Atmospheric Environment 45 (2011) 2017–2020

Contents lists available at ScienceDirect

Atmospheric Environment

journal homepage: www.elsevier.com/locate/atmosenv

Technical note

Use of mixed ion exchange resin and the denitrifier method to determine isotopic values of nitrate in atmospheric deposition and canopy throughfall

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ARTICLE INFO

Article history:
Received 20 October 2010
Received in revised form
11 January 2011
Accepted 14 January 2011

Keywords:
Ecological samples
Natural abundance
Stable isotopes
Partitioning sources of nitrate

ABSTRACT

The objective of this study was to test a technique that utilizes mixed bed resins and the denitrifier method to determine $\delta^{15}N$ and $\delta^{18}O$ of nitrate inputs under varying conditions found in the field. Temperature increases and greater loading of nitrogen led to significant increases in $\delta^{18}O$ values and longer incubation times led to significant increases in $\delta^{15}N$ values, but the changes were relatively small. Results of our study confirm that mixed ion exchange resin can be used to accurately measure natural abundance isotopic values of nitrate in samples of atmospheric deposition and throughfall. However, we also conclude that caution should be applied when using these mixed bed resins in the field if they will be exposed to high temperatures (e.g., >40 °C). This method can increase the feasibility of conducting high density sampling of stable isotopes in atmospheric deposition and canopy throughfall samples with the goal of identifying sources of nitrate, and thus represents an important methodological advance for ecosystem science.

1. Introduction

The use of natural abundance stable isotopes of nitrogen and oxygen is a powerful tool for tracing sources of NO_3^- in water as it moves through an ecosystem (Durka et al., 1994; Kendall and McDonnell, 1998; Elliott et al., 2007). Evaluating $\delta^{15}N$ values in atmospheric deposition has been used to partition sources of nitrogen, such as coal-fired power plant emissions or tailpipe exhaust, since they vary in their isotopic signatures (Moore, 1977; Heaton, 1990). Similarly, the $\delta^{18}O$ signature of NO_3^- in soil solution or stream water can be measured, and using a two end-member mixing model, the predominant source of NO_3^- leached from a terrestrial ecosystem can be determined (e.g., Böttcher et al., 1990; Durka et al., 1994; Kendall et al., 1996). This is because $\delta^{18}O$ values of NO_3^- in precipitation differ from $\delta^{18}O$ values of NO_3^- that is microbially produced during nitrification (Amberger and Schmidt, 1987).

Previous studies have measured the isotopic composition of NO_3^- in atmospheric deposition or canopy throughfall by sampling aqueous solutions in the field (Hoering, 1957; Moore, 1977; Garten, 1992; Pardo et al., 2004; Elliott et al., 2007; NADP, 2007). However, aqueous samples must be collected regularly (<1 week) to avoid chemical transformation (e.g., Butler and Likens, 1998), and frequent collections are often not feasible. Thus, ion exchange resin columns have been used for measurements of atmospheric deposition and below-canopy fluxes in the field (Weathers et al., 2006). With this method, ions in solution that are deposited via atmospheric deposition or canopy throughfall are adsorbed onto resins as water passes over them (e.g., Van Dam et al., 1991; Fenn et al., 2002; Simkin et al., 2004). Theoretically, no chemical transformations occur on resin beads between sampling dates for inorganic N (e.g., Simkin et al., 2004) and therefore resin collectors can remain in the field for a longer period of time than aqueous samples, making them less laborious and costly.

Two common laboratory methods for determining the isotopic composition of NO_3^- adsorbed to resin beads are extraction of NO_3^- from resin beads followed by either (1) diffusions (Garten, 1992; Downs et al., 1999) or (2) purification of NO_3^- as $AgNO_3$ (Aravena and Robertson, 1998; Chang et al., 1999; Mengis et al., 1999; Silva et al., 2000). More recently, the laboratory denitrifier method (Sigman et al., 2001; Casciotti et al., 2002) has been used to determine $\delta^{15}N$ and $\delta^{18}O$ of NO_3^- in a variety of liquid samples, including groundwater, stream water, sediment and septic system samples, as well as 2 M KCl soil extracts (Rock and Ellert, 2007). One study has been published using ion exchange resin with the denitrifier method to analyze the isotopic composition of atmospheric deposition and canopy throughfall in the field (Templer and McCann, 2010). However, we are not aware of any controlled study

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doi:10.1016/j.atmosenv.2011.01.035

that has quantitatively compared the isotopic composition of NO_3^- that is loaded onto the beads with that of the extract from the beads and analyzed using the denitrifier method, which is the focus of this paper.

2. Materials and methods

We conducted a laboratory experiment to compare the isotopic composition of NO_3^- in loading solution to NO_3^- that is extracted from ion exchange resin and analyzed using the denitrifier method. Our treatments included time between loading and extraction, loading rate, moisture (wetting and drying), and the influence of incubation temperature since all of these treatments are relevant for field conditions in which resin columns are used (Fenn et al., 2002; Weathers et al., 2006; Templer and McCann, 2010). Twenty-ml disposable chromatography columns with a 30 μm pore-size filter placed at the bottom were packed with Dowex Monosphere MR-3 UPW Mixed Ion Exchange Resin ($n = 2$ per treatment). A cleaned plastic funnel was attached to the top of each resin column, and nitrogen additions were made in 100 ml volumes to introduce consistent moisture to all resin columns (similar to Simkin et al., 2004 and Weathers et al., 2006). After loading the nitrogen, resin columns were incubated for either 0 (immediately extracted), 14, 30, or 90 days (time between loading of nitrogen and extraction). Resin columns received a one-time nitrogen load of one of five nitrogen treatments: 0, 7.5, 75, 500 or 2500 μmol N as NH_4NO_3. These masses of nitrogen are equivalent to a total of 0, 4.8, 48, 319 and 1592 mol N ha^{-1}, which encompasses the typical range of nitrogen inputs in the northeastern U.S. (NADP, 2007; Clean Air Status and Trends Network; www.epa.gov/castnet). However, some hotspots of deposition can range up to 2855 mol ha^{-1} yr^{-1} in high elevation or polluted ecosystems (Weathers et al., 2000, 2006; Templer and McCann, 2010).

To determine the impact of simulated field conditions on isotopic composition, we included an "ambient" treatment to mimic drying over time and a "wet" treatment to mimic more constant moisture. Half of the resin columns received weekly additions of 100 ml deionized water ("wet"), while the other half did not receive any additional water following initial nitrogen loading ("ambient"). We incubated a subset of resin columns at 40 °C for 30 days to determine the impacts of exposing resin beads to warm, dry conditions ("warmed" treatment) on stable isotope values. The remaining resin columns were stored at room temperature (~22 °C).

Columns were extracted with three sequential additions of 50 ml of 2 M KCl (150 ml total). We determined the $\delta^{15}N$ and $\delta^{18}O$ values of NO_3^- in the loading solution and NO_3^- extracted from the resin beads using the denitrifier method (Sigman et al., 2001; Casciotti et al., 2002) in the Templer Laboratory, Boston University. We incubated 1 ml of either loading solution or KCl extract solution overnight in 20 ml test tubes containing denitrifying bacteria (*Pseudomonas aureofaciens*) that lack active N_2O reductase, which causes all NO_3^- to be converted to N_2O rather than some going to N_2. Before injecting the samples into the test tubes, the vials containing bacteria were crimp sealed, flushed with N_2 for 3 h and antifoam B was added. Following 24 h of incubation, 5 drops (approximately 0.5 ml) of 12 M NaOH was added to kill the bacteria, thereby stopping the reaction. N_2O gas produced by the bacteria was measured on a SerCon Cryoprep trace gas concentration system interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (SerCon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Facility. Standard solutions (USGS standard #32, 34 and 35) were converted to gas as internal standards for solution samples. Each standard was run at three different concentrations to span the range of masses of nitrogen in samples within each batch. Within batches, some standards were used as references to correct the isotopic measurements and others were treated as unknown samples to independently assess the precision of our standards. Within each batch, samples were run in order of increasing nitrogen mass to correct for mass-based isotope effects within each batch. We interpolated values for samples between standard curves to account for any mass-based drift within each batch. Ten percent of samples were run in duplicate, providing us with an estimate of precision (0.015‰ for $\delta^{15}N$ and 0.0093‰ for $\delta^{18}O$; standard error). We used isotopic values for samples whose beam areas (Beam 44) on the isotope ratio mass spectrometer were at least ten times greater than the beam area of our blanks. Blanks composed of water (for batches with loading solution only) or 2 M KCl and resin extract (for batches with samples) were analyzed during each run. Nitrogen isotopic values are expressed as $\delta^{15}N$ values where the standard is atmospheric air, which is defined as 0‰. Oxygen isotopic values are expressed as $\delta^{18}O$ values where the standard is Vienna standard mean ocean water, also defined as 0‰.

We conducted an Analysis of Variance using length of incubation (0, 14, 30 or 90 days), moisture (ambient vs. wet), temperature (ambient vs. warmed) and nitrogen treatment as the main effects and $\delta^{15}N$ and $\delta^{18}O$ as the response variables. We used Tukey's test for post hoc comparisons of means. We used SAS JMP software (Version 8.0.2, 2009) for all statistical analyses.

3. Results and discussion

We found good agreement between our measured values and the known values for USGS standards #32, 34 and 35, as well as between isotopic values of loading solution and NO_3^- extracted from the resin columns (Tables 1 and 2), confirming that the denitrifier method can be used in conjunction with mixed bed resin beads to successfully determine natural abundance $\delta^{15}N$ and $\delta^{18}O$ values of atmospheric deposition and canopy throughfall samples.

The observed differences in $\delta^{18}O$ values between loading solution and resin column extracts were small relative to the variation in $\delta^{18}O$ values of precipitation and compared to differences in $\delta^{18}O$ values of potential sources of NO_3^- (atmospheric deposition vs. nitrification). For example, $\delta^{18}O$ values of NO_3^- in atmospheric deposition collected from watersheds of the northeastern and western United States ranged from +50 to +84‰ (Barnes et al., 2008) and +71 to +78‰ (Nanus et al., 2008), respectively. The largest difference in $\delta^{18}O$ values we observed was 4.41‰ (equal to 25.48‰ in resin extract for 75 μmol N 'wet' treatment at 90 days minus 21.07‰ in resin extract for 7.5 μmol 'wet' treatment at 90 days).

The 40 °C 'warmed' treatment led to a statistically significant increase in $\delta^{18}O$ values in the high nitrogen treatments (500 and 2500 μmol N). The warming treatment may have led to an increase in $\delta^{18}O$ of remaining NO_3^- absorbed to ion exchange resin if some NO_3^- was converted to other forms of nitrogen and lost as gas. Although the effect is relatively small compared to natural variation in $\delta^{18}O$ values of precipitation in nature, caution should be applied in

Table 1
Measured and known $\delta^{15}N$ (‰) and $\delta^{18}O$ (‰) values for USGS standards #32, 34 and 35 (KNO_3, KNO_3 and $NaNO_3$, respectively). "Known" values for $\delta^{15}N$ and $\delta^{18}O$ of USGS standards come from IAEA (2004). Sample size represents number of replicates of each USGS standard treated as an "unknown". Error for measured values is standard error and error for known values is standard deviation.

Standard	Sample Size	Measured $\delta^{15}N$	Known $\delta^{15}N$	Measured $\delta^{18}O$	Known $\delta^{18}O$
USGS-32	12	179.67 ± 0.54	180 ± 1.0	26.83 ± 0.39	25.70 ± 0.4
USGS-34	12	−1.81 ± 0.17	−1.8 ± 0.2	−27.47 ± 0.52	−27.90 ± 0.6
USGS-35	12	3.18 ± 0.17	2.7 ± 0.2	57.19 ± 0.43	57.50 ± 0.6