Biogeosciences Discuss., 7, C1530–C1532, 2010
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Biogeosciences Discussions

Interactive comment on "Tracing carbon assimilation in endosymbiotic deep-sea hydrothermal vent Mytilid fatty acids by ^{13}C-fingerprinting" *by* V. Riou et al.

Anonymous Referee #2

Received and published: 23 June 2010

Riou et al studied physiology of the double symbiosis of methane and sulfur oxidizing bacteria in deep sea hydrothermal vent mussels by fatty-acid 13C-fingerprinting. They also evaluated the potential for heterotrophic growth of the symbionts or mussel by performing an experiment with 13C-labelled amino acids. They studied the metabolic capabilities and further identified the methanotrophic and sulfur-oxidizing symbionts, and the relationship (ie. carbon transfer) between the symbionts and mussels. The study is certainly novel in its approach and experiments were performed very well, which is not easy for these types of organisms. The experiment with labeled amino acids is very interesting and suggests that the mussels are also capable of gaining energy utilizing external organic matter sources besides their bacterial symbionts. Two

related papers on the same experiments were published (Rioux et al 2008, 2010), which however focuses on total 13C incorporation rates of CH4, CO2, and amino acids. In the present manuscript, compound specific 13C data for PLFA and total fatty acids are reported to further identify symbionts and the experiment with labeled amino acids is added.

I had a previous version of the manuscript for review for another journal. The manuscript has certainly been improved and many of my comments on the previous version are covered in the present manuscript. There are however still issues with the presentation of the data that need attention and there are issues the results and their interpretation that need to be addressed if possible.

The description of the labeling experiments is a bit too condensed. They are fully described in previous publications, but it would help the reader to provide a general description of the experiments (experimental set up, actual amounts of label added, conditions during the experiment etc).

It is not clear why PLFA were analyzed for the CO2 and CH4 incubations and total fatty acids (tFA) for the labeling experiment with amino acids. This should be explained in the manuscript.

The data in Fig. 2 are mixed up: black bars present 13CO2+H2S data and grey bars 13CH4 data. In addition, I do not see why the data for some of the highest labeled PLFA (Fig 2) and total FA (Fig 3) are cut off. Labeling levels are apparently higher (y how much?) than the y-axis range, but it seems to me that the highest labeled compounds are the most interesting ones. One may loose some resolution on the lower labeled compounds if the whole range is shown, but these are not that important anyway. Finally, in the heading to Fig 1, NMID is explained but this is not seen in the figure.

I would also add a figure showing the PLFA and tFA concentrations in gill tissue. These are now mentioned in the discussion in several places, but it would help to see the data.

In the amino-acid experiment, labeling was highest in 12:0 and 16:1(n-8)c, and label was also recovered in 16:1(n-6)c. These compounds are also highly labeled in the CH4 experiment and the two mono-unsaturated FA are used as biomarkers for methanotrophs. Doesn't this indicate that methanotrophic symbionts were important in adsorption of amino acids? This is not widely discussed as far as I can tell, and most of the discussion deals with the direct utilization of amino acids by the mussel. Also, the sentence starting on page 3464 line 14, is basically incorrect as it doesn't acknowledge the high labeling in these two methanotrophic marker FA. Finally, given this finding the authors may reconsider some of the discussion on page 3465 about the substrates that can be used by symbiotic bacteria.

Minor comments. P3458L11. " The isotopic composition of individual FAME was. . .." P3458L24. " The d13C ratios of FAMEs were corrected for the addition of one . . .." P3459L27. " to discuss fatty acid synthesis. " P3463L21. NMID P3465L1. delete "switch on/switch off"

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