



Stable isotopes as an effective tool for N nutrient source identification in a heavily urbanized and agriculturally intensive tropical lowland basin

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Abstract We present the application of dual stable isotope analyses of NO_3 ($\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$) to provide a comprehensive assessment of the provenance, partitioning, and conversion of nitrate across the Day River Basin (DRB), Vietnam, which is heavily impacted by agriculture and urbanization. Stable isotope compositions of river water $\delta^{18}\text{O}\text{-H}_2\text{O}$, in addition to their $\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ signatures, were sampled at 12 locations in the DRB. Sample collection was conducted during three different periods to capture changes in regional weather and agricultural fertilization regimes; April (the dry season and key fertilization period), July (the rainy season and another key fertilization period) and October (the rainy season with no regional fertilization). Ranges of

NO_3 stable isotopes are -7.1 to $+9.2\%$ and -3.9 to $+13.2\%$ for $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$, respectively. Interpretation of the stable isotope data characterizes 4 main sources of NO_3 in the DRB; (1) nitrified urea fertilizer derived from an intensive agricultural irrigation network, (2) soil and groundwater leaching from within the basin (3) manure and sewage inputs (which is more prevalent in downstream river sections) and (4) upstream inflow from the Red River which discharges into the Day River through the Dao River. We applied a mixing model for the DRB consisting of 4 variables, representing these 4 different sources. The partition calculation shows that during the fertilization and rainy period of July, more than 45% of river NO_3 is derived from nitrified urea sources. During the other sampling periods (April and October), manure and sewage contribute more than 50% of river NO_3 and are

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derived from the middle portion of the DRB, where the Day River receives domestic wastewater from the Vietnamese capital, Hanoi. Stable isotope data of O and N reveal that nitrification processes are more prevalent in the rainy season than in dry season and that this predominantly takes place in paddy field agricultural zones. In general, data demonstrate that nitrate loss in the DRB is due to denitrification which takes place in polluted stretches of the river and dominates in the dry season. This study highlights that (i) domestic waste should be treated prior to its discharge into the Day River and (ii) the need for better catchment agricultural fertilization practices as large portions of fertilizer currently discharge into the river, which greatly impacts regional water quality.

Keywords Vietnam · Denitrification · Nitrification · Biological assimilation · Dual stable isotopes

Introduction

The global nitrogen (N) cycle has been altered significantly since the mid C20th, in the advent of enhanced agricultural practices and accompanying fertiliser use (Galloway et al. 2004). Together with greater fossil fuel combustion and urbanisation (notably issues surrounding sanitation provision), agriculture has resulted in increased nitrogen fixation, which can ultimately impact upon other global biogeochemical cycles (including phosphorus and silicon) (Turner et al. 2003). Evidence for these impacts (most notably excessive fertilizer use and poor sewage treatment provision) upon the N cycle is documented via the N pollution of riverine environments around the world (Kendall 1998; Duc et al. 2007; Popescu et al. 2015; Vrzal et al. 2016). The consequences of increasingly higher N concentrations of rivers, is already exerting a strong influence on productivity and biodiversity of aquatic ecosystems (Galloway et al. 2004). As a consequence, more recent research has focused on understanding N fluxes in riverine catchments, the key transformation processes that are occurring (e.g. N. retention or denitrification) and thereby the impact that these have on downstream regions (Luu et al. 2012; Do et al. 2014). A comprehensive understanding of these transformation processes is the key to be able to fully budget N fluxes in

these environments, which is particularly important in environments where distinct seasonality will exert a control upon biogeochemical processing (e.g. evidence of denitrification processes dominating in summer months; Panno et al. 2006). This is particularly relevant in tropical regions where strong monsoonal seasonality prevails. Such high rainfall intensities also enhance the potential for the high delivery of N fluxes to coastal regions (Do et al. 2019).

In order to address these issues, the application of nitrate N isotopes ($\delta^{15}\text{N-NO}_3$), and more recently nitrate O isotopes ($\delta^{18}\text{O-NO}_3$) provides an excellent means by which to trace the sources of N pollution in rivers. Furthermore, by applying these analyses in tandem permits an evaluation of the governing biogeochemical processes altering nitrate species in natural environments (Popescu et al. 2015; Vrzal et al. 2016; Ta et al. 2016). The dual isotope approach is based on the principle that nitrate from different origins has distinct $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ isotopic signatures (see Kendall et al. 2007 for a review). For example, inorganic nitrate fertilizers have significantly higher $\delta^{18}\text{O}$ values (+ 17 to + 25‰) compared to most other nitrate sources (e.g. soil, manure, sewage and inorganic NH_4 fertilizers are all not higher than + 15‰), whereas their $\delta^{15}\text{N}$ composition is generally quite low (− 5 to + 6‰). In contrast, nitrate derived from organic sources tends to exhibit elevated $\delta^{15}\text{N}$ signatures (0 to + 26‰), but comparatively low $\delta^{18}\text{O}$ values (− 17 to + 15‰) (Amberger and Schmidt 1987). For simple point-source analyses, these generalizations are useful. However, in anthropogenically impacted systems the situation may be more complex and there is a need to test this application fully. Here we provide one of the first applications of these analyses in a heavily anthropogenically impacted, tropical riverine system, the Day River Basin (DRB) in Vietnam. Here we assume that nitrate isotopic compositions of river waters will reflect both the regional and seasonal differences in nitrate source loading. Furthermore, they will also be modulated by in stream isotopic fractionation via processes such as denitrification, assimilation, and nitrification. The application of dual isotopic analyses of nitrate will allow us to assess isotopic fractionation associated with (1) nitrate removal processes (i.e., denitrification and assimilation), (2) nitrification, as well as to (3) identify inputs from multiple anthropogenic sources (Kendall et al.

2007; Burns et al. 2009; Widory et al. 2013; Michalski et al. 2015).

Indeed, several previous studies have indicated that in the Red River Delta (RRD) in northern Vietnam where the DRB is located, sewage and animal waste (which we also refer to as “anthropogenic organic” sources) inputs of N are greater than the amounts delivered from the river’s upstream watershed sources (Luu et al. 2012). Do et al. (2019) estimate that, annually, manure and sewage contribute more N to the river water than chemical fertilizers running off from regional paddy fields. However, the amounts of N ultimately delivered from the delta to the coastal zone are lower than the amounts carried by the river, showing extremely efficient nutrient retention in both soils and the drainage network of the region (Seitzinger et al. 2006). Although relatively poorly constrained, denitrification and assimilation are considered to be the key nitrogen processes in the delta, with estimates of c. 59% of nitrogen being lost via these means (Quynh et al. 2005). Overall, these studies have used the approaches of subtracting nutrient inputs from the DRB and its output at the river mouth (Quynh et al. 2005; Luu et al. 2012; Do et al. 2014), which cannot estimate (i) the relative proportion of differing NO_3 sources discharging to the river and (ii) the in-stream processes taking place. In this study, we aim to improve these estimates via the use of stable isotope and mass balance approaches. The nitrate isotopes are used here as the key variables to (1) quantify dominant sources of nitrate and (2) to assess the key biogeochemical processes that control the in stream production and reduction of nitrate in the DRB, a highly populous, urbanized landscape set in a tropical, lowland delta region.

Materials and methods

Study site description

Vietnam is the second largest rice exporter in Asia, with its cultivation area having increased from 4.7 Mha in 1961 to 7.3 Mha in 2007. Concurrently, the application of nitrogen fertilizers in Vietnam has increased 7.2% annually since 1985. Such intensive use of chemical fertilizers has been recognized as a potential source of environmental pollution in the region (Luu et al. 2012; Do et al. 2019). This is

particularly notable in the DRB (Fig. 1), where rice cultivation constitutes approximately 50% of land use and the majority of the fertilizer used is applied to paddy fields (DARD-Hanoi 2009; DARD-Namdinh 2011; Kurosawa et al. 2004; Kurosawa et al. 2006). These chemical fertilizers applied within the DRB are the primary sources of N in the Day River (MONRE 2006; Hanh et al. 2010). Along with the expansion of rice agriculture, the country’s production of livestock has also increased 3 times since its economic reform in the 1980s (GSO 2014). On top of that, urbanization in the DRB is rapid, associated with development in and around Hanoi, the capital of Vietnam, is located here.

The DRB covers 7665 km² and includes all of Ha Nam, Nam Dinh, and Ninh Binh Provinces, as well as a part of Hanoi and Hoa Binh Provinces (MONRE 2006). The total population of the area is approximately 11.7 million (GSO 2016). At present, this river system is under considerable pressure from socioeconomic development activities and urbanization, and the basin is experiencing an annual population increase of about 5% (MONRE 2006). However, the region’s infrastructure (irrigation, drainage, traffic systems, urban planning, waste collection and treatment) is not being developed at an equivalent rate and so is unable to accommodate such rapid growth (Do and Nishida 2014). The establishment and operation of industrial zones, including craft villages, factories and agricultural areas, has caused significant changes to the natural environment, especially regional water quality (Duc et al. 2007). Given the existing level of infrastructure and the provision of its resources, most solid and liquid waste is not being treated but rather discharged directly to the surrounding water bodies and waterways. Do and Nishida (2014) reported more than 156,000 industrial, commercial and service establishments discharge about 100,000 m³ of wastewater per day to the river. They also tallied about 133 hospitals in DRB, which discharge a total of 27,686 m³ of hospital wastewater per day to the drainage system without treatment. Rather, only 3.61% of wastewater in the river basin has been treated by wastewater treatment plants (Do and Nishida 2014). Water regimes in the upstream part of Day River are largely controlled by a system of sluice gates and pumping stations, to allocate water for different purposes (e.g. irrigation and draining or preventing urbanized areas from seasonal inundation). Agriculture is also an important activity in this basin.

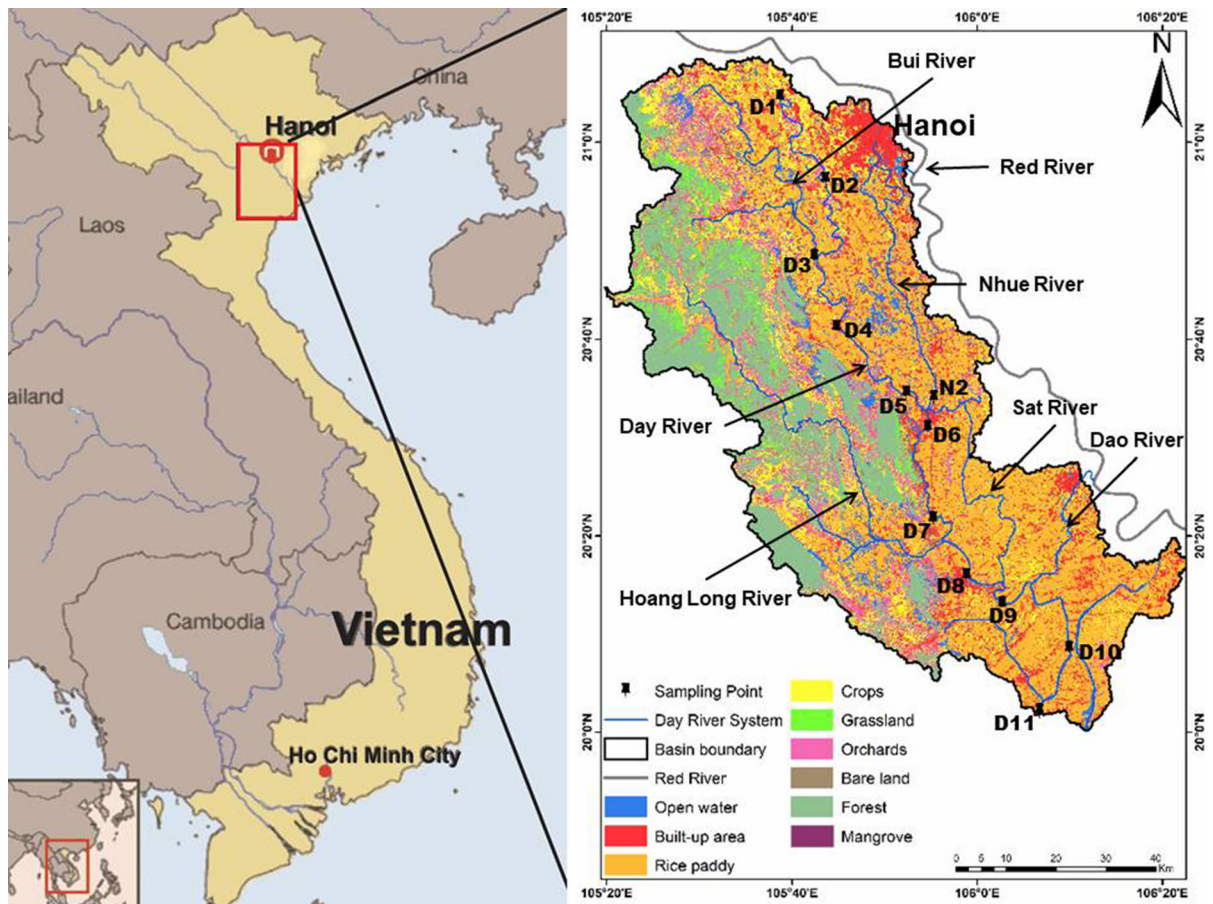


Fig. 1 Map of Day River Basin and the sampling sites of this study

Rice paddies occupy 2,414 km² with two rice seasons; spring fields are cultivated from late January to late May, and summer fields are cultivated from early June to late September. Application rates of chemical fertilizer for spring and summer growing seasons are 13,340–16,100 and 7660–8900 kg N km⁻², respectively (MARD 2008).

Sampling and analyses

In order to identify the sources and assess the processes controlling NO₃ in the DRB, water sampling was conducted at 12 locations upstream and downstream of the Day River's confluences with its tributaries (Fig. 1). The main stream of Day River receives water from 5 main tributaries; named in order from upstream, Bui River, Nhue River, Hoang Long River, Sat River, and Dao River. Among those tributaries, Bui and Hoang Long Rivers bring water

from fairly pristine-mountainous catchments characterized by degraded forests growing on a limestone landscape. The Nhue River is well-known for discharging domestic wastewater from the Hanoi Metropolis (Trinh et al. 2012). The Sat River flows through areas of land irrigated and drained for agricultural purposes in the DRB. The Dao River is a man-made canal connecting the Day and Red Rivers. This man-made canal helps divert a large volume of water from the Red River to the Day River (Luu et al. 2010).

Due to the seasonality of the region (namely a tropical monsoon climate), we selected 3 periods of sampling to capture the variability in regional weather regimes (namely the wet and dry seasons). This is also mirrored in terms of the agricultural fertilization application regimes for the area. This sampling approach permitted a full assessment of nitrate sources to the DRB as well as key transformation processes at different periods of the year. Sampling was conducted

in October 2016 (the rainy season and regional non-fertilization period), July 2017 (the rainy season and regional fertilization period), and April 2018 (the dry season and regional fertilization period). Sampling was not conducted at stations D1-D5 in the July 2017 sampling campaign.

River waters were sampled at a distance of approx. 10 m from river banks and divided into sub-samples for analysis of N nutrient concentrations, Dissolved Organic Carbon (DOC), and stable isotope analyses. These parameters were chosen as it is acknowledged that to assess the sources of NO_3 and to identify the governing processes of NO_3 in stream water, stable isotopes of water ($\delta^{18}\text{O}\text{-H}_2\text{O}$), N species and carbon availability (represented as DOC) should also be analysed (Baker and Vervier 2004; Zarnetske et al. 2011; Ta et al. 2016).

For water stable isotope analyses, sub-samples were filtered in the field with Sartorius technical filter papers (8 μm pore size) and collected in 30 ml HDPE plastic bottles. They were then kept at 20 °C prior to be sent to the Isotope Hydrology Laboratory of the International Atomic Energy Agency (IAEA), Vienna, Austria for analysis. All samples were pipetted into 2 mL laser vials, and high-precision measured using a Los Gatos Research liquid water isotope analyzer model 912-0032 (Los Gatos Research (www.lgrinc.com, California, USA)). The method consisted of 9 injections per vial and ignoring the first 4, with data processing procedures to correct for between-sample memory and instrumental drift, and normalization to the VSMOW-SLAP scale using LIMS for Lasers 2015 as fully described elsewhere (Wassenaar et al. 2014; Coplen and Wassenaar 2015). A 2-point normalization was used using IAEA laboratory standards W-34 (low standard) and W-39 (high standard) to bracket the isotopic composition of the samples. IAEA laboratory standards were calibrated using VSMOW2 and SLAP2 primary reference materials using their assigned values of $0 \pm 0.3\text{‰}$, $0 \pm 0.02\text{‰}$, and $-427.5 \pm 0.3\text{‰}$, $-55.5 \pm 0.02\text{‰}$ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively. The assigned values for the laboratory calibration standards W-39, W-34 and control W-31 were $+25.4 \pm 0.8\text{‰}$ and $+3.634 \pm 0.04\text{‰}$; $-189.5 \pm 0.9\text{‰}$ and $-24.778 \pm 0.02\text{‰}$; $-61.04 \pm 0.6\text{‰}$ and $-8.6 \pm 0.09\text{‰}$ for $\delta^2\text{H}$ and $\delta^{18}\text{O}$ relative to VSMOW, respectively. The control W-31 long-term (1-year running average) analytical

reproducibility (\pm SD) was $\pm 0.11\text{‰}$ and $\pm 0.7\text{‰}$ for $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively.

The sub-samples for dual stable isotope analysis of NO_3 were filtered with GF/F Whatman filters, stored in acid-cleaned, high-density polyethylene (HDPE) bottles and frozen prior to be sent also to the Isotope Hydrology Laboratory of IAEA for analysis. The Cd-azide reduction method to headspace N_2O gas was used as fully described in McIlvin and Altabet (2005). The instrument used was an Isoprime 100 with a Trace Gas (TG) system linked to a continuous flow isotope ratio mass spectrometer (CF-IRMS) system (Isoprime Ltd, Cheadle Hulme, UK). The Isoprime CF-IRMS system operated at an external analytical precision of $\pm 0.2\text{‰}$ ($\delta^{15}\text{N}\text{-N}_2\text{O}$ values) and $\pm 0.3\text{‰}$ ($\delta^{18}\text{O}\text{-N}_2\text{O}$ values) using 2-point normalization using dissolved nitrate reference materials (USGS32, USGS34, USGS35, IAEA NO_3).

The analytical procedures for dissolved nitrogen compounds were conducted in the Institute of Chemistry (ICH), Vietnam Academy of Science and Technology (VAST), in accordance with the Standard Methods for the Examination of Water and Wastewater (Clesceri et al. 1998). The 1 L sub-samples were kept below 4 °C to prevent significant degradation during storage, and analyzed within 48 h. Nitrate was determined by quantitative reduction to nitrite on a cadmium column, followed by colorimetric determination at 540 nm of nitrite using the Griess reaction (Standard method 4500- NO_3 E in Clesceri et al. (1998)). Detection limit (DL) of the NO_3 analysis was $0.02 \text{ mg NO}_3 \text{ L}^{-1}$. Analytical protocols of nitrite (NO_2) was similar to NO_3 but without the reduction step. The DL of this method was $0.005 \text{ mg N L}^{-1}$. Ammonium (NH_4) was determined colorimetrically at 640 nm by the phenol hypochlorite method (Standard method 4500- NH_3 F. phenate in Clesceri et al. 1998). The Kjeldahl digestion method was used for total nitrogen (N_{tot}) analysis as described in the Total Kjeldahl Nitrogen (TKN) method (Standard method 4500-Norg B) of Clesceri et al. (1998). The spectrometer used for colorimetric determination was an UV-VIS GBC Cintra 40 (Australia). Organic nitrogen (N_{org}) was simply subtracted from N_{tot} minus inorganic N species (NO_3 , NO_2 , and NH_4).

Sub-samples for DOC analysis were filtered on Whatman GF/F glass filters (filters were heated at 550°C in a furnace for 4 h to remove organic matter contaminant) and stored in the 10 ml glass tubes prior

to be analyzed in VAST. Each sample tube was doped with 10 µl of concentrated (98%) analytical grade H₃PO₄ and kept in refrigerator prior to analysis. Analysis of DOC was performed on a TOC-Ve (Shimadzu, Japan) with a triplicate reading mode.

Mixing model formulation

We considered the following four main sources of nitrate based on the geographical and natural conditions of the DRB (e.g. Thibodeau et al. 2013; Ta et al. 2016): (1) inputs from soil and groundwater sources (representing natural, background input levels), (2) inputs from the Red River (also considered as natural sources), (3) inputs from regional, excessive application rates of chemical fertilizers (urea), and (4) inputs from organic matter deriving from urban regions and livestock farming (sewage and manure). In order to derive the proportion that these sources derive in the DRB, we used the following partition equations of NO₃, which are based on our stable isotope data:

$$\delta^{18}O = f_S \delta^{18}O_S + f_P \delta^{18}O_P + f_U \delta^{18}O_U + f_M \delta^{18}O_M \quad (1)$$

$$\delta^{15}N = f_S \delta^{15}N_S + f_P \delta^{15}N_P + f_U \delta^{15}N_U + f_M \delta^{15}N_M \quad (2)$$

$$1 = f_S + f_P + f_M + f_U \quad (3)$$

Of which f_S , f_U , f_P , and f_M are respectively the partition coefficients of each of the 4 main sources (as listed above): (1) soil and groundwater input (f_S), (2) Red River inflow (f_U), (3) nitrified urea fertilizer run-off from paddy fields (f_P) and (4) sewage and manure discharge (f_M).

Since the mixing model has three equations (Eqs. 1, 2, and 3) and four variables (f_S , f_P , f_U , and f_M) which mathematically cannot be solved, we had to make further assumptions to deduce a variable (or add an equation). In the DRB, the Red River fraction (f_U) only exists at D10 and D11 (Fig. 1) where the Dao River discharges water into the Day River (Trinh et al. 2017). Thus, from D1 to D9, there are essentially only three variables (f_S , f_P , and f_M) which permit the three equations to be solved. Therefore at sites D10 and D11, we used additional information of water stable isotopes and nitrate concentrations to estimate the variable f_U (Eqs. 4, 5, and 6) so as to calculate the

three other variables (f_S , f_P , and f_M) and apply our mixing model.

$$\delta^{18}O - H_2O_{D10} = f_{D9} \delta^{18}O - H_2O_{D9} + f_{Dao} \delta^{18}O - H_2O_{Dao} \quad (4)$$

$$C_{NO_3, D10} = f_{D9} C_{NO_3, D9} + f_{Dao} C_{NO_3, Dao} \quad (5)$$

$$1 = f_{D9} + f_{Dao} \quad (6)$$

where f_{D9} and f_{Dao} are respectively flow/discharge fractions of D9 and Dao River (Red River input) at D10. C_{NO_3} represents the concentration of NO₃ at different points. In fact, $f_{Dao} * C_{NO_3, Dao}$ and $f_{D9} * C_{NO_3, D9}$ are respectively corresponding to $f_{u, D10}$ and $f_{S, D10} + f_{P, D10} + f_{M, D10}$. In other words:

$$\frac{f_{Dao} C_{NO_3, Dao}}{f_{U, D10}} = \frac{f_{D9} C_{NO_3, D9}}{f_{U, D10} + f_{P, 10} + f_{Ur, D10}} \quad (7)$$

As $\delta^{18}O$ -H₂O in the Dao River is provided here, this equation was used to estimate the Red River input partition coefficient (f_U) at D10 and D11.

Of these 4 different sources applied in our mixing model (our end members), the soil and groundwater (f_S) and the chemical fertilizer (f_P) concentrations and compositions were derived from other studies taken in the RRD. For the soil and groundwater (otherwise naturally released) sources, a comprehensive isotopic analysis of dissolved N species in soil and groundwater for the Hanoi region was reported in Giap et al. (2007). Oxygen isotope ($\delta^{18}O$ -NO₃) signatures were extracted from the work of Saiki et al. (2019) who carried out a comprehensive study of nitrate isotopes within a sub-basin of the RRD, which neighbors the DRB. Nitrogen isotopes of nitrate excess from the application of urea fertilizer in paddy fields were also taken from the study of Saiki et al. (2019), which was in the range of fertilizer nitrogen isotope signatures reviewed in Bateman & Kelly (2007). Oxygen isotope ($\delta^{18}O$ -NO₃) end members were further calculated from the analyzed water stable oxygen isotope ($\delta^{18}O$ -H₂O) and atmospheric oxygen isotope (+ 23.5‰) based on recent studies about isotope fractionation during nitrification (Casciotti et al. 2010; Buchwald and Casciotti 2010). Detailed formulation of this calculation is shown in the appendix. Analytical results of samples taken at N2 in the Nhue River were used as the manure and sewage source based on the

fact that the Nhue River is the main open-air sewer of the Hanoi metropolis (Duc et al. 2007). For the Red River inflow, the additional analysis of 2 samples taken at sites in the Red River (sites of Ha Noi and Nam Dinh as represented in Trinh et al. 2017) provided data and these were used as the Red River inflow end member. All end member isotopic compositions are shown in Table 1.

It should be noted that we acknowledge the uncertainties surrounding source values due to the limited availability of data (on NO_3 isotopes) for this region. In particular, the mean and standard deviations calculated from our small data set do not permit a normal distribution check and so we may only partly represent the true variability of the NO_3 sources in the DRB. However, we highlight the importance of these attempts in elucidating pollution sources and N cycle processing taking place in this highly disturbed catchment of northern Vietnam. In addition, there are other sources of uncertainty which are beyond our control (e.g. the analytical uncertainties of data from

cited literature, and limitations surrounding their differing geographical locations, seasonality and/or time periods).

The Monte Carlo simulation run on the platform of Excel Visual Basic Macro was employed to analyze the uncertainty of the partition computation. As introduced in Do et al. (2014), we first decided the distribution type and specified the standard deviation values for each end member isotope composition based on our literature review (Giap et al. 2007; Bateman and Kelly 2007; Casciotti et al. 2010; Buchwald and Casciotti 2010; Saiki et al. 2019) and our analytical results. Next we reformulated our mixing model equations to embed it into the Excel platform. Then, we ran the Monte Carlo simulation and obtained 1,000 values for nitrate source components and get the uncertainty ranges of target parameters (source component fractions).

Table 1 Names and locations of the sampling stations, refer to Fig. 1 for their special location

Station name	River reach	Longitude (°E)	Latitude (°N)	Altitude (m)	Distance to the next downstream point
Phung (D1)	Upstream	105.64513	21.07521	12	30 km to D2
Mai Linh (D2)	Upstream Bui River's confluence	105.72711	20.93646	11	30 km to D3
Ba Tha (D3)	Confluence with Bui River	105.70722	20.80583	10	25 km to D4
Te Tieu (D4)	Downstream Bui River's confluence	105.74710	20.68646	9	35 km to D5
Que (D5)	Upstream Nhue River's confluence	105.87263	20.57451	8	9 km to D6
Ba Da (N2)	Nhue River mouth	105.92192	20.56717	8	6 km to D6
Do (D6)	Downstream Nhue River's confluence	105.91151	20.51578	7	20 km to D7
Doan Vi (D7)	Upstream Hoang Long River's confluence	105.92081	20.36240	3	20 km to D8
Non Nuoc (D8)	Downstream Hoang Long River's confluence and upstream Sat River's confluence	105.98071	20.26526	3	15 km to D9
Do Thong (D9)	Downstream Sat River's confluence and Upstream Dao River's confluence	106.04511	20.21738	2	25 km to D10
Do Muoi (D10)	Downstream Dao River's confluence (Day River receives the Red River water through Dao River)	106.16600	20.14200	1	20 km to D11
Cua Day (D11)	Day River mouth, estuarine zone	106.10300	19.92800	0	3 km to the Sea

Statistical analyses

Correlation coefficient calculation was used to assess the correlations between (i) isotopic variables and (ii) the isotopic variables and other variables. Principal component analysis (PCA) was used to define the principal processes governing the variability of analytical results (including the isotopes, all N species, and DOC) over the upstream sites (D1–D5). The PCA was run separately for April and October data in order to assess the seasonality of biogeochemical processing. Note, we did not run the PCA analysis for the downstream sites for comparison as NH_4 and NO_2 were, for the most part, below the analytical detection limit. All statistical tests were performed using the statistical Origin software, version 2019b.

Results

Seasonal and spatial distribution of DOC, nitrogen species, and NO_3 stable isotope signatures

The dominant N species was NO_3 (accounting for > 50% of total N, especially in the middle section of the river; Fig. 2a–d). In general, nitrate was higher in the middle section than in the upstream and downstream sections of the Day River, with the highest concentration (4.1 mg-N L^{-1}) at site D6 in the April survey (the dry season) (Fig. 2a). The lowest nitrate concentrations were also found in April at the upstream site of D2 (0.1 mg-N L^{-1}). Nitrite was the least concentrated among N species (Fig. 2b). In general, NO_2 was lower than 0.3 mg-N L^{-1} and spatially there was no clear downstream trend for the Day River. Ammonium was generally lower than detection limit ($0.015 \text{ mg-N L}^{-1}$) in downstream sites (D9–D11) (Fig. 2c). Like NO_3 , total nitrogen was higher in the dry (April) rather than in the rainy season (Fig. 2a, d) while NH_4 was lower in the dry season (rather than in the rainy season months of July and October) (Fig. 2c). Organic nitrogen, like NO_3 and N_{tot} , was higher in middle section than in upstream and downstream sections. DOC concentrations were generally higher in April compared to July and October sampling periods (Fig. 2e). The correlation between DOC and Organic nitrogen was positively weak. The correlation coefficients in April, July, and October were 0.10 ($p_{\text{value}} = 0.78$), 0.17 ($p_{\text{value}} = 0.62$),

and 0.07 ($p_{\text{value}} = 0.85$), respectively, while the DOC/Organic nitrogen ratio averaged at 12.5.

Water oxygen isotope signatures ($\delta^{18}\text{O-H}_2\text{O}$) did not change much between sites D1 to D9 but decreased sharply (nearly 2‰) after site D9, during the rainy season (Fig. 2f). While during the dry season, water oxygen isotope compositions were low in upstream (-5.78‰ at D2) and downstream (-8.6‰ at D10) reaches of the DRB, they remained high in the middle sections (-2.63‰ at D5). Average values of $\delta^{18}\text{O-H}_2\text{O}$ in April, July, and October were -4 , -6.2 , and -8‰ respectively.

In general, the oxygen isotope composition of nitrate ($\delta^{18}\text{O-NO}_3$) varied between -7.1 and $+9.2\text{‰}$ (Fig. 3a). This range of $\delta^{18}\text{O-NO}_3$ was lower than the range usually found in inorganic fertilizer NO_3 ($> +20\text{‰}$, (Amberger and Schmidt 1987)) and atmospheric deposition of NO_x ($> +30\text{‰}$, (Kendall et al. 2007)) sources, corresponding well with urea being the main N fertilizer utilized in this deltaic region. Seasonally, $\delta^{18}\text{O-H}_2\text{O}$ and $\delta^{18}\text{O-NO}_3$ data displayed similar trends, as signatures for both were highest in April, followed by July, and then October (Figs. 2f and 3a).

Overall, the range of $\delta^{15}\text{N-NO}_3$ fell between -3.9 to $+13.2\text{‰}$ which covers most sources of $\delta^{15}\text{N}$ usually found in surface waters (N sources from chemical fertilizers to soil, manure and sewage) (Fig. 3b). Spatially, the variation observed for $\delta^{15}\text{N-NO}_3$ on the upper reaches of watersheds was higher than that observed further downstream (after D8). Seasonal changes in $\delta^{15}\text{N-NO}_3$ were greater in upstream reaches compared to the downstream sites (Fig. 3b).

Correlation coefficient between the nitrate isotopes for the whole dataset was 0.39 ($p_{\text{value}} = 0.05$). For each of the three field campaigns, variations in $\delta^{18}\text{O-NO}_3$ and $\delta^{15}\text{N-NO}_3$ data were not well correlated (Fig. 4). Correlation coefficients between the 2 variables in April, July, and October were -0.21 ($p_{\text{value}} = 0.56$), 0.41 ($p_{\text{value}} = 0.40$), and 0.15 ($p_{\text{value}} = 0.68$), respectively. The correlation coefficient between $\delta^{18}\text{O-NO}_3$ and $\ln(\text{NO}_3)$ was -0.73 ($p_{\text{value}} = 0.01$) in October (wet season). In April, the correlation coefficient was lower ($= -0.47$, $p_{\text{value}} = 0.17$). In addition, correlation coefficients of upstream sites (D1–D5) are shown in Table 2. In both periods, correlation coefficients between $\delta^{18}\text{O-NO}_3$ and NO_3 were negative. Isotopic signatures appeared

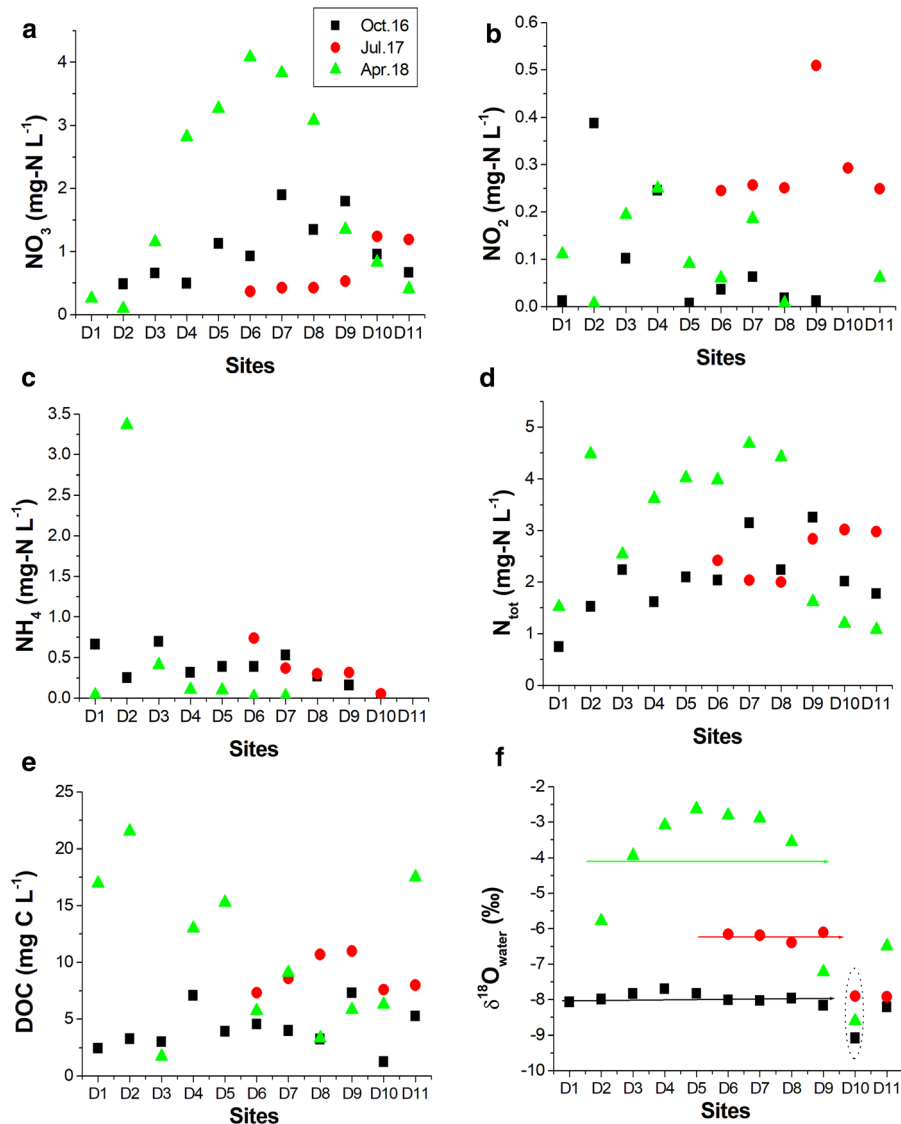


Fig. 2 Concentrations of N species, DOC, and water stable isotopes ($\delta^{18}\text{O}\text{-H}_2\text{O}$) at different sampling sites; April, July, and October are respectively the dry and fertilization period, rainy and fertilization period, and rainy and non-fertilization period

to be more correlated with each other and with DOC in April, than in October.

The PCA for upstream sites (D1–D5) in April and October campaigns (Fig. 5) showed about 90% of data variability was explained by the first 2 components (axes). Statistically, these 2 first axes were sufficient to represent the variability of the whole data set. In both periods, the first axis had high positive loadings of NH_4 and $\delta^{18}\text{O}\text{-NO}_3$ and a high negative loading of NO_3 . Axis 2 had high positive loadings of $\delta^{15}\text{N}\text{-NO}_3$ and DOC. The most noticeable difference between the

2 months is that DOC and $\delta^{15}\text{N}\text{-NO}_3$ changed from high loadings in April to low, negative loadings in October as is demonstrated in their distribution on axis 1.

Partition of N sources

In general, the natural end members (soil/groundwater and Red River inputs) dominated (more than any other anthropogenic end member sources) in the upstream (D1–D5) and downstream (D10–D11) reaches. On the

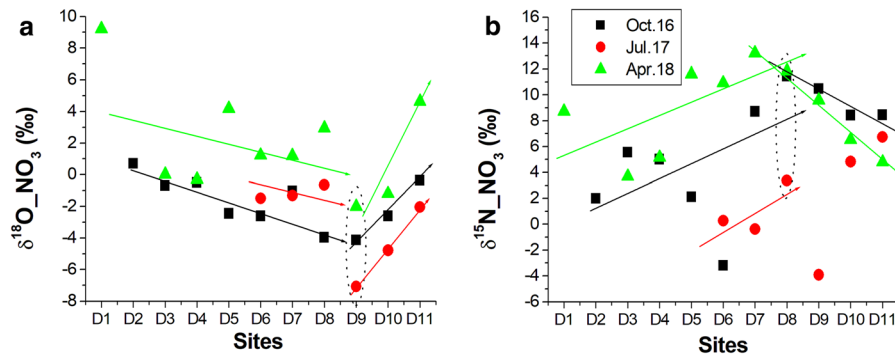


Fig. 3 Variation of nitrate stable isotopes ($\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$); April, July, and October are respectively the dry and fertilization period, rainy and fertilization period, and rainy and non-fertilization period

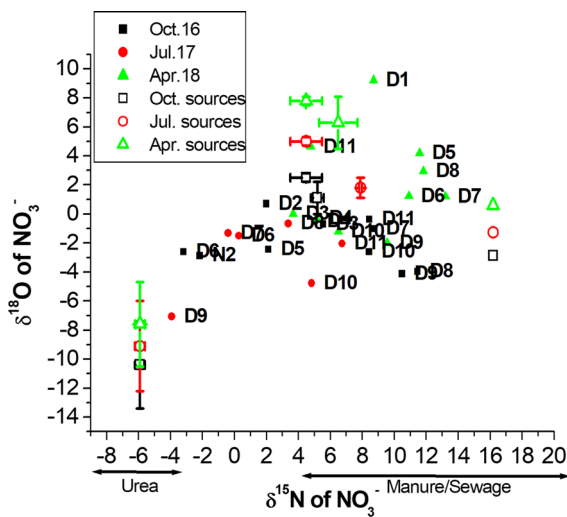


Fig. 4 Crossplot of analytical $\delta^{15}\text{N-NO}_3$ and $\delta^{18}\text{O-NO}_3$ and the mixing model end member compositions; 2-tailed arrows in the horizontal axis represent the ranges of $\delta^{15}\text{N-NO}_3$ if NO_3 is dominated by either urea or manure/sewage

other hand, in the dry season, manure and sewage end member sources were more dominant, particularly in the middle section of the DRB (representing more than $> 50\%$ of total NO_3 at D5–D9). In addition, D9 is located at the point of greatest agricultural impact, especially notable in the fertilization and rainy periods of July ($79 \pm 14\%$). As shown in Fig. 6, for all surveys, the proportion of natural (soil/groundwater and Red River inflow sources) end member inputs to the DRB accounted for not more than $50 \pm 23\%$ of the total NO_3 concentrations in the river. Only in the rainy season and when fertilizers were applied (July), did manure and sewage contributions account for a smaller fraction ($15 \pm 8\%$) than agricultural sources

($45 \pm 10\%$). For other periods (April and October), anthropogenic sources of NO_3 were greater contributors than agricultural sources to the DRB, which is unsurprising given that the DRB is one of the most urbanized catchments in Vietnam.

The results of partition calculations demonstrated that chemical fertilizer was a main source for NO_3 in Day River during the rainy season. Even during the non-fertilization period (October), fertilizer derived NO_3 still accounted for $20 \pm 10\%$ of nitrate sources. This number increased to $45 \pm 10\%$ (mentioned above) when fertilizer was applied during rainy season (July), while in the dry season only $15 \pm 13\%$ of the river NO_3 was derived from nitrified urea fertilizers.

Partition calculations for the downstream sites D10 and D11 showed that the Red River fractions of NO_3 constituted up to about $50 \pm 7\%$ of the total NO_3 (Fig. 6).

Discussion

This manuscript aims to address two central questions, which include where the main sources of river NO_3 come from and what the dominant biogeochemical processes are which control the fate of NO_3 in the DRB. To address these questions, the Discussion is organized in two sections. The first is to examine our applied mixing model results to distinguish if agriculture (rice cultivation and their fertilizer sources) and/or urbanization (anthropogenic sources) are the main sources of NO_3 to the DRB. Our second direction is to correlate isotopic signatures with nitrate and carbon availability so as to estimate the loss (via

Table 2 End member compositions of $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ of nitrate used in this study for the partition calculations. Values for each of the three sampling periods (and seasons) are displayed with their respective standard deviations

	Literature review				This study			
	Soil and ground (natural leaching)		Paddy fields (nitrified urea fertilizer run-off)		Red River (natural inflow)		Manure and sewage (anthropogenic organic N)	
	$\delta^{18}\text{O}$ ^(a)	$\delta^{15}\text{N}$ ^(b)	$\delta^{18}\text{O}$ ^(c,d)	$\delta^{15}\text{N}$ ^(a,e)	$\delta^{18}\text{O}$ ^(c)	$\delta^{15}\text{N}$ ^(c)	$\delta^{18}\text{O}$ ^(c)	$\delta^{15}\text{N}$ ^(c)
Dry, fertilizing	7.8 ± 0.3 (2)	4.5 ± 1.0 (40)	- 7.6 ± 2.9 (32)	- 5.9 ± 0.3 (2)	6.3 ± 1.8 (2)	6.5 ± 1.2 (2)	0.59 (1)	16.2 (1)
Rainy, fertilizing	5.0 ± 0.3 (3)	4.5 ± 1.0 (40)	- 9.1 ± 3.1 (32)	- 5.9 ± 0.3 (2)	1.8 ± 0.7 (2)	7.9 ± 0.1 (2)	- 1.26 (1)	16.2 (1)
Rainy, non-fertilizing	2.5 ± 0.3 (2)	4.5 ± 1.0 (40)	- 10.4 ± 3.0 (32)	- 5.9 ± 0.3 (2)	1.1 ± 1.1 (2)	5.2 ± 0.4 (2)	- 2.87 (1)	16.2 (1)

Numbers in () indicate sample size

^(a)Saiki et al. (2019)

^(b)Giap et al. (2007)

^(c)Our analytical results

^(d)Casciotti et al. 2010 and Buchwald and Casciotti (2010)

^(e)Bateman and Kelly (2007)

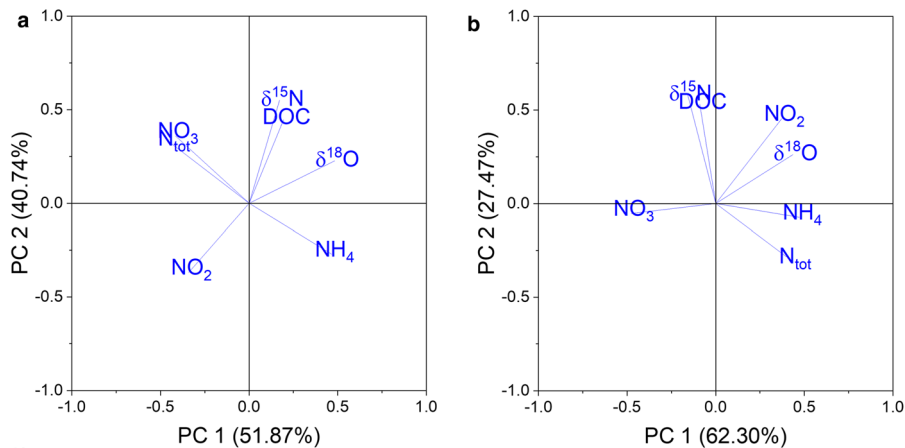


Fig. 5 PCA for the upstream sites (D1–D5) in **a** April and **b** October

denitrification and assimilation) and gain (via nitrification) of NO_3 in the DRB catchment and its streams. Our discussion aims to elucidate these points so as to ultimately propose plans for pollution detection, alleviation and better management practice in the catchment.

Sources of nitrate in the DRB

The weak positive correlation coefficient between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ ($R = 0.39$ ($p_value = 0.05$); Fig. 4) indicates the absence of a simple mixing of sources along the DRB. Nor does it indicate that one single biological process is responsible for the nitrate content in this river network. Indeed, the clear evidence of multiple mixing is further demonstrated by the sharp

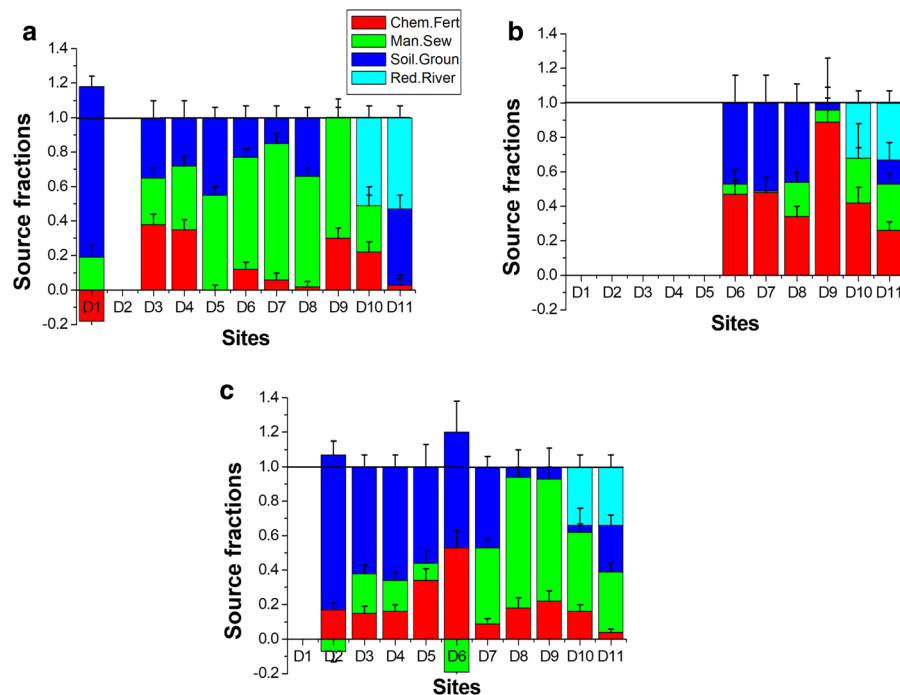


Fig. 6 Fractions of the different N-sources calculated for each site; Chem.Fert: chemical fertilizers (urea) source, Man.Sew: sewage and manure source, Soil.Groun: soil and groundwater sources, Red.River: inputs from the Red River

change in water isotope compositions between sites D9 and D10, and further still between D10 and D11 (Fig. 2f). The first change in these signatures reflects a substantial mixing of waters at D10 with the Red River water, which is delivered to the site via the Dao River (Trinh et al. 2017) and the latter change (at D11) is due to mixing with sea water [with the site located only 3 km from the sea (Table 3)].

Overall, we conclude that the different seasonal surveys capture the dominance of varied N sources at different times of the year (refer to Fig. 4 and the partition calculations). For example, in April, when rain is limited, river water is dominated by manure and sewage derived NO_3 sources. This is a period of (1) limited discharge of chemical fertilizers from paddy fields to water ways and (2) a reduced input of naturally derived NO_3 from soil and groundwater sources (due to the drier climatic conditions). However, during the rainy season (July and October), manure and sewage sources become less dominant due to the increased input of N derived from natural, upstream sources. In addition, nitrified urea fertilizer plays an important role in regulating river nitrate concentrations in July (which is the dominant urea

fertilization period), as indicated by a lower $\delta^{15}\text{N}$ signature than in other periods (Fig. 3b). While nitrate sources in the DRB appear to be dominated by manure and sewage in April, and chemical fertilizer in July, in October neither appear to be most important. Our partition calculation results represented in Fig. 6 also support the findings of Luu et al. (2012) that more than 50% of nutrient input to the RRD (of which the DRB is a sub-basin) is from anthropogenic activities.

Agricultural practices and their impacts on riverine NO_3 in the DRB

Variations in NO_3 stable isotope compositions in the DRB are similar to those in other watersheds (Battaglin et al. 2001; Chen et al. 2009; Lin et al. 2019). For instance, the fractionation effect observed for $\delta^{15}\text{N}\text{-NO}_3$ on the upper reaches of watersheds is often higher than the fractionation observed further downstream. This highlights the importance of the biogeochemical processes taking place in upstream reaches, accounting for these isotopic signatures. Furthermore, seasonal changes in $\delta^{15}\text{N}\text{-NO}_3$ signatures of the DRB are greater than the ranges observed

Table 3 Correlation coefficients among variables for upstream sites (D1–D5)

			October			
			NO ₃	DOC	δ ¹⁸ O-NO ₃	δ ¹⁵ N-NO ₃
April	NO ₃	Pearson Corr.	1	− 0.01	− 0.93	− 0.39
		p_value	–	0.99	0.07	0.61
	DOC	Pearson Corr.	− 0.21	1	− 0.03	0.34
		p_value	0.74	–	0.97	0.66
	δ ¹⁸ O-NO ₃	Pearson Corr.	− 0.50	0.68	1	0.09
		p_value	0.50	0.32	–	0.91
	δ ¹⁵ N-NO ₃	Pearson Corr.	0.31	0.75	0.65	1
		p_value	0.69	0.25	0.35	–

along the upstream–downstream continuum, implying the importance of natural processes (i.e., rainfall and temperature) in generating greater changes in nitrate isotope ratios (point sources and non-point sources) along the river reach. Indeed, in the DRB where agricultural activities dominate and precipitation regimes are seasonal, such seasonal changes of water and nitrate stable isotope signatures between wet and dry seasons and between planting and harvesting periods, are displayed.

There are, on the other hand, some distinctive differences between the DRB and eutrophic river systems elsewhere in the world. For instance, compared to other studies (Battaglin et al. 2001; Chen et al. 2009; Lin et al. 2019), the present study (building on earlier work of Ta et al. 2016) is among the few reporting δ¹⁵N-NO₃ values < 0 (Popescu et al. 2015; Vrzel et al. 2016). A rational explanation is that urea characterized with depleted δ¹⁵N is extensively used in the DRB and agriculture is the dominant source of NO₃ in many parts of this system. In fact, synthetic urea fertilizers of low (negative) δ¹⁵N were reportedly used in paddy fields of the RRD (Ta et al. 2016; Saiki et al. 2019). In all surveys conducted in this study, δ¹⁵N at site D9 was always lower than the preceding upstream site (D8). We argue that this is directly resulting from the large proportion of agricultural water which discharges from the Sat River at site D9. As shown in Fig. 1, the Sat River irrigates and drains large paddy fields and wetlands in Ha Nam and Nam Dinh Provinces. As a result, the composition of the Sat River should be (and is) characterized by low δ¹⁵N. An implication of this finding is that there is a need to re-evaluate the application of fertilizer to paddy fields in the DRB so as to moderate fertilizer application rates, especially during rainy seasons. These arguments are

also based on the partition calculation conducted in this study, which found that at D9, during the rainy season and fertilization period, 79 ± 14% of NO₃ in the river water is derived from chemical fertilizer sources. If one takes into account the water discharge of c. 540 m³ s^{−1} in this middle section during the rainy season (Luu et al. 2010) and a concentration of NO₃ of 0.5 mg N L^{−1} at D9 (derived in this study), we can then estimate the amount of chemical N fertilizers discharging in DRB during this period to be c. 18.66 t d^{−1}. Compared to data published elsewhere (e.g. Luu et al. 2012 argue that total N delivered from DRB as about 30 t d^{−1}), this study shows how substantially large the impact the chemical N fertilizer is to aquatic media (not only riverine but also coastal) in this region.

Manure and sewage NO₃ sources in the DRB

High values of river δ¹⁵N signatures in the middle section of the DRB can be attributed to an increasing impact of domestic waste (manure and sewage) discharge, characterized with a high δ¹⁵N (Bateman and Kelly 2007). Our partitioning calculation shows that the manure/sewage fraction reaches as high as 80% in this middle section (D6–D8) in April (the dry season) and in October (the rainy season) (Fig. 6). However, while the manure and sewage fraction and NO₃ concentration in April are about 1.5 and 2 times higher (respectively) than those values in October, the river discharge in April is only about one third of that in October. We see that there are therefore similar loads of manure and sewage NO₃ between the 2 surveys. In fact, Luu et al. (2010) also reported a seasonally unvarying flow of the main open-air sewer of the north-eastern part of the catchment (Hanoi

Metropolis) which would further support our argument.

Using land use data and Material Flow Analysis, Do et al. (2014, 2019) estimated nitrogen loads in the DRB for the period of 2008–2010 and found that the Day River receives approximately 9600 t year^{-1} of chemical N fertilizer and $12,000 \text{ t year}^{-1}$ of anthropogenic induced organic N (all forms/species of N), respectively. The Day River therefore receives more anthropogenic organic N (e.g. manure, sewage or sludge) than chemical fertilizer N loading. Do et al. (2014) however were not able to provide a seasonal assessment of the changes between chemical and organic N inputs to the river. Here we show (Fig. 6) that the ratio between chemical fertilizer and anthropogenic organic NO_3 sources vary between about 0.3 in the dry season (April) and about 3.0 in the rainy and fertilization period (July). All our results therefore highlight (1) the impact of chemical fertilizer sources of NO_3 on the Day River and (2), more importantly, that there is an absence of seasonal variability in the anthropogenic sourced organic NO_3 end members in the DRB. The larger portion of organic NO_3 (in this case defined as manure and sewage), reflects the impact of urbanization and industrialization in the DRB.

Biogeochemical processing in the DRB

Nitrification processes in the DRB

Nitrate inputs into streams are generally derived from (1) atmospheric deposition, (2) soil/groundwater NO_3 leaching, (3) excessive application of nitrate fertilizers and (4) the nitrification of reduced N species (Kendall 1998). Based on our discussion in the preceding section, it is clear that (1) atmospheric deposition and (2) groundwater NO_3 leaching are minimal sources of NO_3 to the DRB, as is (3) excessive nitrate fertilizer application (Fig. 4). In other words, we argue that NO_3 in the DRB is derived by the nitrification of reduced N species. This is highlighted by the seasonal analogue between $\delta^{18}\text{O-H}_2\text{O}$ and $\delta^{18}\text{O-NO}_3$, which is higher in the dry season and lower in the rainy season (Figs. 2f and 3a), as the *in situ* nitrified NO_3 will consist of one oxygen atom derived from water in its molecule.

Seasonally, our data suggest that nitrification is more dominant in the rainy season than the dry season and more visible at sites with larger agricultural input

(e.g. site D9). This seasonal trend is further supported by Ta et al. (2016) for the Red River, who found a higher negative correlation between $\delta^{18}\text{O-NO}_3$ and $\ln(\text{NO}_3)$ in the rainy season (over the dry season) and concluded therefore that nitrification was more dominant during the former. This statement is corroborated by this study, where the correlation coefficient between $\delta^{18}\text{O-NO}_3$ and $\ln(\text{NO}_3)$ is -0.73 ($p_{\text{value}} = 0.01$, significantly correlated at $p 0.05$) in the October (wet season) sampling. Meanwhile in April, the dry season, the correlation coefficient is lower and insignificant ($= -0.47$, $p_{\text{value}} = 0.17$, insignificantly correlated at $p 0.05$).

Spatially, the downstream-depleted tendency of $\delta^{18}\text{O-NO}_3$ (except for sites D10 and D11 where water is strongly mixed with the Red River water and/or marine water (Trinh et al. 2017 and concluded herein)) may reflect an increasing contribution of in-stream nitrified NO_3 . The prevalence of nitrification in the DRB is evident when examining the nitrate isotopic signatures at site D9, the point of the greatest agricultural impact (the Sat River inflow). Here isotope signatures, especially $\delta^{18}\text{O-NO}_3$, were particularly lower than at all other sites (Fig. 3a). While the low $\delta^{15}\text{N}$ is concluded to be of a chemical fertilizer origin, the low $\delta^{18}\text{O-NO}_3$ compositions could only be a result of nitrification. Indeed, recent studies (e.g. Casciotti et al. 2007, 2010; Buchwald and Casciotti 2010) have presented oxygen isotopic exchange and fractionation during nitrification to form NO_3 , with $\delta^{18}\text{O-NO}_3$ falling between -8.3 and -0.7‰ of the ocean water ($\delta^{18}\text{O-H}_2\text{O}_{\text{VSMOW}}$). By applying the formulas and fractionation coefficients reported in those studies (refer to appendix) to our case study, where $\delta^{18}\text{O-H}_2\text{O}$ of river water signatures range between -8 and -4‰ (Fig. 2f), the $\delta^{18}\text{O-NO}_3$ composition of nitrified NO_3 would be between -10.4 and -7.6‰ . Comparing $\delta^{18}\text{O-NO}_3$ signatures at D9 (which are between -7.07 and -2.01‰ for all three sampling periods, Fig. 3a), we conclude that NO_3 at site D9 is largely a result of the nitrification of urea fertilizers and that isotope enrichment (via assimilation) is minimal (Panno et al. 2006). Rather, we argue that the more positive $\delta^{18}\text{O-NO}_3$ values at sites D9 than the ones calculated for nitrified urea fertilizer sources alone, are due to mixing with other nitrate sources (of other nitrate sources of more positive isotope values) and/or fractionation (NO_3 delivered to

site D9 has already been fractionated (e.g. denitrification) to increase its signature).

The PCA applied to upstream sites (D1–D5) has shown a similarity between DOC and $\delta^{15}\text{N}\text{-NO}_3$ variables in both April and October which we conclude signifies that NO_3 is predominantly derived from soil and domestic organic matter. The opposite positioning of NO_3 and NH_4 vectors for these upstream sites (Fig. 5) also implies that NO_3 is sourced from NH_4 and NH_4 in turn, results from a degradation of organic matter. These PCA results confirm those of the partition calculation which also show a dominance of soil and sewage/manure sources in upstream sites (Fig. 6a,c).

Denitrification and biological assimilation

Overall, the weak positive correlation between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ signatures ($R = 0.39$ ($p\text{-value} = 0.05$); Fig. 4) represents isotopic enrichment in the DRB. The isotopic enrichment is likely a result of denitrification and/or biological assimilation since these processes fractionate nitrate nitrogen and oxygen isotopes equally, leaving behind nitrate that is enriched in both ^{15}N and ^{18}O (Granger et al. 2004, 2008).

Studies have shown that stream systems are particularly efficient at removing and retaining excess nitrogen (N) (Seitzinger et al. 2006) with headwater and mid-network streams being the most effective in the regulation of downstream N exports (Peterson et al. 2001; Alexander et al. 2000; Mulholland et al. 2008). Characteristics of these headwater and mid-network streams is the higher contact (exchange potential) between water and sediment interfaces than in downstream reaches [Anderson et al. 2005], especially during periods of low discharge (Wondzell, 2011). This pattern is particularly evident for the DRB since river flow in the upper reaches of the Day River (sites D1 and D2; Fig. 2f) are lower, resembling a lentic system and thereby providing an environment more conducive to denitrification. Figure 5 also supports these statements, due to strong positive association of $\delta^{18}\text{O}\text{-NO}_3$ and NH_4 with axis 1. The opposite relationship with NO_3 along axis 1, suggests a nitrification-denitrification process is at play, capturing the conversion of N species at sites D1–D5 between oxidized (NO_3) and reduced N (NH_4). This very upstream section is also an urbanized area and

part of the Hanoi Metropolis (Fig. 1). Indeed statistical tests run on data from the upstream sites have confirmed (1) the dominance of organic matter mineralized and nitrified NO_3 (Fig. 5 and discussed above) and more importantly (2) the increased denitrification during the dry season which is demonstrated by the strong association between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ along axis 1 (Fig. 5a) (acting as a nitrification-denitrification scale) and the positive correlation coefficient between the 2 isotopes ($= 0.65$, $p\text{-value} = 0.34$).

The higher signatures seen and the more pronounced increasing trend of $\delta^{15}\text{N}\text{-NO}_3$ in upstream samples (sites D1–D5) in April (Fig. 3b) also suggests a greater denitrification of NO_3 sources. The greater degree of denitrification may indicate relatively longer residence times and more intensified biological activities in these parts of the DRB (as discussed above) during the dry period. Compared to isotopic data obtained in temperate, higher latitude climates, where summer and autumn are seasons of stronger biological activity in upstream regions (e.g. Panno et al. 2006) our data indicates an opposite seasonal change, for denitrification. The difference is because in the DRB the temperature rarely decreases to below 10°C , a condition which would limit biological activity (including plant growth) and denitrification. Even during the monsoon rainy season, when N soil leaching may increase, the intense precipitation dilutes these nutrients and carbon species (indicated by low DOC concentrations; Fig. 2e) to lessen biological activity as well.

Another line of evidence for denitrification occurring at polluted water sites in the DRB is shown via the partition calculation. Negative fractions of chemical fertilizer or manure/sewage end members at sites D1 and D2 (the most upstream sections), where water flows slowly, and site D6, where the Day River receives urban wastewater brought in by the Nhue River (Fig. 1), reflect some strong isotope fractionation. Mathematically, such a negative trend is found if the data points are outside of the end member matrix. This may occur if, (1) by some means, the isotopic fractionation of NO_3 continues substantially downstream (for this case, biological activities will drive fractionation effects to account for the extreme isotopic compositions) or (2) an improper selection of end-member values. When considering option 2, we cannot rule out the possibility of having improperly selected end-member source values, which would

result in negative values. As shown in Table 1, the source values were selected based on a limited number of analytical results or derived from the literature. While future studies should enhance this analytical gap for the DRB, so as to further validate our estimations, we feel confident that our calculations remain valid for this study. As such, we explain that such negative values are due to in-stream isotopic fractionation. In a still water body, that receives a large amount of domestic wastewater (indicated by high DOC concentrations as shown in Fig. 2e), respiration and biodegradation processes would help drive the enrichment of ^{15}N organic N (Kendall et al. 2007) and ^{18}O dissolved oxygen (Quay et al. 1995). Our monthly surveys of water quality show a hypoxic state during dry periods (results not shown). As a consequence, NO_3 produced in such a system should be characterized by elevated $\delta^{15}\text{N}\text{-NO}_3$ and $\delta^{18}\text{O}\text{-NO}_3$ signatures. Furthermore, as denitrification will likely take place in this respiration dominated aquatic medium, NO_3 will be characterized with higher isotopic signatures (Site 1 in Fig. 3). To sum up, in heterotrophic ecosystems, nitrification takes place in an elevated $\delta^{18}\text{O}\text{-O}_2$ media, which will produce NO_3 characterized by a high $\delta^{18}\text{O}\text{-NO}_3$ composition. Following nitrification in such a heterotrophic system, denitrification will lead to a higher $\delta^{18}\text{O}\text{-NO}_3$ signature of the remaining NO_3 pool so that, nitrate isotope compositions will plot outside of the end-member matrix.

Another important observation from the spatio-temporal assessment of in-stream denitrification is whether this has triggered localized within stream hypoxia or anoxia. As denitrification is a reduction process which is intense in parts of the DRB, it is possible that water at certain depth in the river water column has equally been altered to less oxic conditions, which has implications on within stream aquatic ecosystem health. In fact, previous studies on several streams/ivers in the DRB have shown that the rivers running through urban areas are transformed into, and actually function as, open air sewers (Duc et al. 2007; Trinh et al. 2012). At several upstream sites in the DRB where urbanization has increased over the last 20 years, domestic wastewater has notably increased its proportion in the catchment water discharge, which has subsequently augmented the amount of biogeochemical processing which would otherwise not have taken place in surface waters (e.g. denitrification,

methane production), thereby deteriorating the local environment.

Carbon and NO_3 variability in the DRB

Discussions in the precedent sub-sections have pointed to a reality that in tropical aquatic systems, carbon availability (which is related to biodegradable organic matter) plays an important role in NO_3 variability. Many authors have reported the relationship between carbon availability and denitrification/assimilation in streams and rivers (Baker and Vervier, 2004; Zarnetske et al. 2011; Johnson et al. 2012). For instance, Baker and Vervier (2004) highlight that rates of denitrification were driven by the concentration of low molecular weight organic acids, derived from the decomposition of soil organic matter. In particular, DOC availability and river-floodplain connectivity were important factors influencing this process. Similarly, others argued that denitrification in anaerobic portions of the hyporheic zone is limited by labile DOC supply (Zarnetske et al. 2011) and that DOC enrichment in the water column will cause N assimilation to increase (Johnson et al. 2012). As discussed above, our data have highlighted that carbon availability (DOC) has an impact on the NO_3 variability in the DRB, especially in the upstream sites during the dry period. As demonstrated in Fig. 5, DOC is strongly associated with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ at the upstream sites of the DRB. This is further corroborated by correlation coefficients of $\delta^{18}\text{O}\text{-NO}_3 - \delta^{15}\text{N}\text{-NO}_3$, $\delta^{18}\text{O}\text{-NO}_3 - \text{DOC}$, and $\delta^{15}\text{N}\text{-NO}_3 - \text{DOC}$ which are respectively 0.65 (p_value = 0.35), 0.68 (p_value = 0.32) and 0.75 (p_value = 0.25). These data together imply that DOC plays a prominent role in the denitrification process of the DRB in the dry season.

Conclusions

This study highlights the effectiveness of applying stable isotope approaches such as water and dual NO_3 , to assess the provenance and biogeochemistry of an aquatic system impacted by multiple pollution sources. With the use of nitrate isotope data, we show how biological activities drive the isotopic compositions of nitrate in the DRB, Vietnam; these processes include nitrification of urea, NH_4 in autotrophic and heterotrophic media, and denitrification.

We identify 4 different sources of NO_3 in the DRB, with differing importance along the river course. Inorganic urea fertilizer is a major source (particularly at site D9), along with urban sewage, showing a clear impact especially the downstream sections, after the confluence with the Nhue River (at site D6). Indeed, this study shows how strongly anthropogenic activities have impacted the Day River system. In the middle section, after the confluence with the Nhue River, the proportion of natural inputs (soil/ground and upstream Red River inflow sources) of NO_3 rarely contribute more than 50%. Rather, the high proportion of NO_3 deriving from anthropogenic activities in the DRB is apparent. Our study highlights that there is a need to re-evaluate the application of inorganic fertilizers to paddy fields in the DRB region, so as to moderate excessive application. This is particularly important when fertilization practices are taking place in the rainy season, where rapid and large scale delivery of NO_3 (derived from urea end-member sources) is demonstrated.

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Appendix: Calculation of $\delta^{18}\text{O}\text{-NO}_3$ based on water oxygen and dissolved oxygen isotopes

Nitrification occurs as a two-step process whereby ammonia is first converted to nitrite and the produced nitrite is then converted to nitrate. During the bacterial nitrification process, the biogeochemical sources of oxygen atoms are dioxygen (O_2) and water (H_2O). O_2 is incorporated during the oxidation of ammonia to hydroxylamine (NH_2OH), while H_2O is incorporated during the oxidation of both hydroxylamine to nitrite and nitrite to nitrate. While the ratio of 1:2 oxygen atoms from O_2 and H_2O implied by these observations is commonly used to interpret the oxygen isotopic content of nitrate derived from bacterial nitrification (Kendall 1998; Burns and Kendall 2002; Wankel et al. 2006), the utilization of this ratio involves the

assumptions that exchange and fractionation of oxygen isotopes during nitrification are minimal. Recent works (e.g. Casciotti et al. 2007; Casciotti et al. 2010; Buchwald and Casciotti, 2010) have presented oxygen isotopic exchange and fractionation during nitrification. In general, during bacterial ammonia oxidation, the produced $\delta^{18}\text{O}\text{-NO}_2$ is computed as:

$$\delta^{18}\text{O}_{\text{NO}_2} = \left[\frac{1}{2}(1 + x_{\text{AOB}}) \right] (\delta^{18}\text{O}_{\text{H}_2\text{O}}) + \frac{1}{2}(\delta^{18}\text{O}_{\text{O}_2} - \varepsilon_{k,\text{O}_2} - \varepsilon_{k,\text{H}_2\text{O},1}) + (\varepsilon_{eq})(x_{\text{AOB}}) \quad (1)$$

In which χ_{AOB} , $\varepsilon_{k,\text{O}_2}$, $\varepsilon_{k,\text{H}_2\text{O},1}$, ε_{eq} , are respectively the fraction of nitrite oxygen atoms that have equilibrated with H_2O during ammonia oxidation, the kinetic isotope effect for O_2 incorporation, the kinetic isotope effect for H_2O incorporation by hydroxylamine oxidoreductase, and the equilibrium isotope effect for nitrite equilibration with H_2O .

Then, during bacterial nitrite oxidation, $\delta^{18}\text{O}\text{-NO}_3$ is estimated as (exchange of oxygen atoms between nitrite and water is minimal; Buchwald & Casciotti, 2010):

$$\delta^{18}\text{O}_{\text{NO}_3} = \frac{2}{3}\delta^{18}\text{O}_{\text{NO}_2} + \frac{1}{3}(\delta^{18}\text{O}_{\text{H}_2\text{O}} - \varepsilon_{k,\text{H}_2\text{O},2}) \quad (2)$$

whereas $\varepsilon_{k,\text{H}_2\text{O},2}$ is the kinetic isotope effect for water incorporation by nitrite oxidoreductase.

Literature review has shown that χ_{AOB} , $\varepsilon_{k,\text{O}_2}$ + $\varepsilon_{k,\text{H}_2\text{O},1}$, ε_{eq} , and $\varepsilon_{k,\text{H}_2\text{O},2}$ are respectively + 0.15 ± 0.1‰, + 26.3 ± 7.7‰, + 14‰, and + 15.5 ± 3.8‰ (Casciotti et al. 2007; Casciotti et al. 2010; Buchwald & Casciotti, 2010).

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