A Ti(III) reduction method for one-step conversion of seawater and freshwater nitrate into N\textsubscript{2}O for stable isotopic analysis of \( ^{15}\text{N}/^{14}\text{N} \), \( ^{18}\text{O}/^{16}\text{O} \) and \( ^{17}\text{O}/^{16}\text{O} \)

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\textbf{Rationale:} The nitrogen and oxygen (\( \delta^{15}\text{N}, \delta^{18}\text{O}, \text{ and } \delta^{17}\text{O} \) values) isotopic compositions of nitrate (\( \text{NO}_3^- \)) are crucial tracers of nutrient nitrogen (N) sources and dynamics in aquatic systems. Current methods such as bacterial denitrification or Cd-azide reduction require laborious multi-step conversions or toxic chemicals to reduce \( \text{NO}_3^- \) to \( \text{N}_2\text{O} \) for \( ^{15}\text{N} \) and \( ^{18}\text{O} \) isotopic analyses by isotope ratio mass spectrometry (IRMS). Furthermore, the \( ^{17}\text{O} \) composition of \( \text{N}_2\text{O} \) cannot be directly disentangled using IRMS because \( ^{17}\text{O} \) contributes to mass 45 (\( ^{15}\text{N} \)).

\textbf{Methods:} We describe a new one-step chemical conversion method that employs Ti(III) chloride to reduce nitrate to \( \text{N}_2\text{O} \) gas in septum sample vials. Sample preparation takes only a few minutes followed by a 24-h reaction producing \( \text{N}_2\text{O} \) gas (65–75% recovery) which partitions into the headspace. The \( \text{N}_2\text{O} \) headspace was measured for \( ^{15}\text{N} \), \( ^{18}\text{O} \) and \( ^{17}\text{O} \) by IRMS or laser spectrometry.

\textbf{Results:} IRMS and laser spectrometric analyses gave accurate and reproducible N and O isotopic results down to 50 ppb (3.5 \( \mu \text{M} \)) \( \text{NO}_3^-\)-N, similar in precision to the denitrifier and Cd-azide methods. The uncertainties for dissolved nitrate reference materials (USGS32, USGS34, USGS35, IAEA-NO\textsubscript{3}) were \( \pm 0.2\% \) for \( \delta^{15}\text{N} \) values and \( \pm 0.3\% \) for \( \delta^{18}\text{O} \) values using IRMS. For laser-based \( \text{N}_2\text{O} \) isotope analyses the results were similar, with an \( \delta^{17}\text{O} \) uncertainty of \( \pm 0.9\% \) without any need for \( ^{15}\text{N} \) correction.

\textbf{Conclusions:} Advantages of the Ti(III) reduction method are simplicity, low cost, and no requirement for toxic chemicals or anaerobic bacterial cultures. Minor corrections may be required to account for sample nitrate concentration variance and potential chemical interferences. The Ti(III) method is easily implemented into laboratories currently using \( \text{N}_2\text{O} \) headspace sampling apparatus. We expect that the Ti(III) method will promulgate the use of N and O isotopes of nitrate in important studies of nutrient dynamics and pollution in a wide range of aquatic ecosystems.

\section{1 | INTRODUCTION}

Excessive concentrations of nitrate (\( \text{NO}_3^- \)) stemming from agriculture runoff and municipal wastewater entering rivers, lakes, groundwater, and marine estuaries have resulted in increasingly widespread negative impacts that include eutrophication, dead zones in coastal oceans, and drinking water deterioration in many worldwide aquifers\textsuperscript{1-4}. Hence, identifying and quantifying the sources and biogeochemical processing of nitrate in aquatic systems are key to informing water resource management, agricultural nutrient
optimization strategies, and urban pollutant mitigation. Stable isotopes are proven tools in this regard, as the δ15N, δ18O and δ17O isotopic composition of NO3− provides essential information about nitrogen (N) sources and biogeochemical sinks, allowing one to distinguish between organic waste, fertilizers, and atmospheric N sources. Furthermore, isotopic time series and geospatial depictions of nitrate in ground and surface water reveal important changes in agricultural land use, inform optimization of fertilization practices not achievable based on NO3− concentrations alone, and allow for detection of natural bioremediation processes such as bacterial denitrification. NO3− stable isotopes have also been instrumental in studies of the natural N cycle in a variety of systems including the global ocean and large-scale variations in NO3− δ15N and δ18O values have been used to understand the ocean N balance between losses from denitrification and gains from N2 fixation. Unfortunately, for decades, nitrate isotopic assays have been laborious and costly because of the difficulty of quantitatively extracting dissolved NO3− from water with subsequent conversion into N2, CO or N2O gas for N or O isotope-ratio mass spectrometry (IRMS), thereby hampering wide-spread adoption and limiting scientific accessibility. Previous offline NO3− extraction and conversion methods included Kjeldahl distillation for δ15N values, and ion-exchange recovery or micro-diffusion and precipitation to a nitrate salt for δ15N and δ18O determinations by pyrolysis-IRMS analysis. Contemporary methods for IRMS or laser analysis largely favor conversions of NO3− into N2O gas using the bacterial denitrifier or the Cd-oxide reaction methods. Both methods, however, either require laborious multi-step conversion methods and careful maintenance of anaerobic bacterial cultures, or use highly toxic chemicals to reduce NO3− to N2O for N and O isotopic analyses by IRMS and, as a result, are costly and limited to specialized isotope laboratories. In both cases, the δ17O values of NO3− cannot be obtained directly by IRMS due to isobaric interferences between 1H, 17O and 18O in N2O, unless there is subsequent conversion of the produced N2O gas into N2 and O2 and a dual-inlet IRMS analysis of O2 is undertaken. New advances in isotope analysis methodology for nitrate should (i) have N2O as an end product to facilitate dual O/N isotope analysis and cryo-focusing for high sensitivity, (ii) produce quantitative conversion of NO3− into N2O gas or non-quantitative conversion with constant isotope fractionation, (iii) have a correctable or no blank, (iv) be insensitive to major ion composition ranging from fresh to marine waters, and (v) allow for assays on low NO3− concentrations (i.e. nmol/ppb levels in oligotrophic aquatic or ground water systems). Improved methods should also be low-cost and amenable to automation on existing instrumentation, avoiding use of toxic chemicals or maintaining bacterial cultures to help reduce technological barriers to adoption. Finally, new methods should produce accurate and appropriately precise results using IRMS or laser instruments.

NO3− reduction generally proceeds as NO3− ⇌ NO2− ⇌ NO with N2O, NH4+ or N2 as end products with other possible short-lived intermediates. Ti(III) chloride was previously used to convert NO3− into NO (sparged out of solution before further reduction) or NH4+ for colorimetric or chemiluminescent analysis, but was never applied to stable isotope N and O assays. The reaction pathway at low pH, though, leads to N2O as an end product, ideally given by:

\[ 2\text{NO}_3^- + 8\text{Ti}^{3+} + 10\text{H}^+ \rightarrow 2\text{N}_2\text{O}_5 + 8\text{Ti}^{4+} + 5\text{H}_2\text{O} \] (1)

whereby aqueous nitrate is reduced to N2O gas at the expense of trivalent titanium which is accordingly oxidized to tetravalent titanium. A consideration in making use of this reaction for NO3− stable isotope analysis is that the presence of dissolved oxygen and oxy-anions (e.g. SO42−) may cause interferences, incomplete reactions or the formation of Ti dioxide precipitates.

Here we propose a simple conversion method that utilizes Ti(III) chloride in a one-step conversion of aqueous nitrate into N2O headspace gas for δ15N and δ18O analysis using IRMS or N2O laser spectrometers. We report first N and O isotopic results from the Ti(III) technique and propose standardized operational procedures for NO3− in fresh and marine waters (see supporting information). We discuss some of the factors encountered that affected optimal N2O recovery and identified solutions. Finally, we compare our Ti(III)-based N and O isotopic results from contaminated ground water samples with analyses of the same samples obtained using the bacterial denitrifier and Cd method. With further testing and development, we expect that the ease and low cost of the Ti(III) method will foster new N and O isotope studies of nitrate dynamics in a wider range of aquatic ecosystems.

2 | EXPERIMENTAL

2.1 | Overview

The Ti(III) method comprises (1) a sample processing and (2) an analytical component for nitrate N and O isotope determinations. The first component is a simple one-step chemical conversion of aqueous NO3− into headspace N2O using a commercially available Ti(III) chloride reagent (Equation 1). The second component consists of N and O isotopic analysis of the evolved headspace N2O gas by isotope ratio mass spectrometry or by laser spectrometry. For the sample processing component, we utilized Ti(III) chloride in a 1:20 or 1:40 (v/v) proportion to nitrate samples to convert aqueous NO3− to N2O gas. Because we have found that water sample chemistry can affect N2O yield and isotope results, we matched samples and calibration standards with respect to both ionic matrix (i.e. seawater vs freshwater) and NO3− concentration. For the analytical component, we used IRMS systems that employed headspace sampling followed by cryogenic and gas chromatographic purification of the N2O from N2, O2, CO2, H2O with automated sample handling devices (i.e. Trace Gas interface). We also used a N2O isotopic laser instrument, newly available technology that does not require cryogenic purification of N2O for isotopic analyses and permits direct measurements of mixed gas headspace samples. A key advantage of IRMS is automation of the vial headspace N2O.
sampling for isotope analyses, but a distinctive disadvantage is the inability to measure $^{17}$O in N$_2$O. While mass-dependent fractionation of 0.52 relative to $^{18}$O is assumed for $^{15}$N corrections of $^{15}$N in N$_2$O for most environmental samples, it does not hold where NO$_3^-$ has experienced mass-independent fractionation, typically when nitrate has atmospheric sources. Hence, an advantage of using a N$_2$O triple-isotopic laser is the ability to directly measure nitrate $^{17}$O via N$_2$O$^{15}$; however, available laser instrumentation currently lacks automated N$_2$O headspace sampling devices.

2.2 Preparation of the Ti(III) chloride reducing reagent and other materials used

The chemical reduction of aqueous nitrate to N$_2$O utilized Ti(III) chloride in 30% hydrochloric acid (HCl; P/N 8.08308.0500; Merck, Burlington, VT, USA). We found that the magenta colored Ti(III) chloride used “as-is” from the chemical supplier often gave variable N$_2$O yields, depending on the lot number or the supplier (i.e. Merck; Acros Organics; Thermo Fisher). Thus, sub- aliquots of the Ti(III) chloride reagent in enough quantity to process nitrate samples were pre-conditioned using Zn metal powder (ZN006040/7; 150 microns, 99.9% purity; Goodfellow, Huntingdon, UK) to remove potential Ti(III) chloride in 30% hydrochloric acid (HCl; P/N 8.08308.0500; Merck, Burlington, VT, USA). We found that the magenta colored Ti(III) chloride used “as-is” from the chemical supplier often gave variable N$_2$O yields, depending on the lot number or the supplier (i.e. Merck; Acros Organics; Thermo Fisher). Thus, sub- aliquots of the Ti(III) chloride reagent in enough quantity to process nitrate samples were pre-conditioned using Zn metal powder (ZN006040/7; 150 microns, 99.9% purity; Goodfellow, Huntingdon, UK) to remove potential Ti(III) chloride reagent and other materials used.

The reaction of the acidic Ti(III) reagent with Zn was exothermic, foaming and producing H$_2$ gas (hence, the pre-treatment was done in a 250-mL beaker in a fume hood). After cooling to room temperature and double checking by occasional stirring to ensure that the reaction was complete, the Ti(III) chloride solution was used at room temperature. If NO$_2^-$ was not removed, inaccurate NO$_3^-$ isotopic results would be obtained as it is an intermediate in the reduction of NO$_3^-$ to N$_2$O and thereby would also be converted into N$_2$O by Ti(III).

At UMD, nitrate isotope research was focused on marine and estuarine systems, typically having seawater salinity ($>$20–30 g/L) as total dissolved solids (TDS) and low NO$_3^-$ concentrations. To ensure samples and reference matrix equivalence in the reaction procedures, all nitrate isotope reference solutions were made to 0.15 mg L$^{-1}$ NO$_3^-$-N (10 μM) by dissolving or diluting with low nutrient (NO$_3^-$-free) seawater (LNSW). Samples with known NO$_3^-$ concentration were diluted to the target amount using LNSW.

Nitrate reference materials from the United States Geological Survey (USGS, Reston, VA, USA) and the IAEA included USGS34, USGS35 and IAEA-NO$_3$ dissolved in LNSW with assigned δ$^{15}$N$_{AIR}$ values (±SD) of $-1.8$ (±0.1), $+2.7$ (±0.1) and $+4.7$ (±0.1), δ$^{18}$O$_{svmsow}$ values of $-27.9$ (±0.4), $+57.5$ (±0.3) and $+25.6$ (±0.3), and δ$^{17}$O$_{svmsow}$ values of $-14.6$, $+51.5$ and $+13.2$, respectively. An in-house UMD nitrate reference material for δ$^{15}$N$\leq$ values called ‘Labmix’ (δ$^{15}$N = +38.9‰) was produced by gravimetrically mixing solutions of IAEA-NO$_3$ with USGS32 (δ$^{15}$N$_{IAEA}=+180$) in LNSW. In all instances, the use of LNSW ensured that marine or estuarine samples and the isotopic reference materials were similar in their salt type and content, including the HCl and sulfamic acid concentrations.

At the IAEA, the emphasis was on surface, groundwater and precipitation freshwater samples having comparatively higher NO$_3^-$ concentrations than seawater but low in TDS (<3000 mg L$^{-1}$). The IAEA freshwater samples and references for IRMS and laser analysis were targeted to 0.2 mg L$^{-1}$ NO$_3^-$-N (14.3 μM). Reference solutions of USGS32, USGS34, USGS35 and IAEA-NO$_3$ were prepared by dissolving them in DIW to 1 mg L$^{-1}$ NO$_3^-$-N (71.4 μM), preserving them as above, and storing at 5°C in 500-mL glass bottles. The nitrate concentrations of field samples were quantified using a Discrete Analyzer (Seal AQ1; www.seal-analytical.com; US EPA Method 126-A). Before processing samples to N$_2$O with the Ti(III) reagent, the references and samples were gravimetrically diluted by using degassed DIW to the target concentration of 0.2 mg L$^{-1}$ NO$_3^-$-N (14.3 μM) in the sample reaction vial, based on NO$_3^-$ concentration data (supporting information). Field samples with high...
concentrations of NO$_3^-$ (e.g. >5 mg L$^{-1}$ N) were pre-diluted to 5 mg L$^{-1}$ using degassed DIW before final dilution to 0.2 mg L$^{-1}$ and processed as above. This dilution step helped to eliminate the potential effect of interfering SO$_4^{2-}$ (see below).

2.4 | Conversion of NO$_3^-$ by TiCl$_3$ into N$_2$O

For the IRMS analyses, the target sample or reference material was pipetted into a reaction vial as described above and diluted up to 2–4 mL with LNSW or degassed DIW. At UMD, 20-mL crimped sealed headspace glass vials were used with a target of 20 to 40 nmol NO$_3^-$, with a 1:20 or 1:40 (v/v) reagent ratio to NO$_3^-$ for overnight for a minimum of 12 h to complete the reduction of NO$_3^-$ to N$_2$O. The vials containing magenta colored samples plus reagent were gently mixed, and each bottle was over-pressurized with 140 mL of N$_2$O-free air. The samples were then allowed to react for a minimum of 12–24 h to complete the reduction of NO$_3^-$ to N$_2$O. Approximately 30 samples and references could be manually analyzed by laser spectroscopy per day.

2.5 | IRMS $\delta^{15}$N and $\delta^{18}$O analyses

At UMD, an Isoprime™ continuous-flow (CF) isotope ratio mass spectrometer (Isoprime Ltd, Cheddle Hulme, UK) was used for $\delta^{15}$N and $\delta^{18}$O analyses of N$_2$O. The CF-IRMS system utilized a custom purge-and-trap (P&T) system$^{28}$ with N$_2$O headspace samples extracted from 20-mL vials using a PAL autosampler (CTC, Zwingen, Switzerland). A custom double-needle was used to purge the headspace gas with N$_2$O into cold traps for purification. Cryogenically focused N$_2$O peaks were compared with those from N$_2$O uncalibrated reference gas pulses. The UMD sample gas handling system was fitted with a gas injection loop to allow 20 µL (STP) of 1% N$_2$O in N$_2$ to be injected into the same cryo-focus path as the samples in order to check instrumental drift, recovery and internal reproducibility.

At the IAEA, an Isoprime-100™ CF isotope ratio mass spectrometer and Trace-Gas (TG) system was used for automated $\delta^{15}$N and $\delta^{18}$O analyses either (i) by reacting nitrate samples in 40-mL Exetainers or (ii) by injecting N$_2$O headspace obtained from 125-mL laser bottles (below) into pre-evacuated 40-mL Exetainers™ (Labco Ltd, Lampeter, UK). The Isoprime TG system utilized a model GX-271 autosampler (Gilion Inc., Dunstable, UK) with a liquid N$_2$ P&T capillary GC system to carry and cryo-focus N$_2$O from 40-mL mixed gas vials. The sample N$_2$O peak was compared with those from uncalibrated N$_2$O reference gas pulses introduced by a dual-inlet bellows. We found that the delicately machined steel needle-in-needle of the Isoprime TG system quickly corroded when purging the acidic Ti(III) reagent with sample. To overcome this, the purging needle was re-positioned just above the liquid and the headspace N$_2$O trapping time was increased to 300 s. This eliminated corrosion of the needle while trapping the N$_2$O from the sample. Both Isoprime systems had an external analytical precision of better than ±0.1%o ($\delta^{15}$N-N$_2$O) and ±0.2‰ ($\delta^{18}$O-N$_2$O).$^{46}$ An $^{17}$O interference correction for the $\delta^{15}$N value of N$_2$O ($\lambda = 0.52$) was applied to all
samples, except for USGS35 NO₃⁻, a reference material with an anomalous ¹⁷O content relative to ¹⁸O.

2.6 | Laser N₂O isotope spectrometry

At the IAEA, laser-based N₂O stable isotope analysis (δ¹⁵N, δ¹⁸O and δ¹⁷O values) was conducted using a triple isotope N₂O analyzer (23e-EP model 914–0060; Los Gatos Research, Mountain View, CA, USA) fitted with an injection port and using ‘injection-dilution’ mode. The analytical precision for 10-ppm N₂O in dry air was better than ±0.2% (δ¹⁵N-N₂O), ±0.4% (δ¹⁸O-N₂O) and ±1.0% (δ¹⁷O-N₂O).

Sample analysis was performed as described in Wassenaar et al. Briefly, after the 12–24 h nitrate conversion reaction into N₂O, the 125-mL serum bottles were gently swirled to ensure that the N₂O gas was fully equilibrated in the headspace. A 20-mL gastight syringe with two-way valve and needle was used to draw sample headspace gas into the barrel. As the gas overpressure of the sample pushed the plunger out, it was manually held at 3–4 mL (depending on vial initial pressure), to achieve a target 12 mL mixed headspace gas in the syringe (at STP) when the valve was closed. The syringe was taken out of the bottle, the needle removed, and fitted on the injection port of the laser instrument, and N₂O isotopic analysis was conducted. The sample analysis time for N₂O triple isotopic analysis was approximately 12 min. Normalized triple isotope δ values were processed using the algorithms described in Wassenaar et al.

3 | RESULTS

3.1 | Standard operating procedures

The acceptance of new isotopic preparation and analysis methods is ultimately judged on their ease of incorporation, as well as their precision and accuracy performance. Other factors ideally include improved sample throughput, lowered costs and insensitivity to water sample matrix effects. Our Ti(III) method has few procedural steps and is low cost, which makes adoption attractive but, to optimize the isotopic results, we examined several complicating factors influencing isotope results identified during our research, including reagent-to-sample volumes, sample reaction times and sulfate interferences.

Overall, we found that the best isotopic results were obtained when pre-treated TiCl₃ reagent was added to nitrate samples in a 1:20 to 1:40 ratio (v/v) and reacted for 12–24 h without any agitation at room temperature (Figures 1 and 2). Below we document in detail experimental results supporting our procedures for seawater and freshwater as summarized in the supporting information. We assessed the influence of NO₃⁻ concentration variance and sulfate ion composition. Because of the influence on yield and isotopic results, we recommend that NO₃⁻ concentrations are accurately pre-determined and that sample chemistry properties be manipulated to be as similar as possible to those of the

FIGURE 1 | Relative N₂O signal yield (peak height nA on IRMS) and uncorrected δ¹⁵N and δ¹⁸O values for nitrate reference materials as a function of sample to Ti(III) reagent ratios (x-axis), ranging from 10:1 to 40:1. Upper panel is for a 6 M NaCl sample matrix and the lower panel is using DIW. For each symbol n = 2. Reaction time for all samples was >12 h (overnight) [Color figure can be viewed at wileyonlinelibrary.com]
corresponding isotope calibration standards (e.g. saline vs freshwater).

3.2 | N2O yields

The Ti(III) reaction produced N2O recoveries between 65 and 80% for a reagent-to-sample volume ratio of 1:40 with DIW having the highest yield and LNSW and acidified LNSW having lower yields (Table 1). Azide reaction of NO2– reference solutions was used as a yield benchmark (Table 1). The recovery was found to be dependent on the Ti(III) reagent-to-sample volume ratio with higher amounts of Ti(III) reagent (i.e. 1:10 ratio) reducing N2O recovery both in DIW and in a NaCl-saturated solution (Figure 1). The isotope results are discussed in greater detail below, but we also found that lower amounts of Ti(III) reagent (1:40 ratio) within the tested range produced slightly more precise δ15N values but less precise δ18O values. Conversely, the δ18O values were more precise at higher Ti(III) reagent-to-sample ratios (1:10) but the δ15N values less so. A ratio of between 1:20 and 1:40 was therefore deemed practical for overall general performance (see below). Despite the N2O yield not being quantitative, the N and O isotope results were excellent within this range of reagent addition. We did not test the performance of ratios lower than 1:40 as the very small Ti(III) reagent volumes required became difficult to precisely aliquot (e.g. <75 μL) using standard micro-pipettes.

We infer that incomplete N2O recoveries were partly due to the formation of other stable (or steady-state) end products such as N2 or NH4+, mainly because (i) higher Ti(III) reagent additions reduced N2O yields and (ii) increased reaction times beyond 24 h did not show any increase in N2O yield or better stable isotopic results. In addition, a comparative Ti(III)-azide reagent approach (not suitable for δ18O values; not shown) gave ~100% N2O yield, suggesting that the first reduction step of NO3– to NO2– by Ti(III) is quantitative. In the Ti(III)-azide reaction, NO3– is reduced to NO2– which then reacts with the azide to form N2O before further reduction by Ti(III) can occur. Therefore, we suspect that reduced N2O yield may be due to alternative reaction pathways leading to NH4+ or N2 after the formation of NO (its production by Ti(III) reduction of NO3– has also been reported to be quantitative36) or perhaps through formation of unknown solid complexes of N-oxide intermediates with Ti+3 or Ti+4.

Future research considering in more detail the chemistry of Ti(III) reduction of NO3– may help to identify conditions resulting in better quantitative recovery of N2O. We acknowledge that this aspect of our work requires more research.

3.3 | Reaction time, temperature, and salinity

To empirically determine the optimal reaction time for the reduction of NO3– to N2O, a time-series experiment was carried out at room temperature. In one test, the reaction of a nitrate standard multiple vials with Ti(III) was terminated at different times by the addition of NaOH, and the samples were analyzed as a single batch on the next day (not shown). NaOH terminated the Ti(III) reaction because, at high pH, Ti(III) is quickly oxidized to Ti(IV), preventing nitrate reduction. In a second test, samples were sequentially analyzed by IRMS starting immediately following the addition of the Ti(III) reagent and analyzed at 12-min time-steps over a 0 to 96 h period.
(Figure 2). Both approaches, however, showed that the nitrate into N₂O conversion reaction appeared complete (or stable) between 6 and 21 h with respect to N₂O yield (Figure 2). However, the N and O isotope results were found to be more precise when samples were left to react over a longer time (i.e. >12–24 h), possibly because of a longer period required for reaching gas-liquid equilibrium under static conditions. A 96-h reaction gave similar N₂O yields and isotopic results to a 21-h reaction, which suggested that samples can be reacted and held longer without adverse effects for at least 3 days before isotopic analysis. For this reason, an overnight reaction (or >12 h) was found to be convenient, so that samples could be prepared on Day 1 and analyzed for isotopes on the next morning. The long reaction periods (e.g. >96 h) visibly resulted in more Ti(IV) oxide precipitating in the bottom of the reaction vials.

The influence of reaction temperature on the Ti conversion of nitrate into N₂O was investigated by reacting samples for 12 h at 50°C and comparing the results with the room temperature results (20–22°C). No difference in the N₂O yield or isotopic results was observed; hence, we recommend carrying out the reaction at room temperature to avoid unnecessary thermal control.

Tests using nitrate standards prepared in 6 M NaCl showed no adverse performance effects compared with those samples prepared using DIW (Figure 1), but some of the differences observed may account for the N₂O yield reduction in LNSW and HCl-acidified LNSW (Table 1). Comparing the salt and DIW standard preparations also showed both N₂O yield and N and O isotopic differences, but each was internally consistent and reproducible to a high degree of accuracy. This finding supported the idea that sample and reference chemistry matrices should ideally be matched. While the total dissolved salts (~35 g·L⁻¹ TDS) in open oceans vary narrowly in major ion composition (~3500 mg·L⁻¹ SO₄), ‘fresh’ surface and ground waters vary tremendously at lower TDS ranges, from dilute meltwater to >5000 mg·L⁻¹. Ground and surface water samples also vary widely in their Cl⁻, SO₄²⁻ and HCO₃⁻ concentrations depending on watershed geological characteristics. We believe that SO₄²⁻ is of particular importance due to its high concentration in seawater and wide concentration variation in terrestrial freshwaters, as demonstrated previously. Our tests using nitrate reference materials showed that increasing the sample sulfate concentrations from <0 mg·L⁻¹ to >2700 mg·L⁻¹ depressed the N₂O yields by about 10% at the highest concentrations (Table 2) with significant isotope effects. The δ¹⁵N values were reduced by ~2‰ and the δ¹⁸O values became more positive by ~2‰ over this SO₄²⁻ range. Visually, samples with more than ~1000 mg·L⁻¹ sulfate also produced a secondary dark gray precipitate compared with samples with lower SO₄²⁻ or DIW which remained magenta with a white Ti dioxide precipitate forming. The presence of HCO₃⁻ did not have any discernable effect on the N₂O yield or the isotopic composition.

Efforts to remove sulfate from water samples beforehand were unsuccessful. We attempted precipitation of SO₄ to BaSO₄ by adding BaCl₂·H₂O, and by sulfate anion-exchange cartridges (On-Guard II; Dionex, Sunnyvale, CA, USA). In both cases, sample N₂O yields and isotopic results were considerably worse, probably through co-precipitation of NO₃⁻ with BaSO₄. We did not have access to X-ray diffraction or fluorescence instrumentation to determine the composition of the visible gray co-precipitates. Our data also showed there was little effect on δ¹⁸O values for samples with SO₄²⁻ concentrations below ~600 mg·L⁻¹ or for δ¹⁵N values at concentrations below ~300 mg·L⁻¹. The IRMS standard operating procedure (see supporting information) therefore includes a practical step for freshwater samples with SO₄²⁻ concentrations of up to ~5000 mg·L⁻¹ to dilute them with DIW into the <200 mg·L⁻¹ range.

### 3.4 | IRMS ¹⁵N and ¹⁸O Results

The performance of the Ti(III) method for N and O isotope analysis was evaluated in detail in several ways. We considered the reproducibility and accuracy of the results obtained using nitrate isotopic reference material solutions made in DIW and LNSW and the reproducibility of replicate control standards, and compared the Ti results for selected groundwater samples analyzed by the Cd and bacterial methods.

#### Table 2. Effect of SO₄²⁻ concentration and Ti(III) on the yield of N₂O, and the δ¹⁵N and δ¹⁸O values of nitrate. Mix 1 = NaHCO₃ (820 mg·L⁻¹) made with degassed deionized water (DIW). Mix 5 = 4000 mg·L⁻¹ Na₂SO₄ made with degassed DIW. The corresponding mixtures spanned a range of sulfate and bicarbonate concentrations typically encountered in surface, groundwater and seawater. Each mixture had 7.2 mg·L⁻¹ USGS34, USGS35 or IAEA-KNO₃ added (1 mg·L⁻¹ NO₃⁻). Each subsample was acidified to <pH 2 by adding 0.2 mL 10% HCl. The Ti/sample ratio was 1:20.

<table>
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<th>Sample</th>
<th>SO₄ [mg·L⁻¹]</th>
<th>HCO₃ [mg·L⁻¹]</th>
<th>N₂O (nA)</th>
<th>δ¹⁵N USGS34</th>
<th>δ¹⁸O USGS34</th>
<th>δ¹⁵N USGS35</th>
<th>δ¹⁸O USGS35</th>
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<td>Mix3</td>
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<td>293</td>
<td>23.4</td>
<td>−11.9</td>
<td>41.2</td>
<td>−7.8</td>
<td>121.0</td>
<td>−5.5</td>
<td>91.3</td>
</tr>
<tr>
<td>Mix4</td>
<td>2027</td>
<td>146</td>
<td>23.2</td>
<td>−12.3</td>
<td>41.4</td>
<td>−8.2</td>
<td>120.9</td>
<td>−6.2</td>
<td>90.9</td>
</tr>
<tr>
<td>Mix5</td>
<td>2700</td>
<td>0</td>
<td>22.8</td>
<td>−12.2</td>
<td>41.3</td>
<td>−8.3</td>
<td>120.1</td>
<td>−6.5</td>
<td>90.8</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>−11.3</td>
<td>42.0</td>
<td>−7.6</td>
<td>121.4</td>
<td>−5.4</td>
<td>91.7</td>
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<tr>
<td>Stdev.</td>
<td>0.8</td>
<td>0.9</td>
<td>0.5</td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.5 Calibrations using nitrate reference solutions

Cross plots of uncorrected δ¹⁵N and δ¹⁸O IRMS values for evolved N₂O using the Ti(III) reaction versus their accepted δ-values gave strong linear relationships, with \( r^2 > 0.999 \) (Figure 3). In contrast to the Cd-azide method,²⁹ but similar to the bacterial method,²⁶ no extraneous N (e.g. from azide) is added to the product N₂O such that the slopes of δ¹⁵N reference calibrations should be unity. Accordingly, our observed slopes of the raw δ¹⁵N values after blank correction vs the known δ¹⁵N values were close to 1 regardless of whether the reference solutions were made up in DIW, LNSW, or HCl-acidified LNSW (pH 2–3). The δ¹⁸O calibration slope could fall below unity if there were O isotope exchange between reaction intermediates, specifically NO₂⁻ (HNO₂ at acidic pH) and water. However, slopes close to 1 were always observed, suggesting that there was little to no oxygen isotope exchange (see section 3.9) and that the NO₂⁻ reduction step must be very fast.

Intercept values for the δ¹⁵N calibration would be expected to be close to zero if the N₂O yields were 100% quantitative in accordance with conservation of isotope mass balance (δ¹⁵N value of the N₂O product = δ¹⁵N value of the NO₂⁻ source). However, we often observed negative intercepts (δ¹⁵N value of the N₂O product < δ¹⁵N value of the NO₂⁻) probably from incomplete recovery and N isotope fractionation during branching between reduction pathways leading to N₂O vs NH₄⁺ or N₂. The intercept values for the δ¹⁸O calibration were high (74 to 83%) due to oxygen isotope fractionation preferring ¹⁶O during removal of O atoms (5 out of 6). The variability in the proportional yields between these potential pathways and the associated N₂O yield would be expected to degrade the method accuracy and precision for N and O isotopic analyses; however, the high \( r^2 \) values and excellent reproducibility that we observed suggested that this was not the case. In addition to variation between DIW and LNSW, variation in the intercepts for both the δ¹⁵N and the δ¹⁸O values occurred as a function of the reagent to sample volume ratio as well as salinity (or sulfate concentration) or NO₂⁻ concentration, emphasizing that for optimal results, one needs to match samples and standards with respect to sample nitrate concentration, but also the chemical matrix of the samples and references. Fortunately, these can be acceptably controlled, but practitioners need to be aware of these potential (or unforeseen) complications.

3.6 Mixtures of reference solutions

Gravimetric mixtures of nitrate reference materials were made up using DIW and processed as unknown samples by IRMS to obtain a wide range of δ¹⁵N, δ¹⁸O or δ¹⁷O values that reflect a maximal δ-range in nature (Table 3). Mixtures of USGS32 and IAEA-KNO₃ were used to obtain a wide range of δ¹⁵N values in N₂O from +4.7 to +180‰. USGS35 and USGS34 were mixed to produce a wide range of δ¹⁸O and δ¹⁷O values in nitrate, spanning +57.5 to −27.9‰ and +51.5 to −14.8‰, respectively. The four endmembers were made up as 71.4 μM (1 mg L⁻¹-N) solutions in degassed DIW which were gravimetrically mixed in ratios of 0:100, 25:75, 50:50, 75:25 and 100:0, and reacted using the IAEA procedures above. The IRMS results for the δ¹⁵N and δ¹⁸O values were accurate and within acceptable analytical uncertainty (mean SD = ±0.6‰ and ±0.74‰, respectively), which was comparable with the analytical uncertainties reported for the Cd or microbial method.

Isotopic assays using the N₂O laser instrument (Table 3) yielded similar uncertainties for δ¹⁵N values to IRMS (SD = ±0.6‰), but the analytical uncertainty was slightly higher for δ¹⁸O values (±1.3‰). The mean uncertainties for δ¹⁷O values were accordingly higher (±2.6‰) owing to the lower ¹⁷O content and laser instrument detection limits. Nevertheless, the laser outcomes showed accurate results at acceptable precision for N and O isotopes.
TABLE 3 Results of Ti(III) chloride conversion of nitrate into N₂O using mixtures of USGS32 and IAEA-NO₃ or USGS35 and USGS34 in DIW to obtain a wide range of δ-values and using the laser preparative method. Italic denotes gravimetrically estimated nitrate δ-values for δ¹⁵N_air, δ¹⁸O_VSMOW and δ¹⁷O_VSMOW. For all samples n = 2 (±SD). For each sample, the same headspace N₂O gas was subsampled for isotopic analysis by CF-IRMS. A reagent-to-sample ratio (v/v) of 1:40 was used [Color table can be viewed at wileyonlinelibrary.com]

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mixing ratio</th>
<th>δ¹⁵N_air (%)</th>
<th>δ¹⁸O_VSMOW (%)</th>
<th>δ¹⁷O_VSMOW (%)</th>
<th>δ¹⁵N_air CF-IRMS</th>
<th>δ¹⁸O_VSMOW CF-IRMS</th>
<th>δ¹⁷O_VSMOW laser</th>
<th>δ¹⁸O_VSMOW laser</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>¹⁵N mixture test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USGS32:IAEA-NO₃</td>
<td>100.0</td>
<td>+180.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+179.8 ± 0.7</td>
<td>+179.3 ± 0.7</td>
</tr>
<tr>
<td>USGS32:IAEA-NO₃</td>
<td>75.25</td>
<td>+136.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+136.3 ± 0.2</td>
<td>+136.9 ± 0.6</td>
</tr>
<tr>
<td>USGS32:IAEA-NO₃</td>
<td>50.50</td>
<td>+92.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+92.2 ± 0.8</td>
<td>+92.8 ± 0.7</td>
</tr>
<tr>
<td>USGS32:IAEA-NO₃</td>
<td>25.75</td>
<td>+48.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+49.4 ± 0.3</td>
<td>+48.5 ± 0.5</td>
</tr>
<tr>
<td>USGS32:IAEA-NO₃</td>
<td>0.100</td>
<td>+4.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+4.3 ± 0.6</td>
<td>+4.5 ± 0.4</td>
</tr>
<tr>
<td><strong>¹⁸O/¹⁷O mixture test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USGS35:USGS34</td>
<td>100.0</td>
<td>-</td>
<td>+57.5</td>
<td>+51.5</td>
<td>-</td>
<td>-</td>
<td>+57.7 ± 0.3</td>
<td>+58.8 ± 1.2</td>
</tr>
<tr>
<td>USGS35:USGS34</td>
<td>75.25</td>
<td>-</td>
<td>+36.2</td>
<td>+35.0</td>
<td>-</td>
<td>-</td>
<td>+36.7 ± 0.2</td>
<td>+35.3 ± 0.9</td>
</tr>
<tr>
<td>USGS35:USGS34</td>
<td>50.50</td>
<td>-</td>
<td>+14.9</td>
<td>+18.4</td>
<td>-</td>
<td>-</td>
<td>+13.9 ± 0.9</td>
<td>+13.8 ± 2.0</td>
</tr>
<tr>
<td>USGS35:USGS34</td>
<td>25.75</td>
<td>-</td>
<td>-6.5</td>
<td>+1.8</td>
<td>-</td>
<td>-</td>
<td>-6.8 ± 0.4</td>
<td>-7.3 ± 1.0</td>
</tr>
<tr>
<td>USGS35:USGS34</td>
<td>0.100</td>
<td>-</td>
<td>-27.9</td>
<td>-14.8</td>
<td>-</td>
<td>-</td>
<td>-27.2 ± 1.9</td>
<td>-26.4 ± 1.6</td>
</tr>
</tbody>
</table>

Table 4 illustrates the efficacy of this correction to help overcome minor nitrate concentration variances and avoid unnecessary sample repetitions. To evaluate lower nitrate detection limits for N and O isotopic assays, we conducted Ti(III) conversion and isotope analyses of USGS34, USGS35 and IAEA-KNO₃ and reference materials at 50 ppb NO₃⁻. N in practice, field sample nitrate concentrations (determined or provided) are not always as accurate as those of gravimetrically prepared isotopic reference solutions relied upon by the isotope laboratory, resulting in variable sample N₂O signals (peak heights) compared with the reference samples for N and O isotopic assays. The first approach was to reject N or O isotopic results for N₂O yield falls beyond the acceptable target range (adding cost and personnel issue in isotope laboratories conducting N₂O yield analysis). An alternative approach was to determine and apply an isotopic correction based on nitrate concentration. A linear regression of results is illustrated in Table 4. To derive the TSR correction, a linear regression of raw N and O values (y-axis) vs the amount of sample in the Ti(III) preparation and remeasured to the correct target N₂O concentration in the repetition. The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis). The slopes (15N or 18O) were obtained for the 15N and 18O values (y-axis) vs the IRMS N₂O signal (x-axis).
TABLE 4  Correction for isotopic variance due to changes in the TSR using a 1:20 reagent-to-sample volume ratio for lab control standard KNO₃. The regression slopes (m) for δ¹⁵N and δ¹⁸O values on this dataset were 5.29 and 6.10, respectively (see Equations 3–5). The precision of the δ-results was significantly improved to accommodate a 20% range of unexpected nitrate variance and yielded acceptable uncertainties for δ¹⁵N and δ¹⁸O values of ±0.5 and ±0.3 permil, respectively. nA = N₂O signal with IRMS [Color table can be viewed at wileyonlinelibrary.com]

<table>
<thead>
<tr>
<th>NO₃ mgL⁻¹</th>
<th>nA</th>
<th>δ¹⁵N (meas.)</th>
<th>δ¹⁸O (meas.)</th>
<th>(norm. Factor)</th>
<th>δ¹⁵N corr.</th>
<th>δ¹⁸O corr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>21.7</td>
<td>-7.5</td>
<td>90.4</td>
<td>-0.3</td>
<td>-5.8</td>
<td>91.9</td>
</tr>
<tr>
<td>0.8</td>
<td>23.4</td>
<td>-8.5</td>
<td>90.2</td>
<td>-0.2</td>
<td>-7.2</td>
<td>91.4</td>
</tr>
<tr>
<td>0.9</td>
<td>26.8</td>
<td>-8.8</td>
<td>91.4</td>
<td>-0.1</td>
<td>-6.2</td>
<td>92.0</td>
</tr>
<tr>
<td>0.9</td>
<td>26.7</td>
<td>-6.6</td>
<td>91.0</td>
<td>-0.1</td>
<td>-6.0</td>
<td>91.6</td>
</tr>
<tr>
<td>1.0</td>
<td>30.5</td>
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<td>91.5</td>
<td>0.0</td>
<td>-6.8</td>
<td>91.4</td>
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<td>91.3</td>
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<td>91.3</td>
</tr>
<tr>
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<td>-5.9</td>
<td>92.2</td>
<td>0.1</td>
<td>-6.2</td>
<td>91.9</td>
</tr>
<tr>
<td>1.1</td>
<td>30.2</td>
<td>-5.4</td>
<td>92.4</td>
<td>0.0</td>
<td>-5.4</td>
<td>92.4</td>
</tr>
<tr>
<td>1.2</td>
<td>33.9</td>
<td>-5.8</td>
<td>92.4</td>
<td>0.1</td>
<td>-6.6</td>
<td>91.7</td>
</tr>
<tr>
<td>1.2</td>
<td>34.4</td>
<td>-5.4</td>
<td>92.3</td>
<td>0.1</td>
<td>-6.3</td>
<td>91.6</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>-6.5</td>
<td>91.5</td>
<td></td>
<td>-6.3</td>
<td>91.7</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>1.0</td>
<td>0.8</td>
<td></td>
<td>0.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

(3.5 µM) on the Isoprime 100 at the IAEA (not shown). The mean N₂O IRMS signal was 6.7 nA compared with 30 nA for our routine analysis and a blank of 0.8 nA. The results were accurate with acceptable uncertainties of ±0.6 for both δ¹⁵N and δ¹⁸O values. It could be feasible to obtain δ¹⁵N and δ¹⁸O values possibly to 10 ppb (0.7 µM) by increasing the IRMS ion source sensitivity and by pre-evacuating the sample vials to eliminate the blank; however, this aspect of further lowering detection limits requires more testing.

3.9  | Oxygen isotope exchange

The observed δ¹⁸O calibration slopes close to 1 implied little oxygen isotope exchange with water during the Ti(III) reduction of NO₃⁻ to N₂O. Because of the ability to measure ¹⁷O, we further tested for oxygen isotope exchange using the N₂O laser. A KNO₃ laboratory standard was dissolved (at 71.4 µM or 1 mg L⁻¹ N) in nitrate-free tap water having δ¹⁸O values adjusted from −10.2 to +22.0‰ (VSMOW) and an electrical conductivity of −425 µS/cm. The water δ¹⁸O values were adjusted from their initial value (δ¹⁸O = −10.2‰, δ¹⁷O = −5.3‰) by gravimetrically adding small amounts of 97.0 atom % ¹⁸O water (Sigma Aldrich P/N 329878: the ¹⁷O content of the ¹⁸O-enriched water was unknown) to 500 g of tap water. The results from laser spectroscopic analysis showed that, despite the 33‰ range in the δ¹⁸O values (we presume that the ¹⁷O of water varied accordingly) of water of the dissolved NO₃⁻ samples, there was no effect of water oxygen on either the δ¹⁸O or the δ¹⁷O values of the N₂O produced from nitrate through the Ti reaction (Table 5).

<table>
<thead>
<tr>
<th>Water δ¹⁸O_VSMOW</th>
<th>δ¹⁸O-N₂O</th>
<th>δ¹⁷O-N₂O</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>−10.2 ‰</td>
<td>+107.6 ± 0.5 ‰</td>
<td>+59.3 ± 0.4 ‰</td>
<td>IHL-NO₃</td>
</tr>
<tr>
<td>+7.9 ‰</td>
<td>+107.1 ± 0.3 ‰</td>
<td>+59.7 ± 1.5 ‰</td>
<td>IHL-NO₃</td>
</tr>
<tr>
<td>+22.9 ‰</td>
<td>+107.2 ± 0.5 ‰</td>
<td>+58.4 ± 0.5 ‰</td>
<td>IHL-NO₃</td>
</tr>
</tbody>
</table>

3.10  | Comparison of the Ti method vs the microbial and Cd method

For a methodological comparison test, a selection of nitrate-contaminated groundwater samples from IAEA projects in South and Central America (Argentina, Bolivia, Dominican Republic, Chile, Mexico), which had been previously measured for their δ¹⁵N and δ¹⁸O values using the microbial method at the University of Calgary (Calgary, Canada), were re-measured using the Ti(III) method and the Cd-azide method at the IAEA laboratory. These samples had been stored frozen at the IAEA for more than 1.5 years before remeasuring by Cd and Ti(III). This test was undertaken as a preliminary assessment to determine how these different methods compared with each other for the analysis of uncontrolled environmental samples. These samples had NO₃⁻ concentrations ranging between 0.2 and 87 mg L⁻¹ NO₃⁻ (14–6100 µM), with electrical conductivities ranging from 129 to 5340 µS/cm. No SO₄²⁻ concentrations were provided, and the nitrate concentrations provided by the client were frequently inaccurate when the samples were re-tested at the IAEA. Hence, these unknown samples represented a worst-case scenario for a laboratory. The Ti method for these samples was conducted using a 1:20 reagent ratio using the IRMS SOP.
The methodological comparative results for archived ground water samples are summarized in Figure 4. For $\delta^{15}$N values, the calibrated values determined using the Ti method plotted against the Cd and microbial methods agreed well, and for all methods the control standards gave accurate results. The slopes were close to unity ($r^2 > 0.9$) against the Cd and microbial methods. For $\delta^{18}$O values, the comparison was not quite as good. The best comparative result for the Ti(III) method was versus the Cd method; this regression yielded a slope of 0.9 ($r^2 = 0.94$). The microbial comparison was considerably worse with a slope of 0.8 ($r^2 = 0.70$). Correspondingly, the comparative results of the Cd-azide versus microbial method were similar (Figure 4). The intercepts were variable between the N$_2$O preparative methods but, in the case of the $\delta^{18}$O values, heavily influenced by outlier points. Given that the Cd and Ti methods, in general, compared well, it is suggested there can be systematic differences possibly due to sample chemistry that affected the $\delta^{18}$O values of the microbial method. No complete water chemistry (e.g. cations or anions) was provided with these samples other than nitrate and electrical conductivity; it remains unknown if any of the samples had excessive SO$_4^{2-}$ interferences affecting the Ti method for $\delta^{18}$O values. Finally, this comparative test suggests, despite the limitations, that systematic international inter-comparison and proficiency testing for the stable isotopes of nitrates using different preparation method and instrumentation is warranted and overdue.

4 | CONCLUSIONS

Our new Ti(III) method for $\delta^{15}$N, $\delta^{18}$O and $\delta^{17}$O values compares favorably with existing methods for the preparation of water samples for NO$_3^-$ isotopic determination. With respect to minimal effort and low cost per sample, the method excels in comparison with the microbial and Cd-azide methods. The preparation time is only a few minutes per sample and the handling time per sample is 1–2 days including isotopic analysis. Assuming that vials are reused, the material cost up to the point of IRMS or laser analysis is < $2USD for a small amount of reagent and vial septa. The precision and reproducibility of the $\delta^{15}$N and $\delta^{18}$O values using either IRMS or laser analysis are comparable with literature values for the microbial or Cd-azide method. The $\delta^{17}$O results using laser analysis were similar to or better than those from N$_2$O conversions into O$_2$. The calibration plots have high $r^2$ values with slopes near the theoretical value of 1 after blank correction. For environmental samples, a reasonable comparison was obtained with results produced by the microbial and Cd-azide
methods; however, given the uncertainties in storage time and lack of water chemistry, a properly controlled nitrate isotope inter-comparison of various N₂O conversion methods is clearly warranted.

The main caveat of the Ti(III) method, however, is the non-quantitative yield of N₂O resulting in isotopic discrimination and negative intercepts for the calibration cross plots. Nevertheless, the N₂O yields are typically >65% such that analytical sensitivity is minimally impacted. In addition, unexpected variance in sample concentration of NO₃⁻ and ions such as sulfate cause variations in N₂O yield and apparent isotope discrimination. However, as we have in, these factors can be controlled for when reference solutions are matched to samples. The precision and accuracy are within literature values for the microbial and Cd-azide methods. Given the quality of results, ease of use and relatively low cost, the Ti(III) reduction of NO₃⁻ to N₂O is a useful and simpler alternative to existing preparation methods for NO₃⁻ isotope analysis.

4.1 Future research

The chemistry of the Ti(III) reduction of NO₃⁻ and NO₂⁻ to N₂O has been previously investigated and the major intermediates and the kinetics of each reductive step are reasonably well known. It is also known that the end products include N₂O, N₂ and NH₄⁺, verifying our observations that N₂O is a major product at low pH. Hence, we surmise that variations in N₂O yield with variation in sample chemistry are due to changes in reaction kinetics in favor of either N₂ or NH₄⁺ as end products. It remains unclear how these conditions affect reaction pathways and end-product yield, suggesting that future work is warranted to improve or perfect the Ti(III) method by identifying and adopting conditions that can ensure 100% yield of N₂O over the range in composition of environmental samples. We acknowledge that some knowledge gaps remain, particularly concerning the Ti(III) nitrate into N₂O conversion stoichiometry and intermediates. For example, we do not know whether high concentrations of dissolved organic carbon (DOC), for example, in soil porewater leachate would impact the Ti(III) conversion into N₂O. However, we anticipate that future research and efforts may provide insights possibly leading to quantitative conversions of nitrate into N₂O.

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REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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