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Biogeosciences
Discussions

Interactive comment on "Organic exudates promote Fe(II) oxidation in Fe limited cultures of *Trichodesmium erythraeum*" *by* Hanieh T. Farid et al.

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This manuscript describes a (minor) experiment testing the effect of exudes from Trichodesmium cultures on Fe(II) oxidation rate, followed by a (minor) modeling attempt. The most interesting finding/suggestion is that Trichodesmium produces some organic ligands that can bind Fe(II) and hence (slightly) accelerate Fe(II) oxidation rate. However, this suggestion is not pursued further: no attempt is made to quantify these ligands, start characterize them (even basic features), and most importantly conduct speciation measurements (electrochemistry) to demonstrate that they exists, assess the complex strength with Fe(II) and even quantify them. Some basic characterization

of the ligands is easy, while some requires special equipment and knowhow. However, there are plenty of collaborators that the authors can approach to make this scientific contribution more robust for a future submission. In addition, I have various issues with different aspects of the study as described below. One example is that since no Fe replete culture was tested with regards to Fe(II) oxidation, it is not even clear if these presumed ligands are released in respond to Fe limitation. All in all, in my opinion the study is of mediocre quality, too small of a scope, and adds very little to the scientific literature in terms of new concepts, methods or data.

- As the reviewer acknowledged, the main aim of this study was to evaluate how organic exudates released in a batch culture of the marine cyanobacterium T. erythraeum influence Fe(II) oxidation rates. The results suggest an acceleration effect by the organic ligands which is interestingly opposite to what has been previously reported by Santana-Casiano et al. (2014) and Gonzalez et al. (2014) for eukaryotic microorganisms. To the best of our knowledge, until now no study has examine the effect of organic exudates from prokaryotic microorganisms on Fe(II) oxidation rates. Therefore, our study is a significant contribution to our current understanding of Fe acquisition mechanisms by marine microorganisms and should not be considered just 'minor'. Moreover, we were also interested to know if such influence depends on the Fe nutritional status and growth phase of the organism. The results additionally indicate that the acceleration is greater when cells are actively growing, while there was no difference between two Fe treatments, meaning they are possibly releasing the same type of ligands under varying Fe nutritional status. However, we agree that the lack of replication could be an important issue. Hence, we will repeat the experiments for the revised version of the manuscript with two Fe treatments (for details see below) in triplicate, keeping EDTA constant and varying Fe rather than the other way around. The additional experiments are expected to establish more pronounced differences between Fe conditions and allow for more profound statistical testing. - The suggestion made regarding characterization of ligands/speciation measurements are certainly interesting topics to work on as a next step in a follow-up study, but they are well beyond the scope

of the present one. Especially since the suggested methods, e.g. use of electrochemistry for Fe(II)L, or methods to characterize the ligand composition/structure, are not trivial and would require substantial method development. Even when such methods have already been established for Fe(III) (e.g. the LC-ICP-MS method to characterize Fe-binding ligands published recently by Boiteau and Repeta, 2016, Metallomics 7: 877-884) they have not been used to characterize Fe(II)-binding ligands. This brings a whole host of complications that could probably form an entire PhD thesis. - We agree that a clearer distinction in terms of Fe nutritional status, i.e. deplete/ replete, will allow us to draw stronger conclusions about the release of ligands under Fe limitation. The additional experiments are explicitly designed for that purpose.

Specific comments: 1. Replication- I did not see any mention of replication in the paper and hence assume that only 2 cultures were examined (2 levels of Fe') with regard to Fe(II) oxidation rates. This is problematic by all means, as we are dealing with a very complex and sensitive organism, which growth rate is hard to control. So the lack of replication makes the comparison between the cultures questionable (see below) and even cast some shadow on the observed trends with time of Fe(II) oxidation rates (will the next growth curve look the same?, will similar amount of presumed ligands be produced?)

- The referee's point about lack of replication is valid. Hence, we will change the experimental design and replicate Fe treatments (deplete/replete) at one level of Fe(II) addition in the oxidation assays (the three levels previously used did not show any difference).

2. Trichodesmium growth rates under different Fe' conditions- It is unclear why the authors chose to alter EDTA concentrations and not Fe in order to change Fe'. This would have prevented precipitation of Fe, and ease the modeling and the Fe(II) oxidation experimental part (less abiotic background). I think too much effort was put into counting cells (which is very time consuming), instead of following in vivo chlorophyll a (Chl) concentrations. Once the cells are acclimated to a certain level of Fe, the in vivo

chl, measured in a fluorometer is a good, fast and easy marker for growth rate. I do not see how the tedious cell counts contributed to this study. Maybe the authors can think of using these for estimating cell surface area, carbon biomass or anything that can contribute to the discussion of a modified manuscript. On the other hand, not enough effort was invested into establishing the consistency of growth rates (= replicates) between the different conditions. Based on the data in Figure 1, I am not convinced that there are meaningful differenced in the growth rates of both cultures. Note, growth rates for exponential growing cells should be calculated from the slope of Ln(cell) vs time and not the final-initial equation which is given in the appendix. However, since growth rates are not the only way to show limitation by Fe, I think the authors should have attempted probing for Fe limitation by some other means (examples - Fv/Fm; Chl/cell; marked drop in Fe/cell; accelerated Fe uptake rate, growth enhancement after Fe addition, etc). Maybe the differences between the cultures in terms of Fe limitation are not that important, but then a better explanation of why are these chosen is at place. Most importantly, why no high Fe culture was tested?, How can we know that Trichodesmium effect Fe(II) oxidation only when it is Fe limited? Why 2 levels of low Fe' and no high Fe?

- For the revised manuscript Fe' concentrations will be adjusted using varied Fe concentrations at constant EDTA concentration. - The Morphologi G3 (MG3) can be used as an automated cell counter with equivalent accuracy compared to microscopic cell counting methods using Nageotte/Hemocytometer chambers but is less time consuming. While MG3 records actual changes in cell numbers (cells/ml), measuring Chl a concentrations would only be a proxy for cellular growth rates, especially in the stationary phase, which is part of our study. Furthermore, Chl a measurements include several preparation steps which are more time consuming compared to use of the MG3. - However, we agree with the referee that Fv/Fm measurements are a relatively quickly performed indicator for Fe nutritional status. Hence, we will include such measurements in the additional experiments. - Concerning testing for iron nutritional status and a clearer distinction, see comments above and below.

3. Data/figures- In Figure 2 the pseudo first order constant for abiotic conditions is about 3, but in figure 4&5 it is 0.0008 or so. This makes no sense: : : Figure 5 maybe misleading as it is unclear for which sections in time these growth estimates refer. It is best to divide into exponential and stationary phase. In fact this whole figure is probably redundant as it shows exactly the same data as in figure 4 (are these indeed the exact same experiment?). Moreover, when the culture is dying (days 14-20 of the low EDTA culture), things gets messy. What is exactly being released from dying cells, can we treat these as exudes? (not much was found but still: : :). 322-325 Bad phrasing – It reads as if nothing is known, until you get to the end of the sentence. "Since both formation and oxidation rate constants for the Fe(II)EDTA complex were unknown and could not be independently constrained under our experimental conditions due to use of relatively low EDTA concentrations, the oxidation rate constant value was obtained from the literature".

- The units will be checked to maintain consistency between the figures (for both abiotic and exudates graphs) in the revised version. - The suggestion regarding the separation between exponential and stationary phases will be adopted, which will also help to test for potential changes in oxidation rates. - Figure 4 is reporting changes in Fe(II) oxidation rates over time, whereas Figure 5 is evaluating the actual relation between Fe(II) oxidation rates and a physiological parameter, i.e. growth rates. Although they may seem redundant at first glance, they are looking at the Fe(II) oxidation rates from a different perspective. - The sentence 'Since both formation and oxidation rate constants for the Fe(II)EDTA complex ...' will be removed to avoid confusion.

4. Suggestions to improve future manuscript I think that the suggestion that Trichodesmium produces ligands that presumably bind Fe(II) is novel point but is not developed enough. Here are few ideas that can be pursued. Ligand stability with time- In the methods it is mentioned that a biotic background for Fe(II) oxidation was established after keeping the filtrate for 5 hrs in the dark. If this is the case, it is very interesting. Are the exudes themselves short lived? Are they heat stable/sensitive?

(proteins), is it really ligands? Can you show that there are ligands and that they are gone/stop binding (by collaborating with some electrochemistry speciation person?) Ligand concentrations – The authors claim that the concentrations of the exudes are unknown, but they can go after it and make a more meaningful story. Can they saturate them with Fe(II) and see that the Fe(II) oxidation rates reach those of abiotic Fe(II)? Can they dilute the culture and see that it approaches the abiotic background (and then refine the model). Again, can the actual concentrations be evaluated using metal speciation methods (electrochemistry)?

- As mentioned by the reviewer, providing information regarding the existence/release/production of Fe(II) binding ligands in Trichodesmium is a novel finding by itself. But also see our reply to comments regarding ligand characterisation and speciation above. - The use of metal speciation methods such as electrochemistry are well established for Fe(III)L measurements, but to our knowledge have not been used to measure Fe(II)L stability constants in oxic systems. See our reply to comments regarding ligand characterisation and speciation above. - However, the idea regarding saturation of ligands with Fe(II) and comparing with the abiotic oxidation rates is very interesting, and will be tested in the additional experiments.

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