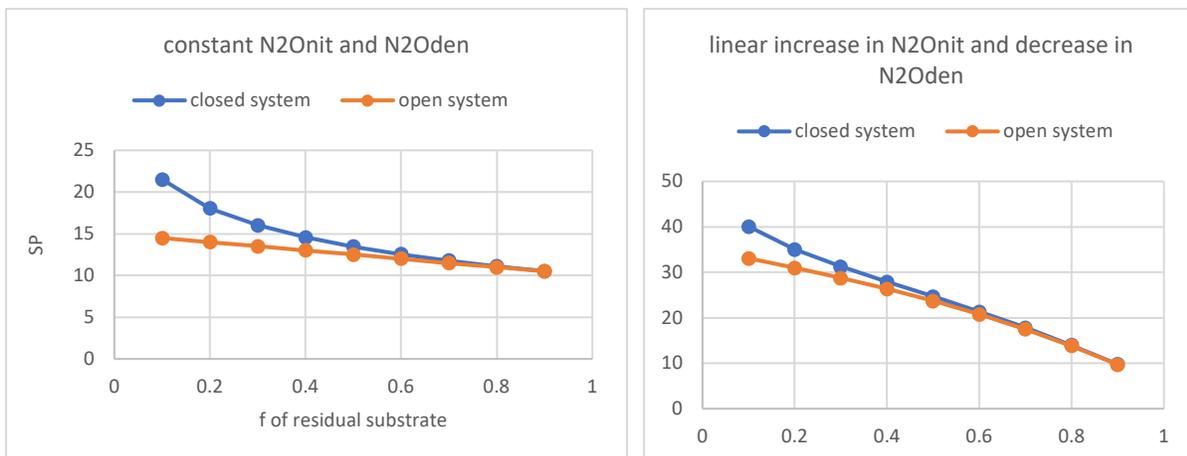


Eq 7,8 – $\eta \cdot F_{\text{reduced/diffused}}$ – you make open system assumption in your equations – definitely right for diffusion, but can be doubt for N_2O reduction, there might be values which cannot be explained with open system equation (Lewicka-Szczebak et al., 2014, 2017), or you can wrongly attribute this to larger proportion of nitrification. Especially given that you have large reduction it makes big difference if using open or closed system equations. I would check what is the difference and report this. You have only justified using the open system equation with the argument of simplified maths, but I think this generates significant bias. The requirement for using open system equation is the equilibrium of the fluxes in and out (Fry, 2006) and you clearly do not have this between N_2O production and reduction.

We acknowledge that carefully considering open versus closed system dynamics is important. If N_2O production and reduction occur simultaneously, both open or closed dynamics could occur. The reviewer refers to the requirement of equilibrium between the fluxes in and out for open system dynamics, and argues this is not the case in our study. We kindly disagree with this assessment. Given the slow but steady change of N_2O and isotope values over time observed in our study, fluxes in and out may well be at near equilibrium in distinct time points. In the study by Lewicka-Szczebak and colleagues (2017), instantaneous N_2O emitted from the soil surface was measured during an incubation, where a N_2O pulse was observed following application of fertilizer N. When plotting isotope values of N_2O in function of the residual N_2O fraction, a logarithmic fit was found, which could be described by the Rayleigh equation that describes N_2O fractionation under closed system dynamics. This clearly illustrates that in their study, closed system dynamics prevailed during N_2O reduction. It should be noted, however, that the patterns observed in Lewicka-Szczebak et al. (2017) are only expected to occur if N_2O source processes remain constant, while there is a progressive reduction of N_2O over time. If N_2O production from nitrification increase or decrease over time, very different patterns would have emerged. The figures below show simulated relationships between SP of the instantaneous product and the residual substrate fraction under scenarios where N_2O production is constant over time, vs. where N_2O production from nitrification and denitrification changes over time, for open vs. closed system dynamics. In all scenarios, we assumed that the residual fraction of N_2O decreases progressively over time.



We argue that, while the study by Lewicka-Szczebak et al. (2017) marked an important milestone in furthering how to interpret N₂O isotope values, the study does not provide unequivocal evidence that closed system dynamics will prevail in all scenarios where N₂O production and reduction occur simultaneously. This leaves the question, when is it appropriate to use open vs. closed system dynamics? In a simulation by Denk et al. 2017, it was shown that the difference between open and closed system dynamics becomes small at small time steps, even when the fraction of residual substrate becomes very small. Given that closed system dynamics would lead to a system of complex non-linear equations that are much harder to solve numerically, we opted to use open system dynamics and a small time step. We will elaborate on this point in the discussion, including recommendations for future research to provide more clarity on this issue.

Denk, T. R. A., et al. (2017). "The nitrogen cycle: A review of isotope effects and isotope modeling approaches." *Soil Biology & Biochemistry* 105: 121-137. (Fig. 7)

L 555 (Appendix) – “With various terms cancelling out, the equation can be rewritten as:” – why can you cancel out “various things”, what are these “various things” and how this cancelling out influences the final result?

The canceling out of terms follows rules in algebra. To make it more clear, we strike through the terms that cancel out below

Equation A.2:

$$\frac{\Delta I}{\Delta t} = (I_0 C_0 + I_{in,1} k_{in,1} \Delta t + I_{in,2} k_{in,2} \Delta t - \eta_{out,1} (C_0 + k_{in,1} \Delta t + k_{in,2} \Delta t - C_0 - k_{in,1} \Delta t - k_{in,2} \Delta t + k_{out,1} \Delta t) - \eta_{out,2} (C_0 + k_{in,1} \Delta t + k_{in,2} \Delta t - C_0 - k_{in,1} \Delta t - k_{in,2} \Delta t + k_{out,2} \Delta t) - I_0 C_0 - I_0 k_{in,1} \Delta t - I_0 k_{in,2} \Delta t) / ((C_0 + k_{in,1} \Delta t + k_{in,2} \Delta t) \Delta t)$$

Equation A.2 with strike-through text for terms that cancel out

$$\frac{\Delta I}{\Delta t} = (\cancel{I_0 C_0} + I_{in,1} k_{in,1} \Delta t + I_{in,2} k_{in,2} \Delta t - \eta_{out,1} (\cancel{C_0} + \cancel{k_{in,1} \Delta t} + \cancel{k_{in,2} \Delta t} - \cancel{C_0} - \cancel{k_{in,1} \Delta t} - \cancel{k_{in,2} \Delta t} + k_{out,1} \Delta t) - \eta_{out,2} (\cancel{C_0} + \cancel{k_{in,1} \Delta t} + \cancel{k_{in,2} \Delta t} - \cancel{C_0} - \cancel{k_{in,1} \Delta t} - \cancel{k_{in,2} \Delta t} + k_{out,2} \Delta t) - \cancel{I_0 C_0} - \cancel{I_0 k_{in,1} \Delta t} - \cancel{I_0 k_{in,2} \Delta t}) / ((C_0 + k_{in,1} \Delta t + k_{in,2} \Delta t) \Delta t)$$

Removing the terms that cancel out result in the following equation:

Equation A.3:

$$\frac{\Delta I}{\Delta t} = \frac{(I_{in,1} k_{in,1} \Delta t + I_{in,2} k_{in,2} \Delta t - \eta_{out,1} k_{out,1} \Delta t - \eta_{out,2} k_{out,2} \Delta t - I_0 k_{in,1} \Delta t - I_0 k_{in,2} \Delta t)}{(C_0 + k_{in,1} \Delta t + k_{in,2} \Delta t) \Delta t}$$

Note that Δt appears in every term in the numerator, and also appears in the denominator. Following algebra, Δt cancels out to the following equation:

Equation A.4:

$$\frac{\Delta I}{\Delta t} = \frac{(I_{in,1}k_{in,1} + I_{in,2}k_{in,2} - \eta_{out,1}k_{out,1} - \eta_{out,2}k_{out,2} - I_0k_{in,1} - I_0k_{in,2})}{(C_0 + k_{in,1}\Delta t + k_{in,2}\Delta t)}$$

We hope this clarifies the math. There are no assumptions applied here, strictly doing algebra.

L563 (Appendix) – “For Δt approaching 0, the equation can be further simplified as:” – why can you assume Δt approaching 0? It is one day, right? When you are using fluxes in your equations you need the time factor to be included in the equations, I guess. What are the units for the fluxes used in your Eq 7 and 8?

The reviewer is referring to the following section of the proof for the equation used in this study:

For Δt approaching 0, the equation can be further simplified as:

Equation A.5:

$$\frac{\Delta I}{\Delta t} = \frac{k_{in,1}(I_{in,1} - I_0) + k_{in,2}(I_{in,2} - I_0) - \eta_{out,1}k_{out,1} - \eta_{out,2}k_{out,2}}{C_0}$$

We argue that $\frac{\Delta I}{\Delta t}$ is considered the infinitesimal change in the isotope value over a short time step. Thus, if Δt is very small, $k_{in,1}\Delta t$ and $k_{in,2}\Delta t$ would approach 0, and the denominator can be reduced to C_0 . In our study, we estimate $\frac{\Delta I}{\Delta t}$ as the first derivative to the smooth curve fitted to the isotope values over time. As such, $\frac{\Delta I}{\Delta t}$ does indeed denote the infinitesimal change in the isotope value as the change in time (the time step) approaches 0. Therefore, we argue that our proof of our equation is valid.