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## On the distribution and characterization of microhabitats in deep soil using soil zymography.

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Still, little is known about the biogeography of microorganisms in soils, especially in subsoils. Soil organic matter distribution in subsoils is characterized by a much higher spatial heterogeneity than in topsoils due to the greater relevance of preferential flow paths, roots, fungal hyphae and animal burrows for fresh substrate inputs. It is suggested that subsoil C-turnover is limited to these microsites of higher nutrient and substrate availability. Thus, it can be expected that such regions are hotspots of microbial life and short C-turnover times, representing spatially restricted microhabitats.

Microbial C and nutrient acquisition is largely determined by extracellular enzymes that are actively excreted to catalyze the degradation of SOM; thus being a suitable proxy for microbial activity and C-turnover. In this study, we mapped three extracellular enzymes (b-glucosidase, chitinase and acid phosphatase) on undisturbed soil samples applying soil zymography. The sampling technique developed by Krueger and Bachmann (2017) was used to obtain undisturbed soil samples with 7 x 11 cm surface area from 0-11, 15-26, 60-71, 80-91 and 150-161 cm depths from a dystric Cambisol covered with an even-aged beech forest stand (Fagus sylvatica L.). Different geostatistical and spatial analyses, such as nearest neighbor ratio or pixel intensity spatial correlation analysis, were applied for gaining more profound knowledge about the distribution of microbial hotspots in deep soil.

Average enzyme activities constantly decreased with depth, as well as the similarity of activity patterns between the three analyzed enzymes. In the topsoil (0-2 cm), no hotspots were identified, but in samples taken from 2-11 and 15-26 cm they represent 2-8% of the total area. In deeper soils, hotspots only made up a proportion of 0.3-2% of the total area and were characterized by average hotspot-sizes of 0.5-2 mm-2. The enzyme activity in such hotspots was not related to depth-specific patterns, showing 2-5 times higher enzyme activities in comparison to non-hotspots. The nearest-neighbor ratio declined with depth from 1.20 in 2-11 cm to 0.85 in 80-91 cm, which indicates clustered arranged hotspots in deep soil, while hotspots found in the upper subsoils were randomly distributed. These results indicate that subsoil provides small areas of heterogeneously restricted habitats for microorganisms, even in 150 cm depth. This supports the assumption that C-turnover and nutrient acquisition in deep soil is limited to small areas, while the major part of the soil volume is not greatly contributing to C- and nutrient-cycling. Further, decreasing pattern similarity may be related to a loss of microbial diversity with depth, meaning that in deep soil microsites are occupied by lower diverse communities that are only equipped with a small set of enzymatic tools.

## Literature

Krueger, J., Bachmann, J., 2017. New Field Sampling Method to Analyze Spatial Distribution of Small-Scale Soil Particle Surface Properties and Processes in Intact Soil. Vadose Zone Journal 16, 0.