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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.

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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 1 main comments 1) 1. The authors applied a 13C label to exposed surface sediments at low tide and then waited 11 h to collect cores (lines 141-143, 144-145).

Approximately 2 h after collection, the cores were placed in the experimental tanks and exposed to the nutrient treatments (lines 150, 154-161). 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 13C 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.

2. Prior to incubation, all cores were maintained in conditions that were as close to identical as possible (both in situ and during transport) to maintain as much homogeneity as possible between the cores. As a result of this care, the carbon exchange between MPB and bacteria are expected to have been similar between cores prior to exposure to the nutrient additions as they were both handled in an identical manner and subsequently randomly allocated (LN 175) to treatment group prior to being placed in the incubation tanks.

As a result of this, carbon processing that occurred after the treatment application should be similar across cores within the treatments, regardless of the microbial processing that occurred in the 13 h prior to nutrient amendment. Any differences among treatments are therefore solely due to the application of different nutrient concentrations. The reviewer is correct that there was processing of newly fixed MPB-C that occurred prior to nutrient additions, but this manuscript focuses on the processing that occurred on the remaining MPB-C that was still labile and present (as evidenced by the 13C found within both bacteria and MPB) within the sediment during our incubations.

3. We have added a clarifying statement about the the effect of the 13 h prior to nutrient amendment. LN 168 now reads: "Processing of newly fixed MPB-C occurred in the 13 h prior to incubation with nutrient amendments, but was likely similar across cores as they were kept in identical conditions prior to incubation before random allocation to treatments. Although MPB-C was not freshly fixed at 13 h and likely more refractory as a result, the available C was still relatively labile and readily processed across all

treatments."

We have also clarified the focus of the study by more clearly stating the focus in the concluding paragraph of the introduction. LN 85 now reads: "In this 13C pulse-chase study we aimed to quantify the short-term effects of increased nutrient concentrations on the processing pathways for MPB-derived C within subtropical intertidal sediments"

2) 1. In addition, it is not clear from S1 that the nutrient treatments were effective.

2. As per text on ln 166-167, figure S1 was included to demonstrate that nutrient additions were incorporated into the biomass and were not sufficient to overload the capacity for nutrient utilization and leave a large concentration of DIN in the water column. We added nutrients in pulses that were processed and removed from the water column at both 0 d and 1.5 d. The sampling at 1.5 d was capped for incubation prior to nutrient addition and therefore was not expected to contain the nutrient addition that occurred at 1.5 d. Nutrient treatments were effective, as evidenced by the increased export of 13C from the sediment within the elevated treatment (LN 471), and the changes in distribution of labeled material as processing pathways were affected (Fig. 7).

3. No changes were made in response to this comment.

3) 1. Further, it seems from the methods that the cores in the same nutrient treatment tank all shared the same water column (lines 176-179). This would affect the independence of processes across cores and could confound the results.

2. The reviewer is correct that the water column was shared by all cores within each treatment tank. It was not logistically possible to separate paired cores into individual treatment tanks for each sampling and adding treatments to sealed cores would have exacerbated any 'bottle effect' given the relatively small volumes of water contained in each core.

Sharing a water column could have been a problem if 13C produced within one core found its way into another through transport of enriched DIC or DOC produced from

MPB-C. However, this was not a problem as the volume in each treatment tank was sufficient to dilute any label coming from these sources. We measured δ13C of water column DIC and DOC; initial values for each light incubation (collected immediately after capping of cores) reflect the treatment tank water. No considerable enrichment in δ13C values for initial DIC or DOC was observed for any of the sampling times across the 3.5 d incubations, confirming that there was no 13C transfer among cores within each treatment tank.

3. No changes were made in response to this comment.

4) 1. 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 186, 188-192). It would be good to clarify the exact duration of light and dark incubations and why the times differed between conditions.

2. The 3 h duration of the light incubation was intended to prevent dissolved oxygen from becoming supersaturated as a requirement for N2/Ar analysis that was being run simultaneously during this experiment as part of another study focused on N.

3. We have clarified that the incubation period was $\sim$15 h (LN 188). LN 192 now reads: "Light incubations were of shorter duration to prevent supersaturation of dissolved oxygen which would have compromised additional analyses required for a complementary study."

5) 1. 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 nonlinear dynamics.

2. Several incubation studies have been performed by this working group across the last 15 years, and while they began with 3 to 5 point incubations (Ferguson et al. 2003, AME 33: 137-154, Veuger et al. 2007, L&O 52(5): 1930-1942) for flux measurements, the dynamics observed were largely linear. More recent studies have used two point

dark and light incubations due to the considerable reduction of cost and sampling effort (e.g., Oakes & Eyre 2014, Biogeosciences 11: 1927-1940) with minimal loss of information and accuracy.

3. We have clarified on LN 300: “Fluxes across the sediment-water interface were calculated from two measured concentrations, at the start and finish of each dark and light period (e.g., Oakes and Eyre 2014), as a function of incubation time, core water volume and sediment surface area.”

6) 1. 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.

2. DO did not fall below 4.85 mg/L (DO% 58.5%) during the dark incubations, minimizing potential long term effects or ‘bottle’ effects. DO consistently increased rapidly when the lights were turned on, resulting in the need to shorten the light period in order to sample below supersaturation as required for N2/Ar analysis for another study occurring simultaneously with this one. The quick recovery of production indicates that any potential bottle effect during the longer incubation was negligible and did not result in considerable lag between respiration and production periods.

3. We have clarified that DO was not allowed either to drop too low or go above saturation. LN 310 now reads: “To prevent the potential development of resource limitations during incubation, O2 concentrations were not allowed to drop below 60% saturation in the dark and light incubations were shortened ($\sim$3 h) to ensure that production was not allowed to become supersaturated.”

7) 1. Additional rationale for the time course of measuring fluxes 6 h after adding nutrients (and 19 h after adding 13C), and then again 1.5 d, 2.5 d, and 3.5 d would also be useful.

2. 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 $\sim$4d (Oakes & Eyre 2014). Sampling was only feasible every $\sim$24 hours, with light/dark periods aligned with in situ conditions, and each light/dark incubation taking $\sim$15h. Acclimation time was allowed for any microclimates or disturbed anaerobic zonation to re-establish after disturbance with coring as this has the potential to influence P/R dynamics.

3. We have added a clarifying statement about the spacing of our sampling times. 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).”

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.”

8) 1. 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 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.

2. We have used a previously published method (Oakes et al. 2016, L&O 61:2296-2308) for calculation of diatom associated 16:1n7. This calculation provides diatom associated 16:1n7 after subtraction of the estimated contribution of 16:1n7 from other

sources, calculated from 18:1n7 using a two source mixing model described on LN 280. 18:1n7 is a monosaturated PLFA that is associated with cyanobacteria, Gram negative bacteria, and sulfate reducing bacteria. By removing the amount of 16:1n7 estimated from 18:1n7, we removed the contribution of these bacterial groups from the total pool of 16:1n7. We did not investigate PLFAs associated with Fe-reducing bacteria as it was outside of the scope of this study. Furthermore, we are confident that pennate diatoms had substantial biomass at the site, and the contribution from other algal species was minimal based on visual analysis of the sediments (via microscopy) that confirmed strong dominance of pennate diatoms (LN 225). On this basis, we conclude that the remaining portion of 16:1n7 is predominately derived from diatoms.

3. We have clarified that 18:1n7 was used to account for the contribution of Gram negative, cyanobacteria, and SRB to 16:1n7 in the initial description of PLFA analysis. LN 227 now reads "The 16:1(n-7) PLFA, which represents 27.4% of total diatom PLFAs (Volkman et al. 1989), was consistently present across all samples and was used as a biomarker for diatoms, following correction for contributions from gram-negative bacteria, cyanobacteria, and sulfate reducing bacteria, determined using 18:1n7 as described in the calculations section below and in Oakes et al. (2016)."

9) 1. 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 13C losses via DIC and DOC (lines 490-491)? If the former, then that should automatically account for losses by respiration and exudation.

2. Neither method that the reviewer suggests reflects the accounting presented in this study.

Figure 6 shows 13C remaining in the sediment, not 13C loss. 13C remaining in the sediment was calculated as the sum of the exported DI13C and DO13C and sediment

derived 13C (bulk OC) divided by the sediment derived 13C and multiplied by 100 for each core at the end of each dark/light incubation. We then went on to use exponential decay functions to explore how the export of 13C from the sediment differed between nutrient amended treatments and ambient treatments.

We feel that we have adequately described how the turnover of 13C was calculated from our response to Reviewer 1 comment 10, briefly repeated here: "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."

To further clarify, the %'s presented in the budget for Figure 7 reflect the portion of the 13C within each pool (MPB, Bacteria, uncharacterized) at each sampling time, with the interpolated fluxes of DIC and DOC included.

3. We have clarified our method of accounting for 13C on LN 261. "Percentages calculated from these pools are presented as portions of the sum of total 13C contained within the sediment and the interpolated fluxes of DIC and DOC that were estimated to have occurred from 0 d until each sampling time."

10) 1. Similarly it is unclear how the exponential decay functions were calculated.

2. Exponential decay functions were fitted using the function Exp2pmod1 in OriginPro 2017. 13C turnover estimates were provided by solving for y = 0 % remaining 13C and x = 30 d.

3. LN 350 now reads: "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."

11) 1. 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 475-478) somewhat unexpected.

2. Some of the error bars in Figs. 5 & 6 are large, as is expected for calculated fluxes across the multiple cores contained in each treatment. We have further investigated the 13C remaining in the sediment (Fig. 6) with a two-way ANOVA and found the elevated treatment to be significantly lower than both the ambient and minimal treatments. We have now included this analysis in our results.

To further investigate the differences between the loss rate constants presented in Fig. 6 resulting from the export of DIC and DOC shown in Fig. 5, we now present an analysis of the transformed data for 13C remaining in the sediment. This compared a model using a single slope for all of the data (i.e. including all treatments) with a model utilizing separate slopes for each treatment. We have added supplemental figure 2 that presents the slopes being compared as well as the underlying data being modeled.

3. LN 471 now describes the two-way ANOVA run for 13C remaining in sediments. LN 471 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)."

LN 482 now describes how the comparison between slopes were analyzed: "Since the intercept is known, i.e., the initial value equals 100% at time 0, linear models where only the slopes were estimable, were fitted to further analyze the differences between slopes. Assuming an exponential decay, the percentage remaining 13C (Y) was log10 transformed and the value 2 was subsequently subtracted (Z=log10(Y) -2), which implies that the intercept of Z versus time equals 0. The model with different slopes

for each treatment fitted significantly better than the model with a single slope (F-test, F3,28=9.84, P <0.001, Supplemental Fig. 2). The analysis was performed in R."

Supplemental Figure 2 has been added to LN 1030 along with a figure caption that reads: "Supplemental Figure 2: Slope comparison between treatments for log10 transformed 13C remaining in sediment. The model with different slopes for each treatment fitted significantly better than the model with a single slope (F-test, F3,28=9.84, P <0.001)."

12) 1. 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.

2. We have examined the discussion and chosen to eliminate some included detail (previously LN 571-574 & LN 587-597) about patterns within the P/R data, as they were covered in too much detail in the text.

The reviewer's description of N stimulating MPB production describes only one side of the interaction that occurs within the intertidal biofilm. Opposite of MPB production, bacteria are working to actively hydrolyze both the EPS and biomass produced by MPB. Bacteria rely heavily on MPB-derived material for N under N-limitation. It may be possible that with increased N availability, bacteria are able to offset increased MPB production through increased hydrolysis and bacterial production. Figs. 3 and 4b support this, with increased incorporation of 13C into both bacteria and the uncharacterized pool (processed C) within the elevated treatments. It appears that the net balance between production and consumption of C within mudflat biofilms regulates whether there is net uptake or net loss of C within the sediment (Cook et al. 2007, Hardison et al. 2013, Spivak et al. 2016).

Labeling applications in coastal sediments with limited fauna and/or macroalgal influence that also include nutrient amendments are sparse in the literature. We have now included a comparison of retention of 13C within the sediment to Hardison et al. (2011). Given that there was very little fauna at our study site, and certainly no macrofauna within our incubated cores, we consider it inappropriate to directly compare to other studies such as Pascal et al. 2013 (6 year enrichment study for both C + N) or Spivak et al. 2016 (seasonal comparison using labeled detritus to work out MPB contribution) due to commentary within both studies about grazing pressure potentially masking bottom up effects of fertilization on MPB due to the faunal abundance found within the study site.

3. We have deleted a substantial amount of text from the discussion of P/R (previously LN 571-574 and LN 587-597).

LN 742 now includes a direct comparison to Hardison et al. 2011 for retention of 13C in sediment during a water column nutrient amended study.

Specific comments

Introduction: 13) 1. 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.

2. The subset of studies that have used both biomarkers and labeled SI additions have not usually gone on to subsequently partition out the DIC and DOC portions of the carbon budget and therefore do not fully discuss these processing pathways. To fully address both portions of the budget that this study examines (sediment and DIC/DOC), we have broadened our focus and included a combination of references for both tracer studies and non-tracer studies that investigated nutrients and MPB on LN 80-84: “Increased autochthonous production driven by nutrient enrichment can lead to increased heterotrophy, as newly produced organic matter is mineralized (Fry et

al. 2015), resulting in increased DIC production. Increased remineralization of newly produced MPB-C will result in greater loss of DIC from intertidal sediment via bacterial respiration (Hardison et al. 2011).” The reason for this diverse focus is that most tracer studies utilizing biomarkers focus solely on partitioning of the budget directly involved with those biomarkers and do not present accounting of the DIC and DOC pathways as a portion of the budget within the framework of the study. Additionally, we have added Pascal et al. (2013) as another relevant stable isotope study in addition to Cook et al. (2007) which was already included (a tracer study examining the effects of nutrient enrichment) and discussed on LN 78: “Both EPS production and bacterial utilization of newly produced EPS may decrease with increasing nutrient availability”. 3. We have added the reference to Pascal et al. (2013) to LN 76.

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

2. We have now provided sed %OC in the study site description in addition to our previous units. Elsewhere in the manuscript we have retained units as per surface area as it is convention for work of this nature (e.g. Oakes et al. 2014, Hardison et al. 2011, and Middelburg et al. 2000) and allows for comparison across studies as well as conversion into %OC if anyone should wish to do so.

3. %OC is now included on LN 113 in addition to the previously provided units for concentration per surface area.

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

2. LN 155 reads: “The treatment tanks were set up at ambient concentration (site water, DIN of 2.5 $\pm$ 0.04 μmol N L-1, measured on incoming tide), and with N (NH4+) and P (H3PO4) amendment to unfiltered site water at 2$\times$ (minimal treatment), 5$\times$ (moderate treatment) and 10$\times$ (elevated treatment) average water column concentrations near the study site (4 μmol L-1 NH4+ and 5 μmol L-1 PO43-; Eyre (1997; 2000).”

The target concentrations for pulsed nutrient amendments were for 8, 25, and 40 μmol L-1 NH4+ and 10, 25, and 50 μmol L-1 PO43- respectively for minimal, moderate and elevated treatment pulses. Site water was unfiltered.

3. We have now specified that the water was not filtered (LN 155). No changes were made to further specify the target concentrations of each treatment, as these can clearly be derived from the text on LN 155 (2x, 5x, and 10x average water column concentrations of 4umol/L NH4 and 5umol/L PO4).

Calculations: 16) 1. 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?

2. This comment addresses concerns about methodology that are not unique to this study, but which are common to methods routinely applied in studies using biomarkers. Conversion factors are frequently applied to FAMEs (fatty acid methyl esters) to estimate and compare biomasses of different microbial groups (bacteria, MPB).

When examining uptake of 13C, we need to account differences in biomass to adequately account for the total 13C present in different pools. It is possible to have a large enrichment of 13C contained in a small pool of biomass that may contain less 13C than a minimally enriched pool of biomass that is considerably larger. Without accounting for the relative biomass that is contained within each pool, it would appear the more enriched biomarker is more involved in the processing of 13C, but upon full accounting of both biomass and enrichment, it becomes abundantly clear that the larger, less enriched pool has a more significant impact.

As such, thorough investigation of "the concentration of lipid per unit biomass change with algal growth or nutrient condition" falls outside of the scope of this study.

3. We have referred to previous work that utilize these conversion estimates on LN 227: "The 16:1(n-7) PLFA, which represents 27.4% of total diatom PLFAs (Volkman et al. 1989), was consistently present across all samples and was used as a biomarker for diatoms, following correction for contributions from gram-negative bacteria, cyanobacteria, and sulfate reducing bacteria, determined using 18:1n7 as described in the calculations section below and in Oakes et al. (2016)."

17) 1. Equations 3-4 are descriptions more so than equations.

2. The reviewer is technically correct, but these serve to efficiently communicate how the fluxes for both respiration and net primary productivity were calculated in order to further calculate gross primary productivity.

3. We consider the current presentation to be both clear and concise; no changes have been made.

18) 1. Line 330: 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.

2. As stated in the previous response to Reviewer 1 comment 3: "Sharing a water column could have been a problem if 13C produced within one core found its way into another through transport of enriched DIC or DOC produced from MPB-C. However, this was not a problem as the volume in each treatment tank was sufficient to dilute any label coming from these sources. We measured δ13C of water column DIC and DOC; initial values for each light incubation (collected immediately after capping of cores) reflect the treatment tank water. No considerable enrichment in δ13C values for initial DIC or DOC was observed for any of the sampling times across the 3.5 d incubations, confirming that there was no 13C transfer among cores within each treatment tank."

3. The reviewer does not provide an alternative analysis for us to investigate, so we have considered their case for a lack of independence and provided refuting evidence. No changes were made as a result of this comment.

19) 1. Line 341: What is the ecological rationale for grouping time points 0.5 and 1.5 vs. 2.5 and 3.5?

2. As stated in the text on LN 341: "To increase replication for statistical analysis, and therefore increase the power to detect a significant difference, we therefore grouped data across times into two levels: before 1.5 d (including 0.5 d and 1.5 d) and after 1.5 d (including 2.5 d and 3.5 d).", the rationale is largely statistically driven. The division provides an equal group weighting between the examined groups. There is some geochemical rationale in doing this as the additions of tracers are often observed to change the most in the earliest part of the experiment, and taper off as the carbon becomes more recalcitrant and dispersed within the system, but primarily, this split was designed to allow for sufficient replication within the groups and, therefore, robust statistical analysis.

3. No changes were made as a result of this comment.

20) 1. Lines 415-426. 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?

2. Saburova et al. 2003 examined diatom migration in detail and found 4 cm migration in sediment with clay sublayers, and up to 8 cm migration in sediments with a coarse sand sublayer, so the reviewer's statement that MPB migration is limited to 2 cm is incorrect. As our site sediments were quite sandy, and the stir bars suspended above the sediments were sufficient to stimulate moderate porewater flow in the upper layer, we consider it plausible that downward transport occurred via MPB transport across depth during the incubations. Saburova et al. 2003 is referenced in the downward transport section of the discussion (LN 717) supporting MPB migration as an effective pathway for downward transport in this study.

Contamination is possible during collection, but would have been minimal and fairly

uniform across the cores since the core liners were placed into the sediment in an identical manner. Additionally, our initial cores displayed minimal downward presence of 13C, which also supports that contamination was minimal during collection as they were collected in exactly the same manner as sample cores.

3. No changes were made as a result of this comment.

21) 1. Line 457: 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.

2. The 13C content of the uncharacterized fraction was calculated by subtracting the 13C in contained microbial biomass (diatom and bacteria calculated from PLFAs) from the 13C contained in the sediment organic carbon. This is now described in the methods section.

3. LN 295 now reads: "Microbial biomass is the sum of calculated diatom and bacterial biomass. Uncharacterized 13C was calculated as: 3. 13Cuncharacterized = 13Csediment organic – 13Cmicrobial biomass where 13Csediment organic represents total 13C in sediment organic carbon and 13Cmicrobial biomass represents the 13C contained in microbial biomass within the same core."

22) 1. Graphs: It would be helpful if the error bars were positive and negative

2. We attempted to add both positive and negative errors bars, but with the complexity of the experimental design that is presented, positive and negative error bars achieve little other than cluttering the bar graphs being presented. This is especially true for figures 1A and 2 where both positive and negative fluxes are presented and with several pools being displayed in the same figure.

3. No changes were made as a result of this comment.

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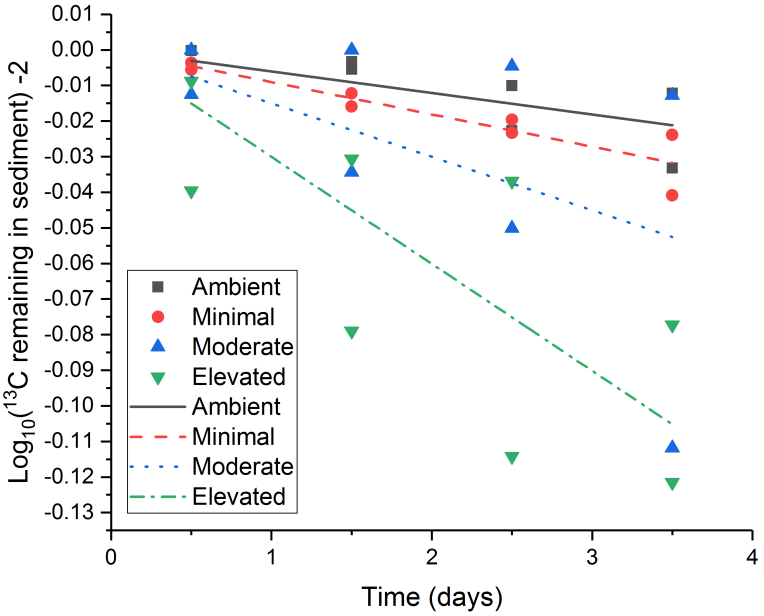
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-0.13
Log10(13C remaining in sediment) -2
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2
3
4
Time (days)
Ambient
Minimal
Moderate
Elevated
Ambient
Minimal
Moderate
Elevated

Fig. 1.