Biogeosciences Discuss., 11, C3499–C3501, 2014
www.biogeosciences-discuss.net/11/C3499/2014/
© Author(s) 2014. This work is distributed under
the Creative Commons Attribute 3.0 License.

Biogeosciences
Discussions
Open Access

Interactive comment on "Taxon-specific responses of Southern Ocean diatoms to Fe enrichment revealed by synchrotron radiation FTIR microspectroscopy" *by* O. Sackett et al.

Anonymous Referee #2

Received and published: 15 July 2014

In this study, cells from 4 taxa of diatoms collected from the Southern Ocean at 4 stations around Kerguelen Island were analyzed with synchrotron radiation Fourier transform infrared microspectroscopy (SR-FTIR) to determine levels of biomolecules and moieties such as carbohydrates, silica, amino acids, lipids and proteins. Resulting data demonstrate differences both between taxa and between stations (which have different nutrient and biogeochemical conditions). The taxa show somewhat different responses to the station gradients, demonstrating the importance and value of measuring the physiology and responses of individual taxa of phytoplankton. The study demonstrates the value of SR-FTIR to understanding changes in chemical composition of phytoplankton in the ocean.

This study presents a unique dataset and therefore is of high novelty. The data appear to be analyzed with skill and rigor, and the resulting statistical analyses appear appropriate and robust. I have two general concerns/comments. First, I am concerned about preservation of the samples prior to analysis. The phytoplankton samples were preserved with 1-2% formaldehyde and stored in the dark for 18 months until analysis. It is important that the authors demonstrate that treatment with formaldehyde does not interfere with or alter the chemical composition of the target cells. Additionally, it seems likely to me that some molecular changes occurred during the >year storage period at room temperature. Please discuss sample preservation and provide additional evidence and/or references demonstrating that these concerns are unfounded.

Second, more information is needed to convince the reader of the quantitative aspect of the data. Much of the discussion is based on an assumption that the data are quantitative but I am not convinced of this. Cells were analyzed with a 5um beam, and only the center of each cell was analyzed (not the edges). This is important to keep in mind with diatoms given that the frustule is under-represented in the center of the cell compared to the edges. The authors state that the multivariate model enables for normalization of sample thickness, but I could not find details for this in the paper. Working in transmission mode, the signals will be dependent on the thickness of the cells at the spot analyzed. Without normalizing for this, it isn't possible to separate differences in cellular concentrations vs. cell thickness between cells. How was normalization for cell thickness accomplished?

Specific comments –please provide a table with the number of cells analyzed for each taxa from each station. It is stated that 20-50 cells were analyzed but it is not clear how many were analyzed for each taxa from each station. This information could be added to Table 3. –p7336, line 20: here and throughout the ms, the authors refer to the S-O peak as representing silicate. However most of the Si in diatoms occurs as solid silica, although there may be some silicate (perhaps more precisely described as silicic acid) in a silica deposition vesicle. Please clarify which Si species is being detected

by the technique and use the appropriate term. –p7337, line 5: following the general comment above, do Fragilariopsis have the highest concentrations of phosphorylate compounds, or are they just the thickest? This needs to be clarified in the methods. –p7337, line 28: the clustering in the pooled taxa scores plot (Fig. 5b) is not apparent to me –P7338, line 7: this sentence ("Models that can robustly…") seems circular to me: if a model can discriminate between spectra from different stations, won't it also indicate that these spectra are different? Please clarify/re-write. –p7338, line 16: where is this observation about intraspecific variability in Fragilariopsis shown and described in the results? –p7339, line 23: what were silicate concentrations in the water at these stations? Was this nutrient not limiting, as inferred here? This information could be included in Table 1. This section is also an example of where the quantitative abilities of the technique need to be demonstrated earlier in order to support this discussion. –p7340, line 8: I suggest saying that "The up-regulation of these proteins would be consistent with observations…", as the current wording makes it sound like these proteins were actually measured in the current study. –p7340, line 16: all of these samples are from KEOPS2, correct? If so, I suggest deleting this phrase here, as it makes it some like they are from a different group of samples than the SR-FTIR ones. –p7341, line 2: "could account for increased concentrations" or "could be caused by increased concentrations"? I think it is the latter. –p7341, line 14: it is problematic to consider the effect of sampling time for only this station. If this is to be considered, it should be added for all stations. When were the others sampled? Can the effects of time and station be pulled apart? –p7341, line 23: delete the third word ("and"). More importantly, this first sentence would be much more strongly supported if more evidence was given for the quantitative nature of SR-FTIR. Although the Beers-Lambert law is discussed in the methods, no evidence is given for the actual quantitative abilities of the technique. Thus, I question the ability of SR-FTIR to quantify nutrient status, much less growth rate, of these target cells. Please add supporting information to buttress this claim.

Interactive comment on Biogeosciences Discuss., 11, 7327, 2014.