Measuring $^{15}$N–$\text{NH}_4^+$ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations

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Abstract

We present a method for measuring $^{15}$N–$\text{NH}_4^+$ in marine, estuarine and fresh waters. The advantage of this method is that it is broadly applicable to all types of water and it allows measurements in samples with lower ammonium concentrations than has previously been possible. The procedure is a modification of the ammonia diffusion method and uses large sample volumes (often 4 l) to obtain sufficient N for isotope ratio mass spectrometric analysis. Large volume samples have not previously been used with the diffusion procedure because isotopic fractionation occurs due to incomplete recovery of ammonium. However, the method we present accounts for this fractionation and allows precise correction of measured $\delta^{15}$N values.

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1. Introduction

The use of stable isotopes in ecological research is becoming increasingly common (Peterson and Fry, 1987; Lajtha and Michener, 1994). Much of this work involves $^{15}$N. The stable isotopic composition of most particulate material is relatively simple to measure, but isotope ratio measurements in dissolved inorganic pools are more difficult. In order to measure $^{15}$N–$\text{NH}_4^+$, the $\text{NH}_4^+$ must be removed from solution and concentrated in a form that can be introduced to a mass spectrometer. During this process, sufficient N must be obtained to make a measurement on a mass spectrometer (1–10 $\mu$mol is typically required) while avoiding contamination and fractionation. These problems have made analysis of $^{15}$N-DIN in low concentration samples difficult or impossible.

Our objective was to develop a relatively simple and general method for measuring $^{15}$N–$\text{NH}_4^+$. Although several methods have been published (Table 1), most require relatively high ammonium concentrations and some are specific to freshwater. Our
emphasis was on devising a procedure that works for marine, estuarine and freshwater samples with a wide-range of ammonium concentrations, particularly samples with less than 2 μM ammonium, the approximate lower limit for published methods. The method we present is a modification of the ammonia diffusion procedure (Adamsen and Reeder, 1983; Brooks et al., 1989; Sørensen and Jensen, 1991; Sigman et al., 1997) and uses large volumes to trap enough N for analysis of samples with low ammonium concentrations.

The diffusion procedure collects ammonium dissolved in water by converting NH$_4^+$ to NH$_3$ under basic conditions and then trapping the NH$_3$ on an acidified glass fiber filter. The method was developed for use with soil extracts where NH$_4^+$ concentrations are typically >50 μM (Adamsen and Reeder, 1983; Brooks et al., 1989; Liu and Mulvaney, 1992). At these concentrations, only small volumes (~25 ml) of sample are required to obtain sufficient NH$_4^+$-N for analysis. To obtain enough N when the NH$_4^+$ concentration is lower (as it is in most natural waters), larger volume diffusions are required.

Larger volume diffusions have not been done in the past because ammonium is not completely recovered. When ammonium recovery is less than 100%, isotope fractionation occurs and measured $^{15}$N:$^{14}$N is erroneously light (depleted of $^{15}$N). The fractionation factor for the conventional ammonia diffusion process had not been defined, so $^{15}$N values for samples with incomplete recovery were not usable. Our modification of the diffusion method allows reliable correction of $\delta$-$^{15}$N--NH$_4^+$ values when ammonium recovery is less than 100%, thus lowering the minimum ammonium concentration that can be analyzed for isotopic composition.

2. Methods

2.1. Ammonia diffusion protocol

The following protocol outlines the general ammonia diffusion procedure (Fig. 1). The protocol is written assuming that diffusions will be started in the field immediately after sample collection, thus avoiding potential changes in the ammonium pool over time. However, if NH$_4^+$ concentration can not be adequately estimated (in order to determine appropriate diffusion volume), samples can be brought back to the lab for measurement of NH$_4^+$ concentration before starting diffusions.

![Outline of NH$_4^+$ Diffusion Procedure](image-url)

**Fig. 1. Outline of ammonia diffusion procedure.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Selected Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distillation</td>
<td>Velinsky et al., 1989</td>
</tr>
<tr>
<td>Diffusion</td>
<td>Sørensen and Jensen, 1991; Lory and Russelle, 1994</td>
</tr>
<tr>
<td>Mercury precipitation</td>
<td>Fisher and Morrissey, 1985</td>
</tr>
<tr>
<td>Ion exchange/diffusion</td>
<td>Downs et al., in review</td>
</tr>
<tr>
<td>Indophenol</td>
<td>Dudek et al., 1986</td>
</tr>
<tr>
<td>Hypobromite oxidation</td>
<td>Risgaard-Peterson et al., 1995</td>
</tr>
</tbody>
</table>

Table 1

$^{15}$N--NH$_4^+$ methods with selected references
Step 1: Field-filter sample (through precombusted GF/F) into diffusion bottle. The bottles may be glass or high density polyethylene (HDPE). The sample volume depends on anticipated NH$_4^+$ concentration. Since it is most convenient to use the same volume for all samples in a given collection, use the smallest volume that will yield sufficient N for mass spectrometric analysis from the sample with the lowest ammonium concentration.

Step 2: Prepare the standards. We take nanopure water to the field in diffusion bottles and then add an ammonium stock solution (of known isotopic composition) to the bottles in the field to achieve an ammonium concentration similar to that expected of the samples. The standard diffusion bottles must be of the same size as the sample diffusion bottles and should be treated identically to the samples because fractionation of standards will be used to correct for fractionation of samples.

Step 3: Add NaCl to the diffusion bottles (if necessary). Freshwater samples receive 50 g NaCl/l. If no salt is added, filter packs (described below) swell and sometimes burst due to water movement into the filter packs via osmosis. Marine samples require no additional salt, whereas estuarine samples of intermediate salinity should be amended with NaCl to achieve a final salinity of approximately 35 ppt. NaCl (reagent grade) should be ashed at 450°C for 4 h prior to addition.

Step 4: Add the ammonia trap (filter pack). Ammonia traps are made with 1 cm diameter GF/D filters (Whatman #1823010), acidified with 25 µl 2 M H$_2$SO$_4$, sandwiched between two 2.5 cm diameter 10 µm pore-size Teflon membranes (Millipore LCWP 02500).

Ammonia trap construction:
(A) Combust GF/D disks at 400°C for 4 h or more.
(B) Use an aluminum foil sheet over several paper towels as a working area. The aluminum foil should be cleaned with ethanol.
(C) Place GF/D on Teflon membrane.
(D) Pipette 25 µl 2 M H$_2$SO$_4$ onto the GF/D.
(E) Place a second Teflon membrane over the GF/D.
(F) Seal the two Teflon membranes together by pressing with a cylinder of about 2 cm diameter. The tops of glass scintillation vials work well. The filter packs can be stored for several weeks without contamination by sealing in an air-tight container (Sigman et al., 1997).

Step 5: Add 3 g/l reagent grade MgO. Diffusion bottles should be sealed tightly immediately after adding MgO. The MgO buffers the samples at pH ~ 9.7, causing NH$_4^+$ to convert to NH$_3$. Prior to use, MgO should be ashed at 450°C for 4 h.

Step 6: Incubate the samples. The diffusion rate depends on temperature as well as how vigorously the samples are shaken. For consistency, we incubate the samples for 2 weeks at 40°C on a custom-made shaker/incubator. This shaker/incubator maintains constant incubation conditions, is high capacity (holds up to 36 4-l diffusions simultaneously) and is relatively inexpensive. The diffusion rate could be increased by incubating at a higher temperature, but high temperatures also increase the likelihood of DON breakdown and thus contamination of the ammonium pool during the diffusion. Alternatively, samples could be incubated at room temperature (while shaking), but this will decrease the rate of NH$_4^+$ recovery.

Step 7: After the incubation, remove the filter pack from the diffusion bottle, place in a desiccator with silica gel and an open container of concentrated sulfuric acid (to remove trace ammonia) and dry (1–2 days).

Step 8: When dry, place the filter packs in individual vials (with sealing caps) for storage.

Step 9: Shortly before combustion for isotopic analysis, separate the Teflon membranes with forceps and remove the glass fiber disk. If analyzing samples by a coupled elemental analyzer-isotope ratio mass spectrometer (Fry et al., 1996), pack disks into tin boats on the day of analysis as the tin reacts with the sulfuric acid in the GF/D disk.

Step 10: Determine the fractionation of standards (observed $\delta^{15}$N – actual $\delta^{15}$N) and add this value to observed sample del values to correct for fractionation.

2.2. Method development: Laboratory experiments

The first experiment examined the relationship between sample volume and fractionation. Triplicate diffusions were done for volumes of 3.0, 1.5, 1.0, 0.5 and 0.2 l. We prepared a 10 µM NH$_4^+$ artificial
seawater standard with $\delta^{15}N-NH_4^+$ equaling $-1.4\%\epsilon$, where $\delta^{15}N = \left(\frac{R_{SA}}{R_{ST}} - 1\right) \times 10^3$. $R_{SA} = ^{15}N/^{14}N$ and the results are expressed as %e deviation of the sample (SA) from the standard (ST), N$_2$ in atmospheric air ($\delta^{15}N_{AIR} = 0\%\epsilon$). Although we were most interested in measuring $\delta^{15}N-NH_4^+$ in samples with ammonium concentrations less than 2 $\mu$M, we used a 10 $\mu$M standard solution in this and the following experiment to insure we would recover enough N in the small volume and short incubation treatments and to minimize the potential problem of blanks at this stage of method development. Later we investigate whether large volume, low concentration samples follow the same relationship.

In the second experiment we investigated the relationship between incubation time and observed $\delta^{15}N$, while holding volume constant. Samples were artificial seawater amended to 10 $\mu$M $NH_4^+$ and incubated on the shaker/incubator at 40°C for 1–14 days.

Finally, the diffusion procedure was tested more generally by using samples with different ammonium concentrations (0.5 to 10 $\mu$M), isotopic enrichments (up to 100%e), volumes (0.2 to 6 l) and incubation periods (1–14 days). Results of these experiments were expressed as percent recovery of ammonium-N versus fractionation (observed minus actual $\delta^{15}N$).

2.3. Method development: Blanks

At low ammonium concentrations, blanks associated with reagents or the sample may significantly influence both the mass and isotopic composition of N recovered during the diffusion procedure. To measure the reagent blank, we ran a series of 4-l diffusions using distilled water. These samples received 200 g NaCl, 12 g MgO and a filter pack and were incubated for 2 weeks at 40°C on the shaker/incubator.

There is also a possibility of a blank associated with the breakdown or conversion of some component of the sample and we will refer to this N as the dissolved organic nitrogen (DON) blank. To estimate the magnitude of the DON blank, we first determined the amount of N we expected to recover based on measured ammonium concentration and expected percent recovery. We subtracted this value from the amount of N we actually recovered (determined with the mass spectrometer) and attributed the remainder to a DON blank. We quantified the DON blank in samples collected from (1) the Sargasso Sea, (2) Great Harbor in Woods Hole, Massachusetts, (3) the Childs River on Cape Cod, Massachusetts and (4) Upper Ball Creek, North Carolina.

2.4. Method development: Field trials

We further tested the method using marine, estuarine and freshwater samples from Cape Cod, Massachusetts. The marine sample came from Great Harbor in Woods Hole, the estuarine sample was from the Quashnet River estuary and the freshwater sample was from Miles Pond. Four replicate diffusions and four standards were done for each sample. Standard concentrations were 0.5, 1 and 3 $\mu$M. Given the low ammonium concentrations of the samples, we were not able to compare the results of our method to the performance of previously published methods; other methods do not allow measurement at these concentrations. Instead, the precision of replicates (for samples and standards) was used as an indicator of method performance. Sample volumes were 4 l and incubations were 2 weeks at 40°C on the shaker/incubator.

3. Results and discussion

3.1. Laboratory experiments

Measured $\delta^{15}N-NH_4^+$ was highly volume-dependent (Fig. 2). Although the 200 ml diffusion was within 0.2%e of the actual value, fractionation increased linearly to 10%e for 3-l samples. If uncorrected, this magnitude of fractionation would be unacceptable in almost all natural abundance studies as well as most $^{15}N$-tracer addition studies. To avoid large errors due to fractionation, diffusions would have to be limited to 200 ml or less. Since our mass spectrometer generally requires 1 $\mu$mol (14 $\mu$g) N per sample, the minimum NH$_4^+$ concentration sample we can analyze for $^{15}N$ without correcting for fractionation is 5 $\mu$M (14 $\mu$g/0.2 l = 5 $\mu$M). This 5 $\mu$M limit is too high to be useful for many natural water samples.
Length of incubation also strongly influenced measured $\delta^{15}$N (Fig. 3). After two weeks incubating 1-l samples, fractionation was approximately 4%, compared to a fractionation of more than 13% after one day. As was the case with the different volume diffusions, fractionation also varied linearly with incubation time.

The relationships between diffusion volume and fractionation, and incubation time and fractionation, reflect an underlying dependence between percent recovery and fractionation. Over a wide range of ammonium concentrations, isotopic enrichments, incubation periods and sample volumes, the relationship between percent recovery and fractionation was linear when percent recovery exceeded 45% ($r^2 = 0.97$, Fig. 4a). Therefore, the regression describing this relationship ($y = 0.2x - 19.95$) allows correction for fractionation based on percent recovery (when recovery exceeds 45%). For example, if recovery was 80%, fractionation would be expected to be $(0.2)(80) - 19.95 = -3.95$.

When recoveries of less than 45% are included, the relationship between percent recovery and fractionation is no longer linear (Fig. 4a). The curvilinear relationship we obtained is as would be expected for an accumulating product in a single step unidirectional reaction (Mariotti et al., 1981). The per mil fractionation factor ($\epsilon$), calculated from the slope of the regression of $-(f)(\ln f)/(1-f)$ versus $\delta^{15}$N–NH$_4^+$ (observed – actual), where $f$ equals the percent of the substrate remaining (in this case, NH$_4^+$) (Mariotti et al., 1981) was $-16.39$ (Fig. 4b). If the four data points where recovery was 100% are excluded from the regression, the slope (i.e. $\epsilon$) increases to $-19.03$ and $r^2 = 0.97$. Interestingly, although the diffusion involves several steps including ammonia diffusion out of the water samples as well as diffusion back onto the filter pack (Fig. 1), this mixed reaction gives a fractionation that approaches that expected for ammonia volatilization under equilibrium conditions (Mariotti, 1982).

There is a suggestion of a sinusoidal shape to the curve in Fig. 4b, indicating a possible variation in mechanism of fractionation as a function of yield. We do not know what the rate-limiting step is in the diffusion procedure, but suspect that it may be the diffusion of NH$_4^+$ across the Teflon membrane. Thus, fractionation may initially be a function of kinetic effects until a quasi-equilibrium between vapor and aqueous phases is established, at which point equilibrium effects may dominate.

The variability about the regression line in Fig. 4a is likely due to the fact that determination of percent recovery using a mass balance approach is quite sensitive to small errors when the quantity of ammo-
Fig. 4. (A) Relationship between percent $\text{NH}_4^+$ recovered during diffusion and isotopic fractionation. (B) Calculation of per ml fractionation factor ($\varepsilon$) based on slope of the regression of $[-f \ln f/(1-f)]$ versus $[\delta^{15}N-\text{NH}_4^+ (\text{observed} - \text{actual})]$, where $f$ equals the percent of the $\text{NH}_4^+$ remaining. All samples were artificial seawater standards with $\text{NH}_4^+$ concentration ranging from 0.5 to 10.0 $\mu M$ and were incubated at 40°C on the shaker/incubator. $\delta^{15}N-\text{NH}_4^+$ of samples ranged from −1.4 to 100‰.

Nium being examined is small. Error in percent recovery estimates arises during mass determination on the mass spectrometer as well as in initial $\text{NH}_4^+$ concentration determination. This is particularly problematic for samples with low ammonium concentrations, as accurate concentration and recovery measurements are more difficult to achieve and errors are proportionally greater.

A recent study involving 106 laboratories clearly demonstrated the difficulty of measuring ammonium, particularly at low ammonium concentrations but also at the relatively high concentration of 4.56 $\mu M$ (Aminot et al., 1997). Since the scientific community does not consistently measure ammonium concentration with high accuracy, correction of fractionation based on calculated percent recovery of ammonium is tenuous. Therefore, we run standards with each set of samples (sample and standard volumes identical, ammonium concentrations similar) and use fractionation of standards to correct sample values. We have shown that fractionation is consistent across samples incubated under identical conditions, so this approach is feasible and eliminates error associated with determination of percent recovery.
Table 2
Dissolved organic nitrogen blanks associated with 4 l diffusions of water samples from the Sargasso Sea, two sites on Cape Cod, Massachusetts, (Woods Hole Great Harbor and Childs River) and Upper Ball Creek, North Carolina. DON blanks were estimated by subtracting the reagent blank (0.25 μmol) and the expected mass of NH₄⁺ to be recovered (at 46% recovery) from the total mass of N collected in acid traps after two week diffusions at 40°C. Nitrogen on the acid traps was measured by mass spectrometry. Values for DON blanks are means ± S.E. for 4 diffusions. Standard errors for the Sargasso Sea and Great Harbor sites reflect variability among sub-samples, while standard errors for Childs River and Upper Ball Creek represent spatial and temporal variability.

<table>
<thead>
<tr>
<th>Site</th>
<th>Conductivity (ms cm⁻¹)</th>
<th>NH₄⁺ (μM)</th>
<th>DON (μM)</th>
<th>DON blank (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sargasso Sea</td>
<td>~ 50</td>
<td>&lt;0.1</td>
<td>5</td>
<td>−0.02 ± 0.09</td>
</tr>
<tr>
<td>Great Harbor, Woods Hole, Massachusetts</td>
<td>~ 50</td>
<td>&lt;0.1</td>
<td>9</td>
<td>0.11 ± 0.08</td>
</tr>
<tr>
<td>Childs River, Massachusetts</td>
<td>&lt;0.5</td>
<td>0.79</td>
<td>40</td>
<td>0.40 ± 0.34</td>
</tr>
<tr>
<td>Upper Ball Creek, North Carolina</td>
<td>&lt;0.5</td>
<td>&lt;0.36</td>
<td>24</td>
<td>0.10 ± 0.09</td>
</tr>
</tbody>
</table>

3.2. Blanks

At low ammonium concentrations, blanks may significantly influence both the apparent percent recovery and measured isotopic ratios. When ammonium diffusions were done on 4-l deionized water samples with no added NH₄⁺, 0.25 ± 0.03 μmol of N was recovered. Assuming that the deionized water contains no ammonium, this result indicates that a blank of 0.25 μmol is associated with the combination of 200 g NaCl, 12 g MgO and a filter pack (reagent blank). Although the reagent blank is small, it will make a significant contribution to the total amount of N recovered when conducting diffusions on samples with sub-micromolar concentrations of ammonium. Hence, the δ¹⁵N of the reagent blank should be measured, and corrected for when estimating the δ¹⁵N of NH₄⁺ at sub-micromolar concentrations.

In addition to the reagent blank, there may also be a blank coming from dissolved organic nitrogen (DON) in field samples (Table 2). Although 4-l diffusions of Sargasso seawater showed no measurable contamination from DON, diffusions of seawater collected near Woods Hole, Massachusetts and samples from Childs River, Massachusetts and Upper Ball Creek, North Carolina, showed evidence of low-level DON contamination (Table 2). As discussed for the reagent blank, the DON blank could make a significant contribution to the total amount of N recovered during diffusion of low concentration samples. It may be difficult to correct for this blank.

Table 3
Application of protocol to a set of marine, estuarine and freshwater samples. Standards were run in three concentrations (0.5, 1.0, 3.0 μM) and both samples and standards were 4-l and had 4 replicates (except for one marine replicate and one 3.0 μM standard that were outliers and were not used). Observed and corrected δ¹⁵N–NH₄⁺ (%) are means ± standard error. Samples collected and diffusions begun on 12 September 1997 and incubations were for two weeks at 40°C on the shaker/incubator.

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>Actual δ¹⁵N–NH₄⁺ (%)</th>
<th>Observed δ¹⁵N–NH₄⁺ (%)</th>
<th>Fractionation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standards</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.6</td>
<td>−10.15 ± 0.23</td>
<td>10.75</td>
</tr>
<tr>
<td>1.0</td>
<td>0.6</td>
<td>−9.55 ± 0.35</td>
<td>10.15</td>
</tr>
<tr>
<td>3.0</td>
<td>0.6</td>
<td>−10.23 ± 0.72</td>
<td>10.83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Description</th>
<th>Concentration (μM)</th>
<th>Observed δ¹⁵N–NH₄⁺ (%)</th>
<th>Corrected δ¹⁵N–NH₄⁺ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Great Harbor, Woods Hole (seawater)</td>
<td>1.1</td>
<td>−3.50 ± 0.70</td>
<td>7.08 ± 0.73</td>
</tr>
<tr>
<td>Quashnet River (estuarine)</td>
<td>2.1</td>
<td>−2.28 ± 0.28</td>
<td>8.30 ± 0.36</td>
</tr>
<tr>
<td>Miles Pond (freshwater)</td>
<td>1.6</td>
<td>−7.75 ± 0.53</td>
<td>2.83 ± 0.57</td>
</tr>
</tbody>
</table>

*Sample δ¹⁵N–NH₄⁺ was calculated by adding mean fractionation of standards (10.58%) to the observed sample δ¹⁵N–NH₄⁺, SE_corrected = sqrt(SE²_standard + SE²_sample).
and, hence, the magnitude of DON contamination may set the lower concentration limit for $^{15}$N–NH$_4^+$ determination at some sites.

3.3. Field trials

The application of the method illustrates a number of important points about the procedure (Table 3). First, fractionation is independent of ammonium concentration, as standards between 0.5 and 3.0 $\mu$M fractionated to similar degrees (10.15–10.83‰). This is important because it demonstrates that correction of the sample $\delta^{15}$N–NH$_4^+$ values is feasible even if standard and sample ammonium concentrations differ. Secondly, it shows that the method performs well at concentrations down to at least 0.5 $\mu$M.

The standard error for the standards was 0.22‰. Measured $\delta^{15}$N–NH$_4^+$ values for the three samples had precisions similar to the standards, with the propagated standard error for corrected sample $\delta^{15}$N–NH$_4^+$ values ranging from 0.36 to 0.73‰. Of the 24 diffusions (samples and standards) in this experiment, two values were outliers (a 3.0 $\mu$M standard and a marine sample) and were discarded. Interestingly, both outliers had anomalously low measured $\delta^{15}$N–NH$_4^+$ values (more than 3.5‰ lower than other replicates) and also had much lower recoveries of ammonium. We do not know what caused the outliers, but sometimes a filter pack sticks to the top of the diffusion bottle and this may slow the diffusion process resulting in increased fractionation.

4. Conclusions

The method we have presented is relatively simple and can be initiated without specialized equipment, thus facilitating sampling at remote locations or at sea. Although diffusions generally last two weeks, the labor involved per sample is minimal which allows many samples to be run concurrently.

Although our method allows measurement of $\delta^{15}$N–NH$_4^+$ at lower ammonium concentrations than has previously been possible, there are still instances when ammonium levels are too low. Our initial thought was that we could further increase diffusion volume in order to increase the amount of N recovered. However, we found that this was not the case, since percent recovery drops faster than the total mass of N captured increases with increasing sample volume. This relationship between percent recovery and total N leads to an upper limit of about 4 l that should be used for diffusion. At this volume, the absolute magnitude of N recovered reaches a maximum.

To measure $\delta^{15}$N–NH$_4^+$ at even lower concentrations, we have three options: (1) incubate samples for longer periods or at higher temperatures in order to increase percent recovery, (2) spike samples with ammonium of known isotopic composition and back-calculate sample isotopic composition using a mass balance approach or (3) improve mass spectrometer methods for measuring low mass N samples. The third approach holds the most promise, as methods have already been developed which allow measurement of $^{15}$N on samples sizes as low as 0.1 $\mu$mol (Fry et al., 1996). These improved mass spectrometer techniques should allow incubation of smaller volume samples for shorter periods of time and at lower temperatures, thus minimizing problems of DON blanks and increasing sample throughput. Ultimately, however, experimental and DON blanks will set the lower limit for $^{15}$N–NH$_4^+$ diffusions.

Acknowledgements

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