



## **Revealing effect of earthworm on C distribution and enzyme activity in top- and sub-soil**

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Despite its importance for terrestrial nutrient and carbon cycling, the spatial organization and localization of microbial activity in soil and in biopores is poorly understood. We hypothesized that biopores created by earthworm play a critical role in reducing the gap of SOM input and microbial activities between topsoil and subsoil.

Accordingly, Carbon (C) allocation by earthworms was related to enzyme distribution along soil profile. For the first time we visualized spatial distribution of enzyme activities ( $\beta$ -glucosidase, chitinase and acid phosphatase) and C allocation (by  $^{14}\text{C}$  imaging) in earthworm biopores in topsoil and subsoil.

Soil zymography (an in situ method for the analysis of the two-dimensional distribution of enzyme activity in soil) was accompanied with  $^{14}\text{C}$  imaging (a method that enables to trace distribution of litter and C in soil profile) to visualize change of enzyme activities along with SOM incorporation by earthworms from topsoil to subsoil. Experiment was set up acquiring rhizoboxes (9\_1\_50 cm) filled up with fresh soil and 3 earthworms (*L. terrestris*), which were then layered with  $^{14}\text{C}$ -labeled plant-litter of 0.3 MBq on the soil surface.  $^{14}\text{C}$  imaging and zymography have been carried out after one month.

Activities of all enzymes regardless of their nutrient involvement (C, N, P) were higher in the biopores than in bulk soil, but the differences were larger in topsoil compared to subsoil. Among three enzymes, Phosphatase activity was 4-times higher in the biopore than in the bulk soil. Phosphatase activity was closely associated with edge of burrows and correlate positively with  $^{14}\text{C}$  activity. These results emphasized especial contribution of hotspots such as biopores to C allocation in subsoil - which is limited in C input and nutrients - and in stimulation of microbial and enzymatic activity by input of organic residues, e.g. by earthworms. In conclusion, biopore increased enzymatic mobilization of nutrients (e.g. P) inducing allocation of nutrients for into deeper soil horizons.