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Isotopic evidence for preferential transport of fertilizer nitrogen into the northern Gulf of Mexico during high water discharge

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Anthropogenic nitrogen inputs from the Mississippi-Atchafalaya River Basin have caused substantial environmental challenges in the northern Gulf of Mexico, such as coastal eutrophication, harmful algal blooms, and seasonal hypoxia. Addressing these issues requires a better understanding of the complex sources of nitrogen, which include fertilizers, groundwater, manure, and sewage. In this study, we analyzed the nitrogen isotopic composition of dissolved nitrate and particulate nitrogen from the Wax Lake Delta, a major distributary of the Mississippi-Atchafalaya River Basin that flows into the Gulf of Mexico. Our findings revealed that during the wet season, $\delta^{15}N$ values of both nitrate and particulate nitrogen were consistently 2-3‰ lower compared to the dry season. This suggests that fertilizer-derived nitrogen, which has lower $\delta^{15}N$, is predominantly exported to the Gulf of Mexico during periods of high water discharge. These findings imply that adjusting fertilizer application timing could help reduce nitrogen loading and mitigate its environmental impact on the Gulf of Mexico.

The Mississippi-Atchafalaya River Basin (MARB) is the largest river basin in North America, accounting for approximately 80% of freshwater discharge and around 90% of total riverine nitrogen (N) input to the northern Gulf of Mexico (GoM)[1,2]. This region is a major agricultural hub, characterized by intensive cultivation and large amount of N fertilizer application[3–5]. Since 1950, the total N load from the Mississippi-Atchafalaya River system into the GoM has increased substantially, peaking in 1993, with fluctuating trends thereafter[6]. This escalation in N input has negatively impacted phytoplankton community composition and the extent of hypoxic zones in the northern GoM[7–9]. Despite decades of efforts to mitigate N loading, hypoxia areas in the northern GoM continue to exceed the target set by the Hypoxia Task Force, which aims to reduce the 5-year average size of hypoxic zone to less than 5000 km^2 by 2035. Current observations indicate that the size of these zones is roughly three times the target[10–12], significantly affecting marine ecosystems, coastal communities and economies dependent on the northern GoM.

Numerous studies indicate that anthropogenic N sources such as synthetic N fertilizers, groundwater, manure and human sewage are the primary contributors to the N load in the MARB[5,6]. Over the past decades, extensive use of N fertilizers in the MARB has significantly elevated N content in soil[13–15]. It is estimated that approximately 50% of the current dissolved nitrate (NO_3^-) load from the MARB to the northern GoM originated from 'legacy N' that has accumulated within the watershed for more than 30 years[14–17]. This 'legacy N' can also leach from soil into groundwater, contaminating water resources in the MARB[12,15–18]. Despite the pressing need to decrease N inputs, research on the transport mechanisms and timing of these anthropogenic N sources reaching the northern GoM remains scarce[3,4,19].

Tracing and quantifying the transport of anthropogenic N to the northern GoM remains challenging due to the complexity of these sources. Although the U.S. Geological Survey provides long-term data on riverine N loads[2], reliance solely on N concentrations and water discharge data is insufficient to distinguish between different anthropogenic N sources. This limitation restricts our understanding of how and when these N sources are transported to the GoM. Empirical modeling approaches have attempted to separate the

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different N sources using bottom-up methods[3,20]; however, these models are constrained by inherent uncertainties, including inadequately captured N biogeochemical processes and ecosystem interactions.

N isotopes are valuable tools for tracing N sources in natural environments[21–24]. Typically, synthetic N fertilizer and atmospheric deposition are characterized by lower $^{15}N/^{14}N$ values (or $\delta^{15}N$, where $\delta^{15}N = [(^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{atm}\ N_2 - 1] \times 1000‰$). In contrast, groundwater, human sewage and manure exhibit higher $^{15}N/^{14}N$ values due to biological processes such as denitrification and trophic enrichment[23], which preferential consumes ^{14}N relative to ^{15}N[23,25]. For example, during denitrification where NO_3^- is consumed, ^{14}N-NO_3^- is preferentially utilized over ^{15}N-NO_3^-, resulting in the residual NO_3^- progressively enriched in ^{15}N. Consequently, $\delta^{15}N$ can help distinguish between low-$\delta^{15}N$ N sources and high-$\delta^{15}N$ endmember[23,26].

Despite its efficacy in identifying N sources, the use of natural-abundance NO_3^- isotopes was only briefly employed in the MARB during the 2000s. These studies suggested similar N sources and N-cycling processes in the Mississippi and Atchafalaya Rivers[27,28]. However, since 2010, there has been a lack of NO_3^- isotope data that can be compared with recent modeling results. This gap highlights the need for updated isotope data to corroborate these models. In addition, recent technological advancements have improved the sensitivity and precision of NO_3^- isotope measurements, offering the potential for more comprehensive studies[29]. In this study, we report the isotopic composition of NO_3^- and particulate nitrogen (PN) collected during two hydrological seasons from the Wax Lake Delta (WLD), a major distributary of the MARB (Fig. 1a; Supplementary Fig. 1).

Results and discussion

Seasonal changes in anthropogenic nitrogen inputs to the WLD and GoM

NO_3^- concentrations in the WLD peak during the wet season when water discharge is high, and decrease during the dry season when discharge is low (Fig. 1). This observation indicates a strong relationship between NO_3^- yield in the upstream MARB and hydrological conditions. To quantify how NO_3^- flux responds to hydrological variations, we examined the concentration-discharge relationships of our samples. In various watersheds, these relationships for different solutes exhibit substantial variations due to hydrological and biogeochemical processes[30–33]. A 'dilution' scenario is characterized by decreasing ion concentrations with increasing water discharge, yielding a power-law exponent of −1[31]. In contrast, in a 'chemostatic' scenario, ion concentrations remain relatively constant despite changes in water discharge, resulting in a near-zero power-law exponent[34]. Notably, our data show that NO_3^- concentrations increase with water discharge (power-law exponent = 0.118, Fig. 2a). This pattern suggests that NO_3^- becomes enriched under high discharge conditions in the Atchafalaya River, and a greater flow of NO_3^--concentrated water is exported to the northern GoM during the wet season (Fig. 2a). This is consistent with the observations in other rivers influenced by anthropogenic N, such as the Seine, Potomac, Connecticut and Mississippi rivers[32,35–38].

The most important finding from the isotope data is the clear distinction between the wet and dry seasons (Figs. 2d, 3). The $\delta^{15}N$ of both NO_3^- and PN are approximately 3‰ lower during the wet season compared to the dry season, suggesting a strong influence of hydrology on the N load to the GoM. Additionally, a statistically significant negative correlation was observed between $\delta^{15}N$-NO_3^- and water discharge ($r^2 = 0.33$, $p < 0.01$; Fig. 2c). These observations mostly likely

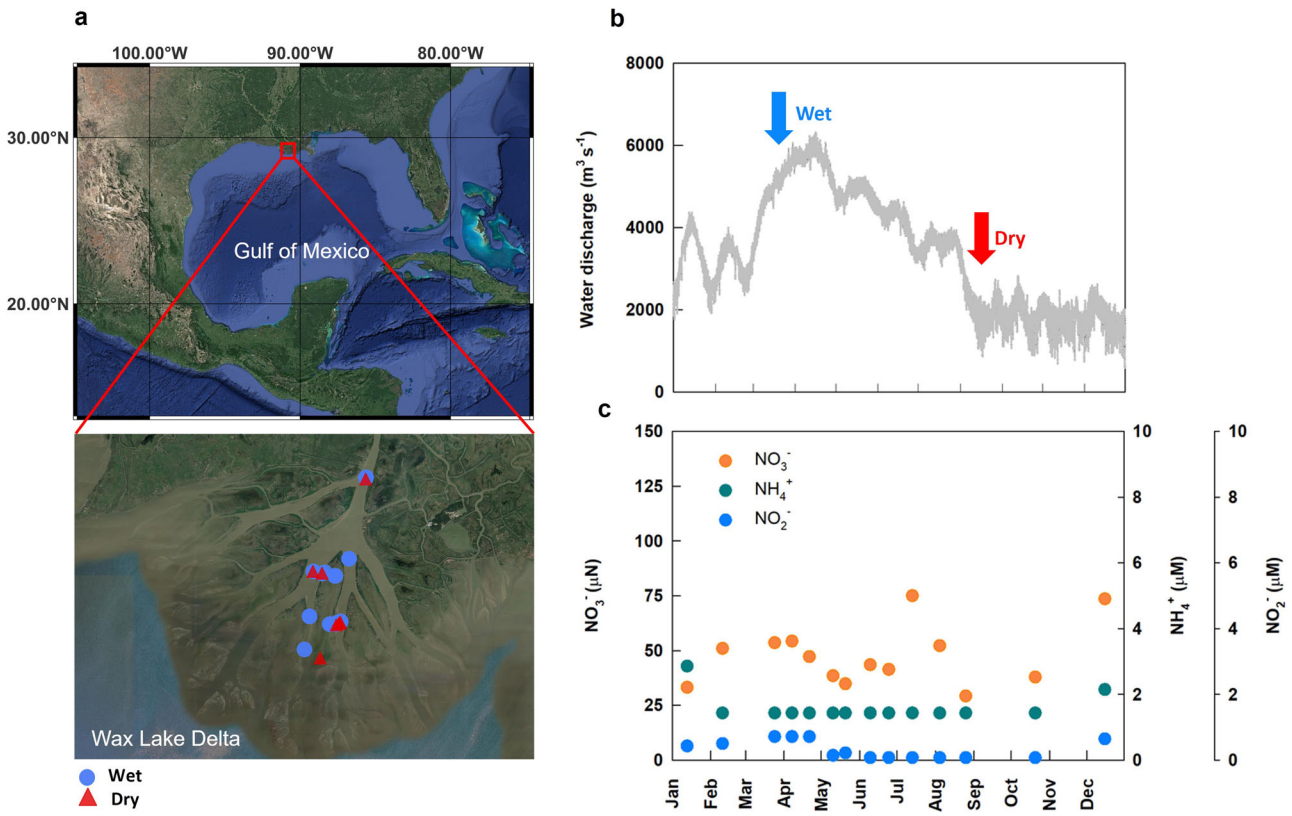

Fig. 1 | Hydrological and nutrient concentrations in the Wax Lake Delta. a Map of the study area in the Wax Lake Delta (WLD), Louisiana, USA. The blue symbols indicate the water and particle isotope sampling locations in the wet season, while the red symbols indicate the dry season. **b** Daily water discharge in 2021 ($m^3\ s^{-1}$), with the arrows indicating the sampling time for this study. **c** Monthly dissolved inorganic nitrogen (NO_3^-, NH_4^+, NO_2^-) in the Wax Lake Delta. Note that NH_4^+ concentrations are consistently lower than 1 μM throughout the year, indicating quantitative nitrification in the WLD watershed. Daily water discharge and nutrient data were obtained from U.S. Geological Survey (USGS). The locations can be found in Supplementary Fig. 1.

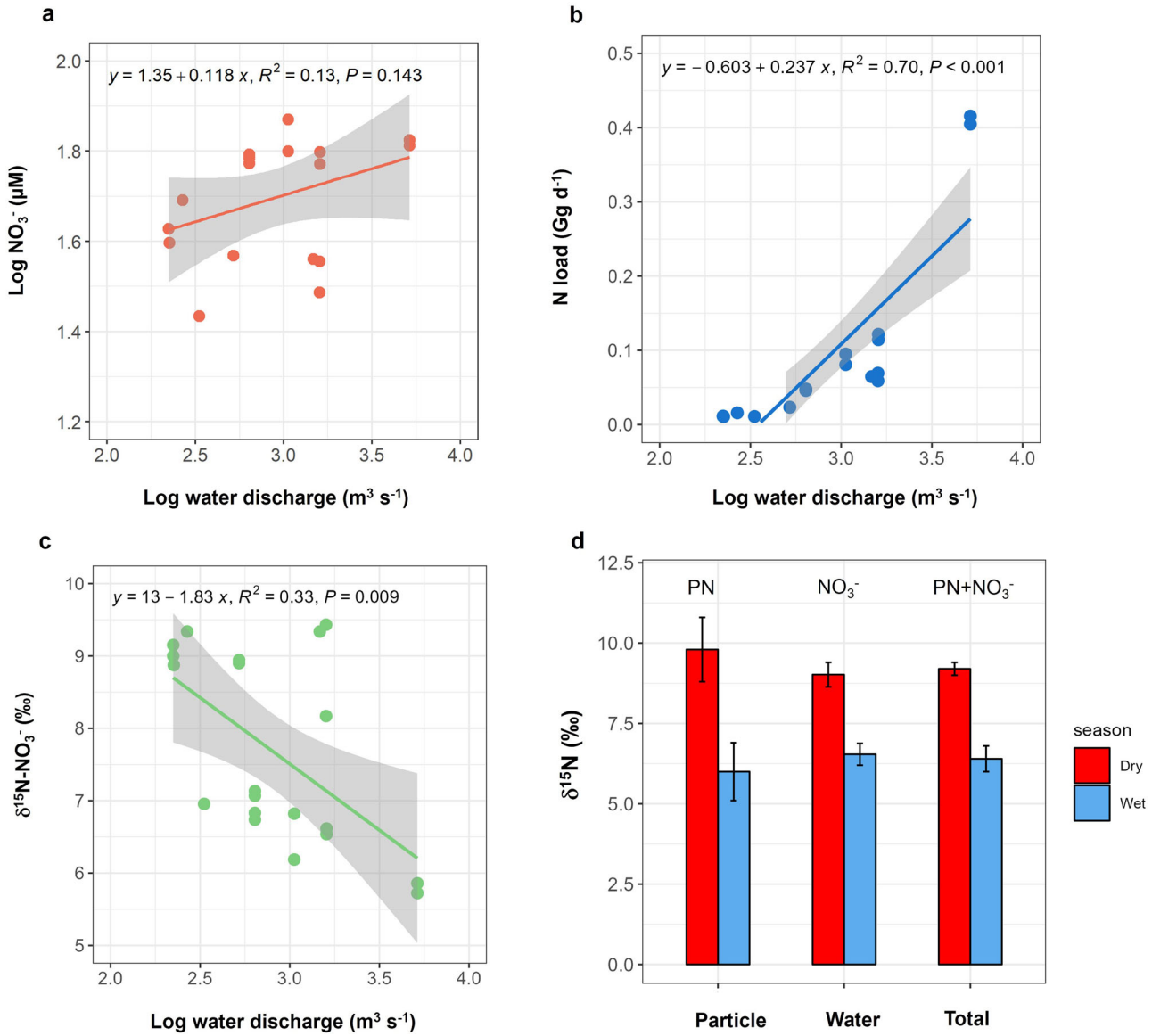

Fig. 2 | Nitrate dynamics and isotopic composition. Relationship between (**a**) log nitrate (NO_3^-) concentrations (μM) and log water discharge (m^{-3} s^{-1}) during the sampling period, (**b**) NO_3^- load (Gg d^{-1}) and water discharge (m^{-3} s^{-1}) during the sampling period, (**c**) $\delta^{15}N$-NO_3^- and log water discharge in the Wax Lake Delta during the wet and dry seasons and (**d**) seasonal $\delta^{15}N$ values of suspended particle, water and concentration weighted nitrate + PN samples. Solid lines indicate linear correlations. Gray shaded areas represent 95% confidence intervals. Error bars represent the standard deviations.

indicate that during periods of high water discharge, anthropogenic N with low $\delta^{15}N$ values was preferentially introduced into the WLD River. However, as biogeochemical processes might also alter the $\delta^{15}N$ values in the river, we first discuss the potential impact of river biogeochemical processes below.

Nitrogen biogeochemical processes in the Wax Lake Delta catchment

When utilizing isotopes to trace the sources of anthropogenic N in a river, it is essential to account for the biogeochemical processes within the river system that may alter the isotopic composition of N sources. As various forms of N, such as NO_3^-, NH_4^+, and organic N, enters the catchment, they undergo a series of N transformation processes influenced by environmental conditions[39,40]. Nitrification and NO_3^- consumption are the primary N transformation processes affecting NO_3^- dynamics in the river. Nitrification involves the oxidation of NH_4^+ to NO_3^-, while NO_3^- assimilation and denitrification consume NO_3^- [41–43]. In the case of the MARB, previous data suggests that NO_3^- is the dominant form of N in the river, which is consistent with our new data from the WLD river and observations from the U.S. Geological Survey in the lower Atchafalaya River at Morgan City (Fig. 1b)[8,14,44]. The water column of WLD is well oxygenated and NH_4^+ concentrations are two orders of magnitude lower than NO_3^- concentrations, suggesting near-complete nitrification and minimal alteration of the isotopic composition of the source N by nitrification or denitrification in the river (Supplementary Figs. 2, 3). Phytoplankton NO_3^- assimilation may influence the isotopic composition of source N in the river, as this process preferentially consumes ^{14}N relative to ^{15}N with an isotope effect of ~5‰[45]. This process is expected to cause a negative correlation between $\delta^{15}N$-NO_3^- and the natural log of [NO_3^-] due to Rayleigh-style fractionation[23,25]. Indeed, during the wet season, we observed this negative correlation observed between $\delta^{15}N$-NO_3^- and the natural log of [NO_3^-], indicative of NO_3^- assimilation in the river (Fig. 2b). However, the change in $\delta^{15}N$-NO_3^- observed along the river is only ~1‰, suggesting that NO_3^- assimilation has a limited impact on altering $\delta^{15}N$-NO_3^- (Fig. 3). Similarly, $\delta^{18}O$-NO_3^- also showed little change along the river within each season (Supplementary Fig. 3).

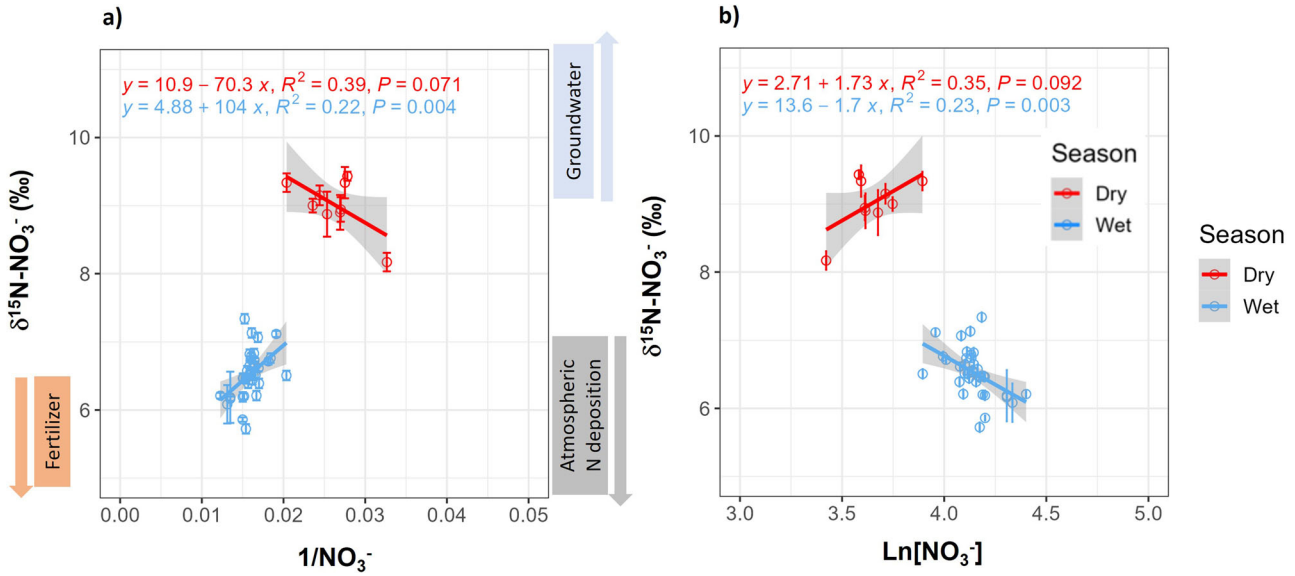

Fig. 3 | Isotopic analysis of nitrate in relation to concentration changes. Relationship between (**a**) δ^{15}N-NO_3^- and the inverse of nitrate concentrations ($1/NO_3^-$) and (**b**) δ^{15}N-NO_3^- and the natural log of NO_3^- concentrations (Ln[NO_3^-]). The δ^{15}N endmembers (fertilizer, atmospheric deposition, and groundwater)[67] were also plotted in **a**. Solid lines indicate linear regressions. Gray shaded areas represent 95% confidence intervals.

Table 1 | Sampling date, water discharge ($m^3\ s^{-1}$), nitrate concentration (μM), particulate nitrogen (PN; %), water ^{15}N-NO_3^-, δ^{18}O-NO_3^-, particle δ^{15}N (δ^{15}N-PN), and δ^{15}N of total N (δ^{15}N-TN)

Date	Discharge ($m^3\ s^{-1}$)	NO_3^- (μM)	PN (%)	δ^{15}N-NO_3^- (‰)	δ^{18}O-NO_3^- (‰)	δ^{15}N-PN (‰)	δ^{15}N-TN (‰)
2021/03/24–2021/03/31	1683	61.5 ± 9	0.21 ± 0.1	6.5 ± 0.3	2.2 ± 0.3	6.1 ± 0.9	6.4 ± 0.4
2021/08/17–2021/08/24	737	38.8 ± 5	0.43 ± 0.1	9 ± 0.3	3.1 ± 0.3	9.8 ± 1	9.2 ± 0.2

In addition, we calculated the concentration-weighted δ^{15}N of NO_3^- plus particulate nitrogen (PN). Since phytoplankton NO_3^- assimilation solely converts NO_3^- into PN, the concentration-weighted δ^{15}N of the PN + NO_3^- should remain unaffected by phytoplankton NO_3^- consumption. Therefore, this parameter predominantly reflects the δ^{15}N of the N sources to the WLD. The average concentration-weighted δ^{15}N-(PN + NO_3^-) values were 6.4 ± 0.4‰ during the wet season and 9.2 ± 0.2‰ during the dry season, similar to those of ^{15}N-PN and δ^{15}N-NO_3^- in the dry and wet seasons, respectively (Fig. 2; Table 1). These results corroborate remarkable seasonal change in the δ^{15}N of the N sources to the WLD (Table 1).

Thus, we conclude that although NO_3^- assimilation occurs in the WLD, its impact on the δ^{15}N of both NO_3^- and PN appears to be limited. The concentration-weighted δ^{15}N-(PN + NO_3^-) is insensitive to NO_3^- assimilation and should therefore reflect the δ^{15}N of N sources. Consequently, the lower δ^{15}N values observed during wet season most likely indicate additional inputs of anthropogenic N with low δ^{15}N values. Synthetic N fertilizer is the most probable candidate for this low-δ^{15}N anthropogenic N source[23], as its application and transformation produce low-δ^{15}N values in both NO_3^- and PN. In the following section, we provide a detailed discussion of anthropogenic N sources in the WLD.

Identification and quantification of nitrogen sources to the WLD and GoM

Our δ^{15}N-NO_3^- values align with those reported in previous N isotopic studies conducted in the MARB[27,46,47]. This study further reveals distinctive δ^{15}N patterns between the wet and dry seasons in the WLD. During the dry season, the WLD exhibits low N concentrations and high δ^{15}N values, suggesting that reduced surface runoff decreased NO_3^- loading from soil to river, and groundwater becomes the dominant N source in the study area. Indeed, a recent study demonstrated that groundwater can be the primary source of NO_3^- during low-flow periods across the United States[18].

In the MARB, groundwater N is likely derived from "legacy" fertilizer N, as a portion of applied fertilizer N is infiltrated into the groundwater through the floodplain soils[16,48]. However, the δ^{15}N of groundwater N is altered by denitrification under low-oxygen conditions, which preferentially removes ^{14}N relative to ^{15}N. As a result, although "legacy" fertilizer N may be the ultimate source of groundwater N, its original δ^{15}N signature is modified. Consequently, the high δ^{15}N values observed in the WLD during the dry season can be attributed to groundwater N[23]. Consistent with this interpretation, the relatively higher δ^{18}O-NO_3^- values during the dry season, compared to the δ^{18}O of the water in the MARB, likely result from denitrification in the groundwater[49–51].

In contrast, during the wet season, WLD is characterized by high N concentrations with lower δ^{15}N values, suggesting an influx of low-δ^{15}N N associated with high water discharge. There are two possible contributors of this low-δ^{15}N N source: fertilizer N and atmospheric deposition. However, the rates of atmospheric N deposition in the MARB region are estimated to be at least one order of magnitude lower than the riverine N flux, suggesting that the atmospheric N deposition should minimally impact the WLD δ^{15}N values[52]. Thus, fertilizer N is the most likely candidate for the low-δ^{15}N N source in the WLD during the wet season. Once applied, fertilizer N, can be integrated into the soil and vegetation (Fig. 4). Intense precipitation during the wet season can flush soil fertilizer N and organic matter into the river, leading to increased N load and decreased in δ^{15}N values of both δ^{15}N-NO_3^- and δ^{15}N-PN. Moreover, while manure and sewage are known sources of anthropogenic N in the region, their δ^{15}N values are generally high (i.e., >9–10‰) and do not explain the low δ^{15}N values observed during the wet season[20,53]. Consequently, we conclude that N sourced from surface

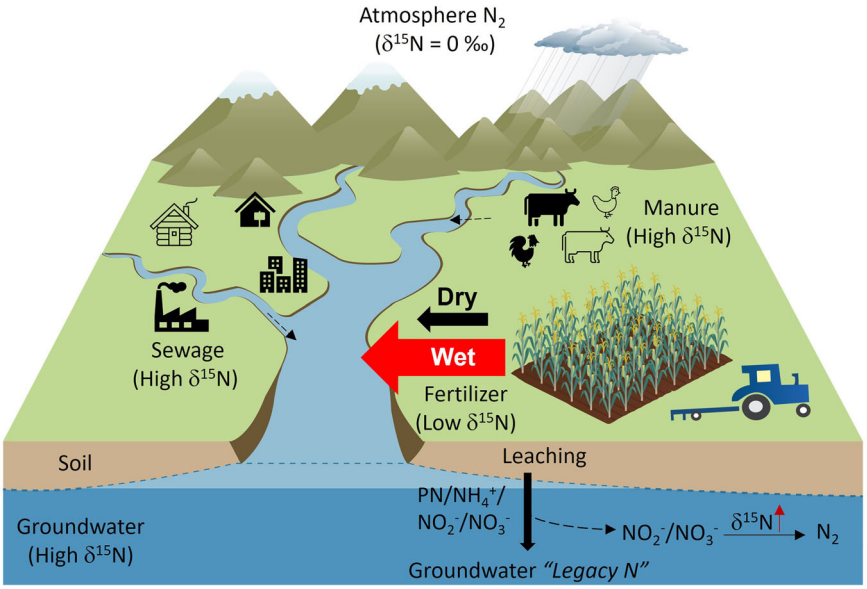

Fig. 4 | A cartoon showing the preferential input of fertilizer-N Input to the MARB during high water discharge. Groundwater legacy N indicates that historical fertilizer N application has leached into groundwater and accumulated within the system.

fertilizers, rather than from manure/sewage (high $\delta^{15}N$), is preferentially exported to the northern GoM during periods of high water discharge.

Based on discussions above, we used a simple isotope mixing model to quantify the contributions of N sources to the WLD (Methods). As manure/sewage N cannot be the cause of low $\delta^{15}N$ signature in the WLD during the wet season, we consider fertilizer and groundwater as the two primary sources to the N to the WLD in the model. Those sources carry distinct $\delta^{15}N$ values, allowing us to quantify their relative contributions to total N load in the WLD (Supplementary Fig. 4). This simple model calculation shows that in the wet season, the contributions from fertilizer (~45%) are slight lower than groundwater (~55%). In the dry season, groundwater (~91%) becomes the dominant N source and fertilizer contributed about (~9%) to the MARB.

A recent modeling study used a bottom-up, spatially explicit distributed model to describe N flow and processes in landscapes in 4 large river basins including the MARB[17]. It was estimated that, groundwater is the dominant source of nitrogen (>50%) in the Mississippi River due to the "legacy N" leached from historical fertilizer application in soils, consistent with our isotope-based top-down estimation in the WLD. One challenge acknowledged by the bottom-up modeling studies is that their sole method of validation is comparing their model outputs with N concentrations and discharge data in rivers. In this context, our isotope data provides new information to validate these model results. In addition, our data revealed that, compared to manure or sewage, fertilizer-derived N is preferentially transported to the WLD during periods of high water discharge. This may be attributed to the relative ease of fertilizer leaching compared to manure or sewage, a factor not yet considered in modeling studies. Future modeling efforts should incorporate different transport mechanisms for various anthropogenic N sources.

Potential applications to historical variations of N sources to the GoM

The $\delta^{15}N$-water discharge relationship observed in the WLD in this study, combined with the isotope mixing model, may enable the estimation of historical anthropogenic N sources in the northern GoM. To demonstrate this approach, we utilized historical N load data from 2010 to 2022 from Atchafalaya River and calculated the contributions of fertilizer and groundwater to the N load entering the GoM (Supplementary Fig. 5, Supplementary Data). The results show that during the wet season, the contribution of N from fertilizer source significantly increased to the overall N load (Supplementary Fig. 6). These findings collectively demonstrate that the N input from various sources are strongly influenced by hydrological conditions in the MARB.

While this approach show promise for quantifying N sources in the MARB, we acknowledge two limitations. First, although our sample set covers multiple sites and water depths in the WLD, it represents only two sampling campaigns and does not capture the full intra-annual variability of $\delta^{15}N$ in the MARB. We recommend future studies to focus on long-term isotope monitoring in the MARB to corroborate and extend the findings of this study. Second, the observed $\delta^{15}N$-discharge relationship might vary across the sub-watersheds and over time in the MARB. Collecting more N isotope data over longer periods would better quantify the spatiotemporal pattern of $\delta^{15}N$-discharge relationship across the MARB.

Implication for nitrogen management in the MARB

The expansion of the eutrophication and hypoxia zones in the northern GoM has been primarily attributed to substantial anthropogenic N loads originating from the MARB[6,8,44]. Despite the growing urgency to reduce N inputs, there remains a lack of adequate research on the transport mechanisms and timing of different anthropogenic N sources reaching the northern GoM. Although our study was limited to two hydrologic seasons within one year, our results provide evidence that fertilizer-derived N is preferentially transported into the GoM during periods of high water discharge, rather than N from manure or sewage N. Future studies should continue to monitor the isotopic composition in the MARB to further constrain the contribution of these anthropogenic N sources to the northern MARB.

Given that climate models project an increase in extreme precipitation events in the MARB under global warming[11,54,55], it is expected that more fertilizer N will be transported from the catchment into the northern GoM. To effectively mitigate and reduce N loading into the northern GoM, it is imperative to improve current N management strategies, and our study provides crucial information towards this goal. For instance, implementing measures such as avoiding N fertilizer application during periods of heavy precipitation, addressing groundwater NO_3^- contamination, and transitioning from monoculture practices (e.g., corn-soybean) to diversified crop systems that rely on N-fixation and require less fertilizer (e.g., incorporating cover crops) should be considered[12,17,56]. Previous work has shown that applied N fertilizer is not fully taken up by plants, with a notable portion being stored in the soil[57]. Therefore, optimizing the timing and efficiency of fertilizer application through strategic management necessary. Implementing these practices is expected to effectively reduce the N load into the northern GoM. Furthermore, conducting comprehensive studies at various temporal and spatial scales, using N isotopes, is crucial for a better understanding of the impact of fertilizer-derived N on N loading in the MARB and to inform future management efforts.

Methods

Study sites

This study was carried out in the Wax Lake Delta (WLD), Louisiana, United States (29.51033°N, 91.4449°W) (Fig. 1a), a relatively modern river-dominated subdelta of the Mississippi and Atchafalaya river systems[28,58]. The Wax Lake Outlet was dredged in 1941 by the U.S. Army Corps of Engineers for flood protection purposes, supplied the WLD with fluvial water and sediment from Mississippi-Atchafalaya River Basin[59]. Annual water discharge was about 3605 m^3 s^{-1} in the WLD and as measured at USGS Calumet, LA, with high water discharge from January to July (wet season) and low water discharge from August to December (dry season) (Fig. 1b)[60,61]. The MARB has been heavily influenced by anthropogenic activities over the last century, which leads to the export a substantial amount of N to the northern Gulf of Mexico[6,54]. To test the whether the water column is well-mixed, we collected water samples from multiple sites and multiple depth during each sampling trip, and no significant differences were observed. This indicates the water column was well-mixed with no significant depth variation, as indicated by the $\delta^{15}N$ results (Supplementary Fig. 7).

Sample collection

Samples and flow data were collected by the NASA Delta-X project. Field sampling in WLD was carried out from March 24–31 (wet season) and August 17–24 (dry season), in 2021. Water discharge and temperature data were collected using a Teledyne RiverPro acoustic Doppler current profiler that was calibrated before the cruise[62]. River water and rainwater samples for nutrient and isotope analysis were collected with a syringe and filtered through a Millipore polytetrafluoroethylene (PTFE) filter (0.2 µm) into a high-density polyethylene bottle (40 ml). The bottles were acid washed in 10% HCl, rinsed, and dried prior to each use. All water samples were stored frozen at −20 °C until analysis. For collecting suspended particulate samples, 8 L of river water was collected using a Van Dorn sampler[63]. Within 24 h after collection, each water sample was filtered through 0.22 µm PES (Polyethersulfone) membrane filters at the field lodging station. The PES filters with suspended particulate samples on them were kept frozen and transported back to the California Institute of Technology. In the laboratory, the suspended particles were separated from the filter by sonicating in MilliQ water and scratching gently using a spatula. The separated sediment samples were oven-dried at 60 °C. The dried samples were ground and homogenized using a mortar and pestle. Splits of each sample were taken for total N and $\delta^{15}N$-PN analysis. In addition to field sampling, the water discharge (daily-monthly resolutions) and nutrients data (monthly resolution) from the Morgan City gaging station (USGS gage 07381600) located downstream of the Atchafalaya River, were used in this study.

Sample analyses

NO_3^- concentrations in the water samples were measured by the chemiluminescence method[64]. Briefly, NO_3^- was reduced to nitric oxide using a vanadium (V^{3+}) solution at 95 °C. The nitric oxide was then quantified with a Teledyne 200 EU chemiluminescence analyzer. The precision of this method is better than 5%. Measurements of $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^- in WLD water samples were performed in the Stable Isotope Biogeochemistry Lab at Boston College, using the "denitrified method" described by Sigman et al.[65,66]. The denitrified method uses cultured denitrifying bacteria (*P. aureofaciens*) that lack the nitrous oxide reductase activity, which quantitively converts NO_3^- to N_2O gas analyte. The N_2O produced from each sample were extracted, concentrated, and purified using a customized Gas Bench and analyzed on a Thermo Delta V plus continuous flow isotope ratio mass spectrometer (IRMS). The international reference materials IAEA ($\delta^{15}N$ = 4.7‰; $\delta^{18}O$ = 25.6‰) and USGS 34 ($\delta^{15}N$ = −1.8‰; $\delta^{18}O$ = −27.9‰) were analyzed in each batch of samples and used for isotope data correction[29]. All stable isotope compositions are expressed as δ values, representing deviations in per mil (‰) from standards for $\delta^{15}N$-NO_3^- and $\delta^{18}O$-NO_3^-.

$$\delta_{\mathrm{sample}}\,(‰) = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (1)$$

Where R_{sample} and $R_{standard}$ represent the measured isotopic ratios (e.g., $^{15}N/^{14}N$ or $^{18}O/^{16}O$) for the sample and standard, respectively. The ratio of $^{15}N/^{14}N$ reference is N_2 in the air and the $^{18}O/^{16}O$ reference is Vienna Standard Mean Ocean Water (VSMOW). Analytical precision for international reference materials for $\delta^{15}N$-NO_3^- was better than 0.2 ‰, and for $\delta^{18}O$-NO_3^- was better than 0.3 ‰.

For PN and $\delta^{15}N$-PN measurements, splits of suspended sediment samples were analyzed using an Elemental Analyser Isotope Ratio Mass Spectrometry at the Marine Science Institute at the University of California Santa Barbara. Analytical uncertainties were within 0.17‰ for $\delta^{15}N$ and 2% (relative percentage error) for the weight percentage of PN from repeated measurements of USGS-40 reference materials.

Isotope mixing model calculations

We employed a simple two end-member isotope mixing model to quantify the contributions of nitrate from fertilizer and groundwater sources in the WLD. During the dry season, low N concentrations and high $\delta^{15}N$ values suggest that reduced surface runoff decreased NO_3^- loading from soil to river, and groundwater becomes the dominant N source in the study area. In contrast, during the wet season, lower nitrate and PN $\delta^{15}N$ values indicate that fertilizer-derived N (characterized by low $\delta^{15}N$) is preferentially transported into the WLD during periods of high water discharge, rather than N from manure or sewage (which have high $\delta^{15}N$). Therefore, our isotopic mixing model considers only fertilizer and groundwater as primary N sources in the WLD. Manure and sewage were excluded from this model because they cannot account for the lower $\delta^{15}N$ observed during the wet season. This is consistent with previous studies that suggest manure and sewage contribute minimally to the total nitrogen load compared to fertilizer and groundwater in the MARB[6,20]. Consequently, the mixing model is described by the following equations:

$$f_e + f_g = 1 \quad (2)$$

$$f_e \times \delta^{15}N_e + f_g \times \delta^{15}N_g = \delta^{15}N \quad (3)$$

where f is defined as the proportional contribution to N from the respective sources. Subscripts e and g represent the NO_3^- sources from fertilizer (e) and groundwater (g), respectively. $\delta^{15}N$ represent the isotope values. Fertilizer $\delta^{15}N$ values were obtained from the literature (0 ± 2.1‰). Recent studies suggest that groundwater contributes significantly to NO_3^- flux during winter low-flow conditions in the United States[18]. Therefore, we used dry season $\delta^{15}N$ values as the groundwater endmember in the mixing model. These values are comparable to previously analyzed groundwater $\delta^{15}N$ values in the MARB, which averaged 9.8 ± 4.9‰, as well as to global nitrate isotope composition in groundwater[67]. To estimate the uncertainty of this two end-member isotope mixing model, we incorporated the variability of $\delta^{15}N$ values measuring during the dry and wet seasons, along with those obtained from the literature and their associated errors.

Calculation of historical variations in N sources in the Atchafalaya River

The $\delta^{15}N$-water discharge relationship obtained from WLD may enable the estimation of historical anthropogenic N sources in the northern GoM. As an example, we applied this relationship to historical N load and discharge data from 2010 to 2022 from Atchafalaya River to calculate the contributions of fertilizer and groundwater to the N load entering the GoM (Supplementary Fig. 5, Supplementary Data). First, the historical N load was calculated by multiplying the historical water discharge by NO_3^- concentrations. obtained from USGS. Second, we used the correlation between water discharge and

$\delta^{15}N$ values to estimate the historical $\delta^{15}N$ values. Next, we applied the isotope two end-member mixing model to determine the N load originating from fertilizer and groundwater sources (Supplementary Fig. 6). While this exercise has inherent uncertainty due to our limited data set, this approach can be extrapolated to more accurately quantify different N sources from the MARB to the GoM as more isotope data becomes available, especially when integrated with more complex biogeochemical models[5,6].

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data sets are available within the Supplementary Data and https://daac.ornl.gov/cgi-bin/dataset_lister.pl?p=41.

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Author contributions

X.T.W. and G.K.L designed the study. G.K.L, J.A.N. and M.P.L. collected the samples used in the study. J.J.C., M.L., T.K. and H.D. analyzed the nitrate isotopes in the Stable Isotope Biogeochemistry Lab at Boston College. G.K.L. and Z.Y. analyzed the particulate nitrogen content isotopes at University of California, Santa Barbara. J.J.C. and X.T.W. wrote the manuscript. All authors contributed to the interpretation of the data and provided input to the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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