Interactive comment on "Sterol preservation in hypersaline microbial mats" *by* Yan Shen et al.

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We sincerely thank referee #2 (Gordon Love) for the helpful comments on our manuscript. Below we list all the points raised by the reviewer (given between quotes), followed by our replies.

1. – "Why do the authors assume that the microbial communities, and hence the lipid composition, should be constant over 1500 years of mat growth and burial? They interpret differences in lipid composition to predominantly taphonomic factors… but this is based on an unsubstantiated assumption that eukaryotic contributions to the mat community were fairly constant over depositional history.

The depletion in sterols in deeper layers (in conflict with the results of Shen et al. that showed abundant steroid lipids in all mat layers from another Kiribati lake) could just as easily indicate a changing mat biological community through time. With higher bacterial contributions to deeper versus shallow layers.

If a "mat-seal" bias was operational and a pervasive diagenetic mechanism for microbial mat remineralization and preservation then why do the results of Shen et al. contradict those found in this investigation? It seems more likely that the differences in lipid composition represent temporal changes in mat community."

REPLY: Although the possibility of a change in microbial communities cannot be excluded, we observed no indications that eukaryotic contributions would have drastically changed over time. So far, the studies on microbial mats from Kiritimati have indicated eukaryotic contributions (Bühring et al., 2009; Blumenberg et al., 2015; Shen et al., 2018), and there are no indications why there should be no eukaryotic inputs at the time when the deeper parts of the mat had been formed. Further, there seem to be no major changes in the texture of the carbonate phases of the mat (except the thin mineral crust representing layer 3), which would suggest major changes in the microbial community. As already discussed, we suggest that the salinity levels and periods of subaerial exposure could have caused the differences observed between the microbial mats from Lake 2 and Lake 22.

Planned changes in manuscript: We will include a brief discussion why we consider a decrease in eukaryotic input over time to be a less likely cause for the fluctuations in steroid distributions as compared to environmental factors (chapter 4.2).

2. – "Given that Shen et al. could not find kerogen-bound steroids in a previous study using this same Py-GC-MS technique then it becomes suspicious that the pyrolysis method used in not optimized to detect bound steroids in degradation products from "young" mat sediments.

There should be no reason from first principles why bound steroids will not be found given that there is ample proto-kerogen in these mat sediments (given that sequestration will begin very early during diagenesis, see point 3 below).

This might be due to high baselines in the ion chromatograms that they are searching for steranes and sterenes. Another reason is that Py-GC-MS will produce a complex mixture of unsaturated steroids and sterols bound within polar moieties after bond cleavage, with no good hydrogen donor in the system to cleave these out as steranes.

The authors could perhaps estimate what their detection limit is for detecting kerogen-bound steroids, giving the analytical complexities?"

REPLY: Please see author's response to referee #1 (5.).

Planned changes in manuscript: Please see author's response to referee #1 (5.).

3. – "I refer the authors to a recent study by Lee et al. (2019) OG, which has only just appeared., in print that showed evidence for early diagenetic incorporation of biomarker lipids by covalent binding into benthic mat sediments from a salt pond Guerrero Negro, Mexico. They used sequential chemolysis and HyPy degradation on extracted microbial mat sediments and found evidence for early diagenetic incorporation of a variety of linear, branched and polycyclic lipid skeletons into proto-kerogen on a timescale of only years to decades. The lipids includes bound hopanoids and bound steroids.

So, this supports the idea that HyPy is an effective method for trying to detect bound steroids in mat proto-kerogen due to the I) high sample capacity and ii) use of reducing conditions that yields appreciable steranes and sterene products. It further supports the idea as described in 2) that the Py-GC-MS method used in this investigation is maybe problematic for detecting immature bound steroids from proto-kerogen.

It is surprising since this group has their own HyPy equipment that they choose an online Py-GC-MS method to try and detect kerogen-bound steroids.

The amount of high mw and polar material in pyrolysates from "young" mat sediments will be appreciable so it is best to choose a method that generates a substantial portion of bound steroids as hydrocarbon products. Even with HyPy, the "polar" fraction dominates the pyrolysate products so this problem will be even more acute with Py-GC-MS performed with an inert gas (rather than high pressure hydrogen and a catalyst as used in HyPy)."

REPLY: We acknowledge the article recently published by Lee et al. (2019) and will refer to it in our revised manuscript. Unfortunately, the HyPy equipment of our group was not available at the time of this project. Nevertheless, we feel that the proven suitability of HyPy does not reject the applicability of Py-GC-MS for this study. As also indicated in the response to referee #1, several studies have demonstrated that Py-GC-MS is a suitable method to investigate steroids in immature kerogen (Gelin et al., 1996; Kruge and Permanyer, 2004).

Planned changes in manuscript: We will discuss the suggested paper (Lee et al., 2019) in the revised version (chapter 4.2). The applicability of Py-GC-MS and the differences compared to HyPy will be also indicated in chapter 3.4 and 4.2.

References cited in the reply:

Bühring S. I., Smittenberg R. H., Sachse D., Lipp J. S., Golubic S., Sachs J. P., Hinrichs K. U. and Summons R. E. (2009). A hypersaline microbial mat from the Pacific Atoll Kiritimati: insights into composition and carbon fixation using biomarker analyses and a ^{13}C-labeling approach. *Geobiology* 7, 308–323.

Gelin F., Sinninghe Damsté J. S., Harrison W. N., Reiss C., Maxwell J. R. and De Leeuw J. W. (1996). Variations in origin and composition of kerogen constituents as revealed by analytical pyrolysis of immature kerogens before and after desulphurization. *Organic Geochemistry* 24, 705–714.

Kruge M. A. and Permanyer A. (2004). Application of pyrolysis-GC/MS for rapid assessment of organic contamination in sediments from Barcelona harbor. *Organic Geochemistry* 35, 1395–1408.

Lee C., Love G. D., Jahnke L. L., Kubo M. D. and Des Marais D. J. (2019). Early diagenetic sequestration of microbial mat lipid biomarkers through covalent binding into insoluble macromolecular organic matter (IMOM) as revealed by sequential chemolysis and catalytic hydropyrolysis. *Organic Geochemistry* 132, 11–22.

Shen Y., Thiel V., Duda J.-P. and Reitner J. (2018) Tracing the fate of steroids through a hypersaline microbial mat (Kiritimati, Kiribati/Central Pacific). *Geobiology* 16, 307–318.