

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 paper “Viable cold-tolerant iron-reducing microorganisms in geographically-isolated subglacial environments” by Sophie Nixon et al. focuses on iron-reducing microorganisms in subglacial sediments from several geographically distinct areas. The authors conducted enrichment incubation experiments (rather than culturing, see below) during which they measured the rates of iron reduction and analysed the microbial communities using pyrosequencing of the 16S rRNA gene. They conclude that microbial iron reduction is widespread in subglacial environments, and may have important implications for global biogeochemical iron cycling and export to marine ecosystems.

The paper addresses an interesting scientific question and presents new and poten-

tially significant data on an important biogeochemical process which had previously been hypothesized to occur in the subglacial environment but has not been directly measured to date.

The methods were adequate and are well described. My only reservation is about calling this approach 'culturing' as environmental samples were used and no attempts to isolate single species/strains were made. Working with real cultures would allow for direct attribution of processes (in this case iron reduction) to specific organisms, which is not quite possible here as the incubations still contain a mixture of microbes. It is known that non-dominant species can significantly contribute to biogeochemical processes (see e.g. Pester et al. 2010 ISME J). This should be acknowledged and discussed in the ms in my opinion. Also, using 16S rRNA instead of DNA would have been useful in terms of providing information on the active part of the community as opposed to "potentially active" DNA-based data.

The hypotheses and aims of the study should be explicitly stated in the introduction (before "Here we present data..." on page 1/line 14).

Why were different primer sets used for different samples? (27-1492 for E, L, FL, R and 357-518 for F and LW; 5/6-15). Are these really bacteria-specific (as is suggested on 5/20) or do they pick up archaea as well? It would have been useful to look at archaea in these samples – potential syntrophies of iron reducers with some archaea could be quite interesting.

There seems to be a discrepancy between what you say on 6/4-6 ("All 4C enrichment tested positive for microbial iron reduction...") and on 6/15 ("The only instance in which statistically significant production of Fe(II) was evident..."). Isn't Fe(II) production what you used as a sign of iron reduction?

Specific/technical comments:

1/2 "geographically-isolated" is a bit confusing. Is geography really the main factor you

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want to emphasize in the title?

1/13 dtto

1/17 italicize *Desulfosporosinus*

1/18 italicize *Geobacter*

1/30, 2/26 the correct citation for this is Stibal et al. 2012 Global Change Biology 18: 3332–3345

2/23, 3/15 the correct citation is O'Donnell et al. 2016 Biogeosciences 13: 3833–3846

4/30 centrifugation

7/15 environments

7/19-22 this section doesn't quite make sense. MIR is characterized by greater metabolic and genetic diversity compared with what exactly? How do the differences between the two temperatures highlight this diversity? Please clarify/rewrite.

7/25 *Rhodoferax*, *Geobacter* and *Desulfosporosinus* have been found in subglacial sediment exported by the river draining Leverett Glacier; *Rhodoferax* in high abundances (>20% of reads in some samples). The results have recently been published (Cameron et al. 2016, Environ Microbiol doi: 10.1111/1462-2920.13483) although the iron reducers are not specifically mentioned in the ms.

8/4 define MIR at first use

8/9 can't iron reducers use legacy OC?

9/1-14 this section is unnecessary as it doesn't add much to the story

9/5 italicize *Thiobacillus*

9/11-13 this statement needs some references

10/12 Mitchell

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11/3 Schulze-Makuch

11/4 Nixon et al. 2012 is not in the reference list

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Interactive
comment