We thank Dr. Treude for handling our manuscript entitled: **Microbial colonization of chasmoendolithic habitats in the hyper-arid zone of the Atacama Desert** and thank referee #1 for her/his insightful comments. Below we have addressed each comment and described the corresponding changes to our manuscript.

<u>Response to Referee #1</u>

General comments

We thank referee #1 for finding our manuscript interesting, the research necessary, and that she/he is looking forward to seeing more articles from our group.

The idea for this work was born from discovering two ecological niches in different lithic substrates in the hyper-arid zone of the Atacama Desert. The colonization was chasmoendolithic, which we described here for the first time in calcite and gypsum-covered rhyolite rocks, leading us to ask two important questions:

1. What was the composition and structure of these newly discovered microbial ecosystems?
2. What key factors were responsible for the differences we observed between the two microbial communities?

Our work definitively provides answers to these questions and we elaborate below to address comments from Reviewer #1.

"what is the nature of what controls microbial colonization of these habitats"

Our data identified several key factors that could explain the observed differences in microbial colonization of the rock substrates. These include (1) the physical structure of the rock itself with networks of deep cracks and fissures in the calcite rock versus shallow fissures in the rhyolite-gypsum rock, having a strong effect on water retention and therefore moisture within the rock environment; (2) the water budget that was augmented in the calcite rock from dew formation as evidenced by the discovery of microrills features at the rock's surface and supported by the high heat capacity of calcite; and (3) the higher solubility rate of the gypsum, suggesting a less stable environment than the calcite substrate; physical stability over long periods of time is a critical factor for microbial communities from extremely arid environments because of their very slow growth rate.

Water availability is therefore a key factor as stated on p. 16 of the manuscript *"Liquid water storage and its retention would be high in this type of fissures and cracks network, suggesting higher water availability in the calcite rock, possibly affecting the microbial diversity we observed in the chasmoendoliths of the Luna rock."*

However, we also recognize that a combination of various deterministic - environmental constraints, substrate mineral composition, and species interactions - and stochastic processes might also control rock colonization and therefore concluded *"rock colonization is controlled by a complex set of factors"*. More on this below.

"Does mineralogy play a role, or is it simply moisture?"
Although we have no evidence that mineralogy played a role in the colonization of our substrate, this aspect cannot be ignored. Different mineral compositions result in different sets of micro-nutrients available to microorganisms and this can have a profound effect on their metabolism. The issue might be best addressed by investigating the mineral-cell interface for evidence of mineral transformation and by using expression profiling of communities to address directly their physiology. These studies are challenging because of the small amount of nucleic acids that can obtained from the rocks but are nevertheless in progress in our lab with the Luna rock.

"How does higher solubility play an important role in possible osmotic stress in this hyper-arid desert environment?"
This question was answer in part in the manuscript, see p 15, lines 23-28: *"Gypsum saturated water solution has a specific conductance value of 2.2 dSm^{-1} compared to only 0.03 dSm^{-1} for a saturated solution in equilibrium with calcite, resulting in a much higher osmotic pressure for the aqueous solution in the rhyolite-gypsum substrate than in the calcite rock."* The higher solution rate of gypsum will produce solutions with higher solutes content that could potentially result in osmotic stress to the community. As with a decrease in stability as a result of gypsum dissolution, these events might be dependent on high moisture events, i.e. rainfalls, but they may have a profound effect on the survival of members of the community.

We followed recommendations from reviewer #1 to "*postulated further … and provide suggestions as to how to better resolve this question*" by making the following changes in the manuscript (in italic):

P 15617, line 25:
"Gypsum saturated water solution has a specific conductance value of 2.2 dSm^{-1} compared to only 0.03 dSm^{-1} for a saturated solution in equilibrium with calcite, *producing an aqueous solution with a much higher osmotic pressure in the rhyolite-gypsum substrate than in the calcite rock and potentially resulting in osmotic stress for members of the community*.

P15620, Line 23:
While the rock mineralogy and physical stability of the substrate are important factors, water availability appeared to be essential in shaping these endolithic microbial communities. In Valle de la Luna, the occurrence of liquid water in the form of scarce precipitations and potential dewfalls, and the increased water retention facilitated by the pervasive network of cracks and fissures in the rock, were associated with a significantly more diverse microbial ecosystem. "*However, water is likely not the only factor shaping these communities. Rock substrates are typically considered low-nutrient environments and the effects of the rock's mineral composition on microbial communities should not be ignored. This question might be best addressed by investigating the mineral-cell interface for evidence of mineral transformation and by using expression profiling of communities to address directly their physiology. The chemical composition of the substrate also affects light transmission, possibly modulating the photosynthetic component of the community. Measurements of*

photosynthetic activity across a range of substrates, under similar climatic conditions, might provide answers to this question. Finally, interactions between members of the community, in particular primary producers and heterotrophs, and the presence of viruses, are likely important factors that should also be addressed. Field studies on community primary productivity coupled with expression profiling under diurnal, seasonal, and water stress might provide the information needed to understand better the functioning of these ecosystems and the factors driving substrate colonization and maintenance of microbial diversity."

Regarding the comment of Referee #1 that perhaps the work described in this manuscript "*does not seem novel*", or that "*we are using the same techniques*" or that "*many of such observations have been described before*" we would like to emphasize the following: (1) chasmoendolithic colonization of rhyolite-gypsum and calcite rock has never been described before in hyper-arid environments, or in any other environments, and is therefore truly novel; (2) while molecular methods have been previously applied to the characterization of endo- and hypolithic environments, the combination of high-throughput methods with electron and photon microscopy techniques - developed over the last 15 years of research in this field - is extremely powerful; this approach allowed us to describe the structure and composition of endolithic microbial communities while providing structural localization of the microorganisms – phototrophs and heterotrophs - within the substrate. However, according to recommendation from reviewer #1, we have removed the term "cutting-edge" from the manuscript.

Responses to "Comments/Corrections":
Page 15605, line 19 – should be "indicate" - done
Page 15605, line 24 – "west coast of South America " - not sure what is to be changed
Page 15606, lines 14-15 – "Here we describe chasmoendolithic photosynthetic…" - done
Page 15606, line 18 – remove "," after "crust" - done
Page 15607, lines 20-21 – should be "…by Wierzchos and Ascaso (1994)." - done
Page 16510, line 5 – "precipitations" should be "precipitation" - done
Page 15610, line 16 – remove "The" before "X-ray" - done
Page 15610, line 17 – remove the word "respectively" - done
Page 15610, line 18 – replace "presented" with "exhibited" - done
Page 15610, line 28 – "This endolithic colonization…"- done
Page 15611, line 14 – "Observations of microbial…"- done
Page 15611, line 15 – remove the word "the" before "autofluorescence" - done
Page 15611, line 22 – remove the word "will" - done
Page 15611, line 23 – should be "petrographic" - done
Page 15611, line 25 – is "detritical" the same as "detrital"? - done
Page 15612, line 23 – is it "baeocyte" or "baeocytes"? - done
Page 15614, line 25 – "No Archaea were found…"- done

Supporting Information:
Page 4 – "cacodilate" is normally spelled "cacodylate" - done
Page 5 – should read "Specific sets of filters…"- done

Page 5 – remove “for” after “and” - done
Page 5 – should read “…were used for green and red signal visualization, respectively.” - done
Page 6 – What were the control DNA extractions based upon? – *No environmental sample added to the extraction – changed in the manuscript.*
Page 6 – remove “The” before “DNA” - done
Page 6 – I think there are only 5 criteria, not 6 – yes, changed to 5
Page 13, Fig. S2 – I think this mineral material might be clays.
We agree that this electron dense structure might be clay minerals and we are conducting additional tests to confirm. In the mean time, we prefer to call this structure an “unidentified mineral.”
Also, I don’t think (d) is required as it is adequately presented in (c).
TEM micrographs S2-d was selected to illustrate better the heterotrophic bacteria relationships among them, and within EPS. As the image is presented in Supplementary Materials, we would like to maintain this image for better description of this unique microbial ecosystem.