

Interactive comment on “Virus mediated transfer of nitrogen from heterotrophic bacteria to phytoplankton” by Emma J. Shelford and Curtis A. Suttle

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Thank you for your review; your comments will help us to improve the manuscript. We will address each of your concerns as outlined below:

General Comments: The introduction is too simple, and subsequent comments: In the Introduction we now emphasize the relationship between primary producers and bacteria through nutrient cycling (especially N), and the role of viruses in the process. As well, we explain the use of *Vibrio* sp. strain PWH3a as a model organism. In the revised Discussion, we explain why *Synechococcus* would not be expected to increase in abundance when lysate was added.

C1

Minor Comments:

P2 L19: We changed “bacteria” to “bacterioplankton” in the abstract to initially clarify, and have pointed out in the Introduction that planktonic bacteria are major players in ocean nutrient cycling.

P2 L4: “Evidence suggests” has been removed from the revised manuscript.

P2 L8: This sentence has been edited in the revised manuscript.

P4 L3: We mention that *Synechococcus* is not axenic in the revised manuscript.

P4 L6-8: The phrasing of “nutrient limitation” has been changed in the revised manuscript.

P5 L3-5: Removal of 15-N from Lys before adding to field experiment: We did not remove the ammonium in the lysate before adding to the experiments. However, the added ammonium is significantly less than the calculated increase in PON in every field experiment. We will make this clear and discuss further in the revised manuscript.

P5 L14: Ultrafiltrate was stored for one year and bacteria grew: We do not claim that the bacteria in the ultrafiltrate are the same as in situ assemblages. Instead, the bacteria in the ultrafiltrate served as a starting bacterial assemblage that was derived from seawater, and survived in the dark, with no nutrient addition for an extended period of time. Albeit the communities will not be the same as the in situ communities, they are derived from the environment and will be more representative than a monoculture. We have explained in the revised Methods the rationale for using the bacterial community that is in the ultrafiltrate. Nutrient limitation condition: We did not directly test for N limitation, although since nitrate was present and there was a strong treatment effect (the bacteria responded to lysate addition by producing ammonium), the bacteria were likely not N limited, and could have been carbon limited. We will clarify these concerns in the manuscript, and emphasize that the experiment was designed to show ammonium production by the bacterial community, and subsequent use by *Synechococcus*.

C2

P9 L13-19: Washing GFF filters: We did not wash the sample at the GFF filter stage. We will clarify in the manuscript how the filters were treated and the effect of any residual PO15N on the results. If there was an effect it would be small, and not affect the interpretation of the results.

P11 L11: Please clarify the demonstration of “N limited *Synechococcus* strain cells”: The media was made with a very low N:P ratio to ensure N limitation. In addition, the positive effect of lysate addition on the abundance of *Synechococcus* is indicative of its N limitation. Dead *Synechococcus* cells releasing nitrogen: Any N that the cells released at the end of exponential growth was not able to be used by either the still-living cells or the contaminating bacteria that co-existed with them, or else the *Synechococcus* would have continued to increase in abundance. In addition, the *Synechococcus* growth was only just beginning to slow from exponential growth, indicating nutrient limitation but not yet producing many dead cells.

P11 L17-18: Possible influence of *Synechococcus*-associated bacteria: Bacteria were present in the *Synechococcus* cultures and were remineralising (see Fig 2C DC2+Lys). The bacteria added with the ultrafiltrate simply added additional “natural” bacteria to the treatment and were associated with higher rates of ammonium regeneration. We have clarified this in the revision.

P12 L2 and Table 2: Perhaps provide total amount (umol) instead of concentration (uM): The revised manuscript now reports N from lysates in umol instead of uM.

P12 L1-15: Why was there no apparent stimulation of picocyanobacteria in field studies: It is likely that picocyanobacteria were outcompeted by larger phytoplankton, as a significant amount of N went into the larger size fraction (>1 um, presumably eukaryotic phytoplankton). We have clarified this in the manuscript.

P12 L21: “Bacterial lysate” has been changed to “*Vibrio* lysate” throughout the revised manuscript.

C3

P17 L1-11: We have reworded this statement in the manuscript to make our meaning clearer.

Table 2: In the field studies, we added far more lysate in two of the experiments than in the other two. We discuss this in the manuscript, but have also emphasized this in the figure legend for clarity.

Fig. 1: DC2 has been changed to Syn throughout the revised manuscript.

Fig. 2: The difference of NH₄ and bacterial abundance at Day 0 should be discussed: There was some ammonium in the lysate that was added, hence the slightly higher concentrations in these treatments. However, this was minor relative to the ammonium that was produced. The ammonium in the lysate was present because the *Vibrio* were still in exponential growth (to facilitate maximum lysis by their viruses), and so not all N had been used. This will be articulated in the revised manuscript.

Fig. 3: An X axis title has been added in the revised manuscript.

Fig. 5: A figure title has been added in the revised manuscript.

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