

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 anonymous Referee, Thank you for your attention to our manuscript, for your accurate reading and comments. Here are the answers: 1. General comment 1. The soil was sampled in the meadow zone in Tatarstan Republic (Russia). Unfortunately, before sampling, we did not analyze the plant species growing in the oil polluted soil. It should be mentioned that the plants were typical for these zone, but they looked more inhibited as compared with those on non-polluted territories. Besides, plants' diversity was visually lower on the polluted sites. The dominating plant species was *Onobrychis arenaria*. 2. General comment 2. We analyzed soil parameters using ISO methods. The manuscript will be corrected as follows (L66-70): The soil showed

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the following characteristics: 40.53. General comment 3. We did not sterilize biochar before 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. Inoculation and further storage of biochar was conducted under sterile conditions. 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”. L. 80: “All the manipulations with biochar before bioremediation were conducted under sterile conditions”. 4. General comment 4. Since we needed about 60-70 g soil for analysis, we mixed the soil, and then took three soil cores about 20-25 g each. We mixed them in order to obtain the sample for further analysis. We tried to sample the soil: i) from the top, ii) from the middle and iii) from the bottom of the container. 5. General comment 5. No, we do not. Therefore we will write microbial biomass carbon. 6. General comment 6. Control variant E was the polluted soil as it was, without any treatment. We wanted to compare the efficiency of bioremediation with no remediation, therefore we did not maintain the moisture level in this variant. In the beginning of the experiment, the moisture content was equal to 377. General comment 7. R function `cmdscale()` used to calculate MDS uses linear method. It calculates an eigenanalysis of the dissimilarity matrix, and does not employ “stress” per se. Rather, it attempts to maximize variability along axes. MDS provide only variability, explained by first two axis: 34.58. General comment 8. We made the plots in Microsoft Excel program and used the smoothed line to connect the dots. Do you suggest to connect the lines without smoothing? 9. General comment 9. We were thinking about the graph instead of Table 2. However, there will be four different graphs instead of one table, since the data presented in the table are measured in different units. Therefore we decided that this table, despite on its complexity, is still better for the reader than 4 graphs. Besides, diversity indexes are presented in table forms in many published

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articles. 10. Technical note 1. The sentence will be rewritten as follows: “It was shown demonstrated that moistening and aeration were the main factors influencing microbial biomass, while implementation of biochar and introduction of microbes were the main factors influencing microbial respiration” 11. Technical note 2. The technical correction will be done as suggested. 12. Technical note 3. The word “indeed” will be removed as suggested. 13. Technical note 4. The sentence will be rewritten as follows: “Poor data concerning the use of biochar for immobilization of hydrocarbon degrading microbes can be found in literature (Qin et al., 2013)” 14. Technical note 5. The word “estimated” will be replaced by “measured” as suggested. 15. Technical note 6. Punctuation will be improved. 16. Technical note 7. “SE” will be replaced by “S.E.” as suggested. 17. Technical note 8. Space will be added. 18. Technical note 8. LSD analysis showed that before day 28 variants are grouped into 2-3 groups because of the high variability of the process. This shows significance of the differences. On the day 84 data of different variants can not be divided into groups which suggests about absence of significant differences. For LSD test, default parameters were used. In order to make these sentences more clear, we will rephrase them as follows: “In the process of bioremediation that lasted for 84 days, hydrocarbon content decreased in all the variants, except the control, as presented in Figure 2. The maximal decrease rate was observed during the first 14 days, but this rate was different for different variants. Thus, on day 14, decrease of hydrocarbon content was estimated to be 13, 42, 49, and 5319. Technical note 9. The idea the biochar helps microbes to overcome the stress was firstly suggested by other authors, we think that our study confirmed this conclusion. It should be also mentioned that the introduced microbes were isolated from the oily waste, but not from that which was later remediated. Therefore, introduced microbes potentially were under stress conditions. 20. Technical note 10. The significant test results will be added. 21. Technical notes 11 and 12. The blue elipses were drawn by hand just to make it easier for the reader to recognize the groups of samples. However, these elipses have statistical basis. Thus, we used time covariate with values 1, 28 and 84 in adonis() function to test the bacterial community structure dependence on time,

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and got p-value less than 1
Call: adonis(formula = spec.mtr.bin env.Time, permutations = 9999)

Permutation: free Number of permutations: 9999

Terms added sequentially (first to last)

Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	env.Time
1	0.12567	0.125673	3.8996	0.28055	0.0011	**
Residuals	10	0.32227	0.032227	0.71945	Total	11
		0.44794	1.00000			

— Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Call: adonis(formula = spec.mtr.bin env.fTime, permutations = 9999)

Permutation: free Number of permutations: 9999

Terms added sequentially (first to last)

Df	SumsOfSqs	MeanSqs	F.Model	R2	Pr(>F)	env.fTime
2	0.18407	0.092033	3.1389	0.41091	0.0014	**
Residuals	9	0.26388	0.029320	0.58909	Total	11
		0.44794	1.00000			

— Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

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