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Hot experience for cold-adapted microorganisms: Temperature sensitivity of soil enzymes

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ABSTRACT

High latitude and cold ecosystems, which constitute the major environment on Earth, are particularly threatened by global warming. Consequently, huge amounts of SOC stored in these ecosystems may be released to the atmosphere by accelerated enzymatic decomposition. Effects of intensive warming on temperature sensitivity and catalytic properties of soil enzymes were tested in cold-adapted alpine grassland of the Tibetan Plateau. We hypothesized that 1) maximal reaction rate will be insensitive to intensive warming at high temperature range ($V_{max} - Q_{10} = 1$); 2) substrate affinity (K_m) remains constant at elevated temperatures due to expression of enzymes with less flexibility. These hypotheses were tested by examining the kinetics of six enzymes involved in carbon (cellobiohydrolase, β-glucosidase, xylanase), nitrogen (tyrosine-aminopeptidase, leucine-aminopeptidase) and phosphorus (acid phosphomonoesterase) cycles after soil incubation at temperatures from 0 to 40 °C.

Q_{10} and E_a decreased at high temperature (25–40 °C). However, enzymes that degrade low quality polymers remained temperature-sensitive even above 25 °C ($V_{max} - Q_{10} = 2$), which explains the faster decomposition of recalcitrant C compounds under warming. Substrate affinity of all enzymes gradually increased up to 20 °C. At 25 °C, however, K_m increased rapidly, leading to an extreme decrease in catalytic efficiency. Above 25 °C, K_m of C and N cycles remained nearly constant, while V_{max} gradually increased from 0 to 40 °C. These results reveal two important implications of warming: 1) there are some temperature thresholds (here 20–25 °C) that lead to sudden reductions in substrate affinity, decreasing temperature sensitivity and catalytic efficiency, 2) decoupled temperature sensitivity of V_{max} and K_m and the resulting maintenance of stable enzyme systems at high temperatures ensured efficient enzymatic functioning and persistent decomposition of SOM at temperatures much higher than the common adaptation range of the ecosystem. Thus, the temperature thresholds of strong changes in enzyme-based processes should be considered and included in the next generation of models in order to improve the prediction of SOM feedbacks to warming.

1. Introduction

Microorganisms in the natural environment cope with changing conditions that demand a wide range of metabolic adaptations (Neidhardt et al., 1990). Among the most challenging environments are high latitude and cold ecosystems, which are threatened by global warming (Davidson and Janssens, 2006). Warming has a fundamental impact on microbial activity, metabolism and enzyme activities (Allison et al., 2010; Van Gestel et al., 2013; Zimmermann and Bird, 2012). Enzymes are essential to microbial metabolism and soil functioning, as they depolymerize large organic compounds and generate soluble oligomers and monomers that can be transported into the cells (Blagodatskaya et al., 2016; Wallenstein et al., 2010). Three mechanisms have been proposed to explain thermal adaptation of enzyme catalyzed processes: 1) change in the enzyme systems 2) the alterations in soil microbial biomass and enzyme expression at higher temperatures and 3) changes in quantity and quality of substrate, affecting reaction rates (Blagodatskaya et al., 2016).

Enzyme activity is a saturating function of substrate concentration and is described by the Michaelis-Menten relationship (Michaelis and Menten, 1913). Enzyme saturation occurs when all

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the enzyme active sites are already occupied by substrate. In this case adding more substrate will not increase the overall rate of the reaction. Both parameters of the Michaelis-Menten equation – the maximal catalytic reaction rate at a given temperature (V_{max}) and the half-saturation constant (K_m), are temperature-sensitive (Davidson et al., 2006; Davidson and Janssens, 2006) and usually increase with temperature (Stone et al., 2012). Various enzymes have different temperature sensitivities and changes in soil temperature may also alter the relative rates of decomposition of different components of organic matter (Koch et al., 2007; Wallenstein et al., 2010; Stone et al., 2012; Razavi et al., 2015). This may affect nutrient availability, for instance it has been observed that N availability may be decoupled from C and P cycling under warming conditions (Allison and Treseder, 2008). Therefore, the temperature sensitivity of enzymes responsible for organic matter decomposition is the most crucial parameter for predicting the effects of global warming on the nutrient and C cycles (Davidson et al., 2006; Davidson and Janssens, 2006).

The temperature sensitivity of V_{max} is directly related to the activation energy for enzyme reaction (Davidson and Janssens, 2006). Activation energies are parameters that mechanistically link enzyme kinetics and temperature response through the Arrhenius equation (Wallenstein et al., 2010). Based on the Arrhenius law, when activation energy is low, the exponential term will tend to 1 and consequently the reaction will become temperature independent (Marx et al., 2007). In the other words, the lower the activation energy, the lower the temperature sensitivity of the reaction rate. Enzymes catalyze biochemical reactions by lowering their activation energy (Gerlt and Gassman, 1993). Thus, a super-efficient enzyme will bring the activation energy to zero (Marx et al., 2007). This is important because, in the context of cold-adapted microorganisms, one way to maintain decomposition processes at low temperatures would be to develop enzymes that are temperature-independent (Marx et al., 2007).

Microbial physiology is evolutionarily selected for the most efficient enzyme systems (Allison et al., 2010; Hochachka and Somero, 2002). Moreover, the activities of hydrolytic enzymes could be adapted to different temperature regimes (Baldwin and Hochachka, 1969; German et al., 2012) with the goal of maintaining critical enzymatic functions. There is evidence for biogeographical patterns in enzyme temperature sensitivity (Huston et al., 2000; Feller, 2003; German et al., 2012). Many studies have observed that cold-adapted microorganisms can produce cold-adapted enzymes that catalyze reactions at lower temperatures with lower activation energy and with higher binding affinity (i.e. low K_m) (Fields, 2001; Bradford, 2013) than their mesophilic counterparts (Gerday et al., 1997). Importantly, microbial adaptation and acclimation strategies have physiological costs (Schimel et al., 2007) and can reduce enzyme catalytic efficiency – determined as V_{max}/K_m (Stone et al., 2012; Tischer et al., 2015).

The parameters of enzyme kinetics – specifically K_m, which determines the binding affinity of the enzyme to substrate – are indicative of enzyme flexibility (the capacity for quick conformation change) (Somero, 1975). The increased flexibility would cause the cold-adapted enzyme to spend more time maintaining conformations that are not optimal for substrate binding (Siddiqui and Cavicchioli, 2006). This can be measured as a gradual increase of K_m with temperature (Fields, 2001). Key to effective enzymatic function is the trade-off between functional capacity and enzyme flexibility, which co-vary with habitat temperature (Somero, 1995; Fields, 2001; Tokuriki et al., 2012). Conformational flexibility and enzyme function are closely related, and organisms have evolved to produce enzymes with thermal optima at their habitat temperature. For example, more flexible enzyme systems are expected under cold conditions, while strongly reduced enzyme flexibility (i.e. low temperature sensitivity of K_m) is predicted in warmer climates (Johns and Somero, 2004; Dong and Somero, 2009; Bradford, 2013).

Furthermore, as enzyme systems are altered by climate warming, different sets of isoenzymes (i.e., enzymes with the same function but different conformations and structures) are expected to be expressed at cold and warm temperatures (Somero, 1978; Bradford, 2013; Razavi et al., 2016). Isoenzymes with higher temperature optima can be produced by the same microbial species adapted to warming (Hochachka and Somero, 2002). Alternatively, isoenzymes can be expressed as a result of changes in microbial community structure caused by warming (Baldwin and Hochachka, 1970; Vanhala et al., 2011). In both cases, temperature sensitivity of catalytic reactions is dependent on enzyme isoforms. Nonetheless, all these mechanisms suggest that microbes prefer to produce enzymes that maintain optimal activity under native soil conditions.

Despite intensive discussion on the mechanisms of enzyme temperature sensitivity, it remains unclear how the functional characteristics of enzymes in cold-adapted soil will be altered by temperature increases. This is extremely important because it provides evidence of the response of cold-adapted soil microbes and the fate of huge amounts of SOC stored in these ecosystems by acceleration of enzymatic decomposition in a warmer world. In addition, there is a lack of studies on the catalytic efficiency of soil enzymes in cold ecosystems as affected by warming.

This study was designed to test the effects of intensive warming on the catalytic properties of soil enzymes in a cold-adapted environment. We hypothesized that maximal reaction rate will be insensitive to intensive warming at high temperature range (H1); and that the substrate affinity (K_m) will remain constant at elevated temperature (H2). To test our hypothesis we collected soil from the Tibetan Plateau and incubated the samples for one month over a temperature range of 0–40 °C (with 5 °C steps) and determined the kinetics, temperature sensitivities and activation energy of six enzymes involved in decomposition of soil organics: cellobiohydrolase and β-glucosidase, which are commonly measured as enzymes responsible for consecutive stages of cellulose degradation (German et al., 2011); xylanase, which degrades xylooligosaccharides into xylose and is thus responsible for breaking down hemicelluloses (Chen et al., 2012); acid phosphomonoesterase, which hydrolyzes (mono) ester bonds of organic P to phosphate under acidic conditions (Eivazi and Tabatabai, 1977; Malcolm, 1983; German et al., 2011). Activities of tyrosine aminopeptidase and leucine aminopeptidase were analyzed to assess the hydrolysis of peptide bonds (Koch et al., 2007; Chen et al., 2012).

2. Material and methods

2.1. Site description and soil collection

The sampling site is located in the upper Kyi Chu catchment north of Lhasa in Pando County, above the Reting Monastery in Qinghai-Tibetan Plateau (south west of China, 4330 m a.s.l.) (Table 1). The mean precipitation during the growing season (from May to October) is 330 mm. The temperature during the growing season ranges between −4 and +17.7 °C. This site has the largest and most sacred *Juniperus* forest in Tibet, diffusely growing in a carpet-like felty turf of *Kobresia pygmaea* C.B. Clarke (Miehe et al., 2008) which is the dominant and eponymous species (covering up to 98% of the root-mat surface).

Four composites of six soil samples each were collected using soil cores (18.5 cm long 4.5 cm diameter). Each composite sample was collected over a 30 m^2 area. A variable-depth sampling scheme was used to obtain the entire A-horizon. This sampling scheme increases our confidence for minimizing random variation in soil