

Interactive comment on “The role of iron species on the competition of two coastal diatoms, *Skeletonema costatum* and *Thalassosira weissflogii*” by S.-X. Li et al.

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Dear Editor and ReviewerijŽ Thanks for your suggestions and the reviewer’s comments. According to the reviewers’ comments and your suggestion, we have revised our manuscript (bg-2013-581) thoroughly. Now we resubmit it to you. I hope it could fit your requirement.

Best Regards, Prof. Dr. Li shun-xing

An itemized response to reviewer’s comments is listed as follows:

General comments (a) Line 16 on page 19609: Authors have to indicate what kind of

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trace metals clean reagent to remove surface-bound Fe you used and references for the trace metals clean reagent.

Thank you for your good suggestion. The trace metals clean reagent to remove surface-bound Fe we used has been shown as follows in Line 95-106 on Page 5: Iron was washed from the cell surface using a trace metal clean reagent (Tovar-Sanchez et al., 2003, 2004). The preliminary experiments indicated that iron adsorbed on the cell surface could be removed using a trace metal clean reagent. In the trace metal clean reagent, oxalate was used as the reductant to remove surface adsorbed trace metals from phytoplankton cells and other particles. To the oxalate solution, hydroxylamine, perchlorate, and 1, 10-phenanthroline were added. Next, the pH was adjusted to 8 with 10 mol L⁻¹ NaOH and the solution was heated in a water bath to 50 °C for 15 min. Immediately, while still hot, the solution was transferred to a 250 mL Teflon separating funnel and extracted twice with 6 and then 4 mL of 1, 2-dichloroethane, and then transferred to a trace metal clean Teflon separating funnel and extracted again with 4 mL of 1, 2-dichloroethane. In each extraction, the organic phase was discharged and aliquots of the reagent were collected. The clean oxalate solution was then transferred to LDPE bottle.

(b) 3.1, 3.2, 3.3, 3.4 in 3 Results and discussion (Pages 19612, 19613, and 19614): Without indicating the cell density (growth rate) and cell size after culture experiment for 4 days, authors should not discuss the iron adsorption and absorption by the cells of *T. weissflogii* and *S. costatum* under different nutrient regimes. After culture experiments for 4 days, the cell density (1*10⁴ cells/ml at start) and cell size of *T. weissflogii* and *S. costatum* may be remarkably different under different nutrient regimes and the cell density and cell size of *T. weissflogii* may be remarkably different from those of *S. costatum*. Therefore, authors need to present the cell density and iron adsorption and absorption per cell surface area and per cell volume, as new Figures, in addition to the cellular iron (fmol/cell, Figs 1 and 2) for the culture experiments of *T. weissflogii* and *S. costatum* under different nutrient regimes. So, you can suggest that “the degree of

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influence of macronutrient additions on the cell size of *S. costatum* was more significant than that of *T. weissflogii*, – (Line 22–25 on page 19612) and “Fe adsorption was most likely to be affected by the following five factors: (1) the amount of surface basic groups on the cell surface, (2) the cell size, – (from line 25 on page 19612 to line 2 on page 19613).

Your good suggestion has been adopted. The cell density (growth rate) after culture experiment for 4 days has been described as follows in Line 222-232 on Page 11: 3.2 Cell density of *T. weissflogii* and *S. costatum* under different nutrient regimes The influence of the additions of nitrate and phosphate on the cell density of *T. weissflogii* and *S. costatum* has shown in Fig.1. The results indicated that N addition in the range of 8.0 to 64.0 $\mu\text{mol L}^{-1}$ could stimulate cell growth and such stimulating effect was the most significant at 32.0 $\mu\text{mol L}^{-1}$ N. However, algal growth could be inhibited by 64.0 $\mu\text{mol L}^{-1}$ N, the influence trends were reverse. When P concentrations from 1.0 to 2.0 $\mu\text{mol L}^{-1}$, the influence on growth of *T. weissflogii* and *S. costatum* was significant, but under high concentration of P ($>2.0 \mu\text{mol L}^{-1}$), algal growth also could be inhibited. So, cell growth rate was controlled by macronutrient concentration, similar results have been reported (Li et al., 2014; Liu et al., 2014). At the same time, the influence of macronutrient on the growth of *T. weissflogii* was more obvious than *S. costatum*, i.e., the cell density was lower.

(c) 3.6 in 3 Results and discussion (Pages 19615 and 19616, Table 1): We are very interested in the distribution (Table 1) of iron species (dissolved, colloidal and particulate Fe concentrations) from the culture medium under different nutrient regimes. We would like to know each iron species amount per cell, which is calculated from each iron concentration and the cell density after culture experiment for 4 days under different nutrient regime. So, we can know which culture media can produce more extracellular organic ligands complexing with iron per cell. Please add the data (cell density and each iron species amount per cell) into Table 1.

Thank you for your good suggestion. The influence of the additions of nitrate and
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phosphate on growth of *T. weissflogii* and *S. costatum* has shown in Fig.1. The data of each iron species amount per cell have been added into Table 1.

Minor comments (a) Line 20 on page 19606: Cancda (Ottawa, Canada) – Canada (Ottawa, Canada)

The “Cancda (Ottawa, Canada)” has been revised as “Canada (Ottawa, Canada)”.

(b) Line 21-22 on page 19612: Iron diffusion decrease (or an increase) with a decreasinge (or increasing) of cell size. – Iron diffusion decreases (or increases) with decreasing (or increasing) cell size.

The “Iron diffusion decrease (or an increase) with a decreasinge (or increasing) of cell size” has been revised as “Iron diffusion decreases (or increases) with decreasing (or increasing) cell size”.

(c) Line 11 on page 19613: *P. donghaiense* – *T. weissflogii*

The “*P. donghaiense*” has been revised as “*T. weissflogii*”.

(d) Line 24 on page 19614: Fig. 3 – Fig. 2

The “Fig. 3” has been revised as “Fig. 2”.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/10/C8628/2014/bgd-10-C8628-2014-supplement.pdf>

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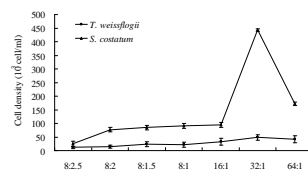


Fig. 1. Influence of the additions of nitrate and phosphate on growth of *T. weissflogii* and *S. costatum*. Data are mean \pm SD ($n=3$). (Fe concentration 1.8 $\mu\text{mol L}^{-1}$)

Fig. 1.

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