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On-line nano-solid phase extraction Fourier-transform ion cyclotron resonance mass spectrometry workflow to analyse soil solution organic matter gradients in the rhizosphere

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Plant-microbe interplay in the rhizosphere generates multi-faceted chemical gradients. The soil solution is a crucial component of the rhizosphere, where chemical gradients of organic molecules first develop upon growth of roots, introduction of plant-derived carbon and microbial turnover. Studying these gradients requires high resolution both in time and space as well as high chemical specificity to resolve the multitude of compounds. Existing methods to probe the rhizosphere soil solution were mostly limited to bulk chemical parameters, inorganic ions or targeted analysis of organic molecules. However, to decipher organic carbon turnover in the rhizosphere the characterization of the complex pool of soil solution organic matter is needed.

Here we present a novel method that combines time-resolved collection of soil solution samples via micro-suction cups in the rhizosphere with ultrahigh chemical resolution provided by Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) to unravel developing pattern of soil solution organic matter.

Zea mays plants were grown in soil columns for three weeks and soil solution samples of undisturbed root-soil system were collected once a week. Growth of the root system and hence position of sampling locations in relation to the distance from the root, were followed by X-ray computed tomography (X-ray CT).

The online sample preparation was optimised to extract and desalt the organic matter from a few microliters of soil solution. The downscaling to a nano-liquid chromatography system for the on-line extraction allowed the analysis of only minute amounts of organic carbon within the samples (down to 10 ng). Given the high background concentration of soil-derived organic carbon, the high mass resolution and sensitivity of FT-ICR-MS enabled to distinguish root derived molecules from soil organic matter based on their exact masses. Molecular formulas of the root derived molecules could be calculated showing distinct chemical characteristics as compared to the bulk soil solution. X-ray CT analyses enabled relating the results from the chemical analysis to distance from the root

and root age. With increasing influence of the roots higher molecular masses and an increasing degree of oxygenation of the molecules could be observed.

Our method is thus capable to show the changing small scale pattern of soil solution organic matter during the early rhizosphere development, closing the knowledge gap between root exudates, soil chemistry and microbial processes.