Biogeosciences Discuss., 11, C5003–C5009, 2014
www.biogeosciences-discuss.net/11/C5003/2014/

Biogeosciences
Discussions
Open Access

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

P. K. Swart et al.

pswart@rsmas.miami.edu

Received and published: 9 September 2014

Reply to Reviewer 2 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 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.

Reply: We thank the reviewer for their comments. In addition the range of concentrations used in previous work has been generally at the higher concentrations used in this study and studies have not been carried out at values approaching those found in the normal marine environment. The one study which we are aware of actually concluded that there was no concentration dependence of the fractionation factor, but they used fairly high concentrations in their experiments.

Specific comments: 1-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 15N 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 15N 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?

Reply: We agree that the inverse isotope effect may be speculative and we did not want to emphasize it too much. However, reviewer 1 felt that we did not emphasize enough. We tend to agree with this reviewer(reviewer 2) that at this point the data only suggest a possibility. We have reworked our interpretation of the Ulva data in the text to cover possible artifacts.

2- 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?

Reply: This may be correct and we have added material to the discussion. The distinction between the new and old growth is clear, but it does not preclude some type of nutrient redistribution in the solid samples and therefore the data on the NO3 is probably better.

3- 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.

Reply: All samples were screened for NO2- and NH4+ but we did not mention the data as none was detected. This has been added to the text.

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 15N and 18O 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?”

Reply: We are not sure why the relationship cannot be explained by quadratic equation. Just like the normal expression is described by a linear relationship Nevertheless the curved or non-linear relationship can be fitted with a quadratic equation, which when one takes the first differential provides the estimate of epsilon at any concentration of NO3-. This is key as figure 3 clearly shows changing slopes with different initial

concentrations.

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.

Reply: We have added some discussion of Cohen and Fong here. Cohen and Fong did experiments in which only NO3 was added at fairly high concentrations. These concentrations are above the level at which our experiments shows little relationship between concentration and fractionation. Hence it is not surprising that they did not find any relationship between the amount of fractionation and the concentration. We have added this to the discussion.

Pg 20 Ln 6-9: Mentioning the Haber-Bosch process here, or the original composition of the NO3-, is irrelevant, right? You just stated that the effects should be seen “regardless of the 15N of the original NO3-.

Reply: We only mention the H-B process as most artificial N fertilizer is produced by this process and since it produces values close to zero. We have removed reference to the HB process from this version.

Pg 20 Ln 11: I don’t follow this exactly. If fractionation is 5‰ and the NO3- 15N is 0‰ then the initial algal biomass would be -5 per mille as NO3- is consumed in the open system described – the NO3- would increase from 0 to +5‰ while the biomass would increase from -5 to 0‰ËŹ here 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.

Reply: The point is that the water containing the nitrate is moving along a path and as the nitrate decreases its d15N increases to value far higher than +5. One can model this using Rayleigh distillation. For example if the fractionation is 5 per mille and one starts with a nitrate concentration of 20 uM with a d15N of zero, then by the time the concentration has been reduced to 2 uM the d15N of the NO3- is+11.5 per mille. Assuming that the fractionation decreases linearly as the concentration decreases, this increase would not be so dramatic. Starting with higher concentrations would produce larger enrichments in the d15N of the NO3.

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.

Reply: We have defined typical marine values. While coastal values are higher the abstract is probably not the place to discuss this.

2. Pg 2 Ln 17 and Ln 22. Sentence is repeated.

Reply: Yes you are correct. This sentence has been deleted.

3. Pg 2 Ln 23: Again – being somewhat specific here about what constitutes 'higher'would be helpful in the abstract.

Reply: Values higher than $\sim$ 10 uM. This has been added.

4. Pg 4 Ln 7: Isotope ratios are expressed:

Reply: This has been fixed.

5. Pg 4 Ln 9: VSMOW?

Reply: This has been fixed.

6. Pg 6 Ln 8: investigations

Reply: This has been fixed. 7. Pg 6 Ln 5: have been

Reply: This has been fixed.

8. 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.

Reply: The justification is that previous work had found a concentration dependence in micro algae, yet Cohen and Fong said that there was none. This has been stated.

9. 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)).

Reply: That has been changed and the term 'f' used throughout.

11. Pg 14 Ln 6: "tend to equal" isn't quite accurate – maybe change to "approach"?

Reply: This has been changed to approach rather than equal

12. Pg 15 Ln 9: " during the experiment as the concentration: : :"

Reply: We are not sure what to change here

13. Pg 19 Ln 21: " have implications for the application: : :" Reply: This has been changed.

14. Pg 20 Ln 5: I wonder in estuaries about the role of NH4+ assimilation, since estuarine sediments typically support a substantial flux of NH4+ into the overlying water.

Reply: We agree but NH4+ is another story.

15. Pg 21 Ln 8: I think "inverse" is the appropriate term.

Reply: This has been changed to inverse 16. Where is the 13C and C:N data?

Reply: As stated in the result section these data are included in the supplemental material. While interesting they were not considered to be integral to the message of the paper.

17. Figure 2: What is the slope of the line?

Reply: I think that was given the result section as being close to unity, but I have now put the exact value into the figure caption.

18. Figure 3: The 60uM treatment seems to be substantially pulled by a single point (d15N 9.5‰. 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 is revisited and addressed in Figure 5 and 6. Also in Figures 3, 5 and 6 – is this both algae or just Ulva?

Reply: The r2 is 0.99,albeit only for three points. Still it is not substantially pulled out by one point. There may be some confusion regarding the points so we changed the symbols to make each one more identifiable. The non-linear model was only applied to the Ulva data as we did not have enough time steps for the other species to make a difference between the non-linear and linear approach

---

Interactive comment on Biogeosciences Discuss., 11, 6909, 2014.