

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

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### **REVIEWER #1**

We thank the reviewer for their constructive comments:

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

In the revised manuscript the introduction will be re-written to include a more comprehensive discussion of seagrass physiology and physiognomy.

C1

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

The different design of the temporal aspect between the different bays was a reflection of the logistical difficulties encountered accessing and sampling at the different sites. The highly tidal nature of the sites selected meant that access to some of the sites proved problematic and were only able to be accessed sporadically – a more detailed description of the sampling design will be provided in the revised manuscript.

**\*\*** 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.

We will re-run our ANOVA ensuring that it takes into account random-effects, the discussion of the model output will also expand upon the results of the interaction term. Currently the outputs of the ANOVA model are presented in the supporting information to the manuscript and these outputs will be updated accordingly.

**\*\*** 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).

We will provide a greater discussion of the slope and intercept values of the figures

C2

presented in the revised version of the manuscript.

\*\* 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?

Whilst this is true, none of the original  $\delta^{15}\text{N}$  signals were <1, hence log-transforming the data didn't yield negative results.

\*\* Describe what you mean by "sediment solid phase".

We were referring to the sediment upon being dried, we agree that this terminology may be slightly confusing and as a result will substitute "sediment solid phase" with "sediment" in the revised manuscript

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

The methods section in the revised manuscript will include a more detailed description of the collection, preservation and analysis techniques used relating to the treatment of the seagrass samples.

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

We will reformat Figure 2 as a table

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