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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

Received and published: 16 June 2010

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 extend. Incubation with labeled amino acids demonstrated incorporation of amino acid-derived carbon in PLFA of endysombionts, which is highly unexpected for methanotrophs. Collectively, the authors have demonstrated the potential of stable-isotope labeling to in-

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vestigate 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 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.

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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Interactive comment on Biogeosciences Discuss., 7, 3453, 2010.