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Interactive comment on “Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments” by A. K. Hardison et al.

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Received and published: 3 May 2013

Response to Anonymous Referee #1 by A.K. Hardison et al. Interactive comment on “Microphytobenthos and benthic macroalgae determine sediment organic matter composition in shallow photic sediments”

We thank the anonymous referee for his/her positive and thoughtful comments on the manuscript. Below we address the reviewer’s general and specific comments:

“There is a lot of data presented in the manuscript. Several times, the authors mention that significant differences were not found between treatments due to high variability among the 4 replicates for each treatment. Was there any consideration for analyzing

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the data using statistical approaches that can account for a low 'n'? Bootstrapping? Or a Bayesian approach?"

Response: We appreciate the suggestions from Reviewer 1 for alternate statistical approaches to analyze our data, which has a relatively low sample size ($n = 4$). Indeed, there were numerous instances when within-treatment variability was higher than between-treatment variability, resulting in a lack of statistical difference between treatments. However, we were encouraged that many treatments did in fact show statistically significant results, given an $n = 4$. We agree with Reviewer 1 that the bootstrapping technique would be appropriate for this dataset; however, we think it is unrealistic to incorporate this approach at this stage. Instead, we focused our discussion and analysis on those parameters that demonstrated the strongest treatment differences given the measured variability. We did use repeated measures analysis of variance (RMANOVA) to account for variability inherent to each mesocosm. Because we repeatedly sampled each mesocosm over time, we could not assume that the random error associated with each sampling was independent. Thus, RMANOVA allowed us to model the covariance structure of the random errors within the data. Given the clear distinction between treatments for many of the parameters measured, we do not feel that additional statistical analysis is necessary. Further, the RMANOVA was used in analysis of other data published for this study (Hardison et al., 2011b), and this approach provides consistency with the previously published studies.

"The expectation is that the Macro treatment was to negatively affect the microphytobenthos because of shading and/or competition for nutrients. Did the authors consider the potentially labile DOM leached or produced by the Macros added to the treatments? Does increased DOM from macroalgal blooms (likely labile DOM) feedback to SOM dynamics similar to what one would expect with the presence microphytobenthos? Is this plausible given that the Macro and Light treatments for sediment C, N, and some of the PLFA results are more similar to one another than the Dark treatment?"

Response: Reviewer 1 brings up a very good point. Numerous previous studies, in-

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cluding some conducted in Hog Island Bay, VA, have shown that macroalgae release dissolved organic matter (DOM) while growing and during senescence (e.g., Tyler et al., 2001; Tyler et al., 2003; Maher and Eyre, 2010). The DOM, in turn, impacts water column carbon and nitrogen cycling. Therefore, we would expect an increase in macroalgal biomass and associated DOM release in our Macro treatment to directly impact the water column bacterial community. It is possible that this water column DOM could also diffuse into the sediments and be available to sediment bacteria. However, we believe the DOM released by microphytobenthos *directly* to the sediment porewater would be more accessible to the sediment bacterial community than macroalgal DOM released to the overlying water. Although the labile DOM produced by macroalgae might be available to the sediment bacteria, we do not expect that it would be a direct substitute for the DOM produced by microphytobenthos in the absence of macroalgae. This is supported by our data, which suggest that buildup of bacterial biomass in the Macro treatment was limited compared with the Light treatment (e.g. BrFA, Figure 5b). We believe that the sediments were more similar for Macro and Light for many of the measured parameters (e.g., TOC, TN, PLFA, etc.) because both Macro and Light treatments equilibrated (without macroalgae added) for two weeks prior to the experiment in natural day/night conditions and thus began the experiment with an active microphytobenthic community while the Dark treatment equilibrated for 2 weeks prior to the experiment in the dark, that is, without an active microphytobenthic community. As the macroalgae grew over the course of the experiment, the microphytobenthos in the Macro treatment were increasingly limited, which is why many of the measured parameters diverged between the Macro and Light treatments around Day 12. Reviewer 2 also mentions the potential importance of water column DOM, and we agree that it warrants further discussion for this study. We will include discussion of the potential importance of water column DOM on the sediment microbial community in the revised manuscript.

“Specific Comments: 1. I understand microphytobenthos is a long word, but I don’t think it is necessary to use an acronym, especially given the high number of acronyms

throughout the manuscript.”

Response: We recognize there are a lot of acronyms in the manuscript. We will write out microphytobenthos in all cases, as suggested, to avoid any confusion.

“2. Page 2804, line 14: Just to stay consistent throughout the manuscript – I assume ‘light treatments’ are both Light and Macro. Give the large amount of data presented here, it would be helpful to always refer to the treatments by “Light”, “Macro”, and “Dark”.”

Response: We will clarify this in all instances, as suggested.

“3. The use of PCA can be useful to elucidate patterns in large data sets with multiple independent factors. However, looking at Figure 7, I don’t see any clear patterns between treatments and time. The PCA results could be found to be more meaningful or helpful to the reader if the variables that make up the various components (or the bulk of the components) were reported in the figure (or within the figure legend). As the figure and the description within the text, stand now – I’m not able to follow any discernable pattern.”

Response: We included the PCA results to illustrate the major underlying trends in our large and complex dataset that occurred between treatments and over time (Day 1 vs. Day 42) in a way that was meant to be easy for the reader to interpret. We thank the reviewer for his/her suggestions that will further improve the presentation of this complex information to readers. As recommended, we will provide additional information in the figure caption describing the major biomarkers that contribute to each principal component.

References: Maher, D.T. and B.D. Eyre (2010). Benthic fluxes of dissolved organic carbon in three temperate Australian estuaries: Implications for global estimates of benthic DOC fluxes. *Journal of Geophysical Research* 115:G04039, doi:10.1029/2010JG001433.

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Tyler, A.C., McGlathery, K.J., Anderson, I.C. (2001). Macroalgae mediation of dissolved organic nitrogen fluxes in a temperate coastal lagoon. *Estuarine, Coastal, and Shelf Science* 53(2):155-168.

Tyler, A.C., McGlathery, K.J., Anderson, I.C. (2003). Benthic algae control sediment-water column fluxes of organic and inorganic nitrogen compounds in a temperate lagoon. *Limnology & Oceanography* 48(6):2125-2137.

Interactive comment on *Biogeosciences Discuss.*, 10, 2791, 2013.

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10, C1486–C1490, 2013

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