Received: 4 September 2017 | Revised: 30 October 2017 | Accepted: 1 November 2017

DOI: 10.1002/rcm.8029

WILEY Rapid Communications in Mass Spectrometry

RESEARCH ARTICLE

N and O isotope ($\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$, $\delta^{17}O$) analyses of dissolved NO_3^- and NO_2^- by the Cd-azide reduction method and N_2O laser spectrometry

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Funding information
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Rationale: The nitrogen and oxygen ($\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$) isotopic compositions of NO_3^- and NO_2^- are important tracers of nutrient dynamics in soil, rain, groundwater and oceans. The Cd-azide method was used to convert NO_3^- or NO_2^- to N_2O for N and triple-O isotopic analyses by N_2O laser spectrometry. A protocol for laser-based headspace isotope analyses was compared with isotope ratio mass spectrometry. Lasers provide the ability to directly measure ^{17}O anomalies which can help discern atmospheric N sources.

Methods: $\delta^{15}N$, $\delta^{18}O$ and $\delta^{17}O$ values were measured on N/O stable isotopic reference materials (IAEA, USGS) by conversion to N_2O using the Cd-azide method and headspace N_2O laser spectrometry. A ^{15}N tracer test assessed the position-specific routing of N to the α or β positions in the N_2O molecule. A data processing algorithm was used to correct for isotopic dependencies on N_2O concentration, cavity pressure and water content.

Results: NO_3^-/NO_2^- nitrogen is routed to the $^{15}N^{\alpha}$ position of N_2O in the azide reaction; hence the $\delta^{15}N^{\alpha}$ value should be used for N_2O laser spectrometry results. With corrections for cavity pressure, N_2O concentration and water content, the $\delta^{15}N^{\alpha}_{AIR}$, $\delta^{18}O_{VSMOW}$ and $\delta^{17}O_{VSMOW}$ values (‰) of international reference materials were +4.8 ± 0.1, +25.9 ± 0.3, +12.7 ± 0.2 (IAEA NO_3), −1.7 ± 0.1, −26.8 ± 0.8, −14.4 ± 1.1 (USGS34) and +2.6 ± 0.1, +57.6 ± 1.2, +51.2 ± 2.0 (USGS35), in agreement with their values and with the isotope ratio mass spectrometry results. The ^{17}O excess for USGS35 was +21.2 ± 9‰, in good agreement with previous results.

Conclusions: The Cd-azide method yielded excellent results for routine determination of $\delta^{15}N$, $\delta^{18}O$ and $\delta^{17}O$ values (and the ^{17}O excess) of nitrate or nitrite by laser spectrometry. Disadvantages are the toxicity of Cd-azide chemicals and the lack of automated sampling devices for N_2O laser spectrometers. The ^{15}N-enriched tracer test revealed potential for position-specific experimentation of aqueous nutrient dynamics at high ^{15}N enrichments by laser spectrometry, but exposed the need for memory corrections and improved spectral deconvolution of ^{17}O.

1 | INTRODUCTION

The nitrogen and oxygen ($\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$) stable isotopic composition of aqueous nitrate (NO_3^-) and the $\delta^{15}N$ value of nitrite (NO_2^-) are well-established and important tracers of nutrient sources and dynamics in soil, rain, surface water, groundwater and seawater. Nitrate isotopes are also used to assess the capacity of aquatic ecosystems to cycle N, and the remediation of nitrate-contaminated aquifers by processes such as subsurface bacterial denitrification.[1-9]

Various preparative sample methods and isotopic techniques have been developed over the decades to measure the stable isotope composition of dissolved nitrate and nitrite in environmental freshwater, seawater and soil water samples. The preparative methods generally involve (i) appropriate field filtration and sample preservation, (ii) extraction and purification of dried nitrogenous salts for isotope ratio mass spectrometry (IRMS) by elemental analysis or thermochemical conversion to N_2 and CO gas[10-12] or (iii) aqueous conversion of sample aliquots by bacterial denitrifiers or by Cd-azide reduction. The latter quantitatively convert NO_3^- and/or NO_2^- to N_2O headspace gas, with $^{15}N/^{14}N$ and $^{18}O/^{16}O$ measurements conducted by purge-and-trap continuous-flow IRMS.[13-16] More recently, the denitrifier approach was adapted for direct injection of microbially produced N_2O headspace and by laser spectrometry. The advantage of using a laser system over IRMS is elimination of the extensive N_2O gas purification requirements by gas chromatography and the ^{17}O corrections needed for $\delta^{15}N$ values.[17]

The direct-conversion denitrifier and Cd-azide approaches for producing N_2O for stable isotopic analyses from dissolved nitrate are both well-established and successful methods, but each has advantages and disadvantages.[18] The primary disadvantages of the microbial denitrifier method, particularly for laboratories without microbiology support, are the need to obtain and maintain anaerobic nitrate-reducing microbial cultures and ensure sterility for all equipment (autoclaving), and the inability of the microbial method to discriminate between dissolved nitrate and nitrite species.[18] As a geochemical approach, the Cd-azide method can easily be set up in new laboratories, and conversion reaction steps are controlled and can separate potential nitrite interferences from nitrate. The sole disadvantage of the Cd-azide approach lies in the toxicity of the reagents used and the need for strict health and safety controls. Here we propose a new application of the Cd-azide method[16] adapted for headspace sampling and injection by N_2O laser spectrometry for triple isotopic ($\delta^{15}N$, $\delta^{18}O$, $\delta^{17}O$) analyses of aqueous NO_3^- and $\delta^{15}N$ analyses of aqueous NO_2^-. We present first results for $\delta^{17}O$ values (and the ^{17}O excess) of dissolved NO_3^- using the N_2O laser-based method, as well as improved laser isotopic data correction procedures that may be applied to N_2O isotopic analyses conducted by both the Cd-azide and the bacterial denitrifier method using off-axis integrated cavity spectrometry (OA-ICOS) laser instruments.

2 | EXPERIMENTAL

2.1 | Instrumentation and modifications

For laser-based isotopic analyses of N_2O samples, we used a N_2O triple isotope analyzer (N_2OIA-23e-EP Model 914-0060; Los Gatos Research, Mountain View, CA, USA) fitted with a septum injection port (Figure 1). The instrument measures N_2O concentrations (0.3–20 ppm), and $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N_{bulk}$, $\delta^{17}O$, $\delta^{18}O$ and H_2O values in air to precisions of 0.03 ppb for N_2O, less than ±1‰ (SEM) for N and less than ±2‰ (SEM) for O isotopes over 300 s of measurement integration. The instrument operates in one of three modes: continuous flowing atmospheric air for large-volume gas samples (at the above specifications), discrete gas sample injections (>60 mL of <10 ppm N_2O) or discrete injection–dilution (<60 mL samples of >3 ppm N_2O). The discrete injections are achieved using a syringe and the injection port (no specifications given by the manufacturer). The laser cavity sample dilution and flush gas is research-grade N_2O-free "Zero Air" (Linde, Munich, Germany), regulated at the inlet port to 15 psi (Figure 1). For instrument initialization and tuning, calibration (either by injection or flowing) and stability testing, we used research-grade 10 ppm N_2O in Zero Air, connected to the flowing gas inlet port (Figure 1). Discrete syringe injections of this 10 ppm gas were

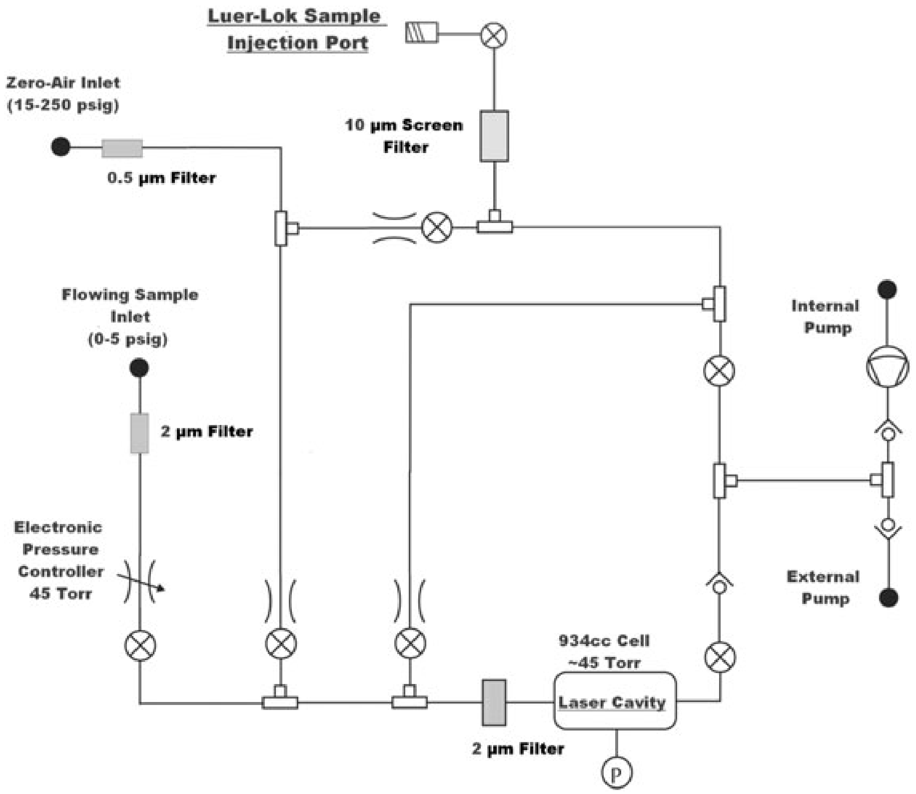

FIGURE 1 Schematic of the Los Gatos Research N_2O triple-isotope analyzer. The only hardware modification was switching the syringe septum port for a Luer Lock sample injection port and isolation valve in order to affix gastight sample syringes to the inlet. See text for details

conducted from 5-L Tedlar® gas sampling bags. For this study, we used the injection–dilution mode (by manual injection) and 300 s integrations, as this mode accommodated a far wider range of sample N_2O concentrations than the other modes. For our targeted concentration (10 ppm N_2O in air), the standard error for injection dilution at a 1 Hz sampling rate (n = 300 s) for N_2O, $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N_{bulk}$, $\delta^{17}O$, $\delta^{18}O$ and H_2O was typically ±0.002 ppm, ±0.07‰, ±0.07‰, ±0.06‰, ±0.3‰, ±0.07‰ and ±0.7 ppm, respectively. The only hardware and software modifications made to the instrument were replacing the 6 mm septum injection port with a stainless steel Luer Lock fitting and a toggle valve to enable gastight sample syringes to be fastened directly to the inlet port (Figure 1), and by removal of default spectral corrections for potential interfering atmospheric trace gases (CO_2, CH_4 and CO) which are present in atmospheric air samples, but not in our samples. The software modification was made by minor changes in several instrument configuration INI files (Los Gatos Research, personal communication). A typical spectral transmittance trace from the laser instrument for N_2O produced from NO_3^- by the Cd-azide method is shown in Figure 2, revealing the relative isotopic abundances (concentrations) and absorbance of the N_2O isotopologues (and H_2O) for these samples, as described in further detail below.

We also used a dual-inlet Isoprime 100 trace gas (TG) continuous-flow isotope ratio mass spectrometry (CF-IRMS) system (Isoprime Ltd, Cheadle Hulme, UK) to conduct a comparative evaluation of our laser-based N_2O $\delta^{15}N$ and $\delta^{18}O$ results with subsampled aliquots of the same N_2O samples used for the laser, injected into pre-evacuated 12-mL Exetainers™ (Labco Ltd, Lampeter, UK). The CF-IRMS automated preparation system utilized a model GX-271 autosampler (Gilson Inc., Middleton, WI, USA) with a dual-core needle connected to the CF-IRMS instrument by a purge-and-trap GC system that cryo-focused N_2O from the 12-mL mixed gas samples. The N_2O pulse from the TG system to the CF-IRMS instrument was compared with a pure N_2O (uncalibrated) 50 s reference gas pulse provided from the dual-inlet bellows. The Isoprime CF-IRMS system operates at an external analytical precision of ±0.2‰ ($\delta^{15}N$ N_2O values) and ±0.4‰ ($\delta^{18}O$ N_2O values); the method that we used is fully described elsewhere[19] and provides a detection limit of ≤0.4 ppm N_2O. An ^{17}O isobaric interference correction for the $\delta^{15}N$ value of N_2O was applied for CF-IRMS assays, as described below.

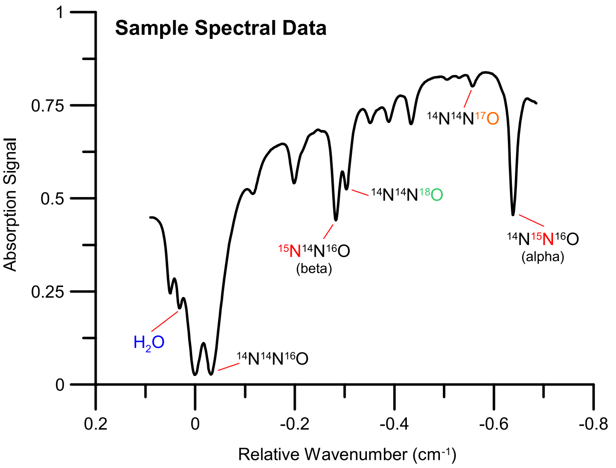

FIGURE 2 Typical spectral isotopologue absorbance signals versus relative wavenumber for a Cd-azide processed $NO_3^- \rightarrow N_2O$ headspace sample (*ca* 10 ppm N_2O in air was injected) showing the relative positions and concentrations of the N_2O isotopologues and H_2O vapor. The most abundant N_2O isotopologue is $^{14}N^{14}N^{16}O$ (>99%) [Color figure can be viewed at wileyonlinelibrary.com]

2.2 | N_2O laser optimization

To further optimize the N_2O laser instrumental performance for the manual injection–dilution mode in routine operation, we empirically predetermined the optimal target sample N_2O concentration that produced the most stable isotopic ratios ($\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{15}N_{total}$, $\delta^{17}O$ and $\delta^{18}O$ values) and the N_2O concentrations at the highest possible precision. We used 100 ppm N_2O gas (mixed using pure N_2O and Zero Air in 5- or 10-L Tedlar® gas sampling bags) to perform a range of injections covering a N_2O concentration range of 1–20 ppm[17] by adjusting the injection–dilution ratios (injecting 1–11 mL of 10 ppm N_2O). Before each isotopic measurement, the laser instrument was pre-evacuated to <1 Torr. Following manual sample gas injection, the laser cavity was automatically filled to an operational target pressure of 45.00 Torr by slowly admitting N_2O-free air (diluent) from the carrier gas cylinder supply. A 20-mL plastic gastight syringe (Beckton-Dickinson, Franklin Lakes, NJ, USA) with Luer Lock fittings was used for all sample gas injections.

Because the Cd-azide headspace samples contained only N_2O gas and H_2O vapor in an air atmosphere (N_2, O_2) and no other spectral interfering gases, we disabled the default suite of instrumental trace gas (CO_2, CO, CH_4) corrections that disentangle spectral line overlap, that would normally be required for atmospheric isotopic measurements of N_2O in more complex gas atmospheres, such as soil, air or biogenic gas samples.[20]

2.3 | Standard calibration and test samples

Currently, no N_2O isotopic reference materials are available that would be practical for calibrating laser instruments by discrete gas injection[21] (https://isotopes.usgs.gov/lab/referencematerials/USGS51-USGS52.pdf). Instead, we used the identical treatment (IT) approach[22] whereby the $\delta^{15}N$ or $\delta^{18}O$ and $\delta^{17}O$ values of N_2O for calibration standards were derived from nitrate or nitrite salts that underwent sample preparation procedures identical to those of N_2O as samples. The IT approach is especially critical for N_2O laser spectrometry because both the sample and the calibration N_2O carrier gas compositions need to be ensured "matrix equivalent" in order to avoid confounding effects of spectral broadening by unequal proportions of the key (N_2, O_2) and interfering species like H_2O, CO_2, CO or CH_4 affecting the accurate determination of N_2O isotopologue concentrations.[20,21] For testing, we prepared nitrate isotopic reference materials (IAEA, Vienna, Austria; USGS, Reston, VA, USA) as well as laboratory standards from the co-authors. For nitrate primary reference materials we used the nitrogenous salts USGS34, USGS35 and IAEA-N3, having assigned $\delta^{15}N_{AIR}$ values (±SD) of −1.8 (±0.1), +2.7 (±0.1) and +4.72 (±0.13), $\delta^{18}O_{VSMOW}$ values of −27.78 (±0.37), +56.81 (±0.31) and +25.32 (±0.29), and $\delta^{17}O_{VSMOW}$ values of −14.8, +51.5 and +13.2, respectively.[23,24] For nitrite, we used University of Massachusetts

Dartmouth (New Bedford, MA, USA) MAA2 and Zh1 NO_2^- laboratory standards, having assigned $\delta^{15}N_{AIR}$ values of +3.9 (±0.3) and −16.4 (±0.3), respectively. Finally, we used research-grade $NaNO_3$ salt denoted IHL-2 (Sigma Aldrich, Vienna, Austria) with unknown N and O stable isotopic composition for use as an internal laboratory standard. All the nitrogenous salts were predissolved in ultrapure deionized water to produce stock solutions of 1 ppm as N and nitrate solutions were preserved with 1 mL of 2.5 mM sulfanilic acid in 10% HCl, and stored refrigerated at 5°C in 500-mL Pyrex bottles.

Finally, to gain a clearer understanding of position-specific routing of sample and reagent N to the α or β positions in the azide reduction step to $^{\beta}N^{\alpha}NO$, and to evaluate possible effects on routine laser analyses of nitrate and nitrate isotopes in natural abundance or tracer studies, we conducted two experiments using highly ^{15}N-enriched sodium azide ($Na^{15}N_3$; >98%, Cambridge Isotope Laboratories, Tewksbury, MA, USA; P/N NLM-2198-PK) and ^{15}N-enriched sodium nitrite ($Na^{15}NO_2$; >98%, Cambridge Isotope Laboratories; P/N NLM-658-1). In the first test, we used our IAEA KNO_3 1 ppm reference solution (as above), and followed the Cd-azide procedure below, but reacted the intermediate NO_2^- sample solution with ^{15}N-enriched azide spiked in the following proportions: 0.06, 0.13, 0.25, 0.5 and 1.5% $Na^{15}N_3$. In the second test, we reversed the spike, using normal NaN_3 for the conversion of NO_2 to N_2O, but using a 1 ppm $NaNO_2$ as N solution gravimetrically ^{15}N-enriched to 0.5, 1.0, 2.0 and 3% (wt% $^{15}NO_2$ enrichment). These ^{15}N-enriched samples were analyzed by laser spectrometry and not by IRMS.

2.4 | Sample preparation for Cd-azide reduction to N_2O

Sample preparation and conversion of nitrate and nitrite to N_2O gas for both laser spectrometry and IRMS followed the Cd-azide method[16,25,26] with minor modifications. For nitrate samples (NO_2- and NH_4-free), 100- to 250-mL field samples were filtered through 0.45-μM pre-combusted GF/F filters and preserved either by (i) freezing or (ii) acidification to pH 2–3 by adding 1 mL of HCl plus sulfanilic acid per 100 mL of sample and thereafter stored at 5°C or frozen (−20°C). Filtered 50-mL subsamples and all our test samples were subjected to NO_3^- and NO_2^- concentration measurements using an AQ1 Discrete Analyzer (Seal Analytical, Southampton, UK).

Quantitative conversion of aqueous NO_3^- or NO_2^- samples to N_2O for N and O stable isotopic analyses followed a two-day preparative routine. A typical sample batch for laser spectrometry consisted of 2 blanks (one each for nitrate/nitrite), triplicates of calibration standards and 10–20 samples. In this paper, we focus primarily on reference and laboratory intercalibration standards with known N and/or O isotopic compositions for demonstration purposes. Results for field samples obtained by laser spectrometry will be presented elsewhere.

2.5 | Cadmium reduction of NO_3^- to NO_2^-

Prior to the conversion of dissolved NO_3^- to NO_2^-, the nitrate or nitrite samples and the calibration standards were subsampled and aliquoted to achieve a target concentration of 0.25 ppm as N by dilution with ultrapure deionized water and 13.5 mL of 6 M NaCl, to make up a final sample volume of 40 mL (10 mL of 1 ppm N, 13.5 mL of 6 M NaCl, 16.5 mL of deionized water). These 40-mL samples were pipetted into 50-mL tapered-bottom plastic centrifuge tubes. Nitrate-/nitrite-free blanks were also prepared. To each nitrate and blank sample tube, we added 100 mg of MgO (Sigma-Aldrich no. 342793; pre-combusted for 4 h at 450°C) and 1 g of cadmium powder (99.5%, Alfa Aesar no. 11891; Ward Hill, MA, USA) to convert nitrate to nitrite. The Cd powder used had been pretreated with 10% HCl to form “spongy Cd,” and thoroughly washed with deionized water to neutral pH. The centrifuge tubes were tightly capped, and laid horizontally on a lateral shaker bench (IKA MTS-4; IKA, Staufen im Breisgau, Germany) with a custom-made cover to keep samples in darkness; samples were gently shaken and allowed to react overnight.

2.6 | Azide conversion of NO_2^- to N_2O

On the following day, the converted nitrate samples and blanks were removed from the shaker and placed in a high-speed centrifuge (Eppendorf 5810; Eppendorf, Hamburg, Germany) for 25 min at 2500 rpm to separate Cd and MgO from aqueous NO_2^-. The samples were pipetted into a precombusted 125-mL Wheaton glass serum bottle (Sigma Aldrich, Z114014) and crimp-sealed with acid-washed thick (14 mm) butyl blue septum (Bellco Glassware, Vineland, NJ, USA; 2048-11800). Each sample bottle was degassed to remove dissolved gases and air headspace by using a rotary vacuum pump to <0.001 atm for *ca* 2–3 min via a 21G needle though the septum.

The final conversion step of NO_2^- to N_2O for isotopic analyses (or initial step in the case of nitrite samples) was completed by the addition of NaN_3 (BioUltra, ≥99.5% (T) Sigma Aldrich no. 71289) added to the sample. Beforehand, a sodium azide/acetic acid buffer solution was prepared (1:1 equal parts 20% acetic acid and 2 M NaN_3, purged of dissolved gases and/or N_2O produced from residual nitrite in the reagents by sparging the reagent with pure N_2 gas (*ca* 50 mL min^{-1} for 10 min) prior to injection into each sample bottle). Using a 2.5-mL plastic gastight syringe and a new 21G needle, a 2-mL aliquot of the azide–acetic acid solution was gently injected through the butyl septum into the pre-evacuated 125-mL sample bottle. The sample was mixed by swirling for 10–15 s, and then allowed to stand for 30 min to finalize the azide conversion of NO_2 to N_2O gas. The reaction was halted by gently injecting 2 mL of 6 M NaOH through the septum, again using a syringe and a 21G needle, followed by gentle hand agitation for 5–10 s. At this point, the N_2O gas samples in these sealed serum bottles could be stored for longer periods of time (weeks) for later headspace extraction and N_2O isotopic analyses. We note that Cd and NaN_3 reagents are toxic; hence, all appropriate health and safety precautions and waste disposal procedures were carefully observed.

2.7 | Laser N_2O isotope spectrometry

Prior to laser stable isotopic analyses of N_2O headspace samples from the serum bottles, the instrument was warmed up and preconditioned by a flow-through calibration using 10 ppm N_2O/air gas cylinder mix, as recommended by the manufacturer. Once the analyzer achieved N_2O stability (*ca* 1 h), it was switched from flowing gas mode to batch injection–dilution mode. Before measuring N_2O gas samples, a further check involved the manual injection (2–3 times) of 5 mL of 100 ppm N_2O obtained from the Tedlar® gas bag using a 20-mL plastic gastight syringe. The N_2O concentration on the laser from this sample was our target of *ca* 10 ppm, and this step was used as a preliminary verification before running any samples.

Immediately prior to sample N_2O isotope analysis, a 140-mL plastic gastight syringe with a 21G needle and a three-way valve was used to overpressure each pre-evacuated N_2O sample bottle with 140 mL of N_2O-free Zero Air, which resulted in a headspace overpressure by *ca* 50 mL. The side port of a three-way Luer Lock valve was attached to a 1/8 inch Tygon® tube to dynamically flush the needle with N_2O-free Zero Air (100 mL min^{-1}) to remove room air from the syringe. The three-way valve was then positioned to enable flushing of the needle, and while flushing, gently inserted mid-point (*ca* 7 mm) into the butyl septum of the sampling bottle, and the three-way valve was closed to the carrier. The three-way valve was then positioned through to the syringe and sample bottle, and the needle was pushed through the septum into the sample bottle and the air injected fully. There were several benefits to the overpressure approach: (i) the initial draw by sample bottle vacuum (*ca* 75 mL) immediately revealed if the bottle had leaked; (ii) we empirically determined that *ca* 8 mL of the overpressured headspace achieved our 10 ppm N_2O target; hence, the excess headspace pressure allowed us flexibility to increase or reduce the sample draw to adjust the amount of N_2O if sample concentrations were unexpectedly low or high (usually stemming from incorrect provided NO_3^- or NO_2^- concentration data); (iii) for our 10 ppm target concentration it provided sufficient N_2O for 2–3 repeat injections (for determining concentration coefficients, below); (iv) the overpressure added dry air as a diluent which further reduced unwanted H_2O by 60% reducing the impact of the H_2O spectral line overlap for $^{14}N^{14}N^{16}O$; and (v) improved spectral fits by ensuring that all sample gas compositions were as "matrix equivalent" as possible (Figure 1). We also note that for demonstration purposes we intentionally processed large NO_3^- and NO_2^- samples in order to facilitate repeated laser analyses and IRMS subsampling of the same N_2O gas from the same sample bottle; for routine operations where our degree of replication is not warranted, the sample size and reagents used can be scaled down by a factor of 3 or more to as low as *ca* 3 μM nitrate or nitrite as N.

To withdraw N_2O from the sample vials for N and O isotopic analysis, we used a 20-mL plastic gastight syringe fitted with a two-way valve and a new 21G needle. After insertion, 2–3 gentle plunger strokes mixed the sample headspace gas into the syringe barrel. The overpressure of the sample bottle pushed the syringe plunger out; hence it was manually restricted to a sample volume of 3–4 mL (depending on initial pressure), to achieve a final, pressure-relaxed, target of 8 mL in the syringe (at STP) when the two-way valve was closed. The syringe was then removed from the bottle, the needle removed and the syringe was fitted to the Luer Lock injection port on the laser instrument (Figure 1). In the same way, smaller 10-mL headspace subsamples were taken from the same reaction vials and injected into pre-evacuated 10-mL Exetainers for comparative analyses by IRMS.

2.8 | N_2O laser isotopic measurements

After the sample syringe was fastened to the Luer Lock inlet port of the N_2O laser instrument, N_2O isotopic analysis was initiated in the instrument software using the batch injection–dilution mode, as described in the instrument user manual. Briefly, the 937-mL laser cavity was flushed several times with N_2O-free Zero Air carrier and then evacuated to <1 Torr. After final evacuation, the valve to the syringe was opened and the sample was expanded by vacuum into the laser cavity. The instrument then "diluted" the sample by admitting pulses of N_2O-free Zero Air to achieve a final operational target pressure of 45.0 Torr. Once pressure and temperature stability was achieved, isotopic analysis proceeded and consisted of 300 s of 1 Hz integration of the N_2O isotopologues (and other gases) (Figure 2). After 300 s, the data were written to a CSV file, and the instrument was evacuated and readied for the next sample. The sample injection and analysis time for each N_2O triple isotopic analysis was approximately 12 min. After all the N_2O samples in the entire batch had been analyzed, the raw CSV files were parsed using a custom R-script into a single Excel table that contained all the key summary data (all N_2O isotopologue concentrations, H_2O, cavity temperature and pressure) for further postprocessing (see Section 3). Uncorrected δ values were obtained by calculating the ratios of each relevant isotopologue concentration (Figure 3) as recorded by the instrument in ppm:

$$\delta^{15}N^{\alpha} = \left[^{14}N^{15}N^{16}O\right]\text{ppm}/\left(\left[^{14}N^{14}N^{16}O\right]\text{ppm}/0.003676{-}1\right)\times 1000$$

$$\delta^{15}N^{\beta} = \left[^{15}N^{14}N^{16}O\right]\text{ppm}/\left(\left[^{14}N^{14}N^{16}O\right]\text{ppm}/0.003676{-}1\right)\times 1000$$

$$\delta^{15}N_{\text{bulk}} = \left(\delta^{15}N^{\alpha} + \delta^{15}N^{\beta}\right)/2$$

$$\delta^{18}O = \left[^{14}N^{14}N^{18}O\right]\text{ppm}/\left(\left[^{14}N^{14}N^{16}O\right]\text{ppm}/0.0020004{-}1\right)\times 1000$$

$$\delta^{17}O = \left[^{14}N^{14}N^{17}O\right]\text{ppm}/\left(\left[^{14}N^{14}N^{16}O\right]\text{ppm}/0.0003799{-}1\right)\times 1000$$

No ^{17}O correction was applied to the $\delta^{15}N$ results because the laser measures ^{15}N concentration directly without any isobaric interference (^{15}N-, ^{18}O- and ^{17}O-containing isotopologues all have distinct absorption peaks; Figure 2). For TG-IRMS, however, $\delta^{15}N$ measurements using N_2O required an empirical correction for the ^{17}O contribution to mass 45 (Equation 2),[16] based on the measured sample $\delta^{18}O$ value and assuming mass-dependent isotope fractionation coefficient (λ) of 0.52 (but see below).[12,16] Normalization of the data to the AIR and VSMOW scales is discussed below.

As reported previously,[17] N_2O triple isotopic determinations by laser analyses cannot yet be easily automated due to extensive chemical sample processing and because automated headspace sampling devices currently do not exist for laser analyzers. Combined with the two-day routine for the Cd-azide preparation steps for nitrate, approximately 15 unknown NO_3^- samples plus standards and blanks per day could be routinely processed by laser spectrometry. Alternatively, the Cd-azide method can be halted at various steps, and the N_2O gas samples from the azide reaction can be stored in the septum sealed bottles for many weeks if accumulated sample batches for isotope analyses are required.

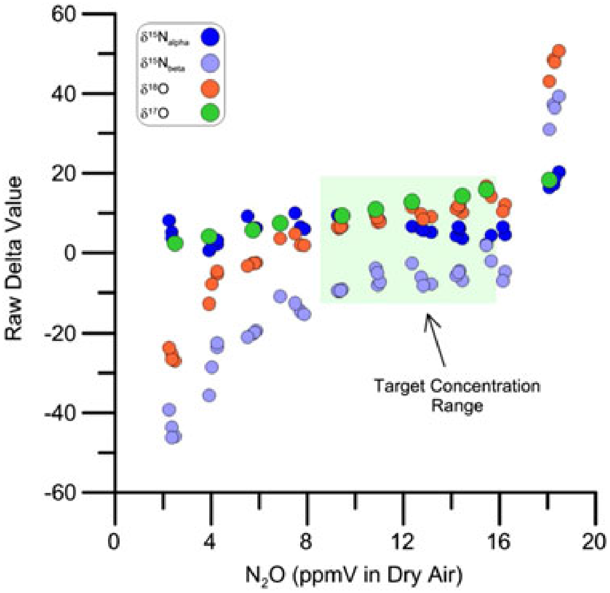

FIGURE 3 Dependence of $\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$, $\delta^{17}O$ instrumental δ values on sample N_2O concentration (ppmV) using injection–dilution mode. The shaded area was our empirically determined target N_2O concentration (10 ppm target; range 8–14 ppm). It was not possible to measure samples >16 ppm N_2O in injection–dilution mode. For samples with higher N_2O concentrations, additional dilution with dry air was required to ensure an appropriate target concentration range [Color figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS AND DISCUSSION

3.1 | Postprocessing corrections

Results of uncorrected $\delta^{15}N^{\beta,\alpha}$, $\delta^{18}O$ and $\delta^{17}O$ measurements of N_2O and their dependency on N_2O concentrations over an instrumental operational range of 0.3–20 ppm N_2O by manual injection–dilution mode are plotted in Figure 3. These data revealed an often nonlinear dependency of each δ value on the associated N_2O concentration, spanning 100‰ over the instrumental operational range. Some δ values ($\delta^{15}N^{\alpha}$, $\delta^{17}O$) showed lower N_2O concentration dependencies than others ($\delta^{18}O$; Figure 3). For the laser instrument used, there was a clear cutoff around 18 ppm N_2O, above which reliable isotopic results were unattainable, revealing a practical upper limit for injection–dilution mode. As noted, we focused our efforts to ensure that the sample concentrations fell within the stable N_2O region between 8 and 16 ppm N_2O (shaded region, Figure 1).

The results of one single representative "batch" of laboratory standards and samples are summarized in Table 1 along with the raw data and corrected isotopic data and other key variables to demonstrate a data post-processing strategy. The uncorrected δ values for each sample in Table 1 do not look very promising, with considerable and unacceptable isotopic variance in the δ values for all isotopologues for both nitrate and nitrite standards, and large differences observed between the $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values. Factor analysis of the measured data revealed that most (*ca* 70%) of the isotopologue variance was strongly, positively or negatively, correlated with three key covariates: (i) the laser cavity pressure (46%, positively), (ii) N_2O concentrations (20%, negatively) and (iii) in some cases H_2O concentration (<4%, negatively).

In order to correct the raw δ values for multivariate isotopic dependencies, we derived a generalized correction algorithm to adjust the raw isotopic data ($\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, $\delta^{18}O$, $\delta^{17}O$ values) for each of the four isotopologues for each analysis:

$$\delta_{Corr} = \delta_{Raw} - [A(P_{meas} - P_{target}) - B(N_{meas} - N_{target}) - C(W_{meas} - W_{median})] \quad (1)$$

where δ_{Raw} is the uncorrected δ value obtained from the instrument, P_{meas} is the measured cavity pressure for each sample, P_{target} ias the target cavity pressure (45.00 Torr), N_{meas} is the measured N_2O concentration for each sample, N_{target} is our target concentration (10.0 ppm), W_{meas} is the measured H_2O concentration of the sample in ppm and W_{median} is the median value of H_2O content in ppm for each daily sample batch (here approx. 3300 ppm; see Table 1). Variables *A*, *B* and *C* are the pressure, concentration and water correction coefficients, which must be determined empirically for each laser instrument and/or each batch of samples.

To determine the *A*, *B* and *C* coefficients, we used the generalized reduced gradient (GRG) algorithm in Excel Solver™ to solve and optimize for each of these coefficients. The solver objective was set to minimize the combined standard uncertainty of all the standards in each batch and to find an optimal solution by iteratively varying each of the three coefficients. To obtain a unique solution, the GRG algorithm required three (preferably more) instances of at least two standards in each batch, with at least one of the standards purposely varied in its N_2O concentrations between 8 and 16 ppm (typically via the user varying the amount of N_2O injected as described above – see USGS34 in Table 1). The solver results of the optimized coefficients were then applied to Equation 1 to obtain "corrected" raw δ values, as summarized in Table 1. The empirical range for each of the correction coefficients over a six-month testing period is summarized in Table 2 for each isotope, and these coefficients were found to be relatively stable and of similar magnitude over time. After this empirical correction, the corrected raw δ value data (Table 1) dramatically improved, and acceptable precisions were obtained for each isotopologue for each isotopic standard. For example, the SD for the $\delta^{15}N^{\alpha}$ value for USGS34 (nitrate) improved from ±9.4‰ for the raw δ results to ±0.4‰ for the corrected results. The SD of the $\delta^{18}O$ value for USGS35 improved from ±9.9‰ for the uncorrected δ results to ±1.2‰ for the corrected results. Improved correction outcomes are apparent overall in Table 1 for all other measured N_2O isotopologues of the laboratory standards.

3.2 | Normalization of the N_2O isotopologue results to AIR and VSMOW

To normalize the $\delta^{15}N$ values in Table 1 to the AIR reference, we found that acceptable results could only be obtained by using a least squares regression of the $\delta^{15}N^{\alpha}$ value versus the known $\delta^{15}N$ value of all the standards (r^2 = 0.998). Interestingly, there was no correlation (r^2 = 0.01) between the $\delta^{15}N^{\beta}$ and the known $\delta^{15}N$ values for any of the N isotopic reference samples, and the measured $\delta^{15}N^{\beta}$ values had a

TABLE 1 Results for a set of potassium nitrate, sodium nitrate and sodium nitrite standards and isotope analyses by Cd-azide reduction and N_2O laser spectrometry (January 25, 2017), with raw δ data, and with pressure, N_2O and H_2O corrections applied. SP (site preference) = $\delta^{15}N^{\alpha} - \delta^{15}N^{\beta}$. The $\delta^{15}N_t$ (bulk) = $(\delta^{15}N^{\alpha} + \delta^{15}N^{\beta})/2$. Corrected data (shaded) were obtained from Equation 1 and coefficients in Table 2. Here, USGS34 had its concentration intentionally varied during N_2O sample injection. The target N_2O concentration was 10.0 ppm. Normalized results are least squares regression median values ± standard error, using the $\delta^{15}N^{\alpha}$ value to derive the sample $\delta^{15}N_{AIR}$ value. Italic values in parentheses are the known reference values for comparison, where known

#	Sample	N_2O (ppm)	$\delta^{15}N^{\alpha}$	$\delta^{15}N^{\beta}$	$\delta^{15}N_t$	$\delta^{18}O$	$\delta^{17}O$	*P* (Torr)	H_2O (ppm)	$\delta^{15}N^{\alpha}$	$\delta^{15}N^{\beta}$	$\delta^{15}N_t$	$\delta^{18}O$	$\delta^{17}O$	SP	$\delta^{15}N^{\alpha}_{AIR}$	$\delta^{18}O_{VSMOW}$	$\delta^{17}O_{VSMOW}$
1	**Blank**	**0.4**	**5.8**	**−74.3**	**−34.3**	**−70.2**	**−106.6**	**45.55**	**2899**									
		Raw data								Conc. *P* and H_2O corrected data						Normalized ± SE vs (*known*)		
	KNO_3																	
2	IAEA-NO3	9.7	3.1	−10.1	−3.5	16.6	4.7	45.06	3396	5.2	−7.1	−0.9	19.2	8.8	12.3	**+4.7±0.1**	**+25.9±0.4**	**+12.5±1.0**
3	IAEA-NO3	9.2	6.1	−6.6	−0.3	23.6	12.2	45.32	4341	5.7	−6.5	−0.4	20.6	11.2	12.2	*(+4.7±0.1)*	*(+25.7±0.2)*	*(+13.2)*
4	IAEA-NO3	9.9	−2.0	−17.5	−9.7	11.5	−4.1	44.60	3406	6.0	−6.7	−0.4	21.0	11.2	12.7			
5	USGS34	9.6	11.5	12.3	11.9	−6.8	11.1	46.15	3207	−0.6	−4.1	−2.4	−20.7	−12.0	3.5	**−1.8±0.1**	**−26.8±0.8**	**−14.5±0.9**
6	USGS34	9.2	−5.5	−11.3	−8.4	−26.1	−18.8	44.88	3600	−0.8	−4.1	−2.4	−20.8	−9.8	3.3	*(−1.8±0.1)*	*(−27.9±0.3)*	*(−14.8)*
7	USGS34	8.2	1.4	−3.5	−1.1	−18.9	−4.6	45.46	3196	−1.6	−5.7	−3.6	−21.4	−10.1	4.1			
8	USGS34	10.8	7.1	9.7	8.4	−9.7	1.6	45.89	3718	−1.3	−3.4	−2.3	−21.2	−14.9	2.1			
9	USGS34	12.9	−8.1	−10.1	−9.1	−27.4	−23.5	44.73	4141	−1.6	−5.4	−3.5	−23.1	−11.7	3.8			
10	USGS34	10.5	−0.1	−2.8	−1.5	−21.5	−9.8	45.28	3540	−0.8	−4.7	−2.8	−23.1	−11.3	3.9			
11	USGS34	10.0	18.3	22.5	20.4	−2.1	27.1	46.71	3286	−0.9	−4.3	−2.6	−25.0	−9.8	3.4			
	$NaNO_3$																	
12	IHL2	9.8	−2.8	−6.8	−4.8	16.0	7.4	45.21	3179	−2.8	−6.8	−4.8	16.7	7.6	4.0	**−4.6±0.1**	**+21.0±0.1**	**+8.9±0.3**
13	IHL2	9.4	18.6	21.9	20.2	42.7	48.7	46.91	3295	−3.1	−7.5	−5.3	17.0	7.0	4.4	-	-	-
14	IHL2	9.7	−5.8	−11.0	−8.4	13.2	2.2	45.00	3364	−2.9	−6.9	−4.9	16.7	7.7	4.0			
15	USGS35	9.2	1.7	−12.2	−5.3	39.5	33.6	45.05	3331	4.0	−8.3	−2.2	42.7	38.1	12.3	**+2.6±0.1**	**+57.6±1.3**	**+51.4±2.5**
16	USGS35	9.2	−1.5	−17.0	−9.2	38.6	31.1	44.78	3360	4.3	−8.3	−2.0	45.9	42.3	12.6	*(+2.7±0.1)*	*(+57.5±0.3)*	*(+51.5)*
17	USGS35	9.9	4.9	−7.9	−1.5	49.0	45.7	45.24	3387	4.7	−8.5	−1.9	48.6	45.2	13.2			
	$NaNO_2$																	
18	MAA2	9.3	7.2	−1.8	2.7	−3.6	0.6	45.49	3219	3.7	−6.1	−1.2	−7.1	−6.0	9.8	**+4.0±0.1**	**−8.9±0.2**	**−7.0±0.7**
19	MAA2	9.2	7.1	−2.8	2.1	−2.1	0.3	45.51	4130	4.0	−6.3	−1.2	−7.7	−5.9	10.4	*(+3.9±0.3)*	-	-
20	MAA2	9.8	13.0	6.5	9.7	4.0	12.9	45.92	3381	4.0	−5.8	−0.9	−6.7	−4.2	9.8			
21	Zh1	10.3	−15.7	−6.3	−11.0	−0.2	−0.9	45.15	3389	−14.8	−5.9	−10.4	0.6	0.7	−8.9	**−16.5±0.1**	**+0.7±0.1**	**+0.10±0.4**
22	Zh1	10.2	−21.3	−13.6	−17.5	−6.2	−11.8	44.76	3589	−15.3	−5.8	−10.5	0.4	−0.2	−9.4	*(−16.4±0.3)*	-	-
23	Zh1	10.4	−18.3	−9.0	−13.7	−2.6	−4.8	44.99	3431	−15.4	−5.8	−10.6	0.6	0.8	−9.6			

TABLE 2 Pressure, N_2O and H_2O correction coefficients (boldface) used for Equation 1 for each of the N_2O isotope ratios to obtain the corrected raw δ values in Table 1. The ranges of these coefficients were observed over a 6-month period. The $\delta^{15}N_{total}$ value may be substituted for the $\delta^{15}N^{\alpha}$ or $\delta^{15}N^{\beta}$ value when using the denitrifier method. These correction coefficients were stable over 6 months; however, they are determined on a batch basis for each instrument

Isotope ratio	*A* (pressure); range	*B* (N_2O); range	*C* (H_2O); range
$\delta^{15}N^{\alpha}$	**12.85**; 12.78–13.11	**−0.13**; -0.61 to 0.26	−0.002 to 0.001
$\delta^{15}N^{\beta}$	**17.67**; 16.28–18.46	**−1.51**; −1.31 to −2.75	0.001 to 0.002
$\delta^{18}O$	**15.43**; 14.51–16.94	**−0.57**; 0.40 to −0.57	−0.002 to −0.006
$\delta^{17}O$	**24.70**; 22.81–29.49	**−0.37**; −6.33 to 3.9	0.023 to −0.008

consistent, single-point, normalized value of −3.1 ± 0.3‰ (versus the offline-measured $\delta^{15}N_{AIR}$ value of −2.9 ± 0.3‰ of the azide used in our sample preparation). These results were not entirely surprising since early experimental isotope studies[27] showed that N in NO_2 during the azide reaction is routed to the terminal α ($N^{15}NO$) position, whereas the N from the azide is routed to the β position (^{15}NNO) (see tracer test below). As noted previously,[16] N_2O bulk samples prepared via the Cd-azide reaction obtain 50% of their N from NO_2 (sample of interest) and 50% from the sodium azide (NaN_3) reagent (fixed $\delta^{15}N$ value of −2.9‰); hence, regression of the measured $\delta^{15}N_{bulk}$ versus the known δ value by IRMS yields a slope of *ca* 0.5 since IRMS cannot distinguish position-specific features as the isotopologues $^{15}N^{14}N^{16}O$ and $^{14}N^{15}N^{16}O$ have the same mass. The N_2O laser isotopologue data verified that N from sample NO_2 is routed predominantly to the α position of NNO, as revealed by an overall slope of 0.998 ± 0.04 on a regression of the $\delta^{15}N^{\alpha}$ value versus the known δ value for all N standards. Accordingly, and akin to IRMS, laser $\delta^{15}N_{bulk}$ analysis yielded a slope of 0.463 given that *ca* 50% of the N was from isotopically uniform azide. Taken altogether, the data suggest that nitrate and nitrite samples should be analyzed and calibrated with similar isotopic standards (nitrate versus nitrite). Following postprocessing data corrections, the and sample $\delta^{15}N^{\alpha}$, $\delta^{18}O$ and $\delta^{17}O$ data were further data normalized to obtain final δ values related to the appropriate isotopic reference scale (AIR, VSMOW). This single batch run in Table 1 yielded accurate results with acceptable uncertainties for $\delta^{15}N^{\alpha}$ (*ca* ±0.1‰), $\delta^{18}O$ (*ca* ±0.5) and $\delta^{17}O$ (±1.0). These results and uncertainties were similar to $\delta^{15}N$ and $\delta^{18}O$ analyses conducted using the denitrifier method by laser,[17] and comparable to other well-performing IRMS nitrate assays such as $AgNO_3$ by TC/EA, or the denitrifier method by CF-IRMS.

The batch of laser-processed N_2O samples (Table 1) was subsampled into Exetainers and re-measured by TG-IRMS; the normalized $\delta^{15}N_{AIR}$ and $\delta^{18}O_{VSMOW}$ results of this comparative test are summarized in Figure 4, and show excellent agreement in final outcomes for $\delta^{15}N$ (slope = 0.992, r^2 = 0.994) and $\delta^{18}O$ (slope = 1.000, r^2 = 0.999). A longer-term summary of excellent N and O results and performance of laser analyses of nitrate and nitrite laboratory standards and samples over a several month period is found in Table 3.

3.3 | ^{17}O anomalies and excess

Of interest is the capability of laser spectrometry to measure ^{17}O concentrations (and excess) of N_2O directly, given the strong interest in mass-independent O isotope fractionations of atmospheric NOX species. Atmospheric processing can lead to $\delta^{17}O$ anomalies in atmospheric nitrate and generally highly positive $\delta^{18}O$ values that can be used to help better distinguish environmental nitrate sources (atmospheric- versus nutrient-derived nitrate). Table 3 clearly demonstrates that laser spectrometry can be used to readily identify oxygen isotope anomalies in nitrate. For example, USGS35 (an atmospherically derived nitrate) yielded a mean ʎ ($\delta^{17}O/\delta^{18}O$) value

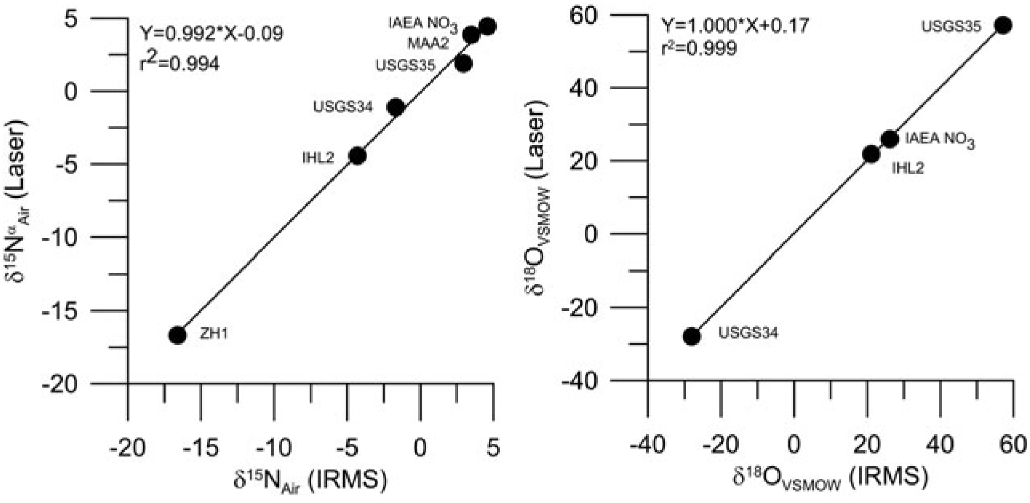

FIGURE 4 Normalized results of laser versus TG-IRMS using the Cd-azide method for NO_3^- and NO_2^- isotope analyses on N_2O using calibration standards and test samples. The left panel shows $\delta^{15}N^{\alpha}_{(AIR)}$ determined by laser spectrometry versus $\delta^{15}N^{bulk}_{(AIR)}$ by IRMS. Right panel shows $\delta^{18}O_{(VMSOW)}$ by laser spectrometry versus TG-IRMS. The $\delta^{15}N_{AIR}$ results for IRMS were ^{17}O-corrected using a global ʎ value of 0.52 for all samples, except for USGS35 (ʎ = 0.88);[18,12] see Table 3. NO ^{17}O correction was required for laser-based $\delta^{15}N$ analyses

TABLE 3 Mean nitrate and nitrite $\delta^{15}N^{\alpha}_{AIR}$ and $\delta^{18}O_{VSMOW}$ and $\delta^{17}O_{VSMOW}$ values for samples (mean ± SD, *n*) prepared with the Cd-azide method and laser spectrometry over a three-month period. Parameter ʎ was the measured ratio of the VSMOW normalized $\delta^{17}O/\delta^{18}O$ N_2O values. $\delta^{17}O = \delta^{17}O_{VSMOW} - (ʎ_{meas} \times \delta^{18}O_{VSMOW})$. The ^{17}O excess was defined by $\delta^{17}O = \delta^{17}O_{VSMOW} - (0.52 \times \delta^{18}O_{VSMOW})$ by applying the terrestrial value of 0.52 for ʎ. Significant excursions in ʎ from 0.52 suggest mass-independent oxygen isotope fractionations occurred (e.g. for USGS35), evident in the laser-measured ʎ value of 0.88. Despite larger uncertainties, the ^{17}O excess values of 0 and +21.2 for USGS34 and USGS35 were in good agreement with their reported values

Sample	$\delta^{15}N^{\alpha}_{AIR}$	$\delta^{18}O_{VSMOW}$	$\delta^{17}O_{VSMOW}$	ʎ (meas)	$\delta^{17}O$	^{17}O excess
KNO_3						
IAEA NO_3	+4.8±0.1 (9)	+25.9±0.3	+12.7±0.2	0.49±0.03	0.0±1.1	−0.8±1.1
USGS34	−1.7±0.1 (13)	−26.8±0.8	−14.4±1.1	0.54±0.10	0.1±9.1	−0.5±9.1
$NaNO_3$						
IHL2	−4.6±0.2 (6)	+22.3±0.3	+10.3±0.3	0.46±0.04	0.0±1.5	−1.3±1.5
USGS35	+2.6±0.1 (11)	+57.6±1.2	+51.2±2.0	0.88±0.06	0.5±9.2	+21.2±9.2
$NaNO_2$						
MAA2	+3.8±0.1 (9)	—	—	—		
Zh1	−16.4±0.1 (9)	—	—	—		

of 0.88, which represented a ^{17}O "excess" of +21.2‰, identical to that reported previously.[12] Although uncertainties are considerable, the ʎ values of nitrate reference and reagents materials ranged between 0.46 and 0.54 for IAEA NO_3 and USGS34 and a reagent nitrate. Despite small ʎ differences having little effect on the $\delta^{15}N$ values of N_2O by IRMS, these results suggested that laser-based assays might allow for future improved refinement of correction algorithms needed for IRMS ^{17}O corrections. This is particularly relevant for newly produced nitrate reference materials and/or for atmospherically dominated nitrate samples, which currently assume mass-dependent ʎ values of 0.52 to infer the $\delta^{17}O$ values. In this example, by applying our measured ʎ value of 0.88 (Table 1) instead of the assumed 0.52 in the ^{17}O correction[16] for the TG-IRMS N_2O assay of USSG35 (Figure 4),

$$\delta^{15}N_{17O\text{-corrected}} = \delta^{15}N_{IRMS} \times 1.051666 - \left(\lambda \times \delta^{18}O_{IRMS}\right) \times 0.051666 \quad (2)$$

the observed USGS35 $\delta^{15}N_{AIR}$ discrepancy of −0.9‰ was brought back "in line" with the laser assay and with other non-N_2O $\delta^{15}N$ IRMS techniques.

3.4 | Routing of N to α and β positons in the azide–N_2O reaction

As noted previously,[27] the reaction of NO_2^- with NaN_3 to N_2O routes sample-derived N mainly into the α positon of $N^{\beta}N^{\alpha}O$. Our ^{15}N tracer experiment verified these findings (Figure 5), with some potential caveats. In Figure 5, the left panel reveals that increasing the ^{15}N content of the azide (0.4 to 1.9% ^{15}N) resulted in a strong linear response (slope = 1.199, r^2 = 0.999) in the corresponding ^{15}N content of the β position of N_2O; but no ^{15}N response was observed in the α N_2O position (slope = 0.002, r^2 = 0.174). Conversely, when azide ^{15}N was held constant and ^{15}N enrichment of NO_2 increased (up to 3.2

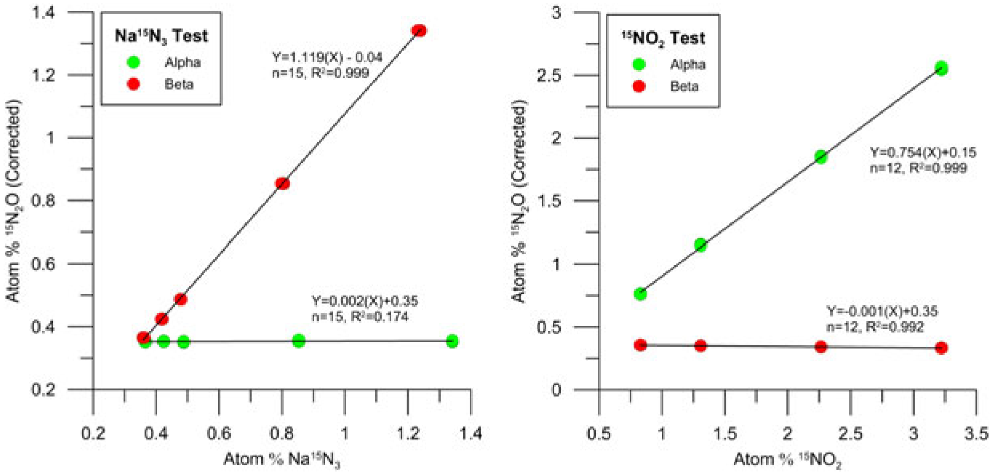

FIGURE 5 Routing of ^{15}N to the α or β position in the $N^{\beta}N^{\alpha}O$ gas molecule in the Cd-azide conversion reaction. Left: samples of IAEA NO_3 (1 ppm) were reacted to N_2O gas using the Cd-azide procedure using 0.4–1.9% (>98 atom%) $Na^{15}N_3$ enrichments. Right: samples of 0 to 3% (>98 atom%) $Na^{15}NO_2$ were reacted with "normal" reagent-grade NaN_3 ($\delta^{15}N_{AIR}$ = −2.9‰) to produce N_2O sample gas. Samples were measured by laser spectrometry and reported with pressure- and concentration-corrected δ values as described (not normalized to the air δ scale, or blank subtracted). The average measurement SD (*n* = 3) for $\delta^{15}N^{\alpha}$ and $\delta^{15}N^{\beta}$ values at these ^{15}N enrichment levels was ±6 and ±7‰ for the azide test, and ±10 and ±3‰ in the NO_2 test, respectively [Color figure can be viewed at wileyonlinelibrary.com]

atom% ^{15}N), there was a linear response in the ^{15}N content in the α position of $N^{\beta}N^{\alpha}O$ (slope = 0.754, r^2 = 0.999), but a negligible response in the β position (slope = −0.001, r^2 = 0.992). Hence, these ^{15}N enrichment tracer tests verified that for routine application of the Cd-azide method for measuring the $\delta^{15}N$ values of nitrate or nitrate by OA-ICOS lasers, one should only use results from $\delta^{15}N^{\alpha}$ in N_2O to obtain the best outcomes.

The ^{15}N tracer also revealed some unresolved (laser) peculiarities that warrant further investigations. The slope of azide ^{15}N versus ^{15}N in N_2O should be unity if *all* the ^{15}N from azide were routed into the β position (Figure 5), as postulated.[27] However, our derived slopes depend on using known ^{15}N-enriched calibration standards (none exist); hence, it was unclear whether the minor slope deviations from unity were partly a result of unaccounted instrumental scaling artefacts. Furthermore, with increasing ^{15}N in azide or NO_2 we observed a concomitant rise in the $\delta^{15}N$ values of concurrently run blank samples (their $\delta^{15}N$ values should have remained constant). This suggested that residual ^{15}N memory became a complicating factor at high ^{15}N enrichments, and this would therefore require development of additional between-sample memory correction strategies as is done for water isotope analyses. Finally, in the NO_2 ^{15}N tracer test we observed an $\delta^{17}O$ decrease in N_2O (by *ca* 100‰; r^2 = 0.96) that linearly correlated with the increasing ^{15}N content of NO_2, but no change was observed in the $\delta^{18}O$ value. Conversely, for the azide ^{15}N experiment we observed a corresponding $\delta^{17}O$ increase in N_2O (by *ca* 100‰, r^2 = 0.8), but no change in $\delta^{18}O$ value despite the proximity of the $^{15}N^{\beta}$ and ^{18}O absorption spectra (Figure 2). Given the proximity of the $^{14}N^{15}N^{16}O$ laser absorbance signal to the $^{14}N^{14}N^{17}O$ absorbance signal (Figure 2), these observations suggest there is a particular need for further improvements to N_2O isotopologue ^{17}O spectral deconvolutions by the N_2O isotope laser instrument manufacturer.

4 | CONCLUSIONS

The Cd-azide method yielded accurate and precise results suitable for adoption in the routine determinations of $\delta^{15}N^{\alpha}$, $\delta^{18}O$ and $\delta^{17}O$ (and ^{17}O excess) values of aqueous nitrate or nitrite by off-axis integrated cavity laser spectrometry. The results showed that NO_3^-/NO_2^- N was routed to the $\delta^{15}N^{\alpha}$ position of N_2O in the NO_2–azide reaction; hence, only $\delta^{15}N^{\alpha}$ values should be used for data interpretation by N_2O laser spectrometry. Following a correction procedure for cavity pressure, concentration dependence and water content, the $\delta^{15}N^{\alpha}_{AIR}$, $\delta^{18}O_{VSMOW}$ and $\delta^{17}O_{VSMOW}$ values (‰) of three international reference materials were +4.8 ± 0.1, +25.9 ± 0.3, +12.7 ± 0.2 (IAEA NO_3), −1.7 ± 0.1, −26.8 ± 0.8, −14.4 ± 1.1 (USGS34), and +2.6 ± 0.1, +57.6 ± 1.2, +51.2 ± 2.0 (USGS35), respectively, all in excellent agreement with their established values, and with the results that we obtained by IRMS for the same samples. The laser-measured ^{17}O excess (relative to ^{18}O, assuming mass-dependent fractionation) for USGS35 was +21.2 ± 9‰, in excellent agreement with previous results.

The proposed Cd-azide laser isotope method has some disadvantages: the toxicity of the Cd method may be of concern for some laboratories and needs appropriate health and safety apparatus and disposal measures. Currently, there is a lack of automated preparative and headspace sampling devices for any commercial N_2O laser spectrometers, resulting in manual and labor-intensive processing of single samples. Finally, whereas the ^{15}N-enriched tracer test revealed potential for position-specific experimentation of N nutrients in field studies at atom% ^{15}N enrichments, the data also exposed the need for the development of robust between-sample memory corrections and improved spectral deconvolution of the $^{14}N^{15}N^{17}O$ absorption spectra.

ACKNOWLEDGEMENTS

We are grateful for the generous support of Los Gatos Research for technical assistance in the configuration of the N_2O laser instrument for optimizing this application (Xu Du, Manish Gupta, Brian Leen, Doug Baer), and for providing ^{15}N-enriched azide for the tracer test. We thank Amanda McIntosh and Mirren Urresti for assistance with sample preparation and running test analyses in the laboratory. David Soto provided the R scripts for laser data extractions. This work was funded by the International Atomic Energy Agency and the University of Massachusetts at Dartmouth.

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How to cite this article: Wassenaar LI, Douence C, Altabet MA, Aggarwal PK. N and O isotope ($\delta^{15}N^{\alpha}$, $\delta^{15}N^{\beta}$, δ^{18}O, δ^{17}O) analyses of dissolved NO_3^- and NO_2^- by the Cd-azide reduction method and N_2O laser spectrometry. *Rapid Commun Mass Spectrom*. 2018;32:184-194. https://doi.org/10.1002/rcm.8029