

## Reply on RC1

Capucine Baubin et al.

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Author comment on "The function and composition of active bacterial communities diverge during the hydration and desiccation of desert biocrust - a field study" by Capucine Baubin et al., SOIL Discuss., <https://doi.org/10.5194/soil-2021-88-AC1>, 2022

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Comment on soil-2021-88  
Anonymous Referee #1

Referee comment on "The response of desert biocrust bacterial communities to hydration-desiccation cycles" by Capucine Baubin et al., SOIL Discuss., <https://doi.org/10.5194/soil-2021-88-RC1>, 2021

General comments:

The manuscript entitled "The response of desert biocrust bacterial communities to hydration-desiccations cycles" is an interesting manuscript which focusing on the effects hydration-desiccations cycles on biocrust bacterial communities. The introduction sets scene very nicely and the authors also clearly show the need for studying hydration-desiccations cycle, but see comments below for several ideas that need justification and/or more details. Some parts of the approaches are not appropriate and need more clarification (see comments below about major concern). Although this paper focused on bacterial communities but the results did not provide typical diversity analysis such as alpha and beta diversity. Discussion and conclusion will need to be revised when the major concern has been addressed. Overall, I think that the manuscript idea is interesting and will be valuable for the scientific community. However, additional work is needed to address major concerns and improve several parts of this manuscript to be more transparency and justified.

Title

- The title indicates what this research is about but it does not provide any information about key results. I would suggest revising the title to be more specific. What is the key result?

*The title was modified to "The function and composition of active bacterial communities diverge during hydration and desiccation of desert biocrust – a field study"*

Abstract

- Line 20: How does rain-mediated dynamics were assessed? Please explain experimental setup briefly? And provide brief details about bacterial communities measurement.  
*The abstract was rewritten adding details (In 25-36): "Here, we assessed rain-mediated dynamics of active bacterial community in the Negev Desert biological soil crust (biocrust) by sampling before, during and after a heavy rainfall, and evaluating the changes in active bacterial composition with amplicon sequencing, potential function, photosynthetic activity, and extracellular polysaccharides (EPS) production. We predicted that rain would resuscitate the biocrust phototrophs (mainly Cyanobacteria), while desiccation would inhibit their activity. In contrast, the biocrust Actinobacteria would decline during rewetting and revive with desiccation. Our results showed that hydration increased chlorophyll content and EPS production. The biocrust rewetting also resuscitated Cyanobacteria, which replaced the former dominant phylum, Actinobacteria, boosting potential autotrophic functions. However, desiccation of the biocrust did not immediately change the bacterial composition or potential function, and was followed by a delayed decrease in chlorophyll and EPS levels. This dramatic shift in the community upon rewetting leads to modifications in the ecosystem services."*
- Line 20: What does active community mean? Active crust community? Active bacterial community? Please be more specific, it is unclear.  
*To clarify, the sentence was changed to "active bacterial community".*
- Line 22-24: Why focus only on phototrophs? What about other bacteria?  
*Following the reviewer comment the sentence was modified (In 24-27): "We predicted that the rain would resuscitate the biocrust phototrophs, mainly the Cyanobacteria, while desiccation would inhibit their activity. In contrast, the biocrust Actinobacteria would decline during rewetting and revive with desiccation."*
- Line 27-28: When Cyanobacteria increased and Actinobacteria decreased, what does it really mean for biocrust?  
*Following the reviewer comment we added a sentence explaining the reported changes (In 31-32): "This dramatic shift in the community upon rewetting led to modifications in ecosystem services."*

## Introduction

- In general, hydration-desiccation cycles were introduced very nicely. However, this paper focused on bacterial communities, but very little information about biocrust bacterial communities was introduced. There are other studies which also focused on biocrust bacterial communities, please introduce them here. What have they done? What were the general bacterial composition? Were there any patterns that other researchers found about biocrust bacterial communities? Is there a reason why this paper should specifically focus on Cyanobacteria and Actinobacteria? Are these the only two phyla found in biocrust?  
*Following the reviewer comment we added the following information: "The harsh desert conditions shift primary production from plants toward oxygenic photosynthetic microorganism, mostly cyanobacteria (Xu et al., 2021). However, biocrusts are dominated by heterotrophic microorganisms, mainly the phyla Actinobacteria and Proteobacteria (Nunes da Rocha et al., 2015; Meier et al., 2021). Members of these phyla can meet their energy demands during the desert prolonged draughts by harvesting sunlight or atmospheric trace gases (Leung et al., 2020). Studies that focused on biocrust community shifts and cyanobacterial response to hydration-desiccation cycles were carried out under controlled conditions (Angel and Conrad, 2013; Oren et al., 2019; Karaoz et al., 2018)."*
- Line 48 "of soil respiration(Castillo-Monroy et al., 2011)" – add space before parenthesis

Line 50 "trace gases(Meier et al., 2021; " – add space before parenthesis  
*Added*

## Materials and Methods

- There are many types of biocrusts. What kind of biocrusts were collected? Were they all the same biocrust types?

*Following the reviewer comment the following sentence was added: "In this site, the biocrust was identified as type 2 and 3 in some places e.g., well-established cyanobacterial dominated biocrust (Veste et al., 2001; Büdel et al., 2009; Kidron et al., 2015).*

- Line 86-87: Please provide a reference.

*The reference "(www.data.lter-europe.net)" was added*

- For section 2.6 Community analysis, please report the number of reads in each step (number of raw reads, filtered reads, after chimera removals, etc.).

*The number of reads at each step were specified in Table S2. To clarify, the following text was modified: "The number of reads at each step can be found in Table S2."*

- What is the average number of reads per sample? And what is the range (min – max)?

*The following was added to the text: "At input, the average number of reads is 86 611 with a minimum number of 22 095 reads and a maximum number of 110 046 reads."*

- MAJOR CONCERN! Since the length of the sequences are different because of different platform, please provide details about how the analysis were performed to account for this issue. This section is also crucial in case other researcher would like to follow or repeat the analysis. Please make sure that all the details required for the experiment are included. Currently, it is not sufficient.

*The following was added to the text: "We note that the despite the differences in sequencing platforms and primers, the community composition obtained matched previous reports performed at the very same site (Meier et al., 2021; Angel and Conrad, 2013; Bay et al., 2021)."*

- Line 172-173: What is the criteria for picking out "genes of interest"?

*The text was modified to explain this more clearly: " We selected these genes in accordance with previous reports (Meier et al., 2021; Bay et al., 2021) and associated them with each step in the KEGG database and built our own database (Table S3)."*

## Results

- Generally, simple diversity analysis (alpha and beta diversity) should be provided for microbiome paper. However, only relative abundance plot was provided. I would suggest including alpha and beta diversity analysis to confirm significant differences that were found in relative abundances comparison.

*Following the reviewer comment, the Alpha diversity (Shannon diversity index), and beta diversity (RDA) were added in the supplementary material (Figure S3 and S4, respectively) with the values and the associated statistics. The following text was added: These patterns were supported by the alpha and beta diversity analyses (Figure S3 and S4). The community diversity significantly differed before the rain (T[0]) and during (T[R]) and after the rain (T[1-3]) (Figure S3,  $p < -0.05$ , F-value = 10.96, Table S10 and S11). The diversity in the later timepoints (T[R, 1-3]) did not differ ( $p > 0.04$ , Table S11). Similarly, the RDA showed that T[0] was separately clustered from the other time points (Figure S4, F-value : 5.75, Table S12 and S13)."*

- Line 190-201: Statistical test must be provided, reporting p-value only is not sufficient. Please check how statistical test results should be reported. Please also indicate significant differences in figures. Currently, the plots do not have these information.  
*Chi2 and F-values were added to the text. Statistical significance marks were added to the figures.*

- Line 209-210: In total, how many bacterial phyla were found in these data?  
*Nine phyla were detected in the biocrust. This information was added to the text: "The community is mostly composed of the phyla Cyanobacteria, Actinobacteria, and Proteobacteria in addition to six other phyla (Figure 3; Table S6)."*

- Line 212-216: Please use appropriate statistical results report format. Reporting p-value only is not sufficient.  
*Chi2 and F-values were added to the text.*

- Figure 3: MAJOR CONCERN! Unless the author provide detailed explanation about how different sequencing platform and primers were accounted for. I think the differences might have been because of different platform used.  
*This was explained in the Materials and Methods section: "We note that the despite the differences in sequencing platforms and primers, the community composition obtained matched previous reports performed at the very same site (Meier et al., 2021; Angel and Conrad, 2013; Bay et al., 2021)."*

## Discussion

- Line 250: What exactly is "cyanobacterial activity" that was measured? How do we know that it is cyanobacterial activity not other bacteria?  
*Chlorophyll content was measured as a proxy of cyanobacterial activity. The text was modified following the reviewer comment: "Though the chlorophyll content, a proxy of cyanobacterial activity, increased with soil moisture (Figure 2A), no significant changes were detected in the total organic carbon C or N (Figure S1 and S2; Table S4 and S5)."*

- Line 276: Perhaps beta diversity might help confirming this claim.  
*Beta diversity helped to confirm the claim and therefore a reference to Figure S4 was added to the text.*

## Conclusion

- This might change when different sequencing platforms were taken into account.  
*The main conclusion was not modified as despite the difference sequencing platforms the community matched previous reports describing the community at the same site (Meier et al., 2021; Angel and Conrad, 2013; Bay et al., 2021).*