Soil Biology & Biochemistry 97 (2016) 15–22

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Temperature selects for static soil enzyme systems to maintain high catalytic efficiency

Bahar S. Razavi [a], Evgenia Blagodatskaya [b, c, *], Yakov Kuzyakov [a, b]

[a] Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany
[b] Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany
[c] Institute of Physicochemical and Biological Problems in Soil Science, 142290 Pushchino, Russia

ARTICLE INFO

Article history:
Received 18 October 2015
Received in revised form
17 January 2016
Accepted 28 February 2016
Available online 10 March 2016

Keywords:
Structural flexibility
Soil enzymes stability
Michaelis–Menten kinetics
Catalytic efficiency
Temperature sensitivity

ABSTRACT

Knowledge on enzymatic mechanisms of acclimation to temperature is required to predict the effects of warming on decomposition of soil organic matter – the largest C stock in terrestrial ecosystems. Based on Michaelis–Menten kinetics we tested the hypothesis that enzyme affinity to substrate (K_m) is more sensitive to warming at cold than at warm temperatures. We also predicted a gradual increase in K_m values with increasing temperature. The kinetic parameters of six enzymes involved in cycles of C, (cellobiohydrolase, β-glucosidase and xylanase), P (phosphatase), and N (leucine-aminopeptidase, tyrosine-aminopeptidase) were determined after one month of soil incubation at a temperature range 0 –40 °C (with 5° increment).

Contrary to our hypothesis, the increase in K_m with temperature was not gradual for most tested enzymes. Within large range of temperatures from 0 to 15 °C (phosphatase), 0–20 °C (enzymes involved in C cycle) and 0–40 °C (proteases) the hydrolytic activity was governed by enzymes with nearly constant substrate affinity. Temperature, therefore, mainly selected for soil enzyme systems maintaining static K_m. The catalytic efficiency of the enzymes (V_{max}/K_m) increased from low to intermediate temperatures (0–20 °C) as a result of linear increase of V_{max} at constant K_m. Static K_m values were explained either by low flexibility (high structural stability) of a single enzyme type, which catalyzed the reaction over a broad temperature range, or by production of multiple isoenzymes each with different temperature optima but with similar affinity to substrate. Thus, maintaining static K_m with temperature increase ensured high enzyme efficiency within a low and intermediate soil temperature range.

1. Introduction

The temperature sensitivity of soil organic matter (SOM) decomposition is crucial for understanding the consequences of global warming (Davidson and Janssens, 2006; Stone et al., 2012). Since microbial enzymes are responsible for SOM decomposition, knowledge on enzymatic mechanisms of acclimation to temperature is required to predict the effects of warming on the cycling of C and major nutrients (Koch et al., 2007; Conant et al., 2011; Birgander et al., 2013).

Hydrolytic enzyme activity is a saturating function of substrate concentration and is described by the Michaelis–Menten relationship (Michaelis and Menten, 1913). 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), which reflects the affinity of the enzyme for the substrate – are temperature sensitive (Davidson and Janssens, 2006; Davidson et al., 2006) and usually increase with temperature (Stone et al., 2012). Temperature sensitivity of potential enzyme activity (V_{max}) is traditionally studied by adding excess substrate. In contrast, little information is available about the temperature sensitivity of K_m in soil enzyme systems; this, however, is especially important for the very low substrate levels common under soil conditions (Hobbie and Hobbie, 2012; Kuzyakov and Blagodatskaya, 2015).

Temperature affects microbial and enzyme activities not only directly (Allison et al., 2010; A'Bear et al., 2012; Wieder et al., 2013), but also indirectly affects enzymatic reactions when a shift in dominating microbial populations changes the temperature sensitivity of the produced isoenzymes (i.e., an enzyme with the

* Corresponding author. Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany.
E-mail address: janeblag@mail.ru (E. Blagodatskaya).

http://dx.doi.org/10.1016/j.soilbio.2016.02.018
0038-0717/

same function but different structure) (Bárcenas-Moreno et al., 2009; Zimmermann and Bird, 2012; Van Gestel et al., 2013). Temperature, therefore, modulates the rate of biogeochemical processes by controlling microbial metabolism (Leroi et al., 1994; Turner et al., 1996; Cooper et al., 2001).

The parameters of enzyme kinetics – specifically, K_m, which determines the binding affinity of enzyme to substrate – are indicative for detecting enzyme flexibility (the capacity for quick conformation change) (Somero, 1975). Flexible enzymes are characterized by a fast rate of conformation change when converting a substrate to a product at an enzyme active site (Fields, 2001; Bradford, 2013). At low temperatures, flexible enzyme structures ensure a fast rate of catalytic reaction at high binding affinity (i.e. low K_m) (Fields, 2001; Bradford, 2013). With temperature increase, the ability of flexible enzymes to maintain binding conformations decreases, reducing the affinity to the substrate (Fields, 2001). This can be measured as an increase of K_m with temperature (Fields, 2001). Therefore, warmer temperatures are favorable for enzymes with lower substrate binding affinity but with higher structural stability (Zavodszky et al., 1998; Bradford, 2013). However, applicability of the hypotheses established on the basis of single enzyme properties needs to be tested under *in situ* soil conditions considering great functional redundancy of microorganisms. Due to functional redundancy, high catalytic efficiency (determined as V_{max}/K_m) is maintained in soil by numerous enzymes with different temperature optima mediating similar functions (Nannipieri et al., 2012). As enzyme systems are altered by climate warming, a different set of isoenzymes is expected to be expressed at cold and warm temperatures (Somero, 1978; Bradford, 2013). Expression of 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, 1969; Vanhala et al., 2011). Therefore, K_m determined in soil reflects simultaneous activity of a suite of isoenzymes with different thermal optima rather than single enzyme properties. It remains to be tested, whether functional capacity of suite of soil enzymes is maintained by increase of K_m with temperature (similar to single flexible enzyme) or whether temperature selects for static K_m through the range of temperatures.

Importantly, microbial adaptation and acclimation strategies have physiological costs (Schimel et al., 2007) and can reduce enzyme catalytic efficiency (Stone et al., 2012; Tischer et al., 2015). Microbial physiology, however, is evolutionarily selected for most efficient enzyme systems (Hochachka and Somero, 2002; Allison et al., 2010). Therefore, the combined thermal response of both parameters of catalytic efficiency needs to be considered to reveal the mechanisms maintaining effective enzymatic functioning through the wide range of increasing temperatures (Jaenick, 1991; Somero, 1995; Fields, 2001).

This study was designed to test the catalytic properties of enzymes involved in the C, P and N cycles, across the environmental temperatures covering psychrophilic, mesophilic and thermophilic ranges. We hypothesized 1) high enzyme flexibility (and gradual increase in K_m with temperature) within a cold temperature range; 2) in contrast, under warm temperatures we expected more static values and low temperature sensitivity of K_m. To test our hypothesis, we incubated soil for one month over a temperature range of 0–40 °C (with 5 °C steps) and determined the kinetic parameters of six enzymes involved in decomposing 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 is responsible for breaking down hemicelluloses (German et al., 2011); acid phosphatase, which mineralizes organic P into phosphate by hydrolyzing phosphoric (mono) ester bonds under acidic conditions (Eivazi and Tabatabai, 1977; Malcolm, 1983). Activities of leucine aminopeptidase and tyrosine aminopeptidase were analyzed to assess the hydrolysis of L-peptide bonds (Koch et al., 2007; Chen et al., 2012).

2. Material and methods

2.1. Soil

Soil samples were taken in the middle of summer from the top 10 cm of the Ap of an arable loamy haplic Luvisol from 4 experimental plots (fallow soil, 5 × 5 m) located on a terrace plain of the Leine River in central Germany (Holtensen, 52°22′40″, 9°41′46″E) (Pausch et al., 2013). The area has a temperate climate with a long-term annual mean precipitation of 645 mm and an air temperature of 8.7 °C (Kramer et al., 2012). The properties of the soil were: pH 6.5; 12.6 g kg^{-1} C, 1.3 g kg^{-1} N, 5.8% sand, 87.2% silt, 5.8% clay; 1.4 g cm^{-3} bulk density, and sampling moisture 60% of WHC (Kramer et al., 2012; Pausch et al., 2013). The samples were kept cold (~4 °C) during transportation to the laboratory. The samples were then frozen at −20 °C until pre-incubation.

2.2. Soil incubation

30 g of soil where incubated in air tight glasses (with rubber seal) with the volume of 125 mL. During the incubation, soil moisture was checked by weighting and was immediately adjusted to 60% of WHC. In order to avoid the anaerobiosis, all the samples have been regularly aerated by opening the cabs for 1 min. After incubation no significant differences were detected in pH of samples. Activity of six enzymes targeting C-, N- and P-containing substrates were determined after incubating the soil at 0, 5, 10, 15, 20, 25, 30, 35 and 40 °C. Nine climate chambers (SBS C120) were used to regulate the temperature (±0.5 °C). The frozen samples were thawed at 4 °C for one day and then pre-incubated at 20 °C for 14 d before the start of 30-day incubation. To minimize the freezing effect on enzymatic activities (Lee et al., 2007; Stone et al., 2012), all samples were frozen similarly, and they were pre-conditioned after thawing. We therefore assume that this pretreatment corresponded to snow thaw in spring and that the freezing effect was strongly reduced after the pre-incubation and was identical for all samples (German et al., 2012).

2.3. Enzyme assays

The kinetics of hydrolytic enzymes involved in C, N and P cycles were measured by fluorimetric microplate assays of 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (AMC) (Marx et al., 2005; Dorodnikov et al., 2009). Four types of fluorogenic substrates based on MUF and two types based on AMC were used to assess enzymatic activities; 4-methylumbelliferyl-phosphate (MUF-P) to detect phosphatase activity, 4-methylumbelliferyl-β-D-glucoside (MUF-G) to detect β-glucosidase activity; 4-methylumbelliferyl-β-D-cellobioside (MUF-C) to detect cellobiohydrolase activity; and 4-methylumbelliferyl-β-D-xylopyranoside (MUF-X) to detect xylanase activity. The activities of leucine aminopeptidase (AMC-L) and tyrosine aminopeptidase (AMC-T) were measured using L-Leucine-7-amino-4-methyl coumarin and L-tyrosine-7-amido-4-methyl-coumarin. All substrates and chemicals were purchased from Sigma (Germany).

We determined enzyme activities in a range of substrate concentrations from low to high (0, 10, 20, 30, 40, 50, 100, 200 μmol g^{-1} soil). Saturation concentrations of fluorogenic substrates were determined in preliminary experiments. Suspensions of 0.5 g soil