



TRANSPORT OF NITRATE IN THE MISSISSIPPI RIVER IN JULY-AUGUST 1999

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ABSTRACT

Lagrangian sampling was conducted on the Mississippi River in late July through early August 1999 to test the hypothesis that nitrate (NO_3^-) is transported conservatively in the Mississippi River. Three different approaches were pursued to test the hypothesis: (1) a mass balance for NO_3^- was evaluated for evidence of net gains and losses, (2) stable isotopes of NO_3^- were measured ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) to determine if fractionation occurred, and (3) the concentrations of dissolved gases (N_2O , N_2 and Ar) in river water were measured and compared to theoretical equilibrium concentrations. Integrated water samples and flow measurements were obtained at 10 sites on the Mississippi River and 7 sites near the mouths of major tributaries from northern Iowa to southern Louisiana, a distance of about 2,250 river kilometers. Mass balance calculations indicate that more than 80 percent of the NO_3^- mass discharged from the Mississippi River (1,930 metric tons/day) during the study period originated in the first 500 river kilometers of the study reach. The mass balance calculations also indicate that NO_3^- was not lost from the water column upstream of Vicksburg, MS, but that there might have

been some loss of NO_3^- in the lower 700 kilometers of the study reach. The stable isotope ratios of N and O ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) of NO_3^- were consistent with mixing and transport in the absence of fractionating gains or losses. The concentrations of nitrogen (N_2) and argon (Ar) dissolved in river water decreased in the downstream direction, approximately in equilibrium with air at increasing temperatures, giving no evidence of gains or losses of N_2 by nitrogen fixation or denitrification. Nitrous oxide (N_2O) concentrations in the Mississippi River were approximately 26 to 200 percent of air saturation, indicating relatively low net production by combination of nitrification and denitrification. Results from this study indicate that most (>90%) of the NO_3^- that entered the Mississippi River during July-August 1999 was transported to the Gulf of Mexico.

Keywords: Mississippi River, denitrification, nitrate, Lagrangian sampling

1. INTRODUCTION

The Mississippi River Basin drains an area of about 3.2 million km^2 in the central United States, about 41 percent of the conterminous States. The Mississippi River Basin is the largest river basin in North America and the third largest river basin in the world, smaller only than the Amazon Basin in South America and the Congo River Basin in Africa. More than 72 million people reside in the Mississippi River Basin, and much of the nation's corn, soybeans, wheat, cattle, and hogs, as well as a substantial amount of the cotton and rice, are grown there.

Large inputs of nitrogen (N) from agriculture, atmospheric deposition, and point sources occur annually in the Basin, especially in Minnesota, Iowa, Illinois, Indiana, and Ohio. A substantial portion of these N inputs eventually discharges to streams, the Mississippi River, and ultimately to the Gulf of Mexico where they are believed to contribute to increases in eutrophication and an expanding hypoxic zone [1-3]. Additionally, there is concern over the high NO_3^- concentrations in the Mississippi River as plans evolve for river diversions to restore Louisiana's coastal wetlands [4].

The average annual flux of nitrate (NO_3^-) from the Mississippi River Basin to the Gulf of Mexico is estimated to be about 900,000 metric tons/year (t/y) [5] for 1979 through 2005. A slightly higher load of 1 million metric t/y was estimated for the period 1980 through 1996 [1]. More than half of the NO_3^- comes from the upper part of the Basin where the N inputs and yields of basins are highest. Source areas were

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determined using a mass balance method and calculating the annual flux from fixed sampling points, subtracting the upstream flux from the downstream flux and assigning the difference as input from the intervening drainage between the two fixed stations [5]. In this way, those areas contributing large amounts of NO_3^- can be identified, and possible mitigation procedures implemented. Other researchers have used spatially referenced regression models such as SPARROW to determine sources areas of nitrate [6].

There are a number of processes that may remove NO_3^- from streams include assimilation and denitrification. Denitrification is a biological process whereby NO_3^- is reduced to nitrogen (N_2) and nitrous oxide (N_2O) and is important because it removes fixed N from the system; whereas other processes that affect NO_3^- , such as assimilation, burial, and reduction to ammonium (NH_4^+), leave N within the potentially reactive N reservoir. These processes, plus nitrification, can cause errors in estimates of source contributions unless accounted for or measured.

There are a number of initiatives in progress with the goal of reducing NO_3^- flux from the Mississippi River [6-7]. To optimize resources, more effort is being applied to important NO_3^- source areas in the Mississippi River Basin. If denitrification is an important NO_3^- removal process in the Mississippi River and is unaccounted for in the determination of the source areas for NO_3^- , then this could lead to a misunderstanding of the system and how to ameliorate the loads of NO_3^- reaching the Gulf of Mexico.

To address this issue, a study was conducted by the U.S. Geological Survey (USGS) in July-August 1999 to determine if significant amounts of NO_3^- were removed in a 2,250-km reach of the Mississippi River.

Changes in the concentration of NO_3^- along a stream reach depend on many factors including input from tributaries or from groundwater discharge with concentrations of NO_3^- either higher or lower than that of the stream, assimilation by plants, production by nitrifying bacteria, and denitrification by denitrifying bacteria in stream sediments. Ascribing gains and losses of NO_3^- to each mechanism is difficult.

Substantial amounts of NO_3^- can be removed by instream processes such as denitrification in small streams [6,10-14], before water reaches major rivers such as the Mississippi River. Studies in the mid-continent corn belt indicate that denitrification is an important process in small streams, but its effect on NO_3^- loads are most likely to be detected during low flow conditions when NO_3^- concentrations are relatively low [14-17]. Up to 50 % NO_3^- loss was measured in a 600-m reach of Prescott Creek in the St. Lawrence lowlands of Quebec, Canada [9], and in another study

some 50 % of the NO_3^- present in the South Platte River was denitrified in a 100-km reach [15]. In a New Zealand headwater stream it was concluded that although denitrification played a small role in the loss of NO_3^- during transport, most of the loss was the result of plant uptake [16]. These and other studies have indicated denitrification and assimilation can remove substantial amounts of NO_3^- from small streams [13,20]; however, little is known about the magnitude and relative importance of instream processes in removing NO_3^- from large rivers such as the Mississippi.

Several studies indicate fractional losses of NO_3^- by denitrification are relatively unimportant in large rivers [10,11,21]. Using a modeling approach, it was demonstrated that the percent of N removed per unit time in a stream reach is inversely related to stream size [22]. It has been suggested that denitrification effects on surface-water NO_3^- loads were limited by the contact time between stream water and sediments, with less fractional loss occurring under high flow conditions [9]; it was demonstrated that denitrification can depend on discharge, travel time, and depth of water [18]. A Lagrangian study that followed a parcel of water from near Baton Rouge, LA, to the Head of Passes, LA, (about 362 km) noted that there was no measureable loss of NO_3^- during the 4-day transit [23].

Other studies have indicated that denitrification in large order rivers is an important NO_3^- sink. An estimated 17% of the annual N load was lost due to denitrification in the Neuse River in North Carolina [24]. A modeling approach indicated that under some scenarios, the proportional removal of upstream input by large streams is several-fold greater than for small streams [25]. In a navigation pool of the Upper Mississippi River, an area with 10,425 ha of wetted area under normal summer flows, denitrification rates of 6.9% of the total annual flux were measured [26]. The same investigators also determined a nitrification rate for Pool 8 of 7% of the annual NO_3^- budget [27]. A denitrification study was conducted on the Swale-Ouse River system in Northeast England (145 km from headwaters to tidal limit) over a 17-month period; the results showed an increase in the denitrification rate moving downstream that was related to an increase in NO_3^- concentration and changes in sediment composition and seasonality related to temperature and NO_3^- concentration [28]. The general increase in denitrification rates in a downstream direction was also noted in a larger study of rivers in England by the same investigators [29].

Using a mass balance approach from data collected in 1997-98 and stable isotopes from a small number of sites, it was estimated that there was a small loss of N in the Mississippi River from the confluence of the

Mississippi and Ohio Rivers to the outflow to the Gulf of Mexico [21]. It was hypothesized that the nitrogen loss was due to assimilation and not denitrification. Water samples collected from three sites in the Mississippi River near Illinois and from two tile drains in central Illinois over a period of nearly 1 year were analyzed for stable isotopes [30]. From these data it was concluded that most of the denitrification occurred in the soil zone and shallow groundwater before discharging to surface water. However, definitive data indicating net NO_3^- losses or gains are scarce in large river systems at the scale presented here.

The principal objective of this study was to test the hypothesis that NO_3^- is transported conservatively through a 2,250-km length of the Mississippi River from Clinton, IA, to near the mouth of the river in southern Louisiana.

2. METHODS

This study was an outgrowth of an earlier investigation [17], but was designed specifically to address the question of the conservative transport of NO_3^- . Three different approaches were pursued to test the hypothesis that NO_3^- is transported conservatively: (1) a mass balance for NO_3^- was determined using samples collected using a Lagrangian sampling approach; (2) stable isotope ratios of N and O ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) in NO_3^- were measured to determine if enrichment was occurring, which might indicate NO_3^- assimilation or denitrification; and (3) the concentrations of dissolved gases (N_2O , N_2 , and Ar) in river water were measured and compared to theoretical equilibrium concentrations.

2.1. Sampling Sites

NO_3^- , stable isotope, chloride (used as a conservative tracer) and dissolved gas data needed to test the conservative nature of NO_3^- were obtained using a Lagrangian sampling approach [24] in which the same mass of water was sampled from the upstream end to the downstream end of the study area. All major tributaries discharging into this water mass were also sampled. Because Lagrangian sampling provides data on the changes in water-quality characteristics as a mass of water moves downstream, these data can be more useful than traditional Eulerian sampling for identifying the chemical, physical, and hydrologic processes that affect stream chemistry [31]. Seventeen (17) sampling sites were selected for this study. Site selection was based on a number of criteria, including: the need to sample the majority of the flow, whether or not the site was already in an existing sampling network, distance to

the nearest USGS field office, drainage area size, and whether there was a gaging station at the site. The chosen sampling sites included nine sites on the main stem of the river from Clinton, IA to Belle Chasse, LA, seven major tributaries to the Mississippi, and one distributary, the Atchafalaya River (Figure 1). A hydrologic 1-dimensional Lagrangian transport model of the Mississippi River [32] was used to estimate the travel time of the water mass and to determine when samples were to be collected at each site (Table 1). The study was conducted from July 20 through August 9, 1999 when water temperatures were high to ensure optimal conditions for denitrification and additionally when the conditions in the Mississippi River Basin were as close to steady state as possible with no large rainfall events occurring in the basin.

2.2. Sample Collection and Processing

Water samples were collected from boats and were depth and width integrated and flow weighted according to standard USGS protocols [33]. Samples from the vertical profiles were composited in glass, polyethylene or Teflon containers. All sampling equipment was cleaned with non-phosphate detergent, rinsed thoroughly with tap water, and then rinsed with distilled/deionized water. Approximately 6 to 9 L of water was collected for each sample. Immediately after collection, the samples were subsampled into appropriate containers. Whole water samples were placed in a dark plastic bottle, capped, and placed on ice. Filtered samples for analysis of dissolved nutrients, isotopes, and major ions were passed through a 0.45- μm filter and immediately chilled. Samples for major ions were preserved with nitric acid. Samples to be analyzed for major ions and nutrients were then shipped chilled with the whole water samples to the USGS National Water Quality Laboratory in Colorado, for analysis by standard procedures [34].

At sites that were co-located with stream-gauging stations, the mean daily flow was used in the analyses; otherwise, an instantaneous measurement of the flow was made using standard USGS protocols [35,36]. The accuracy of discharge measurements, if the measurement is made according to standard procedures, is a few percent. Rantz and others [35] demonstrate that under these circumstances two thirds of the discharge measurements would have an error of 2.2% or less.

2.3. Isotope Analysis

Water samples for isotope analysis were passed through 5 mL of anion exchange resin to isolate the NO_3^- from the sample, and then through a cation column in front of

the anion column to reduce concentrations of adsorbed organic constituents and neutralize HCO_3^- [37]. The cation column protonates and/or adsorbs dissolved organic carbon (DOC), thereby making it less likely for the DOC to compete with NO_3^- for exchange sites on the anion column [37]. About 200 μmol of NO_3^- (equivalent to about 2.8 mg of N) was required for the dual isotope analysis. A special low-level analysis (0.002 mg/L reporting limit) for NO_2^- plus NO_3^- conducted at the USGS Quality of Water Unit in Ocala, FL, was used along with estimated concentrations of other anions from historic data to determine how much water to pass through the exchange column pair and to test for NO_3^- passing through the column pair. Both the minimum volume needed to obtain sufficient NO_3^- and the maximum volume that would avoid exceeding the anion column exchange capacity were considered to determine the sample volume to be processed. To verify that all of the NO_3^- in the sample was adsorbed by the anion column, eluent that passed through the cation and anion exchange columns was saved, and a sub-sample was analyzed for NO_2^- plus NO_3^- . If 95% or more of the NO_3^- was retained by the anion column, the sample was analyzed for $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ of NO_3^- at the USGS stable isotope laboratory in Menlo Park using methods described by [38]. Oxygen-isotope analyses were performed on a Finnigan Mat 251 stable isotope mass spectrometer and nitrogen isotope analyses were performed on an Optima mass spectrometer. N isotope values ($\delta^{15}\text{N}$) are reported in per mil (‰) relative to atmospheric N_2 , which by definition has a $\delta^{15}\text{N}$ of 0‰. Oxygen isotope values ($\delta^{18}\text{O}$) are reported in ‰ relative to the standard VSMOW (Vienna Standard Mean Ocean Water), also defined as 0‰. The $\delta^{18}\text{O}$ values presented here may or may not be consistent with recent data from other studies following introduction of multiple nitrate isotopic reference materials [39,40], but they are considered to be internally comparable for detection of variation in this study.

2.4. Dissolved Gases

Water for dissolved N_2O analysis were collected near the center of flow, midway through the water column, using the following procedure. First, a 25-mL glass bottle was prepared in the laboratory by adding about 100 mg potassium hydroxide (1 pellet) to the bottle, sealing it with a butyl stopper (Bellco Glass, Inc., Vineland, New Jersey), and flushing the bottle headspace with Ar gas (leaving the headspace over-pressured until sampling time). The glass bottles were equilibrated to river temperature by immersing them in river water. Just prior to sampling, the bottle headspace pressure was equilibrated with the atmospheric pressure

at the sampling site by inserting a needle attached to an empty syringe through the stopper and releasing excess Ar into the syringe. Atmospheric pressure was recorded at each site and used in the calculation of dissolved N_2O concentrations [41]. Using a syringe, an additional 10 or 15 mL of Ar gas was then removed from the bottle headspace to make room for the water sample and still maintain the bottle near to atmospheric pressure.

Upon completion of those steps, water for N_2O analysis was collected in a 10- or 30-mL glass syringe with connected needle (21-gauge needle, no syringe valve) by placing the pump discharge line in the bottom of the syringe barrel, allowing water to overflow the syringe barrel and to flush the needle for 1 minute, and then inserting the syringe plunger into the water-filled (no bubbles) syringe barrel. Finally, a 10 or 15-mL water sample was injected into the bottle to replace the final volume of Ar headspace that had been removed. The filled sample bottle was stored at 4 °C until analysis.

Concentrations of N_2O were determined by gas chromatography/ ^{63}Ni -electron capture detection [Hewlett Packard 5890 GC, 183 x 0.32-cm stainless steel column packed with 80/100 Porapak Q, carrier gas was Ar/CH_4 (95/5)]. Dissolved concentrations of N_2O were determined by allowing the liquid and headspace N_2O concentrations to equilibrate at room temperature, analyzing N_2O in the headspace, and back calculating the aqueous N_2O concentration [41]. The precision of the N_2O analysis was $\pm 5\%$, based on replicate analyses of samples and standards. Samples generally were analyzed within 1 week of sample collection. Time-series measurements indicated that N_2O concentrations remained stable in the vials for at least 3 weeks.

Water samples for N_2 and Ar gas analyses were collected in 160 mL bottles without headspace. River water was pumped from about mid depth in the center of flow into a bucket on the boat deck, and the bottles were filled under water, brought to the surface, and 100 mg of potassium hydroxide was added as a preservative. The bottle was sealed with a rubber stopper with a syringe insert to allow excess water to escape. Samples were chilled and shipped to the laboratory.

In the laboratory, approximately 10 mL of water was extracted through a syringe needle with a vacuum pump, leaving low-pressure headspace that was equilibrated with the remaining water. Gas analyses of the low-pressure headspace were done in the USGS Dissolved-Gas Laboratory in Reston, VA with a modified Hewlett-Packard 5890 gas chromatograph (GC) with dual separation columns (<http://water.usgs.gov/lab/dissolved-gas>). Total aqueous gas concentrations were calculated from the headspace concentrations and confirmed by analyses of water

equilibrated with laboratory air, with typical uncertainties ranging from ± 0.5 to 1.0% for Ar and N_2 . Measured concentrations of N_2 and Ar were compared to equilibrium concentrations using solubility data [42].

For isotopic analysis of dissolved N_2 , the low-pressure headspace remaining in a subset of the 160-mL bottles after GC analysis was expanded in a high-vacuum extraction line into a pair of 20-cm quartz glass tubes containing $Cu_2O + Cu$ and CaO [43]. The tubes were sealed, baked, and analyzed by dual-inlet mass spectrometry at m/z 28 and 29. The dissolved N_2 results were calibrated by analyzing aliquots of air N_2 ($\delta^{15}N = 0\%$) and compared to results from laboratory-equilibrated air-saturated water samples that were collected, prepared, and analyzed the same way as the stream samples. Typical $\delta^{15}N[N_2]$ values of laboratory-equilibrated water samples analyzed this way were $+0.7 \pm 0.1\%$, similar to other published experimental results [44].

Analyses of seven pairs of duplicate samples yielded average deviations from the mean values of $\pm 2 \mu\text{mol/L}$ for N_2 concentration, $\pm 0.02 \mu\text{mol/L}$ for Ar concentration, and $\pm 0.12^\circ\text{C}$ for apparent equilibration temperature, indicating the reproducibility of the sampling and analysis under ideal conditions. Data from four of the dissolved gas samples were eliminated from consideration because of anomalously high Ar and N_2 concentrations, indicative of air contamination.

2.5. Quality Assurance

Trip blanks, concurrent replicates, duplicates, and samples collected from multiple locations in the stream cross section were used for quality assurance of the data. Trip blanks that were collected with the N_2O samples did not have detectable concentrations, indicating that the sampling and handling procedures and analysis did not cause contamination.

The precision of the analytical measurements, as well as error associated with sampling, was measured through the collection and analysis of concurrent replicates and duplicates. Concurrent replicates are two or more samples that are collected as closely as possible in time and space, but processed, handled, and analyzed separately. Duplicates are aliquots from the same sample. There were only a few concurrent replicates; therefore, these data are combined with the duplicates and the relative percent difference:

$$\text{relative percent difference} = |A-B|/[(A+B)/2] \quad (1)$$

for N_2 , Ar, N_2O saturation, and N_2O , are shown in Figure 2. The mean difference for $\delta^{15}N$ of 4 replicates was 0.39% , and the mean difference of 3 replicates for

$\delta^{18}O$ was 0.97% . Relative percent differences for the N_2O analyses were high because of the generally small dissolved concentrations in the samples.

In order to evaluate the representativeness of the gas samples (samples were collected from a single point about mid-depth from the centroid of flow), samples from the left and right side of the cross section were collected at two sites; at a third site, samples were collected from the left, center, and right side mid-depth, and also from left, center, and right from as near the bottom of the stream as the sampling apparatus would allow. The results (not shown) indicate that there is no more variability in concentration from within and across the Ohio and Mississippi Rivers than between replicates and duplicates. This is supported by other research [30, 45]. These studies compared single-point pumped samples collected from the center of the river with composite sampling across the width and depth of the Mississippi River and reported that the dissolved composition of the single-point pumped samples was comparable to that of the composite river water samples.

2.6. Data Analysis

In this paper, the NO_3^- flux to the Gulf of Mexico is considered to be the sum of the flux at the Mississippi River at Belle Chasse, LA sampling site and the Atchafalaya River at Melville, LA sampling site. The chloride flux at the Belle Chasse site was much higher than anywhere else in the Mississippi River Basin, indicating possible saltwater intrusion. Therefore, the chloride flux to the Gulf of Mexico is the sum of the flux at Mississippi River at St. Francisville, LA, and the flux at Atchafalaya River at Melville, LA.

The stable isotopes of N and O in the NO_3^- ion might provide evidence for denitrification in the study reach, if it is occurring. Nitrate reduction (by denitrification or assimilation) would result in preferential removal of NO_3^- ions containing the lighter isotopes ^{14}N and ^{16}O over NO_3^- ions containing the heavier ^{15}N and ^{18}O isotopes. As a result, the isotopic ratios, $\delta^{15}N$ and $\delta^{18}O$, of NO_3^- remaining in the river water could increase (enriched with ^{15}N and ^{18}O) downstream through the study reach if NO_3^- reduction were occurring. Isotopic ratios were measured at all sampling sites, but mixing of water in the river with tributary water with differing isotopic ratios also can produce changes in isotopic ratios. Thus, changes in isotopic ratios resulting from NO_3^- reduction may be confounded by changes caused by mixing of waters. However, the $\delta^{15}N$ and $\delta^{18}O$ values that would have resulted from mixing alone can be calculated at each site using a mixing model and compared with the measured values.

Increases in measured $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values over calculated values based on tributary mixing through a study reach could mean isotopes were fractionated by processes such as NO_3^- reduction.

The mixing model values of $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ at each site were calculated with an isotope mass balance equation (2),

$$\Sigma(\delta^{15}\text{N}_i \text{ or } \delta^{18}\text{O}_i * \text{Nmass}_i) / \text{Nmass}_{\Sigma} = \delta^{15}\text{N}_j \text{ or } \delta^{18}\text{O}_j \quad (2)$$

where $\delta^{15}\text{N}_i$ or $\delta^{18}\text{O}_i$ is the measured $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ value of each upstream NO_3^- input; Nmass_i is the measured NO_3^- mass of each upstream input, and $\delta^{15}\text{N}_j$ or $\delta^{18}\text{O}_j$ is the calculated $\delta^{15}\text{N}$ or $\delta^{18}\text{O}$ value of the mixed water.

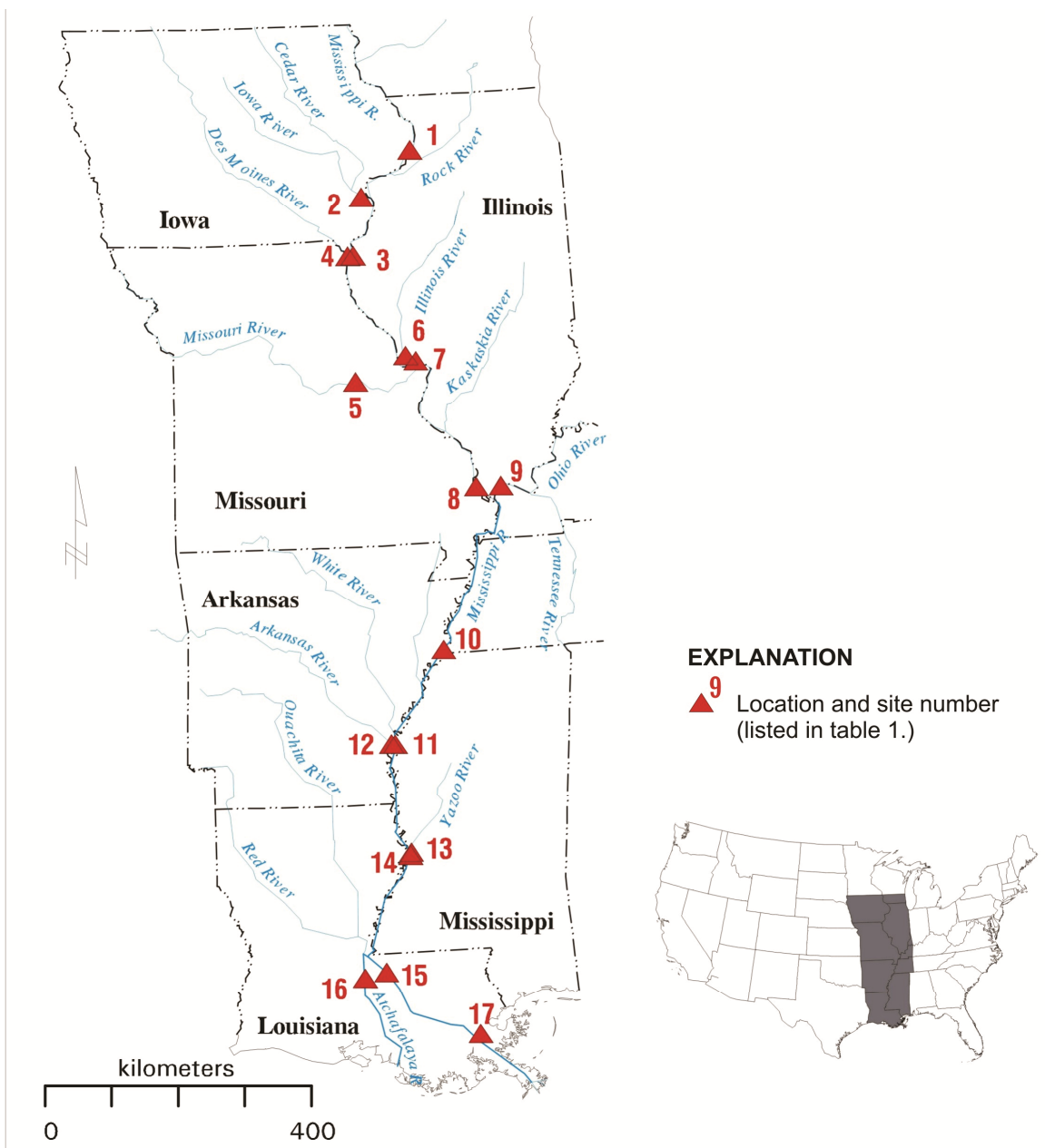
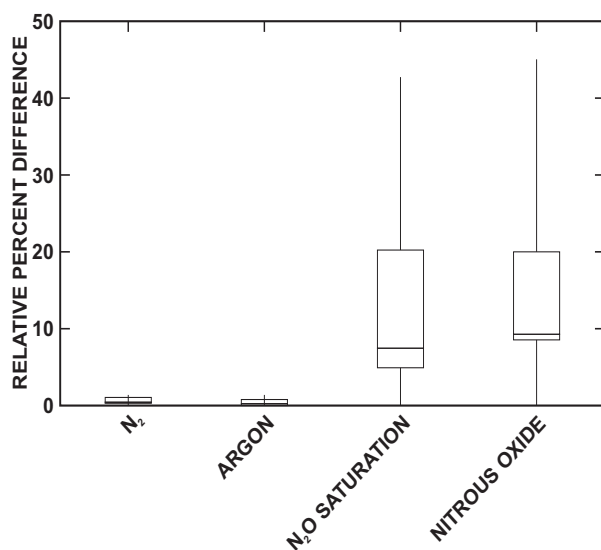


Figure 1 Study area and site locations of Lagrangian sampling July 20 through August 9, 1999 [numbers correspond to site names on Table 1].

Table 1 Selected data for 17 sites sampled during the study period July 20 through August 9, 1999 (*site numbers refer to Figure 1) [other analytical data from these samples can be found at <http://waterdata.usgs.gov>].

Site Number	USGS Number	Site Name	Sampling Date (1999)	Sampling Time	River km	Discharge (m ³ /s)
1	5420500	Mississippi River at Clinton, IA	20-Jul	13:00	2,360	1,930
2	5465500	Iowa River at Wapello, IA	21-Jul	13:00	2,230	388
3	5474500	Mississippi River at Keokuk, IA	22-Jul	7:00	2,140	2,430
4	5492000	Des Moines River near Keokuk, IA	22-Jul	9:00	2,115	535
5	6934500	Missouri River at Hermann, MO	23-Jul	13:10	1,840	2,860
6	5587060	Illinois River near the Mississippi River	24-Jul	10:05	1,885	351
7	5587455	Mississippi River at Grafton, IL	24-Jul	14:30	1,880	3,430
8	7022000	Mississippi River at Thebes, IL	26-Jul	14:30	1,625	6,460
9	3612500	Ohio River at Grand Chain, IL	26-Jul	10:30	1,530	3,030
10	7032000	Mississippi River at Memphis, TN	28-Jul	16:15	1,200	9,430
11	7265402	Mississippi River at Rosedale, MS	30-Jul	13:00	940	10,350
12	7265401	Arkansas River near the Mississippi River	30-Jul	10:30	930	762
14	7289000	Mississippi River at Vicksburg, MS	2-Aug	9:45	702	11,600
13	7288955	Yazoo River near Steele Bayou, MS	2-Aug	12:45	703	251
15	7373420	Mississippi River at St. Francisville, LA	5-Aug	10:30	400	9,090
16	7381495	Atchafalaya River at Melville, LA	5-Aug	10:30	430	4,020
17	7374525	Mississippi River at Belle Chasse, LA	9-Aug	10:00	117	7,650

**Figure 2** Relative percent difference of N₂, argon, N₂O saturation, and nitrous oxide (N₂O) from concurrent replicates and duplicate samples.

For example, the $\delta^{15}\text{N}$ value that could be expected

due to mixing in the Mississippi River at Vicksburg, MS would be calculated by multiplying the mass of NO_3^- by the $\delta^{15}\text{N}$ value for the Mississippi River at Rosedale, the Arkansas River, and the Yazoo River, adding them together and dividing by the sum of the mass of NO_3^- from the Mississippi River at Rosedale, the Arkansas River and the Yazoo River. If the measured values at Vicksburg were different from the values calculated from the upstream contributions, then NO_3^- gains or losses may have occurred within the reach.

An indication of the amount of change in the values for $\delta^{15}\text{N}$ over $\delta^{18}\text{O}$ from a given amount of denitrification can be calculated from a simplified form of the Rayleigh equation (3) [9,16],

$$\delta_f = \delta_0 + \square \ln f \quad (3)$$

where f is the fraction of NO_3^- mass remaining, \square is the isotopic fractionation factor for denitrification, and δ_f and δ_0 are the final and initial isotope values of NO_3^- .

For example, if 10% of the NO_3^- were denitrified, and if \square values [21,30] were -16 ‰ for ^{15}N and -8 ‰ for ^{18}O , then it would be expected that the $\delta^{15}\text{N}$ value would increase by 1.7 ‰ and the $\delta^{18}\text{O}$ value would increase by 0.8 ‰. Values of \square may differ, and the net

effect may approach zero in some situations, but positively correlated variations in these isotope ratios can still provide qualitative evidence for NO_3^- consuming reactions [46,47].

3. RESULTS AND DISCUSSION

The mass balance approach is simple, straightforward and easy to understand; however, NO_3^- production by nitrification and temporary storage of N in a reach by autotrophic uptake or physical retention can affect estimates of denitrification using this approach. It was concluded from an isotope tracer experiment conducted in a small stream, Sugar Creek in northwestern Indiana, that a mass balance approach would have missed the significant denitrification occurring in the stream because of offsetting sources and sinks of NO_3^- [12]. Additionally, the accurate measurement of flow in a river as large as the Mississippi is not a small task and the potential for error exists; even a small error in the flow could lead to masking of the effects of denitrification.

If significant denitrification were occurring in the Mississippi River, increases in the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values could be expected; however, isotopic fractionation by other nitrogen cycling processes may make the results difficult to interpret. Although isotopic fractionation may be expected where NO_3^- reduction occurs, a number of studies have indicated that benthic denitrification of surface-water NO_3^- can occur with little or no net isotopic effect in the overlying water column. If denitrification occurs within the bed sediments and is limited, in part, by transport from the surface water to the reaction sites, then the net isotopic effect on the NO_3^- in the overlying water column can approach zero [48-50].

The use of dissolved gases to measure denitrification rates in an open channel has been accomplished in several rivers much smaller than the Mississippi [52,53]. However, this method normally requires high-precision measurements and may be complicated by uncertainties in air-water exchange rates and by diel changes in air temperature; there could be other sources of these dissolved gasses such as denitrified groundwater, which may obscure instream sources [12,54]. Each of these methods has strengths and weaknesses concerning the assessment of the conservative transport of NO_3^- in the Mississippi River. However, they each point to only a certain set of outcomes and, although confidence in the outcome from a single method would be low, if each method indicates that same outcome then the corresponding confidence would be greater.

3.1. Mass Balance

Figures 3, 4, and 5 show the cumulative transport of water, chloride, and NO_3^- , respectively, through the Mississippi River from Clinton, IA to Belle Chasse, LA from July 20 through August 8, 1999. At the beginning of the study reach (Mississippi River at Clinton, IA), the streamflow, NO_3^- flux, and chloride flux were about 16, 16, and 8 % of the total flux to the Gulf of Mexico, respectively.

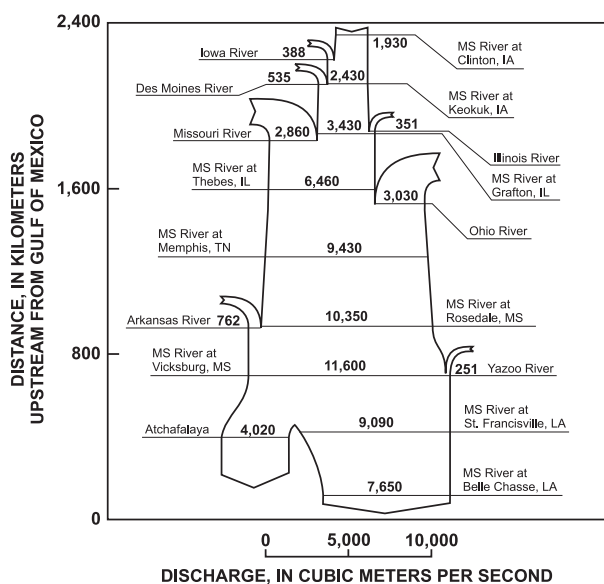


Figure 3 Transport of water in the Mississippi River during the study period July 20 through August 9, 1999.

The input of chloride and water to the river accumulated steadily throughout the 2,250-km length of the Mississippi River in this study. About one third of the flow in the Mississippi River at Memphis, TN originated from the Missouri River, and about one third from the Mississippi River above the confluence of the Mississippi and Missouri Rivers, and about one third comes from the Ohio River. The flux of chloride is similar, with a slightly higher percentage from the upper Mississippi River and less from the Missouri and Ohio Rivers. However, most of the NO_3^- input (almost 90 %) occurred above the confluence of the Mississippi and Ohio Rivers, which includes the Missouri River Basin. About one half of the NO_3^- flux in the Mississippi River at Memphis, TN originated from the Missouri and Des Moines Rivers and another quarter of the flux originated from the Iowa River and the Mississippi River above Clinton, IA. The Ohio and Illinois Rivers contributed little NO_3^- during this time period. The results from the Illinois River may be somewhat anomalous as previous

investigations have indicated that the Illinois River contributed a substantial amount of NO_3^- to the Mississippi River. This anomaly is attributed to the local hydrology at the time of the study, when the Iowa and Des Moines Rivers were flowing well above their mean daily values and the Illinois River was flowing well below its mean daily value.

Below the confluence of the Mississippi and Ohio Rivers, the NO_3^- flux increased slightly in the downstream direction to Vicksburg, MS (Figure 5). Between Vicksburg, MS and St. Francisville, LA, the NO_3^- flux decreased slightly even though the discharge increased. The difference in NO_3^- concentration in the Mississippi River between Vicksburg, MS, and St. Francisville, LA, was within the analytical and sampling variability [55]. The concentration in the Atchafalaya River was less than in the Mississippi River at St. Francisville, LA, probably due to input of low NO_3^- water from the Red River. Historically NO_3^- concentrations in the Red River at Alexandria, La (07255500) (nwis.waterdata.usgs.gov accessed 2/26/08) have been less than the reporting level of 0.02 mg/L during July and August. The NO_3^- flux decreased slightly between Vicksburg, MS and St. Francisville/Atchafalaya less than 10 % and between St. Francisville/Atchafalaya and Belle Chase, LA less than 3%. These decreases are within the precision of discharge measurements ($\pm 5\%$), and the analytical and sampling variability expected for NO_3^- , but they do suggest a loss of NO_3^- from the Mississippi River between Vicksburg, MS and St Francisville, LA.

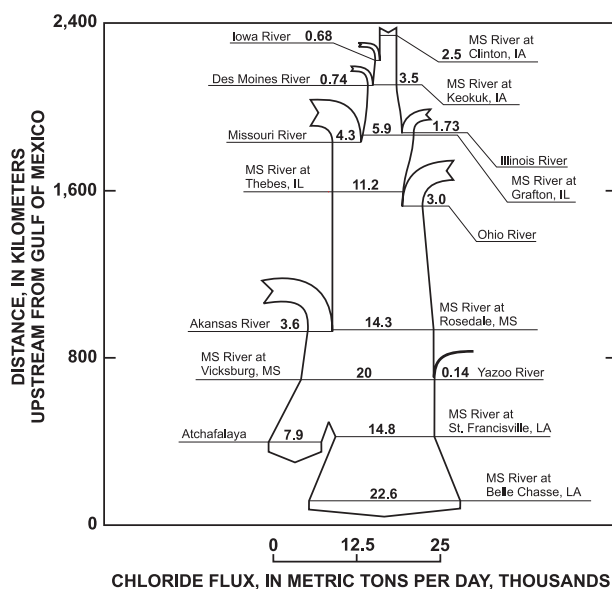


Figure 4 Transport of chloride in the Mississippi River during the study period July 20 through August 9, 1999.

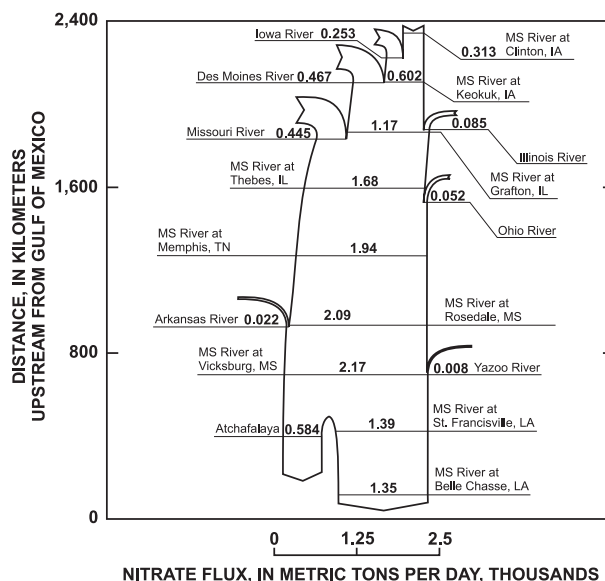


Figure 5 Transport of nitrate in the Mississippi River during the study period July 20 through August 9, 1999.

Table 2 Summary of results for mass balance calculations for streamflow, chloride, nitrate, and total nitrogen (Mt/day, metric tons per day).

Property	Basin Total	Accounted for by measured tributaries	Percent accounted for
Area, km ²	3,207,700	2,686,300	83.7
Streamflow, m ³ /s	11,670 ^a	10,530	86.6
Chloride, Mt/day	22,700 ^b	18,810	83
Nitrate, Mt/day	1,930 ^a	1,680	87
Total nitrogen, Mt/day	2,610 ^a	2,270	87

^a The sum of the flux from the Atchafalaya River and the Mississippi River at Belle Chasse, LA; ^b The sum of the flux from the Atchafalaya River and the Mississippi River at St. Francisville, LA

Table 2 summarizes the flux of water and chemicals to the Gulf of Mexico during the study. Measured inputs from tributaries accounted for 83 and 87% of the chloride and NO_3^- fluxes, respectively, almost 87% of the streamflow and 83% of the basin area. If significant amounts of NO_3^- were being removed from the Mississippi River between measuring points (for any reason such as assimilation or denitrification), then one would expect the percent flux accounted for by the tributaries for NO_3^- to be different than that of the

conservative tracer chloride and streamflow. These data do not suggest a measurable loss of NO_3^- from the Mississippi River.

3.2. Stable Isotopes of Nitrate

The $\delta^{15}\text{N}$ values in the Mississippi River decreased in a downstream direction from a high of about 10.3‰ in the Mississippi River at Clinton, IA, site to a steady 8.8‰ in the Mississippi River until Vicksburg, MS (Figure 6). There is a slight increase in the $\delta^{15}\text{N}$ value between Vicksburg, MS and St Francisville. The tributaries showed much more variability than the Mississippi River, from a low in the Yazoo River of about 4‰ to a high in the Illinois River of about 12‰. With the exception of the Illinois River, the $\delta^{15}\text{N}$ values from other tributaries of the Mississippi River are lower than in the Mississippi River. The $\delta^{15}\text{N}$ values calculated from the mixing model follow the observed values from the Mississippi River (Figure 6), and there is no indication that there is any unknown factor (i.e. denitrification) affecting the $\delta^{15}\text{N}$ values.

The data reported here for $\delta^{18}\text{O}$ of NO_3^- may include systematic offsets with respect to other recent measurements [39], and therefore, should be considered for comparison purposes between sites in this study but not necessarily for comparison to other studies. The

$\delta^{18}\text{O}$ values in the Mississippi River ranged from a high at the northernmost site near Clinton, IA, of 9.35‰ to a low at the southernmost sampling near Belle Chasse, LA of 6.22‰ (Figure 7). The values generally decreased in a downstream direction. The value at Vicksburg, MS was unusually low and not representative of values above or below. The tributaries showed much more variability than in the main stem Mississippi River. The highest value of $\delta^{18}\text{O}$, 16.4‰ was in the Ohio River, and the lowest values, 6.03‰ and 6.2‰, were from the Iowa and Des Moines Rivers, respectively. The $\delta^{18}\text{O}$ values calculated from the mixing model show more variability than for the $\delta^{15}\text{N}$ values, but generally agree well with the measured values from the Mississippi River, except for the value at the Mississippi River at Vicksburg, MS and at St. Francisville, LA (Figure 7). This is probably due to the unusually low value at Vicksburg, MS.

There is no evidence from these stable isotopes that there was significant denitrification occurring in the main stem of the Mississippi River upstream of Vicksburg, MS, as the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ values did not increase even when the tributary input was accounted for in the model. Below Vicksburg, MS the $\delta^{15}\text{N}$ value increased and the $\delta^{18}\text{O}$ value stayed relatively the same as sampling sites just above Vicksburg, MS.

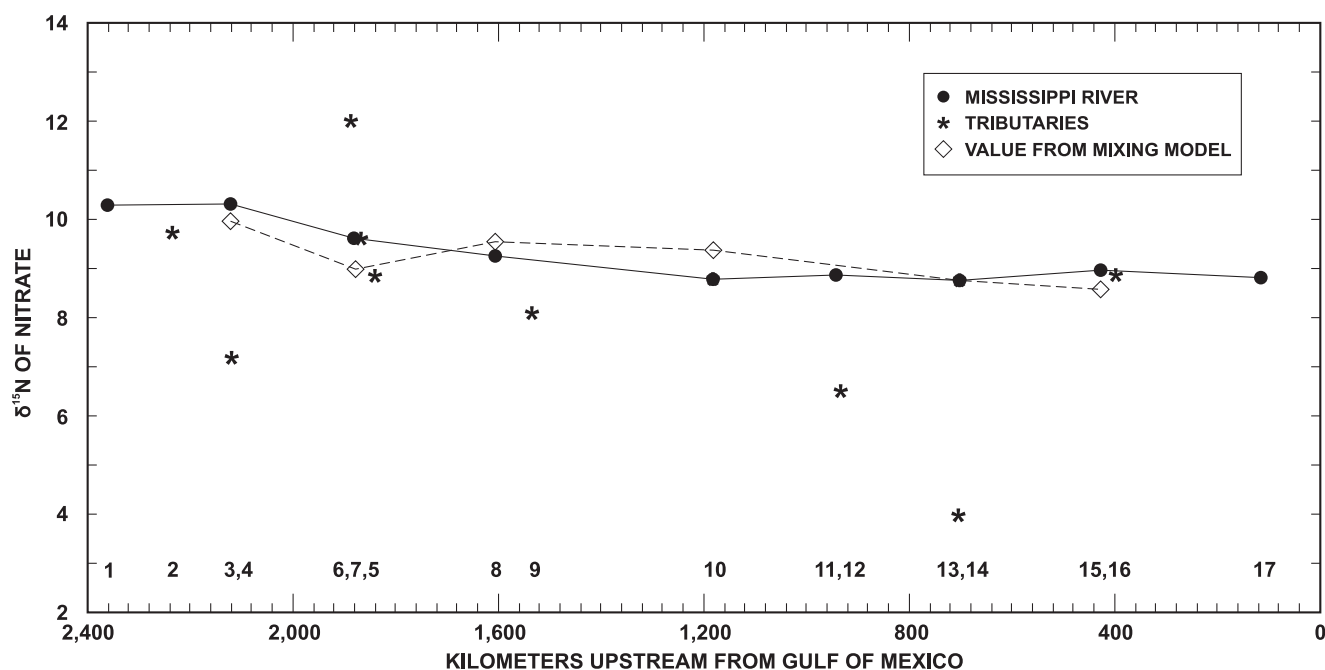


Figure 6 $\delta^{15}\text{N}$ values in the Mississippi River and some of its tributaries July 20 through August 9, 1999, and theoretical values determined from mixing of Mississippi River water with tributary water (numbers are sampling locations and are referenced in table 1).

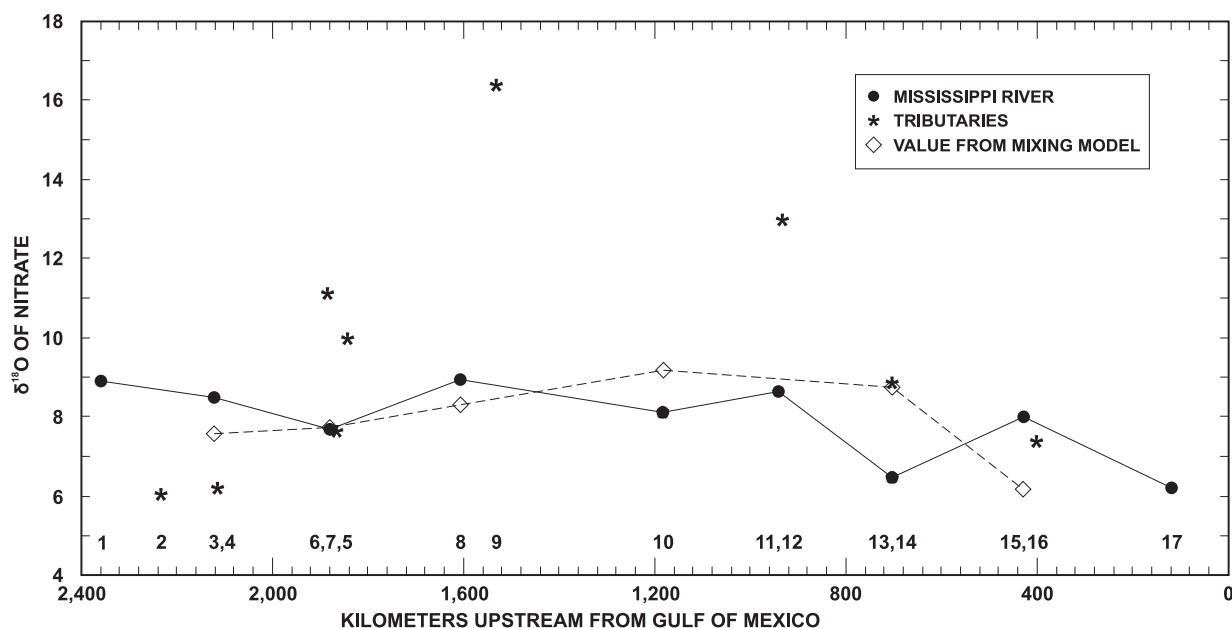


Figure 7 $\delta^{18}\text{O}$ values in the Mississippi River and some of its tributaries July 20 through August 9, 1999, and theoretical values determined from mixing of Mississippi River water with tributary water (numbers are sampling locations and are referenced in table 1).

3.3. Dissolved Gases

Dissolved O_2 , N_2 , Ar, N_2O , and the temperature of air and water, were measured at all sites. O_2 concentrations in the Mississippi River and its tributaries and distributaries ranged from about 165 to 265 $\mu\text{mol/L}$; N_2 concentrations ranged from 421.9 to 482.3 $\mu\text{mol/L}$; Ar concentrations ranged from 10.85 to 12.03 $\mu\text{mol/L}$; the concentration of N_2O ranged from 2.4 to 18.0 nmol/L ; water temperatures ranged from 26.3 to 32.5°C; air temperatures ranged from 28.1 to 35.3°C. Barometric pressure ranged from 748 to 770 mm/Hg.

In the absence of biological activity or groundwater discharge and at constant temperature and pressure, the concentrations of O_2 , N_2 , Ar, and N_2O dissolved in river water should be approximately in equilibrium with the atmosphere. Abrupt changes in air/water temperature and barometric pressure could result in deviations from equilibrium concentrations and deviations could also result from biological processes including photosynthesis, O_2 respiration, denitrification, and nitrification. Short-term variations in temperature and pressure were not monitored in this study. Continuous monitoring of water temperature was conducted by the U.S. Geological Survey NASQAN Program in the late 1970's, 1980's, and early 1990's. These data indicate that on the Mississippi River at Vicksburg, MS, and at New Orleans, LA, the difference between the maximum

and minimum temperature, on a daily basis, in July and August was less than 0.5°C. The minimum air temperature at Greenville, MS, during the study period ranged from 23.3 to 26.7°C, and the maximum ranged from 33.3 to 37.2°C. Diel changes in water and air temperature are likely to be greater in the northern part of the study area (<http://ext.msstate.edu/anr/drec/weather.cgi>). These data indicate that diurnal changes in temperature was not a major factor in causing deviations from equilibrium.

Denitrification produces N_2 , which could cause supersaturation in river water under some conditions, whereas N_2 fixation could reduce N_2 concentrations. Favorable conditions for detecting N_2 excess or deficits from these causes include slow gas exchange between the water and air, and high reaction rates. In this study, the average deviation of N_2 and Ar concentrations from the air-saturation line (expressed as ccSTP/L of excess air) for all samples (minus outliers) was 0.07 ± 0.41 (1 sigma) (Figure 8). The average apparent air-water equilibration temperature based on N_2 and Ar concentrations and measured barometric pressures, assuming 100 % humidity in the boundary layer, was $30.7 \pm 1.7^\circ\text{C}$. The average calculated gas equilibration temperature is indistinguishable from the average measured water temperature ($31.2 \pm 1.5^\circ\text{C}$) (data not shown), but perhaps slightly lower than the average measured air temperature ($32.9 \pm 2.5^\circ\text{C}$). The concentrations of Ar and N_2 decreased systematically

while the temperature of the river increased in the downstream direction. At equilibrium, the overall change in temperature from the northernmost sampling site to the southernmost sampling site of about 6°C would correspond to about a 10% decrease in solubility, which is close to the observed change in concentration. All of these comparisons indicate that the N_2 and Ar were approximately in equilibrium with air when averaged over the whole dataset, but some of the apparent deviations are larger than the reproducibilities of the measurements. The reasons for the deviations are not known, but could be related to degassing, denitrification, temperature changes, or other causes. Recalculating the deviations from the air-saturation line in terms of excess N_2 would yield an average of approximately $2 \pm 14 \mu\text{mol/L}$ (or $4 \pm 28 \mu\text{mol/L}$ as N), which is within the range of uncertainty of the data and assumptions (including variations in humidity, temperature and so on). Isotopic analyses of the dissolved N_2 in a subset of the samples averaged $+0.73 \pm 0.14\text{‰}$ ($n =$

5), in comparison to $+0.74\text{‰}$ for laboratory air-equilibrated water analyzed at the same time. The average $\delta^{15}\text{N}$ value of the N_2 also is consistent with atmospheric equilibrium, but these data are limited. In summary, there is no consistent evidence for excess N_2 attributable to denitrification beyond the uncertainties of the analyses, calculations, and assumptions.

Denitrification can produce substantial amounts of N_2O , which may be supersaturated in surface waters if its production rate exceeds its rate of equilibration with atmospheric N_2O . Because N_2O is an intermediate step in the reaction process, it is also consumed; therefore the concentration of N_2O may be highly variable. Additionally, N_2O is produced in the nitrification of ammonium in rivers [56]. The N_2O concentrations in this study ranged from 1.4 to 13 mg/L and saturation ranged between 26 and 312%, with the two highest values, 312 and 280%, from the Illinois and Yazoo Rivers, respectively (Figure 9).

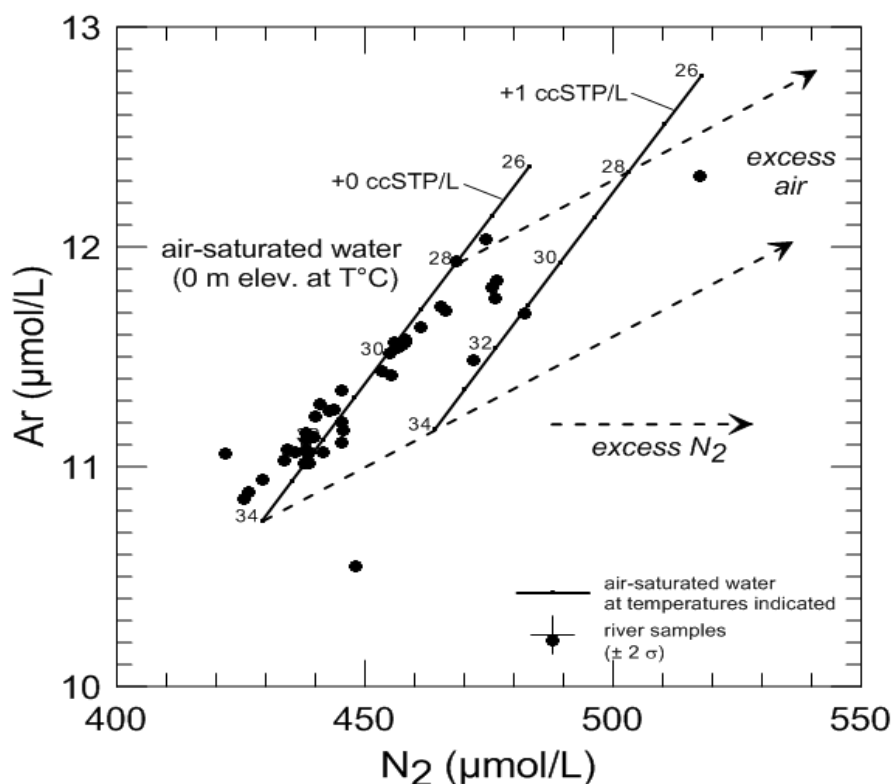


Figure 8 Concentrations of Ar, N_2 in the Mississippi River and major tributaries during the study period July 20 through August 9, 1999, shown with the air-saturation equilibrium line at various temperatures (26 to 34 °C) with no excess air (+0 ccSTP/L) and with 1cc of excess air (+1 ccSTP/L) at 0 m elevation. If there is excess air in the sample, then the Ar and N_2 concentration will increase in tandem along the parallel dashed lines. If N_2 is being produced in the water column, the N_2 concentration will be shifted to the right without an corresponding increase in the Ar concentration.

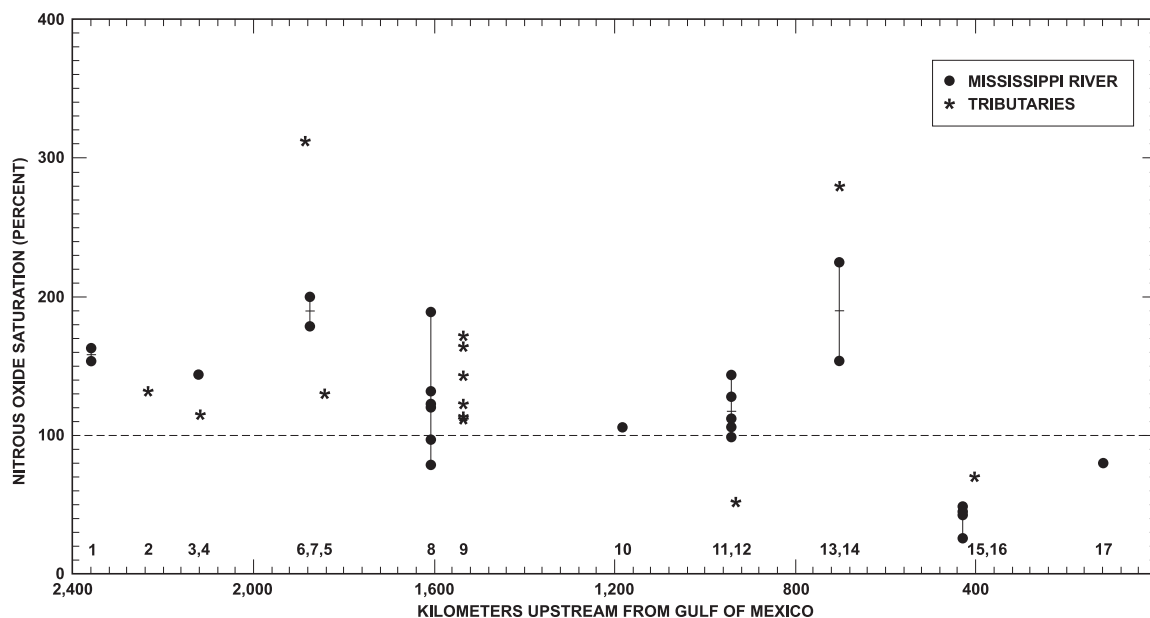


Figure 9 Nitrous oxide percent saturation (all values measured at a sampling site are shown, including concurrent replicates, duplicates, and samples from multiple locations in the stream cross section) versus distance upstream from the Gulf of Mexico during the study period July 20 through August 9, 1999 (numbers are sampling locations and are referenced in table 1).

In a study of three rivers (one in New Jersey and two in Illinois) denitrification rates ranging from 0.31-15.91 mmol N m² h⁻¹ produced a saturation range of N₂O concentrations of 104 to 209% [57]. In a study on 12 headwater streams in southwestern Michigan where denitrification, nitrification, and N₂O saturation were all measured, the streams were nearly always supersaturated with N₂O regardless of whether denitrification or nitrification predominated. The overall mean N₂O saturation across all streams and all samples was 236% [58].

The saturation values in the Mississippi River and its tributaries above St. Francisville, LA, generally are in this range except for the Illinois and Yazoo Rivers, which have much higher saturation values and the Arkansas River which has much lower values. The N₂O results are somewhat ambiguous with respect to the NO₃⁻ and N₂ results. In the tidal freshwater part of the Hudson River in New York, all of the measurements of N₂O in the river were supersaturated [59]. The saturation values ranged from a low of 125% to a high of 385%, similar to those results presented here. But mass balance calculations for this part of the Hudson River (with concentrations of NO₃⁻ similar to those found in the Mississippi River) indicated that some 15 to 20% of the NO₃⁻ entering the river does not exit it: orders of magnitude above what is needed to produce

the observed N₂O flux.

Neither the N₂ nor the NO₃⁻ concentration increases in the water mass in the Mississippi River as it moves downstream. This suggests that the observed supersaturation of N₂O upstream of St. Francisville, LA, probably is a combination of denitrification and nitrification metabolism working in combination to produce supersaturated N₂O concentrations, but no measureable change in N₂ or NO₃⁻.

4. CONCLUSIONS

During July 20 to August 9 1999, a Lagrangian study was conducted on the Mississippi River from Clinton, IA, to Belle Chasse, LA, with the purpose of determining if there was a significant loss of NO₃⁻ occurring. Three lines of evidence point to minimal loss of NO₃⁻ in the 2,250-km reach of the Mississippi River. A mass balance for nitrogen in the Mississippi River indicated that most of the NO₃⁻ enters the Mississippi River above the confluence of the Mississippi and Ohio Rivers, and comparing the mass balance of chloride and water with NO₃⁻ indicates that there is minimal loss of NO₃⁻ from the Mississippi River above Vicksburg, MS. Between Vicksburg, MS and St Francisville, LA the mass balance approach indicates a small loss of NO₃⁻

(about 9%) from the Mississippi River. Stable isotopes of NO_3^- ($\delta^{15}\text{N}$ and $\delta^{18}\text{O}$) point to the same conclusion. The ratio of N_2/Ar and N_2O concentrations did not indicate that NO_3^- was being lost from the Mississippi River by denitrification. The N_2O results indicate some nitrification and/or denitrification was occurring, but the relative importance and rates of these processes cannot be derived directly from the data. Combined results of this study are consistent with the hypothesis that most (>90%) of the NO_3^- that entered the Mississippi River from Iowa, Illinois and the upper Mississippi River Basin was transported directly to the Gulf of Mexico, although minor offsetting gains and losses also may have occurred.

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