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Interactive comment on "Trichodesmium physiological ecology and phosphate reduction in the western Tropical South Pacific" *by* Kyle R. Frischkorn et al.

Anonymous Referee #2

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This is an impressive dataset that has a high potential to offer tantalizing insight into the gene expression of the trichodesmium holobiont in a relatively understudied environment. In spite of my low rating, I actually think it is not far from living up to this potential. That said, I think there is some remaining work that needs to be done with respect to analysis and presentation of these data.

Minor Technical Issues: What methods were used for the biogeochemical measurements? Which of the Outpace articles are these data originally presented? Note: the link to the data from the cruise requires a login and there is no indication in this manuscript as to where these data are published (if they are). If they are not published

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in the other Outpace manuscripts, the methods should be clearly presented here. If they are in other manuscripts, make it clear which manuscript contains the relevant information. What were the methods for the phosphate turnover measurements? I assume these are only presented in this manuscript. While the phosphate uptake measurements are given, the phosphate turnover details are left out.

Results Interpretation: What is different about the 3 samples from LDB? Were they different times? Different colonies? Were they supposed to be replicates? It seems there is as much if not more variability in these three samples as is seen in samples from the other stations, especially with respect to the microbiome, but also with respect to the Trichodesmium expression results. This needs to be addressed somewhere in the manuscript.

What is known about the overall expression levels of the genes that are discussed as marker genes? In other words, is the fact that these genes are among the highest expressed a sign that they are upregulated or because they are constitutively expressed at a high level? The statement on p17 that marker genes are not detectable in cultures grown under replete conditions is false. At least in the citations listed it appears the marker genes are detectable (though significantly downregulated) in replete conditions. Can you use the expression of a housekeeping gene to normalize results in some way? The mix of iron response genes listed are concerning as high expression of all of them is not actually suggestive of iron limitation. Yes, the are all linked to iron metabolism, but previous work has shown that some are upregulated and others are down regulated in situations of iron limitation, and some have shown inconsistent results. For example, Polyvou et al 2018 found that bfr, ftn, fur3, and nifH gene expression was not regulated in response to iron. While examining protein expression, not transcripts, Snow et al 2015 found that ftn was only abundant under iron replete conditions (and absent under iron limitation) and nifH was similarly higher under iron replete conditions as would be expected.

Actually, the high nifH expression and high rates of nitrogen fixation measured on the

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cruise as referenced in the other articles in this special issue would suggest that maybe the Trichodesmium are doing ok. It's very hard to assess the level of iron-limitation, phosphorus-limitation, or co-limitation of the Trichodesmium based on the transcriptome data without some relative measure or other metric to assess what these expression levels mean. It seems as though the authors have potentially relevant data that they could mine to address these issues.

One of the most exciting bit about the paper is the presence of the Trichodesmium gene cassette that appears to allow the organism to perform phosphonate biosynthesis. Right now that exciting result is lost in the weeds of a convoluted discussion of micro-nutrient limitation.

Additionally, in the conclusions the authors suggest that the variable limitation could be influencing growth and nitrogen fixation. It seems that there's a lot of data from this cruise they could examine to see if this is the case. It would be nice to see them include some concrete statements referencing the other manuscripts from the cruise.

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