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Catchment-Scale Quantification of Hyporheic Denitrification Using an Isotopic and Solute Flux Approach

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Ⓢ *Supporting Information*

ABSTRACT: A dual-isotope and solute flux mass-balance was used to elucidate the processes that lead to attenuation of nitrogen contamination in an agriculturally impacted river. The River Wensum drains a lowland catchment with an area of 570 km^2 in East Anglia, eastern England. Analysis of nitrate concentration, $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ of samples from the River Wensum collected from upstream locations to the catchment outlet through all seasons and flow conditions showed a consistent pattern of increasing isotope values with decreasing nitrate concentrations downstream. $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ of catchment surface water and groundwater samples revealed a dominant influence from microbially cycled and nitrified source-nitrogen, which results in high nitrate concentrations in Chalk groundwater and upstream in the River Wensum. Denitrification of Chalk groundwater-baseflow in the hyporheic zone results in the downstream trend observed in the river. Hyporheic denitrification is estimated to remove 931 kg/day of nitrate-nitrogen by the catchment outlet, representing 31% of the potential riverine nitrate load. The use of dual-isotope and solute flux modeling at the catchment scale is a novel application to quantify denitrification within the river valley, demonstrating the importance of hyporheic zone processes in attenuating the impacts of anthropogenic contamination of hydrologic systems.

■ INTRODUCTION

Over the last century, there has been dramatic perturbation of the global nitrogen budget, which has led to a doubling of reactive nitrogen in the environment. Reactive nitrogen of anthropogenic origin now equals that derived from natural terrestrial nitrogen fixation, and is predicted to exceed it by 2020.[1] The majority of the anthropogenic budget is due to fertilizer production,[2] reflecting changes over the last 50 years in agricultural practices to increase food production both in response to population increase and for food security. The accumulation of reactive nitrogen in the environment, however, results in detrimental effects, including increased aquatic biomass productivity leading to hypoxia, eutrophication, and a loss of species diversity.[3−5] A further major result of the doubling of reactive nitrogen in the terrestrial environment is the transport of an increased nitrogen loading through the landscape to rivers, and ultimately to the oceans, with an estimated 6-fold increase in nitrogen export from European rivers over the past 100 years.[6]

Reactive nitrogen may be removed from the terrestrial environment and transformed to N_2 gas through microbially mediated denitrification. While some of the removal and storage of reactive nitrogen occurs within the landscape, a significant proportion is thought to occur within rivers and groundwater.[7,8] The hyporheic zone represents the interface between groundwater and surface water within the fluvial sediments below a river and is a reactive zone characterized by redox and temperature gradients, which has a supply of organic carbon from the river, supporting intense microbial activity including denitrification.[9] This denitrification in the hyporheic zone is thought to provide a major contribution to the reduction of riverine nitrogen exports. However, quantification of hyporheic denitrification is often limited to estimates from streambed sediment cores and studies of short stream transects,[10−14] or is inferred from large-scale nitrogen budget mass-balance modeling, which cannot always pinpoint the location of the denitrification within the catchment.[15]

Natural abundance stable isotopes of nitrogen and oxygen in nitrate have proved a powerful tool to enhance our understanding of nitrogen cycling. Isotopic signatures of nitrate from atmospheric, fertilizer, manure, and wastewater sources have been well characterized,[16] and isotopic fractionations resulting from processes within the nitrogen cycle have been investigated, primarily with respect to nitrogen but also increasingly of oxygen.[16−26] This body of knowledge provides a firm basis for investigations of nitrate cycling and attenuation in the field.

Here, we present a novel application of dual-isotope and solute flux mass-balance at the catchment scale. This utilizes differences in nitrate isotopic composition and solute concentrations from the major contributors to flow in a lowland river draining an agriculturally impacted catchment in eastern U.K. to identify and quantify nitrate removal during hyporheic denitrification of groundwater-supported baseflow.

■ STUDY SITE

The Wensum catchment (570 km^2) is in central Norfolk, East Anglia, and is drained by the Wensum river with a length of 75 km. This calcareous lowland river has protected conservation status and drains into the Broads National Park, a protected wetland habitat and an essential feeding ground for water birds. However, the region's relatively flat terrain (elevation range 0 to 100 m above sea level) and lowland drainage has supported the development of intensive agricultural techniques, making East Anglia one of the most productive agricultural regions in the U.K. Over 80% of land is used for arable and livestock farming, with a heavy reliance on organic and inorganic fertilizers. The significant nitrogen loading accruing from agricultural land-use has led to the classification of over 90% of the land in the catchment as especially vulnerable to nitrate contamination, with the result that the land is subject to European and national legislation aimed at reducing agricultural nitrogen pollution. East Anglia receives on average 601 mm of rainfall falling throughout the year and 1499 h of sunshine per annum. Mean monthly minimum temperatures occur in January and February (0.6 °C) with mean maxima in July and August (21.1 °C).[27]

The Wensum catchment hydrogeology is dominated by the Cretaceous Chalk, a major aquifer comprising a fine-grained white limestone, and a significant groundwater resource for public and irrigation water supply. In the interfluves, the Chalk is confined under the clay-rich Pleistocene Lowestoft Till plateau. The glacial till is thin or absent in the river corridor and valley margins leaving the Chalk unconfined or exposed. Previous research on East Anglian Chalk groundwaters found high concentrations of nitrate in the exposed Chalk (56.6 ± 17.4 mg/L, $n = 48$) [28,29] with $\delta^{15}N_{NO_3}$ 6.9 + 2.4‰ ($n = 29$) and no evidence of denitrification in the relationship between nitrate concentration and $\delta^{15}N_{NO_3}$.[29] Below the till, nitrate concentrations were below the limit of detection ($n = 30$).[29] In the Wensum valley, there are glacial sand and gravel deposits up to 5 m deep that are in hydraulic continuity with the Chalk. Catchment soils comprise freely draining sandy loams in the river corridor, and clay soils with low permeability on the interfluves. The outflow of two sewage-treatment works discharging volumes of effluent into the Wensum via tributaries represents 2% of mean flow at the midriver gauging station. Beyond the headwaters, 50 km of the river to the catchment outlet is gauged at three stations located upstream (GS1), 25km downstream in the midriver (GS2), and at the catchment outlet a further 25km downstream (GS3). The gauging stations record mean flows of 0.87 m^3s^{-1}, 2.64 m^3s^{-1}, and 4.04 m^3s^{-1}, respectively. The river has high a baseflow index (BFI), which represents the proportion of river flow derived from stored groundwater as opposed to rainfall runoff under mean flow conditions. BFIs are calculated as the ratio of the smoothed minimum daily mean flow to the daily mean flow of the total recorded hydrograph for the river reach.[30] BFIs for the Wensum calculated from over 36 years of hydrograph data are: headwaters to GS1: 0.83; upper river reach to GS2: 0.75; and lower river

reach to GS3: 0.74.[31] High BFIs imply a low degree of flashiness. The riverbed of the Wensum comprises gravel and fine sediments, with a high width-to-depth ratio and a high degree of variation in channel width along the river's length. The approximate mean width of the upper Wensum between GS1 and GS2 is 15 ± 5 m, and 25 ± 5 m in the lower Wensum between GS2 and GS3, with mean depths rarely exceeding 0.4 m between GS1 and GS2 during mean flow conditions, and 0.6 m by GS3.[32] The estimated mean travel time of water between GS1 and GS3 is 1.3 days. Water is abstracted from the river at the catchment outlet for public supply.

■ MATERIALS AND METHODS

Samples were collected midstream from 10 bridges using a bucket (locations S1 to S10 between upstream and catchment outlet gauging stations) during seven spatial surveys on the River Wensum in high, medium and low-flow conditions, and across all seasons from February 2007 to May 2009. Because of the shallowness of the river this method sampled across the integrated water-column depth. Locations S1, S5, and S10 were at the gauging-station sites (GS1-S1 52.83′N 0.85′E, GS2-S5 52.73′N 0.99′E, and GS3-S10 52.67′N 1.22′E). Additional samples were collected on nine occasions from up to 13 tributaries and drains feeding into the Wensum, and from Chalk groundwater during the winter at 12 pumped boreholes in the river valley and interfluves. One borehole from the valley and one from the interfluve were sampled twice, in winter and summer. Samples for solutes (NO_3^-, Cl^-, SO_4^{2-}, Na^+), TDN (total dissolved nitrogen), and isotopic analysis were filtered through 0.22 μm cellulose acetate filters. Samples for solute concentration and TDN analysis were stored at 4 °C and analyzed within a week, respectively, using liquid ion chromatography and thermal oxidation with a precision of 2% (Dionex ICS 2000, Dionex DX 600, Thermalox TN). Concentrations of DON (dissolved organic nitrogen) were calculated using the difference between the TDN and solute concentrations. Samples for isotopic analysis were frozen at −20 °C for later preparation using the denitrifier method [33,34] and analysis on a Geo 20:20 GCIRMS with a TG II prep system. Mean precision was ±0.1‰ for $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ with the least precise measurements of ±0.4‰ for both isotopes. [NO_2^-] in all samples was approximately 1% of [NO_3^-], indicating a negligible interference from NO_2^- with the denitrifier method which measures $NO_3^- + NO_2^-$.

■ RESULTS AND DISCUSSION

TDN, Nitrate Concentration and Isotopic Composition, and Chloride Concentration. Nitrate was the primary dissolved nitrogen species in all samples comprising >80% TDN in Wensum river samples ($n = 53$) and tributary and drain samples ($n = 41$), which also had <18% DON, 1% NO_2^- and 1% NH_4^+. Nitrate comprised 98% of TDN in valley Chalk groundwater ($n = 5$), with 2% DON, and NO_2^- and NH_4^+ below the limits of detection. All species of dissolved nitrogen in interfluve Chalk groundwater (ICG) were below the limits of detection ($n = 7$). Generally, for all the samples, a lighter isotopic composition was found with higher concentrations of nitrate. Nitrate from valley Chalk groundwater (VCG) had the highest concentration (56.1 ± 6.8 mg/L) and the lightest isotopic composition ($\delta^{15}N_{NO_3}$ 6.2 ± 0.6‰ $\delta^{18}O_{NO_3}$ 0.8 ± 0.5‰) of all the samples (excepting nitrate from two high-concentration minor drain samples),
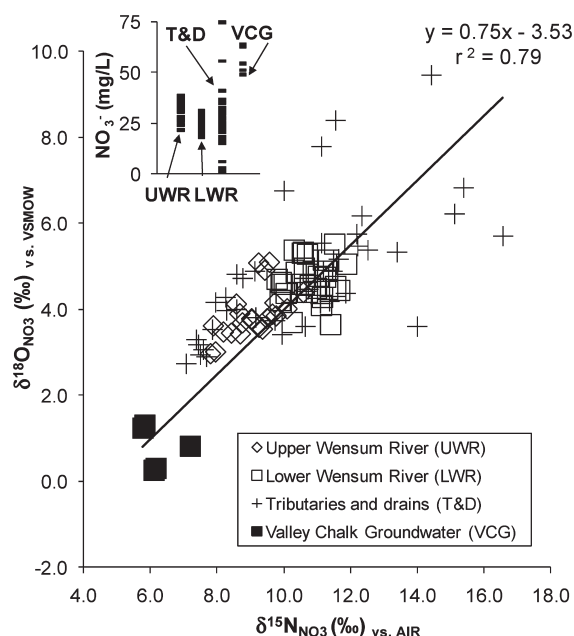
Figure 1. $\delta^{15}N_{NO_3}$ vs $\delta^{18}O_{NO_3}$ (‰) of samples from the upper and lower Wensum river reaches (UWR, LWR), tributaries and drains (T&D), and valley Chalk groundwater (VCG), with the insert showing NO_3^- concentration. Best fit line represents equally weighted groundwater and surface water samples.


Figure 2. $\delta^{15}N_{NO_3}$ vs $\delta^{18}O_{NO_3}$ (‰) of samples from river sampling locations S1, S5, and S10 from the three gauging stations GS1 (upstream), GS2 (mid river), and GS3 (catchment outlet), collected on seven dates, with the insert showing NO_3^- concentration.

clearly differentiating Chalk groundwater nitrate from nitrate in surface water samples (Figure 1). Nitrate from the upper Wensum river (UWR) between GS1 and GS2 had a slightly lighter isotopic composition and higher nitrate concentration than that from the lower Wensum river (LWR) between GS2 and GS3 (UWR NO_3^- 29.8 ± 5.3 mg/L; $\delta^{15}N_{NO_3}$ 9.4 ± 1.0‰; $\delta^{18}O_{NO_3}$ 4.1 ± 0.6‰ versus LWR NO_3^- 24.5 ± 3.6 mg/L; $\delta^{15}N_{NO_3}$ 10.8 ± 0.6‰ $\delta^{18}O_{NO_3}$ 4.7 ± 0.5‰). The isotopic composition and concentration of nitrate from the tributaries and drains (T&D) showed a higher level of variation than the other samples sets (NO_3^- 25.8 ± 13.6 mg/L; $\delta^{15}N_{NO_3}$ 10.6 ± 2.4‰; $\delta^{18}O_{NO_3}$ 4.8 ± 1.5‰). A spatial trend was seen in the river samples, with highest concentrations and the lightest isotopic composition at the uppermost sampling location (S1) and falling concentrations with an increasingly heavy isotopic composition observed to the catchment outlet (S10) with a clear differentiation between nitrate collected from the upper river at S1-GS1 and samples from the other two downstream gauging-station sampling locations S5-GS2 and S10-GS3 (Figure 2). Although samples were collected across all seasons and flow conditions, there were no clear seasonal or flow related trends with respect to nitrate concentration and isotopic composition in the river. Mean concentrations of Cl^- were: UWR 39.9 ± 4.8 mg/L; LWR 40.6 ± 3.6 mg/L; T&D 45.1 ± 15.8 mg/L; VCG 57.8 ± 16.7 mg/L; and ICG 19.5 ± 4.7 mg/L.

Nitrate Concentration and Isotopic Composition to Determine Processes. None of the nitrate sampled reflected the expected isotopic signatures of nitrate inputs from chemical fertilizer, atmospheric dry deposition, and precipitation reported in the literature,[16] all of which have a very heavy oxygen isotopic composition relative to the nitrogen isotopic composition such that $\delta^{15}N_{NO_3} \ll \delta^{18}O_{NO_3}$. In fact, all groundwater and surface water samples had $\delta^{18}O_{NO_3} < \delta^{15}N_{NO_3}$, with a low level of variation in the ratio of the two isotopes ($\delta^{18}O_{NO_3}$: $\delta^{15}N_{NO_3}$ 0.4 ± 0.1).
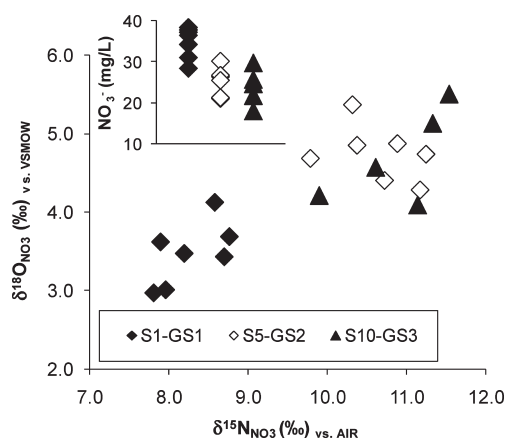
Together this implies that sources of nitrate reaching the river via the land surface are first quantitatively cycled by the biota through the assimilation → remineralization → nitrification pathway. The predicted $\delta^{18}O_{NO_3}$ value for nitrification in East Anglian soils, based on the equation $\delta^{18}O_{NO_3\text{-nitrification}} = {}^2/_3 (\delta^{18}O_{H_2O}) + {}^1/_3 (\delta^{18}O_{O_2})$,[16] using the range of $\delta^{18}O_{H_2O}$ of Wensum catchment waters measured in this study (−7.5 to −6‰) ($n = 106$), is $\delta^{18}O_{NO_3}$ 2.8 to 3.8‰. The lowest $\delta^{18}O_{NO_3}$ values of catchment samples are lower than those predicted for nitrification, seen in the VCG ($\delta^{18}O_{NO_3}$ 0.3 to 1.3‰). This could be due to oxygen exchange between nitrite and water during nitrification.[35,36] The occurrence of VCG nitrate only where the overlying Lowestoft Till is thin and patchy, or absent suggests that recharge waters carry leached nitrate, produced from nitrification in the soil, rapidly from the sandy-loam soil directly to the Chalk, with infiltration unimpeded by the clay-rich till. This rapid transport enables the nitrate to avoid denitrification, and suggests that the well drained soils in the valley do not support significant denitrification under normal conditions. The high concentration of nitrate in valley Chalk groundwater and its relatively light isotopic composition confirm that significant denitrification does not occur within the Chalk itself, perhaps because a source of organic carbon is lacking. The isotopic composition of valley Chalk groundwater, representing the product of cycled and nitrified sources of nitrogen to the catchment, constrains the predicted range of bulk cycled source nitrate in areas of the catchment with freely draining soils to $\delta^{15}N_{NO_3}$ 5.8 to 7.2‰, with a range of oxygen isotopic composition (observed and predicted) of $\delta^{18}O_{NO_3}$ 0.3 to 3.8‰. There is no evidence of denitrification in VCG, which would produce lower nitrate concentrations with a heavier isotopic composition. In contrast to VCG, ICG is fully protected from contemporary nitrate contamination by the overlying clay till deposits on the interfluves, which acts both as a physical barrier and as a zone of denitrification.[29,37]

When the data are weighted so that the number of VCG samples is equal to that of the catchment surface water samples the slope of the regression line of $\delta^{15}N_{NO_3}$ vs $\delta^{18}O_{NO_3}$ is 0.75 (r^2 0.79) with the VCG samples at the base of the line (Figure 1). The slope of the line implies that a fractionating process such as rapid denitrification is affecting the isotopic composition of the
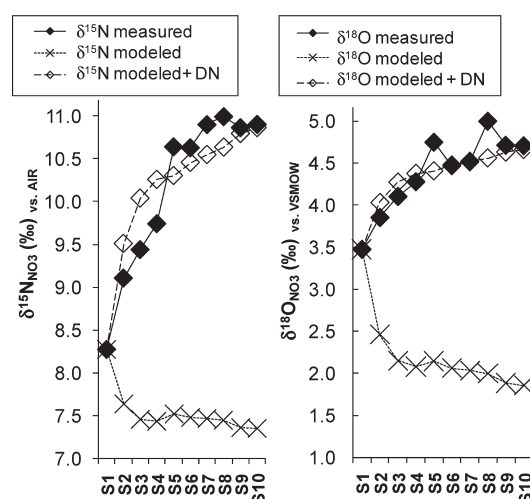
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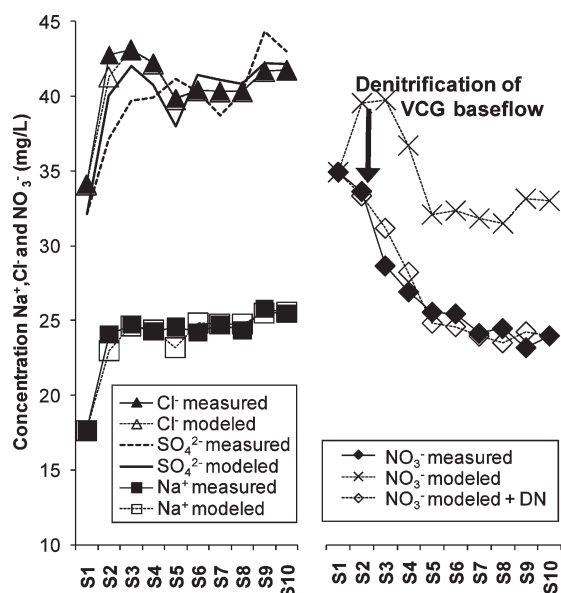
Figure 3. Measured and modeled concentrations of Na^+, Cl^-, and NO_3^- for river sampling locations S1 to S10, also showing NO_3^- modeled using a partially denitrified valley Chalk groundwater end member (arrow) (VCG-DN NO_3^- 31.7 mg/L).

Figure 4. Measured and modeled $\delta^{15}N_{NO_3}$ and $\delta^{18}O_{NO_3}$ (‰) for river sampling locations S1 to S10, also showing NO_3^- modeled using a partially denitrified valley Chalk groundwater end member (VCG-DN $\delta^{15}N_{NO3}$ 12.5‰, $\delta^{18}O_{NO3}$ 5.3‰).

surface water samples,[16,19,21,26,38−43] and that VCG nitrate is the least fractionated/denitrified nitrate. The low level of variation in the ratio of the two isotopes across catchment-water samples also suggests that the ratio is controlled by a process which fractionates both isotopes in tandem, such as denitrification.

Solute Flux Model. To investigate the cause of the observed downstream decrease of nitrate concentration and increase of isotopic values in river samples, a flux model was used to calculate solute flux at sampling locations S1−S10 from the four components of flow: river water at the upstream gauging-station sampling location (S1-GS1); surface accretion from tributaries and drains (T&D); baseflow from valley Chalk groundwater (VCG); and baseflow from interfluve Chalk groundwater (ICG). The calculations were constrained by the baseflow index (0.745) and the mean increase in flow between the gauging stations (GS1 to GS2: 2.31 $m^3\ s^{-1}$; GS2 to GS3: 1.90 $m^3\ s^{-1}$) divided equally between sampling locations, based on the simplifying assumption of an incremental increase in flow. Although flow increase from groundwater and surface water inflow is not likely to be uniform along the river's length, these assumptions will not affect the model results as the relative proportions of groundwater and surface water inflow at each gauging station are constrained by the BFI. Thus, the contributions to flow at sampling location sx are represented by the simple mass balance:

$$Q_{sx} = Q_{US} + Q_{SFsx} + Q_{BFsx} \qquad (\text{eq } 1)$$

where Q_{US} represents upstream flow at S1-GS1, Q_{SFsx} represents surface accretion from the tributaries and drains (T&D) at sampling location sx, and Q_{BFsx} represents baseflow from valley Chalk groundwater and interfluve Chalk groundwater at sampling location sx. The relative proportions of valley Chalk groundwater and interfluve Chalk groundwater in baseflow are controlled by the variables a and b such that:

$$Q_{BFsx} = a(Q_{VCGBFsx}) + b(Q_{IFGBFsx}) \qquad (\text{eq } 2)$$

where $Q_{VCGBFsx}$ and $Q_{IFGBFsx}$ represent baseflow from valley Chalk groundwater and interfluve Chalk groundwater respectively, at sampling location sx.

The flux terms for the four components of flow at each river sampling location were then used with mean measured concentrations of solutes of these four flow components in simple mass balance equations to calculate solute concentrations and nitrate isotopic composition at each sampling location from S1 to S10 for comparison with mean measured values at each river sampling location (eq 3.).

$$C_{sx} = \frac{(C_{US} \times Q_{US}) + (C_{SF} \times Q_{SFsx}) + (C_{VCG} \times aQ_{VCGBFsx}) + (C_{ICG} \times bQ_{IFGBFsx})}{(Q_{US}) + (Q_{SFsx}) + (aQ_{VCGBFsx}) + (bQ_{IFGBFsx})} \qquad (\text{eq } 3)$$

The calculations were calibrated to observed chloride concentrations by increasing the relative proportions in baseflow of ICG to VCG downstream (using the variables a and b) to reflect the expected increasing contribution to baseflow of Chalk groundwater from the interfluves, such that 38% of baseflow is supplied from ICG and 62% from VCG by the catchment outlet. This approach utilized the fact that mean chloride concentrations were distinguishable in these two groundwater types, and also slightly different from those of surface water.

The model reproduced the downstream evolution of concentrations of SO_4^{2-} and Na^+ well (Figure 3). However, the model failed to represent the observed trends in nitrate concentration or isotopic composition, producing higher than observed concentrations with a lighter isotopic composition (at S10, NO_3^- 32.8 mg/L; $\delta^{15}N_{NO_3}$ 7.3‰; $\delta^{18}O_{NO_3}$ 1.9‰) (Figures 3 and 4). To investigate the possibility that this discrepancy was caused by denitrification of the valley Chalk groundwater, this component's concentration and isotopic composition were altered so that the

model fitted the observed data (Figures 3 and 4). For this the valley Chalk groundwater nitrate concentration was reduced by 43% and the isotope values increased to give VCG-DN NO_3^- 31.7 mg/L; $\delta^{15}N_{NO_3}$ 12.5‰; $\delta^{18}O_{NO_3}$ 5.3‰. To determine whether this theoretical concentration and isotopic composition could represent the effects of partial denitrification of valley Chalk groundwater, isotope enrichment factors [21] were calculated between the isotope values of VCG nitrate and VCG-DN nitrate, giving $\varepsilon_{P-S}^{15}N_{NO_3}$ −11.1‰ and $\varepsilon_{P-S}^{18}O_{NO_3}$ of −8.3‰, with a fractionation ratio of O:N of 0.75, in good agreement with literature values.[19,21,22,26,38−43] This suggests that the observed trend in nitrate reduction downstream in the Wensum River is due to the partial denitrification of nitrate in baseflow from VCG. This nitrate reduction is likely to be occurring in the glacial sand and gravel hyporheic deposits at the groundwater/surface water interface below the riverbed as groundwater baseflow advects to the river.

As well as denitrification, aquatic algal assimilation has been found to cause a trend of decreasing nitrate concentration with increasing isotope values.[44] However, a major contribution from algal nitrate assimilation in-stream was discounted due to the fact that these trends were observed all year-round, including in winter, while algal assimilation would be expected to produce a seasonal signal with a loss of the trend in winter. In addition, high-flows mask the effects of algal assimilation by mixing in nitrate of a different isotopic composition and concentration, but the trend observed in the Wensum River is seen even during high-flows. This indicates a hydrologic piston-flow response during high-flows, whereby shallow groundwater, which has been partially denitrified, is displaced into the river by rainfall-runoff entering the sand and gravel deposits, and demonstrates the stability of hyporheic denitrification of groundwater baseflow on this river.

Nitrogen Removal Rates. The difference between the modeled nitrate concentration at the catchment outlet when the measured concentration of VCG is used in the model, and the observed concentration at the catchment outlet with mean flow at S10, gives an estimate of nitrate-nitrogen removal rates. This indicates that 931 kg nitrate-nitrogen per day are removed by the catchment outlet, representing 31% of the predicted load. To test the potential error in nitrogen removal estimates a sensitivity analysis was performed, perturbing the nitrate concentration of each model flow-component, as well as BFI and flow, and including calculations for maximum (Q_{max}) and minimum (Q_{min}) flow. The model showed the highest sensitivity to flow, demonstrating that, along with concentration, it controls the NO_3-N load, which is the product of concentration and flow-volume. The estimated NO_3-N removal rates vary greatly with flow, (Q_{max} 1420 kg NO_3-N/day and Q_{min} 509 kg NO_3-N/day), indicating that high-flows (winter-spring) would be expected to result in a larger amount of nitrate removal than low-flows (summer-autumn). The model's sensitivity to flow supports the use of mean flow conditions for estimated removal rates. The model also showed high sensitivity to nitrate concentration of VCG. The mean VCG concentration used in the model of 56.1 mg/L NO_3^-, is very close to the value of 56.6 mg/L NO_3^- found from measurements of 48 boreholes in previous studies in this region [28,29] suggesting that this parameter value is robust. Using the expected variation of VCG nitrate concentration of ±30% found in the previous studies [28,29] gives maximum estimated removal rates of 1327 kg NO_3-N/day, with minimum rates of 536 kg NO_3-N/day.

The mean estimated nitrogen removal rates are within the range of rates for riverine nitrogen removal reported in other studies of up to 45% predicted for streams with a depth of <1 m,[7] and up to 40% removal found using the in situ acetylene block technique.[45] An upper limit of 20% for within-reach nitrate removal using a global model has been suggested,[8] which is also in fair agreement with the whole-river estimate of 31% found here.

Nitrogen removal rates within the river channel and hyporheic sediments are usually reported in the literature as mg removed per m^2 of riverbed surface area or mg per m^3 of hyporheic sediments. The estimated streambed area between GS1 and GS3 is 1 000 000 ± 250 000 m, giving a calculated rate of removal of 39 mg NO_3-N/m^2/hour (range 31−52 mg NO_3-N/m^2/hour). These rates are within the wide range of values reported in the literature from a variety of approaches which suggests stream-bed removal rates from 10 to 222 mg NO_3-N/m^2/hour.[10,46−50] Using an estimated mean depth of hyporheic sediments in the River Wensum of 1.5 ± 0.5 m the removal rate is 26 mg NO_3-N/m^3/hour (range 16−52 mg NO_3-N/m^3/hour); similar to rates of 28 to 64 mg/m^3/hour found using hyporheic-zone sediment cores.[51]

From the estimated volume of the hyporheic sediments (1 500 000 m^3, range 750 000−2 500 000 m^3), the mean volume of flow at GS3 (5.28 $m^3 s^{-1}$), and the baseflow index (0.745), the calculated Chalk groundwater residence time in the hyporheic sediments is 4.4 days (range 2.2 to 7.4 days). The calculated hydraulic conductivity of the hyporheic sediments based on their estimated volume is 3×10^{-6} m s^{-1}, within the range expected for glacial sands and gravels,[52] supporting the volume estimate used in the model. The presence of deep sand and gravel deposits below the riverbed, with a source of fine sediments including organic carbon from the river, in conjunction with the calculated residence times of Chalk baseflow in the sediments, and the calculated isotope enrichment factors, suggest that a significant proportion of denitrification in this agricultural lowland catchment is occurring in the sand and gravel deposits of the hyporheic zone during baseflow advection. Solute and isotope flux modeling to characterize the dominant influences on nitrate concentrations in-stream represents a simple but effective new approach at the scale of the catchment to identify the dominant interactions between groundwater and surface water, which result in major natural attenuation of anthropogenic nitrogen inputs.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. Location map with surface geology and sampling sites; acronym key; tabulated results; equations; solute flux model; mean hydrograph and error analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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