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Methodological aspects of quantitative membrane-based soil zymography

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Soil zymography is a new technique developed to visualize and analyze two dimensional distributions of enzyme activities. The method consists of placing a membrane saturated with an enzyme-specific substrate on prepared surface of the soil sample, followed by collecting the membrane image in UV light. Substrate diffuses from the membrane into soil surface; there, upon a contact with the enzymes, a fluorescent product (e.g. MUF: methylumbelliferon, AMC: 7-amido-4-methylcoumarin) is released and detected in the UV light. Thus, the fluorescing pattern on the membrane reflects spatial distribution of active enzymes on the studied soil surface. The method is simple, time and labor inexpensive, and nondestructive. These features allow repeated measurements of the same or different enzymes, and thus make the method attractive for studying spatial and temporal variability of enzyme activity in soil, rhizosphere, detritusphere and other hotspots. However, there are several challenges with effective use of zymography not only for qualitative demonstration of enzyme distribution patterns but also for quantitative assessments of their activities. The challenges include: 1) imperfect contact between the membrane and the soil surface leading to underestimation of enzyme activities in locations with low/absent contact; 2) lateral diffusion of the substrate and the fluorescing product inside the membrane decreasing spatial resolution, and 3) backward diffusion of the product from the membrane into the soil during the incubation leading to uncertainties of enzyme activity analysis. The goal of this study was to address these challenges with special focus on the impact of the diffusion on the results of the membrane-based soil zymography. We conducted several experiments to assess diffusion of the enzymes, substrates and products as well as measurements and calculations of contact areas between the membranes and soil surface. Based on these results, a modeling approach was developed for evaluation of enzyme activity obtained from the zymography data, membrane calibration, surface topography measurements, and diffusion experiments. This study will provide deep insights into further development of methodology of membrane-based soil zymography, its quantitative interpretation, and modeling of 3D distribution of microbial activity.