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Biogeosciences Discussions

Interactive comment on "Diversity and abundance of n-alkane degrading bacteria in the near surface soils of a Chinese onshore oil and gas field" *by* K. Xu et al.

Anonymous Referee #2

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The manuscript "Diversity and abundance of n-alkane degrading bacteria in the near subsurface soils of a Chinese onshore oil and gas field", by Xu et al., is a nice, small and straightforward report on how a combination of classical molecular techniques can still be used to answer fundamental ecological questions, even in the "deep sequencing" era. I appreciated the readability of the manuscript and high throughput of the results, confirmed by two different lines of evidences (T-RFLP and qPCR). Therefore, the comments below are aimed to optimize the study, especially concerning some of the experimental procedures.

Major Comments:

- I have a major concern regarding the replication of the experiment. The extracted DNAs were pooled (P. 14871, l. 4), as well as the two separated PCR reactions (technical replicates) for T-RFLP (P. 14872, l. 8). Apparently, no dilutions or technical replicates were used for qPCR (P. 14873). Consequently, what is the explanation for the error bars in Figure 3 and 5? Please clarify. - P. 14871, l. 24-25: "The RDP chimera check...for potential chimeras". The authors should explain this point: RDP has noting like a "chimera check program". It suggests (RDP tutorials), however, 2 options for chimera removal: DECIPHER is an online tool that can detect 16S or eventually 18S rRNA chimeras; the UCHIME algorithm has been validated only on 16S rRNA and fungal ITS. Thus, if the authors used UCHIME with customized database, they should provide details on the procedure. Alternatively, the FUN GENE PIPELINE of RDP has an alkB database but no chimera check, however. So this point must be better elucidated. - P. 14872, l. 13: "...GeneScan software...". GeneScan software is used to analyze the output of capillary electrophoresis. Therefore the sentence is inconsistent. Please rephrase. - P. 14872, l. 18-20: DNASTAR is a package, rather than software. The authors should specify which software within the DNASTAR package was used to predict T-RFs in silico, also including used parameters, as stringency and differences (if any) between observed and predicted T-RFs. - P. 14876, l. 24-25 AND P. 14878, l. 17-19: "Next step,...". Please remove these sentences. Rather, better develop the nice (and then forgotten) concept of the abstract, P. 14868, l. 15-17: "Our finding...". How these finding broaden the field? How your study can be practically applied for MPOG?

Minor Comments:

- P. 14868, l. 13: please change "...soils underlying oil and..." to "...soils above oil and..." - P. 14868, l. 26: change "...can be integrated..." with "...was integrated..." - P. 14869, l. 5: punctuation missed - P. 14869, l. 27: please change "...It is a..." to "...This is a..." - P. 14870, l. 3: It is questionable whether the enzyme is the most important. Please modify to something like: "This enzyme is highlyrelevant/expressed/active/ rep-

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resentative in aerobic oil degrading bacteria". - P. 14870, l. 4: punctuation missed - P. 14870, l. 18: pH values reported here is inconsistent with what reported in table 1. Please correct and remove $\sim$ in between the two values. Replace, instead, with "...is in the range of..." or "...is $\sim$8.59 on average..." - P. 14870, l. 20-21: "...aseptically...under aseptic conditions...", remove redundancy - P14876, l. 6-7: replace "non-hydrocarbon-affected soils" with "hydrocarbon-unaffected soils" - P14876, l. 9: replace "That's why" with "That is why" - P. 14877, l. 1: replace "normalizes" with "normalized" - P. 14877, l. 4: replace "was" with "is"

- Fig. 1: The meaning of thinner lines and related negative 4 digits number is not specified. Please add to the legend or caption. - Fig. 4: How are the arrows generated? Are you sure that is a PCA and not a CCA (canonical correspondence analysis)? - Fig. 5: Please rename the (too long) y axis as: "alkB/16S rRNA ratio (%)"

Interactive comment on Biogeosciences Discuss., 9, 14867, 2012.