nature portfolio

Peer Review File

Spatially consistent microbial biomass and future cellular carbon release from melting Northern Hemisphere glacier surfaces

Web links to the author's journal account have been redacted from the decision letters as indicated to maintain confidentiality

13th Jul 22

Dear Dr Stevens,

Your manuscript titled "The release of microbes from Earth's melting glacier surfaces" has now been seen by 3 reviewers, and I 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. In particular, we ask that you to give more details about the role of the microbial community for the overall glacier carbon budget and how representative these estimates are for other glaciers. Please also comment how this carbon export is related to other relevant environments and what might be the potential fate of this carbon source. Regarding the method you used, please give some more insights about the upscaling of your carbon. 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) and the completed checklist:
[link redacted]
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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.

We understand that due to the current global situation, the time required for revision may be longer than usual. We would appreciate it if you could keep us informed about an estimated timescale for resubmission, to facilitate our planning. Of course, if you are unable to estimate, we are happy to accommodate necessary extensions nevertheless.

Please do not hesitate to contact me 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,

Ilka Peeken, PhD
Editorial Board Member
Communications Earth & Environment
orcid.org/0000-0003-1531-1664

Clare Davis, PhD
Senior Editor
Communications Earth & Environment

EDITORIAL POLICIES AND FORMATTING

We ask that you ensure your manuscript complies with our editorial policies. Please ensure that the following formatting requirements are met, and any checklist relevant to your research is completed and uploaded as a Related Manuscript file type with the revised article.

Editorial Policy: Policy requirements

Furthermore, please align your manuscript with our format requirements, which are summarized on the following checklist:
Communications Earth & Environment formatting checklist

and also in our style and formatting guide Communications Earth & Environment formatting guide .

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All Communications Earth & Environment manuscripts must include a section titled "Data Availability" at the end of the Methods section or main text (if no Methods). More information on this policy, is available at http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf.

In particular, the Data availability statement should include:
- Unique identifiers (such as DOIs and hyperlinks for datasets in public repositories)
- Accession codes where appropriate
- If applicable, a statement regarding data available with restrictions

- If a dataset has a Digital Object Identifier (DOI) as its unique identifier, we strongly encourage including this in the Reference list and citing the dataset in the Data Availability Statement.

DATA SOURCES: All new data associated with the paper should be placed in a persistent repository where they can be freely and enduringly accessed. We recommend submitting the data to discipline-specific, community-recognized repositories, where possible and a list of recommended repositories is provided at http://www.nature.com/sdata/policies/repositories.

If a community resource is unavailable, data can be submitted to generalist repositories such as figshare or Dryad Digital Repository. Please provide a unique identifier for the data (for example a DOI or a permanent URL) in the data availability statement, if possible. If the repository does not provide identifiers, we encourage authors to supply the search terms that will return the data. For data that have been obtained from publically available sources, please provide a URL and the specific data product name in the data availability statement. Data with a DOI should be further cited in the methods reference section.

Please refer to our data policies at http://www.nature.com/authors/policies/availability.html.

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Summary:
Glaciers are highly sensitive biomes that are changing rapidly in response to climatic warming. Increasing meltwater generation going into the 21st century is likely to have dramatic consequences both for the distribution and function of in-situ glacial ecosystems and the downstream environments that receive the large quantities of meltwater. Along with nutrients, dissolved organic matter and sediment, glaciers also export nontrivial quantities of microbial cells, the values of which still poorly constrained in space and time, owing to difficulty of sampling/access, associated lack of data, and standardization of measurement. Here, the authors present a large dataset of microbial cell abundance and size, significantly expanding published datasets. Further, a method for cell counting using flow cytometry is detailed, which includes suggestions for standardization of this technique in the future. The authors demonstrate that microbial cell abundance is remarkably consistent across glacier surface environments (104 cells mL-1) and glaciated regions sampled. The export of microbial cells is expected to equate to ~0.65 M tonnes year-1 over the next 80 years.

General comments:
I enjoyed reading this manuscript. It is an interesting paper, is well written, and contains a large amount of valuable data on microbial abundance and size in a wide range of surface

environments on glaciers across the northern hemisphere. Clearly a lot of effort has gone into data collection and method development, so I commend the authors for their hard work, which looks to have paid off. Given the importance of this subject area in the context of environmental change and regional biogeochemical cycling, and the value of the core dataset as contextual information, I think it will be well cited and that Nat Comms Earth and Environment is a good home for it. I have no major suggestions and my only comments are minor and shouldn't take long to address. I am not an expert in flow cytometry and will therefore allow more qualified reviewers to comment on the main methods, beyond the basics that I understand.

Comment 1) It would be beneficial to provide some additional contextual information for the cell density and size distribution versus other environments (e.g., an additional table for this?). I imagine this would be fairly straightforward to compile and would help further frame the importance of these environments versus those more commonly studied.

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Comment 4) Some additional discussion of nutrient controls would be beneficial. This may be considered outside of the scope of the publication, but is interesting given the similarities between sites, yet presumably variable nutrient conditions (or not?). Some of this is already covered by discussion of particles, but this could be fleshed out a little more to help direct future efforts.

Comment 5) Did the authors carry any process or holding blanks? Is there any evidence of background contamination?

Specific comments:

L26: Also Holland et al. (2019) could be useful here

L87-89: So suspended sediment was measured as number of particles rather than e.g., weight?

L199-200: glaciers outside of the major ice sheets?

L214-215: sterile syringe? Or just well rinsed?

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References:

Holland, A.T., Williamson, C.J., Sgouridis, F., Tedstone, A.J., McCutcheon, J., Cook, J.M., Poniecka, E., Yallop, M.L., Tranter, M., Anesio, A.M., Group, T.B.& B., 2019. Dissolved organic nutrients dominate melting surface ice of the Dark Zone (Greenland Ice Sheet). Biogeosciences 16, 3283–3296. https://doi.org/10.5194/bg-16-3283-2019

Reviewer #2 (Remarks to the Author):

In the paper "The release of microbes from Earth's melting glacier surfaces" the authors measured cell abundance in supraglacial meltwater and the weathering crust (WC) from several Northern hemisphere glaciers using flow cytometry, correlate it with environmental parameters, and estimate cell flux from glacier surfaces in the next 80 years using three climate scenarios (RCPs). The research was carefully designed and performed, the methods used are appropriate and robust, and the results are interpreted appropriately; the paper is well written and easy to read and understand.

I have a couple of questions and comments I would like the authors to address.

First, no significant difference between WC and meltwater was found. This is an interesting and potentially very useful result as it would make all export estimates much easier. However, did the WC samples contain cryoconite or were cryoconite holes avoided during sampling? My concern would be that the main 'fractionation' in the cell mobilisation process might be occurring between cryoconite (where cell abundance can be high) and the surrounding WC ice – in such case the similarity in cell density between WC and meltwater would not be that surprising.

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Third, I assume only the supraglacial habitat is investigated here. If so using the term 'distributed and channelised glacial meltwaters' (line 192) is somewhat confusing as it (at least to me) implies subglacial drainage, which is also important in mobilising and exporting cells.

Minor comments

l. 109 and 111 please correct to 'phosphorus'

l. 109 consider deleting "feeding chemo- and heterotrophic processes' – assimilation of nutrients such as P is universal and not dependent on the trophic strategy

Reviewer #3 (Remarks to the Author):

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RE: Revised submission for Nature Communications Earth and Environment

Dear Reviewers,

We thank the editors and all three reviewers for taking the time to read and provide their kind and useful comments relating to this manuscript. To complement our resubmitted manuscript, please find a point-by-point response to the specific points raised by each reviewer below. Review comments are included in *italics*. In case of inconsistencies due to file formatting, please note that the line numbers herein refer to the "Tracked Changes" MS Word Document, not the PDF version of the manuscript.

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This is more of a question as I did not pick this up in the manuscript or methods. How do the authors deal with dead/live cells. Is this the specific staining used? Does it matter? Is it possible to comment on this in the context of flow cytometry?

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discriminatory stain SYBR Gold. Further detail has been added to the manuscript (lines 310-312) to clarify this point.

Regarding the second point made here, we consider cell viability in these environments to be an important research area, as this may provide an explanation for the upper limit on the cell concentrations observed both within the hydrological system of ice masses and the ice sheet surface. This is a project which is currently being worked on by several of the authors of this manuscript.

Some additional discussion of nutrient controls would be beneficial. This may be considered outside of the scope of the publication, but is interesting given the similarities between sites, yet presumably variable nutrient conditions (or not?). Some of this is already covered by discussion of particles, but this could be fleshed out a little more to help direct future efforts.

We agree with Reviewer 1 that it would be interesting to further investigate nutrient conditions and their influence (or not) on microbial abundance in surface meltwaters, and more widely, on surface ice. However, we are unable to do so within this manuscript as we simply do not have any nutrient data available. We highlight the need to undertake this work in lines 125-128 but wish to avoid any further speculation to avoid over-reaching our dataset; rather encourage readers with interest to follow the works cited in the manuscript.

Did the authors carry any process or holding blanks? Is there any evidence of background contamination?

Field blanks were not collected. Blanks (Ultrapure Water, unfixed and unstained) were run within the sample set, as described in the supplementary information (Figure S2). These blanks were used to identify any background contamination or instrumental drift; neither of which were observed.

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Thank you for highlighting the relevant addition of this citation. It has now been added to the manuscript.

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L199-200: glaciers outside of the major ice sheets?

Thank you for highlighting this missing clause, which has now been added to the manuscript.

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The syringe was pre-rinsed thrice with sample, and the sample storage tubes were sterile. This has been clarified in the manuscript.

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Thank you for highlighting this now corrected typographic error.

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We agree with Reviewer 2 that these results are interesting. None of our WC meltwater samples contained visible cryoconite material, and cryoconite holes were avoided during sampling as the aim of this study was to provide an enumeration of microbes within weathering crust waters and begin to elucidate their transport dynamics. We envisage that future work will look to consider the roles of cryoconite holes and the ice surface itself as potential microbial reservoirs – and agree that this is where fractionation and cell retention in the near-surface hydrological system may occur. However, simplistic numerical comparison suggests that weathering crust (and stream) meltwaters have equivalent cell concentrations to the water phase of cryoconite holes. This is unsurprising given that they are connected hydrologically but agree that considering their role in microbial retention (especially the particulate layer at the base of the holes) present an avenue for future work. To clarify this within the manuscript, a short discussion has been added to lines 71-78.

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This change has been made. Thank you for highlighting this correction, clarifying the manuscript.

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We further thank Reviewer 3 for these suggestions. However, our dataset, providing a geographically dispersed snapshot of microbial abundance in supraglacial meltwaters, unfortunately does not allow for examination of seasonal variability at a single glacier. However, we agree that this is a research priority, and it should be noted that authors involved in the preparation of this manuscript are undertaking work to establish seasonal trends in microbial abundance on glacier surfaces and in glacial meltwaters.

We discuss the limitations of not considering the Himalaya briefly in lines 372-376, outlining why out dataset is not suitable for extrapolation to the Himalayan, ow Latitude or Southern Hemisphere RGI regions. Within this dataset, we cannot consider the role of debris cover, as samples were collected from glaciers which are primarily debris “free” in contrast to the thick, spatially extensive debris mantles observed on Himalayan glaciers. We agree with Reviewer 3 that further work should indeed look to consider these processes; but this is beyond the scope of our work which considerers the ablating near-surface ice environment, rather than the microbial community of surface debris mantles.

3rd Oct 22

Dear Dr Stevens,

We have assessed your revised manuscript titled "The release of microbes from Earth's melting glacier surfaces" and your responses to the earlier reviewer comments. We are happy, in principle, to publish your manuscript 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 comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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

Best regards,

Ilka Peeken, PhD
Editorial Board Member
Communications Earth & Environment

Clare Davis, PhD
Senior Editor
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Season wise changes are to be high lighted , since they have linked to climate change as well , is it also linked to de glaciation or loss of glaciers and how they are correlated with other glaciers to be established, they have to high light the limitations whether is it applicable for Himalayan and other regions , what are the role of debris and non debris in the microbial diversity may be high lighted . Biogeochemical aspects may be highlighted

We further thank Reviewer 3 for these suggestions. However, our dataset, providing a geographically dispersed snapshot of microbial abundance in supraglacial meltwaters, unfortunately does not allow for examination of seasonal variability at a single glacier. However, we agree that this is a research priority, and it should be noted that authors involved in the preparation of this manuscript are undertaking work to establish seasonal trends in microbial abundance on glacier surfaces and in glacial meltwaters.

We discuss the limitations of not considering the Himalaya briefly in lines 372-376, outlining why out dataset is not suitable for extrapolation to the Himalayan, ow Latitude or Southern Hemisphere RGI regions. Within this dataset, we cannot consider the role of debris cover, as samples were collected from glaciers which are primarily debris “free” in contrast to the thick, spatially extensive debris mantles observed on Himalayan glaciers. We agree with Reviewer 3 that further work should indeed look to consider these processes; but this is beyond the scope of our work which considerers the ablating near-surface ice environment, rather than the microbial community of surface debris mantles.