

## ***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 #1**

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### General Comments

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 presenta-

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tion 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.

### Specific Comments

1. In the results section nitrogen isotopes of algae and  $\text{NO}_3^-$  are described separately for the two types of experiments. I would encourage combining these sections, so that the difference between the  $\text{NO}_3^-$  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  $\delta^{15}\text{N}-\text{NO}_3^-$  values to the caption of Table 1.
2. In the discussion section of the *Ulva* experiments it is stated that the  $15\epsilon$  values decrease toward zero with decreasing concentration. It seems more appropriate to say they decrease to a minimum of -3.2.
3. Given the emphasis on concentration dependence- how do you interpret the result that the  $15\epsilon$  in the syringe experiment at  $\sim 3 \mu\text{M}$  concentration and the free drift experiment at  $500 \mu\text{M}$  are so similar for *Ulva* (2.1 ‰ vs 2.0 ‰ respectively).
4. In the discussion section for *Ulva* it states that “although the quadratic equation predicts values less than zero at concentrations less than  $1 \mu\text{M}$ , 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  $15\epsilon$  values measured at concentrations of 60 and  $14 \mu\text{M}$ ? Perhaps this effect begins a higher concentrations.
5. In the discussion section it also states that the syringe experiment yielded a  $15\epsilon$  value 1 ‰ higher than the “values estimated from  $\text{NO}_3^-$  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 ‰.

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in Figure 4.

6. In general I think the discussion of negative values for  $15\epsilon$  could be more clear. It seems that the free drift experiment for *Ulva* yielded negative values at lower concentration and this 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  $15\epsilon$  (quadratic) from  $15\epsilon$ (linear). The caption to Table 5 could specify  $15\epsilon$ (linear).

7. For the discussion of oxygen isotopic composition and  $18\epsilon : 15\epsilon$  values it would be helpful to have  $18\epsilon$  values and the ratios presented in table form. Given that this is 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.

#### 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  $15\epsilon$  calculated from the quadratic fit.

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

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

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*?

5. Figure 3. The black open box in the legend seems not to match the open grey box in the figure. Also it would be helpful to label the trend lines with  $15\epsilon$  values.

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.)

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