Towards a global interpretation of dual nitrate isotopes in surface waters

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Abstract:

Modern anthropogenic activities have significantly increased nitrate (NO_3^-) concentrations in surface waters. Stable isotopes ($\delta^{15}N$ and $\delta^{18}O$) in NO_3^- offer a tool to deconvolute some of the human-made changes in the nitrogen cycle. They are often graphically illustrated on a template designed to identify different sources of NO_3^- and denitrification. In the two decades since this template was developed, $\delta^{15}N$- and $\delta^{18}O$-NO_3^- have been measured in a variety of ecosystems and through the nitrogen cycle. However, its interpretation is often fuzzy or complex. This default is no longer helpful because it does not describe surface water ecosystems well and biases researchers towards denitrification as the NO_3^- removal pathway, even in well oxygenated systems where denitrification is likely to have little to no influence on the nitrogen cycle. We propose a different scheme to encourage a better understanding of the nitrogen cycle and interpretation of NO_3^- isotopes. We use a mechanistic understanding of NO_3^- formation to place bounds on the oxygen isotope axis and provide a means to adjust for different environmental water isotope values, so data from multiple sites and times of year can be appropriately compared. We demonstrate that any interpretation that our data (Canada, Kenya, United Kingdom) show clear evidence of denitrification or a mixture of NO_3^- sources simply because many data points fall outside of arbitrary boxes which cannot be supported once the range of potential $\delta^{18}O$-NO_3^- values has been considered.

Highlights:

- Interpretation of surface water $\delta^{15}N$- and $\delta^{18}O$-NO_3^- requires a more complex framework than currently employed
- Surface water processes alter $\delta^{15}N$- and $\delta^{18}O$-NO_3^- in different ways than the traditional groundwater-denitrification model rendering such frameworks obsolete
- Mechanistic understanding of NO_3^- cycling in surface waters means that the range of $\delta^{18}O$-NO_3^- is constrainable and can be made comparable between sites and across time via concurrent measurements of $\delta^{18}O$-H_2O and $\delta^{18}O$-O_2

Introduction:

Stable isotopes ($\delta^{15}N$ and $\delta^{18}O$) in nitrate (NO_3^-) have been commonly measured for more than 4 decades (see Heaton (1986) and papers therein). Methods have evolved from off-line $AgNO_3$ precipitation (e.g., Chang et al. 1999; Silva et al. 2000), to chemical and microbial reduction to N_2O and subsequent continuous flow – isotope ratio mass spectrometry analyses (Sigman et al. 2001; McIlvin and Altabet 2005). Since NO_3^- is a very common global pollutant, contributes to eutrophication of surface waters (Vitousek et al. 1997) and is the most common groundwater pollutant (Spalding and Exner 1993), a key application of NO_3^- isotopes was to identify NO_3^- sources. Through combining a number of individual studies, this lead to publication of a $\delta^{18}O$-NO_3^- vs $\delta^{15}N$-NO_3^- schematic biplot with suggested ranges for different 'sources' of NO_3^- (Kendall 1998). It has been modified a few times (e.g., Kendall et al. 2008; Xue et al. 2009; Kendall et al. 2015) but the fundamental concept remained the same. Its application for interpreting NO_3^- isotopes has become widespread but this figure is not really fit for this purpose and is commonly over-interpreted. Here, we discuss the assumptions inherent in this figure and key improvements needed for improved understanding of NO_3^- isotopes in surface waters.

Background

The schematic biplot figure was originally designed for interpreting groundwater data where NO_3^- isotope values of different NO_3^- sources are preserved except by (chemo)denitrification (e.g., Böttcher et al. 1990; Aravena et al. 1993; Aravena and Robertson 1998). Some researchers identified that forests receiving a lot of nitrogen deposition export NO_3^- in streams and this NO_3^- does not retain the atmospheric deposition isotope values (e.g., Spoelstra et al. 2001; Pardo et al. 2004). This was early evidence that measured NO_3^- isotopes in surface water showed that they should be carefully used for source identification because of various biological alterations along their flowpath. As method improvements allowed more NO_3^- isotope data to be generated, a schematic figure that recognized biotic and abiotic processing of NO_3^- between its sources and sampling point needed to be developed. However, the commonly used scheme encourages researchers to think only about one process, denitrification (Kendall 1998), although this process may be uncommon in well oxygenated lake surfaces or streams and rivers. In this way, we need a better schematic figure that explicitly recognizes the differences between NO_3^- sources and processes that produce and consume NO_3^-.

The "nitrogen axis" had been used as the primary differentiator between sources. However, given the wide range of possible $\delta^{15}N$ values in manure/sewage and soils (e.g., 30‰ range in soil alone, Craine et al. 2015), and the obvious fact that nitrogen will be biologically cycled in those systems, source identification cannot be done with boxes on a figure. Measuring locally appropriate sources of nitrogen as potential initial $\delta^{15}N$ values is the appropriate way to constrain this axis instead of relying on the broad assumption that a single set of boxes, derived from a limited number of measurements, are globally appropriate (Bateman and Kelly 2007). Without locally appropriate values, the borders between NO_3^- sources become very blurred on the $\delta^{15}N$-NO_3^- axis (Kendall et al. 2015) and this provides no useful resolution in the measured surface water data and no direct ability to identify sources.

In some cases, nitrogen from fertilizers and legumes will be mixed into the soil nitrogen pool (e.g., Oelmann et al. 2007) before NO_3^- is exported to surface waters (e.g., Deutsch et al. 2006). In such cases the exported $\delta^{15}N$-NO_3^- values will be controlled largely by the soil nitrogen pool and land-use history, rather than a single year of precipitation and fertilizer input (e.g., Loo et al. 2017). In this scenario the soil nitrogen averages all of its nitrogen inputs and NO_3^- subsequently exported from the soil to surface water maintains this average unless there is direct input of isotopically district NO_3^- to the surface waters.

The "oxygen axis" has groups that can be defined *a priori*: (i) high $\delta^{18}O$ values from NO_3^- produced in the atmosphere where the $\delta^{18}O$ value depends strongly on latitude (Michalski et al. 2012); and (ii) low $\delta^{18}O$ values where the $\delta^{18}O$ value depends strongly on the $\delta^{18}O$ of H_2O where the NO_3^- is formed (Snider et al. 2010). The $\delta^{18}O$ value of NO_3^- produced by autotrophic and heterotrophic nitrification can be bounded in two ways. First, canonical two-step nitrification (from NH_4^+ to NH_2OH to NO_2^- to NO_3^-) adds one O atom from O_2 in the first step and one O atom from H_2O in each of the next two steps (Hollocher et al. 1981; Andersson et al. 1983; Aleem et al. 1965; Hollocher 1984; DiSpirito and Hooper 1986; Snider et al. 2010). In surface soils, the pore gas $\delta^{18}O$-O_2 value is very likely near the atmospheric value of +23.5‰ (vs SMOW). However, in productive aquatic ecosystems, the diel variability of $\delta^{18}O$-O_2 values can be large (e.g., 23‰ range in Venkiteswaran et al. 2015, 14‰ range in Wassenaar et al. 2010, and 13‰ range in Parker et al. 2005) though this range can be estimated by one set of diel samples during the most productive part of the year and analyzed via a variety of techniques (e.g., Barth et al. 2004; Wassenaar and Koehler 1999). Second, incubation experiments with various levels of $\delta^{18}O$-H_2O indicate that the contribution of $\delta^{18}O$-H_2O values to the final $\delta^{18}O$-NO_3^- value is often greater than the minimum two-thirds and sometimes close to 1. Thus the range of $\delta^{18}O$ values of NO_3^- produced *in situ* can be bounded by knowledge of $\delta^{18}O$-O_2 and $\delta^{18}O$-H_2O values: a minimum of $\delta^{18}O$-H_2O and a maximum of $\frac{1}{2} \times \delta^{18}O\text{-}O_2 + \frac{2}{3} \times \delta^{18}O\text{-}H_2O$.

Site descriptions:

To highlight the need to include nitrogen cycling in surfaces waters into our working interpretation of NO_3^- isotopes, we selected six rivers from Canada, Kenya, and the United Kingdom each with different climate regions, seasonal variation in flow, and $\delta^{18}O$-H_2O values.

The Grand River, Ontario, Canada is the largest river draining into the Canadian side of Lake Erie. There are five cities, 30 wastewater treatment plants, and extensive modern agriculture along the 300km river in its 6800km^2 basin (Venkiteswaran et al. 2015). Climate is humid continental with a warm summer (Köppen–Geiger classification Dfb), average temperature is around 9°C and mean precipitation is 915mm. Samples were collected weekly to monthly from March 2015 to March 2016 from three sites: two sites upstream of the first major city and first large wastewater treatment plant and one below two cities and two large wastewater treatment plants. These sites offer the opportunity to sample from the river largely affected by diffuse non-point sources and after two large point sources. All sites are in the middle of the Grand River.

The Nzoia, Nyando, Sondu Rivers drain from Kenya into the east side of Lake Victoria. Kenyan drainage comprises 40% of the inflows to Lake Victoria (COWI 2002) and is therefore a significant source of the increasing nutrient concentrations in the lake (Juma et al. 2014). Eight sites on the Nzoia River, 11 sites on the Nyando River, and five sites in the Sondu River were sampled from January to April 2015. Sampling sites were selected based on access to the river and upstream land use. Climate in western Kenya is tropical rainforest and tropical monsoon (Köppen–Geiger classifications Af and Am).

The UK study sites compare nitrogen sources from peri-urban and rural river floodplains. Climate is maritime (Köppen–Geiger classification Cfb). Site 1 focuses on a peri-urban section of the River Thames in the vicinity of the city of Oxford in the southern UK. The mean annual flow of the Thames upstream of the study area is 18.48 m^3/s (Marsh and Hannaford, 2008). The baseflow index for the river at this location is 0.67, reflecting the influence of influent groundwater, sourced from the limestone aquifers located in the headwaters, and the extensive floodplain gravel aquifers. During the summer a significant component of flow is supported by effluent from Wastewater Treatment Works (WwTW) (Bowes et al., 2010). Five sites upstream and downstream of a WwTW were selected along the Thames and sampled in April and September 2016 for NO_3^- isotopes. Site 2 is on the River Lambourn in Berkshire. Chalk streams such as this are widespread across southern England (Allen et al., 2010). They are characterised by a high baseflow index (>0.9) and a shallow hyporheic zone. The primary source of nitrogen therefore comes from NO_3^- in groundwater due to fertilizer use.

Methods:

Canadian samples for NO_3^- isotopes were collected in HDPE bottles and filtered in the field to

0.45µm. Samples were kept cold and dark until returned to the lab where they were frozen until analysed. Samples for H_2O isotopes were collected in HDPE bottles without headspace. Canadian analyses were performed at the Environmental Isotope Laboratory at the University of Waterloo. NO_3^- isotope samples were analysed via the chemical denitrifier method where NO_3^- is reduced to N_2O with cadmium and sodium azide (McIlvin and Altabet 2005). The resultant N_2O gas was analysed on an IsoPrime continuous flow isotope ratio mass spectrometer (now Elementar, Cheadle Hulme, UK) with a precision of ±0.3‰ for $\delta^{15}N$-NO_3^- and ±0.5‰ for $\delta^{18}O$-NO_3^-. Water isotopes were measured on a a Los Gatos (Los Gatos Research, San Jose, USA) water isotope analyser with a precision of ±0.2‰ for $\delta^{18}O$-H_2O.

Kenyan samples were filtered to 0.45µm and stored below 4°C in 1L HDPE bottles. Kenyan analyses were performed at the Ghent University Stable Isotope Facility (UGent-SIF). NO_3^- isotopes were analysed by the bacterial denitrification method (Xue et al., 2009) and the resulting N_2O gas analyzed with a SerCon trace gas preparation unit coupled to a SerCon 20-20 isotope ratio mass spectrometer (SerCon, Crewe, UK).

Isotope preparation and analysis for UK samples was carried out at the NERC Isotope Geosciences Laboratory (Keyworth, UK). NO_3^- was separated on anion resins and prepared as $AgNO_3$ using the method of Silva et al. (2000) and $\delta^{15}N$ analysed by combustion in a Flash EA coupled to a Delta Plus XL mass spectrometer (ThermoFinnigan, Bremen, Germany) with precision (1 SD) typically <0.8‰. $\delta^{18}O$ was analysed by thermal conversion to CO gas at 1400°C in a TC–EA online to a Delta Plus XL mass spectrometer with precision (1 SD) typically <1.2‰.

Results and Discussion:

On the traditional biplot, our data from Canada, Kenya, and the United Kingdom fall in a wide swath (Figure 1A). Data from each country has a wider range of $\delta^{15}N$-NO_3^- values than $\delta^{18}O$-NO_3^- values. Additionally, data from each country has a positive relationship between $\delta^{18}O$-NO_3^- and $\delta^{15}N$-NO_3^- (2-tailed parametric $p<0.006$ for each country). But this relationship also contains seasonal changes in ambient $\delta^{18}O$-H_2O values, temperature, and nitrogen sources and processes that confound direct comparison of the data.

This means that without additional independent information, there are several possible explanations for the data that are more complex than simply assigning a source of NO_3^- based on the $\delta^{15}N$ values or assigning a single process based on a simplistic pattern in the $\delta^{18}O$- vs $_3^-$ and $\delta^{15}N$-NO_3^- values. For example, varying contributions of the $\delta^{18}O$-H_2O values, two or more sources of nitrogen, uptake and release of varying amounts of ammonium and NO_3^-, and denitrification in varying combinations may have produced the observed patterns in our data. It is critical to avoid wrongly invoking denitrification as the primary explanation for individual points on the traditional biplot as this risks suggesting nitrogen removal from the ecosystem when other explanations for the data need to be considered.

Certainly, any interpretation that our data show clear evidence of denitrification or a mixture of NO_3^- sources because many data points fall outside of arbitrary boxes with the traditional $\delta^{18}O$ axis (Fig. 1A) cannot be supported once the range of potential $\delta^{18}O$-NO_3^- values has been considered (Fig. 1B). Moreover, almost all measured $\delta^{18}O$-NO_3^- values fall within the range of expected $\delta^{18}O$-NO_3^- values based on nitrification with variable amount of H_2O exchange (Fig. 1B). Thus, the theoretical range of $\delta^{18}O$-NO_3^- values should be generated for each field site rather than a single catch-all approach. Globally, $\delta^{18}O$-H_2O values of surface water vary widely along a meteoric water line, but they can be predicted by latitude and databases such as waterisotopes.org though direct measurement is much simpler than NO_3^- isotopes. Additionally, to make $\delta^{18}O$-NO_3^- data comparable between seasons and sites, $\delta^{18}O$-NO_3^- data should be displayed vs the $\delta^{18}O$-H_2O value from the same sample (i.e., same location and time) rather than vs SMOW. This is akin to the way $\delta^{18}O$-PO_4^{3-} values are plotted relative to their temperature-specific equilibrium point with $\delta^{18}O$-H_2O (e.g., Davies et al. 2014, Paytan et al. 2002) in order to remove the influence of difference $\delta^{18}O$-H_2O values (Figure 1B). Here the differences in $\delta^{18}O$-NO_3^- values between countries is much reduced and most $\delta^{18}O$-NO_3^- values are near the upper-end of the $\delta^{18}O$-NO_3^- values predicted from microbial transformation of nitrogen. There is a positive relationship between $\delta^{18}O$-NO_3^- and $\delta^{15}N$-NO_3^- in the Kenya and UK data ($p<10^{-4}$) but not Canada ($p>0.4$).

Therefore, increases in $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values, which are often interpreted as evidence of denitrification with closed-system assumptions (e.g., Bötttcher et al. 1990), cannot be uniquely separated from multiple processes that recycle nitrogen in surface waters. Necessarily, this requires us to move beyond looking only for denitrification in our $\delta^{15}N$- and $\delta^{18}O$-NO_3^- data and towards how multiple processes and sources interact to produce the values measured in surface waters. Likely, this will ultimately require development of process-based NO_3^- isotope models for surface waters and will be informed by measurements of other nitrogen species, transformation processes and associated isotope enrichment factors (e.g., Venkiteswaran et al. 2018).

Only once the appropriate range of initial $\delta^{18}O$-NO_3^- values has been determined, can processes such nitrification, denitrification, and NO_3^- assimilation be considered. Here, the $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values in the environment will be pulled in multiple directions at the same time. The magnitude of change depends on multiple factors that are difficult or impossible to statically display in a biplot: (1) mineralization of organic nitrogen and subsequent nitrification may decrease $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values depending on if there is a difference between the $\delta^{15}N$ value of organic nitrogen and NO_3^- and the $\delta^{18}O$ contributions of O_2 and H_2O; (2) ammonia and NO_3^- uptake and release by riverine periphyton and macrophytes may have differing impacts since isotope fractionation during ammonia uptake is non-linearly dependant on concentration (Fogel and Cifuentes 1993; Hoch et al. 1992) and denitrification in riparian zones and anoxic river and lake sediments may increase $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values if there is residual NO_3^- to measure. In all cases, changes in the $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values are more complex than a single arrow for denitrification suggests (Kendall 1998). A recent review has summarised the modelling approaches and isotope fractionation factors necessary to interpret measured $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values in soils (Denk et al. 2017). With this process-based understanding it is clear that a single vector or slope on a biplot for denitrification is inappropriate for surface waters.

Summary and Conclusions:

In order to move beyond the simple source apportionment assumptions commonly made in NO_3^- isotope biplots and to explicitly acknowledge that there are a variety of processes that alter the $\delta^{15}N$- and $\delta^{18}O$-NO_3^- values *in situ* we therefore recommend:

- Measuring $\delta^{18}O$-H_2O values at the same time as $\delta^{18}O$-NO_3^- values and report $\delta^{18}O$-NO_3^- values vs $\delta^{18}O$-H_2O instead of V-SMOW to make appropriate comparisons with time and across sites;
- Combining $\delta^{18}O$-H_2O and $\delta^{18}O$-O_2 values to develop appropriate site-specific ranges of $\delta^{18}O$-NO_3^- produced *in situ*; and

- Measuring locally relevant δ^{15}N source values to significantly reduce the range of δ^{15}N values of nitrogen input to aquatic systems.

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Figure

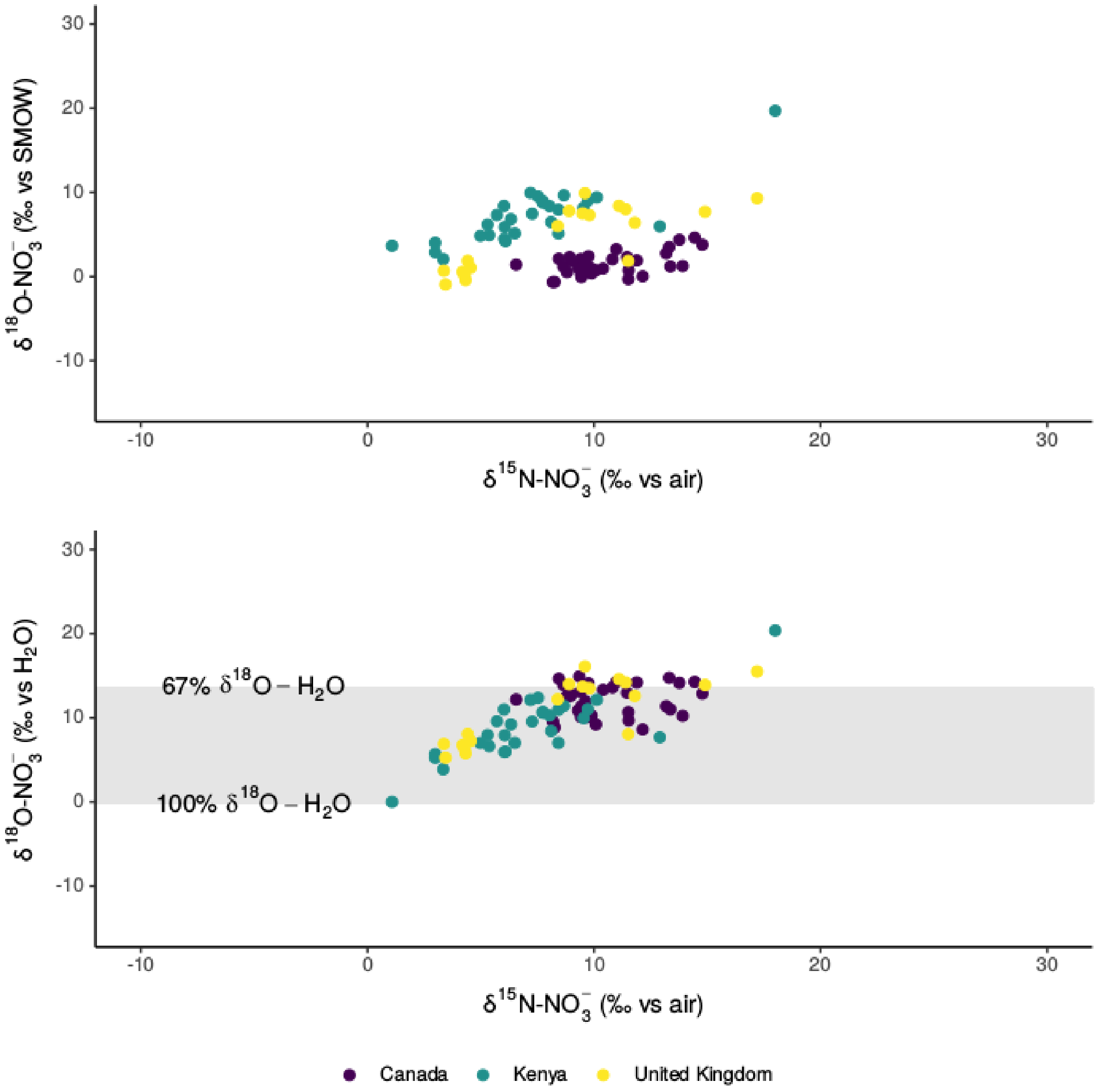

Figure 1 (a): Nitrate isotope biplot of data from three sites in the middle of the Grand River, Ontario, Canada; 11 sites in the Nyando River, Kenya; eight sites in the Nzoia River, Kenya; five sites in the Sondu River, Kenya; eight sites in the River Lambourn near Boxford, United Kingdom; and 11 sites in the River Thames near Oxford, United Kingdom. Comparisons are difficult between seasons at one site and still more difficult between sites because of the variability in δ^{18}O-H_2O since the δ^{18}O-NO_3^- axis is reported relative to the typical standard SMOW.

(b): Nitrate isotope biplot of the same data where the δ^{18}O-NO_3^- axis is reported relative to the ambient δ^{18}O-H_2O values in the river at the time of sampling, as per recommendation A. The grey panel indicates NO_3^- produced with the maximum range of δ^{18}O-NO_3^- values based on a mixture of δ^{18}O-O_2 and δ^{18}O-H_2O values. Here, data are more clearly expressed relative to the appropriate environmental conditions that recognize that nitrogen is biologically cycled and will be largely imprinted with the ambient δ^{18}O-H_2O value. A parsimonious interpretation here is that many data from Kenya and the UK exhibit the range of known contributions of the δ^{18}O-H_2O values, i.e., from two-thirds to one. Most Canadian and some Kenyan and UK data approach the theoretical maximum δ^{18}O-NO_3^- before a requirement of denitrification must be considered.

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