1: Why are the sulfide and methane oxidizers called endosymbionts and why is this relationship regarded as being a symbioses? Has it been proven that both host and endosymbiont can not live without eachother? Do both profit? What does the mussel give to the endosymbionts? I do not have the impression that there is experimental evidence actually proving that this is a symbiosis by definition.

The sulfide and methane oxidizing bacteria are called ENDOsymbionts because they are localized within vacuoles in specialized epithelial cells (bacteriocytes) of the gill, which is an exceptionally developed organ in Bathymodiolids (vent mussels total gill epithelial surface can be 6 times superior to that of littoral mussels, Le Pennec & Hily 1984). This morphological modification is considered as an adaptation to host the endosymbiotic bacteria. However, the nature of this association is indeed not well defined at present. The Mytilid clearly benefits from the organic carbon produced by chemosynthesis by the associated bacteria (e.g. Riou et al. 2008), which therefore play a major role in the host nutrition (Fisher et al. 1987, Fisher & Childress 1992, Jahnke et al. 1995, Pond et al. 1998). Bathymodiolids are thought to acquire organic matter from their associated bacteria by digesting them or using metabolites excreted by the bacteria (Fiala-Médioni et al. 1990, 2002). Starvation experiments on *Bathymodiolus azoricus* specimens kept in aquaria showed that the rapid loss of associated bacteria resulted in a diminished health condition of the mussel (Kàdàr et al. 2006). Sulfur-oxidizing symbionts may also play a role in detoxication because sulfide, their energy source, is known to be extremely toxic for metazoans (Vismann, 1991; Childress and Fisher, 1992).
Although many have tried, it has not yet been possible to culture a chemosynthetic symbiont, which might indicate their dependency on the Mytilid to survive (which is surprising if the hypothesis of vertical transmission is considered, which would suppose a free-living stage of the bacteria before host infection). Bathymodiolid mussels lack specific proteins in their blood that can bind oxygen, sulfide or methane, and are therefore dependent on the diffusion of dissolved gasses from sea water into their gills to take up reductants and oxidants. Mussel beds, however, can disperse the hydrothermal fluids laterally for distances of several meters, resulting in a large increase in the areas in which both dissolved oxygen and hydrogen sulfide are available. The associated bacteria are located at the apical pole of bacteriocytes, closer to the exterior fluids (Le Pennec & Hily 1984), and the Mytilid would provide an ideal shelter for the bacteria.
From all this data, it has been established that the relationship between Bathymodiolids and the associated bacteria was of symbiotic nature (reviewed in Dubilier et al. 2008 and Duperron et al. 2008). However, there are different kinds of symbiotic associations, from mutualistic to parasitic and only genomic and proteomic studies could give further insights into the mechanism of the symbiosis.

2: Page 3455, line 5: In most papers, MOB (methane oxidizing bacteria) is commonly used as abbreviation instead of the MOX used by the authors in this study. The authors may consider using MOB.

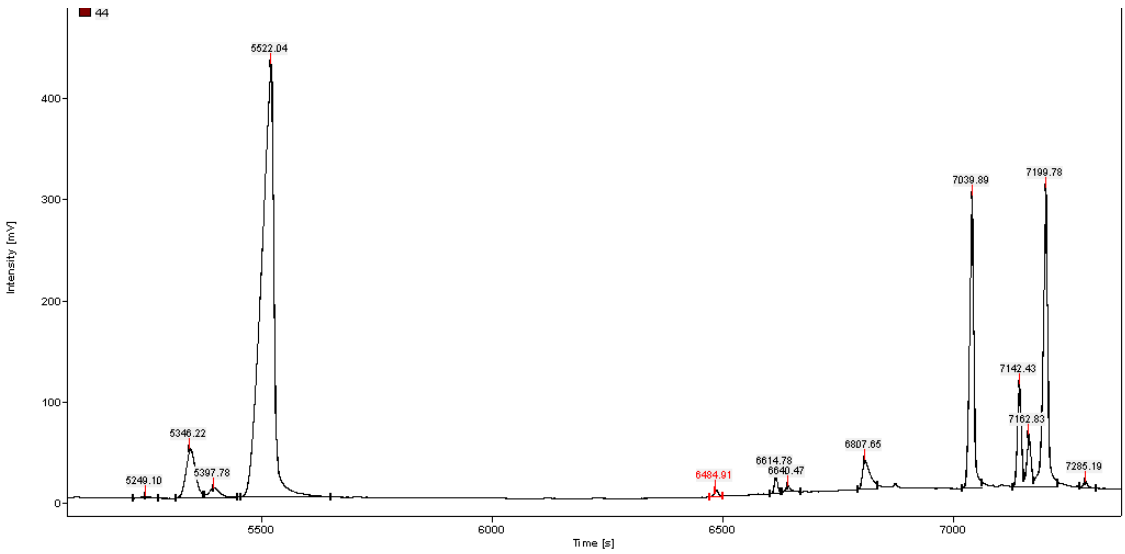
We found it logical to use the abbreviation MOX, since the sulfide oxidizing bacteria are named SOX. However, since reviewer 1 prefers the use of MOB, and to keep it consistent with other publications we replaced MOX by MOB.

3: Page 3455, line 5: Considering the fact that aerobic methane oxidation has also been detected in representatives of the phylum Verrucomicrobia, it is more common now to use gamma- or alpha-proteobacterial methanotrophs when referring to type I and II MOB, respectively.
According to this suggestion, **Page 3455 Line 5**, we substituted:

"for type I methane-oxidising bacteria (MOX; Fiala-Médioni et al., 2002)."
by "for methane-oxidising gamma-proteobacteria (Fiala-Médioni et al., 2002)."
Line 8, "and type I MOX gamma-proteobacteria"
was changed for: "and methane-oxidising gamma-proteobacteria".
Line 25: "PLFA profiles thus allow to distinguish between different MOX species as evidenced for example by the fact that type I MOX bacteria mainly contain fatty acids with 14 and 16 carbon atoms, while type II MOX bacterial PLFA are mainly composed of 18 carbon atoms (Nichols et al., 1985). In addition, MOX bacteria possess fatty acids that are not found in any other known microorganism – type I: 16:1(n-8) and 16:1(n-5)*t*; type II: 18:1(n-8)– and these compounds therefore represent valuable biomarkers (e.g. Nichols et al., 1985)."
was replaced by: "PLFA profiles thus allow to distinguish between different methane-oxidising bacteria (MOB) species as evidenced for example by the fact that methane-oxidising gamma-proteobacteria (formerly named after "Type I MOB") mainly contain fatty acids with 14 and 16 carbon atoms, while methane-oxidising alpha-proteobacteria (formerly "Type II MOB") PLFA are mainly composed of 18 carbon atoms (Nichols et al., 1985). In addition, MOB bacteria possess fatty acids that are not found in any other known microorganism (methane-oxidising gamma-proteobacteria: 16:1(n-8) and 16:1(n-5)*t*; alpha-proteobacteria: 18:1(n-8)) and these compounds therefore represent valuable biomarkers (e.g. Nichols et al., 1985)."
Page 3460 Line 26, the sentence:
"Phylogenetic analyses revealed that the *B. azoricus* methane-oxidising endosymbionts (MOX) is a gamma-proteobacterium related to free living Type I MOX and to MOX symbionts from other Bathymodiolids (Duperron et al., 2006; Nakagawa and Takai, 2008; Spiridonova et al., 2006)."
was replaced by: "Phylogenetic analyses revealed that the *B. azoricus* methane-oxidising endosymbiont is a gamma-proteobacterium related to free living MOB and to MOB symbionts from other Bathymodiolids (Duperron et al., 2006; Nakagawa and Takai, 2008; Spiridonova et al., 2006)."
Page 3465 Line 15:
"In obligate type I MOX bacteria, the chemical conversion of carbohydrates, lipids and proteins…"
was changed for: "In obligate methane-oxidising gamma-proteobacteria, the chemical conversion of carbohydrates, lipids and proteins…"

4: Page 3458, line 20-25: The authors state the chromatographic conditions resulted in baseline separation of most peaks, even C16 and C18 positional isomers. Considering the fact that this is extremely difficult in one-dimensional GC-IRMS, I would really appreciate an example chromatogram showing the baseline separation.

Here is a zoom on the 16:1 (left group) and 18:1 (right group) region from the chromatogram of the Ag-SPE cMUFA fraction from the PLFA extracted from the gills of a specimen exposed 20 days to sulfide. This graph will not be inserted in the paper, unless required by the reviewers.



5: Figure 2: The authors should express the labeling of individual PLFA as percentage excess 13C as compared to the unlabelled control. In this way it is easier to derive for the reader which peaks have actually taken up label.

According to this suggestion, the incorporation of ^{13}C (micrograms of ^{13}C per gram of total PLFA) was calculated for each PLFA, as in Knief *et al.* (2003), and Figure 2 and 3 were modified as shown below.

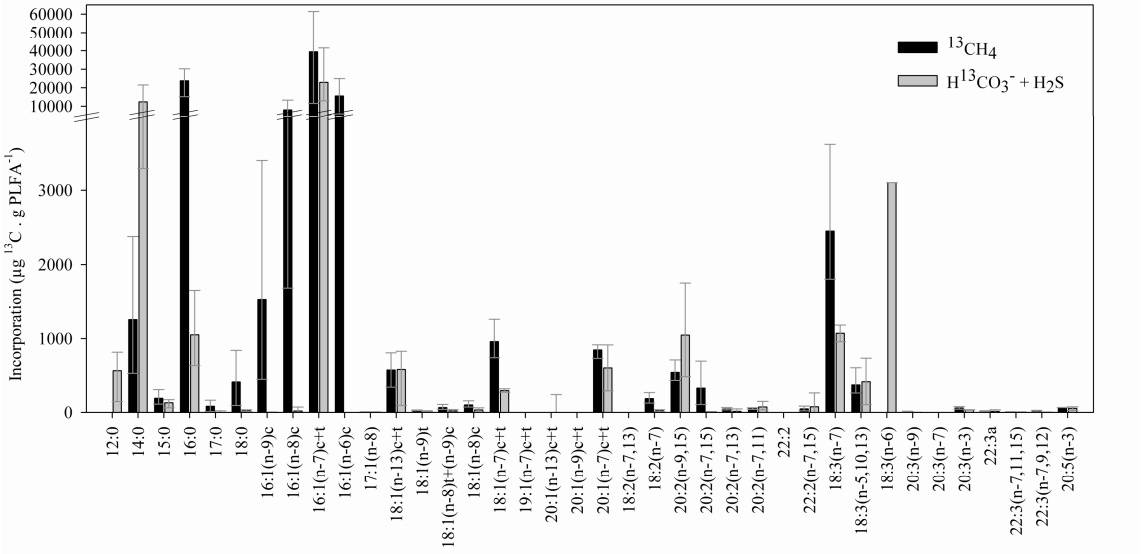


Figure 2: *Bathymodiolus azoricus* gill tissue PLFA (n=3, average±min-max) ^{13}C incorporation (as calculated using formula (3)) after 15 days incubation with $^{13}CH_4$ (black), or 20 days incubation with $H^{13}CO_3^-$ in the presence of H_2S (white).

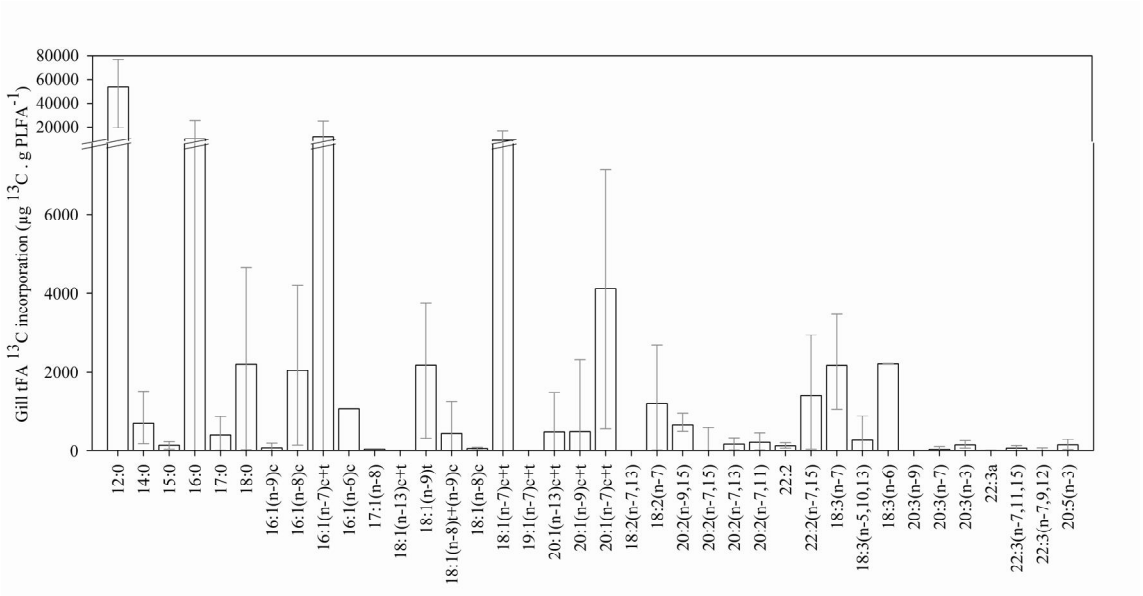


Figure 3: *Bathymodiolus azoricus* gill tissue tFA (n=3, average±min-max) ^{13}C incorporation after 20 days incubation with ^{13}C-amino acids.

The text was modified accordingly. P3463 Line 8: "The experiment with ^{13}C labeled amino acids resulted in significant enrichment of the majority of the total fatty acids (tFA, Fig. 3). Out of the 38 gill tissue tFA only 14 did not show significant enrichment. Among these were the MUFA 16:1(n-9) and 18:1(n-8) (1.6% of the tFA area, Fig. 4), 19:1(n-7) (0.5%), and 20:1(n-13) (4.4%); the non-methylene interrupted di-unsaturated (NMID) fatty acids 18:2(n-7,13) (0.4%), 20:2(n-7,15) (6.2%); and the PUFA 18:3(n-5,10,13) (5.3%), 20:3(n-7) (2.3%) and 22:3 isomers (1.5%). The fatty acid showing most ^{13}C incorporation from the amino acids was 12:0 (representing 2.1% of tFA), followed by 16:1(n-7) (16.0%), 18:1(n-7) (2.7%) and 16:0 (19.1% of tFA)."

The following section was also added to paragraph 2.5:

"For each PLFA, the incorporation of ^{13}C (I, expressed as micrograms of ^{13}C per gram of total PLFA) was calculated as in Knief et al. (2003):

$I = (F_l - F_u) \times (A_x)$ (3)

where A_x is the peak area of $PLFA_x$ divided by the sum of the peak areas of all of the PLFA. F is the fraction of ^{13}C in $PLFA_x$ of samples incubated with ^{13}C (F_l) or in tFA_x of control unlabelled samples (F_u): $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1)$. The carbon isotope ratio (R) was derived from the measured δ^{13}C values as follows: $R = (\delta^{13}C/1000 - 1) \times R_{VPDB}$, with $R_{VPDB} = 0.0112372$."

6: Page 3460, line 26: Which phylogenetic analyses was performed? Please show the results!!!

These analyses were performed in the published studies quoted at the end of the sentence. We could indeed add that the analyses were performed on the DNA sequences encoding the 16S rRNA and functional genes of methane oxidation. Thus, **Page 3460 line 26**, the sentence:
"Phylogenetic analyses revealed that the *B. azoricus* methane-oxidising endosymbionts (MOX) is a gamma-proteobacterium related to free living Type I MOX and to MOX

symbionts from other Bathymodiolids (Duperron et al., 2006; Nakagawa and Takai, 2008; Spiridonova et al., 2006)."

Will be replaced by: "Phylogenetic analyses on sequences of the genes encoding the 16S rRNA subunit and the particulate methane monooxygenase (*pmoA* gene) revealed that the *B. azoricus* methane-oxidising endosymbiont is a gamma-proteobacterium related to free living MOB and to MOB symbionts from other Bathymodiolids (Duperron et al., 2006; Nakagawa and Takai, 2008; Spiridonova et al., 2006)."

7: Page 3461, line 14: The authors conclude that the PLFA labeling patterns indicate the presence of Methylosphaera hansonii. The authors should perform a cluster analyses or another multivariate analyses to specify this result. Please show the result of these analyses in the manuscript.

A cluster analysis is not possible, since we do not have a pure PLFA profile of the symbiont: within the lipids analysed are those of the Mytilid in addition to the symbionts', which would induce a considerable error in the analysis. This is the reason why we opted for a more qualitative representation: we identified the PLFA displaying $^{13}CH_4$ incorporation within the mussel gill tissue (+symbiont). We then screened the data for free living MOB published by Bodelier et al. (2009) searching for a PLFA profile that would come closest to the one of the symbiont marker. We chose to display only the free-living gamma-bacterial species (which is the closest candidate according to 16S rRNA phylogeny; Table 1, *Methylosphaera hansonii*) for which the total PLFA pool shows the largest overlap with the PLFA identified by labeling in the gill tissue.

Here are the results of this analysis: ***Methylohalobium crimeensis 1Ki*** has a PLFA profile which overlaps by 98.1% with the fatty acid profile identified by stable isotope labeling of the *B. azoricus* MOB symbiont, followed by ***Methylosphaera hansonii ACAM 549*** (97.0%: by the way, the error Page 3641 Line 14 was corrected, replacing 95% by 97%), ***Methylocaldum sp. O-12*** (95.8%) and ***Methylocaldum sp. H-11*** (93.0%).

Methylosoma difficle[2]	38.8
Methylococcus capsulatus Bath (ACM332)[3]	66.9
Methylothermus thermalis MYHT[4]	81.9
Methylocaldum sp. E1[5]	88.8
Methylocaldum sp. O-12[6]	**95.8**
Methylocaldum sp. H-11[6]	**93.0**
Methylosarcina fibrata AML-C1[7]	67.4
Methylosarcina quisquiliarum AML-D4[7]	76.7
Methylosarcina lacus[8]	71.1
Methylohalobium crimeensis 1Ki[9]	**98.1**
Methylohalobium crimeensis 4Kr[9]	45.7
Methylosphaera hansonii ACAM 549[10]	**97.0**
Methylomonas pelagica[3]	86.5
Methylomonas methanica ACM337[11]	82.1
Methylomonas fodinarum ACM3268[11]	77.4
Methylomonas aurantiaca ACM346[11]	51.2
Methylomicrobium agile ACM338[11]	64.4
Methylomicrobium album ACM3314[11]	71.1

If Reviewer 1 finds it appropriate (although not as rigorous as a cluster analysis), we could find a way to include these results to the revised manuscript.

8: Page 3463, lines 3-5: This sentence needs some rephrasing.

Rephrased to "The gill tissue showed the most rapid evidence of ^{13}C incorporation from labeled amino acids (Riou et al., 2010): since the symbionts are located in this tissue, they might thus have increased access to this organic food source."