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Associate Editor *Biogeosciences*
Dr. Marcel van der Meer

Göttingen, 20.11.2019

Submission of the revised manuscript ("*Sterol preservation in hypersaline microbial mats*"; bg-2019-214)

Dear Dr. Marcel van der Meer,

also on behalf of my co-authors, I would like to thank you, Dr. Rienk Smittenberg and Dr. Gordon Love for the thorough and constructive comments on the manuscript. All of the comments have been carefully considered, and the suggested corrections have been added into the revised version.

In order to help you to track our changes and modifications, we uploaded the following documents:

1. Reply to your comments
2. Replies to the comments of all reviewers (RC1-2 from Nov 20th)
3. Tracked changes version of the manuscript (main text)
4. The new version of the manuscript + all figures, tables and supplementary documents

We trust that the revised manuscript will meet the requirements and demands of the reviewers. I would appreciate a reply at your earliest convenience.

Thank you for your consideration.

Yours Sincerely,

yanshen

Yan Shen

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

Responses from the authors to the comments of the subject editor (Dr. Marcel van der Meer) on manuscript bg-2019-124

We sincerely thank the associated editor (Dr. Marcel van der Meer) for the helpful and constructive comments that improved our manuscript.

Comment from the editor: "Both reviewers, and myself, have mentioned that this paper seems to be a continuation of already published work with quite some overlap in the author lists as well. I have mentioned before that you have to make it very clear why this manuscript is able to stand on its own and at least one reviewer made a similar remark, so make that absolutely clear in the rebuttal and revised version."

Author's response: The study has been published in Geobiology 2018, at first glance, is similar to the current one. However, in that study we analyzed microbial mat samples from another lake, and there are significant differences in the focus and techniques that we used, and also the outcome of both studies is remarkably different. Furthermore, in the study of Blumenberg et al. (2015), the concentrations of individual steroid compounds have not been provided for our mat in this work.

For the work now under review for Biogeosciences, we explicitly discuss steroid distributions in freely extractable vs. carbonate-bound lipid fractions as well as in the decalcified extraction residues. We also test if calcification within the microbial mat may function as a preservation mechanism for these biomarkers. These points were apparently not included in the 2018 publication. In addition to steroids, new data such as hopanoids and fatty acids requested by reviewer #1 are now included and discussed in the revised version (chapter 3.3, 3.4, 3.5 and 4.2).

Furthermore, in our current study, we observed how total sterol concentrations decreased immediately below the mat surface, thus supporting the "mat-seal effect" hypothesis. This is remarkably different from the 2018 publication, where there was no evidence for such a mat-seal effect. In the discussion, we considered potential mechanisms which might have caused the distinct behavior of sterols and also tried to examine general trends in the preservation of sterols in hypersaline microbial mat systems.

Therefore, this manuscript provides new data and novel insights into the preservation pathways of steroid biomarkers in microbial mats. We trust that these results, together with the analysis of a microbial mat from a different lake, and the different focus and techniques we used, justifies the publication of this work as a stand-alone paper in Biogeosciences.

Comment from the editor: "Both reviewers commented on the detection limit of your method and how this might relate to your detection of sterols or absence thereof. Make sure you address this issue and how this might affect your results and discussion. I am not really happy with your reply to the comment about HyPy versus your Py results of Dr. Gordon Love, please try to come up with a better answer."

Author's response: Indeed, producing reproducible quantitative data with Py-GC-MS is difficult. We further agree with the reviewer that the detection limit of our Py-GC-MS may be higher than the

HyPy-approach and have rephrased the discussion in the revised version. HyPy includes catalytic hydrogenation, chromatographic isolation and concentration of the target analytes, together resulting in less interferences and a relatively higher sensitivity. An estimate for the detection limit of our system can be derived from our analysis of the samples together with an internal standard (*n*-eicosane D42, 120 ng) that was routinely added to check the performance of the chromatographic system. When comparing our steroid peaks to this standard (and neglecting slightly different response factors), the absolute amount of analyte should be ~1 ng to obtain a reliable signal enabling identification via the mass spectrum. The limited sensitivity of our Py-GC-MS system may have resulted in non-detection of small amount of steroids, which are obviously still detectable by HyPy (Blumenberg et al., 2015). Nonetheless, both techniques clearly show that hopanoids are more abundant by far than steroids in the Lake 2 mat. We newly discussed this aspect and also included the findings made by Lee et al. (2019) into the discussion. We do not agree, however, with the reviewer's feeling that our method is generally not useful for our purpose; many studies have demonstrated the capability of Py-GC-MS for analysing steroid moieties in macromolecular OM (e. g. Gelin et al., 1996; Kruge and Permanyer, 2004). Furthermore ,the ability of our instrument for steroid identification was also positively checked using non-extracted original mats and the Eocene Green River Shale as reference materials (see also replies 3, 5 and 6 to reviewer #1).

Comment from the editor: "I do have one other remark concerning microbial mats, degradation and time that I think connects to some of the remarks made by both Dr. Rienk Smittenberg and Dr. Gordon Love. Different from a typical sedimentary setting microbial mats do not simply get buried by material falling on top and therefore getting older with depth. As I understand it, you have photoautotrophic organisms at the top generating biomass and heterotrophic organisms "below" (usually the separations are not so definitive, but to keep it simple). The mats typically grow upwards on top of dead and reworked organic matter from the mat itself. Potentially even similar to say year rings in trees, so new mats, consisting of two layers grown on top of the compacted layers of last year/growing season. The carbonate layer could even be the results of microbial activity, separating layers. This would fit with the observation that age does not increase regularly with depth or that concentration does not decrease linearly with depth, but in steps. High in the fresh photoautotroph layer, lower in the heterotroph layer increasing again into the older photoautotroph layer and lowest in the old heterotroph layer, for instance. This also means that community composition changes with depth, even if it doesn't really change with time (the top layer will always be photoautotrophic). How do your results relate to mat development, is there any information how this mat develops over time?"

Author's response: We agree with the considerations that microbial mats evolve internally through a continuous reworking of organic matter from the own mat, and that microbial activity can be linked to mineral precipitation within the mat. In accordance, we have rewritten and clarified the description of the growth phases of the mat (section 3.1), and we have included (in sections 4.2.1 and 4.2.2) discussion about how some biomarker trends (i.e. stanol/stenol ratios for C_{27}-C_{29} pairs and hopanoids abundance) indicate differences in microbial activity and microbial transformation of stenols in the different growth phases of the mat. Especially interesting, and in agreement with the editor's comments, is the case of the continuous mineral crust of layer 3, where intense microbial activity is recorded by the biomarkers. Concerning the timing of mat development, and in response to comments of Dr. Rienk Smittenberg, we have considered that the exact ages of the individual mat layers are actually not relevant for the purpose of this work and therefore, we have omitted the ^{14}C data and any interpretation or discussion based on them, retaining only the description of the growth phases of the mat, which is more relevant, as indicated by the editor.

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

Responses from the authors to the comments of Referee #1 (Dr. Rienk Smittenberg) on manuscript bg-2019-124

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We are very grateful to referee #1 (Dr. Rienk Smittenberg) for his valuable and thoughtful comments that helped us to improve our manuscript. Below we list all the points raised by the reviewer (given between quotes), followed by our replies.

1. – "The use of TMCS in methanol to methylate fatty acids is rather uncommon. Please provide a reference where this method and its efficiency are described."

REPLY: We now provide a reference describing the use and efficiency of TMCS in methanol for the methylation of fatty acids by Poerschmann and Carlson (2006) in the materials and methods section.

Changes in manuscript: We have cited this reference in the revised version (chapter 2.3).

2. – "How were the ^{13}C contents of the sterols and FAs corrected for the added derivatizing group."

REPLY: We corrected ^{13}C contents for the added derivatizing group following the method described by Goñi and Eglinton (1996). First, we used GC-C-IRMS to analyze *n*-Heneicosanoic acid and Androstanol standards as non-derivatized lipids. Second, they are derivatized as methyl ester (ME-) and trimethylsilyl (TMS-) ethers to obtain the carbon isotopic values of the derivatizing groups. After measuring our samples, we calibrated the carbon isotope values of our derivatized lipids according to the equations provided in that study. This has been indicated in the materials and methods section.

Changes in manuscript: We now have specified the method and added the reference (Goñi and Eglinton, 1996) in the revised version (chapter 2.6).

3. – "I wonder if pyrolysis GC-MS is the best way to assess if steroids make it into the 'kerogen' fraction of recent material. I am not a Py-GC-MS expert but pyrolysis at 560°C is rather high temperature where most organic molecules will break down to smaller pieces; any remaining intact compounds or larger fragments will be low in concentration. The fact that some hopanoids (fragments) may indicate that they are simply more abundant, while any remaining steroids could be below detection limit (i.e. below the background of the 213+215+217 trace). Absence of evidence is not the same as evidence of absence…,"

REPLY: As demonstrated by Gelin et al. (1996), steroids are thermally stable at such high temperatures at 610°C and Py-GC-MS is a suitable method to demonstrate their presence in immature kerogens. Another example proving the value of Py-GC-MS for our objectives is the study by Kruge and Permanyer (2004), who applied Py-GC-MS at 600°C for evaluating steranes/sterenes as tracers for organic contamination in recent sediments. Finally, the results obtained from our Eocene Green River Shale reference material can be taken as proof that the applied method is appropriate for the analysis of kerogen-bound steroids. These points have been indicated in the results and discussion section.

We agree that "*Absence of evidence is not the same as evidence of absence"*. In this regard, we have reworded the discussion chapter and clarified that the kerogen-bound steroids are below the detection

limit for our Py-GC-MS. Regarding the definition of our detection limit, please see author's response below (5.).

Changes in manuscript: We have reworded and add these points in the revised version (chapter 2.5, 3.5 and 4.2.3).

4. – "…Information about the reference kerogen of the Green River shale is lacking (e.g. sample amount, or relative amount of hopanes-steranes in Green River bitumen) so this comparison does not tell very much."

REPLY: Our intention when displaying the reference run was to prove the capability of the system to detect the compounds in question, and to indicate the exact retention times in the chromatograms. For that purpose we used about 0.5 mg of kerogen purified from the Eocene Green River oil shale (Eastern Utah, White River Mine, BLM Oil Shale Research, Development, and Demonstration Lease UTU-84087).

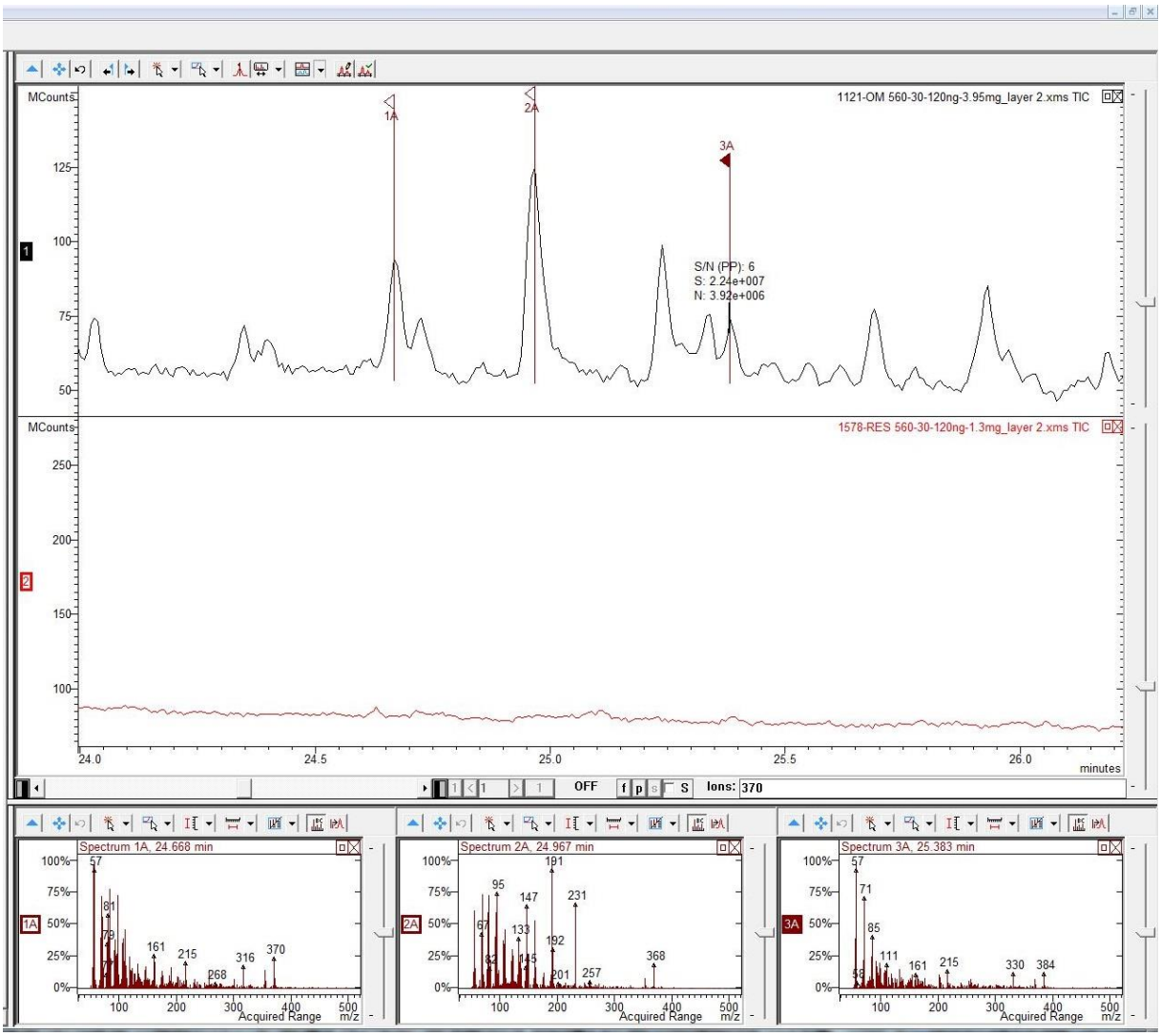
Changes in manuscript: We have now included the detailed information about the reference material to the revised version (chapter 2.5; supplementary information).

5. – "More critically, Blumenberg (2015) could report hopane/sterane ratios for the same mat, which means steranes were present throughout – although they did appear to decrease with depth (at least compared to the hopanes). I would have the same critique to the already published paper about Lake 22 (Shen 2018) when it concerns the use of Py-GC-MS to investigate the presence of steranes in the 'proto-kerogen."

REPLY: The hopane/sterane ratios reported by Blumenberg et al. (2015) showed that hopane concentrations are more than 20 times higher than steranes in the mat that we also analyzed for this work. Moreover, Blumenberg et al. (2015) has not provided abundances of individual steroid compounds, making it difficult to directly compare their data with our results.

In addition, providing sound quantitative data with Py-GC-MS is generally difficult. An estimate for the detection limit of our system can be derived from our analysis of the samples together with an internal standard (*n*-eicosane D42, 120 ng) that was routinely added to check the performance of the chromatographic system. When comparing our steroid peaks to this standard (and neglecting slightly different response factors), the amount of analyte should be about 1 ng (absolute amount) to obtain a reliable mass spectrum that allows for identification. This might be higher than the detection limit of the HyPy-approach (Blumenberg et al. 2015), that includes chromatographic isolation, catalytic hydrogenation and concentration of the target analytes. These fundamental characteristic of HyPy might partly explain the lack of steroid signals in our chromatograms (see also 6.).

To clarify how steroids can be detected by our Py-GC-MS system, we have added a screenshot below showing Py-GC-MS chromatograms (TICs) of the original microbial mat (Layer 2; upper chromatogram), and its extraction residue ('kerogen'; lower chromatogram). Sterenes and hopenes are abundant in the original mat sample, but their concentrations are below detection limit in the kerogen. Mass spectra for some compounds from the upper chromatogram are also given, representing a C_{27} sterene (1A), a C_{27} hopene (2A), and a C_{28} sterene (3A). Compound 3A is just identifiable from the TIC (though some coelution is evident in the mass spectrum) and it was therefore used to define our (conservative) detection limit of ~1 ng (absolute amount) for kerogen-bound steroids.



Changes in manuscript: We have provided the details of our detection limit, and clarified that our pyrolysis results cannot exclude the presence of small amounts of steroids in the kerogen fractions (chapter 3.5 and 4.2.3).

6. – "The paper cannot really be read without also consulting a more comprehensive hypy-GC-MS-biomarker analysis published by Blumenberg (2015), who finds hopane/sterane ratios of 20-100- thus no surprise there are no sterols found by Py-GC-MS while hopanes do show a trace."

REPLY: As detailed above (5.), we agree that small amounts of steroids might not be detected by Py-GC-MS due to our detection limit.

Changes in manuscript: We have provided an improved discussion including a more detailed comparison with the results from Blumenberg et al. (2015), and a discussion of the detection limit (chapter 4.2.3).

7. – "Why did the authors not measure (or present) free hopanoids: Or for that matter a more comprehensive biomarker study (i.e. a free extractable lipid version of the Blumenberg paper). This would give the paper much more body, constraining it to only steroids feels very limited. I strongly recommend expanding the paper in this way."

REPLY: We agree that hopanoids could provide additional information. We have therefore further conducted lab analysis of hopanoids to make comparison with steroids. In addition to hopanoids, we now also add fatty acids data to obtain a complementary picture of individual lipids and to a better understanding of preservation pathways of biomarker study within the mat studied. In order to address the taphonomy of steroids, hopanoids and fatty acids, we have re-structured the manuscript and discussed these three compounds classes in the different lipid fractions, respectively, including freely extractable lipids, carbonate-bound lipids as well as decalcified extraction residues.

Changes in manuscript: We have included detailed information about hopanoids and fatty acids data in the revised version (chapter 3.3, 3.4, 3.5 and 4.2).

8. – "The ^{14}C dating of carbonates on a coral atoll has a large risk of a reservoir effect (the coral is likely from the mid-Holocene sea level high stand thus several 1000 years old). Indeed a ^{14}C age of just -239 years indicates a fraction modern of just over 1, i.e. a mixture of post-bomb atmospheric CO_2 and an ancient source. The downcore increase in age does make sense, but one cannot assign any exact ages to the mat material - for this, one needs to date plant macrofossils. I realize the results were published already by Blumenberg et al but they can only be interpreted as deeper=older."

REPLY: Since we consider the exact ages of these layers irrelevant for our purposes, we have followed the advice of the reviewer and not expanded on the ^{14}C data published by Blumenberg et al. (2015).

Changes in manuscript: We have removed the detailed age information (Figure 1 and chapter 3.1).

9. – "When looking at the depth profiles of the sterols, I do not see a clear decrease with depth, except the large difference between the surface layer and layers below the phototrophic active part. Layer 5 and layer 2 have the same concentration per g TOC, and layer 3 and 6 the same expressed per g dry mat."

REPLY: In the manuscript, we refer to the same "large difference" between the surface and the deeper layers, as noted by the reviewer. We did not mean that there was a gradual decrease with depth.

Changes in manuscript: The respective text passages have been reworded to clarify this (chapter 3.3.1).

10. – "Comparison with Lake 22 (Shen 2018): What is different between the two lakes is a halite-gypsum crust on top of Lake 22 – which must impair oxygen flux to the upper layer. Yet, Lake 22 shows considerably higher steroid concentrations than this Lake 2. This may explain the absence of higher sterol abundances in the upper layer (i.e. absence of eukaryotic sterol-producing photosynthetic organisms in Lake 22 but rather a 'fossil' signal starting already in layer 1 below the halite crust. The higher sterol conc. in Lake 22 may simply be a higher contribution from terrestrial vegetation, but as the authors state it can also be a lower degradation because of ultrahigh salinity. Coprostanol found in Lake 22 could be derived from the abundant land crabs on Kiritimati, which live around the lakes and eat the local vegetation (and each other - personal observation in 2005)."

REPLY: We agree that the halite-gypsum crust on top of the Lake 22 mat may have impaired oxygen flux to the upper layer, thereby reducing the production of sterols in the upper part of the Lake 22 mat. However, it is not related to the current study, when explaining the potential reasons for the distinct degradation patterns of steroids between Lake 2 and 22 mats. On the other hand, we do not consider the higher sterol concentrations in Lake 22 as being caused by a higher terrestrial input. This is demonstrated by the distributions of sterol pseudohomologues (high in C_{27}) as well as the similar sterol concentrations in the top layers of both mats (10^2 μg/g C_{org} range, see chapter 4.3). As discussed in the manuscript, we consider hypersalinity, combined with periods of subaerial exposure, as more

important factors on the degradation rates, and regard this a more likely explanation why steroids showed no overall systematic decrease throughout the mat profile in Lake 22.

11. – "I also agree that the data confirm the hypothesis that microbial mats do not preserve original photosynthetic lipids from the upper very well, and that this signal is overprinted by heterotrophic organisms. However, marine or lake sediments do have the same' problem': organic matter degradation on heterotrophy within (anoxic) sediments, i.e. a diagenetic overprint. That said, these are not really very new insights, the added value compared to the Blumenberg 2015 and Shen 2018 papers is marginal."

REPLY: In the current work, we investigated a microbial mat from a lake with completely different environmental conditions (e.g., salinity and water depth) as compared to the 2018 publication. In addition, the microbial mat studied in 2018 probably experienced periods of subaerial exposure, which is not observed for the currently studied microbial mat. Further, unlike it has been done in the 2018 paper and in Blumenberg et al. (2015), we explicitly analyzed individual lipid distributions, including steroids, hopanoids and fatty acids, in freely extractable and carbonate-bound lipid fractions as well as in decalcified extraction residues. Moreover, we also test whether calcification within the microbial mat may function as a preservation mechanism for eukaryote-derived steroids.

Changes in manuscript: We have reworded and put more emphasis on the novel insights that our new work provided as compared to Blumenberg et al. (2015) and Shen et al. (2018) (chapter 3.3, 3.4, 3.5, 4.2 and 4.3). Please also see reply to the first comment from associated editor.

12. – "I do not agree with the conclusion that steroids are not preserved, in my opinion the authors have not used the right method to investigate this. Blumenberg (2015) found steranes after hypy."

REPLY: The reviewer is correct in that it cannot be concluded that steroids are not preserved in microbial mats, but we did not claim this in our manuscript. For the Lake 2 mat we demonstrate how sterols experienced major *degradation* that largely eliminated the primary eukaryotic signal. In contrast, sterols in the Lake 22 mat (Shen et al., 2018) experienced major microbial *transformation* which greatly preserved their molecular integrity. On the other hand, Blumenberg et al. (2015) has not provided abundances of individual steroid compounds, making it difficult to compare their data with our results.

Our findings highlight that sterols may have contrasting preservation pathways in microbial mats, and that preservation may be much better in mats experiencing higher salinities and/or more desiccated conditions. This is certainly a relevant and novel insight revealed by this study.

Changes in manuscript: We have emphasized on the novel findings and sharpen the respective text passages in abstract, discussion, and conclusions sections (chapters 4.2, 4.3 and 5).

References cited in the reply:

Blumenberg M., Thiel V. and Reitner J. (2015). Organic matter preservation in the carbonate matrix of a recent microbial mat – Is there a 'mat seal effect'? Organic Geochemistry 87, 25–34.

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 desulphnrization. Organic Geochemistry 24, 705–714.

Goñi M. A. and Eglinton T. I. (1996) Stable carbon isotopic analyses of lignin-derived CuO oxidation products by isotope ratio monitoring-gas chromatography-mass spectrometry (irm-GC-MS). Organic Geochemistry 24, 601–615.

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.

Poerschmann J. and Carlson R. (2006) New fractionation scheme for lipid classes based on "in-cell fractionation" using sequential pressurized liquid extraction. Journal of chromatography. A 1127, 18–25.

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.

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

Responses from the authors to the comments of Referee #2 (Dr. Gordon Love) on manuscript bg-2019-124

yshen@gwdg.de

We are very grateful to referee #2 (Dr. Gordon Love) for his helpful and constructive 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.

Changes in manuscript: We have included 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 4.2.1).

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.).

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 have included it in our revised manuscript. However, in contrast to our results, the concentrations of steranes and hopanes in their study are in the same order. On the other hand, the HyPy equipment in our group was broken during 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).

Changes in manuscript: We have discussed the suggested paper (Lee et al., 2019) in the revised version (chapter 4.2.3). The applicability of Py-GC-MS and the differences compared to HyPy have been also indicated in chapter 3.5 and 4.2.3.

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.

Sterol preservation in hypersaline microbial mats

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Abstract. Microbial mats are self-sustaining benthic ecosystems composed of highly diverse microbial communities. It has been proposed that microbial mats were widespread in Proterozoic marine environments, prior to the emergence of bioturbating organisms at the Precambrian-Cambrian transition. One characteristic feature of Precambrian biomarker records is that steranes are typically absent or occur in very low concentrations. This has been explained by low eukaryotic source inputs, or degradation of primary produced sterols in benthic microbial mats ("mat-seal effect"). To better understand the preservational pathways of sterols in microbial mats we analysed freely extractable and carbonate-bound ~~sterols~~lipid fractions as well as decalcified extraction residues in different layers of a recent calcifying mat (~1500 years) from the hypersaline Lake 2 on the island of Kiritimati, Central Pacific. A variety of C_{27}-C_{29} sterols and distinctive C_{31} 4α-methylsterols (4α-methylgorgosterol and 4α-methylgorgostanol, biomarkers for dinoflagellates) were detected in ~~both~~freely extractable and carbonate-bound lipid pools. These sterols most likely originated from organisms living in the water column and the upper mat layers. This autochthonous biomass experienced progressive microbial transformation and degradation in the microbial mat, as reflected by a significant drop in total sterol~~s~~ concentrations, up to 98 %, in the deeper layers, and a concomitant decrease in total organic carbon. Carbonate-bound sterols were generally low in abundance as compared to the freely extractable portion, suggesting that incorporation into the mineral matrix does not play a major role for the preservation of eukaryotic sterols in this mat. Likewise, pyrolysis ~~revealed~~of extraction residues suggested that ~~steroids (i.e., including sterenes, steranes and sterols), in contrast to hopanoids, were not sequestered~~ sequestration of steroid carbon skeletons into insoluble organic matter ~~which may give rise to a further bias in the preservation of steroids vs. hopanoids, particularly in the later stages of burial. While these~~ was low as compared to hopanoids. Taken together, our findings argue for a ~~strong~~ major 'mat-seal effect' affecting the distribution and preservation of steroids in the mat studied.~~,~~ ~~they~~ This result markedly differs from recent findings made for another microbial mat growing in the near-by hypersaline Lake 22 on the same island, where sterols showed no systematic decrease with depth. The observed discrepancies in the taphonomic pathways of sterols in microbial mats from Kiritimati may be linked to multiple biotic and abiotic factors including salinity and periods of subaerial exposure, implying that caution has to be exercised in the interpretation of sterol~~s~~ distributions in modern and ancient microbial mat settings.

1 Introduction

Sterols~~, biological precursors of steroids,~~ are commonly used as biological markers for specific classes of organisms (Atwood et al., 2014; Brocks and Summons, 2004; Rampen et al., 2009; Volkman, 1986; Volkman, 2005). Sterols have been found in many different types of depositional environments such as soils (van Bergen et al., 1997; Birk et al., 2012; Otto and Simpson, 2005), recent lacustrine and marine sediments (Brassell and Eglinton, 1983; Gaskell and Eglinton, 1976; Robinson et al., 1984; Volkman, 1986), as well as microbial mats from meso- to hypersaline conditions (Grimalt et al., 1992; Scherf and Rullkötter, 2009). Further, the hydrocarbon skeleton of sterols is relatively stable, and thus significant amounts can be preserved in the geological record (Brocks et al., 2017; Mattern et al., 1970).

Microbial mats are vertically laminated organo-sedimentary structures, which are primarily self-sustaining ecosystems (Des Marais, 2003), ranging in thickness from millimeters to decimeters. The mineralized fossil product of microbial mats are microbialites, which have a long geological history of over 3 billion years, indicating that microbial mats probably represented the earliest complex ecosystems on Earth (Reitner and Thiel, 2011). Microbial mats typically consist of many different functional groups of microorganisms which control the organic matter (OM) turnover in the microbial mat. Major groups include cyanobacteria, colorless sulfur bacteria, purple sulfur bacteria and sulfate-reducing bacteria, but also eukaryotic organisms (Schneider et al., 2013; van Gemerden, 1993). A large proportion of the OM consists of extracellular polymeric substances (EPS), secreted by the microorganisms, which are crucial for the support and the development of the microbial mat (~~Wingender et al., 1999;~~ Decho, 2011; Reitner and Thiel, 2011; Wingender et al., 1999). EPS are rich in acidic groups that bind cations such as Ca^{2+}, thereby inducing a strong inhibitory effect on the precipitation of common minerals formed within microbial mats, such as $CaCO_3$ (Arp et al., 1999; Dupraz et al., 2009; Ionescu et al., 2015). Consequently, carbonate precipitation often occurs in deeper and older mat layers in which decomposing EPS gradually releases previously-bound Ca^{2+}, thus facilitating carbonate supersaturation (Arp et al., 1999; Dupraz et al., 2009; Ionescu et al., 2015). Previous studies indicate that early sequestration into a mineral matrix may promote the preservation of organic compounds (Summons et al., 2013; Smrzka et al., 2017; Thiel et al., 1999). Hence, microbial mats possibly provide an enhanced chance for OM to survive in the geosphere if carbonate or other mineral precipitation occurs therein.

~~In the Proterozoic, m~~Microbial mats have been proposed to be a predominant life form in the Proterozoic marine environments~~.~~, ~~I~~in contrast, ~~to~~ the Phanerozoic ~~that~~ is characterized by prosperity of biota ~~(~~including fauna and flora~~)~~, and a low abundance of benthic microbial mats (Grotzinger and Knoll, 1999; Riding, 2011; Walter, 1976). One of the characteristic features of the Precambrian biomarker records is that eukaryotic steranes are typically absent or occur in very low concentrations. This may be explained by a limited ecological distribution of eukaryotic algae and thus minor contributions of sterols to sedimentary OM (Anbar and Knoll, 2002; Blumenberg et al., 2012; Brocks et al., 2017; Knoll et al., 2007), and/or by a thermal degradation of sterols during catagenesis, ~~(e.g. in~~ as observed for the 1640 Ma Barney Creek Formation and 1430 Ma Velkerri Formation, Northern Australia~~,~~ (Dutkiewicz et al., 2003; Summons et al., 1988). An alternative explanation would be that eukaryotic lipids have been subject to a preservation bias due to the ubiquity of benthic

microbial mats. It has been hypothesized that these mats would have formed a significant mechanical and chemical barrier against the preservation of eukaryotic lipids sourced from water column and upper mat layers, a phenomenon termed as "mat-seal effect" (Pawlowska et al., 2013). Selective preservation induced by the mat-seal effect would also impart a bias in favour of lipids derived from heterotrophic microorganisms living in the deeper mat layers, and cause a suppression of the primary ecological signal. This is different from the situation in the Phanerozoic, where OM from planktonic primary producers (including algae and bacteria) is more rapidly transferred to the sediment through sinking aggregates (such as crustacean faecal pellets), ~~and~~ without being reworked in benthic microbial mats (Close et al., 2011; Fowler and Knauer, 1986; Logan et al., 1995).

The Kiritimati atoll (Kiribati Republic, Central Pacific, Fig. 1) is an ideal study site for investigating the taphonomy of sterols in microbial mats. The island is covered by c. 500 brackish to hypersaline lakes, most of which are populated by thick and highly developed benthic mats that are clearly laminated and show ongoing mineral precipitation, i.e. microbialite formation (Arp et al., 2012; Trichet et al., 2001; Valencia, 1977). Therefore, Kiritimati enables studies on the behaviour of sterols within various types of microbial mats thriving under different environmental conditions and showing different degrees of mineralization.

A recent study conducted on a microbial mat from Lake 22 on Kiritimati demonstrated that a range of sterols were abundantly present in all parts of that mat (Shen et al., 2018a). The lack of any systematic decrease with depth suggested that the sterols in that particular mat had not been impacted by a major mat-seal effect ~~(Shen et al., 2018a)~~. On the other hand, an earlier study on insoluble OM obtained from a microbial mat from a different lake of the same island (Lake 2, located about 10 km south of Lake 22) reported an increasing trend of hopane/sterane ratios with depth (Blumenberg et al., 2015). In conjunction with other findings, this was considered indicative of a "*suppression of biosignatures derived from the upper mat layers*" and thus, a mat-seal effect (Blumenberg et al., 2015). Since that work had a different focus and did not report detailed sterol data, it is not directly comparable with the results on the Lake 22 mat reported by Shen et al. (2018a). Therefore we revisited the microbial mat from Lake 2 and performed a detailed analysis of sterol compounds, investigating both freely extractable as well as carbonate-bound lipid fractions, and also decalcified extraction residues (here refers to kerogen fraction). Our study was aimed at further examining general trends in the preservation of sterols in hypersaline microbial mat systems by comparing the results from different settings within the same geological and geographical context (i.e. Lakes 2 and 22).

2 Materials and methods

2.1 Location and samples

The atoll of Kiritimati (Republic of Kiribati) is located in the central part of the Pacific Ocean, close to the Equator (Fig. 1). Its surface displays a complex reticular pattern encompassing c. 500 lakes with salinities that range from brackish to hypersaline. Most of the lakes harbour thick microbial mats that show ongoing mineralization processes (Figs. 1, 2) and

generally occur on top of older, more developed microbialites (i.e. already fossilized microbial mats; Arp et al., 2012; Ionescu et al., 2015; Trichet et al., 2001; Valencia, 1977). Vegetation around the lake areas comprises the mangrove *Rhizophoramucronata*, the parasitic climber *Cassytha filiformis*, the grass *Lepturus repens*, and the ironwood *Pemphis acidula* (Fig. 2e; Saenger et al., 2006). The climate of Kiritimati is broadly controlled by the El Niño-Southern Oscillation (ENSO) atmospheric phenomenon. During El Niño wet events, heavy rains occur, decreasing lake salinities; whereas reduced precipitation during La Niña dry events triggers higher evaporation and increasing lake salinities (Arp et al., 2012; Saenger et al., 2006; Trichet et al., 2001). Materials studied in this work were sampled from Lake 2 (Fig. 1), whose salinity was 97 ‰ in 2002 and 125 ‰ in 2011 (own data, unpublished). This high and variable salinity causes low metazoan diversity within Lake 2. Faunal elements include abundant *Tilapia* fish (Fig. 2d) and *Artemia* brine shrimp as well as few land crabs, and unicellular miliolid foraminifera (Saenger et al., 2006; Shen et al., 2018a). Events of mass mortality of fish have been observed in some of the lakes, including Lake 2 (Fig. 2d), which may be linked to extreme hypersaline conditions probably due to heavy evaporation during La Niña dry periods. More detailed information about the environmental setting of Kiritimati can be found elsewhere (Arp et al., 2012; Saenger et al., 2006; Shen et al., 2018a; Trichet et al., 2001).

In this work, a microbial mat from the hypersaline Lake 2, previously studied by Blumenberg et al. (2015), was analysed for ~~sterols~~ steroids, hopanoids and fatty acids (Fig. 1). This mat is 10 cm thick and was sampled from the centre of the lake (water depth c. 4 m) during a field campaign in March 2011 (Figs. 1, 2). Samples were stored at -20°C until laboratory preparation. Based on the macroscopic appearance, Blumenberg et al. (2015) divided the mat in five layers, the topmost layer corresponding to the photosynthetically active mat, and layers 2-5 representing ancient mat generations being degraded by recent anaerobic microorganisms (Figs. 1, 2). For this study, we used the same layer division as Blumenberg et al. (2015). However, a thin but distinctive mineral crust occurring just below layer 2 (Fig. 2c) has not been analysed in the previous study and is additionally included here (corresponding to our layer 3, Figs. 1, 2). Therefore, six layers in total were analysed in this work, each one c. 1-2 cm thick (except layer 3 ~0.15 cm).

2.2 Bulk analysis

Homogenized (mortar) aliquots of the freeze-dried samples (both original mat layers and extraction residues) were subjected to C/N/S analysis, using a Hekatech EA 3000 CNS analyzer and LECO RC 612 multiphase carbon analyser as described elsewhere (Shen et al., 2018a).

2.3 Extraction and derivatization

Aliquots of the freeze-dried samples (5-20 g) were homogenized and extracted using 4×50 ml portions of dichloromethane/methanol (3:1; V/V) (10 min ultrasonication) to obtain the freely extractable lipids. The remaining extraction residues were decalcified using 37 % HCl (dropwise until CO_2 development ceased), and again extracted as described above to yield the carbonate-bound lipids. The remaining extraction residues (after decalcification) were freeze dried for the analysis of bulk C_{org} and pyrolysis.

To make alcohols (including sterols and hopanols) GC-amenable, aliquots of the lipid extracts (both freely extractable and carbonate-bound lipid fractions) were silylated using BSTFA (N,O-bis(trimethylsilyl)trifluoroacetamide) containing 5 % (V/V) trimethylchlorosilane (TMCS) as a catalyser (70°C, 60 min). The resulting trimethylsilyl (TMS-) derivatives were dried under gentle N_2 flow, re-dissolved in *n*-hexane, and analysed by gas chromatography-mass spectrometry (GC-MS).

To make hopanoids and fatty acids GC-amenable, a mixture of TMCS/MeOH (1:9, V:V; Poerschmann and Carlson 2006) was added to all aliquots of lipid extracts and samples were heated at 80°C for 60 min. The resulting fatty acid methyl esters were extracted from the reaction mixture by vigorous shaking with 3×1 ml *n*-hexane. The extracts were combined and evaporated to near-dryness under a gentle stream of N_2, re-dissolved in *n*-hexane, and analysed by gas chromatography-mass spectrometry (GC-MS).

2.4 GC-MS

GC-MS analyses were carried out using a Thermo Fisher Trace 1310 GC coupled to a Thermo Fisher Quantum XLS Ultra MS as described elsewhere (Shen et al., 2018a). Due to low sterol concentrations and co-elutions, particularly in the deeper mat layers, sterols were not quantified via peak integration in the total ion currents (TIC). Instead, the summed ion traces of [m/z 129 + (M^+-90) + M^+] for the TMS-derivatives of Δ^5- and $\Delta^{5,22}$-stenols, and [m/z 215+ (M^+-90) + M^+] for the TMS-derivatives of stanols were used. Appropriate correction factors were applied according to the response of these ions *vs.* concentration in the mass spectra of standard compounds. Average standard deviations of sterol concentrations were determined from repeated analyses of sample material. Hopanoids and fatty acids were identified based on the mass spectra and retention times.

2.5 Pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS)

Aliquots of the decalcified extraction residues were pyrolysed on a fast-heating Pt-filament using a Pyrola 2000 pyrolysis device (Pyrolab SB) coupled to a Varian CP3800 GC and a Varian 1200L MS as described elsewhere (Shen et al., 2018a). An internal standard (*n*-eicosane D42, 120 ng) was routinely added to check the performance of the chromatographic system.

Additionally, the Eocene Green River shale was used as a reference material (Eastern Utah, White River Mine, BLM Oil Shale Research, Development, and Demonstration Lease UTU-84087).

2.6 Compound-specific stable carbon isotopes analysis

Compound-specific stable carbon isotope ratios were measured for sterols and fatty acids in the freely extractable lipid fractions of the microbial mat. Analyses were conducted using a Thermo Scientific Trace gas chromatograph (GC) coupled to a Delta Plus isotope ratio mass spectrometer (IRMS). The conventional CuO/NiO/Pt reactor was used and combusted at 940°C. The GC-column used was an Agilent DB-5 coupled to an Agilent DB-1 (each 30 m length, 250 µm internal diameter, and 0.25 µm film thickness). Lipid fractions were injected into a splitless injector and transferred to the GC column at

290°C. The carrier gas was helium at a flow rate of 1.2 ml/min. The temperature program for analyzing lipid fractions was ramped from 80°C, followed by heating to 325°C (at 5°C/min, held for 60 min). ~~Analysis~~ Analyses of laboratory standards were carried out to control the reproducibility of measuring conditions, and measurements were calibrated by using CO_2 gas of known isotopic composition.

n-Heneicosanoic acid and androstanol standards were analysed by GC-C-IRMS as non-derivatized lipids, and after derivatization as methyl esters (ME-) and trimethylsilyl (TMS-) ethers, respectively, to determine the carbon isotope values of the derivatizing groups. The $\delta^{13}C$-values of derivatized lipids were corrected for the additional carbon according to the equations provided in Goñi and Eglinton (1996).

3 Results

3.1 General characterization of the microbial mat

The microbial mat has a thickness of c.10 cm. Based on its macroscopic appearance ~~it~~, and on data from Blumenberg et al. (2015), the mat shows two major phases of development. The upper, younger growth phase is represented by layer 1 (photosynthetically active mat) and layer 2 (each c. 1 cm thick, Figs. 1, 2). These layers have a cohesive texture, sticking together when handled, due to abundant and relatively fresh organic material (i.e. EPS) of bright orange, green and brown colours. Layer 1 includes small and scarce mineral precipitates, whereas layer 2 shows more abundant whitish minerals within its organic matrix (Fig. 2c). Layer 2 is underlain by a thin but distinctive, laterally continuous mineral crust (layer 3), which separates the two growth phases of the mat. Below the crust, the older growth phase is represented by layers 4, 5 and 6 (c. 7 cm thick in total, Figs. 1, 2). The lower layers are more friable than layers 1-2. ~~,~~ They mainly show brown and beige colours and have ~~having~~ a crumbly appearance, due to a higher abundance of mineral particles as compared to EPS~~. In this older growth phase, brown and beige colours predominate~~ (Fig. 2c). The minerals observed within the mat layers are mainly aragonite ($CaCO_3$), with minor amounts of gypsum ($CaSO_4$) found only in the uppermost layer 1 (Shen et al., 2018b). ~~Previously reported ^{14}C ages of the mat (Blumenberg et al., 2015) show that its upper growth phase formed in approximately 1000 years (62±40 years BP for layer 1, 551±40 years BP for layer 2 and 1111±40 years BP for layer 3; Fig. 1b) whereas the older (and thicker) growth phase formed approximately in the preceding 300 years (1331±40 years BP for layer 5 and 1440±40 years BP for layer 6; Fig. 1b).~~

3.2 Bulk geochemical data

Bulk geochemical data for individual mat layers are shown in Table 1a. In the original ~~mat (i.e. bulk sample before decalcification)~~ non-decalcified mat, relatively high C_{org} contents were observed in layers 1 and 2 (4.7 and 6.2 %, respectively; Table 1a and Fig. 3a), consistent with a more fresh, cohesive appearance of the organic matrix in these layers. ~~Below layer 2, t~~The earlier growth phase ~~consistently~~ below showed ~~low and~~ constant C_{org} contents < 2 %, with the lowest

value found for layer 6 (1.2 %) (; see 3.1Table 1a). The $CaCO_3$ content of the mat increased significantly with depth (Fig. 3a; Table 1a). The lowest value was observed in the top layer 1 (27.1 %), a strong enrichment occurred in layer 2 (73.1 %), and constantly high contents (> 90 %) were found for all deeper mat layers. This is consistent with the observation of more abundant mineral precipitates downwards in the mat. The highest Ssulfur content was detected for layer 1 (9.8 %), due to gypsum precipitates. Below, S decreased sharply (1.2 % in layer 2) and retained low values (< 1 %) in the earlier growth phase of the mat (~0.3-0.5 %). Nitrogen showed generally low contents (0.14-0.75 %) throughout the mat.

In the decalcified extraction residues (i.e., extraction residues after decalcification; Table 1b), C_{org} showed a broad range but increased significantly with depth, with the highest value observed in layer 6 (42.3 %; also see Fig. 3b). N was likewise enhanced in the deeper parts, with the highest amount found in layer 5 (6.7 %). By contrast, a decrease in S content was observed with depth, with highest values occurring in the topmost mat layer 1 (10.4 %).

3.3 Sterol distributions and concentrations

3.3 Freely extractable lipids

3.3.1 Freely extractable sterols

Various sterols were detected within in the matfreely extractable lipid fractions, including saturated sterols (stanols; $C_{27}\Delta^0$, $C_{28}\Delta^0$, $C_{29}\Delta^0$, $C_{31}\Delta^0$) and unsaturated sterols (stenols; $C_{27}\Delta^5$, $C_{28}\Delta^{5,22}$, $C_{28}\Delta^5$, $C_{29}\Delta^{5,22}$, $C_{29}\Delta^5$, $C_{31}\Delta^5$;; see Fig. 4; Table 2a). Based on the retention characteristics and comparison with published mass spectra (Atwood et al., 2014; Houle et al., In Press), In addition, distinctive the C_{31} sterols were also detected and identified as 22,23-methylene-4α,23,24-trimethylcholest-5-en-3β-ol (4α-methylgorgosterol) and 22,23-methylene-4α,23,24-trimethylcholestan-3β-ol (4α-methylgorgostanol), respectively, (see Fig. S1 based on the retention times and comparison with published mass spectra (Fig. S1; Atwood et al., 2014; Houle et al., 2019).

In the freely extractable lipids, stenols were by about an order more abundant than stanols. Both groups showed highest concentrations in layer 1, and a major decrease with depth (see Table 2a). Figure 5a shows the variations of C_{27}-C_{29} sterols (e.g. stenols *vs.*stanols) in the freely extractable lipid fractionss through the mat profile. The highest abundance of sterols occurred in the topmost layer 1 (26.05 µg/g dry mat, see Fig. 5a). Concentrations decreased drastically in the upper layers below, and remained low from layer 3 onwards. C_{28} and C_{29} sterols were the most dominant sterols in layer 1 while the C_{31} sterols dominated in the deeper layers (Fig. S3). The C_{31} sterol distributions differed from Unlike the other sterols, the C_{31} sterols showed enhanced distributions identified in this mat, with concentrations in layer 5, being three to ten times higher than in layers 3, 4 and 6 (Fig. 3c; Table 2a). FurtherIn general, stenols were by about an order more abundant than stanols. Both groups showed highest concentrations in layer 1, and a major decrease within the mat (see Table 2a).; in

In the freely extractable lipids, the ratios of 5α-stanols to their corresponding Δ^5-stenols (stanol/stenol ratios) showed no consistent trend within the profile (Fig. 6a; Table 3). As expected, tThe C_{27}-, C_{28}- and C_{29}- stanol/stenol ratios increased in the upper, younger growth phase of the mat, with the highest value observed for layer 3, but decreased again in the deeper,

older growth phase (Fig. 6; Table 3). In contrast, stanol/stenol ratios for the C_{31} sterols declined from layer 1 to layer 5, and showed a remarkable increase in layer 6.

~~Sterol concentrations in the carbonate bound lipid fractions are given in Table 2b. In the topmost layer 1, no carbonate bound sterols were observed. In mat layers 2 and 3, carbonate bound sterols occurred, but were still much less abundant as freely extractable sterols (below detection limit to ~10^{-2} µg/g dry mat). In the deeper mat layers (4-6), however, sterols showed similar absolute concentrations (~10^{-2} – 10^{-3} µg/g dry mat) in both, the carbonate bound and the freely extractable fractions. Sterenes were also detected in both lipid pools, but only at trace abundances (not discussed further).~~

~~In the carbonate bound lipids, stenols comprised the predominant portion (c. from 65 % up to 90 % of all sterols) (Fig. 5b). Overall low abundances of carbonate bound sterols were observed throughout the mat (10^{-1} µg/g dry mat range). C_{31} groups were the primary contributors in both lipid fractions (ranging up to ca. 85 % in the deeper part of the mat), and C_{29} groups were the second major inputs (Fig. S3). Carbonate bound C_{31} sterols increased in the bottom layer 6, which is distinguished from the other sterols (Fig. 3d). The stanol/stenol ratios in the carbonate bound lipids, increased for the C_{27}, C_{28} and C_{29} pairs between layers 1 and 3, and again decreased downwards as they did in the freely extractable lipids (see Fig. 6b; Table 3). No stanol/stenol ratios could be obtained for the carbonate bound C_{31} sterols, as C_{31} stenols were virtually absent throughout the mat.~~

~~3.4 Pyrolysis~~

~~Ion chromatograms representing steroids (i.e., including sterenes, steranes and sterols) and hopanoids released by pyrolysis of the decalcified extraction residues are shown in Fig. S2. Steroids were not observed in the pyrolysates throughout the mat, while hopanoids were found in the insoluble matter of each mat layer. Notably, only small amounts of hopanoid moieties were observed in the pyrolysates of layer 1, but their abundance gradually increased with depth.~~

~~3.5 Compound specific δ^{13}C values~~

A reliable compound-specific $\delta^{13}C$ value could be obtained for the coeluting C_{31}-sterols from the freely extractable lipids in layer 1. ~~This~~ These compounds showed ~~a~~ strong enrichments in ^{13}C ($\delta^{13}C$ = -7.2 ‰). Fatty acids (including C_{14}-C_{19} homologues) showed similarly high $\delta^{13}C$ values ranging from -4.4 to -11.7 ‰.

3.3.2 Freely extractable hopanoids

Several GC-amenable hopanoids were detected in the freely extractable lipids, with major compounds being hop-22(29)-ene (diploptene), ββ-bishomohopanoic acid and ββ-bishomohopanol. The summed major hopanoids showed highest abundances in layer 1 and 2 (about 15 µg/g dry mat). Below, hopanoid concentrations sharply decreased to < 4 µg/g dry mat in layers 3 and 4, but returned to moderate values in the deeper layers 5 and 6 (see Table S3, Fig. 7a).

3.3.3 Freely extractable fatty acids (FAs)

FAs in the freely extractable lipid pool show carbon numbers ranging from 14 to 30 (Table S1). Short-chain FAs (C_{14}-C_{19}) are predominant, making up > 90 % of the total in the upper layers and 50-60 % in the deeper layers. Further, medium-chain FAs (C_{20}-C_{23}) occur in low abundances (< 10 %) throughout the mat profile. Long chain FAs (C_{24}-C_{30}) made up only a few % of the freely extractable lipids in the upper three layers. However, the relative abundance of these long-chain FAs significantly increased in the deeper part of the mat, with c. 40 % observed for layer 5. Saturated and mono-unsaturated C_{16} and C_{18}, and a cyclopropyl-FA (tentatively identified as *cis*-9,10-methyleneoctadecanoic acid) were the dominant short chain FAs. In addition, terminally branched *iso-/anteiso-* C_{15}- C_{17} FAs were detected, showing a decreasing trend with depth (Table S1). With respect to the total FAs, highest concentrations were found in layer 1 (212.72 µg/g dry mat), and lowest in layer 3 (3.34 µg/g dry mat). Below layer 3, FA concentrations slightly increased again with depth (see Table S3).

3.3.4 Depth distributions of freely extractable compounds

Depth distributions of summed GC-amenable hopanoids, FAs, and steroids in the free lipids are presented in Fig. 7a. In all layers, FAs were by an order more abundant as hopanoids and steroids, but the relative distributions of the three compound classes resembled each other (high amounts in layer 1, low amounts in layers 3 and 4, Table S3). Quite similar distributions were observed when the compound concentrations were plotted against C_{org}, due to relatively low organic carbon throughout the mat (1.20-6.23%, Table 1a, Fig.7a).

3.4 Carbonate-bound lipids

3.4.1 Carbonate-bound sterols

Carbonate-bound sterols include C_{27}-C_{29} conventional sterols as well as C_{31} sterols. When compared to the freely extractable lipids, their concentrations are low, particularly in the surface layers (Fig. 5b; Table 2b). In the topmost layer 1, carbonate-bound sterols were virtually absent. In layer 2, carbonate-bound sterols occurred, but were still much less abundant as freely extractable sterols. In the deeper mat layers (3-6), however, the carbonate-bound and freely extractable sterols were in the same order (~10^{-2} µg/g dry mat range). C_{27}-C_{29} sterenes were also detected in both lipid pools, but only at trace abundances.

C_{31}-sterols were the most abundant sterols in both fractions (ranging up to ca. 85 % in the deeper part of the mat), followed by C_{29}-sterols (Fig. S3). The concentration of carbonate-bound C_{31}-sterols increased in the bottom layer 6, which is distinguished from the other sterols (Fig. 3d). The stanol/stenol ratios in the carbonate-bound lipids increased for the C_{27}-C_{29} pairs between layers 1 and 3, and again decreased further downwards, thus being similar to the freely extractable lipids (see Fig. 6b; Table 3). No stanol/stenol ratios could be obtained for the carbonate-bound C_{31} sterols, as carbonate-bound C_{31} stenols were virtually absent throughout the mat.

3.4.2 Carbonate-bound hopanoids

Small amounts of carbonate-bound hopanoids (mainly hop-17(21)-ene and ββ-bishomohopanoic acid) were observed in all mat layers except layer 1 (Table S2). The concentrations showed no consistent trend with mat depth. In layers 2, 5, and 6, the summed hopanoids were by an order lower than in the free lipids (<1 µg/g dry mat; see Table S4). In contrast, the amount of carbonate-bound hopanoids was markedly enhanced in layer 3 (3.22 µg/g dry mat), thus being similar to the freely extractable lipids (Table S3).

3.4.3 Carbonate-bound fatty acids (FAs)

Carbonate-bound FAs range in chain length from C_{14}-C_{28}, with short-chain saturated and mono-unsaturated homologues predominating (C_{16} - C_{19}). Apart from the straight chain (*n*-) FA, *iso*-/*anteiso*-branched C_{15} - C_{17} FA and a cyclopropyl-FA (tentatively identified as *cis*-9,10-methyleneoctadecanoic acid) were observed (Table S2). The summed concentrations of carbonate-bound FAs were in the same order as in the freely extractable lipid fraction (10^0~10^1 µg/g dry mat range). An exception was found for layer 1, where carbonate-bound FAs were an order less abundant (10^2 µg/g dry mat range; Table S3 and S4). The depth distribution of the summed carbonate-bound FAs showed an increase until layer 3 (highest concentration: 80.45 µg/g dry mat), followed by a sharp decrease in the deeper parts, with the lowest values observed for layer 5 (6.87 µg/g dry mat; Table S4).

A unique feature of the carbonate-bound FA fractions is the occurrence of saturated α,ω-dicarboxylic acids (α,ω-diacids) ranging in carbon numbers from 21 to 28. These diacids were detected in low concentrations (< 1 µg/g dry mat) only in layers 3 and 4, where they make up < 10% of the carbonate-bound FAs.

3.4.4 Depth distributions of carbonate-bound compounds

The depth distributions of the summed carbonate-bound compounds (Fig. 7b) revealed much (by an order) higher concentrations of FAs as compared to steroids and hopanoids. Whereas carbonate-bound steroids displayed no significant changes throughout the profile, summed carbonate-bound FAs and hopanoids showed a decreasing trend with depth, interrupted by a remarkable enrichment in layer 3 (Table S4). Largely identical distributions were observed when these compound classes were plotted against C_{org} (Fig.7b).

3.5 Decalcified extraction residues

Ion chromatograms representing steroids and hopanoids released by pyrolysis of the decalcified extraction residues are shown in Fig. S2. Throughout the mat, steroids were not observed in the pyrolysates, indicating that carbonate-bound steroids released from the macromolecular fraction were below our Py-GC-MS detection limit (~1 ng per analyte, see Fig. S4). On the other hand, hopanoid moieties were pyrolysed from the insoluble matter of each mat layer. Whereas only traces were detected in the pyrolysates of layer 1, their abundance showed a clearly increasing trend with mat depth (Fig. S2).

4 Discussion

4.1 ~~Biological sources~~ Origin of sterols

The studied mat contained a broad variety of C_{27}-C_{29} sterols as well as two C_{31} sterols, indicating potential sources like animals ~~(C_{27})~~, fungi ~~(C_{28})~~, algae ~~including dinoflagellates (C_{27}, C_{29} + C_{31})~~ and terrestrial plants ~~(C_{29})~~ (Atwood et al., 2014; Houle et al., ~~In Press~~2019; Volkman, 1986; Volkman, 2003). The concentrations of freely extractable sterols in the topmost layer 1 in the studied Lake 2 mat are similar to Lake 2A and Lake 22 (~10^2-10^3 µg/g $C_{org;}$ Bühring et al., 2009; Shen et al., 2018a; see Fig. 5a). However, sterols in the deeper layers are much less abundant in the Lake 2 mat as compared to other mats in Kiritimati lakes.

Figure S3 shows the relative distribution of summed C_{27}- vs. C_{28}- vs. C_{29}- vs. C_{31}- sterols in the microbial mat layers. In both lipid fractions, the C_{31}-sterols are predominant, suggesting inputs from dinoflagellates (Atwood et al., 2014; Houle et al., ~~In Press~~2019). C_{29}-sterols make up the next most abundant group of sterols, potentially indicating contributions from either algae or terrestrial plants (Volkman, 1986); moreover, ~~also~~ these compounds are known to be produced by some algae, including diatoms ~~and other algal groups~~ (Rampen et al., 2010; Volkman, 2003).

The high $\delta^{13}C$ value of -7.2 ‰ for the C_{31}-sterols~~,~~ ~~and~~ as well as similarly high values measured for ~~the~~ fatty acids ~~measured~~ from layer 1, imply that the carbon source of these compounds was autochthonous and derived from the hypersaline, CO_2-limited ecosystem of Lake 2 (cf. Schouten et al., 2001). Previous work on carbon isotope compositions of sterols in a mat from the adjacent Kiritimati Lake 2A showed $\delta^{13}C$ values from -19 to -23 ‰ (Bühring et al., 2009). In addition, Trichet et al. (2001) reported $\delta^{13}C$ values for sedimentary bulk OM from -14 to -17 ‰ in Kiritimati Lake 30. ~~Both~~Thus, both studies showed more depleted values than those observed for Lake 2. An explanation could be a better CO_2 exchange in those lakes, due to their shallow water layer (a maximum depth of 0.2 m in Lake 2A, Bühring et al., 2009; depth of 0.9 m in Lake 30, Trichet et al., 2001), leading to the relatively light $\delta^{13}C$ signatures. Another explanation could be that shrinking lake water bodies caused by La Niña dry events are often associated with massive increases in lake salinities (Trichet et al., 2001). For instance, Lake 2A (Bühring et al., 2009) was observed to be nearly dried out during our sampling campaign in 2011. The increasing salinities may result in a CO_2-limited ecosystem, leading to enrichment in ^{13}C. ~~The resulting~~ For Lake 2, such reinforced CO_2-limitation ~~in Lake 2~~ is not only ~~reflected~~ supported by the high $\delta^{13}C$ values of ~~sterols and fatty acids in this work~~ individual biomarkers, but also by $\delta^{13}C$ values of carbonates that were ~~observed~~ reported to be as high as +6 ‰ (Arp et al., 2012).

4.2 Taphonomy of ~~sterols~~lipids

4.2.1 Freely extractable lipids

The sterols in the studied mat are probably sourced from plankton or organisms thriving at the mat surface, because eukaryotes are generally depending on an oxygenated environment and would ~~hardly~~ not thrive in anoxic, deeper parts of the

mat. The abundance of total extractable sterols was high in the top mat but significantly decreased (up to > 90%) immediately below the topmost layer 1, and kept at trace amounts (10^{-1} µg/g dry mat, Fig. 5a) in the deeper part of the mat. Whereas the possibility of a change in microbial vs eukaryotic input over the time of mat deposition has to be considered, there are 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 environmental changes leading to an exclusion of eukaryotes. We therefore interpret this substantial decrease to result from major sterol degradation caused by heterotrophic microorganisms, thus suggesting the existence of a major "mat-seal effect" in the mat studied.

It can furthermore ~~also~~ be expected that most sterols were initially introduced as stenols. Subsequent alteration by early diagenetic processes within the mat would have resulted in a variety of sterol transformation products. Reduction of Δ^5-stenols to 5α-stanols (hydrogenation) is a known result of anaerobic microbial degradation (Rosenfeld and Hellman, 1971; Wakeham, 1989). Consequently, stanol/stenol ratios may reflect the extent of microbial ~~stenol~~ alteration under anoxic conditions (i.e. under low redox potential; Gaskell and Eglinton, 1975; Nishimura, 1977; Wakeham, 1989). Several investigations have reported such conversion in microbial mats (Grimalt et al., 1992; Scherf and Rullkötter, 2009; Słowakiewicz et al., 2016), including some mats from other lakes on Kiritimati (Bühring et al., 2009; Shen et al., 2018a).

~~In the mat studied, s~~Stanol/stenol ratios for C_{27}-C_{29} pairs in the free lipids initially increased with depth as expected, and showed highest values in layer 3, ~~suggesting~~ indicating low redox potentials and a pronounced anaerobic microbial transformation of stenols therein (Fig. 6a). In the deeper layers (4-6), however, ratios decreased again. We interpret this to result from a more efficient microbial OM degradation~~, which occurred~~ under higher redox potentials prevailing during the more rapid accretion of the earlier growth phase of the mat (~~1440–1111 years BP, see Fig. 1b~~Blumenberg et al., 2015). This idea is supported by constantly lower C_{org} contents in the earlier growth phase ~~of the mat (1.20–1.47 %, see~~ (Table 1a). ~~Exclusively for~~ As an exception, the C_{31}-stanol/stenol ratios~~, they~~ showed ~~a steady decrease~~ an outstanding behaviour as they steadily decreased with depth, but sharply increased again in the bottom layer 6. ~~It could be possible that~~ Possibly, primary input variations ~~of the C_{31} stanol and stenol~~ played a ~~more important~~ role ~~than microbial alteration~~ for the distributions of ~~C_{31} sterols~~these compounds. 4α-methylgorgostanol has been reported in a few dinoflagellate species belonging to the genera *Peridinium*, *Alexandrium* and *Pyrodinium*, (Atwood et al., 2014; Houle et al., ~~In Press~~ 2019, and refs therein)~~, and the mass spectra of the sterol we~~ The partly co-eluting stenol has been tentatively identified as 4α-methylgorgosterol ~~is similar to that~~ which has been reported ~~for a sterol occurring~~ in resting cysts but not in the motile cells of the dinoflagellate *Peridinium umbonatum* var. *inaequale* (Amo et al., 2010). ~~As a result~~Consequently, the C_{31} sterols ~~could~~ observed may partly ~~have been derived~~ derive from sedimentary resting cysts ~~that~~ and may have been less affected by ~~degradation during transport or~~ microbial recycling than conventional C_{27}-C_{29} sterols. It may also be speculated that the unusual side-chain structure and methylation pattern of 4α-methylgorgosterols hamper enzymatic microbial degradation (e.g. Giner et al., 2003, and refs therein). The steadily increasing relative abundances of C_{31}- *vs.* C_{27}-C_{29} sterols in the mat profile (Fig. S3) suggest that C_{31} sterols experienced different degradation patterns as compared to conventional sterols.

~~For the total sterols,~~ The GC-amenable hopanoids observed may have largely formed as earliest (eogenetic) products of bacteriohopanepolyols (BHPs; Rohmer, Bouvier-Nave and Ourisson, 1984) via progressive, microbially driven defunctionalisation. In the top layers 1 and 2, these hopanoids showed the highest concentrations within the mat (Fig. 7a). Below, they significantly decreased, suggesting major anaerobic degradation or, alternatively, binding to macromolecules. In the deeper layers 5 and 6, however, concentrations of hopanoids increased again to moderate values. While it can be assumed that a part of the initially produced BHPs has been transformed into the GC-amenable hopanoids observed. Another part has evidently been incorporated into macromolecular organic matter, as revealed by the release of hopanoids from the decalcified extraction residues by Py-GC-MS (Fig. S2). Steroid/hopanoid ratios show a significant drop in the upper two layers (possibly due to a major biodegradation of steroids therein), whereas the ratios keep fairly constant in the deeper mat layers, suggesting a similar degradation of both steroids and hopanoids at depth. However, it should be considered that different input/degradation patterns of C_{31} sterols (see above) may have influenced the steroid/hopanoid ratios observed.

To further check for the sources and additional input of lipids, we observed the FA distributions in the mat profile (Table S1 and S3). The predominance of saturated and mono-unsaturated C_{16} and C_{18} FAs, along with a cyclopropyl-C_{19} FA reveal major contributions from bacteria (Kates, 1964; Kaneda, 1991; Taylor and Parkes, 1985), whereas low amounts of homologues > C_{20} indicate only minor allochthonous inputs derived from higher plant lipids (Brassell et al., 1980; Cranwell, 1982). Fairly constant steroid/FA ratios in layers 1 to 4 (Fig. 7a and Table S3) suggest that both compound classes experienced similar preservation/degradation pathways. Again, there is little evidence for additional production of microbial lipids in deeper parts of the mat. Rather, and unexpectedly, steroid/FA ratios even increased in the deepest part of the mat (see Table S3), which is likely due to additional inputs of resistant C_{31}-sterols derived from dinoflagellate lipids, as discussed above.

4.2.2 Carbonate-bound lipids

~~concentrations of~~Unlike freely extractable sterols, carbonate-bound sterols were ~~high in the top layer but dropped sharply immediately below and kept at very low concentrations throughout the deeper mat (< 1 µg/g dry mat). Carbonate bound sterols, on the other hand, were hardly detectable~~ virtually absent at the top of the mat, and likewise showed constantly low abundances below (Fig. 5b). These observations suggest that ~~(i) freely extractable sterols are rapidly degraded in the upper layers of the mat and (ii)~~ the carbonate matrix played no important role in encasing (i.e., preserving) sterols in this mat~~. This is in agreement with previous reports about minor amounts of such sterols in methane seep microbialites and ooids (Birgel et al., 2006; Thiel et al., 1999; Thiel et al., 2001; Summons et al., 2013). However, in those settings, high concentrations of~~ and may be taken as indicative of a minor role of eukaryotic organisms, and their OM, in carbonate formation. In contrast, studies revealed that distinctive microbial lipids preserved in the carbonate matrix ~~possibly~~ reflect a constructive role of their source organisms in carbonate formation and/or their continuous incorporation~~ into carbonate~~ during the precipitation processes (Peckmann and Thiel, 2004; Summons et al., 2013; O'Reilly et al., 2017). ~~For instance, abundant ^{13}C depleted~~

~~acyclic isoprenoids encased in ancient methane-derived carbonates have been shown to originate from methane oxidizing archaea whose metabolism immediately enhanced calcification (Peckmann and Thiel, 2004).~~

~~Blumenberg et al. (2015) pointed out that organic compounds in the deeper parts of the Lake 2 mat, unlike those at the top, are better preserved in the insoluble OM fraction, which is concordant with the results in this work (Fig. 2S). Specifically, our pyrolysis of the decalcified extraction residues revealed that steroids were not sequestered into insoluble OM, while hopanoids were present in all mat layers (Fig. S2). This phenomenon was also observed for pyrolysates in Lake 22 mat (Shen et al., 2018a). An explanation could be that the low number of reactive sites in common sterols (i.e. one hydroxyl group) would lead to a low tendency to incorporate into macromolecular OM as compared to hopanoids (e.g. highly functionalized bacteriohopanepolyols with several hydroxyl groups). This behaviour may eventually give rise to a further preservational bias of steroids vs hopanoids over geological time.~~

~~In the Lake 2 mat studied here,~~ Stanol/stenol ratios for the carbonate-bound C_{27}-C_{29} sterols were similar as in the free lipids, with the highest value observed in layer 3, which is comprised of a dense mineral crust (Fig. 6b; see section 4.2.1). At the same time, both carbonate-bound hopanoids and microbial FAs were remarkably enriched in layer 3, indicating intensive microbial activity and, seemingly, an enhanced preservation of prokaryotic lipids in the carbonate matrix of this layer.

Steroid/hopanoid ratios in the carbonate-bound fraction showed no consistent trend through the depth profile. After a major drop in layer 3, due to the above mentioned increase in hopanoids, steroid/hopanoid ratios increased again in the lower layers 5 and 6. This may result from a higher abundance of carbonate-bound C_{31}-sterols in the bottom layers (as it was also observed in the freely extractable sterol fraction, see above). Steroid/FA ratios in the carbonate-bound lipids showed very low values through the mat profile. Again, the lowest values were observed for layer 3, due to the highest concentration of FAs observed therein.

A unique feature in the carbonate-bound lipids is the occurrence of α,ω-diacids. Previous work showed that these lipids may have multiple biological sources, e.g., higher plants (Kolattukudy, 1980) and sea-grass (Volkman et al., 1980). Given the presence of our findings of terrestrial biomarkers (albeit in low abundance) in the studied mat, these α,ω-diacids could be sourced from higher plant waxes. On the other hand, α,ω-diacids may also be foming *in situ*, for instance via terminal oxidation of monoacids or other aliphatic moieties such as *n*-alkanes (Ishiwatari and Hanya, 1975; Johns and Onder, 1975). Interestingly, α,ω-diacids were also reported in a recent study on Cretaceous hydrocarbon seep limestones, where they were only detected after the dissolution of the authigenic carbonate minerals (Smrzka et al., 2017). Likewise, α,ω-diacids were reported to be remarkably more abundant in carbonate concretions than in their clastic host rocks (Thiel and Hoppert, 2018). Taken together, these results might indicate that the formation of α,ω-diacids is directly associated with carbonate precipitation, or that these compounds are better preserved in carbonate matrices.

4.2.3 Decalcified extraction residues

Steranes have previously been detected in the insoluble macromolecular OM of benthic mats using catalytic hydropyrolysis (HyPy) (Blumenberg et al., 2015; Lee et al., 2019). In hydropyrolysates of microbial mats from Guerrero Negro in Mexico,

concentrations of steranes and hopanes were in the same range (Lee et al., 2019). Likewise, previously reported HyPy data for our mat from Lake 2 (Blumenberg et al., 2015) also showed the presence of both, hopanoids and steroids, but the latter were more than 20 times less abundant. This finding of predominant hopanoids in the Lake 2 mat is concordant with our Py-GC-MS data, however, steroid moieties were even below detection limit in our analyses (Fig. 2S). The non-detection of steroids here could be due to a lower detection limit in our Py-GC-MS setup, where an absolute amount of >1 ng of the target analyte is required to obtain an interpretable mass spectrum (according to analyses of reference compounds). Further, unlike in HyPy, there is no possibility in Py-GC-MS for a downstream chromatographic separation and concentration of the analytes.

Taken together, the strong decline in freely extractable sterols below the uppermost mat layer, along with ~~the lack of any significant carbonate~~ only minor incorporation~~, suggests~~ into carbonate and macromolecular OM, suggest that major degradation of steroids ~~vs. hopanoids~~ occurred during eogenesis and earliest~~y~~ diagenesis ~~(i.e. on a timescale of 10^2–10^3 years)~~in the Lake 2 mat studied.

4.3 Comparison with sterol taphonomy in other microbial mats

~~The results from~~ Major differences are evident between the depth distributions of steroids in the Lake 2 mat ~~differ significantly from the results of~~ studied here and those reported previously from a ~~previous study on a microbial mat profile in another lake in Kiritimati (~~ Lake 22~~;~~ mat (Shen et al., 2018a). ~~Sterol~~ Whereas the sterol concentrations ~~from~~ in the topmost layers are similar in both mats (10^2 µg/g C_{org} range), ~~but~~ the Lake 22 mat showed no systematic decrease in sterols with depth. Such entirely different behaviour of sterols in ~~these~~ the mats from the two adjacent lakes raises questions about potential mechanisms causing the observed variation.

One explanation for the differences observed ~~between the Lake 2 and 22 mats~~ could be ~~variations~~ differences in salinity. In 2011, the salinity of Lake 22 (Shen et al., 2018a) was 250 ‰, whereas Lake 2 showed only 125 ‰. High salinity may reduce microbial cell growth and reproduction, and limit the metabolism of microorganisms. The resulting decrease in bacterial activity would affect the biodegradation rates of organic compounds (Abed et al., 2006). Several studies reported that the degradation rates of hydrocarbons significantly decrease as salinity increases (~~microbial mat from Saudi Arabia,~~ Abed et al., 2006; ~~water and tar samples from Great Salt Lake,~~ Ward and Brock, 1978). ~~On the other hand~~In turn, lower salinity supports the proliferation of a more diverse microbial community (Bolhuis et al., 2014), thus possibly enhancing OM biodegradation. As a consequence, conditions for ~~mat-forming~~ heterotrophic microorganisms ~~would~~ could be more favourable in Lake 2 as compared to the extremely hypersaline Lake 22, thus accelerating the biodegradation rates of organic molecules, including sterols. ~~Another possible reason~~

A second plausible explanation for the differences between the Lake 2 and 22 mats might be ~~decreased precipitation resulting in the decline~~ associated with the environmental properties of both lakes, particularly water depth. A major drought period prevailed in Kiritimati from 2002 to 2011, as a result of a very strong La Niña dry event. Due to reduced rainfall, the water level of the lakes in Kiritimati generally dropped, so that, in some areas, parts of the lake bottoms became subaerially

exposed (this has been observed for Lake 2A; see section 4.1). The Lake 22 microbial mat was collected at the margin of the lake (Fig. 2f; water depth c. 0.2 m; Shen et al., 2018a). Therefore, mats from this shallow sampling site may have suffered from heavy evaporation due to such major drought events. As pointed out Indeed, the Lake 22 mat studied by Shen et al. (2018a), the Lake 22 mat shows showed an irregular top layer of V-shaped fractures, which are general characteristic features of subaerial exposure in evaporitic settings, thus further demonstrating the repeated occurrence of dry periods in the region. On the other hand, the Lake 2 mat studied here was collected in the lake centre (at the water depth c.of 4 m) which is and would have been clearly less prone to subaerial exposure. These interpretations are further supported by other studies that highlighted how environmental conditions such as highlighting the influence of water depth and salinity may have a significant influence on the microbial and biomarker composition of microbial mats (Pagès et al., 2014). In addition to these environmental conditions, the differences of

Likewise, much higher stanol/stenol ratios between in the two lakes are noteworthy. Much higher ratios were observed in Lake 22 mat (Shen et al., 2018a), indicating indicate a much more intense anaerobic microbial transformation (yet no degradation) as compared to the Lake 2 mat studied here (Fig. 6). Whereas sterols in Lake 22 mat experienced major microbial *transformation* (stenols => stanols => sterenes), sterols in Lake 2 apparently seemingly suffered from major *degradation* that suppressed largely eliminated the primary ecological eukaryotic signal. The contrasting distributions observed for the Kiritimati mats studied also suggest that sterols have a higher preservation potential in microbial mats under stronger salinities and/or more desiccated conditions, such as those of Lake 22. Our finding of such significant differences in two adjacent mat settings on the same island calls for great caution should be considered when studying sterols in microbialites or in modern microbial mats and making generalizations for the fossil record. from studies of sterols in modern microbialites or microbial mats. Sterol preservation within microbial mats is appears to be a complex process that may be strongly influenced by environmental parameters. Therefore, palaeoenvironments must be thoroughly constrained if the presence, or absence, of these compounds is interpreted in the study of ancient deposits.

5 Conclusion

The preservation of primary eukaryotic sterols and their progressive alteration was studied in a c. 1500 years old microbial mat from the hypersaline Lake 2 on Kiritimati. High δ^{13}C values of C_{31} sterols and fatty acids suggest an autochthonous origin for these lipids. TotalConventional C_{27}-C_{29} sterols decreased immediately below the uppermost layerseverely with depth, suggesting a major degradation progressive biodegradation of these compounds within the mat. A different pattern was observed for unusual, isotopically heavy C_{31}-sterols (4α-methylgorgosterol and 4α-methylgorgostanol; $\delta^{13}C$ = -7.2 ‰), which showed increasing abundances in the deeper mat layers. as compared to the conventional C_{27}-C_{29} sterols. These C_{31} sterols This may have partly derived from resting cells of dinoflagellates, or their unusual side-chain might hamper degradation, which may have be explained by an enhanced the resistance of these sterols against degradation. No, possibly due to their unusual side-chain and/or an origin from highly resistant dinoflagellate resting cysts. Separate analysis of

decalcified samples revealed that no significant 'trapping' of sterols into the mineral ~~lattice~~ matrix occurred in this mat. ~~Likewise,~~ Further, Py-GC-MS of decalcified extraction residues showed steroids ~~were not sequestered into insoluble organic matter (as opposed~~ to be below detection limit, in contrast to hopanoids~~). It is therefore suggested~~ which occurred abundantly in the pyrolysates throughout the mat profile. Our combined data suggest that the studied mat might have formed an effective filter against the preservation of sterols in the sedimentary record. ~~The~~ For the studied mat, the results ~~from this microbial mat, therefore,~~ thus support the hypothesis of a 'mat-seal effect' describing the degradation of eukaryote-derived lipids in benthic microbial mats. Our results are markedly different from those recently ~~obtained~~ reported from another microbial mat from ~~Kiritimati~~close-by Lake 22~~,~~ where sterols showed no systematic decrease with depth~~., suggesting that the preservation of sterol carbon skeletons in that microbial mat did not suffer from a mat seal effect.~~ In that ~~lake, an even~~ mat, higher salinity or temporal subaerial exposure ~~may~~ have probably hampered microbial metabolism and ~~instead~~ promoted ~~sterol transformation rather than~~ the preservation of steroids over degradation. The data combined ~~data~~ in this study show that sterol taphonomy may strongly vary between different mat systems, and even contrasting sterol degradation patterns may be expected in response to environmental conditions.

Code and data availability. Data can be found in the Supplement or can be requested from Yan Shen (yshen@gwdg.de).

Author contributions. YS had the main responsibility for analysing the data and writing the manuscript. JR and VT designed the project. JR conducted the field works, collected all the sample materials and participated in the writing process. VT contributed the conceptualisation of this work, participated in constructing the measurement setup and the writing process. PSG had the main responsibility for the bulk data measurements, participated in the writing process. SWP participated in the interpretation of the data and the writing process. All the co-authors contributed to this work.

Competing interests. The authors declare that they have no conflict of interest.

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