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ISOTOPIC BIOGEOCHEMISTRY OF DISSOLVED ORGANIC NITROGEN: A NEW TECHNIQUE AND APPLICATION

By

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ABSTRACT

ISOTOPIC BIOGEOCHEMISTRY OF DISSOLVED ORGANIC NITROGEN: A NEW TECHNIQUE AND APPLICATION

By

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A new technique for isolating and isotopically characterizing dissolved organic nitrogen (DON) for non-marine waters was developed. The technique involves preconcentration of DON via rotary evaporation and removal of dissolved inorganic nitrogen (DIN) via dialysis using a membrane that retains material above 100 daltons. Results demonstrate quantitative removal of DIN and the complete recovery and retention of isotopic integrity of DON. Nitrogen isotope values for DON from Chefswet Basin, Lake Superior were compared to those of other nitrogen reservoirs to infer transformation pathways. Within the euphotic zone, the $\delta^{15}N$ of seston (0.0‰-2.5‰) compared to that of NO_3^- (-5.0‰- -3.5‰) suggests that NO_3^- assimilation is not the primary determinant of the $\delta^{15}N$ of seston. Uptake of ^{15}N-enriched NH_4^+ or loss of ^{14}N through degradation could account for the high $\delta^{15}N$ values of seston. The low $\delta^{15}N$ value of DON (-1.2‰) relative to seston is consistent with the idea that DON at the Lake Superior site is derived from the degradation of seston.

To my future wife Nicole, whose support and patience has kept my spirit alive.

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INTRODUCTION

Dissolved organic nitrogen (DON) is often the most abundant form of nitrogen in aquatic ecosystems (Sharp 1983). Previously DON was perceived to be largely refractory with an age of approximately 6,000 years (Williams and Druffel 1987). More recently, numerous lines of evidence suggest a significant portion of DON is bioactive and a participant in biogeochemical cycles occurring on short time scales (Pomeroy et al., 1990; Bronk and Glibert 1993; Jorgensen et al., 1994; Kroer et al., 1994; Bronk et al., 1994). Uptake of DON by plankton (Bronk and Glibert 1993) and bacteria (Pomeroy et al., 1990; Jorgensen et al., 1994; Kroer et al., 1994) suggest that this dissolved organic reservoir is an available substrate for growth. The significance of DON in nitrogen regeneration is indicated by the observation that 25 to 41% of the dissolved inorganic nitrogen (DIN) assimilated by phytoplankton is released as DON (Bronk et al., 1994). DON can be utilized by phytoplankton (Palenik and Morel 1990a, b) or assimilated by bacteria (Keil and Kirchman 1991, 1993; Wheeler and Kirchman 1986; Simon and Rosenstock 1992) and incorporated back into the food web through the microbial loop (Bronk et al., 1994).

Several important issues regarding the role of DON in nitrogen cycling include: (1) defining rates of DON mineralization, (2) estimating the relative importance of different types of biota in DON cycling, (3) understanding the individual processes with the nitrogen cycle and how they are coupled and what type of feedback loops exist, (4) determining which components of the DON pool are bioavailable and (5) assessing the applicability of short-term rates from uptake experiments to models of DON cycling relating to long-term phenomena. A few studies have begun to address these issues using ^{15}N enrichment approaches (Andersson et al., 1985; Nagata and Kirchman 1991, 1992; Bronk and Glibert 1993; Gardner et al., 1993, 1996; Hoch et al., 1996). An alternative method is the application of natural abundance stable isotope techniques.

Our interest is to apply natural abundance ^{15}N measurements to assess several of the current issues relating to the role of DON in nitrogen cycling. This approach should be informative because measurements of natural abundance isotope ratios are a well-established technique for identifying sources and evaluating transformations of materials within the environment (Macko and Ostrom 1994). For example, near-zero $\delta^{15}N$ values measured for plankton in regions of the North Pacific where nitrogen-fixing cyanobacteria are abundant may reflect incorporation of atmospheric nitrogen (near 0 ‰) because this process because this process is accompanied by little or no isotopic fractionation (Wada and Hattori 1976; Minagawa and Wada 1984). One advantage of the natural abundance isotope approach is that the data reflect long-term processes operating over time periods of weeks to months, rather than the scale of hours to days inherent in enrichment studies. Currently there is only one study that documents natural abundance stable isotope values for DON for the high molecular weight (DON > 1000 daltons) fraction of this reservoir (Benner et al., in press).

We developed a technique that isolates the entire DON pool (100 daltons), and likely minimizes molecular alteration, to obtain measurements of stable nitrogen isotope values in non-marine waters. Data for verification of the technique and preliminary $\delta^{15}N$ analyses for DON from field samples are presented. We verify removal of DIN (in our experiments NH_4^+ and/or NO_3^-) and the recovery and maintenance of isotopic integrity of a DON standard (tripeptide Glu-Gly-Phe; GGP) at each step in the preparatory procedure. We also evaluated reproducibility for isotope ratios and elemental concentrations of natural samples. Data for natural samples include values of the nitrogen isotopic composition of DON from the Chefswet Basin, Lake Superior and Grand Traverse Bay, Lake Michigan as well as DON and DOC concentrations. Additional water column data on other pools of nitrogen in Lake Superior are presented to assist in the interpretation of the DON results and the nitrogen cycling dynamics of this system.

MATERIALS AND METHODS

Technique Overview

Our technique involves filtration, rotary evaporation and dialysis. The following describes the methods used throughout all the experiments mentioned below. A sample of 100-200 mL was filtered through an acid-washed 0.2 μm polysulfone Supor membrane (Gelman Sciences) and concentrated to approximately 5 mL by rotary evaporation. This process was carried out at ca. 10 mm of Hg and a temperature of less than 40 °C. After rotary evaporation the sample was transferred to a dialysis membrane (Spectra/Por CE Membrane) with a nominal molecular weight cutoff (MWCO) of 100 (daltons). The concentrate was diafiltered at 4 °C against a salt solution (5% NaCl, 6.5% $MgSO4{\cdot}7H_2O$ and 4% $CaCl_2$; w/v) for 25 hours and ultra-clean deionized water (Barnstead E-pure) for an additional 75 hours during which time the dialysis solution was changed several times. The sample was then transferred to a pre-combusted (500 °C, 3 hr) quartz ampule (ca. 15 mL). The dialysis membrane was washed with 2 mL of deionized water and the wash was added to the ampule to facilitate quantitative transfer. The sample was dried in-vacuo on a glass high-vacuum line. If the carbon isotope composition was desired, phosphoric acid (100 mL) was added to the sample prior to evacuation to remove dissolved inorganic carbon (DIC).

Upon dryness, the sample was removed from the vacuum line, precombusted copper oxide (Aldrich; 3 g) and pure copper (Alpha Resources; 3 g) were added and the sample re-evacuated. Precombusted copper oxide is obtained by spreading a thin layer of this material in a porcelain dish and heating it to 850 °C for 4 hours; precombusted copper is obtained by heating an evacuated, sealed quartz ampule (30 cm x 10 mm o.d.) filled with CuO and Cu (ca. 1:6 by volume) to 850 °C for 4 hours. After re-evacuating, the sample was sealed under vacuum, heated to 850 °C and allowed to cool gradually following a modified Dumas combustion procedure (Macko, 1981). Nitrogen gas and CO_2 were separated cryogenically from the combustion products and isotope values of purified gases were determined on a PRISM (Micromass) mass spectrometer. The concentration of carbon in the sample was determined using a Baratron capacitance manometer (MKS Inc.) during cryogenic gas separation. Nitrogen concentration was determined based on the ion beam produced (mass 28) within a calibrated volume of the mass spectrometer. Nitrogen and carbon isotope ratios are expressed in per mil:

$$\delta^{I}E = [(R_{sample}/R_{standard}) - 1] * 1000$$

where, I is the heavier isotope of element E and R is the abundance ratio of the heavy to light isotope. The internationally recognized standards for $\delta^{15}N$ and $\delta^{13}C$ are atmospheric nitrogen gas and V-Peedee Belemnite, respectively.

Evaluating DIN Removal

Using our dialysis technique we tested for the removal of DIN (NH_4^+ and/or NO_3^-) amended to (1) deionized water, (2) a solution containing our DON standard, GGP and (3) natural waters. Concentrations of nitrogen in these experiments were chosen to mimic samples containing high concentrations of DIN (100 μM) and/or natural abundance concentrations of DON (20 μM). Removal of DIN was verified by determining the concentration of DIN in the permeate (solution external to the dialysis membrane) at different time intervals during dialysis and/or by evaluating the concentration of DIN in the retentate (solution inside the dialysis membrane at the end of the experiment). In the first experiment 5 mL of 100 μM NH_4Cl was diafiltered against 140 mL of deionized water while stirring at 4°C. Aliquots of permeate (5 mL) were transferred to scintillation vials (25 mL) and frozen immediately. At the completion of the experiment, the retentate was transferred to scintillation vials, aliquots of permeate samples were thawed, and NH_4^+ concentrations were determined using an Orion ion specific electrode (Garside et al., 1978). The detection limit and precision of this technique is 0.1 μM (Ostrom et al., in press).

In the second experiment a 5.25 mL solution containing 100 μM NH_4Cl, 100 μM

KNO_3 and 0.76 mM N from GGP was diafiltered and sampled as mentioned above. Nitrate concentrations were determined by suppresser based anion chromatography (Shipgun and Zolotov 1988) on a high performance liquid chromatograph (Rainin Instruments) with conductivity detection (LDC Analytical). Separation of anions was achieved on a Dionex IonPac column (#AS4A-SC) with suppresser using an eluent consisting of 2.4 mM Na_2CO_3 and 3 mM $NaHCO_3$. The percent NO_3^- removal was estimated by analyzing the abundance of NO_3^- in the permeate at different time intervals during dialysis and by measuring its abundance in the retentate at the end of dialysis. The percent removal of NH_4^+ was estimated by measuring its abundance in the retentate at the end of dialysis using the Orion electrode.

For the last experiment water from Lake Lansing, Michigan was collected at the surface in 1 L Nalgene bottles and filtered. A 200 mL sample was amended with KNO_3 such that the concentration of NO_3^- was 100 μM. The sample was subsequently concentrated to 5 mL by rotary evaporation and diafiltered. Percent removal of NO_3^- and NH_4^+ were estimated by measuring their abundance in the retentate at the end of dialysis.

Recovery and Isotopic Integrity of DOM

We evaluated the recovery and isotopic integrity of GGP by determining the elemental concentration and isotope ratios of nitrogen and/or carbon in GGP standards before and after rotary evaporation, dialysis and the combined procedure. All experiments were run in triplicate and values were corrected for reagent blanks. Isotope values and elemental abundances for procedural blanks (dialysis: 5 mL deionized water; rotary evaporation: 100 mL deionized water; combined procedure: 100 mL deionized water) were also determined. Given the low molecular weight of GGP (351.4 g/mole), this standard offered a rigorous test of our ability to retain DOM without isotopic discrimination.

For the rotary evaporation experiment, 2 mL of a 1.5 mM GGP standard was diluted to 100 mL with deionized water and subsequently concentrated to ca. 5 mL. This solution was dried in-vacuo, analyzed isotopically and the results compared to 2 mL of a 1.5 mM GGP standard that was not subjected to rotary evaporation (non-treated GGP standard). For dialysis 2 mL of the 1.5 mM GGP standard was placed in the dialysis membrane and diafiltered against both the salt solution and deionized water for 25 hours each. The retentate was dried, analyzed isotopically and compared to its non-treated standard. The standard was also subjected to both procedures in sequence for isotopic comparison to the non-treated standard. The recovery of the standard was determined by comparing carbon or nitrogen concentrations for the treated and non-treated standards.

Field Samples

Water column samples were collected from Grand Traverse Bay, Lake Michigan on the RV Northwestern (August 1996) and from the Cheftswet Basin, Lake Superior on the RV Edwin Link (June 1994). The water column at each station was initially characterized by deployment of a SBE-25 conductivity-temperature-depth profiler equipped with a fluorometer and transmissometer (SeaBird Electronics Inc.). All water samples were collected using 5 L lever-action Niskin bottles (General Oceanics) and transferred to acid-washed 1 L Nalgene bottles and stored on ice. Samples were then filtered through an acid-washed 0.2 µm polysulfone Supor membrane in lab within a few hours of collection and frozen in Nalgene bottles priot to isolation and analysis of DON. Methods for sample preparation and isotopic analysis of particulates and DIN from Lake Superior are reported in Ostrom et al., (in press).

RESULTS

Evaluating DIN Removal

We evaluated the removal of DIN (NH_4^+ and/or NO_3^-) during dialysis from 1) 5 mL of deionized water amended with 0.5 µmoles of NH_4Cl (Figure 1), 2) 5.25 mL of deionized water amended with 0.53 µmoles of NH_4Cl, 0.53 µmoles of KNO_3 and 4 µmoles (N) of GGP (Figure 2) and (3) 5 mL of water from Lake Lansing, Michigan that was concentrated by rotary evaporation from an initial solution of 100 mL of lake water that was spiked with 10 µmoles of KNO_3 (Figure 2). Each of these experiments were performed in triplicate. The procedural reproducibility (standard deviation about the mean) for measurements of NH_4^+ abundance in the retentate or permeate was 0.03 µmoles or less and for that NO_3^- was 0.02 µmoles or less.

At the end of the experiment involving NH_4Cl in deionized water, estimates of NH_4^+ concentrations in the permeate indicate greater than 100% removal and those in the retentate suggests nearly 100% removal. The presence of a a small excess of NH_4^+ in the permeate imply minor NH_4^+ contamination external to the dialysis membrane.

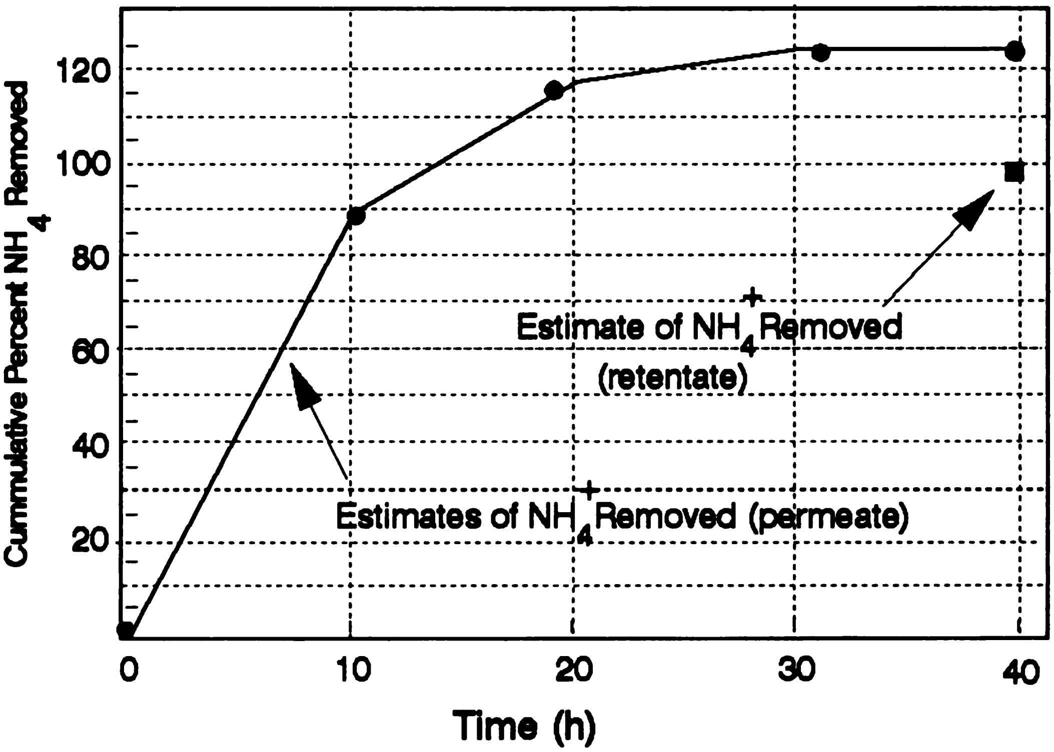

Figure 1 - Estimates of dissolved inorganic nitrogen removal; removal of NH_4^+ from ultra-clean deionized water (Barnstead E-pure)

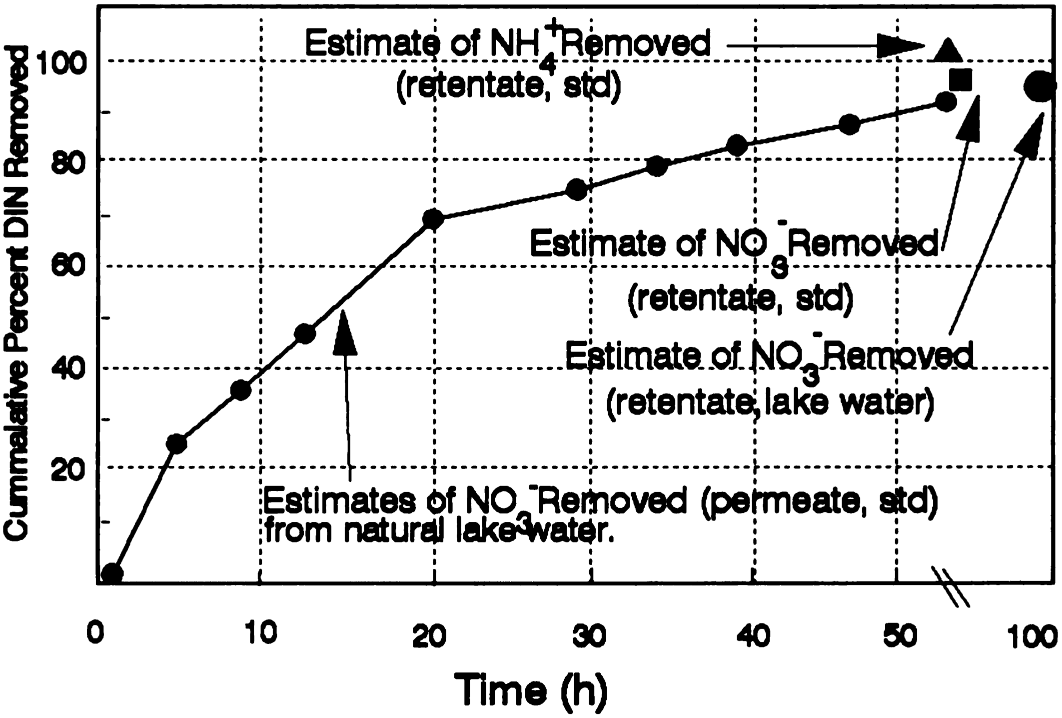

Figure 2 - Estimates of dissolved inorganic nitrogen removal; removal of NH_4^+ and NO_3^- from a solution containing Glu-Gly-Phe (GGP) in ultra-clean deionized water; removal of a NO_3^- spike

In the experiment that evaluated the removal of NH_4^+ and NO_3^- from a solution containing the GGP standard, measurements of NO_3^- in the permeate and retentate at the end of the experiment are in good agreement and show that more than 90% of the nitrate was removed. Similarly, more than 95% of the NH_4^+ was removed based on estimates of the concentration in the retentate.

Removal of NO_3^- from water samples from Lake Lansing were in excess of 95%. Concentrations of NH_4^+ in this sample following dialysis were below our limit of detection (0.1 μM).

Recovery and Isotopic Integrity of DOM

Our first step in addressing recovery during rotary evaporation and dialysis was to evaluate the elemental concentration of both reagent and procedural blanks (Table 1). The reagent blank for carbon was 1.4 μmoles while that for nitrogen was below our limit of detection (0.2 μmoles). The contribution of carbon from the procedural blank for the combined rotary evaporation/dialysis procedure was 1.5 μmoles. A comparison of procedural blanks for rotary evaporation (0.1 μmoles) and dialysis (1.1 μM) indicates that the majority of this carbon was derived during dialysis. The contribution of nitrogen from individual steps or the combined procedure was below our limit of detection

Table 1 - Carbon and nitrogen concentrations for procedural and reagent blanks subjected to each of the three treatments (T); rotary evaporation, dialysis, or the combined procedure and for non-treated (NT) and treated, blank-corrected Glu-Gly-Phe (GGP) standards. Data are reported as average values ± standard deviation with the difference between T and NT designated by Δ; na = not applicable.

Procedure	n	NT (µmole C or N)	T (µmole C or N)	Δ (µmole C orN)
Carbon Recovery				
Reagent blank	2	1.4	na	na
Rotary evaporation				
procedural blank	1	na	0.1	0.1
GGP standard	3	22.5 ± 0.3	22.6 ± 0.9	0.1
Dialysis				
procedural blank	1	na	1.1	1.1
GGP standard	3	23.6 ± 0.1	24.0 ± 0.1	0.4
Combined Procedure				
procedural blank	2	na	1.5	1.5
GGP standard	3	25.0 ± 0.0	25.1 ± 1.4	0.1
Nitrogen Recovery				
Combined Procedure				
procedural blank	2	na	<0.2	<0.2
GGP standard	3	2.0 ± 0.2	1.8 ± 0.2	0.2

(0.2 μmoles).

The next step was to evaluate the recovery of GGP. This was achieved by comparing the concentration of carbon or nitrogen in an aliquot of a standard subjected to a procedure (treated) to that of an equivalent aliquot that had not been submitted to the procedure (non-treated). The difference in the concentration of carbon between the blank-corrected treated and non-treated standard for rotary evaporation, dialysis, or the combined procedure was 0.4 μmoles or less (Table 1). Standards subjected to the combined procedure had nitrogen concentrations that differed by 0.2 μmoles from the non-treated standard. Differences in elemental concentrations between the treated and non-treated standard are small (less than or equal to the standard deviation associated with measurement; Table 1) and, therefore, indicate complete recovery.

To assess isotopic integrity during rotary evaporation and dialysis we began by evaluating the isotope values of reagent and procedural blanks. We were unable to obtain nitrogen isotope ratios for blanks because they were below our limit of detection. Consequently, no isotopic correction was applied to $\delta^{15}N$ values of samples. The average $\delta^{13}C$ value for the reagent blank was -24.1‰ (n=2) and that for the blank associated with the combined procedure was -28.7‰ (n=2). Given isotopic similarity of the blank to that of natural samples (see section below on filed samples) and the low abundance of carbon contributed by the blanks, corrections were not applied to $\delta^{13}C$ values of natural samples.

After assessing blanks, we evaluated the isotopic integrity of GGP during each of our procedures (Table 2). Differences in $\delta^{13}C$ values between the treated and non-treated standard were no more than 0.4‰ for rotary evaporation, dialysis or the combined procedure. The data for the combined procedure shows no difference in nitrogen isotope ratios between the treated and non-treated standard. We therefore concluded that there was essentially no procedural effect on either the $\delta^{15}N$ or $\delta^{13}C$ values.

Field Samples

We determined the elemental concentrations and isotope values of DOM in samples collected from the Cheftswet Basin, Lake Superior and Grand Traverse Bay, Lake Michigan (Table 3). The concentration of DON was 11.0 ± 0.8 μM (n=3) and 6.8 ± 0.2 μM (n=4) in the Lake Michigan and Lake Superior locations, respectively. The concentration of DOC at the Lake Superior site was 126 ± 4 μM (n = 4). The coefficient of variation for DON concentrations at the location in Lake Michigan was less than 8% and in Lake Superior it was less than 5% for both DON and DOC.

The average $\delta^{15}N$ values for DOM at the Lake Michigan and Lake Superior sites were 5.0 and -1.2, respectively. The reproducibility for the $\delta^{15}N$ values of DOM from Lake Michigan was 0.1‰. In Lake Superior, where nitrogen concentrations were about

Table 2 - The $\delta^{15}N$ and $\delta^{13}C$ values for our standard Glu-Gly-Phe (GGP) subjected to each of the three treatments (T); rotary evaporation, dialysis or the combined procedure. Data are reported as average values ± standard deviation with the difference between T and NT designated by Δ.

Procedure	n	NT ($\delta^{15}N$ or $\delta^{13}C$)	T ($\delta^{15}N$ or $\delta^{13}C$)	Δ
Nitrogen isotope data				
Rotary evaporation	3	-0.6 ± 0.0	-0.4 ± 0.0	0.2
Dialysis	3	-4.3 ± 0.3	-3.9 ± 0.1	0.4
Combined procedure	3	-0.7 ± 0.4	-0.7 ± 0.1	0.0
Carbon isotope data				
Rotary evaporation	3	-19.4 ± 0.0	-19.4 ± 0.0	0.0
Dialysis	3	-24.9 ± 0.2	-25.3 ± 0.2	0.4
Combined procedure	3	-19.5 ± 0.1	-19.2 ± 0.1	0.3

Table 3 - **The $\delta^{15}N$ and $\delta^{13}C$ values and nitrogen and carbon elemental concentrations for DOM from Grand Traverse Bay, Lake Michigan and Cheftswet Basin, Lake Superior. Data are reported as average values ± standard deviation; nd = no data.**

Location	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	μM N	μM C
Grand Traverse Bay, Lake Michigan (n=3)	5.0 ± 0.1	nd	11.0 ± 0.8	nd
Cheftswet Basin, Lake Superior (n=4)	-1.2 ± 0.3	-24.6 ± 0.1	6.8 ± 0.2	126 ± 4

half that in Grand Traverse Bay, reproducibility of the isotope values of DOM were 0.3‰ for nitrogen and 0.1‰ for carbon.

DISCUSSION

Applications of natural abundance stable isotope measurements to natural waters offer the potential to provide new perspectives for better understanding nitrogen cycling. This approach overcomes some of the constraints imposed by ^{15}N enrichment incubation experiments such as bottle effects and the use of artificial substrates. In addition, natural abundance data reflect long-term, in-situ phenomena and provide an independent assessment of the source and predominant mechanisms that control nitrogen cycling.

The present study demonstrated the use of rotary evaporation and dialysis as an effective method for the isolation and isotopic characterization of DON from freshwater environments. Verification of our procedure included excellent data for the removal of DIN (greater than 90%), recovery of DON (ca. 100%) and the maintenance of isotopic integrity of DOM (isotopic discrimination less than 0.4‰ for $\delta^{15}N$ or $\delta^{13}C$). We are currently attempting to modify this procedure for marine waters.

There are several positive attributes of our technique. The low temperatures and pressures encountered in rotary evaporation minimize possibilities for molecular alteration. This is clearly an advantage if molecular characterization is of interest. Considering the length of the isolation process, the low temperatures used in dialysis were important to restrict microbial growth. Minimal user intervention and exposure of the sample assisted in minimizing contamination.

Despite the emergence of tangential-flow ultrafiltration as a method for isolating and concentrating DOM, we encountered several problems with ultrafiltration in a preliminary comparison to our technique. The most eminent problems with the tangential-flow technique was adsorption of DOM to the membrane (Molecular/Por 100 MWCO Ultrafiltration Type C) and reduced filtration rates. In addition to poor recoveries of standards, adsorption of DOM was clearly apparent owing to the discoloration of the membrane caused by a yellow nitrofurantoin standard (MW 238.2). Studies employing tangential-flow ultrafiltration for the isolation of DOM commonly use membranes with a MWCO of 1000 and indicate that 20-40% of the organic carbon in seawater is recoverable (Benner et al., 1992; Guo et al., 1995). These recoveries likely reflect the actual carbon abundance of the high molecular weight (>1000) reservoir in addition to smaller amounts of adsorption. This is because the larger MWCO membrane used in these studies may reduce adsorption and associated concentration polarization. Our observations suggest, however, that the tangential-flow ultrafiltration technique will not be appropriate for isolation of the entire DOM reservoir.

The preliminary application of our technique was in the Great Lakes. The difference in $\delta^{15}N$ values of DOM between Grand Traverse Bay and Lake Superior (6.1‰, Table 3), likely reflects unique processes and nitrogen inputs associated with each location. Additional water column data from Lake Superior (Ostrom et al., in press) was used together with these $\delta^{15}N$ values of DOM to assist in a detailed interpretation of this site.

Within Lake Superior, temperature and fluorescence data show a stratified water column and a maximum in chlorophyll-a at approximately 20 m (Figure 3). The concentrations of NH_4^+ and NO_3^- were approximately 0.5 μM and 24 μM, respectively. The concentration of NO_3^- near the surface was slightly lower (ca. 1.5 μM) than it was below 20 m (Figure 3), likely indicating uptake by phytoplankton above 20 m.

Nitrate in Lake Superior was characterized by the lowest $\delta^{15}N$ values ever reported for an aquatic system with an average of -4.1‰ (Figure 3; Liu and Kaplan 1989) and is thought to have an atmospheric origin (Ostrom et al., in press). Ostrom et al. (in press) believe that nitrate is not derived from the sediments or water column because in the sediments, fluxes of nitrate from Great Lakes are low and in the water column, low isotope values derived from fractionation during nitrification are not expected at the low NH_4^+ concentrations present in Lake Superior. The predominance of precipitation to the hydrodynamic balance of Lake Superior and the low isotope values of NO_3^- are consistent with an atmospheric origin (Heaton 1987; Paerl and Fogel 1994; Ostrom et al., in press).

With the exception of one data point, $\delta^{15}N$ values for seston were within the range of 0 to 2.5‰ (Figure 3). Comparison of seston $\delta^{15}N$ values to those of NO_3^- (-5.0 to

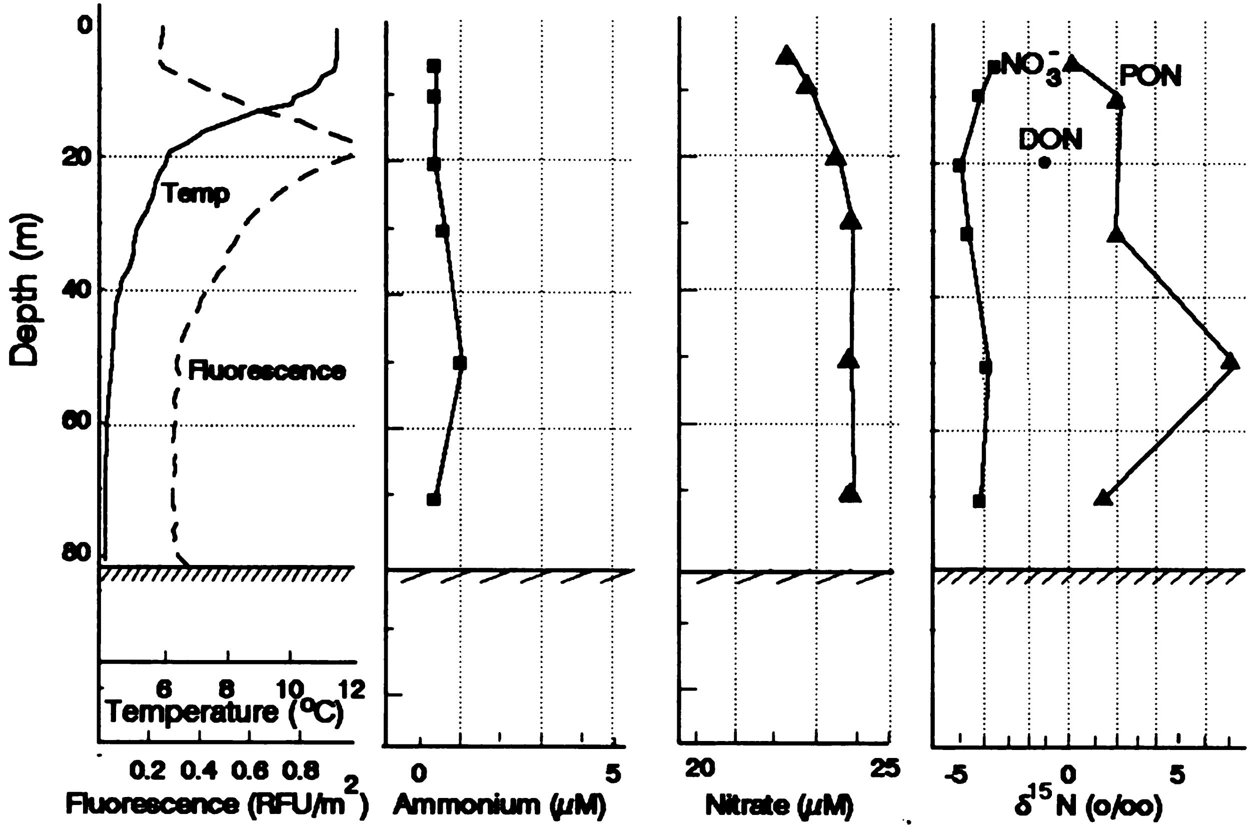

Figure 3 - Temperature, fluorescence (chlorophyll-a), concentrations of NH_4^+ and NO_3^- and $\delta^{15}N$ values of NO_3^-, particulate organic nitrogen (PON) and dissolved organic nitrogen (DON) in Cheftswet Basin, Lake Superior.

-3.5‰) suggests that assimilation of NO_3^- is not the predominant factor controlling the isotopic composition of seston. Fractionation during assimilation should produce seston with $\delta^{15}N$ values equal to or less than that of the substrate, yet the $\delta^{15}N$ values of our seston were always higher than those of nitrate (Wada and Hattori 1978; Macko et al., 1987; Pennock et al., 1988; Cifuentes et al., 1989). This insinuates that processes in addition to uptake and metabolism of nitrate are controlling the $\delta^{15}N$ of seston.

The nitrogen isotope ratios for seston could be explained by either the uptake of ^{15}N enriched NH_4^+ or the loss of ^{14}N through mechanisms that leave the residual reservoir of seston enriched in ^{15}N. Processes that could lead to ^{15}N enrichment include microzooplankton grazing, microbial degradation, peptide bond hydrolysis or the loss of DON. A preliminary result indicates a $\delta^{15}N$ value for DON that is lower than those of seston is consistent with the interpretation that DON in Lake Superior is derived from seston. Therefore the high $\delta^{15}N$ values for seston may, in part, be explained by losses of ^{15}N-depleted DON. A better understanding of the origins of DON will be possible with a more extensive data set.

CONCLUSIONS

The development of techniques to isolate and isotopically characterize DON offers an important advance for understanding nitrogen dynamics in natural waters. With this ability we are now able to quantify and isotopically characterize nitrogen from all reservoirs in aqueous media. We envision studies whose emphasis is on obtaining temporal data sets with the objective of constraining the large number of individual biogeochemical processes that can influence the nitrogen cycle. The application of natural abundance isotope tracing methods on multiple nitrogen reservoirs in such a framework offers an excellent opportunity to determine the predominant controls on nitrogen cycling. We believe temporal isotopic data sets will allow us to better address several outstanding issues such as how individual biogeochemical processes are coupled and what types of feedback loops exist. Furthermore, the technique we describe is particularly suited for an important next step, one that will involve isotopic characterization of individual size classes of DOM to assess their roles in nitrogen cycling dynamics.

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