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***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 #2**

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General Comments

The study by Liu et al. aimed to evaluate the potential for microbial CO<sub>2</sub> fixation and CH<sub>4</sub> production in subsurface oil reservoirs through analysis of functional genes. While the aim of the study is useful for understanding the long-term microbial impacts on CO<sub>2</sub> sequestration, the approach used by the authors fails to provide meaningful data to address the objective as only microbial diversity was assessed. The authors use a cloning and sequencing approach to assess the phylogenetic diversity of five functional genes involved in CO<sub>2</sub> fixation in petroleum reservoirs. While knowledge of the diversity of

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microbial groups involved in CO<sub>2</sub> fixation is useful it does not provide any information about the potential activity of these groups in petroleum reservoirs. Parallel analyses looking at the abundance of genes using quantitative PCR and/or analysis of gene transcripts and cultivation showing that CO<sub>2</sub> fixation can be carried out under reservoir conditions would provide the necessary data needed to understand potential microbial function in petroleum reservoirs. In addition, the authors should perform additional statistical analysis to correlate geochemical conditions in the reservoirs with the different phylogenetic groups detected. The study does show that microbial communities harboring functional genes for microbial CO<sub>2</sub> fixation inhabit four oil reservoirs; however, analysis of only 4 oil reservoirs is not enough to support the statement that these functional groups “inhabit widely in oil reservoirs.” The paper would benefit from having a combined results and discussion section as the results are currently too detailed and the discussion contains a lot of results.

#### Specific Comments:

L. 1-3: The title should be changed to reflect the actual study, which was a phylogenetic assessment of CO<sub>2</sub>-fixation genes in petroleum reservoirs.

L. 16: this was not a comprehensive survey. A comprehensive survey would have included more analyses that provided a full picture of microbial diversity, e.g., quantitative PCR, total community analysis.

L. 23: correct the spelling of Methanomicrobiales

L. 59-61: please reference this statement

L. 62: incorrect usage of “respectively”

L. 82-85: revise

L. 96-97: revise for clarity and please give the volume filtered here

L. 113: correct the reference

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- L. 117-118: please clarify
- L. 130: was bidirectional sequencing performed?
- L. 135: BLAST is not a network service but an algorithm that searches the GenBank database. Please revise.
- L.130-138: please add the methods for translating the nucleotide sequences into amino acid sequences.
- L. 144: please add a section discussing the geochemistry
- L. 145: the heading is incorrect—this is not a section on CO<sub>2</sub> fixation but a section on the diversity of CO<sub>2</sub> fixation functional genes.
- L. 149-150 and elsewhere: please refer to figure 6 for the relative abundance data.
- L. 151 and elsewhere: This is the incorrect nomenclature for the Proteobacteria classes. It should be Betaproteobacteria or  $\beta$ -Proteobacteria.
- L. 151: clone cbbL-XJ6-32 is not in the tree. Please verify that clone names are consistent between the text and the tree.
- L. 164 and elsewhere: it is not necessary to mention GenBank in the results if it is stated in the methods.
- L. 166: add the phylogenetic groups to the tree as brackets for clarity.
- L. 187: this is not acetogenesis but detection of acetogen functional genes. This applies to all headings.
- L. 226-227: this is too strong of a statement—you cannot make global oil reservoir statements from your limited dataset, tone down throughout the paper.
- L. 248 and elsewhere: use proper formatting for genus names (capitalize proper names).

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