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Quantification of transformation rates of soil amino sugars and amino acids by a novel isotope pool dilution approach via liquid chromatography/high resolution mass spectrometry (LC/HRMS)

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Organic nitrogen transformation processes are the key driver of soil nitrogen availability, strongly affecting the nitrogen turnover and carbon cycling of terrestrial ecosystems. Low molecular weight organic nitrogen compounds (e.g. amino acids and amino sugars) that can be directly utilized by plants or microorganisms are released by the extracellular cleavage of high molecular weight organic nitrogen compounds (e.g. proteins, peptidoglycan, and chitin) by hydrolytic enzymes. This decomposition process is believed to be the rate-limiting step in the soil N cycle. Direct measurements of the in situ transformation rates of these small N compounds is highly challenging but can be realized by applying the isotope pool dilution (IPD) technique, in which the target compound pool is labeled with isotopic tracers and subsequently the dilution of the tracers is measured. We have recently pioneered the development of IPD assays to investigate the in situ flux of proteinaceous amino acids and glucose due to decomposition of organic matter and microbial utilization, but the roles of fluxes of amino sugars and amino acid enantiomers in soil nitrogen transformation processes are still unknown due to the lack of feasible extraction, purification, separation and detection methods.

Here we developed a ^{15}N IPD assay by utilizing a novel LC/HRMS (Orbitrap) platform, with the aim to measure transformation rates of amino sugars and amino acid enantiomers. After the tracer experiments soil extracts were purified by solid phase extraction prior to the analysis by MS. The utilization of Orbitrap-HRMS allowed us to resolve the mass signals of unlabeled analytes, and their ^{15}N labeled (tracers) and ^{13}C labeled (internal standards) analogues. The commercially unavailable ^{15}N and ^{13}C labeled amino sugars and amino acid enantiomers were produced from bacterial cell walls after batch culture in labeled growth media. This workflow was validated with soils from two sampling sites, allowing us to successfully investigate the production and consumption of 2 amino sugars, 18 amino acids, and 4 amino acid enantiomers in soils.

We further applied this method to soils from 6 sampling sites differing in geology and land management, after short-term (1-day) temperature (5˚C, 15˚C, 25˚C) pre-incubations. We found that the release of amino sugars (free glucosamine) during the decomposition of peptidoglycan and chitin accounted for approximately 5% to 15% of the total influx into the dissolved organic nitrogen pool (amino acids plus amino sugars). Muramic acid exhibited significantly longer residence times in soils, indicating that free muramic acid was not an important decomposition product of peptidoglycan in soil. We will present further results on potential controls of soil amino sugar fluxes, such as soil temperature, geology and land management, as well as soil peptidoglycan and chitin content, hydrolytic enzyme activity, and microbial community structure. These findings and further ongoing work will greatly advance our knowledge of the transformation processes of soil organic nitrogen and its major controls.