Review of Woulds et al. 2019. Benthic C fixation and cycling in diffuse hydrothermal and background sediments in the Bransfield Strait, Antarctica. DOI: 10.5195/bg-2019-198.

General Comments

The manuscript by Woulds et al. describes a series of stable-isotope pulse chase experiments conducted at three sites, associated with diffuse venting of hydrothermal fluids in Antarctica. The paper is novel and describes some elegant experiments which seek to disentangle the role of chemosynthetic pathways in the benthic carbon cycle at these sites. The paper provides compares benthic fixation of ^{13}C-labelled bicarbonate and processing of 13C-labelled phytodetritus, to determine the relative role that chemosynthetic pathways play within the benthic carbon cycle of diffuse hydrothermal vents and encompasses measurements of both bacterial and macrobenthic ^{13}C-uptake. This provides a very exciting study, which allows the relative roles of the bacteria and fauna to be compared between the study sites. This study is ambitious and provides a valuable contribution to our understanding of the biogeochemistry of deep-sea sediments. However, it also has a number of notable flaws that need to be addressed prior to publication. These are outlined below:

Main points requiring revision.

1. Given that you processed only half a 10 cm core for either bacterial or faunal analysis, care needs to be taken in the interpretation and extrapolation of the data. Each replicate of the experiment sampled only 0.0039 m^2 of the seafloor for either the faunal or bacterial community. Subsequently the data is scaled to units.m^{-2} which is an area ~256 times larger than the area sampled. Like many colleagues in the deep-sea and marine ecology communities, I have made very similar decisions with some of my own papers. However, I wonder whether this is really an appropriate standardization to make. Scaling our data like this inevitably propagates errors from single core incubations up to geographically relevant macro- and meso-scales. I would ask the authors to consider standardizing their data to a smaller areal size (such as cm^2 or 10 cm^2). This would provide a more honest description of the results.
2. Error Bars reporting standard deviations are plotted on Figures 2 and 3, yet only two replicate cores where incubated at each study site. Based upon a sample size of *n*=2

it does not make sense to calculate a mean or standard deviation, as the mean will always be halfway between the two values. Please remove reference to the standard deviations as a measure of variation within the text and revise Figures 2 and 3 to show the individual values for each replicate (as you have done in Figure 5). In terms of future experimental design, *n* = 2 is not really an adequate sample size to allow assessment of differences between sites (for details refer to Sokal and Rolff, 1994, or Underwood, 1997). The novelty of these experiments as an observational study of carbon cycling in a poorly explored region of the oceans, however, warrants their publication.

I also have a number of minor comments which I would ask the authors to address prior to publication.

Minor Comments

Title

Page 1 Line 1: Should read "Benthic carbon fixation…."

Abstract

Page 1 Line 14-15: "There are no previous direct…" This sentence is not required. Please delete the sentence.

Page 1 Lines 15-16: Remove paragraph break.

Page 1 Lines 21-22: Remove paragraph break.

Page 1 Line 22: Revise to: 'Fixation of inorganic C into bacterial biomass was observed in all cores/sites.' Please revise as suggested.

Introduction

Page 3 Line 30 – Page 4 Line 85: Throughout the introduction there are many uses of 'therefore' and 'however'. 90 % of the time these words are superfluous. Please revise the introduction to make less use of them.

Page 3 Line 32. Split this into two sentences. '…dissolved sulphides and methane. This supports microbes that combine…'

Page 4 Line 63-64: Do you have any supporting literature that can be cited to support this sentence.

Page 4 Line 72: 'On the contrary however' please revise, this is not well phrased.

Page 4 Line 77: Delete sub-heading

Page 4 Line 80: Hypotheses should be 'tested' not 'addressed'

Methods

Page 5 Line 108-119: This provides a brief summary of the experimental methods. Please refer to an alternative source as (following…) where a more detailed description of the method can be found.

Page 5 Line 111-112: *Chlorella spp.* phytodetritus would not be representative of the algal material processed in Antarctic systems. A diatom would have been a more appropriate choice of ^{13}C-labelled substrate.

Page 5 Lines 119-119: Half a core seems to be a very small volume of sediment for conducting macrobenthic analysis. Given that the size range of macrobenthic fauna is variable, and species are mobile, is this sample volume appropriate? I get the impression that you may be missing something significant by only focusing on half a core for the bacterial and macrobenthic communities.

Page 6 Lines 151-158: A lot of potential data has been discarded from the PLFAs by just focusing on four 'bacteria-specific' fatty acids. It would be interesting to see the full profiles, particularly as the 13C-labelled bicarbonate treatment may reveal some insight into which PLFAs might be good indicators of microbial carbon fixation.

Results

As previously mentioned, I am not content with the use of standard deviations to describe variation in the data. Where n = 2, you cannot reliably calculate means or standard deviations.

Page 7 Line 168: 'In *the* algae addition experiments…' Please revise.

Page 8 Lines 174-181: I think you could potentially offer more insight into the microbial processes by considering a wider range of PLFAs for each site. Which PLFA groups showed greatest label uptake?

Page 8 Line 175: Normally C19:0 is used as a standard in the PLFA analysis which may explain why it is found in higher concentrations.

Page 8 Line 183: Please revise to 'Faunal uptake of added C differed between the two replicate cores in all experiments…'

Page 8 Line 191-192 and Figure 6: Given the small sample size, I am not convinced that a community level analysis of faunal feeding responses is appropriate. Differences in faunal uptake are likely to be driven by spatial variability, with common taxa such as polychaetes heavily overrepresented. This leads to the 'mixed macrofauna' category essentially consisting of everything except polychaetes.

Page 8 Line 191 – Page 9 Line 199: In light of the small sample size please don't refer to dominance either in terms of faunal abundance or feeding responses. It would be more appropriate to discuss simply which groups were more/less abundant and exhibited greater/weaker uptake of the ^{13}C-label.

Page 8 Line 196- Page 9 Line 199: This last sentence is confusing, please revise and clarify.

Discussion

There is frequent use of 'therefore' and 'however', please remove these where possible.

Page 9 Lines 202-212: This paragraph is a description of the results. Please revise to contextualize your findings.

Page 9 Lines 220-222: Long sentence, requires broken up. Please revise.

Page 9 Lines 223 Page 10 Line 228: Please revise along the lines of "This is supported by a recent modelling study which suggested that…. (Bell et al 2017b). Similar results have also been reported from the methane-rich non-hydrothermal sediments… (Woulds et al., in press).

Page 10 Line 242-248: I am afraid that this is a major flaw in the overall paper. Given that temperature is critical to microbial metabolism, the current paper is likely to seriously

underestimate the level of carbon fixation. This needs to be made clearer earlier in the paper.

Page 11 Lines 273-275: Based on two replicates, it would only be possible to discuss the magnitude of the differences and perhaps compare these between sites. Remove reference to standard deviations from the discussion.

Page 12 Line 285: Delete ‘rather’

Page 12 Line 298: Revise to ‘…Branfield Strait. Therefore…’

Page 12 Lines 298-299: Here you are discussing the effects of temperature on metabolic rates. Here you should consider the impacts of rate limitation and do a quick literature search. There is quite a large body of literature on this topic.

Page 12 Line 302: Delete ‘Thus’

Page 13 Line 324-325: Based on the Q_{10} effect, metabolic activity increases logarithmically with temperature. As such, a change of 1^{o}C may be more significant than you assume. I think this may require further explanation.

Page 13 Line 339: Delete ‘and thus high biomass benthic communities.’

Page 13 Line 341: Delete ‘Further’ and replace ‘while’ with ‘Whilst’

Page 13 Line 341-342: The comparison between sites was limited by the size of each sample (half a core), and lack of replication (n = 2). Your experimental design does not allow you to make any inferences on faunal patchiness.

Page 14 Line 347: You cannot use the term ‘significant’ as this implies the use of inferential statistical tests. Please revise.

Page 14 Line 354: Replace ‘dominant’ with ‘main’

Page 14 Line 357-358: ‘Therefore the hydrothermal site (Hook Ridge) in this study was not the hotspot of C-cycling that we hypothothesised it would be.’ You need to define what is meant here by a ‘hotspot of C-cycling.’ Is this referring to chemosynthetic carbon fixation?

Page 14 Line 358-359: Delete paragraph break.

Page 14 Line 362: Delete the final sentence.

References

Sokal, R.R., Rolff, F.J. (1994). Biometry: The Principles and Practices of Statistics in Biological Research [880 pp.]. New York, USA: W.H. Freeman.

Underwood, A. J. (1997). Experiments in ecology: Their logical design and interpretation using analysis of variance (524 pp). Cambridge, UK: Cambridge University Press.