

## ***Interactive comment on “Iron triggers colony formation in *Phaeocystis antarctica*: connecting molecular mechanisms with iron biogeochemistry” by Sara J. Bender et al.***

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We thank Reviewer #1 for their comments. While the reviewer appreciates the useful data presented in this study, the reviewer is concerned about the evidence to support colony formation in *Phaeocystis antarctica* in response to iron. While we could de-emphasize the notion of this connection and shift focus to overall effects of iron, we were surprised by this comment as we feel there are multiple lines of evidence to support this observation. These include (in order of occurrence): 1) visual (anecdotal) observations of clear differences in colony formation in iron treatments that led to the decision to start measuring colonies (Fig 1a). The first strain became clumpy instead

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of colonial, having apparently lost the ability to complete its colonies, while the second strain made colonies easily discernible by eye in higher iron treatments. While we acknowledge this is anecdotal, it is worth noting how rare it is to have experiments that produce such strong results that they can be clearly observed with the naked eye, resulting in an on-the-fly change in data collection, initiating the cell type microscopy counts. 2) Cell counts in the treatments with the three highest iron treatments had a majority of cells in the colony form (these could be averaged and standard deviation calculated for above and below iron threshold treatments as an additional sentence if desired/appropriate). This change from non-detectable colonial cells to a majority was a very large difference between low and high iron treatments. 3) Increases in cell size across this gradient observed in both *Phaeocystis* strains, consistent with the previously documented larger cell sizes of colony cells (Fig 2e). 4) Numerous protein concentration changes that were consistent with a major differences in ultrastructure proteins across the iron gradient (Supplemental Figure 1; Figure Fig 3b,c,e,f; Fig 4). Specifically, the low iron/flagellate cells of both strains clearly have increased abundance of alpha and beta actin and tubulin proteins that are known to be the major proteins within the flagella and haptonema ultrastructure that occur in flagellated cells but not colonial cells (Figure S1; Figure 1). Similarly, the high iron/colonial cells have clear increased abundances of numerous glycoproteins identified (lectins and von Willebrand proteins) known to be involved in the intercellular colony 'skin' in other colonial organisms such as *Volvox*. The observation of both types of proteins in low/high iron treatments and in both strains is consistent with the clumpy strain being unable to fully complete colony formation likely due loss of some component while in maintenance culture over the years. While we are careful to not exclude the possibility of other types of environmental triggers that could create colonies, in this study we found these four lines of evidence to be convincing and can endeavor to further articulate them in the revision. In addition, recently conducted experiments with strain 1871 in our laboratory have produced similar and consistent results regarding colony formation at varying iron availability.

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The reviewer felt that the connection of the metaproteome to the culture study was not clear. We can work to make this clearer in the revision as well. Figure 9 was intended to make this connection, where proteins described earlier in the culture aspects of the study to be associated with the flagellated (green bars) or colonial (red bars) were observed to both be present in the field *Phaeocystis* net tow metaproteome. The interpretation here is that in order to have an actively growing bloom, both of these diploid cell types are expected to be present and that is consistent with our metaproteome observations of proteins corresponding to each being observed. We acknowledge that there are some methodological aspects of this metaproteome analysis that add some length to this study, but given that metaproteome analysis of eukaryotic algal populations in field samples is relatively new, we feel strongly that it is quite important to have some of this methodological discussion (about database types) included in the manuscript to allow transparency about methodological challenges and successes, and to enable future studies to build on this. We also agree that there are a small number of samples in this metaproteome, but when the samples were collected in 2006, metaproteomic studies were new and studies at that time had few samples included. We recently acquired a large number of samples from this region from a new expedition, and hence we will be able to build on this culture and small metaproteome analysis to interpret large scale temporal and spatial variability dynamics of natural *Phaeocystis* antarctica populations in the future.

We thank the reviewer for their constructive comments, and we look forward to revising and incorporating their suggestions into this manuscript.

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