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The development of a new technical platform to measure soil organic nitrogen cycling processes by microbes

Yuntao Hu, Andreas Richter, and Wolfgang Wanek

Department of Microbiology and Ecosystem Science, Faculty of Life Sciences, University of Vienna, Vienna, Austria(contact.csb@univie.ac.at)

Soil organic matter (SOM) decomposition is one of the most important processes of the global nitrogen cycle, having strong implications on soil N availability, terrestrial carbon cycling and soil carbon sequestration. During SOM decomposition low-molecular weight organic nitrogen (LMWON) is released which can be taken up by microbes (and plants). The breakdown of high-molecular weight organic nitrogen (HMWON, e.g. proteins, peptidoglycan, chitin, nucleic acids) represents the bottleneck of soil HMWON decomposition and is performed by extracellular enzymes released mainly by soil microorganisms. Despite that, the current understanding of the controls of these processes is incomplete. The only way to measure gross decomposition rates of these polymers is to use isotope pool dilution (IPD) techniques. In IPD approaches the product pool is isotopically enriched (by e.g. 15N) and the isotope dilution of this pool is measured over time. We have pioneered an IPD for protein and cellulose depolymerization, but IPD approaches for other polymers, specifically for important microbial necromass components such as chitin (fungi) and peptidoglycan (bacteria), or nucleic acids have not yet been developed.

Here we present a workflow based on a universally applicable technical platform that allows to estimate the gross depolymerization rate of SOM (HMWON) at the molecular level, using ultra high performance liquid chromatography/high resolution Orbitrap mass spectrometry (UPLC/HRMS) combined with IPD techniques. The necessary isotopically labeled organic polymers (chitin, peptidoglycan and others) are extracted from laboratory bacterial and fungal cultures grown in fully isotopically labeled nutrient media (15N, 13C or both). A purification scheme for the different polymers is currently established. Labeled potential decomposition products (e.g. amino sugars and muropeptides from peptidoglycan, amino sugars and chitooligosaccharides from chitin, nucleotides and nucleosides from nucleic acids) are prepared by enzymatic and/or acid digestion of the polymers. Different UPLC separation columns (Hypercarb, HiliC and C18) make it possible to separate more than 100 related monomers and oligomers produced during polymer decomposition, a prerequisite for analyzing the concentrations and isotope kinetics of decomposition products in complex soil samples. The benchtop Orbitrap mass analyzer has a nominal mass resolving power of 100,000 (FWHM at m/z 200), which enables us to separate compounds that are 13C- and 15N-labelled (mass difference: 0.00632) in the same compound, allowing tracing carbon and nitrogen isotopes in the same compound in IPD experiments. With the accurate masses, retention times and the isotopic pattern we can quantify and qualify the target decomposition products and their isotope kinetics during soil incubation experiments. This will enable us to estimate in situ decomposition rates of the major organic nitrogen polymers in soils, allowing new insights into the major controls of the most important step in soil organic nitrogen recycling.