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# Development of analytical method for clumped isotope measurements in soil nitrate

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Stable isotope analysis represents an important indirect measurement for determining the fate of nitrogen in soils, as stable isotope ratios provide a record of past metabolic transformations a sample has undergone. Therefore, stable isotope ratios can be used to distinguish between two major nitrate loss pathways found in soils: microbial denitrification and leaching to groundwater [1]. However, traditional analytical methods for measuring $^{15}N$:$^{14}N$ ratios in nitrate samples produce high isotopic variance at low environmental concentrations, and requires assumptions on the initial nitrate isotope ratios, making this approach uncertain for estimating nitrate losses via these individual pathways [2]. Additionally, to avoid the loss of relevant molecular isotopic (isotopologue) information through the sample conversion process that is necessary with traditional IRMS, electrospray-ionization Orbitrap mass spectrometry has recently been successful in measuring isotopes, including clumped isotopes, of intact nitrate molecules with high precision [3]. With adequate analytical methods for measuring nitrate clumped isotopes now a possibility, isotopologue fractionation occurring during microbial nitrate production (nitrification) and consumption (denitrification) can be quantified to assist in tracing nitrates through soils. This research project aims to develop an analytical method for soil nitrate isotopologue analysis with an emphasis on clumped isotopes using ESI-Orbitrap mass spectrometry. This method can therefore be used to determine the isotopologue fractionation of microbial nitrification and denitrification to assist in distinguishing these nitrate transformation pathways in soils. If successful, clumped isotope analysis will allow for differentiating between source and sink processes of nitrate in soils.

[1] Bachmann et al. (2026), *Geoderma, 467, 117746.*

[2] Biasi et al. (2022), *Rapid Communications in Mass Spectrometry*, *36*(22), e9370.

[3] Hilkert et al. (2021), *Analytical Chemistry, 93*(26), 9139-9148.