

**Anonymous Referee #1**  
**Received and published: 18 August 2017**

This paper reports the food ecology of macrofauna and possible food source, that is microbial communities in the sediments obtained from hydrothermal and on hydrothermal areas in Southern Ocean based on CNS isotope compositions and molecular phylogenetic and PLFA analyses. This study is a sequel to the previous paper about macrofaunal ecology of the same area written by the same authors.

The conclusions led by the analytical results are almost adequate, but the discussion is quite lengthy and is not straightforward. It can be shortened and simplified.

**Owing to the multiple lines of evidence, the discussion as it stands is lengthy. We agree with this appraisal and will ensure that the revised manuscript focuses more strongly upon the hypotheses in order to improve readability, as suggested by the other reviewer.**

Individual points to be improved:

P14 lines 297-304: I cannot find any associated tables and figures mentioned in the texts.

**We will add reference to figure 1 (microbial composition data).**

P17 lines 358-362: What is the “four clusters”? And which figures and tables are related to this paragraph?

**The “four clusters” refer to the Euclidean distance matrix used to delineate sub-structure in the isotopic data. Figure 5 and supplement 3 are related to this paragraph, which we refer to in the text. We will amend the text to improve clarity. We will also expand discussion of the cluster results but will keep in mind not to increase the length of the overall discussion.**

Food ecology of siboglinid species (chemosynthesis-based or not) must be discussed before the section 4.1 (difference of microbial assemblages and those biomass among each site). And this discussion is related to the hypothesis 1, right?

**Discussion concerning food sources of the siboglinids does relate to hypothesis 1, but we would prefer to re-order the hypotheses. We will then have the results and discussion section following a structure of microbial signatures, through individual faunal signatures up to community metrics.**

P21 lines 444-445: Long chain fatty acids originated in land plants are derived as form of triglyceride (wax). They are not PLFA.

**We will correct instances where fatty acids are mislabeled as PLFAs**

P24 lines 545-546: *S. consortium* endosymbiont use only DIC in pore fluid? I think the symbiont use mainly DIC in bottom water. Because the siboglinid worm is not infauna, right?

***Sclerolinum contortum* is an infaunal species so our discussion DIC sources is accurate. We will amend the text to improve clarity of this point.**

P25 lines 548-: The previous studies (Klinkhammer et al., 2001, Aquilina et al., 2013) indicated presence of hydrogen sulfide in the sediments. The H<sub>2</sub>S concentrations were increasing with depth and sulfate concentrations in the pore fluids were decreasing with depth. It possibly suggests that active microbial sulfate reduction is occurred below seafloor. Therefore, very low sulfur isotopic signature of the siboglinid worms mainly associated with microbial sulfide. Mineral sulfide dissolution is not necessary (but hydrothermal fluid input can not be ignored).

**The reviewer’s suggestion is potentially supported by our data and is valid. We will amend the relevant text to include this possibility.**

P26 lines 585-587: If the siboglinid worms harbored thioautotrophic endosymbiont, sulfur isotopic ratios of the worm reflect the ratio of hydrogen sulfide. Therefore, the difference of 6 ‰ is meaningless.

**The 6‰ highlights that the Bransfield Strait are lower than siboglinid worms found in other locations and puts the Bransfield Strait worms in a wider ecological context. The sulphur isotopic ratios of mineralized sulphide in the Bransfield Strait (Petersen et al. 2004) vary widely and their signatures do overlap with those of the siboglinids presented here. However, the reviewer's comment does not consider the role of trophic fractionation, which can easily account for large differences in isotopic signature in sulphur metabolism.**

P27 line 610: "Salp samples were also lighter than...", what is lighter? Carbon isotopic ratio?

**The Salps had a lighter  $d^{13}C$  value than values of sedimentary organic carbon. We will make sure this comparison is added to the text.**

P28 lines 633-635: The sediment samples using this study were not removed pore fluids sulfate before analysis. So the sulfur isotope data include  $34S$  rich sulfate originated in pore fluid. In addition, organic sulfur originated in photosynthetic organic matter, which also enriched in  $34S$ , is main component of the sedimentary sulfur. Possible another sulfur source in the sediment is bacterial and/or hydrothermal sulfide (mainly form of pyrite). Why you mentioned only sulfide oxidation?

**Sediment samples were drained of pore fluids, freeze-dried and then rinsed in de-ionised water, thus traces of sulphate should have been removed as far as possible. Photosynthetic organic sulphur likely remains the major component as the reviewer correctly points out but the vent areas still have lower  $d^{34}S$  values, indicating a source of isotopically light organic (or possibly mineral) sulphur, which we attribute to hydrothermal processes. We will amend text as necessary to improve clarity of this point.**

P30 lines 686-687: methane is not contained nitrogen. Lowest  $d^{15}N$  values cannot explain only methane.

**The text will be amended to remove reference to  $d^{15}N$  values.**

Other minor points The term "vent" means an opening that allows gas or liquid to pass out. This study is not discussed hydrothermal vent, but hydrothermal activity (it include venting and shimmering and any other ascending fluid). So, I think the author change the term "vent" into "activity" or "system (or area)".

**We will change the term "vent" into "activity" as requested by the reviewer. This will better capture the phenomena we are investigating because the manuscript is looking at the ascending fluids derived from sub-surface hydrothermal processes influence microbial and metazoan communities.**

P2 line 20: "among the least studied.." change to "one of the least studied.."

**Text will be amended as recommended by the reviewer.**

P14 line 288: I cannot find "Flavobacteriia" in tables and figures. It should change to "Bacteroidetes". "Sulphate reducing bacteria" should change to "sulphate-reducing bacteria".

**Text will be amended in both cases, as recommended by the reviewer.**