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Biogeosciences
Discussions

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Interactive
Comment

Interactive comment on "Bioavailability of organically bound Fe to model phytoplankton of the Southern Ocean" *by* C. S. Hassler and V. Schoemann

C. S. Hassler and V. Schoemann

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Reply to D. Hutchins (Referee)

We would like to thank the anonymous referee, D. Hutchins and A. Tagliabue for the effort they put in formulating comments that will improve this manuscript. They pointed some important points in the interpretation of our results and some important undiscussed areas that merit more attention. As per guidelines, a revised version of our manuscript will be shortly submitted on-line.

Response to general major comments

Regarding known stability constant of saccharides with iron: Acidic polysaccharides

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are long known to bind cationic metal, including iron (Decho et al. 1990), but measurements of their effect on the chemical speciation of iron are scarce. We indeed were not able to measure any reduction of iron lability in AQUIL or UV-irradiated Southern Ocean seawater (collected during the SAZ-SENSE [Feb. 2007] and CASO/GEOTRACES [April 2008] voyages at 54.0 S, 145.9 W and 45.4 S, 145.4 E, respectively) in presence of 1nM Fe and 15nM GLU or DEX. For this purpose we were using in the competitive exchange ligand adsorptive cathodic stripping voltammetry (CLE-AdCSV) in presence of 10 μM TAC as per Croot and Johansson (2000). The only reported stability constant for saccharides with iron under low iron condition was indeed low (log KFeprimeL 109 M-1 for gluconic acid, Croot and Johansson, 2000), suggesting saccharides are within the class of the low affinity ligands (L2). A recent study showed that bacterial exopolymeric substances (mainly polysaccharides) form labile forms with iron if preequilibrated for 24 h but strong complexes with iron if let to equilibrate for 9 weeks (Hassler et al., submitted to Marine Chemistry). The following text was added in the introduction: To date, the only reported stability constant for a mono-saccharide (gluconic acid) with Fe prime in seawater is 109 M-1 (Croot and Johansson, 2000), suggesting that saccharides are within the class of the low affinity ligands (L2). A recent study showed that bacterial exopolymeric substances (mainly poly-saccharides) form weak complexes (fully labile, log KFe prime L $<$ 11 M-1) with iron if pre-equilibrated for 24 h but strong complexes with iron if let to equilibrate for 9 weeks (non-labile, log KFe prime L $>$ 13 M-1; Hassler et al., submitted). In this case, detection of the stability constant was constrained by the analytical window of the CLEAdCSV.

Regarding the lack of discussion of the negligible bioavailability of the siderophore DFB and the organic amine HBED (Fig 2), coupled with the fact that these are the only two ligands they found to be 100% in the $<$0.02 um soluble size class (Fig. 3): The fact that the two strongest ligands used in this study (DFB and HBED) are relocating iron in the soluble phase but decrease iron bioavailability is now discussed in term of relationship between iron solubility and bioavailability. DFB and HBED are small organic ligands with high stability constant (e.g. log KFe(III)L from 21.6 - 26.5, see

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Maldonado et al., 2005) amongst the stronger ligands class (L1) currently reported in the Ocean. Therefore such strong ligands will relocate iron in the soluble fraction as observed here. Previous studies have demonstrated that siderophores were efficient to solubilise colloidal forms of iron (Borer et al., 2005). With a rapid comparison of the log of the stability constants observed for Fe(III)DFB (21.6 - 26.5 M-1, see Maldonado et al., 2005) and the interaction of Fe(III) with the biological transporters of a diatom (17.6 M-1 for T. weissfloggi; Harrison and Morel 1986, Hudson and Morel 1990), one can easily realise that DFB could be efficient in decreasing iron bioavailability, except in the case that the species specifically excretes siderophores or possess associated receptors. Here, as for previous studies (Maldonado et al., 2005, Hutchins et al., 1999, Wells et al., 1994), we report that DFB and HBED are efficient in decreasing iron bioavailability to phytoplankton. These results suggest that the bioavailability of organic forms of iron is not directly related to iron solubility. From all our results it seems that colloidal iron might be a critical fraction to define its bioavailability to phytoplankton. This is now added in the text (see below answer to comment on page 1689).

Regarding the justification for the use of T. antarctica Comber: As the referee pointed out this strain was isolated from Norway. However, this strain is widely present in the SO (e.g. Sarthou et al. 2005; Armand et al. 2008). In addition it was maintained in SO water (45.4 S, 145.4 E) in our lab for two years before being used for the present study. We therefore consider that this strain is of environmental relevance to the Southern Ocean.

Regarding consideration of the characteristics of the SO regions where the other three cultures were isolated and impact on Fe uptake and Fe:C results: Concerning the variable origin (neritic vs. oceanic) of the strains used, all the strains were at least maintained for 1 year in Southern Ocean water prior to this work (see reply to anonymous referee). In addition, these strains are all reported in pelagic HNLC region of the SO. It is therefore likely that difference related to their place of isolation would have disappeared. Finally, Fe:C ratio of 16-20 μmol/mol were previously reported in the

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Southern Ocean (Schoemann, Hassler et al., in preparation; Hassler and Schoemann, 2009; Twining et al. DSR 2004). This information is now added in the Methods section: All strains were maintained at least for 1-year in open Southern Ocean filtered seawater, collected during the SAZ-SENSE oceanographic voyage using trace metal clean techniques, with dissolved Fe from 0.2 to 0.3 nmol L-1 (Lannuzel et al., submitted). Cultures were transferred into filtered seawater (0.2 μm, Sartorius membrane filter cartridges Sartobran) collected during the ISPOL oceanographic voyage (68 S, 55 W, Lannuzel et al., 2008) at least six weeks prior to experimentation.

Regarding low Fe:C uptake ratios and physiological status of the Fragilariopsis isolate: Similar Fe:C $<$ 1 μmol:mol were previously found (Schmidt and Hutchins 1999, Maldonado and Price, 1996) which is now added in the text. We are therefore confident with such low Fe:C. The Chl a observed by epifluorescence and carbon uptake all showed that Fragilariopsis was active. We discussed the high amount of extracellular Fe in terms of exopolymeric substances production. These references are now added in the revised version of the MS. See below for detailed answers about physiological status and the limitation in the use of short term uptake rate. Similar points were also answered to the anonymous referee. Any link between short-term uptake Fe:C and Fe limitation or requirement has been removed and a statement was added to clearly show this limitation in the Discussion: Because steady-state Fe:C can be quite different that short term uptake Fe:C, the Fe:C ratio determined in this study were not used to discuss Fe biological requirement or strength of limitation.

Response to comments in the Abstracts:

1) The text has been changed, the statement about strength of Fe limitation as been removed see previous answer and the text now reads: Short-term uptake Fe:C ratios were inversely related to the surface area to volume ratios of the phytoplankton.

Response to comments in the Introduction:

1) We agree and this sentence has been removed.

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2) This point was removed as per suggestion.

Response to comments in the Methods:

1) Some text is now added in the methods section: Due to their lower A/V ratio of the colonies, Phaeocystis cells in colonies are expected to have higher half-saturation constants for growth than solitary cells. It was previously observed that Fe addition could have an effect on the morphotype dominance (colonies vs. solitary cells) of Phaeocystis antarctica with proportionnally more solitary cells under low Fe conditions (Becquevort et al., 2007).

Solitary and colonial cells were counted as in Becquevort et al. (2007). Samples to determine P. antarctica abundance were preserved with glutaraldehyde (0.5% final concentration) for free-living cells and with gluteraldehyde-Lugols solution (1% final concentration) for colonies. Free-living cells were enumerated by epifluorescence microscopy after 4-6-diamidino-2-phenylindole (DAPI) staining (Porter and Feig 1980). Colonies were enumerated by inverted light microscopy according to the method of Utermöhl (1958).Colonial cell numbers were estimated according to Mathot et al. (2000).

2) As mentioned above and in the MS, light levels were chosen to be saturating. Please see the answer to following point on how the saturating light level was defined. For Phaeocystis a past study has shown no difference in intracellular iron for 60 and 120 μmol photons m-2 s-1 (Schoemann et al., 2001). In addition, at these low light intensities and absence of UV (white fluorescent light, polycarbonate containers), photo-degradation of organic ligand is unlikely to occur. The text now discusses the potential photo-dissociation of the Fe organically bound considering both the nature of organic ligands and the experimental conditions. The following text was added in the Methods section: Light level was chosen to provide near optimal maximum quantum yield (Fv/Fm) above 0.58 (Water-PAM, Heinz Walz GmbH) under iron replete condition (AQUIL media, Morel et al., 1975); light was adjusted using neutral density filters.

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Some text is added in the discussion section as well to discuss photolability: Photodissociation of inorganic and organic forms of iron (e.g. Barbeau et al., 2001; Borer et al., 2005; Maldonado et al., 2005) was previously shown to improve its bioavailability. However, not all organic ligands are photolabile, amongst siderophores aquachelin is photosensitive (Barbeau et al., 2001) but DFB and CAT are photostable (Maldonado et al., 2005). Alginic acid dissociated when exposed to UV light (313 nm) but not when exposed to visible light (400nm; Kojima et al., 2001). Monosaccharides containing carboxylic group were photostable (Kojima et al., 2001). However, under 50% PAR light and temperature of 1-2C, the presence of μmolar concentrations of mono-saccharides (Glucaric acid; Oztürk et al., 2004) induced an increased concentration of Fe(II) but no difference in H2O2 and organic peroxides concentrations. Under conditions of irradiance and temperature used in this study, using mathematical expression from Kuma et al., 1995, the rate of reduction would be negligible. This suggest that photolability of organically bound Fe would not be significant in the present study and thus cannot explain the enhanced Fe bioavailability measured.

3) Batch cultures were harvested during the exponential growth phase, 15 to 20 days after inoculation. No sufficient counts were done to accurately report a growth rates in the present study, but this was shown to correspond to the exponential phase in previous cultures of the same strains. Moreover, two of the strains used in this study (Chaetoceros sp. and Phaeocystis sp.) were used to study iron bioavailability and physiological responses during acclimation to low iron concentration (Hassler et al., in preparation). These strains were grown under iron-replete condition (AQUIL) and iron-limited conditions (AQUIL low Fe and SO water with [Fe]= 0.3 nM). Under replete Fe conditions, the light level was adjusted to get an Fv/Fm indicative of healthy cells (0.55-0.65). During acclimation to SO water the growth rate, the Fv/Fm and the cell size all decreased by 40-65%, 20-35% and 15-22%, respectively. In addition, Phaeocystis was forming large colonies ($>$ 300 μm) in AQUIL and was mainly present as solitary cells in the SO, a sign that Phaeocystis was indeed iron-limited. The growth rate and Fv/Fm for Phaeocystis and Chaetoceros in SO water were 0.14 and 0.15 d-1 and 0.46 and

0.41, respectively. Here it was assumed that if smaller species were iron-limited then larger species, more prone to iron limitation, were also iron-limited. The text of the MS will be changed accordingly. This information is now stated in the Methods as follow: Two of the strains (Chaetoceros sp. and Phaeocystis sp.) used in this study were used to study iron bioavailability and physiological responses during acclimation from iron-replete (AQUIL) to low iron concentration (SO water with [Fe]dissolved = 0.3 nM; Hassler et al., in prep.). Consistent with the occurrence of iron limitation in SO water, growth rate, Fv/Fm and cell size all decreased by 15-65%; the growth rate and Fv/Fm for Phaeocystis and Chaetoceros in Southern Ocean water were 0.14 d-1 and 0.15 d-1 and 0.46 and 0.41, respectively (Hassler et al. in prep.). In addition, Phaeocystis forming large colonies (> 300 μm) in AQUIL, was mainly present as solitary cells in the SO water as being observed here, a sign that Phaeocystis was indeed iron-limited (Becquevort et al., 2007). It is reasonable to assume that larger species (Thalassiosira and Fragilariopsis), more prone to iron limitation, are also iron-limited in this study.

4) We agree that POC analyses can be planned for future work. However, both diatoms and Phaeocystis produce EPS which will account in the POC and would lead to the problem of normalizing intracellular Fe with extracellular carbon (e.g. EPS from the chain forming diatoms and Phaeocystis colony mucus)

5) We agree with the comment of the referee and organic ligands affinity for Fe measured in surface seawater collected during ISPOL voyage was shown above. However, because the ISPOL water was kept in the dark and room temperature for 4 years prior being used, it is expected that most of organic ligands would have been altered, therefore most likely minimizing their impact on iron chemical speciation in our work. In addition, the important increase of particulate iron, observed following 1nM Fe enriched, suggest that natural ligands would likely be saturated and thus present at lower concentration than 1.3nM (which correspond to the total Fe concentration in this experimental treatment). The organic ligands studied were pre-equilibrated with 55Fe in miliQ water, therefore natural organic ligands (from ISPOL water) were absent. In

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addition Fe was shown to require up to 24h to exchange from organic ligands (e.g. Gerringa et al., 2007; Laglera and van den Berg 2009). For these reasons, it does not seem that the presence of natural organic ligands (from ISPOL) does significantly affect our interpretation.

6) For this type of work only freshly made batches of 55Fe with high specific activity (typically above 80 as stated in the MS) are used. Additional information has been added in the text to provide typical signal in the control treatment, blanks and counting accuracy. Because of the low level of 55Fe used in the control treatment we used relatively long time of incubation (16h) to get enough signal. Under natural conditions (with low level of Chl a) we typically filtered 800 ml up to 1L per treatment. The total amount of 55Fe added compared to dissolved Fe in the control was corrected to 6% as it was between 5.4 and 5.7 %. The added text is: Each sample was analysed three times in presence of four blanks of either non-radioactive seawater or filter. Typical values of blanks were 6 and 10 counts per minutes (cpm) for seawater and filters, respectively. The averaged value of the blanks was susbtracted to the cpm obtained in the sample. Counts per minutes were then converted into disintegration per minutes (dpm) taking into account the radioactive decay and custom quench curves. Counting errors were $<$3% and the maximum standard deviations of 1 % and 5% were obtained on the three readings of seawater and filter samples, respectively.

As an example, in the control treatment, the low level of 55Fe added typically gives 71 plus or minus 3 dpm in solution (2 mL sampled for Fragilariopsis) and 1240 plur or minus 31 dpm in the filter (after the oxalate wash of 200 mL filtered Fragilariopsis culture).

7) The original reference (Tovar-Sanchez et al., 2003) on this washing technique is now added. Hassler and Schoemann 2009 is now published: Limnol Oceanogr. Methods 7:479-489.

8) We agree but we had to deal with space constraints in the incubation cabinet.

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Response to comments in the results and discussion:

1) The fact that 2h and 16h C uptake at not statistically different at a level of 95% of confidence (t-test) as well as Fe:C ratios calculated using the 16h C uptake has been added in the text. Please note that an experimental problem prevented us to obtain a 16h C uptake value for Phaeocystis. The following text as been added in Result section 3.4: No statistical difference ($p > 0.05$) was observed between carbon cellular uptake rate following both 2 h and 16 h incubations. In the control treatment, carbon uptake rate following 16h was between 1.4-fold smaller to 1.2-fold higher than the uptake rate measured following 2 h incubation. An experimental problem prevented us to measure carbon uptake following 16 h incubation for Phaeocystis.

And section 3.5 :Similar results were obtained with Fe:C uptake ratio using the carbon uptake rate measured following 16h incubation. For the control treatment, the short-term Fe:C ratios calculated using the 16 h carbon uptake data were 27.7 μmol:mol for Chaetoceros, 3.2 μmol:mol for Thalassiosira, and 0.7 μmol:mol for Fragilariopsis. Following a 1nmol L-1 Fe addition, the Fe:C uptake ratio increased for Chaetoceros (46.4 μmol:mol), Thalassiosira (14.9 μmol:mol) and Fragilariopsis (1.6 μmol:mol). Please note that the standard deviations are shown in the revised version.

2) Iron uptake normalized using the surface area is now shown in Fig. 1 (panel c). As long as cell density is not too high in the experimental solution to induce any bulk depletion of the iron present in solution during the duration of the experimentation, then Fe uptake should not be dependent on cell density nor surface area. It is true that cell density might affect Fe uptake, if cells are producing significant amount of strong organic ligands (e.g. siderophores) that will affect iron bioavailability. For these reasons, we would suggest that the lowest cell density allowing a good measurement should be preferred.

3) The authors agree here, unfortunately little is known on the bioavailability of Fe bound to CAT or PIX. Report of enhanced iron bioavailability to eukaryotic phytoplank-

ton in presence of PIX and CAT were previously shown (Hutchins et al., 1999; Maldonado et al., 2005). However, the underlying mechanism is still unknown.

4) We decided to adopt the expression of our results in term of Feext:Feint.

5) This results does not seem to be a coincidence as we obtained such results also in the field (Schoemann, Hassler et al. unpublished data from the subantarctic zone; Masson, Schoemann et al., unpubl. data from the Bellingshausen Sea). and other labworks (Hassler, unpubl. data). This stresses that no direct link exist between iron solubility and its bioavailability. In fact, our results suggest that organic ligands relocating iron mostly in the colloidal fraction might be more important in defining its bioavailability. Both inorganic and organic forms of colloidal Fe have been shown to be bioavailable to diatoms (Rich and Morel, 1990; Nodwell and Price, 2001; Chen et al., 2003). Photo- and chemical-reduction as well as siderophores favour the solubilisation of inorganic Fe colloids (Johnson et al., 1994; Borer et al., 2005). Organic colloidal iron is known to be formed as a results of diatom growth and grazing (Zhang and Wang 2004), potentially relieving iron limitation (Barbeau et al., 1996). Organic colloidal iron is present in surface waters (e.g. Hunter and Boyd, 2007). Therefore this suggests that organic ligands, present in the colloidal fraction, might be important for iron bioavailability and limitation. The following text has been added: Previous studies have demonstrated that siderophores were efficient to solubilise inorganic colloidal forms of iron (Borer et al., 2005). By comparing of the log of the stability constants observed for Fe(III)DFB and the interaction of Fe(III) with the biological transporters of a diatom (17.6 M-1 for T. weissfloggi; Harrison and Morel 1986, Hudson and Morel 1990), one can easily realise that DFB could be efficient in decreasing iron bioavailability, except in the case that the species specifically excreted siderophores or associated receptors. Here, as for previous studies (e.g. Maldonado et al., 2005, Hutchins et al., 1999), we report that DFB and HBED are efficient in decreasing iron bioavailability to phytoplankton. These results suggest that the bioavailability of organic forms of iron is not directly related to iron solubility. Relating iron bioavailability with iron solubility for model phytoplankton

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(this study) and natural assemblages from the Southern Ocean (Schoemann et al., in prep.) suggested that colloidal iron might be a critical fraction to define its bioavailability to phytoplankton. Additional work previously published support this observation. Both inorganic and organic forms of colloidal Fe have been shown to be bioavailable to diatoms (Rich and Morel, 1990; Nodwell and Price, 2001; Chen et al., 2003). In addition, organic colloidal iron are known to be released as a result of the growth and grazing of diatoms (Zhang and Wang 2004), potentially relieving iron limitation (Barbeau et al., 1996). Finally, organic colloidal iron is present in surface waters (e.g. Hunter and Boyd, 2007).

Response to comments in the Discussion:

1) Regarding the use of short-term Fe:C to infer iron limitation: we agree with the reviewer and statements relating Fe:C to Fe limitation/requirement have been removed from the text. In fact we now state clearly that Fe:C ratios determined by short term uptakes are not steady state Fe:C ratios and therefore not likely to be directly related to iron limitation/requirement. The relationship between Feext:Feint and Fe:C against A/V is nonetheless interesting, it was thus maintained in the text. Previous studies showed that Fe:C for diatoms could not be solely explained by their provenance (oceanic vs coastal; e.g. Maldonado and Price 1996). In addition, the study of Twining et al. 2004 revealed that pelagic plankton isolated from the Southern Ocean had higher Fe:C ratios for smaller cells. However, the need for further studies and the importance of other factors, as pointed by this reviewer, such as the provenance of the species (neritic vs pelagic, e.g. Sunda and Huntsman 1995; Maldonado and Price 1996), growth irradiance (Sunda and Huntsman 1997; Strzepek and Harrison 2004), iron limitation and requirement (e.g. Maldonado and Price 2001) are now included in the revised version: However, other factors have previously been recognised to affect Fe:C ratio, such as the provenance of the species (neritic vs pelagic, e.g. Sunda and Huntsman, 1995; Maldonado and Price, 1996), growth irradiance (Sunda and Huntsman, 1997; Strzepek and Harrison, 2004), iron limitation and requirement (e.g. Maldonado and Price, 2001).

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2) We agree that several infusions of iron were enough to obtain a large bloom of the strain during SOIREE and this information has been added in the text (Discussion): It is to be noted that four iron infusions were enough during the SOIREE experiment to induce a shift in phytoplankton community towards Fragilariopsis kerguelensis after 6 days (Boyd et al., 2000).

Regarding the use of short-term Fe:C to infer iron limitation: we agree with the reviewer and statements relating Fe:C to Fe limitation/requirement have been removed from the text. In fact we now state clearly that Fe:C ratios determined by short term uptakes are not steady state Fe:C ratios and therefore not necessarily directly related to iron limitation/requirement. The following statement has been added in the Discussion: Because steady-state Fe:C can be quite different that short term uptake Fe:C, the Fe:C ratio determined in this study were not used to discuss Fe biological requirement or strength of limitation.

Because of limited length for our reply, please refer to the response to referee #1 for the list of references cited.

Interactive comment on Biogeosciences Discuss., 6, 1677, 2009.