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Quantification of Nitrate Sources to an Urban Stream Using Dual Nitrate Isotopes

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Supporting Information

ABSTRACT: Human-engineered landscapes and subsequent altered hydrology affect the fate and transport of reactive nitrogen, particularly in urban watersheds. In this study, we used dual-nitrate isotopes and mixing model analysis ($\delta^{15}N$ and $\delta^{18}O$ of NO_3^-) to quantify nitrogen inputs from two sources concentrated in urban systems, sewage and atmospheric deposition. Analysis was conducted on samples collected from Nine Mile Run (Pittsburgh, PA) including over two years of samples collected biweekly and samples collected through the hydrographs of four storm events. Mixing models incorporated uncertainties in the isotopic composition of potential nitrate sources and resolved the relative proportions of nitrate inputs from each source using Bayesian techniques. The results indicate that up to 94% of nitrate in streamwater originated from sewage sources during baseflow conditions. During storms, atmospheric deposition was a substantial nitrate source (~34%) to total event-based nitrate loads, although sewage-derived nitrate remained the dominant source (66%). The potential influence of denitrification was considered by incorporating associated isotopic fractionations into mixing models; up to 19% of sewage-derived samples showed the isotopic effects of denitrification. This study quantitatively delineates proportions of nitrate from different sources to urban streamwater, while incorporating remaining uncertainties in source endmember compositions.

INTRODUCTION

Human-built environments transform hydrologic processes in urban systems by rerouting surface waters, altering topographic variation, influencing local vegetation, microclimate, and atmospheric chemistry, and increasing loading of water pollutants. In particular, urban streams can be partially or completely buried, isolated from groundwater sources, and augmented by sewer and water infrastructure leaks,[1] all of which can contribute to surface water degradation. Surface water impairments can be further compounded by pollutants directly routed to surface water by impervious surfaces and drainage systems.[2] Urban riparian zones may be significantly altered or nonexistent and thus may limit important ecosystem services such as nutrient processing expected in less disturbed systems.[3] As a consequence of these perturbations, urban landscapes and streams with altered hydrology and biogeochemical processes are challenging to characterize with models developed in forested or agricultural areas.[4]

Recognizing the sources and dynamics of biologically available, dissolved nitrogen (i.e., reactive nitrogen) in urban streams is important for nutrient management and groundwater protection in and downstream of urban centers[5−7]. Dissolved inorganic nitrogen (DIN: the sum of ammonium, nitrate, and nitrite) usually comprises over 75% of the total dissolved nitrogen load in streamwater in urban waterways.[8,9] Urban streams show particularly high concentrations of nitrate (>3 mg L^{-1}) in comparison to other species of DIN.[8−13] High concentrations of nitrate indicate a supply that is far greater than the biological demand, a condition referred to as "nitrate hypersaturation" that can have extreme negative consequences for aquatic ecosystems.[8] An important part of addressing problems of hypersaturation in affected streams is understanding the contribution from various nitrate sources and the dynamics particular to each source.

Nitrate in urban areas is derived commonly from sewage and atmospheric deposition,[11,12,14] two sources concentrated in regions with high population densities and significant amounts of human-engineered landscapes. Headspace within sewers provides aerobic conditions necessary for mineralization of organic matter and nitrification of organic N in sewage,[15,16] and aerobic biodegradation in unsaturated zones surrounding sewer leaks creates conditions that are favorable toward converting ammonium to nitrate.[17] Nitrate originating from sewers is often assumed to be directed to streams primarily through sanitary and combined sewer overflows[18] or from wastewater treatment point sources,[20] yet aging, leaking sewer infrastructure

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also contributes potentially significant nitrate loads to urban streams.[21] Atmospherically deposited nitrogen includes both wet (dissolved nitrate (NO_3^-) and ammonium (NH_4^+))[22] and dry atmospheric deposition (particulate nitrate and gaseous nitric acid (HNO_3^-)[23] forms). In urban systems, wet atmospheric deposition combines with accumulated dry atmospheric deposition and is flushed into the stream during storm events.[11,24]

Dual nitrate isotopes have been used to distinguish nutrient sources to urban streams in storms and baseflows.[11,12,24−26] Isotopes of nitrogen and oxygen can distinguish atmospherically deposited nitrate (δ^{15}N: −11‰ to +3.5‰; δ^{18}O: +63‰ to +94‰) from sewage derived nitrate (δ^{15}N: 0‰ to +20‰; δ^{18}O: −15‰ to +15‰).[27,28] Not only do nitrate isotopic compositions reflect nitrate sources, but also they can record transformations of nitrate during transport. For example, increasing δ^{15}N/δ^{18}O values at a ∼ 2:1 ratio indicate denitrification processes, particularly when sample pool nitrate concentrations are decreasing.[29−31] Results from dual-nitrate isotope analysis can be used in mixing models to estimate the proportion contributed by each source;[28] however, due to the ranges of isotopic values observed for each source, mixing model results have large uncertainties.[12] Mixing models utilizing Bayesian techniques, which incorporate this uncertainty by design, can clarify the relative importance of sources and processes.[32−34]

Prior analysis of nutrient budgets in Nine Mile Run (NMR) watershed (Pittsburgh, PA) suggested substantial sewage-sourced nitrogen inputs to stream fluxes, resulting in higher estimates of retention rates than in other studies.[21] Here, our research question was focused on delineating the proportional contribution from sewage and atmospherically derived nitrogen (ADN) to streamwater nitrate concentrations during baseflows and stormflows. This study builds on previous work by attributing in-stream nitrate loads to sources via dual nitrate isotopes and mixing model analysis for two years of biweekly sampling and multiple periods of storm flow.

STUDY LOCATION AND METHODS

NMR is one of the few remaining above-ground streams in Pittsburgh, draining a 1570 ha urban watershed with 38% impervious cover.[35] The upper portions of NMR are buried (Figure S1, Supporting Information), with the stream re-emerging aboveground 3.5 km upstream of the Monongahela River. Human populations in the NMR watershed are served by both sanitary (52% of the total watershed area) and combined sewer systems (36%), with remaining areas (12%) in parkland.[35] Each sewer system is designed to direct waste from households and businesses directly to the sewage treatment plant in dry weather, while in wet weather, combined sewers are designed to direct mixed sewage and stormwater fluxes to rivers and streams.[19]

Field Sampling. Sampling was conducted biweekly between April 2007 and December 2008 at three sampling locations forming a longitudinal transect along NMR (Figure S1, Supporting Information). NMR1 is approximately where the stream emerges from underground storm sewers. NMR2 is located ∼50 m below a combined sewer input. NMR3 is located at the mouth of the watershed. A small ephemeral stream, Fern Hollow (FH), was also sampled when flowing (Figure S1, Supporting Information). Stormflows were grab-sampled at NMR2 during one summer storm (Storm 1: July 20, 2008). Three subsequent storms were sampled at a location ∼50 m below NMR2 with an ISCO 6712 autosampler. Storms sampled at this site include one additional summer storm (Storm 2: July 9−10, 2010) and two winter storms (Storm 3: January 1, 2011; Storm 4: March 22−23, 2011) (Table 1). Stormflow samples were collected before the rainfall began and at intervals throughout the storm (Table 1). Storm water samples were stored frozen until filtered in the lab.

During sampling, instantaneous discharges were measured at each site using the area velocity method. In addition, daily average and 15 min average discharge data (6/14/2006−9/30/2009) were obtained from USGS station 03085049 (Figure S2, Supporting Information). The USGS program "PART" was used for hydrograph separation (storm versus base flows) of the USGS daily average discharge record for 2007 and 2008.[36] During subsequent storm events, a pressure transducer was installed in a stilling well and a rating curve was developed from discharges measured with the area velocity method. Manual discharge measurements were compared with simultaneous stage measurements, and the resulting relation was applied to the continuous stage record (Figure S2, Supporting Information). During Storm 4, the data logger was inadvertently full due to a false download and did not record stages. Discharge during individual sampling events and storms was categorized as "baseflow" or "stormflow" via visual analysis of stream hydrographs from 15 min average discharge data from the USGS station, storm hydrographs recorded by the pressure transducer, or direct observation of stream conditions during sampling. Precipitation data was obtained from 3 Rivers Wet Weather.[37] Dry atmospheric deposition was measured at the Laurel Highlands (LRL117) dry deposition (CASTNET) monitoring site, 75 km from Pittsburgh. Wet deposition was measured at the Piney Reservoir (MD08) National Trends Network (NTN) precipitation monitoring site, 115 km from Pittsburgh.

Biweekly bulk anion samples were vacuum-filtered (0.2 μm nylon filters) within 24 h of collection. Storm samples were frozen immediately and then filtered prior to subsequent analyses. Filtered samples were placed in HDPE bottles and either refrigerated (IC analyses) or frozen (isotopic analyses). Nitrate (NO_3^-) concentrations were measured on a Dionex ICS2000 Ion Chromatograph. Nitrite (NO_2^-) concentrations were measured on a Thermo Scientific Evolution 60S UV−visible Spectrophotometer to evaluate potential interference during isotopic measurements of nitrate.[38]

Isotopic Analysis. Biweekly stream samples for isotopic analysis were filtered in the field (0.2 μm nylon filters) into HDPE bottles triply rinsed with 18 MΩ water during sampling and frozen until subsequent analysis. Storm samples were filtered in the lab and similarly frozen until analysis. In samples with nitrite-N concentrations >3% of total nitrate-N + nitrite-N, aliquots of the sample were pretreated with sulfamic acid to remove nitrite, a potential interference during nitrate isotopic analysis.[38] For isotopic analysis of δ^{15}N and δ^{18}O, a denitrifying bacteria, *Pseudomonas aureofaciens*, was used to convert 20 nmoles of nitrate into $N_2O_{(g)}$, purified in a series of chemical traps and cryofocused.[39−41] The resulting gases were analyzed using an Isoprime Continuous Flow Isotope Ratio Mass Spectrometer (CF-IRMS) equipped with a Gilson GX271 autosampler and a Trace Gas system at the University of Pittsburgh *Regional Stable Isotope Laboratory for Earth and Environmental Science Research*.

Samples are reported relative to Standard Mean Ocean Water (SMOW) for δ^{18}O and atmospheric N_2 (for δ^{15}N):

Table 1. Sampling Data and Results for the 4 Storms[a]

storm event	total rainfall amount (mm)	storm duration	sampling frequency	discharge method	sampling begins	sampling ends	average NO_3^--N (mgL^{-1})	NO_3^--N range (mgL^{-1})	average $\delta^{15}N$ (‰)	$\delta^{15}N$ range (‰)	average $\delta^{18}O$ (‰)	$\delta^{18}O$ range (‰)
1	9.7	1 h 15 min	1/2 h for 1.5 h, then every hour	USGS gauge	7/20/2008 2:15 PM	7/20/2008 7:30 PM	1.0 ± 0.1	0.9–1.1	5.6 ± 0.8	3.9–8.9	28.4 ± 3.0	24.7 to 32.8
2	25.1	6 h 15 min	1/2 h for 6 h, then once an hour	pressure transducer	7/9/2010 2:55 PM	7/10/2010 7:25 AM	0.9 ± 0.05	0.4–1.4	7.3 ± 0.5	4.1–14.2	13.3 ± 1.3	1.0 to 25.7
3	12	6 h 15 min	1/2 h for 6 h, then once an hour	pressure transducer	1/1/2011 3:44 AM	1/1/2011 6:14 PM	0.6 ± 0.02	0.3–1.5	8.0 ± 0.3	6.4–11.4	14.8 ± 1.2	−2.9 to 31.8
4	18	21 h	once an hour	pressure transducer	3/22/2011 10:45 AM	3/23/2011 7:45 PM		0.3–2.3		5.2–10.3		−2.7 to 32.5

[a]Average concentrations reported are discharge-weighted, and average isotope values are flux-weighted. No discharge data is available for Storm 4; therefore, just the ranges are reported.

$$\delta‰ = ((R_{Sample} - R_{Standard})/R_{Standard}) \times 1000$$

where R indicates the ratio of the less abundant isotope to the more abundant isotope (e.g., $^{18}O/^{16}O$). Samples were corrected using international reference standards USGS-32, USGS-34, USGS-35, and IAEA-N3; these standards were also used to correct for linearity and instrument drift. Analytical precision for international reference standards was 0.2‰ and 0.5‰ for $\delta^{15}N$ and $\delta^{18}O$, respectively. To evaluate the potential effect of mass-independent contributions of $\delta^{17}O$ to m/z 45, the increase in $\delta^{15}N$ was estimated by assuming a 1‰ increase in $\delta^{15}N$ corresponds to an 18.8‰ increase in $\delta^{17}O$[42]. Preliminary $\Delta^{17}O$-NO_3^- values, which were analyzed as part of a concurrent, ongoing study (n = 134, $\Delta^{17}O$ range = +0.01‰ to +27.6‰), suggest $\delta^{15}N$ values were 0.0‰ to 1.5‰ lower than uncorrected values. This range of correction factors is small relative to the range of observed values for $\delta^{15}N$ (+2.5‰ to +19.4 ‰); thus, we do not correct for mass-independent contributions of $\delta^{17}O$ to m/z 45.

Markov Chain Monte Carlo Mixing Model Estimates of Source Contributions. Despite extensive characterization of DIN concentrations in NMR, the sources contributing and processes acting on nitrate remain uncertain. For this mixing model analysis, nitrate is assumed to be sourced from ADN, sewage-derived sources, or sewage-derived nitrate that has been partially denitrified. Lawn fertilizer-sourced nitrate was not considered a source in this model due to its unlikely transference to the stream.[12,42,43] Given uncertainty in the isotopic composition of potential endmembers, a Bayesian approach was used to solve the mixing model. Endmember isotopic compositions were defined as follows. $\delta^{15}N$ and $\delta^{18}O$ values in ADN were estimated from NMR precipitation samples collected over a range of seasons and were flux-averaged ($\delta^{15}N = +3 \pm 3‰$ ($n = 8$), $\delta^{18}O = +69.0 \pm 5$ ‰ ($n = 9$)). Sewage endmembers were based on literature values ($\delta^{15}N = +10 \pm 3‰$, $\delta^{18}O = -2 \pm 8‰$).[44,45] Denitrification enrichment factors reported in the literature were used to estimate $\delta^{15}N$ and $\delta^{18}O$ values for sewage-sourced nitrate that has undergone substantial denitrification.[29,31] Large standard deviations were assumed for the denitrified nitrate source to create a range of possible values that incorporates both 2:1 and 1:1 isotope enrichment trajectories[28,44] ($\delta^{15}N = +26 \pm 5‰$, $\delta^{18}O = +12 \pm 5‰$).

Recent advances in Monte Carlo Markov Chain (MCMC) methods for solving mixing models have been successfully used to infer food sources to organisms;[32,33,46] here, these methods are used to elucidate nitrate sources in a geochemical/isotope system. This mixing model analysis was implemented using the "SIAR"(Stable Isotope Analysis in R)[46,47] package where individual water samples were treated as "organisms" and nitrate sources were "food sources." Concentration dependencies were not required in the model; proportional source contributions were the goal. Fractional corrections were not incorporated as the denitrification endmember was estimated on the basis of prior observed fractionations. The SIAR defaults for prior probabilities of source proportions were used; i.e., these proportions were assumed to be vague and assigned a Dirichlet distribution.[46] The model was run for 500 000 iterations, with a burn-in of 5000. Chains were thinned by 15 and convergence evaluated with the diagnostics built into the SIAR package.

RESULTS

Discharge and Nitrate-N Concentrations during Storms. During baseflow and stormflow, nitrate-N concen-

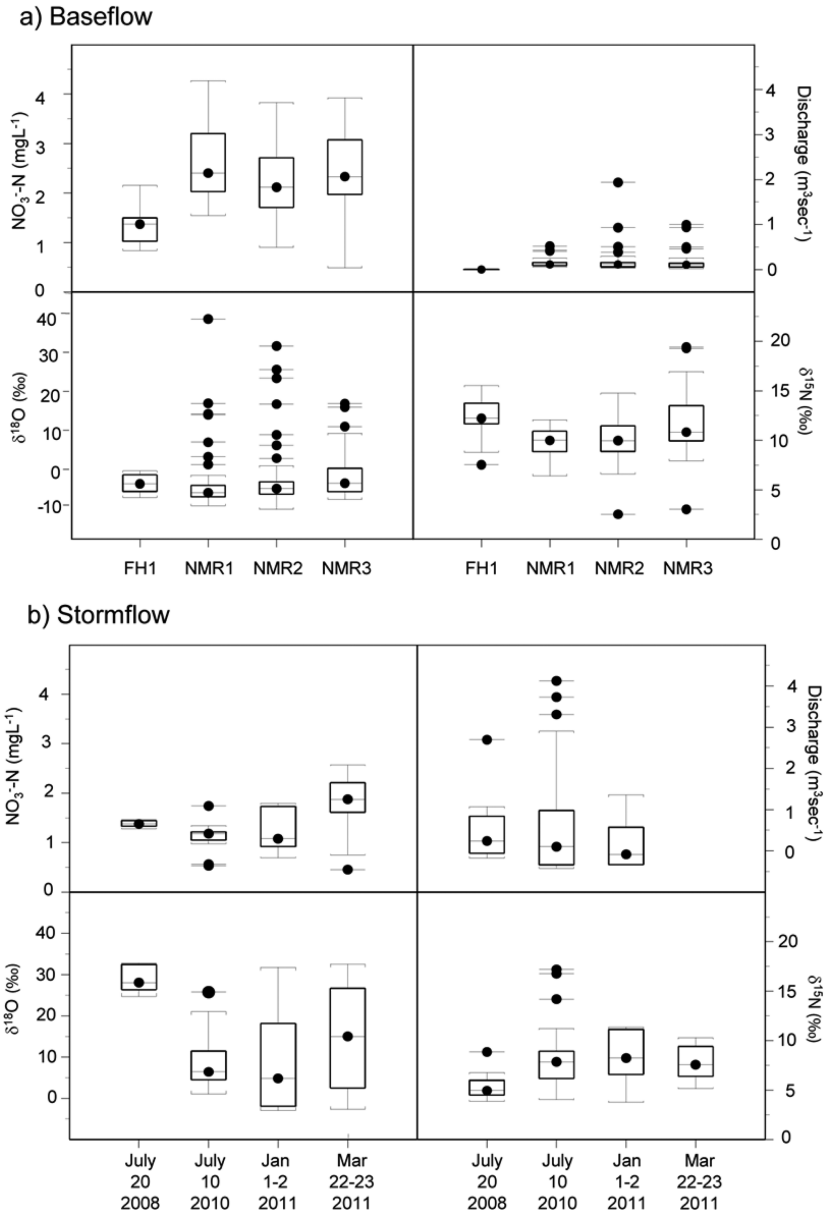

Figure 1. (a) Boxplots indicating median, quartiles, and outliers for each site during biweekly baseflow sampling. n = 38, 49, 38, and 12 for NMR1, 2, 3, and FH, respectively. (b) Boxplots indicating median, quartiles, and outliers for NMR2 during the four storms measured at that site.

trations were similar along the stream course (Figure 1). Discharge-weighted average nitrate-N concentrations were 2.1 ± 0.4, 1.7 ± 0.6, and 1.9 ± 0.7 at NMR1, 2, and 3, respectively, with lower concentrations at FH (0.8 ± 0.07).[21] Additional details regarding discharge and nitrate-N concentrations during baseflow conditions are reported in Divers et al.[21] Discharge-weighted average nitrate-N concentrations for individual storms ranged from 0.6 ± 0.02 (Storm 2) to 1.0 ± 0.1 mg L^{-1} (Storm 1) (Table 1). Nitrate-N concentrations varied across a small range during Storm 1 (0.9−1.1 mg L^{-1}), whereas larger ranges were observed during Storms 2−4 (0.3−2.3 mg L^{-1} (Figure 1)).

Nitrogen and Oxygen Isotope Values during Baseflow and Storms. During baseflows, the δ^{15}N and δ^{18}O values at all sites indicate that the nitrate was sourced primarily from sewage[28](Figure 2). The range in baseflow δ^{15}N values was +6.4‰ to +12.1‰, +2.5‰ to +14.2‰, and +3.0‰ to +19.4‰, at NMR1, 2, and 3, respectively (Figures 1, 2). δ^{18}O values measured during biweekly sampling, which generally captured baseflows, were as low as −2.7‰ (NMR2) and as high as +43.4‰ (NMR1); however, this high sample was taken during higher flows (0.4 m^{3}s^{-1}). Biweekly δ^{18}O values ranged from −1.9‰ to +43.4‰, −2.7‰ to +36.9‰, −0.3‰ to +22.9‰, and +0.1‰ to +19.8‰ at NMR1, 2, 3, and FH, respectively (Figures 1, 2).

δ^{15}N values at NMR2 varied during stormflows (Table 1, Figures 1, 2) where the widest range of isotopic compositions was observed in Storm 2 (13.1‰) and the narrowest range in Storm 1 (5.1 ‰). δ^{18}O values in stormwater nitrate ranged from +1.0‰ to +25.7‰ during Storm 2, −2.9‰ to +31.8‰ during Storm 3, and −2.7‰ to +32.5‰ during Storm 4 (Figure 1). A smaller range in δ^{18}O values was observed during Storm 1, where δ^{18}O values ranged from +24.7‰ to +32.8‰ (Figure 1). The flux-weighted average streamwater nitrate isotopic values measured in the two summer storms and one winter storm (with available discharge measurements) were similar (Table 1).

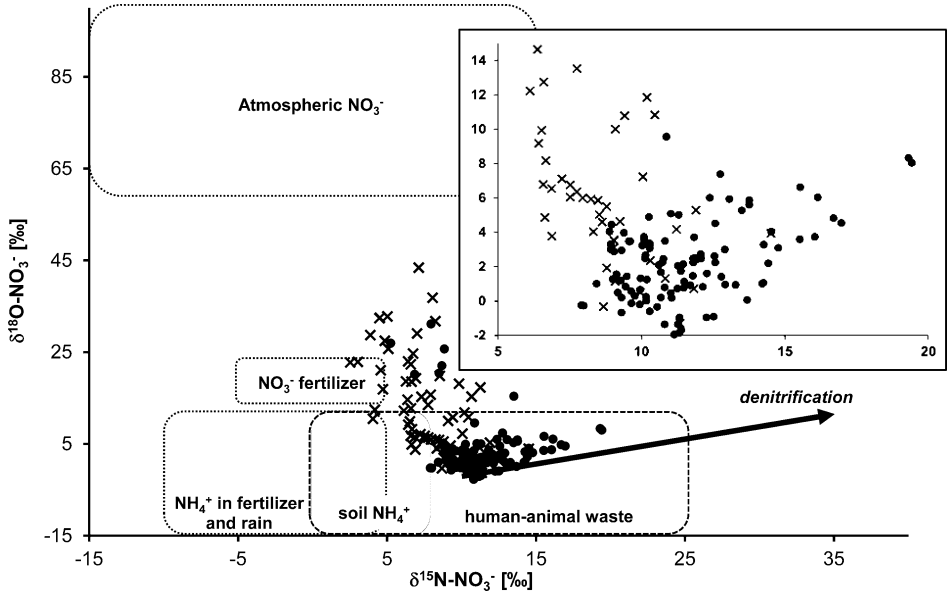

Figure 2. Results from dual isotope analysis of nitrate in baseflow (n = 141, solid dots) and high flow (n = 88, ×) samples. Flow regime was considered "high" as the result of precipitation events and classified by direct examination of the discharge record. Results from all sites (NMR1, 2, 3, and FH 1) are shown. Inset plot shows data cluster in "human-animal waste" section of plot. Figure adapted with permission from ref 28. Copyright 2007 Blackwell Scientific Publications.

MCMC Estimates of Source Proportion. The combination of high nitrate concentrations (Figure 1), high $\delta^{15}N$ values, and low $\delta^{18}O$ values indicates that baseflow nitrate flux was derived primarily from sewage (Figure 2). The SIAR mixing model estimates that sewage-sourced nitrate contributes between 75% and 87% to total baseline nitrate concentrations in the main stem of NMR and 72% in FH (Figure 3). Although sewage contributions appear to decrease moving downstream, this change results from increasing contributions from the denitrification-influenced nitrate endmember (Figure 3). The observed relationship between $\delta^{15}N$ and $\delta^{18}O$ values suggests that the original nitrate source for the denitrification endmember is sewage-derived nitrate (Figure 2). The total percentage of sewage-sourced nitrate in NMR was 93%−94% at each site during baseflows (Figure 3) when the original source of denitrified nitrate is considered. In contrast, at all sites during baseflow, the nitrate contribution from ADN was minor, 6 ± 2% at NMR1, 7 ± 1% at NMR2, and 6 ± 1% at NMR3 (Figure 3).

In comparison, streamwater nitrate during storms has substantial ADN contributions, although the dominant source is still sewage. SIAR results indicate ADN contributions during stormflow of 34 ± 3% of the total nitrate load to NMR2 where the remaining 66 ± 3% is derived from sewage sources (Figure 3). The influence of ADN is evident by higher flux-weighted average $\delta^{18}O$ values and lower $\delta^{15}N$ values at NMR2 relative to those observed during baseflow (+13.3‰ to +28.4‰ and +5.6‰ to +8.0‰ for $\delta^{18}O$ and $\delta^{15}N$, respectively during storms) and indicates mixing between atmospheric and sewage-derived nitrate sources. Sewage contributions during both storm and baseflow likely result from both leaks in the sanitary sewer system[21] and direct inputs from the combined sewer during wet weather.

Although samples were not intentionally collected throughout storm hydrographs at sites NMR1 and NMR3, several high flows were captured during biweekly sampling (n = 8 at NMR1 and n = 7 at NMR3, Figure 2). During these high flow events, NMR1 has a flux-weighted average $\delta^{15}N$ value (+7.7 + 0.5‰) that is lower than the flux-weighted average baseflow value (+9.1 ± 0.2‰) and $\delta^{18}O$ values that are higher during stormflow (+18.8 ± 3.8‰) than during baseflows (+5.6 ± 0.5‰). The lower $\delta^{15}N$ and higher $\delta^{18}O$ values at NMR1 during higher flows indicate a contribution from ADN to this site from storm sewers. In contrast, at NMR3, storm flow flux-averaged $\delta^{15}N$ was +8.0 ± 0.8‰, and $\delta^{18}O$ was +6.9 ± 1.7 ‰, values that overlap with the baseflow values (Figure 2).

DISCUSSION

Export and Flux Estimates by Nitrate Source. Export and flux calculations helped to determine the influence of each source on streamwater loads in different flow regimes. To confirm mass balance analysis that inferred significant inputs of nitrate derived from leaking sewers,[21] dual nitrate isotopes data were combined with flux data to estimate total flux of nitrate. The total export of nitrate from each source was calculated for the years 2007 and 2008 by determining total discharge in base and storm flows, flow-weighted average nitrate concentrations during base and storm flows for each year, and the average proportion of nitrate source during each flow regime.

With this approach, results indicate that 3.5 and 2.7 kg $ha^{-1}yr^{-1}$ of sewage-sourced nitrate was exported from the NMR watershed in 2007 and 2008, respectively. This is similar to previously estimates of sewage-sourced NO_3^--N export for the same years (2007:3.9−5.1 kg $ha^{-1}yr^{-1}$, 2008:2.4−2.7 kg $ha^{-1}yr^{-1}$)[21] based on mass balance models alone. Importantly, the dual isotope data and flux calculations presented here confirm the dominance of sewage-sourced nitrate to DIN loads in NMR streamwater. This observation substantiates the conclusion that sewers leak and sewage-sourced nitrate is not solely from combined sewer overflows. While some disagree with the inference of sewer-derived DIN and exfiltration of sewage from pipes based on mass balance approaches,[48] the isotopic evidence strongly supports this conclusion.

Dual isotope data was also used to calculate the amount of ADN-sourced nitrate exported from the NMR watershed. Total

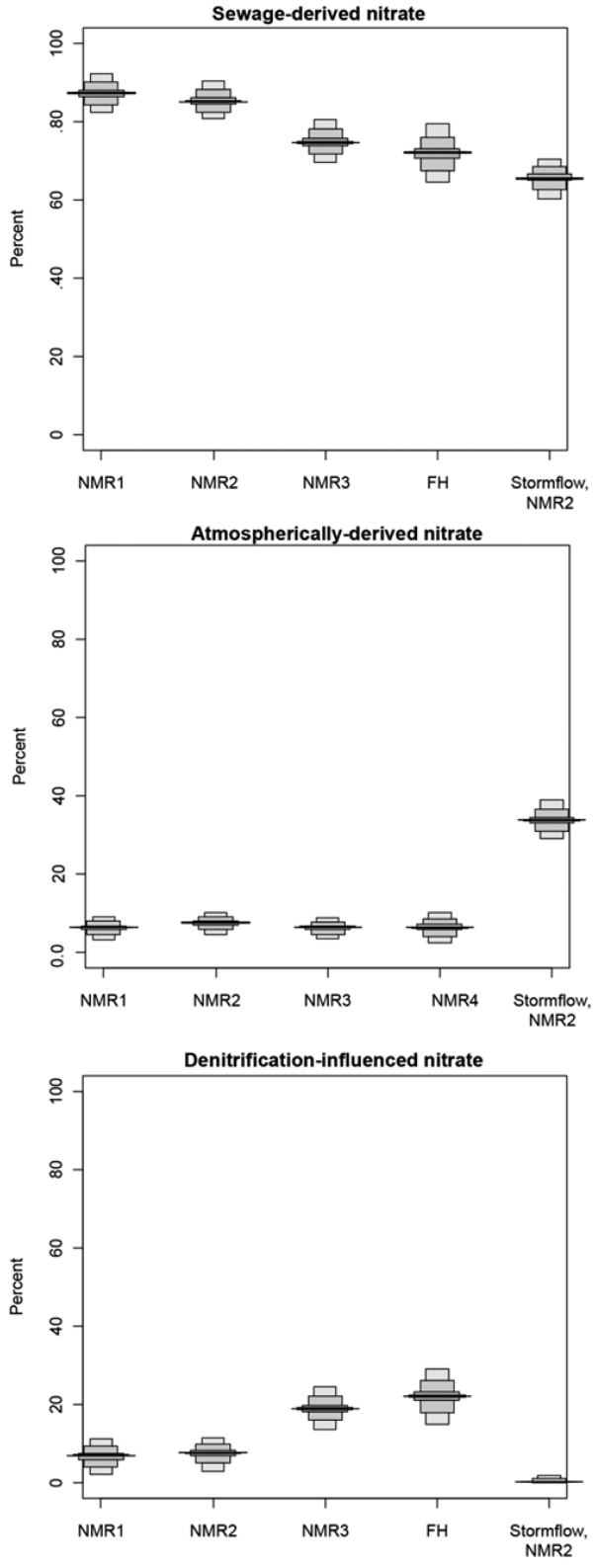

Figure 3. Boxplots of mixing model proportions for each site, categorized by source, during baseflow. Shown are the 5%, 25%, 75%, and 95% Bayesian credible intervals for the probability distribution calculated for each source.

export of ADN was calculated similarly, by multiplying the total stormflow discharge for each year[21] (in m^3) by the average nitrate concentration in streamwater observed during storms (0.27 mg L^{-1}). The estimated export of ADN-sourced nitrate in streamwater was 0.32 kg $ha^{-1}yr^{-1}$ and 0.19 kg $ha^{-1}yr^{-1}$ for 2007 and 2008, respectively. This export constitutes 8% and 5%, respectively, of estimated inputs from wet and dry ADN to the NMR watershed for 2007 (4.0 kg $ha^{-1}yr^{-1}$) and 2008 (3.6 kg $ha^{-1}yr^{-1}$)[49] and supports previous results that indicate significant retention in the NMR watershed.[21] A minimum estimate of retention for ADN is 92−95% of the total flux into the NMR watershed. These results demonstrate that retention of ADN is potentially high in urban systems relative to retention of sewage-sourced nitrate, as there is likely strong subsurface connectivity between sewage-sourced inputs and streamwater. Studies indicate a strong potential for ADN retention in urban lawns, soil,[50,51] and groundwater, where observations indicate that ADN undergoes significant processing.[52] Our results concur with indications of high rates of ADN retention in urban systems and further with these observations by quantifying export, thus allowing for quantitative estimates of actual retention through a budget approach.

Results from this study indicate that 34% of streamwater nitrate loads during storms are sourced from ADN, with the remainder of storm nitrate sourced from sewage. The presence of ADN in suburban/urban streams is expected due to impervious surfaces and storm sewer systems that work together to direct dry and wet deposition to streams during storm events. Comparisons between the percentage of ADN in NMR versus other urban and suburban watersheds using mixing models reveal the strength of the Baysian-based MCMC approach in incorporating uncertainty in source endmember values. Studies in other temperate, Eastern U.S. cities report substantial ADN-sourced nitrate in streamwater, similar to the percentage reported here (New York, 43−50%;[25] Baltimore, MD, 5−94% ADN, with a reported average of 50%[12]). However, endmembers used in the other mixing models do not incorporate the substantial uncertainty in the range of values for each source. It should be noted that newer analytical approaches using O-NO3- take advantage of reduced uncertainty in apportioning atmospheric nitrate inputs to urban systems (for examples, see reference 53). The MCMC analysis used here takes into account the range of values possible for source endmembers, thereby accurately estimating uncertainty in the contribution of ADN-sourced nitrate.

Denitrification in the NMR Watershed. The process of denitrification contributes to overall nitrate retention observed in the NMR watershed. The increase in $\delta^{15}N$ and $\delta^{18}O$ values at downstream NMR3 suggests a portion of the nitrate pool undergoes denitrification (Figure S4, Supporting Information), and SIAR mixing models estimate that 7 ± 2% of streamwater nitrate at NMR1 is contributed by pools of sewage-derived nitrate that exhibit the isotopic effects of denitrification, while downstream at NMR3, 19 ± 3% is contributed by this source (Figure 3). Nitrate in the steam draining the FH subwatershed also shows evidence of denitrification, with 22 ± 3% of streamwater nitrate contributed by denitrified, sewage-sourced nitrate sources (Figure 3). This contrasts with previous work that concludes, on the basis of dual-isotope data, that nitrate sourced from leaky sewers does not undergo processing.[12] The positive slope of $\delta^{15}N$ vs $\delta^{18}O$ suggests denitrification contributions at NMR3, where isotopic values increase linearly, with a ratio of 1:2.3 (Figure S4, Supporting Information). The linear trend indicates systemic enrichment of the remaining nitrate pool, as lighter isotopes are removed via Rayleigh fractionation.[8,27] In the NMR watershed, the weaker isotopic trend at NMR1 may be due to the proximity of the buried portion of NMR with leaking underground sewers that would continue to supply sewer-

sourced nitrate to the stream.[24,54] While precise quantification of denitrification rates will require in situ experiments or other approaches. The application of dual-isotope analysis with MCMC techniques documents denitrification of sewage-sourced nitrate.

IMPLICATIONS

Dual nitrate isotope analysis has refined mass-balance inverse modeling results, attributing up to 94% of in-stream nitrate during baseflow to sewage-derived sources (including sewage that has undergone some denitrification) and an average of 67% during stormflow.[21] Furthermore, this analysis documents and quantifies significant retention of ADN-sourced nitrate in the watershed. This information is important for efforts to reduce nitrate concentrations in urban streams. The impact of reactive nitrogen on downstream systems is strongly dependent on the hydrologic connectivity between nitrogen source and surface waters.[42] In the system described here, both ADN and sewage sources are connected to streamwater via hydrological pathways, thereby leading to significant impacts on streamwater nitrate loads. Both sources, but in particular sewage, contribute to nitrate "hypersaturation" in urban streams with severe negative implications for ecosystem health and in-stream biota.[8] To reduce nutrient loads in NMR and other urban systems with similarly aged sewage infrastructure, focus must be placed on how to reduce these leaks or increase processing of sewer-sourced nitrate before it reaches the stream, where it is quickly exported from the watershed. Source and process information, such as that demonstrated here, should be made part of management decisions in order to effectively mitigate nutrient problems in urban watersheds.[55]

ASSOCIATED CONTENT

Supporting Information

Table S1 and Figures S1–S4. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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