Automated analysis system for coupled δ13C and δ15N measurements. Anal Chem

ARTICLE *in* ANALYTICAL CHEMISTRY · FEBRUARY 1992

Impact Factor: 5.64 · DOI: 10.1021/ac00027a009

5 AUTHORS, INCLUDING:

W. A. Brand

Max Planck Institute for Biogeochemistry Jena

155 PUBLICATIONS **4,716** CITATIONS

(10) Albritton, D. L. *At. Data Nucl. Data Tables* **1978**, *22*, 1-101.
(11) Eisele, F. L.; Tanner, D. J. *J. Geophys. Res.* **1991**, *96*, 9295.
(12) Andreae, M. O. *Marine Chem.* **1990**, *30*, 1.
(13) Charlson, R. J.; Lovelock, J. E.; Andreae, M. O.; Warren, S. G. *Nature* **1987**, *326*, 655.
(14) Thornton, D. C.; Bandy, A. R.; Ridgeway, R. G.; Driedger, A. R., III; Lalevic, M. *J. Atmos. Chem.* **1990**, *11*, 299.
(15) Berresheim, H. *J. Geophys. Res.* **1987**, *92*, 13245.
(16) Ammons, J. M. Ph.D. Thesis, University of Southern Florida, Tampa, 1980.
(17) Andreae, M. O.; Berresheim, H.; Andreae, T. W.; Kritz, M. A.; Bates, T. S.; Merrill, J. T. *J. Atmos. Chem.* **1988**, *6*, 149.
(18) Turco, R. P.; Whitten, R. C.; Toon, O. B.; Pollack, J. B.; Hamill, P. *Nature* **1980**, *283*, 283.
(19) Berresheim, H.; Andreae, M. O.; Ayers, G. P.; Gillett, R. W.; Merrill, J. T.; Davis, V. J.; Chameides, W. L. *J. Atmos. Chem.* **1990**, *10*, 341.
(20) Maroulis, P. J.; Torres, A. L.; Goldberg, A. B.; Bandy, A. R. *J. Geophys. Res.* **1980**, *85*, 7345.
(21) Driedger, A. R., III; Thornton, D. C.; Lalevic, M.; Bandy, A. R. *Anal. Chem.* **1987**, *59*, 1196.

RECEIVED for review May 24, 1991. Accepted October 31, 1991. This work was supported by the National Science Foundation, Grants ATM-8813594, ATM-8717675, and ATM-9021522, and by the Georgia Tech Research Institute, Grant E8904-046.

Automated Analysis System for Coupled $\delta^{13}C$ and $\delta^{15}N$ Measurements

Brian Fry,*[,1] Willi Brand,[2] F. J. Mersch,[1] K. Tholke,[1] and R. Garritt[1]

Ecosystems Center, Marine Biological Laboratory, Woods Hole, Massachusetts 02543, and Finnigan MAT GmbH, Postfach 14 40 62, D-2800 Bremen, FRG

A newly developed machine can analyze >30 samples/day for stable isotopic composition, obtaining both $\delta^{13}C$ and $\delta^{15}N$ values from each sample. Samples are combusted to carbon dioxide and nitrogen gas in an elemental analyzer, cryogenically purified in a custom-built stainless steel manifold, and analyzed with an isotope ratio mass spectrometer. Analysis of standard reference materials (SRMs) distributed by the U.S. National Bureau of Standards showed that the automated system can produce good quality isotopic measurements from representative plant, animal, and sediment samples and that SRMs can be suitable isotopic reference materials for work with complex organic materials.

INTRODUCTION

Carbon and nitrogen isotopic measurements are currently made in many laboratories using a largely manual procedure that involves sample combustion, cryogenic purification of N_2 and CO_2 gases, and separate mass spectrometric determination of $\delta^{13}C$ and $\delta^{15}N$ values (*1*, *2*). We have recently developed an automated instrument for measurement of $\delta^{13}C$ and $\delta^{15}N$ from the same sample, allowing precise and rapid determination of natural abundance level isotopic variation. The report describes developmental problems associated with construction of this system and provides isotopic reference values for three readily available plant, animal, and soil samples.

EXPERIMENTAL SECTION

The automated procedure combines the main steps of the manual procedure. Samples are combusted in a commercial elemental analyzer. The combustion products CO_2, N_2, and H_2O enter a custom-designed automated system of all-metal valves and cold traps where the gases are trapped and cryogenically separated (Figures 1 and 2). The "trapping box" also serves as the inlet reservoir for an isotope ratio mass spectrometer (Finnigan MAT delta S); pure CO_2 and N_2 samples are measured against gas standards maintained in separate bellows. A new design of changeover valves in the mass spectrometer makes it possible to accommodate the four gases involved in the measurements, i.e., N_2 from standard and sample ports and CO_2 from standard and sample ports (Figure 3). The system can be operated in three modes: carbon only, nitrogen only, or carbon and nitrogen. Here we focus on the coupled C + N mode; the other modes are subsets of the C + N procedure.

Sequence of Sample Handling. 1. Combustion. The sample is loaded into a tin boat and dropped from a carousel into a combustion tube. The combustion tube is filled with CuO and maintained at 1000 °C. The sample enters with a carrier stream of helium doped with oxygen and flash-combusts near 1800 °C. Resulting gases are swept over a second column that is filled with elemental copper and maintained at 600 °C. Various nitrogen oxides are converted to N_2 in this reduction furnace, and excess oxygen is scavenged by reaction with copper to form CuO. We have successfully used both Carlo Erba 1500 and Heraeus elemental analyzers to perform these combustion steps, with samples of typically 1–10 mg for plants and animals and 10–60 mg for soils.

2. Collection. Helium containing the combustion gases, H_2O, CO_2, and N_2 is swept into the trapping box through traps T1, T2, and T3 and pumped out with a rotary vacuum pump (Figure 1). Traps T1 and T2 are initially empty to collect H_2O and CO_2, while T3 is filled with Alltech 5-Å zeolite (molecular sieve) to trap N_2. The three traps contain cold fingers that are cooled with liquid nitrogen to capture combustion gases (Figure 2). Liquid nitrogen percolates up through a cone and cascades down around the outside of the cold finger to cool the trap to −196 °C; trapped gases are later released at 20–100 °C using resistance heaters adjacent to the cold fingers (Figure 2).

Helium flow rates in the trapping box are about 75 mL/min and adjusted with needle valves to 0.3 bar inside the trapping box during sample collection. After 4 min of collection, helium is diverted out of the trapping box by closing valve 2 and opening valve 1. Residual helium is pumped out of traps 1–3, and the valves around traps T1–3 are closed.

3a. Cryogenic Distillation—Nitrogen. The T3 trap containing nitrogen is heated to 100 °C to release N_2 from molecular sieve, and the N_2 yield is measured with a capacitance manometer, PM2. To concentrate N_2 prior to $\delta^{15}N$ measurement, N_2 is transferred to a small-volume cold finger T6 that is cooled by liquid nitrogen and filled with Fisher indicator silica gel. After transfer and further mixing of nitrogen in T6 (see N_2 Development Problems, below) $\delta^{15}N$ measurements are begun, with valves 11 and 12 closed for small samples <10 μmol of N, and valve 11 open, valves 10 and 12 closed for samples >10 μmol.

3b. Cryogenic Distillation—Carbon. While N_2 is being processed, CO_2 trapped with water in T1 is distilled into T2 by

[1] Ecosystems Center.
[2] Finnigan MAT GmbH.

0003-2700/92/0364-0288$03.00/0

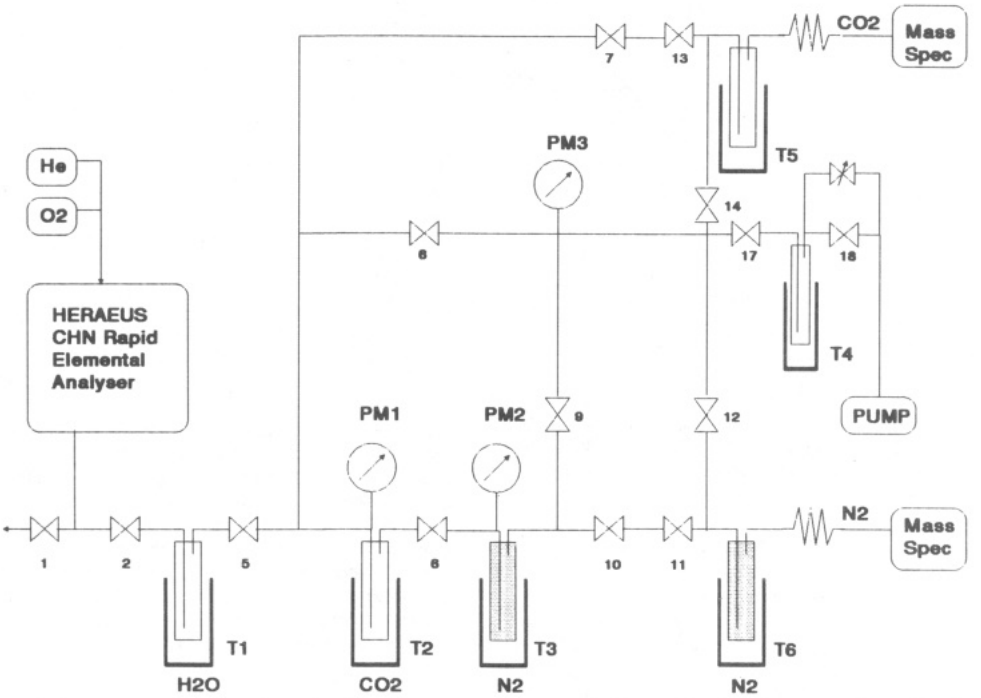

Figure 1. Schematic of the elemental analyzer/trapping box/mass spectrometer analyzer. Combustion gases from the elemental analyzer are separated in traps T1-3, carbon and nitrogen yields are measured with capacitance manometers PM1 and PM2, respectively, and CO_2 and N_2 are separately bled into the mass spectrometer via gas capillaries. Hourglass symbols represent numbered valves and needle valves (with arrows). Traps T3 and T6 are filled with zeolite and silica gel, respectively, for capturing and transferring N_2. In this study, an Heraeus elemental analyzer was coupled to a Finnigan Delta-S mass spectrometer via a trapping box constructed in Bremen, Germany.

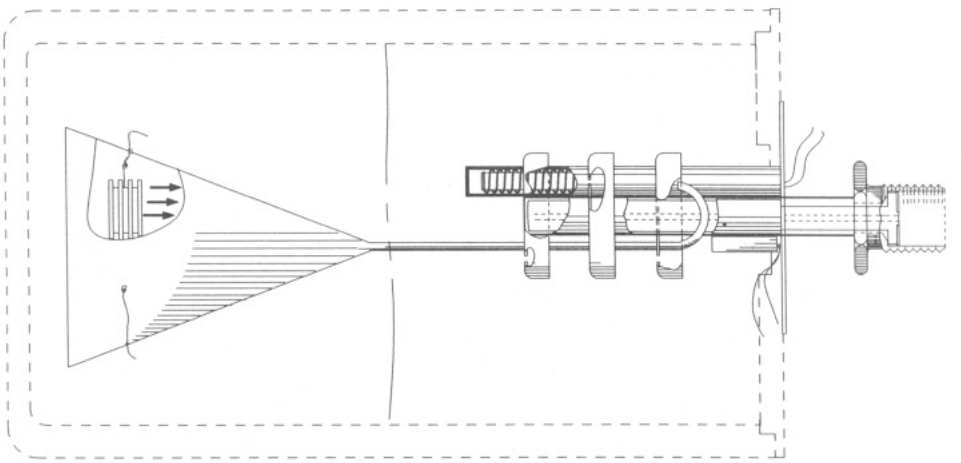

Figure 2. Schematic of a variable-temperature trap used in gas processing. A cold finger is cooled to trap gases and then heated to release gas. Gases pass through the middle of the cold finger (central dashed lines) and back up along outside walls, exiting to the next trap. The trap sits in a dewar (outer dashed lines) filled halfway with liquid nitrogen. Heating elements inside a cone below the trap boil the liquid nitrogen, forcing it up through a tube and into contact with the cold finger. The liquid nitrogen then drips down through three cups back into the dewar, cooling the cold finger to −196 °C. Liquid nitrogen levels in the dewar are controlled by a three-sensor cryostat (right of cold finger). Trapped gases are released upon heating by resistance coils shown in the schematic cutaway to the left of the cold finger.

opening valve 5 and heating T1 to −80 °C, keeping T2 at −180 °C. After 3.5 min, CO_2 transfer is complete, and valve 5 is closed. T2 is heated to 50 °C, and CO_2 yield is estimated with pressure gauge PM1. Large samples are repeatedly split using valves 7, 13, and 14 to obtain the 10–50 μmol of C used in normal mass spectrometric analyses; smaller samples (to 0.2 μmol) can be frozen into a small-volume cold finger, T5, for subsequent analysis.

4. Mass Spectrometry. Nitrogen is first measured with routine programming that includes several comparisons of sample and standard m/e 29/28 and 30/28 ratios, followed by scans for possible interfering gases at m/e 18, 30, 32, 40, and 44. Air leaks appear in elevated argon m/e 40 peaks, and oxygen interference from exhaustion of the copper reduction column results in elevated m/e 30 and 32 peaks. After $\delta^{15}N$ analysis, the mass spectrometer switches to CO_2 reference gas (Figure 2), and $\delta^{13}C$ measurements are initiated. After completion of CO_2 measurements, changeover valves are closed so that no gas enters the mass spectrometer, and CO_2 is pumped away ca. 20 min before the next nitrogen analysis.

5. Pumping Out. At the end of each run, residual combustion gases are pumped through T4. Valve 17 is then closed and trap T4 is heated to 50 °C for removal of water and other gases that otherwise accumulate from sample to sample and eventually clog T4. T3 and T6 are heated to 120 and 50 °C, respectively, between runs for complete evacuation of residual N_2.

6. Data Analysis. Results from each run are printed out and are also stored in a data base that can be later manipulated with, for example, a Lotus spreadsheet.

N_2 Development Problems. The most difficult problems in development involved capturing and processing N_2 from the elemental analyzer. There were four problems: (1) We found it necessary to employ two single-pass traps to capture all contaminating CO_2 and water before collecting nitrogen in a third trap. (2) Few molecular sieves tested were adequate for capturing all nitrogen in T3, a one-pass trap; only 5-Å zeolite from Alltech Associates was effective. (3) To measure $\delta^{15}N$ values are small amounts of nitrogen, we added a cold finger trap T6 filled with

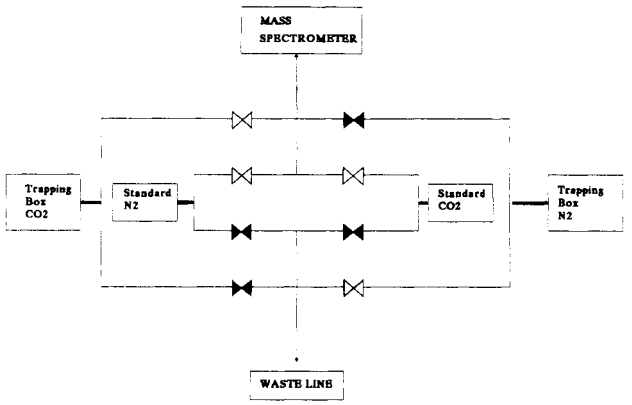

Figure 3. Schematic of four-way changeover valve in the mass spectrometer. Only one gas at a time enters the mass spectrometer, in this case N_2 from the trapping box, while the other three gases go to waste. Hourglass symbols represent valves.

silica gel. The silica gel is adequate for trapping N_2 distilled over 30–90 s from T3 to T6 and has the advantage that it completely releases N_2 near room temperature instead of near 100 °C. (4) Using the combination of zeolite and silica gel molecular sieves, nitrogen gas entering the mass spectrometer was not well mixed at the start of the $\delta^{15}N$ measurement, displaying a 1‰ decline over 30 min to a constant $\delta^{15}N$ value. To ensure isotopic uniformity and a steady 29/28 sample ratio, we found it necessary to mix N_2 in T6 by heating, partial refreezing to −95 °C for 45 s, and reheating to 45 °C before beginning $\delta^{15}N$ measurements.

RESULTS

Automated analyses currently take 43 min/sample and consume approximately 3.5 L of liquid nitrogen. The system produces estimates of carbon and nitrogen mass from PM1 and PM2 pressure readings, C/N ratios, and precise $\delta^{13}C$ and $\delta^{15}N$ values. Blanks are typically <0.03 μmol of C and 0.25–0.50 μmol of N, so that blank corrections are necessary only for small samples. These corrections are made by mass balance:

$$\delta_T = (\delta_o m_o - \delta_B m_B)/(m_o - m_B)$$

where δ values refer to isotopic compositions, m refers to mass measured with PM1 or PM2, and the subscripts o and B refer to values observed for samples and blanks, respectively, while δ_T is the blank-corrected true value of the sample.

Repeated analysis showed that it was difficult to accurately measure the isotopic compositions of small nitrogen blanks, perhaps because trace contaminants assume a larger importance in these small 0.25–0.5-μmol samples. As an alternative to direct measurement, we included a size series of standards in each run of 20–40 samples to estimate the effect and isotopic composition of blanks. Linear regression analysis can be used to determine the isotopic composition of the blank, using a rearrangement of the above equation:

$$\delta_o = \delta_T + [(\delta_B - \delta_T)m_B]/m_o$$

When observed isotope values are plotted versus the inverse of the observed sample size, the y intercept gives the blank-corrected value (δ_T) for the sample, and δ_B can be determined from the slope, $(\delta_B - \delta_T)m_B$, once the size of the blank, m_B, is known from direct measurement. This procedure for estimating δ_B can also be performed graphically (Figure 4, inset; for further details see ref 3).

Our regressions for blank estimation are highly significant ($R^2 > 0.95$) and similar between days, suggesting that blanks are fairly stable within and between runs. After blank correction, even small nitrogen samples of <1 μmol of N yield acceptable $\delta^{15}N$ values, although blank-corrected $\delta^{15}N$ values show more scatter than values for large samples (Figure 4). Carbon and nitrogen elemental composition and C/N ratios

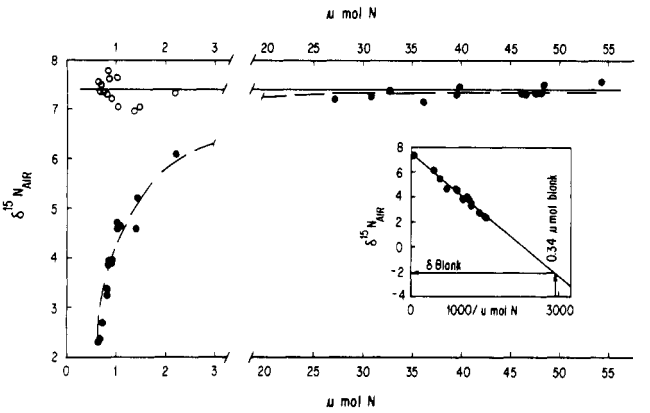

Figure 4. $\delta^{15}N$ values of a laboratory standard, peptone. At small sample sizes, $\delta^{15}N$ declines due to mixing with nitrogen in blanks. In this example, blanks averaged 0.34 μmol of N, with an isotopic composition estimated from the inset regression line as −2.1‰ (see text for details of this procedure). Open circles show blank-corrected values, while solid circles are values actually measured in five runs over 3 weeks, including three C + N runs and two N-only runs. The solid line gives the peptone $\delta^{15}N$ value established by sealed-tube combustion; the dashed line gives the theoretical mixing curve between peptone samples of various sizes and a 0.34-μmol, −2.1‰ nitrogen blank.

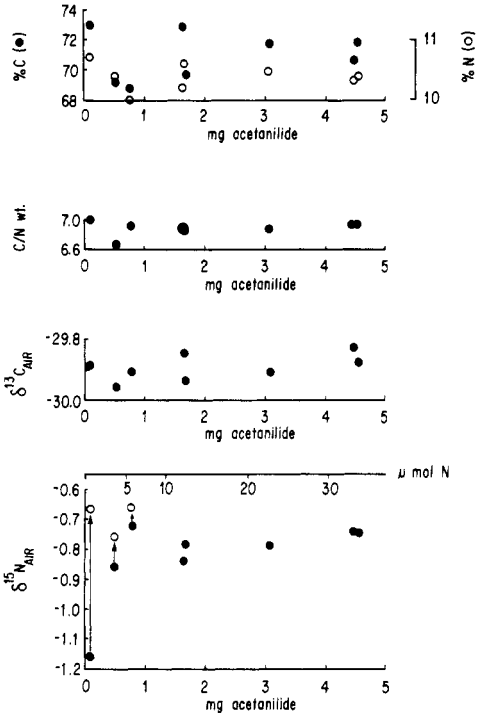

Figure 5. Analytical results for acetanilide, a laboratory standard. Blank corrections were made in calculating % N and $\delta^{15}N$; corrected $\delta^{15}N$ values are shown as open circles.

can also be generated within a C + N run (Figure 5).

We have tested the accuracy of the $\delta^{15}N$ and $\delta^{13}C$ analyses with animal, plant, and soil materials available as Standard Reference Materials (SRMs) from the U.S. National Bureau of Standards. Approximately 1-g aliquots were dried at 60 °C and pulverized to a fine flourlike consistency before subsampling 1–50 mg for analysis. Samples were analyzed with the automated system, with sealed-tube combustions performed at 870–900 °C (*1*) and with Kjeldahl digestion. We followed the recommendations of Mariotti (*4*) and used silica gel to collect and transfer nitrogen gas in work with sealed tubes.

Results from the C + N automated analyzer generally show good agreement with the sealed tube results, with differences

Table I. Carbon and Nitrogen Isotope Compositions of Standard Reference Materials (SRMs) and International Standards Analyzed by Different Methods[a]

	citrus leaves (SRM 1524)	bovine liver (SRM 1577)	river sediment (SRM 1645)	NBS-21 graphite	N-1 ammonium sulfate	N-2 ammonium sulfate
			$\delta^{13}C_{PDB}$			
sealed tubes 1 h, 900 °C	−27.12 ± 0.01 (8)	−21.58 ± 0.01 (4)	−22.21 ± 0.01 (4)			
automated C + N	−27.15 ± 0.03 (14)	−21.51 ± 0.01 (4)	−22.20 ± 0.16 (4)	−28.12 ± 0.05 (4)		
			$\delta^{15}N_{AIR}$			
sealed tubes Kjeldahl[b]	4.86 ● 0.19 (29) 4.56 ± 0.48 (5)	7.58 ± 0.04 (4) 6.66 ± 0.21 (5)	4.34 ± 0.28 (7) 3.77 ± 0.69 (3)		0.56 ± 0.08 (4)	20.42 ± 0.08 (4)
automated N only[c]	4.86 ± 0.07 (4)	7.39 ± 0.08 (4)	4.21 ± 0.21 (4)		0.62 ● 0.04 (2)	20.27 ± 0.04 (3)
C + N[c]	4.70 ± 0.01 (4)	7.48 ± 0.02 (4)	4.40 ± 0.07 (4)		0.56 ± 0.06 (6)	20.30 ± 0.25 (2)

[a] Entries are mean ± standard deviation (*N*). SRMs and NBS-21 graphite are distributed by the U.S. National Bureau of Standards; N-1 and N-2 are distributed by IAEA, Vienna. [b] Courtesy of G. Shearer. [c] After blank correction.

ranging from 0.06 to 0.38‰ (Table I). Noteworthy was the good agreement for the low-nitrogen river sediment (0.09% N) that was the most recalcitrant material analyzed. This agreement was particularly reassuring for the nitrogen work, since relatively small amounts of river sediment were analyzed in the C + N mode (45 mg) and blank corrections for the resulting 3-μmol N samples averaged 0.5‰. Larger 200–350-mg samples combusted in sealed tubes did not require blank correction but gave more variable $\delta^{15}N$ results (Table I).

$\delta^{15}N$ values of the SRMs measured in the C + N mode were 0.15–0.84‰ higher than Kjeldahl values obtained separately by Georgia Shearer of Washington University (Table I). The higher C + N values could possibly be due to trace CO contamination of the automated nitrogen measurements, especially since CO has an effective $\delta^{15}N$ value near +500‰. CO contamination could occur in two ways. CO can be formed during incomplete combustion. Also, if CO_2 travels with and is present in nitrogen samples, it will fragment to form CO in the mass spectrometer.

We evaluated the possibility of CO contamination in three ways. First, we analyzed ammonium sulfate with and without 15 mg of dextrose and observed no detectable isotopic difference between the samples that all fell in a 0.05‰ range. If CO had formed during incomplete combustion of dextrose, higher $\delta^{15}N$ values would have been expected in the dextrose samples. Second, residual CO_2 in the mass spectrometer could contaminate nitrogen samples if it is not pumped away between samples. This was a special concern for the C + N runs since there is always CO_2 in the standard side of the mass spectrometer in the C + N mode and, because in the course of $\delta^{13}C$ measurements, the mass spectrometer also receives CO_2 from a sample every 30 min. To address this concern, we analyzed the SRMs in N-only mode in which there is no CO_2 standard in the mass spectrometer and CO_2 from samples is always pumped to waste instead of entering the mass spectrometer. Values obtained in the N-only and C + N modes are quite comparable (Table I), suggesting little carbon contamination from the CO_2 in the C + N mode. Lastly, scans for CO_2 (m/e 44) made with each sample show that CO_2 comprises less than 0.01% of the nitrogen (m/e 28) signal. In summary, we found no evidence for CO contamination of the C + N results and suspect that higher $\delta^{15}N$ values of the C + N vs Kjeldahl methods are an artifact based on inadequate standardizations between laboratories, or reflect inadequate extraction of nitrogen with the Kjeldahl procedure (*1*).

CONCLUSION

Isotope reference standards for carbon and nitrogen work currently include graphite, inorganic carbonates, nitrogen in air, and ammonium sulfate salts (*4–6*). With this report, we suggest that the Standard Reference Materials of Table I are also suitable isotope reference standards; these materials are readily available from the U.S. National Bureau of Standards. Further interlaboratory comparisons are necessary to establish widely-accepted values for these SRMs. However, supplementing current inorganic standards with soil, plant, and animal standards should be useful for analysts dealing with complex organic materials.

ACKNOWLEDGMENT

Georgia Shearer kindly performed Kjeldahl analyses cited in this work. The research was supported by NSF Grant BSR-8805390 to the LTER Coordinating Committee and by NSF Grant BSR 8806691.

REFERENCES

(1) Minagawa, M.; Winter, D. A.; Kaplan, I. R. *Anal. Chem.* **1984**, *56*, 1859–1861.
(2) Fry, B. *Ecology* **1991**, *72*, 2293–2297.
(3) Gelwicks, J. T.; Hayes, J. M. *Anal. Chem.* **1990**, *62*, 535–539.
(4) Mariotti, A. *Nature* **1983**, *303*, 685–687.
(5) Coplen, T. B.; Kendall, C. *Anal. Chem.* **1982**, *54*, 2611–2612.
(6) Kendall, C.; Grim, Elizabeth. *Anal. Chem.* **1990**, *62*, 526–529.

RECEIVED for review August 15, 1991. Accepted October 21, 1991.