

Review of Harms *et al.* (iteration #2)

General comments:

Overall, Harms *et al.* addressed most of my concerns. The dataset presented is interesting as it focuses on an under-sampled region of the ocean (the subtropical South Indian Ocean). While they now use their dual nitrate isotope data a bit more in their discussion, I was not entirely satisfied. In my opinion, this section of the manuscript remains insufficient. The study would be improved if they could use their dual nitrate isotope data in a simple model to validate their current estimate of newly fixed N derived using Redfield assumptions. I have provided the equations that I use for similar simple isotope box models in my 2009 and 2013 manuscripts below. The authors are welcome to contact me (abourbonnais@seoe.sc.edu) with any questions.

Specific comments and technical corrections:

Abstract:

Page 1, lines 14-15: Change to: "Our results are the first in this ocean region and provide new information on nitrogen sources and transformation processes."

Page 1, line 16: Change to "... IOSG with values of $<3 \mu\text{M}$ for both NO_3^- and PO_4^{3-}"

page 1, lines 23-24: What do they mean by "partial-assimilated organic matter"? Do they mean organic matter with a low $\delta^{15}\text{N}$? Is there any evidence for low $\delta^{15}\text{N}$ of organic material in the SAMW?

Page 1, line: They did not use an isotope budget to derive this estimate (see my general comments above).

Materials and Methods:

page 5, lines 19-20: What was the $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ for the internal standard? What was the size of the blank (if any)?

Results:

Page 7, line 9: Change to ".... of $<3 \mu\text{M}$ for both NO_3^- and PO_4^{3-} "

Discussion:

Page 14, lines 2-3: Change to "and biologically N_2 -fixation are major processes..."

Page 15, lines 9-11: Change to "... N^* . The analytical error on N^* estimate based on the relative error for nitrate and phosphate analysis was below 1.5% for duplicate sample measurements."

Is the error on N^* calculated from propagating the errors for these analysis?

page 15, line 15: Change "characterized" to "affected".

Page 16, lines 11-13: $^{18}\epsilon:^{15}\epsilon$ is associated with both assimilative (nitrate assimilation) and dissimilative (denitrification) nitrate reduction.

Page 16, lines 15-17: This sentence is not clear. The isotope effect associated with ammonium production and nitrification does not affect the $\delta^{15}\text{N-NO}_3^-$ because ammonium and nitrite generally does not accumulate in oxic waters.

Page 16 and 17, last and first sentences: This point needs to be discussed more. How the $\delta^{18}\text{O}$ of seawater is affected by ammonium and nitrite oxidation? For instance, they could add the following sentence: " $\delta^{18}\text{O}$ depends on the ϵ during NH_4^+ and NO_2^- oxidation, water incorporation (with $\delta^{18}\text{O- H}_2\text{O}$ of $\sim 0\text{‰}$), and the exchange of oxygen atoms with water that should generate a $\delta^{18}\text{O}$ of newly produced NO_3^- between -8 and -1‰ (Buchwald and Casciotti, 2010; Casciotti *et al.*, 2010).

Page 19, lines 13-14: Provide an estimate of N atmospheric depositions in this region to support this claim.

Page 19, line 22: Correct "N₂-fixation"

Page 19, section 4.2.2. The manuscript would be improved if they could also derive an N₂ fixation estimate using a simple isotope model, as described below.

Additional references:

Bourbonnais, A., Lehmann, M. F., Hamme, R. C., Manning, C. C., & Juniper, S. K. (2013). Nitrate elimination and regeneration as evidenced by dissolved inorganic nitrogen isotopes in Saanich Inlet, a seasonally anoxic fjord. *Marine chemistry*, 157, 194-207.

Casciotti, K. L., McIlvin, M., & Buchwald, C. (2010). Oxygen isotopic exchange and fractionation during bacterial ammonia oxidation. *Limnology and Oceanography*, 55(2), 753-762.

Example of simple isotope box model (Bourbonnais *et al.*, 2009 and 2013)

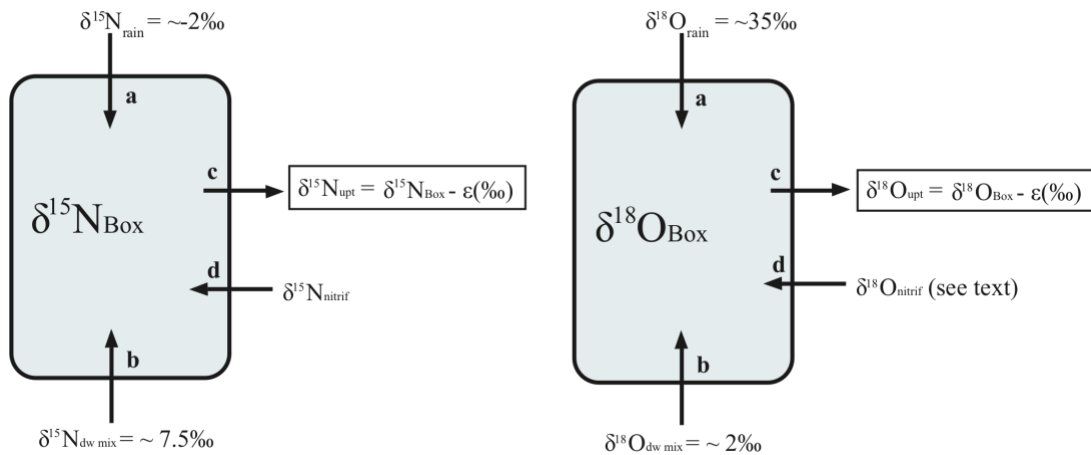


Figure 1. Nitrate isotope box-model for scenario 2a in Bourbonnais *et al.* (2013).

Note: $\delta^{15}\text{N}_{\text{rain}}$ in this model represents the $\delta^{15}\text{N}$ in precipitations, but could be replaced by the $\delta^{15}\text{N}$ for N_2 fixation in the Harms *et al.* paper.

N flux terms:

a = N from precipitation flux

b = seawater mixing

c = nitrate removal (by nitrate assimilation (C_{upt}))

d = organic matter remineralization and nitrification (recycled production)

$\delta^{15}\text{N}_{\text{Box}}$ = steady-state $\delta^{15}\text{N-NO}_3^-$ (under model assumptions)

$\delta^{18}\text{O}_{\text{Box}}$ = steady-state $\delta^{18}\text{O-NO}_3^-$ (under model assumptions)

$\delta^{15}\text{N}_{\text{rain}}$ = $\delta^{15}\text{N-NO}_3^-$ from precipitation (2‰)

$\delta^{18}\text{O}_{\text{rain}}$ = $\delta^{18}\text{O-NO}_3^-$ from precipitation (35‰)

$\delta^{15}\text{N}_{\text{dw mix}}$ = $\delta^{15}\text{N-NO}_3^-$ added from vertical mixing with deep-water (7.5‰)

$\delta^{18}\text{O}_{\text{dw mix}}$ = $\delta^{18}\text{O-NO}_3^-$ added from vertical mixing with deep-water (2‰)

$\delta^{15}\text{N}_{\text{upt}}$ = $\delta^{15}\text{N}_{\text{Box}} - \epsilon_{\text{upt}}$ (‰) = $\delta^{15}\text{N-NO}_3^-$ of nitrate assimilated

$\delta^{18}\text{O}_{\text{upt}}$ = $\delta^{18}\text{O}_{\text{Box}} - \epsilon_{\text{upt}}$ (‰) = $\delta^{18}\text{O-NO}_3^-$ of nitrate assimilated

where

ϵ_{upt} (‰) = nitrate assimilation isotope effect (5 ‰)

$\delta^{15}\text{N}_{\text{nitrif}}$ = $\delta^{15}\text{N}$ of nitrate generated in the process of organic matter remineralization and nitrification.

$\delta^{18}\text{O}_{\text{nitrif}}$ = $\delta^{18}\text{O}$ of nitrate generated in the process of organic matter remineralization and nitrification (-3.8 ‰).

The equation for $\delta^{15}\text{N}_{\text{Box}}$ is derived from:

$$\delta^{15}\text{N}_{\text{Box}} = [\delta^{15}\text{N}_{\text{rain}} \times a] + [\delta^{15}\text{N}_{\text{dw mix}} \times b] - [(c_{\text{upt}} - d) \times (\delta^{15}\text{N}_{\text{Box}} - \epsilon_{\text{upt}})]$$

$$\delta^{15}\text{N}_{\text{Box}} = [\delta^{15}\text{N}_{\text{rain}} \times a] + [\delta^{15}\text{N}_{\text{dw mix}} \times b] - [(c_{\text{upt}} - d) \times \delta^{15}\text{N}_{\text{Box}}] + [(c_{\text{upt}} - d) \times \epsilon_{\text{upt}}]$$

$$\delta^{15}\text{N}_{\text{Box}} + [(c_{\text{upt}} - d) \times \delta^{15}\text{N}_{\text{Box}}] = [\delta^{15}\text{N}_{\text{rain}} \times a] + [\delta^{15}\text{N}_{\text{dw mix}} \times b] + [(c_{\text{upt}} - d) \times \epsilon_{\text{upt}}]$$

$$\delta^{15}\text{N}_{\text{Box}} + [(c_{\text{upt}} - d) \times \delta^{15}\text{N}_{\text{Box}}] = [\delta^{15}\text{N}_{\text{rain}} \times a] + [\delta^{15}\text{N}_{\text{dw mix}} \times b] + [(c_{\text{upt}} - d) \times \epsilon_{\text{upt}}]$$

$$\delta^{15}\text{N}_{\text{Box}} \times [1 + (c_{\text{upt}} - d)] = [\delta^{15}\text{N}_{\text{rain}} \times a] + [\delta^{15}\text{N}_{\text{dw mix}} \times b] + [(c_{\text{upt}} - d) \times \epsilon_{\text{upt}}]$$

The final equation for $\delta^{15}\text{N}_{\text{Box}}$ is:

$$\delta^{15}\text{N}_{\text{Box}} = [(\delta^{15}\text{N}_{\text{rain}} \times a) + [\delta^{15}\text{N}_{\text{dw mix}} \times b] + [(c_{\text{upt}} - d) \times {}^{15}\epsilon_{\text{upt}}] / [1 + (c_{\text{upt}} - d)]$$

The equation for $\delta^{18}\text{O}_{\text{box}}$ is derived from:

$$\delta^{18}\text{O}_{\text{Box}} = [\delta^{18}\text{O}_{\text{rain}} \times a] + [\delta^{18}\text{O}_{\text{sw mix}} \times b] - [c_{\text{upt}} \times (\delta^{18}\text{O}_{\text{Box}} - {}^{18}\epsilon_{\text{upt}})] + [d \times \delta^{18}\text{O}_{\text{nitrif}}]$$

$$\delta^{18}\text{O}_{\text{Box}} = [\delta^{18}\text{O}_{\text{rain}} \times a] + [\delta^{18}\text{O}_{\text{sw mix}} \times b] - [c_{\text{upt}} \times \delta^{18}\text{O}_{\text{Box}}] + [c_{\text{upt}} \times {}^{18}\epsilon_{\text{upt}}] + [d \times \delta^{18}\text{O}_{\text{nitrif}}]$$

$$\delta^{18}\text{O}_{\text{Box}} + [c_{\text{upt}} \times \delta^{18}\text{O}_{\text{Box}}] = [\delta^{18}\text{O}_{\text{rain}} \times a] + [\delta^{18}\text{O}_{\text{sw mix}} \times b] + [c_{\text{upt}} \times {}^{18}\epsilon_{\text{upt}}] + [d \times \delta^{18}\text{O}_{\text{nitrif}}]$$

$$\delta^{18}\text{O}_{\text{Box}} \times [1 + c_{\text{upt}}] = [\delta^{18}\text{O}_{\text{rain}} \times a] + [\delta^{18}\text{O}_{\text{sw mix}} \times b] + [c_{\text{upt}} \times {}^{18}\epsilon_{\text{upt}}] + [d \times \delta^{18}\text{O}_{\text{nitrif}}]$$

The final equation for $\delta^{18}\text{O}_{\text{Box}}$ is:

$$\delta^{18}\text{O}_{\text{Box}} = [\delta^{18}\text{O}_{\text{rain}} \times a] + [\delta^{18}\text{O}_{\text{sw mix}} \times b] + [c_{\text{upt}} \times {}^{18}\epsilon_{\text{upt}}] + [d \times \delta^{18}\text{O}_{\text{nitrif}}] / [1 + c_{\text{upt}}]$$

The $\Delta(15,18)$ is calculated according to the following equation:

$$\Delta(15,18) = (\delta^{15}\text{N} - \delta^{15}\text{N}_{\text{deep}}) - [({}^{18}\delta/{}^{15}\delta) (\delta^{18}\text{O} - \delta^{18}\text{O}_{\text{deep}})]$$