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Comparative evaluation of pooling strategy in soil metaproteomics

Dong Liu (1), Katharina Maria Keiblinger (1), Stephan Fuchs (2), Uwe Wegner (2), Christian Hentschker (2), Dörthe Becher (2), Kathrin Riedel (2), and Sophie Zechmeister-Boltenstern (1)

(1) Institute for Soil Research, Department of Forest and Soil Sciences, Vienna, Austria (ld19871015@hotmail.com), (2) Institute of Microbiology, University of Greifswald, Friedrich-Ludwig-Jahnstrasse 15, 17489 Greifswald, Germany

Metaproteomics allow the simultaneous mapping of peptides of all known genomes and proteomes to disclose the identity of different organisms present in a sample; the simultaneous examination of microbial community structure and various protein functions is possible. However, metaproteomic studies of soils present a major challenge since (i) sample complexity hamper protein identification, and (ii) soil contains high microbial diversity but low protein amounts. The soil matrix is heterogeneous over diverse scales in space and time.
Within the present study we test a pooling strategy for standardization of soil sample protein extraction. To this end we conducted a pooling experiment to evaluate the applicability and suitability for metaproteome analysis of the soil samples. Five individual replicate soil samples from small plots (1 m x 1 m size) on the larger plot scale (with the size of approximately 10 m x 10 m in a mature common beech stand) were analyzed and for pooling purposes a fraction of each individual sample was used to create a pooled soil sample prior to extraction. Extracted soil proteins were subject to protein separation on a 1D-SDS-PAGE, to remove interfering substances and reduce soil sample complexity. After trypsin digestion, the resulting peptide mixtures were analyzed on a LTQ-Orbitrap Velos mass spectrometer.
Comparison of peptide mass spectra to protein groups for individual and pooled soil samples resulted in similar abundances of microbial taxa and functions. Our results indicate that pooling is a time- and cost-efficient practice for proteomic analyses of soils if the research questions are focusing on the variability of most abundant taxa and functions.
In general, we think it will be necessary to test soils from each site or experiment individually if pooling is not suitable. We conclude that, the application of pooling for extraction purposes should be considered for metaproteomics from field studies that do not focus on within-site variability.