

***Interactive comment on “Impact of nutrient
starvation on the biochemical composition of the
marine diatom *Thalassiosira weissflogii*: from the
whole cell to the frustule fraction” by C. Soler et al.***

Anonymous Referee #3

Received and published: 1 September 2010

This manuscript reports on the biochemical composition of the whole cell, and chemically treated frustules of the diatom *T. weissflogii* under various nutrient-depleted conditions. The authors applied state-of-art technique of FTIR spectroscopy to analyze biochemical composition of the diatoms which give us informative aspects on the marine biogeochemistry. I find the data and applicability of FTIR technique presented to be a valuable addition to our understanding of how the diatom frustule affects ocean biogeochemistry and I feel this manuscript is worthy of publication in Biogeoscience. However, I do not agree with some of the method and discussion made in the manuscript and

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stated my concerns below. Several shortcomings should be adequately addressed before acceptance of publication.

General Comments:

1) Even under your complete manipulations for nutrient-depleted medium, why the diatoms did not show the decrease in photosynthetic parameters under Si- and N-starved conditions for 7–12 days? It can be recognized that (1) inadvertent nutrient contaminations in the bottle or (2) N and Si-starvations were farther related to photosynthetic parameters than P-starvation. General figure of the relationships between each nutrient-depletion or limitation and photosynthetic parameters should be given in Introduction section to improve our understanding. In addition, the relationship between nutrient concentrations and cell density, and critical threshold of cell quota of each depleted element are the most important indicators to assess nutrient stress. Why you choose photosynthetic parameters as an indicator of nutrient stress? I also strongly recommend showing nutrient concentrations and cell numbers of each batch in the manuscript.

2) Please clarify the accuracy of FTIR-spectroscopy and variation/range of the duplicate bottles. Because this manuscript refers the difference of 1 to several% in diatom biochemical compositions, I often feel too small to distinguish the characteristics among nutrient-starvations. Even the composition seems valid by cluster analysis, the validation of apparent concentration or composition of each element/component should describe in the manuscript. Please show the variation or range of the data obtained from duplicate bottles throughout the manuscript especially for the value on biochemical compositions.

3) I feel the composition of organic matters within the frustules are too much high. In general, mineral ballast contains only several percent organic carbon per ballast mineral (e.g. De La Rocha et al., 2008, *Global Biogeochem. Cy.*, 22, GB4005, doi:10.1029/2007GB003156, references therein). In addition, silaffin (NatSil-1A,

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6.5kDa) react with silicic acid at molar ratio of 86.5 (mol SiO₂:mol silaffin) (Kröger et al., 2002, Science, 298, 584–586); i.e., 37% is consisted by silicon. Please compare to the previous studies. For example, reaction containers should shake to allow dissolution of all frustules when digestion of the frustules by HNO₃ or NaOH because simple centrifugation should make unavoidable adhesion of frustules on the tube walls. Such contamination may affect conclusion of this manuscript. Please describe thoroughly in Material and Methods section. In addition, it is interesting to note that your data imply the effect of burial diagenesis is stronger than heating with strong acid or base treatment when compare the data of diatomite and FAW fraction. After overcoming the shortcomings of the methodology, it would be worth spending time to address this issue.

4) What is less reactive bSi? As discussed in the manuscript (e.g. page 5979), such protein-rich Si can change the remineralization process in the water column, but proteins are less preserved in the diatomite. It is clearly observed in the FTIR-spectra (Fig. 5b, 5c). The peak of 850 cm⁻¹ is major concern (Fig. 5b: absent; Fig 5c: prominent and among highest in 800–1000 cm⁻¹ in FAW). It seems to be changed chemical structure by NaOH digestion. What amount of NaOH used for digesting bSi? Probably, small NaOH addition relative to particulate Si make unintended polycondensation of silicon or particulate amorphous silica (e.g. Loassachan and Toda, 2008, J. Oceanogr., 64, 657-662). In addition, how to rinse the FBW fraction? (Page 5959 lines 7–9) According to Fig. 5, absorbance of proteins (bands 3/4) increased in FBW fraction compared to FAW and TF fraction (summing absorbance 3 and 4), indicating all proteins were remained in FBW with modification of chemical structure. I was confused. This is critically important to this manuscript. I think that simple filtration or centrifugation of FBW could not remove organic substances completely which dissociated from TF fraction due to organic matter adsorption (filtration) or adhesion of the cells on tube wall (centrifugation). Please describe all procedures in the Methods section. If such refractory bSi is valid, you can refer the shortcomings of traditional bSi analysis. I feel that it is the very glorious achievement in this manuscript.

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5) Concerning EPS or TEP production (Pages 5976 lines 2–6; Page 5976 lines 23–26; Page 5977 lines 26–28; Page 5978 line 26–page 5979 line 3). This manuscript repeatedly discuss about organic excretions such as TEP. Co-author Claquin et al. (2008: *Aquat. Microbiol. Ecol.*, 51, 1–11) reported that TEP production is positively correlated with ETR_{max}. The ETR_{max} decreased due to prolonged nutrient-starved conditions especially for PO₄-starvation, however, you referred some reserve products is released in the extracellular media. I thoroughly confused because extracellular C release should be small in the present study based on the previous report (Claquin et al., 2008). Please revise.

6) Page 5975 lines 27–29; page 5978 lines 17–19: I argue that change in amides and amine composition within TF (i.e. Fig. 5a) and/or organic coating can really impact "greatly" on the frustule architecture or physical and chemical properties? Simply thinking, structural proteins may be involved within the frustules, i.e., such proteins can also be seen in the FAW fraction (pending within FBW fraction as General comment 4). Are there any adequate references or supporting data?

Specific Comments:

Abstract

Page5954 line 8: *T. weissflogii* is not ubiquitous diatom but brackish-temperate species (Tomas ed., *Identifying Marine Phytoplankton*, Academic Press).

Page5954 lines 25–27: The last sentence of "...which could imply mechanical and chemical resistance" seems not to be discerned from this manuscript.

INTRODUCTION

Page 5955 line 6: Probably "...is composed of amorphous silica called as biogenic silica".

Page 5955 lines 21–25: The description of the relationship between photosynthesis and nutrient depletions in this section is unclear. I feel that statements in unexpected

fashion, and then please reconstruct the sentences of the importance of measuring photosynthesis under nutrients-depleted conditions.

Page 5955 line 25: I would propose to insert line feeds before “Macromolecules.”

Page 5956 line 23: mesozooplankton

MATERIAL and METHODS

1. The material of culture vessel should be given in Methods section.

Page 5958 line 5: A 5,000 cells mL⁻¹ should not be low abundance. Probably up to several μg Chl-a L⁻¹, i.e., mild blooming condition. Please delete “low-cell-density inoculums”.

Page 5958 line 11: You mentioned 3 growth phases in p.5957 lines 23–25 probably to determine the timing of sampling. I cannot recognize measuring growth phase in this manuscript. I strongly recommend showing cell numbers and nutrient concentrations of each batch in the manuscript. Please revise.

Page 5959 lines 7–9: Simple question. Do heating and NaOH (or HNO₃) treatments on a certain proteins change FTIR-spectrum? It may also affect result. Please spend some time.

Page 5962 line 17: The manuscript mentioned that “...was estimated by integrating the surface area of each band”. How to measure or quantify surface area of the skirt of band distributions?

RESULT

Page 5963 lines 15–16: Probably, “The impact of PO₄ starvation was severer effects on photosynthetic parameters than those of Si(OH)₄ and NO₃.” is more precise phrase. I would argue against the statement of comparing the nutritional status of each element-starvation by measuring only photosynthetic parameters (see also General Comment 1).

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Page 5965 lines 16–25: This paragraph is not result. Already wrote in Introduction and Material and Methods sections. Please delete.

Page 5968 lines 2–4: I lost sight of "as previously described" of labile and refractory bSi fractions. Please revise or add precise references.

Page 5968 lines 11–17: Such short organized results, discussions and cues of next paragraph are completely unnecessary in Result section. Please delete such sections from the Results. There are the others elsewhere.

Page 5968 line 20: 74% and 79%..... What is this two values represent? Meaning unclear.

Page 5968 line 22: Si(OH)₄-starved

Page 5968 lines 23–27; Page 5969 lines 23–26; page 5971 lines 1–5 : The relative composition of amide I, amide II and amine cannot be recognized from the figure 7. Please delete the (Fig. 7) or refine figure 7.

Page 5969 line 2: NO₃ starved

Page 5970 line 11: Refer appropriate figure(s), not Fig. 6.

Page 5970 line 20: ...PO₄-starved cells (Fig. 7: 11%) compared to... control, NO₃-....

Page 5970 line 24: NO₃ starvation was less sever effect on photosynthesis....

Page 5971 lines 10–13: Again, what is this refractory bSi fraction?

Page 5971 lines 27–28: Meaning unclear. "**variations... was** observed ... this ratio was **equal...**"

DISCUSSION

Page 5972 line 2: I would suggest slightly change the subtitle to: Nutrient starved photosynthesis effects on biochemical composition

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Page 5972 lines 3–10: Probably, these sentences are appropriate in Introduction.

Pages 5972–5973 line16: The logic of first paragraph may be poorly constructed. Result, discussion and references shuffle back and forth. Please revise.

Page 5973 lines 1–2: Insert (unpublished data) after "NO₃ starvation".

Page 5973 lines 17–: PO₄ starvation had drastic effects on diatom photosynthetic activities, such as ETRmax decreased That is that N and Si starvations have also drastic effect (ceased cell growth!!) but not detectable as drastic by measuring photosynthetic activities.

Page 5974 line 16: Kröger and Poulsen (2008) noted frustulin and pleuralin are also bSi-related proteins.

Page 5974 line 17: reduction of the relative biogenic silica to what? Please clarify this sentence.

Page 5974 lines 25–29: Too bad, the same two sentences "As cell division....in the cells" are coexistent. Please check.

Page 5975 lines 7–14: I would suggest again delete these Introduction-like and short result sentences. (note: line 9: biogeochemical cycle of **carbon** and especially in **carbon**?)

Page 5975 lines 26–29: I cannot find out that such a shift from Figures 5a and 7. Please describe in the manuscript. I also argue that such change in amides and amine composition within TF (i.e. Fig. 5a) can really impact "greatly" on the frustule architecture?

Page 5976 lines 3–5: "The fact....architecture." This sentence underspecify the impact on frustule composition and architecture. Please revise.

Page 5976 lines 20–23: The manuscript mentioned as "stopped the cell division". Please show the result of change in cell number during incubation as described in

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General comment 1.

Page 5976 lines 16–23: I don't make sense these statements concerning "Carbohydrates were probably stored in the cells" because carbohydrates were relatively rich in FAW fraction of all nutritional status of the cells (Fig. 7).

Page 5977 line 1: In the result section (page 5965), you mentioned the decrease in bSi contribution of the whole diatom cells grown only under Si-depleted medium. Please revise.

Page 5978 line 26–page 5979 line 3: I cannot make any sense of those sentences. For example, the TEP cloud enhance the growth of bacteria and subsequently other protist or viruses, and hence, it seems less protective mechanism. Such stressed diatoms increase sinking rate by producing TEP or heavy frustule to sink the ocean depths to sequester such parasites and to protect healthy cells in the upper mixed layer (see Raven and Waite, 2004, *New Phytol.*, 162, 45–61).

Moriceau et al., 2007 cannot find in the reference list.

Page 5980 lines 2–3: Suggest as: PO_4 starvation is an important factor for the change in the composition of organic matters in the frustules. (see also General Comment 2)

Page 5980 lines 10–11: What was drastic effect of PO_4 starvation compared to N or Si starvation? A several percent changes in the amine composition seem small.

REFERENCES

Please capitalize the initial of the phytoplankton genus.

I glanced over the reference, Moriceau et al. (2009), Osborne and Geider (1986), Staats et al. (2000), Theodorou et al. (1991) and Vartanian et al. (2009) are not good.

Passow (2002): (TEP)

Please check thoroughly!

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Thank you.

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