

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

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REVIEWER #2

We thank the reviewer for their constructive comments:

** There are several key publications in the area that the authors did not mention in the introduction. Therefore, it is not very convincing and is not giving an overall view to the readers. For example, Papadimitriou et al. (2006) have already reported $\delta^{15}\text{N}$ in *Zostera noltii* meadows and $\delta^{15}\text{N}$ in porewater ammonium with a conclusion that reflected each other. So the “no studies” at line 9, page 1 does not appear justified. Also, the “previous studiES” at line 13, page 1 showing a fractionation of 2‰ of N fractionation during OM mineralization could not be only related to the SINGLE study on

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sapropels. The authors are invited to consult Lehmann et al. (2002); Rooze and Meile (2016) where a full description of the N fractionation process during OM mineralization was provided in either marine/lacustrine environments. Therefore, the “uncertainty” mentioned at line 14 is also not justified. These two examples justified the main problems of this manuscript which are the lack of literature documentation leading to the excessive confirmation of confidence (i.e., “no studies” at line 9 page 1, line 17 page 2). The authors are therefore invited to revise the introduction and provide further details on how and why N isotopes are fractionated by geochemical but also biological processes. This lack of a good literature review is also imputable to the quality of the discussion which is not novel and convincing.

In the revised manuscript both the introduction and discussion will be rewritten to provide the reader with a more comprehensive literature review, with specific references to the manuscript that the reviewer has suggested. We agree that we missed out on one reference that has described nitrogen fractionation processes in seagrass meadows (Papadimitriou et al. 2006). However, as none of the other papers mentioned here deal with nitrogen fractionation specifically in seagrass meadows means that a far greater body of work is required here to understand these fractionation processes. We feel that whilst this study isn't the first to tackle this problem, the lack of overwhelming evidence means that this study is nonetheless an important contribution to the ‘limited’ body of work that currently exists, and therefore our use of “uncertainty” in this context is justified.

In hindsight we should have reworded the section dealing with the fractionation effect due to mineralisation, however the papers cited here have values between 2.5-4‰ (for Lehmann et al. 2002) and generally 2-3‰ (for Rooze and Meile 2016). Therefore, in the revised manuscript we will revise this statement about mineralisation fractionation to ~3‰ and add in the references previously mentioned.

** Section 2.2, 2.3 and elsewhere, the authors are invited to mention the number of samples/replicate collected and number of observation each time a statistical test has

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been done.

Revised manuscript will have the numbers of samples/replicates and observations each time a statistical test were been done.

** Which reference materials were used in sections 2.3 to 2.5?

Revised manuscript will list all reference materials used

** Page 6 line 11: Again, the authors are invited to revise the “no studies” as it is not quite true.

In the revised version of this manuscript, this statement will be revised

** The section 4.1 is very hard to follow. The aim of this section is, so far as I understand, is to attribute a reason for the 1.6 ‰ shift in average between seagrass root and porewater. With the approach used and the way the data are shown by the authors, the difficulty is obvious to find a single reason explaining this shift. In fact, the literature shows clearly the vertical gradient of ammonia in porewater, and the bio-irrigation amplifies the heterogeneity of diagenetic reactions. Therefore, a single sample of a broad sediment depth (20 cm) could not be explanatory for the change in N and N isotope for each seagrass root. The correlation in Fig 4 may be enhanced if the authors correct porewater ammonia concentration by the sediment porosity which may give a better idea of the whole N pool, accessible to the plants. However, the section 5.2.2. in Pa padimitriou et al. (2006) has very well discussed the N isotope composition in *Z. noltii* leaves and porewater ammonia. The authors are therefore recommended to shorten and clarify the current section 4.1..

We feel that by using bulk porewater samples over a broad depth give an overall indication of the processes occurring in the sediment, which was the intent of our study; not a fine scale description of fractionation processes. Furthermore, the range of seagrass $\delta^{15}\text{N}$ was rather narrow (between $\sim 3\text{-}8\text{‰}$, in comparison our study encompassed a wider range of seagrass $\delta^{15}\text{N}$ ($\sim 2\text{-}16\text{‰}$). As mentioned by the reviewer, there may be

very localised reactions taking place but the question is whether they significantly contribute to the overall system or are they in effect insignificant on the larger scale? In the revised manuscript we will better articulate our reasoning for looking at the bulk sediment and porewater pools instead of a smaller scale. We will also revise the correlations in Figure 4 as per the reviewer's suggestions to investigate whether stronger correlations are obtained.

** Similarly, the section 4.2. shows that key publications in the area are missed. Contradictory to what mentioned, there are several work and models on C, N, and their isotopes during mineralization, e.g., (Lehmann et al., 2002; Bouillon et al., 2012; Rooze and Meile, 2016). -Finally, what is interesting in this study is the correlation between seagrass root, sediment N, and porewater ammonia. Correlations in Fig 3a and 3b show a very similar slope (0.786 vs. 0.773), that means plotting seagrass roots vs. sediment may give a slope of 1. That may lead to a more straightforward conclusion that seagrass roots take the same N isotope signature than sediments rather than trying to explain roots vs. porewater and sediment vs. porewater.

We agree that we could be clearer about how our study is different to the publications that were listed by the reviewer; in essence, very few studies have used an experimental approach to look at the differences in $\delta^{15}\text{N}$ between the sediment and porewater NH_4^+ pools. Section 4.2 will be revised to include a more thorough discussion of the isotopic fractionation effects of mineralisation, with specific references made to the manuscripts that you mentioned. In the revised manuscript, we will investigate the relationship suggested by the reviewer and include a thorough discussion of these results.

** Technical comments: -Page 5, line 32: The subtraction sign is not a good idea to use in the text as it is confusing with a simple hyphen or a minus, one alternative is using a big delta and having "porewater-seagrass" in subscript.

Revised manuscript will be changed to reflect this suggestion

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