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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.

Anonymous Referee #6

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General comment: The work submitted by Galitskaya et al. described an investigation of the impacts of oil and radionuclides on soil bacterial communities. Although the idea is interesting, some of the experimental and analytical methods are not solid and some of the conclusions are not accurate.

The major problems: 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).

2. "DNA extracts were stored at 20 degree C for further analysis." The DNA would be degraded at this temperature.

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").

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?

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)?

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.

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

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.

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.

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.

Specific problems: 1. Some of the sentences are not clear: such as Page 1756, Lines 5-6; Page 1767, Lines 20-22; Page 1769, Lines 26-27. 2. The conclusion section can be made shorter and more concise.

Interactive comment on Biogeosciences Discuss., 12, 1753, 2015.