biomass did not vary among sites along the gradient in either the dry or the wet season. In contrast, extracellular enzyme V_{max} and K_m varied as a function of fungal biomass and substrate availability. We also found that V_{max} of most enzymes was more sensitive to temperature in colder sites than in warmer sites, though only in the dry season. In contrast, K_m of multiple enzymes was more sensitive to temperature in warmer sites across both seasons. Observed enzyme V_{max} and K_m were indicative of extracellular enzyme accumulation in the drier sites along the gradient, which may contribute to the large pulses of respiration that follow rewetting events in these xeric systems. Variation in enzyme characteristics along the gradient indicate that as these systems become more arid in the future, enzyme dynamics will shift from smaller, potentially more active pools to larger, potentially less active enzyme pools that accumulate over dry periods. In addition, rates of enzymatic decomposition will likely be most sensitive to rising temperatures in the coldest sites along our gradient.

1. Introduction

Many microbes secrete extracellular enzymes (EE) capable of degrading complex biological polymers into bio-available compounds that fuel metabolism and respiration (Burns et al., 2013; Sinsabaugh et al., 1994). These processes account for a substantial fraction of ecosystem respiration from soils and litter (Raich and Schlesinger, 2002) and are affected by abiotic climate variables such as moisture and temperature that alter diffusion, reaction rates, and osmotic potential. In addition to these direct effects, climate indirectly shapes microbial communities by exerting strong control on the composition of plant communities (IPCC, 2014), thereby determining substrate availability for microbial decomposers (Saleska et al., 2002).

EE catalysis of complex organic substrate degradation is the rate-limiting step in returning C from plant detritus to the atmosphere (Sinsabaugh and Shah, 2011), though physical protection of C and diffusion constraints can supersede the importance of enzyme catalysis in mineral soils (Schimel and Schaeffer, 2012). These substrates vary, from highly accessible disaccharides and starches to more chemically complex compounds such as hemicellulose, cellulose, and lignin. In the last few decades, decomposition dynamics have been related to microbial activity and assays of EE kinetics in a host of studies (Allison et al., 2007; see also references in Burns et al., 2013; Sinsabaugh et al., 2008), but investigations of how EE characteristics vary in xeric ecosystems is lacking. In a 2008 global meta-analysis of EE activity in soils, 10% or fewer of the sites were located in dryland ecosystems (Sinsabaugh et al., 2008), even though drylands make up ~40% of terrestrial ecosystems by land area (MEA, 2005).

This knowledge gap is significant because decomposition models validated in mesic ecosystems and built around

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temperature, moisture, and litter chemistry consistently underestimate rates of decomposition in more xeric drylands ecosystems, such as semiarid Mediterranean grasslands and arid deserts (Whitford et al., 1981). As such, conclusions drawn from decomposition dynamics and EE kinetics observed in mesic ecosystems may not be applicable to more xeric ecosystems. This uncertainty complicates efforts to predict future carbon dynamics, especially given that xeric ecosystems are projected to become hotter and drier. This is especially true for the American Southwest, where models are remarkably consistent in predicting a shift to a more arid climate beginning in the early part of the 21st century (Seager et al., 2007) and where temperatures are projected to rise by 2.5–5.5° in the next fifty years if global emissions continue to increase (Garfin et al., 2014). Determining how enzyme kinetics vary with climate in such drylands ecosystems is therefore a necessary step in predicting how decomposition rates in these systems may be affected by future climate change.

EE kinetics can be described by the Michaelis-Menten model, whereby activity (V) of an individual enzyme is described as a saturating function of substrate (S) concentration:

$$V = V_{max}[S]/(K_m + [S])$$

where V_{max} is the enzyme's maximum reaction rate and K_m, the half-saturation constant, is the substrate concentration at which the reaction rate is one-half V_{max}. Given that enzyme concentrations *in situ* are controlled by feedbacks between microbial activity and substrate availability, conditions that are conducive to high EE V_{max} should therefore also be conducive to high K_m (Wallenstein et al., 2011). This is because high substrate availability makes enzymatic reactions proceed more quickly, resulting in more products that can be absorbed by microbial cells to fuel further enzyme biosynthesis. While we expect EE kinetic parameters to vary as a function of microbial activity (Sinsabaugh et al., 1994), thermodynamic theory predicts that V_{max} and K_m will also increase with increasing temperature (Davidson and Janssens, 2006), which has potential implications for future C-cycling. Increasing temperature can allow more reactants to attain their activation energies, increasing V_{max}. At the same time, the stability of the substrate-enzyme complex may be reduced, causing decreased substrate affinity and higher observed K_m (Johns and Somero, 2004; Sørensen et al., 2015).

Increases in temperature should have reduced effect on the kinetic parameters of EEs from colder environs (Siddiqui and Cavicchioli, 2006), especially if enzymes are locally adapted (Belotte et al., 2003). This is because cold-adapted organisms optimize enzyme efficacy at low temperatures by minimizing reaction activation energy, or E_a (Georlette et al., 2004; Lonhienne et al., 2000), and V_{max} temperature sensitivity increases as E_a increases according to the Arrhenius relationship (Davidson et al., 2006). Organisms optimized for higher temperatures have relaxed selection for minimizing E_a because their enzymes and substrates have greater kinetic energy. Given that changes in V_{max} and K_m are generally correlated (Sinsabaugh et al., 2014), it is reasonable to assume that K_m temperature sensitivity may exhibit similar patterns as those hypothesized for V_{max} based on thermodynamic theory. Evidence from natural systems is generally lacking in the literature, but several published studies seemingly contradict these expectations: Koch et al. (2007) found that EE temperature sensitivities increased at lower temperatures in alpine soils assayed across three seasons, and both they and Wallenstein et al. (2009) found that EE temperature sensitivity declined over the growing season in alpine and arctic tundra soils, respectively. However, this hypothesis has never been tested along a regional climate gradient, where microbial communities are unlikely to be dispersal limited with regards to the pool of regional taxa (Kivlin et al., 2011), and local climate variation is likely to be a strong filter.

The goal of this study is to use a climate gradient across xeric ecosystems of southern California to determine how microbial EEs might respond to long-term climate change. Along this gradient, temperature and moisture co-vary, such that hotter, drier sites at low elevations contrast with colder, wetter sites at higher elevations. As such, moving from higher to lower elevations mimics the shift to more arid climates expected in the American Southwest. Litter lignin content and the size of the litter pool also generally decrease when transitioning from higher to lower elevations. We aimed to quantify the environmental drivers of enzyme kinetic parameters along the climate gradient to advance knowledge of biogeochemical mechanisms in xeric ecosystems. Based on the above theory, we formulated the following hypotheses:

1. Microbial biomass, EE V_{max}, and EE K_m will increase with increasing precipitation, as moisture and substrate availability limit microbial activity which in turn limits EE production.
2. V_{max} and K_m of enzymes from colder, wetter sites will be less temperature sensitive, and enzymes assayed in the wet season will be less temperature sensitive than those assayed in the dry season.

We tested these hypotheses by measuring microbial properties, litter substrates, and enzyme kinetics along a climate gradient spanning 12.5 °C and 300 mm precipitation in southern California.

2. Materials and methods

2.1. Site description

To test how EE kinetic parameters and thermodynamics varied with climate, we assayed plant litter from five sites representing five biomes in southern California – Colorado desert (lat, long: 33.652, −116.372), pinyon-juniper scrubland (33.605, −116.455), coastal grassland (33.737, −117.695), pine-oak forest (33.808, −116.772), and subalpine forest (33.824, −116.755). All five sites are located on granitic parent material and experience Mediterranean precipitation patterns (cool, wet winters; hot, dry summers). The desert is on a deposit of Carrizo stony sand, and is dominated by desert perennials and annuals. The scrubland is on an Omstott coarse sandy loam, and is dominated by pinyon pine, juniper, and desert perennials and annuals. The grassland is on a Myford sandy loam, and is dominated by annual grasses and forbs, particularly *Bromus* and *Avena* spp. The pine-oak forest is on a Pacifico-Preston families soil complex and is dominated by pines as well as evergreen and deciduous oak. The subalpine site is on a rocky outcrop of granite that lies among a Pacifico-Wapi families soil complex, and is dominated by lodgepole and limber pine. The gradient spans a range of ~12.5 °C in mean annual temperature (MAT), from 22.8 ± 0.8 °C at the desert site to 10.3 ± 1.8 °C at the subalpine site (Table 1). The desert site experienced the least mean annual precipitation in the form of rainfall over the five years prior to this study (100 ± 24 mm), and the pine-oak forest (hereafter referred to as "pine-oak") site experienced the most (400 ± 120 mm). Standing litter pools are largest in the grassland and pine-oak site, reduced in the subalpine site, significantly reduced in the scrubland site, and negligible in the desert site (personal observation). Air temperature, soil temperature, rainfall, and solar radiation data for all sites other than the subalpine site come from eddy covariance towers at each site (Goulden et al., 2006). Two iButton temperature sensors (Maxim Integrated)