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Visualization of enzyme activities inside earthworm biopores by in situ soil zymography

Thi Thu Duyen Hoang (1), Bahar.S Razavi (2), Evgenia Blagodatskaya (1), and Yakov Kuzyakov (2)

(1) Soil science of temperate ecosystem, Göttingen University, Göttingen, Germany, (2) Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany

Earthworms can strongly activate microorganisms, increase microbial and enzyme activities and consequently the turnover of native soil organic matter. In extremely dynamic microhabitats and hotspots as biopores made by earthworms, the in situ enzyme activities are a footprint of complex biotic interactions. The effect of earthworms on the alteration of enzyme activities inside biopores and the difference between bio-pores and earthworm-free soil was visualized by in situ soil zymography (Spohn and Kuzyakov, 2014). For the first time, we prepared quantitative imaging of enzyme activities in biopores. Furthermore, we developed the zymography technique by direct application of a substrate saturated membrane to the soil to obtain better spatial resolution.

Lumbricus terrestris L. was placed into transparent box (15×20×15cm). Simultaneously, maize seed was sown in the soil. Control soil box with maize and without earthworm was prepared in the same way. After two weeks when bio-pore systems were formed by earthworm, we visualized in situ enzyme activities of five hydrolytic enzymes (β-glucosidase, cellobiohydrolase, chitinase, xylanase, leucine aminopeptidase) and phosphatase. Followed by non-destructive zymography, biopore samples and control soil were destructively collected to assay enzyme kinetics by fluorogenically labeled substrates method.

Zymography showed higher activity of β-glucosidase, chitinase, xylanase and phosphatase in biopores comparing to bulk soil. These differences were further confirmed by fluorimetric microplate enzyme assay detected significant difference of Vmax in four above mentioned enzymes. Vmax of β-glucosidase, chitinase, xylanase and phosphatase in biopores is 68%, 108%, 50% and 49% higher than that of control soil. However, no difference in cellobiohydrolase and leucine aminopeptidase kinetics between biopores and control soil were detected. This indicated little effect of earthworms on protein and cellulose transformation in soil. In conclusion, earthworms contribute to the decomposition of carbohydrates through promoting enzyme activities involved in the C-cycle except for leucine aminopeptidase and cellobiohydrolase.

References

Spohn M, Kuzyakov Y. (2014) Spatial and temporal dynamics of hotspots of enzyme activity in soil as affected by living and dead roots – a soil zymography analysis, Plant Soil 379: 67-77