

Peer Review File

Biogeochemical explanations for the world's most phosphate-rich lake, an origin-of-life analog



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Decision letter and referee reports: first round

6th Oct 23

Dear Dr Haas,

Your manuscript titled "Biogeochemical explanations for the world's most phosphate-rich lake, an origin-of-life analog" has now been seen by 2 reviewers, and we include their comments at the end of this message. They find your work of interest, but some important points are raised. We are interested in the possibility of publishing your study in *Communications Earth & Environment*, but would like to consider your responses to these concerns and assess a revised manuscript before we make a final decision on publication.

We therefore invite you to revise and resubmit your manuscript, along with a point-by-point response that takes into account the points raised. Please highlight all changes in the manuscript text file.

We are committed to providing a fair and constructive peer-review process. Please don't hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to any cover letter), a tracked-changes version of the manuscript (as a PDF file) and the completed checklist:

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We hope to receive your revised paper within six weeks; please let us know if you aren't able to submit it within this time so that we can discuss how best to proceed. If we don't hear from you, and the revision process takes significantly longer, we may close your file. In this event, we will still be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at *Communications Earth & Environment* or published elsewhere in the meantime.

Please do not hesitate to contact us if you have any questions or would like to discuss these revisions further. We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Best regards,

-Mojj

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Senior Editor
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Decision letter and referee reports: first round

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Review of Haas et al 2023

Overall comments:

This is an important paper in my view. Tackling the ways in which prebiotically interesting environments are today shaped by biology, in order to try and reconstruct their prebiotic

Decision letter and referee reports: first round

equivalents, is quite urgent in the quest for the origins of life. Haas et al seek to unpick the mechanisms that produce the extreme compositions of soda lakes, which have in recent years garnered increasing attention as possible environments for fostering prebiotic chemistry. The present study combined fieldwork, sample analysis, and modelling to better understand how soda lakes on Earth today come into being, and discusses the implications of that new understanding for how we think about stockpiling prebiotically relevant species in analogous environmental contexts on early Earth. I find the approach, results, and discussion to be compelling, on which basis I recommend publication with minor revisions. I have a few specific comments, outlined below, which I would like to see addressed before the article is accepted.

Craig Walton // ETH Zürich

Specific comments:

"...bioavailable, physical and geochemical factors largely determine dissolved P levels."

- I find this to be a confusing oversimplification. The focus of the present paper is the fact that biology massively perturbs dissolved P levels by consumption. Of course, biology also returns P to the water column during respiration of sinking organic matter. It is therefore not so simple to contrast N and P levels as being set by biology vs abiotic mechanisms, respectively. I would advise revising the language here to help readers stay on track.

"Phosphorus or authigenic iron evaporite minerals were not found with XRD and their low abundance in LCL evaporites was confirmed by ICP-MS (P: $0.13 \pm 0.08\%$)."

- What do the authors envisage did bury the P? I couldn't seem to find a clear statement on this. Can we demonstrate the pathway (iron oxide binding, nanoparticulate apatite, organic matter)? Doing so seems crucial to understand how different the system would be without life.

"LCL is not more phosphate-rich simply because its water is more evaporated than GL's"

- If LCL is not more evaporated than GL, why is it more saline? Or, is the point here that whilst it is more evaporated and more saline, that is not the only reason phosphate is high? I believe it is the latter, consistent with the small geological sinks argument being made, but if so this sentence could perhaps do with rewording.

- I find the evidence that salinity is the key factor impacting N fixation evidence to be fairly weak. The data presented is scattered and with huge variability in salinity without change in N fixation, and vice versa. Is there any other hypothesis that could easily be tested here with available data? What about the fact that sulfide is so abundant in LCL? Could this inhibit N-fixing activity? It seems plausible to me, and it could be informative to see some figures produced if the data is already in hand for sulfide concentrations over time in the two study sites.

- I found it generally quite unclear what the main P inputs to the lakes are. There were mentions of anthropogenic runoff. However, clearly we would prefer our prebiotic analogue system to have only geological inputs of P. Can the authors clarify at some point in the paper the absolute + relative magnitude of inputs of P to each lake?

- The citation of 'J. Sutherland; pers. comm' seems like an inappropriate way to evidence the need for high phosphate concentrations to drive phosphorylation reactions, etc. There are plenty of papers to choose from that make similar statements or indeed provide the experimental evidence for that claim. From the top of my head, what about Patel et al 2015, Islam and Powner 2017, and/or Morasch et al 2019?

- I am curious to know what the rate of carbon burial is overall in these systems. Does it just correlate with nitrogen fixation rates? This also comes back to the point of trying to figure out if biology, despite being slow in LCL, still dominates the P sink or not, which seems relevant. If biology is only a minor P sink in this system (relatively speaking) then presumably removing biology would have not so much of an impact when revising P concentrations for a prebiotic

Decision letter and referee reports: first round

setting. If biology still dominates the minor P sink, then geology is hugely inefficient and P levels will necessarily be much higher without any life.

Reviewer #2 (Remarks to the Author):

The manuscript titled "Biogeochemical explanations for the world's most phosphate-rich lake, an origin-of-life analog" presented research about rich dissolve phosphate salt in soda lake and its connection with prebiotic phosphorylation in early Earth. In this research, the authors highlighted that nitrogen-limitation of biological production and high rich calcium carbonate (not calcium phosphate) precipitation may support high dissolve phosphate salt plausible in soda lake. This is an interesting topic. The manuscript is well written, but I find the structure confusing at certain points. I suggest the manuscript need do more expanding work before published.

First, the manuscript mentioned that biological N₂-fixation (BNF) plays a significant role to support calcium cation to form calcium carbonate sediment, not apatite sediment. It's a strong condition in nowadays Earth system. However, in Hadean Eon there is no biological N₂-fixation condition, hence how did those soda lake rich in dissolve P in the 1st Ga of Earth (without BNF at that time)? In another word, I believe the conditions of prebiotic phosphorylation in early Earth is non-biological, and life biomolecules should arise from abiotic processes (from compound to organic), not BNF or other bio conditions.

Then, the manuscript stated that dissolve phosphate salts in LCL and GL are mostly monovalent phosphate (Na⁺ phosphate or K⁺ phosphate). However, these phosphates are low reactive and thus this made prebiotic phosphorylation is thermodynamically unfavored. Hence, the manuscript required more evidence or details to explain how dissolve phosphate salts phosphorylation into organophosphate.

I also suggest the authors do more literature review work for the prebiotic phosphorylation in the manuscript. Previous research investigated that phosphorylated molecules, were they present, must be a product between a phosphorus mineral and with an organic substrate (see Gull et al. 2017 life). Moreover, some researchers suggested that alternatives to phosphate at the onset of the origin of life, like phosphide (see Pasek 2008, PNAS), glyoxylate (Bean et al. 2006, OLEB) or arsenate (Wolfe-Simon et al. 2011, Science). These works may support the manuscript to be more reasonable.

Author Responses: first round

Response to the Reviewer comments

We would like to thank the reviewers for their constructive feedback, which has resulted in this improved, revised version of manuscript. Below, we address each point raised by the reviewers in detail with our response in blue italics. Please note that line numbers refer to the manuscript version containing tracked changes.

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Review of Haas et al 2023

Overall comments:

This is an important paper in my view. Tackling the ways in which prebiotically interesting environments are today shaped by biology, in order to try and reconstruct their prebiotic equivalents, is quite urgent in the quest for the origins of life. Haas et al seek to unpick the mechanisms that produce the extreme compositions of soda lakes, which have in recent years garnered increasing attention as possible environments for fostering prebiotic chemistry. The present study combined fieldwork, sample analysis, and modelling to better understand how soda lakes on Earth today come into being, and discusses the implications of that new understanding for how we think about stockpiling prebiotically relevant species in analogous environmental contexts on early Earth. I find the approach, results, and discussion to be compelling, on which basis I recommend publication with minor revisions. I have a few specific comments, outlined below, which I would like to see addressed before the article is accepted.

Craig Walton // ETH Zürich

Response: We thank the reviewer for the encouraging comments and helpful feedback!

Specific comments:

“...bioavailable, physical and geochemical factors largely determine dissolved P levels.”

- I find this to be a confusing oversimplification. The focus of the present paper is the fact that biology massively perturbs dissolved P levels by consumption. Of course, biology also returns P to the water column during respiration of sinking organic matter. It is therefore not so simple to contrast N and P levels as being set by biology vs abiotic mechanisms, respectively. I would advise revising the language here to help readers stay on track.

Response: We thank the reviewer for pointing out this oversimplified statement and have adjusted and amended it accordingly (line 68-72).

“Phosphorus or authigenic iron evaporite minerals were not found with XRD and their low abundance in LCL evaporites was confirmed by ICP-MS (P: $0.13 \pm 0.08\%$).”

- What do the authors envisage did bury the P? I couldn't seem to find a clear statement on this. Can we demonstrate the pathway (iron oxide binding, nanoparticulate apatite, organic matter)? Doing so seems crucial to understand how different the system would be without life.

Response: We, discuss this (somewhat implicitly, previously) in the middle of the last discussion section (“Implications for the origin of life”), where we talk about sediment-bound P that might be bound in ways unrepresentative of early-Earth conditions and that have the potential to further increase the P in a prebiotic analog of LCL: iron-oxide-bound or organic-bound. We now made this more explicit by adding sentences pointing out the presence of P in LCL sediments and discussing various forms it may be in (lines 328-336). We very much agree with the reviewer that this is a crucial point, and in fact have another paper in preparation that focuses on resolving the forms of phosphorus in the lake sediments and quantifying the organic-phosphorus sink with the goal to understand how much more P could be dissolved in LCL without that sink. Quantifying P speciation with specialized lab analyses and modeling the P fluxes are extensive tasks that are beyond the scope of the present paper.

“LCL is not more phosphate-rich simply because its water is more evaporated than GL's”

- If LCL is not more evaporated than GL, why is it more saline? Or, is the point here that whilst it is more evaporated and more saline, that is not the only reason phosphate is high? I believe it is the latter, consistent with the small geological sinks argument being made, but if so this sentence could perhaps do with rewording.

Response: The point is indeed the latter. We rephrased the sentences in the manuscript to make this clearer (line 155-158).

- I find the evidence that salinity is the key factor impacting N fixation evidence to be fairly weak. The data presented is scattered and with huge variability in salinity without change in N fixation, and vice versa. Is there any other hypothesis that could easily be tested here with available data? What about the fact that sulfide is so abundant in LCL? Could this inhibit N-fixing activity? It seems plausible to me, and it could be informative to see some figures produced if the data is already in hand for sulfide concentrations over time in the two study sites.

Response: This study does not claim to conclusively identify the reason for BNF limitation in LCL, and hence the abstract has the qualifier “probably” for high salinity. In the absence of a dedicated study addressing this question, we argue that the salinity-inhibition hypothesis is the most plausible, given the evidence across soda lakes reviewed in Figure 5 and the known molecular mechanism (Apte 2001). We concur that the data in Figure 5 is scattered but we do not claim a clean correlation (as low BNF rates can be caused by other reasons) but a conspicuous absence of the very high BNF rates seen at lower salinities at salinity >100-150g/L

(line 296). And it is these very high rates that are necessary to create sufficient biological P demand for the relatively low P levels in Goodenough Lake (line 258-275).

We agree, however, that inhibition by sulfide is a possible alternative (or additional) explanation that is consistent with much higher dissolved sulfur in LCL than GL. Note that it might be related to salinity because sulfate reduction tends to scale with sulfate concentration, which tends to scale with salinity. In any case, we appreciate the reviewer suggestion and added the sulfide hypothesis to the discussion (line 297pp).

- I found it generally quite unclear what the main P inputs to the lakes are. There were mentions of anthropogenic runoff. However, clearly we would prefer our prebiotic analogue system to have only geological inputs of P. Can the authors clarify at some point in the paper the absolute + relative magnitude of inputs of P to each lake?

Response: We now added a sentence to identify P inputs to the lakes (line 111) and thank the reviewer for pointing out this gap. For more details, we have a paper in preparation (as mentioned above) that will detail and quantify phosphorus sources and sinks of the lakes beyond the present study. Note that the mentioned runoff (line 359) is not necessarily anthropogenic (we acknowledge that the word ‘runoff’ without a specifying adjective was potentially misleading) – there is no wastewater or similar entering the lakes, instead we were referring to water (potentially containing nutrients from soil) entering the lakes via the surrounding hillslopes during strong rainfall. In fact, we decided to delete this mentioning of ‘runoff’ entirely as it could be misleading based on our analyses for that in-preparation paper done in the meantime, which suggest no important role for surface-runoff.

- The citation of ‘J. Sutherland; pers. comm.’ seems like an inappropriate way to evidence the need for high phosphate concentrations to drive phosphorylation reactions, etc. There are plenty of papers to choose from that make similar statements or indeed provide the experimental evidence for that claim. From the top of my head, what about Patel et al 2015, Islam and Powner 2017, and/or Morasch et al 2019?

Response: We were able to remove the ‘...pers. comm.’ citation. The lower bounds of phosphate required for the prebiotic nucleoside phosphorylation are not explored thoroughly by organic synthesis chemists, including by the cited authors who use ~100 mM phosphate (e.g., Morasch et al.: 240 mM phosphate, Patel et al.: 100 mM) without trying to go lower. However, Xu et al., 2019 (Nat. Chem. 10.1038/s41557-019-0225-x) successfully use phosphate concentrations as low as 40 mM and this is now cited instead of the ‘...pers. comm.’ (line 314). The entire topic of prebiotic phosphorylation is now introduced in more detail (lines 37-50). Also, please note that the question regarding the concentration threshold allowing prebiotic phosphorylation is mostly interesting for orienting our comparison with the environmental phosphate levels. Ultimately, the chemistry of these lakes provides a way for phosphate to remain in solution to very high levels (probably even well beyond the observed levels; see Toner&Catling 2020, Postberg et al. 2023) and it is obvious that “the more phosphate the better” for abiogenesis, because (i) higher product (nucleotide) yield allows better RNA primer extension implying more RNA (line 44-47), and (ii) nucleoside phosphorylation is a condensation reaction and the concentrations used in

these experimental studies are the initial concentrations often prior to dehydration leading to further solute concentration (line 48).

- I am curious to know what the rate of carbon burial is overall in these systems. Does it just correlate with nitrogen fixation rates? This also comes back to the point of trying to figure out if biology, despite being slow in LCL, still dominates the P sink or not, which seems relevant. If biology is only a minor P sink in this system (relatively speaking) then presumably removing biology would have not so much of an impact when revising P concentrations for a prebiotic setting. If biology still dominates the minor P sink, then geology is hugely inefficient and P levels will necessarily be much higher without any life.

Response: As mentioned above in response to a similar comment, the question on the size of the biological P sink in LCL will be quantitatively addressed in a future paper with the goal to estimate how much higher P levels could be in an analog prebiotic setting. Careful analyses and budgeting of P fluxes are necessary for this, necessitating a separate manuscript. However, there is an aspect to this interesting question that does not considerably exceed the scope of the present manuscript, and that is an attempt at comparing the biological (photoautotrophic) productivity of GL and LCL. Rates of carbon burial in these systems are unknown, but our chlorophyll a data can be used as a proxy for water-column based photo-autotrophy. We now discuss this more explicitly in the Supplementary Text "Seasonal variations in chlorophyll a".

Reviewer #2 (Remarks to the Author):

The manuscript titled "Biogeochemical explanations for the world's most phosphate-rich lake, an origin-of-life analog" presented research about rich dissolve phosphate salt in soda lake and its connection with prebiotic phosphorylation in early Earth. In this research, the authors highlighted that nitrogen-limitation of biological production and high rich calcium carbonate (not calcium phosphate) precipitation may support high dissolve phosphate salt plausible in soda lake. This is an interesting topic. The manuscript is well written, but I find the structure confusing at certain points. I suggest the manuscript need do more expanding work before published.

Response: We thank the reviewer for the constructive feedback and address the individual points below. Overall, we improved the manuscript structure by inserting guiding sentences (e.g., line 244, line 337, line 350) and expanded upon the introduction with a paragraph on prebiotic phosphorylation (line 37-50).

First, the manuscript mentioned that biological N₂-fixation (BNF) plays a significant role to support calcium cation to form calcium carbonate sediment, not apatite sediment. It's a strong condition in nowadays Earth system. However, in Hadean Eon there is no biological N₂-fixation condition, hence how did those soda lake rich in dissolve P in the 1st Ga of Earth (without BNF at that time)? In another word, I believe the conditions of prebiotic phosphorylation in early

Earth is non-biological, and life biomolecules should arise from abiotic processes (from compound to organic), not BNF or other bio conditions.

Response: We are not suggesting that BNF plays any role in causing calcium carbonate formation in soda lakes nor at the origin of life (line 64). Instead, we analyze BNF as a key mechanism enabling a significant biological sink for phosphorus in modern-day aquatic systems (line 53). Identification of BNF and its modern role and rate is needed to understand phosphorus accumulation and -cycling in modern-day soda lakes and how they differ from ideal prebiotic analogs. If we understand the effect of BNF allowing phosphate use by biological production today, then we can make better inferences about likely higher levels of phosphate in a prebiotic analog soda lake. This is now discussed more clearly (line 64, line 91-94).

Then, the manuscript stated that dissolve phosphate salts in LCL and GL are mostly monovalent phosphate (Na^+ phosphate or K^+ phosphate). However, these phosphates are low reactive and thus this made prebiotic phosphorylation is thermodynamically unfavored. Hence, the manuscript required more evidence or details to explain how dissolve phosphate salts phosphorylation into organophosphate.

Response: While dissolved inorganic phosphate is somewhat unreactive, sodium phosphate is a demonstrated viable substrate for prebiotic phosphorylation either when the initially concentrated phosphate solution is further dried (Xu et al. 2019), which could occur in nature in further drying of an evaporative lake or part of the lake such as the shoreline or outlier pool, or in concentrated solution with other reagents (Powner et al., 2009*). We now explicitly name sodium phosphate as demonstrated viable substrate in prebiotic phosphorylation of nucleosides (line 47). Although other possibilities for phosphorus have been proposed in the prebiotic chemistry literature, such as phosphide delivered from schreibersite in iron meteorites (Gull et al., 2017*), the average flux of such delivery is many orders of magnitude smaller than the continuous flux of phosphate from weathering. Specifically, the early Earth meteorite flux is $\sim 10^6$ - 10^7 kg P/yr (Hess et al., 2021, Nat. Comm. 12, 1535), which averages $\sim 10^5$ - 10^6 times less than prehuman geologic weathering $\sim 10^{12}$ kg P/yr (Compton et al., 2000, Variations in the global phosphorus cycle. In: C. R. Glenn, et al., (Eds.), Marine Authigenesis: From Global to Microbial. SEPM Society for Sedimentary Geology, Tulsa, OK, 2000, pp. 21-33.). Nonetheless, we have added description of this alternative (or additional) phosphorus reagent (line 367).*

**please find detailed reference in the bibliography of the main manuscript*

I also suggest the authors do more literature review work for the prebiotic phosphorylation in the manuscript. Previous research investigated that phosphorylated molecules, were they present, must be a product between a phosphorus mineral and with an organic substrate (see Gull et al. 2017 life). Moreover, some researchers suggested that alternatives to phosphate at the onset of the origin of life, like phosphide (see Pasek 2008, PNAS), glyoxylate (Bean et al. 2006, OLEB) or arsenate (Wolfe-Simon et al. 2011, Science). These works may support the manuscript to be more reasonable.

Response: We agree that some more context about prebiotic phosphorylation in the introduction is helpful and thank the reviewer for this suggestion. A paragraph about prebiotic phosphorylation was added to lines 37-50. A possible role for phosphate alternatives in prebiotic phosphorylation are now mentioned in the discussion (line 365-371). However, we do not cite the paper by Wolfe-Simon et al. 2011 about arsenic replacing P in some microbial DNA because this paper is incorrect in its methods, interpretation, and conclusions and has been thoroughly refuted (see Reaves et al., 2012, Science, DOI: 10.1126/science.121986, Erb et al., 2012, Science, DOI: 10.1126/science.121845).

Decision letter and referee reports: second round

23rd Nov 23

Dear Dr Haas,

Your manuscript titled "Biogeochemical explanations for the world's most phosphate-rich lake, an origin-of-life analog" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

Please note that it may still be possible for your paper to be published before the end of 2023, but in order to do this we will need you to address these points as quickly as possible so that we can move forward with your paper.

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Decision letter and referee reports: second round

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Mojtaba Fakhraee, PhD
Editorial Board Member
Communications Earth & Environment

Clare Davis, PhD
Senior Editor
Communications Earth & Environment

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

[No further comments for the author]

Reviewer #2 (Remarks to the Author):

The revision manuscript is clearly written and overall, the quality of the study is high. I believe this paper is qualified to be published

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The revision manuscript is clearly written and overall, the quality of the study is high. I believe this paper is qualified to be published

AUTHOR'S RESPONSE TO THE REVIEWER COMMENTS:

We would like to thank the editor and the reviewers for their constructive feedback, which helped to improve this manuscript!