Response to anonymous referee no. 1 (BGD 9, C2825-C2828, 2012)
From Buford Price, first author of manuscript BGD 9, 6535-6577, 2012

Under referee's Specific Comments:

Added at end of Introduction:

"Our first goal was to use our scanning fluorescence spectrometer in the dark at -25°C to measure chlorophyll (Chl) and tryptophan (Trp) as a function of depth in Greenland and Antarctic ice cores with high statistics. We found that those autofluorescing compounds decrease only about a factor three over a depth of ~2300 m and that they show an annual modulation of ~25% between local summer and winter. Our next goal, using differential interference contrast microscopy and epifluorescence microscopy, was to show that the summer-winter modulation of Chl is due to Chl-containing cells rather than to organic slime swept up from ocean surfaces. Our next goal was to compare unstained cells from melted ice with cultures of Chl-rich *Prochlorococcus* and *Synechococcus*, from which we concluded that those submicron-size cyanobacteria bleached rapidly while being viewed in epifluorescence microscopy. With flow cytometry, which enables one to study even rapidly bleaching autofluorescing cells, we showed that every sample of melted ice contains cells that emit red fluorescence when excited at 488 nm and orange fluorescence when excited at 567 nm. Next, comparing stained and unstained cells, we found that ~10% or more of the micron-size cells in polar ice exhibit the two types of autofluorescence. We then did flow cytometric studies of 12 types of micron-size mineral grains commonly found in glacial ice melts, from which we concluded that their autofluorescence was too weak to interfere with our flow cytometric study of bacterial cells in ice. Finally, we evaluated the importance of wind transport of cells from temperate ocean waters onto polar ice, which led us to conclude that the summer-winter modulation of *Prochlorococcus* and *Synechococcus* concentrations in ice cores is best explained by wind transport from mid-latitudes."

Added in red font in section on Materials and Methods:

"Kathryn Johnson, a student of Lisa Moore (U. of Southern Maine), prepared cultures of *Prochlorococcus* MED4, MIT9313, SS120, and *Synechococcus* WH8102(described in Moore et al., 1995). Kate Mackey (Stanford U.) provided *Synechococcus* cultures from five marine locations(to be published). Alex Worden (MBARI)provided cultures of *Ostreococcus* OT495 P67 and OT495 P118(Worden et al., 2004).We stored a few vials of cultures at 4°C for quick access and froze the remainder at -30°C without preservatives for long-term storage.None of the media were found to show autofluorescence."

"We obtained BFS measurements at mm depth intervals along ice cores from GISP2 and D4 (Greenland); from WAIS Divide, Siple Dome, and RIDS (West Antarctica); and from Vostok and South Pole (East Antarctica).

We carried out flow cytometric analyses of samples cut and melted from ice cores at GISP2 and D4 (Greenland); WAIS Divide, Siple Dome, and RIDS (West Antarctica); and South Pole, Dome C, and a USA-Norwegian traverse from South Pole into East Antarctica."

"Figure 2b shows Chl in Greenland ice for three years; the wiggly red line is a spline fit."

"We did not obtain ice from enough depths at Dome C or the U.S.-Norwegian traverse to provide useful statistics on depth-dependence."

"*Pro* cells have not been identified at high latitude (Vincent, 2000). The total populations of *Syn* cells and larger cyanobacteria (Vincent, 2000) found in isolated high-latitude sites such as lakes and cryoconite holes (Christner et al., 2003) are far smaller than could account for the *Syn*-like cells we found at all depths and in all Arctic and Antarctic ice cores." The added reference is to Christner, B. C., Kvitko, B. H., and Reeve, J. N.: Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole, Extremophiles, 7, 177-183, 2003.

Suggested technical corrections:

I followed exactly the referee's suggestions on subtitles; numbering of sub-sections; adding of subtitles; I spelled out phycoerythrin; I added the requested references; I described the wiggly red line in the caption to Fig. 2 (wiggly red line is a spline fit to the data.); and I corrected the axes on Fig. 8.

To clarify some statements, I added the following text in the Discussion:

"They concluded that the excess methane had been generated by methanogens metabolizing duringmore than one hundred thousand years in the ice at a temperature of -10°C."

To explain how one can study evolution over 300 million generations, I added:

"Depending on microbial concentration and availability of large enough quantities of ice at the greatest depths, it may eventually be possible to extend the evolutionary study to 300million generations (for a generation time of 1 day) or720,000 years back in time by analyzing genomes of bacterial cells in ice from Dome Fuji(Motoyama, 2007)."

The changes of labels on the axes in Fig. 8 are shown in the attached figure.

Under references, I added

Christner, B. C., Kvitko, B. H., and Reeve, J. N.: Molecular identification of Bacteria and Eukarya inhabiting an Antarctic cryoconite hole, Extremophiles, 7, 177-183, 2003.

Under Figure Legends, I made the changes indicated in red:

1. (A) The Berkeley Fluorescence Spectrometer (BFS) maps fluorescence of Trp and Chl with 1400 spectra/m in 2 min. Ice protects phototrophic cells from photobleaching. (B) Intensity of Chl autofluorescence in ice cores from 7 polar ice locations: 2 sites in Greenland (GISP2 and site D4), 3 sites in West Antarctica (WAIS Divide, Ross Ice Drainage System (RIDS), and Siple Dome), and 2 sites in East Antarctica (Vostok and South Pole). Depths extend from 120 m to 2300 m; each point is an average over ~10^3 depths per meter and over the 7 locations.

2. …summers; wiggly red line is a spline fit to the data.

8. (A) to (J) show FCM plots of unstained cells from ice cores. Points in gray at low SSC were gated out before plotting Chl *vs* PE. The gain on the Chl phototube was set higher for plots (A), (B), (E), (F), (I), and (J). Plots of kaolinite grains with trigger on 100 units of SSC are shown in (K) and (L). See text for discussion.

9. Concentrations of cells from 8 different locations (including South Pole, Dome C, and the U.S.-Norway traverse in E. Antarctica), taking into account centrifuging and sample volume…

Figure 8 with changes in axes is attached.