**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.

K. Xu et al.

xukewei8201@gmail.com

Received and published: 5 February 2013

Authors' Comments to Referee 2

The authors wish to thank referee 2 for his/her efforts in reviewing our manuscript and for the helpful and constructive comments provided. Below are our point by point responses to all issues raised by the referee. The manuscript has been revised accordingly.

Referee: 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 tech-

niques 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: Referee: 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.

Response: Sorry for this confusion. In fact, as the kindly referee said, the extracted DNAs were pooled, as well as the two separated PCR reactions. However, aliquots of the amplicons were then digested with restriction enzyme in triplicate. Real-time PCR of tenfold dilutions of extracted DNA was carried out using the SYBR Premix Ex Taq Perfect Real Time (TaKaRa) in triplicate. We will clarify the protocol in our revised manuscript.

Referee: 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.

Response: Thanks for the referee's careful review. Initially, as the referee said, we tried to use UCHIME algorithm to screen potential chimeras. However, the reference database was difficult to construct. Therefore, alkB sequences were then aligned

manually with alkB sequences obtained from the GenBank database and checked for chimeras by bisecting and drawing two sub-phylogenetic trees from the bisects of each sequence. The sequences that showed different topologies among the two sub-trees were regarded as chimeric and removed from the libraries. When we submitted the first edition of the manuscript, we forgot to change the description. Sorry for this confusion. We will add the annotation to "Materials and methods" in our revised manuscript.

Referee:P. 14872, l. 13: ": : :GeneScan software: : :". GeneScan software is used to analyze the output of capillary electrophoresis. Therefore the sentence is inconsistent. Please rephrase.

Response: Thank you for this suggestion. After electrophoresis, the sizes of the 5'-terminal restriction fragments (T-RF) and the intensities of their fluorescence emission signals were automatically calculated by the GeneScan analysis software (Applied Biosystems). This will be changed in the revised version.

Referee: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.

Response:The size of each n-alkane degrading species T-RF peak corresponded to the value for that species determined by in silico analysis of clone library with EditSeq software (by searching for the first restriction enzyme site "C/CGG" of Msp I) of DNAS-TAR package. All predicted and observed T-RF matches were within 2 bp from each other. We will add the annotation in our revised manuscript.

Referee: 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?

Response: We followed your suggestion. These sentences will be removed in the revised manuscript.

Minor Comments: Referee: P. 14868, l. 13: please change ": : :soils underlying oil and: : :" to ": : :soils above oil and: : :"

Response:The "underlying" have been changed to " above".

Referee: P. 14868, l. 26: change ": : :can be integrated: : :" with ": : :was integrated: : :"

Response: This sentence has been rewritten in our revised manuscript.

Referee: P.14869, l. 5: punctuation missed

Response: The full stop has been added.

Referee: P. 14869, l. 27: please change ": : :It is a: : :" to ": : :This is a: : :"

Response: This item has been changed.

Referee: P. 14870, l. 3: It is questionable whether the enzyme is the most important. Please modify to something like: "This enzyme is highly relevant/expressed/active/ representative in aerobic oil degrading bacteria".

Response: We followed your suggestion. This sentence has been rewritten.

Referee: P. 14870, l. 4: punctuation missed

Response: The full stop has been added.

Referee: P. 14870, l. 18: pH values reported here is inconsistent with what reported in table 1. Please correct and remove _ in between the two values. Replace, instead, with ": : :is in the range of: : :" or ": : :is _8.59 on average: : :"

Response: Thanks for the referee's careful review. This item has been changed.

Referee: P. 14870, l. 20-21: ": : :aseptically: : :under aseptic conditions: : :", remove

redundancy

Response: The redundant "aseptically " have been removed.

Referee: P14876, l. 6-7: replace "non-hydrocarbon-affected soils" with "hydrocarbon-unaffected soils"

Response: This sentence has been rewritten.

Referee: P14876, l. 9: replace "That's why" with "That is why"

Response: This item has been changed.

Referee: P. 14877, l. 1: replace "normalizes" with "normalized"; P. 14877, l. 4: replace "was" with "is"

Response: This item has been changed.

Referee: Fig. 1: The meaning of thinner lines and related negative 4 digits number is not specified. Please add to the legend or caption.

Response: Thanks for the referee's careful review. We will add the annotation to the caption.

Referee: Fig. 4: How are the arrows generated? Are you sure that is a PCA and not a CCA (canonical correspondence analysis)?

Response: In this study, the canonical correspondence analysis was firstly run to estimate the gradient length of variables. However, it was found that the longest gradient was shorter than 3.0. Thus, the principal component analysis (PCA) was chosen for analysis, because it performed better than the unimodal approaches under such conditions according to the CANOCO manual (Ter Braak and Smilauer, 1998). Here, the settings of CANOCO software are as follows: inter-sample distance scaling, no post-transformation of scores, log data transformation (no offset), and center by species. The arrows represent n-alkane degrading species. We will add the annotation in our

revised manuscript.

Referee: Fig. 5: Please rename the (too long) y axis as: "alkB/16S rRNA ratio (%)"

Response: The name of y axis of Fig. 5 has been changed to "alkB/16S rRNA ratio (%)"

—————————————————————

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