

## ***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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Received and published: 14 October 2017

Thank you for your careful review. Please see below for how we will address your concerns.

General Comments:

1. The lack of DON measurements in the laboratory study may change the quantitative claims of N uptake and flux by viruses. In the field studies, uptake of DON concurrent with  $15\text{NH}_4$  could have meant we underestimated N uptake from lysate. Therefore, we can update our quantitative claims with these caveats. However, just because we are

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not able to report the total N taken up from lysates (and those lysates are a laboratory construct, in any event), our experiment still shows that N uptake from the production of lysates occurs. We can't be certain whether the phytoplankton are taking up  $15\text{NH}_4$  or  $15\text{N}$ , but they are taking up N from lysate in either case. The ability of phytoplankton to take up DON directly varies greatly dependent on factors such as trophic status of the environment and the species of phytoplankton present (Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. *Aquat Microb Ecol* 31:279-305), but it is generally less than inorganic sources of N.

2. Effect of DOP: We chose to focus our study on N, as N is typically limiting to phytoplankton growth in coastal BC waters (e.g. Yin K, Liu H, Harrison PJ (2017) Sequential nutrient uptake as a potential mechanism for phytoplankton to maintain high primary productivity and balanced nutrient stoichiometry. *Biogeosciences* 14:2469-2480). We are not aware of evidence for P limitation in these waters. We address this in the revised paper.

3. Effect of viruses on Bac+lysate treatment: Although viruses would be released it is unlikely that they would infect members of the bacterial community. *Vibrio* phages are typically species specific, and often strain specific (Comeau AM, Buenaventura E, Suttle CA (2005) A persistent, productive and seasonally dynamic vibriophage population within Pacific Oysters (*Crassostrea gigas*). *Appl Environ Microbiol* 71:5324-5331), and experiments have shown that there was undetectable binding of PWH3a-P1 to natural populations of bacteria, even in the environment from which it was isolated (Hennes KP, Suttle CA, Chan AM (1995) Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities. *Appl Environ Microbiol* 61:3623-3627). Finally, even if it was able to bind to *Vibrio* spp., in general, the estimated abundance in BC coastal waters ranges from  $\sim 1/\text{mL}$  to several hundred  $/\text{mL}$  (Comeau AM, Suttle CA (2007) Distribution, genetic richness and phage sensitivity of *Vibrio* spp. from coastal British Columbia. *Environ Microbiol* 9:1790-1800), and is not enough to significantly affect N release. Regardless, this would not affect the

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observation that N released by viral lysis is ultimately incorporated into cells. We have addressed this in the revised manuscript.

4. Possible need for a Lys-only treatment: There were bacteria in the Lys addition as well as in the DC2+Lys treatment, which could have led to nutrient regeneration, and is likely the reason that growth occurred in the DC2+Lys treatment. The increase in DC2 could be from remineralisation by these contaminating bacteria, or potentially from DOM use by DC2 (although preliminary experiments with filtered lysate indicated that DC2 was unable to grow on lysate directly). We have adjusted the manuscript to indicate that it could be possible that some growth of DC2 occurred as the result of direct uptake by DON.

5. Section 4.3 is speculative: Indeed, this is speculative, and is included to emphasize that the N released by viral lysis and its subsequent remineralization can be quantitatively significant. We have emphasized, in the revised manuscript, that these estimates are a “best guess”.

Specific Comments:

Page 5 line 13 and 14: The bacteria in the ultrafiltrate are an essential aspect of the experiment, as they serve as the remineralisers in the laboratory study. We have emphasized this more strongly in the revised manuscript.

Page 12 line 16 and 17: This sentence has been edited in the revised manuscript.

Page 18 section 4.3: We have clarified how we calculated the yearly loss rate from the bacterial loss rates in the revised manuscript.

Page 27 Table 2: Upon reflection, we have removed the MOI information from the revised manuscript. It is unnecessary information to provide the MOI, as we incubated the infected cultures for 7 h until lysis was complete.

Page 4 line 4: 5 M bicine is correct.

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Page 26 line 4: This error has been fixed in the revised manuscript.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-276>, 2017.

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