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

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The manuscript, "The Fractionation of nitrogen and oxygen isotopes in macroalgae during the assimilation of nitrate" by Swart et al., presents the results of a laboratory study of isotopic fraction by macroalgae over a range of nitrate concentrations. This topic is relevant to a broad audience in biogeochemistry and environmental science, which makes Biogeochemistry an appropriate journal for this work. The subject of nitrogen isotope fractionation during assimilation is an area of active research, and a more complete understanding of the process is critically important to interpreting nitrogen stable isotope data in the environment, therefore this study is a valuable contribution. The

methods and tools used in the study are not particularly novel, however the presentation of fractionation data for macroalgae is new. The methods and results sections are clearly outlined. The results are appropriately interpreted in the context of recent work on microalgae and bacteria. Overall this is a well written paper.

Reply: We thank the reviewer for their comments and agree that such work has not been generally carried out with rigor using macroalgae. In addition the range of concentrations used in the study of microalgae in previous work has been generally at high values. Hence studies on the assimilation in both micro and macro algae has not been carried out at concentrations approaching those found in the normal marine or even coastal environments. Therefore we believe our paper presents valuable insight into the processes involved in assimilation.

Specific Comments 1. In the results section nitrogen isotopes of algae and NO3– are described separately for the two types of experiments. I would encourage combining these sections, so that the difference between the NO3– and the algal material can be understood quickly. I would suggest combining Tables 1, 2 and 3, into a single table. Or at a minimum adding the average initial d15N-NO3– values to the caption of Table 1.

Reply: Tables 1-3 have been now combined into one table.

2. In the discussion section of the Ulva experiments it is stated that the 15e values decrease toward zero with decreasing concentration. It seems more appropriate to say they decrease to a minimum of -3.2.

Reply: The text has been changed to say that the values decrease to -3.2‰

3. Given the emphasis on concentration dependence- how do you interpret the result that the 15e in the syringe experiment at ∼3 uM concentration and the free drift experiment at 500 mM are so similar for Ulva (2.1 ‰ vs 2.0 ‰ respectively).

Reply: In the syringe experiments during which a constant concentration of ∼ 3 uM was maintained, the e15N of the algae decreased from 3.1 to 1.3 equivalent to about 2 per mille fractionation. This decrease was similar to that observed in the 500 uM treatment. We have added a section to the text to discuss this, but it should be pointed out that the free drift showed a decreasing fractionation with decreasing concentration to that the extent that the values fell significantly below zero at low concentrations. While this may be real we are not as confident about the solid tissue data and we have added a small discussion of the pitfalls of using the tissue numbers. The discussion of this has been expanded(see page 15 (ln 20-24) and 16 (ln 1-11)). Also page 13 lines 18-22 and page 14 lines 1-13.

4. In the discussion section for Ulva it states that "although the quadratic equation predicts values less than zero at concentrations less than 1 uM, none of the experiments were actually performed at these low concentrations and there for this observation will need to be confirmed" Isn't it somewhat confirmed by the negative 15e values measured at concentrations of 60 and 14 uM? Perhaps this effect begins a higher concentrations.

Reply: We did not want to over interpret the data (see response to previous query). The negative e values in the Ulva experiments certainly suggest the preferential incorporation of 15N, but such values have not been seen previously and were not observed in the case of the other algae. Hence we want to err on the side of caution. Nonetheless your statement is true so we have added something to the text here to explain the results of the apparent negative epsilon values for the solid tissues in the case of Ulva. See also the comments of reviewer 2.

5. In the discussion section it also states that the syringe experiment yielded a 15e value 1 ‰ higher than the e15N estimated from NO3– draw down and from the solid free drift experiments" What is the difference between draw down and free drift experiments? It seems only fair to compare solids from the syringe experiment to solids from the free drift experiments in which case the difference looks like nearly 6 ‰

Reply: In the case of Ulva the 15e values calculated from the solids seem to indicate values of less than unity. The discrepancy with the free drift and the syringe data may suggest that there is a problem with the solid Ulva data. This comment is also pertinent to issue 4 (see above). Therefore we trust the syringe experiment and the NO3- data rather than the solid d15N values. We have emphasized this in the text and the reasons why we trust that data to a great extent.

6. In general I think the discussion of negative values for 15e could be more clear. It seems that the free drift experiment for Ulva yielded negative values at lower concentration and this is can be modeled with both the linear and quadratic approximations, therefore it is only the syringe experiment that is not consistent with this result. One thing that might help clarify the discussion would be to consistently use nomenclature that differentiates 15e (quadratic) from 15e (linear). The caption to Table 5 could specify 15e (linear).

Reply: We have attempted to clarify the discussion here. This comment is also covered by the previous three comments of this reviewer and we thank them for pointing this out. The caption for Table 3 (old Table 5) no indicates the linear values.

7. For the discussion of oxygen isotopic composition and 18e:15e values it would be helpful to have 18e values and the ratios presented in table form. Given that this a main conclusion of the paper it is odd not to see the values. Similarly the trend lines on Figure 2 should be labeled with their respective slopes.

Reply: I believe that 18e values were already in the table (old Table 5, new Table 3). (Note that tables have been renumbered). Also slopes are now given in Figure 2.

Technical Corrections 1. In the caption for Table 5, add text to explain the calculated fractionation factors using eqns 3 and 4. To differentiate from 15E calculated from the quadratic fit.

Reply: This table presents the fractionation using the linear model. If the quadratic approach is used then the slope can be calculated using the first differential at a specific NO3 concentration.

2. Line 5, pg 6922 “a more refine of equation”

Reply: This has been fixed.

3.Line 6-8, pg 6922, awkward repetition of “As in the case”

Reply: This has been fixed.

4.Figure 2. It appears that there was more analytical error in measurements of Ulva compared to Agardhiella- is that right or are the error bars just absent from Agardhiella?

Reply: Error bars have been removed for clarity. We have added this to the figure caption.

5. Figure 3. The black open box in the legend seems not to match the open grey box in the figure. Also it it would be helpful to label the trend lines with 15E values. Reply: Symbols changed and lines labeled.

6. For figure captions 4, 5, 6 it would be easier to grasp quickly if the caption include the experiment, species, etc. (currently Fig 6 says data in Fig 5, and Fig 5 says data in Fig 4 and so on.)

Reply: This has been changed.

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