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## PROTOCOL

# Nitrogen isotope analysis of aqueous ammonium and nitrate by membrane inlet isotope ratio mass spectrometry (MIRMS) at natural abundance levels

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**Rationale:** Existing methods for the measurement of the  $^{15}\text{N}/^{14}\text{N}$  isotopic composition of ammonium and nitrate are either only suitable for labelled samples or require considerable sample preparation efforts (or both). Our goal was to modify an existing analytical approach to allow for natural abundance precision levels.

**Methods:** Published reaction protocols were used to convert ammonium into  $\text{N}_2$  by  $\text{NaOBr}$  and nitrate into  $\text{N}_2\text{O}$  by  $\text{TiCl}_3$ . A membrane inlet system was developed and coupled to an isotope ratio mass spectrometer to allow precise determination of the analytes.

**Results:** Concentrations of  $\geq 35 \mu\text{mol/L N}$  for both ammonium or nitrate could be analysed for  $\delta^{15}\text{N}$  values with precisions of better than 0.9 mUr. While ammonium analyses exhibited a small concentration dependency and an offset of 2.7 mUr at high ammonium concentrations irrespective of the standard isotopic composition, nitrate analysis showed no offset but a blank contribution visible at very low concentrations.

**Conclusions:** The presented method is capable of fast measurement of  $\delta^{15}\text{N}$  values in ammonium and nitrate from aqueous samples with reasonable accuracy at natural abundance levels. It will thus facilitate the application of isotopic methods to studies of nitrogen cycling in ecosystems.

## 1 | INTRODUCTION

Ammonium and nitrate are among the key reactive N-species in the terrestrial nitrogen cycle. The measurement of their isotopic composition can help in many approaches to the study of the dynamics of the nitrogen cycle, either after tracer addition or at natural abundance levels.<sup>1,2</sup>

Numerous approaches have been published and are being used to determine the isotopic composition of ammonium and nitrate, mostly applying some kind of chemical conversion<sup>3–14</sup> or

microbiological methods<sup>15</sup> to convert nitrate or ammonium into gaseous analytes suitable for isotope analysis.

Only a few of these methods are suitable for stable isotope analysis at natural abundance levels.<sup>8,10,13–15</sup> Here we present a modification of an existing method<sup>9,12</sup> of chemical conversion, to extend it to natural abundance level. The membrane inlet isotope ratio mass spectrometry (MIRMS) method presented here allows fast measurement of  $\delta^{15}\text{N}$  values in nitrate or ammonium in an online approach that requires no sample preparation and takes about 15 min per analysis.

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## 2 | MATERIALS AND METHODS

### 2.1 | Chemical reactions

For the conversion of ammonium<sup>9</sup> (NH<sub>4</sub><sup>+</sup>) and nitrate<sup>13</sup> (NO<sub>3</sub><sup>-</sup>), the following reactions were used:

1.  $2 \text{NH}_4^+ + 3 \text{BrO}^- + 2 \text{OH}^- \rightarrow \text{N}_2 + 5 \text{H}_2\text{O} + 3 \text{Br}^-$
2.  $2 \text{NO}_3^- + 8 \text{Ti}^{3+} + 10 \text{H}^+ \rightarrow \text{N}_2\text{O} + 8 \text{Ti}^{4+} + 5 \text{H}_2\text{O}$

### 2.2 | Reagents

Reagents were of reagent grade and used without purification. The NaOBr solution for the oxidation of ammonium to N<sub>2</sub> was prepared by adding 20 mmol Br<sub>2</sub> dropwise to 100 mL 2.5 mol/L NaOH solution in an ice/water bath. The resulting solution has an approximate concentration of 0.2 mol/L sodium bromide (NaBr), 0.2 mol/L NaOBr and 2.1 mol/L NaOH. The solution was stored overnight at approx. 8°C, and 25 mL 0.03 mol/L KI solution was then added. The TiCl<sub>3</sub> solution for the reduction of nitrate to N<sub>2</sub>O was prepared by mixing 10% TiCl<sub>3</sub> in HCl (10 vol%, Merck, Darmstadt, Germany) with 10 % HCl solution in a 1:4 ratio.

### 2.3 | Experimental setup

The sample preparation unit for inorganic nitrogen (SPIN) setup described in Eschenbach et al.<sup>9</sup> was used for the analyte preparation in aqueous solution for both nitrate and ammonium analysis with some important modifications. In brief, sample and reagents are pumped at a rate of 0.4 and 0.2 mL/min, respectively, through separate degassing units (4 m silicone-elastomer tubing, i.d. 0.51 mm; Silastic, Dow Corning, Midland, MI, USA, in 100-mL Schott flasks) that were held under vacuum for the sample side and flushed with He (7 mL/min) for the reagent side. After mixing in a mixing tee, the analytes are converted into N<sub>2</sub> or N<sub>2</sub>O, respectively, in a reaction capillary (i.d. 0.53 mm, length 6.5 m, sample travel time 3 min, for details see below). The solution then enters the membrane unit.

The membrane unit consists of a gas-permeable silicone elastomer tube (length 580 mm, id 0.51 mm; Silastic) held in a glass tubing (length 600 mm, od 6 mm, id 1.5 mm) that is flushed with He (0.8 mL/min) to strip the analytes from the aqueous phase. The He stream passes two Nafion traps (length 200 mm, id 6 mm, od 2 mm) and finely powdered ascarite in a borosilicate tube (length 75 mm,

id 1.4 mm, od 2 mm) before entering the isotope ratio mass spectrometer (Delta plus; Thermo Finnigan, Bremen, Germany) via a ConFlo III interface (Thermo Finnigan).

Different reaction capillaries were used for ammonium and nitrate analysis. For ammonium (which is oxidised to N<sub>2</sub> by NaOBr), a stainless steel capillary (i.d. 0.53 mm, o.d. 0.8 mm) was used at room temperature. For the conversion of nitrate into N<sub>2</sub>O, a fused-silica capillary (i.d. 0.53 mm) was coiled on an aluminum cylinder (diameter 15 cm) that was mounted in a metal box and heated to 80°C. The use of a fused-silica capillary is possible for ammonium analysis; however, the lifetime of the capillary will be limited to weeks. In contrast, the TiCl<sub>3</sub> solution will destroy the stainless-steel capillary within hours.

It is noteworthy that the TiCl<sub>3</sub> solution must be degassed before it enters the heated reaction capillary because insoluble TiO<sub>2</sub> will be formed in the presence of dissolved molecular oxygen and capillary blockages will result.

### 2.4 | Standard materials

Two commercially available salts of ammonium [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with δ<sup>15</sup>N = 0.59 ± 0.06 mUr and NH<sub>4</sub>Cl with δ<sup>15</sup>N = -2.76 ± 0.03 mUr] and nitrate [Ca(NO<sub>3</sub>)<sub>2</sub> with δ<sup>15</sup>N = 7.01 ± 0.08 mUr and KNO<sub>3</sub> with δ<sup>15</sup>N = 0.68 ± 0.11 mUr], respectively, were used as standards to test the presented setup. The salts differ in their isotopic composition by more than 3 mUr to allow for separation of fractionation and blank contribution effects on the results determined by SPIN-MIRMS. It should be noted, however, that for precise determination of correction factors for both fractionation and blank effects, standards with a higher isotopic distance will be needed.

The isotopic composition of the salts as given above was determined by IRMS coupled to an elemental analyser (EA/IRMS).

## 3 | RESULTS

### 3.1 | Conversion efficiency

The conversion efficiency at different temperatures was tested for the oxidation of ammonium to N<sub>2</sub>. The peak areas (i.e. amount of N<sub>2</sub> produced), isotopic composition and its repeatability of the analyte gas were comparable for all tested temperatures (Table 1).

The reaction of nitrate with TiCl<sub>3</sub> yields several products, the most important ones being NO and N<sub>2</sub>O. A quantitative conversion into one analyte gas is therefore not possible. To test the

**TABLE 1** Signal height on *m/z* 28 and δ<sup>15</sup>N values for a standard solution of ammonium (0.7 mmol/L) at different reaction temperatures (n = 3, mean and standard deviation)

		25°C		50°C		80°C	
Ampl. 28	(mV)	2193	± 57	2134	± 32	2263	± 61
δ <sup>15</sup> N	(mUr)	-5.59	± 0.08	-5.51	± 0.20	-5.28	± 0.05

completeness of the reaction (i.e. all nitrate is consumed), we increased the reaction time from 4 min to 6 and 10 min but found no increase in  $\text{N}_2\text{O}$  yield (data not shown).

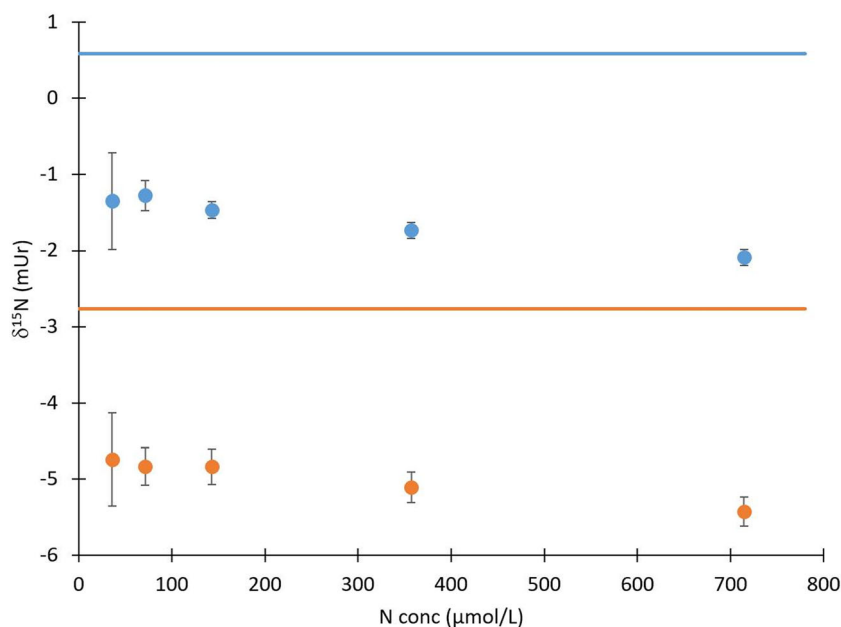
### 3.2 | Isotope analysis

The conversion of ammonium from aqueous solutions into  $\text{N}_2$  as proposed here is subject to an amount-dependent change in isotope delta values (Figure 1). The  $\delta^{15}\text{N}$  results show a concentration dependence, with 0.7 mUr higher values at 35  $\mu\text{mol/L}$  N than at 700  $\mu\text{mol/L}$  N, independent of the isotopic composition of the standard. The relationship appears to be linear ( $r^2 > 0.98$ ) and not

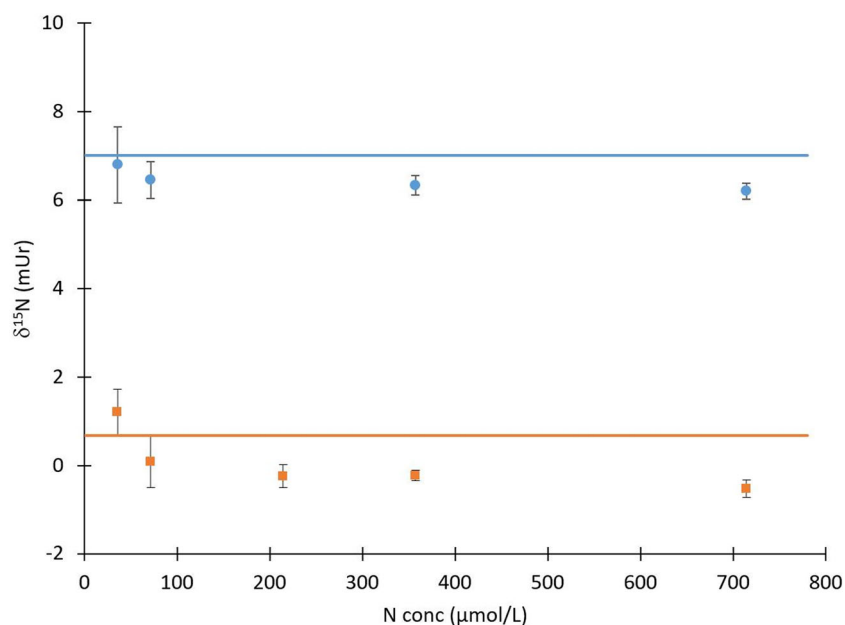
reciprocal. For both salts there was an offset in  $\delta^{15}\text{N}$  of 2.7 mUr between the results of the SPIN-MIRMS measurement at 700  $\mu\text{mol/L}$  and the EA/IRMS measurement of the respective solid material. For nitrate conversion into  $\text{N}_2\text{O}$ , a correlation of the isotope ratio with the reciprocal concentration was observed ( $r^2 > 0.95$ ), which results in a tendency towards higher values for concentrations <70  $\mu\text{mol/L}$  N (Figure 2).

The standard deviation of repeated analyses of a sample was relatively high at 35  $\mu\text{mol/L}$  N for ammonium (0.6 mUr,  $n = 5$ ), but was <0.25 mUr for concentrations between 70 and 700  $\mu\text{mol/L}$  N. For nitrate, the standard deviation was <0.9 mUr for 35  $\mu\text{mol/L}$  N, and <0.5 mUr for concentrations between 70 and 700  $\mu\text{mol/L}$  N.

**FIGURE 1** Apparent isotopic composition of two different standard solutions of ammonium at different concentrations. Mean and standard deviation ( $n = 5$ ). Solid lines give the reference value of the ammonium salts measured by EA/IRMS



**FIGURE 2** Apparent isotopic composition of two different standard solutions of nitrate at different concentrations. Mean and standard deviation ( $n = 5$ ). Solid lines give the reference value of the nitrate salts measured by EA/IRMS



## 4 | DISCUSSION

The setup presented here is a modification of the SPIN-MIMS system described by Eschenbach et al.<sup>12</sup> that has been shown to accurately determine ammonium and nitrate isotopic composition in  $^{15}\text{N}$ -labelled liquid samples using a quadrupole mass spectrometer. The modified system presented here in contrast uses an isotope ratio mass spectrometer for the analysis to allow for higher accuracy and thus analysis also at natural abundance levels. Another important modification made is the transfer of the measurement gas into the mass spectrometer, which is now advectively transported (in a stream of He) at atmospheric pressure but not in a high vacuum. The main reason for this modification was to enable removal of water from the gas stream via Nafion and ascarite traps and avoid the use of cold traps (ethanol/liquid  $\text{N}_2$  at  $-110^\circ\text{C}$ ). The latter led to considerable isotope effects because the system could not be operated at a stable temperature for longer periods of time (e.g. for overnight batch analysis). However, advective transport has the additional advantage that isotope fractionation due to diffusion is minimised.

In addition to the system setup, the reaction conditions were also modified in the present method. For ammonium analysis, we could show that the reaction yield and precision of analysis do not differ substantially when the reaction temperature is decreased from  $80^\circ\text{C}$  to room temperature (Table 1) – it is noteworthy that offline sample preparation to measure N isotopic composition from solids was also performed at room temperature.<sup>16,17</sup> The benefit of the lower reaction temperature is that elevated temperatures lead to a considerable buildup of contaminants on (among others)  $m/z$  29, which hampers precise isotope ratio determination. The nature of this contaminant(s) could not be elucidated, but it has been proven to be quite persistent if once present in the sample preparation unit (flushing with He for more than 5 days is necessary to remove these contaminants). Therefore, the reaction at room temperature was chosen even though the reaction yield was marginally lower than at  $80^\circ\text{C}$ .

For the analysis of nitrate as  $\text{N}_2\text{O}$ , a reaction using  $\text{VCl}_3$  with subsequent alkalisation was proposed by Eschenbach et al.<sup>18</sup> While this reaction is in principle usable for the determination of isotope ratios of nitrate at natural abundance (data not shown), the use of  $\text{TiCl}_3$  avoids the mid-reaction addition of  $\text{NaOH}$ , which simplifies the method. Moreover, titanium salts are less poisonous than vanadium salts. Therefore, the reaction with  $\text{TiCl}_3$  as proposed by Altabet et al.<sup>13</sup> was used to produce  $\text{N}_2\text{O}$  from nitrate. While Altabet et al.<sup>13</sup> used this reaction at room temperature for 24 h, we could show that this reaction proceeds much faster at elevated temperatures and the product yield does not increase after 3 min at  $80^\circ\text{C}$ . With our setup we cannot evaluate the completeness of the reaction because the transfer of the analyte gas from the liquid phase into the gas phase in the membrane unit is not quantitative, and we therefore cannot differentiate between the reaction and gas transfer yield, respectively. We do, however, observe a peak on  $m/z$  30 which indicates that  $\text{NO}$  is also being formed with an estimated yield of about 30%.

As we use a high  $\text{HCl}$  concentration and high temperatures to speed up the reaction, exchange of nitrate oxygen with water does take place, and the measurement of  $\delta^{18}\text{O}$  values is not possible with our reaction scheme (data not shown). This is in contrast to the approach of Altabet et al.<sup>13</sup> which allows for the determination of the nitrate oxygen and nitrogen isotopic composition in one single run.

The isotope composition of ammonium measured by SPIN-MIRMS has an offset of about 3 mUr to the values of the respective solid materials measured by EA/IRMS. Furthermore, there is a linear relationship between the measured isotopic composition and the ammonium concentration in the solution. For both ammonium standards, the difference in the apparent  $\delta^{15}\text{N}$  value is 0.7 mUr between the 0.5 and 10 mg/L N samples. We can exclude this difference being due to an  $\text{N}_2$  blank, because this would lead to a linear relationship for the reciprocal sample amounts.<sup>19</sup> As it has been shown that the reaction yield is not affected by the sample/reagent ratio,<sup>12</sup> we speculate that the offset and amount dependency observed here could be a result of fractionation due to incomplete gas transfer in the membrane inlet, with lower yields and thus increasing apparent fractionation for higher sample amounts.

The nitrate isotopic composition measured by SPIN-MIRMS is very similar to the values determined by EA/IRMS. The correlation of the isotope ratios with the reciprocal concentration indicates a blank contribution with high delta values. A reason for this blank could be the presence of  $\text{CO}_2$ . However, due to the ascarite trapping this blank is very low and only affects measurements at concentrations  $<70 \mu\text{mol/L}$ .

It has been shown previously that cross-contamination from ammonium and nitrate is not observed,<sup>9</sup> but  $\text{TiCl}_3$  will convert nitrite into  $\text{N}_2\text{O}$  along with nitrate. The analysis of ammonium may be affected by the presence of amines; while urea will be converted as efficiently as ammonium, free amino acids give about 20% of the ammonium yield (data not shown). This finding is especially important for studies with manure application. We suspect that other methods using  $\text{NaOBr}$  as reactant<sup>9–11</sup> are subject to the same limitation. Matrix effects for chloride or sulfate at a concentration of 0.5 mM were not observed (data not shown). However, the offset and amount dependency of the SPIN-MIRMS measurements clearly show that careful referencing (ideally matrix sensitive) with standard material is necessary when using this method.

While the setup has been specifically developed for the analysis of natural abundance samples, it can also be used for the analysis of labelled samples. For labelled samples, nitrite can also be analysed after conversion into nitrous monoxide ( $\text{NO}$ ) by  $\text{KI}$  (see Eschenbach et al.<sup>12</sup>) while the  $\text{NO}^+$  fragment ion that is produced from the  $\text{N}_2\text{O}$  reference gas in the ion source can be used for referencing.

## 5 | CONCLUSIONS

The method presented here allows fast, reliable and accurate measurement of the nitrogen isotope composition of ammonium and nitrate from aqueous samples at concentrations of  $35 \mu\text{mol/L}$  N or



higher. Although there are methods that are more precise and work at lower concentrations,<sup>8,10,13,15</sup> the key benefit of our method is the speed of analysis as there is no sample pretreatment required. This will be beneficial for using isotopic methods to study nitrogen cycling in ecosystems receiving anthropogenic input of reactive N as atmospheric N deposition or fertiliser<sup>20</sup> where ammonium and nitrate are typically above our detection limits. Moreover, our improved analytical speed will help to better assess the complex spatial and temporal dynamics of nitrogen turnover processes<sup>21</sup> in future studies. As no sample pretreatment is necessary, our method may even be used for quasi real-time analysis of dynamic experiments. However, the alternating analysis of nitrate and ammonium requires two separate sample preparation lines because we could not identify a reaction capillary that is compatible with both  $\text{TiCl}_3$  and  $\text{NaOBr}$ .

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## PEER REVIEW

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