ASSESSMENT OF STABLE NITROGEN ISOTOPES IN FINGERPRINTING SURFACE WATER INORGANIC NITROGEN SOURCES

C. W. LINDAU, R. D. DELAUNE, W.H. PATRICK, Jr., and E. N. LAMBREMONT

Nuclear Science Center/Laboratory for Wetland Soils and Sediments, Louisiana State University, Baton Rouge, LA 70803, U.S.A.

(Received July 17, 1989; revised September 20, 1989)

Abstract. In many coastal areas of Louisiana, surface water quality is deteriorating rapidly due to elevated nutrient input from agricultural, domestic and industrial sources. This study investigates the potential use of natural abundance variations in $^{15}N/^{14}N$ ratios for identification and tracing surface water inorganic N sources. Surface water samples were collected from streams and point sources in Louisiana and analyzed for NH_4^+-N, NO_3^--N and associated $^{15}N/^{14}N$ ($\delta^{15}N$ ‰) concentrations. Ammonium-N from domestic sewage and industrial discharge point sources was found to have distinct $\delta^{15}N$ ranges. Domestic sewage discharge into a slow flowing stream was traced for about 30 km downstream using $^{15}N/^{14}N$ ratios. At the sewage point source NH_4^+-$\delta^{15}N$ values averaged +43‰ and increased linearly to +162‰ with distance from the discharge. In a larger stream with a greater flow velocity the NH_4^+-$\delta^{15}N$ surface water signature of an industrial discharge source was identifiable for approximately 1 km from the point source. Surface water NO_3^--$\delta^{15}N$ values generally ranged from +1 to +99‰ and no significant association was observed between $\delta^{15}N$ values with distance from the domestic sewage and industrial point sources. The discrete NH_4^+-$\delta^{15}N$ signatures of domestic sewage and industrial point sources compared to downstream surface water NH_4^+-$\delta^{15}N$ values suggest that N isotopic ratios have the potential to be used as tracers in surface waters contaminated with inorganic N.

1. Introduction

In many regions of the Gulf Coast, including Louisiana, surface water quality is deteriorating. Water bodies in the northern portions of these coastal areas are rapidly becoming eutrophic and anoxic. Domestic waste discharge is becoming an increasing problem to surface water quality because of inadequate sewage treatment and population increases. Agricultural runoff and industrial waste discharge also results in large quantities of N entering surface water bodies. Source identification of this N is needed to restrict or eliminate future N loading.

Natural variations in N stable isotopic ratios ($^{15}N/^{14}N$) have been used successfully in source identification of N contaminated surface- and ground waters (Kreitler, 1974; Kreitler and Browning, 1983; Heaton, 1984; Lindau and Spalding, 1984; Mariotti *et al.*, 1988). Three main sources of NO_3^--N pollution to the hydrosphere are nitrogenous fertilizers, animal wastes and soil organic matter mineralization (Heaton, 1986). These N sources can produce NO_3^--N with distinguishable $^{15}N/^{14}N$ isotopic ratios or *$\delta^{15}N$ (‰) values. Nitrogen fertilizers are derived from industrial

---

* $\delta^{15}N$ (‰) = (R sample/R standard −1) × 1000 where R = $^{15}N/^{14}N$ ratio and the standard is atmospheric N_2, with a ^{15}N abundance of 0.3663 % (Junk and Svec, 1958).

Water, Air, and Soil Pollution **48**: 489–496, 1989.

fixation of atmospheric N_2 and nitrification produces NO_3^--$\delta^{15}N$ values close to zero (Shearer *et al.*, 1974; Kreitler, 1979; Heaton, 1986). Nitrates produced by the oxidation of animal wastes have $\delta^{15}N$ values in the range of +10 to +22‰ (Kreitler, 1974; Lindau and Spalding, 1984) and NO_3^--N produced from mineralization of soil organic N has a characteristic $\delta^{15}N$ range of about +4 to +9‰ (Gormly and Spalding, 1979; Mariotti, 1984). These variations in $\delta^{15}N$ ranges should be used as semi-quantitative source interpretations due to the unpredictable magnitude and complexities of N stable isotope fractionations in natural systems (Hauck, 1973; Mariotti *et al.*, 1988).

Very little research has been published on the use of natural variations in $^{15}N/^{14}N$ ratios for source identification of inorganic N in surface waters (Heaton, 1986). The objectives of this note were 1) to assess the potential of natural variations of N stable isotopes for identifying sources of inorganic N and 2) determine if $^{15}N/^{14}N$ isotopic values could be used to trace inorganic N from point source waste discharge entering Louisiana surface waters.

2. Materials and Methods

Surface water from streams and discharge from known point sources were collected

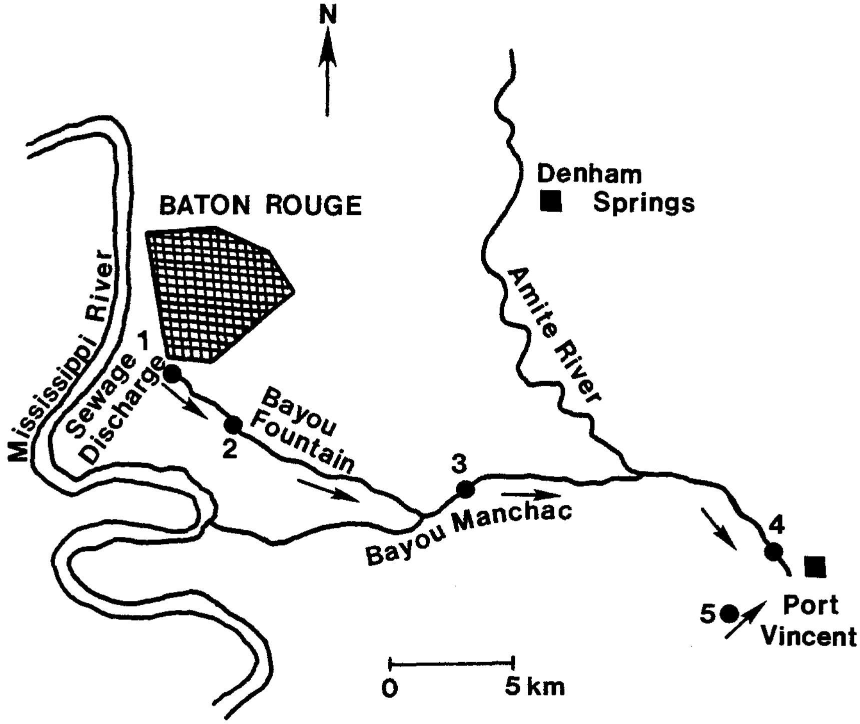

Fig. 1. Municipal sewage discharge and surface water sample locations (●) in the Bayou Fountain-Amite River drainage basin, Louisiana.

on three sampling dates and analyzed for NH_4^+- and NO_3^--N and ^{15}N content. Sample areas included sewage discharge into the Bayou Fountain-Amite River system and industrial NH_4^+-N input into the Calcasieu River, Louisiana. Stations 2, 3, and 4 were located downstream from the sewage treatment outfall (Figure 1). Sampling sites 1 and 2 were established above and sites 5, 6, and 7 below the industrial discharge point at the Calcasieu site (Figure 2).

For each sampling location and sampling date 4 to 8 L of surface water or discharge was collected in precleaned plastic containers. Water and discharge samples were preserved with concentrated H_2SO_4 and transported to the laboratory and stored at 4°C (EPA, 1983). Samples were filtered through 0.45 µm membrane filters and evaporated down to 100 to 200 mL at 50 °C. The NH_4^+- and NO_3^--N concentrations were determined by steam distillation techniques (Hauck, 1982; Keeney and Nelson, 1982; EPA, 1983). Dilute NaOH was added to the NH_4^+-N fraction to raise the solution pH and Devarda's alloy (<300 mesh) reduced NO_3^--

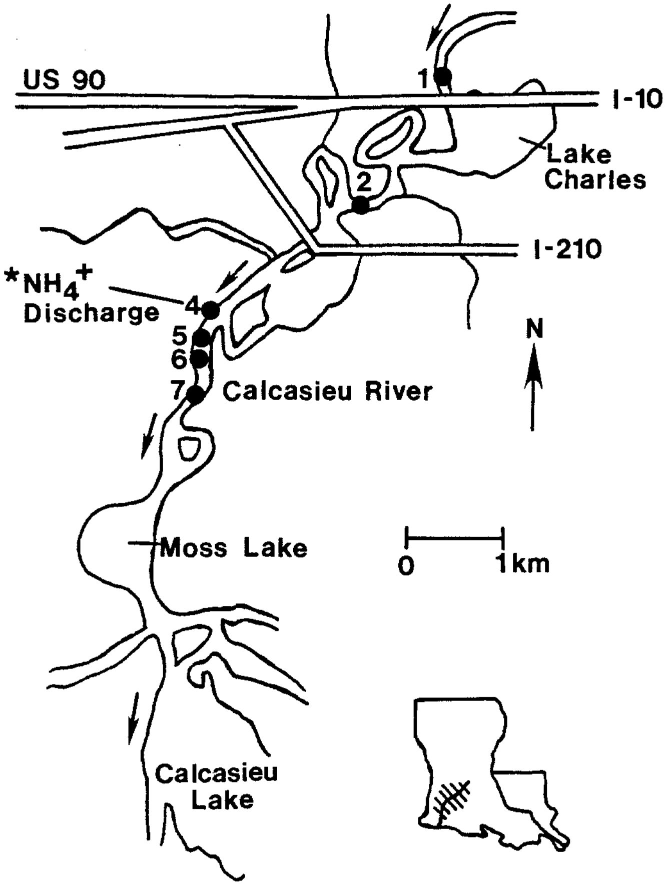

Fig. 2. Industrial ammonium discharge and surface water collection sites (●) in the Calcasieu River, Louisiana.

N to NH_3. The ammonia from each inorganic N fraction was collected in a boric acid solution and titrated to determine N concentration. The titrated samples were acidified (H_2SO_4) and evaporated to dryness and stored in 20 mL glass scintillation vials (Hauck, 1982). For $^{15}N/^{14}N$ ratio determination the NH_4^+-salt of each fraction was converted to N_2 by reacting with NaOBr. The N_2 gas samples were purified through a LN_2 trap and a hot Cu furnace. The purified gas samples were analyzed on a Finnigan Mat Delta E mass spectrometer equipped with a dual inlet and triple collector. The $^{15}N/^{14}N$ ratio of the sample gas was compared to the $^{15}N/^{14}N$ ratio of atmospheric N_2 and sample $\delta^{15}N$ values computed.

Atmospheric N_2 standards were prepared 10 times and the calculated $\delta^{15}N$ standard deviation was ± 0.05‰. A second working standard of $(NH_4)_2SO_4$ was prepared 12 times by steam distillation and had a mean $\delta^{15}N$ value of + 0.40‰ and a standard deviation of ± 0.34‰.

To check if fractionation was occurring during the evaporation and concentration step of the water samples 4 mg N each of the $(NH_4)_2SO_4$ and KNO_3 standards was added to 8 L of acidified deionized H_2O. The 8 L was evaporated and distilled as outlined for the surface water samples and replicated 3 times. Mass spectrometer analysis showed a 1 to 2‰ ^{15}N enrichment of the evaporated + distilled standards compared to steam distillation of the same standards. The 2‰ enrichment was associated with the NO_3^--N standard.

To minimize ^{15}N cross-contamination, plastic containers, glassware and steam distillation apparatus were cleaned with dilute HCl, 1 M formic acid and 95% ethanol. This cleaning procedure was used after each sample collection and between distillations (Mulvaney, 1986).

3. Results and Discussion

The inorganic N content and corresponding $\delta^{15}N$ values of the sewage and surface water samples collected downstream from the sewage discharge site into Bayou Fountain are shown in Table I for the three sampling dates. Ammonium and NO_3^--N concentrations varied considerably and were higher at the sewage discharge site and decreased in surface water samples with distance from the point source. The mean sewage discharge NH_4^+- and NO_3-N concentratrions over the three sampling dates were 3.2 and 1.2 mg L^{-1}, respectively. This compares to surface water values of about 0.3 (NH_4^+) and 0.2 (NO_3^-) mg N L^{-1} 32 km downstream. The decrease in sewage NH_4^+-N with distance was apparently due to nitrification and/or mixing with other surface water sources draining into the stream. Mixing, uptake and denitrification were also causing the sewage NO_3^--N concentrations to decrease downstream. The NH_4^+-$\delta^{15}N$(‰) values varied considerably and ranged from +23 at the sewage discharge to +221 in the surface water sample collected from site #3 (Table I). The range of surface water NO_3^--$\delta^{15}N$ values was narrower (+10 to +99‰) except on the second sampling date at site #3 (+170‰).

Surface water NH_4^+-$\delta^{15}N$ values increased linearly with distance from the sewage

TABLE I

Ammonium, NO_3^-–N and $\delta^{15}N$ (‰) concentrations of surface water samples with distance from a sewage point source discharge into Bayou Fountain, Louisiana

Sampling site	Date of sampling[a]	Distance from point source (km)	N concentration		N-15 content	
			NH_4^+ (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ ($\delta^{15}N$‰)	NO_3^- ($\delta^{15}N$‰)
1	1	0	7.9	0.3	23	24
(point source	2		0.5	2.9	61	10
discharge)	3		1.3	0.4	46	55
2	1	10	0.6	0.2	63	28
	2		1.0	0.7	45	13
	3		1.4	0.7	37	18
3	1	19	0.3	0.1	153	99
	2		0.3	0.2	221	170
	3		0.4	0.8	60	30
4	1	32	0.3	0.1	132	29
	2		0.2	0.1	183	74
	3		0.3	0.3	170	30
5[b]		35	0.2	0.1	214	99

[a] 02–25–88, 05–01–88, 08–23–88, respectively.

[b] Runoff from swamp forest draining into Bayou Fountain-Amite River system, sampled 09–15–88.

point source. The association was significant at the 0.05 level of probability with an r value of 0.92. The significant correlation would suggest that the mixing of sewage NH_4^+-N discharge with surface water NH_4^+-N could be predicted downstream. No correlation was found between surface water NO_3^--$\delta^{15}N$ values with distance from the sewage source.

Swamp forest surface water runoff into the Bayou Fountain-Amite River system (below site #4) was collected on 09-15-88 (Table I). This was a nonsewage source and the NH_4^+- and NO_3^--N fractions had $\delta^{15}N$ values of +214 and +99‰, respectively.

Nitrogen and $\delta^{15}N$ analysis of the industrial discharge and Calcasieu River surface water samples collected above and below the NH_4^+-N discharge site are shown in Table II. Ammonium and NO_3^--N concentrations of the upstream and downstream surface water samples were low and rarely exceeded 0.2 mg N L^{-1} except at or near the discharge site. On the first sampling date discharge into the Calcasieu River was observed and discharge concentrations of NH_4^+- and NO_3^--N were 25.4 and 14.6 mg N L^{-1}, respectively (Table II). Discharge (site 4) was restricted on the remaining 2 sampling dates and N concentrations never exceeded 1.6 mg L^{-1}. Discharge sample $\delta^{15}N$‰ values were +7 and +19 for NH_4^+- and NO_3^--N, respectively but showed a much wider range (+1 to +43‰) for the remaining two dates (Table II). Ammonium and NO_3^--N isotopic values above and below the discharge site were highly variable and ranged from +1 to +164‰. Generally the NH_4^+-$\delta^{15}N$ surface

TABLE II

Ammonium, NO_3^--N and $\delta^{15}N$ (‰) concentrations of surface water samples above and below an industrial NH_4^+-N discharge site into the Calcasieu River, Louisiana

Sampling site	Date of sampling[a]	Distance from point source[b] (km)	N concentration		N-15 content	
			NH_4^+ (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ ($\delta^{15}N$‰)	NO_3^- ($\delta^{15}N$‰)
1	1	3.2	0.1	ND	164	ND
	2		0.3	ND	134	ND
	3		0.2	0.1	75	21
2	1	1.6	ND	ND	ND	ND
	2		ND	ND	ND	ND
	3		0.2	0.1	55	56
4 (point source discharge)	1	0.0	25.4	14.6	7	19
	2		0.7	0.4	43	1
	3		1.1	1.6	17	27
5	1	0.1	ND	ND	ND	ND
	2		0.4	0.3	66	1
	3		1.0	1.4	18	28
6	1	0.5	ND	ND	ND	ND
	2		0.2	0.1	84	16
	3		0.2	0.1	41	32
7	1	1.0	0.1	ND	145	ND
	2		ND	ND	ND	ND
	3		0.1	0.1	47	28

[a] 10-23-87, 03-07-88, 07-02-88, respectively.
[b] Sites 1 and 2 are upriver and 5, 6, and 7 downriver from the point source.
ND = Not determined, concentrations below detection limits.

water values increased with distance (up to 3.2 km) from the discharge point but no trend was observed for the NO_3^--$\delta^{15}N$ surface water values. The range in surface water NH_4^+-$\delta^{15}N$ values was much larger than that observed for the NO_3^--N isotopic values (Table II). Correlations were not calculated between NH_4^+- and NO_3^--$\delta^{15}N$ surface water values and distance from the industrial point source because many surface water N concentrations were below analytical detection limits.

Many of the surface water, sewage and industrial discharge $\delta^{15}N$ values reported in this paper are much higher than the $\delta^{15}N$ range (–20 to +30‰) reported in the literature for terrestrial N compounds (Kreitler, 1974; Heaton, 1986). The surface water N compounds are undergoing transformations and are constantly being reworked by bacteria to cause the N isotopic fractionations observed. Microbial-mediated reactions may be responsible for the large kinetic isotopic fractionations observed for the inorganic N in the surface water samples collected downstream from the point sources (Macko, 1981; Heaton, 1986; Mariotti, 1988; Shearer and Kohl, 1988). It appears that for some N transformations bacteria selectively use the ^{14}N isotope which would result in the residual N material being enriched with ^{15}N.

The large increase (+43 to +162‰) in surface water NH_4^+-$\delta^{15}N$ values with distance (up to 32 km) from the sewage point source was thought to be due to mixing with isotopically heavier sources entering the sewage contaminated stream and/or bacterially-mediated reactions. In this case, mixing was thought to be more significant since swamp forest surface water runoff into the slow flowing Bayou Fountain-Amite River system had a NH_4^+-$\delta^{15}N$ value of +214‰. The industrial point source NH_4^+-$\delta^{15}N$ signature in the Calcasieu River was not identifiable with as great a distance from the discharge site. Larger stream flow and rapid mixing with isotopically different surface waters masked the $\delta^{15}N$ signature.

Ammonium-N from sewage and industrial point sources was found to have distinct $\delta^{15}N$ signatures different from downstream surface water NH_4^+-N samples. The large range in surface water NH_4^+-$\delta^{15}N$ values and significant $\delta^{15}N$ correlation with distance from the sewage discharge site suggest that N isotopic ratios have the potential to be used as tracers in surface waters contaminated with inorganic N. The lack of association between surface water NO_3^--$\delta^{15}N$ values with distance from the discharge points may limit its use as a N tracer in contaminated surface waters.

Acknowledgments

This work was supported in part by the Louisiana Water Resources Research Institute and the National Science Foundation (BSR–8806601).

References

Gormly, J. R. and Spalding, R. F.: 1979, *Ground Water* **17**, 291.

Hauck, R. D.: 1973, *J. Environ. Qual.* **2**, 317.

Hauck, R. D.: 1982, 'Nitrogen-Isotope-Ratio Analysis', in A. L. Page, R. H. Miller and D. R. Keeney (eds.), *Methods of Soil Analysis, Part 2 – Chemical and Microbiological Properties*, American Society of Agronomy, Madison, WI, p. 735.

Heaton, T. H. E.: 1984, *J. Hydrol.* **67**, 249.

Heaton, T. H. E.: 1986, *Chem. Geol.* **59**, 87.

Junk, G. and Svec, H. J.: 1958, *Geochim. Cosmochim. Acta* **14**, 234.

Keeney, D. R. and Nelson, D. W.: 1982, 'Nitrogen-Inorganic Forms', in A. L. Page, R. H. Miller and D. R. Keeney (eds.), *Methods of Soil Analysis, Part 2 – Chemical and Microbiological Properties*, American Society of Agronomy, Madison, WI, p. 643.

Kreitler, C. W.: 1974, Determining the Source of Nitrate in Groundwater by Nitrogen Isotope Studies. Ph.D. thesis, University of Texas, Austin, Tex.

Kreitler, C. W.: 1979, *J. Hydrol.* **42**, 147.

Kreitler, C. W. and Browning, L. A.: 1983, *J. Hydrol.* **61**, 285.

Lindau, C. W. and Spalding, R. F.: 1984, *Ground Water* **22**, 273.

Macko, S. A.: 1981, Stable Nitrogen Isotope Ratios as Tracers of Organic Geochemical Processes. Ph.D. thesis, University of Texas, Austin, Tex.

Mariotti, A.: 1984, 'Utilisation des varaitons naturelles d'abondance isotopique en ^{15}N pour tracer l'origine des pollutions des aquiferes par les nitrates', in Int. At. Energy Agency (ed.), *Isotope Hydrology* 1983, I.A.E.A., Vienna, p. 605.

Mariotti, A., Landreau, A., and Simon, B.: 1988, *Geochim. Cosmochim. Acta* **52**, 1869.

Mulvaney, R. L.: 1986, *Soil Sci. Soc. Am. J.* **50**, 92.

Shearer, G. and Kohl, D. H.: 1988, *J. Biol. Chem.* **263**, 13231.

Shearer, G. B., Kohl, D. H., and Commoner, B.: 1974, *Soil Sci.* **118**, 308.

U.S. Environmental Protection Agency: 1983, *Methods for Chemical Analysis of Water and Wastes*, revised March 1983. EPA 600/4-79-020, U. S. Environmental Protection Agency, Cincinnati, OH.