

***Interactive comment on* “The fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate” by P. K. Swart et al.**

Anonymous Referee #2

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General comments:

Swart et al present data for the N and O isotopic fractionation imparted during NO₃-assimilation by a species of green and red macroalgae (*Ulva* sp. and *Agardhiella* sp., respectively). Similar studies have been conducted for years – but are an important component of a foundational understanding of the behavior of stable isotopes in the environment. Without such fundamental studies – field data can be severely confounded by factors that are overlooked or misunderstood. Overall – the paper is well presented (a few minor typos) and the data appear robust and of high quality. The general finding that the apparent isotope fractionation decreases at lower concentrations has been observed before in other microalgae and bacterial system. However, this work represents

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the first dual isotopic study of macroalgae fractionation during assimilation – and therefore fills an important gap in our overall understanding of nitrogen isotope dynamics in marine systems.

Specific comments:

The prediction of an inverse isotope effect at the lowest NO₃⁻ concentrations is interesting (although necessarily adequately supported by the data). There could be very important implications for this in oligotrophic ecosystems. Given the fact that the Ulva experiment with 3uM did not show this, however, I suspect that this is probably due to either error in sampling the new algal growth (giving the observed $\delta^{15}\text{N}$ values that were higher than NO₃⁻) and/or an artifact of the quadratic fit to the ‘free drift’ experiments. Additionally – the experiments in which the algal tissue $\delta^{15}\text{N}$ was higher than the NO₃⁻ - were performed at 14 and 60uM – hardly ‘low’ levels – and so I find this to be perhaps a little bit overly speculative. What did the ‘old growth’ look like? Were its values measured?

Since isotopic fractionation factors were calculated using both NO₃⁻ and algal biomass – the authors argue that the two methods give ‘statistically’ the same results. This may be true – but I wonder about the validity of sampling ‘new growth’ – and indeed the results presented in Table 5 show a consistent offset between the fractionation calculated between the two approaches, with the biomass approach regularly giving lower results in the Ulva experiments (but not in the Agardhiella). I think this may be revealing something important – either about the fidelity of sampling ‘new biomass,’ nutrient translocation in algae (?), or perhaps some other physiological explanation? I

Apparently NH₄⁺ and NO₂⁻ were also measured? Where are the data? Was any NH₄⁺ or NO₂⁻ observed? This would be an important component to constrain.

Pg 18 Ln 23: Granger et al 2010 actually revised this argument to consider that diffusion is likely not the major cause of the 2:1 slope – but rather transport effects are the primary driver. Karsh et al., 2014 of course elaborate on this as well.

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Pg 19 Ln 11: While I think that the quadratic fit to the data may accurately represent the observations and be useful for predicting the isotope effects, I think it is inaccurate to state that the relationship between $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ is mathematically described by a quadratic equation. Indeed – the results indicate that a non-linear (or curved) evolution is predicted – I agree. Maybe consider replacing “quadratic” here with “curved?”

Pg 20 Ln 1: Is this in contrast to Cohen and Fong, 2005? If so – I think there needs to be a little bit of direct discussion for addressing the differences between the findings of the two studies.

Pg 20 Ln 6-9: Mentioning the Haber-Bosch process here, or the original composition of the NO_3^- , is irrelevant, right? You just stated that the effects should be seen “regardless of the $\delta^{15}\text{N}$ of the original NO_3^- .”

Pg 20 Ln 11: I don’t follow this exactly. If fractionation is 5‰ and the NO_3^- $\delta^{15}\text{N}$ is 0‰ then the initial algal biomass would be -5‰. As NO_3^- is consumed in the open system described – the NO_3^- would increase from 0 to +5‰ while the biomass would increase from -5 to 0‰. Where in this scenario would “isotopically positive algal material be formed?” I agree that isotopic fractionation and differential drawdown and mixing and convolute any simple source signatures – but I don’t quite understand the argument as it’s presented.

Minor comments:

1. Pg 2 Ln 17: It would be nice to define what is meant here by ‘typical’ – these algae are commonly found in coastal systems – and often these systems may see higher concentrations than coral reef, oligotrophic or open water systems.
2. Pg 2 Ln 17 and Ln 22. Sentence is repeated.
3. Pg 2 Ln 23: Again – being somewhat specific here about what constitutes ‘higher’ would be helpful in the abstract.
4. Pg 4 Ln 7: Isotope ratios are expressed. . .

5. Pg 4 Ln 9: VSMOW?
6. Pg 6 Ln 8: investigations
7. Pg 6 Ln 5: have been
8. Pg 6 Ln 10-14: Given the findings of concentration independence by Cohen and Fong (2005) – I think the justification for this study should be more strongly justified here. In particular – are there findings of Cohen and Fong that are suspect or misinterpreted?
9. Pg 8 Ln 11: I assume it is well established that new macroalgal growth is comprised of the ‘new’ nitrogen – and that there is no translocation of internal N pools in these species? The results seem consistent with this – but maybe it should be stated for clarity.
10. Pg 14 Ln 4: Equation 6. Since you’ve already defined ‘f’ – I feel this equation could/should be written in its more familiar form (e.g., $f^* \ln(f)/(1-f)$).
11. Pg 14 Ln 6: “tend to equal” isn’t quite accurate – maybe change to “approach”?
12. Pg 15 Ln 9: “ during the experiment as the concentration. . .”
13. Pg 19 Ln 21: “ have implications for the application. . .”
14. Pg 20 Ln 5: I wonder in estuaries about the role of NH_4^+ assimilation, since estuarine sediments typically support a substantial flux of NH_4^+ into the overlying water.
15. Pg 21 Ln 8: I think “inverse” is the appropriate term.
16. Where is the $\delta^{13}\text{C}$ and C:N data?
17. Figure 2: What is the slope of the line?
18. Figure 3: The 60uM treatment seems to be substantially pulled by a single point ($\delta^{15}\text{N} \sim 9.5\text{‰}$). The other 60uM data seems to line up well with the 103uM data – suggesting a similar mechanism at work at both of these concentrations? I guess this

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is revisited and addressed in Figure 5 and 6. Also in Figures 3, 5 and 6 – is this both algae or just Ulva?

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