

Response to anonymous referee #3 (bgd, 9, C2951-2012) of manuscript by Price and Bay

First paragraph: See the section on transport of picocyanobacteria (and also of similar-size volcanic ash particles and mineral dust from deserts and grasslands) over thousands of kilometers... There is no direct evidence for aerial transport of the specific genera *Pro* and *Syn*. See, however, papers on capture of bacteria in airplanes, balloons, and nets, referenced on P 6553, lines 18-29.

P 6536, line 19-22 (abstract): see response to referee 2.

P 6539, line 11-12: there is extensive evidence for survival of more than a week of atmospheric transport, deposition onto snow, and gradual compaction, with adaptation to subfreezing T and acidic veinover several winter seasons of snowfalls. Many or most probably die when they become incorporated into snow, firm, and then ice without going through the liquid water phase. Many may die when at high enough ice density to become incorporated into liquid (acidic) veins, where they may not be able to survive at very low pH. Up to half of the cells die when the ice melts, due to salinity shock. Our BFS data suggest that at least a third of the Chl survives for many thousand years; in view of the very similar magnitude of Chl and PE intensity seen in FCM at depths from 19 m to 3037 m in GISP ice, it seems more likely that cells die during 1e5 years rather than bleach while in the ice.

P 6540, l 10-13: I replaced heterotrophs with non-cyanobacteria.

P 6542, line 7: I inserted “The BFS distinguishes the autofluorescence of Trp in cells from free Trp by virtue of the fact that, when excited at 224 nm, free Trp has a peak at ~350 nm, whereas Trp bound in cells has an emission peak shifted shortward by up to ~40 nm (Bramall, 2007).”

P 6542, line 17-19: Insert new paragraph at end of text on line 24. “Although the physiology is unclear, it would appear that micron-size cyanobacteria that survive gradual compaction into ice without disruption may recover from sunlight-induced bleaching. Compaction from surface snow into fully dense ice, perhaps ending up in an unfrozen vein, may provide not only the habitat proposed by Price (2000) but also provide protection from photobleaching.”

P 6542, line 22-24: Referee 3 pointed out that non-cellular Chl is lipophilic and cannot fluoresce as a dissolved impurity in the ice. Insert “We plan to use a series of live/dead staining tests over one year to see if the decreasing Chl fluorescence with depth might be due to gradual death of cyanobacteria in ice.”

P. 6544, line 16-17: Here again, we invoke Alex Glazer’s proposal that phototrophs resist photobleaching by de-exciting non-radiatively while frozen in ice.

P. 6546, line 6-9: Rewrite this text: “Using Ruzin’s live/dead stain we found (Fig. 4) that more than half of the cells from cultures of *Pro*, *Syn*, and *Ostreo* retained their autofluorescence (i.e., appeared green in the live/dead test) when samples were removed from the freezer at intervals during a period of one year. Thus, they survived freezing at -30°C without having been in contact

with liquid water, which suggests that wind-borne cells would survive the shock of deposition onto polar snow.

P. 6551, line 16-25: Add the following at the beginning of Conclusions, P. 6557, line 13: “We considered two possible sources of the picocyanobacteria that we studied in glacial ice from Arctic and Antarctic sites: (1) Picocyanobacteria in the marine polar environment are rare, and *Pro* cells are absent. In their cultures and 16S rDNA analyses of picocyanobacteria in polar lakes, Vincent et al. (2000) found oxyphotobacteria they ascribed to the genera *Synechococcus* and *Synechocystis* and occasionally to phycoerythrin-rich isolates similar to *P. marinus*. The problem with this hypothetical source is that the total volume of polar lakes in the Arctic and Antarctic ice is extremely small, and the ratio of *Pro*-like to *Syn*-like cells is extremely low. (2) *Pro* and *Syn* cells in roughly the proportions we observed with FCM are carried by winds from oceans at temperate latitudes and could account for our observation of an annual summer/winter modulation. It is difficult to model accurately the fluxes of wind-borne *Pro* and *Syn* cells that reach the polar regions from mid-latitudes. Nevertheless, we believe the second explanation is the more likely of the two.”

P. 6554, line 24-26: Add the following in line 26: “Protection against bleaching by sunlight during a several-day trip from mid-latitude to polar ice would be offered by the coating of slime on cells lofted from ocean surfaces and by their zeaxanthin pigment.”

P. 6557, line 2: Referee no. 2 raised this same point. We elaborated on the text in response to the question by referees 2 and 3. Ed Brook’s table of gas measurements was shown as having a depth resolution of 10 cm.

P. 6557, line 8-10: This comment by referee 3 does not require a response.