

# ***Interactive comment on “Enhanced microbial nitrogen transformations in association with intertidal macrobiota” by Catherine A. Pfister and Mark A. Altabet***

## **Anonymous Referee #2**

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The manuscript addresses roles of microbes associated with mussels, *Mytilus californianus*, and macro algae, *Prionitis sternbergii*, in nitrogen processing in coastal environment by experimental approach using enclosed chambers. The approach used in this study and presented results are not novel. Furthermore it is difficult to apply the knowledge obtained from a particular experimental condition to other environments because of lack of description of environmental and experimental condition. Although this topic fits well to the scope of the journal BGD, authors need to improve the discussion experimental condition and limitation of application to other environments. Further, the data presentation is inappropriate (see General comments). Thus the manuscript need major revision before publishing Biogeoscinece.

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General comments: A) Was biomass of animals or macroalgae uniform in each chamber? I guess that they were not uniform because authors presented rates in per gram in the different paragraph. Units of Y-axis in Figs 1, 2 and 4 are not in per gram. Does the mean values of rates or differences in concentration obtained from chamber containing different biomass make sense? Whether does the variations in the figures depend on the difference in metabolic rate per unit biomass among individuals or in total biomass? Further, it is hard to understand aim of box in Fig 1 combining data obtained from different experimental conditions. How readers compare the rates and the differences obtained bioballs with those of macrobiota? In biomass of microbes dwelling on the surface or surface area? Similarly, the rates and the differences obtained mussel shell should be compared with those of living mussel after normalization with the surface area or with dry mass of shell. Because the data treatment could influence following statistical analysis comparing mean values, authors clarify/improve the data presentation. I cannot decide whether conclusion is based on appropriate analysis or not in the present style.

B) Addition of glucose might be one of the extreme case of DOC enrichment. C/N ratio of bacterial biomass and organic substrate affect uptake/release of DIN by heterotrophic bacteria (Kircham 2012 Processes in microbial ecology. Oxford University Press). Why authors choose glucose, which does not contain nitrogen? Is glucose major component of macroalgal exudate? I feel mismatch DOC between the term DOC used thorough the manuscript and glucose although glucose is DOC. Authors should clarify the aim of experimental addition DOC in the last part of introduction or discuss the difference between algal exudate reported in literature and glucose.

Specific comments: 1) Abstract Lines 26-27: "When we experimentally added DOC (glucose) as a carbon source, there was no change to nitrification rates." As described in general comments, please discuss rates in per gram of biomass.

2) Introduction: I feel that this section could be shortened.

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- 3) Lines 38-42: This sentence is too long. Please separate the sentence.
- 4) Lines 83-85: Add reference.
- 5) Lines 89-90: Add reference.
- 6) Lines 130-132: Because incubation period did not cover day time, time should be described. Since photosynthesis affects nitrogen cycling in the chamber as authors state, light condition should be described as if authors compare the results of experiments conducted at the same time. And depth level at which the chambers were put should be added.
- 7) Lines 134-135: Biomass of macrobiota and mass/area of mussel sell should be added.
- 8) Lines 135-139: Add total amount or total surface area put into the chamber.
- 9) Lines 151-152: Did incubation conducted at in situ temperature?
- 10) Lines 159-161: Is there reference showing that nitrogen metabolism of microbes are saturated in these concentration?
- 11) Lines 176-178: What is final concentration of  $\text{NH}_4^+$  in the chamber after addition of  $15\text{NH}_4\text{Cl}$ ?
- 12) Lines 217-219: The flux in inorganic nitrogen from where to where? Please clarify them.
- 13) In Materials and Methods section: Statistical analysis should be explained.
- 14) In result section: all of rates and difference should be presented in per gram and with SD or SE. In my opinion the results should be presented with its variation. Thus SD is appropriate rather than SE.
- 15) Line 269: Which graph should be referred to compare with data at night? In the next section? Please rearrange the paragraphs.

16) Section 3.4: Description of biomass of microbes and DIN concentration should be presented. The analysis is poor in the present style.

17) Lines 294-295: the DOC concentration? 9.3 mmol C L<sup>-1</sup> per hour?

18) Lines 306-307: Assimilation rather than respiration with glucose enrichment.

19) Lines 321-323: Could amounts of substrates (ammonium and nitrate) support these potential consumption rate? The determined rates were obtained in enriched experimental condition in the chamber.

20) Line 393: the C:N of what?

21) Figures 1, 2 and 4: Values should be presented in per gram or area.

22) Figure 1: Each group of macrobiota should be presented separately.

END OF REVIEW

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