Towards a global interpretation of dual nitrate isotopes in surface waters

Venkiteswaran JJ[1], Boeckx P[2], Gooddy DC[3]

[1] Department of Geography and Environmental Studies, Wilfrid Laurier University, Waterloo, Ontario, Canada, jvenkiteswaran@wlu.ca, orcid: 0000-0002-6574-7071
[2] Isotope Bioscience Laboratory - ISOFYS, Ghent University, Coupure Links 653, 9000 Gent, Belgium, orcid: 0000-0003-3998-0010
[3] British Geological Survey, Maclean Building, Crowmarsh, Oxfordshire OX10 8BB, United Kingdom, orcid: 0000-0002-6015-1332

Keywords: nitrate, isotopes, lakes, rivers

EarthArXiv preprint DOI: 10.17605/OSF.IO/CZT8P

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 of our example datasets (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. Knowledge of isotope fractionation during NO_3^- production and consumption was summarized in Kendall (1998) yet, despite the many figures in this chapter, one figure described as "simplified" has become the ubiquitous interpretation scheme. This figure visually summarizes a compilation of NO_3^- isotope data with boxes by "dominant sources of nitrate" and encourages researchers to think only about one process, denitrification, 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. Moreover a system with three NO_3^- sources and only one measurement, $\delta^{15}N$, is underdetermined. 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 (e.g., 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. Hence the large overlap in the NO_3^- sources boxes that does not contribute to source identification (e.g., Kendall et al. 2015).

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). Isotope fractionation during these steps occurs but is not always expressed, such as when NO_2^- is fully consumed (Buchwald and Casciotti 2010; Casciotti et al. 2010; Snider et al. 2010). Abiotic equilibrium of oxygen may occur between H_2O and NO_2^- and increase the $\delta^{18}O$ value of the NO_2^- (Casciotti et al. 2007). In surface soils, the pore gas $\delta^{18}O\text{-}O_2$ value is very likely near the atmospheric value of +23.5‰ (vs SMOW). However, in productive aquatic ecosystems, the diel variability of $\delta^{18}O\text{-}O_2$ values can be large (e.g., 26‰ range in Gammons et al. 2011, 23‰ range in Venkiteswaran et al. 2015, 18‰ range in Hotchkiss and Hall, Jr 2014, 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 $\delta^{18}O\text{-}H_2O$ indicate that the contribution of $\delta^{18}O\text{-}H_2O$ values to the final $\delta^{18}O\text{-}NO_3^-$ value is often much greater than the minimum two-thirds and sometimes close to 1 (Snider et al. 2010). Thus the range of $\delta^{18}O$ values of NO_3^- produced *in situ* can be bounded by knowledge of $\delta^{18}O\text{-}O_2$ and $\delta^{18}O\text{-}H_2O$ values: a minimum of the $\delta^{18}O\text{-}H_2O$ value and a maximum of $\frac{1}{3} \times \delta^{18}O\text{-}O_2 + \frac{2}{3} \times \delta^{18}O\text{-}H_2O$. However abiotic exchange of oxygen between H_2O and NO_2^- may increase this theoretical minimum value. When the diel range in $\delta^{18}O\text{-}O_2$ values is considered the maximum $\delta^{18}O$ values of NO_3^- produced *in situ* will vary by upwards of 10‰ (i.e., ⅓ of the diel range of $\delta^{18}O\text{-}O_2$ values, e.g., 9‰ in Gammons et al. 2011, 8‰ in Venkiteswaran et al. 2015, 6‰ in Hotchkiss and Hall, Jr 2014, 5‰ in Wassenaar et al. 2010, and 4‰ range in Parker et al. 2005). Data in Silver Bow Creek, Montana, USA exhibit synchronous diel $\delta^{18}O\text{-}NO_3^-$ and $\delta^{18}O\text{-}O_2$ cycles (Gammons et al. 2011).

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 $\delta^{18}O\text{-}H_2O$ 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 (Hood et al. 2014; Venkiteswaran et al. 2018). All sites are in the middle of the Grand River and were sampled at baseflow.

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 at steady-state flow. 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. Samples where collected at steady-state flow.

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 $\delta^{15}N\text{-}NO_3^-$ and ±0.5‰ for $\delta^{18}O\text{-}NO_3^-$. 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 $\delta^{18}O\text{-}H_2O$.

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).

UK samples were also filtered to 0.45 μm and stored below 4°C in 1L HDPE bottles. 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 $\delta^{15}N\text{-}NO_3^-$ values than $\delta^{18}O\text{-}NO_3^-$ values. Additionally, data from each country has a positive relationship between $\delta^{18}O\text{-}NO_3^-$ and $\delta^{15}N\text{-}NO_3^-$ (2-tailed parametric $p<0.006$ for each country). But this relationship also contains seasonal changes in ambient $\delta^{18}O\text{-}H_2O$ 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 ($p>0.4$).

Some variability due to watershed size and seasonality can also be considered with this approach. First, as watershed size increases above a river sampling point the average duration the nitrogen spends in the watershed increases and thus the likelihood that the sampled NO_3^- had been assimilated and released multiple times approaches 100%. Second, initial $\delta^{18}O$-NO_3^- values entirely depend on the ambient $\delta^{18}O$-H_2O and $\delta^{18}O$-O_2 at the time of nitrification and not the $\delta^{18}O$ value of the NO_3^- added to the watershed at some point upstream if the nitrogen has been cycled at least once. Thus changes in $\delta^{18}O$-H_2O between seasons or throughout watersheds are accounted for by reporting $\delta^{18}O$-NO_3^- relative to the H_2O. The implication here is that identifying the source of the NO_3^- cannot be done with $\delta^{18}O$-NO_3^- values.

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., Böttcher 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 $\delta^{18}O\text{-}NO_3^-$ values has been determined, can processes such nitrification, denitrification, and NO_3^- assimilation be considered. Here, the $\delta^{15}N$- and $\delta^{18}O\text{-}NO_3^-$ 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 $\delta^{18}O\text{-}NO_3^-$ 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 $\delta^{18}O\text{-}NO_3^-$ values if there is residual NO_3^- to measure. In all cases, changes in the $\delta^{15}N$- and $\delta^{18}O\text{-}NO_3^-$ 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 $\delta^{18}O\text{-}NO_3^-$ 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 $\delta^{18}O\text{-}NO_3^-$ values *in situ* we therefore recommend:

- Measuring $\delta^{18}O\text{-}H_2O$ values at the same time as $\delta^{18}O\text{-}NO_3^-$ values and report $\delta^{18}O\text{-}NO_3^-$ values vs $\delta^{18}O\text{-}H_2O$ instead of V-SMOW to make appropriate comparisons with time and across sites;
- Combining $\delta^{18}O\text{-}H_2O$ and $\delta^{18}O\text{-}O_2$ values to develop appropriate site-specific ranges of $\delta^{18}O\text{-}NO_3^-$ produced *in situ*; and
- Measuring locally relevant $\delta^{15}N$ source values to significantly reduce the range of $\delta^{15}N$ values of nitrogen input to aquatic systems.

Acknowledgements

This work was completed under the auspices of the International Atomic Energy Agency's Coordinated Research project 'Isotopes to Study Nitrogen Pollution and Eutrophication of Rivers and Lakes' (F32007). Samples in Canada were collected under the auspices of the Natural Science and Engineering Research Council (NSERC) grant number STPGP-447692-2013. Samples in the UK were funded by the Natural Environment Research Council (NERC) National Capability resources devolved to the British Geological Survey. DCG publishes with permission of the Executive Director, British Geological Survey (NERC).

Figure

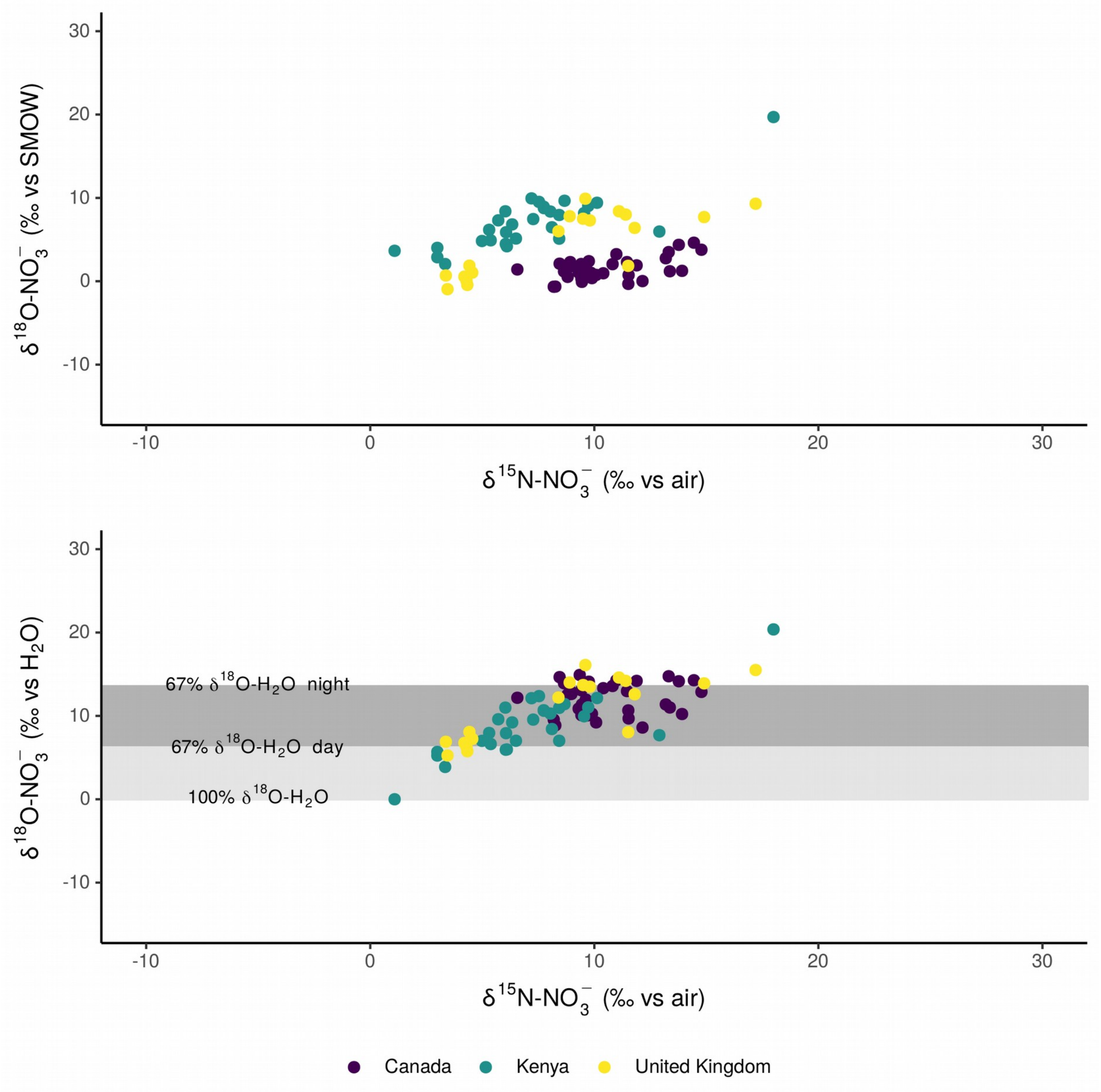

Figure 1 (a): Nitrate isotope biplot of data from three sites in the middle of the Grand River, Ontario, Canada; 11 sites in the Nyando River, Kenya; eight sites in the Nzoia River, Kenya; five sites in the Sondu River, Kenya; eight sites in the River Lambourn near Boxford, United Kingdom; and 11 sites 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 $\delta^{18}O\text{-}H_2O$ since the $\delta^{18}O\text{-}NO_3^-$ axis is reported relative to the typical standard SMOW.
(b): Nitrate isotope biplot of the same data where the $\delta^{18}O\text{-}NO_3^-$ axis is reported relative to the ambient $\delta^{18}O\text{-}H_2O$ values in the river at the time of sampling, as per recommendation A. The grey bands indicates NO_3^- produced with a range of $\delta^{18}O\text{-}NO_3^-$ values based on a mixture of $\delta^{18}O\text{-}O_2$ and $\delta^{18}O\text{-}H_2O$ values. The minimum value is where the $\delta^{18}O\text{-}H_2O$ is entirely retained in the $\delta^{18}O\text{-}NO_3^-$value and without isotope fractionation associated with abiotic oxygen exchange (Casciotti et al. 2007). The light grey band covers the range expected when $\delta^{18}O\text{-}O_2$ values are lowest during the day. The dark grey band extends the range expected when $\delta^{18}O\text{-}O_2$ values are greatest during the night

(Venkiteswaran et al. 2015). Thus the δ^{18}O value of newly producted NO_3^- in these rivers may cycle through these ranges on a diel basis. 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 with a minor contribution from the variable δ^{18}O-O_2 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.

References

Aleem MIH, Hoch GE, Varner JE. 1965. Water as the source of oxidant and reductant in bacterial chemosynthesis. Biochemistry, 54, 869–873, DOI: 10.1073/pnas.54.3.869

Allen DJ, Darling WG, Gooddy DC, Lapworth DJ, Newell AJ, Williams AT, Allen D, Abesser C. 2010. Interaction between groundwater, the hyporheic zone and a Chalk stream: a case study from the River Lambourn, UK. Hydrogeology Journal 18(5): 1125-1141, DOI: 10.1007/s10040-010-0592-2

Andersson KK, Hooper AB. 1983. O_2 and H_2O are each the source of one O in NO_2^- produced from NH_3 by Nitrosomonas: ^{15}N-NMR evidence. FEBS Lett. 164, 236–240, DOI: 10.1016/0014-5793(83)80292-0

Aravena R, Evans ML, Cherry JA. 1993. Stable Isotopes of Oxygen and Nitrogen in Source Identification of Nitrate from Septic Systems. Groundwater, 31: 180-186, DOI: 10.1111/j.1745-6584.1993.tb01809.x

Aravena R. Robertson WD. 1998. Use of Multiple Isotope Tracers to Evaluate Denitrification in Ground Water: Study of Nitrate from a Large-Flux Septic System Plume. Groundwater, 36: 975-982, DOI: 10.1111/j.1745-6584.1998.tb02104.x

Bateman AS, Kelly SD. 2007. Fertilizer nitrogen isotope signatures, Isotopes in Environmental and Health Studies, 43:3, 237-247, DOI: 10.1080/10256010701550732

Barth JAC, Tait A, Bolshaw M (2004) Automated analyses of ^{18}O/^{16}O ratios in dissolved oxygen from 12-mL water samples. Limnol Oceanogr Meth 2:35–41, DOI: 10.4319/lom.2004.2.35

Böttcher J, Strebel O, Voerkelius S, Schmidt H-L. 1990. Using isotope fractionation of nitrate-nitrogen and nitrate-oxygen for evaluation of microbial denitrification in a sandy aquifer, Journal of Hydrology, 114, 413-424, DOI: 10.1016/0022-1694(90)90068-9

Bowes MJ, Neal C, Jarvie HP, Smith JT, Davies HN. 2010. Predicting phosphorus concentrations in British rivers resulting from the introduction of improved phosphorus removal from sewage effluent. Science of The Total Environment 408(19): 4239-4250, DOI: 10.1016/j.scitotenv.2010.05.016.

Buchwald C, Casciotti KL. 2010. Oxygen isotopic fractionation and exchange during bacterial nitrite oxidation Limnol. Oceanogr. 55, 1064– 1074, DOI: 10.4319/lo.2010.55.3.1064

Casciotti KL, Böhlke JK, McIlvin MR, Mroczkowski SJ, Hannon JE. 2007. Oxygen Isotopes in Nitrite: Analysis, Calibration, and Equilibration. Anal. Chem. 79(6), 2427–2436, DOI: 10.1021/ac061598h

Casciotti KL, McIlvin M, Buchwald C. 2010. Oxygen isotopic exchange and fractionation during bacterial ammonia oxidation. Limnol. Oceanogr. 55(2), 753-762, DOI: 10.4319/lo.2010.55.2.0753

Chang CCY, Langston J, Riggs M, Campbell DH, Silva SR, Kendall C. 1999. A method for nitrate collection for $\delta^{15}N$ and $\delta^{18}O$ analysis from waters with low nitrate concentrations. Can. J. Fish. Aquatic Sci., 56, 1856-1864, DOI: 10.1139/f99-126

COWI Consulting Engineers, 2002. Integrated water quality/Limnology study for Lake Victoria. Lake Victoria Environmental Management Project, Part II Technical Report.

Craine JM, Elmore AJ, Wang L, Augusto L, Baisden WT, Brookshire EN, Cramer MD, Hasselquist NJ, Hobbie EA, Kahmen A, Koba K (2015) Convergence of soil nitrogen isotopes across global climate gradients. Scientific Reports 5:8280, DOI: 10.1038/srep08280

Davies CL, Surridge DWJ, Gooddy DC (2014) Phosphate oxygen isotopes within aquatic ecosystems: Global data synthesis and future research priorities. Sci Total Environ 496:563–575, DOI: 10.1016/j.scitotenv.2014.07.057

Denk TRA, Mohn J, Decock C, Lewicka-Szczebak D, Harris W, Butterbach-Bahl K, Kiese R, Wolf B. 2017. The nitrogen cycle: A review of isotope effects and isotope modeling approaches. Soil Biology and Biochemistry. 105, 121-137, DOI: 10.1016/j.soilbio.2016.11.015.

Deutsch B, Mewes M, Liskow I, Voss M. 2006. Quantification of diffuse nitrate inputs into a small river system using stable isotopes of oxygen and nitrogen in nitrate. Organic Geochemistry, 37(10): 1333-1342, DOI: 10.1016/j.orggeochem.2006.04.012.

DiSpirito AA, Hooper AB. 1986. Oxygen exchange between nitrate molecules during nitrite oxidation by Nitrobacter. J. Biol. Chem. 1986, 261, 10534–10537.

Fogel M L, Cifuentes LA. 1993. Isotope Fractionation during Primary Production. In Organic geochemistry; Engel, H. M., Macko, S. A., Eds.; Plenum Press: New York; pp 73–98.

Gammons CH, Babcock JN, Parker SR, Poulson SR. 2011. Diel cycling and stable isotopes of dissolved oxygen, dissolved inorganic carbon, and nitrogenous species in a stream receiving treated municipal sewage. Chem. Geol. 283(102), 44-55, DOI: 10.1016/j.chemgeo.2010.07.006

Hannaford J, Marsh TJ. 2008. High-flow and flood trends in a network of undisturbed catchments in the UK. International Journal of Climatology 28(10): 1325-1338, DOI: 10.1002/joc.1643

Heaton THE. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. Chem Geol 59:87–102, DOI: 10.1016/0168-9622(86)90059-X

Hoch MP, Fogel ML, Kirchman DL. 1992. Isotope Fractionation Associated with Ammonium Uptake by a Marine Bacterium. Limnol. Oceanogr., 37(7), 1447-1459, DOI: 10.4319/lo.1992.37.7.1447

Hollocher TC. 1984. Source of the oxygen atoms of nitrate in the oxidation of nitrite by Nitrobacter agilis and evidence against a P-O-N anhydride mechanism in oxidative phosphorylation. Arch. Biochem. Biophys. 233, 721–727, DOI: 10.1016/0003-9861(84)90499-5

Hollocher TC, Tate ME, Nicholas DJD. 1981. Oxidation of ammonia by *Nitrosomonas europaea*. J.

Biol. Chem. 256, 10834–10836.

Hood JLA, Taylor WD, Schiff SL. 2014. Examining the fate of WWTP effluent nitrogen using δ^{15}N–NH_4^+, δ^{15}N–NO_3^- and δ^{15}N of submersed macrophytes. Aquat. Sci. 76(2), 243-258, DOI: 10.1007/s00027-013-0333-4

Hotchkiss ER. Hall Jr, RO. 2014. High rates of daytime respiration in three streams: Use of $\delta^{18}O_{O2}$ and O_2 to model diel ecosystem metabolism. Limnol. Oceanogr. 59(3), 798-810, DOI: 10.4319/lo.2014.59.3.0798

Juma DW, Wang H, Li F. 2014. Impacts of population growth and economic development on water quality of a lake: case study of Lake Victoria Kenya water. Environ. Sci. Pollut. R. 21: 5737–46, DOI: 10.1007/s11356-014-2524-5

Kendall C. 1998. Tracing Nitrogen Sources and Cycling in Catchments. In: Kendall C, McDonnell JJ (eds) Elsevier Science, Amsterdam, pp 519–576

Kendall C, Elliott EM, Wankel SD. 2008. Tracing Anthropogenic Inputs of Nitrogen to Ecosystems. In Stable Isotopes in Ecology and Environmental Science (eds) R. Michener and K. Lajtha, doi: 10.1002/9780470691854.ch12

Kendall C, Young MB, Silva SR, Kraus TEC, Peek S, Guerin M. 2015. Tracing nutrient and organic matter sources and biogeochemical processes in the Sacramento River and Northern Delta: proof of concept using stable isotope data. U.S. Geological Survey, Data Release, DOI: 10.5066/F7QJ7FCM

Loo SE, Ryan MC, Zebarth BJ, Kuchta SH, Neilsen D, Mayer B. 2017. Irrigated Crops: A Cautionary Vadose Zone Tale. Journal of Environmental Quality, 46: 528–536, DOI: 10.2134/jeq2016.08.0294

McIlvin MR, Altabet MA. 2005. Chemical Conversion of Nitrate and Nitrite to Nitrous Oxide for Nitrogen and Oxygen Isotopic Analysis in Freshwater and Seawater. Analytical Chemistry 2005 77 (17), 5589-5595, DOI: 10.1021/ac050528s

Michalski G, Bhattacharya SK, Mase DF. 2012. Oxygen isotope dynamics of atmospheric nitrate and its precursor molecules. In: Baskaram M (ed) Handbook of environmental isotope geochemistry, Springer, Berlin Heidelberg, pp 613–635

Oelmann Y, Kreutziger Y, Bol R, Wilcke W. 2007. Nitrate leaching in soil: Tracing the NO3− sources with the help of stable N and O isotopes. Soil Biology and Biochemistry, 39(12): 3024-3033, DOI: 10.1016/j.soilbio.2007.05.036.

Pardo LH, Kendall C, Pett-Ridge J, Chang CC. 2004. Evaluating the source of streamwater nitrate using δ^{15}N and δ^{18}O in nitrate in two watersheds in New Hampshire, USA. Hydrol. Process., 18: 2699-2712. DOI: 10.1002/hyp.5576

Parker SR, Poulson SR, Gammons CH, DeGrandpre MD. 2005. Biogeochemical Controls on Diel Cycling of Stable Isotopes of Dissolved O2 and Dissolved Inorganic Carbon in the Big Hole River, Montana. Environmental Science and Technology 39(18), pp 7134–7140, DOI: 10.1021/es0505595

Paytan A, Kolodny Y, Neori A, Luz B. 2002. Rapid biologically mediated oxygen isotope exchange between water and phosphate, Global Biogeochem Cy, 16, DOI: 10.1029/2001GB001430

Sigman DM, Casciotti KL, Andreani M, Barford C, Galanter M, Böhlke JK. 2001. A Bacterial Method for the Nitrogen Isotopic Analysis of Nitrate in Seawater and Freshwater. Analytical Chemistry 2001 73 (17), 4145-4153, DOI: 10.1021/ac010088e

Silva S, Kendall C, Wilkison D, Ziegler A, Chang CC, Avanzino R. 2000. A new method for collection of nitrate from fresh water and the analysis of nitrogen and oxygen isotope ratios. J. Hydrol., 228, 22-36, DOI: 10.1016/S0022-1694(99)00205-X

Snider DM, Spoelstra J, Schiff SL, Venkiteswaran JJ. 2010. Stable oxygen isotope ratios of nitrate produced from nitrification: ^{18}O-labelled water incubations of agricultural and temperate forest soils. Environ Sci Technol 44:5358–5364 DOI: 10.1021/es1002567

Spalding RF, Exner ME. 1993. Occurrence of nitrate in groundwater: A review. Journal of Environmental Quality, 22, 392–402, DOI: 10.2134/jeq1993.00472425002200030002x.

Spoelstra J, Schiff SL, Elgood RJ, Semkin RG, Jeffries DS. 2001. Tracing the Sources of Exported Nitrate in the Turkey Lakes Watershed Using ^{15}N/^{14}N and ^{18}O/^{16}O isotopic ratios. Ecosystems 4: 536-544, DOI: 10.1007/s10021-001-0027-y

Venkiteswaran JJ, Schiff SL, Ingalls BJ. 2018. Quantifying the Fate of Wastewater Nitrogen Discharged to a Canadian River. EarthArXiv, doi:10.17605/OSF.IO/MQ2GN.

Venkiteswaran JJ, Schiff SL, Taylor WD. 2015. Linking aquatic metabolism, gas exchange, and hypoxia to impacts along the 300-km Grand River, Canada. Freshwat Sci 34:1216-1232, DOI: 10.1086/683241

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tillman DG. 1997. Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7, 737-750, DOI: 10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Wassenaar LI, Koehler G. 1999. An On-Line Technique for the Determination of the δ^{18}O and δ^{17}O of Gaseous and Dissolved Oxygen. Anal Chem 71:4965–4968, DOI: 10.1021/ac9903961

Wassenaar LI, Venkiteswaran JJ, Schiff SL, Koehler G. 2010. Aquatic community metabolism response to municipal effluent inputs in rivers quantified using diel δ^{18}O values of dissolved oxygen. Can J Fish Aquat Sci 67:1232-1246, DOI: 10.1139/F10-057

Xue D, Botte J, De Baets B, Accoe F, Nestler A, Taylor P, Van Cleemput O, Berglund M, Boeckx P. 2009. Present limitations and future prospects of stable isotope methods for nitrate source identification in surface- and groundwater. Water Res. 43:1159–1170, DOI: 10.1016/j.watres.2008.12.048