



From isotopes and biomarkers to metabolic flux tracing: New insights into soil carbon and nutrient cycling in soil

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To disentangle fluxes and biogeochemical cycling of OC and nutrients (N, P) in soil a detailed knowledge on the biogeochemical pathways and its controlling factors is required. Over the last decades, biomarker approaches and isotope analyses became the most applied and promising tools to study SOM cycling.

We will firstly review current knowledge on combining biomarker with isotope analyses (^{13}C , ^{15}N , ^{18}O , ^{33}P) for identifying the mechanisms and rates of OC and nutrient transformation and cycling. As compound-specific isotope studies on uniformly labeled substances do not allow the differentiation of the intact use of the initial substances from its transformation to metabolites, we will introduce position-specific labeling to trace molecule atoms separately - a prerequisite for metabolic flux tracing. This together with the quantification of isotope incorporation in CO_2 and phospholipid fatty acids (PLFA), enables tracing specific metabolic pathways of individual microbial communities in situ.

We then discuss changes in the transformation pathways caused by sorption. Sorption to mineral surfaces is one of the most important processes contributing to soil organic matter (SOM) stabilization. While this stabilization is commonly attributed to inaccessibility of the sorbed SOM to microorganisms, recent findings have revealed increased metabolic efficiency from sorbed substrates. The application of position-specifically ^{13}C labeled sorbed tracer, combined with compound-specific ^{13}C analysis of PLFA, enables determination of whether this higher efficiency is due to a metabolic shift in a) the whole microbial community or b) microbial specialists adapted to sorbed C sources.

To assess metabolic changes induced by sorption in individual microbial groups, we added uniformly and position-specifically ^{13}C labeled alanine to a loamy Luvisol and incubated the soil samples for 10 days. Two different amendments were compared: i) alanine sorbed to sterilized soil, and ii) free alanine in solution. Incorporation of C from individual alanine positions was evaluated in distinct microbial groups classified by ^{13}C -PLFA analysis.

Most of the microbial groups took up sorbed and free alanine equally. The metabolic pattern was also similar in most microbial groups: incorporation of C from carboxylic C-1 into PLFA was negligible, whereas C from the amino-bound C-2 and the methyl C-3 were preferentially incorporated into PLFA. This pattern reflects the basic microbial metabolism of C3 molecules - fast mineralization of C-1 via pyruvate decarboxylation. Only fungi incorporated more C from sorbed than free alanine into their PLFA. Their metabolic pattern also revealed a more complex metabolization via gluconeogenesis, followed by pentose-phosphate pathway.

While the incorporation into most microbial groups' PLFA remained stable or decreased over 10 days, the ^{13}C in Actinobacteria's PLFA increased up to 7 times from day 1 to day 10. This strong ^{13}C increase in Actinobacteria was explained by their uptake of secondary substrates derived from microbial necromass or secretion products.

This experiment showed that the more efficient metabolization of sorbed substrates is the work of microbial specialists: While uniform labeling revealed the higher incorporation of sorbed than free substrate into fungus PLFAs, position-specific labeling allowed the reconstruction of metabolic changes triggered by sorption of the substrate.