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Membrane inlet mass spectrometry method (REOX/MIMS) to measure ^{15}N-nitrate in isotope-enrichment experiments

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Using ^{15}N stable isotope as a tracer to quantify N transformation rates in isotope-enrichment experiments improves understanding of the N cycle in various ecosystems. However, measuring ^{15}N-nitrate ($^{15}NO_3^-$) in small volumes of water for these experiments is a major challenge due to the inconvenience of preparing samples by traditional techniques. Lin et al. (2021) developed a "REOX/MIMS" method by applying membrane inlet mass spectrometry **(MIMS, Hiden HPR-40, Hiden Analytical Ltd., Warrington, UK)** to determining $^{15}NO_3^-$ concentrations in a small volume of water from isotope-enrichment experiments after converting the dissolved inorganic N to N_2. The nitrates (NO_3^- + NO_2^-) were reduced to NH_4^+ with zinc powder, and the ammonium (NH_4^+) was then oxidized to N_2 by hypobromite iodine solution. The resulting $^{29}N_2$ and $^{30}N_2$ were measured via MIMS. This optimized protocol provides a sensitive (~0.1 μM) and precise (relative standard deviation = 0.1–4.37%) approach to quantify $^{15}NO_3^-$ concentrations (0.1–500 μM) in water samples over a wide range of salinities (0–35 ‰) and in 2 M KCl solution with excellent calibration curves ($R^2 \geq 0.9996$, $p < 0.0001$). The method was combined with $^{15}NO_3^-$ isotope-enrichment incubation experiments to measure gross nitrification and gross NO_3^- immobilization rates in various ecosystems. It was rapid, accurate, and cost-effective. Future applications of this efficient approach will inform scientists, modelers and decision makers about mechanisms, sources, fates, and effects of NO_3^- delivered to or produced in numerous aquatic and terrestrial ecosystems. The privous OX/MIMS method has be expanded successfully to determine DNRA, N fixation, mineralization and immobilization with isotope tracer or dilution techniques in sediments of aquatic environments (Yin, et al, 2014; Hou et al., 2018; Lin et al., 2016a; Lin et al., 2016b; Lin et al., 2017). Extending use of the MIMS to measurements of $^{15}NO_3^-$ using the described REOX/MIMS method provides a convenient and cost-effective approach to determine gross nitrification, gross N mineralization and immobilization, and N fixation rates, and extends the application field of MIMS to terrestrial ecosystems. In addition, REOX/MIMS has been extended to determine DO^{15}N concentration using UV oxidation (Lu et al., 2020). With this method, we can quantify the main N-transformation processes in the soils/sediments from various ecosystems using a MIMS.

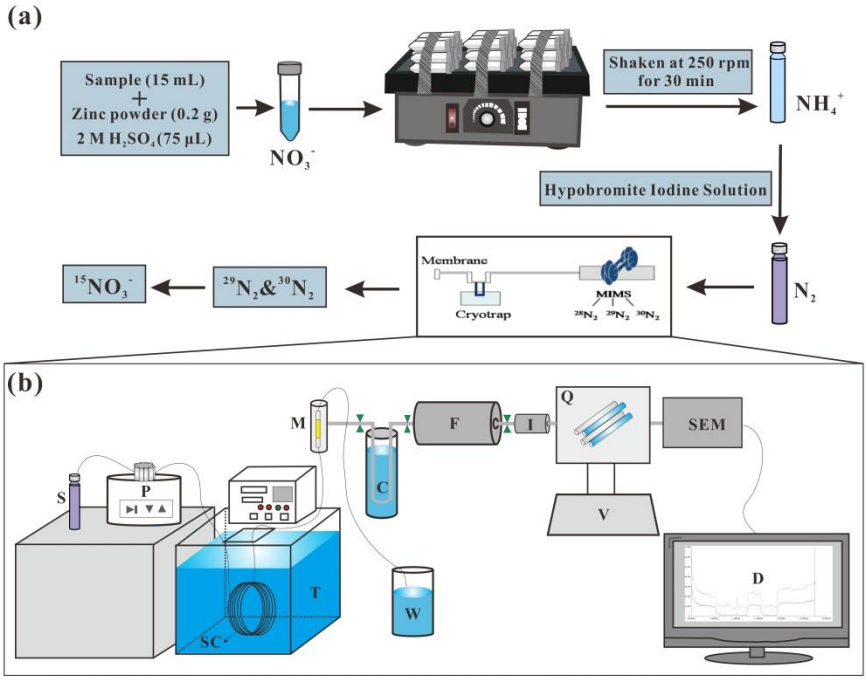

Fig. 1. *The general procedure of the "REOX/MIMS" method for determination of $^{15}NO_3^-$ in aqueous samples (a) and the schematic diagram of self-assembled membrane injection mass spectrometry system (b); The main components of this system are: sample vial (S), injection peristaltic pump (P), constant temperature water bath (T), stainless steel capillary (SC), membrane injector (M, including a gas-permeable silicone elastomer tube and a thick glass tubing), waste recovery bottle (W), cold trap (C), copper reduction furnace (F, containing a quartz tube with reduced copper wire), vacuum system (V), Ion source (I), quadrupole mass analyzer (Q), secondary electron multiplier (SEM), data processing system (D).*

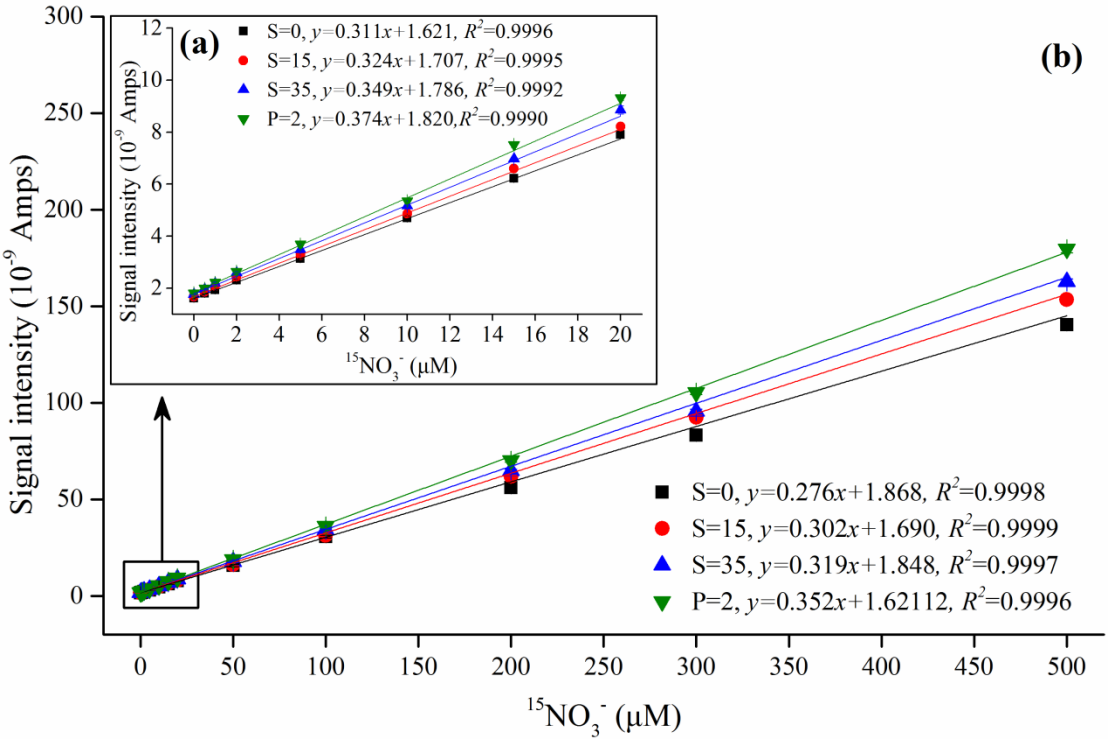

Fig. 2. *Relationships of the known $^{15}NO_3^-$ concentrations with measured signal intensities of total ^{15}N ($^{29}N_2$+2×$^{30}N_2$) under optimal condition at salinity of 0, 15, and 35 ‰, as well as at solution of 2 M KCl. Vertical bars denote the standard errors (n = 3). S and P represent salinity and 2 M KCl, respectively.*

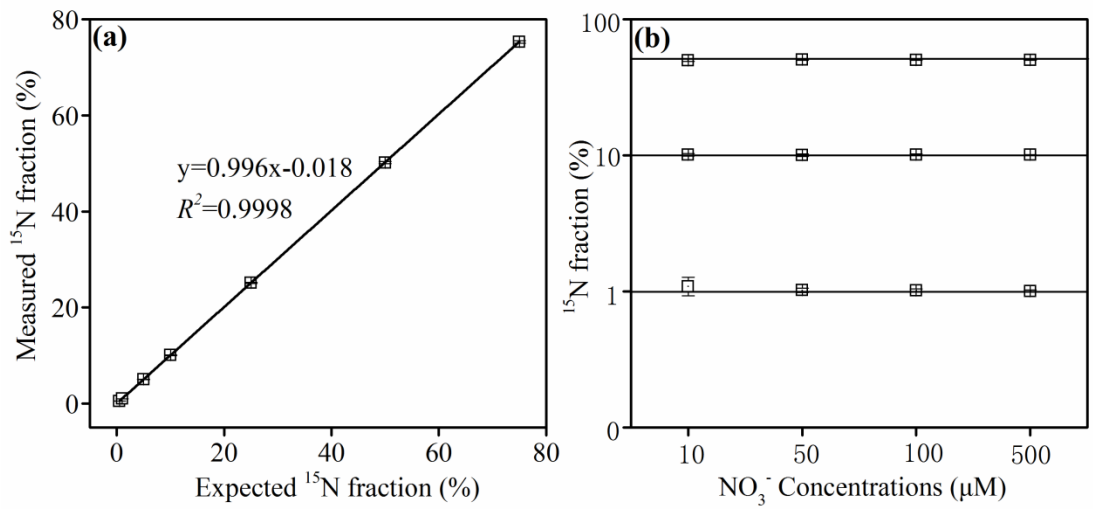

Fig. 3. *(a) Relationships of the measured ^{15}N fraction (0.5, 1, 5, 10, 25, 50, and 75%) with expected ^{15}N fraction for standards at 500 μM, (b) ^{15}N abundances measured by at different NO_3^- concentrations (n = 3, mean and standard deviation)*

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