PLAN OF STUDY TO DETERMINE IF THE ISOTOPIC RATIOS $\delta^{15}N$ AND $\delta^{18}O$ CAN REVEAL THE SOURCES OF NITRATE DISCHARGED BY THE MISSISSIPPI RIVER INTO THE GULF OF MEXICO

By William A. Battaglin, Carol Kendall, Donald A. Goolsby and Laurie L. Boyer

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CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
cubic meters per second	3.531×10^{1}	cubic foot per second (ft^3/s)
metric ton	2.205×10^{3}	pound
square kilometer	3.861×10^{-1}	square mile (mi^2)
kilogram	2.2046	pounds
hectare	2.471	acres
micron	2.0×10^{-6}	meters
liter	2.642×10^{-1}	gallons

Temperature can be converted from degrees Celsius (°C) to Fahrenheit (°F) by using the following equation:

$$°F = 9/5(°C)+32.$$

Abbreviations

NASQAN II -- National Stream Quality Accounting Network
NAWQA -- National Water Quality Assessment
microgram per liter (µg/L)
microsiemens per centimeter at 25 degrees Celsius (µS/cm)
milligram (mg)
milligram per liter (mg/L)
milliliter (ml)

Plan of Study to Determine if the Isotopic Ratios $\delta^{15}N$ and $\delta^{18}O$ Can Reveal the Sources of Nitrate Discharged by the Mississippi River into the Gulf of Mexico

By William A. Battaglin, Carol Kendall, Donald A. Goolsby *and* Laurie L. Boyer

Abstract

Nitrate and other nutrients discharged from the Mississippi River basin are suspected of causing a zone of depleted dissolved oxygen (hypoxic zone) in the Gulf of Mexico each summer. The hypoxic zone may have an adverse effect on aquatic life and commercial fisheries. Commercial fertilizers are the dominant source of nitrogen input to the Mississippi basin. Other nitrogen sources include animal waste, fixation of atmospheric nitrogen by legumes, precipitation, domestic and industrial effluent, and the soil. The inputs of nitrogen from most of these sources to the Mississippi basin can be estimated and the outputs in surface water can be measured. However, nitrogen from each source is affected differently by physical, chemical, and biological processes that control nitrogen cycling in terrestrial and aquatic systems. Hence, the relative contributions from the various sources of nitrogen to nitrate load in the Mississippi River are unknown because the different sources may not contribute proportionally to their inputs in the basin.

It may be possible to determine the relative contributions of the major sources of nitrate in river water using the stable isotopic ratios $\delta^{15}N$ and $\delta^{18}O$ of the nitrate ion. A few researchers have used the $\delta^{15}N$ and/or $\delta^{18}O$ isotope ratios to determine sources of nitrate in ground water, headwater catchments, and small rivers, but little is known about the isotopic composition of nitrate in larger rivers. The objective of this study is to measure the isotopic composition of nitrate and suspended organic matter in the Mississippi River and its major tributaries, in discharge to the Gulf of Mexico, and in streamflow from smaller watersheds that have distinct sources of nitrogen (row crops, animal wastes, and urban effluents) or are minimally impacted by man (undeveloped). Samples from seven sites on the Mississippi River and its tributaries and from 17 sites in smaller watersheds within the Mississippi River basin will be analyzed for $\delta^{15}N$ and $\delta^{18}O$ of dissolved nitrate. Suspended sediment collected from these sites will also be analyzed to determine the $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ of the suspended organic material. Six samples will be collected at each site during the winter, spring, and summer of 1996-97. Results from these samples will be used to identify seasonal and flow-related variability in $\delta^{15}N$ and $\delta^{18}O$ ratios from each site and may also help determine the principal sources of the nitrate entering the Gulf of Mexico.

Introduction

The Mississippi and Atchafalaya Rivers are the primary riverine sources of fresh water and nutrients discharged to the Gulf of Mexico. The combined annual mean streamflow for the Mississippi and Atchafalaya Rivers (21,800 cubic meters per second) represents about 80 percent of the estimated freshwater discharge to the Gulf (Dunn, 1996). These two rivers account for an estimated 90 percent of total nitrogen load and 87 percent of the total phosphorus load discharged annually to the Gulf (Dunn, 1996). The average annual transport of nitrogen in all forms (total N) from the Mississippi River Basin to the Gulf of Mexico is about 1.5 million metric tons per year, based on data collected since 1980. Nitrate-N accounts for about 60 percent of the total N transport. The remainder is dissolved organic N (about 26%) and particulate N (about 13%). In

recent years, as much as one million metric tons of dissolved nitrate-nitrogen are discharged annually into the Gulf of Mexico by the Mississippi and Atchafalaya Rivers (Goolsby and Battaglin, 1995). The flux of dissolved nitrate tends to peak in the spring and early summer months when daily flux rates can exceed 5,000 metric tons per day (fig. 1).

Nitrate along with other nutrients are suspected of being responsible for a large zone along the Louisiana-Texas coast where dissolved oxygen (DO) levels in bottom water of the Gulf are seasonally lowered (zone of hypoxia) (Justic and others, 1993; Justic and others, 1994; Turner and Rabalais, 1991; Rabalais and others, 1996). The seasonal reduction in DO occurs each year during late spring and summer following high inflows of freshwater and nutrients to the Gulf. The zone of hypoxia covered nearly 17,000 square kilometers following the 1993 flood, twice the size of Chesapeake Bay. The zone of hypoxia was reported to be as large or larger in 1994 and 1995 (Rabalais and others, 1995). Estimates of the size of the zone of hypoxia prior to the 1993 flood (1985-1992) averaged about 10,000 square kilometers (Rabalais and others, 1995).

Nitrogen Sources

Agriculture, specifically the increased use of nitrogen and phosphorus fertilizer, is the probable cause of the Gulf of Mexico zone of hypoxia (Rabalais and others, 1996). Fertilizer use in the basin has increased over the past 25 years, as have the concentrations and resulting flux of nitrate in the Mississippi River (fig. 2)(Goolsby and Battaglin, 1995). This increase in nitrate flux may contribute to the hypoxia problem, but fertilizer is not the only input of nitrogen in the basin. Other inputs include animal manure, fixation of atmospheric nitrogen by legumes (soybeans and alfalfa), domestic and industrial effluents, atmospheric deposition in precipitation, and soil nitrogen. Estimates of some of these inputs of nitrogen to the Mississippi basin and its major tributary basins are given in table 1. Estimates of nitrogen fertilizer inputs for 1987 are given both as a total and by major type, based on data provided by the U.S. Environmental Protection Agency (1990) and summarized by Battaglin and Goolsby (1995). Estimates of manure nitrogen inputs for 1987 were computed by R. B. Alexander (USGS, written commun., 1992) from livestock population estimates in the 1987 Census of Agriculture (U.S. Department of Commerce, 1989) and estimates of the nutrient content of wastes produced by livestock as listed in the National Resource Conservation Service Agricultural Waste Management Field Handbook. Estimates of nitrogen input from legumes for 1987 were calculated using information on soybean and alfalfa acreage from the 1987 Census of Agriculture and nitrogen replacement rates (nitrogen fixed - nitrogen in harvested crop) of 35 kg/ha for soybeans and 65 kg/ha for alfalfa (Board on Agriculture, National Research Council, 1993). Estimates of nitrogen input in wet deposition for 1987 were calculated from estimates of annual mean nitrate deposition at 188 National Atmospheric Deposition Program stations across the United States (Alexander, R.L., USGS, written commun., 1995). Estimates of nitrogen input from human domestic waste for 1990 were calculated from population estimates (U.S. Department of Commerce, 1990) and an estimated per capita loading of nitrogen in untreated municipal waste of 8.65 kg per year. Estimates of municipal and industrial point loadings of nitrogen are typical for 1977-81 and were reported originally as total Kjeldahl nitrogen (Gianessi and Peskin, 1984). Estimates of industrial point sources of nitrogen were included in the total inputs reported in table 1. Estimates of municipal points sources of nitrogen were considered to represent a subset of human domestic waste and were not included in the total inputs.

While most of the inputs of nitrogen to the Mississippi basin can be estimated and the outputs in surface water can be measured, the actual sources of the nitrate transported by the Mississippi River are unknown. How much is from this year's fertilizer? from last year's

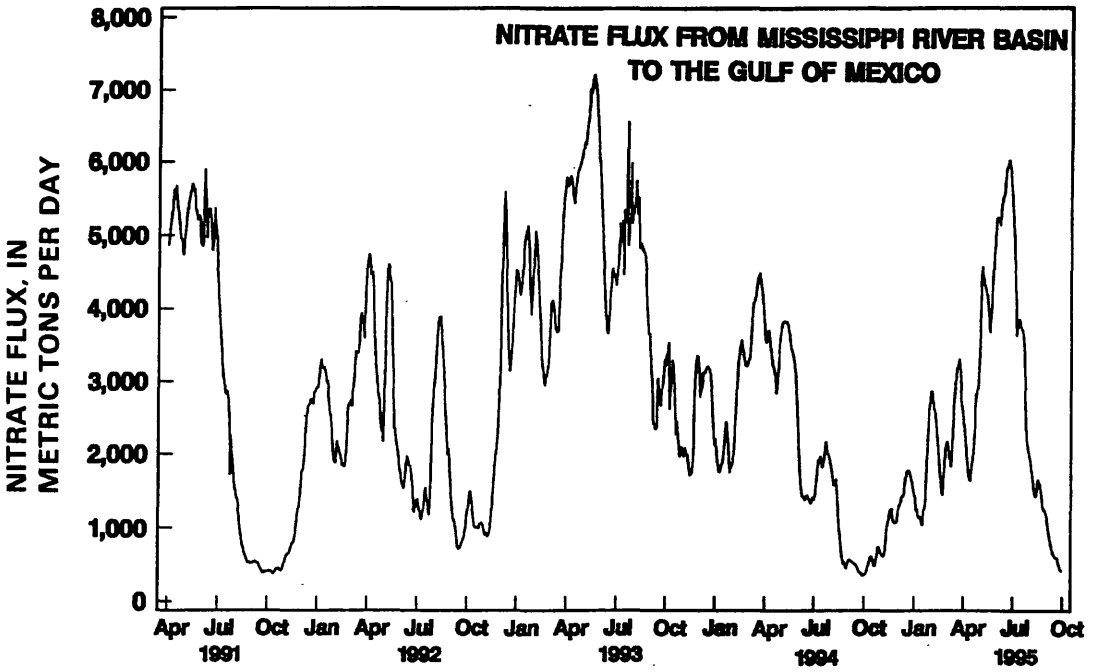

Figure 1. Estimated flux of dissolved nitrate as nitrogen from the Mississippi River Basin to the Gulf of Mexico, April 1991 through September 1995, in metric tons per day.

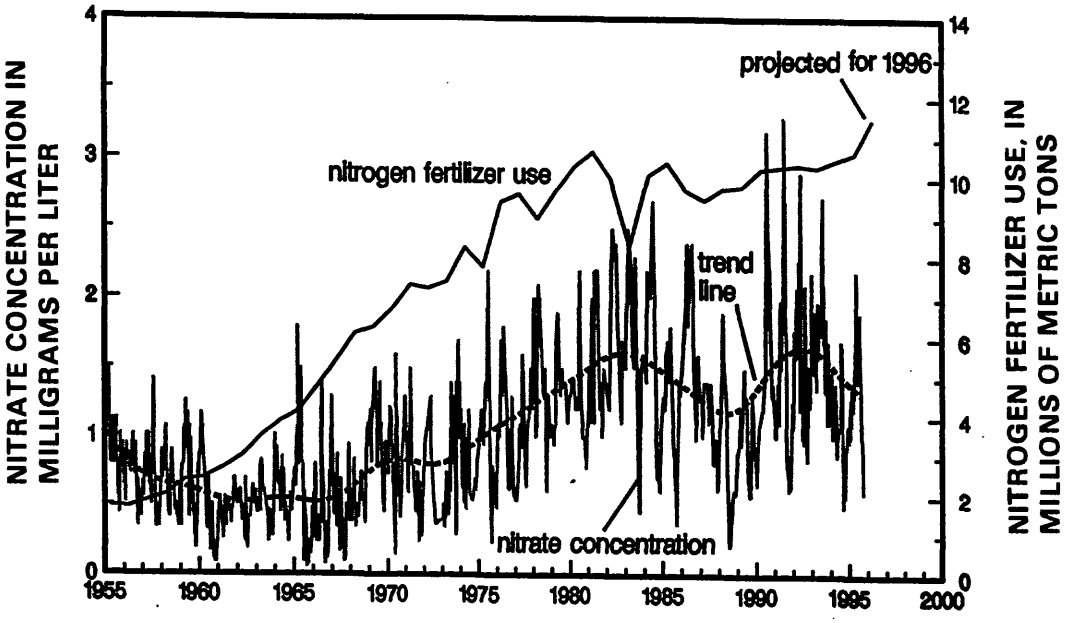

Figure 2. Estimated nitrogen fertilizer use in the United States, and nitrate concentrations in the Mississippi River at St. Francisville, La., 1955-95.

Table 1. Estimates of annual nitrogen inputs in metric tons to land in the Mississippi and Atchafalaya River basins in 1987.

Source of nitrogen	Ohio at Grand Chain, Ill.	Missouri at Hermann, Mo.	Mississippi at Clinton, Iowa	Mississippi at Thebes, Ill.	Mississippi at St. Francisville, La.	Mississippi and Atchafalaya Basins
Commercial fertilizer, all forms as N	1,058,200	1,684,900	562,200	3,583,700	5,529,100	5,87?,800
as anhydrous ammonium	336,700	871,800	248,500	1,772,900	2,411,200	2,518.700
as urea	156,800	251,900	127,100	523,300	907,300	97?.700
as nitrogen solutions	256,000	305,100	79,500	666,200	1,066,300	1,142,300
as miscellaneous forms	256,800	181,200	97,300	510,200	905,700	974,000
as ammonium nitrate	51,900	74,900	9,800	111,100	238,700	268,100
Livestock manure	547,600	1,173,300	400,300	2,087,400	3,196,300	3,451,300
Legumes (soybeans and alfalfa)	169,500	324,700	129,900	700,200	1,001,600	1,031,900
Atmospheric wet deposition of nitrate as N	130,000	130,200	38,900	237,900	456,400	512,300
Human domestic waste	222,900	83,300	45,600	271,900	581,200	627,800
Municipal point sources	73,100	39,800	14,800	136,400	245,200	264.000
Industrial point sources	52,100	2,800	2,800	15,400	73,000	105,800
Nitrification of soil N	?	?	?	?	?	?
Total, all sources	2,180,300	3,399,200	1,179,700	6,896,500	10,837,600	11,602.900

fertilizer flushed from the soil zone? from manure? legumes? natural sources? Of an estimated 11.6 million metric tons of nitrogen added annually to the Mississippi and Atchafalaya basins, approximately 51 percent is from commercial fertilizer, 30 percent is from livestock manure, 9 percent is from fixation by legumes, 5 percent is from human domestic waste, and 4 percent is deposited by precipitation. Municipal and industrial point discharges of nitrogen to rivers are estimated to contribute only 2 and 1 percent, respectively, to the total annual loading of nitrogen in the Mississippi basin. Municipal and industrial point discharges of nitrogen are often directly to rivers, whereas the other potential nitrogen sources are applied or generated at the land surface. Municipal and industrial point discharges of nitrogen could be the source of as much as 37 percent of the nitrate discharged to the Gulf of Mexico.

The contribution from nitrification of soil nitrogen to surface water or ground-water nitrate is difficult to estimate. Although the soil is a large reservoir of N, the amount lost or gained by this reservoir on an annual basis is unknown. Contributions of soil organic nitrogen to surface

water or ground-water nitrate are difficult to quantify because they are highly variable and dependent on climatic and cultural factors (Cheng and others, 1964; Bremner and Tabatabai, 1973: Edwards, 1973; Heaton, 1986; Hubner, 1986). Results of laboratory analysis of soil samples are often not representative of field conditions (Letolle, 1980). Several researchers have argued that mineralization and nitrification of soil organic nitrogen is the predominant source of nitrate to surface waters (Bremner and Tabatabai, 1973; Edwards, 1973; Heaton, 1986). Nitrogen in mineral soils can be in the inorganic form, but generally most is organic nitrogen in the form of plant debris that must be nitrified before it can be utilized by plants (Buckman and Brady, 1970). If nitrification of soil organic nitrogen was a significant source of nitrate to surface waters, one would expect that substantial concentrations of nitrate would be observed in some surface waters draining undeveloped land. However, elevated nitrate concentrations in water associated with natural organic nitrogen sources in undisturbed environments are documented in only a few exotic settings such as deserts and limestone caves populated by large numbers of bats (Hem, 1985). Mueller and others (1995) reported that concentrations of nitrate in surface waters of the United States draining undeveloped land exceeded 1 mg/L in less than 10 percent of 3,751 samples, whereas nitrate in surface waters draining agricultural and urban land exceeded 1 mg/L in about 50 percent of 7,656 samples. Smith and others (1993) reported that less than 10 percent of 171 sites that drain forest and range land had average nitrate concentrations that exceeded 1 mg/L, whereas average nitrate concentrations at sites that drain agricultural and urban land exceeded 1 mg/L at 35 percent of 112 agricultural and urban sites. Beisecker and Leifeste (1975) observed that nitrate concentrations in samples collected at "hydrologic benchmark stations" had median nitrate concentrations substantially below those in samples from major streams in the same general region. Benchmark stations were selected to represent conditions uninfluenced by human activity. Together, these studies indicate that natural soil organic nitrogen is not the source of elevated nitrate concentrations in rivers of the United States, and that inputs of nitrogen from agricultural and urban sources result in leaching or runoff of excess as nitrate in many watersheds. The bulk of plants grown in agricultural areas use commercial fertilizers or animal manure as a supplement to the nitrogen present in the soil, so in agricultural areas mineralization and nitrification of soil organic nitrogen from plant debris may contribute more significantly to nitrate concentrations in surface waters.

If the soil nitrogen reservoir was in a natural steady-state condition (that is, prior to any agricultural impacts), then the contribution of soil nitrogen to surface and ground water would equal the amount of nitrogen added by precipitation plus the amount fixed by native plants minus that utilized by native plants. The concentration of nitrogen in pre-industrial age precipitation is unknown, but is almost certainly less than the amount in present-day precipitation (Lynch and others, 1996). The amount of nitrogen entering tallgrass prairie streams has been estimated by Tate (1989) and Dodds and others (1996). Tate (1989) observed that nitrogen concentrations in streams increased during storm events, but that overall mean total nitrogen concentrations were small and similar between growing (0.087 mg/L) and dormant seasons (0.082 mg/L). Dodds and others (1996) estimated that the total annual export of nitrogen from a tallgrass prairie watershed via streams ranged from 0.01 to 6 percent of the nitrogen input from precipitation. Ten percent of current nitrogen inputs from precipitation in the Mississippi basin would represent a mass of nitrate equal to about 5 percent of the current flux to the Gulf of Mexico.

Previous Investigations

Because different sources of nitrogen may not contribute to nitrate flux in the Mississippi River and its tributaries in proportion to their inputs, a technique is required that can provide

estimates of the relative contributions of the major sources of nitrate. One approach for estimating the contributions of various sources of nitrate in ground water utilizes the stable isotopic ratios $\delta^{15}N$ and/or $\delta^{18}O$ of the nitrate ion (Amberger and Schmidt, 1987; Aravena and others, 1993; Blevins and others, 1996; Bottcher and others, 1990; Durka and others, 1994; Heaton, 1986; Komor and Anderson, 1993; Kreitler, 1975; and Wassenaar, 1995). Although there are some problems associated with denitrification causing enrichment of ^{15}N in the remaining nitrate, thus obscuring the source, Kendall and others (1995b) showed that the relative contribution of two sources of nitrate can be identified using a combination of $\delta^{18}O$ and $\delta^{15}N$ data, even if the extent and timing of denitrification of nitrate from the two sources is not equal.

Few studies have attempted to identify sources of nitrate in surface water using stable isotopic ratios. Kohl and others (1971) used $\delta^{15}N$ ratios in a reduced soil sample and in raw fertilizer as end members and compared the values to the $\delta^{15}N$ in water samples collected from the Sangamon River near Lake Decatur, Ill. A simple mixing model suggested that the fertilizer contribution to nitrate in the river varied seasonally and peaked at 55% during the spring months. Their work was criticized for a lack of detail in the determination of the $\delta^{15}N$ value for soil N, failure to account for transformation of fertilizer nitrogen in the soil zone, failure to account for variability in the $\delta^{15}N$ of different fertilizers, and for failure to account for other sources of nitrogen such as precipitation (Edwards, 1973; Hauck and others, 1972, Freyer and Aly, 1974; Bremner and Tabatabai, 1973). Some of these criticisms appear justified, but statements such as "...there is no question that human and animal wastes and soil organic nitrogen all outweigh inorganic fertilizers as contributors to the general nitrate load of surface waters, even in agricultural counties..." (Edwards, 1973) are not substantiated.

Showers and others (1990) used the $\delta^{15}N$ ratio of nitrate in the Neuse River, N.C., to determine that the relative contributions from point and nonpoint sources varied by season and discharge rate and that the isotopic composition of nitrate was exponentially related to river discharge. They concluded that the mixing of point and non-point source nitrogen reservoirs was not entirely controlled by surface-water runoff of agricultural fertilizer and excess soil nitrate, but that non-point source nitrate passed through a reservoir (either ground water or wetlands) that modulated the mixing. Cravotta (1995) attempted to use the stable isotopes of carbon, nitrogen, and sulfur to identify sources of nitrogen in the Susquehanna River and found that variations in source isotopic compositions and transformation and fractionation during natural cycling of nitrogen prevented the accurate estimation of relative contributions of multiple nitrogen sources to nitrogen loads in streams. Kendall and others (1995b) used $\delta^{18}O$ and $\delta^{15}N$ to determine sources of nitrate in snowmelt runoff from three watersheds in the USA. They determined that most of the nitrate in early runoff was derived from the soil, and not from atmospheric nitrate released from the current year's snowpack. Kellman and Hillaire-Marcel (1996) used $\delta^{15}N$ to determine the importance of in-stream denitrification on the N-budget of a small watershed. They found that denitrification could be identified and was significant during dry conditions in late summer, but that on a yearly basis, in-stream denitrification did not significantly affect the N-budget. Ging and others (1996) used $\delta^{15}N$ and $\delta^{18}O$ of nitrate to determine sources of nitrate in two small streams in Austin, Tex. They concluded that the most likely sources were atmospheric nitrate, soil nitrate, and ammonium fertilizer.

Reliable identification of nitrogen source contributions in both two- and three-component mixing models require that the isotopic composition of the sources are stable and distinctive from one another. Various researchers (Aravena and others, 1993; Brandes and others,

1996; Heaton, 1986; Hubner, 1986; Kendall and others, 1995a; 1995b; and Letolle, 1980) have identified ranges for isotopic compositions of potential sources of nitrate (table 2; fig. 3). Nitrogen isotope values ($\delta^{15}N$) are reported in per mil (‰) relative to the standard air (atmospheric nitrogen isotopic ratio) defined as 0 ‰; oxygen isotope values ($\delta^{18}O$) are reported relative to the standard V-SMOW (Vienna Standard Mean Ocean Water) defined as 0 ‰. The $\delta^{18}O$ and $\delta^{15}N$ of nitrate from samples collected in several previous investigations are plotted on figure 3. Values from surface water and precipitation samples are shown on figure 3a, and values from groundwater samples are shown on figure 3b. The ranges of isotopic ratios for the sources of nitrogen in table 2 and figure 3 show some overlap that might prevent accurate modeling of nitrogen sources.

Effects of Nitrogen Transformations

Transformations of nitrogen that result in fractionation of the stable isotopes will confound efforts to utilize $\delta^{18}O$ and $\delta^{15}N$ to determine the relative contributions from major nitrogen sources to nitrate in streams. Transformation of nitrogen can take place in the soil zone, unsaturated zone, saturated zone, or within the stream. In-stream biological transformations of nitrogen can significantly affect the $\delta^{15}N$ and $\delta^{18}O$ of the residual nitrate. Nitrification of dissolved ammonium in the stream probably poses the most serious potential problem because this process may fractionate the ammonium, resulting in heavier $\delta^{15}N$ values of the residual ammonium, and the newly formed nitrate will probably be lighter in ^{15}N and depleted in ^{18}O relative to nitrate from the same source. However, if little dissolved ammonium reaches the stream relative to the amounts of nitrate, this process will not be important. Denitrification is unlikely to be an important process in well oxygenated streams, but when significant, it will leave a "fingerprint" of higher $\delta^{15}N$ and $\delta^{18}O$ values in the residual nitrate. On figure 3b, samples with $\delta^{18}O$ greater than ~10 and $\delta^{15}N$ greater than ~20 were likely to have been affected by denitrification (Bottcher and others, 1990; Wassenaar, 1995). Uptake by organisms probably has minimal effect on isotopic composition.

Table 2. Ranges of isotopic compositions of potential sources of nitrate.

Source of nitrate*	$\delta^{18}O$ of nitrate	$\delta^{15}N$ of nitrate
Atmospheric NO_3^-	23 to 75	-10 to 9
Nitrate fertilizer	18 to 24	-5 to 5
Ammonium fertilizer	-5 to 7	-5 to 0
Animal waste	-5 to 7	10 to 20
Poultry manure	-5 to 7	7.9 to 8.6
Legumes and other nitrogen fixers	-5 to 7	0 to 2
Nitrification in soils	-5 to 7	0 to 17
Non-contaminated soil	-5 to 7	2 to 5

*Data from Amberger and Schmidt (1987), Aravena and others (1993), Heaton (1986), Kendall and others (1995b), Kendall, C., USGS, written commun., 1997, and Wassenaar (1995).

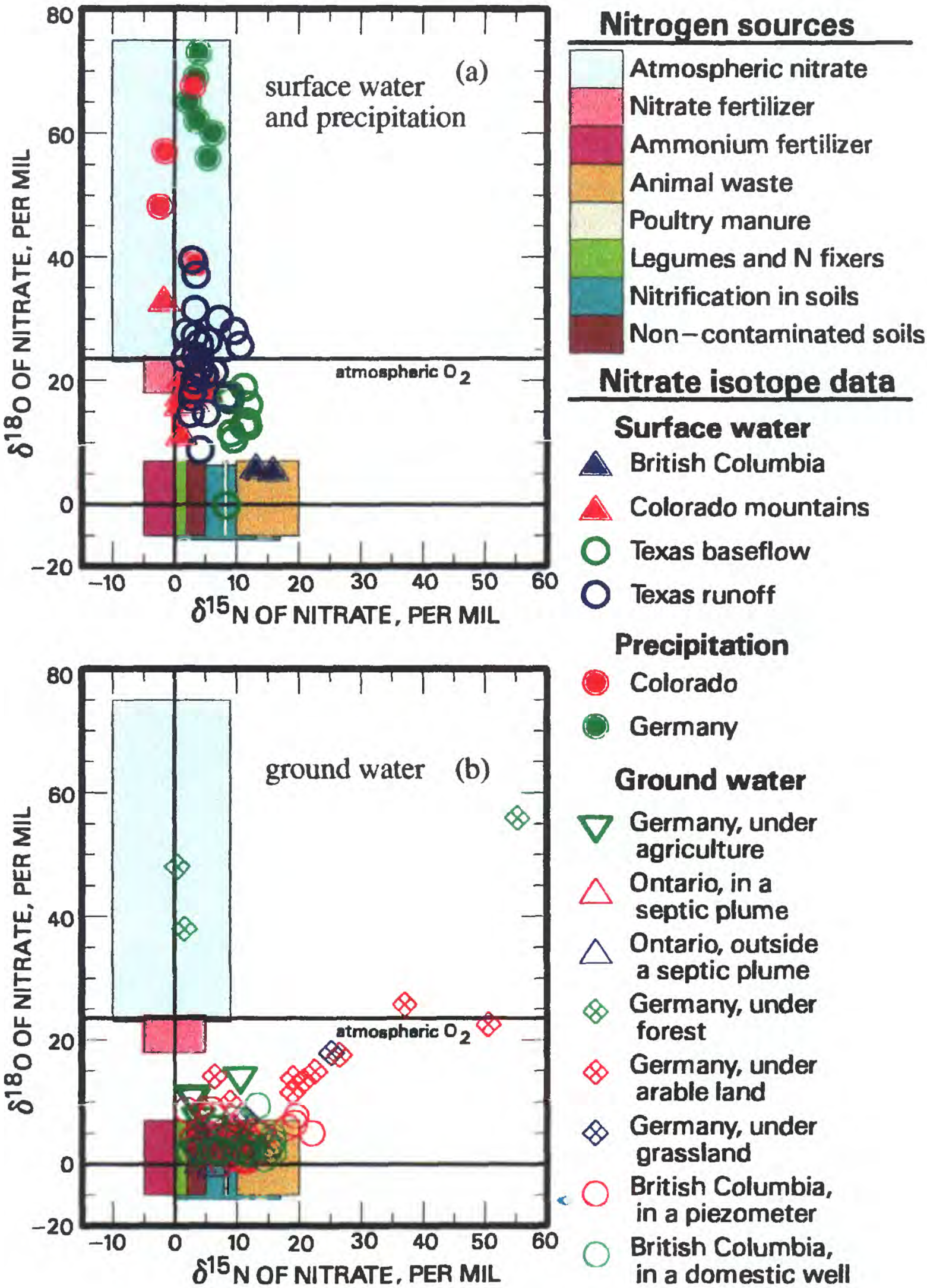

Figure 3. Expected ranges of $\delta^{15}N$ and $\delta^{18}O$ for various sources of nitrogen and values in nitrate samples from recent investigations of (a) surface water and precipitation, and (b) ground water.

The extent to which in-stream processes such as nitrification and denitrification affect the $\delta^{18}O$ and $\delta^{15}N$ of nitrate in large rivers like the Mississippi has not been determined. Analysis of data collecting in previous investigations suggests that there is not a significant loss in NO_3 in river water as it flows down stream. Results from six Mississippi River cruises that employed a Lagrangian sampling strategy showed little change in nitrate concentrations in the Mississippi River from its confluence with the Ohio River to New Orleans (Moody, 1993; Brinton et al., 1995; Garbarino et al., 1995). The transport (flux) of nitrate continued to increase down river. These results suggest that denitrification or other nitrogen transformations do not significantly alter the mass of nitrate as it is transported through the lower Mississippi River.

One way to assess whether the isotopic composition of nitrate has been affected by in-stream transformations is to analyze the isotopic composition of the suspended organic matter. The $\delta^{15}N$ values of organic matter from sewage, fertilizer, and soils show about the same values as the nitrate from these sources. However, since the particulate and dissolved organic matter that washes off the landscape is less biologically labile than the nitrate, the isotopic compositions of this material is less likely to be affected by the above processes. Hence, the $\delta^{15}N$ values of the organic matter can provide additional data about nitrate sources. The organic matter associated with different land uses can have very distinct isotopic compositions. Analysis of the carbon, nitrogen, and sulfur isotopic compositions of the organic matter may provide a more precise characterization of the land use where the material originated than can be obtained using the $\delta^{18}O$ and $\delta^{15}N$ of dissolved nitrate. For example, organic matter associated with corn fields has a $\delta^{13}C$ different from organic matter from wheat fields, legumes have a lower $\delta^{15}N$ value than other crops, and rice fields probably have lower $\delta^{34}S$ values than crops grown under drier conditions.

In the proposed study, both water samples and suspended organic matter will be analyzed for stable isotopes. Water samples will be analyzed for $\delta^{18}O$ and $\delta^{15}N$ in the nitrate ion. Suspended sediment samples will be analyzed for $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ in the organic matter. Information on the stable isotopic composition of the organic matter will allow an independent assessment of the extent of biological transformations, and will provide additional information on the sources of the suspended organic loads that are transported to the Gulf along with nitrate. These data will also provide an important link between the USGS study of nutrient sources in the Mississippi River Basin and the study proposed by Dr. Cifuentes at Texas A&M, for identifying sources of nutrients responsible for oxygen consumption in the Gulf itself.

Purpose and Scope

The principal goal of this study is to determine if stable isotopes can be used to determine the dominant sources of nitrate discharged to the Gulf of Mexico. Currently, little is known about the isotopic composition of nitrate or suspended organic matter in surface waters in the Mississippi Basin. Thus, the first step in accomplishing this goal is to characterize the isotopic composition of nitrate and suspended organic matter in streamflow in the Mississippi River and its major tributaries, in discharge to the Gulf of Mexico, and in streamflow from smaller drainages that have land uses representing distinct sources of nitrate (row crops, animal wastes, urban areas) or are minimally impacted by man (undeveloped areas). To accomplish this objective, samples will be collected in the winter, spring, and summer of 1996-97. Until these data have been collected and examined, it will not be known whether stable isotopes can help determine nitrate sources. If the characterization study is successful, a more detailed study will be proposed. Specific hypotheses to be tested are:

1. There are significant temporal and spatial variations in observed $\delta^{15}N$ and $\delta^{18}O$ of nitrate in water and the $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ of suspended

organic matter from the Mississippi River and its major tributaries.

2. Small streams draining areas of distinctly different land use (corn and soybean production, livestock production, urban land, or undeveloped land) will have distinctly different isotopic ratios.

3. The $\delta^{15}N$ and $\delta^{18}O$ ratios of nitrate in water and the $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ ratios of suspended organic matter from the Mississippi River can be used to determine the principal sources of the nitrate entering the Gulf of Mexico.

Plan of Study

In the first phase of this project, samples will be collected and processed from 7 sites representing large tributaries to the Mississippi River system and from 17 sites representing smaller watersheds within the Mississippi River basin. Whenever possible, samples will be collected in conjunction with NASQAN II and NAWQA sampling activities, both to reduce the cost of sample collection and to insure availability of other water-quality data (nitrate concentration, major ion, chemistry, field parameters). Water samples will be extracted and analyzed for $\delta^{15}N$ and $\delta^{18}O$ of nitrate at a USGS National Research Program lab in Menlo Park, CA. The methods of sample collection and processing as described by D. Wilkison (USGS, written commun., 1996) will be followed.

Sampling Sites

Samples from the Mississippi River and its major tributaries will be collected at five NASQAN II sites, one NAWQA integrator site, and one former NASQAN site. These samples will represent water containing nitrate from several sources. The information collected at these sites will be used to define the range of isotopic ratios expected in large rivers and will provide necessary background data to determine if there is temporal and spatial variability in isotopic ratios at these sites. The sites to be sampled and the purpose of sample collection are listed in table 3. Site locations and associated drainage basins are shown in figure 4.

Table 3. Sampling sites on the Mississippi River and major tributaries, drainage basin area, and purpose for sample collection.

Sampling site	Site number	Drainage area in square kilometers	Purpose of sample collection
Mississippi River at Belle Chasse, La.	07374525	2,926,500	Identify $\delta^{15}N$ and $\delta^{18}O$ for water entering the Gulf of Mexico; investigate isotopic changes in nitrate in Baton Rouge to New Orleans reach
Mississippi River at St. Francisville, La.	07373420	2,914,500	Identify $\delta^{15}N$ and $\delta^{18}O$ for water in the lower Mississippi River above Baton Rouge, La.
Mississippi River at Thebes, Ill.	07022000	1,847,200	Identify $\delta^{15}N$ and $\delta^{18}O$ for water entering the Mississippi River system between Clinton, Iowa and the confluence with the Ohio River
Missouri River at Hermann, Mo.	06934500	1,357,700	Identify $\delta^{15}N$ and $\delta^{18}O$ for water leaving the Missouri River system
Ohio River at Grand Chain, Ill.	03612500	526,000	Identify $\delta^{15}N$ and $\delta^{18}O$ for water leaving the Ohio River system
Mississippi River at Clinton, Iowa	05420500	221,700	Identify $\delta^{15}N$ and $\delta^{18}O$ for water leaving the Upper Mississippi River system
Yazoo River below Steele Bayou near Long Lake, Miss.	07288955	34,590	Identify $\delta^{15}N$ and $\delta^{18}O$ for water leaving the Yazoo River system

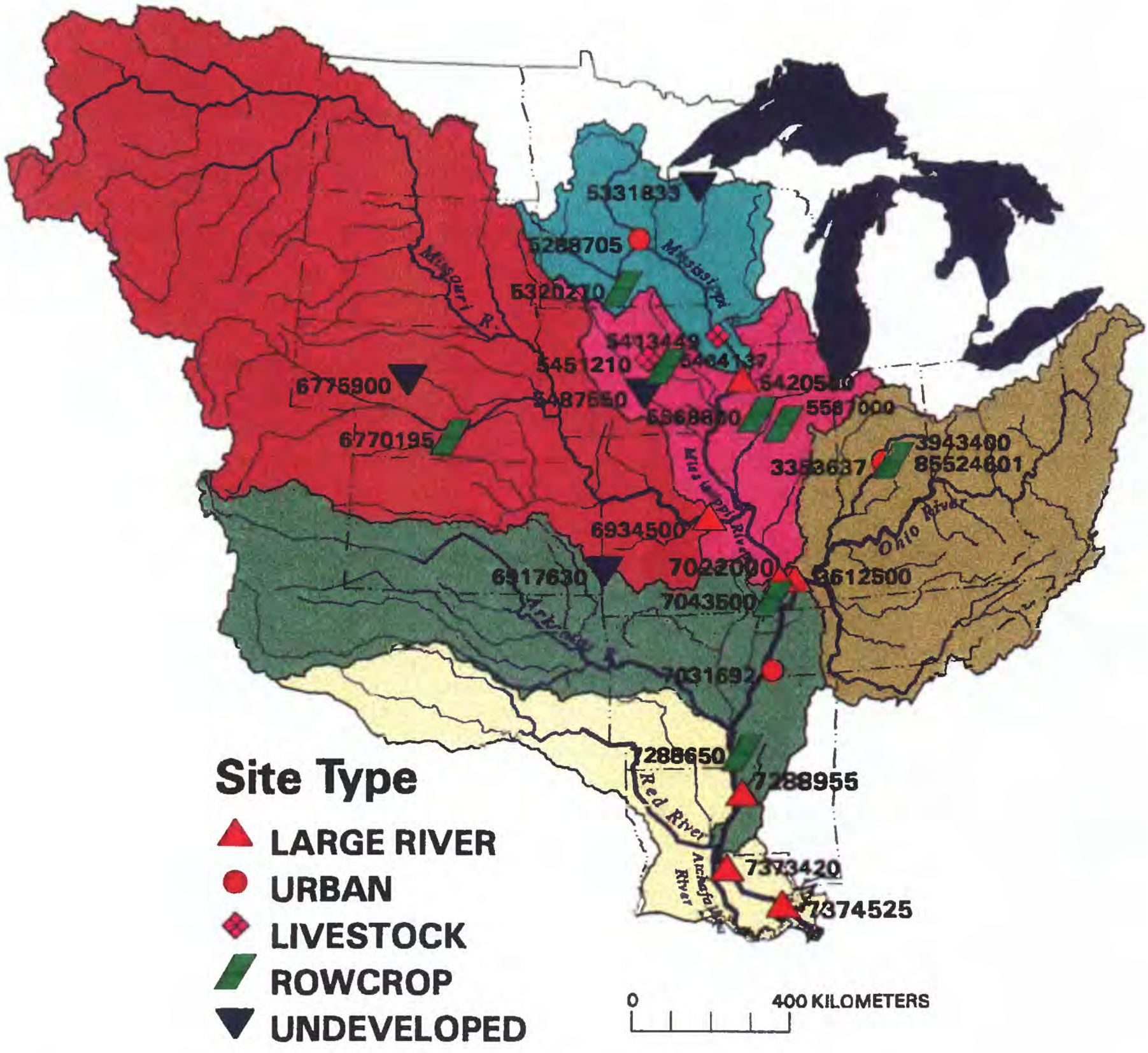

Figure 4. Study area and location of sampling sites.

Samples will also be collected from smaller basins most of which are within NAWQA study units in the Mississippi River basin. These sites were selected to distinctly represent one of four land-use classes: land in rowcrop production; land in hog, cattle, or poultry (livestock) production; urban land; or undeveloped land. Isotopic ratios from samples collected at these sites will define the ranges of isotopic ratios expected in smaller rivers dominated by a single source of nitrate. Sites that represent each of the four land-use classes will be distributed among the Ohio, Missouri, and Mississippi River basins. Sites to be sampled are listed in table 4 and shown in figure 4.

Sample Collection and Processing

Six samples will be collected at each site during the winter, spring, and summer of 1996-97. Results from these samples will be used to identify seasonal and flow related variability in $\delta^{15}N$ and $\delta^{18}O$ ratios in nitrate and $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ ratios in suspended organic matter from each site. The first set of samples will be collected in December 1996 and January 1997, when base flow conditions are generally prevalent in streams and rivers. A second set of samples will be collected in February and March 1997, prior to application of fertilizers to fields. A third set of samples will be collected during a significant runoff event in April/May 1997, after 50 percent or more of the crops in the basin have been planted. A forth and fifth set of samples will be collected during significant runoff events in May/June and June/July 1997, respectively. Finally, a sixth set of samples will be collected in September/October 1997, after harvest, when rivers are again at base flow conditions.

Dissolved Nitrate: Samples will be collected with a depth integrating sampler (where conditions are appropriate for this collection method) from three or more verticals using NASQAN/NAWQA protocols (Shelton, 1994). Samples from the vertical profiles will be composited in a glass, polyethylene, or Teflon container. All sampling equipment will be cleaned with non-phosphate detergent, rinsed thoroughly with tap water, and then rinsed with distilled/deionized water. Samples will be filtered through a 0.45-micron cartridge filter into 1-liter or 1-gallon pre-cleaned polyethylene bottles, chilled without preservative, and sent on ice to the USGS laboratory in Missouri. If the filters are clogging, samples can be pre-filtered using a glass-fiber filter. All sample bottles will be labeled "ISO", and should include the site name, site id, date, and time of sample collection.

About 200 µmol of nitrogen as N_2 (about 5 mg of nitrogen) are required for the isotopic analysis. Most of this is needed for determining the $\delta^{18}O$ of nitrate. Table 5 indicates the volume of sample to be collected and shipped to the Missouri laboratory for various expected concentrations of nitrate. This table should be used to determine sample volumes required for the sites listed in table 4, whereas table 6 indicates the volume of sample to be collected at the sites listed in table 3, by sampling set. Sample collection will not be concurrent with NAWQA or NASQAN activities at a few sites; therefore, other water-quality data will not be available for these samples. Samples will be collected for nutrient analysis (schedule 2702, requires two 125-ml bottles, one filtered and one unfiltered), major ions (schedule 2701, requires one 250-ml filtered acidified bottle, one 250-ml raw untreated bottle, and one 500-ml filtered untreated bottle), and dissolved organic carbon (schedule 2085, requires one 100-ml bottle) at these sites. Sample bottles will be labeled "ISO_NUT", "ISO_ION, and "ISO_DOC", respectively, and include the site name, site id, date, and time of sample collection. Samples will be sent to the USGS National Water Quality Lab (NWQL) using the standard procedure.

Suspended Organic Material: Suspended sediment for isotopic analysis of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ of the suspended organic material will be collected at each site. Approximately 1 liter of water will be filtered through a 0.7 micrometer heat-cleaned glass-fiber filter (142 mm diameter) using a peristaltic pump and an aluminum plate

Table 4. Sampling sites on smaller rivers in the Mississippi Basin, subbasin, drainage basin area and purpose for sample collection

Sampling site name	Subbasin	Site number	Drainage area, square kilometers	Purpose of sample collection
S. Fork Iowa River NE of New Providence, Iowa	Mississippi at Thebes	05451210	596	high density of hog farms within an agricultural (corn/soybeans) basin
Fourmile Creek near Traer, Iowa	Mississippi at Thebes	05464137	50.5	intensive corn and soybean production in the basin
Walnut Creek near Vandalia, Iowa	Mississippi at Thebes	05487550	52.6	large area reverting back to prairie, undeveloped land
Panther Creek near El Paso, Ill.	Mississippi at Thebes	05567000	243	intensive row crop agriculture
Indian Creek near Wyoming, Ill.	Mississippi at Thebes	05568800	162	intensive row crop agriculture
Sugar Creek at Co Rd 400 S at New Palistine, Ind.	Ohio at Grand Chain	39434008 5524601	243	intensive corn and soybean production in the basin
Little Buck Creek near Indianapolis, Ind.	Ohio at Grand Chain	03353637	242	suburban Indianapolis ~70% urban area, many septic systems
North Dry Creek near Kearney, Nebr.	Missouri at Hermann	06770195	~100	intensive row crop and irrigated corn
Dismal River near Thedford, Nebr.	Missouri at Hermann	06775900	2,500, 80 contributing	undeveloped land mostly in the Sand Hills
East Fork Drywood Creek in Praire State Park, Mo.	Missouri at Hermann	06917630	?	basin largely undeveloped prairie land
Shingle Creek at Queen Ave. N., Minn.	Mississippi at Clinton	05288705	73	city of Brookland Park, urban area
Little Cobb River, Minn.	Mississippi at Clinton	05320270	337	intensive row crop agriculture
Namekagon River, Wis.	Mississippi at Clinton	05331833	311	basin largely undeveloped
Rattlesnake Creek near North Andover.Wis.	Mississippi at Clinton	05413449	110	high density of dairy and feedlot operations
Fletcher Creek, Tenn.	Mississippi at St. Francisville	07031692	~77	suburban Memphis, urban area
Bogue Phalia near Leland, Miss.	Mississippi at St. Francisville	07288650	1,254	intensive rice, corn, and soybean agriculture
Little River Ditch No. 1 near Morehouse, Mo.	Mississippi at St. Francisville	07043500	1,166	intensive row crop agriculture

Table 5. Volume of sample to be collected for various ranges in expected nitrate plus nitrite as nitrogen concentrations.

Concentration range, in milligrams per liter	Volume of sample, in liters
Less than 0.5	15
0.5 to 1.0	10
1.1 to 2.0	5
2.1 to 3.0	3
More than 3.0	2

Table 6. Volume of sample to be collected for sites listed in table 3, by sampling set

	Volume of sample, in liters					
Site number	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
07374525	-	-	5	5	5	5
07373420	10	5	5	5	10	10
07022000	5	3	3	3	5	5
06934500	10	5	5	5	15	15
03612500	10	10	10	10	15	15
05420500	5	5	5	5	10	10
07288955	15	15	12	12	12	12

filter. After filtration of the sample the glass fiber filter will be placed on a small sheet of clean aluminum foil using tweezers. The filter will be folded in half, and then into quarters using the tweezers, keeping the sediment on the inside. The filter will be wrapped in the aluminum foil, and labeled with the station name, ID number, sampling date, and time. The filter will be placed in a ziplock bag, chilled after collection, and frozen upon returning to the office. After several filters have been collected, they will be shipped to the USGS Regional Office in Denver, Colo. for cataloging, and then on to the National Research Program laboratory in Menlo Park, Calif. for isotopic analysis.

Quality Assurance: Quality assurance and quality control (QA/QC) samples will be collected at selected sites to provide information on the variability and bias of the measured isotopic ratios. These samples will consist exclusively of concurrent replicates, which are two or more samples that are collected as closely as possible in time and space, but processed, handled, and analyzed separately. Collection of concurrent replicate samples requires two separate passes at each vertical in the cross section, to be composited in separate vessels. Table 7 gives the schedule for collection of QA/QC samples. QA/QC samples should be labeled with sampling times that are later than the primary sample in order to distinguish them from each other.

Analytical Methods

Samples sent to the USGS laboratory in Missouri will be passed through anion-exchange

columns to extract the nitrate from water samples and ease the subsequent transport and processing of samples. The anion-exchange columns will be sent to the USGS laboratory in Menlo Park, Calif. for $\delta^{15}N$ and $\delta^{18}O$ analysis. Nitrate will be stripped from the columns, neutralized, filtered, and split into two aliquots for analysis of the two isotopes. All other O-bearing chemical species are removed before $\delta^{18}O$ analysis, and the nitrate is then combusted with graphite in sealed quartz tubes; the resulting CO_2 is purified and the $\delta^{18}O$ analyzed on a Finnigan 251 stable-isotope mass spectrometer. The nitrate is combusted in silver boats (with sugar) in a Carlo Erba elemental analyzer for $\delta^{15}N$ analysis, and the resulting N_2 is analyzed on an attached Micromass Optima continuous-flow stable-isotope mass spectrometer.

Data Analysis Methods and Products

Results of the isotopic analysis will be analyzed graphically and statistically. Plots similar to figure 3, showing the values of $\delta^{18}O$ and $\delta^{15}N$ of nitrate for the collected samples will be constructed. These plots may indicate how the isotopic ratios from samples collected in the Mississippi River differ from samples collected at the "end-member" sites, and from other known

Table 7. Schedule for collection of concurrent replicates at sampling sites (X, replicate to be collected)

Sampling site name	Sample set					
	1	2	3	4	5	6
Mississippi River at Thebes, Ill.		x				
Missouri River at Hermann, Mo.						
Ohio River at Grand Chain, Ill.			x			
Mississippi River at Clinton, Iowa						
Mississippi River at Belle Chasse, La.					x	
Yazoo River below Steele Bayou near Long Lake, Miss.					x	
S. Fork Iowa River NE of New Providence, Iowa	x					
Fourmile Creek near Traer, Iowa		x				
Walnut Creek near Vandalia, Iowa			x			
Sugar Creek at New Palistine, Ind.						
Little Buck Creek near Indianapolis, Ind.				x		
North Dry Creek near Kearney, Nebr.					x	
Dismal River near Thedford, Nebr.						
Shingle Creek at Queen Ave. N., Minn.						
Little Cobb River, Minn.						x
Namekagon River, Wis.						
East Fork Drywood Creek in Praire State Park, Mo.	x					
Rattlesnake Creek near North Andover.Wis.		x				
Panther Creek near El Paso, Ill.			x			
Indian Creek near Wyoming, Ill.				x		
Fletcher Creek, Tenn.						
Bogue Phalia near Leland, Miss.						x
Little River Ditch No. 1 near Morehouse, Mo.						

nitrate sources (for example, fertilizer). Statistical techniques will be utilized to determine if samples from the various subbasins differ significantly from each other. Mixing models will be applied to quantify relative contributions from various nitrate sources (Kendall et al., 1995b; Kohl, et al., 1971). Expected $\delta^{18}O$ and $\delta^{15}N$ ratios of nitrate will be calculated for the Mississippi River sites based upon the relative magnitude of nitrogen loading within the associated drainage basins. These expected values will be compared with the measured values to determine if the nitrate present in the Mississippi River and its tributaries is proportional to gross nitrogen loading within the associated drainage basins.

A Geographic Information System (GIS) will be used to manage, analyze, and display data on site locations, isotopic ratios, and nitrogen sources. Information on several nitrogen sources will be updated, with newly available data in an effort to improve the nitrogen loading estimates given in table 1.

The results of the analyses and evaluations of their interpretative value will be made available to the public. Results from the first phase of this project will determine if a second phase will be undertaken.

References

Amberger, A., and Schmidt, H. L., 1987, Naturliche isotopengehalte von nitrat als indikatoren fur dessen herkunft: Geochimica Cosmochimica Act., v. 51, p. 2699-2705.

Aravena, R., Evans, M. L., and Cherry, J. A., 1993, Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems: Ground Water, v. 31, no. 2, p. 180-186.

Battaglin, W.A., and Goolsby, D.A., 1995, Spatial data in geographic information system format on agricultural chemical use, land use, and cropping practices in the United States: U.S. Geological Survey Water-Resources Investigations Report 94-4176, 87 p.

Bremner, J.M., and Tabatabai, M.A., 1973, Nitrogen-15 enrichment of soils and soil-derived nitrate: Journal of Environmental Quality, v. 2, no. 3, p. 363-365.

Brinton, T.I., Garbarino, D.B., Peart, D.B., Taylor, H.E., and Antweiler, R.C., 1995. Concentration and transport data for dissolved inorganic constituents in water collected during seven cruises on the Mississippi River and some of its tributaries, July 1987- June 1990: U.S. Geological Survey Open-File Report 94-524, 102 p.

Biesecker, J.E. and Leifeste, D.K., 1975, Water quality of hydrologic bench marks--an indicator of water quality in natural environments: U.S. Geological Survey Circular 460-E, 21 p.

Blevins, D.W., Wilkison, D.H., Kelly, B.P., and Silva, S.R., 1996, Movement of nitrate fertilizer to glacial till and runoff from a claypan soil: Journal of Environmental Quality, v. 25, p. 584-593.

Board on Agriculture, National Research Council, 1993, Soil and Water Quality--An Agenda for Agriculture: Washington, D.C., National Academy Press, 516 p.

Bottcher, J., Strebel, O., Voerkelius, S., and Schmidt, H., 1990, Using isotope fractionation of nitrate - nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer: Journal of Hydrology, v. 114, p. 413-424.

Brandes, J.A., McClain, M.E., and Pimentel, T.P., 1996, ^{15}N evidence for the origin and cycling of inorganic nitrogen in a small Amazonian Catchment: Biogeochemistry, v. 34, p. 45-56.

Buckman, H.O., and Brady, N.C., 1970, The nature and properties of soils: London, The Macmillan Company, 653 p.

Cheng, H.H., Bremner, J.M., and Edwards, A.P., 1964, Variations in the nitrogen-15 abundance in soils: Science, v. 146, p. 1574-1575

Cravotta, C.A., 1995, Use of stable isotopes of carbon, nitrogen, and sulfur to identify sources of nitrogen in surface waters in the Lower Susquehanna River Basin, Pennsylvania: U.S. Geological Survey Open-File Report 94-510, 103 p.

Dodds, W. K., Blair, J. M., Henebry, G. M., Koelliker, J. K., Ramundo, R., and Tate, C. M., 1996, Nitrogen transport from tallgrass prairie watersheds: Journal of Environmental Quality, v. 25, no. 5, p. 973-981.

Dunn, D. D., 1996, Trends in nutrient inflows to the Gulf of Mexico from streams draining the conterminous United States, 1972-93: U.S. Geological Survey Water-Resources Investigations Report 96-4113, 60 p.

Durka, W., Schulze, E.D., Gebaure, G., and Voerkelius, S., 1994, Effects of forest decline on uptake and

leaching of deposited nitrate determined from ^{15}N and ^{18}O measurements: Nature, v. 372, p. 765-767.

Edwards, A.P., 1973, Isotopic tracer techniques for identification of sources of nitrate pollution: Journal of Environmental Quality, v. 2, no. 3, p. 382-386.

Freyer, H.D., and Aly, A.I., 1974, Nitrogen-15 variations in fertilizer nitrogen: Journal of Environmental Quality, v. 3, no. 4, p. 405-406.

Garbarino, J.R., Antweiler, R.C., Brinton, T.I., Roth, D.A., and Taylor, H.E., 1995. Concentration and transport data for selected dissolved inorganic constituents and dissolved organic carbon in water collected from the Mississippi River and some of its tributaries, July 1991- May 1992: U.S. Geological Survey Open-File Report 95-149, 149 p.

Gianessi, L.P., and Peskin, H.M., 1984, An overview of the RFF environmental data inventory: Methods, sources and preliminary results. v. 1: Washington, D.C., Renewable Resources Division, Resources for the Future; 111 p.

Ging, P.B., Lee, R.W., and Silva, S.R., 1996, Water chemistry of Shoal Creek and Waller Creek, Austin, Texas, and potential sources of nitrate: U.S. Geological Survey Water Resources Investigations Report 96-4196, 24 p.

Goolsby, D. A., and Battaglin, W. A., 1995, Effects of episodic events on the transport of nutrients to the Gulf of Mexico: *in* Proceedings of First Gulf of Mexico Hypoxia Management Conference, Dec. 5-6, Kenner, La., p. 8. On-line at: http://pelican.gmpo.gov/gulfweb/hypoxia/hypoxia.html#abstract15

Hauck, R.D., Bartholomew, W.V., Bremner, J.M., Broadbent, F.E., Cheng, H.H., Edwards, A.P., Keeney, D.R., Legg, J.O., Olsen, S.R., and Porter, L.K., 1972, Use of variations in natural nitrogen isotope abundance for environmental studies: A questionable approach: Science, v. 177, p. 453-454.

Heaton, T.H.E., 1986, Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: a review: Chemical Geology (Isotope Geoscience Section) v. 59, p. 87-102.

Hem, J.D., 1985, Study and interpretation of the chemical characteristics of natural water: U.S. Geological Survey Water-Supply Paper 2254, 264 p.

Hubner, H., 1986, Isotope effects of nitrogen in the soil and biosphere, *in* Fritz, P. and Fontes, J. eds., Handbook of Environmental Isotope Geochemistry, v. 2: Amsterdam, Elsevier, p. 361-425.

Justic, D., Rabalais, N. N., Turner, R. E., and Wiseman, W.J., 1993, Seasonal coupling between riverborne nutrients, net productivity and hypoxia: Marine Pollution Bulletin, v. 26, no. 4, p. 184-189.

Justic, D., Rabalais, N. N., and Turner, R. E., 1994, Riverborne nutrients, hypoxia and coastal ecosystem evolution: biological responses to long-term changes in nutrient loads carried by the Po and the Mississippi Rivers, *in* Dyer K.R. and Orth, R.J., eds., Changes in Fluxes in Estuaries: Implications from Science to Management, Proceedings of ECSA22/ERF Symposium, Sept. 13-18, 1992: Fredensborg, Denmark, Olsen & Olsen, Academic Publishers, p. 161-167.

Kellman, L., and Hillaire-Marcel, C., 1996, Moritoring in-stream denitrification using natural abundances of $^{15}N\text{-}NO^3$ in a small agricultural watershed (St. Lawrence Lowlands) [abs.]: EOS, Transactions of the American Geophysical Union, v. 77, no 46:F201.

Kendall, C., Sklash, M.G., and Bullen, T.D., 1995a, Isotope tracers of water and solute sources in catchments, *in* Trudgill, S.T. ed., Solute modelling in catchment systems: West Sussex, England, John Wiley, p. 261-303.

Kendall, C., Campbell, D.H., Burns, D.A., Shanley, J.B., Silva, S.R., and Chang, C.Y., 1995b, Tracing sources of nitrate in snowmelt runoff using the oxygen and nitrogen isotopic compositions of nitrate, *in* Biogeochemistry of seasonally snow-covered catchments: International Association of Hydrological Sciences Publication no. 228, p. 339-347.

Kohl, D.H., Shearer, G.B., and Commoner, B., 1971, Fertilizer nitrogen: Contribution to nitrate in surface water in a corn belt watershed: Science, v. 174, p. 1331-1334.

Komor, S. C. and Anderson, H. W., 1993, Nitrogen isotopes as indicators of nitrate sources in Minnesota sand-plain aquifers: Ground Water, v. 31, no. 2, p. 260-270.

Kreitler, C.W., 1975, Determining the sources of nitrate in ground water by nitrogen isotope studies: Austin, University of Texas, Bureau of Economic Geology, Report of Investigations No. 83, 57 p.

Letolle, R., 1980, Nitrogen-15 in the natural environment, *in* Fritz, P., and Fontes, J., eds., Handbook of environmental isotope geochemistry, v. 1: Amsterdam, Elsevier, p. 407-434.

Lynch, J.A., Bowersox, V.C., and Grimm, J.W., 1996, Trends in precipitation chemistry in the United States, 1983-94: An analysis of the effects in 1995

of Phase I of the Clean Air Act Amendments of 1990, Title IV: U.S. Geological Survey Open-File Report 96-346, 100 p.

Moody, J.A., 1993. Evaluation of the Lagrangian scheme for sampling the Mississippi River during 1987-90: U.S. Geological Survey Water-Resources Investigations Report 93-4042, 31 p.

Mueller, D.K., Hamilton, P.A., Helsel, D.R., Hitt, K.J., and Ruddy, B.C., 1995, Nutrients in ground water and surface water of the United States--An analysis of data through 1992: U.S. Geological Survey Water-Resources Investigations Report 95-4031, 74 p.

Rabalais, N. N., Turner, R. E., and Wiseman, W. J., 1995. Hypoxia in the Northern Gulf of Mexico: Past, Present and Future: *in* Proceedings of First Gulf of Mexico Hypoxia Management Conference, Dec. 5-6, Kenner, LA. On-line at: http://pelican.gmpo.gov/gulfweb/hypoxia/hypoxia.html#abstract4.

Rabalais, N. N., Turner, R. E., Justic, D., Dortch, Q., Wiseman, W. J., and Gupta, B. K. S., 1996, Nutrient changes in the Mississippi River and System Responses on the Adjacent Continental Shelf: Estuaries, v. 19, no. 2b, p. 386-407.

Shelton, L. R., 1994, Field guide for collecting and processing stream-water samples for the National Water-Quality Assessment program: U.S. Geological Survey Open-File Report 94-539, 57 p.

Showers, W.J., Eisenstein, D.M., Paerl, H., and Rudek, J., 1990, Stable isotope tracers of nitrogen sources to the Neuse River, North Carolina: Raleigh, North Carolina Water Resources Research Institute, Completion Report No. 253, 28 p.

Smith, R.A., Alexander, R.B., and Lanfear, K.J., 1993, Stream water quality in the conterminous United States--Status and trends of selected indicators during the 1980's, *in* Paulson, R.W., Chase, E.B., Williams, J.S., and Moody, D.W., comps., National Water Summary 1990-91--Hydrologic Events and Stream Water Quality: U.S. Geological Survey Water-Supply Paper 2400, 590 p.

Tate, C.M., 1989, Patterns and controls of nitrogen in tallgrass prairie streams: Ecology, v. 71, no. 5, p. 2007-2018.

Turner, R. E., and Rabalais, N. N., 1991, Changes in Mississippi River water quality this century: Implications for coastal food webs: BioScience, v. 41, p. 140-147.

U.S. Department of Commerce, 1989, Census of agriculture, 1987--Final county file: U.S. Department of Commerce, Bureau of the Census [machine-readable data file].

_____, 1990, Census of population and housing, 1990: U.S. Department of Commerce, Bureau of the Census, Data Users Service Division, Washington, D.C.

U.S. Environmental Protection Agency, 1990, County-level fertilizer sales data: U.S. Environmental Protection Agency, Office of Policy, Planning, and Evaluation, PM-221.

Wassenaar, L.I., 1995, Evaluation of the origin and fate of nitrate in the Abbotsford aquifer using the isotopes of ^{15}N and ^{18}O in NO_3: Applied Geochemistry, v. 10, p. 391-405.