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Hotsphere illumination

Bahar S. Razavi (1) and Yakov Kuzyakov (1,2)

(1) Göttingen university, Büsgen-Institut, Agrarpedologie, Göttingen, Germany (bs.razavi@gmail.com), (2) Department of Soil Science of Temperate Ecosystems, University Göttingen, Germany (kuzyakov@gwdg.de)

Soils are the most heterogeneous parts of the biosphere, with an extremely high differentiation of properties and processes at all spatial and temporal scales. Importance of the hotspheres such as rhizosphere, detritusphere, porosphere (including drilosphere and biopores), hyphasphere and spermosphere, calls for spatially explicit methods to illuminate distribution of microbial activities in these hotspheres (Kuzyakov and Blagodatskaya, 2015). Zymography technique has previously been adapted to visualize the spatial dynamics of enzyme activities in rhizosphere (Spohn and Kuzyakov, 2014).
Here, we further developed soil zymography to obtain a higher resolution of enzyme activities by enabling direct contact of substrate-saturated membranes with soil. For the first time, we aimed at quantitative imaging of enzyme activities in various hotspheres. We calculated and compared percentage of enzymatic hotspots of five hotspheres: spermosphere, rhizosphere, detritusphere, drilosphere and biopores.
Spatial distribution of activities of two enzymes: β-glucosidase and leucine amino peptidase were analyzed in the spermosphere, rhizosphere and detritusphere of maize and lentil. Zymography has been done 3 days (spermosphere), 14 days (rhizosphere) after sowing and 21 days after cutting plant (detritusphere). Spatial resolution of fluorescent images was improved by direct application fluorogenically labelled substrates on the soil surface. Such improvement enabled to visualize enzyme distribution of mycorrhiza hypha on the rhizobox surface. Further, to visualize the 2D distribution of the enzyme activities in porosphere, we placed earthworms (Lumbricus terrestris), (drilosphere) and ground beetle species Platynus dorsalis Pont. (Coleoptera; Carabidae), (biopore), in transparent boxes for 2weeks.
The developed in situ zymography visualized the heterogeneity of enzyme activities along and across the roots. Spatial patterns of enzyme activities as a function of distance along the root demonstrated plant specific patterns of enzyme distribution: it was uniform and homogenous along the lentil roots, whereas the enzyme activities in maize rhizosphere were higher at the apical or proximal root parts. The activity of leucine-aminopeptidase was higher at the apical parts and β-glucosidase activity was higher at both apical and proximal part of individual maize roots. Much higher activity of leucine-aminopeptidase and β-glucosidase per mm2 of hotspots were found for rhizosphere (12-5 fold), drilosphere (10-4), spermosphere (9-4), biopore (9-1), hyphasphere (8-3) and detritusphere (5-2) compared to the bulk soil. Despite the transient nature of spermosphere, its microbial activities had long-lasting impact. We conclude that improved zymography is promising in situ technique to identify, analyze, visualize and quantify temporal-spatial distribution of enzyme activities in the various hotspheres.

Key words: hotsphere, enzyme distribution, temporal-spatial, zymography

Reference:
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