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A novel fluxomics approach to decipher the flux partitioning between anabolic and catabolic processes in soil microbial communities

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The activities of soil microorganisms drive soil carbon (C) and nutrient cycling and therefore play an important role in local and global terrestrial C dynamics and nutrient cycles. Unfortunately, soil microbial activities have been defined mostly by measurements of heterotrophic respiration, potential enzyme activities, or net N processes. However, soil microbial activities comprise more than just catabolic processes such as respiration and N mineralization. Recently anabolic processes (biosynthesis and growth) and the partitioning between anabolic and catabolic processes in soil microbial metabolism have gained more attention as they control microbial soil organic matter formation. Understanding the controls on these processes allows an improved understanding of the key roles that soil microbes play in organic matter decomposition (catabolic processes) and soil organic matter sequestration (anabolic processes leading to growth, biomass and necromass formation), and their potential feedback to global change.

Generally, there are two approaches to study the metabolism of soil microbial communities: First, position-specific isotope labeling is a tool that allows the tracing of ^{13}C-atoms in organic molecules on their way through the network of metabolic pathways and second, metabolomics and fluxomics approaches can enable disentangling the highly complex metabolic networks of microbial communities, which however have rarely (metabolomics) or never (fluxomics) been applied to soils.

In this study we developed a targeted soil metabolomics approach coupled to ^{13}C isotope tracing (fluxomics), in which we extract, purify and measure a preselected set of key metabolites. Our aim was to cover the wide spectrum of soil microbial metabolic pathways based on the analysis of biomarker metabolites being unique to specific metabolic pathways such as glycolysis/gluconeogenesis (e.g. fructose 1,6-bisphosphate), the pentose phosphate pathway (ribose-5-phosphate), the citric acid cycle (α-ketoglutaric acid), purine and pyrimidine metabolism (UMP, AMP, allantoin), amino acid biosynthesis and degradation (10proteinogenic amino acids and their intermediates), the urea cycle (ornithine), amino sugar metabolism (N-Acetyl-D-Glucosamine and –muramic acid) and the shikimate pathway (shikimate). The minute concentrations of these primary metabolites are extracted from soils by 1 M KCl including 5 % chloroform, salts are

removed by freeze-drying, methanol dissolution and cation-/anion-exchange chromatography and the metabolites and their isotopomers quantified by UPLC-Orbitrap mass spectrometry. To cover the wide range of metabolites, compound separations are performed by hydrophilic interaction chromatography (HILIC) for metabolites such as amino acids, (poly-)amines, nucleosides and nucleobases and by Ion chromatography (IC), to separate charged molecules like amino sugars, sugar phosphates and organic acids. Here we will show fluxomics results from a laboratory soil warming experiment where we added ^{13}C-glucose to a temperate forest soil as a proof of concept.