

Hotsphere illumination

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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 hotspots- environment with abundant high microbial activity- i.e.: rhizosphere, detritosphere, biopores, spermosphere and hyphasphere calls for spatially explicit methods to illuminate distribution of microbial activities (Kuzyakov and Blagodatskaya, 2015). Zymography technique has previously been adapted to visualize the spatial dynamics of enzyme activities in rhizosphere.

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 hotspots. We calculated and compared percentage of enzymatic hotspots of four hotspots: Spermosphere, rhizosphere, detritosphere and biopores.

Spatial distribution of activities of two enzymes: β -glucosidase and phosphatase were analyzed in the spermosphere and rhizosphere of maize and lentil. Zymography has been done 3 days (spermosphere), 14 days (rhizosphere) after sowing. Further, manure was placed on surface of rhizoboxes to visualize spatio-temporal distribution of the enzyme activities in detritosphere after 25 days. Biopores were produced by earthworms (*Lumbricus terrestris* L.) in transparent boxes for 2 weeks and enzyme distribution were measured by zymography thereafter.

The developed *in situ* direct soil 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.

For the first time were applied “spatial point pattern analysis” to determine whether the pattern of hotspot distribution is localized (aggregated) or dispersed.

Under effect of earthworms (in biopores), the hotspots were dispersed in whole soil profile but the distribution of hotspots in rhizosphere and spermosphere were localized and mainly associated with root or with manure layer (in detritosphere). Much higher enzyme activities per mm^2 of hotspots were found in rhizosphere (12-5 fold), detritosphere (10-4), spermosphere (9-4) and biopore (9-1), compared to the bulk soil. Despite the transient nature of spermosphere, its microbial activities had long-lasting impact. We conclude that plant induced hotspots with high enzyme activity; while, earthworms spread out enzyme activities in the whole soil profile. Concluding, direct zymography enabled to illuminate the contribution pattern of hotspots to emerging soil properties and ecosystem processes.