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Interactive comment on "Viable cold-tolerant iron-reducing microorganisms in geographically-isolated subglacial environments" *by* Sophie L. Nixon et al.

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The article "Viable cold-tolerant iron-reducing microorganisms in geographically-isolated subglacial environments" by Nixon and colleagues addresses a significant, poorly studied aspect of biogeochemical cycling in subglacial environments; namely, iron cycling, and specifically focuses on microbially-driven iron reduction. I find several aspects of this work quite commendable; specifically, the inclusion of samples from multiple, geographically widespread and geologically distinct subglacial environments, the enrichment culture approach (which is somewhat less biased than pure culture approaches), and the clarity of the writing.

However, I have multiple reservations as well, some of them serious and some more

technical, about the methodological approach and interpretation of the data. First, I am quite concerned that there were no killed controls included in their analyses; thus, they have no way of telling whether the iron reduction that they observed during their enrichments was biological or chemical in nature. It also appears that the only chemical species that they followed was iron; thus, there is no way to tell from this data whether the iron reduction observed was direct (i.e. microbial iron reduction) or indirect (i.e. microbial sulphate reduction, which produces sulphide, which could then secondarily reduce iron oxides to produce iron (II), or other means). The possibility of indirect iron reduction is particularly problematic because both iron reducing and sulphate reducing bacteria (amongst others) utilize the organic carbon substrates provided in the enrichment cultures. While this concern does not invalidate their observation that iron reduction occurs and thus could have downstream implications, much of their discussion relies on the assumptions that the iron reduction is biological and direct. Thus, the language needs to be seriously toned down throughout the manuscript regarding how confident they are in their results.

Second, they identify their bacteria in their enrichment cultures by 16S rRNA gene analysis and then proceed to discuss their potential role as iron reducing bacteria. This approach is based on two unwarranted assumptions: 1) because a sequence is abundant, it is carrying out the metabolism of interestâĂŤthis is not necessarily true, even in an enrichment culture, 2) taxonomy is equivalent to physiologyâĂŤjust because a sequence is related to a known iron reducing species does not necessarily mean that the sequence originates from an iron reducing bacterium. I am a professor and my sister is a bank loan officerâĂŤwe do very different things, but we are closely related. Thus, the data presented do not directly demonstrate which bacteria may be carrying out iron reduction in subglacial sedimentsâĂŤthey only provide indirect evidence that would need to be confirmed more directly. This holds for the previous papers that they cite as observing iron-reducing bacteria in non-culture based approachesâĂŤthese papers are quite careful not to claim that they have identified iron-reducing bacteria in subglacial environments (unless they have actually done culture work); only that they have

found relatives of iron-reducing species in subglacial environments. I would strongly recommend modification of the discussion to reflect these concerns.

Third, it is difficult to determine whether this process might be cold-adapted in all systems as there are no intermediate temperatures between 15 and 30C. This is a big jump and a large transition zone for many bacteria. Furthermore, there was no work beyond the first round of enrichment for the 30C samples. Thus, it is unclear whether other conditions may have produced different outcomes. In at least one case reported here, the final iron concentrations are much higher at 15C than at 4C; thus, more work is needed to determine whether this process is cold adapted or not. Generally, their language is fairly circumspect with regard to this issue, but they should be cautious in their interpretation.

The major concerns above lead to further questions. The section of the discussion addressing the possible role of Desulfosporosinus in iron reduction relies on the assumptions that the iron reduction was direct, biological, and carried out by Desulfosporosinus, none of which can be confirmed for the reasons outlined above. I would suggest removing this paragraph, or expanding it to address the possibility that they are acting as SRB and indirectly reducing the iron.

Some less critical, but still significant concerns I have with the manuscript include:

1. There was no discussion of the overall biodiversity or comparison between treatments (i.e. temperatures) of the enrichments. Since they have this data (from the high throughput sequencing), why is it not included?

2. For Figure 2, it is unclear why they are only reporting a subset of the data (“only genera known to include strains capable of microbial iron reduction, and other major taxa, are included in the legend"). Why not report all data? And what do relative abundances mean if not all data is reported?

3. For figure 1: why are there two lines for each treatment? Are these replicates? If so,

why are they showing both replicates rather than a mean and standard error? Also, it would be useful if they would calculate the rate of iron reduction so that they could be compared between sample types.

4. They use an amorphous iron oxyhydroxide as the source of Fe(III) for their enrichments. Does the source of the iron matter? They don’t discuss crystalline or chelated iron or whether that might make a difference in the outcomes, except to discuss crystalline iron as a possible source in the subglacial environment.

5. As mentioned above, they don’t report data for tracking other chemical species. Did they measure degradation of the carbon source? It would be useful to know if the iron reduction is stoichiometric with the carbon utilization. If that data is available, please report it. Not required, but useful.

6. They argue that H2, derived from chemical reactions with rocks, could be a source of reductant in subglacial environments with low organic carbon levels. This is a possibility, but there are several issues with this argument. First, the concentrations of H2 that would be produced in this way are likely to be quite low. Second, a lot of other biological and chemical pathways would be competing for that H2 (sulphate reduction, nitrate reduction, etc.). Third, and most importantly, they have not demonstrated that their enrichments can utilize H2. So, I would like to see that part of the discussion toned downâĂŤelectron source may well be a limiting factor for iron reduction in the subglacial.

7. It is unclear to me what the relative abundance of their proposed iron reducers is in unenriched samples. How many fold enrichment do they see? Are these abundant in “normal” subglacial environments, or did they grow “weeds” in their enrichment culture? Is there any direct indication that these microbes are important in these environments? If not, they need to be careful in their interpretations of how significant their findings are to the actual identity of iron reducers in subglacial systems.

8. Phosphate adsorbs to iron oxides, it is not “coupled” (p. 10, line 29)

In sum, this is an interesting initial attempt at examining iron reduction in subglacial sediments. The enrichment culture approach is appropriate; however, there are important missing pieces of the puzzle here. I would strongly recommend assessing the confidence they have in their data and it's relevance to the real world systems they are discussing. The language of the manuscript needs to be toned way down and other data should be included, if available. Other analyses of the sequence data and further analysis of the iron reduction data would be helpful.

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