

## ***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 #2**

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The manuscript by Riekenberg et al. describes data from  $^{13}\text{C}$ -incubation experiments whereby microphytobenthos was labeled with  $^{13}\text{C}$  in situ, and then incubated under controlled conditions over a period of 3.5 days; either with background nutrient levels or with higher than ambient N &/or P concentrations.

While generally a well performed study, I am surprised by the short duration of the experiments (3.5 days). When fitting exponential decay functions on the resulting data (Figure 6), I feel this is somewhat thin ice – the data should be spread more in time for a convincing exponential fit.

-Abstract, line 30 and in Discussion: clearly define in the manuscript how you define

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and calculate the turnover time, to avoid any ambiguity. I find these turnover times surprisingly high (i.e., long), and in line with other comments, wonder whether the short incubation period did not lead to a bias in this estimate – with 3 time points very early on it seems not ideal to fit an exponential fit to these data. Also, it is not unambiguously clear what your  $t=0$  is (after the 6 hour ‘acclimation period’ ? See next comment).

On page 9, line 179, the authors mention that the cores were allowed to ‘acclimate for 6 hrs prior to the start of the incubation’. I’m not sure what this means, it’s not as if no microbial activity would take place during this period, hence for me it would seem to be an integral part of the incubation period. Why not simply define  $t=0$  as the moment the cores were no longer exposed to  $^{13}\text{C}$ -DIC labeling ? Are these 6 hours part of the incubation times mentioned throughout the ms ? If not, this may bias the estimates of turnover times.

In the abstract (line 26-27), the authors mention that treatments with higher nutrient levels showed higher loss of  $^{13}\text{C}$  label, “supporting increased production of extracellular enzymes and storage products”. I have two reservations here: First, this pattern would equally be consistent with a scenario in which the heterotrophic bacterial community was N and/or P-limited ? Eg Keuskamp et al. *Sci Total Environ.* 2015 doi: 10.1016/j.scitotenv.2014.11.092. I would suggest to add this as a possible mechanism in the introduction on page 4 (section starting at line 68). Secondly, this conclusion contradicts the statements in the introduction that “EPS production and bacterial utilization of newly produced EPS may decrease with increasing nutrient availability’ (page 5, first lines). It is indeed generally assumed that extracellular release is a higher fraction of total primary production under nutrient-limiting conditions. On page 5 line 92-93 you write that you expected that increased nutrient availability would stimulate EPS production – I don’t see why you would assume this, it is the opposite of what the literature suggests?

I feel the quantitative handling of the data is not always transparent or easy to follow. For the overall budgets in Figure 7, it is not clear to me how these were closed: you

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have concentrations and  $\delta^{13}\text{C}$  data on all these compartments, so you can calculate them individually – but they add up to 100% each time; you could add confidence to these numbers by verifying which % of the initial  $^{13}\text{C}$ -labeled biomass you can account for.

Figure 6: why are these first ‘accounted for by loss of  $^{13}\text{C}$  in DIC & DOC’ ? My first impression would be that you should simply look at the amount of  $^{13}\text{C}$  remaining in the sediment, without this ‘correction’ ? Please explain the rationale behind this in the text.

Towards the end of the discussion (line 704), the authors mention estimates of C retention at 30 days. This is odd, as the experiment ran over only 3.5 days and I would not consider extrapolations to 30 days very reliable (see also first comments).

#### Minor corrections

Abstract, line 15: what is meant with ‘over-enrichment’ ? I assume ‘enrichment’ suffices. Line 147: chlorophyll a (not alpha) Line 46-47: re-write this sentence, structure is odd.

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