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Interactive comment on “Tracing carbon assimilation in endosymbiotic deep-sea hydrothermal vent Mytilid fatty acids by ¹³C-fingerprinting” by V. Riou et al.

Anonymous Referee #1

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General assessment

The present paper describes the incorporation of carbon into endosymbionts of the deep sea mussel *Bathymodiolus azoricus*. By incubation with labeled methane and combined sulfide+ labeled bicarbonate, PLFA characteristics of sulfide and methane oxidizers, respectively, residing in mussel gill-tissue were labeled. The labeling patterns allow the authors to indicate the identity of the endosymbionts to a certain extent. Incubation with labeled amino acids demonstrated incorporation of amino acid-derived carbon in PLFA of endosymbionts, which is highly unexpected for methanotrophs. Collectively, the authors have demonstrated the potential of stable-isotope labeling to in-

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investigate mussel-endosymbiont relationships and have got a glimpse on the identity of the organisms involved. Next to this, the results indicate that the endosymbionts may also utilize multi-carbon substrates, shedding light on important new pathways of carbon transfer to the host. The study is executed in a sound way and the results have been described satisfactorily. The conclusions drawn are justified and supported by the results. However, I have some specific comments the authors should attend to make the paper clearer to the reader.

Specific comments:

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.

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.

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 typel and II MOB, respectively.

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.

5: Figure 2: The authors should express the labeling of individual PLFA as percentage excess ^{13}C as compared to the unlabelled control. In this way it is easier to derive for

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the reader which peaks have actually taken up label.

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

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.

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

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7, C1396–C1398, 2010

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