Minor revisions for bg-2017-558

We are very appreciative of the reviewers' and editor's constructive suggestions and have followed all suggestions. The changes to the manuscript text were relatively minor (small edits or changing phrases for clarity or softening of message based on reviewer comments). Below is a summary of changes made the manuscript.

Reviewer #1:

The reviewer's concerns regarding colony formation were addressed in the response to reviewer comment. Specific changes resulting from the reviewer's comments are:

Given the reviewer's skepticism we have added an additional experiment that showed the same trends to the supplemental materials and this sentence was added to the manuscript discussion: "This influence of iron on colony abundance was observed in an additional experiment, where colonial cells were again absent at the lowest three iron concentrations and were present at the three higher concentrations (Fig. S10)." This new Fig S10 is added at the end of this document as well.

A clause was added to describe the ability to observe colonies visually in the high iron treatments, and on subsequent experiments. The title was changed (see editor's request below).

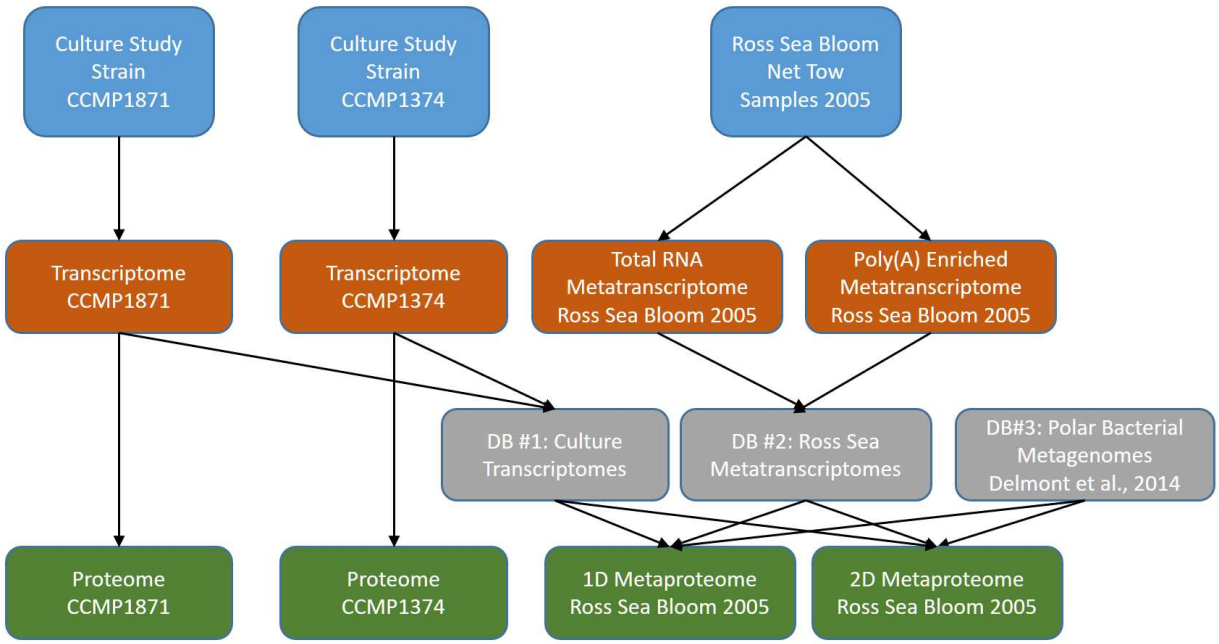
Information regarding the cut-off stringency was included in the supplemental materials.

Reviewer #2:

All changes requested by reviewer #2 were made to the manuscript and supplemental materials with the minor exception of the semantics of the verb "produced" comment, which we were unsure what the reviewer intended. We tried to clarify this sentence as well.

The mistake in Table 1 legend that described RNA data was removed.

The suggested workflow figure was added as a new Figure 2:



Associate Editor's report:

The title was modified as requested to "Colony formation in *Phaeocystis antarctica*: connecting molecular mechanisms with iron biogeochemistry". Description of the iron-induced colony formation observed in this study was checked for any overstatements and/or softened.

The Luxum et al. reference was added.

Changes were made to state that the Ross sea is *one* of the most productive regions as requested in the editor's initial review: (Editor's comments: P. 4 Line 86-88: Not really correct: " The Ross Sea is one of the most productive regions". Check the productivity values in Arrigo (2008), and Arrigo et al. (2015; J. Geophys. Res. Oceans, 120, 5545–5565, doi:10.1002/2015JC010888.). Lovenduski and Sarmiento do not refer to the Ross Sea but the Southern Ocean in general.)

Additional changes in revision:

Figure 6 lines and symbols made darker for improved readability.

In the intervening time during since submission, an additional re-analysis of the metaproteome Ross Sea sample was completed. These were the two Ross Sea 2005 samples presented in the original submission, but now also analyzed by 2-dimensional chromatography (8 hour runs) in addition to the 1-dimensional chromatography runs (3h). The resulting dataset has significantly more peptide and protein identifications (an increase in peptides from 2013 to 3816 using database #1 for example). While not removing any original data from the original submission, we have now supplemented this minor revision version with this deeper analysis, keeping the original reviewed 1D dataset for comparison in the text and supplemental materials. Notably, the increased depth of the proteome in the 2D additional data *does not alter the conclusions of the manuscript at all*, but will serve to provide readers greater supplemental information to explore the metabolism of the region more fully and allow the development of future mass spectrometry based targeted assays by having nearly double the amount of discovered peptides that are being reported in the supplemental materials.

Specific changes that were made by the addition of the 2D data are listed here:

- Additional values added to the metaproteome discussion providing both 1D and 2D values. (format: "### (in 2D; ### in 1D)".
- Additional filled boxes added to Figure 5 the # of field proteins went from 50 to 61.
- Additional filled boxes added to Supp Figure 1, the # of field proteins went from 26 to 30.
- Figure 9 a) peptide numbers in Venn diagram updated to 2D values, with trends are very similar despite ~2-fold increase in peptide identifications. b) no change (RNA data), c) updated to 2D data, slight expansion of *Oceanospirillaceae* (not this database search produced few hits in either 1D or 2D likely due to minimal bacterial recovery likely caused by the use of a coarse net. The text has been updated to include this methodological interpretation.), d) updated to 2D data, very similar trend.
- Additional metaproteome statistics added to Table 2
- Methods paragraph added to supplemental:

Samples were analyzed with a Thermo Fusion mass spectrometer following online 2-dimension active modulation liquid chromatography using a Dionex Ultimate3000 RSLCnano system with an additional RSLCnano pump. The first column separation utilized a nonlinear 8 hour pH = 10 gradient (10 mM Ammonium Formate and 10mM ammonium

Formate in 90% acetonitrile) on a PLRP-S column (200 μm x 150 mm, 3 μm bead size, 300Å pore size, NanoLCMS Solutions). The eluent was diluted inline (10 μL/min 0.1% Formic acid) then trapped and eluted every 30 min on alternating dual traps (300 μm x 5 mm, 5 μm bead size, 100 Å pore size, C18 PepMap100, Thermo Scientific). The alternating traps were eluted at 500 nL/min onto a C18 column (100 μm x 150 mm, 3 μm particle size, 120 Å pore size, C18 Reprosil-Gold, Dr. Maisch GmbH packed in a New Objective PicoFrit column) with a 30 min nonlinear gradient (0.1% Formic Acid and 0.1% Formic Acid in 99.9% Acetonitrile) on a Thermo Flex ion source attached to the mass spectrometer.

- Supplemental Figure S3 added addition panels B and D for the 2D metaproteome datasets in addition to 1D datasets.
- Supplemental dataset 2 The additional 2D dataset and its peptides found from the three databases were added to as three new tabs, the metadata and summary tab were updated.
- Corresponding additional Venn diagram peptide count data was added to the supplemental dataset 2 (used in the Figure 9a, original 1D data presented as well).

Figure S10. An additional experiment on *Phaeocystis antarctica* strain 1871 across six iron treatments using the same approach and methods as that shown in Figure 3 showing the same trend in colony formation at higher iron concentrations. In this experiment, cells were counted at the time of harvest. Note that the number of cells is higher in the lowest three iron treatments due to being given a longer growth period prior to harvest, since biomass is greater in colonial cells due to their larger cell size (see Figure 3e).