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Spatial distribution of enzyme activities along the root and in the rhizosphere of different plants

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Extracellular enzymes are important for decomposition of many biological macromolecules abundant in soil such as cellulose, hemicelluloses and proteins. Activities of enzymes produced by both plant roots and microbes are the primary biological drivers of organic matter decomposition and nutrient cycling. So far acquisition of *in situ* data about local activity of different enzymes in soil has been challenged. That is why there is an urgent need in spatially explicit methods such as 2-D zymography to determine the variation of enzymes along the roots in different plants. Here, we developed further the zymography technique in order to quantitatively visualize the enzyme activities (Spohn and Kuzyakov, 2013), with a better spatial resolution

We grew Maize *(Zea mays L.)* and Lentil *(Lens culinaris)* in rhizoboxes under optimum conditions for 21 days to study spatial distribution of enzyme activity in soil and along roots. We visualized the 2D distribution of the activity of three enzymes:β-glucosidase, leucine amino peptidase and phosphatase, using fluorogenically labelled substrates. Spatial resolution of fluorescent images was improved by direct application of a substrate saturated membrane to the soil-root system.

The newly-developed direct zymography shows different pattern of spatial distribution of enzyme activity along roots and soil of different plants. We observed a uniform distribution of enzyme activities along the root system of Lentil. However, root system of Maize demonstrated inhomogeneity of enzyme activities. The apical part of an individual root (root tip) in maize showed the highest activity. The activity of all enzymes was the highest at vicinity of the roots and it decreased towards the bulk soil. Spatial patterns of enzyme activities as a function of distance from the root surface were enzyme specific, with highest extension for phosphatase. We conclude that improved zymography is promising *in situ* technique to analyze, visualize and quantify spatial distribution of enzyme activities in the rhizosphere hotspots.

References

Spohn, M., Kuzyakov, Y., 2013. Phosphorus mineralization can be driven by microbial need for carbon. *Soil Biology & Biochemistry* 61: 69-75