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Peer Review

Review of: "Microbial Mediation in Gypsum Dissolution and Its Role in Evaporitic System Transformation in Northern Chilean Salt Flats"

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This manuscript examines the influence of microbial consortia on gypsum dissolution and secondary mineral precipitation under controlled laboratory conditions, using synthetic gypsum crystals incubated with microbial mats and natural brines from three Andean salt flats (Llamara, Pajonales, and Gorbea), and compares biotic and abiotic systems to assess microbe-mineral interactions and associated textural and mineralogical changes.

The manuscript presents an experimental dataset addressing microbe-mineral interactions in evaporitic systems, combining laboratory incubations, microscopy, and geochemical analyses.

Importantly, the topic is highly relevant and timely for geomicrobiology and evaporite research, particularly given the still limited understanding of gypsum-microbial community interactions and their consequences for mineral dissolution and secondary mineral formation.

In this context, the study has strong potential to provide genuinely valuable insights into how microbial activity may influence gypsum alteration pathways and related mineralogical transformations. However, in its current form, the manuscript exhibits several issues related to structure, clarity, and presentation that hinder its overall readability and impact. These include an imbalanced organization of background and contextual sections, limited separation between descriptive results and interpretation, and inconsistencies in figure and table presentation.

I recommend a major revision. The manuscript needs substantial revision and reorganization to improve clarity, coherence, and scientific communication; only once these issues have been thoroughly addressed should the study be considered for publication.

doi.org/10.32388/S9NF4X

General Comments

Introduction

The Introduction is very brief relative to the length and complexity of the manuscript and does not provide sufficient conceptual framing for the study. In particular, it does not adequately review the current state of the art on microbially mediated gypsum dissolution, existing geomicrobiological models in evaporitic environments, or clearly articulate the specific research gap that this work aims to address. As a result, the scientific motivation of the study and the rationale for the experimental approach are not fully established at the outset. Moreover, the objectives of the study are not explicitly stated in the Introduction, making it difficult for the reader to clearly identify the research questions or hypotheses guiding the work.

Sections 1.1-1.3 contain extensive geological, geomorphological, and microbiological background information; however, much of this material is introduced before the conceptual framework is clearly defined. I recommend condensing and reorganizing these sections to improve narrative flow and coherence. In particular, Section 1.2 (“Salt flats and previous evaporite formation models”) would be more appropriately placed outside the Introduction and reorganized as a separate section (e.g., “Geological Setting” or “Study Area”). This restructuring would allow the Introduction to remain focused on the scientific context and research motivation, while positioning regional geological descriptions where readers would more naturally expect them.

Several passages in Section 1.3 adopt an interpretative or evaluative tone that is more typical of a Discussion than of an Introduction. In particular, strong critiques of traditional evaporite models, normative statements regarding how evaporite formation “should” be studied, and broad interpretative syntheses are introduced before the research objectives are clearly defined. I recommend rephrasing these sections in a more neutral, background-oriented manner and/or relocating some of the broader interpretations and methodological critiques to the Discussion, in order to maintain a clear separation between background, results, and interpretation.

In addition, Figure 1 and its caption require revision for clarity and consistency with the text. The caption refers to a panel labeled “A” (“A) Geolocation of the studied salt flats”), but this label does not appear in the figure, making it difficult to interpret the relationship between the different panels. All subfigures should be clearly labeled within the figure itself. Furthermore, the figure does not sufficiently contextualize the study area, as the regional map lacks clear geographic references (e.g., northern Chile, the Atacama Desert, major physiographic units). I recommend revising the figure to include appropriate

subfigure labels, geographic context (such as regional outlines, key cities, scale bar, and north arrow), and expanding the caption to clearly describe each panel and its relevance to the geological setting discussed in the text.

Methodology

Figure 2 is potentially useful to summarize the workflow; however, the current caption is not adequate as a standalone description and should be thoroughly rewritten. First, the caption contains grammatical and structural issues and reads as a sequence of partially incomplete sentences, which affects clarity. Second, it omits key experimental details that are necessary to interpret the figure (experimental unit, number of containers per treatment/site, and replication), and it does not clearly distinguish between t = 0 reference crystals and abiotic controls. Finally, there is a mismatch and ambiguity between what is stated in the caption ("2 crystals of each morphology intact as control" and the general sampling scheme) and what is described in the Methods regarding how many crystals were actually selected, stored as t=0 controls, and incubated per condition. I strongly recommend rewriting the caption and ensuring full consistency with Sections 2.1–2.4.

Evaporation control may alter brine chemistry and should be addressed. The water level was "maintained through replenishment with distilled and autoclaved water," which can change salinity and ionic strength and thus the gypsum saturation state. This needs either (i) justification, and/or (ii) reporting of how brine chemistry and salinity were monitored and maintained.

Results

3.1. Brine geochemistry and gypsum initial conditions

Figure 3 is useful to document the initial gypsum morphologies prior to incubation; however, in its current form, the figure and caption require substantial improvement to be clear and reproducible. First, panel labeling is inconsistent and incomplete: the caption refers to SEM panels (D–F), but the figure as presented does not clearly show all corresponding labels (e.g., panel "E" is not explicitly identified), making it difficult to match images to sites and observations.

The caption is not sufficiently informative: it describes A–C but does not provide a panel-by-panel description for the SEM images (D–F), nor does it explicitly state whether the SEM micrographs correspond to the same representative crystals shown in A–C and what exact features are being highlighted (cleavage, overgrowths, aggregates, early dissolution textures). Some statements in the caption are overly assertive and subjective (e.g., "no signs of dissolution") and should be rephrased as

observational (“no macroscopic dissolution features were observed under the imaging conditions”) or supported with clearer criteria.

Finally, there are consistency issues with the Methods and Results that should be resolved: the Methods describe the selection of “well-formed, non-twinned crystals measuring 20 mm in length,” whereas Fig. 3 includes a “twinned” GOR crystal, and the size context in the Results spans mm to cm-scale crystals (e.g., PAJ up to ~3 cm), which is not readily reconciled with the representative images shown and the stated selection criteria. I recommend: (i) correcting/standardizing panel labels; (ii) rewriting the caption to be fully self-contained (explicit A-C = stereomicroscope/binocular images; D-F = SEM; site assignment for each panel; features highlighted; representative nature); and (iii) ensuring full alignment between crystal selection criteria (size, twinning) and what is illustrated/discussed.

3.2. Culture evolution over time

Below are the main critical issues:

1. The section discusses growth curves and presents Table 2 and Fig. 4, yet Fig. 4 explicitly refers to “gypsum dissolution under microbial growth in BG-11 medium… with constant agitation of 100 rpm.” This is difficult to reconcile with the microcosm setup described in the Methods (natural brines, separate inoculated/abiotic containers, and no clear indication of agitation during the incubation). Please clarify whether Section 3.2 reports (i) the pre-experiment enrichment step, (ii) the actual microcosm experiment, or (iii) a combination of both, and ensure the text/figure captions are fully consistent with the described experimental conditions.
2. The study design repeatedly refers to 14/30/60/90 days, but Table 2 lacks some time points for GOR (e.g., day 60 is missing) and uses “n.a.” for non-fluorescent cells without explanation. This should be completed and standardized across all cultures.
3. Table 2 separates “photosynthetic,” “active photosynthetic,” “inactive photosynthetic,” and “non-fluorescent” cells, and reports “Living [52]” as a percentage, but the operational criteria used to classify cells (and to compute the “Living” fraction) are not defined in the Results and are not sufficiently explicit in the Methods. Please provide clear definitions (e.g., based on autofluorescence intensity thresholds, staining, or microscopy criteria), and indicate how counting was performed (replicates/fields, counting error, and whether values are means ± SD).

Overall, I recommend reorganizing Section 3.2 to explicitly: (i) state which experimental stage is being reported, (ii) link Table 2/Fig. 4 to the main microcosm timeline, and (iii) define counting/classification

criteria and replication, so that the reported culture evolution can be directly interpreted in relation to subsequent mineral/EPS observations.

Discussion

The Discussion is generally well-structured. However, several interpretations are currently overstated relative to the strength of the evidence and should be revised to more clearly separate observations from hypotheses. In addition, some internal inconsistencies (tables/terminology) reduce clarity and scientific rigor. The key concerns are summarized below.

1. The manuscript repeatedly refers to differences in dissolution/precipitation rates and to a measurable influence of microbes, yet the dataset presented is largely qualitative (SEM textures) and the quantitative descriptors (porosity, frequency, axis size) are not reported with replication/uncertainty. Please either (i) provide quantitative rate estimates (or clearly defined proxies with statistics), or (ii) rephrase these statements as qualitative trends.
2. Section 4.1 interprets smooth/rounded cavities as consistent with endolithic colonization and "microbial boring". This is a reasonable hypothesis, but the current evidence (surface SEM) does not unambiguously demonstrate boring into gypsum (e.g., subsurface tunnels, depth profiles, cross-sections). Please provide supporting analyses.
3. The authors attribute dissolution in controls to brines being undersaturated/near-zero SI, but the Results report gypsum SI values close to equilibrium (Table 1). Please reconcile these statements and explicitly discuss how the water-level maintenance via the addition of distilled/autoclaved water may have altered salinity and saturation state over time (potentially affecting both dissolution and precipitation).
4. The controls are described as abiotic and are said to show no microbial growth by microscopy, yet the Figure 6 caption refers to "EPS-embedded" phases in controls. This (biotic vs abiotic) should be clarified. If organic films exist in controls, they should not be labelled "EPS" without evidence of biological origin.
5. In Section 4.1, the text links "reduced porosity" to "Table 1", but Table 1 reports brine geochemistry rather than porosity metrics. Please correct table references throughout the Discussion (and ensure consistency with Tables 3-4) to avoid confusion and improve the traceability of claims.

Declarations

Potential competing interests: No potential competing interests to declare.