

## ***Interactive comment on “Negligible isotopic fractionation of nitrogen within temperate *Zostera* spp. meadows” by Douglas G. Russell et al.***

**Anonymous Referee #1**

Received and published: 29 May 2018

The manuscript by Russell and colleagues reports on a set of field surveys designed to examine patterns in  $\delta^{15}\text{N}$  of seagrass, and organic matter and porewater in the sediments underneath seagrass. This is a useful line of inquiry for several reasons, including that seagrasses often suffer from the effects of excess nitrogen, and understanding the mechanisms that generate patterns in  $\delta^{15}\text{N}$  of plants will ultimately help us understand broad biogeochemical and ecological patterns. Below I list a set of issues that probably should be resolved before publication, but I suggest that overall the study is a useful contribution.

- It would be good to see some consideration of seagrass physiology and physiognomy in the Introduction. This will help understand why results mostly focused on roots, and also set the context for some of the interpretations and inferences that are consid-

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ered in the Discussion. This could include what we know about nitrogen species that seagrasses use, and how they get them.

- Why was the design of the temporal aspect different between the two bays?

- Think more deeply about your hypotheses, and whether the statistical methods used are appropriate. Take the ANOVA: the method is largely inappropriate here, because a two-way fixed factor analysis was used, meaning that the results cannot be broadened beyond the sites and dates surveyed, and the  $p$  is largely uninteresting (being overly influenced by sample size). If an ANOVA model is appropriate, I suggest it would be better to use a random-effects model (so that the sites and dates surveyed are considered only a selection of the possible sites and dates that could have been surveyed), and use variance components to examine the importance of spatial and temporal variation. Don't forget the interaction term, which is largely ignored here. Also, give the  $MS$  in your tables, or the reader doesn't have the information needed to fully examine the results if they wish.

- Also, think about the regression and the paired  $t$ -tests. I think the regressions are good, but I also think you can get deeper insights by looking at slope and intercept values, not just  $r^2$ . From the figures, it seems that confidence intervals around the slope do not overlap 1 – so there isn't a 1:1 relationship, which is very interesting (and probably invalidates the use of paired  $t$ -tests).

- Also, log-transformation on data  $<1$  will yield negative results, which probably isn't what you want, did you check that the transformed data make sense?

- Describe what you mean by “sediment solid phase”.

- In the methods, more information is needed about the sample collections and preservation, and the seagrass analysis – give the information needed to allow others to repeat the methods, much like you have done for the  $\text{NH}_4$  analyses. For example, give the sample size ( $n$ ) for seagrass, describe in more detail how porewater was sepa-

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rated from the water column, how epiphytes were dealt with and how the seagrass was cleaned, what standards were used for the stable isotope analysis.

- Lastly, I would find Figure 2 more useful as a table – think about it.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-154>, 2018.