Replies to Referee #2

Thank you very much for your helpful suggestions and critical comments. Below are our responses to all your comments.

Comments:

The relationship between available Fe and dissolved Fe (measured in the paper) is complex to define - hence there are always issues in relating dFe to nutrient status. In addition a paper by Ryan-Keogh et al 2013 (L&O) demonstrates how the iron-stress is often only apparent when nitrate is also available in the system - i.e if both Nitrate and Fe are low the system may reduce biomass without showing significant iron-stress. This should be discussed in this paper.

We totally agree on your comment that the relationship between bioavailable Fe and D-Fe is complex. For example, particulate Fe can also support for the growth of phytoplankton assemblages in the Sea of Okhotsk (e.g., Sugie et al., 2013) – This has already been mentioned in the Sect. 4.2. Ryan-Keogh et al. (2013) found that phytoplankton Fe stress developed during the transition from the pre-bloom to peak bloom conditions in the high-latitude North Atlantic and was more severe for larger cells. Also, Fe stress was reduced in regions where macronutrients were depleted following the bloom. However, it is difficult for us to refer this paper to our manuscript, because their study area and analytical techniques differed from ours. Additionally, Ryan-Keogh et al. (2013) did not mention phytoplankton species in their bottle incubation experiments. Therefore, we gave up on discussing this issue in our revised manuscript.

Much weight is given to the Fd/Fld ratio - not only to other phytoplankton species display this switch - but we should consider that there will be a mixed response of different diatoms to this ratio as well. A genetic analysis of Fd and Fld gene diversity in the communities would be of benefit in this study and may help explain situations where the community does not respond as predicted.

Yes, there could be a mixed response of different diatoms to the Fd index. To minimize the effect of differences in diatom species on the index, we discriminated micro-sized diatoms from the total phytoplankton in terms of Fe requirements depending on cell size. However, we do not agree on the comment that genetic analyses of Fd and Fld gene diversity in the communities would be of benefit in this study. Since the Fd and Fld gene sequences derived from marine diatoms have still been very scarce – In particular, little sequence data are available for the diatoms' Fld genes (see the NCBI nucleotide database with BLAST), such genetic analyses in the community level are impracticable at present. Therefore, we have little discussed this issue in our manuscript (also see our response to your next comment).

Section 4.1 the relationship between dFe and Fd index is presented as being a possible marker for in situ fe stress. I feel this comment is a little strong – there is a high range (from 0.2-0.6 in Fd index with little variation in dFe) i.e there are many different Fd index's at the same dFe concentration. This should be addressed.

We have modified the sentences in the revised manuscript:
Values of the Fd index significantly correlated with levels of D-Fe (Fig. 8a), indicating that, in general, the Fd index could be used as a diagnostic Fe stress marker for the micro-sized diatoms in this area. However, it should be noted that, in principle, the Fd index should be varied with intracellular Fe levels rather than D-Fe concentrations in seawater. Additionally, the Fd index can also be changed among species in the micro-sized diatoms. Therefore, there could be various Fd index values at a D-Fe concentration.

Figure 12 should perhaps lead to a discussion on id Fd index is better than Fv/Fm as a maker of Fe-stress. Potentially both require the artificial addition of Fe in bottle experiments to demonstrate they both increase with increasing available Fe to fully interpret these indices in complex systems?

We agree on your comment. However, in practice, it would be infeasible to collect sufficient amount of pellets for the protein analyses from such incubation experiments, unless a number of huge carboys (≥ 20 L) prepared with a trace metal clean technique

are used. Unfortunately, we were not able to prepare these carboys in this study.

Specific comments:

Section 2.3 – error in describing fraction of phytoplankton

This sentence is correct. The seawater concentrated with a plankton net of 20 μm nylon mesh contained phytoplankton with ≥ 20 μm in size, and then it was filtered through 200 μm mesh.

Figure 2 should refer to Table 1 not Table 2.

Corrected .

What is the recovery of protein in your samples? This should be reported - are there stations where recovery is lower - does this effect the sensitivity of blotting.

The recovery cannot be estimated for our samples, because no protein standard was added to them. As far as we know, such procedure has never been conducted for SDS-PAGE or western blots. As mentioned in the text, every sample containing 10 μg of protein was added to each lane for SDS-PAGE. Therefore, we believe that relative estimates of Fd and Fld levels (i.e., Fd index) would be valid in our western blots as well as other studies, assuming the recovery of proteins was the same among samples.

What evidence is there that the antibody reacts to all Fd/Fld in all diatoms species and does not cross react with other species (an example blot would be useful?)

It is incapable of examining the cross-reactivity of anti-Fd or anti-Fld antibodies against all diatoms – The number of extant species of diatoms is estimated to be at least 30,000 and probably ca. 100,000 (Mann and Vanormelingen, 2013, J. Eukaryot. Microbiol., 60, 414–420). As written in the text, the anti-Fd antibody used in this study was raised against an antigen of the synthetic peptide corresponding to the C-terminal end of Fd encoded by the *petF* gene of diatoms (Suzuki et al., 2009). The amino acid sequence of Fd is completely conserved in not only the chloroplast-genome sequenced marine diatoms *Odontella sinensis*, *Thalassiosira weissflogii* and *Th. pseudonana*, but also the freshwater pennate diatom *Synedra acus* (Galachyants et al., 2012, Int. J. Biol., 4, 27–35). The results indicate that the sequence is widely conserved both in marine and

freshwater diatoms. The cross-reactivity of the anti-Fd antibody was also confirmed for various marine diatom species by Hattori-Saito (2010, Ph.D. dissertation, Hokkaido University). The anti-Fld antibody used in this study has also been allowed to react with diatoms specifically in laboratory experiments (LaRoche et al., 1995), and it has also shown cross-reactivity with various diatom species (LaRoche et al., 1995; McKay et al., 1997, 2000).