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

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We thank Arwyn for his constructive criticisms. We answer his specific comments on the acetylene assays and qpCR work as follows.

Calibration of acetylene reduction assays:

We acknowledge that ideally the acetylene assay should be calibrated to the 15N technique. For the current manuscript we agree that some caveat should be stated in the text, and we propose the following: "However we acknowledge that in natural freshwater environments ratios may differ from this theoretical 1:3 ratio (e.g. range of 1:2.2 to 1:11.9 in freshwater lakes; Howarth et al., 1988). In the absence of calibration to the 15N technique (Howarth et al., 1988), the calculated nitrogen fluxes from N2 fixation

should therefore be considered order of magnitude estimates."

qPCR:

To answer Arwyn's comments, we should first state that qPCR was only used as a supporting method in this biogeochemical study, in order to determine if microbes capable of N2 fixation (using the nifH gene as a proxy) are present along the whole transect, and whether their abundance changes along the transect (as N2 fixation rates do). Therefore, we used more primer sets so as not to miss any important group of N2 fixers. We were not attempting to answer questions about the diversity of N fixers in the samples, and therefore did not perform any further molecular work (cloning etc.). For the same reason, the suggested issue of specificity of the primer sets was not significant. Second, we appreciate the concerns about the linearity of the response and the efficiency of the assay. However, due to technical problems and time constrains we could not perform calibrations with nifH standards (The machine-generated efficiencies were satisfactory, usually >80%, while procedural blanks were never above noise values). We appreciate that the data is currently presented in an unorthodox manner, and have now revised the figure to show nifH gene abundance in units of log 1/(2ˆCT) values rather than relative units normalised to the 0 km site of each run. We will add an additional line to the figure legend stressing that the data does not allow quantitative assessment of the relative abundance of nifH genes for gamma proteobacteria, trichodesmium-type and heterocystic nifH genes at the same sites. And last, we only extracted DNA from one pooled sample from each transect site, and so our qPCR are replications are indeed only machine replicates. This will be clarified in the text.

We believe that despite the shortcomings the qPCR data are worth including in the ms, as they help illustrate the point we are making in the paper – namely that nifH genes, and therefore N fixers, are present even at sites with no detected N2 fixation, and that there is the potential for N2 fixation to occur at those sites.

Additional references Howarth, R.W., R. Marino, J. Lane, and J.C. Cole: Nitrogen Fix-

ation in Freshwater, Estuarine, and Marine Ecosystems. 1. Rates and Importance, Limnol. Oceanogr., 33, 669-687, 1988.

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