

The perennially ice-covered Lake Enigma, Antarctica supports unique microbial communities

Corresponding Author: Dr Michail Yakimov

This file contains all editorial decision letters in order by version, followed by all author rebuttals in order by version.

Version 0:

Decision Letter:

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Dear Dr Yakimov,

Your manuscript titled "Lake Enigma, a microbially unique perennially ice-covered Antarctic lake" 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. Specifically, please discuss your findings more comprehensively in the context of the existing literature and deepen your analysis in line with the suggestions from the reviewers, to maximise the impact of your paper.

Please highlight all changes in the manuscript text file.

Please submit your point-by-point responses as a separate file, distinct from your cover letter where you can add responses to the Editors' comments that you do not want to be made available to the reviewers. Word files are preferred.

Important: The response to reviewers must not include any figures, tables or graphs. If you wish to respond to the reviewer reports with additional data in one of these formats, please add them to the main article or Supplementary Information, and refer to them in the rebuttal. Due to current technical limitations, any figures, tables, or graphs embedded in your rebuttal will not be included in the peer review file, if published.

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,

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Please refer to our data policies at

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REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

The study describes the physical, chemical and 16S rRNA gene microbial diversity of Lake Engima. The characterization of the lake is interesting and important to understand the variety of lakes in Antarctic and type of life supported by the lake. The

presentation of the results could have been of higher standard. The discussion picks up many topics but only covers them at a superficial level.

The study could have explained objectives and hypothesis in more detail.

The findings are compared with studies on other ice-covered lakes but conclusions based on comparison with other lakes are overstated in some cases and contain some inaccuracies.

Most recent publications on microbial diversity and ecology in the water column and benthic microbial mats from Antarctic ice-covered lakes are missing from the introduction and discussion of the new findings from Lake Engima. It therefore requires more detail to demonstrate that the novelty of the microbiology communities.

Introduction

The introduction is very general and lacks overview of current understanding of microbial diversity and ecology in ice-covered lakes in Antarctica in particular McMurdo Dry Valleys and Dronning Maud Land.

Line 65. The MDV have many more meltwater ponds. Please revised section according to current literature

Lines 68-71: The references are limited to geochemical studies and not microbiology. The microbiology is linked to physical and chemical gradients and not phenology. Please revisit literature and revise according to studies with focus on microbial diversity and ecology

Results and Discussion

Lines 152-168: Detailed description of drilling of holes can be transferred to supplement.

Line 173. Please revisit literature. Lake Engima is not one of the deepest lakes, e.g. Lake Joyce is 35 m and Lake Vanda is more than 60 m

Lines 270-273. Please verify references. The provided references are for fossils in Australia and not Antarctica microbial mat comparison with fossil structures. It would require macroscopic study of the microbial mats in order to allow conclusion that they are analogs to the fossils found in Australia.

Reference 63 focuses on 16S rRNA gene meta analysis and not survey of cyanobacteria in water column.

The quality of the figures could be better.

Please use English punctuation for numbers e.g. 1.2 not 1,2

Figure 1A: Please add a map of Antarctica to show location, and add scale bar.

Figure 1B: It would be easier to show the different measurements in separate graphs

Table 6: The low number of ASV is quite striking.

Figure 10 in supplement: Was chloroplast data used to assign the algae? The chloroplast 16S rRNA gene is not a good marker for algae, and should be at least indicated in the figure legends, or considered to remove it. Relative abundance data from bacteria and algae is not comparable due to multicellular morphology of algae versus mostly unicellular bacteria.

Reviewer #2 (Remarks to the Author):

Smedile describe a perennial ice covered lake in Antarctica. The research uniquely combines spectroscopy, mineralogy, geochemistry, and microbiology to describe this unique ecosystem. The authors describe a stratified lake with distinct assemblages of communities in the overlying ice, upper and lower water columns, and benthic microbial mats. The mats form interesting morphological structures and while the communities are not particularly diverse or interesting, the authors noted an interesting presence of CPR bacteria that are likely to be ultrasmall. Based on previous characterization of CPR lineages, the authors point out their potentially interesting contribution to lake ecosystem function.

Overall, I enjoyed reading this paper. The presentation was clean and clear and the writing was organized. My only major comment would be that metagenomic sequencing and analyses of the CPR would have been very informative and would have strengthened the conclusions of the paper. If DNA remains, I would strongly suggest doing at least this to remove any concerns about these CPR (a very large and diverse groups) are similar to other characterized CPR genomically/metabolically.

I have only a few minor comments that need to be corrected before I would recommend the paper for publication.

Line 39: Remnant seems like the wrong word choice here and seems to imply that these cells are not doing anything, etc. if the authors truly think that the lake is this isolated as to not incorporate new cells from melting ice, etc., then I would think that the relatedness of those cells relative to cells from other modern environments would be more distinct.

Line 42: The ultrasmall bacteria seems implied from phylogenetic relatedness when presented in this context. Yet, you have data to indicate that this is not true.

Line 43: The average reader will not understand why Patescibacteria (CPR) point to an overlooked complexity of the food webs in these systems. In fact, I don't know that I agree that their presence does... Also, I wonder why the authors decided to not point out the large cyanobacterial mat structures in the abstract – this would be of interest to the Comms Earth and Environ readership.

Line 44: The authors do not really have evidence to suggest symbiotic or predatory strategies exist in the populations in the lake.

Line 59: What is near zero?

Lines 76-77: Odd sentence structure

Line 79: hover

Line 311 and elsewhere: 16S rRNA gene ...

Line 321: ASV

Line 373: concerned

Lines 419-423: I think it is unnecessary to assume that these are nitrogen fixing or nitrite oxidizing/reducing. If you absolutely must keep these in, then at least qualify by stating they are putatively involved in these processes.

Line 422-423: It would be odd for any ecosystem to not cycle nitrogen

Line 436: You have not yet shown they are tiny bacteria, would reserve this until you show your differential filtration/sequencing data or at least better qualify.

Congratulations on an interesting study

Eric Boyd

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Version 1:

Decision Letter:

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Dear Dr Yakimov,

Your manuscript titled "Lake Enigma, a microbially unique perennially ice-covered Antarctic lake" 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.

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.

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We have made some requests for your consideration while revising the manuscript. You may find them in the Editorial Request Table, highlighted in yellow.

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

Best regards,

Somaparna Ghosh, PhD
Associate Editor,
Communications Earth & Environment

REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

The authors have sufficiently addressed all of my previously raised concerns.

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Responses to the Reviewer #1.

First of all, we would like to thank Reviewer #1 for helpful criticism and suggestions. Taking them into account and adding and changing some parts, the manuscript, in our opinion, has been significantly improved.

The study describes the physical, chemical and 16S rRNA gene microbial diversity of Lake Enigma. The characterization of the lake is interesting and important to understand the variety of lakes in Antarctic and type of life supported by the lake. The presentation of the results could have been of higher standard. The discussion picks up many topics but only covers them at a superficial level.

The study could have explained objectives and hypothesis in more detail.

The findings are compared with studies on other ice-covered lakes but conclusions based on comparison with other lakes are overstated in some cases and contain some inaccuracies.

Most recent publications on microbial diversity and ecology in the water column and benthic microbial mats from Antarctic ice-covered lakes are missing from the introduction and discussion of the new findings from Lake Enigma. It therefore requires more detail to demonstrate that the novelty of the microbiology communities.

Following these critical observations, we have added 19 updated references and revised the Results / Discussion and Conclusions sections to highlight the obvious novelties we have discovered while studying the microbiota of Lake Enigma (see our detailed responses below). Among the most important are: (i) the presence of huge and complex microbial mats in Lake Enigma, reminiscent of a tree canopy (as seen in one of the underwater movies), never observed anywhere else before; (ii) the absence of planktonic forms of cyanobacteria in the water column of Lake Enigma; and finally, (iii) the presence and even abundance of ultra-small bacteria belonging to the superphylum *Patescibacteria*. Such organisms were found in Lake Enigma but not in MDV lake waters, further evidence of the novelty of the Lake Enigma ecosystem and the apparent absence of ultra-small bacteria in the MDV lakes.

Introduction

The introduction is very general and lacks overview of current understanding of microbial diversity and ecology in ice-covered lakes in Antarctica in particular McMurdo Dry Valleys and Dronning Maud Land.

As we mentioned above, we have added 19 updated references that provide an overview of current knowledge of the microbial diversity and ecology of ice-covered lakes in Antarctica particularly, in McMurdo Dry Valleys and Dronning Maud Land.

Line 65. The MDV have many more meltwater ponds. Please revised section according to current literature.

We have revised this section and without, repeating ourself, provide a detailed response below.

Lines 68-71: The references are limited to geochemical studies and not microbiology. The microbiology is linked to physical and chemical gradients and not phenology. Please revisit literature and revise according to studies with focus on microbial diversity and ecology

We have added a clear statement on the Objectives of our study (starting on Line 215). There are many specialized papers that could be cited as to the microbiology of Antarctic lakes although several of these are now dated, and many did not employ the molecular tools deployed in the study of Lake Enigma. Nevertheless, several fairly recent MDV lake diversity papers are now cited, but the list is not exhaustive. Rather than lakes from other areas of Antarctica, we focused on comparing Lake Enigma with MDV lakes. Hence, the molecular survey of these lakes by Kwon et al. is probably the best comparison to our work in Lake Enigma.

Results and Discussion

Lines 152-168: Detailed description of drilling of holes can be transferred to supplement.

We feel that the text mentioned here by the reviewer reads as results rather than methods. For example, there is no description here of the equipment used, the drilling methodology, limnological equipment and measurements, microscopy, etc. All of these are described in the Materials and Methods. The text in question describes what was *observed* during drilling, not the drilling mechanics *per se*. We feel these are results and thus no changes were made here.

Line 173. Please revisit literature. Lake Engima is not one of the deepest lakes, e.g. Lake Joyce is 35 m and Lake Vanda is more than 60 m

The reviewer is correct and this is now clearly stated in line 240 of the revised ms.

Lines 270-273. Please verify references. The provided references are for fossils in Australia and not Antarctica microbial mat comparison with fossil structures. It would require macroscopic study of the microbial mats in order to allow conclusion that they are analogs to the fossils found in Australia.

The text has been revised here to simply state that the benthic microbial mats in Lake Enigma are reminiscent of microbial assemblages from thermal regions and shallow marine basins that are widespread on Earth today; we make no specific mention of Australia in the text. Microbial mats have been proposed by many scientists as models of early Earth's biosphere.

Reference 63 focuses on 16S rRNA gene meta-analysis and not survey of cyanobacteria in water column.

The reference 63 was replaced with this one: Singh & Elster (2007). *Cyanobacteria in Antarctic lake environments: a mini-review*.

The quality of the figures could be better.

We are not quite sure what to make of this comment. All figures in the main text of the article were submitted separately at the required resolution (300 dpi). Supplementary materials were submitted in Word and pdf formats. Which figures in particular need improvement? Could the problem be that the journal has reduced the size of the figures too much?

Please use English punctuation for numbers e.g. 1.2 not 1,2

We corrected these errors in punctuations in Supplementary Table 7 and 9.

Figure 1A: Please add a map of Antarctica to show location, and add scale bar.

We have added a map of Antarctica showing all the areas discussed, as requested.

Figure 1B: It would be easier to show the different measurements in separate graphs

We have modified Figure 1B, as requested.

Table 6: The low number of ASV is quite striking.

This finding can be explained by at least two reasons. First, it is clear that there is a generally low diversity of organisms able to thrive in the harsh environmental conditions of Lake Enigma. Second, we apply a species-genus threshold (<97% identity) to distinguish ASVs.

Figure 10 in supplement: Was chloroplast data used to assign the algae? The chloroplast 16S rRNA gene is not a good marker for algae, and should be at least indicated in the figure legends, or considered to remove it. Relative abundance data from bacteria and algae is not comparable due to multicellular morphology of algae versus mostly unicellular bacteria.

We changed the figure legend to "*Plot of relative abundance (%) of cyanobacteria and unicellular algae (assigned by their plastid 16S rRNA genes) found in the upper layers of microbial mats collected at different drilling sites.*" All of the assigned algae are unicellular organisms. Based on this fact, we felt it reasonable to assume that the relative abundance data for bacteria and algae may be at least somewhat comparable.

Responses to the Reviewer #2.

Smedile et al. describe a perennial ice-covered lake in Antarctica. The research uniquely combines spectroscopy, mineralogy, geochemistry, and microbiology to describe this unique ecosystem. The authors describe a stratified lake with distinct assemblages of communities in the overlying ice, upper and lower water columns, and benthic microbial mats. The mats form interesting morphological structures and while the communities are not particularly diverse or interesting, the authors noted an interesting presence of CPR bacteria that are likely to be ultrasmall. Based on previous characterization of CPR lineages, the authors point out their potentially interesting contribution to lake ecosystem function.

Overall, I enjoyed reading this paper. The presentation was clean and clear and the writing was organized. My only major comment would be that metagenomic sequencing and analyses of the CPR would have been very informative and would have strengthened the conclusions of the paper. If DNA remains, I would strongly suggest doing at least this to remove any concerns about these CPR (a very large and diverse groups) are similar to other characterized CPR genomically/metabolically.

We appreciate this positive validation of our work. As Reviewer #2 stated, the work is very comprehensive, combining spectroscopy, mineralogy, geochemistry, and microbiology. We also made underwater movies showing the presence of huge and complex microbial mats in Lake Enigma, resembling a tree canopy, which have never been observed anywhere else before. The manuscript as it stands already contains too much information, and adding metagenomic sequencing and analysis, we believe, would overwhelm an already very complex framework. Just to reassure Reviewer #2, we are currently running this analysis focusing on CPR bacteria, and we are also doing a virome analysis, both of which are very impressive, and we hope that the second part of the Enigma study will also be reviewed by Reviewer #2. At the very least, we will include Reviewer #2 in our list of desired reviewers.

I have only a few minor comments that need to be corrected before I would recommend the paper for publication.

Line 39: Remnant seems like the wrong word choice here and seems to imply that these cells are not doing anything, etc. if the authors truly think that the lake is this isolated as to not incorporate new cells from melting ice, etc., then I would think that the relatedness of those cells relative to cells from other modern environments would be more distinct.

The word “remnant” has been changed to “legacy”.

Line 42: The ultrasmall bacteria seems implied from phylogenetic relatedness when presented in this context. Yet, you have data to indicate that this is not true.

We are deeply sorry, but we do not quite understand what exactly the reviewer is asking here.

Line 43: The average reader will not understand why *Patescibacteria* (CPR) point to an overlooked complexity of the food webs in these systems. In fact, I don't know that I agree that their presence does... Also, I wonder why the authors decided to not point out the large cyanobacterial mat structures in the abstract – this would be of interest to the Comms Earth and Environ readership.

This section of the abstract has been modified to better reflect the microbial diversity present (and absent) from Lake Enigma. We have not included the description of giant microbial mats in Abstract simply for two reasons: (i) these formations were only observed at the bottom of the shallowest drilling point and (ii) explaining this would exceed the words limit. We have discussed this in the main text of manuscript, moreover, we expect readers to be more impressed taking by the underwater movies provided.

Line 44: The authors do not really have evidence to suggest symbiotic or predatory strategies exist in the populations in the lake.

The wording in the abstract has been changed to indicate that predatory and symbiotic lifestyles may exist. The reason this is brought up is that some *Patescibacteria* have been shown to have such a lifestyle. Just an example, a recent paper by the corresponding author describing the cultivation and physiological characterization of one of the CPR bacteria can be seen: Growing a Vampire: '*Candidatus Absconditicoccus praedator*'.

Line 59: What is near zero?

We have excluded this part of the Introduction as irrelevant to the actual messages we wish to convey to COMMS ENV readers.

Lines 76-77: Odd sentence structure

The paragraph beginning with “Limnologically studies of perennially ice-covered freshwater Antarctic lakes” has been extensively edited and the odd sentence structure fixed.

Line 79: hover

Changed to “hovering”

Line 311 and elsewhere: 16S rRNA gene ...

Line 321: ASV

Fixed to read “16S rRNA gene...”

The acronym ASVs is defined in the previous paragraph (Amplicon Sequence Variants).

Line 373: concerned

The first sentence of this paragraph has been rewritten and the word “concern/concerned” is no longer used.

Lines 419-423: I think it is unnecessary to assume that these are nitrogen fixing or nitrite oxidizing/reducing. If you absolutely must keep these in, then at least qualify by stating they are putatively involved in these processes.

The wording of “likely indicates” has been changed to “suggests” in order to soften our statement here according to the reviewer’s suggestion.

Line 422-423: It would be odd for any ecosystem to not cycle nitrogen

We agree with the Reviewer#2 and thus the wording has been changed to say that nitrogen cycling “occurs” rather than “likely indicates that nitrogen cycling is occurring”.

Line 436: You have not yet shown they are tiny bacteria, would reserve this until you show your differential filtration/sequencing data or at least better qualify.

***Patescibacteria* are now described in the previous paragraph as “ultrasmall bacteria” and so the reader will know this before the use of “tiny” in the lead sentence of the next paragraph.**

Congratulations on an interesting study

We appreciate the constructive comments of this reviewer.