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Interactive comment on "Shifts in organic sulfur cycling and microbiome composition in the red-tide causing dinoflagellate *Alexandrium minutum* during a simulated marine heat wave" *by* Elisabeth Deschaseaux et al.

Anonymous Referee #1

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General comments

The manuscript by Deschaseaux et al presents a study of how two different levels of temperature change affected 1) the growth and physiological state of the cultured dinoflagellate Alexandrium minutum. 2) the concentrations of the phytoplankton osmolyte DMSP and its degradation products, DMS and DMSO in the cultures, and 3) the taxonomic composition of the bacterial community associated with the cultures, over a six-day period after the temperature shifts. The goal was to assess how temperature increases that might be representative of marine heat waves would affect the phyto-

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plankton and the associated sulfur biogeochemistry and microbial ecology. Marine heat waves are certainly a topic worthy of study, and their effects need investigation.

The authors chose as their control temperature, 20 oC and acclimated the Alexandrium cultures to that temperature before shocking them with +4 and +12 oC increases. The authors don't really justify the choice of their temperatures very well, and their relevance to potential changes in the natural habitats where Alexandrium minutum is found is not evident. The +4 degree temperature shift caused little effects. The +12 degree shift caused effects but what is the environmental relevance of a sudden 12 degree shift? It seems doubtful that a heat wave of that magnitude in a marine system would happen in a short period, if at all. The choice of control temperature of 20 deg was unfortunate. It seems it should have been higher and perhaps the temperature upshift less dramatic. That would have been more realistic.

While there was a clear response of the +12 deg temperature on growth, Fv/Fm and cellular ROS, the effects on DMSP, DMS and DMSO were less clear. There were just a few points with significant differences - not very convincing that it was experimental effect. Most of the discussion is speculation in trying to explain the odd points of higher or lower parameters at particular time points.

In my opinion, the changes in the microbiome were not particularly informative for interpreting the DMS/P/O data. It seems the authors can only speculate on what drove the changes; the MDS analyses are not very convincing for firm conclusions. I know they replicated the treatments in this experiment, but to be really convincing that temperature effects microbiome shifts reproducibly, the entire experiment should be repeated. Also, the bacterial populations would respond to dissolved materials released from the phytoplankton, but there were no measurements aimed at quantifying those releases, making interpretations difficult.

Overall, I feel that the manuscript does not make a substantial contribution as it is, primarily because of the extreme temperature used to produce effects.

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Specific comments.

Title. They really didn't study sulfur cycling so I suggest changing the wording.

In Figure 4, the DMSP per cell (0.5 to 1.6 pmol per cell) for Alexandrium minutum is much lower than you report in Introduction for A. minutum (14.2 pmol/cell; line 68). Is there an explanation for that?

L90. When mentioning the 2016 Marine Heat Waves associated with El Nino, give some indication of the temperature increases that occurred.

L131. Julabo, country??

L178. 10 ul of H2O2. Give the concentration of H2O2 added and the final concentration in the sample.

L185. The DMS samples were unfiltered. Were they purged for analysis or did you do static headspace? The static headspace would have a relatively high detection limit. Please provide that value.

L188. From the description, the "DMSP" samples would include DMS that was already in the sample. Was this subtracted from the total DMS after the NaOH?

L192. The transition here to "after the experiment DMSP samples were opened. . ." is awkward because they didn't describe yet how the DMSP samples were measured. They did this by headspace analysis, which is described further down. I suggest re-organizing to make it clearer. It should be mentioned in methods that all the sulfur compounds were normalized to cell number. But normalizing these parameters to the cells may be misleading. While most of the DMSP will be in the cells, the DMS is most certainly not in the cells. The DMSO has an unknown dissolved and particulate partitioning in their cultures. Referring to them as "cellular" concentrations is not correct.

L225. The description of which samples were sequenced is a little vague. They say they sequenced the three highest DNA samples from each treatment at time zero (so

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6 samples) and at T=120 h (6 samples). So, a total of 12 samples were sequenced. Is that correct? By choosing the three samples with the highest DNA could that bias the results?

They filtered 400 ml onto a 0.22 μm filter, so this would capture both prokaryote and eukaryote DNA. Any interference from all the phytoplankton DNA? They mention removing the chloroplast DNA sequences later on. If the focus here is only the bacteria then the description should be clarified.

L248. I am not an expert in statistics so I can't comment on the approaches used here. But I will say that it wasn't clear to me whether the relative abundance of bacterial groups in each independent replicate was averaged to obtain an error term.

L287 Add word . . .compared to the 20°C CONTROL at all time points. . .

L 289. You say the 32 deg cultures increased to close to those of the control, but were they still significantly lower?

L396. It should be a negative correlation, not positive. L436. The statement that algal DMSP lyases seem to be exclusively extracellular, is not correct. The Stefels paper is the only one that reported extracellular lyase activity, and that study might have methodological issues that led to that conclusion. Evidence against extracellular lyase in Phaeocystis (the same genus studied by Stefels) was presented in del Valle et al (2011, Marine Chemistry, 124: 57-67). Admittedly, few studies have looked at this directly, but even from the bacterial side, most of the evidence from natural water samples (algae and bacteria present) points to intracellular degradation of DMSP. This is based on the fact that an inhibitor of DMSP uptake (e.g. glycine betaine), which does not inhibit DMSP lyases, is nearly 100% effective at blocking DMSP degradation (e.g. Li et al. 2016, Environ. Chem. 13: 266) . If extracellular lyases were important, DMSP degradation would not be blocked by glycine betaine. Furthermore, the bacterial taxa that were identified to have an extracellular lyase (Alcaligenes sp), and its lyase type (dddY), are not prevalent in marine systems (Moran et al Ann Rev Marine Sci, 2012, 4:

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523).

L534. In this conclusion section the authors need to make it clear that the effect was with the extreme 12-degree upshift.

Figures 1 and 2. If you are going to connect the data points as a time trend, you should plot them on a linear x-axis rather than a categorical axis, as presently done. The categorical axis gives a misleading impression of the time trend.

Figure 3. The x scale is screwed up. Fv/Fm should be less than 1. It seems they have multiplied it by 100. Please fix.

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