

Interactive comment on “Identify the core bacterial microbiome of hydrocarbon degradation and a shift of dominant methanogenesis pathways in oil and aqueous phases of petroleum reservoirs with different temperatures from China” by Zhichao Zhou et al.

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In this manuscript, Zhichao Zhou et al describe the geochemistry and microbial community diversity of several petroleum reservoirs characterized by a broad range of temperatures. For microbial community analyses, the aqueous and oil phases were separated and treated independently. Analyses were essentially based on 16S rRNA and mcrA gene sequencing. In addition, qPCR for the mcrA gene were done, although

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the results of this analysis do not appear to be extensively discussed in the main text. A major conclusion of the work is that despite the distinct geographical locations and different physical-chemical parameters, the analyzed reservoirs shared a common microbiome represented by a small number of OTUs of high relative abundance. Shifts of the methanogenesis mechanisms between the aqueous and oil phase were observed in low and high temperature reservoirs (but not in those of moderate temperature).

Major concerns: Overall, the findings are interesting, but there are, however, several aspects that need more careful consideration. The Introduction makes the case for MEOR: however, in the study rationale (line 95-98) there is not apparent connection to MEOR, and this is also not mentioned again in the conclusions. Is there an impact of the findings of this work on MEOR? Could the observed similarities between the microbial communities of the reservoirs be actually caused by the many years of enhanced oil recovery applied at those sites?

Reply: Thanks for the comments. These reservoirs have been under enhanced oil recovery (EOR) practice for many years, such as flooding with chemical and water. We could not rule out the possibility completely in that the long-time EOR could result in microbial community similarity observed in this study. There is a reasonable possibility in that substantial portions of aerobic bacteria discovered in the core bacteria microbiome across different petroleum reservoirs could be introduced by the exogenous sources through the water flushing. We added this into the Discussions (Lines 263-266). Meanwhile, as indicated by the reviewer, there are not so many interpretations on the connection between MEOR and the microbial community and function investigated in this study. We also added some information into the Discussions on this in the manuscript (Lines 97-99, 257-262).

Overall, the manuscript needs some revision with respect to writing. For some sentences, it is not right away clear if they refer to previous papers, or to results of the present study (for ex. line 30-32, 271-272).

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Reply: Thanks for the suggestions. We added “previous reports” to the sentences to make it clear (Lines 29, 278)

It was demonstrated in many other studies that the PCR reaction is introducing biases: clone libraries and MiSeq data should be therefore interpreted as ‘relative abundance’. With the exception of methanogenesis, which benefits from *mcrA* analyses, all other function assignments are done based on short 16S rRNA gene sequences which give taxonomic resolution at phylum level. This should be revised, and sequence data should be more cautiously interpreted. For example, there is no solid basis to assign all those detected phyla as hydrocarbon degraders (line 28-30).

Reply: Thanks for the comments. We used the 515F/909R based-MiSeq sequencing to generate short 16S rRNA gene reads (which is the longest read length that could be achieved by High-throughput Sequencing method currently, see <http://www.earthmicrobiome.org/protocols-and-standards/16s/>). The others would be even shorter, such as 300bp or 200 bp). We could obtain the taxonomic assignments down to the level of genus for certain groups by using QIIME and the SILVA database (the most comprehensive 16S rRNA database better than others, such as Greengenes and etc). We did not simply assign the function to the microbes at the phylum level but we picked the most abundant genera/core OTU, and found their functional roles according to the reported cultured strains (Tables S5 and S6) (We will also have a review paper on this in the near future). Nevertheless, it is not allowed to assign any functions to certain microbial groups simply based on the closest cultured strains in the same genus/family/order; at the current stage, we think that this is the most careful or reasonable way to deal with the “digital datasets”.

It is stated that methanogenesis is a dominant process (Abstract), but there are not too many arguments provided for this; MiSeq analysis show a high relative abundance of Proteobacteria in all samples (Fig. 1), and these are not methanogens. Also, some samples contain very high amounts of sulfate, comparable to sea water (P1 and P5); in the same samples, nitrate is not detectable. If one assumes oxygen is also absent,

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conditions are favorable for sulfate reduction rather than for methanogenesis. This is not discussed in the manuscript.

Reply: Thanks for the comments. The archaeal methanogenesis is not the most dominant process, and their relative abundance could not compete with bacterial ones. We rephrase the sentence here, that, the methanogenesis process shifts from the dominant hydrogenotrophic pathway in aqueous phase to acetoclastic pathway in oil phase in high-temperature reservoirs (Line 32, 284).

In the low oxygen subsurface petroleum environments, as the reviewer suggested, high sulfate concentrations could fuel the growth and activity of sulfate-reducing bacteria, and this will compete with methanogenesis process. We do find sulfate reducer increase in these samples, e.g., a large proportion of Firmicutes and Deferribacteres in the P5A for the sulfate reduction. We added this part of discussion to the manuscript (Line 300-307).

Currently the reader cannot appreciate if the method used to separate aqueous and oil phases (line 109-110) may alter the community structure. More details should be given, especially the times employed. Was this method tested to make sure cells are not lysed during heating? For how long was the mixture kept at 50C? What exactly are the ‘undetermined results’ (line 210-211) that were omitted?

Reply: Thanks for the comments. In the actual operations, there is not a defined time for the heating. The purpose of this step is to heat the cool and solidified petroleum fluids into semi-fluids, and to benefit for the downstream fractionation. We added the details into the manuscript (Line 114-117).

Normally, we did three replicates for one qPCR to determine the gene quantities. However, for certain experiments, there will be one data that is significantly deviated from others, and we deleted these data from the replicate groups. The undetermined result means a result that is under the detection limit. We added the interpretation in the manuscript (Line 218).

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'Core microbiome' is used often in the text: this should be defined. Does it refer to all taxa with a relative abundance over 0.1%? What does 'quantity requirement for quality control' means exactly? Other inconsistencies: in the Abstract, enrichment cultures are mentioned (line 29), but there is nothing mentioned about enrichments in the rest of the manuscript; Salmonella and tuberculosis are associated with archaeal communities (line 355 – 359)? The equilibrate claim at line 304-305 is at odds with the conclusion at line 371-372. Figure 3, header is missing for the last column?

Reply: The core microbiome actually indicates the OTUs that are shared among all petroleum components from all the samples in this study. The "quantity requirement for quality control" means that we pool the DNA extraction from several repetitive experiments to meet the quantity requirement and then use the DNA to conduct quality control (Line 128).

In the abstract, the enrichment cultures are referring to the previously reported studies (We added this information accordingly, see Line 29). Salmonella and tuberculosis are usually not associated with archaeal community; however, this is the interpretation result from the Tax4Fun and LEfSe. Since FTU values of archaeal communities (fraction of taxa that could be mapped to existing KEGG pathway) were unevenly distributed from sample to sample, the reliability of these functional predictions is in question (Line 393-395). So, I didn't go much into the result discussion for this part, and also state the shortage of this analysis which is based on the current collected database that is far from completeness (Line 395-397).

In this study, we observed the shift of dominant methanogenesis pathway between aqueous and oil phases within samples by temperature, but we do not know whether the temperature is directly/indirectly involved with the shift and influence mechanisms. This needs further observations and studies. We change the expression of related contents (Line 333-334).

The last column is for sample P7A and P8O, which are from different petroleum reser-

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voirs. Fig 3 reflects the compositional shift of methanogenic archaeal community of aqueous/oil phases of individual samples. So, we did not include the last two separated samples which lacked the corresponding aqueous/oil phase samples.

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