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Development of micro-zymography: microscopic visualization of enzymatic activity in soil aggregates and Zea mays L. root

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Enzymes are secreted by microbial cells into the soil to catalyze the acquisition of carbon or nutrients like nitrogen and phosphorus from soil organic matter. Apart from microorganisms and soil fauna, roots also secrete enzymes to mobilize nutrients from the soil nutrient pool. Thus, living plants and microorganisms are considered the main sources of soil enzymes in agroecosystems. To understand how exo-enzymes are distributed in soil microhabitats, and whether enzymatic activity is higher in soil solution or on particle surfaces, we visualized enzymatic activity at the corresponding scale. Visualization of enzymatic activity links microbial functioning to localization in heterogeneous soil habitats. To assess enzymatic reactions in soil at the microscopic level, we developed a micro-zymography approach based on fluorogenically-labeled substrate (phosphomonoesterase) in the rhizosphere soil of *Zea mays* L. For this, first we compared different fixatives required to prevent sample drying and found super transparent silicon as the most appropriate one. Then we evaluated micro-zymography i) on individual soil aggregates, ii) on thin layers of aggregates (≈ 500 µm) to assess the dynamics of phosphomonoesterase activity, and iii) on maize roots under laser scanning microscope. The results demonstrated that the main fluorescence signal shifted from the soil solution to the interface between the soil solution and aggregates within 30 min after substrate addition and was finally only detectable on the surface of a few aggregates. This was probably due to higher microbial abundance and enzymatic activity on the soil aggregates compared to the soil solution. The enzymatic activity appeared patchy on the aggregate surfaces indicating heterogeneous distribution of microorganisms. Similarly, a patchy distribution of enzymatic activity was detected on maize root surfaces. This work was conducted within the framework of the priority program 2089 “Rhizosphere spatiotemporal organization – a key to rhizosphere functions”, funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project number: 403664478. Seeds of the maize were provided by Caroline Marcon and Frank Hochholdinger (University of Bonn).

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