

1 Towards a global interpretation of dual 2 nitrate isotopes in natural water

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11 Abstract:

12 Modern anthropogenic activities have significantly increased nitrate concentrations in natural waters.
13 Stable isotopes of nitrate (NO_3^-) offer a tool to deconvolute some of these man-made changes in
14 the nitrogen cycle. They are often graphically illustrated on a template designed to identify different
15 sources and denitrification in groundwater. In the two decades since this template was developed,
16 NO_3^- isotopes have been measured in a variety of ecosystems and through the nitrogen cycle, however
17 the interpretation of NO_3^- isotopes has not advanced. This default is no longer helpful because it does
18 not describe non-groundwater ecosystems well and encourages researchers to think solely about
19 denitrification even in well oxygenated systems where denitrification is likely to have little to no
20 influence on the nitrogen cycle. We propose a different scheme to encourage a better understanding of
21 the nitrogen cycle and interpretation of NO_3^- isotopes. We use a mechanistic understanding of NO_3^-
22 formation to place bounds on the oxygen isotope axis and provide a means to adjust for different
23 environmental water isotope so data from multiple sites and times of year can be appropriately
24 compared.

25 Background:

26 Stable isotopes in nitrate (NO_3^-) have been commonly measured for more than 4 decades (see Heaton
27 (1986) and papers therein). Methods have evolved from off-line AgNO_3 precipitation, to chemical and
28 microbial reduction to N_2O and subsequent CF-IRMS analyses. Since NO_3^- is a very common global
29 pollutant and contributor to eutrophication in water (Vitousek et al. 1997), a key application of NO_3^-
30 isotopes was to identify the NO_3^- source or sources. Through combining a number of individual
31 studies, this led to publication of a $\delta^{18}\text{O}\text{-NO}_3^-$ vs $\delta^{15}\text{N}\text{-NO}_3^-$ co-plot with suggested ranges for
32 different 'sources' of NO_3^- (Kendall 1998). It has been modified a few times but the fundamental
33 concept remained the same. Its application for interpreting NO_3^- isotopes has become widespread but
34 this figure is not really fit for this purpose and commonly over-interpolated. Here, we discuss the
35 assumptions inherent in this figure and key improvements needed improved understanding of NO_3^-
36 isotopes in the environment.

37 The figure was designed for interpreting groundwater data where NO_3^- isotope values of different
38 NO_3^- sources are preserved except by (chemo)denitrification. Some researchers identified forests that
39 receive a lot of nitrogen deposition export NO_3^- in streams and this does not retain the atmospheric
40 deposition isotope values. This was early evidence that measured NO_3^- isotopes in surface water
41 showed that they should not be used for source identification because of various biological alteration
42 along their flowpath. As method improvements allowed more NO_3^- isotope data to be generated, a
43 schematic figure that recognized biological processing of NO_3^- between its sources and sampling point
44 needed to be developed. However, this commonly used figure encourages researchers to think only
45 about one process, denitrification (Kendall 1998), although it may be uncommon in well oxygenated
46 lake surfaces or streams and rivers. In this way, we need a better schematic figure that explicitly
47 recognizes the differences between NO_3^- sources and processes that produce and consume NO_3^- .

48 Interpretation:

49 The "nitrogen axis" had been used as the primary differentiator between sources. However, given the
50 wide range of possible $\delta^{15}\text{N}$ values in manure/sewage and soils (Craine et al. 2015), and the obvious
51 fact that nitrogen will be biologically cycled in those systems, source identification cannot be done

52 with boxes on a figure. Since the $\delta^{15}\text{N}$ scale is typically defined against atmospheric N_2 , this means
53 the data are already adjusted for the high levels of synthetic nitrogen produced and used globally.
54 Measuring locally appropriate sources of nitrogen as potential initial $\delta^{15}\text{N}$ values would be a very
55 useful contribution to each study instead of relying on the broad assumption that a single set of boxes,
56 derived from a limited number of measurements and locally constrained, are globally appropriate.

57 The “oxygen axis” has groups that can be defined a priori: (i) high $\delta^{18}\text{O}$ values from NO_3^- produced in
58 the atmosphere where the $\delta^{18}\text{O}$ value depends strongly on latitude (Michalski et al. 2012); and (ii) low
59 $\delta^{18}\text{O}$ values where the $\delta^{18}\text{O}$ value depends strongly on the $\delta^{18}\text{O}$ of H_2O where the NO_3^- is formed
60 (Snider et al. 2010). The $\delta^{18}\text{O}$ value of NO_3^- produced by nitrification, including nitrification of nitrogen
61 ammonified from organic nitrogen, can be bounded in two ways. First, canonical two-step
62 nitrification (from NH_4^+ over NH_2OH to NO_2^- to NO_3^-) adds one O atom from O_2 in the first step and
63 one O atom from H_2O in each of the next two steps (Snider et al. 2010). In surface soils, the pore gas
64 $\delta^{18}\text{O}\text{-O}_2$ value is very likely near the atmospheric value of +23.5‰ (vs SMOW). In productive aquatic
65 ecosystems, the diel variability of $\delta^{18}\text{O}\text{-O}_2$ values can be large (e.g. Venkiteswaran et al. 2015,
66 Wassenaar et al. 2010) though this range can be estimated by one set of diel samples during the most
67 productive part of the year and analyzed via a variety of techniques (e.g., Barth et al. 2004; Wassenaar
68 and Koehler 1999). Second, incubation experiments with various levels of $\delta^{18}\text{O}\text{-H}_2\text{O}$ indicate that the
69 contribution of $\delta^{18}\text{O}\text{-H}_2\text{O}$ values to the final $\delta^{18}\text{O}\text{-NO}_3^-$ value is often greater than the minimum two-
70 thirds and sometimes close to 1. Thus the range of $\delta^{18}\text{O}$ values of NO_3^- produced in situ can be
71 bounded by knowledge of $\delta^{18}\text{O}\text{-O}_2$ and $\delta^{18}\text{O}\text{-H}_2\text{O}$ values: a minimum of $\frac{2}{3} \times \delta^{18}\text{O}\text{-H}_2\text{O}$ and a
72 maximum of $\frac{1}{2} \times \delta^{18}\text{O}\text{-O}_2 + \frac{2}{3} \times \delta^{18}\text{O}\text{-H}_2\text{O}$. These are the logical values that should be generated for
73 each field site rather than a single catch-all approach. Globally, $\delta^{18}\text{O}\text{-H}_2\text{O}$ values of surface water vary
74 widely but they can be predicted by latitude and databases such as waterisotopes.org though direct
75 measurement is much simpler than NO_3^- isotopes. Additionally, to make data $\delta^{18}\text{O}\text{-NO}_3^-$ comparable
76 between seasons and sites, displaying the data vs the $\delta^{18}\text{O}\text{-H}_2\text{O}$ value from the same sample rather
77 than vs V-SMOW. This is akin to the way $\delta^{18}\text{O}\text{-PO}_4^{3-}$ values are plotted relative to their temperature-
78 specific equilibrium point with $\delta^{18}\text{O}\text{-H}_2\text{O}$ (Davies et al. 2014, Paytan et al. 2002) in order to remove
79 the influence of difference $\delta^{18}\text{O}\text{-H}_2\text{O}$ values (Fig. 1).

80 Only once the appropriate range of initial $\delta^{18}\text{O}\text{-NO}_3^-$ values has been determined can processes, such
81 nitrification, denitrification, and NO_3^- assimilation be considered. Here, the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of
82 NO_3^- in the environment will be pulled in multiple directions at the same time. The magnitude of
83 change depends on multiple factors that are difficult to statically display in a biplot: mineralization of
84 organic nitrogen and subsequent nitrification may decrease $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of NO_3^- , ammonia
85 and NO_3^- uptake and release by riverine periphyton and macrophytes may have differing impacts, and
86 denitrification in riparian zones and anoxic river and lake sediments may increase $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$
87 values of NO_3^- if there is residual NO_3^- to measure. In all cases, changes in the $\delta^{18}\text{O}$ and $\delta^{15}\text{N}$ values of
88 NO_3^- are more complex than a single arrow for denitrification suggests (Kendall 1998).

89 At our sites in Canada, Kenya, and the United Kingdom, almost all data fall within the range of $\delta^{18}\text{O}\text{-}$
90 NO_3^- values produced in site from O_2 and H_2O values (Fig. 1). This means that without additional
91 independent information, there are several possible explanations for the data that are more complex
92 than simply assigning a source based on the $\delta^{15}\text{N}$ values. For example, varying contributions of the
93 $\delta^{18}\text{O}\text{-H}_2\text{O}$ values, two or more sources of nitrogen, uptake and release of varying amounts of ammonia
94 and NO_3^- , and denitrification in varying combinations may have produced the observed patterns in our
95 data.

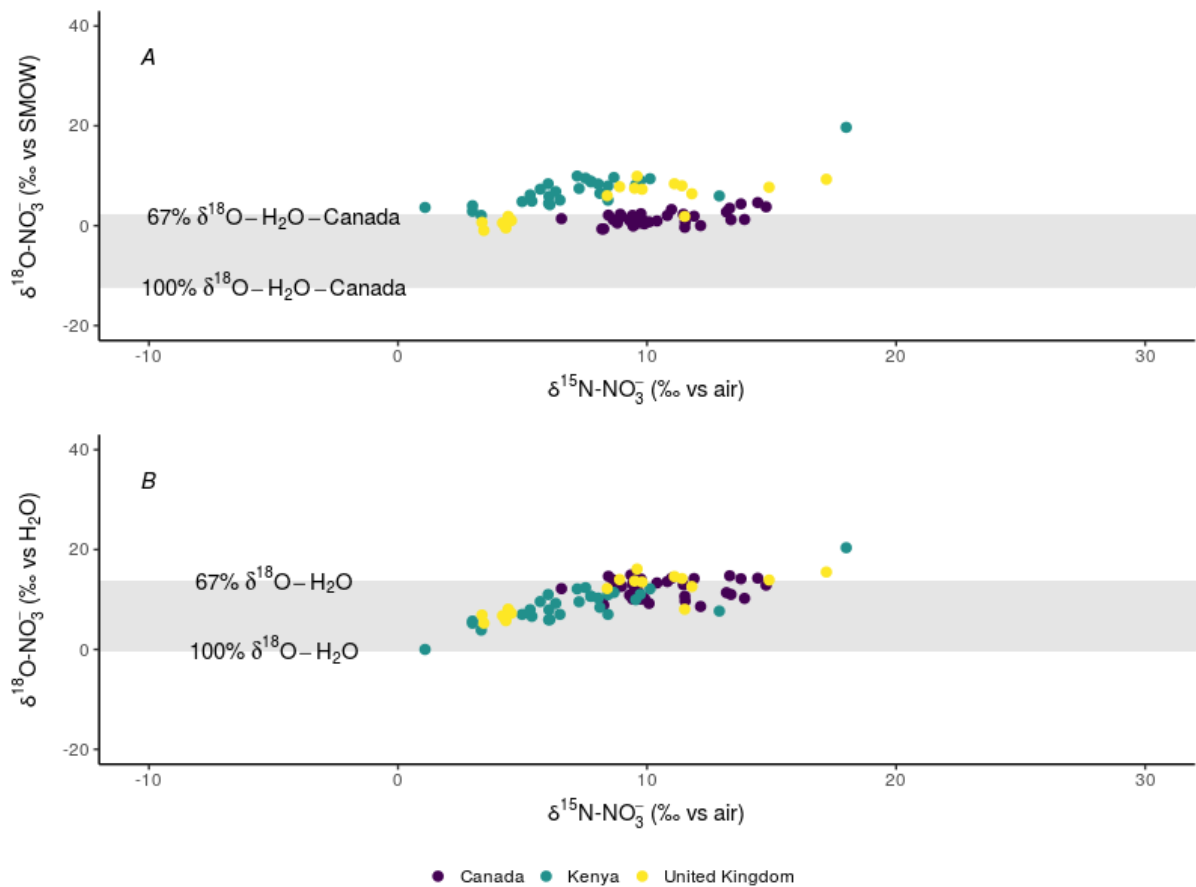
96 Recommendations:

- 97 In order to move beyond the simple source apportionment assumptions commonly made in NO_3^-
98 isotope biplots and to explicitly acknowledge that there are a variety of processes that alter the $\delta^{15}\text{N}$
99 and $\delta^{18}\text{O}$ values of NO_3^- we recommend:
- 100 A. Measuring $\delta^{18}\text{O}\text{-H}_2\text{O}$ values at the same time as $\delta^{18}\text{O}\text{-NO}_3^-$ values and report $\delta^{18}\text{O}\text{-NO}_3^-$ values
101 vs $\delta^{18}\text{O}\text{-H}_2\text{O}$ instead of V-SMOW to make appropriate comparisons with time and across
102 sites;
 - 103 B. Combining $\delta^{18}\text{O}\text{-H}_2\text{O}$ and $\delta^{18}\text{O}\text{-O}_2$ values to develop appropriate site-specific ranges of $\delta^{18}\text{O}\text{-}$
104 NO_3^- produced *in situ*; and
 - 105 C. Measuring locally relevant $\delta^{15}\text{N}$ source values to significantly reduce the range of $\delta^{15}\text{N}$ values
106 of nitrogen input to aquatic systems.

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112 Figure



113 *Fig. 1 (a)*: Nitrate isotope biplot of data from three sites in the middle of the Grand River, Ontario,
 114 Canada; 11 sites in the Nyando River, Kenya; eight sites in the Nzoia River, Kenya; five sites in the
 115 Sondu River, Kenya; eight sites in the River Lambourn near Boxford, United Kingdom; and 11 sites
 116 in the River Thames near Oxford, United Kingdom. The grey panel indicates NO_3^- produced with the
 117 maximum range of $\delta^{18}\text{O-NO}_3^-$ values based on a mixture of $\delta^{18}\text{O-O}_2$ and $\delta^{18}\text{O-H}_2\text{O}$ values for the
 118 Canadian sites. Comparisons are difficult between seasons at one site and still more difficult between
 119 sites because of the variability in $\delta^{18}\text{O-H}_2\text{O}$ since the $\delta^{18}\text{O-NO}_3^-$ axis is reported relative to the typical
 120 standard SMOW.
 121 *(b)*: Nitrate isotope biplot of the same data where the $\delta^{18}\text{O-NO}_3^-$ axis is reported relative to the ambient
 122 $\delta^{18}\text{O-H}_2\text{O}$ values in the river at the time of sampling, as per recommendation A. Here, data are more
 123 clearly expressed relative to the appropriate environmental conditions that recognize that nitrogen is
 124 biologically cycled and will be largely imprinted with the ambient $\delta^{18}\text{O-H}_2\text{O}$ value. A parsimonious
 125 interpretation here is that many data from Kenya and the UK exhibit the range of known contributions
 126 of the $\delta^{18}\text{O-H}_2\text{O}$ values, i.e. from two-thirds to one. Most Canadian and some Kenyan and UK data
 127 approach the theoretical maximum $\delta^{18}\text{O-NO}_3^-$ before a requirement of denitrification must be
 128 considered.

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