

Interactive comment on “Marine bacteria in deep Arctic and Antarctic ice cores: a proxy for evolution in oceans over 300 million generations” by P. B. Price and R. C. Bay

Anonymous Referee #2

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The authors report an interesting study on chlorophyll (Chl) and other biological compounds preserved for thousands of years in different ice cores of Greenland and Antarctica. Briefly, the results demonstrate that abundance of Chl fluctuate along ice depth, which corresponds to annual modulation of local summers, and therefore deep ice cores could be used as a proxy for quantifying marine picocyanobacteria transported from lower to higher latitudes in the past. The authors used cultivation-independent methods (fluorescence spectrometer, flow cytometry, epifluorescence microscopy and differential interference contrast microscopy) to analyze melted filtered ice samples, which go beyond the limitations of using traditional culture methods. Experimental controls are well suited for the methods, results are well interpreted and

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discussed, and the conclusions are supported by the results. I recommend the publication of this manuscript in Biogeosciences, but also I have a few suggestions for improvements.

Major comments:

1. The manuscript title says the polar ice cores can work as a proxy for marine bacterial evolution over 300 million generations. It is unclear to me how you calculated this number of generations. As described in the manuscript, ice cores up to ~150.000 years (GISP, Greenland) were studied, but there is no reference or explanation how you extrapolated the core ages to microbial generations (e.g. which cell doubling time was used?). Please explain.
2. Counting cells from melted ice using EFM seems to me highly influenced by the amount of volume of ice that is filtered. If very low concentrations of cells are found, one must filter enough volume to have a more accurate count (if enough sample is available). On P6545 L15 it is said that melted ice passed through a 0.2 μm filter, but how much volume was filtered? The result of ~10 cells . mL⁻¹ reported on same page Line 19 is maybe on the lower threshold for counting total cells in these samples. Therefore I suggest reporting the volume of melted ice used for filtrations.

Minor comments:

1. P6540, L26: FSC and SSC abbreviations appear the first time, but are defined only on P6541 L6. I suggest you define abbreviations the first time they appear.
2. P6542, L14: Replace “BSF” by “BFS”.
3. P6546, L6: Regarding “more than half of the cells”, please be more specific reporting how much cells survived after freezing at -30°C. Did Pro, Syn and Ostreo endured the same after freezing?
4. P6546, L12: Correct reference is Marie et al. (1999) (and not “1990”).

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5. P6556, L28: "Furthermore, we can set an experimental upper limit of . . .". Please add reference. I suppose it is Price et al. (2009).
6. P6558, L26: Replace reference Bramall 2007 (PhD Thesis) for his/her published paper, if available.
7. P6566, Fig. 1a caption: Replace "BSF" by "BFS".
8. P6567, Fig. 2: Please add the units for y-axis in Figure 2a and 2b.
9. P6568, Fig. 3a: Although (A) shows the stained-filtered melted ice (for total cell count), and (B) and (C) comes from a *Prochlorococcus* and *Synechococcus* culture, comparisons between the three figures is difficult because the scale bar is different on (A). I suggest changing the scale bar from 2 μm to 10 μm on (A).
10. P6569, Fig. 4: Please add scale bar.
11. P6572, Fig 6 caption: There is no description of (I) to (L), please add description of these figures.
12. P6573, Fig 7: Please note that some axes values are missing or displayed incorrectly. (I) y-axis values are missing. (I) to (L) x-axis values are displaced to the right. Please correct.

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