A Sequential Diffusion Method for ^{15}N Natural Abundance Measurement of Ammonium, Nitrate and Total Dissolved Nitrogen in Water Samples

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Abstract
Natural abundance of nitrogen-15 ($\delta^{15}N$) is a useful tool to estimate sources of nitrogen (N). The objective of the study was to clarify practical conditions of a sequential diffusion-based method for ^{15}N natural abundance of inorganic N and total dissolved N (TDN) with relatively high concentration. We tested the sequential diffusion method at 40ºC for 24 hours for recovery of NH_4-N and NO_3-N (0–40 mg L^{-1}) and for that of TDN (0–4 mg L^{-1}). Results showed that the complete recovery was achieved for inorganic N with 0.3–30 mg L^{-1} and for TDN 0–3 mg L^{-1}. Furthermore, recovery rates for TDN declined when the amount of N exceeded 120 µg N. The time required for N recovery can be shortened to 24 hours by increasing temperature to 40ºC. No discrimination of ^{15}N occurred during the whole process under the above conditions. In conclusion, the sequential diffusion method for ^{15}N natural abundance measurement can be applied to water samples including 0.2–20 mg L^{-1} for NH_4-N or NO_3-N, and 0.25–3 mg L^{-1} for TDN. The volume for TDN recovery must be adjusted so that amount of TDN in solution is less than 90 µg N.

Key Words
PTFE, Devarda's alloy, MgO, agricultural area

Introduction

Nitrogen pollution of ground or surface waters is a serious matter of concern in the world. In agricultural areas, N in water exists in forms of NH_4-N, NO_3-N and organic N, and the concentration is relatively higher than in forest areas or in sea water. Natural abundance of ^{15}N has been used to estimate sources of N in water or soil samples and to understand the occurrence of denitrification in agricultural land (Maeda et al., 2003; Maeda et al., 2011).

Solid samples are subjected to measurement of $\delta^{15}N$ using a mass spectrometer with an element analyzer. The diffusion method developed by Sorensen and Jensen (1991), uses a polytetrafluoroethylene (PTFE) tape that encloses a sulfuric-acid-pipetted filter to absorb NH_3 from the solution. Sorensen and Jensen (1991) proposed a sequential diffusion method for collecting ^{15}N-lableled NH_4 and NO_3. Stark and Hart (1996) measured ^{15}N concentration of TDN in water samples for the first time by recovering NO_3-N converted from TDN by the persulfate digestion. Holmes et al. (1998) and Sigman et al. (1997) applied the sequential diffusion method to NH_4-N and NO_3-N at natural abundance levels.

Previous studies on diffusion-based methods have some drawbacks. Because of a long period required for N recovery (3–14 day), N may be transformed to another by bacteria during the process. Holmes et al. (1998) proposed the temperature of 40ºC to increase a N recovery rate but they did not consider the time for the N recovery. The accuracy of $\delta^{15}N$ measurement is reduced by contamination with inorganic N derived from the air-born NH_3 or impurities in chemical reagents (Sigman et al., 1997; Stephan and Kavanagh, 2009). Since the diffusion methods have been applied to relatively low N concentration samples (< 3 mg L^{-1}), the optimal ranges of higher N concentration remains uncertain.

In this study, we find out practical conditions for the sequential diffusion-based method to recover different forms of N as pretreatments of ^{15}N natural abundance measurement. We examined recovery rates of NH_4-N, NO_3-N or TDN with relatively high concentrations at 40ºC for 24 hours. The recovery time can be shorter if the recovery of N is complete under these conditions.

Materials and Methods

Manufacturing PTFE Traps

According to Holmes et al. (1998), a glass fiber filter (Whatman GF/D, φ 10 mm, combusted at 450ºC for 4

hours) was placed on a piece of PTFE tape (Sigma-Aldrich, 25×50 mm). Twenty microliters of 2 *M* H_2SO_4 were pipetted onto the center of the GF/D filter, then the PTFE tape was immediately sealed with tweezers. The PTFE traps were kept in a high density polyethylene bottle to prevent NH_3 contamination from the atmosphere.

Recovery of Inorganic N

Recovery rates of NH_4-N and NO_3-N were examined using the PTFE trap (Sorensen and Jensen, 1991). The solution samples include known concentrations of NH_4Cl or KNO_3 (0–40 mg L^{-1}). In the subsequent procedure, NH_4-N recovery was followed by NO_3-N. The isotopic discrimination of each form of N during the process was also checked for 15 mg L^{-1} samples. A volume of each sample for N recovery was determined so that the solution contained 40–200 µg N, which is required for the mass-spectrometry analysis.

Different volumes of samples (1–10 mL) with known concentrations of NH_4-N and NO_3-N, and NaCl (0.5 g in 10 mL, combusted at 450ºC) was added into a 100 or 200 mL vial to increase the osmotic potential to prevent the PTFE traps from swelling. After addition of a PTFE trap and MgO (0.03 g in 10 mL, combusted at 450ºC), the vials were immediately sealed with a butyl rubber cap, then shaken at 90 rpm at 40ºC for 24 hours. After removing the PTFE trap from the solution, a new PTFE trap and DA (0.08 g in 10 mL) were added into the solution for NO_3-N recovery. The vials were again shaken at 90 rpm at 40ºC for 24 hours. High pressure gas inside the vials was released to the air by inserting a needle into the rubber at 8 hour of the shaking. The PTFE trap that was taken out from the solution was rinsed with distilled water, and kept in a desiccator for 3 days until being dry.

Recovery of TDN

Recovery rates of TDN were tested using NH_4Cl and glycine. Ten milliliters of samples containing NH_4-N or glycine (0–4 mg L^{-1}) were mixed with 2 mL of the persulfate oxidation reagent (4 mg NaOH and 3 mg $K_2S_2O_8$ in 100 mL of deionized water) for the digestion to NO_3-N for 30 minutes at 121ºC. The digested samples were transferred to 100 or 200 mL vials and NO_3-N derived from NH_4-N or glycine was collected in the same manner as the NO_3-N recovery except for the timing of gas release (at 30 min of the shaking). Because the persulfate oxidation reagent increases the osmotic potential, we tested for NH_4-N samples whether or not NaCl should be added to the samples as used for the inorganic N recovery procedure. The ^{15}N discrimination during the whole process was checked for 3 mg N L^{-1} of glycine samples. A volume of samples were calculated according to the amount of N (40–200 µg).

Chemical Analyses

Collected PTFE traps were subjected to measurements of N concentration and $\delta^{15}N$. The GF/D filter containing NH_4-N was taken out of the PTFE tape. The GF/D filter was added into distilled water in a polyethylene bottle, then shaken for 1 hour at 25ºC. The extracts from all samples were analyzed for NH_4-N concentration using a continuous flow analyzer (Auto analyzer, QuAAtro 2-HR, BLTEC). For the isotopic analysis, the GF/D filter was enclosed into a tin cup (φ 5 mm, 9 mm deep). The enclosed samples were analyzed with a mass spectrometer (DELTA V ADVANTAGE, Thermo Scientific) combined with an element analyzer (FLASH2000, Thermo scientific). This procedure was done within 24 hours because the tin cups were corroded with H_2SO_4. The original chemical reagents were analyzed for ^{15}N to compare with those of recovered N in samples.

Results and Discussion

Recovery rates were nearly 100% for NH_4-N and NO_3-N samples including < 150 µg N (Figs. 1 and 2), which is corresponding to N concentrations < 30 mg L^{-1}. The results indicate that the recovering period can be shortened to 24 hours from 3–14 days (Sorensen and Jensen, 1991; Koba et al., 2010). On the other hand, recovery rates declined for samples including 150 µg N (N concentrations of 30 mg L^{-1}) for NH_4 and 210 µg (40 mg L^{-1}) for both inorganic N. Koba et al. (2010) reported that 4200 µg NH_4-N was completely recovered from 100 mL. The different results should be further investigated. In blank tests, NH_4-N was detected with 1.4–2.5 µg N (Fig. 1), which is consistent with Stephan and Kavanagh (2009). This contamination would be derived from the impurities in MgO or air-born NH_3. This level of NH_4-N contamination can be negligible when analyzing water samples with relatively high N concentration.

Recovery rates of TDN inputted as NH_4Cl were significantly improved by no addition of NaCl ($p < 0.05$, Fig. 3), when the NH_4-N concentration was less than 3 mg L^{-1}. Even without NaCl addition, PTFE traps were not broken for all samples. Accordingly, NaCl should not be added to TDN samples.

Recovery rates for TDN were nearly 100% for samples with < 90 µg N (Figs. 4–6). The largest standard deviations were found for 2 mg N L^{-1} samples (150 and 120 µg N for 80 and 60 mL, respectively, Figs. 4 and 5). Recovery rates of TDN derived from glycine declined with larger standard deviations when the amount of N exceeded 120 µg (Fig. 7), presumably due to the limited capacity of NH_4-N absorption by the PTFE trap used. Koba et al. (2010) reported that 4200 µg N was collected in the same PTFE trap during the process of NH_4-N recovery from freshwater and 2 *M* KCl solution. The persulfate reagent with pH 13 would neutralize the acid in the PTFE trap, resulting in lower NH_4-N absorption by the trap.

There was no significant difference in $\delta^{15}N$ values between the original reagents and recovered N ($p > 0.05$, Table 1). These results agreed with previous studies (Koba et al., 2010; Sigman et al., 1997).

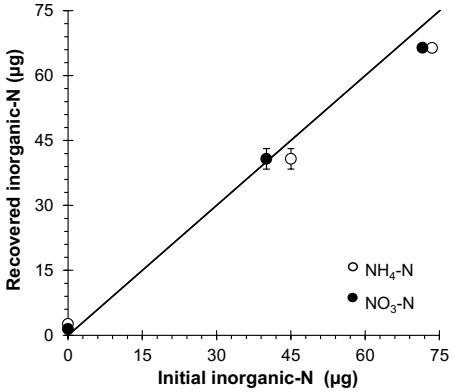

Figure 1 Initial vs. recovered inorganic N in low N concentration samples (Water volume: 150 mL).

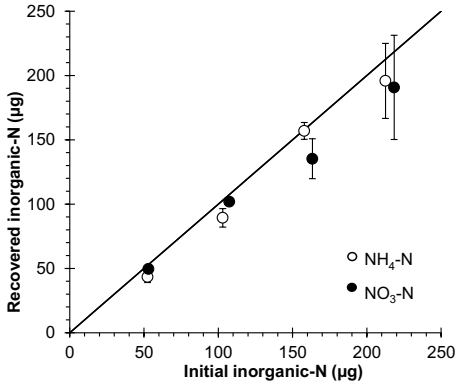

Figure 2 Initial vs. recovered inorganic N in high N concentration samples (Water volume: 5 mL).

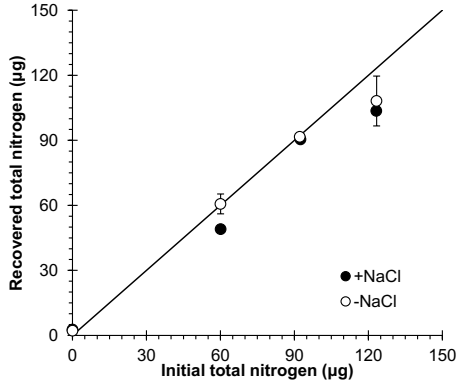

Figure 3 Effect of NaCl addition on the recovery process of TDN inputted as NH_4Cl (Water volume: 30 mL).

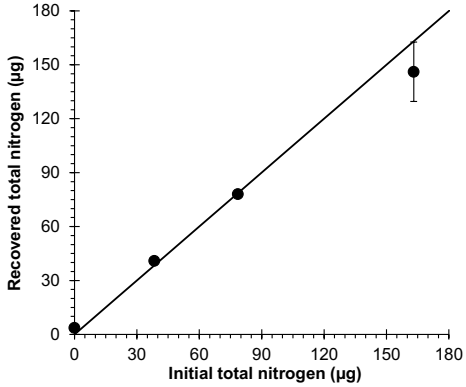

Figure 4 Initial vs. recovered TDN inputted as glycine with N concentration of 0.5–2 mg L^{-1} (Water volume: 80 mL).

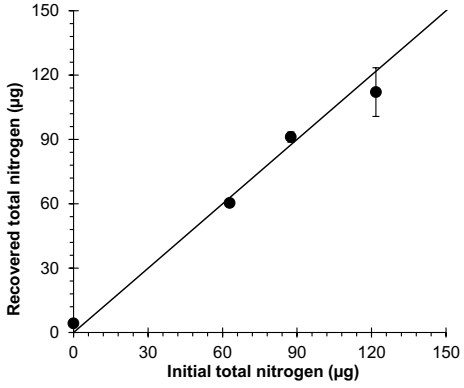

Figure 5 Initial vs. recovered TDN inputted as glycine with N concentration of 1–2 mg L^{-1} (Water volume: 60 mL).

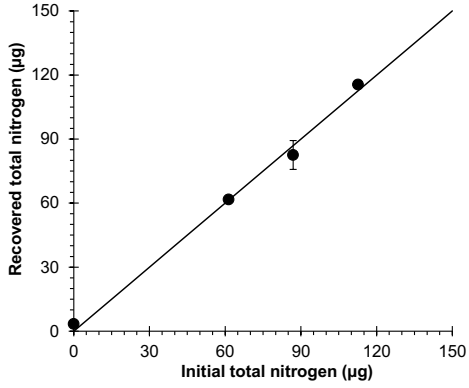

Figure 6 Initial vs. recovered TDN inputted as glycine with N concentration of 2–4 mg L^{-1} (Water volume: 30 mL).

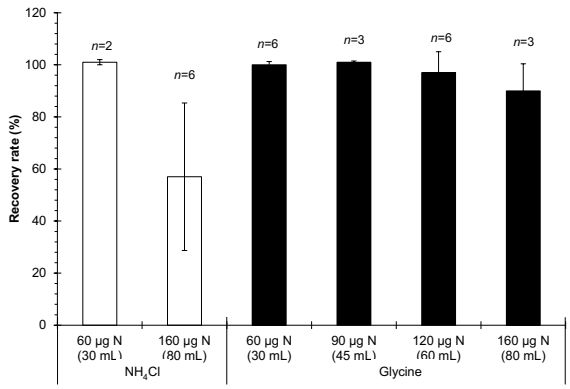

Figure 7 Effect of the amount of N on the recovery rates in NH_4Cl and glycine solutions with N concentration of 2 mg L^{-1}.

Table 1 Comparison of $\delta^{15}N$ values between the original reagents and recovered N.

Sample	N concentration (mg L^{-1})	$\delta^{15}N$ (‰)
NH_4-N		
Original NH_4Cl	–	-0.9±0.1*
Recovered N	15.0	-1.0±0.1*
NO_3-N		
Original KNO_3	–	-1.8±0.0*
Recovered N	15.0	-1.9±0.2*
TDN		
Original Glycine	–	+0.1±0.0*
Recovered N	3.0	+0.2±0.1*

Note. * indicates that there is no significant difference in $\delta^{15}N$ values between the original reagent and recovered N. The analytical errors for $\delta^{15}N$ values are less than 0.2‰.

Conclusion

We examined practical conditions for the sequential diffusion-based method at 40 ºC for 24 hours to recover N with different forms and high concentration. The time required for N recovery can be shortened to 24 hours by increasing temperature (40ºC) and additionally by releasing the pressure inside the vial with a needle 1 hour after the DA addition. Each concentration of NH_4-N and NO_3-N should be 0.3–20 mg L^{-1} and TDN 0.25–3 mg L^{-1}. For the $\delta^{15}N$ analysis of TDN, the amount of N should be less than 90 µg in a vial. If a water sample includes more than the ranges of N suggested above, the sample can be simply diluted before the N recovery procedures.

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