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Biogeosciences
Discussions
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Interactive comment on "Controls on microalgal community structures in cryoconite holes upon high Arctic glaciers, Svalbard" *by* T. R. Vonnahme et al.

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Referee comment: general comments: This is an interesting and overall well written manuscript describing the physical and community characteristics of cryoconite holes. The manuscript further attempts to determine how both the physical features of the environment and tropic level interactions may affect the biology of the system. Few previous studies have treated cryoconite holes in this manner and this manuscript compliments these earlier works well.

Author's response: Dear Referee #1, We want to thank you for the detailed feedback and comments, which helped to improve the manuscript a lot. We considered the

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comments and changed the new version of the manuscript accordingly. Please, find our specific responses below feel free to add comments and suggestions for further revisions.

specific comments

Page 11752 Line 6 Referee comment: Suggest at examples of the “grazers”

Author’s response: We agree, that these details are helpful here. We changed the sentence in the following way: . . . relations to their potential grazers, such as tardigrades and rotifers . . .

Line 11 Referee comment: Add comment mentioned in the conclusions that the positive relationship could be caused by similar environmental requirements of grazers and microalgae

Author’s response: We agree, that more details are helpful here. Similar environmental requirements is one possibility, but a positive control via nutrient recycling is another one, mentioned in the discussion. We changed the sentence in the following way: . . .not show any significant negative correlation with microalgal abundances, but a positive correlation with eukaryotic microalgae. Shared environmental preferences and a positive effect of grazing are the proposed mechanisms to explain these correlations.

Line 18 Referee comment: Bird guano is a nutrient input not just a proxy.

Author’s response: We agree and changed the sentence in the following way: . . . and a high impact of nutrient input by bird guano. , as a proxy for nutrients.

Page 11753 Referee comment: Suggest a comment on the life span of a cryoconite hole, i.e. do they form in the same location each year forming around the dark cryoconite on the glacier surface? Can they be considered a “semipermanent” habitat?

Author’s response: Thanks for the comment, we can add this information in the following way:. Cryoconite holes are usually open and photosynthetically active for a few months in summer. During this time they are highly dynamic systems with occasional stripping events during which they can be cleared and the newly distributed sediment starts forming new cryoconite holes nearby (personal observations; MacDonell and Fitzsimons, 2008). During this time several cryoconite holes are connected hydrologically. Most of the year, they are sealed with an ice lid and covered by snow, which protects them from stripping events, but which also inhibits the photosynthetic activity (Jesamine Bartlett, personal communication). However, the cited study relates to cryoconite holes in Antarctica, which are quite different from the cryoconites in our study. We couldn't find a specific study for the Arctic, but during our observations in the current study, we observed a rapid exchange of meltwater in the cryoconite holes and a few stripping events. Some of the data are given as the changing dimensions (depth, diameter) of the cryoconite holes on Hørbyebreen and Nordenskiöldbreen in the attachment. We could also discuss these data in this manuscript, but we don't think that it adds much relevant information to the topic of this study.

Lines 16-18. Referee comment: Delete the truism that only organisms adapted to the cryoconite holes can survive there.

Author's response: We agree that this statement is too generalized and not all organisms, living in cryoconite holes are specifically adapated to this habitat. We changed the sentence in the following way: Cryoconite holes represent ultraoligotrophic environments (Hodson et al., 2008) inhabited only by microorganisms, which are able to cope with many environmental challenges associated with a life on the surface of glaciers.

Page 11754 Line 12 Referee comment: Give some idea of sizes. Small is a relative term.

Author's response: We agree that is information is important. We added the typically observed maximum size of the grazers in our study. Only very few tardigrades reached larger sizes. We changed the sentence in the following way: . . . to consist of much smaller grazers, usually shorter than 200 μm (personal observations).

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Line 18 Referee comment: Expand on the “adaptation”. In what way?

Author’s response: Thanks for the comment. We clarified the “adaptation” in the following way: . . . enlarged colonies of a Coenobium species as possible adaptation to grazing. Larger colonies are proposed to outgrow the maximum food size of filtration feeders.

Page 11755 Line 3. Referee comment: Suggest beginning each “group” with a Roman numeral, i). .

Author’s response: We agree, that this helps to clarify the structure of this section. microalgae can be classified into four dominant groups . . . . i) Filamentous cyanobacteria. . . . ii) Nostocales, ... iii) Chlorophyceae, ... iv) Zygnematophycea . . . .

Page 11756 Referee comment: Include in the Site description something on the life span of the cryoconite holes. Are they formed new each year or does the cryoconite ensure they form in the same location each year? How many months of the year are they present? When does the surface snow clear from these glaciers?

Author’s response: Please, see also the comment about page 11753 in addition to the following comment. Thanks for this comment. Unfortunately, it is hard to generalize the life span and the length of the summer season. To our knowledge, no study covered this life time dynamics for Arctic cryoconites. Hence, the following section is mainly based on personal observations during the current study. A series of pictures was taken during the present study and could demonstrate the explained dynamics. However, we do not think that this information would add much crucial information to the focus of this manuscript. Thus, we add one figure here in the method section, but won’t focus on it in the results and discussion section. Most cryoconite holes form a dynamic system with hydrologically interconnected cryoconites holes. The dimensions are frequently changing and some cryoconite holes may experience stripping events, whereby the sediment content is transported downstream and builds a new cryoconite hole nearby.

The time, when the surface snow clears from the glacier is highly depended on the altitude, thus we can only give the usual start of the snowmelt. Close to the equilibrium line altitude, the time of snow-free days can be as short as a few days. We tried to add the required information in the following paragraph: The cryoconite holes are rather unstable habitats with a life span often shorter than one summer season. The closer the cryoconite hole to the glacier margin, the shorter the life span (personal observations). Hence, the cryoconite holes on the Plateau on Nordenskiöldbreen have the longest life span and the cryoconite holes near Retrettøya the shortest one. During the current study twenty cryoconite holes were monitored continuously with depth measurements and photography. We could show that three cryoconite holes experienced a complete stripping event and that nine of them drained, but regrew at the same place (Figure S3). Cryoconite holes on the present glaciers are only open for one to three months in summer, depending on their altitude. They remain rather stable after an ice lid gets formed in atumn until the snow starts melting in late June and the first parts of the glacier clear from the snow in July (personal observations). The current study focusses on the summer months, because only during the summer season, a significant photoautotrophic activity is expected.

We could add figure 1 in the supplement to show the life times of some of the studied cryoconite holes. However, it is not too closely related to the story of the current paper. Thus it will appear in the supplement, rather than in the manuscript.

Line 10. Referee comment: Not very clear how many samples taken on the Ebbabreen.

Author's response: We tried to clarify the total number of samples, taken on Ebbabreen in the following way: On Ebbabreen, in total 6 samples were collected every 25–100 m in height.

Page 11757 Section 2.2. Referee comment: State where the lab work was undertaken. At field camp or were the samples returned to the mainland?

Author's response: We added this information in the following sentence: All density estimations were done in the field station in Petuniabukta. The species determinations were done on fixed cryoconite samples (4% Formaldehyde) back in the lab in the Czech Republic.

Line 5. Referee comment: State that there were no organisms in the supernatant. Was this examined?

Author's response: We screened the supernatant in some of the samples to exclude the possibility of abundant planktonic grazers. We included this information in the following way: The supernatant was screened randomly for planktonic individuals, but no grazers have been found.

Referee comment: Please state what keys were used for the identifications. How were these ids performed? Where is the identified material deposited?

Author's response: The missing information are added in the following section: The rotifers have been identified, using the monograph of Donner (1965). Tardigrades were identified, using the key to world tardigrade by Ramazotti and Maucci (1983) and by comparisons with other original papers (Dastych, 1988; Miller et al., 2005). The identified material is deposited in the Biology Centre AS CR, Institute of Soil Biology in Ceske Budejovice in the Czech Republic.

Dastych, H.: The Tardigrada of Poland. Monografie Fauny Polski 17. Donner, J., 1965. Ordnung Bdelloidea (Rotatoria). Akademie-Verlag, Berlin, 1988.

Ramazzotti, G., and Maucci, W. 1983. Il Philum Tardigrada (III. edizione riveduta e aggiornata). Memorie dell'Istituto italiano di idrobiologia, 41, 1-1016.

Section 2.3 Line 18. Referee comment: How was "wet supernatant" judged? Small differences in water content will have large differences on the determined densities.

Author's response: The wet sediment is defined as the sediment that settled after more than 30 minutes. The supernatant was removed completely with a syringe, and only the water saturated sediment was used for microalgae density estimations. The wa-

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ter content of this saturated wet sediment was measured later and the wet weight per area was calculated as the total weight of the wet sediment, which was collected in a defined area. We added this information in the following way: After settling of the sediment for at least 30 minutes the supernatant was removed with a sysinge and kept for further dilutions. Due to the current of meltwater through cryoconite holes, the sediment is already well selected towards high sedimentation rates and the supernatant appeared clear and no remaining paricles have been observed. The remaining water saturated wet sediment was used for estimations of the microalgae densities and the water content. For the counting, 0.25 g of wet . . .

Line 19. Referee comment: Diluted with "meltwater"? Where did this originate? From collected ice?

Author's response: The meltwater is the supernatant from the same cryoconite. See comment for 11757 Line 18.

Page 11758 Lines 1-4. Referee comment: Some references are required to support these divisions of filtering classes. Especially as these become a major point in the ms later.

Author's response: The divisions of filtering classes is mainly based on measurements of the feeding apparatuses in our own samples. We can add some figures to the supplement to show how we measured the sizes. Since, most tardigrades are not filter feeders the food size is not as important. But 25 μm as the maximum size of ingestible particles for the rotifers is proposed to be an important value. Later it will be shown, that most microalgae in the sampled cryoconites are larger than that. We will add the following figure in the supplement as an example how we measured the buccal tube and filtrating apparatus lengths and we add the following example of Hino and Hirano (1980) in the manuscript. The division of filtering classes is mainly based on measurements of the feeding apparatuses in our own samples (Figure S4). Additionally, Hino and Hirano (1980) found a linear relationship between the maximum ingestible particle

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size and the body length in the rotifer Brachionus pricatilis. For 200 μm long specimen they found a maximum ingestible particle size of about 21 μm.

Hino, A., & Hirano, R. (1980). Relationship between body size of the rotifer Brachionus plicatilis and the maximum size of particles ingested. Bull. Jpn. Soc. Sci. Fish, 46(10), 1217-1222.

Line 6. Referee comment: Reference required for photosynthetic activity occurring only in the first few μm of the sediment.

Author's response: For sediments oxygen profiles, measured with microsensors support this statements. For cryoconites one study by Telling et al. (2011) can support the the idea. We will add the following information. General oxygen profiles in sediments, obtained with microsensors showed photosynthetic activities at sediment depths only below 0.5-1mm (E.g. Revsbech et al., 1986). For cryoconite sediments a study by Telling et al. (2011) showed that only in sediment layers <3 mm a net autotrophic system is maintained. . . .

Revsbech, N. P., Madsen, B., & Jørgensen, B. B. (1986). Oxygen production and consumption in sediments determined at high spatial resolution by computer simulation of oxygen microelectrode data. Limnol. Oceanogr, 31(2), 293-304.

Line 15. Referee comment: Has the work in 2012 been published? If not, some details on the sequencing of the 16S rRNA required.

Author's response: No, it has not been published. We added the following method section for the sequencing details:

2.4 16S rRNA gene sequencing and sequence analysis

The highly variable V3/V4 region of the 16S rRNA gene was amplified with the bacterial primers S-D-Bact-0341-b-S-17 forward and S-D-Bact-0785-a-A-21 reverse, with overhang Illumina adaptors attached to the primer sequences, creating a single amplicon of about 460 bp (Klindworth et al., 2013). The reaction was carried out in 50

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μl volumes, containing 0.3 mg ml-1 Bovine Serum Albumin, 250 mM dNTPs, 0.5 mM of each primer, 0.02 μl Phusion High-Fidelity DNA Polymerase (Finnzymes OY, Espoo, Finland) and 5x Phusion HF Buffer, containing 1.5mM MgCl2. The following PCR conditions were used: initial denaturation at 95°C for 5 min., followed by 25 cycles consisting of denaturation (95°C for 40 s=, annealing (55°C for 1 min.) and extension (72°C for 1 min.) and a final extension step at 72°C for seven minutes. The amplified DNA was sequenced using the Illumina MiSeq platform at Liverpool Centre for Genomics Research and generated 2 x 300 bp overlapping pairs-end reads. The 16S sequences were further processed, using the mothur (v. 1.35) pipeline (Schloss et al., 200). Chimeric sequences were identified and removed using UCHIME (Edgar et al., 2011). Reads were clustered into operational taxonomical units (OTUs), based on at least 97% sequence similarity, and assigned taxonomically against the SILVA database (Quast et al., 2013).

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R.: UCHIME improves sensitivity and speed of chimera detection, Bioinformatics, 27(16), 2194–2200, doi:10.1093/bioinformatics/btr381, 2011.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M. and Glöckner, F. O.: Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies, Nucleic Acids Res., 41(1), 1–11, doi:10.1093/nar/gks808, 2013.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. a., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J. and Weber, C. F.: Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities, Appl. Environ. Microbiol., 75(23), 7537–7541, doi:10.1128/AEM.01541-09, 2009.

Line 26. Referee comment: When were these measured?

Author's response: The measurements included in this study were done immediately after sampling. In the cryoconite holes, which were sampled more than one time the depth was measured continuously (See FigS3) to observe the overall stability of these cryoconite holes and to detect stripping events. We changed the sentence in the following way: As proxies for the age and stability of the hole, water depth was measured with a ruler immediately after the sampling of the sediment.

Line 28. Referee comment: Please define "saturated sediment" more clearly. How was the excess water removed first?

Author's response: See comment for 11757 Line 18.

Page 11768 Line 21. Referee comment: Please explain 'lateral thermal conductivity' and how this results in a thin grain layer.

Author's response: We clarified it in the following way: . . .by lateral thermal conductivity if time allows. Thereby, the absorbed solar radiation is conducted laterally to the ice walls of the cryoconite hole, resulting in an increasing area and a decreasing sediment thickness.

Page 11769 Line 2. Referee comment: Consider using full site names in the text rather than abbreviations (e.g. HC and NC). It is easier for the reader to follow.

Author's response: Some of the site names are rather long (e.g. Plateau on Nordenskiöldbreen, main site on Nordenskiöldbreen), but we can change it.

Page 11770 Lines 3-7. Referee comment: This is a rather awkward sentence.

Author's response: We changed it in the following way: Previous sentence: The finding that all cyanobacteria identified have had heterocysts or close relatives with the nifH gene and their dominance in often nitrogen depleted cryoconites supports the hypothesis that sediment associated cyanobacteria act as drivers of this ecosystem in respect to inorganic carbon and nitrogen fixation in nutrient depleted areas. Changed sentences: All cyanobacteria found in the current study are known to have hetero-

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cysts or to have close relatives with the nifH gene for nitrogen fixation. These potential diazotrophs were often dominating in nitrogen depleted cryoconites. These findings indicate that sediment associated cyanobacteria are highly important as ecosystem engineers in cryoconites in respect to inorganic carbon and nitrogen fixation, especially in nitrogen depleted areas.

Page 11771 Line 13. Referee comment: Define more clearly what the 'strong selective pressure' is to etc.

Author's response: We defined it more detailed. One possibility for this temporal homogeneity is the short summer season and the strong selective pressure, such as cold temperatures, high light intensities and unstable habitats which are rather constant over the summer season.

Section 4.6 Referee comment: This is rather awkward to read and I suggest a re-write.

Author's response: We re-wrote the whole section in the following way:

4.6 Microalgae size and grazing resistance The formation of large cyanobacteria colonies (< 10 cells, or > 25 μm) observed in the studied cryoconite holes may have several benefits for the organisms. Firstly, the colony size most likely becomes larger than the maximum prey size of the present filtration feeders (Sand-Jensen, 2014). A previous study by Vanormelingen et al. (2009) showed that the increasing colony size of a Coenobium species can be an effective defense strategy against filtration feeders. The habitat of closely connected freshwater ponds studied by Vanormelingen et al. (2009) is well comparable to cryoconite holes in regard to their size and connectivity. In the current study, the negative correlation between the average length of Oscillatoriales trichomes and the abundance of filtrating rotifers indicates that this may also be true for cryoconites. We propose that with increasing length of the trichomes, rotifers have a decreasing amount of ingestible food available in the system, which yields in a smaller density. Secondly, a large colony size may be an adaptation to the typical environmental stressors in cryoconites. Previously, large colonies of Nostoc sp. have

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been shown to be more tolerant to freezing and desiccation than smaller colonies (Li and Gai, 2007). Also a nutrient storage mechanism via extracellular mucus has been proposed to be an effective strategy to cope with nutrient pulses in otherwise ultra-oligotrophic environments (Li and Gao, 2007). Both mechanisms are good strategies to live with the environmental stressors in cryoconites. Another indirect advantage of long filaments is their importance in stabilizing large granules, which are important for possibly symbiotic heterotrophic bacteria (Takeuchi et al., 2001). The overall reason for the formation of large colonies in cryoconites can be related to both, environmental and predation based stressors. Ciliates are most likely unimportant as predators for microalgae due to their small size and usually bacterivorous diet. The positive relation between ciliate abundance and Oscillatoriales trichome length can be explained by several indirect effects. One possible explanation is that ciliates can act as food source for larger grazers. If the larger grazers are absent, the microalgae and ciliates have an advantage. Another reason could be that a lack of competition for bacteria as diet with the filtrating rotifers increases the number of ciliates. Green microalgae are, in general, relatively large and occur mainly as single cells. Grazer abundances were not correlated to their sizes (Table 7). Thus, it is proposed that grazing as a minor impact on the morphology of green microalgae.

Page 11772 Section 4.7 Referee comment: This sections feels a bit repetitive from earlier sections and would benefit from reducing or focussing more clearly.

Author's response: We agree, that this section is rather repetitive. Thus, we will remove the section and add the additional information to the sections about Microalgae distribution (4.1) and geographic properties (4.2), where appropriate.

Page 11773 Line 13. Referee comment: Grazer abundances are related to the impact of birds not impact of birds to grazers as the text currently implies.

Author's response: We switched it in the following way:. The latter is more likely . . . and grazer abundance and green microalgal densities are positively related to the impact

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of birds.

technical corrections

Referee comment: The English is generally very good but there are some grammar errors that should be addressed. Here are a few examples.

Author's response: Thanks for the grammatical corrections. We corrected all the errors and looked for additional errors.

Table 2 Referee comment: It is unclear to me why site NR appears in the column but not the row and NL occurring in a row but not a column?

Author's response: We assumed that NR and NR are per definition the same. But for clarification we made sure to add the NR to the rows. NL is already in the columns and in the rows.

Fig 1. Referee comment: Suggest a map locating Svalbard. Suggest simplifying the map, e.g. less detail, fewer contours, to enable the site locations and names to be more easily read.

Author's response: Ok, we can increase the font size and reduce the topographic lines of the surrounding mountains. We can also add a general map of the location of Svalbard in the upper right corner.

Figure 2 Referee comment: can be deleted. This system is basically a large pooter and could be referenced to Southwood and P A Henderson 2000 Ecological Methods. Blackwell.

Author's response: Thanks for the reference. We removed the figure and cited the method instead. Cryoconite sediment was collected into a 0.5 l polyethylene bottle with a pooter (Southwood and Henderson 2000). Sediments in a defined area within a 4.5 cm plastic ring were taken. All sampling equipment was washed with meltwater from the sampling site prior to the sampling. Southwood, T. R. E., & Henderson, P. A.

(2009). Ecological methods. John Wiley & Sons p.269.

Figures 3 and 5a Referee comment: are only understandable in colour. Can these be adjusted to be clear in B&W?

Author's response: We adjusted the figures with patterns and colors included and we changed the colors to avoid using the colors of green and red in the same figure.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/12/C6114/2015/bgd-12-C6114-2015-supplement.pdf

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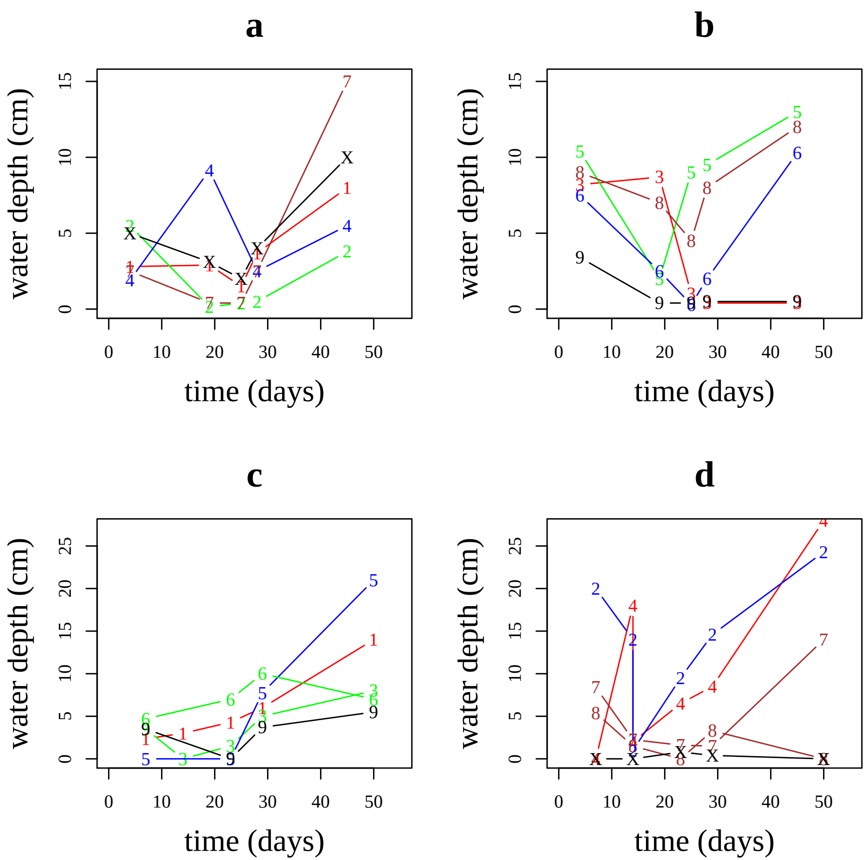

Fig. 1. Water depth in cryoconite holes on Hørbyebreen (a,c) and on Nordenskiöldbreen (c,d). The numbers represent the ID of the continuously samples cryoconite holes. X refers to the ID number 10.

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Fig. 2. Typical tardigrade (left) and rotifer (right) specimen of our study and an example of the measurements of the diameter of the buccal tube (tardigrade) and filtration apparatus (rotifer).

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