

Interactive comment on “The importance of nitrogen fixation to a temperate, intertidal embayment determined using a stable isotope mass balance approach” by Douglas G. Russell et al.

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*We thank the reviewer for their comments. In reading these comments, we realise we have not articulated the model or the key assumptions behind it clearly enough. In the revised version of the paper we will stress that the model is essentially an isotope mass balance model used routinely in ecological studies. The relative contribution to each end-member is estimated by comparing mass weighted isotope sources (the rivers, nitrogen fixation, the ocean and the atmosphere). The key assumptions are as follows:

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1. All sources of nitrogen are assimilated into the solid pool which is well mixed and then some loss through denitrification. This is justified by the fact that it is a macrotidal system and that it has a relatively uniform marine salinity. 2. Algae/vegetation assimilates all dissolved nitrogen hence there is no fractionation associated with this process, and eventually all the nitrogen is returned to the sedimentary nitrogen pool. 3. We note that steady state conditions are not required for this model. A mixing model such as ours does not require steady state, but does assume the nitrogen pool is well mixed in this system, and therefore the isotopic values will not be affected if there is export of material from the bay. Our measurements of the outgoing N flux support this. For more information on mixing models such as this, please see Fry (2006).

This manuscript estimated the N supply by nitrogen fixation under an assumption of balanced nitrogen influx (source) and outflux (sink) in the intertidal embayment, and addressed its validity by comparing $\delta^{15}\text{N}$ of sediments between the model derived and measured values. It's interesting approach, but I have serious concerns about the steady state in the study area, and the fluxes and isotopes used in the model (see the following comments). While I appreciate the effort of the work presented, I cannot recommend this manuscript for publication unless these concerns are solved clearly. Major comments:

1. I doubt the steady state within the small and complicated bay that authors assumed. This requires no change of nitrogen budget (pool size) in the system. Authors should discuss about major nitrogen pools, their sizes and temporal change of them.

*The key assumption in this study is that the nitrogen pool in the bay is well mixed. Consequently, we do not need to assume that steady state conditions exist within the bay, this is because once the isotopes are well mixed, and this will not affect isotope values in the case of export of material from the bay. The revised manuscript will clearly articulate this point and provide evidence in the support of this assumption. This includes: 1. The observation that the $\delta^{15}\text{N}$ of sediment within the bay was relatively uniform. 2. The macrotidal and uniformly marine nature of the bay.

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2. Authors also assumed the pseudo closed system in which all nitrogen supplied is consumed by the sink processes (sediment burial, denitrification, algal assimilation) within the study area. This implied that any riverine nitrate is not transported out of the bay. Although authors pointed extremely low concentration of dissolved inorganic nitrogen in the bay, Russell et al. (2016) reported the range of 0.2-5 μM of nitrate. The spatial distribution of nitrate should be presented along the rivers-the inside bay-out of the bay, then mixing of freshwater and seawater to be discussed.

*Samples collected at the ingoing and outgoing tide suggest no significant DIN import or export from the bay. Therefore, we do not believe that discussion of nitrate gradients from rivers to bay to out-of-bay are relevant. The revised manuscript will illustrate this point more clearly.

Authors also suggested no flux between inside and out of the bay for the particulate nitrogen because of similar concentration and its isotope between them. However, many scientists (e.g. Sukigara & Saino, 2006, *Geophysical Research Letters*, 33, L09607) have stressed a significant transport of resuspended sediments as nitrogen flux from the bay to open ocean. This possibility should be examined in this study. If any riverine materials including nitrogen is not transported, dissolved inorganic nitrogen out of the bay should originate from open ocean. Is it true?

*Whilst the above scenario is possible, our measurements of particulate N as described by the reviewer suggest that this is not the case for our system. Instead, we suggest that exported particulate nitrogen originates from sources such as remineralised and nitrified ammonium from the buried/sediment pools. As a consequence, it will have been well mixed into the bay isotope pool, and therefore even if there is net import/export of material this will not affect the outcome of our model. Nonetheless, the reviewers point is a valid one, and we will include discussion of resuspension, and why it does not need to factor in our model, in the revised manuscript.

3. Authors assumed that almost riverine nitrate are assimilated by phytoplankton and

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seagrass. Ultimately, I think, these organic nitrogen is decomposed into inorganic nitrogen (ammonium and nitrate). A part of them can be buried into the bottom. The nitrate regenerated from algal organic nitrogen can be consumed by denitrification. These processes links each other complicatedly. It's impossible to estimate their independent fluxes, especially in annual scale.

*We assumed that the riverine nitrogen was either assimilated, then mixed into the sediment pool, or that refractory particulate nitrogen was mixed into the sediment pool, contributing to the isotope signature in that manner. There are many cycling processes – including decomposition to organic N as the reviewer suggests, but so long as the assimilated N forms a well-mixed pool, these processes do not fractionate much and hence do not affect the d15N of the isotope pool.

4. Authors seem to confound the 15N of removed nitrogen with the isotopic fractionation associated with the removal (sink) processes. The isotopic fractionation (ε) is expressed as 15N difference between substrate and product of the process. Therefore, equation (3) was inadequate.

*We thank the reviewer for pointing this out, equation 3 will be re-written to accurately reflect the removal of nitrogen due to denitrification. We also note that there is an error in Figure S3 regarding the effect of the denitrification fractionation factor on the d15N of the sediment pool (the axes are reversed). This mistake will also be rectified in the revised manuscript.

Furthermore, I have some concerns about 15N of removed nitrogen used in the model. As for denitrification, 15N of 3.5‰ is used by referring Brandes & Devol (2002) in which they assumed 15N of typical oceanic nitrate with 5‰ and ε with 1.5‰. Meanwhile, authors suggested that riverine nitrate 15N was 12.6‰ (P7_L24), which looks to conflict with nitrate 15N of 5‰. Authors should explain the origin of nitrate in the bay. As for algal assimilation, it's okay with ε of 4‰. The 15N of assimilated nitrogen, however, to be calculated from nitrate 15N minus ε . If assuming riverine nitrate with 12.6‰ as a major

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substrate, it corresponds with 8.6 ‰ (= 12.6 - 4.0). This would lower the sediment 15N estimated from your model.

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*We thank the reviewer for raising this point, however on reflection, the effect of algal assimilation is likely to be insignificant. This is because there is little evidence of riverine-derived nitrogen being exported from the bay (see above), which suggests that it is either being directly bound up in this sedimentary pool, or there is algal assimilation. If algal assimilation is occurring, this will have limited effects as ultimately this nitrogen will re-enter the sediment pool as algae die and are buried. A similar argument also exists for vegetative assimilation. In our revised manuscript, this point along with all relevant assumptions will be more clearly articulated and the proposed isotope balance modified accordingly.

5. I'm afraid I can't understand the model calculation in this study. The sediment 15N derived from this model were shown in Fig. 3 and S1, which are the output of 10,000 iterations (P10_L16). I suspect that these outputs are same with the result of sensitivity analysis, illustrated in Fig. S2 and S3. If so, I cannot find any significance of the average and the standard deviation of this result because, I think, they do not support the validity of model output.

*We agree with the reviewer the overarching rationale behind what was attempted here has not be sufficiently explained, and as a consequence the approach used and its meaning are confusing. At its core, this study was using an isotope mixing model to estimate the nitrogen isotope value of the sedimentary nitrogen pool based upon prior measurements of nitrogen processing rates in the bay. A comparison between these estimated isotope values and values that were taken independently will be undertaken to assess how good the previous rate measurements were (particularly nitrogen fixation). This in turn will also allow for a commentary surrounding the importance of nitrogen fixation in coastal embayments to be undertaken. The revised version of this manuscript will ensure that these points are clearly addressed.

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References Cited:

Fry, B.: Stable Isotope Ecology, Springer, New York, 2006.

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