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

Anonymous Referee #2

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This manuscript seeks to relate microbial nitrogen cycling, specifically N₂ fixation, with measurements of a number of geochemical and physical processes. The authors then use the derived data in several modeling scenarios in an attempt to understand the extent of N cycling on glacial surfaces and their potential to contribute to the subglacial ecosystem, and global biogeochemical processes. The authors are noted authorities in glaciology and geochemistry and have extensive knowledge of the Greenland system.

I have several reservations regarding the experimental design, which in my opinion, reduce the impact that this paper may have on the field - these will be outlined in a list of major and minor points. Secondly, the manuscript is fairly short and fails to integrate several relevant studies into their introduction of the topic and their discussion of their results. Thirdly, attention was only paid to dinitrogen reduction, and assessments were

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based on primers that miss a significant majority of diazotrophs as well as acetylene reduction assays that were allowed to incubate in a refrigerator for up to 2 months - how can one really assess the relevance of these results to ecosystem function and/or dynamics. Finally, from the perspective of the potential shortcomings of the experimental design, I will wait to comment on the modeling studies, as their merit is potentially confounded by the specifics listed below.

Major:

Page 10424, Line 26: Why is nitrogen the most important nutrient. Don't microorganisms need a number of nutrients (N, C, P, S, etc.), which without any of these, limit their growth?

Page 10435, Lines 5 to 10. Fought et al., 2004 isolated N₂ fixing and NO₃ reducing populations from glacial subsurface and surface environments. This is relevant and is not mentioned. Boyd et al., 2011 describe N cycling in subglacial and surface environments - this is very relevant but was not cited or discussed.

Page 10425, Lines 15-18. It is not clear how this study will address this issue since measurements of anthropogenic N were not considered, per se.

Page 10428, Section 2.3. It is not clear how any of the activity assays reflect 'in situ' activities, especially considering the fact that samples were incubated in the refrigerator for up to 2 months prior to GC analyses. No killed controls were included and thus matrix-based, abiotic reduction cannot be ruled out as the source of ethylene. How were rates normalized to area of cryoconite - some additional text would help in reducing the confusion of this statement.

Page 10429, Section 2.4. There are potentially serious shortcomings with the molecular studies performed. Why were primers that target only a subset of diazotrophs chosen when primers that capture the known diversity of diazotrophs are available and have been employed in glaciated systems (Hamilton et al., 2011). Secondly, the qPCR

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is flawed in that apparently nothing was done to normalize for DNA template concentration and standardization across samples. How were the standard curves constructed - what was used as a standard. What was the detection limit of the assay in templates/ng DNA. What range of template abundances was used to assess unknown sample abundances. What about negative controls?

Page 10439-10436: My expertise is not modeling so i will not comment. However, i do think that the results of the models will be significantly impacted by the issues commented on above.

Page 10436, Section 4.1. No evidence for 'active' N₂ fixation is presented. RNA based studies would get you much closer to saying this, but over a 24 hr to 2 month period (potential length of the experiment) one would expect the system to change dramatically and may induce nitrogenase. Also, no evidence for in situ nitrogenase activity is presented.

Page 10436, line 21: but the cryoconites are where the activity is. Thus, this would be expected to be the source of DON and if these systems are indeed N limited, then would presumably be a significant source of N in the systems.

Page 10336, line 24: Without taking into consideration flux, i am not sure how the authors can make this statement. Secondly, the authors do not consider N₂O, NO, and/or N₂ loss from the system due to biological or abiological reactions, as far as i can tell.

Page 10437, line 30. denitrification is not inhibited by oxygen, just is outcompeted as a process in oxygenated environments

Page 10440, line 22. No details of the statistical analysis was given. Seems that this correlation would be dependent on only several points if it is a linear regression. Might try a non-parametric regression approach

Page 10440, line 27. Probably more appropriate to say that ammonia suppresses

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nitrogenase rather than inhibits it.

Figure 3. While it is suggested that DON does not contribute much to TIN, the overall trend (acknowledging SD) is that up to 10-20% of TN cannot be explained by DIN. Rarely does the trend fall outside of the 1:1 line where TN is greater than DIN, suggesting this is not an analytical problem but rather is likely to be real.

Entire discussion is primarily speculation. There is no evidence presented for denitrification, ammonification, nitrification, etc., yet discussion of these potentially important processes is given nearly as much treatment as the entire results section. Likewise, why is so much discussion given to anthropogenic N, since this paper does not directly assess this?

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