Geochemistry, faunal composition and trophic structure in reducing sediments on the southwest South Georgia margin

James B. Bell, Alfred Aquilina, Clare Woulds, Adrian G. Glover, Crispin T. S. Little, William D. K. Reid, Laura E. Hepburn, Jason Newton and Rachel A. Mills

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Final acceptance: 25 August 2016

Review History
RSOS-160284.R0 (Original submission)

Review form: Reviewer 1 (David Pond)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Adequate
Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept as is

Comments to the Author(s)
The paper provides a comprehensive albeit largely descriptive study of the faunal and
geochemical characteristics of reducing sediments of the south west South Georgia margin.

Determining the carbon isotope composition of source methane would have been a valuable
addition to the paper since as the authors acknowledge, geothermally produced methane can
have quite enriched isotope signatures comparable with signatures of POC. Line 500 in the paper
'Methane with similarly heavy isotope values is generally associated with unsedimented
hydrothermal vents. The Menez Gwen site in the Atlantic is characterised by heavy methane and
also quire highly sedimented, at least in locations where the vent mussels are abundant.

Another valuable addition to the paper would be have been to conclusively establish the presence
or otherwise of chemoaotrophic symbionts in tissue sections of invertebrates and reducing
sediments by well-established molecular and microscopic techniques.

These criticisms aside, the paper represents a substantial body of new information on polar biota
associated with reducing sediments and will be a substantial contribution to the literature of a
developing topic of research. I also appreciate the difficulties of undertaking research in the
Southern Ocean where sea conditions often limit what scientific objectives can be achieved.

Review form: Reviewer 2 (Lisa Levin)

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Is it clear how to access all supporting data?
Yes supporting data are clear although column headings are needed on supplement 1

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept with minor revision (please list in comments)
Comments to the Author(s)

This study contributes to the growing literature on the transition zones between chemosynthetic and background communities, as well as on systems that appear to be hybrids. Reducing sediments are widespread in the ocean – from estuaries to the deep sea. This paper makes a valuable contribution to the inventory and description of such systems, and should be of broad interest to readers working on sediment benthos.

The paper is written clearly, with methods well described.

Some specifics should be included:
Methods: what are the dimensions of the megacorer tubes? What was the total area sampled?
Was temperature of sediments measured?

Isotopes:
Preservation in ethanol is known to change isotope signatures of animal tissues. There are a variety of papers that discuss the magnitude of the effect – these should be cited and discussed. Can you give the precision of the instruments for each isotope measured?
Was there any thought given to running compound-specific isotopes to better constrain the base of the food web?

Results:
Fauna: Figures and tables with species lists and their densities belong in the main paper. (e.g., Table S1)
Was there discolored sediment seen in cores or visual surveys?

Oxygen: I did not see the oxygen results presented in the text. Could the aeration activities of the large number of tube builders and burrowers (cirratulids, oligochaetes, bivalves) promote aerobic methane oxidation subsurface? Are the Aphelochaeta mudball builders?

Discussion:
Lines 399-406. Be more explicit about what differences there were in this comparison, specific depths and sampling equipment.

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Can you discuss the signature of methane in the area? The signature of surface water POC? – if it is not known what is it likely to be?

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You do not mention the possibility of aerobic methane oxidation. Is this likely? Or does methane seep too slowly for this?

Line 435 I think you mean Order Phyllodocida? Normally phylodocids refer to the family Phyllodocidae – but you clearly are talking about other families here.

Line 438-9 Sphaerosyllis and Exogoninae are common on carbonates at Costa Rica (e.g., Levin et al. 2015 DSR I) and Hydrate Ridge seeps (Levin et al. 2016 DSR II) and in Chile Margin hydrothermal sediments (Thurber, Levin unpublished)
Deposit feeders usually have high δ15N because they are eating sediments whose N has been heavily recycled on the way down from the surface.

Explain - why is microbial AOM associated with low δ34S? use of sulfide generated by sulfate reducers?

how much lower δ13C?

produced from A combination of…

Doesn’t the signature of the C go up when it is oxidized?

What methane has a signature of -100? Isn’t this the fractionated signature of archaeal lipids?

Fig. 3, 4, captions +/- 1? SD

Table 1 clarify if there is one core taken per substation or how many?

Decision letter (RSOS-160284)

19-Aug-2016

Dear Mr Bell

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-160284 entitled "Geochemistry, faunal composition and trophic structure in reducing sediments on the southwest South Georgia margin" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been...
deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link: http://datadryad.org/submit?journalID=RSOS&manu=RSOS-160284

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days (i.e. by the 28-Aug-2016). If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees.

When uploading your revised files please make sure that you have:
1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document".
2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format)
3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript
5) Included your supplementary files in a format you are happy with (no line numbers, vancouver referencing, track changes removed etc) as these files will NOT be edited in production

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,
Andrew Dunn
Senior Publishing Editor
Royal Society Open Science
openscience@royalsociety.org

on behalf of Jon Blundy
Subject Editor, Royal Society Open Science
openscience@royalsociety.org

Associate Editor Comments to Author:
Associate Editor: 1
Comments to the Author:
Both referees support publication of this well generally written manuscript. The first referee is happy with the MS as it stands, whilst the second referee raises some helpful specific points that need to addressed.

Reviewer comments to Author:
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The paper provides a comprehensive albeit largely descriptive study of the faunal and geochemical characteristics of reducing sediments of the south west South Georgia margin.

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Another valuable addition to the paper would be have been to conclusively establish the presence or otherwise of chemoaotrophic symbionts in tissue sections of invertebrates and reducing sediments by well-established molecular and microscopic techniques.

These criticisms aside, the paper represents a substantial body of new information on polar biota associated with reducing sediments and will be a substantial contribution to the literature of a developing topic of research. I also appreciate the difficulties of undertaking research in the Southern Ocean where sea conditions often limit what scientific objectives can be achieved.

Reviewer: 2

Comments to the Author(s)

This study contributes to the growing literature on the transition zones between chemosynthetic and background communities, as well as on systems that appear to be hybrids. Reducing sediments are widespread in the ocean – from estuaries to the deep sea. This paper makes a valuable contribution to the inventory and description of such systems, and should be of broad interest to readers working on sediment benthos.

The paper is written clearly, with methods well described.

Some specifics should be included:
Methods: what are the dimensions of the megacorer tubes? What was the total area sampled?

Was temperature of sediments measured?

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Preservation in ethanol is known to change isotope signatures of animal tissues. There are a variety of papers that discuss the magnitude of the effect – these should be cited and discussed. Can you give the precision of the instruments for each isotope measured?

Was there any thought given to running compound-specific isotopes to better constrain the base of the food web?

Results:
Fauna: Figures and tables with species lists and their densities belong in the main paper. (e.g., Table S1)

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Discussion:
Lines 399-406. Be more explicit about what differences there were in this comparison, specific depths and sampling equipment.

Trophic section:
Can you discuss the signature of methane in the area? The signature of surface water POC? – if it is not known what is it likely to be?

Line 425 Give water depth of the CA seep
You do not mention the possibility of aerobic methane oxidation. Is this likely? Or does methane seep too slowly for this?

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Line 438-9 Sphaerosyllis and Exogoninae are common on carbonates at Costa Rica (e.g., Levin et al. 2015 DSR I) and Hydrate Ridge seeps (Levin et al. 2016 DSR II) and in Chile Margin hydrothermal sediments (Thurber, Levin unpublished)

Line 449 omit “not consistent with morphology” Deposit feeders usually have high d15N because they are eating sediments whose N has been heavily recycled on the way down from the surface.

Line 457 Explain - why is microbial AOM associated with low d34S? use of sulfide generated by sulfate reducers?
Line 475 how much lower d13C?
Line 499 produced from A combination of…
Line 500-2 Doesn’t the signature of the C go up when it is oxidized?

Line 526 – What methane has a signature of -100? Isn’t this the fractionated signature of archaeal lipids?

Line 552 missing word after although?
Line 564 off seep and near-seep?
Line 567 role of microbial sub seafloor methane consumption

Fig. 3, 4, captions +/- 1? SD
Table 1 clarify if there is one core taken per substation or how many?

Author's Response to Decision Letter for (RSOS-160284)

See Appendix A.
Reviewer response letter in support of Royal Society Open Science Submission RSOS-160284:

Dear Royal Society Open Science Editors,

The following details the changes we have made to the manuscript in response to the reviewer comments. We have addressed each comment (reviewer comment in bold) individually and refer directly to changes we have made as excerpts from the text. Thank you for agreeing to accept this article (subject to the corrections outlined below).

Sincerely,

James Bell (on behalf of the authors)

Associate Editor Comments to Author:
Associate Editor: 1
Comments to the Author:
Both referees support publication of this well generally written manuscript. The first referee is happy with the MS as it stands, whilst the second referee raises some helpful specific points that need to be addressed.

Reviewer comments to Author:

Reviewer: 1

The paper provides a comprehensive albeit largely descriptive study of the faunal and geochemical characteristics of reducing sediments of the southwest South Georgia margin.

Determining the carbon isotope composition of source methane would have been a valuable addition to the paper since as the authors acknowledge, geothermally produced methane can have quite enriched isotope signatures comparable with
signatures of POC. Line 500 in the paper 'Methane with similarly heavy isotope values is generally associated with unsedimented hydrothermal vents. The Menez Gwen site in the Atlantic is characterised by heavy methane and also quire highly sedimented, at least in locations where the vent mussels are abundant.

The carbon isotopic signature of the sediment methane would certainly have been a valuable source of information for this paper. Unfortunately, suitable samples were not available at the time that data were being collected and the measurements had not been made at an earlier date so we were unable to determine the isotopic values of sediment methane from these sites. There was some consideration as to whether samples could be collected retrospectively but it cannot be guaranteed that subsequent measurements would have been representative of the sampling time of the rest of the samples. We have amended line 500 to clarify, following the reviewer's suggestion. The sentence now reads “Methane with similarly heavy isotopic values is more commonly associated with a hydrothermal origin (89)...”.

Another valuable addition to the paper would be have been to conclusively establish the presence or otherwise of chemoautotrophic symbionts in tissue sections of invertebrates and reducing sediments by well-established molecular and microscopic techniques.

The reviewer is correct to point out that these data would strengthen the conclusions of the paper. During the data collection, we undertook microscopic techniques for the dominant bivalve and oligochaete species and consulted with taxonomists with the aim of detecting symbiotic relationships with microbial symbionts. These efforts did not identify any species with obvious populations of endosymbiotic fauna. We also, in collaboration with Christer Erséus at the University of Gothenburg, attempted to sequence some of the oligochaete specimens. Sequencing efforts failed, likely because of treatment effects and the storage time of the samples.

These criticisms aside, the paper represents a substantial body of new information on polar biota associated with reducing sediments and will be a substantial contribution to the literature of a developing topic of research. I also appreciate the difficulties of undertaking research in the Southern Ocean where sea conditions often limit what scientific objectives can be achieved.

We thank the reviewer for their comments and acknowledge the limitations that they have identified. As detailed above, these were unfortunately unavoidable and the present submission represents the best available data that could be included in the manuscript.

Reviewer: 2

This study contributes to the growing literature on the transition zones between chemosynthetic and background communities, as well as on systems that appear to be hybrids. Reducing sediments are widespread in the ocean from estuaries to the deep sea. This paper makes a valuable contribution to the inventory and description of such systems, and should be of broad interest to readers working on sediment benthos. The paper is written clearly, with methods well described.

Some specifics should be included:
**Methods:** what are the dimensions of the megacorer tubes? What was the total area sampled?

We have amended line 124 to clarify. It now reads “Fauna were all sampled during JC55 using a Bowers-Connelly dampened megacorer fitted with eight 10cm diameter tubes (44). Between 1 and 6 deployments were taken from each site (Table 1) giving a total area quantitatively sampled of ~1490 cm$^2$.”

**Was temperature of sediments measured?**

Bottom water temperatures were 1.2°C and there is no reason to think that sediment pore waters would have been significantly different. We have added temperature data on line 241.

**Isotopes:**
*Preservation in ethanol is known to change isotope signatures of animal tissues. There are a variety of papers that discuss the magnitude of the effect - these should be cited and discussed.*

We have expanded the last paragraph of the discussion before the PLFA section (beginning ~line 476) to include more detailed discussion as the reviewer suggests.

**Can you give the precision of the instruments for each isotope measured?**

Precision for sediment PLFAs added on line 217 and bulk isotopic data on line 183. Bulk isotopic precision data from the internal reference material used in this study.

**Was there any thought given to running compound-specific isotopes to better constrain the base of the food web?**

The reviewer correctly identifies a further source of data that would have been useful to this paper. However, nitrogen compound-specific techniques require considerable mass of faunal samples that were unfortunately not available during data collection.

**Results:**
*Fauna: Figures and tables with species lists and their densities belong in the main paper. (e.g., Table S1)*

We have added the species list and density data from supplementary file 1 as a table (table 3) but have kept the information in the supplementary file, to make it easier to download for interested users.

**Was there discolored sediment seen in cores or visual surveys?**

A representative example of the visual survey data is presented in figure 2. No discolouration of sediment was noted in the cores at the time of collection.

**Oxygen: I did not see the oxygen results presented in the text. Could the aeration activities of the large number of tube builders and burrowers (cirratulids, oligochaetes, bivalves) promote aerobic methane oxidation subsurface? Are the Aphelochaeta**
mudball builders?

Oxygen data is presented in supplementary figure three and are presented in Figure 5 (bottom-right panel) and discussed on lines 354 – 356 in the results. Oxygen penetration depth (~2cmbsf) was not sufficient to allow for much aerobic methanotrophy, given the distribution of elevated methane concentrations (>10 cmbsf). We have added a sentence to line 539 to clarify this: “Oxygen penetration depth (Fig. 5) was less than 2cmbsf, suggesting that aerobic oxidation of methane was probably very limited.” We did not observe areas of extensive mud balls during towed video transects of the area.

Discussion:
Lines 399-406. Be more explicit about what differences there were in this comparison, specific depths and sampling equipment.

We have clarified differences in depth and sampling equipment. The section now reads “Samples from comparable depths (300 – 500 m) around the South Sandwich Islands (80) were different to the South Georgia assemblages, being instead dominated by molluscs and malacostracans, with annelids comprising a relatively small component of the assemblages. These samples, whilst not directly comparable owing to differences in sampling techniques (collected by epibenthic sled, as opposed to megacoring), provide evidence of differences between South Georgia and other shelf areas of the East Scotia back-arc basin at several taxonomic levels and support the suggestion that these sediments represent a continuum between seep and background sediments.”

Trophic section:
Can you discuss the signature of methane in the area? The signature of surface water POC, or if it is not known, what is it likely to be?

As mentioned in the response to the comments of reviewer 1, we were unfortunately unable to acquire data for methane carbon isotopic signatures (though we do certainly acknowledge that it would have been very valuable). We have also added an estimate for the $\delta^{13}$C signature of surface primary productivity on line 429, which reads “Estimates of the $\delta^{13}$C signatures of surface POC at this latitude approximately range between -29 and -24 ‰ (81).”

Line 425: Give water depth of the CA seep

Depth of California margin seep (520m) added to text

You do not mention the possibility of aerobic methane oxidation. Is this likely? Or does methane seep too slowly for this?

As mentioned above, oxygen penetration depth was insufficient to allow for aerobic methane oxidation. We cannot comment on the rate of methane seepage, as suitable measurements were not available.

Line 435: I think you mean Order Phyllodocida? Normally phylodocids refer to the family Phyllodocidae - but you clearly are talking about other families here.

The reviewer is correct and we have amended the sentence to redress this confusion. It now...
reads “Polychaetes of the order Phyllodocida (e.g. alciopids, hesionids, nephtyids and
polynoids) were among the taxa with the highest δ¹⁵N values”

Line 438-9: Sphaerosyllis and Exogoninae are common on carbonates at Costa Rica (e.g.,
Levin et al. 2015 DSR I) and Hydrate Ridge seeps (Levin et al. 2016 DSR II) and in Chile
Margin hydrothermal sediments (Thurber, Levin unpublished)

We have added the sentence and references at the reviewers’ suggestion.

Line 449: omit ‘not consistent with morphology’. Deposit feeders usually have high d15N
because they are eating sediments whose N has been heavily recycled on the way down
from the surface.

We have amended the line, which now reads “…strategies that favour OM that had been
recycled by microbial activity, resulting in a relatively high trophic level.”

Line 457: Explain - why is microbial AOM associated with low d34S? Use of sulfide
generated by sulfate reducers?

We have amended the sentence at the reviewer’s suggestion, to clarify the decrease in δ³⁴S. The
sentence now reads “Low δ³⁴S values are associated with microbial AOM (24, 31) as sulphate
reduction, which results in low δ³⁴S signatures, co-occurs with methane oxidation.”

Line 475: How much lower d13C?

Sentence corrected to give more detail. The sentence now reads “. Untreated sediment samples
had δ¹³C values that were 0.66 ‰ greater than acidified sediment samples (82).”

Line 499: Produced from A combination of

Correction made as suggested by reviewer.

Line 500-2: Doesn’t the signature of the C go up when it is oxidized?

We have amended the sentence to clarify. The sentence now reads “hence it is unlikely that
methane was the sole source of carbon in MUFAs without a very substantial metabolic
fractionation effect.”

Line 526: What methane has a signature of -100‰? Isn’t this the fractionated signature
of archaeanal lipids?

We have included an additional reference (Whiticar, 1999. Chemical Geology) which discusses
isotopic characteristics of sedimentary methane in some detail. Methane carbon isotopic values
in the range we present include methane oxidation and carbonate reduction (see. Whiticar, Fig.
4). The range likely exceeds the realistic limits of methane carbon isotopic signatures but in
this context, serves to emphasise how small an impact methanotrophy could possibly have had
on the δ¹³C of sediment organic carbon. We have however amended the range given to reflect
the reviewers’ suggestion. The sentence now reads “We estimate that, if entirely converted into
POC, this amount of methane would only have changed the δ¹³Corg by between 10⁻² and 10⁻¹⁴
‰ in each core section, depending upon the isotopic signature of the methane which was unknown, but estimated between approximately -80 and -50 ‰ (96).”

**Line 538: Omit and before not on other**

Amendment made as suggested by reviewer.

**Line 552: Missing word after although?**

Amendment made at reviewer suggestion. The sentence now reads: “These deposits were attributed to a γ-proteobacterial community, utilising iron and were not linked to methanotrophy or AOM, but were similar to hydrothermal vent communities (111-113).”

**Line 564: Off seep and near-seep?**

Amendment made as suggested by reviewer.

**Line 567: Role of microbial sub seafloor methane consumption**

Amendment made as suggested by reviewer.

**Fig. 3, 4, captions +/- 1? SD**

Figure captions amended according to reviewer suggestion.

**Table 1: Clarify if there is one core taken per substation or how many?**

Table caption amended according to reviewer suggestion.

This concludes our response to the reviewer comments. We thank both reviewers for their helpful responses and the editors for the timely handling of our manuscript and look forward to seeing it published.