Here follows the reviewer comments from reviewer 3 (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. We thank the reviewer for their insightful comments and have endeavored to fulfill their suggestions in the revised manuscript.

Interactive comment on "Hydrothermal activity lowers trophic diversity in Antarctic sedimented hydrothermal vents" by James B. Bell et al.
Anonymous Referee #3
Received and published: 29 November 2016

General comments:

I think the data reported in this study is still valuable, but it is not suitable for publication at this time.

There are no previous studies that have presented comparable data from the Southern Ocean as detailed in this manuscript. Given the novelty of these data and the relatively minor comments offered by the reviewer, we find it surprising that the reviewer feels that the present manuscript is unsuitable. Both reviews have been broadly positive and we have taken all steps to address the concerns that they have raised. By making these alterations and expanding upon the manuscript where suggested, we believe that the revised manuscript represents a substantial improvement upon the original submission. This manuscript represents an important step forward in our understanding of the structure and function of deep-sea biology communities in the Southern Ocean and at sedimented hydrothermal vents and is widely relevant to several marine science disciplines.

This paper reports microbial and biogeochemical data obtained from the recovered sediments at and near hydrothermal vent and non-vent fields in Bransfield Basin. Such data is quite limited from the basin, even Southern Ocean, so this is a possible first systematic report.

These data have been collected as part of the ChEsSo (chemosynthetically-driven ecosystems south of the polar front) research programme and are part of a suite of papers that are the first to examine the ecology of these systems. The reviewer is correct that this is the first time that microbial data and metazoan stable isotope data have been reported from this site (or indeed from any sediment-hosted vent system in the Southern Ocean). Assemblage data and pore water geochemistry have been reported in previous studies (Dahlmann et al. 2001; Klinkhammer et al. 2001; Aquilina et al. 2013; 2014; Bell et al. 2016b) and we encourage interested readers to refer to these studies that expand upon the scope of the present paper.

The authors mentioned that such sedimented hydrothermal systems (or commonly called sediment-hosted hydrothermal system, not only vent!) are the least studied deep-sea ecosystems, however, Okinawa Trough, which is a similar sedimented basin involved hydrothermal activities, have been studied for a long time. I cannot understand why authors ignore a lot of previous studies performed in Okinawa Trough, Southwestern Japan. For example, recently many related studies have been published as an open access book from Springer (http://www.springer.com/jp/book/9784431548645). Sedimentary fatty acids (not only PLFAs) were also studied by Yamanaka and Sakata (Org. Geochem. 35: 573-582, 2004). Authors should compare their data with those previous studies.

We have included several references from Okinawa trough sediment-hosted vent systems as suggested by the reviewer. However, we had not originally included much of the material in the TAIGA book as it is more relevant to hard substratum vent systems, which have some

considerable physical dissimilarities to the vent system discussed in the present paper. It was not our intention to overlook the valuable studies in the TAIGA group and we acknowledge that there are several instances of papers that are now rightfully cited in the present paper. We thank the reviewer for alerting us to these studies. We have also endeavoured to expand discussion of various aspects in the light of the data presented from the western Pacific vent systems to redress this oversight.

Specific comments:

In the sediment-covered hydrothermal field the physicochemical condition of surface sediment is quite heterogeneous. So more careful consideration is required. In addition, description of core samples was almost lack. Authors cited previous study (Bell et al., 2016), but some parameters such as Cl-, H2S, and methane should be provided for reader. This information is related to evaluation of sample heterogeneity. And also please show bathymetric map of the Bransfield Basin with sampling sites.

Sediment geochemistry is discussed in detail in the references by Aquilina et al. (2013; 2014). In our paper from earlier this year (Bell et al. 2016b), we discuss in detail the impacts of the sediment geochemical heterogeneity upon faunal assemblages. We recommend that interested readers refer back to these studies for this information, since it would not be appropriate to repeat it in the present paper. Bell et al (2016b) also contains the bathymetric map figure, but please note we have now included this as Figure 1. We have also added an additional table giving information information on the various sites, rather than referring to original papers, at the reviewers' suggestion.

Authors displayed the isotopic data to two decimal places, but S.D. of instrumental analysis is not so small. The last decimal is so significant?

We have converted isotopic values given in the text to 1 decimal place at the reviewer's suggestion.

For sulfur isotope analysis of organisms and sediments it is quite important for complete removal of seawater sulfate. I could not find any description about sample preparation for sulfur isotope analysis in the manuscript. Sulfur data in this study, especially sediment data, is incredible for me.

All of the details of sample preparation are given in section 2.4. Faunal samples were preserved in ethanol or formalin prior to sorting and selection for isotopic analyses. Sediment samples were freeze-dried. All samples (faunal and sediment) were rinsed in de-ionised water to remove any precipitates formed during drying. Rinsed samples were then dried and analysed according to the instrumental set up detailed, using a tri-isotope analysis procedure at the Natural Environment Research Council facility (i.e. each sample was converted into CO_2, N_2O and SO_2). This is consistent with previous CNS measurements (e.g. Reid et al. 2013; Bell et al. 2016a) at this facility and error in $\delta^{34}S$ measurements was broadly similar to $\delta^{13}C$ and $\delta^{15}N$.

A typical faunal sample (2.5 mg) was comprised of approximately 0.62 % sulphur by weight and contained around 0.015 – 0.030 mg of sulphur. If there had been contamination from a seawater sulphate endmember $\delta^{34}S$ value of ~+20 ‰ (similar to sinking OM in our paper), we would not expect to see such a wide range of $\delta^{34}S$ values within our dataset (range = 46.7 ‰). Contamination would also have meant that many of the samples with more depleted $\delta^{34}S$ measurements would had to have had exceptionally low organic $\delta^{34}S$ signatures, which seems unlikely. We are therefore confident that the sulphur data presented are accurate and of sufficient reliability. We hope that this has clarified our analyses.

Authors performed PLFA analysis and identified many PLFAs, but discussion of those origins is insufficient. I think this data contain some important information of organic matter sources necessary to discuss.

We have expanded the results and discussion of the PLFA analysis at the reviewer's suggestion, including the references listed in the earlier comment. We also invite readers to view the supplementary material, which includes a full graph of the PLFA suite and a PCA ordination that compares the PLFA data from our study to other sites (both hydrothermal and background sites). However, it is difficult to discuss the organic sources in great detail, as there remains considerable uncertainty in the specificity of PLFAs to particular synthetic processes. Data such as are presented here are therefore very important to improving the knowledge base of these potential biomarkers.

Line 328: Authors could not avoid the possibility of inorganic carbonate contamination, why did not authors treat the samples with acid?

We have amended the text to explain more fully the nature of the possible carbonate contamination (see comments from Reviewer 1 and our response for more details). As detailed in section 2.4, we elected not to acidify the samples. This was owing to the small size of the faunal specimens (and thus low sample mass) and results from a pilot study, which indicated that acidifying specimens did not have a consistent effect upon carbon isotope ratios, but risked adverse impacts on the S and N isotopic data. Additionally, the low sediment porewater pH meant that there was very little naturally occurring sediment carbonate.

Line 435: Do authors have any other evidence of nitrogen fixation? Such negative values is often found in chemosynthesis-based animals.

We have provided several references that corroborate our assertion that the very low nitrogen isotopic signatures are likely to be associated with nitrogen fixation. Further evidence is that only the obligate chemosynthetic species (i.e. the siboglinid polychaetes) had such low $\delta^{15}N$ values. Background fauna at vent and non-vent sites all had much higher $\delta^{15}N$ signatures. We also present evidence of microbial lineages in sediments that are relevant to biological nitrogen processing (e.g. *Nitrospira* or *Nitrosomas*). We have amended this section to clarify this point.

Line 438-439: Really SRB facilitate nitrogen fixation? Please indicate reference.

Nitrogen fixation has been demonstrated concurrently with various chemoautotrophic processes. It is difficult to be confident with the data available that this was definitely the case, but the combination of the isotopic values of *Siboglinum*, relative to sediment OM seemed to suggest that such processes were active. We have amended the sentence to improve clarity. Please see the following reference for details:

Desai MS, Assig K, Dattagupta S. Nitrogen fixation in distinct microbial niches within a chemoautotrophy-driven cave ecosystem. Isme Journal. 2013; 7(12): 2411-23.

Line 444-453: Discussion about carbon isotope ratio of DIC is thrown into confusion.

Here we have shown that the approximate isotopic signature of the DIC is known in the Southern Ocean and that it can be related to carbon isotopic signatures of *Sclerolinum contortum*. However, because DIC $\delta^{13}C$ can change via various processes in hydrothermal sediments (Walker et al. 2008), we cannot confidently assert which chemosynthetic reaction pathways were most active in the Bransfield Strait. We have amended the relevant section

to attempt to improve clarity of this point.

Line 455-458: re-dissolved sulfide means the following reaction?

$$2FeS2 + \text{organic matter } (CH2O) + 2H2O = 2FeS + 2H2S + H2CO3$$

This reaction is expected to occur at high temperature (>300-dgree C) (Seewald et al., 1994 GCA 58: 5065-5082). So it is expected high temperature fluid discharging near the sampling point. Maybe hydrothermal precipitate, which have quite low _34S values (< -5‰) originate in bacterial pyrite dissolution, but. . .

In addition to the reaction pathway described by the reviewer, sulphide mineral dissolution and contribution to metazoan food webs has been found at inactive (and thus low temperature) hydrothermal vent fields (Erickson et al. 2009). The hydrothermal precipitates at Hook Ridge are thought to originate from a previous period when high-temperature venting was active at this site (Klinkhammer et al. 2001). We have amended these lines to improve clarity of this point.

Line 474-475: Carbon isotope ratio of methane is easily changed by bacterial consumption (enriched in 13C). Those methane values were reported from the same core samples?

We have provided a reference (Whiticar & Suess 1990) that sampled methane isotopic values from several sites across the Bransfield Strait and we have given the range of these values in the text. These values correspond very closely to the *Siboglinum* carbon isotopic signatures, meaning that this is in our opinion the most likely source of carbon for this species. Given the strong difference in carbon signatures between this obligate chemosynthetic species and the other members of the food web (~15 ‰), it is unlikely that *Siboglinum* was relying upon the same carbon source.

Line 482: sulfate reducer? Really??

Given the very low $\delta^{34}S$ signatures of this species (-22.85 ‰), it seems likely that sulphate reduction was active amongst its endosymbionts. We have provided supporting references and are not aware of other sulphur reaction pathways that would have been likely to elicit such signatures in this context. We have also presented evidence of a wide range of microbial lineages associated with sulphur cycling.

Fig. 3: This figure makes no sense to me. It is difficult to compare the difference because X-Y scales vary among species.

We appreciate the reviewer's concern but feel that this figure is still correct. The point of this particular figure is not to facilitate comparisons between species, rather comparisons within species from vent and non-vent areas of the Bransfield Strait. The X-Y scales are intentionally varied to allow the reader to see clearly where there are differences in isotopic signatures of a particular species between sites. Figures 2 and 6 show the bivariate data in a more standard format.

Line 607: What is the cause of the environmental toxicity? Hydrogen sulfide? Low DO? Heavy metals?

It is likely to be a combination of these effects and thus is difficult to say confidently which of the stressors is most influential. We have amended the text to clarify this point.

Other comments:
This manuscript contains many typos. Please check carefully.

We have corrected several instances of typographic and grammatical errors.

In section 1, I cannot find subsection 1.1 and 1.2.

We have corrected this error at the reviewer's suggestion.

Line 416: 19:1w8 PLFA is not PUFA (poly-unsaturated fatty acid). It is monounsaturated fatty acid. And also indicate PUFA stands for. . .

We have corrected this error and added the explanation of the acronyms PUFA and MUFA.

We thank the reviewer for their helpful comments regarding the manuscript and hope that we have taken adequate steps to mitigate their concerns regarding the points raised above.