



## **Kinetics and spatial distribution of enzymes of carbon, nitrogen and phosphorus cycles in earthworm biopores**

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Earthworms boost microbial activities and consequently form hotspots in soil. The distribution of enzyme activities inside the earthworm biopores is completely unknown. For the first time, we analyzed enzyme kinetics and visualized enzyme distribution inside and outside biopores by in situ soil zymography. Kinetic parameters ( $V_{max}$  and  $K_m$ ) of 6 enzymes  $\beta$ -glucosidase (GLU), cellobiohydrolase (CBH), xylanase (XYL), chitinase (NAG), leucine aminopeptidase (LAP) and acid phosphatase (APT) were determined in biopores formed by *Lumbricus terrestris* L.. The spatial distributions of GLU, NAG and APT become visible via zymograms in comparison between earthworm-inhabited and earthworm-free soil. Zymography showed heterogeneous distribution of hotspots in the rhizosphere and biopores. The hotspot areas were 2.4 to 14 times larger in the biopores than in soil without earthworms. The significantly higher  $V_{max}$  values for GLU, CBH, XYL, NAG and APT in biopores confirmed the stimulation of enzyme activities by earthworms. For CBH, XYL and NAG, the 2- to 3-fold higher  $K_m$  values in biopores indicated different enzyme systems with lower substrate affinity compared to control soil. The positive effects of earthworms on  $V_{max}$  were cancelled by the  $K_m$  increase for CBH, XYL and NAG at a substrate concentration below 20  $\mu\text{mol g}^{-1}$  soil. The change of enzyme systems reflected a shift in dominant microbial populations toward species with lower affinity to holo-celluloses and to N-acetylglucosamine, and with higher affinity to proteins as compared to the biopores-free soil. We conclude that earthworm biopores are microbial hotspots with much higher and dense distribution of enzyme activities compared to bulk soil.

### References

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