The authors have addressed many of the questions from the previous round of reviews. Overall, I think they have begun to strengthen their case for the biogenicity of moonmilk in Tarquinia, but there are still three major areas of revision that need to happen before publication. In short, the argument for biogenicity is divided into three parts- environment, cultures, and RNA.

First, the authors note that meteoric waters in the tombs should be undersaturated with respect to calcite (Section 3.1), generally fostering dissolution. I broadly agree with this point, but it should be noted that meteoric waters dissolving calcite will gradually increase in saturation, re-precipitating crystals elsewhere without the need for life. The authors discuss these potentials, but the text contains several contradicting statements about the presence/absence of carbonate dissolution and abiotic precipitation of cements, noted in detail below.

Second, the text compares culturing experiments between living and sterilized bedrock- this is the strongest evidence for biogenic moonmilk in the paper. However, as noted below, this argument relies on data from an unpublished paper. Furthermore, the culturing experiments from this research are missing from the Methods section, and need an expanded discussion to compare lab conditions with in-situ environments.

Finally, the text asserts that similar microbial communities in bedrock and moonmilk is a sign of biogenicity. In the detailed notes below, I repeat the argument that there are many abiotic scenarios where similar microbial communities can be preserved in different parts of the same rock. In short, the similarity alone cannot be a biosignature.

1) Section 3.1

In my first comments, I mentioned that minerals can entomb cells without requiring microbial metabolisms, especially in supersaturated environments. The authors responded that the tombs are in the vadose zone, that there's no carbonate-rich groundwater source nearby, and that any water in the system is likely meteoric. I agree with the authors on all these points. The authors added these points in Lines 265-280, and they strengthen the manuscript.

However, the processes of carbonate dissolution and precipitation in the tomb walls need to be clarified. At the moment, there are a few contradicting statements about dissolution and precipitation in the bedrock. For example, in Lines 249-250 describing Macco facies,

> "*In the inner walls of the intergranular voids* ***microsparite cement precipitation and/or recrystallization*** *often occurs, due to* ***diagenetic processes of dissolution****."*

This sounds reasonable to me- the vadose zone often shows signs of dissolution followed by precipitation, as previously undersaturated waters become concentrated in Ca and CO3. However, in Lines 267-9 describing the general bedrock:

> *"Noteworthily, the characteristics of bedrock porosity indicate that the* ***dissolution processes prevail*** *on those of* ***inorganic carbonate precipitation****; indeed,* ***meteoric cements*** *as well as speleothems in the largest cavities* ***are absent.*** *Moreover, the bedrock where the tombs are carved is located in the shallow vadose zone (few meters below the surface) and* ***it is barely susceptible to dissolution-reprecipitation processes,…"***

In short, Section 3.1 says that the bedrock 1) contains evidence for dissolution and cement precipitation, 2) mostly evidence for dissolution with no cement precipitation, and 3) barely any dissolution or precipitation. These contradictions either need to be corrected, or if the authors are talking about different locations, that information needs to be clarified.

Here's why the discussion of dissolution and precipitation is important. A convincing argument for biogenic moonmilk requires: 1) a source of calcium, provided from calcite dissolution somewhere in the vadose zone, and 2) undersaturated meteoric waters which are less likely to precipitate calcite, requiring microbial metabolisms to foster precipitation. The authors address these points (end of 3.1), but there are two major questions left to address:

If the bedrock has no evidence for calcite dissolution, where is the calcium coming from to fuel biogenic moonmilk formation? On the other hand, if abiotic cements are present elsewhere in the bedrock, indicating periods of supersaturation, why does moonmilk need to be biogenic?

For publication to continue, the authors should:

1: clarify their interpretations on patterns of precipitation and dissolution in the bedrock- is it an environment that contains both dissolution and precip? Is it dominated by dissolution? Or are both dissolution and precip limited?

2: once the authors have a consistent interpretation of precip/dissolution, clearly describe how such groundwaters create conditions where only microbes can make moonmilk (the authors start to do this in Lines 278-279).

3: (Optional) If the authors have any data on local groundwater, especially pH, Ca, and estimates for calcite saturation, that would greatly help build their case for a subsurface environment where only microbes could make moonmilk. This data could be from the authors themselves, or collected from the literature.

2) Section 3.3- cultures

The strongest evidence for biogenic moonmilk at Tarquinia is presented in Line 329:

> *"Indeed, under laboratory conditions, we have evidence that the grinded calcarenite, with its entire microbial community, when present in a medium containing urea and CaCl2 produced calcite.* ***In the same conditions, calcium carbonate is not produced with sterile (autoclaved) grinded calcarenite*** *(Benedetti et al. 2023)."*

This is exactly the type of experiment needed to show the biogenicity of carbonate precipitation. However, I have a few notes about the culture experiments and references.

First, Benedetti et al., 2023 is still in review. I don't know Biogeosciences' citation policies, but many journals require such references to be cited as (Benedetti et al. in review). More importantly, if this crucial data is rejected by the scientific community, then the argument for moonmilk biogenicity in this paper becomes much weaker.

This paper provides its' own culture experiments, as mentioned in Lines 304-329 and shown in Supp. Fig. 9. The methods for these experiments must be described in the Methods section- my apologies for missing that note last time. How much CaCl2 was used? How much urea? What was the temperature and carbonate saturation of the experiments? Most importantly, were sterilized experiments run during the research for this paper (not the unpublished work)? If so, sterilized comparisons would greatly help convince an audience that moonmilk is biogenic.

The paper also needs to describe differences between the culturing experiments and the natural conditions in the bedrock. While it is impossible to exactly replicate the tomb environment inside a culture, the differences must still be addressed in the discussion. For example- do the authors think that Ca and urea are abundant in the bedrock environment? Especially important: What was the saturation of calcite in these experiments, and how does that relate to potential saturation states in the bedrock?

On a similar note, the authors provide a nice description of urea hydrolysis in the author reply. They mentioned leaving it out of the manuscript to avoid confusion, but I think this metabolism should be included in the discussion. Otherwise, the reader is missing crucial information on a potential process behind moonmilk formation.

For publication to continue, the authors should:

1: Describe culturing experiments in the Methods.

2: Change Benedetti references to "in review".

3: Provide extra discussion on the differences between culturing experiment conditions and conditions in the tombs- especially for chemical conditions (temperature, Ca, urea, calcite saturation).

4: Include a description of urea hydrolysis in the discussion. The best location is probably alongside the discussion of culturing experiments.

3) Section 3.3- RNA

The paper asserts that similar 16S RNA in bedrock and moonmilk are evidence for moonmilk biogenicity. While the results are interesting, and belong in the paper, such similarities alone are not enough to determine biogenicity, for one main reason.

There are other scenarios where moonmilk and bedrock communities are similar, but do not require biogenic moonmilk. For example, if the same microbes inhabited the entire porous bedrock sample before moonmilk formation, and then moonmilk formed abiotically through environmental change, both locations should have the same microbial populations. Therefore, similar populations can not distinguish biogenic vs abiogenic moonmilk.

When I brought this idea up in the previous round of reviews, the authors responded that the environment was unlikely to precipitate calcite abiotically. However, my issue here is not with

environmental saturation- I've already addressed that topic. My issue is with the claim that similar RNA data can be used as evidence for biogenic moonmilk, as stated in Line 346:

> *These results show that **in moonmilk and rocks the microbial composition is similar,** irrespective of rock type (calcarenite and hybrid sandstone) or the environment where the samples were collected (outdoor or indoor). It should be noted that 16S SSU rRNA analysis does not provide information about metabolic activity, thus these data do not identify microorganisms that are active in CaCO3 deposition, but **the overall data demonstrate that the endolytic community of the rocks is promoting moonmilk deposition.***

In short, if the response to the question "Are similar communities in moonmilk and bedrock a biosignature?" is "Yes, but only because the environment cannot precipitate moonmilk", then the biosignature is not the RNA itself, but the presence of moonmilk in an undersaturated environment.

For publication to continue, the authors should:

1: Keep the RNA data and keep the comparisons of similar communities in the bedrock and moonmilk. However, any sentences that mention the RNA itself as evidence for biogenic moonmilk need to be removed (Lines 338-339, 350-352).