ROUTINE MEASUREMENT OF DISSOLVED INORGANIC ^{15}N IN PRECIPITATION AND STREAMWATER

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Abstract. The difficulty of measuring ^{15}N in dilute solutions has limited the potential of ecosystem labeling experiments and model comparisons. By concentrating the N from large (up to 20 L) water samples on ion-exchange resin columns, we obtained enough N for accurate and reproducible ^{15}N measurement using the Teflon tape diffusion method. Analysis of standards demonstrated >95% recovery of inorganic N from samples, and $\delta^{15}N$ values comparable to those obtained using standard distillation methods. The value of the blank at our laboratory was 0.16 μmoles N. Analytical precision was within 2 ‰ $\delta^{15}N$ when samples of streamwater from the Bear Brook Watersheds (1800 eq · ha^{-1} · yr^{-1} N addition at 192 ‰ $\delta^{15}N$) were kept frozen until analysis. The analytical process is lengthy, but once set up can easily be performed for large numbers of samples.

1. Introduction

Whole ecosystem and plot-level ^{15}N addition experiments have become more common in recent years. (e.g., Fry *et al.*, 1995; Nadelhoffer *et al.*, 1995; Kjønaas *et al.*, 1993a,b). This is due, in large part, to the increasing availability of high-precision automated ^{15}N analysis. Analysis of bulk soil and plant material is now routine (Jensen, 1991), and several diffusion methods have been published which are suitable for soil extracts and digested soils and plant tissues (Kelley *et al.*, 1991; Brooks *et al.*, 1989; Burke *et al.*, 1990). However, methods for measuring ^{15}N contents of surface waters with low N concentration are labor-intensive and subject to contamination. Ecosystem model validation using ^{15}N (Currie *et al.*, 1998) requires reliable estimates of ^{15}N inputs and outputs through time. Thus, improved methods of determining the ^{15}N concentration of rainwater and streamwater are essential.

Fifteen-N labeled $(NH_4)_2SO_4$ was applied to one of two paired ~10 ha catchments at the Bear Brook Watersheds in Maine, USA (BBWM) from April 1991 through December 1992 (Kahl *et al.*, 1993; Norton *et al.*, this volume). Fate of the ^{15}N - labeled fertilizer added to the West Bear Brook catchment is presented in a companion paper (Nadelhoffer *et al.*, this volume). In this paper, we report results of total N and ^{15}N analysis of laboratory solutions and of streamwater sampled at the outlet of the treated catchment in the years during and immediately following ^{15}N additions. Ammonium and nitrate concentrations in East and West Bear Brooks are commonly less than 5 μeq · L^{-1}, requiring that N from 1 to 10 L of solution be concentrated to acquire the minimum of 5 μmol N required for reliable ^{15}N measurement. The method we describe here combines a concentration step using ion exchange resins, based on

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the methods of Hoering (1957), Garten and Hanson (1990) and Garten (1992), in conjunction with a diffusion step based on the work of Sørensen and Jensen (1991).

2. Methods

2.1. Site

The Bear Brook watersheds (Norton *et al.*, this volume) are in eastern Maine, on the southeast facing slope of Lead Mountain. Forest cover on the paired 10.2 and 10.9 ha watersheds is composed of 40 to 60 year old mixed northern hardwoods with abundant older red spruce (*Picea rubens*) at higher elevations. Dry NH_4SO_4 fertilizer was applied bimonthly, by helicopter, starting in November, 1989 and continuing through 1995. Annual N loading has been increased from background levels of 600 eq $\cdot$ ha^{-1} $\cdot$ yr^{-1} (wet + dry) to 2400 eq $\cdot$ ha^{-1} $\cdot$ yr^{-1} in 1990-1995. Fertilizer applied to the snowpack in 1989 was included in the 1990 allocation. Fertilizer added to the West Bear catchment from April 1991 through December 1992 was labeled with ^{15}N, thereby increasing the ^{15}N concentration of fertilizer from $\delta\ ^{15}N = 0$ ‰ (0.3663 atm% ^{15}N) to $\delta\ ^{15}N = 192$ ‰ (0.4366 atm% ^{15}N).

2.2. Samples

Streams were generally sampled weekly. During periods of higher flow, sampling frequency was increased to daily and even hourly during high-flow episodes. After subsampling for chemical analysis, stream samples were combined into a large (1 to10 L) biweekly sample which was kept refrigerated if analysis could be completed within a few weeks or frozen for later analysis.

2.3. Concentration of N

Ion exchange resins were pre-treated by soaking in 1N HCl, and rinsed with deionized water until rinse water pH was 6 or greater. Resin columns consisted of a 10 mL plastic syringe, packed with a small wad of glass wool, 5 mL of either anion exchange resin (Dowex 1X-8) or cation exchange resin (Dowex 50X-8), followed by another small wad of glass wool. We arranged the columns in series with the cation exchange column first and the outlet maintained at or above the level of the resins in the first column (Figure 1), so that resins did not run dry. We introduced samples to the columns, using either gravity flow from an elevated reservoir or a peristaltic pump. When gravity flow was used, flow was regulated by a stopcock on the outlet tubing. Flow rates were kept below 0.5 drop $second^{-1}$. The volume of sample needed was determined by the concentrations of NO_3^- and NH_4^+ in streamwater. We processed enough water to capture a minimum of 5 μmol N (60μg N) sample for ^{15}N analysis,

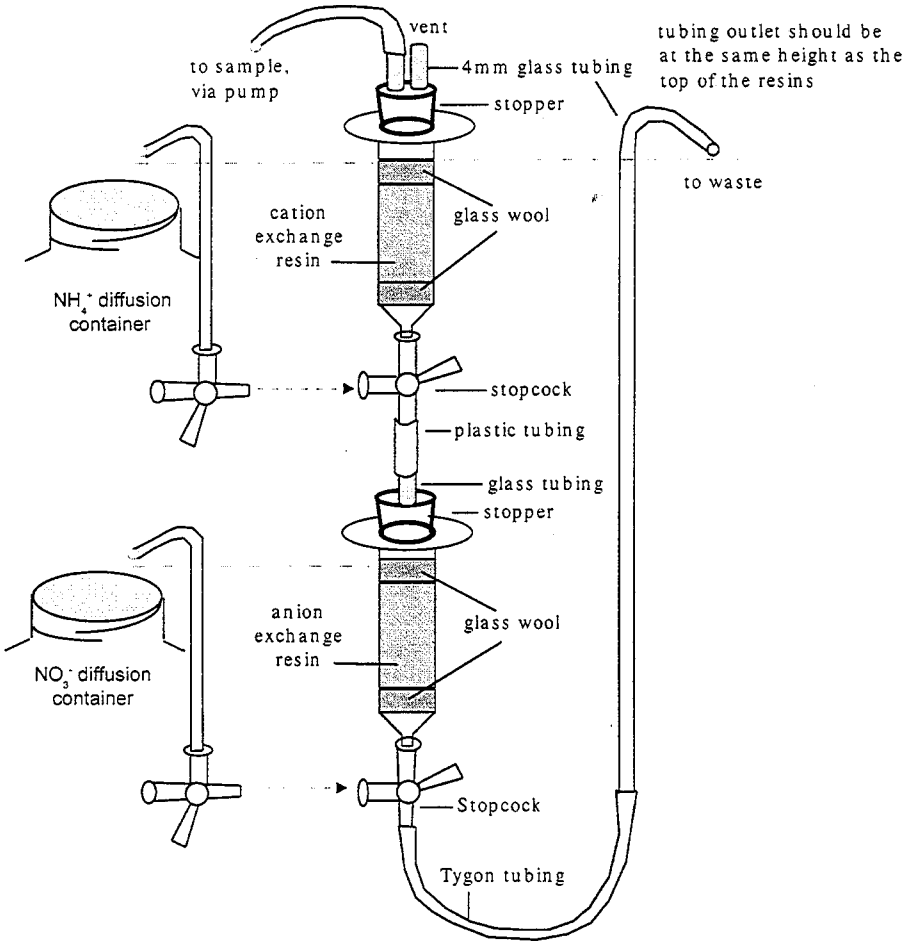

Fig. 1. Set-up for concentrating inorganic N on resin columns.

but not enough to saturate the exchange capacity of the resins (2.3 meq · mL^{-1}). To test for complete removal of inorganic N, we analyzed solutions leaving the resin beds.

2.4. RESIN ELUTION

After concentrating dissolved inorganic N (DIN) on resins, we eluted cation and anion exchange columns separately. First, any bubbles which formed in the columns during concentration were removed by gently backflushing with a small amount of deionized water from a syringe. We used 2 N KCl for an eluant, which we allowed to flow over the columns at 0.5 drop $second^{-1}$ and captured in the diffusion containers (250 mL polypropylene square bottles). We found that 100 mL KCl was sufficient to completely elute 5 mL resin columns at this flow rate. We analyzed subsamples of the eluant for NO_3^- and NH_4^+ and calculated recovery of dissolved inorganic N (DIN).

We pumped eluant onto the column and diverted the flow from the outlet stopcock of the cation exchange resin column into a collection container which was later used for diffusion (Figure 1). When the cation exchange column was completely eluted, it was removed from the series and eluant was pumped onto the anion exchange column. Outflow was diverted into a separate diffusion container. This procedure produces two separate solution samples for each water sample, one containing the eluted NO_3^- and the other NH_4^+. In some cases, we eluted the columns in series, yielding one sample for diffusion containing both NO_3^- and NH_4^+.

2.5. DIFFUSION

We converted NH_4^+ in eluant from cation exchange resins to NH_3 by increasing pH using MgO additions. Ammonia diffusing out of solution was captured on Teflon™ enclosed, acid-soaked glass fiber filter strips. Nitrate in solutions eluted from anion exchange resins was first reduced to ammonium by addition of Devarda's alloy. We prepared glass fiber filter strips by cutting (Whatman GF-F) into small (~5 mm x 12 mm) pieces and ashing at 450° C overnight. Packets were made from strips of polytetrafluoroethylene (Teflon™) tape (Fisher # 14-831-300B) approximately 2.5 cm x 6 cm. We placed a filter strip on one side of the tape, added 35 μL 2 N H_2SO_4, then quickly and carefully folded the Teflon back over the filter strip and sealed the edges firmly, doubling the tape on the sealed edges (Figure 2). Packets were immediately placed into 250 mL wide-mouth polypropylene bottles and the tops were sealed. When all solutions were ready to be diffused, we added an excess of MgO (~0.2 g) to all containers and ~0.4 g of Devarda's alloy to NO_3^- solutions, quickly recapped the containers and swirled them. Diffusion continued for 7 days at room temperature (~ 25° C), and bottles were swirled and inverted at least 3 times daily or placed on a gently oscillating shaker table. After 7 days, we removed the Teflon™ packets, opened them and allowed the filter strips to dry in a dessicator with an open container of concentrated H_2SO_4. When dry, filter strips were packed in tin boats for analysis and stored in individual air-tight vials until they were analyzed.

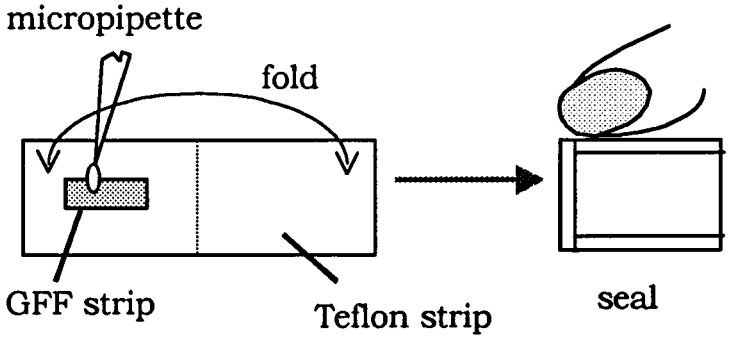

Fig. 2. Packing acid-soaked filter papers into Teflon packets.

When samples for diffusion contained both NH_4^+ and NO_3^- in solution, we employed a sequential diffusion technique. First, NH_4^+ was diffused and captured, using only MgO. After 7 days we removed those traps and replaced them with fresh ones, then added Devarda's alloy, converting and trapping the remaining NO_3^-.

2.6. Total N and ^{15}N analysis

Ammonium and NO_3^- in solution were analyzed on an Alpkem 300 rapid flow analyzer, using the phenol-alkaline hypochlorite (A303-S020) and the N-(1-napthyl)ethylenediamine dihydrochloride-sulfanilamide (A303-S170) methods respectively (Alpkem Standard Methods Manual, 1986). We also analyzed ammonium in solution by the phenate method (Standard Methods for the Examination of Water and Wastewater, American Public Health Association, 1980). Total N recovery on filters was measured on a Perkin-Elmer 2400 elemental analyzer, using acetanilide as a standard.

All streamwater ^{15}N analyses were conducted in the stable isotope laboratory at the Ecosystems Center in Woods Hole, Massachusetts and at the Department of Biology, Boston University, Boston, MA. Samples were analyzed for ^{15}N using a Finnigan MAT delta S isotope ratio mass spectrometer according to the methods of Fry *et al.* (1992). Combined analytical errors for sample combustion and measurement are generally less than 0.2 ‰ $\delta^{15}N$.

2.7. Comparison with distillation method

We performed a comparison of the standard distillation method with the resin/diffusion method presented here, using samples from a fertilization experiment which were of unknown enrichment between 50 and 100‰ δ ^{15}N. We removed 4

aliquots containing approximately 100 μmoles N, from each of 4 different samples of NH_4NO_3 fertilizer, enriched with $^{15}NO_3^-$ (1 & 2) or $^{15}NH_4^+$ (3 & 4). Two aliquots were distilled according to methods of Horrigan *et al.* (1990). Two other aliquots were diluted, then re-concentrated on resin columns, using the method described above. One of these eluants was then distilled and the other was diffused. In this experiment, the cation and anion exchange columns were eluted together into a single diffusion container, then NH_4^+ and NO_3^- were diffused sequentially from the single sample.

3. Results

3.1. Solution Concentration

We attempted to concentrate dilute solutes first by simply mixing approximately 5 mL of cation-exchange resin into 1 liter of solution, mixing over a period of 4 to 12 hours, filtering out resin, then adding anion-exchange resin and mixing for 4 to 12 hours. For 12 samples of 30 μm NH_4-N/30μm NO_3-N, removal of NH_4^+ averaged 96.2 ± 0.5% and removal of NO_3^- averaged 97.1 ± 0.5% (Table I). We were able to improve removal efficiency to 99.5 ± 0.1% (n=4) by passing solutions through 5 mL resin columns. Using 50 mL of 2 N KCl eluant, at a drip rate of 0.5 drops/second, we achieved an elution efficiency of 102.2 ± 2.75% (n=3).

TABLE I

Removal of 30 μmoles NH_4^+ and 30 μmoles NO_3^- from 1 liter of solution, using 1 to 4 (~0.2g) scoops of resin and shaking for 4 to12 hours. Results are expressed as percent of original N removed. *nt*=not tested

		Resin Addition (*scoops*)			
		1	2	3	4
ion	*hours*	N removal (%)			
NH_4^+	4	93.43%	96.80%	96.80%	97.67%
NH_4^+	8	95.50%	96.63%	97.00%	97.30%
NH_4^+	12	93.07%	96.17%	97.00%	97.47%
NO_3^-	4	97.50%	*nt*	*nt*	96.77%

3.2. Diffusion

To test for complete recovery of N on filters, we diffused 20 μeq NH_4^+ and 20 μeq NO_3^- samples and analyzed the filters for N. We recovered 98.4 ± 1.7% of the NH_4^+ and 99.0 ± 2.8% of the NO_3^- in solution. We also diffused 2 sets of natural abundance standards, at two different laboratories (Table II). These were internal laboratory

standards, which have been analyzed repeatedly. Recoveries were consistent, but slightly above 100%, even when the small blank associated with the KCl was considered. Isotope results were consistent within 1 ‰ $\delta\ ^{15}N$ and matched direct measures of standard salts within 1.5 ‰ $\delta\ ^{15}N$. We were unable to measure the mass of N associated with KCl only, but even a very small mass can distort ^{15}N results when its ^{15}N abundance is quite different from the sample. To address this problem, we analyzed a size series of slightly ^{15}N-enriched samples (Figure 3), and determined the blank by regressing the inverse of sample size against the measured $\delta\ ^{15}N$ value (Fry *et al.*, 1992). The blank is estimated from this regression by assuming the $\delta\ ^{15}N$ of the blank is at natural abundance level, *i.e.*, 0 ‰. For the sample containers that we used and 100 mL of 2 N KCl, the blank was 0.16 μmol N.

TABLE II

Direct analysis of natural abundance standards compared with diffusion of separate NH_4^+ and NO_3^- standard solutions and sequential diffusion of combined NO_3^- and NH_4^+ standard solutions. Direct analysis was the long-term average of ^{15}N results from these internal laboratory standards. *nt*=not tested

Sample	lab.	direct (‰)	Separate diffusion (‰)	sequential diffusion (‰)	recovery (%)
$(NH_4)_2SO_4$	MBL	-1.7	-3.0 (± 0.0)	-3.0 (±0.3)	*nt*
KNO_3	MBL	4.4	3.5 (± 0.6)	4.2 (±0.7)	*nt*
$(NH_4)_2SO_4$ *	BU	-1.5	-1.5 (± 0.0)		103.8 (± 0.8)
KNO_3*	BU	2.3	2.1(± 0.1)		103.4 (± 0.2)

* Standards run at BU and standards run at MBL came from different lots.

3.3. Comparison with distillation method

Samples concentrated on resin columns and then either diffused or distilled produced $\delta^{15}N$ values which were quite similar, but generally somewhat less than those of samples which had been directly distilled (Table III). Dowex 1X8 anion exchange resins consist of a trimethylbenzylammonium exchange group on a polystyrene base and thus contain a large amount of N. Nitrogen released from the resins could have diluted ^{15}N in the eluant as the cation and anion exchange resin columns for these samples were eluted together, rather than eluting each column into a separate diffusion container.

3.4. Analytical variability in natural samples

When we had large enough water samples or high enough N concentrations, we made replicate resin runs and/or replicate diffusions from the same samples. The average standard error for 7 sets of triplicate and 10 sets of duplicate diffusion samples was 0.7 ‰. Replicate resin runs fell into two classes. Some samples were run quite early and the replicates were refrozen and run up to 2 years later. Those samples commonly had

large variations in ^{15}N. Samples for which we ran duplicates in the same resin run had variability similar to that found for diffusions (0.8 ‰). We recommend against

TABLE III

A comparison of direct distillation and resin concentration, using slightly ^{15}N enriched fertilizer.

Fertilizer samples	Distillation (not diluted)	resins with distillation	resins with diffusion
	‰		
1 ($^{15}NH_4^+$)	72.2, 75.7	71.5	71.6
2 ($^{15}NH_4^+$)	93.7, 95.5	89.8	91.3
3 ($^{15}NO_3^-$)	54.1, 54.1	48.7	51.3
4 ($^{15}NO_3^-$)	106.1, 115.8	108.6	113.7

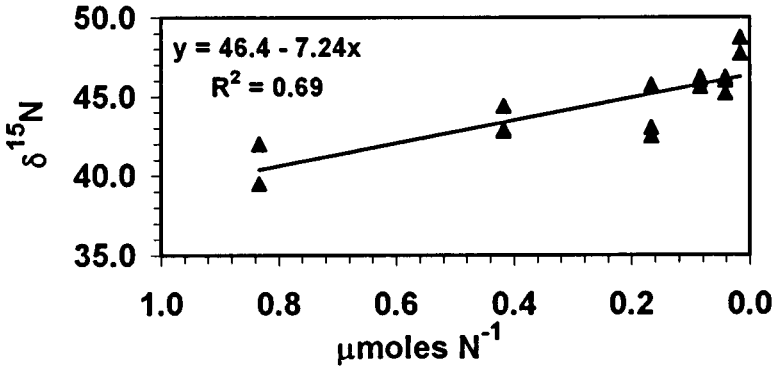

Fig. 3. Regression of inverse sample size vs. $\delta\ ^{15}N$ (expected $\delta\ ^{15}N \approx 50$ ‰) for estimation of blank.

refreezing samples, and all water samples are now kept refrigerated while thawing and are kept on ice during analytical procedures. The diffusion method (without resin concentration) has also been used on groundwater samples at the Boston University laboratory. For these groundwater samples, duplicates differed by an average of 0.2 (± 0.15) ‰ $\delta\ ^{15}N$.

4. Conclusion

It is often not practical to distill the large quantities of water needed to obtain enough N for ^{15}N analysis from streamwater or precipitation samples. By concentrating N on resin columns combined with Teflon tape diffusions, we measured ^{15}N abundance in dilute samples which otherwise could not have been estimated. With careful sample storage and attention to the hazard of NH_4^+ contamination from anion exchange resins, this combined method yields results which are satisfactory for a low-level enrichment experiment. Results would be more than adequate for more highly enriched systems. For the more demanding requirements of natural abundance level work, we believe further testing of this procedure will be necessary. Blanks should be determined in each laboratory. Some potential exists for contamination by dissolved organic N, and this should be examined more fully.

By using a multichannel pump to deliver samples to the resin columns, many samples can be handled simultaneously. The process of concentration and diffusion can be quite long (samples are processed over a 7 to10 day period), but once the system is in place samples require little attention and several batches can be run at the same time.

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