

Interactive comment on “The composition of endolithic communities in gypcrete is determined by the specific microhabitat architecture” by María Cristina Casero et al.

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We like to thank the reviewer for his/her comments, which helped us to improve our manuscript.

1. Abstract: suggesting that the lithic substrate “might” be an essential factor does not instill confidence in the results and conclusions, which contrasts with the term “confirms” in the Conclusions. In addition, the abstract should be more concise in describing the results of this work, not general observations of the results. For example, it currently points out that the hypoendolithic community was the least diverse and hosted unique taxa; explaining “why” here is important for the reader.

C1

Authors: We agree with Reviewer 3 regarding of the use of the term “might” in the abstract. We changed the text and now reads: “These results show, for the first time, that the differences in the architecture of a microhabitat, even within the same piece of lithic substrate, plays an essential role in shaping the diversity and composition of endolithic microbial communities”. Also, to better describe the results of the work, as suggested by the reviewer, we added “as a result of a lower access to sun radiation” after that sentence.

2. What is the significance of “Preandean Atacama Desert” within the context of this study?

Authors: Due to the huge extension of the Atacama Desert, the term Preandean is used in this manuscript to better localize the region of the sampling site, which also determines the climatic conditions of the area of study. The Preandean region of the Atacama Desert has been well defined in several Chapters of the book: “Microbial Ecosystems in Central Andes Extreme Environments. Biofilms, Microbial Mats, Microbialites and Endoevaporites” edited by M.E. Fariás, Springer Nature Switzerland AG, 2020. In this book the Part II is referred to Preandean and Andean Atacama Desert: Life at Limits with Chapter 3: The Desert Polyextreme Environment and Endolithic Habitats by Jacek Wierzchos, Carmen Ascaso, Octavio Artieda, and María Cristina Casero and Chapter 4: Preandean Atacama Desert Endolithic Microbiology by María Cristina Casero, Victoria Meslier, Jacek Wierzchos, and Jocelyne DiRuggiero. We are grateful to Referee #3 for this comment and bibliographic reference of elsewhere mentioned book (chapter 4) was introduced to the manuscript were Preandean region of the Atacama Desert was mentioned.

3. Section 2.2: It is unclear how this climate data is directly relevant to the results of this manuscript. Other than thermal measurements, it does not appear to have been collected specifically for this work and so only needs to be mentioned in the Discussion

Authors: Our paper is focused on microbial ecology of very singular endolithic microbial

C2

communities within gypcrete rocks in an extreme environment. Indeed, microclimate parameters such as air temperature and RH over a period of 22 months in the sampling place was only described once by Wierzchos et al. (2015). We consider these data of interest to the readers, such as microbial ecologists of extreme environments. Moreover, we have measured colonized gypcrete surface temperature revealing maximum of 68°C, which is very high temperature, even for desiccated endolithic microbial communities. Also this data was considered by us as of interest to the readers. We preferred to introduce detailed values of climatic and thermal measurements data in description of Sampling Site section (Results) as also these data were discussed in Discussion section.

4. Section 2.5: Title should include “DNA extraction procedures” to be consistent with Section 2.6.

Authors: Included in the title.

5. Section 3: Results – Lines 139-141 are not necessary, nor is Section 3.1 with exception of gypcrete surface temperatures if they were measured for this study.

Authors: Please, consider as correct our response as in point 3.

6. Section 3.3: Use present tense to describe observations, such as “. . . colonization zone is close. . .” Authors: We agree with Reviewer 3 and so we changed observations to present tense.

7. Section 4: What is the distance between the cryptoendolithic/chasmoendolithic habitats in the upper part of the substrate and the hypoendolithic habitat in the lower part of the substrate? Are they separated by millimetres? Centimetres?

Authors: Cryptoendolithic/chasmoendolithic microhabitats and hypoendolithic microhabitat are separated by centimetres (~ 4 cm).

Line 21 – “. . . a combination of microscopic investigations and. . .” Line 22 – “. . . the endolithic communities and their habitats at the microscale. . .” Line 23 – replace “lithic

C3

substrate” with “gypcrete”

Authors: It now reads: “A combination of microscopy investigation and high-throughput sequencing approaches were used to characterize the endolithic communities and their habitats at the microscale within the same piece of gypcrete”

Line 39 – replace “noticeable” with “plausible” Line 39 – “. . . only by microorganisms that can survive and/or thrive under physical or geochemical extremes such as temperature. . .”

Authors: It now reads: “Regarding the second half of the statement (but, the environment selects) extreme environments present some of the most plausible scenarios since they are inhabited only by microorganisms that can survive and/or thrive in their respective physical or geochemical extremes such as temperature, solar radiation, pressure, desiccation, pH”

Line 43 – replace “stress” with “limitations”:

Authors: Replaced

Line 45 – “. . . able to survive under such conditions”

Authors: accepted and changed

Line 46 – “The Atacama Desert. . . on Earth, with scarce precipitation events. . . and extremely low mean annual relative humidity”

Authors: accepted and changed

Line 54 – replace “living inside the rock but close to the bottom” with “living on the underside of the rock”

Authors: We agree with Reviewer 3 that the expression used is not clear. However we can not change it by the expression “underside of the rock” since it could be understood as the hypolithic colonization. Thus, we changed it and now it reads “hypoendolithic

C4

(living inside pores in the bottom part of the rock)”.

Line 56 – “. . .PAR radiation levels. . . ”

Authors: accepted and changed

Line 67 – “. . .architecture on the diversity. . .”

Authors: accepted and changed

Line 71 – “. . .the microscale dimensions. . .”

Authors: accepted and changed

Line 71 – How do you define “peculiar”?

Authors: The term “peculiar” in this context is ambiguous so that we decided to change it by a term that better describes what we want to say. Now it reads: “The microscale dimensions and differential diversity distribution in this unique environment has led us to coin the new term “microbiogeography”

Line 72 – Can you provide a definition for the term “microbiogeography”?

Authors: Biogeography is a known term. However, our investigation describes for the first time the composition of endolithic communities at microscale, namely within few cm³ of lithic substrate. For this reason, we introduced in our manuscript the term “microbiogeography”, since the composition of microbial communities is changing even at microscale, what was demonstrated in our work.

Line 75 – “The area experiences. . .”

Authors: accepted and changed

Line 78 – “. . .we sampled gypcrete. . .”

Authors: accepted and changed

Line 80 – dry and dark environment – a lab drawer?

C5

Authors: Thank to Reviewer . #3 for this indication. Exactly, samples were stored in a lab drawer. However, to clarify the description we changed the sentence, that now reads: “. . .dry and dark conditions. . .”.

Line 92 – “Light microscopy (LM) was used to examine cell aggregates. . .” Line 93 – “. . .on cyanobacterial isolates cultured from. . .”

Authors: accepted and changed

Line 97 – “. . .were run on pieces. . .”

Authors: accepted and changed

Line 101 – “. . .to reduce beam hardening.”

Authors: accepted and changed

Line 102 – “. . .performed using VG Studio Max Version 2.2 software.”

Authors: accepted and changed

Line 105 – “Biological material removed from gypcrete. . .”

Authors: accepted and changed

Line 105 – Was it BG11+N or BG11-N?

Authors: The culture medium used was BG11 with nitrogen (NaNO₃). When BG11 culture medium contains no NaNO₃, it would be called BG110. (sensu Rippka et al. 1979)

Line 107 – Include a period after “Philips”

Authors: accepted and changed

Line 107 – “After incubation for 15 days, visible. . .”

Authors: Accepted and changed. It now reads: “After incubation for 15 days, visible

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cyanobacterial growth appeared. Colonies were isolated by repeated plating on 0.8%-agar with BG11 medium (Rippka et al. 1979), and successfully isolated colonies were transferred to liquid BG11 medium”

Line 116 – “Colonization zones were scrapped. . .” (not scrapped)

Authors: accepted and changed

Line 130 – “Sequences of 16S rRNA genes. . .”

Authors: accepted and changed

Line 151 – Replace “visualization” with “representation”

Authors: accepted and changed

Line 152 – Replace “following” with “exhibit”

Authors: accepted and changed

Line 155 – Can you better describe “undulated furrows”?

Authors: We agree with Reviewer 3 that the description should be clarify. After consulting the literature, a better term for the observed dissolution features over gypsum was found. Thus, we changed it by “microrills weathering features (DiRuggiero et al. 2013)”

Line 160 – How did you differentiate pigmentation in layers? Light microscopy?

Authors: The observation of different pigmentation layer was performed by stereoscopic microscopy

Line 161 – “. . .with high carotenoid content closest to. . .”

Authors: accepted and changed

Line 219 – “. . .included only one. . .”

Authors: accepted and changed

C7

Line 220 – Which Chinese desert are you referring to?

Authors: After revising the literature corresponding to the cited sequence and GenBank database, it is not possible to assign that *Chroococciopsis* sequence to a specific location of those studied by Pointing et al. (2007): Qaidam Basin, Turpan Depression and Taklimakan Desert. However, to clarify the climatic conditions of the studied deserts by those authors, the sentence now reads: “*Chroococciopsis* sp. strains isolated from quartz hypolithic communities from hyperarid Chinese deserts (Pointing et al. 2007)”

Line 221 – Please provide a reference for University Valley

Authors: Included. Cumbers, J. and Rothschild, L. J.: Salt tolerance and polyphyly in the cyanobacterium *Chroococciopsis* (Pleurocapsales)., *J. Phycol.*, 50(3), 472–482, doi:10.1111/jpy.12169, 2014.

Line 222 – “. . .no sequences from isolates. . .”

Authors: accepted and changed

Line 230 – “. . .with our isolate sequences. . .”

Authors: accepted and changed

Line 234 – Can you think of another way to say “differentially abundant”?

Authors: “Differentially abundant taxa” is a common term in this type of studies to define those taxa/features whose different abundance across samples is statistically significant. Examples: Taye et al. (2020) <https://doi.org/10.3389/fmicb.2019.03007> Shatzkes et al. (2017) <https://doi.org/10.1038/srep43483> Jiang et al. (2017) <https://doi.org/10.1128/mSystems.00092-17>

Thus, we consider that the term is correctly used in this study.

Line 235 – “Both OTU11. . .”

Authors: accepted and changed

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Line 255 – The last sentence of this paragraph is not necessary.

Authors: We removed that sentence

Line 259 – “Both substrates show. . .”

Authors: accepted and changed

Line 263 – “. . .based on the ratio of mean. . .”

Authors: accepted and changed

Lines 265-267 – I do not know if you can compare temperatures on terrestrial rock surfaces with those in hot springs as an approximation for the upper temperature limit for photosynthesis. Can you estimate the temperatures within the endolithic habitats?

Authors: We agree with the reviewer that the comparison of rock surface temperatures with those in hot springs is inaccurate and that its relation to the temperature in the endolithic microhabitats should be mentioned. Thus, we revised that paragraph and now it reads:

"Specific measurements of surface temperature on gypsum revealed values of close to 70°C. This value was detected at zenith, when microbial communities are desiccated and metabolically inactive (Cockell et al. 2008). The temperature within the endolithic habitats is expected to be close to that in the rock surface as shown by Wierzchos et al. (2012a) for halite endoliths."

Line 273 – “: :water to metabolise and grow.” Line 273 – “: : gypsum restricts water loss”

Authors: accepted and changed

Line 280 – I don't think “consolidates” is the correct word in this sentence

Authors: It now reads “supports the proposal of Wierzchos et al. (2015)”

Line 282 – “following water gravity flow” is unclear

C9

Authors: We agree with Reviewer 3 and this phrase was corrected as follow: “. . .” This structure reveals different dissolving and crystallization processes of the gypsum following the water displacement from the surface to the bottom of the rock (gravity flow). This water gravity flow giving rise to the cave-shaped pores, thus providing this HE microhabitat with a hard permeable bottom gypsum layer” . . .

Line 295 – end the sentence with a period

Authors: accepted.

Line 297 – “EPSs” should be “EPS” Line 300 – see line 297

Authors: accepted and changed

Line 302 – “The aggregate-like structure. . .”

Authors: accepted and changed

Line 303 – “. . .and heterotrophic bacteria also helps. . .”

Authors: accepted and changed

Line 308 – “. . .in such an oligotrophic environment.”

Authors: accepted and changed

Line 330 – can you provide a reference that would support the statement that light intensity should be considered a crucial factor in understanding differences in community composition between top and bottom habitats?

Authors: Recently the light intensity, as driving factor of spatial heterogeneity within halite endolithic microbial communities was reported by Uritskiy et al. (2020). This phrase and ref. was introduced to the manuscript text.

Line 342 – replace “unidentification” with “lack of positive identification”

Authors: Changed. It now reads “the inability to identify the UC-OTU”

C10

Line 353 – Replace “confirmed” with “hypothesized”; I would not say that this work confirms that liquid water availability is a driver of community composition, as no experimental evidence was provided in the manuscript to substantiate this claim. A more convincing argument for how microenvironmental conditions determines microbial distribution would strengthen the manuscript.

Authors: We agree with Reviewer 3 that confirmed is not an appropriate word to define the findings included in this work. Thus, the term it was changed and now it reads “In this study, liquid water availability was proposed to be a driver of community composition. . .”

Line 369 – “. . .draw conclusions. . .”

Authors: accepted and changed.

Figure 1 – Latitude and longitude markers should be included in the study area map

Authors: GPS coordinates are already included in site description and sampling section

Figure 3 – It would be helpful to point out what samples are polished blocks/thin sections vs whole mounts for SEM work.

Authors: no thin sections were included in Figure 3. All samples are polished blocks

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