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Peer Review File

Morphology shapes microbial ecosystems and carbon cycling within cryoconite holes on a Greenland outlet glacier

Corresponding Author: Professor Nozomu Takeuchi

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

Version 0:

Decision Letter:

** Please ensure you delete the link to your author home page in this e-mail if you wish to forward it to your coauthors **

Dear Professor Takeuchi,

Your manuscript titled "Morphological Control of Microbial Ecosystems and Carbon Cycling in Greenlandic Cryoconite Holes" has now been seen by 3 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.

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. We recommend that any figures, tables or graphs that are included in the response to reviewers are also included in the main article or Supplementary Information.

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:
Link Redacted
** This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage first **

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,

Jose Luis Iriarte Machuca, PhD
Editorial Board Member
Communications Earth & Environment

Somaparna Ghosh, PhD

Associate Editor - Communications Earth & Environment
Consulting Editor - Communications Sustainability

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In particular, the Data availability statement should include:
- Unique identifiers (such as DOIs and hyperlinks for datasets in public repositories)
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- 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.

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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):

Takeuchi et al. describe the microbial communities and biogeochemical parameters of an outlet glacier of the Greenland Ice Sheet, focusing on the shallow cryoconite holes found on the marginal rougher surface of the glacier with the deeper holes found on more stable ice near the glacier's midline. Their hypothesis is that the hole depth controls the microbial community present within the cryoconite. I found their argument compelling, not least as they tested the plausible alternatives (for example, mineralogical or ice facies driven differences) and the paper is the product of a large amount of carefully conducted work. I have no major recommendations for improving the paper as the work is competently performed and well presented. I would however encourage the authors to consider two papers before revisiting their discussion section and clarify/qualify

their interpretation a little:

1. Vonhamme et al, cited as reference 7. This is cited in passing, but closer inspection shows a comparable result in a very different context - microalgae associated with unstable holes. Differences in dispersal potential are inferred from the abundance of oscillatorales in more stable, distal holes. Perhaps similar phenomena are at play here?

2. Cook et al DOI:10.1111/1462-2920.13349. While reference 39 elaborates the concept of biocryomorphic evolution, this paper may have been missed by the authors and provides primary data detailing the mechanisms and adaptations underlying the concept. It is the product of studies inland on the Greenland Ice Sheet and it too documents the stable hole communities described here. For transparency's sake I will sign the review as I am an author on this paper.

Minor comments

L14: here I think midline would be clearer than centre?
L28: not all cryoconite holes contain stagnant meltwater. Their position within the perched aquifer of the weathering crust indicates percolative exchange of meltwater with the surrounding interstitial spaces of the ice surface. See your text ca. L46-49 and citation of ref 27 in support of this point.

Dr Arwyn Edwards
Aberystwyth University and UNIS, Svalbard

Reviewer #2 (Remarks to the Author):

This manuscript by Takeuchi et al. provides the first insight to the role of cryoconite hole morphology upon the microbial communities habituated within, and their biogeochemical condition. They present a suite of biogeochemical data collected from a transect on the margin of the southwestern Greenland Ice Sheet. They associate the composition of the phototrophic community within cryoconite holes with hole morphology by binning the 20 sampling locations on their transect into ‘deep’ and ‘shallow’ holes, which lie on ‘flat’ and ‘crevassed’ terrain respectively, with deep holes hosting cyanobacteria dominated phototrophic communities, and shallow holes hosting glacier ice algae dominated photoautotrophic communities.

This is a suitable approach to the analysis of the data, due to the large difference in glacier surface topography and the statistical separation of the two hole morphologies. Coupled with the geochemical data, the authors robustly demonstrate that the cryoconite holes in the flat area are more stable, and hence host a ‘typical’ cryoconite community, whilst those in the crevasse area are more transitory, primarily hosting glacier ice algae. Interestingly, the authors demonstrate that there is no significant difference in mineralogy between the two types of cryoconite holes, and that there are no significant differences in nutrient availability – and as such assert that neither of these two variables influences the phototrophic community composition.

However, there are some limitations to the study and the manuscript. Primarily, I believe the study would have benefited from an additional transect, potentially at another field site with differing surface topography, such as a marginal zone without extensive crevassing – the relationships identified herein may only be relevant to this field site. I also think the addition of non-cryoconite hole ice samples would have provided useful context, especially for the geochemical data. Moreover, there are sections of the manuscript where, I believe, arguments made ‘over-reach’ the data – but it should be noted that I do not think these prohibit publication of the study in its current form, rather that the arguments made require a degree of moderation. These points are as follows:

1. The authors state that “glacier surface topography primarily drives cryoconite hole development” (L18-19), but I do not think the authors have suitable evidence to support this claim. Whilst they observation an association between cryoconite hole morphology and glacier topography, they do not provide a proposed mechanism whereby the topography of the glacier surface controls hole morphology. Furthermore, they do not consider the potential role of other processes, such as distance from the glacier margin, variable SWR receipt of each surface (arguably a function of topography, i.e. slope and aspect, on this spatial scale), or the role of potential disturbances such as differential flow through the weathering crust. For example, the authors could consider modelling SWR receipt for each hole morphology with consideration of surface slope, to assess if this is a potential driver behind the differing hole morphologies. They could also present broad slope and aspect data for each site, perhaps by using ArcticDEM. However, the authors are limited by only having a sample set from a single location, and I do agree that, for this glacier, the two distinct cryoconite hole morphologies are associated with differing glacier surface topographies. Whilst there is an association with topography, I don’t think the manuscript addresses whether the hole morphology controls the community within, or whether the community controls the hole morphology (see Langford 2010, ref 10, who argue that cryoconite holes form in part because cyanobacteria mechanically bind particles and dark humic substances).

2. In line 151, the authors assert that the presence of different cyanobacterial OTUs and ASVs contained within in different holes “…demonstrates that cryoconite hole dimensions influenced bacterial lineage composition…”. Whilst several discussion points are made to expand this argument, none are conclusive enough, and rather serve to highlight the need for further understanding (e.g. L228). Differing hole morphologies are associated with different OTUs and ASVs, but there is no identification of a process or an expansion of the argument to assert how the hole morphology “influences” the different community composition at the OTU and ASV level. The finding itself is very interesting, but this argument reaches a little beyond the data.

3. In the final section, which acts as a de-facto conclusion, the authors argue that future glacier change could increase crevassing and reduce flat surface topography, consequently changing the photoautotrophic community on a glacier-wide scale. As per point 1, I think this argument needs more support.

Overall, the manuscript is well written and presents significant advances in our understanding of cryoconite holes; I thoroughly enjoyed reading it. Key findings are typically well argued and supported by a suite of robust and appropriate data. The figures are generally well presented, and the relevant literature is cited to place the study into suitable context. Furthermore, the authors highlight several opportunities for further work, which I believe are suitable suggestions based upon the findings herein. I would, however, suggest the following minor revisions:

-The title of the manuscript implies that the work represents cryoconite holes across the Greenland Ice Sheet, rather than in one location on an outlet glacier of the Greenland Ice Sheet. I think this should be moderated somewhat, to be more site-specific.
-The sampling interval along the transect is not stated – it would be nice to include this information. If the sampling interval along the transect is not consistent, then it would be beneficial if the x-axis of each plot could be adjusted to reflect this. Equally, I would prefer to see points not connected with lines in the plots.
-It is unclear how many holes were measured at each site along the transect (L315) – was it 20 at each location, or 20 in total? Assuming it was >1 per site, it would be nice to see some of the uncertainties or some information on how the samples/data were pooled.

-L28: “stagnant” is not the correct term to describe cryoconite hole water, which is referred to as flowing (i.e. through the weathering crust, which such holes punctuate) elsewhere in the manuscript. Please change, or simply remove, this word.
-L49 and 50: The latter part of this claim is not supported by the cited reference (i.e. that density and spatial arrangement of cryoconite holes can affect…patterns of glacier discharge). If this refers to ice discharge, then the latter claim should be removed. If it is referring to meltwater discharge – which is supported by the citation – then the sentence requires clarification.
-L58: Please expand on ‘specific weather conditions’ to enhance readability for non-cryoconite-experts.
-L80, Fig 1. Typographic – “Crevass Zone”.
-L87-94, 161-161: Please state the stats tests used and the number of data for each statistical analysis.
-L96, 105, 309, 311: Issunguata Sermia Glacier is a tautology, given that ‘sermia’ crudely translates to ‘glacier’ in English. Please change this to Issunguata Sermia, but please note that I appreciate the efforts of the authors for usage of local name.
-L264: citation needed to support “nutrient limitation influences phototrophic communities”.
-L346: Please specify the nature of “blank controls without samples”. I assume deionised/ultrapure water was used in place of sample material?
-L389: “20 mg of the samples was” is unclear and grammatically incorrect (samples were/sample was). Please clarify/correct this sentence.

Please note that typographic suggestions are not comprehensive, rather they are the errors I spotted in reviewing the manuscript.

Reviewer #3 (Remarks to the Author):

Manuscript#: COMMSENV-25-3128

Corresponding Author: Nozomu Takeuchi

Title: Morphological Control of Microbial Ecosystems and Carbon Cycling in Greenlandic Cryoconite Holes

Cryoconite holes on glaciers have been recognized as microbial hotspots in a harsh environment which undergo seasonal variations due to changes in light and nutrient availability.
The manuscript submitted by Takeuchi et al. is tackling in issue which is often at discussion, sometimes also heavily speculated but has never been seriously investigated. Nowadays, with this rapid dynamics of glacial surfaces and landscapes, it is useful to learn more about the fate of microbial composition structure and also nutrient cycling since it is crucial for downstream habitats.
Despite being quite logical that cryoconite holes with shallower depth provide better light conditions for phototrophic organisms there was a still a lack of a solid study. Hence, this submitted manuscript would deserve a publication, however, I would suggest some minor changes to clarify some issues beforehand.

I find the manuscript well-written in a clear language with a clear structure. The background of the authors is clearly given by numerous publications being game-changing in the emerging field of cryoconite ecology.
The concept of their study is comprehensible where they select cryoconite holes at 20 sites along a horizontal transect from the ice margin to the center. They also clearly distinguish these zones as flat zones versus crevasse/marginal zone and they select similar cryoconite types for their study.
It is clear that it might be very tricky to collect samples from randomized cryoconite holes in size, diameter, position, size, etc. Here, they clearly announce that they excluded larger cryoconite holes to avoid too much bias. They also clearly focused on stagnant and stable cryoconite holes.
By focusing exclusively on stagnant/stable cryoconite holes I would recommend also to mention this more clearly in the title.

It could be a bit misleading since those two types clearly show distinct morphologies, community composition and dynamics. Additionally, here I find it very difficult to argument out of the following reasons: the sample collection took place during 2 days in summer during ablation phase where all holes have been stagnant. It cannot be guaranteed that the same stable hole is not subject to a meltwater dynamics the next day? This could be elaborated a bit more in the text.

Additionally, the results / diving factors could be demonstrated a bit better with statistics.

All in all I find the manuscript convincing, timely, well-written and backed up with the relevant literature.

Additional observations and recommendations:
Figure 2: It is very hard to see in the picture if one hole is deeper than the other. I find the panel c and d obsolete due to the lack of optical difference.
Figure 6: there is no indication of the procedures of why just uneven sampling spots are shown
Line 292: The authors state that the presence of crevasses clearly shapes the holes – it goes (to my understanding) also hand in hand with the slope of the terrain. It would be helpful also to take the slope into account. Has this parameter been measured in the field?
Line 284: the authors speak about the relatively small study area. Albeit it is roughly shown in Figure 1 it would be helpful explain the area and/or the distance between the sampled 20 cryoconite holes.
Line 316: “holes with irregular shapes have been excluded from this study”: please explain why (would they not fulfil the “random” or general criteria if they show stagnant melt water?
Line 318: Have the samples been collected in random plastic bottles or were the containers sterile? Would be helpful for DNA analysis…
Line 355: please indicate the pore size of the filters
Line 356: please indicate the type of epifluorescence microscope
Line 353: Which PCR products have been pooled? Please indicate
Line 475 and line 484: please correct the abbreviation of the Journal Arc. Antarc. Alp. Res. into Arct. Antarct. Alp. Res.
Line 554: odd writing of author MacAYEAL

Version 1:

Decision Letter:

<*** REMEMBER TO ATTACH REVISIONS CHECKLIST (WORD)***>

Dear Professor Takeuchi,

Your manuscript titled "Morphological Control of Microbial Ecosystems and Carbon Cycling in Cryoconite Holes on an outlet glacier of the Greenland Ice Sheet" 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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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table".

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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
Consulting Editor,
Communications Sustainability

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have revised the manuscript in line with the recommendations from my previous review and provide careful justifications in their response letter. I think this paper will make an interesting addition to the literature and have nothing further to raise to improve the manuscript.

Reviewer #2 (Remarks to the Author):

I thank the authors for making the changes and corrections they describe. I look forward to the publication of the manuscript.

Reviewer #3 (Remarks to the Author):

Dear Authors,

I have read the revised version with great interest and noticed a substantial improvement of the manuscript. Hence, I do not see any pending issues with this article.

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Sep-26 2025

Takeuchi et al.

Morphological Control of Microbial Ecosystems and Carbon Cycling in Greenlandic Cryoconite Holes

Dear Editors:

We greatly appreciate the constructive comments on our manuscript from the three reviewers. We carefully considered each comment of the reviewers and have revised the manuscript. Our responses to each comment are as follows:

Reviewer #1 (Remarks to the Author):

1. Vonhamme et al, cited as reference 7. This is cited in passing, but closer inspection shows a comparable result in a very different context - microalgae associated with unstable holes. Differences in dispersal potential are inferred from the abundance of oscillatorales in more stable, distal holes. Perhaps similar phenomena are at play here?

We thank the reviewer for the insightful comment. We agree that the hole stability and dispersal potential are also potential factors in the community structure in the holes. We have cited Vohamme et al (2016) in the first section of the discussion (L238-239) to support the dominance of glacier algae in unstable holes.

2. Cook et al DOI:10.1111/1462-2920.13349. While reference 39 elaborates the concept of biocryomorphic evolution, this paper may have been missed by the authors and provides primary data detailing the mechanisms and adaptations underlying the concept. It is the product of studies inland on the Greenland Ice Sheet and it too documents the stable hole communities described here. For transparency's sake I will sign the review as I am an author on this paper.

We would think that the biocryomorphic equilibrium state, suggested by Cook et al (2016) is represented as a state of the deep holes described in this study. We have revised the text to clarify this idea in the last paragraph of the discussion section (L305-308).

Minor comments

L14: here I think midline would be clearer than centre?

The “centre” has been replaced to “midline”. (L15)

L28: not all cryoconite holes contain stagnant meltwater. Their position within the perched aquifer of the weathering crust indicates percolative exchange of meltwater with the surrounding interstitial spaces of the ice surface. See your text ca. L46-49 and citation of ref 27 in support of this point.

The word “stagnant” has been removed. (L29)

Reviewer #2 (Remarks to the Author):

However, there are some limitations to the study and the manuscript. Primarily, I believe the study would have benefited from an additional transect, potentially at another field site with differing surface topography, such as a marginal zone without extensive crevassing – the relationships identified herein may only be relevant to this field site. I also think the addition of non-cryoconite hole ice samples would have provided useful context, especially for the geochemical data. Moreover, there are sections of the manuscript where, I believe, arguments made 'over-reach' the data – but it should be noted that I do not think these prohibit publication of the study in its current form, rather that the arguments made require a degree of moderation.

We appreciate the insightful comments and agree that the additional transect studies and non-hole samples could strengthen the claims of our study. However, as we investigated one of the typical outlet glaciers of the Greenland Ice Sheet, we believe that this study could provide a useful context for the ice sheet ecosystem.

1. The authors state that "glacier surface topography primarily drives cryoconite hole development" (L18-19), but I do not think the authors have suitable evidence to support this claim. Whilst they observation an association between cryoconite hole morphology and glacier topography, they do not provide a proposed mechanism whereby the topography of the glacier surface controls hole morphology. Furthermore, they do not consider the potential role of other processes, such as distance from the glacier margin, variable SWR receipt of each surface (arguably a function of topography, i.e. slope and aspect, on this spatial scale), or the role of potential disturbances such as differential flow through the weathering crust. For example, the authors could consider modelling SWR receipt for each hole morphology with consideration of surface slope, to assess if this is a potential driver behind the differing hole morphologies.

They could also present broad slope and aspect data for each site, perhaps by using ArcticDEM. However, the authors are limited by only having a sample set from a single location, and I do agree that, for this glacier, the two distinct cryoconite hole morphologies are associated with differing glacier surface topographies. Whilst there is an association with topography, I don't think the manuscript addresses whether the hole morphology controls the community within, or whether the community controls the hole morphology (see Langford 2010, ref 10, who argue that cryoconite holes form in part because cyanobacteria mechanically bind particles and dark humic substances).

We agree that there is still uncertainty on the mechanisms of the association between the hole morphology and glacier topography, and that further studies are needed to fully elucidate the relationship between them. However, the possible mechanisms of the presence of shallow holes in the crevasse area are increases of radiatively driven surface ablation due to surface roughness and/or alternation of the light exposure due to irregular surface slopes, which have already been explained in the second paragraph of the last sub-section. We have revised the text to clarify the explanation. (L299-300)

The distance from the glacier margin and the presence of the weathering crust may partially affect the hole depth, but they are unlikely to explain the two distinct depths of holes along the transect.
Regarding the slope and aspect of the surface, the total elevation difference across the 4 km transect line was only 95 m, suggesting that variable SWR receipt due to the difference in surface slope had a negligible impact. Although the surface near the margin (S1 – S3) seems to be slightly steeper than other sites, it cannot explain the hole depth distribution. We have added the elevation data at each site in the supplementary table S1
Langford et al (2010) certainly argued the role of the cyanobacteria and EPS to form "cryoconite granules", but did not exactly argue their role in forming "cryoconite holes". As far as we know, there has been no published paper showing that the microbial community controls the hole morphology, although it is an interesting topic.
We have additionally discussed these possible factors in the first paragraph of the last sub-section of the discussion. (L286-292)

2. In line 151, the authors assert that the presence of different cyanobacterial OTUs and ASVs contained within in different holes "…demonstrates that cryoconite hole dimensions influenced bacterial lineage composition…". Whilst several discussion points are made to expand this argument, none are conclusive enough, and rather serve to highlight the need for further understanding (e.g. L228). Differing hole morphologies are associated with different OTUs and ASVs, but there is no identification of a process or an expansion of the argument to assert how the hole morphology "influences" the different community composition at the OTU and ASV level. The finding itself is very interesting, but this argument reaches a little beyond the data.
We agree that the mechanisms by which hole morphology affects OTU/ASV-level composition remain unclear. As discussed in the first subsection of the Discussion, possible explanations include differences in light exposure at the hole bottom, microbial inputs from adjacent ice surface, and the relative hydrologic stability of holes. Because our dataset cannot distinguish among these, we have revised L240-242 to state that the observed patterns suggest an association between hole dimensions and lineage composition, while noting that the underlying processes require further study.

3. In the final section, which acts as a de-facto conclusion, the authors argue that future glacier change could increase crevassing and reduce flat surface topography, consequently changing the photoautotrophic community on a glacier-wide scale. As per point 1, I think this argument needs more support.
We have cited an additional supporting paper (Chuldley et al., 2025), showing the recent ice-sheet-wide increase of crevassing (L311-315). The study suggests that the increases in crevasse extent result from a steeping ablation area and outlet glacier acceleration, and also from an increase in meltwater available for hydrofracture. Both of them are likely to continue in the future.

Minor revisions:

-The title of the manuscript implies that the work represents cryoconite holes across the Greenland Ice Sheet, rather than in one location on an outlet glacier of the Greenland Ice Sheet. I think this should be moderated somewhat, to be more site-specific.
The title has been revised to be more site-specific as suggested.

-The sampling interval along the transect is not stated – it would be nice to include this information. If the sampling interval along the transect is not consistent, then it would be beneficial if the x-axis of each plot could be adjusted to reflect this. Equally, I would prefer to see points not connected with lines in the plots.
The sampling interval was almost constant, 200±5 m, which has been mentioned in the method section (L324-325), so that we don't think that the x-axis needs to be adjusted. The points in the Figure 1 have been revised to be separated on the map (L81).

-It is unclear how many holes were measured at each site along the transect (L315) – was it 20 at each location, or 20 in total? Assuming it was >1 per site, it would be nice to see some of the uncertainties or some information on how the samples/data were pooled.
The sentence in the method section has been revised to clarify the number of holes measured at each location (L323-324). The sample numbers and data have been added as Supplementary Table S1.

-L28: "stagnant" is not the correct term to describe cryoconite hole water, which is referred to as flowing (i.e. through the weathering crust, which such holes punctuate) elsewhere in the manuscript. Please change, or simply remove, this word.
The word "stagnant" has been removed. (L29)

-L49 and 50: The latter part of this claim is not supported by the cited reference (i.e. that density and spatial arrangement of cryoconite holes can affect…patterns of glacier discharge). If this refers to ice discharge, then the latter claim should be removed. If it is referring to meltwater discharge – which is supported by the citation – then the sentence requires clarification.
This is referring to meltwater discharge. The text has been revised to clarify it. (L51-52)

-L58: Please expand on 'specific weather conditions' to enhance readability for non-cryoconite-experts.
We have already explained the weather conditions in the next line (L60), "warm, windy, and/or cloudy conditions", which are probably sufficient in the context.

-L80, Fig 1. Typographic – "Crevass Zone".
The typo has been corrected. (L81)

-L87-94, 161-161: Please state the stats tests used and the number of data for each statistical analysis.
We have added the text about stat in the method section (L229-230, 411-412, 420-421), and a table showing the number of data in Supplementary Table S1.

-L96, 105, 309, 311: Issunguata Sermia Glacier is a tautology, given that 'sermia' crudely translates to 'glacier' in English. Please change this to Issunguata Sermia, but please note that I appreciate the efforts of the authors for usage of local name.
We have removed "Glacier" from the "Issunguata Sermia Glacier" throughout the text. (L82 and others)

-L264: citation needed to support "nutrient limitation influences phototrophic communities".
We have cited Vonhamme et al (2016) in the text. (L267)

-L346: Please specify the nature of "blank controls without samples". I assume deionised/ultrapure water was used in place of sample material?
We have added the specific explanation about the blank controls. (L360-361)

-L389: "20 mg of the samples was" is unclear and grammatically incorrect (samples were/sample was). Please clarify/correct this sentence.
Corrected. (L404)

Please note that typographic suggestions are not comprehensive, rather they are the errors I spotted in reviewing the manuscript.
We have corrected all typographical errors we could find.

Reviewer #3 (Remarks to the Author):

By focusing exclusively on stagnant/stable cryoconite holes I would recommend also to mention this more clearly in the title. It could be a bit misleading since those two types clearly show distinct morphologies, community composition and dynamics. Additionally, here I find it very difficult to argument out of the following reasons: the sample collection took place during 2 days in summer during ablation phase where all holes have been stagnant. It cannot be guaranteed that the same stable hole is not subject to a meltwater dynamics the next day? This could be elaborated a bit more in the text.
As claimed by the reviewers #1 and #2, meltwater in cryoconite holes is unlikely to be stagnant. Nevertheless, the consistent weather conditions during the two-day sampling period (sunny and light wind) allowed us to assume that the hole conditions—including meltwater dynamics and geochemistry—were in a steady state. We have mentioned the consistent weather conditions during the fieldwork in the methods section. (L322-324)

Additionally, the results / diving factors could be demonstrated a bit better with statistics.
As we have added more data about the cryoconite holes in supplementary table S1, and descriptions of the statistics in the method. (L329-330)

Additional observations and recommendations:
Figure 2: It is very hard to see in the picture if one hole is deeper than the other. I find the panel c and d obsolete due to the lack of optical difference.
We have noted in the caption that the photos highlight the visual similarity between the shallow and deep holes, despite their distinct depth measurements. (L99)

Figure 6: there is no indication of the procedures of why just uneven sampling spots are shown
We have explained in the caption and method section that 10 out of the 20 sites were analyzed in this study. (L360-361)

Line 292: The authors state that the presence of crevasses clearly shapes the holes – it goes (to my understanding) also hand in hand with the slope of the terrain. It would be helpful also to take the slope into account. Has this parameter been measured in the field?
As shown in Table S1, the total elevation difference across the 4 km transect line was only 95 m, suggesting that the slope had a negligible impact. We have mentioned it in the text. (L286-287)

Line 284: the authors speak about the relatively small study area. Albeit it is roughly shown in Figure 1 it would be helpful explain the area and/or the distance between the sampled 20 cryoconite holes.
The length of the transect line (4 km) has been added in the text. (L286)

Line 316: "holes with irregular shapes have been excluded from this study": please explain why (would they not fulfil the "random" or general criteria if they show stagnant melt water?
As claimed by other reviewers, the hole-meltwater was not stagnant. We selected the regular cylindrical shape of holes to avoid the effect of irregular meltwater or sediment in/outflows in the holes. (L328)

Line 318: Have the samples been collected in random plastic bottles or were the containers sterile? Would be helpful for DNA analysis…
The sample collection and preservation procedures have been revised. (L336-337)

Line 355: please indicate the pore size of the filters
We have added the pore size of the filters. (L349)

Line 356: please indicate the type of epifluorescence microscope
The specifications have been added in the text. (L350)

Line 353: Which PCR products have been pooled? Please indicate

We have added the explanation about the PCR products. (L368-369)

Line 475 and line 484: please correct the abbreviation of the Journal Arc. Antarc. Alp. Res. into Arct. Antarct. Alp. Res.

Corrected. (L466, 492, 501)

Line 554: odd writing of author MacAYEAL

The typo has been corrected. (L571)

10-Nov- 2025

Takeuchi et al.

Morphology shapes microbial ecosystems and carbon cycling within cryoconite holes on a Greenland outlet glacier

Dear Editors:

We greatly appreciate the feedback on our revised manuscript from the three reviewers. No further revisions were made to the text, as all three reviewers indicated that no additional revisions were required. We changed the title as suggested by the editorial comments and revised Figure 1 to comply with the journal's publication requirements. Thank you very much for your consideration of this manuscript for publication in the Communications Earth and Environment.

The following are the final comments from the three reviewers:

Reviewer #1 (Remarks to the Author):

The authors have revised the manuscript in line with the recommendations from my previous review and provided careful justifications in their response letter. I think this paper will make an interesting addition to the literature and has nothing further to raise to improve the manuscript.

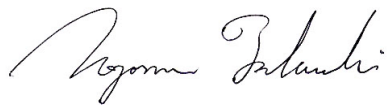
Reviewer #2 (Remarks to the Author):

I thank the authors for making the changes and corrections they describe. I look forward to the publication of the manuscript.

Reviewer #3 (Remarks to the Author): Dear Authors,

I have read the revised version with great interest and noticed a substantial improvement of the manuscript. Hence, I do not see any pending issues with this article.

Sincerely,



Nozomu Takeuchi

Corresponding Author