

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 #1

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General overview

Held et al., present a metaproteomic analysis of *Trichodesmium* isolates mostly collected in the Tropical and Subtropical Atlantic and use targeted proteomics to quantify putative markers of P and Fe stress to assess correlations with Nitrogenase. Given that *Trichodesmium* is a dominant fixer of nitrogen in the oceans, mechanisms controlling the abundance the major nitrogen fixing enzyme within this cyanobacter is of great interest to ocean biogeochemists (and should be of interest to all). The study involves a survey of *Trichodesmium* populations and across multiple cruises and dates which supports a more generalizable perspective of *Trichodesmium* response to low Fe

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or P. Rather than simply reporting data and immediate findings regarding correlations between proteins between P and Fe levels, the authors make a considerate attempt to extend hypotheses into the biophysical realm by setting up hypothetical scenarios of protein space competition that may lead to reduction in N fixation via membrane overcrowding. Despite lack of experimental evidence and sometime speculative, the authors cite precedent in many cases and have written a thought-provoking manuscript that should lead to testing of alternative hypotheses with regards to how *Trichodesmium* counters P and Fe limitations at the membrane in addition to regulatory gene expression.

Major comments

Abstract line 19, 20 the authors make reference to a ‘...specific physiological state under nutrient stress’. Given the fact that everything will have a specific physiological state under stress or non-stress conditions, this statement does not carry much impact. Perhaps the authors could be more specific. Did they intend to say that there is a generalized stress response regardless of stressors and that nitrogenase appears to comprise this stress response?

Intro Line 47 – The use of the term ‘established’ when describing biomarkers is a bit subjective and loses impact when not followed by multiple citations, which in turn, lends credence to the idea that the protein markers are routinely accepted and utilized by the scientific community. Its reasonable for the authors to state that these proteins were utilized as biomarkers in this study based upon scientific precedent from expression studies, but the discriminatory power of these proteins to classify has not been thoroughly validated. The rationale provided in line 47-59 is sufficient for inclusion in the study. The word putative or candidate in lieu of ‘established’ seems a better fit.

Line 151 -157. Point of clarification. The authors state that standard MS2 peak area was linear between 1 amol and 20 fmol per uL. Further that samples were spiked with standard to 10 fmol per uL and 10uL injected. The assumption is that 10uL was also

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injected for linearity testing of the standards. Please confirm.

Methods: There is no mention of transition ions in the PRM section of the methods. For the sake of reproducibility, the authors should make reference to which transition ions were utilized for quantification (can be supplemental). Further the *Trichodesmium* genome and version should be referenced that was used for searching.

Line 196 “This clustering indicated direct regulatory links between C and N fixation..” perhaps use suggests in lieu of indicates due to probabilistic nature of the association of protein covariance. Similar strong language should be avoided without follow-up direct experimentation which is beyond the scope of the study.

Line 228 – 229 “This observation contrasts with the current paradigm that *Trichodesmium* down regulates nitrogen fixation when it is Fe or P stressed. . .” This statement needs to cite the current published paradigm.

Line 267 – 278 when considering membrane protein space, the authors make a considerate attempt. Space and physics are often ignored; however, because the authors measured whole cell protein abundance and did not attempt to isolate the plasma membrane fraction, the % occupancy estimates are bound to be overestimates. A statement providing limitations of the estimate are needed here. Limitation of space and crowding on a membrane makes sense. Assigning the protein number to the membrane alone is not accurate.

Line 321 “Thus, we conclude that in certain scenarios, lack of membrane space could indeed limit Fe and perhaps P acquisition by *Trichodesmium*.” There is no disagreement with the rationale and calculations that led to this statement, but the statement is hypothetical in nature and the term ‘hypothetical’ should be included. The authors do a nice job in addressing model limitations in the next paragraph and go on to describe an artificial scenario where space limitation could produce further nutrient uptake limitation as additional proteins are made and transported to the membrane. One has to assume uptake activity does not change or is influenced by intracellular events or

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membrane compositional changes that lead to conformational changes; however, the idea that a generalized stress response to Fe or P could lead to a negative effect on uptake and more limitation due to space limits is fun to ponder.

Line 369 – 385, Conclusions. The conclusion section is written as a perspective which is fine given that the conclusions linked specifically to the data are stated within the results and discussion. In line 372, the use of the word ‘norm’ is understandable given the common phrase ‘the norm rather than the exception’, but this might be contentious because normal is being assigned to all *Trichodesmium* based on 16 sampling sites mainly focused in the Atlantic. Fe and P stress may be more common than previously accepted or realized. If the authors feel strongly about this phrasing and believe the audience will be receptive and not over-interpret, then it is fine. Otherwise, perhaps it can be a bit more tempered. The same phrase was used in the Abstract, but due to space limitations the fact that authors were inferring norm based on their samples seems reasonable.

Conclusion Line 378-385, membrane space limitation is likely to be confronted by all cells, not only *Trichodesmium*, but the idea is understood. The authors make a case for including co-stress based on protein observations and if nutrient stress is truly occurring (which can be difficult to define to everyone’s liking and measure in situ; hence use of protein markers) then including these parameters in biological models makes sense.

Figure 3. This is a very informative and nice figure. If possible it would be great to see Figure S2 incorporated for ease of reading and comparison.

Figure 9 is unnecessary, but certainly would be welcomed by visual learners if space is not an issue. Otherwise the concepts are described in the discussion.

Minor comments

Line 63-64“..suggesting nutrient stress was driven not only by biogeochemical gradi-

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ents but also by Trichodesmium's inherent physiology". The term 'inherent physiology' is very broad and does not add substance to the sentence. Trichodesmium is responding to stress in the study and saying something like "...but also by Trichodesmium's response to stress" puts the sentence in a category that doesn't contain every biochemical reaction in the organism.

Line 75. Table S1 does not correspond to the supplemental table indicated. Please correct the designation.

Line 100 "...vacuum centrifugation to 1 100 $\mu\text{g } \mu\text{L}^{-1}$ concentration." Assume this was estimated based on starting concentration of protein and not actually measured?

Line 107 "C18 columns packed in house." Please add column size, diameter, c18 particle size and supplier.

Line 110 A little more detail regarding the parameters would be useful. Based on the search parameters the instrument was likely operated in orbitrap/ion trap mode with HCD? This would be of interest to include and assume more details are in located in Pride.

Line 120 should include the term "local FDR" if local FDR was used.

Line 179-180 is repeated from methods section. Can be deleted.

Line 185 ribosomal and phycobilisomal

Line 237 – 257 This is quite fascinating although not the focus of the study. urtA substrate specificity is poorly defined outside of urea. Curious if the authors also found urease protein elevation in stressed samples.

Figure S2 "Note that the phosphate concentrations from the Tricolim cruise were not measured at the..." Do the authors mean to say Tricolim_13?

Supplemental figures. Please read through legends for spelling errors.

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