Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2017-448-AC2, 2018


Biogeosciences Discussions

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

**Philip M. Riekenberg et al.**

phrieken@gmail.com

Received and published: 1 March 2018

We thank the two anonymous reviewers for their feedback. The suggested changes have helped to improve this manuscript.

The structure of the response is: 1) Reviewer comment number 1. Reviewer comment, 2. Author response 3. Changes to manuscript.

Highlighted text reflects portions of the text added as a result of comment.

Reviewer 2 main comments

1) 1. The manuscript by Riekenberg et al. describes data from 13C-incubation exper-



iments whereby microphyobenthos was labeled with 13C 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.

2. The reviewer is correct, the short time frame and relatively few data points means that the rate of loss may not be entirely representative of longer time loss at the site. However, the aim of this part of the study was to determine the relative differences in loss rates between the nutrient amended treatments and the ambient treatment. The difference between treatments is clear (now supported with a two-way ANOVA on LN 471) and similar whether an exponential or linear fit is required. The use of an exponential relationship was supported given that previous studies using a similar method of 13C application have found robust exponential relationships when describing the loss of 13C from sediment over a longer period, including data over the first few days following labeling (Oakes et al. 2014 Fig. 4; Veuger et al. 2012). Oakes et al. 2014 is a labeling study that occurred in the intertidal range within this same site that found exponential loss to be adequate to model 13C loss from this site.

In response to the 'thin ice' comment, we have more thoroughly investigated the differences in sediment retention of 13C between treatments using a two-way ANOVA. This analysis indicated significant differences between the means for the elevated and both the ambient and minimal treatments. We subsequently explored the relationships between treatments and found the difference observed between the elevated and ambient treatments to be robust as demonstrated by the analysis now provided (LN 471) that examines the differences between the slopes of loss found for each treatment (Supplemental Figure 2). It is also interesting to note the apparent dose-effect relationship within the data set, as the moderate treatment appears to fall in between that of the

minimal and elevated treatment.

Regarding the short duration of this study, we have addressed this in a previous comment from Reviewer 1 comment 7 briefly repeated here: “This project focused on initial processing of C, with multiple incubations over a relatively short period (3.5d after label addition), due to the observation in a comparable previous study at the same site that most 13C transformation occurs within this ∼4d (Oakes & Eyre 2014).”

3. As a result of this comment, we have now performed a two-way ANOVA on the % of 13C retained in the sediment provided on LN 471 and we have also included an analysis comparing the differences between slopes for our loss rates. LN 471 now reads: “The total 13C remaining in sediment (Fig. 6) varied significantly among treatments (two-way ANOVA: F3, 31= 5.7, p=0.008) and across sampling times (F3, 31= 3.9, p=0.03). Throughout the study, there was generally less 13C remaining within the elevated treatment than in than either the ambient (p=0.008) or minimal treatments (p=0.02), and there was significantly less 13C remaining within the sediment at 3.5 d than at 0.5 d (p=0.02).”

Our response to Reviewer 2’s second comment also further addresses the relevance of the turnover times calculated from this study and how they compare to previous labeling experiments performed in similar environments.

We have also added a clarifying statement about the duration of the study in response to Reviewer 1 comment 7 repeated here: LN 204 now reads: “These sampling time periods were chosen to capture the active dynamics of 13C processing that were expected to occur over the first few days of the study, based on previous work by Oakes and Eyre (2014).”

2) 1. Abstract, line 30 and in Discussion: clearly define in the manuscript how you define 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.

2. We disagree with the reviewer that turnover times are high. Our estimates of retention at 30 d (18-58%, LN 757) fall around the range found for other studies in unvegetated sediments (30-50%) as stated on LN 740:

“Although the focus has primarily been on vegetated environments (Duarte et al. 2005), which store the most carbon, unvegetated sediments also have capacity for longer-term retention (e.g. ∼50% after 21 d Hardison et al. 2011, 30% after 30 d Oakes and Eyre 2014; 31% after 30 d Oakes et al. 2012).”

The relatively short incubation times were ideal to explore the short-term fate of MPB-C which was the primary focus of this study. We then tried to use that data to see what implications these dynamics may have for longer-term retention. This is clearly not ideal, as it requires extrapolation from a small data set, but the relative differences between treatments are robust. Future studies examining nutrient effects on 13C retention should examine this relationship across a longer time period if possible.

3. We have now clearly defined how we have calculated turnover time with the inclusion of our clarifying statement on LN 350: “The data for 13C remaining in sediment OC were further examined by fitting an exponential decay function for each treatment across 3.5 d using the Exp2pMod1 function in OriginPro 2017 and 13C turnover estimates were then determined by solving for y = 0.05% remaining 13C (a value close to 0) and x = 30 d for each treatment.” We have included a statement supporting our utilizing exponential functions on LN 527: “The focus of this study was short-term fate, but our findings also show potential implications for longer- term retention. Our calculated retention times may be under or over-estimated due to their reliance on short-term data. However, the relative differences between treatments (decreased retention with increased nutrient amendment) are clear. The rationale for utilizing exponential functions in this study follows previous findings in Oakes et al. (2014) that 13C export from subtidal sediments at this site were well-described by an exponential decay function

across a longer time period (31 d). Additionally, the 30 d estimates provided within this study (18-58%) fall across a range similar to that of other previous labeling experiments (30-50%; Hardison et al. 2011; Oakes and Eyre 2014; Oakes et al. 2012), leading the authors to conclude that the use of exponential functions to describe this relationship was valid in this study."

3) 1. Also, it is not unambiguously clear what your t=0 is (after the 6 hour 'acclimation period' ? See next comment). On page 9, line 184, 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 13C-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.

2. The purpose of the acclimation period was to allow for re-establishment of any disturbed sediment redox zonation that occurred during coring (see changes due to reviewer 1 comment 7). Therefore, we omitted the 6 h period prior to measuring in an effort to obtain more robust measurements for P/R and water column fluxes for DOC and DIC. As this study was focused primarily on the development of differences between the treatments kept under the same conditions, we do not agree with the reviewer that this 6 h period is integral to the results presented.

The described shift used with the exponential functions from the current 0 to 19 h before (when the 13C was at its maximum) describes the mathematical parameter of an x transform shift by 0.8 d. Calculation of the resulting change results in materially the same estimates for turnover time (shifted longer by 0.8 d). There is no apparent bias within the dataset resulting from this as the functions used to model loss rates were not forced to 100% at the starting point (x=0).

3. This comment further supports the previous changes that resulted from Reviewer



1, comment 7, copied here: We have added a clarifying statement about the rationale behind the 6 h acclimation period. LN 184 now reads: "Cores were allowed to acclimate in tanks for 6 h prior to the start of incubation to allow for the re-establishment of microclimates and anaerobic zonation that was potentially disturbed by coring."

4) 1. In the abstract (line 26-27), the authors mention that treatments with higher nutrient levels showed higher loss of 13C 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 67).

2. We agree with the reviewer that relaxation of nutrient limitation may have increased the extracellular products being released. We previously postulated that relaxation of nutrient limitation had potential to affect microbial biomass on LN 67.

3. We have added this possibility to our previous statement in LN 67: "A major source of environmental change in coastal systems is nutrient over-enrichment (Cloern et al. 2001), which may affect the assimilation and flux pathways of MPB-derived carbon through 1) increased microbial biomass or an increase in production of extracellular enzymes resulting from relaxation of nutrient limitation, 2) increased algal production that drives elevated heterotrophic processes as bacteria utilize newly produced C, and 3) increased loss of C as DIC via respiratory pathways as heterotrophic processes."

5) 1. 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?

2. The reviewer is correct that the fraction of primary productivity that EPS represents is higher in nutrient limited settings as MPB produce EPS as a way to manage excess C. We agree that the nutrient addition should stimulate overall MPB-C production and not just that of EPS and have changed the wording to reflect that.

3. LN 92 now reads: "We expected increased nutrient availability to stimulate production of MPB-C after initial labeling, resulting in decreased turnover times for MPB-C as well as a shift towards dominance of heterotrophic processes as bacteria utilize this additional labile C"

6) 1. 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 have concentrations and d13C 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 13C-labeled biomass you can account for.

2. We closed the budgets in Figure 7 by accounting for the total 13C contained in the bulk sediment organic C and including the calculated fluxes for DIC and DOC that were interpolated from measurements across the 3.5 d for both DIC and DOC.

3. To make it clear how the budgets were constructed and closed, we have included a brief statement in the methods section about how the budget was calculated. LN 326 now reads: "Because all 13C was contained within the cores, values for 13C budgets add to 100%. Starting values were estimated by looking at how much 13C remained in the sediment and how much was lost to the water column (initial 13C= 13C remaining + 13C lost)."

7) 1. Figure 6: why are these first 'accounted for by loss of 13C in DIC & DOC" ? My first impression would be that you should simply look at the amount of 13C remaining



in the sediment, without this 'correction'? Please explain the rationale behind this in the text.

2. Since these core incubations are a contained system, the estimate of original 13C present adds to 100%, with the measured fluxes of DIC and DOC needing to be incorporated in order to correctly portion the amount of 13C still contained in the sediment versus the amount exported to the water column across the incubation period.

3. We have further clarified how the 13C budgets were constructed as a result of Reviewer 2 comment 6, briefly repeated here: To make it clear how the budgets were constructed and closed, we are including a brief statement in the methods section about how the budget was calculated. LN 326 now reads: "Because all 13C was contained within the cores, values for 13C budgets add to 100%. Starting values were estimated by looking at how much 13C remained in the sediment and how much was lost to the water column (initial 13C= 13C remaining + 13C lost)."

8) 1. Towards the end of the discussion (line 757), 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).

2. We address this with quoted text in reviewer 2's comment 2, but will expand further here. On LN 740, we detail findings of other studies that determined 13C retention across 30 days. Further in that paragraph (LN 757) we provide our estimates of retention at 30 d taken from our exponential functions. Our ambient and minimal estimates fall close to the range of these previous studies. This finding allows us to feel reasonably confident that we are not too far out of the ballpark working with our exponential fits in the data set. Being able to gauge our results against the findings of other studies that extended over this period (e.g. Oakes & Eyre 2014) was the goal behind extrapolating our data to 30 d. We realize that extrapolation is not terrifically reliable when based on a data set formed over a short time period, but extrapolation in this case allows us to compare our short-term loss rates to other longer-term studies in an effort

to gauge whether the rates we have found agree and are in a reasonable range.

3. A clarifying statement about the 30 d comparisons is now provided, pointing out that substantial extrapolation is required in order for us to compare to other studies looking at longer term retention of MPB-C. LN 752 now reads: “The primary focus of this study was short-term fate of MPB-C, but the significant decrease in retention observed with nutrient amendments imply that short-term processes may have implications for longer term retention. It is interesting to consider how these short-term changes may affect the longer-term retention (30 d) reported by previous studies (e.g., Oakes & Eyre 2014), with the caveat that the substantial extrapolation required could introduce considerable error to estimates of retention.”

Minor corrections 9) 1. Abstract, line 15: what is meant with ‘over-enrichment’ ? I assume ‘enrichment’ suffices.

2. Enrichment does suffice, corrected as suggested.

10) 1. Line 148: chlorophyll a (not alpha)

2. Fixed throughout manuscript.

11) 1. Line 46-47: re-write this sentence, structure is odd.

3. LN 46 now reads: “Application of rare isotope tracers can render fractionation effects and variability that affect natural abundance stable isotope techniques negligible and has been useful for elucidating pathways for the processing and loss of MPB-derived C within estuarine sediments.”

---

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2017-448, 2017.



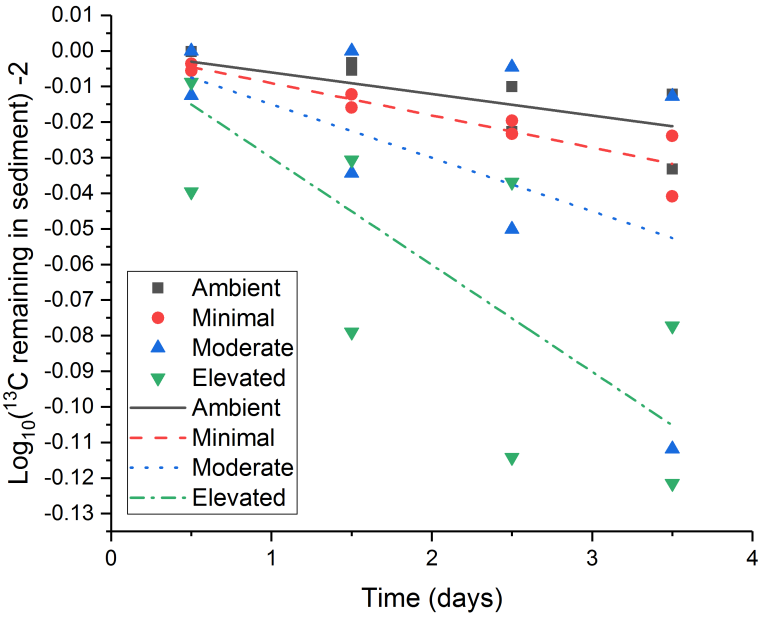


**Fig. 1.**