Towards a global interpretation of dual nitrate isotopes in surface waters

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Abstract:

Modern anthropogenic activities have significantly increased nitrate (NO\textsubscript{3}⁻) concentrations in surface waters. Stable isotopes (δ\textsuperscript{15}N and δ\textsuperscript{18}O) in NO\textsubscript{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\textsubscript{3}⁻ and denitrification. In the two decades since this template was developed, δ\textsuperscript{15}N- and δ\textsuperscript{18}O-NO\textsubscript{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\textsubscript{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\textsubscript{3}⁻ isotopes. We use a mechanistic understanding of NO\textsubscript{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\textsubscript{3}⁻ sources simply because many data points fall outside of arbitrary boxes which cannot be supported once the range of potential δ\textsuperscript{18}O-NO\textsubscript{3}⁻ values has been considered.

Highlights:

- Interpretation of surface water δ\textsuperscript{15}N- and δ\textsuperscript{18}O-NO\textsubscript{3}⁻ requires a more complex framework than currently employed
- Surface water processes alter δ\textsuperscript{15}N- and δ\textsuperscript{18}O-NO\textsubscript{3}⁻ in different ways than the traditional groundwater-denitrification model rendering such frameworks obsolete
- Mechanistic understanding of NO\textsubscript{3}⁻ cycling in surface waters means that the range of δ\textsuperscript{18}O-NO\textsubscript{3}⁻ is constrainable and can be made comparable between sites and across time via concurrent measurements of δ\textsuperscript{18}O-H\textsubscript{2}O and δ\textsuperscript{18}O-O\textsubscript{2}

Introduction:

Stable isotopes (δ\textsuperscript{15}N and δ\textsuperscript{18}O) in nitrate (NO\textsubscript{3}⁻) have been commonly measured for more than 4 decades (see Heaton (1986) and papers therein). Methods have evolved from off-line AgNO\textsubscript{3} precipitation (e.g., Chang et al. 1999; Silva et al. 2000), to chemical and microbial reduction to N\textsubscript{2}O and subsequent continuous flow – isotope ratio mass spectrometry analyses (Sigman et al. 2001; McIlvin and Altabet 2005). Since NO\textsubscript{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\textsubscript{3}⁻ isotopes was to identify NO\textsubscript{3}⁻ sources. Through combining a number of individual studies, this lead to publication of a δ\textsuperscript{18}O-NO\textsubscript{3}⁻ vs δ\textsuperscript{14}N-NO\textsubscript{3}⁻ schematic biplot with suggested ranges for different ‘sources’ of NO\textsubscript{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\textsubscript{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 recognised 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 summarised 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 summarises 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 recognises 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 δ$^{15}$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, δ$^{15}$N, is underdetermined. Measuring locally appropriate sources of nitrogen as potential initial δ$^{15}$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 δ$^{15}$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 δ$^{15}$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 δ$^{18}$O values from NO$_3^-$ produced in the atmosphere where the δ$^{18}$O value depends strongly on latitude (Michalski et al. 2012); and (ii) low

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δ¹⁸O values where the δ¹⁸O value depends strongly on the δ¹⁸O of H₂O where the NO₃⁻ is formed (Snider et al. 2010). The δ¹⁸O value of NO₃⁻ produced by autotrophic and heterotrophic nitrification can be bounded in two ways. First, canonical two-step nitrification (from NH₄⁺ to NH₂OH to NO₂⁻ to NO₃⁻) adds one O atom from O₂ in the first step and one O atom from H₂O 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 NO₂⁻ is fully consumed (Buchwald and Casciotti 2010; Casciotti et al. 2010; Snider et al. 2010).

Abiotic equilibrium of oxygen may occur between H₂O and NO₂⁻ and increase the δ¹⁸O value of the NO₂⁻ (Casciotti et al. 2007). In surface soils, the pore gas δ¹⁸O-O₂ value is very likely near the atmospheric value of +23.5‰ (vs SMOW; Kroopnick and Craig 1972). However, in productive aquatic ecosystems, the diel variability of δ¹⁸O-O₂ 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 δ¹⁸O-H₂O indicate that the contribution of δ¹⁸O-H₂O values to the final δ¹⁸O-NO₃⁻ value is often much greater than the minimum two-thirds and sometimes close to 1 (Snider et al. 2010). Thus the range of δ¹⁸O values of NO₃⁻ produced in situ can be bounded by knowledge of δ¹⁸O-O₂ and δ¹⁸O-H₂O values: a minimum of the δ¹⁸O-H₂O value and a maximum of ⅓ × δ¹⁸O-O₂ + ⅓ × δ¹⁸O-H₂O. However, abiotic exchange of oxygen between H₂O and NO₂⁻ may increase this theoretical minimum value. When the diel range in δ¹⁸O-O₂ values is considered the maximum δ¹⁸O values of NO₃⁻ produced in situ will vary by upwards of 10‰ (i.e., ⅓ of the diel range of δ¹⁸O-O₂ 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 δ¹⁸O-NO₃⁻ and δ¹⁸O-O₂ cycles (Gammons et al. 2011).

Site descriptions:

To highlight the need to include nitrogen cycling in surface waters into our working interpretation of NO₃⁻ isotopes, we selected six rivers from Canada, Kenya, and the United Kingdom each with different climate regions, seasonal variation in flow, and δ¹⁸O-H₂O 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 6800km² 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 were 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$_2$O 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$_2$O with cadmium and sodium azide (McIlvin and Altabet 2005). The resultant N$_2$O gas was analysed on an IsoPrime continuous flow isotope ratio mass spectrometer (now Elementar, Cheadle Hulme, UK) with a precision of ±0.3‰ for δ$^{15}$N-NO$_3^-$ and ±0.5‰ for δ$^{18}$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 δ$^{18}$O-H$_2$O.

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$_2$O 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 δ$^{15}$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‰. δ$^{18}$O was analysed by thermal conversion to CO gas at 1400°C in a TC–EA online to a Delta Plus XL

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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 δ¹⁸N-NO₃ values than δ¹⁸O-NO₃ values (ranges of δ¹⁸N-NO₃ and δ¹⁸O-NO₃ are 6.6‰ to 14.8‰ and -0.66‰ to 4.6 in Canada, 1.1‰ to 18.0‰ and 2.1‰ to 19.7‰ in Kenya, and 3.4‰ to 17.2‰ and -0.95‰ to 9.9‰ in the United Kingdom). Additionally, data from each country has a positive relationship between δ¹⁸O-NO₃ and δ¹⁸N-NO₃ (2-tailed parametric p<0.006 for each country). But this relationship also contains seasonal changes in ambient δ¹⁸O-H₂O 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 NO₃⁻ based on the δ¹⁸N values or assigning a single process based on a simplistic pattern in the δ¹⁸O-NO₃ vs δ¹⁸N-NO₃ values. For example, varying contributions of the δ¹⁸O-H₂O values, two or more sources of nitrogen, uptake and release of varying amounts of ammonium and NO₃⁻, 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 NO₃⁻ sources because many data points fall outside of arbitrary boxes with the traditional δ¹⁸O axis (Fig. 1A) cannot be supported once the range of potential δ¹⁸O-NO₃ values has been considered (Fig. 1B). Moreover, almost all measured δ¹⁸O-NO₃ values fall within the range of expected δ¹⁸O-NO₃ values based on nitrification with variable amount of H₂O exchange (Fig. 1B). Thus, the theoretical range of δ¹⁸O-NO₃ values should be generated for each field site rather than a single catch-all approach. Globally, δ¹⁸O-H₂O 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 NO₃⁻ isotopes. Additionally, to make δ¹⁸O-NO₃ data comparable between seasons and sites, δ¹⁸O-NO₃ data should be displayed vs the δ¹⁸O-H₂O value from the same sample (i.e., same location and time) rather than vs SMOW¹. This is the δ¹⁸O/δ¹⁶O ratio of NO₃⁻ divided by the δ¹⁸O/δ¹⁶O ratio of H₂O rather than by the δ¹⁸O/δ¹⁶O ratio of SMOW. This is akin to the way δ¹⁸O-PO₄³⁻ values are plotted relative to their temperature-specific equilibrium point with δ¹⁸O-H₂O (e.g., Davies et al. 2014, Paytan et al. 2002) in order to remove the influence of difference δ¹⁸O-H₂O values (Figure 1B). Here the differences in δ¹⁸O-NO₃ values between countries is much reduced and most δ¹⁸O-NO₃ values are near the upper-end of the δ¹⁸O-NO₃ values predicted from microbial transformation of nitrogen. There is a positive linear relationship between δ¹⁸O-NO₃ and δ¹⁸N-NO₃ in the Kenya and UK data (p<10⁻⁶) but not Canada (p>0.4).

Some variability due to watershed size and seasonality can also be considered with this approach.

¹ Unitless δ values are converted from ‘relative to SMOW’ to ‘relative to H₂O’ as:

\[ \delta^{18}O - NO_{3}^{-,H_{2}O} = \frac{\delta^{18}O - NO_{3}^{-,SMOW}}{\delta^{18}O - H_{2}O^{18}O_{SMOW}} + 1 - 1 \]

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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 δ$^{18}$O-NO$_3^-$ values entirely depend on the
ambient δ$^{18}$O-H$_2$O and δ$^{18}$O-O; at the time of nitrification and not the δ$^{18}$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
δ$^{18}$O-H$_2$O between seasons or throughout watersheds (e.g., Yue et al. 2018) are accounted for by
reporting δ$^{18}$O-NO$_3^-$ relative to the H$_2$O.

We recognise that in our approach that the δ$^{18}$O-H$_2$O measured concomitantly with the δ$^{18}$O-NO$_3^-$ does
not completely represent the H$_2$O that relevant during the most recent production of each NO$_3^-$
molecule. Indeed, the δ$^{18}$O-H$_2$O during NO$_3^-$ formation is not necessarily that which is found in the
river during sampling due to mixing of a plethora of sources of N and H$_2$O. Similarly, small or slow
flowing rivers maybe subject to significant seasonal evaporation resulting in increases in ambient
δ$^{18}$O-H$_2$O values that may temporally differ from when NO$_3^-$ was formed. These issues reinforce the
need to collect samples of waters where NO$_3^-$ is formed, to recognise that NO$_3^-$ is continuously cycled
in surface waters, and to explicitly make a distinction between N sources and processing. The
implication here is that identifying the source of the NO$_3^-$ cannot be done with δ$^{18}$O-NO$_3^-$ values.

Increases in δ$^{15}$N- and δ$^{18}$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 δ$^{15}$N- and δ$^{18}$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 δ$^{18}$O-NO$_3^-$ values has been determined, can processes such
as nitrification, denitrification, and NO$_3^-$ assimilation be considered. Here, the δ$^{15}$N- and δ$^{18}$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 δ$^{15}$N- and δ$^{18}$O-NO$_3^-$
values depending on if there is a difference between the δ$^{15}$N value of organic nitrogen and NO$_3^-$ and
the δ$^{18}$O contributions of O$_2$ and H$_2$O; (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 δ$^{15}$N- and δ$^{18}$O-NO$_3^-$
values if there is residual NO$_3^-$ to measure. In all cases, changes in the δ$^{15}$N- and δ$^{18}$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 δ$^{15}$N- and δ$^{18}$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 δ$^{15}$N- and δ$^{18}$O-NO$_3^-$ values in situ we therefore recommend:

- Measuring δ$^{18}$O-H$_2$O values at the same time as δ$^{18}$O-NO$_3^-$ values and report δ$^{18}$O-NO$_3^-$ values vs δ$^{18}$O-H$_2$O instead of V-SMOW to make appropriate comparisons with time and across sites;
- Combining δ$^{18}$O-H$_2$O and δ$^{18}$O-O$_2$ values to develop appropriate site-specific ranges of δ$^{18}$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 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 $\delta^{18}O-H_2O$ since the $\delta^{18}O-NO_3^-$ axis is reported relative to the typical standard SMOW.

(b): Nitrate isotope biplot of the same data where the $\delta^{18}O-NO_3^-$ axis is reported relative to the ambient $\delta^{18}O-H_2O$ values in the river at the time of sampling, as per recommendation A. The grey bands indicate NO$_3^-$ produced with a range of $\delta^{18}O-NO_3^-$ values based on a mixture of $\delta^{18}O-O_2$ and $\delta^{18}O-H_2O$ values. The minimum value is where the $\delta^{18}O-H_2O$ is entirely retained in the $\delta^{18}O-NO_3^-$ value and without isotope fractionation associated with abiotic oxygen exchange (Casciotti et al. 2007). The

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light grey band covers the range expected when δ^{18}O-O_{2} values are lowest during the day. The dark grey band extends the range expected when δ^{18}O-O_{2} values are greatest during the night (Venkiteswaran et al. 2015). Thus the δ^{18}O value of newly produced 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 recognise that nitrogen is biologically cycled and will be largely imprinted with the ambient δ^{18}O-H_{2}O 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_{2}O 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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