Dear Dr. Jack Middelburg:
Below are the authors combined responses to the comments posted by the two referees to the manuscript “Chemosymbiotic species from the Gulf of Cadiz (NE Atlantic): distribution, life histories and nutritional patterns. We feel that we have answered all the reviewers’ comments and have carefully checked the manuscript; we now hope it is acceptable for publication in Biogeosciences. We believe that the latest version is improved on the original and we would like to thank you and the two referees for providing such helpful and constructive criticism.

Clara F. Rodrigues
Ana Hilário
Marina R. Cunha

Reply to RC C7395

This paper samples the species diversity and stable isotope content of symbiotic species around mud volcanoes in the Gulf of Cadiz. The sampling encompasses a wide depth range and varying environmental and chemical characteristics of the sediments. From this, the authors describe the distribution of chemosymbiotic species (which have an unusually high diversity compared with other seep regions of the world) in the context of the known environmental characteristics if the different sites to infer why species may occur at certain places and why this region may support such high diversity. Stable isotope analysis of tissues from select species gives insight into the primary inorganic carbon, nitrogen, and sulfur sources and the mode of carbon fixation used by their symbionts. This work forms a basis for future studies on trophic ecology and resource partitioning among species, especially those that appear ecologically similar, inhabit the same space, but have different isotope contents.

I found the methods, analysis, and conclusions of this paper to be appropriate for the purposes of this study. I have made a few minor comments about the scientific content of the paper and many edits for spelling, grammar, and syntax. Besides the errors due to the authors not being native English speakers, the manuscript is generally clear and well-organized. Though I have pointed out a few of the errors, a more thorough reading by a native English speaker would help to put all of the commas in the right places. I recommend this manuscript for publication in Biogeosciences.

The authors wish to thank both reviewers for the overall positive comment and many useful suggestions. Please see below our detailed rebuttal to all the points raised by the two reviewers.

General Comments:
The use of “life styles”. I think it is more appropriate to call this “life histories”, which essentially means the lifestyle of an organism, but is specifically used in a biological (scientific) context. Life history traits of an animal, for example, could be things like the animal lays eggs, lives 12 years, eats algae, etc. Lifestyle (one word) is more often used for people.
Authors: the authors prefer to keep this terminology life style as it is frequently used in invertebrate biology/ecology – it refers particularly to the life history traits related to mobility and relationship to the substrata (e.g. sedentary or sessile lifestyle)

Table 2: why not just report the difference between gill and tissue instead of standard error. Standard error does not have much meaning when you are only dealing with two data points and it would be good to show that one tissue is or is not consistently higher or lower in isotope value than the other (using positive and negative signs for the difference). Also, please align the numbers to the right. Ideally the numbers would align by the decimal point.
Authors: It’s true that when possible we have separated on board gill and foot tissue for further analysis, but this was not possible for all the specimens (in these cases the whole animal was analyzed); for some samples several specimens were pooled together. In addition the differences between tissues were not coherent among all cases. Since our study focused on interspecific and intraspecific spatial variability rather than among tissues we decided to use the mean isotope values for both tissues. The word “significant” has been replaced by “coherent”

Figure 2: in panel C, the colors of two of the green shades are not the same in the legend and the figure. Consider using different colors with more contrast and making the symbols in the legend larger. In the caption, saying “mean values” is confusing. I think it is fine to state once that you averaged the value for the two tissues, and thereafter refer to these as individual values instead of mean values. Mean implies averaging among individuals as is the meaning in the second sentence of the caption.

Authors: The figure has been changed (also according to suggestion of the Reviewer #2) as suggested with larger symbols and different colors. The second mean refers to a global mean for each species. This has been rephrased.

Comments by Line:

Page 17348:
Line 4: change “life styles of those species” to “life histories of the species”
Authors: Changed to “these” – “these” refers specifically to the chemosymbiotic species referred in the previous line, while “the” is more general (can be interpreted as referring to a wider set of species in the study area)
Lines 7-9: Change “Twenty siboglinid and nine bivalve chemosymbiotic species have been identified and were found living in fifteen mud volcanoes during our studies.” To “During our studies, we identified 20 siboglinid and 9 bivalve chemosymbiotic species living in 15 mud volcanoes.” Or “20 siboglinid and 9 bivalve species were identified.”
Authors: This has been changed, as suggested but we kept the numbers written in full as the final format will depend on the “Biogeosciences” editorial options.
Lines 14-15: change “Isotopic values found for selected species” to “Tissue stable isotope contents for select species”
Authors: This has been changed to: “Tissue stable isotope signatures for selected species”
Line 21 and 22: should be “highlights” and “provides”
Authors: The sentence was changed according to the comments of reviewer #2.
Line 25: “tubeworms” should be “mussels”. That’s what the Rau and Hedges paper was about and vent tubeworms sometimes have enriched δ13C.
Authors: The “tubeworms” were replaced by “mussels”-Thanks for the correction

Page 17349:
Line 3: citations?
Authors: Citations have been added
Line 5: change to “and in exchange the symbionts provide nutrition to the host”
Authors: To avoid losing information this sentence was not changed. “in exchange” was replaced by “in return”.
Line 8: DIC doesn’t always come from pore water. Maybe just add “or bottom water”
Authors: “or near-bottom water” has been added to the sentence
Line 17: phytoplankton, (comma)
Authors: This has been changed, as suggested
Line 18-19: change to “relative to heterotrophs and animals with methanotrophic symbionts”
Page 17350:
Line 13: “a major influence on the quantity”
Authors: This has been changed, as suggested
Line 15: column, (comma)
Authors: This has been changed, as suggested
Line 20: organization,
Authors: This has been changed, as suggested
Line 21: gill,
Authors: This has been changed, as suggested
Line 22: also have very different life histories
Line 23: “bysate” I have never heard this word before. Do you mean to say “epibenthic” instead of epifaunal?
Authors: Lines 22-23 were changed to: “They also have very different lifestyles ranging from deep sediment burrowers to epibenthic byssus-tethered species”.
Line 25: “methane-oxidizing symbionts... and that they have retained”
Authors: This has been changed

Page 17351:
Line 3: bottom water, where oxygen
Authors: This has been changed, as suggested
Line 4: poseidoni, which; Line 6: methane is transported; Line 8: their symbionts,
Authors: Lines 4-8 have been changed to: Hydrogen sulphide or methane in the case of Siboglinum poseidoni that harbours methane-oxidizing endosymbionts (Schmaljohann and Flügel, 1987), is transported,...
Line 21: sampling devices
Authors: This has been changed, as suggested
Line 22: on board as described in Rodrigues et al. (2011a).
Authors: This has been changed to: on board as described by Rodrigues et al. (2011a).
Line 25: “new code names when species hadn't previously been collected (described?)”
Is this your meaning? Same line change “more” to “most abundant”
Authors: The sentence has been rephrased to: “or given new codenames consistent throughout the samples when they did not yet matched a known species”.

Page 17352
Line 3: “freeze-dried” was this really what you did? Or did you dry the samples in a drying oven. Same line: mortar and pestle.
Authors: “freeze-dried” is correct, not changed;
Line 4: tool, and subsamples were separated for... The ground subsample
Line 7 and 10: reground instead of re-grinded
Line 8: shaken not shacked
Authors: Lines 3-10 have been changed to: The samples were freeze-dried and homogenized with a mortar-and-pestle grinding tool, and subsamples were separated for $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S analyses. The ground subsample for carbon analysis was acidified with HCl (1M) until no further bubbling occurred; it was re-suspended in distilled water, centrifuged and the supernatant was discarded, finally the subsample was dried at 60ºC and reground. The ground subsample for sulphur analysis was re-suspended in distilled water, shaken for 5 minutes, centrifuged and the supernatant was discarded; this procedure was repeated 3 times and finally the subsample was dried at 60ºC and reground.
Line 13-14: change to “are reported in standard delta notation relative to a standard material according to the following equation.”
Authors: This has been changed to: The isotope compositions are reported in delta notation relative to standard material according to the following equation:
Line 17: and y the molecular weight of the lighter
Authors: This has been changed, as suggested

Page 17353
Line 3: identified from
Authors: This has been changed, as suggested
Line 19: were always found
Authors: This has been changed, as suggested

Page 17354
Line 3: multitenticate (add an L)
Authors: This has been changed, as suggested
Line 6: undetermined not undermined
This has been changed, as suggested
Line 8-9: “…long tubes that continuously cover the crater…”
Authors: This has been changed, as suggested
Line 14: “on the gill tissue and foot tissue, no difference larger than XX was found between the two tissues”. It is not clear what “significant” means here. Did you do a statistical test? Did you use all species together or each species separately? Might be safer to just say the largest difference instead of using “significant”. I make a note about this for Table 2 as well.
Authors: already explained above - “significant” has been replaced by “coherent”

Line 21: “Table 2), which never had δ13C values...” and “-36.8‰” (permil)
Authors: The % sign was added
Line 22: values,
Authors: This has been changed, as suggested
Line 24: (27.5‰),
Authors: This has been changed, as suggested
Line 25: showing (not shoing)
Authors: This has been changed, as suggested

Page 17355
Line 7-8: Siboglinum lb, which......MVs,
Authors: Lines 7-8 changed to: Except for Siboglinum lb specimens from the shallow Gemini and Lazarillo de Tormes MVs that had values ranging from 8.2 to 12.2‰, the majority of the species showed δ15N values lower than 6 ‰.
Line 14: “with a clear separation of values” might be worth making a figure that shows this visually. There are, after all, only two figures in this manuscript and this spatial difference is interesting.
Authors: a new figure was added.
Line 17: site,
Authors: Not changed
Line 21: MVs,
Authors: This has been changed, as suggested

Page 17356
Line 20: “between a eukaryote” Even though eukaryote starts with an e it has a y sound so is preceded by a.
Because the association of a eukaryote and its symbionts can be seen as an adaptation to bridge oxic-anoxic interfaces, the chemistry.

Solemyid, lucinid, and thyasirid

Only been found in the Porto, Bonjardim, and Carlos Ribeiro MVs, respectively, where...

Regarding Vesicomyidae, sediment,

Flexibility: some species are capable of hosting multiple symbioses, including the co-occurrence of sulfur- and methane-oxidizers, and can receive additional nutrition...

Posterior tube and body

Only been found in the Porto, Bonjardim, and Carlos Ribeiro MVs, respectively, where...

Hydrate not hydrates

Sustaining a higher biomass... Capt. Arutyonov MV,

May have more flexible requirements of reduced compounds, allowing them to exploit even lower concentrations that diffuse to the upper sediments

Rare,

The juveniles and the smaller mixotrophic species may occur in relatively high densities

Surface, while the larger species Thyasira

The larger species Christineconcha regab was

I believe it is the other way around, biogenic is more negative than thermogenic. Check Schoell again carefully (the figures are a bit confusing) and see Roberts and Aharon 1994. Hydrocarbon-derived carbonate buildups of the northern Gulf of Mexico continental slope: A review of submersible investigations. This paper is about the Gulf of Mexico, but there is a nice schematic drawing of thermogenic and biogenic methane with the δ13C ranges.
Nevertheless, the values reported here are compatible with methanotrophic nutrition, and have already shown bacteria in...

The average δ34S value (below 6‰) found in S. cf. poseidoni was unexpected as it may indicate thiotrophy. A possible explanation for this value is a putative contamination of the sample by specimens belonging to other species. Because of their small size and morphological similarity it is possible that co-occurring juveniles of Polybrachia sp. 2 may have been pooled together in the analysis, which complicates the interpretation of the results and may explain this apparent contradiction.

"B." mauritanicus, which have highly depleted δ13C values and δ34S values lower than 5‰, have only methane-oxidizing symbionts. The other species in the Gulf of Mexico, B. heckerae and B. brooksi, have dual symbioses. The highly depleted δ13C isotope signature of "B." mauritanicus, (species confirmed as part of the "childressi" group; Génio et al., 2008) are in accordance with values previously reported for B. childressi (a known methanotrophic mussel; see Table S1). Molecular studies for “B.” mauritanicus revealed the occurrence of a dual symbiosis with the presence of two phylotypes of methane-oxidising bacteria and a less abundant phylotype of a sulphur-oxidising bacterium (Rodrigues et al., 2013). The low abundance of sulphur-oxidizing bacteria can explain the δ34S values above 5‰ found in our study, limit that is usually taken as evidence for methanotrophy (Vetter and Fry 1998). Isotopic values for mytilids are variable and dependent upon their nutrition, symbionts, ontogeny and local environmental conditions.

The all paragraph has been rephrased. It is now "The highly depleted δ13C isotope signature of “B.” mauritanicus, (species confirmed as part of the “childressi” group; Génio et al., 2008) are in accordance with values previously reported for B. childressi (a known methanotrophic mussel; see Table S1). Molecular studies for “B.” mauritanicus revealed the occurrence of a dual symbiosis with the presence of two phylotypes of methane-oxidising bacteria and a less abundant phylotype of a sulphur-oxidising bacterium (Rodrigues et al., 2013). The low abundance of sulphur-oxidizing bacteria can explain the δ34S values above 5‰ found in our study, limit that is usually taken as evidence for methanotrophy (Vetter and Fry 1998). Isotopic values for mytilids are variable and dependent upon their nutrition, symbionts, ontogeny and local environmental conditions.
Line 25: high values found in (delete “were”); Line 27: can exploit different sources of nitrogen (delete “since”) in addition to the nutrition provided by their symbionts, because they may be able to take up and metabolise dissolved organic compounds
Authors: Lines 25-27: This has been changed to: Similar relatively high values were found in *Siboglinum* Iib collected from three of the shallowest mud volcanoes are probably related to the fact that these animals can exploit different sources of nitrogen. *Siboglinum* species are known to be able of taking up and metabolizing dissolved organic compounds in addition to the nutrition provided by their symbionts (Southward and Southward, 1981)

Page 17363
Line 2: surficial not superficial
Authors: This has been changed to surface water
Line 4: possibly not possible
Authors: This has been changed, as suggested
Line 7: in this study results
Authors: Not changed “in this study may result”
Line 9: fractionation factors during
Authors: This has been changed, as suggested
Line 13: MVs, which could be due to a higher variation
Authors: Not changed “is plausibly explained” is correct
Line 15: differences may be lower at shallow MVs, because
Authors: Not changed – phrasing is correct

Page 17364
Line 5: the variations in environmental setting and AOM...
Authors: This has been changed, as suggested
Line 10: chemosymbiotic animals
Authors: Not changed – metazoans is correct
Line 18: in the Gulf of Cadiz, thiotrophy
Authors: This has been changed, as suggested

Reply to RC C7900

The Manuscript “Chemosymbiotic species from the Gulf of Cadiz (NE Atlantic): distribution, life styles and nutritional patterns” by Rodrigues at al. presents the distribution and dominant microbial processes used by symbiont-bearing fauna in the Gulf of Cadiz (GoC). The manuscript found that there was a diversity of different microbial metabolic pathways that fueled these communities and highlighted the differences among sites especially in regards to species composition. While the data presented is quite useful and exciting, the authors could do a better job of explaining how the results expand beyond the GoC, something that is possible from their research but would require a mild rewrite and some restructuring.

The greatest challenge for this manuscript is getting it to transcend from a report of values and into a test of hypotheses with broader scale appeal than those working in the GoC. This is made more difficult by the numerous species and sites and variance in isotopic signatures - which cannot be avoided. However, if a series (or one) hypotheses was presented that could be tested with these data (trophic diversity is a function of species diversity; species richness is greater in areas where there is greater methane input as it allows methane to fuel symbionts in addition to thiotrophy, etc.) it would add not only to the broader appeal but also linearize the structure of the results and discussion making it easier to follow. The GoC is a very exciting place due to the close proximity of many seep settings and that should be stressed before the conclusions. An attempt should be made to make the figures more interpretable as well.
there is a great amount of data presented in figure 1, it takes quite a bit of time to understand the patterns being presented. As and idea, species ranges by depth (species x-axis and depth y-axis) could be presented in a bar graph with carbon isotopic ranges for each species as a point with error bars over the top and its value on a second y-axis. That would allow the reader to quickly see what species were where and what that species was likely using as an energy source in relation to depth.

Authors: Several important changes were made in the manuscript to accommodate the valuable comments of the reviewer. Among other things these include the reformulation of the objectives (in Introduction) rephrased as working hypotheses and two new figures – one with the bathymetric ranges of the species will be included as supplementary material; the other will hopefully illustrate better inter and intraspecific variation in isotopic signatures of the different species in the studied MVs. Figure 2 was also modified.

Abstract: The abstract does a good job of summarizing the findings. However the last sentence falls a bit flat as it doesn’t really state how exciting it is to have this information on so many sites and species in a small spatial area. As a suggestion, I would state that these patterns highlight how trophic fueling changes on a small spatial scales with direct ramifications to seep communities and potentially biodiversity of margins.

Authors: The last sentence has been rephrased: “This study increases the knowledge on distributional patterns and resource partitioning of chemosymbiotic species and highlights how trophic fuelling changes on spatial scales with direct implications to seep assemblages and potentially biodiversity of margins.”

Introduction: The introduction introduces the site and the background for the rest of the paper. What it does not do is place this study in a wider context other than understanding the GoC (Gulf of Cadiz). 15 mud volcanoes with many species symbionts bearing bivalve and frenulates means that the presented research of intra and inter specific relationships of trophic support could be used to get an idea of how small scale features and history (i.e. larval supply and survivorship) of a particular site can lead to increased diversity and trophic diversity – this is discussed later but should be here to get the reader in that mindset while looking at the results. If the symbionts are all eating different things then that is important or if the symbionts are all eating the same thing that is also important and that all pertains to niche overlap and competition which could be discussed to expand the appeal of the manuscript to a broader audience.

Authors: Objectives were framed by a hopefully wider context. Also the new figure will better show the segregation of trophic niches and the differences among MVs.

Results: It seems as if a discussion of mean isotopic signature among the different sites could provide some insight into the factors controlling the distribution of fauna and the trophic signatures (ignoring species specific isotopic signatures but just the mean of the sites studied).

Authors: The authors feel that one of the strong points of this study is the diversity (in morphology, size, metabolism, relation with the substrate) using a single mean by study site would mask all this diversity. In fact, the sites are different not only by their mean isotopic signature but also be the range and diversity of exploited chemical resources.

Discussion: In general the discussion touches on all of the important points of the manuscript. However it is quite long and could be restructured to have better flow. There are many short paragraphs that seemed like they could be lumped into larger paragraphs based around ideas. The initial paragraph seemed almost like it should go at the end after the trophic introduction as it brought in a broader perspective than the later paragraphs. Having this near the end would also allow trophic relationships to be placed in context of the distribution of fauna. As a reader, it is easy to get lost in the names and values and places (which also have names) and to lose sight of the overall point of the study. Again this could be made simpler by having a more
clearly stated hypothesis or hypotheses that this could be based around, allowing a more straight forward discussion.

Authors: Several changes were made in the discussion and conclusion aiming to accommodate the comments of the reviewer.

Tables: Table 2 – the sample size should be given and “species code” should likely be the code given in figure 1 as that is the code that identifies the species. A separate identifier (e.g. site-species code) would be more appropriate for those numbers presented.

Authors: Table 2 was changed (and Table 1 also for coherence)

Figure 2: in this particular case – I feel that having the same axis on all plots is less important than being able see the points more clearly (i.e. ‘A’ is very clumped to keep the y-axis the same as on ‘C’ making it hard to see the nuances). The size of points on ‘B’ are far better than those on the other panes and yellow should be avoided for points on a white background.

Authors: The figure has been changed, with larger symbols and different colors; an additional figure was drawn

Specific comments:

17349 L6 – while the Review by Fisher is a good choice for this reference, I do not think that this particular work by MacAvoy et al (in this particular instance) is as good as the physiological studies at demonstrated the roll of symbionts to hosts – the MacAvoy work demonstrated it to the predators of the system.

Authors: The reference MacAvoy has been removed


Authors: This information has been added, as the respective reference

17351 – Line 24 “code names” should probably be “currently undescribed putative species”

Authors: This has been changed to: “given new codenames consistent throughout the samples when they did not yet matched a known species”

17352 0 line1 – instead of different tissues state the tissues.

Authors: This has been changed as suggested: “were dissected on board and the different tissues (e.g. gill and foot for bivalves)

Line 7 – re-ground rather than re-grinded

Authors: This has been changed as suggested

Line 8 - shaken instead of shacked (which is not the past tense of to shake.)

Authors: This has been changed as suggested

Line 10 – re-ground not grounded.

Authors: This has been changed as suggested

Line 11 – specify which ea-irms and the word method can be removed, it is an instrument not a method.

Authors: The word method and been replaced by the word technique (referring to mass spectrometry)

Page 17353 lines 8 on – when referring to depth (either shallow or deeper) indicate that it is sediment depth or water depth.

Authors: Modified accordingly throughout the text.

Page 17354 line 17 – depleted in 13C – depleted in d13C is vague since that refers to the ratio of both isotopes (even though it is often treated that way in the literature). For both the most depleted and the most enriched individuals and sites (i.e. the part of the sentence after the semi-colon put in the mean value for that species at that site to be specific and save the reader from having to look in the table)
The change was not made as too many numbers will make the text less readable

line 21 – per mil sign needed after -36.8

Authors: The sign has been added

L25 – showing instead of shoing

Authors: This has been changed as suggested

Line 27 – when starting a sentence the genus should be spelled out even if it has been used previously.

Authors: This has been changed as suggested

P17355 L8 and 9 – presented is not quite the right word. “had” would be better.

Authors: This has been changed as suggested

P17358 L1-3 a reference would be good for this statement The words “many” and “most” are very often used throughout the manuscript – I would evaluate each one and decide if it is possible to replace them with the actual value rather than these vague descriptors. In addition, they are occasionally used twice in a sentence which is not necessary.

Authors: The word many was found only once in the text. The word most/mostly was changed whenever possible – there are only seven matches throughout the text

Page 17359 – L15 - if one of the findings is that there is no difference among the foot and the gill then this needs to be tested statistically – would suggest a paired T-test and include in the methods and results beyond stating no difference.

Authors: It's true that when possible we have separated on board gill and foot tissue for further analysis, but this was not possible for all the specimens (in these cases the whole animal was analyzed); for some samples several specimens were pooled together). In addition the differences between tissues were not coherent among all cases. Since our study focused on interspecific and intraspecific spatial variability rather than among tissues we decided to use the mean isotope values for both tissues. The word “significant” has been replaced by “coherent”

Line 20 signs are backwards. Lighter isotopic signature of methane is biogenic.

Authors: This has been changed. It was the other way around indeed

P17361 L3 – The isotopic composition of methane in addition to the isotopic composition of the fauna should be stated whenever the former is available.

Authors: There are very few available data on methane – these can be very variable across each MV and between MVs and therefore their utility is reduced. However, we may compile the available data in a table to be include in supplementary material

L13-15 – sentence is missing a word or two “and the GoC has co-occurring and closely related species of both : : :” – also why is it interesting?

Authors: This has been changed

17362 – L5 – “may have factors other than this”

Authors: This has been changed as suggested

L20 “very ‘Missing a word’ 15N”

Authors: The word rich has been added

L29 of taking up and metabolizing

Authors: This has been changed as suggested