Interactive comment on "Co-occurrence of Fe and P stress in natural populations of the marine diazotroph *Trichodesmium*" *by* Noelle A. Held et al.

Anonymous Referee #2

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General

The authors present a metaproteomic study of field-collected Trichodesmium colonies, focused on phosphate and iron stress markers, and complement that study with a membrane crowding model, which I think is a nice approach to try and understand the observed co-limitation patterns for iron and phosphate. The study is comprehensive in that samples from multiple cruises and years are used; with all but one station (HOT) located in the Atlantic Ocean. Increasing the knowledge of nutrient limitation in natural Trichodesmium populations is certainly of interest, given that it seems to be connected to aggregation of Trichodesmium in some way, either directly or through a general

stress response. While the study as such is valuable and should be published, I have a few major remarks that I think should be addressed before it is ready.

Major Remarks

1. The whole conclusion of co-occurring phosphate and iron stress relies on the assumption that protein abundances of IdiA and SphX are good proxies for iron or phosphate limitation, respectively. The authors do cite the relevant literature that showed upregulation of the respective markers under the corresponding nutrient stress. What I am missing is information on which fold-changes in protein abundance were measured in the cited studies under the respective nutrient limiting conditions. For example, what are the base levels of IdiA and SphX protein in the cell? If there is three times more SphX than IdiA, such as in Fig 3 for some of the Tricolim samples, does that really indicate co-limitation, or does that just reflect the base level of IdiA? For example, Snow et al, 2015 (Fig. 4) only report a two-fold change for IdiA from $\sim$100 fmol/ug to $\sim$200 fmol/ug under iron stress. I suggest presenting the evidence for IdiA and SphX being markers for the respective stresses clearly in a table, including the type of experiment (culture or field), absolute quantifications if stated, and fold-changes measured. I also suggest then being a little more careful in wording throughout the paper, and differentiating better how the results from different stations could be interpreted.

2. Figure 3, and the corresponding Figure S2 are nice and the basis for some important claims being made in part 3.2. of this manuscript. However, these claims should be supported with the necessary statistics, and it would help if Fig S2 was not in the Supplement, but presented together. For example, in line 210 ff, the authors claim that a) "Biomarkers for iron (IdiA) and phosphate (SphX) stress were highly abundant and positively associated with surface Fe or P concentrations" and b) "IdiA varied up to 8 fold, and increased moving West to East across the JC150 transect, consistent with an observed decrease in dFe concentrations" . For a) I think the authors mean "negatively", not "positively", correlated. And while I believe this correlation for SphX, it is not obvious for IdiA. For b) I cannot see increasing protein abundance from west to

east at all. Please prove this statistically before claiming it.

3. Given that only 1 sampling station is NOT in the Atlantic, please remove all claims that generalize the findings, e.g. "co-stress is the norm rather than the exception" (l. 18) → add "in the Atlantic", if wanting to keep this. Or in line 60f: "simultaneously Fe and P stressed throughout the worlds oceans" – this statement cannot be made with just one station outside the Atlantic.

Specific comments

Abstract

The abstract is missing some specificity. line 19: nitrogenase was most abundant – compared to what? Please rephrase: more abundant than under . . .

line 22: is confronted by the biophysical limits – when? Under which conditions is it confronted by this?

line 24f: be more specific. The last sentence is true for any microbe.

Introduction

Line 36: colloquialism

Line 58: add . . .Pho box, a regulatory DNA sequence, which is necessary. . .

Line 65f: Fe and P stress were positively associated – only as co-stress? If yes, say so. Also say how Fe, P, and N statuses are closely linked.

Methods

Line 119: what does that mean? Which precursors, of what? Was every protein normalized to the top 3 precursor intensities? Make this clear also to a reader who is not familiar with the specifics of proteomics analysis.

Line 120: How is the FDR defined? What does "0.1% peptide" and "1.2% protein" mean?

Line 128: Which peptides were selected?

Results and Discussion

Line 178: change "most" to "all but one"

Line 232: please rephrase. What exactly is common in marine bacteria. For sure, all bacteria have regulatory networks.

Line 255ff: skip this justification sentence

Section 3.4. Throughout this section, I think the use of the term ligand is not the norm. For ABC transporters, the word "ligand" is typically used for whatever binds to and is transported by the transporter. The part of the transporter binding the substrate is usually called "ligand-binding protein".

Line 260: change to ". . .required for both iron and phosphate uptake"

Line 305ff: rewrite sentence. Hard to understand.

Line 316ff on cylinders: Shouldn't the Trichodesmium filament, instead of a single Trichodesmium cell, be considered for these models? The effective cell surface of a Trichodesmium cell is reduced by its contact to the neighboring cells.

Line 361: Reference missing for mucus production being a "hallmark of Trichodesmium colony formation"

Line 362f: If mucus acts as a diffusive barrier, it also does the opposite of "protecting them [the cells] from oxygen", namely preventing O2 to diffuse out of the cells during photosynthesis, which was also shown in Eichner et al, 2019.

Line 384: Which specific regulatory systems should be characterized? What do you mean by chemical phases?

Figures

Figure 1: Please use the same numbers on the figure and the legend, or at least also

add the figure numbering top the legend.

Figure 2: Please increase the font size on the legend, and add a legend name like "# of times a protein appeared in the same cluster" – consider changing the legend to a percentage. Please also say in the caption what the color legend shows.

Figure 3: Please state in the caption how the protein abundance values were normalized.

Figure 4: Please adjust font sized throughout panels. How were the dashed lines in c and d defined? Based on what do they denote Fe- or P-stress? And why are they different in c and d?

Figure 5: Does not necessarily need to be a figure if wanting to save space.