Virus mediated transfer of nitrogen from heterotrophic bacteria to phytoplankton

Emma J. Shelford and Curtis A. Suttle

Response to Reviews and Relevant Changes Made:

Referee #1

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, in the Methods we now 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.

Minor Comments:

P2 L19: We changed "bacteria" to "bacterioplankton" in the Abstract and Introduction to initially clarify, and have pointed out in the Introduction that planktonic bacteria are major players in ocean nutrient cycling. We also clearly state that "bacteria" in the manuscript is used to refer to "heterotrophic bacterioplankton"

P2 L4: "*which evidence suggests can support*". "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. This is clarified and discussed 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 clarified these concerns in the manuscript, and emphasized that the experiment was designed to show ammonium production by the bacterial community, and subsequent use by *Synechococcus*.

P9 L13-19: *Washing GFF filters*: We did not wash the sample at the GF filter stage. We clarified in the manuscript how the filters were treated and the effect of any residual $PO^{15}N$ on the results. If there was an effect it would be small, and would 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. This is mentioned in the revised manuscript. *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. This is discussed in the revised Discussion.

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)*: We have kept µM, when we are referring to the concentration of a molecule, and µmol N l^{-1} when referring to the concentration of DON, which is not a specific molecule. The revised manuscript now reports N from lysates in umol, where it may be useful in estimating mass balance.

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: We have continued to use "bacterial lysate" in the manuscript, but have emphasized why it is reasonable to assume that lysate from *Vibrio* is a reasonable proxy for coastal marine bacteria.

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: We have made it clear that *Synechococcus* sp. strain DC2 is now referred to as *Synechococcus* in the revised manuscript, and not as DC2.

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 is articulated in the revised manuscript.

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

Fig. 5: We have removed this figure in the revised manuscript.

Referee #2

Thank you for your comments that have improved the manuscript.

General Comments:

Lack of 1999 paper by Proctor and Fuhrman: Thank you for pointing out this important oversight (actually Noble RT, Fuhrman JA (1999) Breakdown and Microbial Uptake of Marine Viruses and Other Lysis Products. Aquat Microb Ecol 20:1-11) that we have incorporated into our revised manuscript.

Relationship between ambient nutrient concentration and ammonium uptake: we did not find a relationship between ambient concentrations of nitrate, phosphate, or ammonium, and ammonium uptake. We have discussed this in the revised manuscript.

Further Comments:

Figure 1: We appreciate that the reviewer found it difficult to follow the Venn diagram. We have put this information into a table in the revised manuscript.

Figure 2A*: Grey lines in figures*: The figure lines are more discernable in the revised manuscript.

Pg 15, lines 9-12: The statement has been edited in the revised manuscript to reduce quantitative claims.

Pg 12, lines 20-21: This sentence has been edited in the revised manuscript.

Figure 5: Upon reflection, we have deemed this figure unnecessary to the manuscript, and have removed it.

Referee #3

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 $^{15}NH4$ could have meant we underestimated N uptake from lysate. Therefore, we updated our quantitative claims

with these caveats. However, just because we are 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 DO^{15}N or ^{15}NH4, 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. This is discussed in the revised Discussion.

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 ~1/mL to several hundred /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 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 Syn+lysate treatment, which could have led to nutrient regeneration, and is likely the reason that growth occurred in the Syn+lysate treatment. The increase in Syn could be from remineralisation by these contaminating bacteria, or potentially from DOM use by Syn (although preliminary experiments with filtered lysate indicated that Syn was unable to grow on lysate directly). We have adjusted the manuscript to discuss the implications of bacteria in Syn treatments.

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 simplified the calculations in this section to provide an estimate of daily rates of N availability from viral lysis.

Page 27 Table 2: Upon reflection, we have removed the MOI information from the revised Table. 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: This error has been fixed in the revised manuscript.

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

Virus mediated transfer of nitrogen from heterotrophic bacteria to phytoplankton

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Abstract

Lytic infection of bacteria by viruses releases nutrients during cell lysis and stimulates the growth of primary producers, but the path by which these nutrients flow from lysates to primary producers has not been traced. This study examines the remineralisation of nitrogen (N) from ~~bacterial lysate~~*Vibrio* lysates by heterotrophic bacterioplankton~~a~~ and its transfer to primary producers. In laboratory trials, *Vibrio* sp. strain PWH3a was infected with a lytic virus (PWH3a-P1) and the resulting 36.0 µmol l^{-1}~~M~~ of dissolved organic N (DON) in the lysate was added to cultures containing cyanobacteria (*Synechococcus* sp. strain DC2)~~,~~ and a natural bacterial assemblage. Based on the increase in cyanobacteria, 74% (26.5 µmol l^{-1}~~M~~ N) of the DON in the lysate was remineralised and taken up ~~by *Synechococcus* sp. strain DC2 cells~~. Lysate from *Vibrio* sp. strain PWH3a labeled with $^{15}NH_4^+$ was also added to seawater containing natural microbial communities, and in four field experiments, stable isotope analysis indicated that the uptake of ~~labeled~~ ^{15}N was 0.09 to 0.70 µmol N per µg of chlorophyll *a*. The results from these experiments demonstrate that DON from ~~bacterial lysate~~~~*Vibrio*~~ lysate can be efficiently remineralised and transferred to phytoplankton, and provides further evidence that the viral shunt is an important link in nitrogen recycling in aquatic systems.

1 Introduction

Nutrient recycling is an important link between phytoplankton and heterotrophic bacterioplankton (henceforth, referred to as bacteria) in the ocean. Cell death of p~~P~~hytoplankton ~~die after blooms and~~ and bacteria release dissolved ~~exude~~ organic material~~carbon~~ (DOM)

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products during growth, which is rich in, both of which can provide a source of carbon for bacterioplankton (e.g. Brussaard et al., 1996, Fouilland et al. 2014). Bacterioplankton contribute to ocean nutrient cycling by degrading waste products from other microbes. Major constituents of the released material are free and combined amino acids (Middelboe and Jørgensen, 2006) that, which are taken up and metabolised by bacteria (Middelboe et al., 1996, 2003). They canWhen the C:N of DOM is low relative to bacterial nutritional requirements, bacteria deaminate DOM and release ammonium (Goldman et al., 1987; Hollibaugh, 1978), to acquire carbonDOC for energy and growth (e.g. Brussaard et al., 1996, Fouilland et al. 2014); remineralise nutrients such as nitrogenthe release of ammoniumwhich can support phytoplankton growth (Haaber and Middelboe, 2009; Shelford et al., 2012; Weinbauer et al., 2011)and those nutrients are then available for uptake by phytoplankton.

But these two groups are not the only players in the microbial world. Viruses are significant mortality agents of bacterioplanktonaa and phytoplankton in the ocean, and consequently of DOM release (Gobler et al., 1997; Middelboe and Jørgensen, 2006; Noble and Fuhrman, 1999), thereby affecting ; consequently, affecting pathways and rates of nutrient cycling (Fuhrman, 1999; Suttle, 2005, 2007; Wilhelm and Suttle, 1999) when particulate and dissolved organic matter (DOM) is released into the water during cell lysis (Gobler et al., 1997; Middelboe and Jørgensen, 2006; Noble and Fuhrman, 1999). Although many nutrients are released during cell lysis, nitrogen is typically limiting to phytoplankton growth in coastal BC waters (e.g. Yin et al., 2017), which is the location of the current study. Major constituents of the released material are free and combined amino acids (Middelboe and Jørgensen, 2006), which are taken up and metabolised by bacteria (Middelboe et al., 1996, 2003). When the C:N of DOM is low relative to the nutritional requirements of heterotrophic bacteria, ammonium may be released (Goldman et

~~al., 1987; Hollibaugh, 1978), which~~ ~~evidence suggests~~ ~~can support phytoplankton growth~~ ~~(Haaber and Middelboe, 2009; Shelford et al., 2012; Weinbauer et al., 2011)~~.

High rates of bacterial mortality from viral lysis imply a continuous and substantial flux of DOM from ~~lysates~~ cells into seawater. Weinbauer et al. (2011) provided evidence of the importance of this flux by showing that reducing viral abundance decreased the growth of *Synechococcus*, the dominant primary producer during their experiments in the Gulf of Mexico and Mediterranean Sea. It was postulated that *Synechococcus* growth may have been directly stimulated by uptake of dissolved organic nutrients released by lysis, or indirectly through the incorporation of these organics by uninfected bacteria and subsequent remineralisation of inorganic nutrients. Evidence that mineralis~~z~~ation of DOM and release of ammonium by uninfected bacteria stimulates phytoplankton growth was shown by Shelford et al. (2012).

The present contribution demonstrates, in the laboratory and field, that uninfected bacteria metabolise dissolved organic N (DON) released as the result of viral lysis of bacteria, and ~~release~~ produce ammonium that supports the growth of phytoplankton.

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2 Methods

2.1 Laboratory cultures

A non-axenic semi-continuous culture of *Synechococcus* sp. strain DC2 ~~*Synechococcus* sp. strain DC2~~ (Bigelow, CCMP #1334; WH7803); henceforth, referred to as *Synechococcus*, was grown

on artificial seawater (Berges et al., 2001), modified by adding 5 mM bicine (Healey and Hendzel, 1979), 124 µM NH_4Cl instead of nitrate, and 13 µM K_2HPO_4, ~~in order~~ to ensure a ~~low concentrations and a~~ low N:P ratio and N-limited growth. ~~.~~ Cultures were maintained at 19°C and continuous light (42 µmol quanta m^{-2} s^{-1} photosynthetically active radiation). Experiments were started when cultures entered ~~nutrient~~ N limitation near the end of exponential growth, as determined by epifluorescence microscopy counts.

The gram-~~-~~negative marine bacterium *Vibrio* sp. strain PWH3a (henceforth referred to as *Vibrio* PWH3a), also known as *Vibrio natriegens* strain PWH3a (Suttle and Chen, 1992; Weinbauer et al., 1997) and *Vibrio alginolyticus* strain PWH3a (Poorvin et al. 2011), was grown on artificial seawater with 5 m~~mol l^{-1}~~M bicine, 500 ~~µmol l^{-1}~~µM NH_4Cl, 100 ~~µmol l^{-1}~~µM K_2HPO_4, and 1 m~~mol l^{-1}~~M glucose as a carbon source for a C:N:P ratio of 60:5:1. The cultures were grown at 25°C and continuously mixed at 100 rpm. This bacterium was chosen as a model to produce lysates for the current study because it originated from a coastal marine source, and because it has an isolated lytic virus (PWH3a-P1). It is assumed that lysate from *Vibrio* PWH3a is a reasonable proxy for ~~dissolved organic material (~~DOM~~)~~ produced by viral lysis of marine heterotrophic bacteria, and throughout the manuscript is referred to as bacterial lysate.

Bacteriophage PWH3a-P1 was added in eight-fold excess abundance (multiplicity of infection of 8:1) to cultures of ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a at the end of exponential growth, as determined by absorbance at 660 nm (Ultrospec spectrophotometer, Biochrom, United Kingdom). The culture was incubated with the virus until absorbance decreased to 20% of the initial value~~absorbance~~ (~7 h). The lysate ~~(Lys)~~ was filtered through a 0.22-~~-~~µm pore-size Durapore membrane (Millipore, Billerica, MA) and kept at 4°C for approximately 20 h. The number of cells lysed prior to filtration was determined by flow cytometry as described below

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(section 2.4.1). The amount of DON released was determined by the number of cells lysed multiplied by the measured cellular N quota for ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a, 2.54 fmol cell^{-1} as described below (section 2.4.4). The result is the amount of total N released by lysis of ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a.

Lysates for field experiments were prepared as above, except that ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a was grown on $^{15}NH_4Cl$ instead of $^{14}NH_4Cl$ (90+ atom % ^{15}N, Isotec, Miamisburg, OH), and the filtered lysate was kept at 4°C for 2 to 5 d until the experiments were initiated. Excess ammonium in the lysate was not removed before adding to the experiments; h~~. H~~owever, the ~~concentration of~~ added ammonium was ~~significantly~~ less than the ~~calculated~~ increase in particulate organic N (PON) in every field experiment.

2.2 Growth of *Synechococcus* ~~sp. strain DC2~~ on lysate from *Vibrio* ~~sp. strain~~ PWH3a

Water was collected from Queen Charlotte Sound (51° 02.37N, 127° 52.38W) on 2 Oct 2011. Temperature and salinity were 11°C and 32, respectively. Nitrate and phosphate concentrations were 21.3 and 1.9 µM, respectively. The water was ultrafiltered using a 30 kDa molecular weight cut-off tangential flow cartridge (Prep/Scale, Millipore, Billerica, MA) after pre-filtration through a 0.45 µm pore-size polyvinylidene fluoride (PVDF) Durapore membrane (Millipore, Billerica, MA) as outlined in Suttle et al. (1991). The ultrafiltrate was stored in the dark at 4°C for a year until used in experiments. Bacteria in the ultrafiltrate grew to a density of 4.18×10^5 cells ml^{-1}, as determined by flow cytometry (described in section 2.4.1), and were used for the ~~regeneration~~ remineralisation experiment described below. The bacteria in the ultrafiltrate were ~~not in situ assemblages; however, they were~~ derived from the environment and persisted at low

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nutrient concentrations for an extended period, and hence were more representative of in situ communities than a monoculture. They were an essential component of the laboratory study, where they served as remineralisers.

The experiment ~~used~~ combined *Synechococcus* (Syn) ~~sp. strain DC2~~, lysate from ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a, and the bacterial assemblage ~~(Bac)~~ in ultrafiltrate from Queen Charlotte Sound (Bac) in the following six combinations (Table 1~~(Figure 1~~): 1) Syn~~DC2~~+Bac+lysate was the experimental treatment with DON from lysate; 2) ~~DC2~~Syn+Bac was a control for ~~*Synechococcus* sp. strain DC2~~*Synechococcus* growth in the presence of the bacterial assemblage without a DON source from lysate; 3) Syn~~DC2~~+lysate was a control for bacterial remineralisation in the non-axenic ~~*Synechococcus* sp. strain DC2~~*Synechococcus* culture; 4) Bac+lysate was a control to quantify ammonium remineralisation by the bacterial assemblage with the addition of lysate; 5) Bac only was a control to determine the ammonium concentration of the bacterial assemblage by itself; 6) ~~DC2~~ Syn only was a control to determine the ammonium concentration and increase in cell number ~~activity~~ of ~~*Synechococcus* sp. strain DC2~~*Synechococcus* by itself. All treatments were in triplicate in 1 l~~L~~ polycarbonate Erlenmeyer flasks (Corning, New York). To each appropriate treatment was added 10 ml of ~~*Synechococcus* sp. strain DC2~~*Synechococcus* culture, 100 ml of bacterial assemblage, and/or 10 ml of lysate. The experimental treatment volume was 200 ml, and volumes of control treatments were topped up to 200 ml by adding nitrate- and phosphate-free artificial seawater to control treatments.

Treatments were incubated in the laboratory at 19°C under continuous light (42 µmol quanta m^{-2} s^{-1} of photosynthetically active radiation) and sampled daily for cell abundance and ammonium concentration.

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2.3 Uptake of ^{15}N from lysates in the field

Water was collected from the surface at Saanich Inlet (SI), the Fraser River Plume (FRP), Semiahoo Bay (SB) and Jericho Pier (JP) in southern coastal British Columbia (Table 1

Treatments in the laboratory experiment to examine increase in *Synechococcus* abundance (Syn), in the presence of bacterial lysate from *Vibrio* PWH3a (Lys) and an environmental bacterial assemblage (Bac).

Treatment	Syn (ml)	Bac (ml)	Lysate (ml)	Media (ml)	Aim
Syn+Bac+lysate	10	100	10	80	Experimental treatment
Syn+Bac	10	100	-	90	Control for Syn growth with bacterial assemblage but not lysate
Syn+lysate	10	-	10	180	Control for remineralisation in non-axenic Syn culture
Bac+lysate	-	100	10	90	Control for remineralisation by bacterial assemblage
Bac only	-	100	-	100	Control for remineralisation in bacterial assemblage
Syn only	10	-	-	190	Control for Syn growth and remineralisation

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Table 2). SI and FRP samples were collected using Go-Flo bottles mounted on a rosette, which held a SBE 25 CTD (Sea-Bird, Bellevue, WA) for measuring temperature and salinity. SB and JP samples were collected by hand using a 20 l carboy rinsed with 10% HCl and ultrapure water, and temperature and salinity were measured with a hand-held thermometer and refractometer. Samples for phytoplankton identification were collected from the whole water and preserved with Lugol's acidic iodine solution (Edler and Elbrächter, 2010). The water was filtered through 118-µm mesh-size Nitex screening to remove large particulates.

For each experiment, 0.22-µm filtered lysate from ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a was added to Nitex-filtered seawater (SW+lysate) and compared to a control containing 0.22-µm filtered seawater (SW). For SB and JP, a third treatment included 0.22-µm filtered seawater and 0.9 µM $^{15}NH_4Cl$ (SW+N), to confirm that N stimulated production and that another factor was not limiting phytoplankton growth, and to mirror the estimated N contributed by the lysate in the SW+lysate treatment. Treatments were in triplicate. SI and FRP experiments were done in an on-deck flow-through incubator with neutral density screening to reduce sunlight to approximate in situ irradiance. For SB and JP, experiments were done at 19°C and 42 µmol quanta m^{-2} s^{-1} continuous irradiance. Samples for cell abundance and ammonium concentration were collected every 6 to 9 h for 1 to 2 d; samples for chlorophyll *a* were collected at the initial and final time points, and samples for $PO^{15}N$ were collected at the final time point.

2.4 Sample Analysis

2.4.1 Cell and virus counts

One ml samples were fixed with a final concentration of 0.5% gluteraldehyde for bacteria and viruses, and 2% formaldehyde for phytoplankton, and flash frozen in liquid nitrogen and stored

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at -80°C. Bacteria and viruses were stained with SYBR Green I (Sigma-Aldrich, St. Louis, MO), while picophytoplankton were left unstained, immediately before counting by flow cytometry (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ) following the procedures of Brussaard (2004), Gasol and Del Giorgio (2000), and Olson et al. (1993). Larger phytoplankton were preserved with Lugol's solution, and the dominant taxa identified using a settling chamber (Edler and Elbrächter, 2010) and light microscope (Axiovert 10, Zeiss, Canada).

2.4.2 Nutrient and chlorophyll *a* collection and analysis

Samples of 25 ml were filtered through acid-rinsed syringes fitted with MilliQ-soaked 0.45-µm pore size, 25-mm diameter, cellulose-nitrate filters (Whatman, United Kingdom) in a Swinnex filter holder (Millipore, Billerica, MA). The first 15 ml were used to rinse the ~~15 ml~~15-ml acid-rinsed polypropylene screw-cap collection tubes (Sarstedt, Germany), before collecting and freezing the final 10 ml at -20°C for subsequent nutrient analysis. The filters were folded, placed in aluminum foil packages, and frozen ~~dessicated~~desiccated at -20°C until chlorophyll *a* was extracted using 90% acetone and sonication, and analysed fluorometrically (Turner Designs, 10AU fluorometer, Sunnyvale, CA) following Parsons et al. (1984).

Nitrate+nitrite and phosphate concentrations were analyzed on a Bran & Luebbe AutoAnalyzer 3 using air-segmented continuous-flow analysis (Technicon, Oakland, CA), while ammonium concentrations were determined fluorometrically (Holmes et al., 1999) using a TD-700 fluorometer (Turner Designs, Sunnyvale, CA).

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2.4.3 $PO^{15}N$ size-fractionation, collection and determination of ^{15}N enrichment

For the field experiments, the ^{15}N incorporated was determined by collecting the particulate material onto combusted (450°C for 4 h) glass-fibre (GF) filters (nominal pore size of 0.7 µm, Whatman, United Kingdom), which were subsequently dried at 50°C for 2 d. Prior to collection onto the GF filters, the SI and FRP samples were poured into a Sterifil (Millipore, Germany) filtration tower fitted with a 1-µm pore-size polycarbonate filter (AMD Manufacturing, Canada). While the sample was being gently filtered, the phytoplankton were kept in suspension and rinsed, while the volume was maintained by adding 200 ml of ultrafiltrate. In this way, cells captured by the 1-µm filter were retained for analysis of isotopic enrichment, while smaller cells passed through. Samples for cell counts were taken before and after washing to determine the proportion of cells lost by this process. Samples that were not rinsed with ultrafiltrate were also filtered onto combusted GF filters to estimate the amount of ^{15}N uptake that was due to bacteria that passed through the 1-µm pore size membrane. These GF filters were not rinsed with ultrafiltrate after collecting the~~filtering with~~ sample; hence, if there was~~which may have overestimated the uptake of ^{15}N for the <1-µm size fraction from residual~~ noncellular $PO^{15}N$ in the <1-µm size fraction, uptake by the bacterial size fraction would be overestimated. H~~;~~ ~~h~~owever, there is no reason to expect significant noncellular $PO^{15}N$ in the <1-µm size fraction, and it is uptake by the ~~uptake of interest is by~~ >1-µm size fraction, which was washed, that is the focus of this study. The SW+N treatment (SB and JP) was filtered directly onto GF filters without rinsing. The $\delta^{15}N$-PON and total PON on the filters was determined at the Stable Isotope Laboratory at Boston University on a GV Instruments IsoPrime isotope-ratio mass spectrometer and a Eurovector elemental analyzer, calibrated against atmospheric N_2 and IAEA standards N-1, N-2, and N-3 (replicate analysis within ± 0.2‰). Due to instrument error, the

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stable-isotope data for SI and FRP are not replicated; whereas, duplicates for JP and triplicates for SB were measured.

2.4.4 Particulate C and N analysis

N cell quotas of *Synechococcus* ~~sp. strain DC2~~ and ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a were determined from cultures grown using the same media and conditions as described in section 2.1. Cultures at the ~~entering stationary~~end of exponential phase for ~~*Synechococcus* sp. strain DC2~~*Synechococcus* as determined by epifluorescence microscopy counts, and mid-exponential phase for ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a as determined by absorbance, were filtered onto combusted GF filters, dried at 50°C for two days, and placed in a desiccator until packaged into aluminum foil and analyzed on a CHN Elemental Analyser (Carlo Erba NA-1500).

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2.4.5 Calculations and statistical analysis

Total uptake rates of ^{15}N in the field experiments were calculated by dividing the total particulate ^{15}N on the filters by the volume filtered. Normalised uptake rates were calculated by dividing the total uptake rate by the initial chlorophyll *a* concentration for each incubation. The percent contribution of cells >1 µm to total ^{15}N uptake was calculated by dividing the total particulate ^{15}N on the filters of the 'washed' samples (adjusted for decrease in phytoplankton abundance) by the ^{15}N on the unwashed samples.

The differences between initial and final time points for *Synechococcus* abundances and ammonium concentrations were normally distributed and with equal variances according to Shapiro-Wilk and Levene's tests, respectively; hence, the significance of the results was analysed using one-way analysis of variance (ANOVA). The differences between initial and

final time points for bacterial abundances in the laboratory experiment, while normally distributed, had unequal variances, and therefore a Kruskal-Wallis test was performed to test for significant differences.

The differences between initial and final time points for ammonium and chlorophyll *a* concentrations and bacteria and picophytoplankton abundances were normally distributed (except for the picophytoplankton data for treatment SW in SI, which had only two samples), and with equal variances (except the bacteria data for SI and JP). Significant differences between treatments were determined using two-tailed Student's *t* tests. Data which did not meet the assumptions for the Student's *t* test were analysed for significant differences using the non-parametric Mann-Whitney *U* test.

Because the final time point of the bacterial data for the SW+lysate treatment in SI was approximately six-fold lower than the previous time point, concurrent with a significant increase in viral abundance (data not shown), significance between the two treatments was calculated for the differences between the initial and the fourth time points. The data were normally distributed and had equal variances (Shapiro-Wilk and Levene's tests), and so a Student's *t* test was run.

3 RESULTS

3.1 Response of N-limited *Synechococcus* ~~sp. strain DC2~~ to the addition of lysate

There was a greater increase in the abundance of N-limited ~~*Synechococcus* sp. strain DC2~~*Synechococcus* cells exposed to lysate and a remineralising bacterial assemblage than when grown with each component separately; the greatest increase occurred in the treatment Syn~~DC2~~+Bac+lysate (Figure 1A), with final abundances significantly different among all treatments ($p<0.05$). Ammonium decreased from ~1.0 ~~µmol l^{-1}~~µM to less than 0.2 ~~µmol l^{-1}~~µM except in the Bac+lysate treatment, in which it increased to 8.24 ± 0.04 ~~µmol l^{-1}~~µM (Figure 1B), significantly higher than in the other treatments ($p<0.05$). Bacterial abundance increased in all treatments, but the greatest increases occurred in treatments with lysate addition (Figure 1C). Bacterial abundance increased in the Syn+lysate treatment, because bacteria were present in the non-axenic ~~*Synechococcus* sp. strain DC2~~*Synechococcus* and were remineralising (see Figure 1C Syn+lysate). The bacteria added with the ultrafiltrate (Bac) simply added additional "environmental" bacteria to the treatment and were associated with higher rates of ammonium regeneration.

The proportion of N released by lysis of ~~cultures of *Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a that was taken up by ~~*Synechococcus* sp. strain DC2~~*Synechococcus* cells was calculated using the N cell quotas for ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a of 2.54 fmol cell^{-1}, and for *Synechococcus* ~~sp. strain DC2~~ of 7.83 fmol cell^{-1}. Based on the decrease in ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a cells from viral infection, multiplied by the N cell quota of ~~*Vibrio* sp. strain~~

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~~PWH3a~~*Vibrio* PWH3a, approximately 36.0 µmol l^{-1} ~~µM~~ N was added to each incubation from bacterial lysis (Table 3). The mean percent of N from lysate that was taken up by ~~*Synechococcus sp. strain DC2*~~*Synechococcus* cells via remineralisation by bacteria was 74%.

3.2 Uptake of N from bacterial lysate by primary producers in field studies

Lysate from ^{15}N-labe~~l~~led ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a cultures was added to seawater from Saanich Inlet (SI), the Fraser River Plume (FRP), Semiahoo Bay (SB), and Jericho Pier (JP;

Table 1 Treatments in the laboratory experiment to examine increase in *Synechococcus* abundance (Syn), in the presence of bacterial lysate from *Vibrio* PWH3a (Lys) and an environmental bacterial assemblage (Bac).

Treatment	Syn (ml)	Bac (ml)	Lysate (ml)	Media (ml)	Aim
Syn+Bac+lysate	10	100	10	80	Experimental treatment
Syn+Bac	10	100	-	90	Control for Syn growth with bacterial assemblage but not lysate
Syn+lysate	10	-	10	180	Control for remineralisation in non-axenic Syn culture
Bac+lysate	-	100	10	90	Control for remineralisation by bacterial assemblage
Bac only	-	100	-	100	Control for remineralisation in bacterial assemblage
Syn only	10	-	-	190	Control for Syn growth and remineralisation

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Table 2). Changes were followed in ammonium concentrations and in abundances of bacteria (~~Figure 3~~Figure 2). Bacterial abundances in SW+lysate treatments were significantly higher than in samples without lysate (SW) by the final time point for FRP, SB, and JP ($p<0.05$). In the SI sample, bacterial abundances in the treatment with lysate added (SW+lysate) increased almost tenfold before decreasing to below initial values by the final time point, concurrent with an increase in viral abundance (data not shown). There were no significant differences between experimental and control treatments for picophytoplankton abundances, or for chlorophyll *a* concentrations at~~in~~ the other stations (data not shown). ~~A lack of picophytoplankton abundance increase may be due to competition by the larger size fraction (>1 µm, presumably eukaryotic phytoplankton), and an increase in chlorophyll *a* concentrations may have been present, but not significant over the experiment time.~~ Ammonium concentrations in SI, SB, and JP decreased to less than 0.2 ~~µmol l^{-1}~~µM. In FRP, ammonium concentrations in the SW+lysate treatment decreased to less than 0.04 µ~~mol l^{-1}~~M by 19.75 h ~~to less than 0.04 µM~~ before both ammonium and bacterial abundance increased concurrently~~climbing again to 0.25 µM, correlated with a spike in bacterial abundance~~. There was significantly greater drawdown of ammonium in the SW+lysate treatment at~~in~~ every station except SB ($p<0.05$). The increase of bacteria and greater ammonium uptake at most stations indicates that bacterial growth and overall uptake of ammonium ~~was~~ were enhanced in these samples by the addition of bacterial lysate.

There was uptake of lysate-derived N by cells >1 µm, which would be comprised primarily of phytoplankton. The addition of lysate to the SW+lysate treatment for each station contributed approximately 67.7 µmol l^{-1}~~µM~~ N for SI and FRP, and 0.44 µmol l^{-1}~~µM~~ N for SB and JP (Table 3). ~~In order to~~To more closely reflect ambient~~add a more reasonable~~ concentrations ~~of N to the samples~~, the ~~concentration~~ amount of lysate N added to SB and JP was greatly reduced compared

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to the ~~other~~ two previous experiments. Stable isotope data ~~collected~~ from PON ~~particulate organic material~~ collected on 0.7-µm pore-size glass-fibre filters indicated ~~that there was~~ uptake of lysate by the particulate fraction (Table 4; Figure 3). From the ^{15}N data, the calculated contribution to the total uptake by cells in the >1 µm size fraction was 46.3% (SI), 47.6% (FRP), and 100% (SB and JP). The large (>50%) contribution of the bacterial size fraction to ^{15}N uptake in SI and FRP (Figure 3) corresponded to an increase in bacterial abundances (~~Figure 3~~Figure 2).

There was ~~confirmed~~ uptake of $^{15}NH_4^+$ in the SW+N treatment ~~with ^{15}N addition (SW+N)~~, with 0.61 ± 0.20 ~~µmol l^{-1}~~ µmol l^{-1} N (SB) and 0.44 ± 0.26 ~~µmol l^{-1}~~ µmol l^{-1} N (JP) taken up into the particulate fraction (corrected for 90 atom % ^{15}N, ~~;~~ data not shown); therefore, a ~~. Any a~~mmonium produced by remineralisation of lysate in the SW+Lys treatment ~~, therefore,~~ c ~~w~~could be used by the microbes at these two stations.

4 DISCUSSION

Experiments in the laboratory and with natural systems ~~show~~ demonstrate that N in viral lysates can be remineralised by bacterial communities to fuel primary production. Studies have shown that lysis by viruses produces bioavailable ~~dissolved organic matter~~DOM (e.g. Middelboe et al. 2003, Poorvin et al. 2004), and that phytoplankton lysate can be remineralised (Gobler et al., 1997). Other studies have provided evidence that ammonium from remineralisation may stimulate primary production (Haaber and Middelboe, 2009; Shelford et al., 2012; Weinbauer et

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al., 2011); yet, data are lacking on the mechanism involved in the transfer of N to primary producers from bacterial lysate~~*Vibrio* lysate~~s produced by viral infection. This study shows that phytoplankton in culture and in environmental samples take up N from bacterial lysate~~*Vibrio* lysate~~s, and that ammonium produced through bacterial remineralisation of DON enables that uptake.

4.1 Remineralisation of nitrogen in bacterial lysate~~*Vibrio* lysate~~s

The increase in bacterial abundance in all experiments, along with the production of ammonium in the laboratory experiment, establishes that ~~bacterial lysate~~bacterial lysates produced through viral infection are available for bacterial growth and ~~potential~~ ammonium remineralisation. In the laboratory ~~experiment~~, every treatment with added lysate had a greater increase of bacteria than treatments without lysate, indicating that the bacteria used the added lysate for growth. For all field studies, bacterial abundances increased significantly in the treatments with lysate over the controls ($p<0.05$; ~~Figure 3~~Figure 2), demonstrating uptake of lysis products by the bacterial communities.

Ammonium concentration increased eightfold in laboratory treatments with lysate added to bacteria (Bac+lysate; Figure 1B) from remineralisation of N in the lysate by the bacterial community. In the other treatments, either lysate was not added as a source of DON (treatment Bac), or ~~*Synechococcus* sp. strain DC2~~*Synechococcus* was present and consumed the ammonium that was produced (the remainder of the treatments). There was no measured ammonium production in the field studies except in the Fraser River Plume (FRP;~~-~~ Figure 2~~Figure 3~~). The increase in ammonium at the final time point in FRP may be due to the concurrent ~~sharp~~ rapid increase in bacteria, which likely resulted in increased ~~ing~~ ammonium remineralisation. There

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was some ammonium in the added lysate (see Figure 1B, T=0), which was present because the ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a was in exponential growth to facilitate maximum lysis by PWH3a-P1, and so not all the ammonium in the medium was ~~had been~~ taken up by the bacteria prior to lysis. However, the~~is~~ amount that was added was minor relative to the ammonium that was produced by remineralis~~z~~ation. The data clearly show that~~Viral~~ lysates ~~can be~~were used by the bacterial community for growth, and excess N remineralised to produce ammonium.

Although viruses (PWH3a-P1) were present in the bacterial lysate, it is unlikely that they would infect members of the bacterial community. *Vibrio* phages are typically species specific, and often strain specific (Comeau et al. 2005), 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 et al. 1995). Finally, even if PWH3a-P1 was able to bind to *Vibrio* spp., the estimated abundance of *Vibrio* spp. in BC coastal waters ranges from ~1 ml^{-1} to several hundred ml^{-1} (Comeau and Suttle 2007), and is not enough to significantly affect N release. Moreover~~Regardless~~, this would not affect the observation that N released by viral lysis is ~~ultimately~~ incorporated into cells.

4.2 Phytoplankton uptake of remineralised nitrogen

This study shows that remineralised N from viral~~us~~ lysis of bacteria can fuel the growth of primary producers. Observations of increased ammonium production in the presence of viral lysis (Haaber and Middelboe, 2009; Shelford et al., 2012), are extended by this study, which demonstrates that bacteria remineralise the organic N in lysates and produce ammonium, which is then taken up by ~~phytoplankton~~primary producers. The use of a cultivated bacterium for lysate

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production limits generalisation of quantitative data to environmental systems; however, the observation that 74% of the N in bacterial lysates was taken up by cultures of ~~*Synechococcus* sp. strain DC2~~*Synechococcus* provides strong evidence that N from lysates ~~are~~is available to~~may provide a large portion of the N taken up by~~ phytoplankton.

DON from bacterial lysates was remineralised by the bacterial assemblage into ammonium and used to fuel primary production. In the laboratory experiment, the increase in ammonium in the Bac+lysate treatment did not occur in the treatment with ~~*Synechococcus* sp. strain DC2~~*Synechococcus* (Syn~~DC2~~+Bac+lysate; Figure 1B), indicating that the remineralised ammonium was taken up and le~~a~~d~~ing~~ to ~~by *Synechococcus* sp. strain DC2; this is consistent with~~ the concurrent increase in ~~*Synechococcus* sp. strain DC2~~*Synechococcus* cells (Figure 1A). The increase in *Synechococcus* ~~sp. strain DC2~~ in treatment Syn+lysate may have resulted from remineralisation by contaminating bacteria (bacteria in the non-axenic ~~*Synechococcus* sp. strain DC2~~*Synechococcus* culture, or from uninfected ~~*Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a in the lysate), or possibly from direct DON uptake, although preliminary experiments ~~suggest~~indicated that direct uptake of DON was not significant ~~that this is unlikely~~. ~~The uptake of ^{15}N in seawater samples by organisms >1 µm (Figure 4) also demonstrates that remineralised N in bacterial lysate supports primary production, as suggested previously (Shelford et al., 2012; Weinbauer et al., 2011).~~

All four field studies showed that ^{15}N from labeled ~~bacterial lysate~~bacterial lysate was taken up by the >1-µm fraction (Figure 3), which ~~,~~demonstrates that remineralised N in ~~*Vibrio*~~ lysate supports primary production, as suggested previously (Shelford et al., 2012; Weinbauer et al., 2011). The reason that increases in phytoplankton or chlorophyll were not observed is likely because there are often significant lags between incorporation of ammonium and growth in N-

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limited phytoplankton (Collos, 1986, Davidson et al., 1992). ~~This is likely largely the result of uptake by phytoplankton which dominate this size fraction.~~ As well, these rates represent minimum estimates, as ^{15}N released through rapid recycling of lysis products (Noble and Fuhrman, 1999) ~~bacterial remineralisation~~ or phytoplankton exudation (Bronk and Ward, 2000) is not included.~~-~~ Uptake of DO^{15}N concurrent with $^{15}NH_4$ could also have resulted in an underestimation of N uptake from remineralisation. The ability of phytoplankton to take up DON directly varies greatly, and is dependent on factors such as trophic status of the environment and the species of phytoplankton present (Berman and Bronk, 2003), but ~~it~~ is generally less important~~taken up with less frequency~~ than ~~are~~ inorganic sources of N. Although the field experiments do not provide quantitative estimates of the amount of N in lysate that ~~can be~~was taken up, the results corroborate those from the laboratory experiment and indicate that N uptake from ~~the production of~~ lysates occurs. ~~An~~ The anomalous result in~~for~~ the SB experiment ~~was~~ that the estimated uptake ~~amount of N taken up~~ (0.67 ± 0.02 µmol l^{-1}~~µM~~ N; corrected for 90 atom % ^{15}N; Figure 3) was ~~higher~~ more than the calculated N added~~amount of N in the lysate addition~~ (0.44 µmol l^{-1}~~µM~~ N; Table 3) suggests that ~~; h~~the~~owever, the~~ N in the lysate was underestimated. This is plausible, as lysate N was calculated~~contribution is an estimate calculated~~ from the cellular N quota multiplied by~~and~~ the decline in abundance of~~reduction in cell number of *Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a, and cell count data were not replicated~~some error may derive from these calculations and from a lack of replicates in the cell number data~~. Nonetheless, the data are convincing~~it is reasonable to conclude~~ that most of the lysate added in the SB and JP experiments was incorporated by primary producers, since at both stations 100% of the estimated ^{15}N uptake was into the >1 µm fraction. Interestingly, we did not find a relationship between ambient concentrations of nitrate, phosphate, or ammonium, and ammonium uptake despite

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evidence of an increase in uptake of lysis products with lower trophic status in previous studies (e.g. Noble and Fuhrman, 1999), although this could be due to ~~all of~~all the stations in these studies being relatively productive~~to the current study only having four data points~~.

Nonetheless, not all ~~of~~ the N in lysates was taken up by primary producers. Although bacteria can remineralise lysate (Bac+lysate; Figure 1B), and ~~*Synechococcus* sp. strain~~ ~~DC2~~*Synechococcus* can use remineralised N from co-occurring bacteria (Syn~~DC2~~+lysate; Figure 1A), in the laboratory only 74% of the N in the lysate contributed to an increase in ~~*Synechococcus* sp. strain DC2~~*Synechococcus* abundance. As well, in the FRP and SI samples, only about half of the ^{15}N uptake was into the >1 µm fraction. Discrepancies between N uptake and phytoplankton growth is likely because of the lag between ammonium uptake and growth that is observed in many N-limited phytoplankton (Collos, 1986), and~~may be in partly~~ ~~be~~ because phytoplankton can take up substantially more N than they incorporate because of DON release~~have been observed to take up almost 74% more N than the net amount incorporated~~ (Bronk et al., 1994)~~; thus, in our experiments more N may have been taken up than was measured, because of DON release~~. ~~Bronk et al. (1994) also measured DON turnover times in seawater samples of 4 to 18 d, much longer than in our incubations; however, the DON in seawater would also include phytoplankton exudates and refractory DON that would have a different composition than bacterial lysate~~*Vibrio* lysates~~.~~ As well, longer~~Hence, the~~ incubation times may have allowed the in situ bacterial communities to break down more complex DON in the lysate and regenerate additional ammonium that could be~~in our study may not have been long enough to allow for uptake of more recalcitrant DON, which might have been~~ incorporated ~~with time~~ (Middelboe and Jørgensen, 2006), but would add the additional complication of secondary release and uptake of labelled DON. For SI and FRP, ~~the~~ lysate N was added at ~~a~~ relatively high

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concentrations (Table 3), which may ~~also~~ have ~~contributed~~ led to incomplete incorporation. In contrast, ~~the~~ lysate N was added at ~~a~~ much lower concentrations in the SB and JP experiments, and the ^{15}N in the lysate was completely recovered in the particulate fraction~~, indicating that the DON in the lysate N was incorporated when added at more natural concentrations~~. Finally, the incomplete transfer of N from lysate ~~of *Vibrio* sp. strain PWH3a~~ to ~~*Synechococcus* sp. strain DC2~~*Synechococcus* in the laboratory, relative to the complete uptake of ^{15}N at SB and JP, may indicate that complete remineralisation of the DON depends on the make-up of the bacterial assemblage, or ~~else~~ that ~~the~~ N uptake of ~~*Synechococcus* sp. strain DC2~~*Synechococcus* was saturated. ~~some phytoplankton in natural waters may take up DON directly (Bronk et al., 2007).~~

Laboratory experiments were conducted on ~~The *Synechococcus* sp. strain DC2~~*Synechococcus* cultures ~~were~~ grown under a low N:P ratio and which ~~were~~ transitioning from exponential growth, to ensure N limitation~~would be the limiting nutrient~~ but not starvation and potential cell death with release of DOM. ~~. The positive effect of *Vibrio* lysate addition on the abundance of *Synechococcus* sp. strain DC2 is indicative of its N limitation. The *Synechococcus* sp. strain DC2 cultures were added as the cells were beginning to slow from exponential growth, indicating nutrient limitation but minimizing cell death to prevent dead cells from releasing N. 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 co-existing contaminating bacteria, or else the *Synechococcus* sp. strain DC2 would have continued to increase in abundance.~~

~~We did not directly test for N limitation of t~~The bacterial community (Bac) in these experiments was unlikely N-limited, and most likely C-limited, given nitrate in the medium and the production o~~n~~f ammonium in the~~prior to treatment preparation, although since nitrate was present and there was a strong treatment effect in~~ Bac+lysate treatment~~(the bacteria responded to~~

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~~lysate addition by producing ammonium), the bacteria were likely not N limited, and could have been carbon limited~~. The experiment was designed to show ammonium production by the bacterial community (Bac+lysate), and its subsequent use ~~of that ammonium~~ by ~~*Synechococcus* sp. strain DC2~~*Synechococcus* (Syn+Bac+lysate).

The increase in *Synechococcus* ~~sp. strain DC2~~ abundance in the laboratory experiment (Figure 1A) can be explained by the different sources of N present in the experimental treatment (Syn~~DC2~~+Bac+lysate). ~~A portion of t~~The increase can be attributed as follows (calculated from the increase in cell number multiplied by cell quota) ~~to~~: 1) 16.7 µmol l^{-1} N from the 21.3 µM of nitrate ~~present~~ in the Bac ultrafiltrate, 2) ~~a portion to~~ 9.2 µmol l^{-1} N from ~~the~~ remineralisation of ammonium by the added bacterial assemblage, and 3) 9.8 µmol l^{-1} N from the ~~another portion to~~ uptake of ammonium from remineralisation by ~~contaminating~~ heterotrophic bacteria in the ~~non-axenic *Synechococcus* sp. strain DC2~~*Synechococcus* culture. Despite evidence for direct uptake of DON ~~directly~~ by phytoplankton ~~in field experiments~~ (see Bronk et al. 2007 for a review), preliminary ~~studies~~ experiments ~~to the current one~~ showed no evidence for uptake of N from ~~uptake of *Vibrio* sp. strain PWH3a~~*Vibrio* PWH3a lysate ~~uptake~~ by axenic ~~*Synechococcus* sp. strain DC2~~*Synechococcus*. Despite the addition of N from other sources, the results clearly show that remineralization of N from the lysate contributed to ~~If considered together,~~ the increase of ~~*Synechococcus*~~*Synechococcus* sp. strain DC2 in the ~~experimental treatment (~~Syn~~DC2~~+Bac+lysate~~)~~ treatment ~~can be accounted for by the effects from each of these control treatments~~.

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4.3 Ecological implications and future directions

Some studies have focused on the influence of phytoplankton lysates on fueling bacterial production (Gobler et al., 1997; Haaber and Middelboe, 2009), which can be especially~~.~~ ~~Phytoplankton lysates can be very~~ important in certain situations, such as ~~during a~~ bloom termination (Brussaard et al., 2005). In contrast, ~~but bacterial lysates are produced constantly throughout the ocean. Suttle (1994) estimated that~~ 10 to 20% of heterotrophic bacteria are estimated to be lysed every day by viruses (Suttle 1994), although it~~which~~ can vary widely ~~dependent on location and conditions~~ (e.g. Wilhelm et al. 1998, 2002). With an estimated 3.6 x 10^{28} prokaryotic cells in the upper 200 m of the ocean and an average carbon quota of 2.0 x 10^{-14} g cell^{-1} (Whitman et al., 1998), a bacterial C:N of approximately 5 (Goldman et al., 1987), and estimated loss rates of 10% d^{-1} from viral lysis, approximately 1.44 x 10^{13} g N d^{-1} is released from viral lysis of bacteria in the photic zone. Primary production is estimated to be ~1.35 x10^{14} g C d^{-1} (Ducklow and Carlson, 1992), corresponding to ~2.04 x10^{13} g N d^{-1} assuming the Redfield ratio (106C:16N). Therefore, an estimated 71% of global primary production could be supported by N released by viral lysis of bacteria. While these estimates need to be verified, evidence suggests that viral lysis supplies a substantial portion of the N required for primary production, and emphasizes the importance of the viral shunt, especially in N-limited regions.~~With an estimated 26-70 Pg C yr^{-1} of bacterial production in the euphotic zone (Ducklow and Carlson, 1992), a bacterial C:N of approximately 5 (Goldman et al., 1987), and loss rates of 10-20% from viral lysis, approximately 0.52-2.8 Pg N yr^{-1} is released from viral lysis of bacteria in the photic zone. Primary production C demand is estimated to be ~49.3 Pg C yr^{-1} (Ducklow and Carlson, 1992), corresponding to ~7.4 Pg N yr^{-1} assumccording to the Redfield ratio (106C:16N). Therefore, an estimated 7-38% of global primary production can be supported by N

~~released by viral lysis of bacteria from bacterial lysates from viral infection (Figure 5). estimates need to be verifiedviral lysis suppliesThis is a substantial portion of the N required for primary production, and emphasizes the importance of the viral shuntsource of recycled N available to fuel primary production, especially in N-limited regions that are N-limited.~~

The ~~ability of primary producers to~~ use of N by phytoplankton from bacterial remineralisation of DOM originating from viral lysis of bacteria indicates that viruses are not simply C sinks that disrupt trophic levels (Azam and Worden, 2004), but are important facilitators in N recycling pathways. Traditional food chain models state that C and other nutrients flow from primary producers to higher trophic levels. The introduction of the microbial loop (Azam et al., 1983) included bacterial dynamics, ~~;~~ whereby~~,~~ DOM produced by the members of the traditional food chain is taken up by bacteria and reintroduced to the food web instead of being lost to the system. The viral shunt (Wilhelm and Suttle, 1999) introduced viruses as a 'short-circuit,' removing particulate C from primary producers, consumers, and bacteria to the pool of organic matter. This model emphasises viruses as a loss mechanism of food web C; however, implicit in these models is that nutrients, as well as C, must also be released by viral lysis, and that N and P are likely recycled with greater efficiency than C (Suttle, 2007). The loss of C from the particulate pool is clear, but there is evidence of increased productivity in the presence of viruses. In Fe-limited regions of the eastern Pacific Ocean, viruses were shown to liberate Fe into the DOM pool, where it was available for uptake by primary producers (Poorvin et al., 2004). Weinbauer et al. (2011) provided evidence for this phenomenon when they removed the viral fraction from a cyanobacteria bloom and primary production ceased. Shelford et al. (2012) confirmed that observation by demonstrating a reduction in both ammonium remineralisation and phytoplankton abundance with removal of viruses. The current study demonstrated in four

separate field experiments that N is transferred from bacterial ~~bacterial lysate~~*~~Vibrio~~* lysates to phytoplankton ~~biomass~~. Weinbauer et al. (2011) hypothesised that ~~primary production decreased in their experiments due to either~~ lysates either directly provided~~ing a direct source of~~ nutrients supporting~~for the~~ *Synechococcus* growth~~blooms~~, or that~~rough~~ bacteria provided inorganic nutrients~~!~~ by remineralis~~z~~ing the products of viral lysis~~sation providing inorganic nutrients~~. This study demonstrates~~supports the interpretation~~ that remobilization of nutrients by viral lysis of bacteria and subsequent remineralisation by uninfected bacteria can fuel the growth of primary producers~~supported contributed to the observed growth of *Synechococcus*~~.

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Author contributions

E. J. S.helford and C. A.S. Suttle designed the experiments. E. J. S.helford performed the experiments and analysed the samples except for the stable isotope data which was analysed by the Stable Isotope Facility at UC Davis, and the nitrogen cell content which was analysed by Maureen Soon at UBC. E. J. S.helford prepared the manuscript and analyzed the data with contributions and guidance from C. A. Suttle.

Competing Interests

The authors declare that they have no conflict of interest.

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References

Azam, F., Fenchel, T., Field, J. G., Gray, J. S., Meyer-Reil, L. A. and Thingstad, F.: The ecological role of water-column microbes in the sea, Mar. Ecol. Prog. Ser., 10, 257–263, 1983.

Azam, F. and Worden, A. Z.: Microbes, molecules, and marine ecosystems, Science, 303, 1622–1624, 2004.

Berges, J. A., Franklin, D. J. and Harrison, P. J.: Evolution of an artificial seawter medium: improvements in enriched seawater, artificial water over the last two decades, J. Phycol., 37, 1138–1145, 2001.

Berman, T., and Bronk, D. A.: Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems, Aquat Microb Ecol, 31, 279-305, 2003.

Bronk, D. A., Glibert, P. M. and Ward, B. B.: Nitrogen uptake, dissolved organic nitrogen release, and new production., Science, 265, 1843–1846, doi:10.1126/science.265.5180.1843, 1994.

Bronk, D. A., See, J. H., Bradley, P. and Killberg, L.: DON as a source of bioavailable nitrogen for phytoplankton, Biogeosciences, 4, 283–296, 2007.

Bronk, D. A. and Ward, B. B.: Magnitude of dissolved organic nitrogen release relative to gross nitrogen uptake in marine systems, Limnol. Oceanogr., 45, 1879–1883, 2000.

Brussaard, C. P. D.: Optimization of procedures for counting viruses by flow cytometry, Appl. Environ. Microbiol., 70, 1506–1513, doi:10.1128/AEM.70.3.1506, 2004.

Brussaard, C. P. D., Gast, G. J., van Duyl, F. C. and Riegman, R.: Impact of phytoplankton bloom magnitude on a pelagic microbial food web, Mar. Ecol. Prog. Ser., 144, 211-221, 1996.

Brussaard, C. P. D., Kuipers, B. and Veldhuis, M. J. W.: A mesocosm study of *Phaeocystis globosa* population dynamics, Harmful Algae, 4, 859–874, doi:10.1016/j.hal.2004.12.015, 2005.

Collos, Y.: Time-lag algal growth dynamics: biological constraints on primary production in aquatic environments, Mar. Ecol. Prog. Ser., 33, 193-206, 1986.

Comeau, A. M., Suttle, C. A.: Distribution, genetic richness and phage sensitivity of Vibrio spp. from coastal British Columbia. Environ Microbiol, 9, 1790-1800, 2007.

Comeau, A. M., Buenaventura, E., and Suttle, C. A.: A persistent, productive and seasonally dynamic vibriophage population within Pacific Oysters (Crassostrea gigas), Appl Environ Microbiol, 71, 5324-5331, 2005.

Davidson, K., Flynn, K. J., Cunningham, A.: Non-steady state ammonium-limited growth of the marine phytoflagellate, Isochrysis galbana Parke, New. Phytol., 122, 433-438, 1992.

Ducklow, H. W. and Carlson, C. A.: Oceanic bacterial production, in advances in microbial ecology, vol. 12, edited by K. Marshall, pp. 113–181, Plenum Press, New York, 1992.

Edler, L. and Elbrächter, M.: The Utermöhl method for quantitative phytoplankton analysis, in Microscopic and molecular methods for quantitative phytoplankton analysis, edited by B. Karlson, C. Cusack, and E. Bresnan, pp. 13–20, UNESCO, Paris, 2010.

Fouilland, E., Tolosa, I., Bonnet, D., Bouvier, C., Bouvier, T., Bouvy, M., Got, P., Le Floc'h, E., Mostajir, B., Roques Richard Sempéré, C., Sime-Ngando, T., Vidussi, F.: Bacterial carbon

dependence on freshly produced phytoplankton exudates under different nutrient availability and grazing pressure conditions in coastal marine waters, FEMS Microb. Ecol., 87, 757-769, doi.org/10.1111/1574-6941.12262, 2014.

Fuhrman, J. A.: Marine viruses and their biogeochemical and ecological effects, Nature, 399, 541–548, 1999.

Gasol, J. M. and Del Giorgio, P. A.: Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities, Sci. Mar., 64, 197-224, 2000.

Gobler, C. J., Hutchins, D. A., Fisher, N. S., Cosper, E. M. and Sañudo-Wilhelmy, S.: Release and bioavailability of C, N, P Se, and Fe following viral lysis of a marine chrysophyte, Limnol. Oceanogr., 42, 1492–1504, 1997.

Goldman, J. C., Caron, D. A. and Dennett, M. R.: Regulation of gross growth efficiency and ammonium regeneration in bacteria by substrate C:N ratio, Limnol. Oceanogr., 32, 1239–1252, 1987.

Haaber, J. and Middelboe, M.: Viral lysis of *Phaeocystis pouchetii*: implications for algal population dynamics and heterotrophic C, N and P cycling, ISME J., 3, 430–441, doi:10.1038/ismej.2008.125, 2009.

Healey, F. P. and Hendzel, L. L.: Indicators of phosphorus and nitrogen deficiency in five algae in culture, J. Fish. Res. Board Canada, 36, 1364–1369, 1979.

Hennes, K. P., Suttle, C. A., Chan, A. M.: Fluorescently labeled virus probes show that natural virus populations can control the structure of marine microbial communities, Appl Environ Microbiol, 61, 3623-3627, 1995.

Hollibaugh, J. T.: Nitrogen regeneration during the degradation of several amino acids by plankton communities collected near Halifax, Nova Scotia, Mar. Biol., 45, 191–201, 1978.

Holmes, R. M., Aminot, A., Kerouel, R., Hooker, B. A. and Peterso, B. J.: A simple and precise method for measuring ammonium and marine and freshwater ecosystems, Can. J. Fish. Aquat. Sci., 56, 1801–1808, 1999.

Middelboe, M. and Jørgensen, N. O. G.: Viral lysis of bacteria: an important source of dissolved amino acids and cell wall compounds, J. Mar. Biol. Assoc. UK, 86, 605–612, 2006.

Middelboe, M., Jørgensen, N. O. G. and Kroer, N.: Effects of viruses on nutrient turnover and growth efficiency of noninfected marine bacterioplankton, Appl. Environ. Microbiol., 62, 1991–1997, 1996.

Middelboe, M., Riemann, L., Steward, G., Hansen, V. and Nybroe, O.: Virus-induced transfer of organic carbon between marine bacteria in a model community, Aquat. Microb. Ecol., 33, 1–10, 2003.

Noble, R. T., and Fuhrman, J. A.: Breakdown and microbial uptake of marine viruses and other lysis products, Aquat. Microb. Ecol., 20, 1-11, 1999.

Olson, R. J., Zettler, E. R. and D, D. M.: Phytoplankton analysis using flow cytometry, in handbook of methods in aquatic microbial ecology, pp. 175–186, Lewis Publishers, Boca Raton., 1993.

Parsons, T. R., Maita, Y. and Lalli, C. M.: Manual of chemical and biological methods for seawater analysis, Pergamon Press., 1984.

Poorvin, L., Rinta-Kanto, J. M., Hutchins, D. A. and Wilhelm, S. W.: Viral release of iron and its bioavailability to marine plankton, Limnol. Oceanogr., 49, 1734–1741, 2004.

Poorvin L., Sander S. G., Velasquez I., Ibisanmi E., LeCleir G. R., Wilhelm S.W.: A comparison of Fe bioavailability and binding of a catecholate siderophore with virus-mediated lysates from the marine bacterium Vibrio alginolyticus PWH3a. J. Exp. Mar. Biol. Ecol., 399, 43-47, 2011.

Shelford, E. J., Middelboe, M., Møller, E. F. and Suttle, C. A.: Virus-driven nitrogen cycling enhances phytoplankton growth, Aquat. Microb. Ecol., 66, 41–46, doi:10.3354/ame01553, 2012.

Suttle, C. A.: The significance of viruses to mortality in aquatic microbial communities, Microb. Ecol., 28, 237–243, doi:10.1007/BF00166813, 1994.

Suttle, C. A.: Viruses in the sea., Nature, 437, 356–361, doi:10.1038/nature04160, 2005.

Suttle, C. A.: Marine viruses--major players in the global ecosystem., Nat. Rev. Microbiol., 5, 801–812, doi:10.1038/nrmicro1750, 2007.

Suttle, C. A., Chan, A. M., and Cottrell, M. T. Use of ultrafiltration to isolate viruses from seawater which are pathogens of marine phytoplankton. Appl. Environ. Microbiol. 57, 721-726, 1991.

Suttle, C. A. and Chen, F.: Mechanisms and rates of decay of marine viruses in seawater., Appl. Environ. Microbiol., 58, 3721–3729, 1992.

Weinbauer, M. G., Bonilla-Findji, O., Chan, A. M., Dolan, J. R., Short, S. M., Šimek, K., Wilhelm, S. W. and Suttle, C. A.: *Synechococcus* growth in the ocean may depend on the lysis of heterotrophic bacteria, J. Plankton Res., 33, 1465–1476, doi:10.1093/plankt/fbr041, 2011.

Weinbauer, M. G., Wilhelm, S. W., Suttle, C. A. and Garza, D. R.: Photoreactivation compensates for UV damage and restores infectivity to natural marine virus communities~~.~~, Appl. Environ. Microbiol., 63, 2200–2205, 1997.

Whitman, W. B., Coleman, D. C., Wiebe, W. J.: Prokaryotes: the unseen majority, P. Natl. Acad. Sci. USA, 95, 6578-6583, 1998.

Wilhelm, S. W., Brigden, S. M. and Suttle, C. A.: A dilution technique for the direct measurement of viral production: a comparison in stratified and tidally mixed coastal waters., Microb. Ecol., 43, 168–173, doi:10.1007/s00248-001-1021-9, 2002.

Wilhelm, S. W. and Suttle, C. A.: Viruses and nutrient cycles in the sea, Bioscience, 49, 781–788, doi:10.2307/1313569, 1999.

Wilhelm, S. W., Weinbauer, M. G., Suttle, C. A. and Jeffrey, W. H.: The role of sunlight in the removal and repair of viruses in the sea, Limnol. Oceanogr., 43, 586–592, 1998.

Yin, K., Liu, H., Harrison, P. J.: Sequential nutrient uptake as a potential mechanism for phytoplankton to maintain high primary productivity and balanced nutrient stoichiometry, Biogeosciences, 14, 2469-2480, 2017.

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Table 1 Treatments in the laboratory experiment to examine increase in *Synechococcus* abundance (Syn), in the presence of bacterial lysate from *Vibrio* PWH3a (Lys) and an environmental bacterial assemblage (Bac).

Treatment	Syn (ml)	Bac (ml)	Lysate (ml)	Media (ml)	Aim
Syn+Bac+lysate	10	100	10	80	Experimental treatment
Syn+Bac	10	100	-	90	Control for Syn growth with bacterial assemblage but not lysate
Syn+lysate	10	-	10	180	Control for remineralisation in non-axenic Syn culture
Bac+lysate	-	100	10	90	Control for remineralisation by bacterial assemblage
Bac only	-	100	-	100	Control for remineralisation in bacterial assemblage
Syn only	10	-	-	190	Control for Syn growth and remineralisation

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Table 2 Locations and environmental parameters for field sampling stations: Saanich Inlet (SI), Fraser River Plume (FRP), Semiahoo Bay (SB), and Jericho Pier (JP). Environmental and biological conditions measured include temperature (Temp), salinity (Sal), nitrate (NO_3^-), phosphate (PO_4^{2-}), ammonium (NH_4^+), and the dominant phytoplankton.

Station	Location	Date	Temp (°C)	Sal	NO_3^- (~~µmol l^{-1}~~ µM)	PO_4^{2-} (~~µmol l^{-1}~~ µM)	NH_4^+ (~~µmol l^{-1}~~ µM)	Dominant phytoplankton
SI	48.592°N, -123.505°W	13 Sep 2012	14	27.9	1.24	0.75	0.54	Mixed assemblage
FRP	49.072°N, -123.402°W	13 Sep 2012	11	28.0	10.9	1.12	0.07	*Phaeocystis*, *Skeletonema*, *Leptocylindrus*
SB	49.013°N, -123.037°W	26 Aug 2013	20.2	29	0.00	2.40	0.08	Cyanobacteria Unknown ciliate
JP	49.277°N, -123.202°W	27 Aug 2013	19.5	21	0.00	0.20	0.11	Diatoms (mixed assemblage)

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Table 3 *Vibrio* sp. strain PWH3a dynamics during the creation of lysates by viral infection (Laboratory = experiments with cultured *Synechococcus* ~~sp. strain DC2~~, SI = Saanich Inlet, FRP = Fraser River Plume, SB = Semiahoo Bay, JP = Jericho Pier). N was added at lower concentrations for stations SB and JP to approximate in situ ~~N~~ concentrations.

	Laboratory	SI and FRP	SB and JP
Vibrio ~~sp. strain~~ PWH3a abundance pre-virus (cell l^{-1})	3.96×10^{11}	7.87×10^{11}	7.89×10^{10}
Vibrio ~~sp. strain~~ PWH3a abundance post-virus incubation (cell l^{-1})	1.12×10^{11}	9.43×10^{10}	6.38×10^{10}
~~Multiplicity of infection (MOI)~~	~~1~~	~~0.41~~	~~5~~
Total N release (µmol l^{-1} ~~M~~)	721	1760	38.4
N addition to experimental incubations (µmol l^{-1} ~~M~~)	36.0	67.7	0.44
N addition to experimental incubations (µmol)	7.21	33.8	0.31

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Table 4 Uptake by the particulate fraction of ^{15}N from ~~bacterial lysate~~bacterial lysate from four field stations, and the same uptake normalised by initial chlorophyll *a* concentrations. Error measurements are standard error of triplicate incubations for SB, and range of duplicate incubations for JP. SI and FRP are singleton measurements.

	Total uptake of ^{15}N (µmol)	Total uptake of ^{15}N (µmol l^{-1}~~M~~)	Uptake of ^{15}N normalised by chlorophyll *a* (µmol $µg^{-1}$)
Saanich Inlet (SI)	0.83	1.67	0.27
Fraser River Plume (FRP)	0.88	1.76	0.70
Semiahoo Bay (SB)	0.42 ± 0.01	0.60 ± 0.02	0.70 ± 0.16
Jericho Pier (JP)	0.35 ± 0.04	0.50 ± 0.1	0.09 ± 0.00

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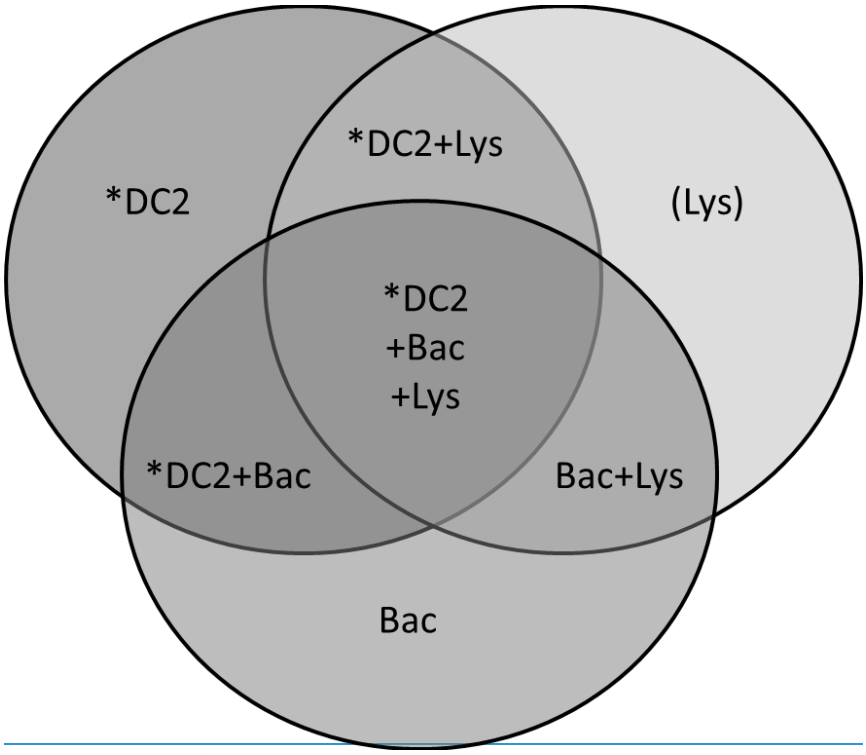
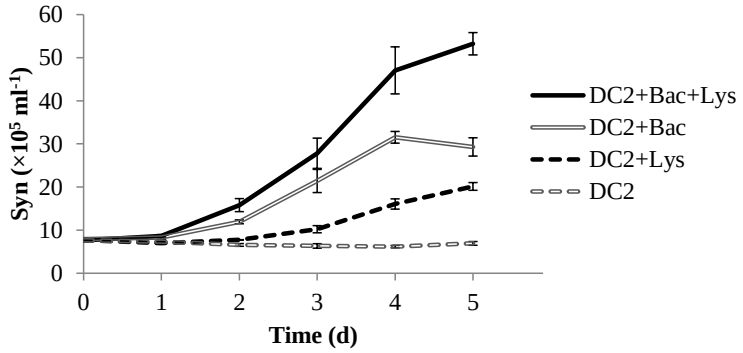


Figure 1 Diagram of treatments in the laboratory experiment examining increase in *Synechococcus* sp. strain DC2 abundance, in the presence of bacterial lysate from *Vibrio* sp. strain PWH3a (Lys) and an environmental bacterial assemblage (Bac). Lys, while a component of some of the treatments, was not a treatment by itself (indicated by parentheses). The * is an indication that DC2 is not axenic, and therefore contains some heterotrophic bacteria.



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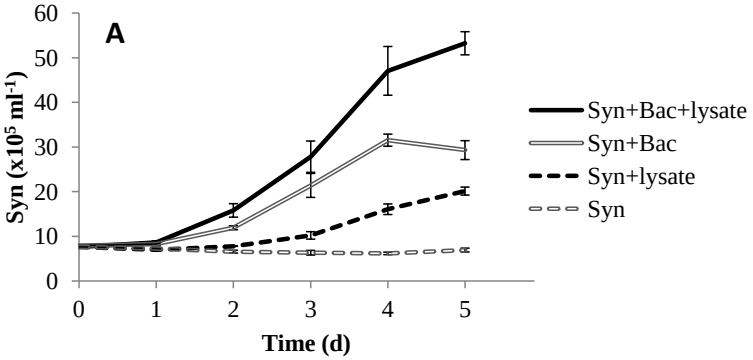

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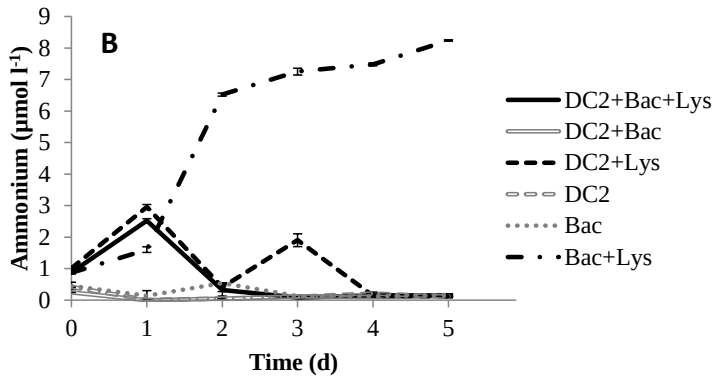
B
Ammonium (µmol l-1)
Time (d)
DC2+Bac+Lys
DC2+Bac
DC2+Lys
DC2
Bac
Bac+Lys

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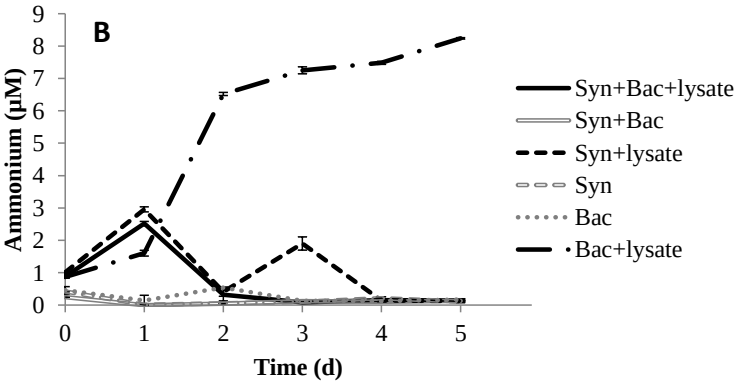
B
Ammonium (µM)
Time (d)
Syn+Bac+lysate
Syn+Bac
Syn+lysate
Syn
Bac
Bac+lysate

2

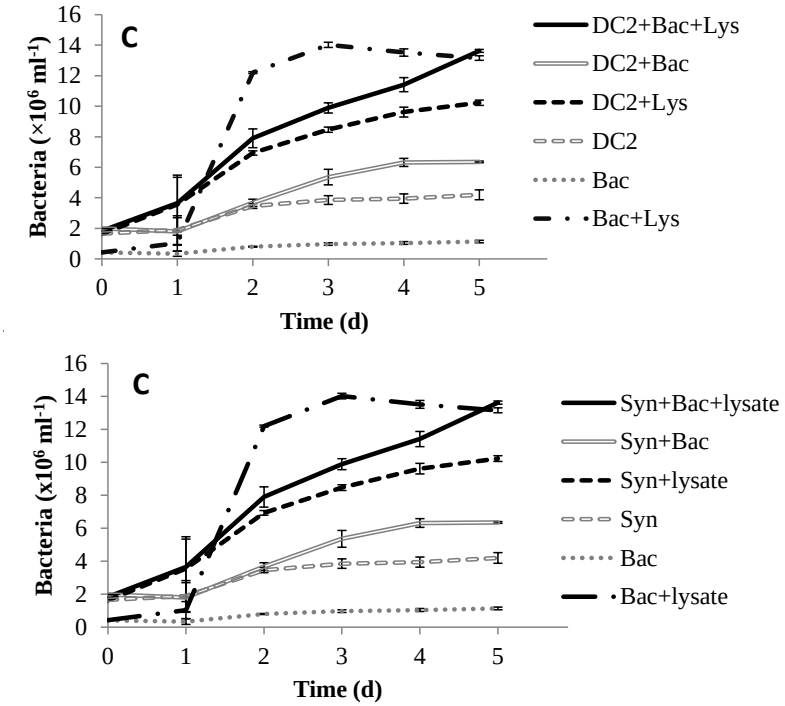

Figure 1 Time series of (A) *Synechococcus* ~~sp. strain DC2~~ abundance, (B) ammonium concentration, and (C) bacterial abundance in the laboratory experiment. Error bars are standard error from triplicate incubations.

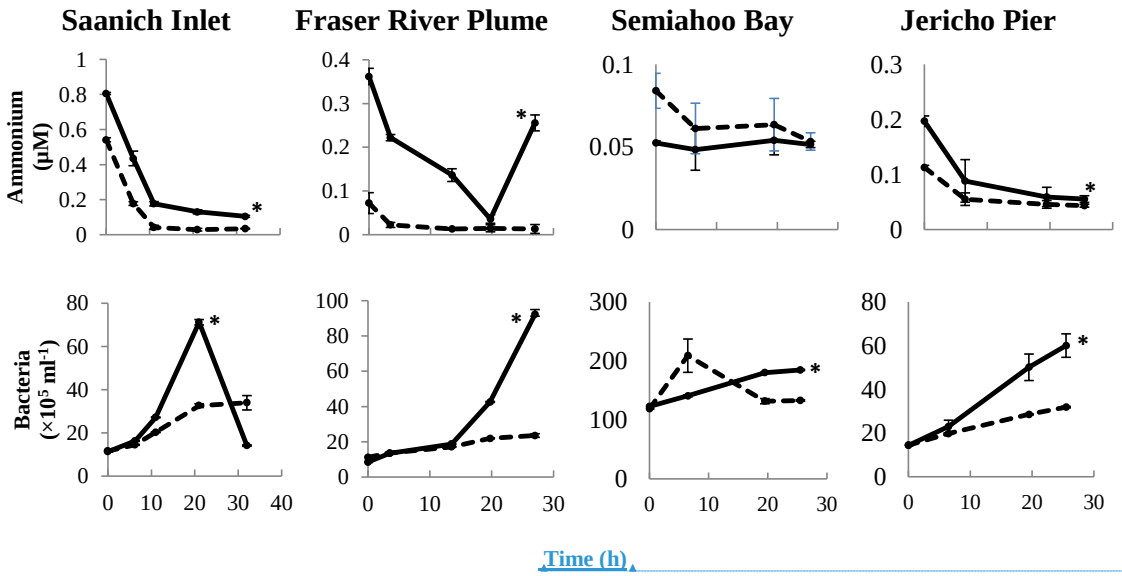

Figure 2 Time-course of ammonium concentrations and bacterial abundances in the field experiments. Solid and dashed lines indicate treatments with lysate addition (SW+lysate), or without (SW), respectively. Error bars are standard errors of triplicate incubations. Asterisks indicate significant differences between treatments. Note that the scales are different among plots.

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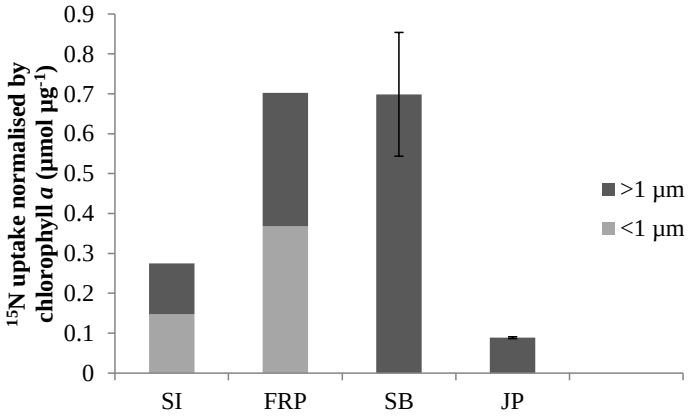

Figure 3 Uptake of ^{15}N normalised by initial chlorophyll *a* concentrations ~~during the experiment~~ by the >1 µm and <1 µm fractions in treatments with lysate addition for Saanich Inlet (SI), Fraser River Plume (FRP), Semiahoo Bay (SB), and Jericho Pier (JP). Error bars are standard error of triplicate incubations for SB, and range of duplicate incubations for JP. SI and FRP are singleton measurements.

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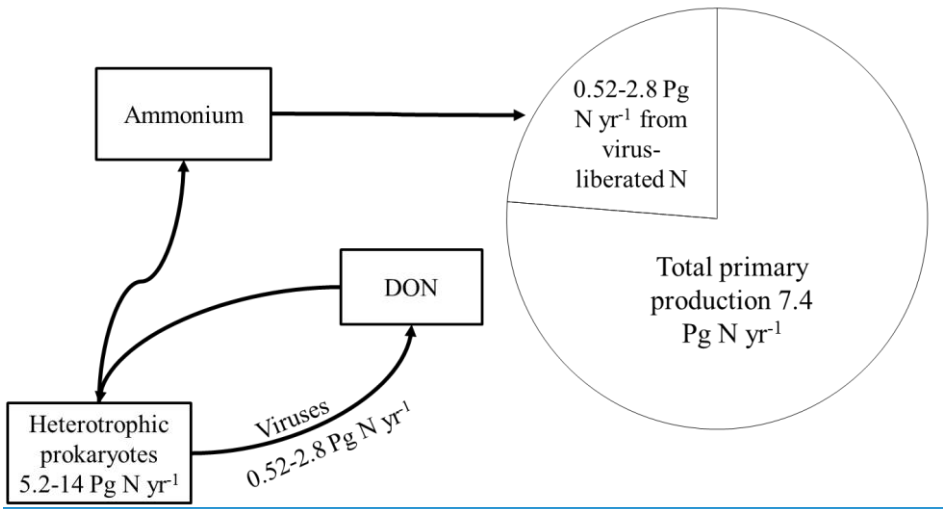

Figure 5 Viruses release 0.52–2.8 Pg N yr^{-1} globally in the euphotic ocean from lysis of heterotrophic prokaryotes. That dissolved organic N (DON) is available for remineralisation by other prokaryotes into ammonium, which constitutes a large proportion of the total N demand of primary producers.

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