James Bell
University of Leeds
Leeds
LS2 9JT
UK

2nd October 2017

**Author Response to BG-2017-288 reviews**

Dear Biogeosciences Editors,

We are pleased to see that our manuscript 'Hydrothermal Activity lowers Trophic Diversity in Antarctic Sedimented Hydrothermal Vents' was well received by both reviewers, building upon the improvements made during the previous round of reviews. Many of the comments are technical issues, which are simple to rectify, and we will be pleased to make these changes, pending the editor's decision.

We agree that in places, the structure of the manuscript could be improved, particularly so for the discussion and we will focus the revisions upon improving the flow and readability of the manuscript as outlined below.

We propose to make the following changes (in bold, following each of the reviewer comments), and thank both the reviewers for their considered and helpful comments.

Thank you for your continued consideration of this article.

**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 have made efforts to ensure that the revised manuscript focuses more strongly upon the hypotheses presented in order to improve readability, as suggested by both reviewers.**

Individual points to be improved:

P14 lines 297-304: I cannot find any associated tables and figures mentioned in the texts.
**We have added more references to figure 1 (microbial composition data) in section 3.1.**

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 have amended the text to improve clarity (~Line 365). We have also expanded discussion of the cluster results (~Line 680) keeping in mind the feedback to reduce 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 (~Line 117-19). We now have the hypotheses, 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 have corrected several instances instances where other fatty acids are mislabeled as PLFAs or the entire FA suite has been referred to 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 have amended the text to improve clarity of this point (Section 4.2).**

P25 lines 548-: The previous studies (Klinkhammer et al., 2001, Aquilina et al., 2013) indicated presence of hydrogen sulfide in the sediments. The H2S 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 have amended the relevant text to include this possibility (~Line 599).**

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. We address the amendments more fully later in response to the editor's additional comment.**

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 macrofauna and sedimentary organic carbon. We have amended the text to improve clarity of this point (~Line 660).**

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 have amended the text in section 4.3 (~lines 688 – 702) to improve clarity of this point.**

P30 lines 686-687: methane is not contained nitrogen. Lowest d15N 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 have changed the term “vent” into “activity” or “hydrothermal” 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 has been amended as recommended by the reviewer.**

P14 line 288: I cannot find “Flavobacteriia” in tables and figures. It should change to "Bacteroidetes”.
**Bacterial genera have been added to a new table (see also Reviewer 2: comment 2).**

“Sulphate reducing bacteria” should change to “sulphate-reducing bacteria”.
**Text amended as suggested.**

**Anonymous Referee #2**
**Received and published: 9 September 2017**

I was asked to review the paper "Hydrothermal activity lowers trophic diversity in Antarctic sedimented hydrothermal vents" by James B. Bell, William D. K. Reid, David A. Pearce, Adrian G. Glover, Christopher J. Sweeting, Jason Newton, and Clare Woulds.

I find the paper well in the scope and focus of the Journal and the scientific work carried out is surely of high quality. Data are abundant, protocols and procedures of sampling and analysis are adequate and the techniques used are relevant. This manuscript is the natural continuation of the previous paper written by the same author pool on the same site and it completes the previous findings.

Although the results are interesting and well supported, I find the manuscript very long and often difficult to follow and wearisome. In particular, the discussion in not straightforward, lengthy and, in my opinion, it lacks a strong structure. Too often it winds and results tortuous, forcing the reading to go back in order to find the "fil rouge" to follow. I would warmly suggest to shorten the whole manuscript and in particular the discussion. In my opinion, the discussion should follow fewer clear, strong and important points, starting from hypothesis moving through the results and finally offering the conclusions and the answers to the main scientific questions.
**This point has been fairly raised by both reviewers. We agree that the discussion could be structured better and shortened in length and have addressed this point in the revision, through a clearer focus upon the hypotheses presented and reduction in overall length.**

I would suggest to insert some more tables and figures that better present the results: for instance, the data reported in the paragraph 3.1 lines 297-304 are not listed in any table nor well represented in a figure and this is a pity. Since the scientific and technical effort behind this work is huge, I would suggest trying to valorize it more by showing all the numbers and cite tables and figures more in the text than in the supplementary material.
**We have added a table detailing the major microbial genera sequenced from each site, complementing figure 2, as recommended by the reviewer. The present manuscript comprises 6 figures and 6 tables and is supplied with 3 additional supplementary figures. We believe that this covers the breadth of the key points and, with respect to the comments raised concerning the length of the manuscript, would recommend that no additional figures/ tables are necessary. We would however welcome the Associate Editor’s opinion on this point.**

I have only one strictly scientific comment to make: in lines 686-687 the authors say "Neotanaids from the off-axis site had the lowest d13C and d15N values of any non-siboglinid taxon (Fig. 5), suggesting a significant contribution of methane-derived carbon". This sentence may be misleading: while I agree that a lower d13C may suggest the metabolism of methane-derived carbon, I fail to see how a lower d15N signature may support this hypothesis, since methane does not contain N. It would be better to reformulate the sentence.

**We have removed reference to nitrogen isotopic values as suggested by the reviewer, so as to avoid confusion.**

**Associate Editor Comment**
**Received: 17 October 2017**

Thank you for your series of answers and discussions and also making revision in response to reviewers comments. Most of revisions are satisfied for us except for one point.

As Reviewer 1 made a comment,

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.

You have answered as follows.

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.

Trophic fractionation of sulfur isotope is small as similar to carbon isotopes. this is well known phenomena as that has already described in Fry, 1983; 1988 and Peterson and Howarth, 1988. If you do not agree on their opinion, you should refer following papers.

Fry B. (1983) Fishery Bulletin 81: 789-801
Fry B. (1988) Limnology and Oceanography 33: 1182-1190
Peterson B.J. and Howarth R.W. (1987) Limnology and Oceanography 32: 1195-1213

Then, you are requested to change following sentence to suggested one.

(your revision)
Sulphur isotopic signatures in Siboglinum spp. from Atlantic mud volcanoes ranged between -16.8 ‰ to 6.5 ‰ (Rodrigues et al. 2013) with the lowest value still being 6 ‰ greater than that of Bransfield strait specimens.

(recommended correction)
Sulphur isotopic signatures in Siboglinum spp. from Atlantic mud volcanoes ranged between -16.8 ‰ to 6.5 ‰ (Rodrigues et al. 2013), whereas the lowest value of this study was still 6 ‰ lower. It reflects the relative lower sulphur isotopic ratios of hydrogen sulphide yielding in the study sites (that is also suggesting that bacterial sulphide is main source of hydrogen sulfide).

**We have amended the sentence as suggested by the editor but would like to clarify that, whilst sulphur does not fractionate substantially between faunal trophic levels, there are a number of metabolic processes that are involved in sulphur cycling, which can result in substantial shifts in sulphur isotopic composition (e.g. Canfield DE (2001) Isotope fractionation by natural**

**populations of sulfate-reducing bacteria. Geochimica Et Cosmochimica Acta 65:1117-1124 or Habicht KS, Canfield DE (1997) Sulfur isotope fractionation during bacterial sulfate reduction in organic-rich sediments. Geochimica Et Cosmochimica Acta 61:5351-5361).**

End of comments

**Once again, we thank both the anonymous reviewers, Professor Kitazato, and the editorial staff for their handling of this manuscript and we look forward to concluding this submission.**

**Regards,**

**Dr James Bell (on behalf of the authors)**

# Hydrothermal activity lowers trophic diversity in Antarctic ~~sedimented hydrothermal vents~~hydrothermal sediments

James B. Bell[1,2,3], William D. K. Reid[4], David A. Pearce[5], Adrian G. Glover[2~~6~~], Christopher J. Sweeting[4~~6~~], Jason Newton[7~~6~~], & Clare Woulds[1]*

[1]School of Geography & Water@Leeds, University of Leeds, LS2 9JT, UK.

[2]Life Sciences Dept., Natural History Museum, Cromwell Rd, London SW7 5BD, UK

[3]Centre for Environment, Fisheries and Aquaculture Science, Lowestoft, NR34 0HT, UK

[4]~~Ridley~~Marine Sciences- -School of Natural and Environmental Sciences, Ridley Building, ~~School of Biology,~~ Newcastle University, NE1 7RU, UK

[5]Applied Sciences, Northumbria University, Newcastle, NE1 8ST, UK

~~[6]Ridley Building, School of Marine Science and Technology, Newcastle University, NE1 7RU, UK~~

[7~~6~~]NERC Life Sciences Mass Spectrometry Facility, SUERC, East Kilbride G75 0QF, UK

* E-mail: c.woulds@leeds.ac.uk

Keywords: Stable Isotopes; Trophic Niche; Sedimented; Hydrothermal; Southern Ocean; Microbial; 16S; PLFA

Abstract

~~Sedimented hydrothermal vents~~Hydrothermal sediments are those in which hydrothermal fluid is discharged through sediments and are ~~among~~ one of the least studied deep-sea ecosystems. We present a combination of microbial and biochemical data to assess trophodynamics between and within hydrothermal ~~ly active~~ and ~~off-vent~~background areas of the Bransfield Strait (1050 – 1647m depth). Microbial composition, biomass and fatty acid signatures varied widely between and within ~~vent~~hydrothermally active and ~~non-vent~~background sites, ~~and~~ providinged evidence of diverse metabolic activity. Several species ~~showed diverse feeding strategies and occupied~~ had different feeding strategies and trophic positions between~~in~~ ~~vent~~hydrothermally active and inactive ~~and non-vent~~ areas and~~.~~ s~~S~~table isotope values of consumers were ~~generally~~ not consistent with feeding ~~structure~~ morphology. Niche area and the diversity of microbial fatty acids was lowest at the most hydrothermally active site, reflecting~~ed~~ trends in species diversity, ~~and was lowest at the most hydrothermally active site.~~ Faunal ~~utilisation~~ uptake of chemosynthetically produced organics ~~activity~~ was relatively limited but was detected at both ~~vent~~hydrothermal and non-~~vent~~hydrothermal sites,~~s~~ potentially ~~as evidenced by carbon and sulphur isotopic signatures,~~ suggesting ~~that~~ hydrothermal activity can affect trophodynamics over a much wider area than previously thought.

## Section 1. Introduction

Hydrothermal sediment~~Sedimented hydrothermal vents~~ (~~SHVs,~~ a.k.a. Sediment-hosted/ sedimented hydrothermal vents), the product of subsurface mixing between hydrothermal fluid and ambient seawater within the sediment, are physically more similar to ~~non-hydrothermal~~background deep-sea habitats than they are to high temperature, hard substratum vents (Bemis et al. 2012, Bernardino et al. 2012). This means that, whilst they can host chemosynthetic obligate species, they can also be more easily colonised by non-specialist fauna and~~,~~ potentially offer~~ing~~ an important metabolic resource in the nutrient-limited deep-sea (Levin et al. 2009, Dowell et al. 2016). ~~Sedimented vents~~Hydrothermal sediments have also been suggested to act as evolutionary bridges between hard substratum vents and methane seeps (Kiel 2016). To utilise in situ production in~~at~~ ~~SHV~~hydrothermal sediments~~s~~, fauna must overcome the environmental stress associated with high-temperature, acidic and toxic conditions (Levin et al. 2013, Gollner et al. 2015). The combination of elevated toxicity and in-situ organic matter (OM) production results in a different complement of ecological niches between hydrothermal~~vents~~ and background conditions that elicits compositional changes along a productivity-toxicity gradient (Bernardino et al. 2012, Gollner et al. 2015, Bell et al. 2016b). Hydrothermal sediments offer different relative abundances of chemosynthetic and photosynthetic organic matter, depending upon supply of surface-derived primary productivity~~, which may vary with depth and latitude,~~ and levels of hydrothermal activity (Tarasov et al. 2005). In shallow environments (<200 m depth), where production of chemosynthetic and photosynthetic organic matter sources can co-occur, consumption may still favour photosynthetic OM over chemosynthetic OM as this does not require physiological adaptions to environmental toxicity (Kharlamenko et al. 1995, Tarasov et al. 2005, Sellanes et al. 2011). The limited data available concerning trophodynamics at deep-sea ~~SHV~~hydrothermal sediments,

from in the Arctic, indicate that diet composition can varyies widely between speciestaxa, ranging between 0 – 87 % contribution from chemosynthetic OM (Sweetman et al. 2013). Thus, understanding of the significance of chemosynthetic activity in these settings is very limited.

Sedimented hydrothermal ventsHydrothermal sediments host diverse microbial communities (Teske et al. 2002, Kallmeyer & Boetius 2004). Microbial communities are a vital intermediate between hydrothermal fluidinorganic substrates and metazoan consumers, and thus their composition and isotopic signatures are of direct relevance to metazoan food webs. The heat flux associated with hydrothermal activity provides thermodynamic both benefits and constraints to microbial communities (Kallmeyer & Boetius 2004, Teske et al. 2014) whilst as well as accelerating the degradation of organic matter, giving rise to a wide variety of compounds including hydrocarbons and organic acids (Martens 1990, Whiticar & Suess 1990, Dowell et al. 2016). Microbial aggregations are commonly visible on the sediment surface at SHVin hydrothermal sedimentss (Levin et al. 2009, Sweetman et al. 2013, Dowell et al. 2016) but . However, active communities are also distributedmicrobial activity also occurs throughout the underlying sediment layers, occupying a wide range of geochemical and thermal niches (reviewed by Teske et al. 2014). This zonation in microbial function and composition is very strong and has been extensively studied in Guaymas basin hydrothermal sediments. Sedimented chemosynthetic ecosystems may present several sources of organic matter to consumers (Bernardino et al. 2012, Sweetman et al. 2013, Yamanaka et al. 2015) and the diverse microbial assemblages can support a variety of reaction pathways, including methane oxidation, sulphide oxidation, sulphate reduction and nitrogen fixation (Teske et al. 2002, Dekas et al. 2009, Jaeschke et al. 2014). Phospholipid fatty acid (PLFA) analysis can be used to describe recent microbial activity and $\delta^{13}C$ signatures (Boschker & Middelburg 2002, Yamanaka & Sakata 2004, Colaço et al. 2007). Although it can be difficult to ascribe a PLFA to a specific microbial group or process,

high relative abundances of certain PLFAs can be strongly indicative of chemoautotrophy (Yamanaka & Sakata 2004, Colaço et al. 2007), and can support an understanding of microbial ecosystem function in hydrothermal sediments (e.g. in western pacific vents, see Yamanaka & Sakata 2004).

Macrofaunal assemblages ~~of the~~in Bransfield ~~SHV~~hydrothermal sediments~~s~~ were strongly influenced by hydrothermal activity (Bell et al. 2016b, Bell et al. 2017). Bacterial mats were widespread across Hook Ridge, where variable levels of hydrothermal activity were detected (Aquilina et al. 2013). Populations of siboglinid polychaetes (*Sclerolinum contortum* and *Siboglinum* sp.)~~,~~ were found at Hook Ridge and non-hydrothermally active sites (Sahling et al. 2005, Georgieva et al. 2015, Bell et al. 2016b) and~~. These species are known to~~can harbour chemoautotrophic endosymbionts (Schmaljohann et al. 1990, Eichinger et al. 2013, Rodrigues et al. 2013).

Stable isotope analysis (SIA) is a powerful tool to assess spatial and temporal patterns in faunal feeding behaviour and has been used to study trophodynamics and resource partitioning in other ~~SHV~~hydrothermal sediments~~s~~, predominately in the Pacific (Fry et al. 1991, Levin et al. 2009, Portail et al. 2016). 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~~to estimate ~~of~~ trophic position. The signature of source isotope ratios ($\delta^{13}C$ & $\delta^{34}S$) is influenced by the isotopic ratio of the chemical substrate, and the fractionation associated with the metabolic process involved and thus, different fixation pathways can elicit different isotopic signatures, even when ~~they utilise~~derived from a single ~~the same~~ source (e.g. DIC) (Fry et al. 1991). Possible $\delta^{13}C$ isotopic values of sources in the Bransfield Strait include: ~-40 ‰ for thermogenic

methane; ~-27 ‰ for suspended particulate matter or ~-15 ‰ for ice algae (Whiticar & Suess 1990, Mincks et al. 2008, Henley et al. 2012, Young et al. 2013). As an example, *Siboglinum* spp. can use a range of resources, including methane or dissolved organic matter (Southward et al. 1979, Schmaljohann et al. 1990, Thornhill et al. 2008, Rodrigues et al. 2013), making SIA an ideal way in which to examine resource utilisation in these settings (Levin et al. 2009, Soto 2009). We also apply the concept of an isotopic niche (Layman et al. 2007) whereby species or community trophic activity is inferred from the distribution of stable isotopic data in two or three dimensional isotope space.

Hypotheses

We used a combination of microbial diversity data based sequencing and compound specific isotopic analyses and bulk isotopic data from sediment, microbial, macro- and megafaunal samples to investigate resource utilisation, niche partitioning and trophic structure at ~~vent~~hydrothermal and background sites in the Bransfield Strait to test the following hypotheses: 1) ~~Siboglinid species subsist upon chemosynthetically-derived OM; 2)~~ Chemosynthetic organic matter will be a ~~significant~~ n important food source in~~at~~ hydrothermal sediment~~SHV~~s; 2) Siboglinid species subsist upon chemosynthetically-derived OM 3) Stable isotope signatures will reflect a-priori functional designations defined by faunal morphology and 4) Fauna will have distinct niches between ~~vent~~hydrothermal sites and background areas.

## Section 2. Materials and Methods

### 2.1. Sites and Sampling

Samples were collected; during RRS *James Cook* cruise JC55 in the austral summer of 2011 (Tyler et al. 2011), from three raised edifices along the basin axis (Hook Ridge, the Three Sisters and The Axe) and one off-axis site in the Bransfield Strait (1024 – 1311m depth; Fig. 1; Table 1). We visited two sites of variable hydrothermal activity (Hook Ridge 1 and 2) and three sites where hydrothermal activity was not detected (Three Sisters, the Axe and an Off-Axis site) (Aquilina et al. 2013). Of the two hydrothermal sites, Hook Ridge 2 ~~was~~ had higher ~~hydrothermal~~ fluid advection rates and pore fluid temperature but lower concentrations of sulphide and methane (Dählmann et al. 2001, Aquilina et al. 2013, Aquilina et al. 2014).

Samples were collected with ~~a series of megacore deployments, using~~ a Bowers & Connelly dampened megacorer (1024 – 1311 m depth) and a single Agassiz trawl at Hook Ridge (1647 m depth). With the exception of salps, all microbial and faunal samples presented here were from megacore deployments. For a detailed description of the megacore sampling programme and macrofaunal communities, see Bell et al. (2016b). Sampling consisted of 1 – 6 megacore deployments per site, with 2 – 5 ~~tubes~~ cores pooled per deployment (Bell et al. 2016b). Cores were sliced into 0 – 5 cm and 5 – 10 cm partitions and macrofauna were retained on a 300µm sieve. Residues were preserved in either 80 % ethanol or 10 % buffered formalin initially and then stored in 80% ethanol after sorting (Bell et al. 2016b). Fauna were sorted to species/ morphospecies level (for annelid and bivalve taxa); family level (for peracarids) and higher levels for less abundant phyla (e.g. echiurans). Salps were collected using an Agassiz trawl and samples were immediately picked and frozen at -80 ˚C and subsequently freeze-dried.

392

393 2.2. Microbiology Sequencing

394

395 Samples of surface sediment (0 – 1 cm below seafloor (cmbsf)) were taken from megacores the
396 two Hook Ridge sites and the off-axis site and frozen (-80˚C). DNA was extracted from the
397 sediment by Mr DNA (Shallowater, TX, USA) using an in-house standard 454 pipeline. The
398 resultant sequences were trimmed and sorted using default methods in Geneious (v.9.1.5 with
399 RDP v.2.8 and Krona v.2.0) and analysed in the Geneious '16 Biodiversity Tool'
400 (https://16s.geneious.com/16s/help.html; (Wang et al. 2007, Ondov et al. 2011, Biomatters
401 2014).

402

403 2.3. Phospholipid Fatty Acids

404

405 Samples of 3 – 3.5 g of freeze-dried sediment from Hook Ridge 1 & 2, the off-vent site and the
406 Three Sisters were analysed at the James Hutton Institute (Aberdeen, UK) following the
407 procedure detailed in Main et al. (2015), ~~which we~~ summarised below. Samples were from the
408 top 1 cm of sediment for all sites except Hook Ridge 2 where sediment was pooled from two core
409 slices (0 – 2 cm), due to sample mass limitations. Lipids were extracted following a method
410 adapted from Bligh (1959), using a single phase mixture of chloroform: methanol: citrate buffer
411 (1:2:0.8 v-v:v). Lipids were fractionated using 6 ml ISOLUTE SI SPE columns, preconditioned
412 with 5 ml chloroform. Freeze-dried material was taken up in 400 µL of chloroform; vortex mixed
413 twice and allowed to pass through the column. Columns were washed in chloroform and acetone
414 (eluates discarded) and finally 10 ml of methanol. Eluates were collected, allowed to evaporate
415 under a $N_2$ atmosphere and frozen (-20 ˚C).

416

Fatty acidPLFAs were derivitised with methanol and KOH to produce fatty acid methyl esters (FAMEs). Samples were taken up in 1 mL of 1:1 (v:v) mixture of methanol and toluene. 1 mL of 0.2 M KOH (in methanol) was added with a known quantity of the C19an internal standard (C19 – nonadecanoic acid), vortex mixed and incubated at 37 ˚C for 15 min. After cooling to room temperature, 2 mL of isohexane:chloroform (4:1 v:v), 0.3 mL of 1 M acetic acid and 2 mL of deionized water was added to each vial. The solution was mixed and centrifuged and the organic phase transferred to a new vial and the remaining aqueous phase was mixed and centrifuged again to further extract the organic phase, which was combined with the previous. The organic phases were evaporated under a $N_2$ atmosphere and frozen at -20 ˚C.

Samples were taken up in isohexane to perform gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS). The quantity and $\delta^{13}C$ values of individual FAMEs were determined using a GC Trace Ultra with combustion column attached via a GC Combustion III to a Delta V Advantage isotope ratio mass spectrometer (Thermo Finnigan, Bremen). The $\delta^{13}C_{VPDB}$ values (‰) of each FAME were calculated with respect to a reference gas of $CO_2$, traceable to IAEA reference material NBS 19 TS-Limestone. Measurement of the Indiana University reference material hexadecanoicacid methyl ester (certified $\delta^{13}C_{VPDB}$ -30.74 ± 0.01‰) gave a value of 30.91 ± 0.31‰ (mean ± s. d., n = 51). Combined areas of all mass peaks (m/z 44, 45 and 46), following background correction, were collected for each FAME. These areas, relative to the internal C19:0 standard, were used to quantify the 34 most abundant FAMEs and related to the PLFAs from which they are derived (Thornton et al. 2011).

Bacterial biomass was calculated using transfer functions from the total mass of four PLFAs (i14:0, i15:0, a15:0 and i16:0), estimated at 14 % of total bacterial PLFA, which in turn is estimated at 5.6 % of total bacterial biomass (Boschker & Middelburg 2002).

442

443 2.4. Bulk Stable Isotopes

444

445 All bulk isotopic analyses were completed at the East Kilbride Node of the Natural Environment
446 Research Council Life Sciences Mass Spectrometry Facility. Specimens with carbonate structures
447 (e.g. bivalves) were physically decarbonated and all specimens were rinsed in de-ionised water
448 (e.g. to remove soluble precipitates such as sulphates) and cleaned of attached sediment before
449 drying. Specimens dried for at least 24 hours at 50˚C and weighed (mg, correct to 3 d.p.) into tin
450 capsules and stored in a desiccator whilst awaiting SIA. Samples were analysed by continuous
451 flow isotope ratio mass spectrometer using a Vario-Pyro Cube elemental analyser (Elementar),
452 coupled with a Delta Plus XP isotope ratio mass spectrometer (Thermo Electron). Each of the
453 runs of CN and CNS isotope analyses used laboratory standards (Gelatine and two amino acid-
454 gelatine mixtures) as well as the international standard USGS40 (glutamic acid). CNS
455 measurements used the internal standards (MSAG2: (Methanesulfonamide/ Gelatine and M1:
456 Methionine) and the international silver sulphide standards IAEA-S1, S2 and S3. All sample runs
457 included samples of freeze-dried, powdered *Antimora rostrata* (ANR), an external reference
458 material used in other studies of chemosynthetic ecosystems (Reid et al. 2013, Bell et al. 2016a),
459 used to monitor variation between runs and instruments (supplementary file 1). Instrument
460 precision (S.D.) for each isotope measured from ANR was 0.42 ‰, 0.33 ‰ and 0.54 ‰ for
461 carbon, nitrogen and sulphur respectively. The reference samples were generally consistent
462 except in one of the CNS runs, which showed unusual $\delta^{15}N$ measurements (S1), so faunal $\delta^{15}N$
463 measurements from this run were excluded as a precaution. Stable isotope ratios are all reported
464 in delta ($\delta$) per mil (‰) notation, relative to international standards: V-PDB ($\delta^{13}C$); Air ($\delta^{15}N$)
465 and V-CDT ($\delta^{34}S$). Machine error, relative to these standards ranged 0.01 – 0.23 for $\delta^{13}C$, for 0.01
466 – 0.13 $\delta^{15}N$ and 0.13 – 3.04 for $\delta^{34}S$. One of the Sulphur standards ($Ag_2S$ IAEA: S2) had a notable

difference from the agreed measurements, suggesting either a compromised standard or poor instrument precision. This error was not observed in other standards, or the reference material used, but given the uncertainty here; only $\delta^{34}S$ differences greater than 3 ‰ are considered as being significant.

A combination of dual- ($\delta^{13}C$ & $\delta^{15}N$, 319 samples) and tri-isotope ($\delta^{13}C$, $\delta^{15}N$ & $\delta^{34}S$, 83 samples) techniques was used to describe bulk isotopic signatures of 43 species of macrofauna (35 from non-~~vent~~hydrothermal sites, 19 from ~~vent~~hydrothermal sites and 11 from both), 3 megafaunal taxa and sources of organic matter. Samples submitted for carbon and nitrogen (CN) analyses were pooled if necessary to achieve an optimal mass of 0.7 mg (± 0.5 mg). Where possible, individual specimens were kept separate in order to preserve variance structure within populations but in some cases, low sample mass meant individuals had to be pooled (from individuals found in replicate deployments). Optimal mass for ~~Carbon-Nitrogen-Sulphur~~tri-istope (CNS) measurements was 2.5 mg (± 0.5 mg) and, as with CN analyses, specimens were preferentially submitted as individual samples or pooled ~~where~~ if necessary. Samples of freeze-dried sediment from each site were also submitted for CNS analyses (untreated for NS and acidified with 6M HCl for C). Acidification was carried out by repeated washing with acid and de-ionised water.

Specimens were not acidified. A pilot study, and subsequent results presented here, confirmed that the range in $\delta^{13}C$ measurements between acidified (0.1M and 1.0M HCl) was within the untreated population range, in both polychaetes and peracarids and that acidification did not notably or consistently reduce $\delta^{13}C$ standard deviation (Table 2). In the absence of a large or consistent treatment effect, the low sample mass, (particularly for CNS samples) was dedicated to increasing replication and preserving integrity of $\delta^{15}N$ & $\delta^{34}S$ measurements instead of

492 separating carbon and nitrogen/ sulphur samples (Connolly & Schlacher 2013).

493

494 Formalin and ethanol preservation effects can both influence the isotopic signature of a sample
495 (Fanelli et al. 2010, Rennie et al. 2012). Taxa that had several samples of each preservation
496 method from a single site (to minimise intra-specific differences) were examined to determine
497 the extent of isotopic shifts associated with preservation effects. Carbon and nitrogen isotopic
498 differences between ethanol and formalin preserved samples ranged between 0.1 ‰ – 1.4 ‰
499 and 0.4 ‰ – 2.0 ‰ respectively. Differences across all samples were not significant (Paired t-
500 test, $\delta^{13}C$: t = 2.10, df = 3, p = 0.126 and $\delta^{15}N$: t=1.14, df = 3, p = 0.337). Given the unpredictable
501 response of isotopic signatures to preservation effects (which also cannot be extricated from
502 within-site, intraspecific variation) it was not possible to correct isotopic data (Bell et al. 2016a).
503 This contributed an unavoidable, but generally quite small, source of error in these
504 measurements.

505

506 2.5. Statistical Analyses

507

508 All analyses were completed in the R statistical environment (R Core Team 2013). ~~Carbon and~~
509 ~~nitrogen~~ CN stable isotopic measurements were divided into those from ~~vent~~hydrothermal or
510 non-~~vent~~hydrothermal sites and averaged by taxa and used to construct a Euclidean distance
511 matrix (Valls et al. 2014). ~~This matrix was used to conduct~~A ~~a~~ similarity profile routine
512 (SIMPROF, 10 000 permutations, p = 0.05, Ward linkage) was applied to the distance matrix in
513 ~~using~~ the clustsig package (v1.0) (Clarke et al. 2008, Whitaker & Christmann 2013) to ~~test for~~
514 detect significant structure ~~within the matrix~~. The resulting cluster assignations were compared
515 to a-priori feeding groups (Bell et al. 2016b) using a Spearman Correlation Test (with 9 999
516 Monte Carlo resamplings) using the coin package (v1.0-24) (Hothorn et al. 2015). Isotopic

signatures of species sampled from both ~~vent~~hydrothermal and non-~~vent~~hydrothermal sites were also compared with a one-way ANOVA with Tukey's HSD pairwise comparisons (following a Shapiro-Wilk normality test).

Mean faunal measurements of $\delta^{13}C$ & $\delta^{15}N$ were used to calculate Layman metrics for each site (Layman et al. 2007), sample-size corrected standard elliptical area (SEAc) and Bayesian posterior draws (SEA.B, mean of $10^5$ draws ± 95 % credibility interval) in the SIAR package (v4.2) (Parnell et al. 2010, Jackson et al. 2011). Differences in SEA.B between sites were compared in mixSIAR. The value of p given is the proportion of ellipses from group A that were smaller in area than those from group B (e.g. if p = 0.02, then 2 % of posterior draws from group A were smaller than the group B mean) and is considered to be a semi-quantitative measure of difference in means (Jackson et al. 2011).

Section 3. Results

3.1. Differences in microbial composition along a hydrothermal gradient

A total of 28,767, 35,490 and 47,870 sequences were obtained from the off-axis site and the ventthydrothermal sites, Hook Ridge 1 and 2, respectively. Bacteria comprised almost the entirety of each sample, with aArchaea being detected only in the Hook Ridge 2 sample (< 0.1 % of sequences; Fig. 21). Hook Ridge 1 was qualitatively more similar to the off-axis site than Hook Ridge 2. Both Hook Ridge 1 (venthydrothermal) and the off-vent site, BOV (non-vent), were dominated by pProteobacteria (48 % and 61 % of reads respectively; Fig. 21), whereas flFlavobacteriia dominated Hook Ridge 2 (43 %, 7 – 12 % elsewhere) with pProteobacteria accounting for a smaller percentage of sequences (36 %; Fig. 21). By sequence abundance, fFlavobacteriia were the most clearly disparate group between Hook Ridge 2 and the other sites. fFlavobacteriia were comprised of 73 genera at Hook Ridge 2, 60 genera at BOV and 63 genera at HR1, of which 54 genera were shared between all sites. Hook Ridge 2 had 15 unique flavobacteriial genera but these collectively accounted for just 0.9% of reads, indicating that compositional differences were mainly driven by relative abundance, rather than taxonomic richness.

The most abundant genus from each site was *Arenicella* at BOV and HR1 (7.1 and 5.2 % of reads respectively) and *Aestuariicola* at HR2 (6.9 % of reads) (Table 3). The four most abundant genera at both BOV and HR1 were *Arenicella* (γ-proteobacteria), *Methylohalomonas* (γ-proteobacteria), *Pasteuria* (bBacilli) & *Blastopirellula* (pPlanctomycetacia), though not in the same order, and accounted for 17.2% and 16.0 % of reads respectively. The four most abundant genera at HR2, accounting for 20.2 % of reads were *Aestuariicola*, *Lutimonas, Maritimimonas* & *Winogradskyella*

(fall Flavobacteriia). The genera *Arenicella* and *Pasteuria* were the most relatively abundant across all sites (2.2 % – 7.1 % and 1.7 % – 5.0 % of reads respectively; Table 3).

3.2. Microbial fatty acids

A total of 37 sedimentary PLFAs were identified across all sites, in individual abundances ranging between 0 % – 26.4 % of total PLFA (Table 43; Supplementary Fig 1). All lipid samples were dominated by saturated and mono-unsaturated fatty acids (SFAs and MUFAs), comprising 91 % – 94 % of PLFA abundance per site. The most abundant FPLFAs at each site were 16:0 (15.7 % – 26.4 %), 16:1ω7c (11.5 % – 20.0 %) and 18:1ω7 (4.8 % – 16.9 %; Table 43). PLFA profiles from each of the non-venthydrothermal sites sampled (Off-axis and the Three Sisters, 33 and 34 PLFAs respectively) were quite similar (Table 43) and shared all but one compound (16:1ω11c, present only at the non-venthydrothermal Three Sisters site). Fewer PLFAs were enumerated from Hook Ridge 1 and 2 (31 and 23 respectively), including 3 PLFAs not observed at the non-venthydrothermal sites (br17:0, 10-Me-17:0 & 10-Me-18:0), which accounted for 0.5 % – 1.2 % of the total at these sites. Poly-unsaturated algal biomarkers (20:5ω3 and 22:6 ω3) were only detected at the non-venthydrothermal site (0.83 – 1.57 % of total FA abundance). Hook Ridge 2 had the lowest number of PLFAs and the lowest total PLFA biomass of any site, though this was due in part to the fact that this sample had to be pooled from the top 2 cm of sediment (top 1cm at other sites). Bacterial biomass was highest at Hook Ridge 1 and ranged 85 mg C $m^{-2}$ – 535 mg C $m^{-2}$ (Table 3).

PLFA carbon isotopic signatures ranged -56 ‰ to -20 ‰ at non-venthydrothermal sites and -42 ‰ to -8 ‰ at venthydrothermal sites (Table 43). Weighted average $\delta^{13}C$ values were quite similar between the non-venthydrothermal sites and Hook Ridge 1 (-30.5 ‰ and -30.1 ‰

respectively), but were heavier at Hook Ridge 2 (-26.9 ‰; Table 43). Several of the ~~PL~~FAs identified had a large range in $\delta^{13}C$ between samples (including 16:1ω11t $\delta^{13}C$ range = 17.2 ‰ or 19:1ω8 $\delta^{13}C$ range = 19.1 ‰), even between the non-~~vent~~hydrothermal sites (e.g. 18:2ω6, 9, $\Delta\delta^{13}C$ = 24.4; Table 43). Of the 37 ~~PL~~FAs, 7 had a $\delta^{13}C$ range of > 10 ‰ but these were comparatively minor and individually accounted for 0 % – 4.9 % of total abundance. Average $\delta^{13}C$ range was 6.3 ‰ and a further 11 ~~PL~~FAs had a $\delta^{13}C$ range of > 5 ‰, including some of the more abundant ~~PL~~FAs, accounting for 36.8 ‰ – 46.6 % at each site. ~~PL~~FAs with small $\delta^{13}C$ ranges (< 5 ‰) accounted for 44.6 % – 54.4 % of total abundance at each site.

3.3. Description of bulk isotopic signatures

Most faunal isotopic signatures were within a comparatively narrow range ($\delta^{13}C$: -30 ‰ to -20 ‰, $\delta^{15}N$: 5 ‰ to 15 ‰ and $\delta^{34}S$: 10 ‰ to 20 ‰) and more depleted isotopic signatures were usually attributable to siboglinid species (Fig. 3). *Siboglinum* sp. (found at all non-~~vent~~hydrothermal sites) had mean $\delta^{13}C$ and $\delta^{15}N$ values of -41.4 ‰ and -8.9 ‰ respectively and *Sclerolinum contortum* (predominately from Hook Ridge 1 but found at both ~~vent~~hydrothermal sites) had values of -20.5 ‰ and -5.3‰ respectively. Some non-endosymbiont bearing taxa (e.g. macrofaunal neotanaids from the off-axis site and megafaunal ophiuroids at Hook Ridge 2) also had notably depleted $\delta^{15}N$ signatures (means -3.6‰ to 2.6 ‰ respectively; Fig. 3).

Isotopic signatures of sediment organic matter were similar between ~~vent~~hydrothermals~~s~~ and non-~~vents~~ hydrothermal sites for $\delta^{13}C$ and $\delta^{15}N$ but $\delta^{34}S$ was significantly greater at non-~~vent~~hydrothermal sites ($p < 0.05$, Table 54; Fig. 4). Variability was higher in ~~vent~~hydrothermal sediments for all isotopic signatures. Faunal isotopic signatures for $\delta^{13}C$ and $\delta^{34}S$ ranged much more widely than sediment signatures and indicate that sediment organics were a mixture of

two or more sources of organic matter. A few macrofaunal species had relatively heavy $\delta^{13}C$ signatures that exceeded -20 ‰ that suggested either a heavy source of carbon or marine carbonate in residual exoskeletal tissue, particularly for peracarids (~0 ‰). Samples of pelagic salps from Hook Ridge had mean values for $\delta^{13}C$ of -27.4 ‰ (± 0.9) and $\delta^{34}S$ of 21.5 ‰ (± 0.8).

3.4. Comparing macrofaunal morphology and stable isotopic signatures

Isotopic data (mean of each species for each of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) ~~Isotopic data~~ were used to construct a Euclidean distance matrix and the resultant hierarchy was compared to classifications based upon morphology. Species were~~each~~ assigned to one of four clusters (SIMPROF, p = 0.05; Supplementary Figure 3). No significant correlation between a-priori (based on morphology) and a-posteriori cluster~~s~~ assignations (based on isotopic data) was detected (Spearman Correlation Test: Z = -1.34; N = 43; p = 0.18). Clusters were mainly discriminated based on $\delta^{15}N$ values and peracarids were the only taxa to be represented in all of the clusters, indicating relatively high trophic diversity.

Several taxa found at both ~~vent~~hydrothermal and non-~~vent~~hydrothermal sites were assigned to different clusters between sites. A total of eleven taxa were sampled from both ~~vent~~hydrothermal and non-~~vent~~hydrothermal regions, of which four were assigned to different clusters at ~~vent~~hydrothermal and non-~~vent~~hydrothermal sites. Neotanaids (Peracarida: Tanaidacea) had the greatest Euclidean distance between ~~vent~~hydrothermal/ non-~~vent~~hydrothermal samples (11.36), demonstrating clear differences in dietary composition (Fig. 5). All other species were separated by much smaller distances between regions (range: 0.24 to 2.69). Raw $\delta^{13}C$ and $\delta^{15}N$ values were also compared between ~~vent~~hydrothermal and non-~~vent~~hydrothermal samples for each species (one-way ANOVA with Tukey HSD pairwise

comparisons). Analysis of the raw data indicated that $\delta^{13}C$ signatures were different for neotanaids only and $\delta^{15}N$ were different for neotanaids and an oligochaete species (*Limnodriloides* sp.) (ANOVA, $p < 0.01$, Fig. 5).

3.5.Community-level trophic metrics

All site niches overlapped (mean = 50 %, range = 30 – 82 %) and the positions of ellipse centroids were broadly similar for all sites (Table 6~~5~~; Fig 6). ~~Vent~~Hydrothermal site ellipse areas were similar but significantly smaller than non-~~vent~~hydrothermal ellipses (SEA.B, $n = 10^5$, $p = < 0.05$). There were no significant differences in ellipse area between any of the non-~~vent~~hydrothermal sites. Ranges in carbon sources (dCr) were higher for non-~~vent~~hydrothermal sites (Table 6~~5~~) indicating a greater trophic diversity in background conditions. Nitrogen range (dNr, Table 6~~5~~) was similar between ~~vent~~hydrothermals and non-~~vent~~hydrothermal sites suggesting a similar number of trophic levels within each assemblage. All site ellipses had broadly similar eccentricity (degree of extension along long axis), ranging 0.85 – 0.97 (Table 6~~5~~), however theta (angle of long axis) differed between ~~vent~~hydrothermal and non-~~vent~~hydrothermal sites (-1.43 to 1.55 at Hook Ridge, 0.67 to 0.86 at non-~~vent~~hydrothermal sites). Range in nitrogen sources was more influential at ~~vent~~hydrothermal sites as *Sclerolinum contortum*, which had very low $\delta^{15}N$ signatures but similar $\delta^{13}C$ values, when compared with non-endosymbiont bearing taxa from the same sites. The strongly depleted $\delta^{13}C$ measurements of *Siboglinum* sp. meant that ellipse theta was skewed more towards horizontal (closer to zero) for non-~~vent~~hydrothermal sites.

## Section 4. Discussion

### 4.1. Microbial signatures of hydrothermal activity

Fatty acidPLFA profiles between at the non-hydrothermal off-axis site and the tThree sSisters sites indicated similar bacterial biomass at each of these non-vent sites, and that Bbacterial biomass varied much more widely at Hook Ridge (Table 43). The Hook Ridge 2 sample is not directly comparable to the others assince it was sampled from sediment 0 – 2 cmbsf (rather than 0 – 1 cmbsf, owing to sample mass availability), though Oorganic carbon content, hydrogen sulphide flux and taxonomic diversity were all lower at this site and may support suggestion of a lower overall bacterial biomass (Aquilina et al. 2013, Bell et al. 2016b). The very high bacterial biomass at Hook Ridge 1 suggests a potentially very active bacterial community, comparable to other hydrothermal sediments (Yamanaka & Sakata 2004) but $\delta^{13}C_{org}$ was qualitatively similar to non-venthydrothermal sites, implying that chemosynthetic activity was comparatively limitednot the dominant source of organic carbon, or that the isotopic signatures of the basal carbon source (e.g. DIC) and the fractionation associated with FA synthesis resulted in similar $\delta^{13}C$ signatures.

Formatted: Superscript

Hook Ridge 1 PLFA composition was intermediate between non-vent sites and Hook Ridge 2 (Supplementary Fig. 2) but the PLFA suite was quite similar between Hook Ridge 1 and the off-axis site (Fig. 2). A small number of the more abundant PLfatty acidFAs had notable differences in relative abundance between venthydrothermal/ non-vent and background sites (Table 43). For example, 16:1ω7, which has been linked to sulphur cycling pathways (Colaço et al. 2007) comprised 14.0 % – 15.2 % of abundance at non-venthydrothermal sites and 20.0 % – 23.5 % at venthydrothermal sites. However, 18:1ω7, also a suggested PLFA linked to thio-oxidation

(McCaffrey et al. 1989, Colaço et al. 2007) occurred in lower abundance at ~~vent~~hydrothermal sites (4.8 % – 11.1 %) than non-~~vent~~hydrothermal sites (15.9 % – 16.9 %), and was also abundant in deeper areas of the Antarctic shelf (Würzberg et al. 2011). Heavier carbon isotopic signatures (> -15 ‰) are generally associated with rTCA cycle carbon fixation (Hayes 2001, Hugler & Sievert 2011, Reid et al. 2013), suggesting that this pathway may have been active at the hydrothermal sites, albeit at probably quite low rates. Conversely, many of the lightest $\delta^{13}C$ signatures (e.g. 19:1ω8, -56.6 ‰, off-axis site) were associated with the non-hydrothermal sites, although it should be noted that 19:1ω8 has not been definitively linked to a particular bacterial process (Koranda et al. 2013, Dong et al. 2015). Lower FA carbon isotope signatures with small ranges (e.g. -60 ‰ to -50 ‰) could also be indicative of methane cycling, but most FAs at all sites had $\delta^{13}C$ of > -40 ‰. These results further suggest that chemosynthetic activity was relatively limited and support a rejection of hypothesis one, –since, although there were differences between sites in PLFAs that are potentially indicative of chemosynthetic activity, these were not necessarily consistent between different PLFAs. The metabolic provenance of several of the more abundant PLFAs is also still uncertain. A number of fatty acids have been linked, though not exclusively, to chemoautotrophy, such as 10-Me-16:0 (*Desulfobacter* or *Desulfocurvus,* s~~S~~ulphate reducers) and 18:1ω7 (Yamanaka & Sakata 2004, Colaço et al. 2007, Klouche et al. 2009, Boschker et al. 2014) and their presence ~~presence of these FAs~~ may be consistent with the hydrothermal signature of the sediment microbial community. There were notable proportions of compounds normally associated with sulphate-reducing bacteria (Kohring et al. 1994, Boschker et al. 2014). These included iC15:0, aiC15:0, 1C17:0 and aiC17:0, which together constituted ~8-12 % of the FA suite. In addition, C16:1ω5c was relatively abundant (Supplementary figure 1), and minor amounts of 10MeC16:0, C17:1ω8c, and cycloC17:0 were present. These have also been used as indicators of sulphate-reducing bacteria, and sometimes of particular groups (e.g. Guezennec & Fiala-Medioni 1996, Boschker et al. 2014).

These compounds indicate the presence of sulphate-reducing bacteria, although perhaps not as the dominant group. Although the FA suite was indicative of active sulphur cycling activity, it remains difficult to be conclusive about the origin of most FAs even those which have been regularly observed in chemosynthetic contexts (e.g. 18:1ω7) may still be abundant elsewhere (Würzberg et al. 2011).

Formatted: Font: Font color: Auto

Together C16:1ω7c and C18:1ω7 accounted for ~25-35% of the total ~~PL~~FA suite~~. While~~ and, although they can be more generally associated with gram-negative eubacteria, ~~these PLFAs in sediment samples~~ have frequently been linked to sulphur oxidising bacteria in sediment samples (Pond et al. 1998, Yamanaka & Sakata 2004, Boschker et al. 2014). Their dominance of the suite in the Bransfield Strait is similar to sediments from a vent in the Barbados Trench, where together C16:1ω7 and C18:1ω7 contributed up to 50% of ~~PL~~FAs (Guezennec & Fiala-Medioni 1996).

~~They have also been shown to be dominant in the PLFA suites of sulphur oxidising bacteria such as *Beggiatoa* (e.g. Guezennec et al. 1998). The PLFA suites also contained notable proportions of compounds normally associated with sulphate reducing bacteria (Kohring et al. 1994, Boschker et al. 2014). These included iC15:0, aiC15:0, 1C17:0 and aiC17:0, which together constituted ~ 8-12 % of the PLFA suite. In addition, C16:1ω5c was relatively abundant (Supplementary figure 1), and minor amounts of 10MeC16:0, C17:1ω8c, and cycloC17:0 were present. These have also been used as indicators of sulphate reducing bacteria, and sometimes of particular groups (e.g. Guezennec & Fiala-Medioni 1996, Boschker et al. 2014). These compounds indicate the presence of sulphate reducing bacteria, although perhaps not as the dominant group. Although the PLFA suite was indicative of active sulphur cycling activity, it remains difficult to be conclusive about the origin of most PLFAs even those which have been regularly observed in chemosynthetic contexts (e.g. 18:1ω7) may still be abundant elsewhere (Würzberg et al. 2011).~~

Formatted: Font: (Default) Cambria

Unsurprisingly, Llong chain fatty acids (>C22) indicative of land plants (e.g. Yamanaka & Sakata 2004) were negligible or absent. More notably, the and typical indicators of marine phytoplankton production (e.g. C20:3ω5 and C22:6ω3) were very minor constituents, never accounting for more than 3% of total PLFA masss and only detected at the non-venthydrothermal sites. Off- Vent and Middle Sister. While their low abundance is at least partially accounted for by rapid degradation of polyunsaturated fatty acids during sinking through the water column (Veuger et al. 2012), it also suggests that sedimentary PLFAs weare predominantly of bacterial origin, whether that be due to bacterial reworking of photosynthetic organic matter, or in situ production, and that this influence of bacterial activity is greater at vent sites. than at non-vent sites.

Heavier carbon isotopic signatures (> -15 ‰) are generally associated with rTCA cycle carbon fixation (Hayes 2001, Hugler & Sievert 2011, Reid et al. 2013), suggesting that this pathway may have been active at the vent sites, albeit at probably quite low rates. Conversely, many of the lightest $\delta^{13}C$ signatures (e.g. 19:1ω8, -56.6 ‰, off-axis site) were associated with the non-vent sites, however 19:1ω8 has not been directly associated with a particular bacterial process (Koranda et al. 2013, Dong et al. 2015). Lower PLFA carbon isotope signatures with small ranges (e.g. -60 ‰ to -50 ‰) could also be indicative of methane cycling, but most PLFAs at all sites had $\delta^{13}C$ of > -40 ‰.

Several PLFAs had isotopic signatures that varied widely between sites, demonstrating differences in fractionation and/ or source isotopic signatures. Fang et al. (2006) demonstrated that depth (i.e. pressure) can exert an influence upon PLFA fractionation, but at these sites, depth varied only by a small amount (1045 – 1312 m), meaning that this effect should have been quite

~~limited. The heaviest PLFA $\delta^{13}C$ signatures were associated with Hook Ridge sites (e.g. 16:1ω11t at HR2, $\delta^{13}C$ = -8.7 ‰, ~-24 ‰ to -25 ‰ elsewhere). This suggests isotopic differences in the sources or fractionation by the metabolic pathways used to synthesise these FAs. However, bacterial fractionation of organic matter can have substantial variation in $\delta^{13}C$ signatures, depending upon variability in the composition and quality (e.g. C: N ratios) of the source (Macko & Estep 1984) and growth of the organism (Fang et al. 2006), which makes it difficult to elucidate the specific nature of the differences in substrates between sites.~~

~~*Siboglinum* isotopic data demonstrates that methanotrophy was probably occurring at the off axis sites (Supplementary Figure 1), and depleted PLFA isotopic signatures (e.g. 19:1ω8 - $\delta^{13}C$: -56.6 ‰; Table 3) provide further suggestion of methanotrophy amongst free-living sedimentary bacteria.~~ Chemotrophic bacterial sequences, such as *Blastopirellula* (Schlesner 2015) or *Rhodopirellula* (Bondoso et al. 2014) were found at all sites in relatively high abundance, suggesting widespread and active chemosynthesis, though the lack of a particularly dominant bacterial group associated with chemosynthetic activity suggested that the supply of chemosynthetic OM was likely relatively limited. It remains difficult however to determine which ~~PL~~FAs these bacterial lineages may be have been synthesising.

Some ~~PL~~FAs also had marked differences in $\delta^{13}C$ signatures, even where there was strong compositional similarity between sites (i.e. the non-~~vent~~hydrothermal sites). This suggested that either there were differences in the isotopic values of inorganic or organic matter sources or different bacterial metabolic pathways were active. Between the non-~~vent~~hydrothermal sites, these ~~PLFAs~~ included PUFAs and MUFAs (p~~P~~oly- and m~~M~~ono-unsaturated fatty acids) such as 18:2ω6, 9 ($\Delta\delta^{13}C$ 24.4 ‰) and 19:1ω8 ($\Delta\delta^{13}C$ 19.1 ‰). Differences in PLFA $\delta^{13}C$ between ~~Hook Ridge~~the hydrothermal sites also ranged widely, with the largest differences being associated

with PLFAs such as 16:1ω11t ($\Delta\delta^{13}C$ 17.2 ‰) and 10-Me-16:0 ($\Delta\delta^{13}C$ 11.0 ‰). However, it should be stressed that all PLFAs with larger $\delta^{13}C$ differences between sites were comparatively rare and never individually exceeded 5% of total abundance. ~~This provides further evidence of limited chemosynthetic activity at all sites and is consistent with the presence of bacteria associated with methane and sulphur cycling.~~ Microbial signatures, whilst supporting the suggestion of chemosynthetic activity, are not indicative of chemosynthetic OM being the dominant source of organic matter to food webs at any site (hypothesis ~~four~~one). It is not possible to assess from PLFA data the relative importance of chemoautotrophic and photosynthetic OM sources, since PLFAs degrade quickly and therefore surface FA abundances are inevitably underestimated in deep water samples. ~~Abundance of PLFAs associated with surface production, such as 15:0, 20:5ω3, C22:ω6 (Colaço et al. 2007, Parrish 2013) were low (max 1.8 %), which is consistent with the expected degradation rates during sinking. Further, piezophillic bacteria have been shown to synthesise some long chain PUFAs (20:5ω3 and 22:6ω3), which were previously thought to be algal markers (Fang et al. 2006).~~

4.2. Siboglinids

Both species of infaunal siboglinid (*Sclerolinum contortum* from Hook Ridge and *Siboglinum* sp. from the non-~~vent~~hydrothermal sites) appeared to subsist upon chemosynthetically derived organic matter, as evidenced by their morphology, and also by their strongly $^{15}N$-depleted isotopic signatures (see values with $\delta^{15}N$ of < -2 ‰ in Fig. 3). Low $\delta^{15}N$ signatures have also been observed in other siboglinids in a range of hydrothermal settings, such as *Riftia pachyptila* at the East Pacific Rise hard substratum vents (Rau 1981). Diazotrophy has been detected previously in hydrothermal vents and cold seeps, ~~and has been associated with~~typified by low $\delta^{15}N$ values (e.g. Rau, 1981; Desai et al., 2013; Wu et al., 2014; (Yamanaka et al. 2015). Diazotrophy in various

reducing settings has been found associated with anaerobic oxidation of methane (Dekas et al., 2009), methanotrophy (Mehta & Baross 2006) and (in a non-marine cave) sulphate reduction (Desai et al. 2013). The latter is also consistent with the low $\delta^{34}S$ signatures of both siboglinid species (Fig. ~~3;~~ 4), but gene expression analysis and/or isotopic tracing would be required to confirm this suggestion. ~~The low $\delta^{34}S$ may also be explained by assimilation of bacterial sulphide, which also gave rise to metal sulphides (e.g. pyrite) at the vent sites (Petersen et al. 2004).~~ Alternately, low $\delta^{15}N$ signatures may be explained by ~~endosymbionts conducting~~uptake of ammonium produced through dissimilatory nitrate reduction ~~to ammonium~~ (Naraoka et al. 2008, Liao et al. 2014, Bennett et al. 2015), or strong isotopic fractionation during utilization of ammonia (Naraoka et al. 2008, Liao et al. 2014, Bennett et al. 2015). Bulk faunal isotopic signatures are inadequate to determine which of these chemosynthesis-related mechanisms is responsible for *Siboglinum* $\delta^{15}N$ values, which would require analysis of the functional genes in the *Siboglinum* endosymbionts.

~~Whichever pathway is dominant,~~ $\delta^{15}N$ values for both ~~species~~ siboglinids ($\delta^{15}N$ *Sclerolinum* = -5.3 ‰ ± 1.0, *Siboglinum* = -8.9 ‰ ± 0.8) ~~seem to~~ indicated reliance upon locally fixed $N_2$ (Rau 1981, Dekas et al. 2009, Dekas et al. 2014, Wu et al. 2014, Yamanaka et al. 2015), rather than utilisation of sediment organic nitrogen ~~sources within the sediment~~ ($\delta^{15}N$ = 5.7 ‰ ± 0.7). These values were also in contrast to the rest of the non-chemosynthetic obligate species, which generally had much heavier $\delta^{15}N$ values. This supports hypothesis two~~hree~~, that the siboglinid species were subsisting upon chemosynthetic OM, most likely supplied by their endosymbionts.

Carbon isotopic signatures in chemosynthetic primary production depend upon the mode of fixation and the initial $^{13}C$ of the ~~available DIC~~inorganic substrate. *Sclerolinum contortum* $\delta^{13}C$ (-20.5 ‰ ± 1.0 ‰) was depleted in $\delta^{13}C$ relative to Southern Ocean DIC by around 10 ‰ (Henley

et al. 2012, Young et al. 2013), giving it a signal within the fractionation range of the reverse tricarboxyclic acid cycle (Yorisue et al. 2012). Regional measurements of surface ocean DIC $\delta^{13}C$ have an average isotopic signature of -10.4 ‰ (Henley et al. 2012, Young et al. 2013) but the concentration and isotopic composition of DIC can undergo considerable alteration in hydrothermalat sediented ventss (Walker et al. 2008). Therefore, without measurements of $\delta^{13}C$ in pore fluid DIC, it was not possible to determine which fixation pathway(s) were being used by *S. contortum* endosymbionts.

Sulphur isotopic signatures in *S. contortum* were very low, and quite variable (-26.7 ‰ ± 3.5 ‰). *Sclerolinum* endosymbionts may have been utilising sulphide either from hydrothermal fluid, microbial sulphate reduction or re-dissolved from hydrothermal precipitates. Mineral sulphide was present at Hook Ridge that ranged between -28.1 ‰ to +5.1 ‰ (Petersen et al. 2004), consistent with the relatively high $\delta^{34}S$ variability in *S. contortum*. (but $\delta^{34}S$ measurements were subject to higher error between replicates of standards). These precipitates at Hook Ridge are thought to originate from a previous period of high-temperature venting at this site (Klinkhammer et al. 2001). Alternatively, sulphide supplied as a result of microbial sulphate reduction (Canfield 2001) may have been the primary source of organic sulphur, similar to that of solemyid bivalves from in reducing sediments near a sewage pipe outfall (mean $\delta^{34}S$ of ranged -30 ‰ to -20 ‰; Vetter and Fry (1998) and in cold seep settings (Yamanaka et al. 2015). Sulphate reduction can also be associated with anaerobic oxidation of methane (Whiticar & Suess 1990, Canfield 2001, Dowell et al. 2016), suggesting that methanotrophic pathways could also have been important at Hook Ridge. (e.g. abundance of *Methylohalomonas*, 2.1 % – 4.3 % of sequences at all sites; Table 3). Although endosymbiont composition data were not available for the Southern Ocean population, *Sclerolinum contortum* is also known from hydrocarbon seeps in the Gulf of Mexico (Eichinger et al. 2013, Eichinger et al. 2014, Georgieva et al. 2015) and the

Håkon Mosby mud volcano in the Arctic ocean, where *S. contortum* $\delta^{13}C$ ranged between -48.3 ‰ to -34.9 ‰ (Gebruk et al. 2003) demonstrating that this species ~~is capable of~~can occupy~~ing~~ several reducing environments and us~~e~~ing a range of chemosynthetic fixation pathways, including sulphide oxidation and methanotrophy (Eichinger et al. 2014, Georgieva et al. 2015).

*Siboglinum* sp. $\delta^{13}C$ values (mean -41.4 ‰, range -45.7 ‰ to -38.1 ‰, n = 8) corresponded very closely to published values of thermogenic methane (-43 ‰ to -38 ‰) from the Bransfield Strait (Whiticar & Suess 1990), strongly suggesting ~~. This suggested~~ that methanotrophy was the ~~likely~~ dominant carbon source for this species. Biogenic methane, although present in the Bransfield Strait, typically has much lower $\delta^{13}C$ values (Whiticar 1999, Yamanaka et al. 2015), indicating a hydrothermal/ thermogenic source of methane in the Bransfield Strait (Whiticar & Suess 1990). ~~Sources of microbially-mediated methane were also present in the Bransfield Strait (Whiticar & Suess 1990) but these $\delta^{13}C$ values were far lower than any of the faunal signatures observed here.~~ Sulphur isotopic signatures were also very low in *Siboglinum* sp. ($\delta^{34}S$ -22.9 ‰, one sample from 15 pooled individuals from the off-axis site), the lowest measurement of $\delta^{34}S$ reported for this genus (Schmaljohann & Flügel 1987, Rodrigues et al. 2013). ~~The low $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ signatures of *Siboglinum* sp. suggest that its symbionts may have included methanotrophs (Thornhill et al. 2008) and diazotrophic/ denitrifying bacteria (Boetius et al. 2000, Canfield 2001, Dekas et al. 2009).~~ Methanotrophy in *Siboglinum* spp. has been previously documented at seeps in the NE Pacific (Bernardino & Smith 2010) and Norwegian margin ($\delta^{13}C$ = -78.3 ‰ to -62.2 ‰) (Schmaljohann et al. 1990) and in Atlantic mud volcanoes ($\delta^{13}C$ range -49.8 ‰ to -33.0 ‰) (Rodrigues et al. 2013). ~~Sulphur isotopic signatures in *Siboglinum* spp. from Atlantic mud volcanoes ranged between -16.8 ‰ to 6.5 ‰ (Rodrigues et al. 2013) with the lowest value still being 6 ‰ greater than that of Bransfield strait specimens.~~ Rodrigues et al.

(2013) also reported a greater range in $\delta^{15}N$ than observed in the Bransfield siboglinids ($\delta^{15}N$ -1.3 ‰ to 12.2 ‰ and -10.2 ‰ to -7.6 ‰ respectively). This suggests that, in comparison to *Siboglinum* spp. in Atlantic Mud volcanoes, which seemed to be using a mixture of organic matter sources (Rodrigues et al. 2013), the Bransfield specimens relied much more heavily upon a single OM source, suggesting considerable trophic plasticity in this genus worldwide.

Off-vent methanotrophy, using thermogenic methane, potentially illustrates an indirect dependence upon hydrothermalism (Whiticar & Suess 1990). Sediment methane production is thought to be accelerated by the heat flux associated with mixing of hydrothermal fluid in sediment (Whiticar & Suess 1990) and sediment and *Siboglinum* isotopic data suggest that the footprint of hydrothermal influence may be much larger than previously recognised, giving rise to transitional environments (Bell et al. 2016a, Levin et al. 2016). Clear contribution of methane-derived carbon to consumer diets was limited predominately to neotanaids, consistent with the relatively small population sizes (64 ind. $m^2$– 159 ind. $m^2$) of *Siboglinum* sp. observed in the Bransfield Strait (Bell et al. 2016b).

4.3. Organic Matter Sources

Pelagic salps, collected from an Agassiz trawl at Hook Ridge (1647m), were presumed to most closely represent a diet of entirely surface-derived material and were more depleted in $^{13}C$ and more enriched in $^{34}S$ than were sediments (Salp $\delta^{13}C$ = -27.4 ‰ & $\delta^{34}S$ = 20.1; Hook Ridge sediment $\delta^{13}C$ = -26.2 ‰ & $\delta^{34}S$ = 14.3) Salp ~~samples~~ carbon isotopic signatures were also lighter than the majority of macrofauna or sedimentary organic carbon, both at Hook Ridge and the non-~~vent~~hydrothermal sites (Fig. 3) and similar to other suspension feeding fauna in the Bransfield Strait (Elias-Piera et al. 2013).

~~Sediment bulk organic C ($\delta^{13}C$ -25.8 to -26.2) was similar to but nonetheless isotopically heavier than the salp samples. Sediment PLFA data shows that 20.8 - 29.9 % were attributed to bacteria (summed contributions of i15:0, ai15:0, 16:1ω5c, i17:0, ai17:0, 17:0, and 18:1ω7; Parrish (2013)), while only 1.0 - 3.8 % were indicative of algal inputs (summed contributions of 15:0, 20:5ω3, 22:6ω3; Parrish (2013)). Thus, while the C isotopes suggest that sedimentary OM was dominantly derived from surface photosynthesis, the material deposited in the sediment was likely strongly reworked by bacterial activity.~~

F~~This suggests that f~~auna with more depleted $\delta^{34}S$/ more enriched $\delta^{13}C$ values we~~a~~re likely to have derived at least a small amount of their diet from chemosynthetic sources (potentially indirectly through non-selective consumption of detrital OM), both at ~~vent~~hydrothermal~~s~~ and background regions (Bell et al. 2017). Carbon and sulphur isotopic measurements indicated mixed sources for most consumers between chemosynthetic OM and surface-derived photosynthetic OM. ~~Sediment OM was likely a combination of these two sources, making both available to non-specific deposit-feeding fauna and suggesting that consumption of chemosynthetic OM may even have been incidental in some cases.~~ The low content of algal biomarkers (particularly at the ~~vent~~hydrothermal sites) suggests that phytodetritus was probably quite degraded and thus challenging to detect using short-lived fatty acids. However, the Bransfield Strait can be subject to substantial export production and it is probable that surface production contributes much more to seafloor OM than is evident from the fatty acid composition. Non-~~vent~~hydrothermal sediments were more enriched in $^{34}S$ than ~~vent~~hydrothermal sediments, an offset that probably resulted from greater availability of lighter

926 sulphur sources such as sulphide oxidation at Hook Ridge, even if surface-derived OM remained
927 the dominant source of organic matter at the hydrothermal sites (Bell et al. 2017).
928
929 Samples of bacterial mat could not be collected during JC55 (Tyler et al. 2011) and without these
930 endmember measurements, it was not possible to quantitatively model resource partitioning in
931 the Bransfield Strait using isotope mixing models (Phillips et al. 2014). Bacterial mats from high-
932 temperature vents in the Southern Ocean had $\delta^{34}S$ values of 0.8 ‰ (Reid et al. 2013) and at
933 sedimented areas of the Loki's Castle hydrothermal vents in the Arctic Ocean has $\delta^{34}S$ values of
934 -4.9 ‰ (Bulk sediment; Jaeschke et al. 2014). Therefore it is probable that low faunal $\delta^{34}S$ values
935 represent a contribution of chemosynthetic OM (from either siboglinid tissue or free-living
936 bacteria). Inorganic sulphur can also be a source to consumers when sulphide is utilised by free
937 living bacteria ($\delta^{34}S$ ranged -7.3 ‰ to 5.4 ‰; Erickson et al. (2009)) and, although we could not
938 analyse the $\delta^{34}S$ of fluid sulphide, sulphide crusts have been found at Hook Ridge and may
939 provide a proxy for typical isotopic composition ($\delta^{34}S$ -28.1 ‰ to 5.1 ‰; Petersen et al. (2004)).
940 There were several species (e.g. Tubificid oligochaetes) that had moderately depleted $\delta^{34}S$
941 signatures, such as *Limnodriloides* sp. ($\delta^{34}S$ 7.6 ‰ at ~~vent~~hydrothermal sites, -1.2 ‰ at non-
942 ~~vent~~hydrothermal sites~~,~~, Fig. 4) further supporting the hypothesis of different trophic positions
943 between ~~vent~~hydrothermal/ non-~~vent~~hydrothermal regions (hypothesis two). This provides
944 evidence of coupled anaerobic oxidation of methane/ sulphate reduction but overall, the
945 contribution of $\delta^{34}S$-depleted bacterial production did not seem widespread (further rejecting
946 hypothesis four).
947
948 Without samples of all OM sources we cannot quantitatively assert that faunal utilisation of
949 chemosynthetic OM was low in the Bransfield Strait. Although isotopic data were consistent with
950 several OM sources, it seemed unlikely that chemosynthetic OM was a dominant source of OM

to the vast majority of taxa. The apparently limited consumption of chemosynthetic OM suggested that either it was not widely available (e.g. patchy or low density of endosymbiont-bearing fauna (Bell et al. 2016b)), or that the ecological stress associated with feeding in areas of in situ production was a significant deterrent to many species (Bernardino et al. 2012, Levin et al. 2013).

### 4.4. A-priori vs. a-posteriori trophic groups

Classifications based upon m~~M~~orphology did not prove to be an accurate predictor of ~~trophic associations~~isotopic data, suggesting that faunal behaviour is potentially more important in determining dietary composition than morphology (e.g. having/ lacking jaws). Peracarid species that possessed structures adapted to a motile, carnivorous lifestyle were assigned to a carnivore/ scavenger guild (Bell et al. 2016b) and were distributed throughout the food web both at ~~vent~~hydrothermal sites and background regions, indicating more diverse feeding strategies than expected. Taxa presumed to be deposit feeders (largely annelids) also had a ~~surprisingly~~ large range of $\delta^{15}N$ values. This may reflect the consumption of detritus from both 'fresh' and more recycled/ refractory OM sources as observed in other non-~~vent~~hydrothermal sedimented deep-sea habitats (Iken et al. 2001, Reid et al. 2012) or reflect variability in trophic discrimination related to diet quality (Adams & Sterner 2000). ~~Another possibility is taxa feeding on foraminifera conducting denitrification.~~ A range of foraminifera have now been shown to ~~conduct this process,~~utilise denitrification which results in them ~~showing elevated~~having heavier $\delta^{15}N$ ~~leading to heavy $\delta^{15}N$~~ values (Pina-Ochoa et al. 2010, Jeffreys et al. 2015). The result is high $\delta^{15}N$ values in taxa without predatory morphology (e.g. oligochaetes) ~~(Bell et al. 2016a).~~ Tubificid oligochaetes had higher $\delta^{15}N$ values at the ~~vent~~hydrothermal sites, suggesting that they fed upon more recycled organic matter, possibly owing to greater microbial activity at

~~vent~~hydrothermal sites. ~~Bacterial biomass was very variable at the vent sites (86 mg C m$^{-2}$ – 535 mg C m$^{-2}$, compared with 136 mg C m$^{-2}$ – 197 mg C m$^{-2}$ at non-vent sites; Table 3) and so it is possible that at Hook Ridge 1 bacterial assemblages could have had a greater influence upon $\delta^{15}$N of organic matter.~~

~~Neotanaids from the off-axis site had the lowest $\delta^{13}$C and $\delta^{15}$N values of any non-siboglinid taxon (Fig. 5), suggesting a significant contribution of methane-derived carbon. The clustering of the neotanaids together with endosymbiont-bearing taxa is far more likely to be an artefact of the cluster-linkage method, introduced by consumption of low $\delta^{13}$C methanotrophic sources (e.g. *Siboglinum* tissue), rather than suggesting symbionts in these fauna (Larsen 2006, Levin et al. 2009).~~

Several taxa (e.g. ~~neotanaids from the off-axis site and~~ ophiuroids at Hook Ridge) had low $\delta^{15}$N values, relative to sediment OM, suggesting preferential consumption of chemosynthetic OM (Rau 1981, Dekas et al. 2014). In these taxa, it is likely that the widespread, but patchy bacterial mats or *Sclerolinum* populations at Hook Ridge (Aquilina et al. 2013) were an important source of organic matter ~~to fauna with low $\delta^{15}$N values (e.g. ophiuroids)~~. Fauna from the non-~~vent~~hydrothermal sites with low $\delta^{15}$N (e.g. neotanaids) were likely subsisting in part upon siboglinid tissue (*Siboglinum* sp.). There were no video transects over the off-axis site but footage of the Three Sisters, which was similar in macrofaunal composition (Bell et al. 2016b), did not reveal bacterial mats (Aquilina et al. 2013), hence it is unlikely that these were an important resource at non-~~vent~~hydrothermal sites.

It is clear that some fauna can exhibit a degree of trophic plasticity, depending upon habitat (supporting hypothesis four~~two~~). This is consistent with other ~~SHV~~hydrothermal sediments~~s~~

where several taxa (e.g. *Prionospio* sp. – Polychaeta: Spionidae) had different isotopic signatures, depending upon their environment (Levin et al. 2009), demonstrating differential patterns in resource utilisation. Alternatively, there could have been different $\delta^{15}N$ baselines between sites, though if these differences were significant, we argue that it likely that more species would have had significant differences in tissue $\delta^{15}N$. Conversely, samples of *Aurospio foodbancsia* at both ~~vent~~hydrothermal and non-~~vent~~hydrothermal sites had broadly similar $\delta^{15}N$ values to that of the west Antarctic Peninsula; 8.1 ‰ and 7.9 ‰ respectively, albeit with a higher variability (Mincks et al. 2008). $\delta^{13}C$ values of *Aurospio* were also broadly similar, implying that this species occupied a detritivorous trophic niche, irrespective of environmental conditions.

4.5. Impact of hydrothermal activity on community trophodynamics

Standard ellipse area was lower at Hook Ridge than ~~at non-vent sites~~elsewhere (Table ~~6~~5), analogous to trends in macrofaunal diversity and abundance in the Bransfield Strait (Bell et al. 2016b) and changes in SEA.B along a gradient of methane flux at vent and seep ecosystems in the Guaymas Basin (Portail et al. 2016). This demonstrates that at community level, ellipse area can be associated with other macrofaunal assemblage characteristics. C~~This c~~oncurrent decline in niche area and alpha diversity is consistent with the concept that species have finely partitioned niches and greater total niche area permits higher biodiversity (McClain & Schlacher 2015). ~~The decline in alpha diversity and niche area is~~This relationship may also suggest ~~consistent with~~ that the influence of disturbance gradients created by hydrothermalism can ~~that~~ result in an impoverished community (McClain & Schlacher 2015, Bell et al. 2016b). Productivity-diversity relationships, whereby higher productivity sustains higher diversity, have also been suggested for deep-sea ecosystems (McClain & Schlacher 2015, Woolley et al. 2016) ~~but in the absence of measurements of in situ organic matter fixation rates at Hook Ridge,~~

~~it is unclear whether such relationships exist in the Bransfield Strait~~this is not supported by the Bransfield Strait sites (Bell et al. 2017)~~{Gollner, 2015 #1747}~~. ~~Sediment organic carbon content was similar between Hook Ridge 1 and non-vent sites but was slightly lower at Hook Ridge 2 (Bell et al. 2016b), which is not consistent with variation in niche area. The decline in alpha diversity and niche area is consistent with the influence of disturbance gradients created by hydrothermalism that result in an impoverished community (McClain & Schlacher 2015, Bell et al. 2016b).~~We suggest that, in the Bransfield Strait, the environmental toxicity ~~at SHV~~in hydrothermal sediments (from differences in temperature and porewater chemistry) causes a concomitant decline in both trophic and species diversity (Bell et al. 2016b), in spite of the potential for increased localised production (Bell et al. 2017). However, we acknowledge that, owing to the high small-scale habitat heterogeneity apparent from video imagery over the ~~vent~~hydrothermally influenced area, that it is likely that the contribution of chemosynthetic organic matter varies widely over 10s of metres at Hook Ridge.

Community-based trophic metrics (Layman et al. 2007) indicated that, although measures of dispersion within sites were relatively similar between ~~vent~~hydrothermal sites and background areas (Table 6~~5~~), trophic diversity, particularly in terms of range of carbon sources (dCr) and total hull area (TA) were higher at background sites, owing to the more depleted carbon and nitrogen signatures of *Siboglinum* spp.. ~~It was expected that trophic diversity would be greater at Hook Ridge but the greater dCr at non-vent sites (owing to the methanotrophic source) meant that the isotopic niches at these sites were larger. Range in nitrogen values (dNr) was also greater at non-vents, driven by the more heavily depleted $\delta^{15}$N values of *Siboglinum* sp.~~ It is ~~of course debatable~~still unclear whether th~~eis~~ assemblage isotopic niche really corresponds to ~~the assemblage's~~ its actualised trophic niche and, although the niche space was smaller at the ~~vent~~hydrothermal sites, the potential for different trophic strategies was still potentially greater

(Bell et al. 2017)~~than at non-vent sites. Differences in eccentricity are more heavily influenced by the spread of all isotopes used to construct the niche space (where E = 0 corresponds to an equal influence of both carbon and nitrogen) whereas theta (the angle of the long axis) determines which, if any, isotope is most influential in determining ellipse characteristics (Reid et al. 2016). For the non-vent sites, the dominant isotope was carbon, owing to the relatively light $\delta^{13}C$ of methanotrophic source utilised by *Siboglinum*. Some sites, particularly the Axe, had several fauna with heavy $\delta^{13}C$ values (Fig. 6), which could be explained by either contamination from marine carbonate (~ 0 ‰), as specimens were not acidified, or a diet that included a heavier source of carbon, such as sea ice algae (Henley et al. 2012).~~

## Section 5. Conclusions

In this study, we demonstrate the influence of sediment-hosted hydrothermal ~~venting~~ activity upon trophodynamics and microbial populations. Low activity ~~vent~~hydrothermal microbiota were more similar to the non-~~vent~~hydrothermal site than to high activity populations, illustrating the effect of ecological gradients upon deep-sea microbial diversity. Despite widespread bacterial mats, and populations of ~~vent~~hydrothermal-endemic macrofauna, utilisation of chemosynthetic OM amongst non-specialist macro- and megafauna seemed relatively low, with a concomitant decline in trophic diversity with increasing hydrothermal activity. Morphology was also not indicative of trophic relationships, demonstrating the effects of differential resource availability and behaviour. We suggest that, because these sedimented hydrothermal site~~vent~~s are insufficiently active to host large populations of vent-endemic megafauna, the transfer of chemosynthetic organic matter into the metazoan food web is likely to be more limited than in other similar environments.

1074 6. Acknowledgements
1075
1076 JBB was funded by a NERC PhD Studentship (NE/L501542/1). This work was funded by the
1077 NERC ChEsSo consortium (Chemosynthetically-driven Ecosystems South of the Polar Front,
1078 NERC Grant NE/DOI249X/I). Elemental analyses were funded by the NERC Life Sciences Mass
1079 Spectrometry Facility (Proposal no. EK234-13/14). We thank Barry Thornton and the James
1080 Hutton Laboratory, Aberdeen for processing the PLFA samples. We also thank Will Goodall-
1081 Copestake for assistance in processing the 16S sequence data. We are grateful to the Master and
1082 Crew of RRS *James Cook* cruise 055 for technical support and the Cruise Principal Scientific
1083 Officer Professor Paul Tyler.
1084
1085 7. Ethics Statement
1086
1087 In accordance with the Antarctic Act (1994) and the Antarctic Regulations (1995), necessary
1088 permits (S5-4/2010) were acquired from the South Georgia and South Sandwich Islands
1089 Government.
1090
1091 8. Author contributions
1092
1093 Conceived and designed the sampling programme: WDKR, DAP, AGG, CJS & CW. Sample
1094 laboratory preparation and isotopic analyses: JBB, JN & CJS. Microbial sequencing: DAP.
1095 Statistical analyses: JBB. Produced figures: JBB. Wrote the paper: JBB, CW & WDKR, with
1096 contributions and comments from all other authors.

1097 9. References

1098


1099 Adams TS, Sterner RW (2000) The effect of dietary nitrogen content on trophic level $^{15}N$
1100 enrichment. Limnology & Oceanography 45:601-607
1101 Aquilina A, Connelly DP, Copley JT, Green DR, Hawkes JA, Hepburn L, Huvenne VA, Marsh L, Mills
1102 RA, Tyler PA (2013) Geochemical and Visual Indicators of Hydrothermal Fluid Flow
1103 through a Sediment-Hosted Volcanic Ridge in the Central Bransfield Basin (Antarctica).
1104 Plos One 8:e54686
1105 Aquilina A, Homoky WB, Hawkes JA, Lyons TW, Mills RA (2014) Hydrothermal sediments are a
1106 source of water column Fe and Mn in the Bransfield Strait, Antarctica. Geochimica et
1107 Cosmochimica Acta 137:64-80
1108 Bell JB, Aquilina A, Woulds C, Glover AG, Little CTS, Reid WDK, Hepburn LE, Newton J, Mills RA
1109 (2016a) Geochemistry, faunal composition and trophic structure at an area of weak
1110 methane seepage on the southwest South Georgia margin. Royal Society Open Science 3
1111 Bell JB, Woulds C, Brown LE, Little CTS, Sweeting CJ, Reid WDK, Glover AG (2016b) Macrofaunal
1112 ecology of sedimented hydrothermal vents in the Bransfield Strait, Antarctica. Frontiers
1113 in Marine Science 3:32
1114 Bell JB, Woulds C, van Oevelen D (2017) Hydrothermal activity, functional diversity and
1115 chemoautotrophy are major drivers of seafloor carbon cycling. Scientific reports 7
1116 Bemis K, Lowell R, Farough A (2012) Diffuse Flow On and Around Hydrothermal Vents at Mid-
1117 Ocean Ridges. Oceanography 25:182-191
1118 Bennett SA, Dover CV, Breier JA, Coleman M (2015) Effect of depth and vent fluid composition
1119 on the carbon sources at two neighboring deep-sea hydrothermal vent fields (Mid-
1120 Cayman Rise). Deep Sea Research Part I: Oceanographic Research Papers 104:122-133
1121 Bernardino AF, Levin LA, Thurber AR, Smith CR (2012) Comparative Composition, Diversity and
1122 Trophic Ecology of Sediment Macrofauna at Vents, Seeps and Organic Falls. Plos ONE
1123 7:e33515
1124 Bernardino AF, Smith CR (2010) Community structure of infaunal macrobenthos around
1125 vestimentiferan thickets at the San Clemente cold seep, NE Pacific. Marine Ecology-an
1126 Evolutionary Perspective 31:608-621
1127 Biomatters (2014) Geneious.
1128 Bligh EG (1959) A rapid method of total lipid extration and purification. Canadian Journal of
1129 Biochemistry and Phsiology 37:911-917
1130 Bondoso J, Albuquerque L, Lobo-da-Cunha A, da Costa MS, Harder J, Lage OM (2014)
1131 Rhodopirellula lusitana sp. nov. and Rhodopirellula rubra sp. nov., isolated from the
1132 surface of macroalgae. Syst Appl Microbiol 37:157-164
1133 Boschker HT, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. FEMS
1134 Microbiology Ecology 40:85-95
1135 Boschker HT, Vasquez-Cardenas D, Bolhuis H, Moerdijk-Poortvliet TW, Moodley L (2014)
1136 Chemoautotrophic carbon fixation rates and active bacterial communities in intertidal
1137 marine sediments. PLoS One 9:e101443
1138 Canfield DE (2001) Isotope fractionation by natural populations of sulfate-reducing bacteria.
1139 Geochimica Et Cosmochimica Acta 65:1117-1124
1140 Clarke KR, Somerfield PJ, Gorley RN (2008) Testing of null hypotheses in exploratory community
1141 analyses: similarity profiles and biota-environment linkage. Journal of Experimental
1142 Marine Biology and Ecology 366:56-69

1143 Colaço A, Desbruyères D, Guezennec J (2007) Polar lipid fatty acids as indicators of trophic
1144 associations in a deep-sea vent system community. Marine Ecology 28:15-24
1145 Connolly RM, Schlacher TA (2013) Sample acidification significantly alters stable isotope ratios
1146 of sulfur in aquatic plants and animals. Marine Ecology Progress Series 493:1-8
1147 Dählmann A, Wallman K, Sahling H, Sarthou G, Bohrmann G, Petersen S, Chin CS, Klinkhammer
1148 GP (2001) Hot vents in an ice-cold ocean: Indications for phase separation at the
1149 southernmost area of hydrothermal activity, Bransfield Strait, Antarctica. Earth and
1150 Planetary Science Letters 193:381-394
1151 Dekas AE, Chadwick GL, Bowles MW, Joye SB, Orphan VJ (2014) Spatial distribution of nitrogen
1152 fixation in methane seep sediment and the role of the ANME archaea. Environ Microbiol
1153 16:3012-3029
1154 Dekas AE, Poretsky RS, Orphan VJ (2009) Deep-sea archaea fix and share nitrogen in methane-
1155 consuming microbial consortia. Science 326:422-426
1156 Desai MS, Assig K, Dattagupta S (2013) Nitrogen fixation in distinct microbial niches within a
1157 chemoautotrophy-driven cave ecosystem. Isme Journal 7:2411-2423
1158 Dong L-J, Sun Z-K, Gao Y, He W-M (2015) Two-year interactions between invasive Solidago
1159 canadensis and soil decrease its subsequent growth and competitive ability. Journal of
1160 Plant Ecology:rtv003
1161 Dowell F, Cardman Z, Dasarathy S, Kellerman M, Lipp JS, Ruff SE, Biddle JF, McKay L, MacGregor
1162 BJ, Lloyd KG, Albert DB, Mendlovitz H, Hinrichs KU, Teske A (2016) Microbial
1163 communities in methane- and short chain alkane- rich hydrothermal sediments of
1164 Guaymas Basin. Frontiers in microbiology
1165 Eichinger I, Hourdez S, Bright M (2013) Morphology, microanatomy and sequence data of
1166 *Sclerolinum contortum* (Siboglindae, Annelida) of the Gulf of Mexico. Organisms Diversity
1167 & Evolution 13:311-329
1168 Eichinger I, Schmitz-Esser S, Schmid M, Fisher CR, Bright M (2014) Symbiont-driven sulfur
1169 crystal formation in a thiotrophic symbiosis from deep-sea hydrocarbon seeps. Environ
1170 Microbiol Rep 6:364-372
1171 Elias-Piera F, Rossi S, Gili JM, Orejas C (2013) Trophic ecology of seven Antarctic gorgonian
1172 species. Marine Ecology Progress Series 477:93-106
1173 Erickson KL, Macko SA, Van Dover CL (2009) Evidence for a chemotrophically based food web
1174 at inactive hydrothermal vents (Manus Basin). Deep Sea Research Part II: Topical Studies
1175 in Oceanography 56:1577-1585
1176 Fanelli E, Cartes JE, Enric J, Papiol V, Rumolo P, Sprovieri M (2010) Effects of preservation on the
1177 δ13C and δ15N values of deep sea macrofauna. Journal of Experimental Marine Biology
1178 and Ecology 395:93-97
1179 Fry B, Jannasch HW, Molyneaux SJ, Wirsen CO, Muramoto JA, King S (1991) Stable Isotope
1180 Studies of the Carbon Nitrogen and Sulfur Cycles in the Black Sea and the Cariaco Trench.
1181 Deep-Sea Research Part A Oceanographic Research Papers 38:S1003-S1020
1182 Gebruk A, Krylova E, Lein A, Vinogradov G, Anderson E, Pimenov N, Cherkashev G, Crane K
1183 (2003) Methane seep community of the Håkon Mosby mud volcano (the Norwegian Sea):
1184 composition and trophic aspects. Sarsia: North Atlantic Marine Science 88:394-403
1185 Georgieva M, Wiklund H, Bell JB, Eilersten MH, Mills RA, Little CTS, Glover AG (2015) A
1186 chemosynthetic weed: the tubeworm *Sclerolinum contortum* is a bipolar, cosmopolitan
1187 species. BMC Evolutionary Biology 15:280
1188 Gollner S, Govenar B, Fisher CR, Bright M (2015) Size matters at deep-sea hydrothermal vents:
1189 different diversity and habitat fidelity patterns of meio- and macrofauna. Marine Ecology
1190 Progress Series 520:57-66

1191 Guezennec J, Fiala-Medioni A (1996) Bacterial abundance and diversity in the Barbados Trench
1192 determined by phospholipid analysis. Microbiology Ecology 19:83-93
1193 Hayes JM (2001) Fractionation of carbon and hydrogen isotopes in biosynthetic processes.
1194 Reviews in Mineralogy & Geochemistry 43:225-277
1195 Henley SF, Annett AL, Ganeshram RS, Carson DS, Weston K, Crosta X, Tait A, Dougans J, Fallick
1196 AE, Clarke A (2012) Factors influencing the stable carbon isotopic composition of
1197 suspended and sinking organic matter in the coastal Antarctic sea ice environment.
1198 Biogeosciences 9:1137-1157
1199 Hothorn T, van de Wiel MA, Zeilis A (2015) Package 'Coin': Conditional Inference Procedures in
1200 a Permutation Test Framework. cranr-projectorg
1201 Hugler M, Sievert SM (2011) Beyond the Calvin cycle: autotrophic carbon fixation in the ocean.
1202 Annual review of marine science 3:261-289
1203 Iken K, Brey T, Wand U, Voight J, Junghans P (2001) Trophic relationships in the benthic
1204 community at Porcupine Abyssal Plain (NE Atlantic): a stable isotope analysis. Progress
1205 in Oceanography 50:383-405
1206 Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and
1207 within communities: SIBER - Stable Isotope Bayesian Ellipses in R. The Journal of animal
1208 ecology 80:595-602
1209 Jaeschke A, Eickmann B, Lang SQ, Bernasconi SM, Strauss H, Fruh-Green GL (2014) Biosignatures
1210 in chimney structures and sediment from the Loki's Castle low-temperature
1211 hydrothermal vent field at the Arctic Mid-Ocean Ridge. Extremophiles 18:545-560
1212 Jeffreys RM, Fisher EH, Gooday AJ, Larkin KE, Billett DSM, Wolff GA (2015) The trophic and
1213 metabolic pathways of foraminifera in the Arabian Sea: evidence from cellular stable
1214 isotopes. Biogeosciences 12:1781-1797
1215 Kallmeyer J, Boetius A (2004) Effects of Temperature and Pressure on Sulfate Reduction and
1216 Anaerobic Oxidation of Methane in Hydrothermal Sediments of Guaymas Basin. Applied
1217 and environmental microbiology 70:1231-1233
1218 Kharlamenko VI, Zhukova NV, Khotimchenko SV, Svetashev VI, Kamenev GM (1995) Fatty-acids
1219 as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya
1220 Bight, Yankich island, Kurile Islands). Marine Ecology Progress Series 120:231-241
1221 Kiel S (2016) A biogeographic network reveals evolutionary links between deep-sea
1222 hydrothermal vent and methane seep faunas. Proceedings of the Royal Society B:
1223 Biological Sciences 283
1224 Klinkhammer GP, Chin CS, Keller RA, Dahlmann A, Sahling H, Sarthou G, Petersen S, Smith F
1225 (2001) Discovery of new hydrothermal vent sites in Bransfield Strait, Antarctica. Earth
1226 and Planetary Science Letters 193:395-407
1227 Klouche N, Basso O, Lascourréges J-F, Cavol J-L, Thomas P, Fauque G, Fardeau M-L, Magot M
1228 (2009) Desulfocurvus vexinensis gen. nov., sp. nov., a sulfate-reducing bacterium isolated
1229 from a deep subsurface aquifer. International Journal of Systematic and Evolutionary
1230 Microbiology 30:3100-3104
1231 Kohring L, Ringelberg D, Devereuk R, Stahl DA, Mittelman MW, White DC (1994) Comparison of
1232 phylogenetic relationships based on phospholipid fatty acid profiles and ribosomal RNA
1233 sequence similarities among dissimiltory sulfate-reducing bacteria. Fems Microbiology
1234 Letters 119:303-308
1235 Koranda M, Kaiser C, Fuchslueger L, Kitzler B, Sessitsch A, Zechmeister-Boltenstern S, Rickhter
1236 A (2013) Fungal and bacterial utilization of organic substrates depends on substrate
1237 complexity and N availability. In: Dieckmann U (ed). International Institute for Applied
1238 Systems Analysis, Laxenburg, Austria

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can Stable Isotope Ratios Provide For Community-Wide Measures of Trophic Structure? Ecology 88:42-48
Levin LA, Baco AR, Bowden D, Colaço A, Cordes E, Cunha MR, Demopoulos A, Gobin J, Grupe B, Le J, Metaxas A, Netburn A, Rouse GW, Thurber AR, Tunnicliffe V, Van Dover C, Vanreusel A, Watling L (2016) Hydrothermal Vents and Methane Seeps: Rethinking the Sphere of Influence. Frontiers in Marine Science 3:72
Levin LA, Mendoza GF, Konotchick T, Lee R (2009) Macrobenthos community structure and trophic relationships within active and inactive Pacific hydrothermal sediments. Deep Sea Research Part II: Topical Studies in Oceanography 56:1632-1648
Levin LA, Ziebis W, Mendoza GF, Bertics VJ, Washington T, Gonzalez J, Thurber AR, Ebbed B, Lee RW (2013) Ecological release and niche partitioning under stress: Lessons from dorvilleid polychaetes in sulfidic sediments at methane seeps. Deep-Sea Research Part II-Topical Studies in Oceanography 92:214-233
Liao L, Wankel SD, Wu M, Cavanaugh CM, Girguis PR (2014) Characterizing the plasticity of nitrogen metabolism by the host and symbionts of the hydrothermal vent chemoautotrophic symbioses Ridgeia piscesae. Mol Ecol 23:1544-1557
Main CE, Ruhl HA, Jones DOB, Yool A, Thornton B, Mayor DJ (2015) Hydrocarbon contamination affects deep-sea benthic oxygen uptake and microbial community composition. Deep Sea Research Part I: Oceanographic Research Papers 100:79-87
Martens CS (1990) Generation of short chain organic acid anions in hydrothermally altered sediments of the Guaymas Basin, Gulf of California. Applied Geochemistry 5:71-76
McCaffrey MA, Farrington JW, Repeta DJ (1989) Geo-chemical implications of the lipid composition of *Thioploca* spp. from the Peru upwelling region - 15°S. Organic Geochemistry 14
McClain CR, Schlacher TA (2015) On some hypotheses of diversity of animal life at great depths on the sea floor. Marine Ecology:12288
Mehta MP, Baross JA (2006) Nitrogen Fixation at 92°C by a Hydrothermal Vent Archaeon. Science 314:1783-1786
Mincks SL, Smith CR, Jeffreys RM, Sumida PYG (2008) Trophic structure on the West Antarctic Peninsula shelf: Detritivory and benthic inertia revealed by δ13C and δ15N analysis. Deep Sea Research Part II: Topical Studies in Oceanography 55:2502-2514
Naraoka H, Naito T, Yamanaka T, Tsunogai U, Fujikura K (2008) A multi-isotope study of deep-sea mussels at three different hydrothermal vent sites in the northwestern Pacific. Chemical Geology 255:25-32
Ondov BD, Bergman NH, Phillippy AM (2011) Interactive metagenomic visualization in a Web browser. BMC Bioinformatics 30:385
Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672
Petersen S, Herzig PM, Schwarz-Schampera U, Hannington MD, Jonasson IR (2004) Hydrothermal precipitates associated with bimodal volcanism in the Central Bransfield Strait, Antarctica. Mineralium Deposita 39:358-379
Phillips DL, Inger R, Bearhop S, Jackson AL, Moore JW, Parnell AC, Semmens BX, Ward EJ (2014) Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92:823-835
Pina-Ochoa E, Koho KA, Geslin E, Risgaard-Petersen N (2010) Survival and life strategy of the foraminiferan *Globobulimina turgida* though nitrate storage and denitrification. Marine Ecology Progress Series 417:39-49
Pond DW, Bell MV, Dixon DR, Fallick AE, Segonzac M, Sargent JR (1998) Stable-Carbon-Isotope Composition of Fatty Acids in Hydrothermal Vent Mussels Containing Methanotrophic

and Thiotrophic Bacterial Endosymbionts. Applied and environmental microbiology 64:370-375
Portail M, Olu K, Dubois SF, Escobar-Briones E, Gelinas Y, Menot L, Sarrazin J (2016) Food-Web Complexity in Guaymas Basin Hydrothermal Vents and Cold Seeps. PLoS One 11:e0162263
R Core Team (2013) R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria http://www.R-project.org/.
Rau GH (1981) Low $^{15}N/^{14}N$ in hydrothermal vent animals: ecological implications. Nature 289:484-485
Reid WDK, Sweeting CJ, Wigham BD, Zwirglmaier K, Hawkes JA, McGill RAR, Linse K, Polunin NVC (2013) Spatial Differences in East Scotia Ridge Hydrothermal Vent Food Webs: Influences of Chemistry, Microbiology and Predation on Trophodynamics. Plos One 8
Reid WDK, Wigham BD, McGill RAR, Polunin NVC (2012) Elucidating trophic pathways in benthic deep-sea assemblages of the Mid-Atlantic Ridge north and south of the Charlie-Gibbs Fracture Zone. Marine Ecology Progress Series 463:89-103
Rennie MD, Ozersky T, Evans DO (2012) Effects of formalin preservation on invertebrate stable isotope values over decadal time scales. Canadian Journal of Zoology 90:1320-1327
Rodrigues CF, Hilário A, Cunha MR (2013) Chemosymbiotic species from the Gulf of Cadiz (NE Atlantic): distribution, life styles and nutritional patterns. Biogeosciences 10:2569-2581
Sahling H, Wallman K, Dählmann A, Schmaljohann R, Petersen S (2005) The physicochemical habitat of *Sclerolinum sp.* at Hook Ridge hydrothermal vent, Bransfield Strait, Antarctica. Limnology & Oceanography 50:598-606
Schlesner H (2015) Blastopirellula. Bergey's Manual of Systematics of Archaea and Bacteria. John Wiley & Sons, Ltd
Schmaljohann R, Faber E, Whiticar MJ, Dando PR (1990) Co-existence of methane- and sulphur-based endosymbioses between bacteria and invertebrates at a site in the Skagerrak. Marine Ecology Progress Series 61:11-124
Schmaljohann R, Flügel HJ (1987) Methane-oxidizing bacteria in Pogonophora. Sarsia 72:91-98
Sellanes J, Zapata-Hernández G, Pantoja S, Jessen GL (2011) Chemosynthetic trophic support for the benthic community at an intertidal cold seep site at Mocha Island off central Chile. Estuarine, Coastal and Shelf Science 95:431-439
Soto LA (2009) Stable carbon and nitrogen isotopic signatures of fauna associated with the deep-sea hydrothermal vent system of Guaymas Basin, Gulf of California. Deep Sea Research Part II: Topical Studies in Oceanography 56:1675-1682
Southward A, J., Southward EC, Brattegard T, Bakke T (1979) Further Experiments on the value of Dissolved Organic Matter as Food for *Siboglinum fjiordicum* (Pogonophora). Journal of Marine Biological Asociation of the United Kingdom 59:133-148
Sweetman AK, Levin LA, Rapp HT, Schander C (2013) Faunal trophic structure at hydrothermal vents on the southern Mohn's Ridge, Arctic Ocean. Marine Ecology Progress Series 473:115
Tarasov VG, Gebruk AV, Mironov AN, Moskalev LI (2005) Deep-sea and shallow-water hydrothermal vent communities: Two different phenomena? Chemical Geology 224:5-39
Teske A, Callaghan AV, LaRowe DE (2014) Biosphere frontiers of subsurface life in the sedimented hydrothermal system of Guaymas Basin. Frontiers in microbiology 5:362
Teske A, Hinrichs KU, Edgcomb V, de Vera Gomez A, Kysela D, Sylva SP, Sogin ML, Jannasch HW (2002) Microbial Diversity of Hydrothermal Sediments in the Guaymas Basin: Evidence for Anaerobic Methanotrophic Communities. Applied and environmental microbiology 68:1994-2007

1336 Thornhill DJ, Wiley AA, Campbell AL, Bartol FF, Teske A, Halanych KM (2008) Endosymbionts of
1337 *Siboglinum fjordicum* and the Phylogeny of Bacterial Endosymbionts in Siboglinidae
1338 (Annelida). Biological Bulletin 214:135-144
1339 Thornton B, Zhang Z, Mayes RW, Högberg MN, Midwood AJ (2011) Can gas chromatography
1340 combustion isotope ratio mass spectrometry be used to quantify organic compound
1341 abundance? Rapid Communications in Mass Spectrometry 25:2433-2438
1342 Tyler PA, Connelly DP, Copley JT, Linse K, Mills RA, Pearce DA, Aquilina A, Cole C, Glover AG,
1343 Green DR, Hawkes JA, Hepburn L, Herrera S, Marsh L, Reid WD, Roterman CN, Sweeting
1344 CJ, Tate A, Woulds C, Zwirglmaier K (2011) RRS *James Cook* cruise JC55: Chemosynthetic
1345 Ecosystems of the Southern Ocean. BODC Cruise Report
1346 Valls M, Olivar MP, Fernández de Puelles ML, Molí B, Bernal A, Sweeting CJ (2014) Trophic
1347 structure of mesopelagic fishes in the western Mediterranean based on stable isotopes of
1348 carbon and nitrogen. Journal of Marine Systems 138:160-170
1349 Vetter RD, Fry B (1998) Sulfur contents and sulfur-isotope compositions of thiotrophic
1350 symbioses in bivalve molluscs and vestimentiferan worms. Marine Biology 132:453-460
1351 Veuger B, van Oevelen D, Middelburg JJ (2012) Fate of microbial nitrogen, carbon, hydrolysable
1352 amino acids, monosaccharides, and fatty acids in sediment. Geochimica Et Cosmochimica
1353 Acta 83
1354 Walker BD, McCarthy MD, Fisher AT, Guilderson TP (2008) Dissolved inorganic carbon isotopic
1355 composition of low-temperature axial and ridge-flank hydrothermal fluids of the Juan de
1356 Fuca Ridge. Marine Chemistry 108:123-136
1357 Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian Classifier for Rapid Asssignment
1358 of rRNA sequences into the New Bacterial Taxonomy. Applied Environmental
1359 Microbiology 73:5261-5267
1360 Whitaker D, Christmann M (2013) Package 'clustsig'. cranr-projectorg
1361 Whiticar MJ (1999) Carbon and Hydrogen isotope systematics of bacterial formation and
1362 oxidation of methane. Chemical Geology 161:291-314
1363 Whiticar MJ, Suess E (1990) Hydrothermal hydrocarbon gases in the sediments of the King
1364 George Basin, Bransfield Strait, Antarctica. Applied Geochemistry 5:135-147
1365 Woolley SNC, Tittensor DP, Dunstan PK, Guillera-Arroita G, Lahoz-Monfort JJ, Wintle BA, Worm
1366 B, O'Hara TD (2016) Deep-sea diversity patterns are shaped by energy availability.
1367 Nature
1368 Wu Y, Cao Y, Wang C, Wu M, Aharon O, Xu X (2014) Microbial community structure and
1369 nitrogenase gene diversity of sediment from a deep-sea hydrothermal vent field on the
1370 Southwest Indian Ridge. Acta Oceanologica Sinica 33:94-104
1371 Würzberg L, Peters J, Schüller M, Brandt A (2011) Diet insights of deep-sea polychaetes derived
1372 from fatty acids analyses. Deep Sea Research Part II 58:153-162
1373 Yamanaka T, Sakata S (2004) Abundance and distribution of fatty acids in hydrothermal vent
1374 sediments of the western Pacific Ocean. Organic Geochemistry 35:573-582
1375 Yamanaka T, Shimamura S, Nagashio H, Yamagami S, Onishi Y, Hyodo A, Mampuku M, Mizota C
1376 (2015) A Compilation of the Stable Isotopic Compositions of Carbon, Nitrogen, and Sulfur
1377 in Soft Body Parts of Animals Collected from Deep-Sea Hydrothermal Vent and Methane
1378 Seep Fields: Variations in Energy Source and Importance of Subsurface Microbial
1379 Processes in the Sediment-Hosted Systems. In: Ishibashi J, Okino K, Sunamura M (eds)
1380 Subseafloor Biosphere Linked to Hydrothermal Systems. SpringerOpen, Tokyo
1381 Yorisue T, Inoue K, Miyake H, Kojima S (2012) Trophic structure of hydrothermal vent
1382 communities at Myojin Knoll and Nikko Seamount in the northwestern Pacific:
1383 Implications for photosynthesis-derived food supply. Plankton and Benthos Research
1384 7:35-40

1385 Young JN, Bruggeman J, Rickaby REM, Erez J, Conte M (2013) Evidence for changes in carbon
1386 isotopic fractionation by phytoplankton between 1960 and 2010. Global Biogeochemical
1387 Cycles 27:505-515
1388
1389

10. Figure captions

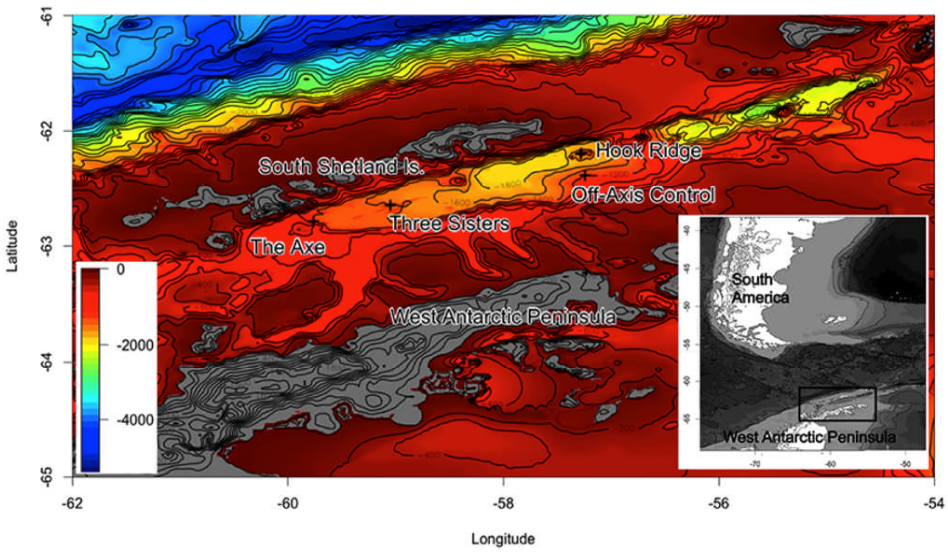
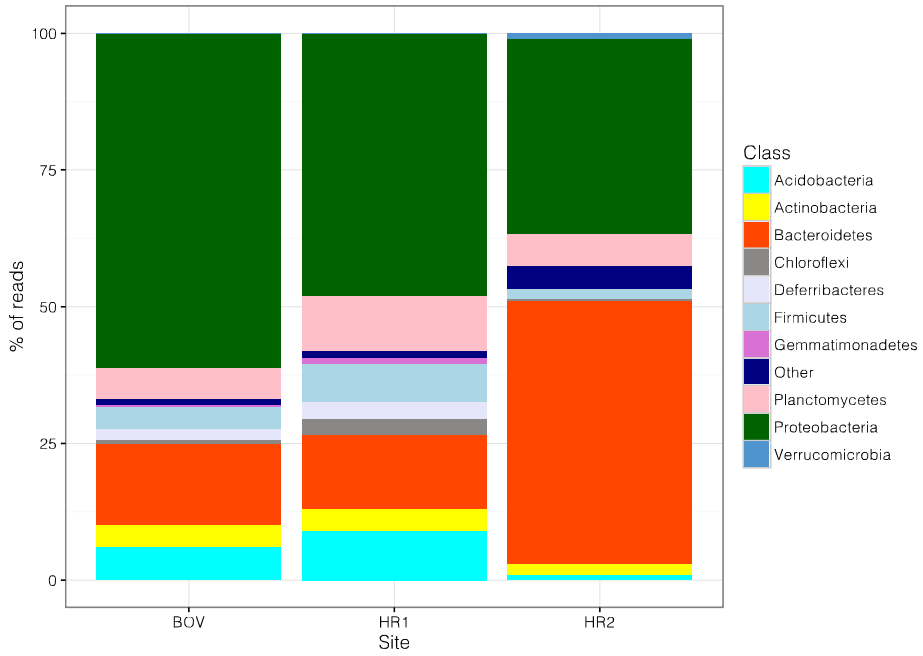



Figure 1 – Sampling sites (after Bell et al. 2016b)

1394




1395

1396 Figure 2 – Microbial composition (classes) at the off-vent/ off-axis site (BOV) and the two Hook
1397 Ridge sites (HR1 and HR2). Archaea excluded from figure as they only accounted for 0.008 % of
1398 reads at HR2 and were not found elsewhere.

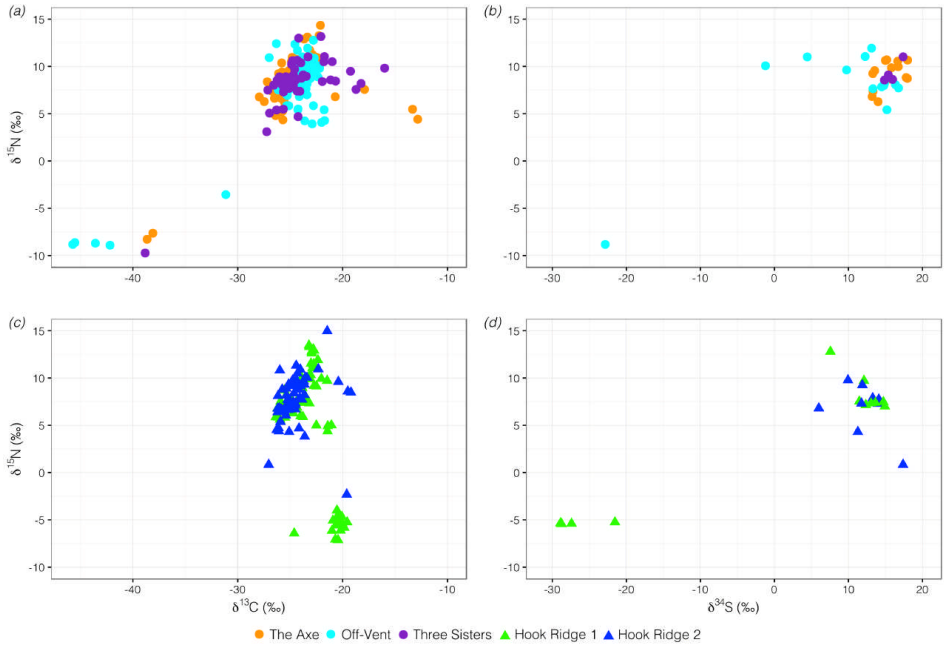



Figure 3 – Carbon-Nitrogen and Sulphur-Nitrogen biplots for bulk isotopic signatures of benthos, separated into non-~~vent~~hydrothermal (top) and ~~vent~~hydrothermal sites (bottom). Excepting one value from the off-vent site (for a peracarid species), all values with $\delta^{15}N$ of < 0 were siboglinid species (*Sclerolinum contortum* from the ~~vent~~hydrothermal sites and *Siboglinum* spp. from the non-~~vent~~hydrothermal sites).

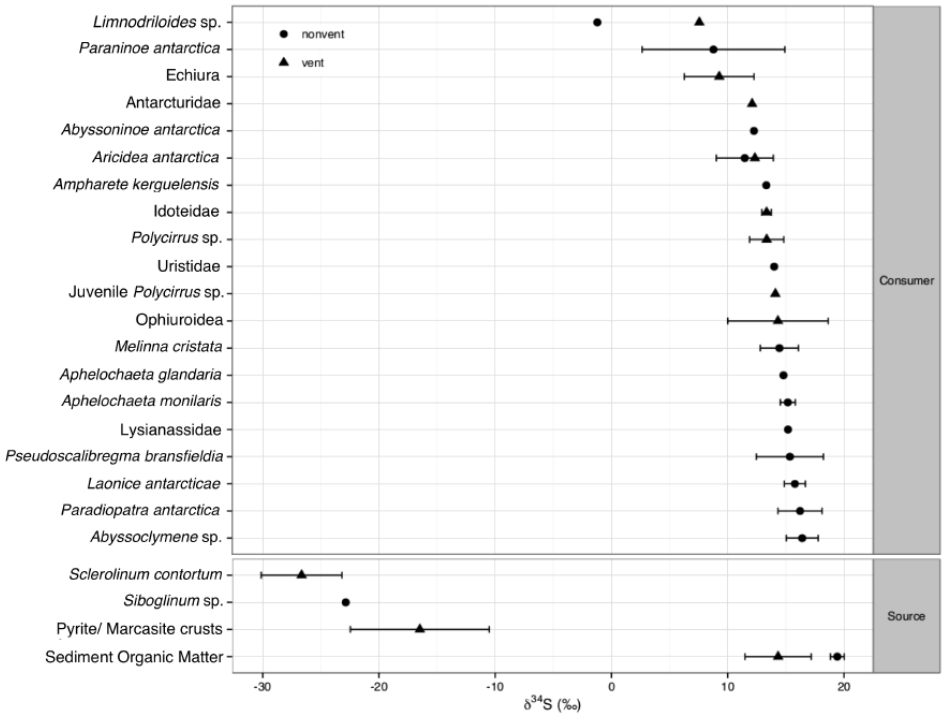



Figure 4 – Plot of $\delta^{34}S$ measurements by discriminated by species and habitat (hydrothermally active vents & sediments/ non-hydrothermally active sediments~~vent~~ ± 1 s.d.). Data for $\delta^{34}S$ in crusts from Petersen et al. (2004)

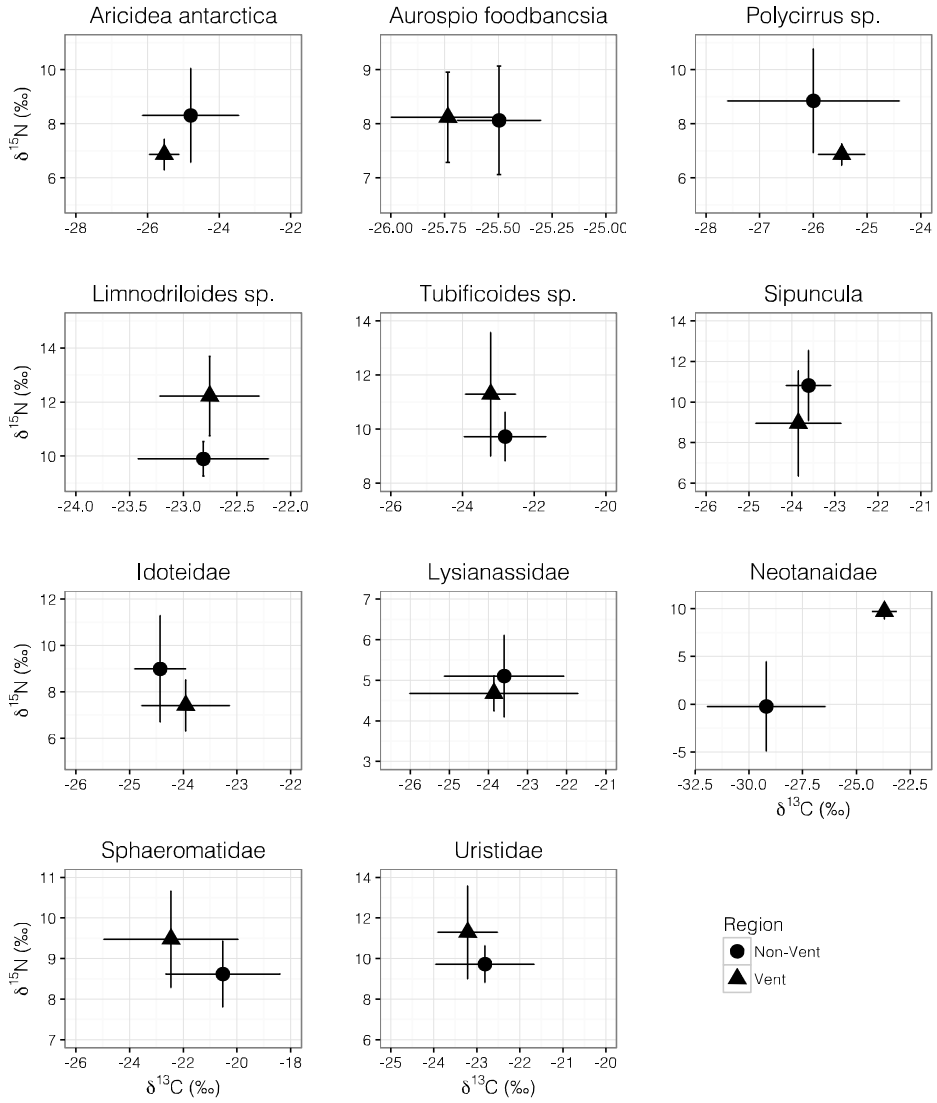



Figure 5– Biplot of CN isotopic data from species sampled both at ~~vent~~hydrothermal sitess and non-~~vent~~hydrothermal background regions. Mean ± standard deviation, X-Y scales vary

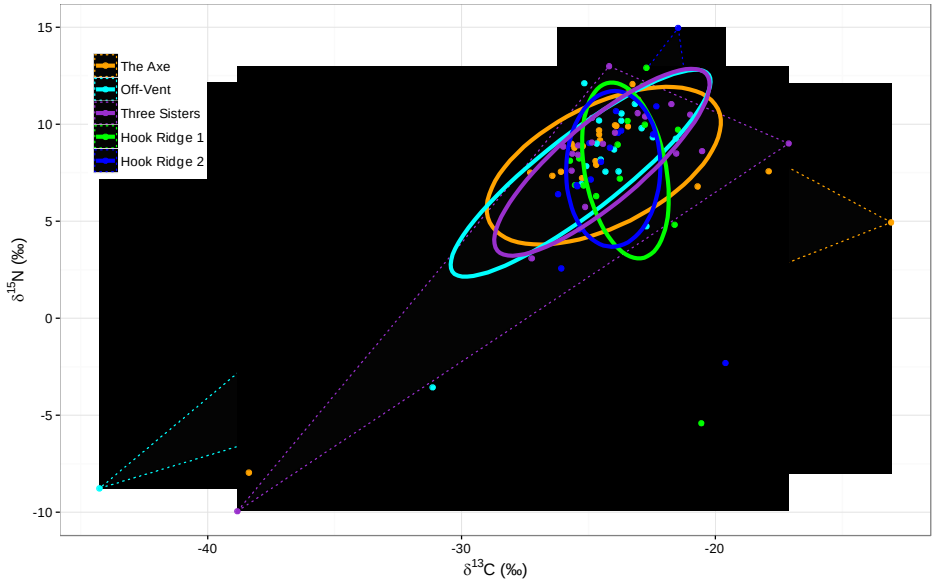



Figure 6 – Faunal isotopic signatures (mean per species), grouped by site with total area (shaded area marked by dotted lines) and sample-size corrected standard elliptical area (solid lines)

1419 11. Tables

| Site | Depth (m) | Hydrothermally active? | References |
|---|---|---|---|
| The Axe (AXE) | 1024 | No | (Dählmann et al. 2001, Klinkhammer et al. 2001, Sahling et al. 2005, Aquilina et al. 2013, Aquilina et al. 2014, Bell et al. 2016b) |
| Off-Vent (BOV) | 1150 | No | |
| Three Sisters (TS) | 1311 | No | |
| Hook Ridge 1 (HR1) | 1174 | Low activity (9 cm $yr^{-1}$) | |
| Hook Ridge 2 (HR2) | 1054 | High Activity (34 cm $yr^{-1}$) | |

1420

1421 Table 1 – Site descriptions and associated references

| Isotope | Species | Idoteidae | *Polycirrus* sp. | *Aphelochaeta glandaria* | Phyllodocida sp. |
|---|---|---|---|---|---|
| | Treatment | 0.1M HCl | 0.1M HCl | 0.1M HCl | 1.0M HCl |
| $\delta^{13}C$ (‰) | Difference in mean | 1.6 | 0.2 | 0.4 | 0.9 |
| | σ untreated | 0.7 | 0.3 | 0.2 | 0.5 |
| | σ treated | 0.7 | 0.3 | 0.2 | 0.2 |
| | Population range | 2.9 | 3.0 | 2.7 | - |
| $\delta^{15}N$ (‰) | Difference in mean | 0.9 | 0.2 | 0.1 | 0.9 |
| | σ untreated | 0.2 | 0.3 | 0.2 | 0.4 |
| | σ treated | 1.0 | 0.2 | 0.2 | 0.3 |
| | Population range | 3.4 | 4.6 | 5.8 | - |
| $\delta^{34}S$ (‰) | Difference in mean | - | - | 0.4 | 1.1 |
| | σ untreated | - | - | 0.4 | 0.8 |
| | σ treated | - | - | 0.7 | 1.4 |
| | Population range | - | - | 2.3 | - |

Table 2 – Differences in isotopic values and standard deviation (σ) of ethanol preserved fauna sampled during JC55 in response to acid treatment, compared with population ranges of untreated samples. Phyllodocida sp. was a single large specimen, used only as part of preliminary experiments. Data rounded to 1 d.p. to account for measurement error.

| Genera | Class | Off-Vent % | Hook Ridge 1 % | Hook Ridge 2 % |
|---|---|---|---|---|
| *Aestuariicola* | Flavobacteria | 1.37 | 0.53 | 6.89 |
| *Arenicella* | Gammaproteobacteria | 7.14 | 5.17 | 2.24 |
| *Blastopireulla* | Planctomycetacia | 2.50 | 3.01 | 1.92 |
| *Denitrovibiro* | Deferribacteres | 1.72 | 2.54 | 0.27 |
| *Geothermobacter* | Deltaproteobacteria | 2.40 | 1.90 | 0.52 |
| *Lutimonas* | Flavobacteria | 0.45 | 0.42 | 4.87 |
| *Maritimimonas* | Flavobacteria | 1.10 | 0.15 | 4.32 |
| *Methylohalomonas* | Gammaproteobacteria | 4.29 | 2.78 | 2.08 |
| *Pasteuria* | Bacilli | 3.30 | 5.02 | 1.67 |
| *Tenacibaculum* | Flavobacteria | 0.26 | 0.04 | 3.36 |
| *Winogradskyella* | Flavobacteria | 0.99 | 0.90 | 4.09 |

Formatted Table

Formatted: Font: Font color: Auto

Formatted: Font: +Body (Cambria), 12 pt

Formatted: Font: Italic

Formatted: Font: Font color: Auto

Formatted: Font: Font color: Auto

Formatted: Font: Font color: Auto

Table 3 – Most dominant bacterial genera (covering the top 5 at each site), with percent of total sequenced reads.

| | Bransfield Off-Vent | | | Three Sisters | | |
|---|---|---|---|---|---|---|
| PLFA | nM $g^{-1}$ | % | $\delta^{13}C$ (‰) | nM $g^{-1}$ | % | $\delta^{13}C$ (‰) |
| i14:0 | 0.03 | 0.12 | -22.0 | 0.02 | 0.09 | -28.0 |
| 14:0 | 0.80 | 3.04 | -31.2 | 0.83 | 3.43 | -30.9 |
| i15:0 | 0.76 | 2.89 | -28.6 | 0.76 | 3.13 | -28.1 |
| a15:0 | 1.06 | 4.03 | -28.4 | 1.06 | 4.39 | -27.7 |
| 15:0 | 0.30 | 1.13 | -29.3 | 0.19 | 0.77 | -29.8 |
| i16:1 | 0.11 | 0.44 | -31.4 | 0.02 | 0.10 | -20.3 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 16:1w11c | 0.00 | 0.00 | n.d. | 0.06 | 0.24 | -23.1 |
| i16:0 | 0.34 | 1.30 | -28.5 | 0.30 | 1.24 | -27.8 |
| 16:1w11t | 0.78 | 2.98 | -24.4 | 0.66 | 2.75 | -25.0 |
| 16:1w7c | 3.98 | 15.19 | -28.9 | 3.37 | 13.95 | -28.1 |
| 16:1w5c | 1.12 | 4.27 | -34.1 | 0.96 | 3.99 | -34.0 |
| 16:0 | 4.29 | 16.37 | -31.1 | 3.80 | 15.73 | -30.0 |
| br17:0 | 0.00 | 0.00 | n.d. | 0.00 | 0.00 | n.d. |
| 10-Me-16:0 | 0.46 | 1.77 | -28.5 | 0.45 | 1.87 | -29.1 |
| i17:0 | 0.08 | 0.32 | -33.2 | 0.20 | 0.84 | -29.8 |
| a17:0 | 0.25 | 0.97 | -31.9 | 0.21 | 0.87 | -31.3 |
| 12-Me-16:0 | 0.25 | 0.94 | -32.9 | 0.21 | 0.86 | -31.6 |
| 17:1w8c | 0.13 | 0.50 | -34.1 | 0.11 | 0.44 | -31.3 |
| 17:0cy | 0.33 | 1.26 | -36.2 | 0.27 | 1.10 | -32.8 |
| 17:0 | 0.15 | 0.56 | -40.0 | 0.08 | 0.33 | -50.4 |
| 10-Me-17:0 | 0.00 | 0.00 | n.d. | 0.00 | 0.00 | n.d. |
| 18:3w6,8,13 | 0.67 | 2.55 | -34.6 | 0.69 | 2.87 | -33.8 |
| 18:2w6,9 | 0.12 | 0.46 | -27.8 | 0.09 | 0.36 | -52.2 |
| 18:1w9 | 1.13 | 4.30 | -30.0 | 1.33 | 5.50 | -29.9 |
| 18:1w7 | 4.42 | 16.85 | -29.0 | 3.84 | 15.91 | -29.1 |
| 18:1w(10 or 11) | 2.33 | 8.88 | -30.1 | 2.26 | 9.36 | -29.9 |
| 18:0 | 0.66 | 2.50 | -30.6 | 0.54 | 2.22 | -30.6 |
| 19:1w6 | 0.03 | 0.12 | -23.5 | 0.03 | 0.12 | -30.1 |
| 10-Me-18:0 | 0.00 | 0.00 | n.d. | 0.00 | 0.00 | n.d. |
| 19:1w8 | 0.11 | 0.42 | -56.6 | 0.17 | 0.69 | -37.5 |
| 19:0cy | 0.20 | 0.77 | -35.6 | 0.20 | 0.83 | -34.8 |
| 20:4(n-6) | 0.14 | 0.55 | -40.0 | 0.20 | 0.83 | -34.1 |
| 20:5(n-3) | 0.41 | 1.57 | -38.0 | 0.30 | 1.23 | -39.3 |
| 20:1(n-9) | 0.42 | 1.60 | -31.5 | 0.41 | 1.71 | -33.7 |
| 22:6(n-3) | 0.22 | 0.83 | -34.1 | 0.43 | 1.77 | -30.0 |
| 22:1(n-9) | 0.10 | 0.39 | -31.3 | 0.10 | 0.41 | -29.9 |
| 24:1(n-9) | 0.03 | 0.12 | -28.7 | 0.02 | 0.07 | -29.7 |
| | | | | | | |
| Total | 26.23 | | | 24.15 | | |
| Average | 0.71 | | -30.5 | 0.65 | | -30.1 |
| | | | | | | |
| | | **mg C $m^{-2}$** | **$\delta^{13}C$ (‰)** | | **mg C $m^{-2}$** | **$\delta^{13}C$ (‰)** |
| Bacterial Biomass | | 134.50 | -26.8 | | 197.12 | -26.4 |

1434

1435

| | **Hook Ridge 1** | | **Hook Ridge 2** | | | **Range** |
|---|---|---|---|---|---|---|
| **PLFA** | **nM $g^{-1}$** | **$\delta^{13}C$ (‰)** | **nM $g^{-1}$** | **%** | **$\delta^{13}C$ (‰)** | **$\delta^{13}C$** |

| | | | | | | (‰) |
|---|---|---|---|---|---|---|
| i14:0 | 0.03 | -15.7 | 0.10 | 0.80 | -28.8 | -13.1 |
| 14:0 | 0.80 | -32.7 | 0.80 | 6.40 | -29.6 | -3.1 |
| i15:0 | 0.76 | -29.7 | 0.40 | 3.20 | -28.1 | -1.7 |
| a15:0 | 1.06 | -29.1 | 0.90 | 7.20 | -28.9 | -1.4 |
| 15:0 | 0.30 | -29.0 | 0.30 | 2.40 | -28.3 | -1.5 |
| i16:1 | 0.11 | -27.6 | 0.00 | 0.00 | n.d. | -11.1 |
| 16:1ω11c | 0.00 | -17.4 | 0.00 | 0.00 | n.d. | -5.7 |
| i16:0 | 0.34 | -29.4 | 0.20 | 1.60 | -28.8 | -1.6 |
| 16:1ω11t | 0.78 | -25.8 | 0.30 | 2.40 | -8.7 | -17.2 |
| 16:1ω7c | 3.98 | -29.2 | 2.50 | 20.00 | -22.9 | -6.3 |
| 16:1ω5c | 1.12 | -31.2 | 0.30 | 2.40 | -24.3 | -9.7 |
| 16:0 | 4.29 | -31.8 | 3.30 | 26.40 | -29.3 | -2.5 |
| br17:0 | 0.00 | -22.9 | 0.00 | 0.00 | -15.8 | -7.2 |
| 10-Me-16:0 | 0.46 | -30.3 | 0.20 | 1.60 | -41.3 | -12.8 |
| i17:0 | 0.08 | n.d. | 0.00 | 0.00 | n.d. | -3.4 |
| a17:0 | 0.25 | -29.0 | 0.20 | 1.60 | -28.6 | -3.4 |
| 12-Me-16:0 | 0.25 | -28.6 | 0.10 | 0.80 | -28.2 | -4.7 |
| 17:1ω8c | 0.13 | -27.1 | 0.10 | 0.80 | -27.2 | -6.9 |
| 17:0cy | 0.33 | -32.3 | 0.20 | 1.60 | -27.7 | -8.5 |
| 17:0 | 0.15 | -40.0 | 0.20 | 1.60 | -30.8 | -19.6 |
| 10-Me-17:0 | 0.00 | -35.0 | 0.00 | 0.00 | n.d. | 0.00 |
| 18:3ω6,8,13 | 0.67 | -31.2 | 0.50 | 4.00 | -29.0 | -5.6 |
| 18:2ω6,9 | 0.12 | -30.0 | 0.30 | 2.40 | -26.7 | -25.5 |
| 18:1ω9 | 1.13 | -29.6 | 0.40 | 3.20 | -25.6 | -4.4 |
| 18:1w7 | 4.42 | -29.9 | 0.60 | 4.80 | -24.7 | -5.1 |
| 18:1ω(10 or 11) | 2.33 | -31.9 | 0.00 | 1.60 | n.d. | -2.0 |
| 18:0 | 0.66 | -29.4 | 0.30 | 0.00 | -29.9 | -1.2 |
| 19:1ω6 | 0.03 | -26.2 | 0.00 | 2.40 | n.d. | -6.6 |
| 10-Me-18:0 | 0.00 | -25.4 | 0.00 | 0.00 | n.d. | 0.0 |
| 19:1ω8 | 0.11 | -41.2 | 0.00 | 0.00 | n.d. | -19.1 |
| 19:0cy | 0.20 | -30.5 | 0.10 | 0.00 | -28.7 | -6.9 |
| 20:4(n-6) | 0.14 | n.d. | 0.00 | 0.80 | n.d. | -5.9 |
| 20:5(n-3) | 0.41 | n.d. | 0.00 | 0.00 | n.d. | -1.3 |
| 20:1(n-9) | 0.42 | n.d. | 0.00 | 0.00 | n.d. | -2.2 |
| 22:6(n-3) | 0.22 | n.d. | 0.00 | 0.00 | n.d. | -4.2 |
| 22:1(n-9) | 0.10 | n.d. | 0.00 | 0.00 | n.d. | -1.4 |
| 24:1(n-9) | 0.03 | n.d. | 0.00 | 0.00 | n.d. | -1.0 |
| | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| Total | 26.23 | | 12.30 | | | |
| Average | 0.71 | -30.3 | 0.33 | | -26.9 | |
| | | | | | | |
| | **mg C m$^{-2}$** | **$\delta^{13}$C (‰)** | | **mg C m$^{-2}$** | **$\delta^{13}$C (‰)** | |
| Bacterial Biomass | 534.55 | -26.6 | | 85.45 | -23.1 | |

Table ~~43~~ – PLFA profiles from freeze-dried sediment (nM per g dry sediment). PLFA names relate to standard notation (i = iso; a = anti-iso; first number = number of carbon atoms in chain; ω = double bond; Me = methyl group). N.P. = Not present in sample. Total PLFA $\delta^{13}$C measurements weighted by concentration Bulk bacterial $\delta^{13}$C estimated from average conversion factor of 3.7 ‰ (Boschker & Middelburg 2002). No data = n.d. Range measurements may be subject to rounding error. N. B. Table split to conform to submission portal requirements.

| Isotope | ~~Vent~~Hydrothermal sites ‰ (± S.D.) | Non-~~Vent~~Hydrothermal sites ‰ (± S.D.) | Different? (T-Test, df = 3) |
|---|---|---|---|
| $\delta^{13}C$ | -26.2 (± 0.4) | -25.8 (± 0.3) | No |
| $\delta^{15}N$ | 5.7 (± 0.7) | 5.0 (± 0.3) | No |
| $\delta^{34}S$ | 14.3 (± 2.9) | 19.4 (± 0.6) | Yes (T = 3.49, p < 0.05) |

Table 54 – Mean isotopic signatures of sediment organic matter.

1447

| | Ellipse | | | | | | | Nearest Neighbour Distance | |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **SEAc (‰²)** | **SEA.B (‰²)** | **Cred. (95% ± ‰²)** | **TA (‰²)** | **Θ** | **E** | **CD** | **Mean** | **S.D.** |
| The Axe | 49.3 | 45.0 | 19.9 | 161.6 | 0.67 | 0.85 | 3.59 | 1.76 | 4.17 |
| Off-Vent | 39.8 | 36.5 | 16.8 | 139.1 | 0.81 | 0.97 | 4.34 | 2.13 | 3.88 |
| Three Sisters | 35.5 | 32.6 | 14.7 | 110.2 | 0.86 | 0.95 | 3.85 | 1.93 | 3.78 |
| Hook Ridge 1 | 23.1 | 20.7 | 11.2 | 42.6 | -1.43 | 0.94 | 3.30 | 1.64 | 2.60 |
| Hook Ridge 2 | 23.4 | 21.1 | 10.7 | 61.8 | 1.55 | 0.89 | 3.17 | 1.52 | 2.03 |
| **Mean** | | | | | | | | | |
| **Non-~~Vent~~Hydrothermal** | **41.5** | **38.0** | **17.2** | **137.0** | **0.78** | **0.92** | **3.93** | **1.94** | **3.94** |
| **~~Vent~~Hydrothermally active** | **23.2** | **20.9** | **11.0** | **52.2** | **0.10** | **0.91** | **3.23** | **1.58** | **2.31** |

1448

| | Centroid | | | | |
|---|---|---|---|---|---|
| **Site** | **$\delta^{13}C$ (‰)** | **$\delta^{15}N$ (‰)** | **$\delta^{34}S$ (‰)** | **dNr (‰)** | **dCr (‰)** |
| The Axe | -24.4 | 7.9 | | 20.0 | 25.3 |

| | | | | | |
|---|---|---|---|---|---|
| Off-Vent | -25.3 | 7.5 | 8.1 | 20.9 | 22.7 |
| Three Sisters | -24.5 | 8.0 | | 22.9 | 21.7 |
| Hook Ridge 1 | -23.5 | 7.6 | 5.4 | 18.3 | 5.2 |
| Hook Ridge 2 | -24.0 | 7.7 | | 17.3 | 6.6 |
| **Mean** | | | | | |
| **Non-~~Vent~~Hydrothermally active** | **-24.7** | **7.8** | | **21.3** | **23.2** |
| **~~Vent~~Hydrothermally active** | **-23.8** | **7.7** | | **17.8** | **5.9** |

Table 6~~5~~ – Ellipse Area & Layman Metrics of benthos by site. SEAc = Sample-sized corrected standard elliptical area; SEA.B = Bayesian estimate of standard elliptical area; TA = Total hull area; E = Eccentricity; dNr = Nitrogen range; dCr = Carbon range; dSr = Sulphur range; CD = Centroid distance. Note: dSR reported only for Hook Ridge 1 and the off-vent site since $\delta^{34}S$ values of siboglinids were only measured from these sites; hence dSr at other sites would be a considerable underestimate. As $\delta^{34}S$ values were comparatively under-representative, these values were not used in calculation of any other metric. Data rounded to 1 d.p. N. B. Table split to conform to submission portal requirements.

Supplementary Information

Supplementary Figure 1 – PLFA Abundances by site.

Supplementary Figure 2 – nMDS plot of PLFA composition, with reference to PLFA suites from the Goban Spur (NE Atlantic) (Main et al. 2015) and Loki's Castle hydrothermal sediments (Jaeschke et al. 2014).

Supplementary Figure 3 – Cluster dendrogram (Euclidean distance) for averaged CN isotopic signatures for species from vent and non-vent areas.

Supplementary File 1 – Bulk isotopic data.

Supplementary File 2 – PLFA data.