Here follows the reviewer comments from reviewer 1 (black) along with our responses (blue). We thank the reviewer for their helpful comments and for their detailed attention to the present manuscript. These comments were prepared by the lead author (JB Bell), with all co-authors given opportunity to comment.

Interactive comment on "Hydrothermal activity lowers trophic diversity in Antarctic sedimented hydrothermal vents" by James B. Bell et al. Anonymous Referee #1

Received and published: 4 October 2016

General comments: This is an interesting paper and is within the remit of Biogeosciences journal. This paper presents new data on the trophic ecology of Southern Ocean macrofaunal communities in sedimentary environments where hydrothermal activity is present. The manuscript gives a very thorough description of the trophic ecology, encompassing bacteria and the fauna spanning a range of analytical and statistical techniques. The manuscript is well written. I have listed more specific and technical comments below.

Specific comments: Throughout the manuscript you refer to vent and non-vent sites and there are a few sampling sites within each of these descriptors. I would suggest that each time before you mention a specific site e.g. Hook 1 you state whether it is a vent or non-vent site. You have done this most of the time but not all of the time and it will provide further clarity for a reader not familiar with this area.

We have amended several instances of the site nomenclature for clarity.

Introduction

Line 89 you suggest that SIA is a powerful tool to assess spatial and temporal trends in faunal behaviour, I think this should be clarified e.g. with the word 'feeding' before behaviour.

Line amended according to reviewer suggestion

Line 91-93: you suggest that stable isotopes can be used to distinguish between methane or dissolved organic matter as a food source for macrofauna. Has this been done for natural abundance isotopes? If so could you provide a reference?

The references provided on line 95-96 give examples of where SIA has been previously used for *Siboglinum*. We request that interested readers refer to these for relevant detail.

I would like to see further explanation of the use of stable isotopes in trophic ecology e.g. how different fixation pathways influence the isotopes you use (CNS: see Levin et al. 2002).

We have added some additional detail to lines 91-94 for clarity, which read: "Stable isotopic analyses provide inferential measures of different synthesis pathways and can elucidate a wide range of autotrophic or feeding behaviours. Carbon and sulphur isotopes are used here to delineate food sources and nitrogen is used as a measure of trophic position (Fry et al. 1991, Levin & Michener 2002)."

Some inclusion of natural sources of OM would also be good e.g. if there is data for CN isotopes of ice algae, POM or phytodetritus in the area. It would also be good to explain how this changes with trophic position. I realise that this is repeated often in the literature however, not all of your readers will be experts in isotope ecology.

We have added some additional discussion of the literature on lines 95-97 for carbon isotopic signatures of possible food sources in the Bransfield Strait.

The hypotheses section is good. However, you do not refer to them in the same order in the discussion. I would suggest changing the order of the hypotheses in the introduction to reflect the order in which they are referred to in the discussion.

Hypotheses re-ordered as per reviewer suggestion

Methods

Line 143: you state you summarise the PLFA method of Main et al. (2015) method, yet the methodology you give is lengthy. Is there a way to shorten it?

To summarise the method further would inevitably miss methodological details, after which it may just as well be reduced to one sentence referencing Main et al. For the time being, we have decided to keep this section as is and if further comments suggest that it is not necessary, we will consider removing this section and replacing it with the reference only.

Lines 198-200: This is too much detail. If you do not include these data in the paper you do not need to state why here. You give precision data for the reference materials I presume this is from the laboratory and international standards. What was the precision for the *Antimora*?

The precision data for the *Antimora rostrata* reference material is given on lines 198-199. We have amended line 198 to clarify.

You calculate Layman metrics for C and N isotopes. Could you also have done this with S and N isotopes, I realise you have fewer data points for S. It would be interesting to compare the trophic niches for different isotopes.

As detailed in the legend for Table 4, we did not calculate Layman metrics for S vs. N, since the problem with those data is more fundamental than a low number of replicates. Owing to the increased sample mass required, we could not obtain as representative a proportion of the total assemblage as for CN isotopic data. Therefore, the resultant metrics would be heavily biased towards those fauna with higher mass. We also could not obtain sulphur isotopic measurements for siboglinids from Hook Ridge 2, the Three Sisters or the Axe, meaning that assemblage level metrics of sulphur isotopic signatures for these sites would not be meaningful.

That said, we agree that a comparison of layman metrics between CN and SN would be a useful exercise (or even a tri-isotope layman metric format), but unfortunately we feel that it is beyond the reasonable reach of the data we present here.

Results

General comment, where you state a numerical result e.g. 84% or -15‰ always give the unit after the figure/number, even when you state a range e.g. 64% - 95%. You have done this some of the time so just remember to be consistent.

We have corrected several instances of this error according to the reviewer's suggestions.

Line 272: Is it necessary to state 0.85% of the reads to two decimal places? And also for the figures stated in the next paragraph.

Corrected to one decimal place at the reviewer's suggestion

Line 289: I could not find the supplementary figures. I had the supplementary tables but not the figures.

This is surprising as all three supplementary figures were uploaded into the sample .zip file as the tables. I have just successfully downloaded the entire package from the manuscript page on the Biogeosciences website so perhaps there was some fault within the reviewer portal. Please access the data <u>here</u>.

Line 328: how were the samples contaminated with marine carbonate?

Since we could not acidify samples for the reasons detailed in the manuscript, specimens with carbonate structures were physically decarbonated. Although every effort was made to use tissue with no carbonate material, it is possible that carbonate on some samples (e.g. peracarids) may have still remained attached. We suggest this only as a means to highlight that these data should be interpreted with caution since we believe that this effect may be noticeable in a minority of samples but was generally negligible. This is not to declare that these data are definitely erroneous. We have amended lines 337-339 to clarify.

Line 335: I cannot review the supplementary figures.

See earlier comment, regarding Line 289.

Line 337: rejection of hypothesis 1, I would leave this for the discussion.

Sentence amended at reviewer suggestion.

Discussion

You mention in the methods that the two Hook sites had variable hydrothermal activity. It would be nice to have a short description of this e.g. temperatures, methane concentrations etc. I found this paper below, which could help with this.

G. Bohrmann, C. Chin, S. Petersen, H. Sahling U. Schwarz-Schampera, J. Greinert, S. Lammers, G. Rehder, A. Daehlmann, K. Wallmann, S. Dijkstra, H.-W. Schenke Hydrothermal activity at Hook Ridge in the Central Bransfield Basin, Antarctica. Geo-Marine Letters (1999) 18: 277-284

We have added the extra detail requested by the reviewer on lines 123-126, immediately following the site description.

Line 378: could you state whether this is high or low hydrogen sulphide flux.

We have amended the sentence to clarify. It now reads "organic carbon content, hydrogen sulphide flux and taxonomic diversity were all lower at this site and may support suggestion of a lower overall bacterial biomass"

Line 383: I would state 'basal carbon source e.g. DIC

Amended according to reviewer suggestion

Line 390-391: Is this in your study? If so start with in this study or we have shown..., if this has been shown elsewhere as well give a supporting reference.

We have amended line 390 to clarify that the following statement is based upon our own results. We are not aware of similar results, as PLFAs have not so far been widely used in chemosynthetic ecosystems.

Line 393: do you mean isotopic signatures, when you refer to signatures here? Or do you mean

distributions of PLFAs? I am guessing the former.

We have amended line 392 to clarify. The statement refers to isotopic results as the reviewer guessed.

Line 396: Macko and Estep (1984 Organic geochemistry 6: 787-790) demonstrate nicely the high variability associated with bacterial remineralisation of organic matter. It might be a useful reference to include here and to think about for this section of your discussion.

We have added some discussion of Macko and Estep (1984) to section 4.1 (line 409 onwards) to provide some experimental context for the PLFA discussion.

Line 401: There must be an earlier paper describing the carbon isotope signatures associated with the rTCA cycle.

We argue that the review article 'Hugler & Sievert (2011)' is a valid reference in this case. Reid et al., (2013) is provided for hydrothermal vent context.

Line 424: You suggest that PLFAs with large ranges d13C ranges are indicative of methane and sulphur cycling, however small ranges around e.g. -50 per mil could also be indicative of methane cycling...

The reviewer is correct to suggest that range may be a misleading term. The sentence referred to the ranges in isotopic values between sites for the same PLFA (hence a wide range is likely to mean that the synthesis pathway may have been different in each case). We have amended the sentence to clarify, which now reads "However, it should be stressed that all PLFAs with larger δ^{13} C differences between sites were comparatively rare"

Line 433: you discuss the isotopes of Sclerolinum and Siboglinum and refer to figure 2 here, however it is not possible to easily pick out, which points on the plot are in fact these two species.

We have amended the reference to figure 2 to provide clarification. It now reads: "Both species of siboglinid (*Sclerolinum contortum* from Hook Ridge and *Siboglinum* sp. from the non-vent sites) were clearly subsisting upon chemosynthetically derived organic matter, as evidenced by their morphology and strongly depleted isotopic signatures (see values with δ^{15} N of < -2 ‰ in Fig. 2).

Lines 625: You mention sea-ice algae here. Perhaps you could mention this in the introduction.

We have added mention of sea ice algae in the discussion

Conclusion

I think you are well aware of the patchiness of deep-sea soft sediments. Although you have processed a considerable number of cores for this study, due to the nature of the high heterogeneity in the deep-sea this made it unlikely you would hit a bacterial mat or an area that may be more heavily dominated by chemosynthetically driven food webs. Perhaps this should be taken into account in the conclusion section.

We have added comments to this effect on lines 615-618 (discussion section 4.5) as we feel that, although the reviewer makes a valid point, that the discussion is a more suitable point at which to elaborate upon this potential oversight.

Once again, we would like to thank the reviewer for their time and insight. The resultant manuscript is certainly clearer and comprehensive.

Technical comments

Line 19: change to 'Sedimented hydrothermal vents are those in which hydrothermal fluid is discharged through sediments and...'

Amended according to reviewer suggestion

Line 30: remove 'the' after 'suggesting that'

Amended according to reviewer suggestion

Line 64: change 'but also accelerates' to 'whilst accelerating'

Amended according to reviewer suggestion

Line 68: change 'but active communities...' to ' however, active communities are also...'

Amended according to reviewer suggestion

Line 81: place the reference Bell et al. 2016 at the end of the sentence.

Amended according to reviewer suggestion

Line 114: I would move the three sites in brackets to the line below before the 'Aquilina et al' reference.

Amended according to reviewer suggestion

Line 117: change to 'With the exception of salps,'

Amended according to reviewer suggestion

Line 183: I do not think you need to provide an acronym for East Kilbride and I would remove it from line 187 so it should read 'Samples were analysed by continuous flow...'

Amended according to reviewer suggestion

Line 217: you can remove EK here.

Amended according to reviewer suggestion

Line 290: change to (4.8% - 16.9%, Table 2).

Amended according to reviewer suggestion

Line 315: in brackets I would change to (non-vent sites)

Amended according to reviewer suggestion

Line 320: change to (means x to x, respectively, Fig. 2).

Amended according to reviewer suggestion

Line 347: replace 'but' with 'however'

Amended according to reviewer suggestion

Line 359: change to ' between any of the non-vent sites.'

Amended according to reviewer suggestion

Line 363: replace 'but' with 'however'

Amended according to reviewer suggestion

Line 375: replace 'but' with 'and'

Amended according to reviewer suggestion

Line 385: insert 'the' after 'but'

Amended according to reviewer suggestion

Line 387: remove 'for' after notable

Amended according to reviewer suggestion

Line 408: remove a bracket

Amended according to reviewer suggestion

Line 413: refer to as non-vent sites

Amended according to reviewer suggestion

Line 446: need a space between 'around 10'

Amended according to reviewer suggestion

Line 455-457: missing units here.

Amended according to reviewer suggestion

Line 461: too many brackets around reference

The brackets also encompass some detail on the reference, the double brackets are correct in this instance Line 463: suggest dropping some references here

We have removed two references from this line that only appeared once in the manuscript.

Line 465: insert 'abundance of Methylohalomonas, constituting 2.1 to 4.3% of sequences...'

Amended according to reviewer suggestion

Line 495-496: place reference at end of sentence.

Amended according to reviewer suggestion

Line 529 and 530: too many brackets around reference

As in the example on line 461, these references are double bracketed because they are accompanied by relevant data from each of the references.

Line 534: This is the first time you mention 'AOM' can you spell out the abbreviation at first mention please

Amended according to reviewer suggestion

Line 552: replace but with and

Amended according to reviewer suggestion

Line 558: missing bracket.

Amended according to reviewer suggestion

Line 575: replace 'was' with 'were'

Amended according to reviewer suggestion

Line 579: too many brackets around reference

Amended according to reviewer suggestion

Line 599: change to 'suggested for deep-sea ecosystems. . .'

Amended according to reviewer suggestion

Line 603: too many brackets

Brackets and contents therein removed for clarity

Line 613: replace 'was' with 'were'

Amended according to reviewer suggestion

Line 617: insert 'heavily' before 'influenced'

Amended according to reviewer suggestion

Figure 2 and 4: could you be consistent with figure 4 and use triangles and circles to represent vent and non-vent sites?

We note the lack of continuity between the figures and have amended to make the figures more consistent. We have also altered figure 3 to the same end.

Figure 4: what are the error bars?

Amended according to reviewer suggestion

Line 955: too many brackets around reference.

Amended according to reviewer suggestion