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Combining position-specific 13C labeling with compound-specific isotope analysis: first steps towards soil fluxomics

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Understanding the soil organic matter (SOM) dynamics is one of the most important challenges in soil science. Transformation of low molecular weight organic substances (LMWOS) is a key step in biogeochemical cycles because 1) all high molecular substances pass this stage during their decomposition and 2) only LMWOS will be taken up by microorganisms. Previous studies on LMWOS were focused on determining net fluxes through the LMWOS pool, but they rarely identified transformations. As LMWOS are the preferred C and energy source for microorganisms, the transformations of LMWOS are dominated by biochemical pathways of the soil microorganisms. Thus, understanding fluxes and transformations in soils requires a detailed knowledge on the biochemical pathways and its controlling factors.

Tracing C fate in soil by isotopes became on of the most applied and promising biogeochemistry tools. Up to now, studies on LMWOS were nearly exclusively based on uniformly labeled organic substances i.e. all C atoms in the molecules were labeled with 13C or 14C. However, this classical approach did not allow the differentiation between use of intact initial substances in any process, or whether they were transformed to metabolites. The novel tool of position-specific labeling enables to trace molecule atoms separately and thus to determine the cleavage of molecules - a prerequisite for metabolic tracing. Position-specific labeling of LMWOS and quantification of $13CO_2$ and 13C in bulk soil enabled following the basic metabolic pathways of soil microorganisms. However, only the combination of position-specific 13C labeling with compound-specific isotope analysis of microbial biomarkers and metabolites allowed 1) tracing specific anabolic pathways in diverse microbial communities in soils and 2) identification of specific pathways of individual functional microbial groups. So, these are the prerequisites for soil fluxomics.

Our studies combining position-specific labeled glucose with amino sugar 13C analysis showed that oxidizing catabolic pathways and anabolic pathways, i.e. building-up new cellular compounds, occurred in soils simultaneously. This involved an intensive C recycling within the microorganisms that was observed not only for cytosolic compounds but also for cell wall polymers. Fungal metabolism and fluxes were slower than bacterial intracellular C recycling and turnover. Furthermore, position-specific labeling of glutamate and subsequent 13C analysis of microbial phospholipid fatty acids (PLFA) revealed starvation pathways, which were only active in specific microbial groups in soils. These studies revealed that position-specific labeling enables the reconstruction of metabolic pathways of LMWOS within diverse microbial communities in complex media such as soil. Processes occurring simultaneously in soil i.e. 1) within individual, reversible metabolic pathways and 2) in various microbial groups could be traced by position-specific labeling in soils in situ.

Tracing these pathways and understanding their regulating factors are crucial for soil C fluxomics, the extremely complex network of transformations towards mineralization versus the formation of microbial biomass compounds. Quantitative models to assess microbial group specific metabolic networks can be generated and parameterized by this approach. The submolecular knowledge of transformation steps and biochemical pathways in soils and their regulating factors is essential for understanding C cycling and long-term C storage in soils.