egusphere-2024-1663 R2 - authors' response

We would like to thank Reviewer #2 for a thorough and constructive review. We appreciate the attention devoted to our manuscript, particularly in identifying limitations that warranted further clarification. Below is a detailed, point-by-point response to the review, with our replies highlighted in color for clarity.

The authors study the impact of ureolytic biostimulation on the soil microbiome in Aridisols. The approach of the authors is scientifically sound and the results are worth to be published. The manuscript is well-written and shows only minor slips. In addition, the manuscript is structured in a comprehensible and clear way.

Thank you for the constructive and positive assessment.

However, it is not totally clear why the authors took samples from three sites, but in large parts only refer to two (undisturbed) sites and did not treat the samples from all three sites in the same way - this needs to be explained.

The authors should also - at least shortly - discuss the reasons why soil depths has an impact on microbial communities (litter input, soil moisture...).

Please see our reply in the detailed comments

And while the authors mention that they analyzed the chemical and physical properties of the soils, this is completely neglected in the Results/Discussion. The authors should therefore include in their Discussion other factors that could have had an impact on the microbial communities.

We added the following sentence in the Results and Discussion section (3.2.2)

"Given the small variation in mineralogical and textural properties across the soil profile, physical factors alone are unlikely to explain the differences in microbial communities. The observed shifts in microbial community composition likely result from vertical environmental gradients—specifically temperature, UV radiation, and oxygen availability—given that the particle size distribution and mineralogical composition remain largely consistent between surface and subsurface layers."

Overall, the authors should tighten their manuscript, some parts are repetitive. Especially the last part of the Results/Discussion section should be tightened and focus more on the authors' results.

Thank you. We followed the suggestion and believe that the revised manuscript is now a tighter and more focused version of the original.

The authors should also stick to basic rules of writing: Do not start a sentence with an abbreviation, write numbers up to ten as words etc.

Thank you. We have revised the manuscript as suggested. We kept the use of MICP abbreviation as it is abundant in the text.

Please see my detailed comments below:

Title:

The title could be more precise: What are the responses? How do they change with depth?

In response to R1 we suggested the following title " *Differential responses of Aridisol microbiomes from different depths to ureolytic biostimulation*".

We further revise the title to "*Depth-Dependent Loss of Microbiome Diversity and Firmicutes Compositional Shift Induced by Ureolytic Biostimulation in Aridisol* "

Abstract

l. 25 I suggest to replace "possess" by "fulfill", "play" or something similar.

Changed as suggested.

l. 44 Do you mean that members of the Planococcaceae family replaced Paenibacillaceae and Bacillaceae as most prevalent Firmicutes?

Yes. We rewrote the sentence for clarity

"We also found that biostimulation lead to a shift in the composition of the Firmicutes family, where specific members of the Planococcaceae family became the most prevalent Firmicutes, replacing Paenibacillaceae and Bacillaceae as the dominant families."

Introduction

l. 62-62 I suggest to rephrase this sentence or delete it.

We have revised the first two sentences of the paragraph to make clearer and consIstent:

"Anthropogenic soil erosion may lead to changes in soil biodiversity, essential ecosystem traits, and impact human well-being (Delgado-Baquerizo et al., 2014; Maestre et al., 2013; Rodriguez-Caballero et al., 2022). Chemo-physical stabilization of the soil surface is often used to mitigate soil erosion (Zuazo and Pleguezuelo, 2009)."

l. 63 This reference (Zuazo and Pleguezuelo, 2009) is not in your list of references.

Thank you, added to references list.

Durán Zuazo, V.H., Rodríguez Pleguezuelo, C.R. Soil-erosion and runoff prevention by plant covers. A review. *Agron. Sustain. Dev.* **28**, 65–86 (2008). https://doi.org/10.1051/agro:2007062

l. 62-69 In this passage, the connection between the relevance of biological soil crusts and the role of MICP should be made clearer

The passage was rewritten to make clearer connections.

l. 83-92 You should at least shortly indicate why soil depth has such an impact on microbial communities - litter input, temperature, moisture regime in the soil...

We added the following paragraph to the Introduction section:

"Soil microbial communities in arid and semi-arid environments exhibit strong vertical stratification, driven by steep environmental gradients. Surface layers (0–5 cm) typically harbor the highest microbial diversity, dominated by phototrophic and heterotrophic organisms such as cyanobacteria, actinobacteria, and fungi, which are well adapted to desiccation, ultraviolet radiation, and rapid shifts in temperature and moisture (Pointing & Belnap, 2012; Steven et al., 2018). Subsurface soils (5–30 cm and deeper) tend to exhibit reduced richness and compositional shifts toward oligotrophic taxa that can survive under limited energy and carbon availability (Fierer et al., 2003; Barnard et al., 2013). While microbial biomass and activity decrease with depth, subsurface communities may exhibit functional specialization, including resilience to long-term dormancy and adaptation of metabolic pathways."

Barnard, R. L., Osborne, C. A., & Firestone, M. K. (2013). Responses of soil bacterial and fungal communities to extreme desiccation and rewetting. *ISME Journal*, 7(11), 2229–2241. , https://doi.org/10.1038/ismej.2013.104

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Influence of drying–rewetting frequency on soil bacterial community structure. *Microbial Ecology*, 45(1), 63–71. https://doi.org/10.1007/s00248-002-1007-2

Pointing, S. B., & Belnap, J. (2012). Microbial colonization and controls in dryland systems. *Nature Reviews Microbiology*, 10(8), 551–562. doi: 10.1038/nrmicro2831

Steven, B., Gallegos-Graves, L. V., Belnap, J., & Kuske, C. R. (2013). Dryland soil microbial communities display spatial biogeographic patterns associated with soil depth and soil parent material. *FEMS Microbiology Ecology*, 86(1), 101-113. https://doi.org/10.1111/1574-6941.12143

l. 106 What do you mean by "microbiology of MICP"? Why is knowledge "particularly lacking" in the Negev? You stated before that it is widely lacking.

The sentence, and the following, was rewritten to make clearer connections:

"In this study, we aimed to investigate the consequences of ureolytic biostimulation in Aridisol, on a microbiome level. Specifically, we study soils from the Negev Desert (Israel) to: i) characterize the native microbial community at depths relevant to soil surface (top 1 meter) stabilization; ii) establish the efficiency of ureolytic biostimulation using native microbiomes, and iii) study the effects of the treatment on microbial diversity."

Materials and methods

l. 119 Please include a reference for the soil classification (USDA soil taxonomy).

Added as suggested

Soil Survey Staff. 1999. Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys. 2nd edition. Natural Resources Conservation Service. U.S. Department of Agriculture Handbook 436.

l. 123 20 what prior to your study?

20 years. Corrected. Thank you.

l. 123-124 Please indicate cleary from which depths you took samples - what do you mean by surface? 0-5 cm? 0-10 cm? You should give the precise range, especially as you refer to "soil layers" later.

0-5 cm. We added the definition in the text.

l. 125 Why n=12 samples? You have three sites and samples from three depth per layer in duplicates? Should be n=18?

Please note that only the two undisturbed sites (1 and 2) were sampled for characterization using 16S DNA sequencing. The n = 12 was misplaced in the text and represents duplicates of soil samples sampled from two sites and three depths. We rewrote the sentence for clarity:

"Within each site, soil was sampled from three depths: surface, topsoil at 5 cm depth, 50 cm below the surface, and 100 cm below the surface. These depths were sampled in duplicates within each site, with approximately 10 m separating between replicates The microbial communities of the soils of sites 1 and 2 were chosen for characterization using 16S DNA sequencing (overall, n = 12 samples representing native Negev soil),..."

l. 126 Why did you not include site 3 here?

We focused on sites 1 and 2 as they are undisturbed soils with natural stratification. Site 3 was mechanically disturbed and most likely homogenized, as evidenced by the ureolytic biostimulation rates presented in Figure 1. Knowing a priori that this was the case we decided to "blind test" this notion.

l. 127 You do not need "refridgerated" here.

Corrected. Thank you

l. 131 Please include a short description and not only a reference to another publication.

Thank you. We added D_{50} and Ca content for the soils.

l. 131-132 How do you explain the huge standard deviation and why do you add this information here and not in the Results section?

Soil properties are described in the Materials section, as this study focuses primarily on microbiological and chemical parameters.

l. 149 At which temperature?

We added the requested information in text

Results and discussion

l. 178-181 You do not need that additional introductory part here.

Thank you, we rewrote the first paragrpah.

l. 183-184 The first sentence is also not needed here.

Deleted. Thank you

l. 189 It should be "...than in the topsoil..."

Corrected. Thank you

l. 198 Why do you use passive here ("...has yet to be recovered.")?

Rewritten as suggested.

l. 200-202 How do you know that the decisive factor is the disturbance? You do not present the results of the chemical and physical characterization of the soils, so it is not clear if there are differences that could also have an impact.

We didn't claim that mechanical disturbance is "the decisive factor". Rather, we write "*our results provide evidence for functional consequences of mechanical disturbance to the soil microbiome*".

Also please see our reply on page 1 above.

l. 224 I suggest to rephrase this part and write something like "...and hosted similar communities..." instead of "...and did not host distinct communities...".

Rewritten as suggested:

"Prior to treatment, the two sites did not differ in their overall local microbial diversity ($F_{1,6}$ = 3.06, *P* = 0.13) nor beta-diversity (Fig. 2), hosting similar communities without clear compositional distinctions (ANOSIM: R = -0.04, *P* = 0.56)."

l. 234-235 Please rephrase this sentence.

Rewritten as suggested.

Figure 3 Why did you put the panels in this order? I suggest to start with the surface on the left and end with the 100 cm depth on the right.

We have reordered the figure as suggested (see revised version at the end of this reply). Thank you.

l. 253-254 You do not have to announce what you are going to discuss later.

We chose to inform the reader that these findings will be addressed later in the text

Figure 4 Please take care that your labels are consistent. Here, you use "hndred cm" and "fifty cm", while you use "100 cm" and "50 cm" everywhere else.

We have revised the figure legend as suggested (see revised version at the end of this reply). Thank you.

l. 319 What do you mean by "it" here? ("...we found it had a more prominent influence...")

We rewrite to: “In contrast, at our mechanically disturbed site, disturbance had a more pronounced impact on the ureolytic response, which was both delayed at the surface and enhanced in the deeper layers relative to two nearby undisturbed sites”.

Conclusions

Please take care that your Conclusions are coherent, at the moment some sentences seem not be related to the sentences before and after them.

The conclusions were rewritten as suggested to be more coherent

“In this study, we demonstrated the feasibility of inducing effective ureolytic biostimulation using native microbiomes that inhabit different depths within the upper 1 meter of an Aridisol. Effective ureolysis was achieved at the depths of interest, regardless of the pre-existing differences between the microbial communities. However, vertical microbial heterogeneity influenced the intensity of the ureolytic response, with depth-dependent variations likely reflecting differences in functional potential and environmental constraints.

While biostimulated MICP shows promise for stabilizing soil surfaces in arid environments, our results highlight potential ecological trade-offs. Specifically, the treatment consistently altered microbial community composition, enriching specific heterotrophic taxa while suppressing autotrophs and other functionally important groups. Given that autotrophs are central to the integrity of biological soil crusts, their decline may undermine long-term soil stability and ecosystem function.

Taken together, these findings underscore the importance of incorporating environmental heterogeneity and microbial biodiversity into the design and assessment of MICP-based interventions. Future studies should evaluate both the engineering effectiveness and the ecological consequences of biostimulation in arid soils to ensure sustainable implementation."

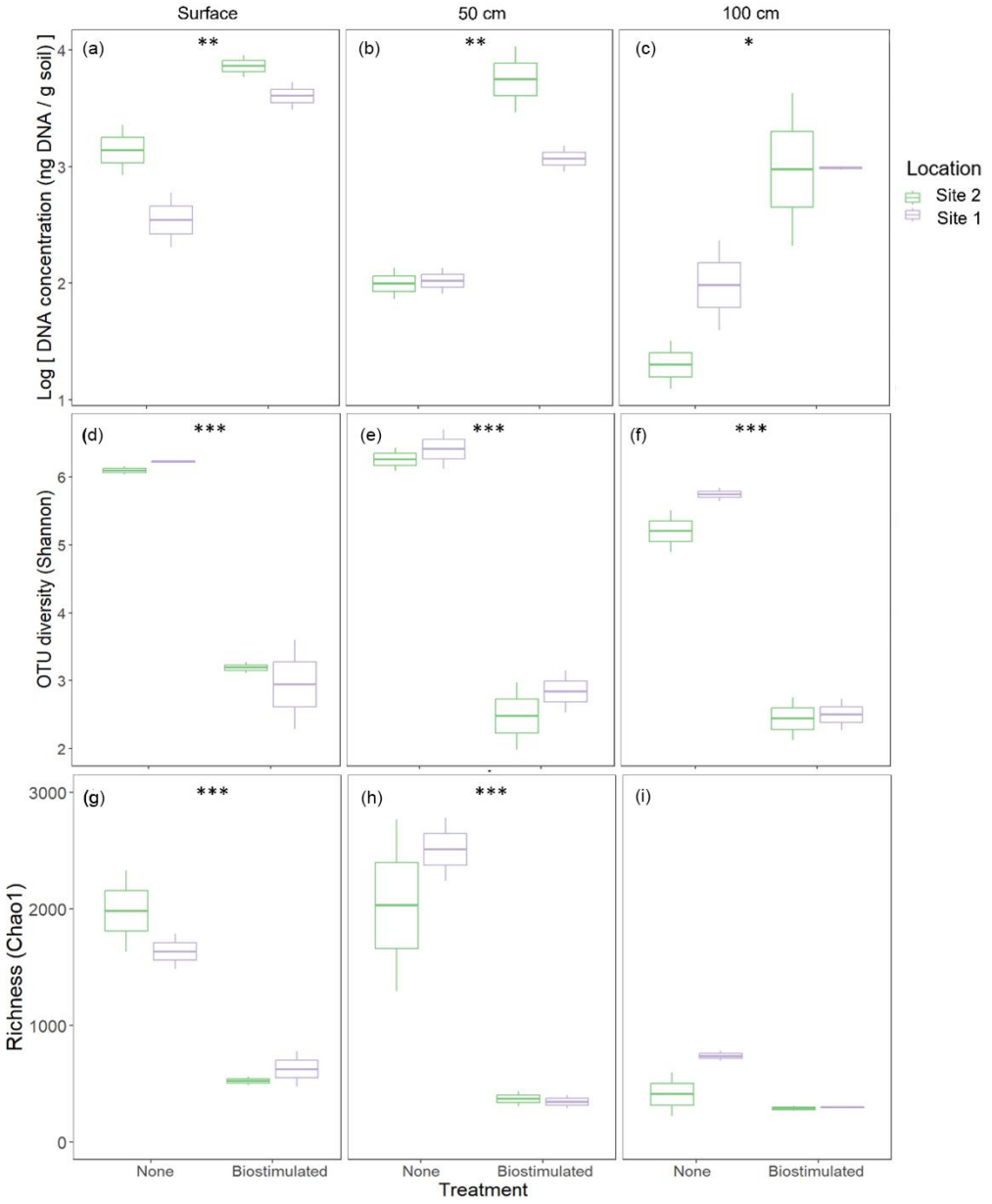

Fig 3 Revised

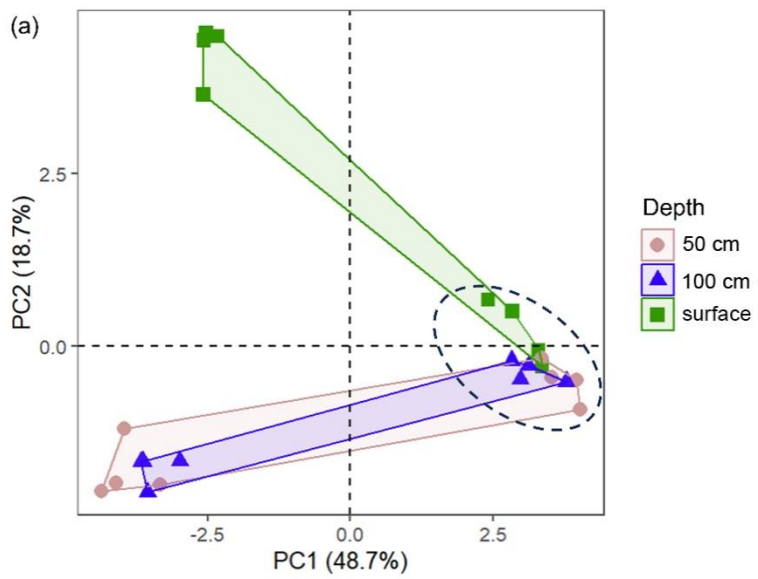

Figure 4a Revised