



## **Combining X-ray computed micro-tomography and zymography to understand spatial variability patterns of soil extracellular enzymes: The first steps to 3D zymography**

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Heterogeneity in physico-chemical micro-habitats is one of the key drivers of variability in microbial activity and functioning. However, direct experimental evidence of specific micro-environmental influences on microorganisms at their scale is only beginning to emerge. This is in part due to technical challenges associated with observing activities of microorganisms within their native habitats at spatial scales relevant for their biology and ecology. Recent analytical advances opened new opportunities for in situ micron-resolution descriptions and monitoring of various soil properties. Among such advances are (1) X-ray computed micro-tomography ( $\mu$ CT) for 3D characterization of soil pores and particulate organic matter (POM) and (2) in situ soil zymography for 2D mapping of enzyme activities. Here we combined the two techniques to explore linkages between X-ray  $\mu$ -CT micro-scale observations of soil properties with in situ micro-scale maps of enzyme activities. Intact soil cores (5 cm diameter x 5 cm height) were collected from 5-10 cm depth at Great Lakes Bioenergy Research experimental site located in Southwest Michigan, USA. Soil cores were collected from five land use and management practices, i.e. conventionally managed continuous corn, continuous corn with cover crops, switch grass, poplar trees, and native succession vegetation, which have been in place since 2008. The cores were first subjected to  $\mu$ CT scanning at 29  $\mu$ m resolution; then, 2D zymographic images of 7 extracellular enzymes were obtained on geo-referenced cut slices of the scanned cores. The studied enzymes were beta-glucosidase, cellobiohydrolase, xylanase, N acetyl-beta-glucosaminidase, leucine amino peptidase, tyrosine amino peptidase, and acid phosphatase. Relationships between pores of different size ranges and POM with enzyme activities were analyzed using multiple regression and principal component analyses. Presence of pores was associated with the activity of the most enzymes, but these relationships vary depending on enzyme type and pore size. Land use and management practices were additional secondary modifiers of these regressions between pore size and enzyme activities. We conclude that land use and management affect the 3D distribution of enzyme activities in soil due to modification of the size and type of pores as well as POM contents.