

Response to Referee #2

General comments

We thank referee #2 for his/her general impression that our work “is a worthy study”.

We are, however, quite surprised by the referee's statement “the communities are not significantly different from those previously reported from this region”. While a handful of studies have reported endolithic microbial communities from the Atacama Desert, none before addressed chasmoendolithic colonization of calcite and of the interface between gypsum and rhyolite. Chasmoendolithic colonization of these substrates has not been described in hyper-arid environments - or in any arid or mild environment – and as such our report is the first.

Regarding endolithic microbial communities from the Atacama Desert, very few studies have been published:

- Wierzchos et al. 2006 and de Los Rios (2010): halite endolithic communities dominated by *Halotheca*-like cyanobacteria and archaea; in de Los Rios (2010), conventional clone libraries.
- Warren-Rhodes et al. 2006 and 2008: hypolithic communities colonizing the underside of quartz rocks; conventional clone libraries.
- Kuhlman et al. 2008: communities from rock varnish; conventional clone libraries.
- Dong et al. 2007: soil gypsum crust; conventional clone libraries.
- Wierzchos et al. 2011: epilithic lichen and endolithic cyanobacteria and non-photosynthetic bacteria of Ca-sulfate crusts; no molecular characterization.
- Wierzchos et al. 2012 (in press): cryptoendolithic colonization of volcanic rock (Ignimbrite); DGGE and clone sequencing for cyanobacteria only.

All these studies differ significantly from the work we reported in our manuscript (MS) as follows:

- With the exception of Wierzchos et al. 2012 (in press), none of these studies described microbial communities from the Lomas de Tilocalar and Valle de la Luna regions, where we collected our samples.
- None of the studies characterized chasmoendolithic colonization of rhyolite-covered gypsum and of calcite rocks.
- None of the studies addressed the following questions by comparing two endolithic substrates from similar climatic regions: (1) What was the composition and structure of these newly discovered microbial ecosystems? And (2) What key factor(s) might be responsible for the significant differences we observed between the two microbial communities?
- None of these studies used high-throughput molecular methods to characterize endolithic microbial colonization (see more below about this issue).

“However, I would like to see more understanding of the data than presented. For example, are there things that we know now from high throughput sequencing which we did not know before from conventional clone libraries?”

- Using high-throughput sequencing allowed for an in-depth characterization of two completely new microbial communities, colonizing cracks and fissures of calcite and rhyolite-covered gypsum rocks. As a result we identified bacterial families not-previously reported in Atacama endoliths and provided a solid basis for the statistical analyses of alpha and beta diversity, including richness (number of distinct taxa), evenness (equitability of taxa), structure (representation of dominant and rare taxa), composition (relative abundance of specific taxonomic groups), and phylogenetic diversity (number and relationship of phylogenetic lineages). This level of analysis was necessary to determine that the two communities were significantly different in terms of community structure and composition and to evaluate key factors responsible for the higher microbial diversity found in the calcite rocks.
- These molecular methods, combined with mineralogy analyses (DRX and FRX) and an array of microscopy analyses (SEM-BSE, SEM-SE, TEM, FM) provided an integrated approach for the characterization of endolithic communities. This multidisciplinary approach provided an in-depth characterization of not only the colonizing lithobionts but also the lithic substrates, the localization of the colonization inside the substratum, the interactions between microorganisms and with their mineral environment.

Based on the points we made above, and the new insights we provided with this study (see below), we would like to retain sentences such as “for the first time” and “novel”. However, according to recommendation from reviewer #1, we removed the term “cutting-edge” from the MS.

Regarding Referee #2’s question [“How this site compare to the previously studied sites in terms of climate characteristics?”](#), we would like to point out that this has been extensively addressed in the MS, in particular the similarity and differences to Atacama sites previously studied in terms of climate and geology – described page 15609, lines: 21-23 and lines 26-27. A number of bibliographical references also referenced the climate characteristics in the Atacama hyper-arid zone and are all cited in the MS.

Regarding Referee #2’s comment [“The paper set out to address the question of what factors determine species compositions, but did not answer it or provide new insights”](#), here again we would like to point out what is extensively discussed in the manuscript and the answers to the question “what factors are responsible for the significant differences we observed between these two chasmoendolithic microbial ecosystems”?

In Discussion, 4.1 Microclimatic and physical differences in the chasmoendolithic microbial colonization of rhyolite-gypsum and calcite rocks:

- differences in mineralogy: page 15616, lines: 7-21
- differences in light scattering and UV attenuation: page 15616, lines 22-29 and page 15617, lines: 1-21;
- differences in physical stability of substrates (solubility): page 15617, lines 22-29 and page 15618, lines 1-3;

- differences in thermal conductivity and potential dew formation of substrates: page 15618, lines 4-23;
- differences in substrate structure: page 15618, line 23-28 and page 15619, line 1-3;

In Conclusion:

- “water availability appeared to be essential in shaping these endolithic microbial communities” and following statements in page 15620 and 15621.

Specific comments:

Referee’s comment: Page 15604 line 23. You characterize cryptoendolithic and chaesmolithic [should be “chasmoendolithic”] as “indirectly connected” and “connected to the rock surface”, respectively. I understand it, but not sure if everyone would. According to my understanding, the difference is whether or not the colonized pore spaces are covered by a surface crust (hence the prefix crypto-,) or the difference can be characterized as whether or not pore spaces are directly open to the outside environment.

Response: We provided definitions for cryptoendolithic and chasmoendolithic habitats that are commonly accepted by the scientific community and reported by Golubic et al. (1981) (reference cited in the text), Nienow (2009), and more recently by Wierzchos et al. (2012). The two latter references were added to the MS.

Referee’s comment: Page 15605 line 2. I would suggest dropping “hypoendolithic”. I know that this was published, but I am not convinced. Small stone flip all the time as a result of perturbation by animals or humans.

Response: We suggest leaving to readers of this work the choice of being convinced or not by the newly described hypoendolithic habitat (cited by Wierzchos et al. 2011). Work describing this habitat was recently been published in Vitek, P., Cámara-Gallego, B., Edwards, H.G.M., Jehlička, J., Ascaso, C., and Wierzchos, J. Phototrophic community in gypsum crust from the Atacama Desert studied by Raman spectroscopy and microscopic imaging. Geomicrobiol. J., 30, 1-12, 2013; this reference was added to the MS.

The argument that ...”Small stone flip all the time as a result of perturbation by animals or humans”... should be ruled out since there is no animal or human activity (tourisms, mining, etc) in the area where these hypoendolithic habitats were discovered. Another similar habitat, the hypolithic habitat, is well recognized and has not been invalidated because of stone flips or animal perturbations.

Referee’s comment: Page 15609 line 7 “the occurrence of rare precipitations, a lack of fog, and the absence of moisture (dewfall) on rock surfaces, except during rainfall events.” This needs rephrasing. I read this as saying “fogs and dewfalls occur only during rainfall events”.

Response: No, we meant to say that we did not detect moisture on rock surfaces except during rainfall events in the Tilocalar area. This was rephrased properly in the MS.

Referee's comment: Page 15609 line 9. potential evapotranspiration or PET is a good gauge for higher plants, as plants have to stay hydrated all the time and deal with evapotranscription [evapotranspiration?]. Desert cyanobacteria are poikilohydric and don't have to, so PET is not a useful indicator of their stress. I still prefer Imre Friedmann's concept of total wet duration to be useful and would suggest using that instead of PET.

Response: The aridity index (AI) defined as the ratio between mean annual rainfall and mean annual evapotranspiration is commonly accepted as a main indicator of the dryness according to the United Nations Environment Program (UNEP), which describes and classifies the world deserts as arid ($AI = 0.2 - 0.05$) and hyper-arid ($AI < 0.05$). This classification has been used in many publications and was recently published in three reviews by: Nienow (2009), Pointing SB, Belnap J (2012) Microbial colonization and controls in dryland systems. *Nat Rev Microbiol* 10:551-562 and Wierchos, J., de los Ríos, A. and Ascaso, C.: Microorganisms in desert rocks: the edge of life on Earth, *Int. Microbiol.*, 15, 171-181, 2012. The point here is not to relate to the physiology of the organisms we studied but rather to provide a commonly used indicator of dryness, as a climate category, which can then be compared to other studies on the world arid regions.

Calculating "total wet duration" (for cyanobacteria?) as suggested by this Referee could only be possible (with challenges) in laboratory conditions but not in natural habitats.

Referee's comment: Page 15609 line 20. I find the presentation of climate data inadequate. The annual average RH does not tell me when the organisms become active and for how long. Why not display the data the traditional way, like McKay et al. *Astrobiology* 2003 3:393-406. It would be much more meaningful.

Response: There might be a misunderstanding here, since our data (Table 1) display minimum, maximum and annual averages for air temperature, air RH, and annual averages for rain (and PAR values). This is exactly the way McKay et al. (2003) displayed their data in their Table 1.

The Referee #2's statement that "The annual average RH does not tell me when the organisms become active and for how long" is correct. However, the objective of this paper was not to measure microorganisms activity and how metabolic activity related to RH. This is interesting subject, requiring specific and complex methodology, and it is out of the scope of this paper.

Referee's comment: Page 15610 line 13. These organisms live under gypsum or calcite crusts that happen to form on rhyolite. The physical and chemical properties of the crusts are relevant, but I don't see the need for the underlying rhyolite to be described in detail. Elsewhere in the paper, descriptions should also be revised so that the reader understands that these communities are associated with the translucent crust, not living within the opaque rhyolite.

Response: We disagree with this referee's comments and uphold that we found microorganisms not only under the gypsum crust but also within the gypsum crust and within the rhyolite minerals (rhyolite is a common volcanic porous substrate). On page 15611 (lines 8-13), we described the localization of the microbial cell aggregates (see also Figure 1) and in line 13 the reader can find that "...cells were also found in fissure

and crack spaces with gypsum crystals and cristobalite (Fig. 1d)”. Cristobalite is a constituent of the rhyolite rock and therefore our data showed the association of microbial cells with rhyolite bearing minerals. This type of colonization has not been reported before and it is essential that it should be described in detail in the MS.

Furthermore, the rhyolite described here is not an opaque mineral; it is very porous and similar in color to the colonized ignimbrite substrate we found at a nearby location (Wierzchos et al., 2012).

Referee’s comment: Page 15617 line 13 The difference in colonization depth between the two specimens is attributed to water filled cracks serving as optical fibers. This is a puzzling (I know this is based on a published paper, but published papers can be wrong). Why don’t attribute the greater depth to calcite crystals transmitting light?

Response: We agree that published papers can sometimes be wrong, but in this case we would like to leave it to the readers to form their own opinion. Because we did not find hypoliths under calcite layers, we cannot uphold the statement that calcite crystals can transmit light and support phototrophic growth.

Referee’s comment: Page 15617 line 22. Sorry, but I don’t get how substrate solubility affects the species composition of the community. If an otherwise suitable substrate is not colonized, you can speculate whether or not it is because it dissolves too fast. If the rock is colonized, then it is stable enough. Who gets to live in it therefore has nothing to do with solubility. Your calculation of the age of the calcite coating, 12,000 years, supports my point.

Response: We did not explain how substrate solubility affects the composition of the community. We only suggested that lower substrate solubility make the substrate more physically stable, which might explain the more diverse community we observed in the calcite chasmoendoliths. Moreover we suggested that higher osmotic pressure (more soluble substrate) might affect the diversity and survival of microorganisms within the substrate, as high osmotic pressure can be stressful for life.

Referee’s comment: Page 15618 line 4. you interpret the micro scale weathering features in the calcite sample as being due to dewfalls and wet dry cycles. This seems to contradict your climate data where you say dewfalls are rare, except in days following a rainfall. More importantly, why can’t be rain that caused these weathering features?

Response: We interpreted the micro-scale weathering features in the calcite sample as being due to dewfalls according to well-established references from the literature (see MS references of Smith, 1988; Laudermilk and Woodford, 1932, Smith et al., 2000). More importantly, to our knowledge, there are no scientific report or study that attribute these weathering features to rainfall.

The climate data for Valle de la Luna (air T, air RH and rainfall) cannot be used to confirm or rule out the dew formation on calcite layer surfaces (for this reason in 2012 we have installed a microclimatic station in Valle de la Luna with a dew detection sensor). We indicated that higher RH values for Valle de la Luna (remarkably higher than in Tilocalar) might potentially enhanced dew formation on calcite rock surfaces; the presence of the microrills features supports this hypothesis.

We did say that “dewfalls are rare, except in days following a rainfall”. This statement is correct but only for the Tilo rock (p. 15609, lines: 6-9), where we have measured the frequency of this phenomenon.

Referee’s comment: Page 15620 line 23. “Rock mineralogy as a nutrient source”. This is contrary to the general understanding... Essential nutrients come from dissolved minerals in rain water or in some cases from the biological weathering of the rock as well.

Response: We agree that essential nutrients come from dissolved minerals in rainwater and that the source of those minerals can be atmospheric deposition or dust precipitation. However, a large parts of these minerals come directly from the weathering of the rock itself (Herrera, A., Cockell, C.S., Self, S., Blaxter, M., Reitner, J., Arp, G. et al. (2008) Bacterial colonization and weathering of terrestrial obsidian in Iceland. *Geomicrobiology Journal* **25**: 25-37) and a number of studies have shown that the addition of host rock extracts to defined media improves the growth of the organisms (Siebert J, Hirsch P, Hoffmann B, Gliesche CG, Peissl K, Jendrach M. 1996. Cryptoendolithic microorganisms from Antarctic sandstone of Linnaeus Terrace (Asgard range): diversity, properties and interactions. *Biodivers. Conserv.* 5:1337–63). We modified our statement in the conclusion of the MS and expended on the idea.

Referee’s comment: Page 15621 line 5-12. The statements made in the last paragraph of the discussion are not supported by the data presented.

Response: The last paragraph was modified to reflect potentials for future studies.

Referee’s comment: Figure 3 is not necessary, in my opinion. I did not learn anything that I did already know from Figure 2.

Response: We disagree with Referee #2’s suggestion. Not all readers will be familiar with the concept of chasmoendolithic rock colonization and the models depicted in Figure 3 outline the major differences in colonization patterns between the two substrates described in this MS. Our objective here is to reach a wide audience and in this case, we believe that “a picture is worth a thousand words”.

Referee’s comment: I would turn Figure 4 into a table. The point here is to compare and contrast, something is better suited for a table than a bar graph. I believe interesting details are lost because of the presentation.

Response: In contrast to this reviewer’s opinion, we would like to keep Figure 4. Here again, the color bar graph and the pie charts readily convey the differences in community composition, which is the main finding from the molecular analysis of these microbial communities. This is also the most common way this type of data is presented in microbial ecology publications. We understand that one might want to compare and contrast these communities at a more detailed level and, to that effect, we added a large table in supplementary materials. This table contains the phylogenetic affiliation for all OTUs, classified by families, and the number of each OTU per rock sample; the cyanobacteria OTUs were highlighted.

Referee’s comment: I also suggest that the climate data be moved from supplemental to the manuscript, but presented in the traditional way.

We agree with Referee #2's suggestion; we have moved Table 1 to the main text of the MS and presented the data as in McKay et al. (2003).