

# ***Interactive comment on “Patterns of carbon processing at the seafloor: the role of faunal and microbial communities in moderating carbon flows” by C. Woulds et al.***

## **Anonymous Referee #1**

Received and published: 11 March 2016

This paper described carbon processing at two different sites (fine-grained, high TOC concentration site and coarse-grained, low TOC site) at shallow water depths. Although the experiments were not totally comparable for some reasons (see details below), the experimental results are clear and meaningful; comparable respiration rates at both sites, but high macrofaunal uptake at the high TOC site while high bacterial uptake at the low TOC site. However, I think the discussion regarding carbon processing categorization has critical problems and needed to be removed, or presented after considerable, extensive modification.

Major comments:

1. Carbon processing categorization The discussion 4.5. based on many uncertainty

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and speculations, and need to remove from the manuscript. The authors proposed the categorization of C processing using data in this study and references. However, there is no mention on how and why authors selected specific time scale of the incubation duration. In Woulds et al. (2009), there were circle graphs of carbon fate for both ~2 days and ~5 days. However, in this paper, only one of them (I guess so) are shown. It is expected that the respired C increases with time (as mentioned in the line 563) while macrofaunal and bacterial  $^{13}\text{C}$ -label will be respired and decreased. Further, the faunal uptake and bacterial uptake also showed different patterns with time between taxa: for instance, macrofauna responded quicker than foraminifera (Witte et al. 2003, Nature), bacterial assimilation decreased after 1 or 2 days (Middelburg et al. 2000) whereas foraminiferal uptake showed increasing pattern during similar time scale (Moodley et al. 2000). It is thus obvious that the time scale selection is the most important factor to properly categorize the carbon processing. In this manuscript, data from different time scales (hours to 23 days) were combined without description what time scale of incubation was selected in the categorization from several different incubation periods (e.g. Moodley et al. 2002, Witte et al. 2003a, b, Bhuring et al. 2006). Also, there is no discussion on the effect of time scale (except line 563, which mentioned as to explain the irregular pattern of the categorization). I therefore recommend to remove discussion 4.5 from the manuscript and just discuss Loch Etive was macrofauna dominated C processing and Ythan sand flat was bacteria dominated. The manuscript itself can withstand as research paper without the chapter 4.5.

2. Differences in light condition. The authors performed the  $^{13}\text{C}$ -labeled phytodetritus experiments with and without light (with light: Loch Etive, without light: Ythan sand flat). The authors validate the different conditions because natural environments are dark and light conditions, respectively. However, I believe that the incubation with light makes complicated pathways. Without light, the  $^{13}\text{C}$ -phytodetritus is incorporated into heterotrophic microbes or eukaryotes, and either assimilated into their biomass or respired as  $^{13}\text{CO}_2$ . With light, however, the respired  $^{13}\text{CO}_2$  can be assimilated into photoautotrophic microbial biomass via photosynthesis. This leads underestima-

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tion of respired carbon and overestimation of bacterial assimilation. Without light, chemolithoautotrophic microbes can also cause same process, but the contribution must be smaller than photosynthesis. How much proportion of CO<sub>2</sub> was labeled with <sup>13</sup>C? If the <sup>13</sup>C concentrations in CO<sub>2</sub> is almost negligible (few %), then the bacterial assimilation via photosynthesis may also be negligible. This can be calculated from the DIC-d<sup>13</sup>C data of the study. Or, if there are literature which investigated bacterial community at this area, then the authors may validate that photoautotrophic bacteria was minor.

3. Uptake calculation The authors calculated the Carbon uptake by sample with the equation (3), line 253. However, the At% phytodetritus must be subtracted by At% background. I understand that the extent of <sup>13</sup>C-label in this study (25% and 34%) are high and the re-calculated values using subtracted value may change only 2 or 3 % (considering 25 become 23.9 and 34 become 32.9). However, the it is necessary to indicate appropriate values as much as possible.

Other specific comments. Line 32 Did the accessibility by bacteria to added C similar between two sites? Please show the vertical profiles of <sup>13</sup>C if possible.

Line 145. Figure 1 does not show any sills or geographical names. Please include these information to the figure or delete the citation (Fig.1 ) from the end of this sentence.

Line 163. While the Loch Evive site has 70 m water depth, the Ythan estuary site exposed during low tide. This is a great difference between two sites, in addition to sediment grain size and OC concentrations. The authors need to discuss the potential impacts of these differences of OC cycling and validate why the authors did not perform the experiment at coarse grained, OC poor site having similar water depths (or vice versa).

Line 171. What exactly was the phytodetritus labeled with <sup>13</sup>C? Was that degraded in some way? Or some sort of algal species? Was this same to the one which was added

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to Ythan sand flat? Please clarify these details.

Line 173. How much volume was the overlying water in the core?

Line 185 150  $\mu\text{m}$  sieve is not typical size separation for meiofauna. Why did the authors choose this size?

Line 189 Why the authors used milliQ water instead filtered seawater of artificial seawater? MilliQ water may had elution of organic matters from fauna due to osmotic shock (although the results showed insignificant effect).

Line 196 Bubbling with air in this experiment while the Loch Etive site cores were maintained with oxystat system. How did this affect to  $^{13}\text{C}$ - $\text{CO}_2$  amounts?

Line 253 The equation is not presented in correct way (no under bar below “C Uptake sample”. What the unit of “C Uptake sample”?

Line 263 It is not clear about the linear regression. Do the authors mean linear regression of different incubation periods? It is also important to show the changes in  $\text{d}^{13}\text{C}$ -DIC (or  $^{13}\text{C}$ -respiration rates) with time, because the changes in  $^{13}\text{C}$ -respiration with time should give crucial info regarding faunal or bacterial responses and C processing.

Line 267 It is necessary to show the respiration data of Ythan sand flat, too, as Table or supplementary figure.

Line 274 Please describe the centrifuge condition (  $\times g$ , how long, and what temperature etc). It will help to guess the potential effects of centrifuge on bacterial PLFA loss.

Line 279. Did the authors examine the  $\text{d}^{13}\text{C}$  of bulk sediments? If so, please include as Table etc.

Line 282 Again, it is important to show temporal changes in  $\text{d}^{13}\text{C}$  (or respired  $^{13}\text{C}$ ).

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Line 326. 0.00023 mgC per mgC corresponds  $\sim 5$  or 10 per mil of  $\delta^{13}\text{C}$ , which is relatively low labeling. What were the variation in  $\delta^{13}\text{C}$  of natural PLFA and labeled PLFA? Can you add as Table?

Lines 347 to 353. Whatever the C dose amounts were similar, the authors should think about the difference in natural phytodetritus supply rates at two sites. The same amount of  $^{13}\text{C}$ -phytodetritus input should have completely different effects on between originally eutrophic (in terms of OM) site and oligotrophic site. The authors should discuss these point of view by referring the primary production rates at two sites.

Line 368 Can you cite any paper which dealing different size screens?

Lines 376 to 380. Due to the osmotic shock by milliQ water (according to M&M), the fauna may be dead and did not have a time to void the gut.

Line 431. Gooday et al. 2008 represent biomass-uptake relationships with different symbols for bacteria, fauna, foraminifera. Can you also make such kind of Figure 4 for better comparison?

Line 438. This may suggest that the macrofauna of Ythan sand flat has low background metabolism than Loch Etive.

Line 459. I cannot follow why the authors said “macrofaunal biomass” in this sentence whereas the line 456 mentioned “biomass (faunal plus bacterial)”. Please describe more in detail if the authors actually intended to say “macrofaunal biomass”.

Chapter 4.4. can be combined to 4.3.

Line 520. Both methods (Total respiration rate measurements and bacterial C assimilation rates) has considerable uncertainty. Thus the discussion here, dealing bacterial growth efficiency, is somewhat over-interpretation. Also, as mentioned earlier, because the incubation of Ythan sand flat sediment was carried out under light condition, it is possible that some  $^{13}\text{C}$ -bacterial lipids were originated from the photoautotrophic microbes, not by heterotrophic bacteria which incorporated  $^{13}\text{C}$ -labeled phytoplankton.

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Line 571 Again, temporal changes in DIC-13C at both site may give better idea about these interpretations.

Line 673 Hunter et al. 2012b. There is no Hunter et al. 2012a, thus deleted “b”.

Table 1 Please add a new column showing incubation periods.

Figure 2. Please add “n.d.” for meiofauna and foraminifera of Ythan sand flat.

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