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Biogeosciences Discussions

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 #3

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This manuscript provides evidence for annual fluctuations in the concentration of microbial cells in Greenland and Antarctic ice cores based on autofluorescence of specific amino acids (Trp) and pigments (Chl b and phycoerythrin). These fluorescence signatures can be detected in the ice, and to some degree, in melt samples of the ice. The samples analyzed ranged from a variety of high latitude sites, and in short segments of the WAIS Divide and GISP2 cores, a pattern of Trp and Chl autoflourescence is observed that appears to co-vary with annual cycles.

The smoking gun would be if there was molecular evidence for these specific cyanobacteria in ice cores. According to their arguments, *Prochlorococcus* and *Syne-*

chococcus are continually transported to the polar regions from mid-latitudes and deposited in snow that transforms into glacial ice (the authors provide evidence for seasonal fluctuation but this is only $\sim$25%). In this scenario, such species would be expected to be very well represented in molecular surveys of marine/terrestrial aerosols and glacial ice. I was curious if there is any evidence for the aerial transport of marine *Prochlorococcus* and *Synechococcus* species.

Their technological approach is attractive and the results and quite interesting. In certain cases the authors have over interpreted the data, but nevertheless, I would recommend publishing this article if the authors would kindly clarify some of the points raised below.

Pg 6536, line 19-22 (abstract): The "300 million generation" number is in the title and abstract, but is never described or explained. This seemed important enough to include in the title so please can this be elaborated on in the text.

Pg 6539, line 11-12: Comment and clarification here. First, it is interesting that the cells presumably survive atmospheric transport, thousands of years in the ice, and then almost die instantly when they are released from the ice. My real point here is that the authors interpret the absence of Chl autofluorescence as an indication of cell death. Instead it may simply mean that the fluorescent compounds being measured in the cells simply became bleached or degraded over time, which may not necessarily indicate cell death.

Pg 6540, line 10-13: I don't follow the experimental logic here. What compound is it exactly that they are using as a specific biosignature for heterotrophs? Heterotrophs are organisms that use organic carbon as their electron donor and carbon source. N.B. there may also be lithotrophic microorganisms in the ice.

Pg 6542, line 7: Can the authors please briefly comment on how different the fluorescent properties of "protein-bound tryptophan" are versus that of the free amino acid.

Pg 6542, line 17-19: Interesting idea, but the cells must also travel through the atmosphere to be deposited in glacial ice. This would mean that reversion to a photoactive state would have to occur in situ, i.e., in the ice? Price has been one of the leading proponents for the idea of microbial metabolism under such conditions, but is this what the authors are actually suggesting here?

Pg 6542, line 22-24: Comment on statement "Chl that is either not in cells"; chlorophyll is a lipophilic pigment that will not exist in a functional state as a dissolved impurity in the ice. Secondly, the authors used live/dead staining that measures cell membrane integrity. Hence, there should be data to test the hypothesis they put forward regarding time and membrane integrity.

Pg 6544, line 16-17: Here (and elsewhere) they are discussing how rapid the cells bleach when released from the ice. They also see rapid bleaching when looking at any of the cultures tested. If the ice is transparent to their laser, what is different about being hit with UV when you are in the ice? In other words, how can it be explained that the autoflourescent signatures, presumably cyanobacteria, found are not bleached when they are in the ice (see pg 6542, lines 12-15)?

Pg 6546, line 6-9: Were the 1 year frozen cells tested to see if they were still autofluorescent?

Pg 6551, line 16-25: *Prochlorococcus* and *Synechococcus* do not exist in high latitude marine waters, that much is clear. What cyanos and other chlorophyll –containing phototrophs are common to these regions? I just want to understand how more local environments can they be excluded from this analysis. It seems counterintuitive that the majority of microbes deposited in the ice are coming from the furthest points possible. In describing the results obtained, there seems to be no leeway for an explanation that entertains microbial sources that are in much closer proximity to the high latitude sites examined.

Pg 6554, line 24-26: Perhaps they are protected in the ice (see comment above for

Pg 6546, line 6-9), but what about in the atmospheric trip from mid-latitudes? Would their pigments be expected to survive bleaching on this extended journey in the atmosphere?

Pg 6557, line 2: "depths within 20 um" Can these two datasets really be compared with such accuracy? What was the resolution of the gas measurements?

Pg 6557, line 8-10: The authors are a little ahead of themselves here, but these are precisely the kinds of approaches that are needed *to confirm* that the signals they are observing are picocyanobacteria.

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