

## ***Interactive comment on “Microbial communities responsible for fixation of CO<sub>2</sub> revealed by using *mcrA*, *cbbM*, *cbbL*, *fthfs*, *fefe-hydrogenase* genes as molecular biomarkers in petroleum reservoirs of different temperatures” by J.-F. Liu et al.***

**Anonymous Referee #1**

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In this manuscript the authors analyzed the diversity of potentially CO<sub>2</sub>-fixing microorganisms using PCR amplification and cloning of marker genes involved in the Calvin-Benson-Bassham cycle, reductive acetyl-CoA pathway, and methane Synthesis in samples of Petroleum reservoirs. They were able to amplify all of these genes from the petroleum reservoir samples they obtained, and discussed the potential role of the different phylotypes for CO<sub>2</sub>-fixation in the petroleum reservoirs. This manuscript deals with a rather limited dataset, and I have some concerns regarding the general experimental outline and if the conclusions drawn are really fully justified by the data

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provided in this study. (1) The authors state at the end of their introduction that they want to evaluate the potential of in situ microbial CO<sub>2</sub>-fixation in the different petroleum reservoirs. However, since they did not use a quantitative but only a qualitative approach to analyze the different marker genes, it is difficult to estimate the genetic potential for the different CO<sub>2</sub>-fixation pathways. Here, at least quantitative PCR targeting the different marker genes should be performed to gain insight into the abundance of the different genes and of the corresponding microbial populations. Such quantitative data would provide a more suitable data set to estimate which CO<sub>2</sub>-fixation pathways might be quantitatively relevant in the reservoirs. (2) Some conclusions are based on the relative fraction of certain phylotypes within the microbial communities based on each marker gene. What was the coverage of the clone libraries, how well did the authors cover the groups involved in a certain CO<sub>2</sub>-fixation pathway? (3) Regarding the rather limited set of results, the discussion is much too long and should be shortened substantially. Results are repeated in the discussion and some of the background information placed here should rather be moved to the introduction. (4) The introduction lacks information about the motivation of this study and especially about the authors' expectations which CO<sub>2</sub>-fixation pathways they expect to be represented in the petroleum reservoirs and under which conditions a particular pathway would be favoured. As it is now, the introduction primarily lists the different pathways without a clear link to the system investigated in this study. The authors should also elaborate more on the effect of the different temperatures, which is mentioned in the title but not explained in the introduction. What would be the mechanism behind a relationship between CO<sub>2</sub>-fixation pathways or the composition of the CO<sub>2</sub>-fixing communities on the one hand and temperature on the other hand? What are the authors' expectations regarding the effect of temperature? Regarding the effect of temperature, the authors should be careful with their conclusions, since no replication or time series of samples was performed. The observed differences between the communities at different temperatures could also be of a rather random nature, or the observed differences could be linked to another factor. It is obvious from table 1 that the water chemistry of the four

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reservoirs was different with regard to several parameters. Here, multivariate statistics could help to identify other possible relationships.

Specific comments: p. 1878, l. 4: The CBB cycle is known... p. 1882, l. 7: The headers also of the following sections are misleading since only sequencing results of certain marker genes but not results of processes or activities are presented. Please replace by a more appropriate title. p. 1884, l. 12-14 and l. 27: How much is "less" or "low" similarity"? Please be more specific. p. 1885, l. 15-16: This sentence is not clear, please rephrase. p. 1885, l. 24-25: How can the authors be sure that temperature is the only factor underlying the observed differences in community composition? p. 1888, l.6-11: Should this not rather be placed at the beginning of the discussion? p. 1890, l. 4-10: In order to fully understand these relationships, quantitative data are needed.

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