

Interactive comment on “Biochar carrying hydrocarbon decomposers promotes degradation during the early stage of bioremediation” by P. Galitskaya et al.

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Dear Dr. Guenet, thank you for your comments and for your accurate studying of the manuscript. Here are the author's responses: 1. General comment 1. We extracted DNA from each independent sample (see below). This means that for each variant of treatment (A, B, C, D and E) we had 6 independent DNA extracts. Before performing Illumina sequencing, we screened diversity of the 16S rRNA gene fragments for soils sampled on the 7th, 42th and 84th days, using PCR-DGGE method (data not presented in the manuscript). Since no significant differences between the samples were observed, we mixed DNA from the samples of each variant and sequenced them as one replicate. 2. General comment 2. We did not sterilize biochar before

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amendment. However, we sampled biochar immediately after its preparation from the pyrolysis chamber directly into sterile containers. Therefore we suppose that biochar was free of microbes. Indirectly, sterility of biochar is proved by SEM pictures, both presented and not presented in the manuscript. We will add this information into the manuscript, L. 70-71: “Biochar used in the study was obtained by slow pyrolysis of birch wastes at 450°C. Immediately after preparation and cooling, biochar was sampled from the pyrolysis chamber under sterile conditions. Biochar characteristics are presented in Table S1.” 3. L 92-96. In our study, 4 variants of treatments of oil polluted soil (A, B, C, D) as well as one non-treated sample (variant E) were analyzed. For each variant, we had 3 independent containers, and two samples were taken from each container for further analysis. Further, we analyzed these samples in replicates as described on L.136. According to your comment, we will rewrite the sentence on L.92 as follows: “The remediated soil samples were taken on days 1, 7, 14, 28, 42, 56, 70 and 84 of the study. On each sampling day, thirty samples (5 variants of treatments (A-E) x 3 containers for each treatment x 2 samples from each container) were examined, . . .” 4. L103. Indeed, there are several understandings, what the germination index (GI) is. In our study we understood GI as relative seed germination (sample to control) multiplied by relative root elongation (sample to control), expressed in %. According to your comment, we will improve the text as follows: “Germination index (GI) was calculated as described by Zucconi et al. (1981) and used as a phytotoxicity parameter. GI (%) combined measures of relative seed germination and relative root elongation.” 5. L163. We consider the differences to be significant, when the p-value is ≤ 0.05 . According to your comment, we will add “(p ≤ 0.05)” into the sentence on L. 163 and in the other parts of the manuscript, when we are writing about significant differences. 6. L167. Here we mean that the petroleum concentration rapidly decreases in soil in the process of biodegradation. If biodegradation will not be performed, no decrease may happen. Besides, we mention that after this rapid decrease, the concentration does not fall at all or does slowly. 7. L198-L200. The microbial stress was not measured in our study indeed. However here we just cite the work of Tahhan et al.,

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2011, who suggested that low effect of bioaugmentation may be caused by microbial stress. We think that being introduced on biochar, microbes can survive better in the soil as compared with direct addition of microbial cultures to soil. 8. L212. In the work of Labud et al., 2007 two types of soil (sandy and clayey ones) contaminated by 5% and 10% of gasoline, 5% and 10% of diesel, and 5% and 10% of petroleum were analyzed. The microbial biomass carbon on the first day of experiment was about 0.08 mg g⁻¹ in all the samples in the clayey soil, and ranged between 0.025 and 0.08 mg g⁻¹ in the sandy soil. In the work of Tejada et al., 2008 soil was contaminated by 5% and 10% of gasoline, and microbial biomass level was measured during 270 days. As a whole, 14 values of microbial biomass were obtained, the minimum level was equal to 0.025 mg g⁻¹, and the maximum level – 0.093 mg g⁻¹. Since both works cited contain a lot of data (many numbers that are not readable), we did not find it relevant to give the values in the manuscript. 9. L252-254. According to your suggestion, we will add one sentence on the L256. “Besides, increase of microbial respiration could be caused by massive death of the introduced bacteria, and decomposition of the dead biomass by soil indigenous microflora.”

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