

## ***Interactive comment on “Short-term fate of intertidal microphytobenthos carbon under enhanced nutrient availability: A $^{13}\text{C}$ pulse-chase experiment” by Philip M. Riekenberg et al.***

### **Anonymous Referee #1**

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#### General comments

The goal of this study was to quantify the effects of nutrient additions on carbon transformations in coastal sediments dominated by MPB and bacteria. Understanding the effects of nutrient loading on benthic microbes is important because coastal ecosystems are increasingly impacted by eutrophication and MPB are the dominant primary producer in unvegetated, shallow sediments. Consequently the authors are addressing questions that are likely of interest to readers of Biogeosciences. Although the research questions are interesting and timely, there are several issues that the authors should address.

C1

The authors applied a  $^{13}\text{C}$  label to exposed surface sediments at low tide and then waited 11 h to collect cores (lines 128-130, 143-144). Approximately 2 h after collection, the cores were placed in the experimental tanks and exposed to the nutrient treatments (lines 150, 155-161). Carbon exchange, recycling, and loss between MPB and bacteria occurs quickly – over just a few hours. My concern is that at least 13 h passed between the  $^{13}\text{C}$  label addition and the application of the nutrient treatments. It seems that carbon exchange between MPB and bacteria that occurred prior to nutrient additions would confound the effects of the nutrient treatments. In addition, it is not clear from S1 that the nutrient treatments were effective. Further, it seems from the methods that the cores in the same nutrient treatment tank all shared the same water column (lines 171-173). This would affect the independence of processes across cores and could confound the results.

For the incubations, cores were sealed for 30 mins before initial samples were collected. Final samples were collected 3 h (light), 12 h (dark), or up to 16 h later (lines 182, 184-185). It would be good to clarify the exact duration of light and dark incubations and why the times differed between conditions. The authors should also clarify whether rates were calculated based on concentration changes between two time points. Generally, at least 3 time points are needed to calculate fluxes and capture non-linear dynamics. Moreover, long incubations, particularly under dark conditions, likely created ‘bottle’ effects that could affect metabolic rates. The authors should discuss potential artifacts of the sampling approach – particularly the potential impact of low DO on sediment respiration rates. Additional rationale for the time course of measuring fluxes 6 h after adding nutrients (and 19 h after adding  $^{13}\text{C}$ ), and then again 1.5 d, 2.5 d, and 3.5 d would also be useful.

The authors need to provide more rationale for using  $16:1n7$  as a marker for diatoms. While this compound is an important component of diatom lipids, it is also produced by other groups of algae as well as by iron reducing bacteria and sulfate reducing bacteria. These communities are likely active in shallow coastal sediments and could be

C2

in close proximity to MPB if the sediments are anoxic. The absence of polyunsaturated C20 and C22 PLFAs seems to suggest that diatoms and other microalgae were at low abundances. I am not convinced that the current approach adequately isolates contributions from diatoms vs. non-diatoms.

The carbon mass balance calculations should be clarified. For instance, the authors report the %C loss from the sediments, but it is unclear how this was calculated. Did this reflect the % change between the initial core collections and each sampling time point (0.5, 1.5, 2.5, and 3.5d)? Or was this calculated by subtracting out <sup>13</sup>C losses via DIC and DOC (lines 446-448)? If the former, then that should automatically account for losses by respiration and exudation. Similarly it is unclear how the exponential decay functions were calculated. The error bars in Figures 5 and 6 are large, particularly for the moderate and elevated treatments; this variability makes the substantial differences across treatments (lines 473-476) somewhat unexpected.

The discussion should be more concise and focused. It would have been helpful if the authors considered processes driving variability across the treatments and compared their findings to other studies examining MPB-bacterial responses to surface water nutrient additions – particularly other stable isotope labeling experiments. Along these lines, I was somewhat surprised that nutrient additions would not stimulate MPB production and thereby promote C retention in the sediments.

Specific comments Introduction: The authors acknowledge that other studies have examined the effects of nutrient loading on MPB (lines 73-77), but should discuss the subset of studies that used isotope tracer techniques in more depth, as these are directly relevant to the current manuscript.

Line 113 and elsewhere: instead of reporting carbon concentration per surface area, please report % organic carbon.

Lines 155-160. What were the target concentrations of each of the treatments and was the site water filtered before the treatments were applied?

C3

Calculations: The biomass calculations use several conversion factors to scale from lipid concentrations to bacterial and diatom biomass. It is unclear how well constrained the conversion factors are and the amount of uncertainty they introduce into the calculations. For instance, does the concentration of lipid per unit biomass change with algal growth or nutrient condition? It is unclear why the lipid concentrations need to be scaled to biomass – why not compare the ratio of bacterial to microalgal lipids?

Equations 3-4 are descriptions more so than equations.

Line 309: ANOVA is not the most appropriate test because the cores within a treatment were not independent of one another as they shared a water column.

Line 318: What is the ecological rationale for grouping time points 0.5 and 1.5 vs. 2.5 and 3.5?

Lines 388-400. How did downward transport occur in these cores? MPB are generally restricted to the top 2 cm and it does not seem that there was pore water flow during the lab incubations. Was there mixing by animal communities? Is it possible that contamination occurred during core collection in the field?

Line 431: Was the uncharacterized fraction defined as PLFAs that were not i,a-15 or 16:1n7? If so, additional rationale is needed to justify this approach.

Graphs: It would be helpful if the error bars were positive and negative

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