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Interactive comment on "Response of soil microorganisms to radioactive oil waste: results from a leaching experiment" *by* P. Galitskaya et al.

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Dear Referee, we are very thankful for your comments. Below you will find our answers on them. In attachment, there is the revised manuscript where changes are tracked by red color.

1. In the section of estimation of waste toxicity, only one species (Bacillus pumilus) was selected for the bacterial assay. In natural bacterial community, some bacteria may use hydrocarbons, some may be sensitive to hydrocarbons. It may be a similar situation for radionuclides, some bacteria are radiation resistant, some may be sensitive. Due to the lack of systematic assay, this part of the experiments did not contribute to the main theme of the work very much, and it is actually misleading (by showing only the toxic or inhibitory effect) Bacillus pumilus toxicity bioassay is one of standardized

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methods recommended for toxicity characterization of the wastes. We included the data obtained by this assay together with data from the two other bioassays in order to show that this waste is hazardous for the environment, so may cause negative effects. However, taking into account this comment and comments of the other reviewers, we excluded the data concerning toxicological characterization of the waste samples, from the article.

2. "DNA extracts were stored at 20 degree C for further analysis." The DNA would be degraded at this temperature. Technical mistake, improved. The temperature was of course -20 °C. 3. The quality of SSCP experiment is problematic (see Figure 4). The low quality of the electrophoresis gel picture may prevent accurate analyses, especially for those using the "quantitative" data ("band areas and integrated intensities"). The analysis of electrophoresis gel was carried using the programe with Quantity One 1-D Analysis Software (Biorad, Hercules, CA, USA). This software permits to get the quantitative data for further analysis. The quality of the picture is better than shown in the manuscript where the quality was reduced to minimize the size (Mb) of the document. Moreover as you can see in the lower part of the figure 4 the Quantity One 1-D software was able to recognize and quantify a lot more bands than visible by eyes indicating the good quality of the picture in the software analysis.

4. Only 4 bands were excised from the SSCP gels and sequenced. How about the other bands? Is it possible that some of the other bands are chimeras (which should be excluded from analyses)? In the Materials and Methods section, "Sequences were analyzed for chimeras with the Pintail program ..., and putative chimeras were removed from the data set." How many chimera sequences were found? We only choose 4 strong DNA bands to concentrate on the dominate bacteria. The selection criteria were described in the text of the manuscript. Bands 1 and 2 were considered stable, as they were present in all samples (except Ru) in relatively large amounts. Band 3 dominated in control samples, and its relative abundance expressed in term of area and band intensity was 1.5- to 3.8-fold higher in control samples than in H- and R-samples. Band

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4 was present in all samples, but was predominant in H-samples. To avoid chimeras we chose the cloning approach. Theoretically every DNA-band in the SSCP gel is DNA from on single species. But by comigration and by using universal primers in a highly diverse environment sometime DNA from more than 1 species is inside of one band. If the DNA of the band is sequence directly without cloning the chance to get chimeras is very high. By choosing the cloning approach we avoid this problem. Nevertheless also by cloning one can get chimera sequences e.g. two different E. coli clones located close together and if they grow together in one colony. If the DNA of this colony is sequenced one would also get some chimeras. Therefore we chose 4 different clones from each SSCP-DNA band and sequence the clones and checked for chimera. Only one clone of the 16 clones showed a chimera sequence and was sorted out.

5. For the phylogenetic tree construction method, the length of the PCR products sing the Com1/Com2 primers is about 400 bp, how the tree could be constructed with nearly full-length sequences (> 1.300 bp)? The reviewer is right that is not possible. In a draft version a tree was included in the manuscript but we deleted the tree because of shortage of space and the low informative value of the tree for the paper. The phylogenetic tree of the 16S rRNA partial gene sequences was constructed by using the maximum likelihood algorithm for the nearly full-length sequences out of the SILVA Database and the clone sequences (about 400 bp) were added by using the ARB parsimony tool to this tree. As in the material & methods correctly described the similarity values were calculated using the PHYLIP neighbor-joining algorithm (Felsenstein, 1989) implemented in the ARB software package (Ludwig et al., 2004). The generated similarity matrix was used to identify the next relatives to the 16S rRNA gene sequences of the clones. We rephrased the paragraph in the “Result and Discussion” part.

6. Figure 5: this figure indicates that the bacterial assemblages of the control soils and the R samples (excluding the deepest layer) could not be separated. For answer see comment 7 below

7. Figure 6: the bacterial assemblages of the control soils and most of the R samples

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are quite closely positioned in the plot. So the sentences "It is important to note that communities from the R-columns were separated from the communities from Ccolumns despite the fact that the activity concentration of 226Ra was below the recommended level (IBSS, 2001) and not in line with the estimates for functional characteristics of the microbial community. This confirmed that PCR-based estimates of environmental influence can be more sensitive than traditional methods (Lin et al., 2012; Bialek et al., 2011)" are not correct. We think that it is correct since the groups can be linearely discriminated, using, for example, Fisher's LDA (linear discriminant analysis). It is what we mean under "separated" Answers to both Fig 5 and Fig 6 comments: The analysis of electrophoresis gel was carried using the program with Quantity One 1-D Analysis Software (Biorad, Hercules, CA, USA). This software permits to get the quantitative data for further analysis. The figure 5 presented the data of cluster analysis where the three-dimensional volume picture is located on the plane. In this case some time the distances between objects are unevident. Besides the differences in bacterial assemblages of control and R samples were proved by the biodiversity indexes, presented in Table 8 Figures 6 and 7 and some other results from the current work may indicate that the radionuclides from the treated oil wastes may not have a big impact on the soil bacterial assemblage. We agree, hydrocarbons play more essential role, both for functions and structure of microbial community. However, using biodiversity indexes and cluster analysis we can prove that soil sample from the R-columns differ significantly from the corresponding control samples, which means that impact of radionuclides exists.

9. It would be helpful to follow the change of the detail chemical composition of the waste oils added to the soils during the experiment, to see which compounds may be utilized by the soil microbes and which compounds may be inhibitory. We did measure content of different fractions in the sample H: we found there 36% aromatics, 27% alphaltenes, 16% aliphatics, 21% resins, so the content of heavy biodegradable fractions (asphaletenes in resins) was higher than in the crude oil. This information is included into “Materials and Methods” section (paragraph 2.1). However we did not analyze the content of specific compounds, or changes of fractions’ content over time and in de-

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pendence on soil layer. These questions are important and may become objectives of the new study.

10. "... the cellulase enzyme complex was sensitive to hydrocarbon contamination", is there any genetic, molecular or enzymatic mechanistic explanation for this? Hydrocarbons may also influence the gene expression of microbial cellulases. Surely the enzymes activity may be regulated by inhibition of the gene expression or by the inhibition of its activities. However the learning of mechanism of these effects was not in the focus of this investigation, but thank you for the comments, we can do it in the future. According to the comment from the Referee, we slightly changed the paragraph concerning CA in “Results and Discussion” section (3.4.1).

11. Some of the sentences are not clear: such as Page 1756, Lines 5-6; Page 1767, Lines 20-22; Page 1769, Lines 26-27. Rephrased

12. The conclusion section can be made shorter and more concise. Shortened (we excluded paragraph concerning toxicity assessment of the raw waste)

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C1629/2015/bgd-12-C1629-2015-supplement.pdf

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