

**Review comments on “Particulate organic matter controls benthic microbial N retention and N removal in contrasting estuaries of Baltic Sea” by Bartl et al.**

This study by Bartl et al. aims to address the role of two temperate estuaries with contrasting geomorphological, hydrological, biogeochemical features in processing riverine N and their efficiency as coastal filters for excessive land derived N in two different seasons. It is an honest attempt to emphasize the role of biological and microbial transformation processes which make these estuaries as traps for anthropogenic N. On a bird’s-eye view, I see that the authors have tried to do a comparative analysis of various biogeochemical processes in Ore estuary and Vistula estuary in relation to their capacity to process anthropogenic N. Interestingly, the authors have used the available data on water column and sediment characteristics, and sedimentary denitrification rates already reported by Helleman et al. (2017) for Öre estuary but they have additionally carried out ammonium assimilation and nitrification study in benthic boundary layer (BBL). Similarly, for Vistula estuary, the authors have used the available data on water column characteristics, and ammonium assimilation and nitrification rates in BBL already reported by Bartl et al. (2018; *Estuarine coastal shelf science*) but they have additionally carried out sediment characteristics and sedimentary denitrification study in order to address their central issue.

Broadly, I see that the manuscript has not been written properly. It has a lot of unnecessary repetition of published stuffs by Helleman et al. (2017) and Bartl et al. (2018, ECSS). There are some gaps in terms of information and interpretation at certain sections and it has missed the focus at times. The major weakness of this manuscript is the discussion part which needs to be non-repetitive (in relation to Helleman et al. 2017 & Bartl et al.2018), novel on its own right and robust. Thus, the manuscript needs a lot of modification and re-interpretation at several sections to bring it to a decent publishable form. The study has the potential to contribute to our understanding of the role of estuaries as ecosystem service providers through the inter-connected biological-microbial-geochemical transformation processes. I would recommend it for publication, provided the authors make a major revision of the manuscript based on my critical observations as follows.

1. *Line 34*: A reference is needed for the last part of the sentence.
2. *Line 85*: Patsuszak et al. (2012) is used twice. Does this same reference give two different conclusions? If not, then two references can be given i.e. Patsuszak et al. (2012a) and Patsuszak et al. (2012b).
3. *Section 2.1*: This study emphasizes N transformation processes such as nitrification and denitrification in the benthic boundary layer which are sensitive to O<sub>2</sub>. But O<sub>2</sub> regime of the estuarine water columns are not described. The authors should describe the variability of oxygen condition of two estuaries throughout year or at least from spring to summer based on previous study or this study.

*Line 121*: .....to fill 30% (silt) to 50% (sand) of each one.....What does this sentence mean? It sounds confusing. Please clarify by rephrasing the sentence.

4. *Section 2.2.1*: Was O<sub>2</sub> measured in the water column? If yes, then how? By sensor coupled with CTD or measured analytically? Please give a brief description.

The authors should mention the thickness of BBL for both the estuaries in both spring and summer.

Line 129-130: *Water samples were.....*If BBL thickness is just 20-40cm (If I understand correctly from Line 115) and sampler length is 0.5-1m. Then, how can you possibly say that the water sampler was completely inside BBL? Apparently, the sampler could also enclose the water above BBL.

5. *Line 163*: The authors mentioned that porewater was extracted at 2 cm interval from 5 cm to 11 cm depth by Rhizon tubings. But Seeburg-Elverfeldt et al. (2005) says that Rhizon tubings can extract porewater with a vertical resolution of 1 cm only. Please explain.

6. *Section 2.3.1*: It is not clear whether 100-170ml from BBL and 625 ml from water column were mixed together prior to <sup>15</sup>NH<sub>4</sub><sup>+</sup> enrichment or they were separately enriched with the substrate and incubated. If they were mixed, then what was the reason for that? BBL and the overlying water column can have different biogeochemical properties. So, if they were mixed and incubated with <sup>15</sup>NH<sub>4</sub><sup>+</sup>, it cannot represent nitrification rates of BBL only and the aim of the study is to determine nitrification rate in benthic system not in water column. Please explain. O<sub>2</sub> content of BBL and water column is not mentioned. Was O<sub>2</sub> measured in sealed gas tight bags just prior to the experiment? Moreover, why were nitrification rates in the top oxic sediments not measured in both the estuaries and both seasons? The authors have emphasized the role of coupled nitrification-denitrification in these sediments. Then it makes sense to discuss benthic nitrification here which can have much higher rates compared to BBL nitrification due to higher availability of NH<sub>4</sub><sup>+</sup> diffusing from deeper sediments and its oxidation in top layer.

7. *Section 2.3.2*: The authors have not given a diagram for diffusive experimental set-up.

8. *Line 194-200*: For ÖE I and ÖE II, 4 replicates were made for each concentration but 12 replicates were made for 120µM VE I and 3 replicates for VE II. Why? Moreover, for ÖE I, ÖE II and VE I, three concentrations i.e. 40, 80, 120 µM <sup>15</sup>NO<sub>3</sub><sup>-</sup> were used but for VE II, four concentrations i.e. 30, 60, 90, 120 µM <sup>15</sup>NO<sub>3</sub><sup>-</sup> were used. Again, for permeable sediments of VE, three concentration treatments were given with 5-7 replicates. Why were <sup>15</sup>NO<sub>3</sub><sup>-</sup> concentrations different and why were no. of concentration treatments different? What was the rationale behind such varied no. of treatments, replicates and concentrations between ÖE I, ÖE II, VE I and VE II? Why didn't the authors use same concentrations treatments and no. of replicates? For example, let's say, why couldn't they use 40, 80, 120µM <sup>15</sup>NO<sub>3</sub><sup>-</sup> treatments for all types of sediments with 4 replicates?

9. *Line 204-205*: Was the overlying water drawn only from the ports that were 5mm above oxic-anoxic interface or from all the ports lie above at 5mm resolution?

10. *Line 212-213*: What are the sampling time points? Was O<sub>2</sub>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup> measured in the overlying water at different time points?

11. Line 220-228: This paragraph needs to be rephrased. Risgaard-Petersen (2003) talks about the contribution of anammox to total  $N_2$  production from slurry incubation. But this study was based on intact core incubation. So first of all, please justify well that it can be applied to this study, given that the availability of  $^{14}NH_4^+$  can be less in case of intact core incubation compared to slurry incubation which can affect p14 and p15 values described by Risgaard-Petersen et al (2003).

Also I see that the first sentence of this paragraph i.e. from *According to.....till...1992*) is a word to word copy from a sentence from Helleman et al (2017). This is not acceptable. Please rephrase the sentence.

12. Line 230: Replace it with *significance of difference or variability*.

13. Line 250 and Line 253: Both sentences contradict each other. Please rephrase the sentences. Sentence in line 250 means in both spring and summer POM in Öre river is dominated by terrestrial fraction but sentence in line 253 says in both spring and summer, POM is largely phytoplankton derived.

14. Line 273: It doesn't look so from the rates presented.

15. Line 273-276: These two sentences look contradictory. How can nitrification be positively correlated with POC if it shows negative trend with particulate C:N in case of Öre estuary? How can nitrification be positively correlated with PON, if it shows positive trend with particulate C:N?

16. Line 276-280: Same contradiction as in the case of nitrification. How can  $NH_4^+$  assimilation be positively correlated with POC if it is negatively correlated with C:N? What is the logical explanation?

17. Section 3.2.2: The authors clearly concluded that there was no anammox and denitrification was the sole N loss process. What about DNRA? The authors didn't mention anything about it although it is only an N transformation process. I think the authors are coming to conclusion here rather abruptly without considering findings of Jensen et al. (2011) in the Arabian Sea. Coupling of DNRA-Anammox can happen which can create an impression of denitrification signal and hence the conclusion can be misleading. Thus, the authors should relook at their incubation data and reinterpret if necessary. Again, the authors have not given any figure on  $^{15}N$ -labelled intact core incubation which is very important. Please present few figures depicting increase in  $^{15}N-N_2O$  and  $^{15}N-N_2$  with time to support your conclusion on denitrification being a major N loss pathway. Similarly, if you find anammox and DNRA upon re-analysis of the incubation data, then please show the proof in terms of additional figures. Why coupled nitrification-denitrification was not correlated negatively particulate C:N in case of Vistula estuary?

18. Line 302-303: It doesn't look so. I don't see  $NO_3^-+NO_2^-$  in BBL of estuaries differing significantly if we strictly consider standard deviation (SD) given in Table S1. On the contrary, POC and PON in BBL of Vistula estuary are much higher than that in BBL of Öre estuary (Table 2). Please rephrase these sentences.

19. Line 306-309: This is true only for spring where we see high POC and PON in BBL of Öre compared to Vistula. But again on closer look, if we take SD and no. of replicates into account, POC in BBL of Öre is similar to that in Vistula and interestingly PON in BBL of Öre is higher than that in Vistula. This claim is anyway not true for summer.
20. Line 328: Delete Fig.4 from the sentence as it does not show C:N.
21. Line 330-332: Not a satisfactory explanation. Öre estuary has a sill and thus restricted exchange of estuarine water with seawater can likely cause more sedimentation within the estuary.
22. Line 333-334: C:N in Öre is higher than that in Vistula but POC:Chla in Vistula vary from 5.4 to 33.2 which is  $\ll 200$ . How can it indicate degraded POM? Only because of  $C:N < 12$ ?
23. Line 336-338: Summer time POC:Chla in Ore varies from 12.6 to 140 that is  $< 200$ . How can the POM be in degraded state?
24. Line 401 and Line 397: Please show the  $r$  and  $p$  values of the correlation.
25. Line 398: *because the less-degraded POM in.....*This is questionable as POC:Chla is not above 200 rather  $\ll 200$ .
26. Line 399: *By contrast, the more degraded POM in.....*First of all, POC:Chla in both Öre and Vistula estuary are much lower than 200. So can we call it degraded POM? Even though we assume higher POC:Chla ( $> 200$ ) as indicator for highly degraded POM, POM in Vistula estuary looks more degraded compared to that in Öre estuary. Not the other way.
27. Line 401: How significant is this correlation? What are the  $r$  and  $p$  values?
28. Line 402-404: These two sentences contradict each other. First sentence is questionable. I see a significant seasonal difference in the rates in both the estuaries. Please clarify the role of trophic state on these two processes.
29. Line 407-409: *Difference in denitrification.....*How do you know that? Where is sedimentary  $C_{org}$  data and  $\delta^{13}C-C_{org}$  for both the estuaries?
30. Line 415-419: *While newly produced.....*If higher POM availability increased denitrification rates in sediments, then why not in water? Especially in BBL?
31. Line 421-423: what are the  $r$  and  $p$  values of the correlation?
32. Line: 425-427: Not written properly. OPD itself can get  $NO_3^-$  from BBL. Nitrification is significant in BBL and  $NO_3^-$  is not that low.
33. Line 427: *Hence, only a small.....*How did the authors calculate that? Is there any nitrification rate measurement in the top oxic layer of the estuarine sediments?

34. Line 429: This sentence contradicts the previous sentence. If the dominant  $\text{NO}_3^-$  source is controversial, then how can you say that <10% of  $\text{NO}_3^-$  from BBL was removed by denitrification in permeable sediments of Vistula estuary?
35. Line 449: Please write it as *During summer.....*
36. Line 450-452: How is that possible? What about denitrification rate during spring?
37. Line 457-458: *Despite their.....*What about spring?
38. Line 461-463: These two statements are contradictory. How would the authors reconcile these statements vis-a-vis their observation?
39. Line 465-468: What about DNRA? That would show that how much riverine N is preserved in estuarine sediments through DNRA. It is necessary to discuss that here.
40. 471-473: *Through close.....*It is not necessary that only POM controlled benthic nitrification. What about benthic  $\text{NH}_4^+$  efflux?
41. Line 482: What are the DNRA rates?
42. Line 490-492: *We thus hypothesize.....* How do the authors say it is a coast parallel transport? Is there any reference? The riverine flow may be perpendicular to the coast into the Baltic.
43. *References:* Holtermann et al. (2014), Risgaard-Petersen et al. (2004) and Schultz (2000) are not cited in the text. Schultz (2005) is missing in the reference list.
44. *Table 2:* The authors need to show C:N in a column here.
45. *Table 3:* How have  $\text{NH}_4^+$  surface pool and  $\text{NH}_4^+$  deep pool been defined? Up to what depth you consider it as surface pool? Please mention clearly in the table caption.
46. *Table 4:* I don't see any denitrification rate in permeable sediments of Öre estuary. Was it not measured or it is not detectable? “-” symbol doesn't mean anything. Please clarify.
47. *Figure 2 & 3:* The PON plots for Öre estuary are reproduced from Helleman et al (2017). So please mention the reference clearly in the figure captions.
48. *Figure 4:* This figure contradicts the data in Table 2 and Table S1. If we calculate POC:Chla from Table 2 and S1, they range from 5.4 to 140. How come Fig.4 shows such higher POC:Chla values then?
49. *Figure 5:* Shows vertical  $\text{O}_2$  profile of Vistula estuary sediments. But what about that of Öre estuary sediments? The authors should show that also.
50. *Figure S1:* The authors should point out the ports through which water sample was collected. Please point out the water above the sediments.

51. *Table S1*: Looks a bit confusing and unexplained. River plume very much prevails within these two estuaries and occupies a depth range of up to 3m in case of Öre estuary and up to 12m in case of Vistula estuary. So when we say river plume here that actually means surface water of estuary. So, why can't the authors consider the depth from the river plume till bottom? If they do so, then I believe the so-called surface here would actually be a depth of 3m in case of Öre and 12m in case of Vistula. The authors should clear the confusion and mention terms in a logically correct way. Additionally, I believe a column for POC:Chla is necessary in this table.

*Overall comments & suggestions:*

I suggest the authors to be careful about not repeating the description of sampling methods, analysis/experiment methods and results which are already reported by Helleman et al. (2017) and Bartl et al. (2018) for these two estuaries. For example: Do not describe the water column sampling methods, sediment sampling methods, analysis methodology, denitrification experiment method and their results in details for Öre estuary because these are already published by Helleman et al. (2017). But you can retain everything about  $\text{NH}_4^+$  assimilation and nitrification in Öre estuary. Similarly, for Vistula estuary, avoid detailed description of column sampling methods, analysis methods and ammonium assimilation and nitrification experiment methods in BBL and their results because these are already published by Bartl et al. (2018). But you can retain everything about sediment sampling and analysis methods, denitrification experiment methods and their results. However, the authors can use the published data and their own generated data for the discussion since it's a comparative account study.

The authors have not measured DNRA rates and have not discussed its role in transforming riverine N to  $\text{NH}_4^+$  in the estuarine sediments. They have not also measured sedimentary nitrification rates which is very important. I did not see any discussion on benthic N ( $\text{NO}_3^-$  uptake or  $\text{NH}_4^+$  release) exchange. All these could have made the discussion on benthic N cycling robust. However, the authors should use the published data (if any) on benthic nitrification, benthic DNRA and benthic N exchange and thoroughly discuss the interplay of all N cycling processes in relation to net N loss/ immobilization in these sediments in the discussion section in general and section 4.2.4 and section 4.3 in particular. I suggest the authors to relook into the classic integrated discussion on benthic N cycling in the Gulf of Bothnia by Bonaglia et al. (2017). In order to show the efficiency of these two estuaries as coastal filters, the authors should mention how much % of riverine N is ultimately lost in estuarine sediments through denitrification and/or anammox (if any), how much % is immobilized in sediments through DNRA and how much % is transported out of estuary to the coastal sea. Overall, I would suggest the authors to revise the manuscript by showing novelty of their study objectives, approach and findings which would make it appear as different from studies by Helleman et al. (2017) and Bartl et al. (2018).