

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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We kindly thank you for your time and your opinion! *Bacillus pumilus* toxicity bioassay is one of standardized 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. We disagree with the Referee that changes of cellulase activity together with qCO₂ can't characterize the microbial community as a whole. We analyzed these parameters in the column soil layers, and obtained the differences caused by depth and pollution factors. We suggest that these parameters

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can be used as integrative ones for soil microbial community. We agree with the Referee, that by waste disposal on the soil surface, we observed the shifts in microbial community structure. These shifts are described as follows: Abstract: “PCR-SSCP (polymerase chain reaction – single strand conformation polymorphism) analysis followed by MDS (metric multidimensional scaling) and clustering analysis revealed that the shifts in microbial community structure were affected by both hydrocarbons and radioactivity”. Results and Discussion: “In contrast, PCR-SSCP demonstrated that both oil compounds and radioactive elements could cause shifts in the microbial community structure”. As for method suggested by the Referee “prepare microcosms with sterile soil and add a mixture of known microbial strains known to be present in these soils and see how the presence of the contaminant would affect their activity (e.g. changes in the 16S rRNA gene expression)”, it concerns changes of activity of the community only on the basis of changes in gene expression. For functional characterization of microbial community, estimations of respiration and enzyme activities are widely used. As for “out of date methods”: You are totally right, the modern methods of sequencing permit to investigate the microbial composition of soils in more details, to identify the strains and to make deep conclusions about the shifts in microbial community and reaction of different OTUs on pollution. Understanding that, we still found it possible to use PCR-SSCP method to distinguish effects of the main components presented in the raw waste, on soil in case of waste disposal. Using PCR-SSCP method we managed to reveal the presence/absence of influence of hydrocarbons+radionuclides (containing in the sample H) or radionuclides only (containing in the sample R) on soil microbes. Fingerprinting methods based on gel electrophoresis are still used by many authors for the similar goals: - Gao et al., 2015. Effects of salinization and crude oil contamination on soil bacterial community structure in the Yellow River Delta region, China. *Applied Soil Ecology*, 86, 165-173). - Yu et al., 2015. Changes in soil microbial community structure and functional diversity in the rhizosphere surrounding mulberry subjected to long-term fertilization. *Applied Soil Ecology*, 86, 30-40. - Cattaneo et al., 2014. Perennial energy cropping systems affect soil enzyme activities and bacterial

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community structure in a South European agricultural area. *Applied Soil Ecology*, 84, 213-222; - Wang et al., 2014. Immobilization of Cd in soil and changes of soil microbial community by bioaugmentation of UV-mutated *Bacillus subtilis* 38 assisted by biostimulation. *European Journal of Soil Biology*, 65, 62-69;

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