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Biogeosciences Discussions

Interactive comment on "Microbial nitrogen cycling on the Greenland Ice Sheet" *by* J. Telling et al.

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General comments: This is an interesting paper which provides a timely and valuable insight into the functionality of supraglacial microbial ecosystems on the Greenland Ice Sheet (GrIS). Specifically, the authors provide evidence for the cycling of nitrogen by cryoconite microbiota on the periphery of the GrIS. On the whole the methodology and conclusions are acceptable and when combined with other recent work (e.g Stibal et al Microb. Ecol. in press; Hodson et al. (2010) Annals Glaciol. 51:56) advances our understanding of microbial processes on the surface of the GrIS. Although a few of the very early entries into the cryoconite literature concerned Greenland cryoconite, for example the work of Gaijda, Gerdel, Drouet, and Gribbon, little has been reported in recent years, which is regrettable considering the increasing interest in the

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dynamics of the GrIS. The authors are to be commended for the skilled integration of work in glaciology, microbial physiology and molecular biology to provide a study of biogeochemical cycling on GrIS which would in my opinion be broadly appropriate for publication. I would, however, like to provide specific comments for consideration on two important issues relating to the experimental work yielding the data presented on nitrogen fixation.

Specific comments:

Calibration of acetylene reduction assays:

In this study, the authors cite Stewart et al.'s 1967 paper reporting the utility of the acetylene reduction (AR) technique as a convenient assay for nitrogen fixation to support the assumption of a 3:1 molar ratio between micromoles ethylene produced to micromoles nitrogen fixed (p10431 L19) to permit estimation of nitrogen fixation. However the 1967 paper cited is also the first to caution that as AR is an indirect measure of nitrogen fixation and that workers should verify nitrogen fixation is occurring by occasional recourse to 15N2 reduction assays. While the 3:1 rule of thumb holds well for many experimental systems, other habitats have described considerable divergences from the 3:1 ratio, which could lead to considerable errors in quantitative estimates of nitrogen fixation made from uncalibrated AR data. Neither this paper, the previous report of AR activity by Arctic cryoconite (Telling et al. [2011] J Geophys Res. 116:G3039), nor any other publication to my knowledge provides N fixation data for cryoconite from 15N2 reduction assays or AR assays calibrated by 15N2 reduction data. Since these papers constitute early reports of N fixation in an unusual habitat I am concerned that the AR data do not appear confirmed or calibrated by 15N2 reduction assays, even by cryoconite in cold laboratory incubations. As such the 3:1 molar ratio should be treated with care when using it as the quantitative basis for arguments developed at length in the paper, e.g. Section 4.4. I would recommend that the authors either revise the manuscript to provide the appropriate health warnings or to provide calibrated AR data.

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Real Time PCR:

The inclusion of PCR assays for nifH genes in cryoconite along the sample transect is most welcome, not least as several independent workers have previously experienced difficulty in amplifying nifH genes in cryoconite by PCR. A strength of the work is the selection of three primer sets targeting cyanobacteria and proteobacteria to ensure better coverage of potential sources of nifH. While the primers used are derived from the previously published work of Church et al. (2005) and appear specific for nifH in oligotrophic ocean waters when used with TaqMan probes as reporters, no data is available on their specificity and sensitivity when applied to a different habitat (cryoconite) using a DNA intercalating dye as the reporter chemistry (which are often considered less specific than probe-based qPCR assays – if only by vendors of probe-based chemistry qPCR products!). I consider it would be best practice to confirm the assay specificity in this context by the cloning and sequencing of some Real Time PCR products generated from the sampled cryoconite. This would have the additional benefit of furnishing the authors with some data to examine phylogenetic affiliations of nifH genes, and providing clones to generate standards for the calibration of the qPCR assay. This leads me to my major reservation with the qPCR data reported here, or rather how it is reported. Sites are reported as abundances of nifH relative to the site with the lowest abundance. Care should be exercised in using this somewhat unorthodox means of quantification. The paper does not report the calibration of the assay to ensure a linear response and a high qPCR efficiency over the range of target gene abundances measured, therefore the use of threshold cycle values corrected in this manner is risky. The assay could be validated by using a tenfold dilution series of a standard of known concentration, for example a plasmid bearing nifH, which would also enable the reporting of the number of gene copies per nanogram of template DNA or ultimately unit mass of sample. However, if the qPCR data are reported as either copies/ng DNA or copies/g dry or fresh weight of sample, this still conceals a further issue with the data when attempting to illustrate the functional potential of cryoconite communities along the transect. As P10439 L10 notes the organic matter content (or

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is it TOC as per Stibal et al. 2011? –values and units suggest so) varies from site to site, and as described by Stibal et al. 2011 so does the abundance of cells at each site. Therefore either the relative abundance as reported in the paper or abundance as copies per unit mass of template DNA or sample could conceal the correct interpretation of the functional potential of a site. It would not be possible to distinguish between a community with a high abundance of nifH where that is incidental to a high total microbial abundance, and that where a high abundance of nifH is relative to a low total abundance, which would more likely reflect a community where the genetic potential for N fixation is key to community functionality. Finally, differences in organic matter content may result in variable DNA extraction efficiencies. Therefore if the authors seek to evaluate the functional potential of each community to fix nitrogen the numbers of nifH copies could be expressed as a ratio of the copy number of a ubiquitous housekeeping gene e.g. the 16S rRNA gene, which is a common practice in qPCR experiments where the copy number of a target gene requires normalisation to account for other factors. In summary I would recommend the presentation of qPCR data which is calibrated and normalised in this manner. Additionally the authors may find the recent work of Brankatschk et al. ([2011] ISME J. 5:1025) insightful reading on the relation between qPCR data and measurements of nitrogen cycling in glacial environments. Finally, while the number of machine replicates (replicate reactions per sample) is reported (P10429 L22), the number of biological replicates (independent DNA extractions from different cryoconite holes at the transect sites) does not appear to be. Without this information the mean value and 1SD error bars in Fig4e are potentially misleading: do they refer to mean and SD of machine or biological replicates? I would recommend the revision of the manuscript to clarify this. One would typically expect 3-4 machine replicates per DNA extract and several separate cryoconite holes sampled at each site to approximate biological replicates. I appreciate the considerable constraint imposed by limited heli-time to conduct the deeper traverse so it may be the authors collected from a single cryoconite hole at each site or pooled cryoconite into a single tube to save on the dwell time-per-site. If this is the case it should be made clear

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in the text.

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