To the Editor: 28 Jul 2018

We were grateful to receive such thorough reviews of our ms bg-2018-198, "*Enhanced microbial nitrogen transformations in association with macrobiota from the rocky intertidal*." We have responded to all comments below using italicized font for our reply.

Sincerely,
Catherine Pfister & Mark Altabet

The response below is to Rev 2 only.
Received and published: 14 July 2018

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.

We welcome the opportunity to revise the manuscript with these comments in mind, including the description of the local environment. Indeed, we thought a strength of the work was the fact that many experiments were done in situ in tidepools, while others were done immediately adjacent to the site where the organisms were collected; all used seawater collected immediately on site.

C1
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.
Biomass was not uniform among individual animals and algae and there was thus natural variability in mass and we do present rates per gram. We put in more information on the mass in the Methods.

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.

We agree with the reviewer that it is difficult to know whether mass, surface area, or the area of a particular surface is the best determinant of microbial activity. Area estimates can be particularly hard to quantify. Instead, we used a metric of the size of a species that we could scale-up based on data from the shore. Thus, biomass allowed us to scale up these rates to Paine's algal densities and Wootton's mussel densities. Because we were always comparing our addition of a seaweed, animal or inert substrate to seawater, it was appropriate to compare the rates in a volume of seawater. Indeed, it was our goal to investigate how species influence the processes in the water column. Thus, we show the rates in the figure as a volume measurement, but use the per mass estimates to scale up.
While Fig 1 showed all macrospecies together to illustrate the point that species have an effect, we appreciate that both reviewers want them separated and we have now done so in a revised Figure 1. As for comparing with bioballs, we agree that this is difficult comparison. However, bioballs are manufactured as microbial habitat for nitrogen transformations in commercial aquaria and yet their microbial activity is several orders of magnitude less than an individual alga.

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 (Kirchamn 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.

As mentioned to Reviewer 1, we added rationale to the Methods, Section 2.2. Glucose is common to many species and used in other studies (e.g. Zhang et al. 2013), so it allowed comparison. We recognize, however, that there are many other potentially important and relevant compounds (laminarin, mannitol, and more) and we hope to incorporate this in future research.

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.

We think it is important here to keep the units as per volume in our graphical presentation in order to compare to seawater. Seawater is well-studied with respect to microbial nitrogen processing and our point here is that macrospecies can be loci for these transformations and they will affect the surrounding seawater. We do use rates in terms of biomass when we scale up to rocky shores (Discussion). Also see below.

2) Introduction: I feel that this section could be shortened.
Specific editorial suggestions from Rev 1 led us to shorten this (see Rev 1 response too).

3) Lines 38-42: This sentence is too long. Please separate the sentence.
Done.
4) Lines 83-85: Add reference.
Done.

5) Lines 89-90: Add reference.
Done.

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.
Chambers always had ~15 cm of water depth in coolers and tidepools. We did not, however, continuously monitor light levels. Daytime incubations always started by 6 am and ended by 6 pm, while nighttime incubations were under full darkness.

7) Lines 134-135: Biomass of macrobiota and mass/area of mussel sell should be added.
Biomass data is now more complete.

8) Lines 135-139: Add total amount or total surface area put into the chamber.
Inserted. Each ceramic ring has a surface area of approximately 6 cm2. We estimated a surface area for the bioballs between 15-20 cm2.

9) Lines 151-152: Did incubation conducted at in situ temperature?
Yes. More detailed added on temperature Line 168.

10) Lines 159-161: Is there reference showing that nitrogen metabolism of microbes are saturated in these concentration?
No. The nitrogen concentrations that determine microbial activity is unknown in these systems and we note that nitrate concentrations are high (at least 20 uM) due to upwelling and ammonium can reach 5 uM and more due to animal activity.

11) Lines 176-178: What is final concentration of NH4+ in the chamber after addition of 15NH4Cl?
The concentration of NH4+ that was added as 15NH4Cl was trivial (<.01uM) compared to the amount already there (~1-2uM or more). Thus, the addition of 15NH4Cl did not markedly change concentration.

12) Lines 217-219: The flux in inorganic nitrogen from where to where? Please clarify them.
Oxidation and reduction. Clarified.
13) In Materials and Methods section: Statistical analysis should be explained.

More detail now put in at the end of Section 2.3. In general, the statistical analyses were ANOVA or paired t-tests and are stated in each part of the Methods.

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.

Through our presentation of the data, we have shown the variation in all data and in all Figures using boxplots. We now provide a key to the boxplots in Figure 1 caption to make this clear. Specifically, we state that the box shows 50% of the data, the horizontal line is the median, and the vertical lines represent the first and fourth quartiles. Where vertical lines are absent, they are contained within the boxes. Outliers are shown as individual points and are 1.5 times the value beyond the top or bottom of the box. Note that the only place we use SE is in our estimates of per gram rates nitrogen metabolism for mussels and Prionitis in Section 3.2. We report the SE here because we then use these mean estimates to scale up and a measure of the dispersion around the mean was appropriate. Again, we report the measurements here in per g to scale up to previous censuses of mussels (Wootton) and algae(Paine) in this region. Otherwise, we report rates in per L to compare with seawater. Thus, rates in both metrics are available to the reader.

15) Line 269: Which graph should be referred to compare with data at night? In the next section? Please rearrange the paragraphs.

Agreed that this paragraph is out of place. We moved it to be the second paragraph in Section 3.3 and made clear that we are referencing Figure 2.

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

This paragraph has more detail to make clear the ammonium oxidation, and changes in DIN and Silica did not differ. We have no data on the biomass of microbes.

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

Clarified, per L.

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

Or perhaps both? Reworded.

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.

It seems that it could. We did not increase ammonium or nitrate concentrations in the chambers; we used ambient levels. Further, the chamber has no flow or new nutrient supply as you would find in a natural coastal setting. Thus, it seems unlikely that we are overestimating the process.

20) Line 393: the C:N of what?
Clarified. Strauss and Lamberti termed this 'environmental' C:N, which presumes whatever substrate the microbial assemblages are interacting with.

21) Figures 1, 2 and 4: Values should be presented in per gram or area.
As mentioned above, we think it is important and logical to present the data as per Liter in the Figures that compare with seawater, but continue to use the per gram estimates for scaling up to the rocky shore.

22) Figure 1: Each group of macrobiota should be presented separately.
Done. Figure 1 is now revised to show each species.

END OF REVIEW