Geophysical Research Abstracts
Vol. 21, EGU2019-4238, 2019
EGU General Assembly 2019

Development and validation of a two point normalisation method for determination of 15N-enriched amino sugars by gas chromatography-combustion-isotope ratio mass spectrometry

Michaela Reay (1), Timothy Knowles (2), David Jones (3,4), and Richard Evershed (1)

(1) Organic Geochemistry Unit, School of Chemistry, University of Bristol, Cantock's Close, Bristol, BS8 1TS, U.K. , (2) Bristol Radiocarbon Accelerator Mass Spectrometer, University of Bristol, 43 Woodland Road, Bristol, BS8 1UU, U.K , (3) UWA School of Agriculture and Environment, University of Western Australia, Crawley, WA 6009, Australia, (4) Environment Centre Wales, Deiniol Road, Bangor University, Bangor, Gwynedd, LL57 2UW, U.K.

15N-stable isotope probing (SIP) is a powerful tool to elucidate the active nitrogen (N) cycle in soil. Amino sugars (AS) in soil can be attributed to bacterial or fungal sources, providing insight into the role of these communities in nitrogen assimilation in soil. A 15N-tracer approach has been applied to AS using gas chromatography-mass spectrometry (GC-MS). This technique requires high application rates of environmentally relevant substrates and high 15N-enrichments perturbing the soil ecosystem and observed community shifts may be artefacts of the incubation set-up. GC-C-IRMS is a more sensitive technique which enables the investigation of N-assimilation into the microbial community in soil using environmentally relevant conditions. Such a technique has been previously applied to amino acids and development and validation of a GC-C-IRMS method for AS would provide novel insight into microbial N assimilation. This study details the assessment for the suitability of GC-C-IRMS to determine δ15N values of 15N-enriched AS prior to application in an environmental setting. An efficient derivatisation and clean-up and chromatographic separation for alditol acetate derivatives of the four most abundant AS in soil (glucosamine, GlcN; galactosamine GalN; mannosamine ManN and muramic acid MurN) was optimised. Subsequently, the first application of a two-point normalisation for compound specific δ15N value determinations was achieved using in-house laboratory standards of known δ15N values (four natural abundance AS and 15N-enriched GlcN (up to 469 ‰. The optimal carrier gas flow rate found to be 1.7 ml min-1. Linearity of δ15N value determinations up to 469 ‰ was confirmed when 30 nmol N was injected on-column. At low analyte amount, the determined δ15N value deviated depending on the 15N-abundance of the analyte, due to the relative sizes of peaks in the m/z 28 and m/z 29 traces and sensitivity of Faraday cups used in the IRMS. Finally, significant between- and within-run memory effects were observed when a highly enriched standard (469 ‰ was run therefore analytical run order and variation in 15N-enrichment of analytes within the same sample must be considered. The investigated parameters have confirmed the isotopic robustness of alditol acetate derivatives of amino sugars for the GC-C-IRMS analysis of 15N-enriched AS in terms of linearity over an enrichment range (natural abundance to 469 ‰ with on column analyte amount over 30 nmol N. Furthermore, this study is the first to apply a two-point normalisation for compound-specific δ15N determinations, which will ensure robust determination of δ15N values when this method is applied in environmental settings.