

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

Shibin Liu (1), Baharsadat Razavidezfuly (2), Yakov Kuzyakov (1,2)

(1) Department of Soil Science of Temperate Ecosystems, University of Göttingen, Germany (sliu3@gwdg.de), (2) Department of Agricultural Soil Science, University of Göttingen, Germany (brazavi@gwdg.de, kuzyakov@gwdg.de)

The temperature sensitivity of enzymes responsible for organic matter decomposition in cold environment soil, where warming is expected to be greatest is crucial. Based on Michaelis-Menten kinetics and Arrhenius function, we hypothesized that cold-adapted microorganisms will produce high efficient enzymes at cold temperatures (enzymes with lower apparent activation energy ( $E_a$ ) at cold temperature ranges).

To test our hypothesis, 30 g soil of Tibetan Plateau (4100 m a.s.l., annual temperature 2.4 °C) in 4 replicates were incubated 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 organics: cellobiohydrolase and  $\beta$ -glucosidase, which are commonly measured as enzymes responsible for consecutive stages of cellulose degradation; xylanase, which is responsible for breaking down hemicelluloses; acid phosphatase, which mineralizes organic P to phosphate by hydrolyzing phosphoric (mono) ester bonds under acidic conditions. Activities of leucine aminopeptidase and tyrosine aminopeptidase were analyzed to assess the hydrolysis of L-peptide bonds.

The apparent activation energy varied between enzymes from 42 (phosphatase) to 54 (cellobiohydrolase) kJ mol<sup>-1</sup> corresponding to the Q<sub>10</sub> values of the enzyme reactions of 1.8–2.3. The increase of substrate affinity ( $K_m$ ) with temperature was gradual for most tested enzymes from 0–20 °C (enzymes involved in C cycle), (proteases) and 0–40 °C (phosphatase). However, within a high range of temperatures (25–40 °C) the hydrolytic activity was governed by enzymes with nearly constant substrate affinity.

Overall, for enzymes involved in C cycle and proteases, a strong increase (30–40%) in  $K_m$  at high temperatures (25 °C) reflects an expression of multiple isoenzymes each with different temperature optima and probable shift of microbial community. The general trend of catalytic efficiency ( $V_{max}/K_m$ ) demonstrated a gradual increase with temperature both at cold and at warm ranges. The only remarkable exception occurred at 25 °C, where a strong increase in  $K_m$  was accompanied by a significant decrease in catalytic efficiency. In agreement with our hypothesis, high catalytic efficiency was accompanied by low  $E_a$  at cold temperature ranges. We conclude that predicting and modeling the consequences of warming for C, N and P cycles should consider possible temperature thresholds triggering strong changes in catalytic efficiency and, thus, in the process rates.

Keywords: Tibetan plateau, cold-adapted enzymes, Arrhenius function, Michaelis-Menten kinetics, catalytic efficiency, temperature sensitivity.