Sensitive Measurement of NH4+ 15N/14N (δ15NH4+) at Natural Abundance Levels in Fresh and Saltwaters

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We report a new method for determining the $^{15}N/^{14}N$ of NH_4^+ at natural abundance level in both freshwater and seawater. NH_4^+ is first quantitatively oxidized to NO_2^- by hypobromite (BrO^-) at pH $\sim$ 12. After the addition of sodium arsenite to consume excess BrO^-, yield is verified by colorimetric NO_2^- determination. NO_2^- is further reduced to N_2O using a 1:1 sodium azide and acetic acid buffer solution using previously established procedures. The product N_2O is then analyzed for isotopic composition using a continuous flow purge and cryogenic trap system coupled to an isotope ratio mass spectrometer. Reliable $\delta^{15}N$ values (standard deviation is 0.3‰ or better) are obtained over an NH_4^+ concentration range of 0.5–10 μM using 20 mL volumes of either freshwater or seawater samples. Higher concentration samples are readily diluted to lower concentration. Preexisting NO_2^- is removed by treatment with sulfanilic acid. There is no interference from any of the nitrogen-containing compounds tested except short-chain aliphatic amino acids (i.e., glycine) which typically are present at very low environmental concentrations. As compared to published methods, our approach is more robust, readily applicable at low concentrations and small sample volumes, and requires less time for preparation and analysis.

Ammonium (NH_4^+) is a key dissolved inorganic nitrogen (DIN) species for aquatic nitrogen cycles. Though not necessarily dominant by mass, it is often predominant with respect to flux. NH_4^+ is produced primarily by the breakdown and remineralization of organic forms of nitrogen and consumed by autotrophic assimilation and reincorporation into organic molecules, chemosynthetic nitrification to NO_2^-, and anammox oxidation by NO_2^- to N_2.[1] Recent analytical innovations have stimulated rapid expansion of natural abundance nitrogen isotope ratio ($^{15}N/^{14}N$; measured as $\delta^{15}N$) for DIN species such as NO_3^- as an in situ tracer of N cycle processes. However, application to NH_4^+ has by comparison lagged behind. Several methods for $NH_4^{+-}\delta^{15}N$ ($\delta^{15}NH_4^+$) determination have been developed over the past several decades, but none are robust at the low concentrations usually found in natural waters.

Quantitative extraction of NH_4^+ from solution and conversion to a gaseous species suitable for analysis by an isotope ratio mass spectrometer (IRMS; see the Supporting Information) has been the principal analytical impediment. Distillation under alkaline conditions of NH_3 with subsequent conversion to N_2 gas is time-consuming, technically demanding, and subject to cross-contamination and fractionation.[2–5] The diffusion method is similar in concept except NH_3 passes through a membrane into an acidic medium and is trapped as an NH_4^+ salt.[4,6–8] However, this approach is both labor- and time-intensive and not very reliable at low concentrations due in part to dissolved organic nitrogen (DON) breakdown.[4] Mercury precipitation and cation exchange are reported to be only suitable for freshwater samples.[5,9,10] An organic reaction with NH_3 producing indophenol permits organic or solid-phase extraction but was shown to have a significant reagent blank (0.2 μmol N) and was not reliable at $[NH_4^+]$ < 5 μM.[9] When analyzed using GC/MS, this approach works well for ^{15}N-enriched samples[5] but appears to not be applicable for natural abundance level analysis.

Most of these methods produce N_2 gas as an end-product analyte for IRMS using either the Rittenberg oxidation or elemental analyzer (EA) combustion. As a result, they require relatively large amounts of material ($\sim$1 μmol N) due to problems associated with incidental atmospheric contamination and the relative difficulty of trapping and concentrating N_2. Accordingly, there has been no robust and sensitive method available for $\delta^{15}NH_4^+$ determination at the low natural concentrations found in freshwater and marine environments.

N_2O is an excellent alternative to N_2 as an IRMS analyte due to ease of cryogenic concentration/purification, relative inertness, as well as scarcity in the atmosphere. The isotopic composition of N_2O can now be routinely measured[11–14] using commercially

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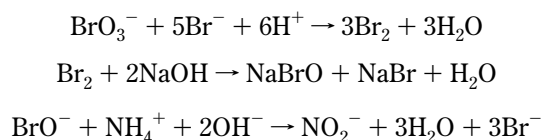* Corresponding author. Phone: 508-292-2520. E-mail: linzhang0508@gmail.com.
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(1) Altabet, M. A. Isotopic Tracers of the Marine Nitrogen Cycle. In *Marine Organic Matter: Chemical and Biological Markers*; Volkman, J., Ed.; Springer-Verlag: Berlin, Heidelberg, 2005; pp 251–293.

(2) Feast, N. A.; Dennis, P. F. *Chem. Geol.* **1996**, *129*, 167–171.
(3) Lehmann, M. F.; Bernasconi, S. M.; McKenzie, J. A. *Anal. Chem.* **2001**, *73*, 4717–4721.
(4) O'Deen, W. A.; Porter, L. K. *Anal. Chem.* **1980**, *52*, 1164–1166.
(5) Preston, T.; Bury, S.; Presing, M.; Moncoiffe, G.; Slater, C. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 959–964.
(6) Holmes, R. M.; McClelland, J. W.; Sigman, D. M.; Fry, B.; Peterson, B. J. *Mar. Chem.* **1998**, *60*, 235–243.
(7) Risgaard-Petersen, N.; Rysgaard, S.; Revsbech, N. P. *Soil Sci. Soc. Am. J.* **1995**, *59*, 1077–1080.
(8) Sebilo, M.; Mayer, B.; Grably, M.; Billiou, D.; Mariotti, A. *Environ. Chem.* **2004**, *1*, 99–103.
(9) Dudek, N.; Brezinski, M. A.; Wheeler, P. *Mar. Chem.* **1986**, *18*, 59–69.
(10) Fisher, T. R.; Morrissey, K. M. *Mar. Chem.* **1985**, *16*, 11–21.
(11) Casciotti, K. L.; Sigman, D. M.; Hastings, M. G.; Bohlke, J. K.; Hilkert, A. *Anal. Chem.* **2002**, *74*, 4905–4912.
(12) McIlvin, M. R.; Altabet, M. A. *Anal. Chem.* **2005**, *77*, 5589–5595.

available purge and trap systems (PT) coupled via continuous He carrier flow (CF) to an IRMS. Furthermore, a robust and simple chemistry exists using azide to convert NO_2^- to N_2O with low N mass requirement for this application (10 nmol[12]). Due to the nature of the reagent and mechanism of the reaction this approach is virtually blank free and highly specific.

Here we report a robust and quantitative method for conversion of NH_4^+ to NO_2^- suitable for subsequent azide conversion to N_2O and $\delta^{15}N$ analysis. Previous workers have investigated the oxidation of NH_4^+ by hypobromite (BrO^-) and found a variety of reaction products depending on experimental conditions.[15] The formation of N_2 by the Rittenberg technique is only one of these.[16] With a much lower final BrO^- concentration (50 μM as compared to ~3 M for the Rittenberg reaction), produced from bromate and bromide as shown below, NH_4^+ can be oxidized to NO_2^- with >90% recovery in freshwater and seawater samples.[17]

$$BrO_3^- + 5Br^- + 6H^+ \rightarrow 3Br_2 + 3H_2O$$

$$Br_2 + 2NaOH \rightarrow NaBrO + NaBr + H_2O$$

$$BrO^- + NH_4^+ + 2OH^- \rightarrow NO_2^- + 3H_2O + 3Br^-$$

Our study focused on modifying and testing the experimental parameters necessary to achieve the required precision for natural abundance $^{15}N/^{14}N$ measurement under oceanographic and other environmental conditions. To increase the usefulness of this technique we have also adapted the method of Yakushiji and Kanda[18] to remove any naturally occurring NO_2^- prior to NH_4^+ oxidation. We have found the method to be reliable over the NH_4^+ concentration range of 0.5–20 μM in deionized distilled water (DIW) and 0.5–10 μM in seawater (SW) with little interference from DON.

MATERIALS AND METHODS

The overall protocol for determining $\delta^{15}NH_4^+$ is schematicized in Figure 1. The two primary reactions of the method, NH_4^+ to NO_2^- and NO_2^- to N_2O, require relatively little time and effort. For low-concentration samples, it is therefore recommended to perform them in the field to avoid artifacts associated with sample preservation. We have found, though, that higher concentration samples (>2 μM) preserve well when filtered and mildly acidified (pH 2–3).

The first step in the procedure is colorimetric determination of NH_4^+ and NO_2^- concentration to determine if sample dilution or NO_2^- removal is necessary. The indophenol method[19] was used for $[NH_4^+]$ and NO_2^- concentration determination followed the protocol of Strickland and Parsons[19] except the sulfanilamide reagent was prepared with 2.5 g sulfanilamide (Fisher, lot 061592) in 250 mL of 6 M HCl. We have found that samples should be
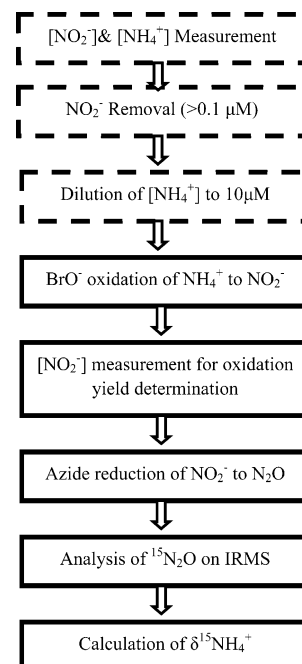
(13) McIlvin, M. R.; Casciotti, K. L. *Anal. Chem.* **2006**, *78*, 2377−2381.
(14) Sigman, D. M.; Casciotti, K. L.; Andreani, M.; Barford, C.; Galanter, M.; Böhlke, J. K. *Anal. Chem.* **2001**, *73*, 4145−4153.
(15) Riley, R. F.; Richter, E.; Rotheram, M.; Todd, N.; Myers, L. S., Jr.; Nusbaum, R. *J. Am. Chem. Soc.* **1954**, *76*, 3301−3303.
(16) Sprinson, D. B.; Rittenberg, D. *J. Biol. Chem.* **1949**, *180*, 707−714.
(17) Lu, X.; Kang, D.; Hong, W. *Haiyang Yu Huzhao* **1991**, *22*, 389−395.
(18) Yakushiji, H.; Kanda, J. *J. Oceanogr.* **1998**, *54*, 337−342.
(19) Strickland, J. D. H.; Parsons, T. R. *A Practical Handbook of Seawater Analysis*; Alger Press: Ottawa, Canada, 1972.

Figure 1. Flowchart of procedure for $\delta^{15}NH_4^+$ determination.

diluted to 10 μM $[NH_4^+]$ for SW and freshwaters to ensure quantitative yield (see below). If NO_2^- is detectable, sulfanilic acid should be used for its removal prior to NH_4^+ oxidation. Sulfanilic acid (Sigma, lot 129H008) was dissolved in HCl-acidified DIW. All DIW used was passed through an ion-exchange cartridge (Barnstead, cat. no. D8911) just before use. Sufficient sulfanilic acid is added to equal 1.5 times the NO_2^- concentration and allowed to react for at least 30 min followed by heating in a boiling water bath for 15 min to destroy the resulting complex.

For BrO^- oxidation of NH_4^+ to NO_2^-, a bromate/bromide stock solution is made by mixing 0.6 g of sodium bromate (Fisher, lot 037708) and 5 g of sodium bromide (Mallinckrodt) in 250 mL of DIW. This reagent has a shelf life of at least 6 months. A BrO^- working solution is prepared daily by adding 1 mL of stock solution to 50 mL of DIW followed by 3 mL of 6 M HCl to produce Br_2. After reacting in the dark for 5 min, 50 mL of 10 M NaOH is added quickly to produce BrO^-. Arsenite is used to remove excess BrO^- after NH_4^+ oxidation. This reagent is made by dissolving 5.1 g of sodium arsenite (EM, lot 30159) in 100 mL of DIW.

After NO_2^- removal, 20 mL samples are placed in 60 mL vials (Kimble, no. 60958A 16) with PTFE-lined polypropylene closures (Kimble, no.73808 24400). Vials were previously acid rinsed and combusted in the oven at 450 °C for 4 h, and caps were rinsed with DIW several times before using. A volume of 2 mL of BrO^- working solution is added to each sample followed by vigorous shaking. After reacting for 30 min at room temperature, 0.4 mL of arsenite reagent is added to remove remaining BrO^- which would react with the azide reagent in the next step to produce an N_2O blank. Prior to the azide reaction, NO_2^- produced from the oxidation of NH_4^+ may be measured colorimetrically to check yield.

Basic hypochlorite (ClO^-) oxidation using Br^- as a catalyst[19] is an alternative to BrO^- at concentrations between 5 and 40 μM. However, ClO^- oxidation is not as robust as BrO^- and has low NO_2^- yields at low concentrations. Also it was subject to greater interference from DON than the BrO^- method.

After BrO⁻ oxidation of NH_4^+ to NO_2^-, NO_2^- is subsequently reduced to N_2O using sodium azide buffered to pH 4–5[12]. Here we used 100% instead of 20% acetic acid to buffer the solution to the proper pH. $\delta^{15}N_2O$ analysis by IRMS is also as described previously using an automated PT-CF-IRMS (GV IsoPrime). In every sample batch, unknowns are run together with replicates of three sets of working standards with established $\delta^{15}N$ values spanning the expected range in natural $\delta^{15}N$. For NO_2^-, we use working standards whose $\delta^{15}N$ has been determined by EA-CF-IRMS. (no. 1, 3.8‰; no. 6, −18.4‰; no. 7, 13.5‰), For NH_4^+, internationally recognized standards are used (IAEA N1, 0.5‰; NITS USGS25, −29.4‰; NITS USGS26, 52.9‰). Our accepted $\delta^{15}N$ values for these standards were carefully determined in our laboratory by EA-IRMS using atmospheric N_2 as the ultimate reference material. For the IAEA- and NITS-provided materials, differences with previously published values are within 1‰.

$\delta^{15}NH_4^+$ is determined through three levels of standardization and quality control. First, N_2O isotopic ratio for all samples and standards is normalized to an N_2O reference gas to eliminate analytical variation associated with any drift in IRMS response over the course of long analytical runs:

$$\delta^{45}N_2O = (R - R_{reference})/R_{reference} \times 1000 \qquad (1)$$

where R is the ratio of the areas of masses 45/44. The contribution of $N_2^{17}O$ to mass 45 is corrected for using the mass 46/44 ratio with a "Craig-type" correction.

NO_2^- standards nos. 1, 6, and 7 are included in every sample batch as a check on the performance of the conversion of NO_2^- to N_2O and long-term consistency in the technique. In parallel, NH_4^+ standards N1, USGS25, USGS26 in a matrix representative of the samples are treated as unknowns to check and calibrate the oxidation of NH_4^+ to NO_2^-. In theory, the relationship between the $\delta^{15}N$ of standards and the $\delta^{15}N$ of the final product N_2O should be the same whether the starting point is NH_4^+ or NO_2^- given that the oxidation of NH_4^+ to NO_2^- is quantitative. As discussed in McIlvin and Altabet,[12] the theoretical slope is 0.5 since one N atom in the product N_2O is from the azide reagent. The intercept is a function of both the initial $\delta^{15}N$ of the azide and isotopic fractionation associated with its reaction with NO_2^-. After a calibration curve is determined, $\delta^{15}NH_4^+$ is calculated:

$$\delta^{15}N_{NH_4^+sample} = (\delta^{15}N_{N_2O\,sample} - b_{NH_4^+STD})/a_{NH_4^+STD} \quad (2)$$

where $b_{NH_4^+}STD$ and $a_{NH_4^+}STD$ are the intercept and slope, respectively, derived from the linear regression of the NH_4^+ standard calibration curve.

RESULTS AND DISCUSSIONS

Optimal Reaction Parameters. To avoid inconsistent isotope fractionation due to variable conversion yield of NH_4^+ to NO_2^-, several experiments were conducted to optimize experimental parameters for maximizing NH_4^+ oxidation yield. First, the oxidation time course for 4 μM USGS26 NH_4^+ was examined in both DIW and SW. The relative oxidation yield was calculated by comparing oxidation yields at different time points to the highest yield obtained in the time course experiment. Results which were
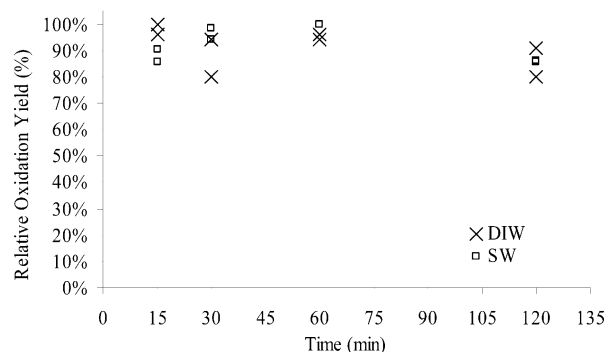

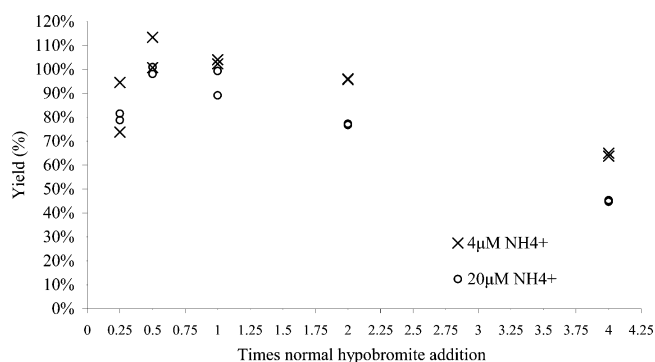**Figure 2.** Effect of reaction time on NH_4^+ oxidation yield.

Figure 3. Effect of hypobromite addition on oxidation yield.

expressed as percent relative yield versus time (Figure 2) showed 30 min to be the optimal reaction time for both DIW and fresh SW samples. However, experience indicates that optimal reaction time should be checked for other sample matrices to ensure maximal recovery.

In a second experiment, the effects of oxidizer concentration on yield were investigated while holding the reaction time constant at 30 min. Concentrations of 4 and 20 μM USGS26 NH_4^+ standard solutions in DIW were examined with BrO⁻ ranging from 0.25 to 4 times the normal addition. Within analytical error, halving the oxidizer addition did not change yield (Figure 3). However, in subsequent experiments, reducing the oxidizer addition did not give consistent results (data not shown), such that we kept using the original amount of BrO⁻ working solution.

Working [NH_4^+] Range in Freshwater and Seawater. The oxidation yields of NH_4^+ to NO_2^- are more than 90% over a [NH_4^+] range of 0.5–20 μM in DIW and 0.5–10 μM in fresh SW. (Figure 4). Beyond these ranges, there is a reduction in yield that produces an undesirable isotope fractionation effect (see the next section). Any samples with [NH_4^+] above this range should be diluted to around 10 μM. Since NO_2^- is easily and quantitatively reduced to nitrous oxide throughout the range of conditions explored here, it is the oxidation yield of NH_4^+ to NO_2^- that determines the performance of this method and is thus the benchmark for it.

Isotope Results $\delta^{15}N$ of Added NH_4^+ Standards. Assuming NH_4^+ is quantitatively converted to N_2O via NO_2^-, the linear regression slope and intercept for the relationship between $\delta^{15}N_2O$ and $\delta^{15}NH_4^+$ should be the same as for $\delta^{15}N_2O$ and $\delta^{15}NO_2^-$ when NO_2^- standards are run. Theoretically, the slope should be 0.5 in all instances with the intercept a function of both the $\delta^{15}N$ of the azide reagent, which contributes one of the N atoms in the product N_2O, as well as the degree of isotopic fractionation with respect
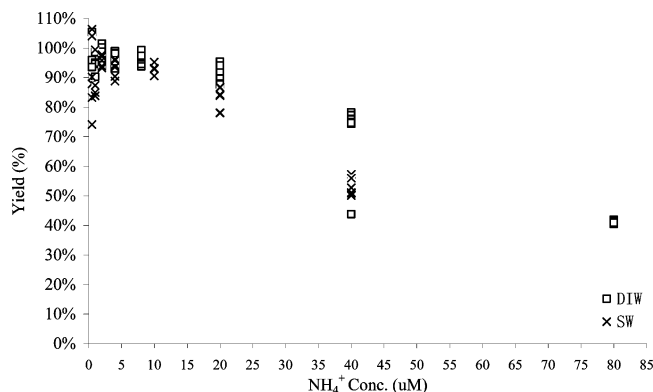
Figure 4. Effect of $[NH_4^+]$ on oxidation yield in DIW and fresh SW.

Table 1. Liner Regression Slopes and Intercepts for NH_4^+ and NO_2^- $\delta^{15}N$ Standard Calibration Curves in DIW and SW

ammonium standards concn (μM)	DIW		SW	
	slope	intercept	slope	intercept
0.5	0.36	−7.72	0.29	−8.23
1	0.44	−6.12	0.38	−8.17
2	0.47	−5.20	0.42	−7.93
4	0.48	−4.88	0.46	−7.26
8	0.50	−4.90	N/A	N/A
10	N/A	N/A	0.48	−5.21
20	0.49	−5.18	0.49	−8.13
40	0.49	−9.56	0.49	−13.73
80	0.49	−14.80	N/A	N/A
NO_2^- standards	0.50	−5.32	0.50	−5.58

to azide-N during this reaction[12]. NO_2^- standards run in the same batch with NH_4^+ standards have a slope of 0.5 and an intercept of about −5 ‰ similar to the long-term average in our laboratory (Table 1; Figure 5). For $[NH_4^+]$ below 20 μM in DIW and 10 μM in SW, NH_4^+ standards have slopes close to the theoretical value 0.5 and intercepts close to −5, showing that the method works well at concentrations down to 0.5 μM for natural abundance N isotopic ratio determination (Table. 1). The systematic deviations in slope and intercept with progressively lower $[NH_4^+]$ are consistent with influence from a low-level NH_4^+ blank in the DIW water used to prepare the standards and perhaps the reagents (~ 0.1 μM, discussed below). The SW used had a somewhat higher preexisting $[NH_4^+]$ (~ 0.3 μM) as seen in the larger deviation in slope and intercept of linear regressions at low added $[NH_4^+]$. SW cannot be treated with a deionization cartridge, and this was the lowest preexisting $[NH_4^+]$ we could achieve from local sources. In practice, SW samples will have the same true blank effects as other samples (see the next section).

When above the working concentration range, linear regressions between $\delta^{15}N_2O$ and $\delta^{15}NH_4^+$ for NH_4^+ standards are still parallel to that of NO_2^- standards. However, intercepts are biased to lower values because of isotopic fractionation associated with reduced oxidation yield (Figure 6 and the Supporting Information). Although these regressions may be used for calibration at reduced yield, for greatest precision it is best to dilute NH_4^+ samples with DIW to 10 μM to maintain quantitative recovery.

Reproducibility of the $\delta^{15}NH_4^+$ determination for replicates was also very good with standard deviations of $\sim 0.3‰$ ($n = 5$) even where $[NH_4^+]$ varied over the working range.
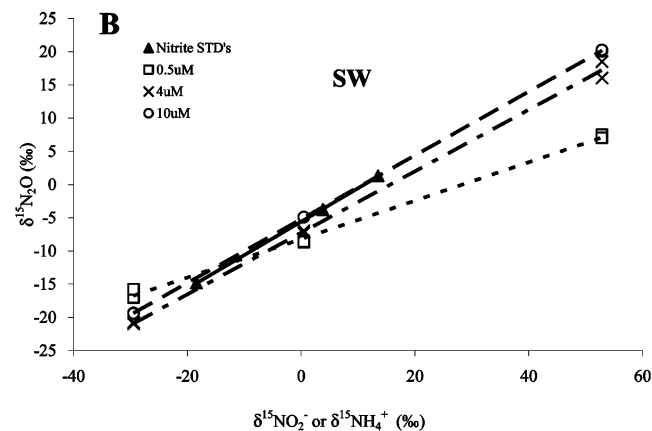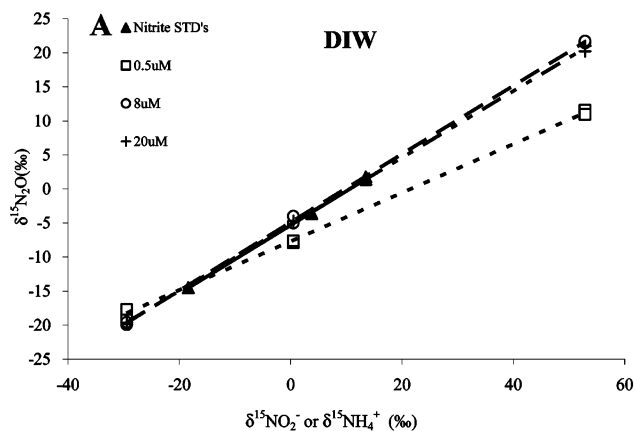

Figure 5. Isotope calibration curves for three NH_4^+ standards at different concentrations in (A) DIW and (B) fresh SW. Lines are linear regressions for each.

Blank Effects. With decreasing $[NH_4^+]$, observed $\delta^{15}NH_4^+$ converge to a single value regardless of the $\delta^{15}NH_4^+$ for the standard used (Figure 7). Using a mass balance equation, we calculate expected $\delta^{15}NH_4^+$ as a function of concentration for each of the three standards assuming that blank $\delta^{15}NH_4^+$ and concentration is close to that observed in our experiments. Expected $\delta^{15}NH_4^+$ is plotted together with observations (Figure 7). Excellent agreement confirms that progressive deviation in $\delta^{15}NH_4^+$ with decreasing concentration is mostly due to blank NH_4^+.

$$[NH_4^+]_{observed}\delta^{15}N_{observed} =$$
$$[NH_4^+]_{STD}\delta^{15}N_{STD} + [NH_4^+]_{blk}\delta^{15}N_{blk} \quad (3)$$

In principle, there are two likely sources of blank NH_4^+: the crystalline reagent stocks and the DIW used to make up the reagents as well as to dilute NH_4^+ standards to specified concentrations. The blanks effects thus observed in Figure 7 are the sum of the reagent and dilution blanks, though in practice it is only the reagent blank which would affect the determination of $\delta^{15}NH_4^+$ in undiluted unknowns.

Although colorimetric concentration determination does not appear sensitive enough to quantify the reagent blank, it is readily monitored by the deviation in the linear regression slope of the $\delta^{15}NH_4^+$ standard calibration with decreasing $[NH_4^+]$. To determine the reagent blank source, the relative additions of BrO^- and NaOH stock solutions were varied. Half-addition of the BrO^- stock
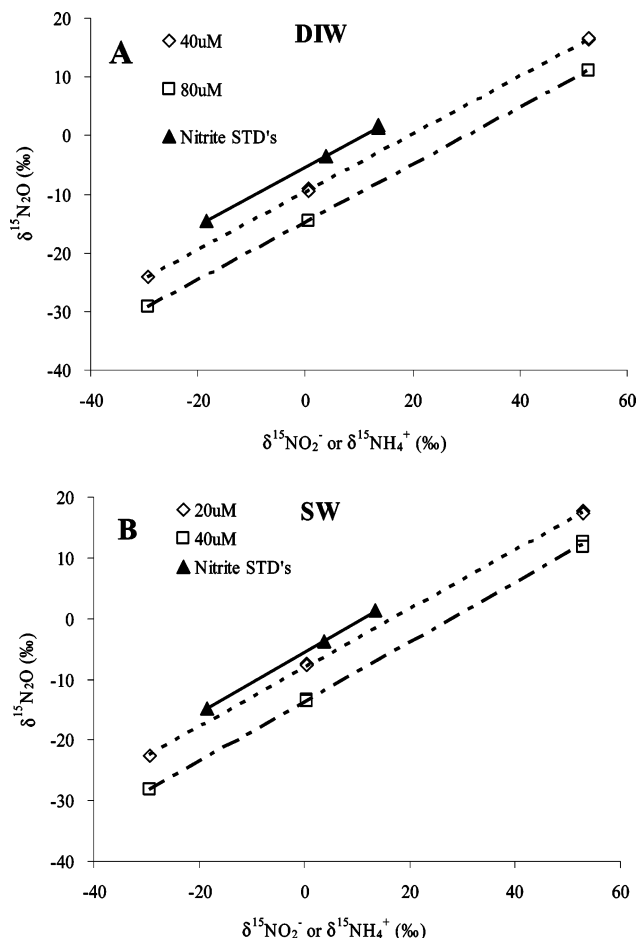
Figure 6. Effects of low oxidation yield at high [NH$_4^+$] on isotopic fractionation in (A) DIW and (B) SW.

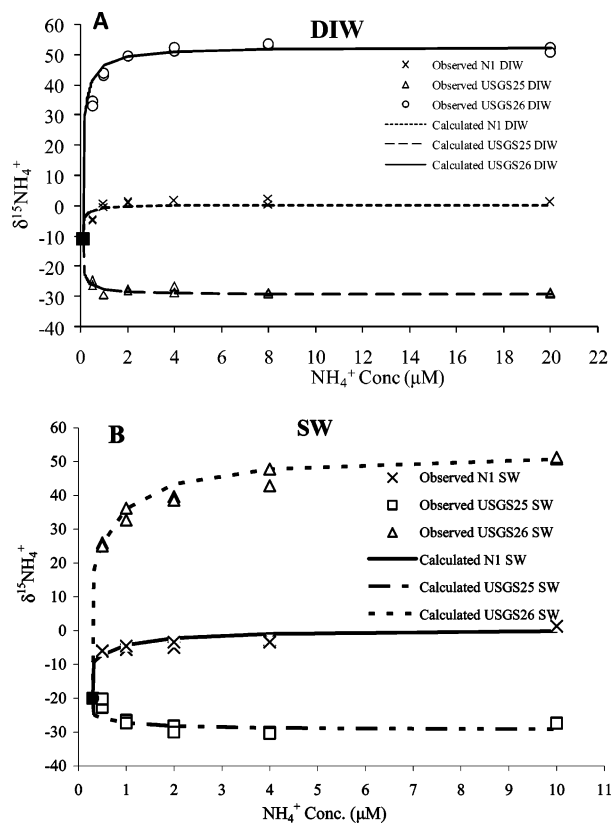
Figure 7. Comparison of observed and calculated δ^{15}NH$_4^+$: (A) DIW blank [NH$_4^+$] and δ^{15}NH$_4^+$ used in the calculation are 0.1 μM and −11‰, respectively; (B) fresh SW blank [NH$_4^+$] and δ^{15}NH$_4^+$ used in the calculation are 0.3 μM and −20‰, respectively.

solution produces the same calibration slope as the normal addition (see the Supporting Information) showing no significant blank NH$_4^+$ associated with this reagent. Unexpectedly, half-addition of NaOH produces a reduced slope for the standard linear regression. If there were contaminating NH$_4^+$ in the NaOH, reduced NaOH addition should have increased the value of the slope. The reagent blank is evidently so small that it is difficult to manipulate, and we were not able to directly determine reagent blank level. Blank [NH$_4^+$] (reagent and standard) estimated by deviations from expected δ^{15}N in DIW and SW are 0.1 and 0.3 μM, respectively (Figure 7). We therefore assume that most of the blank NH$_4^+$ comes from the water used to dilute standard NH$_4^+$ to the desired concentration rather than from the reagents themselves.

With such small reagent blanks as observed here, there is effectively no influence for real samples with >0.5 μM [NH$_4^+$] within the natural range in δ^{15}NH$_4^+$ as verified by simple calculation. Assuming DIW as the only source for blank NH$_4^+$, reagent addition introduces only ∼0.2 nmol/sample or about 2% of a 20 mL, 0.5 μM NH$_4^+$ sample. Given our calculated blank δ^{15}N of −11‰ (Figure 7), a −0.2‰ deviation for a sample δ^{15}N of 0‰ would be expected. If high-concentration samples are diluted to 10 μM, NH$_4^+$ introduced by the DIW diluent is 2 nmol, accounting for only 1% of the sample NH$_4^+$. Our results specifically showed the blank effect was trivial when standard [NH$_4^+$] was >10 μM in both DIW and SW (Figure 7) even for very high standard δ^{15}N.

Therefore, in practice, 10 μM NH$_4^+$ standards should be used to calibrate real samples since at this concentration oxidation yields are optimal (Figure 4) and blank effects on measured δ^{15}NH$_4^+$ are insignificant.

DON Interference in DIW. Several amino acids differing in side-chain structure, a typical protein, and other nitrogen-containing compounds were subjected to hypobromite oxidation to test for potential interferences (see the Supporting Information). Oxidation yields in DIW with respect to NO$_2^-$ production were generally below 15% except for the short-chain aliphatic amino acid, glycine, which had a yield of 30%. However, free glycine is found at such low environmental concentrations that no detectable interference is expected. Results for SW were similar.

Preexisting NO$_2^-$ Removal. Preexisting sample NO$_2^-$ would contribute to the δ^{15}N$_2$O measured at the end of the procedure since it would also react with azide to produce N$_2$O leading to artifacts. We adapted the method of Yakushiji and Kanda[18] which uses sulfanilic acid at low pH to form a complex with NO$_2^-$ that is subsequently destroyed by heating. This treatment does not affect NO$_2^-$ subsequently produced by BrO$^-$ oxidation since solution pH stays high until the reaction with azide. We added sulfanilic acid at a concentration slightly higher than that of the preexisting NO$_2^-$ since excessive sulfanilic acid does appear to reduce the BrO$^-$ oxidation yield of NH$_4^+$ to NO$_2^-$. In most natural waters, NO$_2^-$ rarely exceeds 10 μM. To test the upper limit required, we used 14.45 μM sulfanilic acid to remove 9.75 μM NO$_2^-$. Results show that satisfactory oxidation yield (>90%) and δ^{15}NH$_4^+$ (about 1‰ difference with theoretical value) were

Table 2. Effect of Adding Sulfanilic Acid to Remove Preexisting NO_2^- on NH_4^+ Oxidation Yield and $\delta^{15}NH_4^+$

concn of sulfanilic acid (μM)	concn of no. 6 NO_2^- (μM)	concn of NH_4^+ USGS26 (μM)	NH_4^+ oxidation yield (%)	measured $\delta^{15}NH_4^+$
72.3	9.75	10	81.8	N/A
72.3	9.75	10	74.8	N/A
14.45	0	10	90.2	51.8
14.45	0	10	91.9	51.7
14.45	9.75	10	93.0	51.7
14.45	9.75	10	89.5	51.4

Table 3. Ammonium Ion (NH_4^+) Concentration (μM) and $\delta^{15}N$ (‰) Results of Water Samples from Lake Kinneret (Israel) Measured by BrO^- Oxidation and Diffusion Methods

	7/14/2004				8/11/2004			
	NH_4^+ concn (μM)		$\delta^{15}NH_4^+$			NH_4^+ concn (μM)		$\delta^{15}NH_4^+$
depth (m)	BrO^-	indo-phenol	BrO^-	diffu-sion	depth (m)	BrO^-	indo-phenol	BrO^-	diffu-sion
3	1.3	1.0	−5.5		1	2.7	1.3	−2.5	
15	9.4	9.1	17.2	16.9	5	1.5	0.9	−6.5	
17	19.6	20.5	13.3	12.5	15	2.9	1.9	−4.5	8.3
20	18.8	19.6	14.7	14.3	16	3.2	3.1	8.5	11.1
25	19.6	20.7	13.8	14.1	17	6.0	4.3	15.1	12.9
30	34.4	33.5	12.8	12.3	20	22.3	21.6	15.3	13.8
40	37.5	35.7	11.9	12.3	30	35.4	35.5	13.8	12.8
					39	47.4	43.9	13.0	12.6

Note: Table 3 header has one extra column than shown; values aligned per depth/BrO/indophenol/BrO diffusion layout.

maintained (Table 2). Quantitative yield of N_2O also showed that remaining unreacted sulfanilic acid did not compete for substrate during the reaction with azide even with pH reduced to between 4 and 5. With a much higher sulfanilic acid addition (72.3 μM), oxidation yields were indeed reduced to unacceptable levels (Table. 2). We conservatively recommend that this treatment be used for samples with $[NO_2^-] > 0.1$ μM. This removal procedure may need to be further checked in the rarer case where preexisting NO_2^- is much more abundant.

Comparison with NH_3 Diffusion Method for Lake Kinneret Samples. As a demonstration of the BrO^- oxidation method on environmental samples, three depth profiles of water samples from Lake Kinneret (Israel) were analyzed with this method as well as the NH_3 diffusion method (Figure 8). The NH_3 diffusion method was chosen for comparison as the most common method in the literature for $\delta^{15}NH_4^+$ determination. Quantitative BrO^- oxidation yield was verified by comparison of the resulting $[NO_2^-]$ with $[NH_4^+]$ determined by the indophenol method. Concentration determined by two methods fall on a 1:1 line showing sample NH_4^+ was quantitatively converted to NO_2^- (see the Supporting Information). Isotopic results also agree for the two methods (Table 3), but because of higher blanks and sample size requirements the diffusion method could not be applied to samples with $[NH_4^+] < 3$ μM.

The lake samples cover a large concentration range from <1 μM at the surface to more than 40 μM near-bottom (Figure 8; Table 3) as documented by previous studies.[20] During this summertime period, phytoplankton in the euphotic zone (<15 m depth) utilize NH_4^+ as rapidly as it is produced by heterotrophs

(20) McCarthy, J. J.; Wynne, D.; Berman, T. *Limnol. Oceanogr.* **1982**, *27*, 673−680.
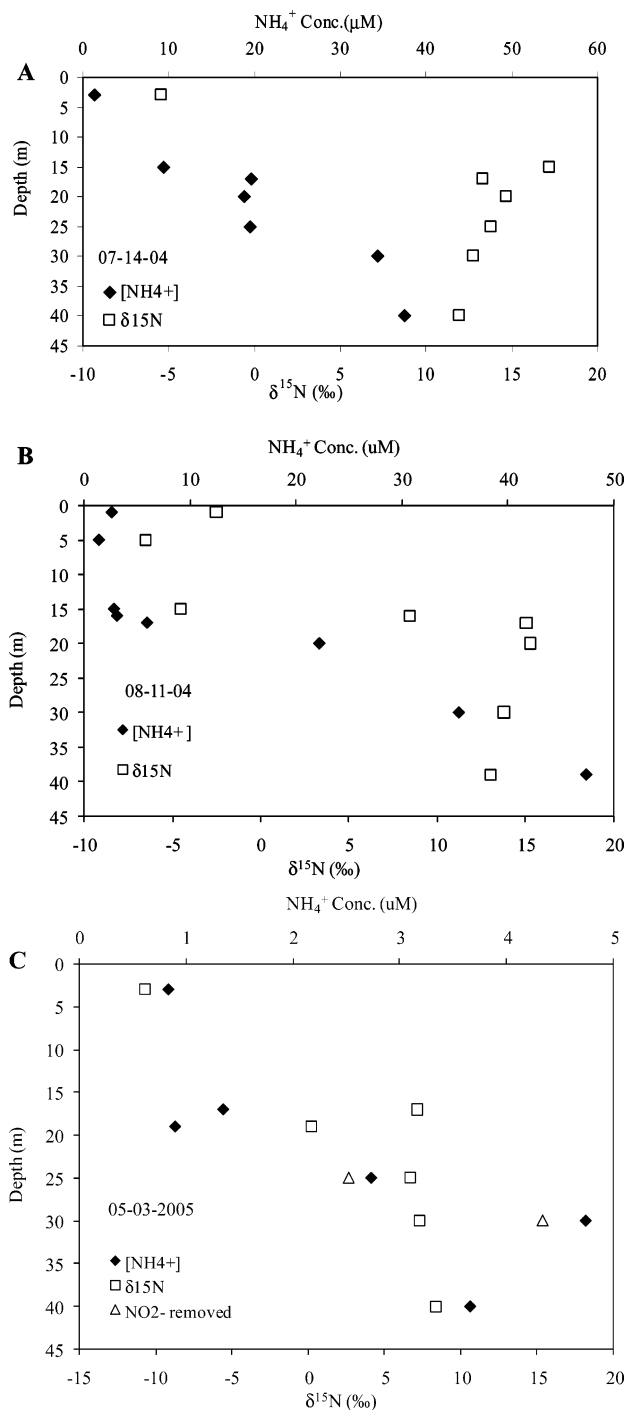
Figure 8. Lake Kinneret $[NH_4^+]$ (μM) and $\delta^{15}NH_4^+$ (‰) depth profiles at (A) July 14, 2004, (B) Aug 11, 2004, and (C) May 3, 2005.

leading to rapid turnover.[20] Thermal stratification restricts vertical exchange permitting development of anoxia and accumulation of NH_4^+ at depth. High NH_4^+ turnover rates may be responsible for the negative $\delta^{15}NH_4^+$ in the euphotic zone as a result of the ubiquitous ^{15}N trophic enrichment effect. Much higher $\delta^{15}NH_4^+$ associated with high concentrations in the hypolimnion likely reflects remineralization from high $\delta^{15}N$ organic matter (known from prior observations). At 25 and 30 m, NO_2^- was detectable, posing a potential interference for $\delta^{15}NH_4^+$ determination. Before NO_2^- removal, the apparent $\delta^{15}NH_4^+$ at 25 m ($[NO_2^-] = 0.4$ μM) and 30 m ($[NO_2^-] = 0.6$ μM) was 6.7‰ and 7.3‰, respectively.

However, actual $\delta^{15}NH_4^+$ was found to be 2.7‰ and 15.4 ‰, respectively (Figure 8C). Clearly, prior removal of NO_2^- is critical for accurate $\delta^{15}NH_4^+$ determination but relatively easy to carry out using our methodology.

SUMMARY

Our method allows, for the first time, robust natural abundance level measurement of the nitrogen isotopic ratio of NH_4^+ in both SW and freshwater samples at low environmental concentrations (0.5 μM or lower). This method involves oxidation of NH_4^+ to NO_2^- and subsequent reduction to N_2O followed by automated PT-CF-IRMS analysis. Our results show that the BrO^- method provides reliable and precise isotope data in the range of 0.5−20 μM in DIW (10 μM in SW). Replicate NH_4^+ standards ($n = 5$) varying in concentration had a standard deviation of 0.3‰. For samples with higher concentrations, it is recommended to simply dilute the samples with DIW to 10 μM a level at which the blank effect is nil. Although we could not quantitatively determine the reagent blank, it is sufficiently low that there is no significant influence for samples with $[NH_4^+] > 0.5$ μM at moderate $\delta^{15}N$ values. The NO_2^- yield from the tested N-containing organic compounds is low, suggesting little interference from DON in environmental samples. Sulfanilic acid can be used to remove preexisting NO_2^-, eliminating this potential interference. In comparison to the NH_3 diffusion approach, our method is superior in requiring both lower $[NH_4^+]$ and less volume (down to 0.5 μM in a 20 mL sample or less) which greatly facilitates field sampling and in situ measurement. It is also less prone to DON interference and requires substantially less processing time.

At this point, it is the magnitude of the reagent blank and not sample mass that determines the detection limit of this method. We anticipate that with further purification of reagents and improvement of the sensitivity of IRMS it will be applicable at even lower NH_4^+ concentrations.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.