



Methods for the Determination of Stable Isotope Ratios of Multiple Nitrogen Species in Rainwater Using Distillation and Evaporation

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Nitrogen (N) isotope ratios ($\delta^{15}\text{N}$) of multiple N species including ammonium (NH_4^+), nitrate (NO_3^-), dissolved organic N (DON), and total dissolved N (TDN) can serve as indicators of the sources of wet N deposition. However, methods for sequential analysis of these multiple N species are not well developed. In this study, methods for the determination of $\delta^{15}\text{N}$ of these multiple N species were proposed through a traditional distillation method for NH_4^+ and NO_3^- , direct evaporation for TDN, and an isotope mass balance equation for $\delta^{15}\text{N}$ -DON. A series of experiments were conducted 1) to find evaporation conditions including pH (<3.5 vs. 5.4) and evaporation methods (oven drying at 60 and 100°C, infrared chamber, and freeze-drying), 2) to investigate precision and accuracy of distillation for $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- , and 3) to determine $\delta^{15}\text{N}$ -TDN by direct evaporation and $\delta^{15}\text{N}$ -DON with mass balance equation. $(\text{NH}_4)_2\text{SO}_4$ ($-4.0 \pm 0.03\text{‰}$), KNO_3 ($-4.2 \pm 0.03\text{‰}$), and $\text{CO}(\text{NH}_2)_2$ ($-5.4 \pm 0.04\text{‰}$) were used as reference materials for NH_4^+ , NO_3^- , and DON, respectively. In this study, for the analysis of $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- , the conventional distillation method was tested in an effort to save cost for laboratories equipped with the distillation system. Evaporation using oven at 60°C (but not 100°C), infrared chamber, or freeze-drier after acidification to < pH 3.5 prevented $^{14}\text{NH}_3$ loss. Analysis of the $\delta^{15}\text{N}$ - NO_3^- ($-4.4 \pm 0.1\text{‰}$ to $-3.9 \pm 0.5\text{‰}$) was reliable for a wide range of N content (0.1–0.5 mg), but analytical errors for $\delta^{15}\text{N}$ - NH_4^+ were as high as 2.1‰ when N content was small (e.g., 0.1–0.3 mg N) due to background contamination and potential interference by co-existing DON. Direct evaporation of solution containing NH_4^+ , NO_3^- , and DON to dryness produced reliable $\delta^{15}\text{N}$ -TDN with accuracy <0.15‰ and precision <0.21‰. However, the analytical errors of $\delta^{15}\text{N}$ -DON were highly dependent on the content of co-existing NH_4^+ as well as DON content. Therefore, the proposed protocol can be applied for rainwater containing a high NH_4^+ concentration (>2.0 mg N L⁻¹ assuming that 200 ml of sample is used for distillation).

Keywords: ammonium, dissolved organic nitrogen, nitrate, nitrogen isotope fractionation, stable nitrogen isotope

INTRODUCTION

Atmospheric nitrogen (N) deposition is an important N source in terrestrial ecosystems (Fowler et al., 2015). During the last four decades, global N deposition in terrestrial ecosystems increased from 87 Tg Nyr⁻¹ in the 1980s to 93 Tg Nyr⁻¹ in the 2010s, and it is expected to increase to 125 Tg N yr⁻¹ by 2050 (Bobbink et al., 2010; Ackerman et al., 2019). Though the contribution of wet and dry deposition to total N deposition varies with countries, wet N deposition such as ammonium (NH₄⁺) and nitrate (NO₃⁻) through rainfall is substantial (Li et al., 2016; Felix et al., 2017). Increased N deposition may impact terrestrial ecosystems through soil acidification and associated changes in soil environment and vegetation compositions (Phoenix et al., 2012). Therefore, to estimate the environmental and ecological impacts of N deposition, it is necessary to distinguish the sources of N and to trace deposited N in ecosystems (Ohte, 2012).

The stable N isotope ratios ($\delta^{15}\text{N}$) of NH₄⁺ (e.g., Felix et al., 2017) and NO₃⁻ (e.g., Nanus et al., 2018) have extensively been used for source appointment of wet N deposition. For example, the $\delta^{15}\text{N}$ of NH₄⁺ originated from agricultural sources such as livestock waste and fertilizer is generally lower than those from coal combustion (Felix et al., 2017), and the $\delta^{15}\text{N}$ of NO₃⁻ derived from coal combustion is higher than that from diesel and gasoline combustions (Nanus et al., 2018). Though analytical accuracy slightly differs, many methods are available for the analysis of $\delta^{15}\text{N}$ of NH₄⁺ and NO₃⁻. For example, the $\delta^{15}\text{N}$ of NH₄⁺ is often analyzed through distillation (Hauck, 1982), diffusion (Cao et al., 2018), or oxidation to NO₃⁻ followed by reduction to N₂O (Lachouani et al., 2010). For the analysis of $\delta^{15}\text{N}$ of NO₃⁻, chemical (McIlvin and Altabet, 2005) and bacterial (Sigman et al., 2001) reduction of NO₃⁻ to N₂O followed by gases measurement of $\delta^{15}\text{N}$ of N₂O and distillation after reduction of NO₃⁻ to NH₄⁺ by addition of Devarda's alloy (Hauck, 1982) are frequently used.

Method selection for $\delta^{15}\text{N}$ measurement of water samples is largely dependent on the resource (apparatus and instruments) availability of laboratories as each method has both advantages and disadvantages. For example, the NH₄⁺ diffusion method is less labor-intensive but requires a long time (several days) for complete NH₃ diffusion due to a slow rate of diffusion (Cao et al., 2018). Distillation is quick but labor-intensive and has the potential of cross-contamination between samples (Chen and Dittert, 2008). Reduction of NO₃⁻ to N₂O is accurate but requires several hours for completion of denitrification (Sigman et al., 2001).

The accuracy of diffusion (Chen and Dittert, 2008; Zhang et al., 2015; Cao et al., 2018), chemical reduction (McIlvin and Altabet, 2005; Liu et al., 2014), and bacterial reduction (Sigman et al., 2001; Böhlke et al., 2007) methods for measurement of $\delta^{15}\text{N}$ of NH₄⁺ and NO₃⁻ have been extensively tested, and many advanced modifications have been proposed. However, unexpectedly, there are very few studies on the distillation method (Feast and Dennis, 1996) despite distillation systems are still widely used for the determination of N concentration and thus are available in many laboratories (Sáez-Plaza et al., 2013). If the $\delta^{15}\text{N}$ of NH₄⁺ and NO₃⁻ could be measured accurately using

the distillation method, it may save cost to install additional equipment for the $\delta^{15}\text{N}$ analysis in laboratories equipped with the traditional distillation system.

Analytical uncertainties of sample preparation using a distillation method for $\delta^{15}\text{N}$ measurement typically arise from NH₃ volatilization during evaporating NH₄⁺ solution (in H₂SO₄ matrix) to obtain (NH₄)₂SO₄ salt as preferential losses of ¹⁴NH₃ result in overestimation of the $\delta^{15}\text{N}$ (Feast and Dennis, 1996). Typically, NH₃ volatilization is affected by pH, and thus acidification (pH < 3–4) of the solution before evaporation is required (Buresh et al., 1982; Hauck, 1982; Feast and Dennis, 1996). Evaporation methods using an oven, an infra-red chamber, or a freeze-drier may also affect the accuracy of $\delta^{15}\text{N}$ measurement due to the temperature dependency of NH₃ volatilization (Emerson et al., 1975; Hales and Drewes, 1979). Despite the critical effects of NH₃ volatilization on the accuracy of $\delta^{15}\text{N}$, no study has investigated the combined effects of pH adjustment and evaporation methods on $\delta^{15}\text{N}$, highlighting the necessity of relevant studies.

Compared to NO₃⁻ and NH₄⁺, DON has received less research interest despite that DON is a dominant N form and thus a key component in the global N cycle (Russow et al., 2002; Lee et al., 2012; Nikolenko et al., 2018; Cao et al., 2021). The contribution of DON to total dissolved N (TDN) is also substantial up to >40% (Cornell et al., 2003; Lee et al., 2012). The $\delta^{15}\text{N}$ of DON can be determined after removing NH₄⁺ and NO₃⁻ *via* ion retardation columns (Bronk and Gilbert, 1991), membrane filters (Feuerstein et al., 1997), and diffusion of NH₄⁺ (and NO₃⁻ after reduction of NO₃⁻ to NH₄⁺) using MgO and Devarda's alloy (Slawyk and Raimbault, 1995). However, the direct measurement of the $\delta^{15}\text{N}$ of DON is often being questioned due to low accuracy (Cao et al., 2021). Alternatively, the $\delta^{15}\text{N}$ of DON can be obtained indirectly with isotope mass balance equation using the concentrations and $\delta^{15}\text{N}$ of NH₄⁺, NO₃⁻, and TDN (Liu et al., 2017). The $\delta^{15}\text{N}$ of TDN is often determined through alkaline-persulfate digestion followed by reduction of NO₃⁻ to N₂O (Lachouani et al., 2010; Liu et al., 2017). Recently, Cao et al. (2021) has successfully proposed a protocol for the determination of $\delta^{15}\text{N}$ -DON using an isotope mass balance equation through analyses of $\delta^{15}\text{N}$ of NH₄⁺, NO₃⁻, and TDN. However, in the proposed procedures, $\delta^{15}\text{N}$ of each N species is measured independently; diffusion for NH₄⁺, denitrification for NO₃⁻, and persulfate-oxidation followed by denitrification for TDN. Therefore, a simple method to determine $\delta^{15}\text{N}$ of NH₄⁺, NO₃⁻, and TDN may be of help to determine $\delta^{15}\text{N}$ of DON; however, the relevant study is not available.

Therefore, the literature indicates that a method for analysis of these multiple N species is not well developed. This study was conducted to establish a procedure for sequential measurement of $\delta^{15}\text{N}$ of NH₄⁺, NO₃⁻, DON, and TDN in rainwater samples through distillation and evaporation. To achieve the objective, firstly, we investigated the combined effects of acidification and evaporation methods on the $\delta^{15}\text{N}$ measurement to suggest suitable evaporating conditions of H₂SO₄ solution containing NH₄⁺ (experiment 1). Secondly, the precision and accuracy of measurement of $\delta^{15}\text{N}$ of NH₄⁺ and NO₃⁻ through sequential distillation were investigated using samples with different N content (0.1–0.5 mg N) (experiment 2). Thirdly, the precision

and accuracy of direct evaporation of water samples to determine δ¹⁵N of TDN were tested (experiment 3). Finally, by combining the results of experiments 1–3, we suggested a protocol for the simultaneous measurement of δ¹⁵N of NH₄⁺, NO₃⁻, DON, and TDN.

MATERIALS AND METHODS

Reference Materials

All the experiments were conducted using chemical-grade ammonium sulfate ((NH₄)₂SO₄, 7783-20-2, Junsei, Japan), potassium nitrate (KNO₃, 7757-79-1, Junsei, Japan), and urea (CO(NH)₂, 57-13-6, Junsei, Japan) as reference materials for both concentration and δ¹⁵N of NH₄⁺, NO₃⁻, and DON, respectively, throughout the experiments. Though nitrite (NO₂⁻) may be also be contained in rainwater, NO₂⁻ was not considered as the concentration of NO₂⁻ is reported to be as low as < 1% of NO₃⁻ (Sa et al., 2022). The δ¹⁵N of the compounds were analyzed using a stable isotope ratio mass spectrometer linked to an elemental analyzer (EA-IRMS) (VisION, Isoprime Ltd., Cheadle Hulme, United Kingdom), and reported as

$$\delta (\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000$$

where R is the atom % of ¹⁵N/(¹⁴N + ¹⁵N), and the standard was atmospheric N₂ (R = 0.3663%). Accuracy of the measurement by the EA-IRMS tested using IAEA-N1 and N2 (both ammonium sulfate, +0.4‰ and +20.3‰, respectively) was <0.2‰. The δ¹⁵N of the reference materials was calibrated against the IAEA-N1 and N2. The mean and standard errors of the δ¹⁵N of (NH₄)₂SO₄, KNO₃, and CO(NH)₂ measured in 10 replicates were -4.0 ± 0.03‰, -4.2 ± 0.03‰, and -5.4 ± 0.04‰, respectively.

Setting pH and Selecting Evaporation Method for Evaporation After Distillation

To set pH and select evaporation method to obtain (NH₄)₂SO₄ salt from distillates for measurement of δ¹⁵N using the EA-IRMS, the effects of pH adjustment (pH < 3.5 and 5.4) and evaporation methods (oven at 60°C, oven at 100°C, infra-red chamber, and freeze-drying) were investigated using (NH₄)₂SO₄ as reference material. (NH₄)₂SO₄ solutions with two different concentrations (2 and 4 mg N L⁻¹ for low and high N, respectively) were prepared. Solution (100 ml) of (NH₄)₂SO₄ (0.2 and 0.4 mg N for low and high N contents, respectively) was transferred to a 250-ml beaker, and 10 mg sodium sulfate (Na₂SO₄) (1 ml of 10 g Na₂SO₄ L⁻¹ solution) was added to the beaker as a bulking agent to make sure sufficient amount of salt recovered after evaporation. Therefore, the N solutions are comprised of NH₄⁺, Na⁺, and SO₄²⁻, which are the ion compositions of the solutions when the distilled NH₄⁺ is collected in H₂SO₄ and back-titrated with NaOH (Hauck, 1982). The pH of the solution measured with a pH meter (Orion 3 Star, Thermo Fisher Scientific Inc., United States) was 5.4 ± 0.1. Another set of the N solution was acidified to pH < 3.5 by adding 0.2 ml of 0.1 N H₂SO₄.

To select evaporation methods, the beakers containing (NH₄)₂SO₄ solution were placed into a drying oven (WOF-155, Daihan Scientific, Korea), a house-made infra-red chamber, and a freeze drier (FD5508, Ilshin, Korea). The infra-red chamber (56 × 58 × 53 m³) was equipped with two infra-red lamps (Dr. Fischer 177.5 mm and 500 W). Each experiment was repeated four times for 2 N concentration levels, pH adjustment, and evaporation methods. The N solution was evaporated to dryness for 48 h. The dried salts were crushed to fine powder using a spatula. Powder containing approximately 0.1 mg N (²⁸N₂ beam area: 2.0E-07) was wrapped into a tin capsule and analyzed for δ¹⁵N using the EA-IRMS.

To determine the recovery of NH₄⁺ after evaporation, another set of four replicates was processed following the same procedures described above. The dried salts were re-dissolved by adding 50-ml of deionized water, and N content was determined using the indophenol method (Mulvaney, 1996) to calculate the loss of N during evaporation. The overall pattern of temperature change during evaporation was monitored using 1 L of deionized water contained in a 1 L beaker. Tip of temperature sensors (TR-52, T&D Cooperation, Japan) was installed at 3.5 cm above the bottom and 3.5 cm below the water surface, and the temperature was monitored for 10 h. After 10 h, it was not possible to measure temperature at the position due to decreased sample volume.

δ¹⁵N Measurement of NH₄⁺ and NO₃⁻ Using Distillation

Nitrogen mixture solutions containing NH₄⁺, NO₃⁻, and CO(NH)₂ (0.5, 1, 1.5, 2, and 2.5 mg N for each N species) were prepared by dissolving the reference (NH₄)₂SO₄, KNO₃, and CO(NH)₂ in 1 L deionized water. The concentrations of reference N were set to include the range of N concentration of rainfall (<0.1 to >1.0 mg N L⁻¹) reported in the literature (Lee et al., 2012; Zeng et al., 2020; Chen et al., 2022). Distillation was performed following the standard distillation procedures of Mulvaney (1996) with some modifications to distillate a large quantity of samples. Briefly, 200 ml of the solution (containing 0.1, 0.2, 0.3, 0.4, and 0.5 mg N for each N species) was transferred to 500-ml distillation flasks, and the flasks were placed on a heating mantle (GLHMP-F100, Global Lab, Korea). NH₄⁺ was liberated by distillation with addition 0.5 g of carbonate-free MgO (1309-48-4, Junsei, Japan) for 4–5 min to collect 30–40 ml of distillates. Carbonate-free MgO was prepared by heating MgO at 700°C for 2 h and used following the suggestion of Mulvaney (1996). In a preliminary experiment, 4–5 min distillation was sufficient to recover NH₄⁺ (>99%). The liberated NH₃ was collected in a 250 ml beaker containing 10 ml of 0.01 N H₂SO₄. The (NH₄)₂SO₄ solution was titrated to pH 5.4 using 0.01 N NaOH and further acidified to pH < 3.5 by adding approximately 0.2 ml of 0.01 N H₂SO₄ and dried to salt under the infra-red lamps.

The distillation flasks were cooled down to room temperature, and the distillation apparatus was washed by distilling 30 ml of 90% ethanol for 5 min using another set of distilling flasks. For

distillation of NO_3^- , 50 ml of deionized water was added to the flasks to restore sample volume. To convert NO_3^- to NH_3 , 0.3 g of Devarda's alloy (8049-11-4, Kanto Chemical, Japan) was added and distilled again following the procedure for NH_4^+ . The $(\text{NH}_4)_2\text{SO}_4$ solution was acidified to $\text{pH} < 3.5$ following the procedure described above. The acidified solution was dried through evaporation under infra-red lamps as it was found that oven drying at 60°C , freeze-drying, and drying under infra-red lamps were all suitable (see the results). The $\delta^{15}\text{N}$ of dried powder was analyzed using the EA-IRMS. The experiments were replicated four times.

Determination of $\delta^{15}\text{N}$ -TDN Using Direct Evaporation and Calculation of $\delta^{15}\text{N}$ -DON

The same N mixture solutions containing NH_4^+ , NO_3^- , and $\text{CO}(\text{NH}_2)_2$, which were used for the distillation experiment were prepared. The theoretical values of $\delta^{15}\text{N}$ -TDN calculated from NH_4^+ , NO_3^- , and $\text{CO}(\text{NH}_2)_2$ was $-4.5 \pm 0.03\text{‰}$. An aliquot (200 ml) of the standard samples were transferred to 250-ml beaker, and 10 mg Na_2SO_4 (1 ml of $10 \text{ g Na}_2\text{SO}_4 \text{ L}^{-1}$ solution) was added to the beaker as a bulking agent followed by addition of 0.2 ml of 0.1 N H_2SO_4 to adjust the pH of the solution < 3.5 . The mixture was dried under infra-red lamps, and the dried powder was analyzed for $\delta^{15}\text{N}$ of TDN using the EA-IRMS. All experiments were replicated four times.

The $\delta^{15}\text{N}$ of DON was calculated using the following isotope mass balance equation (Karamanos and Rennie, 1981):

$$\delta^{15}\text{N}_{\text{DON}} = \left[(\delta^{15}\text{N}_{\text{TDN}} \times C_{\text{TDN}}) - (\delta^{15}\text{N}_{\text{NH}_4} \times C_{\text{NH}_4} + \delta^{15}\text{N}_{\text{NO}_3} \times C_{\text{NO}_3}) \right] / [C_{\text{TDN}} - (C_{\text{NH}_4} + C_{\text{NO}_3})]$$

where C_{DON} , C_{TDN} , C_{NH_4} , and C_{NO_3} are contents of DON, TDN, NH_4^+ , and NO_3^- , respectively, and $\delta^{15}\text{N}_{\text{DON}}$, $\delta^{15}\text{N}_{\text{TDN}}$, $\delta^{15}\text{N}_{\text{NH}_4}$, and $\delta^{15}\text{N}_{\text{NO}_3}$ are their respective $\delta^{15}\text{N}$. The standard deviation (SD) of $\delta^{15}\text{N}_{\text{DON}}$ ($\text{SD}_{\delta\text{DON}}$) was calculated by using the following equation (Cao et al., 2021):

$$\text{SD}_{\delta\text{DON}} = \left[\left((C_{\text{TDN}}/C_{\text{DON}}) \times \text{SD}_{\delta\text{TDN}} \right)^2 + \left((C_{\text{NO}_3}/C_{\text{DON}}) \times \text{SD}_{\delta\text{NO}_3} \right)^2 + \left((C_{\text{NH}_4}/C_{\text{DON}}) \times \text{SD}_{\delta\text{NH}_4} \right)^2 \right]^{1/2}$$

where $\text{SD}_{\delta\text{TDN}}$, $\text{SD}_{\delta\text{NO}_3}$, and $\text{SD}_{\delta\text{NH}_4}$ are the standard deviations of $\delta^{15}\text{N}$ of TDN, NO_3^- , and NH_4^+ , respectively.

Validation of $\delta^{15}\text{N}$ Analyses Using the Proposed Methods

The methods for the determination of $\delta^{15}\text{N}$ of NH_4^+ , NO_3^- , DON, and TDN using distillation and evaporation developed in the present study were validated using reference solutions. The reference solutions were prepared by dissolving the reference $(\text{NH}_4)_2\text{SO}_4$, KNO_3 , and $\text{CO}(\text{NH}_2)_2$ in distilled water at two levels of N concentrations (2 and 3 mg N L^{-1} for each N species). 200 ml of the solution (containing each 0.4 or 0.6 mg N of NH_4^+ , NO_3^- , and DON) was distilled for the analysis of the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- , and another 200 ml of the solution was directly dried for the analysis of $\delta^{15}\text{N}$ of TDN under infra-red following the

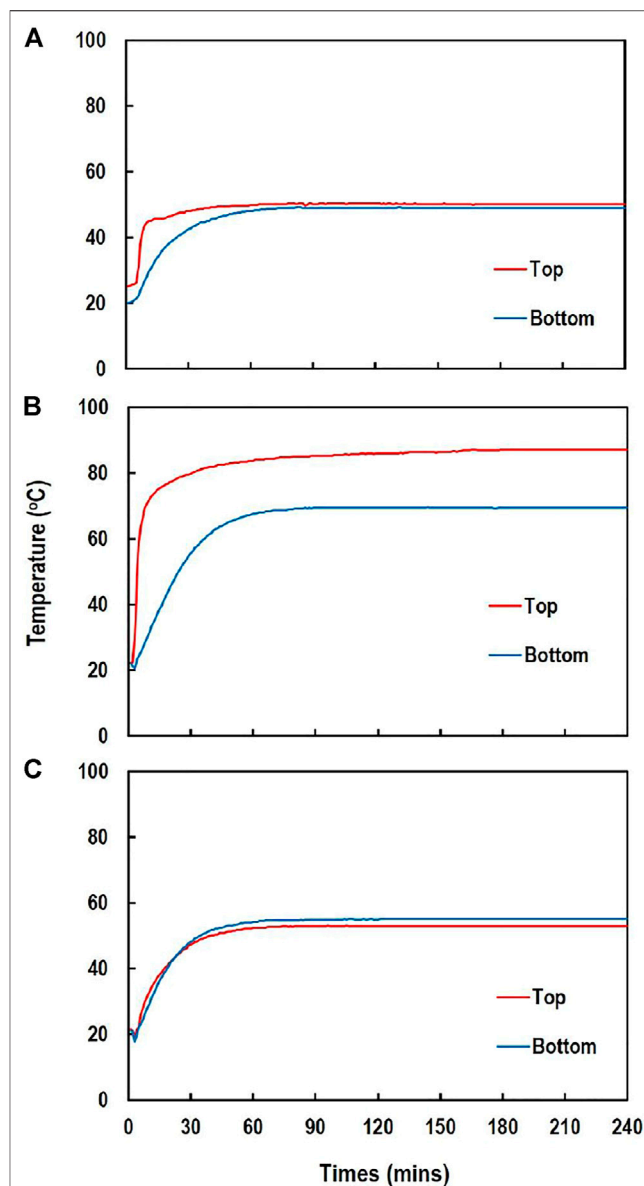


FIGURE 1 | Changes in temperature of the $(\text{NH}_4)_2\text{SO}_4$ solution during evaporation measured at 3.5 cm below the water surface (top) and 3.5 cm above the bottom (bottom) for 10 h: (A) oven at 60°C (B) oven 100°C , and (C) infra-red chamber.

procedures described above. All experiments were replicated four times. The $\delta^{15}\text{N}$ of DON was calculated using the isotope mass balance equation.

Statistical Analyses

All statistical analyses were performed using IBM SPSS Statistics 23 (IBM Corp., Armonk, New York, United States) at a significance level of 0.05. Data were tested for normality of distribution and homogeneity of variance with Shapiro-Wilk test and Levene's test, respectively. Data transformation was not needed as no heterogeneity was detected and the distribution was normal. In each experiment, the changes in

TABLE 1 | Effects of N content, pH, and evaporating methods on the N recovery and δ¹⁵N of (NH₄)₂SO₄ (−4.0 ± 0.03‰) solution.

Nitrogen content (mg N)	pH	Evaporation method	N recovery (%)	δ ¹⁵ N (‰)	Isotope enrichment (‰) ^a
0.2	3.5	Oven at 60°C	98.7 (2.2)ef ^b	−4.1 (0.2)a	−0.1 (0.2)a
		Oven at 100°C	95.0 (1.9)def	−3.5 (0.0)ab	0.5 (0.0)ab
		Infra-red chamber	103.6 (1.9)f	−4.1 (0.2)a	−0.1 (0.2)a
		Freeze drier	96.8 (6.2)def	−4.0 (0.0)a	0.0 (0.0)a
	5.4	Oven at 60°C	44.4 (3.2)b	17.8 (1.0)d	21.8 (0.2)d
		Oven at 100°C	31.5 (3.6)a	17.1 (2.6)d	21.1 (0.1)d
		Infra-red chamber	46.0 (5.9)b	17.3 (0.4)d	21.3 (0.0)d
		Freeze drier	85.6 (1.9)d	−1.4 (0.1)b	2.6 (0.0)b
0.4	3.5	Oven at 60°C	91.0 (1.2)de	−3.9 (0.2)a	0.1 (1.0)a
		Oven at 100°C	95.0 (4.1)def	−3.1 (0.1)b	0.9 (1.1)b
		Infra-red chamber	95.6 (4.1)def	−3.9 (0.0)a	0.1 (0.3)a
		Freeze drier	96.2 (1.7)def	−4.0 (0.0)a	0.0 (0.1)a
	5.4	Oven at 60°C	67.8 (5.0)c	9.0 (0.1)c	13.0 (0.1)c
		Oven at 100°C	44.0 (2.7)b	15.4 (1.8)d	19.4 (1.8)d
		Infra-red chamber	59.2 (4.5)c	7.8 (1.2)c	11.8 (1.2)c
		Freeze drier	89.4 (1.6)de	−2.6 (0.1)ab	1.4 (0.1)ab
Effects			Probability > F		
Nitrogen content (N)		0.014	<0.001	<0.001	<0.001
pH (P)		<0.001	<0.001	<0.001	<0.001
Evaporation method (E)		<0.001	<0.001	<0.001	<0.001
N × P		<0.001	<0.001	<0.001	<0.001
N × E		0.598	<0.001	<0.001	<0.001
P × E		<0.001	<0.001	<0.001	<0.001
N × P × E		0.063	<0.001	<0.001	<0.001

Values are the means (n = 4) with standard deviations in the parentheses.

^aCalculated as the difference between δ¹⁵N measured after evaporation (A) of (NH₄)₂SO₄ solution and that of solid (NH₄)₂SO₄ determined directly with a combustion method (B) (A−B).

^bDifferent lowercase letters indicate that the values are significantly different among the samples at α = 0.05.

the δ¹⁵N among the treatments (e.g., N content, pH adjustment, and evaporating method) were assessed by the analysis of variance (ANOVA). The precision of the δ¹⁵N measurement was assessed by calculating the standard deviation for the replicated treatments. The accuracy of the δ¹⁵N measurement was evaluated by calculating isotope enrichment, the differences between δ¹⁵N obtained from distillation-evaporation methods and that of reference materials determined with the direct combustion method. A t-test was performed to examine the difference in δ¹⁵N between distillation-evaporation and direct combustion methods.

RESULTS AND DISCUSSION

Evaporation Conditions: pH and Evaporating Method

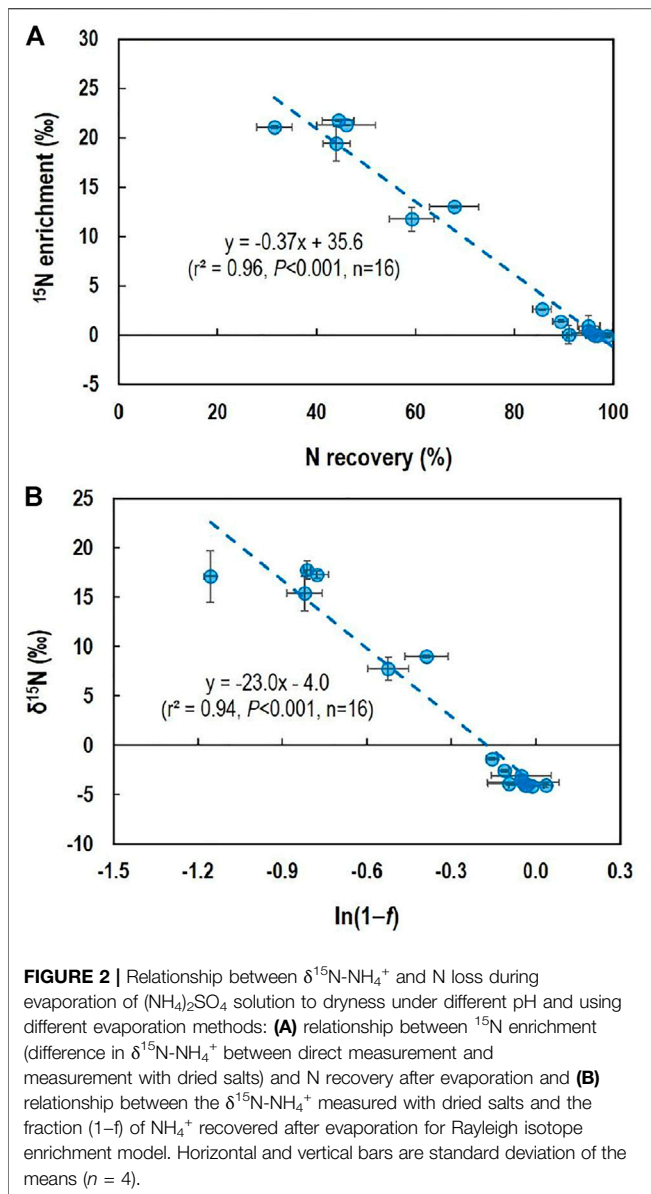
The temperature of the solutions quickly increased to the maximum temperature within 1 h, and was maintained at the temperature thereafter (Figure 1). The maximum temperatures of the top and bottom sides of the solution were 50.1 and 49.0°C for oven drying at 60°C, 87.0 and 69.3°C for oven drying at 100°C, and 53.0 and 53.2°C for infra-red chamber, respectively. The δ¹⁵N of (NH₄)₂SO₄ measured after evaporation was affected by N content and pH of the solutions and evaporation method (Table 1). Though the effects of these three factors on δ¹⁵N

were complicated as inferred from the significant interactions among the experimental factors, evaporation at pH < 3.5 produced more reliable δ¹⁵N than pH 5.4 across the N contents and evaporation methods. Among the evaporation methods, oven at 60°C, infra-red chamber, and freeze drier, but not oven at 100°C resulted in the δ¹⁵N comparable to the initial δ¹⁵N (−4.0‰) at pH < 3.5 regardless of N contents. Evaporating the solutions at pH 5.4 resulted in significant errors (1.4–21.8‰) regardless of N contents and evaporation methods including freeze-drying due to N loss as inferred from the low N recoveries (30–89%) (Table 1).

Isotope enrichment during evaporation, which was calculated as the difference between the δ¹⁵N measured after evaporation and the initial δ¹⁵N of solid (NH₄)₂SO₄ before dissolving and evaporation (Table 1), was negatively correlated with N recovery (Figure 2A). The relationship between N recovery and δ¹⁵N followed the Rayleigh isotope enrichment model (Mariotti et al., 1981):

$$\delta^{15}\text{N}_s = \delta^{15}\text{N}_i - \varepsilon \ln(1 - f)$$

where δ¹⁵N_s is δ¹⁵N of the remaining substrate (i.e., N in dried salts), δ¹⁵N_i is the initial δ¹⁵N (−4.0‰) of the substrate, ε is the isotope enrichment factor (the difference in δ¹⁵N between the substrate and its instantaneous product), and *f* is the fraction of the substrate that is consumed in the reaction (i.e., the fraction of N lost during evaporation). The slope of the regression equation indicated that ε is 23.0‰ and thus isotope fractionation factor is



1.023 (**Figure 2B**). This value is within the isotope fractionation factors (1.005–1.031) reported for NH_3 volatilization (Cejudo and Schiff, 2018).

Acidification (pH 3–4) of the distillate solution containing NH_4^+ is conventionally recommended for evaporation using an oven or infra-red chamber (Buresh et al., 1982; Feast and Dennis, 1996; Lee et al., 2012). Our results provide quantitative data on ^{15}N enrichment of the samples caused by NH_3 volatilization. Notably, it was found that acidification did not completely prevent NH_3 from volatilization under oven drying at 100°C , resulting in ^{15}N enrichments (0.5–0.9‰). This result is interesting as it is believed that acidulated $(\text{NH}_4)_2\text{SO}_4$ solution is stable at high temperature up to 235°C (Hauck, 1982). It is also notable that freeze-drying at pH 5.4 resulted in significant ^{15}N enrichments (1.4–2.6‰), suggesting that freeze-drying does not prevent NH_3 from volatilization and thus that acidification is still

necessary even when the samples are evaporated using a freeze drier (Stock et al., 2019). Therefore, our results suggest that evaporation using an oven at 100°C should be avoided and that acidification of NH_4^+ solution is essential regardless of evaporation methods (including freeze-drying).

$\delta^{15}\text{N}$ of NH_4^+ and NO_3^- Determined With Distillation Method

The $\delta^{15}\text{N-NH}_4^+$ measured using the distillation method varied ($p = 0.022$) with N content (**Figure 3A**). When N content was ≤ 0.3 mg, $\delta^{15}\text{N-NH}_4^+$ was underestimated by 1.0–2.1‰ (**Figure 3A**); meanwhile, when N contents were 0.4 and 0.5 mg, $\delta^{15}\text{N-NH}_4^+$ ($-4.0 \pm 0.5\text{‰}$ for both) was comparable to the reference $\delta^{15}\text{N}$. The poor accuracy for low- NH_4^+ samples

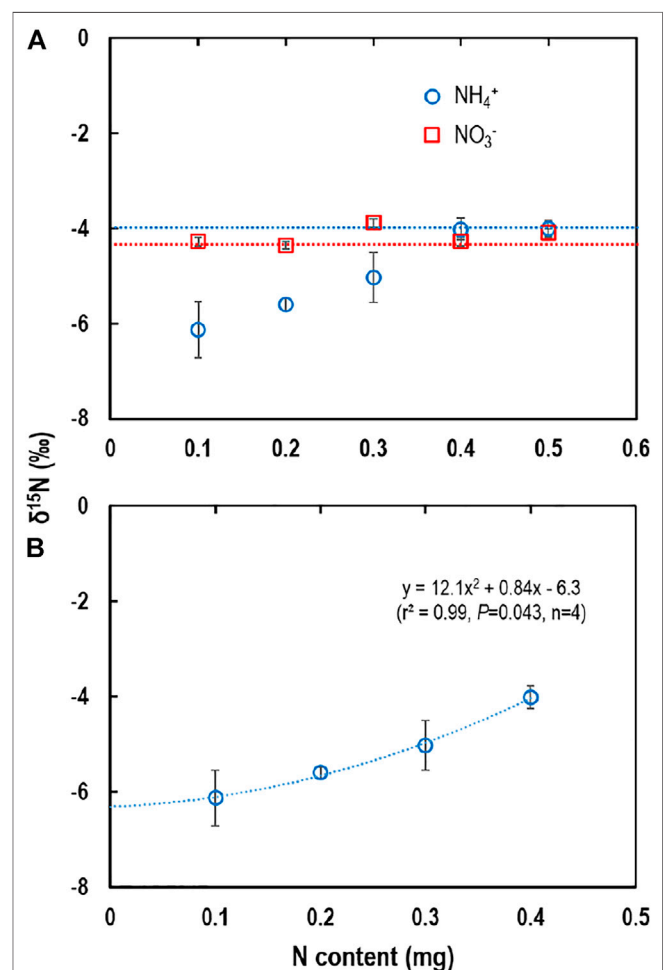
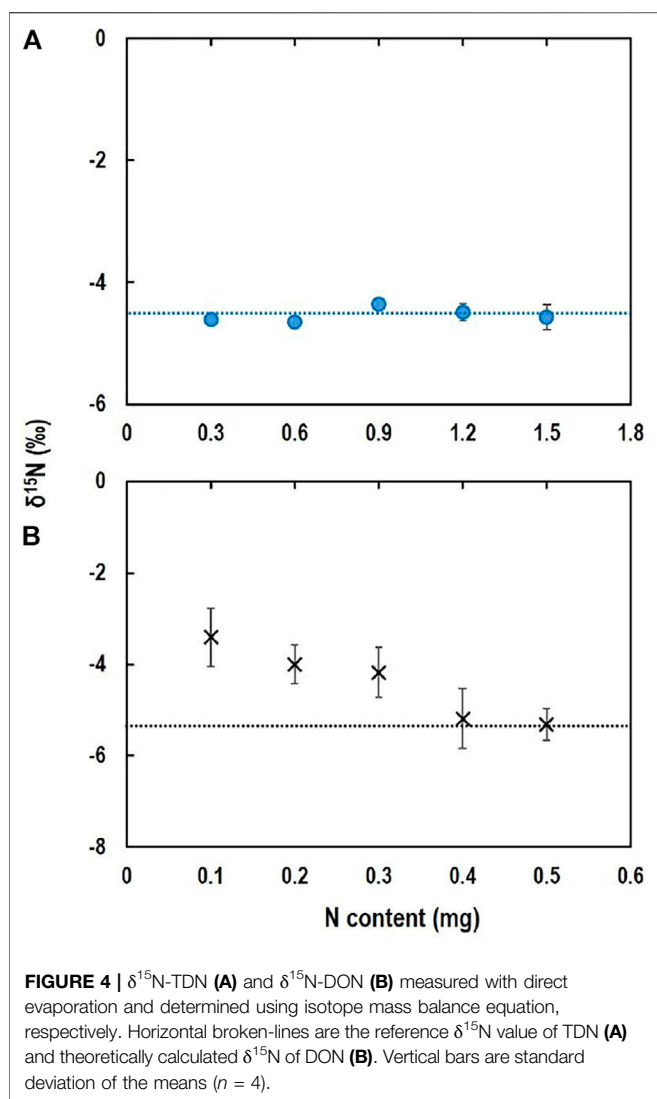


FIGURE 3 | $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ measured with distillation: **(A)** $\delta^{15}\text{N-NH}_4^+$ and $\delta^{15}\text{N-NO}_3^-$ with increased N content from 0.1 to 0.5 mg N and **(B)** regression between N content and $\delta^{15}\text{N}$ of NH_4^+ for 0.1–0.4 mg N sample (0.5 mg N was not included as accuracy was high enough). Vertical bars are the standard deviation of the means ($n = 4$). For **(A)**, horizontal broken-lines are the reference $\delta^{15}\text{N}$ values of NH_4^+ (blue one) and NO_3^- (red one) determined directly with EA-IRMS. For **(B)**, the y-intercept of the regression equation indicates that the $\delta^{15}\text{N}$ of background contamination is -6.3‰ .



could be attributed to the sample size effect on EA-IRMS measurement and/or inevitable background N contamination (Stark and Hart, 1996). However, in the present study, a similar amount of N (ca. 0.1 mg N) was used for the $\delta^{15}\text{N}$ analysis on EA-IRMS for all the samples, and thus the sample size effect might be negligible. Therefore, background contamination arose from impurities of reagents and unintended trapping of NH_3 from laboratory air during evaporation might be more critical (Stark and Hart, 1996; Jensen, 1991; Sakata, 2001). In the present study, air NH_3 contamination was not detected in the evaporation experiment (Table 1), and thus the background contamination should be ascribed to impurities in the reagents. The underestimated $\delta^{15}\text{N}$ - NH_4^+ together with the positive correlation between N contents and $\delta^{15}\text{N}$ - NH_4^+ (Figure 3B) indicates potential contamination by NH_4^+ impurity of which $\delta^{15}\text{N}$ is lower than the sample NH_4^+ (-4.0‰). The $\delta^{15}\text{N}$ of background NH_4^+ contaminants could be estimated using a regression equation between N content and $\delta^{15}\text{N}$ of NH_4^+ (Figure 3B). The regression

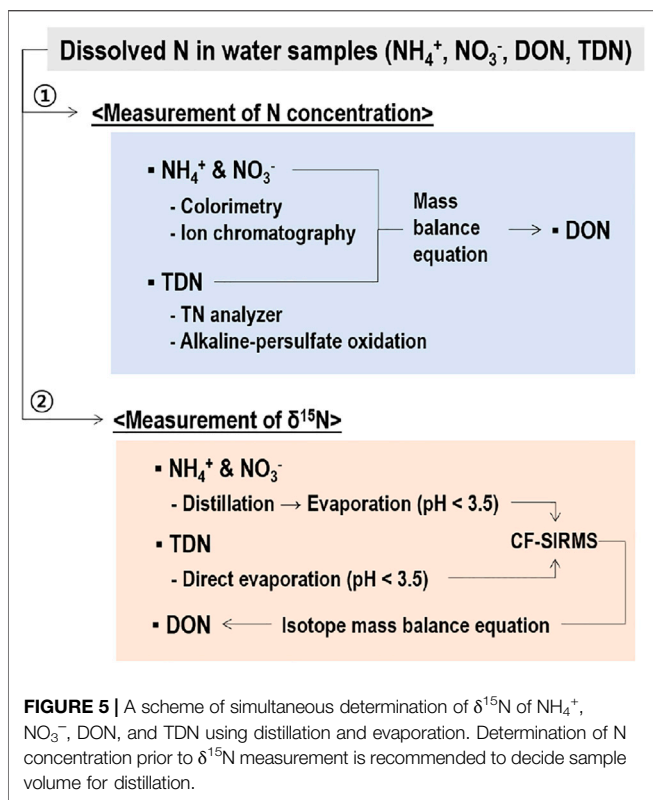
equation indicated that the $\delta^{15}\text{N}$ of background NH_4^+ was -6.3‰ (y -intercept; i.e., the $\delta^{15}\text{N}$ value when no sample NH_4^+ was added). Theoretically, background correction can be made using isotope dilution of a known ^{15}N -enriched standard (Stark and Hart, 1996; Chen and Dittert, 2008; Cao et al., 2018) though this was not possible in the present study. As the magnitude of background contamination may vary with the experimental batch, we suggest that a laboratory-specific minimum N requirement (0.4 mg in the present study) needs to be determined prior to sample analyses for reliable measurement of $\delta^{15}\text{N}$ of NH_4^+ using distillation. For samples containing a low NH_4^+ concentration, therefore, either increasing sample volume or sample concentration is necessary for distillation though this process may require an additional time. However, it is necessary to notice that other method for the determination of the $\delta^{15}\text{N}$ of NH_4^+ , such as diffusion methods, also takes several days (up to 7 days) for complete diffusion (Cao et al., 2018).

The $\delta^{15}\text{N}$ - NO_3^- fluctuated from $-4.4 \pm 0.1\text{‰}$ to $-3.9 \pm 0.5\text{‰}$, but the values were not statistically different ($p > 0.05$) from the reference $\delta^{15}\text{N}$ across the contents of NO_3^- (Figure 3A). This result indicates that any trace background NH_4^+ was removed from the distillation system during the first distillation for sample NH_4^+ under alkaline conditions using MgO. Therefore, $\delta^{15}\text{N}$ of NO_3^- can be measured with a high accuracy using the distillation method across a wide range of N content.

$\delta^{15}\text{N}$ -Total Dissolved N Determined With Direct Evaporation and $\delta^{15}\text{N}$ -Dissolved Organic N Calculated Using the Isotope Mass Balance Equation

The $\delta^{15}\text{N}$ -TDN was consistent across N contents from 0.1 to 0.5 mg N, and the averaged $\delta^{15}\text{N}$ -TDN ($-4.5 \pm 0.1\text{‰}$) (Figure 4A) was not different ($p > 0.05$) from the theoretical $\delta^{15}\text{N}$ -TDN ($-4.5 \pm 0.03\text{‰}$). Some studies have investigated $\delta^{15}\text{N}$ -TDN using alkaline-persulfate oxidation of TDN to produce NO_3^- followed by reduction to gases N_2O for $\delta^{15}\text{N}$ measurement (Lachouani et al., 2010; Cao et al., 2021). Our results show that direct evaporation of water samples after acidification is also a feasible and simple method to determine $\delta^{15}\text{N}$ -TDN. Though a small segment of CO_2 contained in water sample may be decomposed to CO ($^{12}\text{C}^{18}\text{O}$) to interfere with $^{30}\text{N}_2$ in the IRMS (Russow et al., 2002), acidification of the sample could eliminate the potential interference as CO_2 is removed under acidic conditions.

$\delta^{15}\text{N}$ -DON calculated using the isotope mass balance equation showed a high variability with errors of 1.2–2.0‰ when DON content was 0.1–0.3 mg N, suggesting that the accuracy of $\delta^{15}\text{N}$ -DON is dependent on the DON content (Figure 4B). Cao et al. (2021) reported that accuracy of indirect determination of $\delta^{15}\text{N}$ -DON by measuring $\delta^{15}\text{N}$ -TDN through alkaline-persulfate digestion method depended on DON content as analytical errors for determination of $\delta^{15}\text{N}$ -DON were as high as 0.7–1.4‰ for samples with a low DON content (<0.2 mg N). It is also obvious that $\delta^{15}\text{N}$ -DON is dependent on $\delta^{15}\text{N}$ of other N species used in the isotope mass balance equation (Cao et al.,



2021). In the present study, among N species, the variability of δ¹⁵N-NH₄⁺ was highest for the samples containing 0.1–0.3 mg N (Figure 3A). Therefore, the accuracy of determination of δ¹⁵N-DON with the isotope mass balance equation using δ¹⁵N-NH₄⁺ and δ¹⁵N-NO₃⁻ (distillation) and δ¹⁵N-TDN (direct evaporation) is dependent on NH₄⁺ content. Therefore, indirect determination of δ¹⁵N-DON might be feasible for water samples with a high NH₄⁺ concentration.

Suggestions of the Procedure for Determination of δ¹⁵N of NH₄⁺, NO₃⁻, Dissolved Organic N, and Total Dissolved N of Rainwater Samples

Based on the results of this study, a procedure was suggested for determination of δ¹⁵N of multiple N species including NH₄⁺,

NO₃⁻, DON, and TDN of rainwater samples using distillation for NH₄⁺ and NO₃⁻, direct evaporation for TDN, and indirect calculation using isotope mass balance equation for DON (Figure 5). Prior to δ¹⁵N measurement, it is necessary to determine N concentration of each N species to determine the sample volume to be used for distillation, particularly for NH₄⁺. The N concentrations of samples could be determined with the non-distillation method more accurately with colorimetry (Mulvaney, 1996) and ion chromatography (Mou et al., 1993). Though the concentrations of NH₄⁺ and NO₃⁻ could be determined through distillation and acid-base titration, it is known that the precision and accuracy of distillation for the determination of NH₄⁺ and NO₃⁻ are not reliable particularly when H₂SO₄ is used as an absorbent of NH₃ instead of boric acid (APHA-AWWA-WEF, 1998). The concentrations of NH₄⁺ and NO₃⁻ can be easily determined accurately with colorimetry (Mulvaney, 1996) and ion chromatography (Mou et al., 1993). The concentration of TDN can be easily determined using TN auto-analyzer or alkaline-persulfate oxidation followed by manual or automated determination of NO₃⁻ (Ebina et al., 1983). The concentrations of DON can be calculated as difference between TDN and inorganic N (NH₄⁺ + NO₃⁻).

The δ¹⁵N-NH₄⁺ and δ¹⁵N-NO₃⁻ could be determined through distillation and evaporation following the procedure described above. For δ¹⁵N-NH₄⁺, it is important to make sure that NH₄⁺ content of the samples subject to distillation should be at least 0.4 mg N. However, a minimum requirement of NH₄⁺ for reliable measurement of δ¹⁵N may differ with laboratory conditions, and thus it is necessary to determine the minimum NH₄⁺ requirement using a standard NH₄⁺ chemical for each laboratory following the procedure used in the present study. For samples containing a low NH₄⁺ concentration, a larger volume of samples needs to be used for distillation. As the concentrations of NH₄⁺ in rainwater are highly variable depending on the sites and seasons, ranging from <0.1 to >1.0 mg N L⁻¹ (Lee et al., 2012; Zeng et al., 2020; Chen et al., 2022), however, water samples with an extremely low NH₄⁺ may be concentrated to reduce the volume of water sample being added to distillation flasks, which have a confined volume capacity (e.g., 500 ml in the present study). Water samples containing a low N are often concentrated using a freeze-drier (Chen et al., 2022). In this context, our results further suggest that acidification of water samples is essential to prevent ¹⁴NH₃ loss even under freeze-drying. The δ¹⁵N-TDN can be directly determined using salts obtained from evaporation of the

TABLE 2 | Results of the analyses of δ¹⁵N of reference N solution containing NH₄⁺, NO₃⁻, DON, and TDN using the proposed methods.

Reference N solutions ^a	δ ¹⁵ N (‰)			
	NH ₄ ⁺	NO ₃ ⁻	DON	TDN
Each 2 mg N of (NH ₄) ₂ SO ₄ , KNO ₃ , and CO(NH ₂) ₂ dissolved in 1 L distilled water	-4.2 (0.7)	-4.4 (0.4)	-5.2 (0.6)	-4.6 (0.3)
Each 3 mg N of (NH ₄) ₂ SO ₄ , KNO ₃ , and CO(NH ₂) ₂ dissolved in 1 L distilled water	-4.3 (0.7)	-4.4 (0.3)	-5.1 (0.6)	-4.6 (0.5)

Values are the means (n = 5) with standard deviations in the parentheses.

^aThe δ¹⁵N values of (NH₄)₂SO₄, KNO₃, and CO(NH₂)₂ determined with direct combustion using the EA-IRMS were -4.0 ± 0.03‰, -4.2 ± 0.03‰, and -5.4 ± 0.04‰, respectively, and the theoretical values of δ¹⁵N-TDN calculated from NH₄⁺, NO₃⁻, and CO(NH₂)₂ was -4.5 ± 0.03‰.

acidified samples to dryness. Finally, $\delta^{15}\text{N}$ -DON is determined with an isotope mass balance equation using N concentration and $\delta^{15}\text{N}$ of N species.

When the methods were tested using the reference solutions, the measured $\delta^{15}\text{N}$ was not statistically ($p > 0.05$) different from the values determined by the direct combustion method using the EA-IRMS (Table 2). However, the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- were slightly lower than the reference $\delta^{15}\text{N}$, while the $\delta^{15}\text{N}$ of DON was higher than the reference $\delta^{15}\text{N}$, probably due to the potential influence of hydrolysable DON (i.e., $\text{CO}(\text{NH}_4)_2$ in the present study) on the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- . In the present study, pH was raised by using MgO rather than NaOH to minimize the interference of N liberated from DON under alkaline conditions (Mulvaney, 1996; Sakata, 2001). However, such variations in the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- suggest that $\text{CO}(\text{NH}_2)_2$ used as a DON reference might be partially subject to hydrolysis during distillation. As such interference of DON was not detected in the early experiment, it was suspected that alteration of the $\delta^{15}\text{N}$ of NH_4^+ and NO_3^- by hydrolyzable DON is not systematic but random. In the natural water environment, however, DON compounds present as more complex and recalcitrant compounds associated with lipids, proteins, amino sugars, lignins, and tannins (Lusk and Toor, 2016; Zhang et al., 2021), and thus the interference caused by DON during distillation of natural water samples might be less significant than the experimental conditions in the present study.

CONCLUSION

In this study, we proposed a protocol for sequential determination of $\delta^{15}\text{N}$ of NH_4^+ , NO_3^- , DON, and TDN through the distillation and direct evaporation of rainwater samples. To obtain salt samples for EA-IRMS after distillation, it was found that acidification to $\text{pH} < 3.5$ is essential regardless of evaporation methods including freeze-drying, but evaporation using an oven at 100°C should be avoided. The $\delta^{15}\text{N}$ - NO_3^- analyzed with the distillation method was reliable regardless of N content. However, there was uncertainty for $\delta^{15}\text{N}$ - NH_4^+ that was affected by sample N content, background contamination, and potential interference by co-existing DON. Despite those, the

REFERENCES

- Ackerman, D., Millet, D. B., and Chen, X. (2019). Global Estimates of Inorganic Nitrogen Deposition across Four Decades. *Glob. Biogeochem. Cycles* 33, 100–107. doi:10.1029/2018GB005990
- APHA-AWWA-WEF (1998). *Standard Methods for the Examination of Water and Wastewater*. 20th ed. Maryland: United Book Press Inc.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., et al. (2010). Global Assessment of Nitrogen Deposition Effects on Terrestrial Plant Diversity: a Synthesis. *Ecol. Appl.* 20, 30–59. doi:10.1890/08-1140.1
- Böhlke, J. K., Smith, R. L., and Hannon, J. E. (2007). Isotopic Analysis of N and O in Nitrite and Nitrate by Sequential Selective Bacterial Reduction to N_2O . *Anal. Chem.* 79, 5888–5895. doi:10.1021/ac070176k

distillation of >0.4 mg N of NH_4^+ produced stable and reliable $\delta^{15}\text{N}$. For $\delta^{15}\text{N}$ -TDN, direct evaporation to dryness was proven to be a reliable method. Therefore, the analytical reliability of the determination of $\delta^{15}\text{N}$ -DON using the isotope mass balance equation largely depends on the accurate measurement of $\delta^{15}\text{N}$ - NH_4^+ that was directly affected by the content of NH_4^+ -N in the present study. Therefore, for samples containing a low NH_4^+ concentration, either increasing sample volume for distillation or using an alternative method such as diffusion is recommended for analysis of $\delta^{15}\text{N}$ - NH_4^+ . Though the time and cost-effectiveness of the proposed methods were not analyzed in the present study, the proposed protocol can be considered in the laboratories equipped with distillation apparatus for the determination of N of soil, water, and plant samples to save cost of installing additional experimental apparatus for the analysis of the $\delta^{15}\text{N}$ of multiple N species.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

AUTHOR CONTRIBUTIONS

Y-JJ, Investigation, Writing-drafts; B-SS, Investigation; NB, Investigation; J-HK, Writing-editing; S-ML, Investigation and Writing-editing; H-JP, Conceptualization and Writing-editing; W-JC, Conceptualization and Writing-editing.

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- Bronk, D., and Glibert, P. (1991). A ^{15}N Tracer Method for the Measurement of Dissolved Organic Nitrogen Release by Phytoplankton. *Mar. Ecol. Prog. Ser.* 77, 171–182. doi:10.3354/meps077171
- Buresh, R., Austin, E., and Craswell, E. (1982). Analytical Methods in ^{15}N Research. *Fertilizer Res.* 3, 37–62. doi:10.1007/BF01063408
- Cao, H. T. T., Nakamura, T., Toyama, T., and Nishida, K. (2021). A Protocol for Nitrogen Isotopic Measurement of Dissolved Organic Nitrogen with a Combination of Oxidation-Denitrification and Gas Phase Diffusion Methods. *Isotopes Environ. Health Stud.* 57 (6), 576–584. doi:10.1080/10256016.2021.1948411
- Cao, T. T. H., Nakamura, T., Saiki, M., Ta, T. T., Toyama, T., and Nishida, K. (2018). Effect of Dissolved Organic Nitrogen Contamination on $\delta^{15}\text{N}$ - NH_4^+ Determination in Water Samples by Modification of the Diffusion Method with Gas-phase Trapping. *Rapid Commun. Mass. Spectrom.* 32 (8), 635–638. doi:10.1002/rcm.8091

- Cejudo, E., and Schiff, S. L. (2018). Nitrogen Isotope Fractionation Factors (α) Measured and Estimated from the Volatilisation of Ammonia from Water at pH 9.2 and pH 8.5. *Isotopes Environ. Health Stud.* 54 (6), 642–655. doi:10.1080/10256016.2018.1526792
- Chen, R. R., and Dittert, K. (2008). Diffusion Technique for ^{15}N and Inorganic N Analysis of Low-N Aqueous Solutions and Kjeldahl Digests. *Rapid Commun. Mass. Spectrom.* 22 (11), 1727–1734. doi:10.1002/rcm.3525
- Chen, Z., Huang, X., Huang, C., Yang, Y., Yang, H., Zhang, J., et al. (2022). High Atmospheric Wet Nitrogen Deposition and Major Sources in Two Cities of Yangtze River Delta: Combustion-Related NH_3 and Non-fossil Fuel NO_x . *Sci. Total Environ.* 806, 150502. doi:10.1016/j.scitotenv.2021.150502
- Cornell, S. E., Jickells, T. D., Cape, J. N., Rowland, A. P., and Duce, R. A. (2003). Organic Nitrogen Deposition on Land and Coastal Environments: A Review of Methods and Data. *Atmos. Environ.* 37, 2173–2191. doi:10.1016/S1352-2310(03)00133-X
- Ebina, J., Tsutsui, T., and Shirai, T. (1983). Simultaneous Determination of Total Nitrogen and Total Phosphorus in Water Using Peroxodisulfate Oxidation. *Water Res.* 17 (12), 1721–1726. doi:10.1016/0043-1354(83)90192-6
- Emerson, K., Russo, R. C., Lund, R. E., and Thurston, R. V. (1975). Aqueous Ammonia Equilibrium Calculations: Effect of pH and Temperature. *J. Fish. Res. Bd. Can.* 32, 2379–2383. doi:10.1139/f75-274
- Feast, N. A., and Dennis, P. (1996). A Comparison of Methods for Nitrogen Isotope Analysis of Groundwater. *Chem. Geol.* 129 (1-2), 167–171. doi:10.1016/0009-2541(95)00186-7
- Felix, J. D., Elliott, E. M., and Gay, D. A. (2017). Spatial and Temporal Patterns of Nitrogen Isotopic Composition of Ammonia at U.S. Ammonia Monitoring Network Sites. *Atmos. Environ.* 150, 434–442. doi:10.1016/j.atmosenv.2016.11.039
- Feuerstein, T. P., Ostrom, P. H., and Ostrom, N. E. (1997). Isotopic Biogeochemistry of Dissolved Organic Nitrogen: A New Technique and Application. *Org. Geochem.* 27 (7-8), 363–370. doi:10.1016/S0146-6380(97)00071-5
- Fowler, Z. K., Adams, M. B., and Peterjohn, W. T. (2015). Will More Nitrogen Enhance Carbon Storage in Young forest Stands in central Appalachia? *For. Ecol. Manag.* 337, 144–152. doi:10.1016/j.foreco.2014.10.023
- Hales, J. M., and Drewes, D. R. (1979). Solubility of Ammonia in Water at Low Concentrations. *Atmos. Environ.* 13 (8), 1133–1147. doi:10.1016/0004-6981(79)90037-4
- Hauck, R. D. (1982). “Nitrogen-Isotope-Ratio Analysis,” in *Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties*. Editor A. L. Page (Madison, WI: SSSA and ASA), 735–779. doi:10.2134/agronmonogr9.2.2ed.c36
- Jensen, E. S. (1991). Evaluation of Automated Analysis of ^{15}N and Total N in Plant Material and Soil. *Plant Soil* 133, 83–92. doi:10.1007/BF00011902
- Karamanos, R. E., and Rennie, D. A. (1981). The Isotope Composition of Residual Fertilizer Nitrogen in Soil Columns. *Soil Sci. Soc. America J.* 45, 316–321. doi:10.2136/sssaj1981.03615995004500020018x
- Lachouani, P., Frank, A. H., and Wanek, W. (2010). A Suite of Sensitive Chemical Methods to Determine the $\delta^{15}\text{N}$ of Ammonium, Nitrate and Total Dissolved N in Soil Extracts. *Rapid Commun. Mass. Spectrom.* 24 (24), 3615–3623. doi:10.1002/rcm.4798
- Lee, K.-S., Lee, D.-S., Lim, S.-S., Kwak, J.-H., Jeon, B.-J., Lee, S.-I., et al. (2012). Nitrogen Isotope Ratios of Dissolved Organic Nitrogen in Wet Precipitation in a metropolis Surrounded by Agricultural Areas in Southern Korea. *Agric. Ecosyst. Environ.* 159, 161–169. doi:10.1016/j.agee.2012.07.010
- Li, Y., Schichtel, B. A., Walker, J. T., Schwede, D. B., Chen, X., Lehmann, C. M. B., et al. (2016). Increasing Importance of Deposition of Reduced Nitrogen in the United States. *Proc. Natl. Acad. Sci. U.S.A.* 113, 5874–5879. doi:10.1073/pnas.1525736113
- Liu, D., Fang, Y., Tu, Y., and Pan, Y. (2014). Chemical Method for Nitrogen Isotopic Analysis of Ammonium at Natural Abundance. *Anal. Chem.* 86, 3787–3792. doi:10.1021/ac403756u
- Liu, X.-Y., Xiao, H.-W., Xiao, H.-Y., Song, W., Sun, X.-C., Zheng, X.-D., et al. (2017). Stable Isotope Analyses of Precipitation Nitrogen Sources in Guiyang, Southwestern China. *Environ. Pollut.* 230, 486–494. doi:10.1016/j.envpol.2017.06.010
- Lusk, M. G., and Toor, G. S. (2016). Biodegradability and Molecular Composition of Dissolved Organic Nitrogen in Urban Stormwater Runoff and Outflow Water from a Stormwater Retention Pond. *Environ. Sci. Technol.* 50, 3391–3398. doi:10.1021/acs.est.5b05714
- Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., et al. (1981). Experimental Determination of Nitrogen Kinetic Isotope Fractionation: Some Principles; Illustration for the Denitrification and Nitrification Processes. *Plant Soil* 62, 413–430. doi:10.1007/BF02374138
- McIlvin, M. R., and Altabet, M. A. (2005). Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. *Anal. Chem.* 77, 5589–5595. doi:10.1021/ac050528s
- Mou, S., Wang, H., and Sun, Q. (1993). Simultaneous Determination of the Three Main Inorganic Forms of Nitrogen by Ion Chromatography. *J. Chromatogr. A.* 640 (1-2), 161–165. doi:10.1016/0021-9673(93)80178-B
- Mulvaney, R. L. (1996). “Methods of Soil Analysis,” in *Methods of Soil Analysis. Part 3: Chemical Methods*. Editors D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, et al. (Madison, WI: SSSA and CSA), 1123–1184. doi:10.2136/sssabookser5.3
- Nanus, L., Campbell, D. H., Lehmann, C. M. B., and Mast, M. A. (2018). Spatial and Temporal Variation in Sources of Atmospheric Nitrogen Deposition in the Rocky Mountains Using Nitrogen Isotopes. *Atmos. Environ.* 176, 110–119. doi:10.1016/j.atmosenv.2017.12.023
- Nikolenko, O., Jurado, A., Borges, A. V., Knöller, K., and Brouyère, S. (2018). Isotopic Composition of Nitrogen Species in Groundwater under Agricultural Areas: A Review. *Sci. Total Environ.* 621, 1415–1432. doi:10.1016/j.scitotenv.2017.10.086
- Ohte, N. (2012). Tracing Sources and Pathways of Dissolved Nitrate in forest and River Ecosystems Using High-Resolution Isotopic Techniques: a Review. *Ecol. Res.* 28, 749–757. doi:10.1007/s11284-012-0939-3
- Phoenix, G. K., Emmett, B. A., Britton, A. J., Caporn, S. J. M., Dise, N. B., Helliwell, R., et al. (2012). Impacts of Atmospheric Nitrogen Deposition: Responses of Multiple Plant and Soil Parameters across Contrasting Ecosystems in Long-Term Field Experiments. *Glob. Change Biol.* 18, 1197–1215. doi:10.1111/j.1365-2486.2011.02590.x
- Russow, R., Kupka, H.-J., Götz, A., and Apelt, B. (2002). A New Approach to Determining the Content and ^{15}N Abundance of Total Dissolved Nitrogen in Aqueous Samples: TOC Analyser-QMS Coupling. *Isotopes Environ. Health Stud.* 38 (4), 215–225. doi:10.1080/10256010208033267
- Sá, T. S. W., Najjar, M. K., Hammad, A. W. A., Vazquez, E., and Haddad, A. (2022). Assessing Rainwater Quality Treated via a green Roof System. *Clean. Techn Environ. Pol.* 24 (2), 645–660. doi:10.1007/s10098-021-02144-6
- Sáez-Plaza, P., Navas, M. J., Wybraniec, S., Michałowski, T., and Asuero, A. G. (2013). An Overview of the Kjeldahl Method of Nitrogen Determination. Part II. Sample Preparation, Working Scale, Instrumental Finish, and Quality Control. *Crit. Rev. Anal. Chem.* 43 (4), 224–272. doi:10.1080/10408347.2012.751787
- Sakata, M. (2001). A Simple and Rapid Method for $\text{DELTA}^{15}\text{N}$ Determination of Ammonium and Nitrate in Water Samples. *Geochem. J.* 35 (4), 271–275. doi:10.2343/geochemj.35.271
- Sigman, D. M., Casciotti, K. L., Andreani, M., Barford, C., Galanter, M., and Böhlke, J. K. (2001). A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. *Anal. Chem.* 73, 4145–4153. doi:10.1021/ac010088e
- Slawyk, G., and Raimbault, P. (1995). Simple Procedure for Simultaneous Recovery of Dissolved Inorganic and Organic Nitrogen in ^{15}N -Tracer Experiments and Improving the Isotopic Mass Balance. *Mar. Ecol. Prog. Ser.* 124, 289–299. doi:10.3354/MEPS124289
- Stark, J. M., and Hart, S. C. (1996). Diffusion Technique for Preparing Salt Solutions, Kjeldahl Digests, and Persulfate Digests for Nitrogen-15 Analysis. *Soil Sci. Soc. America J.* 60 (6), 1846–1855. doi:10.2136/sssaj1996.03615995006000060033x
- Stock, P., Hösch, A., and Burghardt, D. (2019). $\delta^{15}\text{N}$ Analysis of Ammonium in Freeze-dried Natural Groundwater Samples by Precipitation with Sodium Tetrphenylborate. *Rapid Commun. Mass. Spectrom.* 33 (22), 1730–1738. doi:10.1002/rcm.8526
- Zeng, J., Yue, F.-J., Li, S.-L., Wang, Z.-J., Qin, C.-Q., Wu, Q.-X., et al. (2020). Agriculture Driven Nitrogen Wet Deposition in a Karst Catchment in

- Southwest China. *Agric. Ecosyst. Environ.* 294, 106883. doi:10.1016/j.agee.2020.106883
- Zhang, S., Fang, Y., and Xi, D. (2015). Adaptation of Micro-diffusion Method for the Analysis of ^{15}N Natural Abundance of Ammonium in Samples with Small Volume. *Rapid Commun. Mass. Spectrom.* 29 (14), 1297–1306. doi:10.1002/rcm.7224
- Zhang, L., You, Y., Gao, C., Peng, Y., and Cao, Z. (2021). Dissolved Organic Nitrogen Structural and Component Changes in Overlying Water along Urban River at Molecular and Material Levels - Beiyun basin Case Study. *J. Clean. Prod.* 287, 125570. doi:10.1016/j.jclepro.2020.125570

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