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LETTER

Rapid evolution buffers ecosystem impacts of viruses in a microbial food web§

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Abstract

Predation and parasitism often regulate population dynamics, community interactions, and ecosystem functioning. The strength of these top-down pressures is variable, however, and may be influenced by both ecological and evolutionary processes. We conducted a chemostat experiment to assess the direct and indirect effects of viruses on a marine microbial food web comprised of an autotrophic host (*Synechococcus*) and non-target heterotrophic bacteria. Viruses dramatically altered the host population dynamics, which in turn influenced phosphorus resource availability and the stoichiometric allocation of nutrients into microbial biomass. These virus effects diminished with time, but could not be attributed to changes in the abundance or composition of heterotrophic bacteria. Instead, attenuation of the virus effects coincided with the detection of resistant host phenotypes, suggesting that rapid evolution buffered the effect of viruses on nutrient cycling. Our results demonstrate that evolutionary processes are important for community dynamics and ecosystem processes on ecologically relevant time scales.

Keywords

Bacteria, ecosystems, food webs, host–parasite, microbial diversity, nutrients, phage, resource, top-down, trade-off.

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INTRODUCTION

It is well established that 'top-down' forces influence a suite of ecological patterns and processes in nature including producer biomass, competitive interactions, and ecosystem functioning (Oksanen *et al.* 1981; Leibold 1996; Schindler *et al.* 1997). However, the magnitude and persistence of top-down effects tend to vary through space and time (Chase 2003; Borer *et al.* 2005). This variability may be influenced by both ecological changes in community structure and evolutionary changes in the phenotype of keystone species. For example, complex food webs tend to have more indirect trophic interactions than simple food webs (e.g. Menge 1995), which can dampen the effects of predation and parasitism on prey and host populations, respectively (Wootton 1994). Likewise, a growing number of studies have shown that the importance of top-down effects vary over time because rapid evolution can generate feedbacks that modify community dynamics (Yoshida *et al.* 2003; Fussmann *et al.* 2007). For example, the evolution of predator defence strategies can reduce population fluctuations (Yoshida *et al.* 2007) and may affect some ecosystem processes, such as nutrient cycling (Loeuille & Loreau 2004; Fussmann *et al.* 2007).

Although historically overlooked, it is now recognized that viruses play an important role in regulating population, food web, and ecosystem dynamics (Wren *et al.* 2006; Suttle 2007). As obligate parasites, viruses have direct negative effects on their host populations, which include altered behaviour, nutritional deficits, and reduced fitness (Hurst 2000). For example, in aquatic ecosystems viruses attain high densities (10^7 mL^{-1}) and are responsible for lysing up to 40% of the resident prokaryotes per day (Suttle 1994), which can equal the losses imposed by grazing (Fuhrman & Noble 1995). In addition to regulating host population densities, virus-induced mortality can indirectly affect other populations within diverse microbial assemblages. Through mechanisms akin to apparent competition and keystone predation, viruses can reduce the densities of dominant host populations and provide opportunities for partially resistant

or rare non-target populations to increase in abundance (e.g. Bohannan & Lenksi 2000; Brockhurst *et al.* 2006; Bouvier & del Giorgio 2007). Lastly, the impacts of viruses on microbial communities can affect ecosystem processes (Fuhrman 1999; Wilhelm & Suttle 1999; Brussaard *et al.* 2008). For example, viral lysis of microbial biomass alters the quantity and quality of growth-limiting resources (Middelboe *et al.* 1996; Gobler *et al.* 1997; Poorvin *et al.* 2004), which may influence the stoichiometry and carbon sequestering capacity of aquatic ecosystems (Suttle 2007).

The impacts of viruses on microbial food webs may be contingent upon the evolutionary dynamics of the system, however. It has been argued that the observed effects of viruses on ecosystem processes may be transient owing to the rapid evolution of host resistance (Olofsson & Kjelleberg 1991). Indeed, many strains of aquatic bacteria are either naturally resistant or have the ability to evolve resistance in response to the strong selective pressure of an infectious virus (Waterbury & Valois 1993; Middelboe *et al.* 2001; Stoddard *et al.* 2007). Recent evidence suggests that the evolution of virus resistance in some strains of aquatic bacteria comes at a cost (Lennon *et al.* 2007). The resulting fitness trade-off provides a mechanism allowing for the coexistence of multiple host phenotypes, which can in turn generate population dynamics that are not predicted by traditional ecological theory (Yoshida *et al.* 2007). Ultimately, the rapid evolution of bacteria and viruses is predicted to buffer resource fluctuations and enhance food web stability (Bohannan & Lenksi 2000).

In this study, we examined the direct and indirect effects of a lytic virus on the ecology and evolution of a simplified aquatic microbial food web consisting of autotrophic and heterotrophic bacteria. We used marine *Synechococcus*, an autotrophic picocyanobacterium, as the host population because it is known to experience virus predation in nature (Waterbury & Valois 1993) and because it plays an important role in nutrient cycling and carbon flux at a global scale (e.g. Richardson & Jackson 2007). Heterotrophic bacteria form close associations with photosynthetic microorganisms like *Synechococcus*. On the one hand, heterotrophs compete with autotrophs for shared inorganic nutrients (e.g. nitrogen and phosphorus); on the other hand, they rely on photosynthetically derived organic matter (e.g. exudates, lysates, and dead cells) as their principal source of carbon and energy (Grover 2000; Bertilsson *et al.* 2005). This autotrophic–heterotrophic coupling is a fundamental attribute of food webs that may be important for understanding how microbial communities respond to virus predation. At the same time, we know that bacteria often evolve resistance to viruses on ecologically relevant time scales, which can have consequences for community dynamics and possibly ecosystem processes (Bohannan & Lenksi 2000). Therefore, we conducted experiments to assess whether indirect ecological interactions (compensation by heterotrophic bacteria) or rapid evolution (compensation by resistant hosts) could buffer the fluctuations in nutrient dynamics that we predicted would be caused by virus-induced mortality of *Synechococcus*.

METHODS

Experimental system

Our experimental system consisted of chemostats containing photosynthetic *Synechococcus* (Cyanobacteria), an infectious virus (Myoviridae), and heterotrophic bacteria. A peristaltic pump continuously supplied the chemostats vessels with a modified version of 'AN' artificial seawater medium (see Lennon *et al.* 2007) at a dilution rate of 1 day^{-1}. Due to slight evaporation caused by vacuum back pressure, we reduced the NaCl concentration in the AN medium to maintain a salinity of 30 ppt. In addition, we adjusted nitrogen (N) and phosphorus (P) concentrations to 1100 μmol L^{-1} N ($NaNO_3$) and 11 μmol L^{-1} P (K_2HPO_4) to achieve an N : P supply ratio of 100. Separate experiments confirmed that the equilibrium cell density of *Synechococcus* in our chemostats was P-limited (data not shown). Contents of the chemostat (40 mL) were homogenized by magnetic stir bars at the bottom of each vessel placed on stir plates in a Percival growth chamber at 25 °C on a 14 : 10 light : dark cycle at 10 μM m^{-2} s^{-1}. To quantify the densities of microorganisms, samples were taken directly from the chemostats using sterile transfer pipettes. Larger sample volumes, however, were needed to measure dissolved and particular nutrient concentrations. Therefore, our experimental system included sterile collection vessels (40 mL) located downstream from each chemostat vessel that could be emptied for sampling. The chemostat experiments were run for just over 4 months.

Synechococcus and virus

The *Synechococcus* strain (WH7803) used as the host in this study was obtained from the Woods Hole Collection of Cyanobacteria (Woods Hole Oceanographic Institution, Woods Hole, MA,USA). We inoculated eight replicate chemostats with a suspension of *Synechococcus* that had been recently derived from a single colony isolate. We introduced virus ('+V') to four of the eight chemostats 29 days after *Synechococcus* inoculation to achieve a multiplicity of infection (i.e. virus to host ratio) of 10. The remaining chemostats without virus ('−V') served as controls. The myovirus used in this study (S-RIM8) was previously isolated from Mount Hope Bay, Rhode Island using *Synechococcus* WH7803 as the host (Marston & Sallee 2003). We quantified *Synechococcus*

and virus densities 3 days week^{-1} over the duration of the experiment using epifluorescent microscopy. For *Synechococcus*, chemostat samples were filtered onto 0.2 μm black polycarbonate filters (Osmonics, Minnetonka, MN, USA) and quantified as the number of autofluorescent cells using a Rhodamine filter set (546 nm excitation, 590 nm emission). We enumerated viruses using the SYBR Green staining procedures of Noble & Fuhrman (1998) after treating samples with DNAse 1.

Heterotrophic bacteria

The *Synechococcus* culture used to inoculate the chemostats was non-axenic. We isolated six strains of heterotrophic bacteria from the original inoculum and the chemostats that were capable of growing on Lauria–Bertani (LB) plates (a carbon-rich medium) in the dark. Based on morphological differences, we estimated the abundance of heterotrophic bacteria in the chemostats over time by calculating the number of colony forming units (CFU) via dilution plating onto LB agar medium. To explore the resource requirements of the heterotroph bacteria, we performed growth curve experiments with five of the above strains by inoculating them into LB and AN liquid media. In addition, we introduced the dominant heterotrophic population by itself into two replicate chemostats receiving AN medium under the same experimental conditions described above to determine whether it could persist in the absence of *Synechococcus*.

We identified the heterotrophic bacteria associated with *Synechococcus* by 16S rDNA sequencing. We extracted DNA from single colony isolates by lysing cells in distilled H_2O at 100 °C for 10 min. We PCR-amplified DNA using universal bacterial primers (8F: AGAGTTTGATCCTGGCTCAG; 1492R: GGTTACCTTGTTACGACTT; Weisburg *et al.* 1991), Platinum *Taq* Polymerase, and MasterAmp reagents. Template DNA was denatured at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 3 min. A final extension at 72 °C for 7 min was followed by a hold at 4 °C. We purified PCR products using the MoBio Clean-Up kit (Carlsbad, CA, USA). Sequencing was performed at the Research Technology Support Facility (RTSF) at Michigan State University. We determined the closest relatives of the heterotrophic sequences by conducting BLAST searches in GenBank (http://www.ncbi.nlm.nih.gov).

We evaluated the composition of the bacterial community in each chemostat at the end of the experiment using terminal restriction fragment length polymorphisms (T-RFLP; Liu *et al.* 1997). DNA was extracted from bacteria retained on 0.2 μm polycarbonate filters (Whatman) using a MoBio Ultra Clean DNA extraction kit (Carlsbad, CA, USA). The PCR conditions for T-RFLP were identical to those conducted on the single colony isolates above, except that the forward primer was fluorescently labelled (FAM-6). In duplicate, we digested 250 ng of the resulting PCR product with 20 U of the enzyme Hha 1 for 4 h. Bands from the T-RFLP analysis were examined using GENESCANVIEW software (http://bmr.cribi.unipd.it/). We excluded bands that were < 5% of the summed peak height for a given chemostat sample. In addition, we excluded *Synechococcus* bands from our analyses using Virtual Digest (http://mica.ibest.uidaho.edu/).

Sensitivity and virus-resistance of *Synechococcus*

We monitored the changes in sensitivity and resistance of *Synechococcus* populations in each +V chemostat by challenging them against virus populations collected from the same chemostat over the duration of our experiment (see Buckling & Rainey 2002). We enriched *Synechococcus* populations from each chemostat approximately every 2 weeks by transferring and incubating 2 mL of a chemostat sample in an Erlenmeyer flask (25 mL) containing sterile AN media under experimental conditions. We collected virus populations at the same time by passing a chemostat sample through 0.1 μm filters (Whatman) to remove all bacteria and then stored the filtrate containing viruses in glass vials at 4 °C. After *Synechococcus* achieved relatively high population density (~10^8 mL^{-1}), we exposed host cells (1 mL of culture) to virus (50 μL of 0.1 μm-filtrate) from different sampling times in replicate wells of a microtitre plate containing 1 mL of AN media for 3 weeks. We concluded that a *Synechococcus* population was 'sensitive' if it grew in control wells (i.e. no virus), but not in wells containing a virus population. Conversely, we concluded that a *Synechococcus* population had evolved to be 'resistant' if the enriched hosts grew in wells containing a virus population and in the control wells (see Stoddard *et al.* 2007). For the purposes here, the growth of the host population was determined by visual inspection; unlysed cultures of *Synechococcus* WH7803 appeared bright pink-red because they contain phycoerythrin, a photosynthetic accessory pigment (e.g. see Fig. 2).

Virus impact on nutrient cycling

We evaluated the impact of viruses on the availability of the limiting resource by measuring concentrations of total dissolved phosphorous (TDP), which comprised free PO_4^- and dissolved organic phosphorus (DOP) that might be released during cell lysis. We measured TDP based on the absorbance of phosphomolybdate and malachite green (Vanveldhoven & Mannaerts 1987). We also evaluated the impact of viruses on the allocation of carbon (C), N, and P to microbial biomass. To do so, we filtered chemostat samples onto pre-combusted Whatman GF/F (0.7 μm) filters. Particulate C and N were measured on these filters using a CE Instruments Model NC2100 elemental analyzer.

Particulate P was measured on separate filters using the colorimetric method described above. We expressed the C, N, and P concentrations in microbial biomass as C : N, C : P, and N : P ratios on a molar basis. Dissolved and particulate nutrients were measured weekly from samples in the collection vessels attached downstream of each chemostat.

Statistical analyses

We determined the effects of viruses on microbial and nutrient response variables using repeated measures ANOVA (SAS PROC MIXED) with covariance structures selected using the Bayesian Information Criterion (BIC; Wolfinger & Chang 1999). In addition, we estimated the temporal coherence between *Synechococcus* and virus densities in the +V chemostats by calculating the cross correlation coefficients on prewhitened data using the Auto Regressive Integrated Moving-Average (ARIMA) procedures in SAS. Lastly, using PRIMER software (v. 6.0, Lutton, Ivybridge, UK) we tested whether the assemblages of heterotrophic bacteria were affected by viruses using analysis of similarity (ANOSIM) with a Jaccard's similarity matrix generated from the T-RFLP data.

RESULTS

Synechococcus and virus dynamics

The population dynamics of *Synechococcus* were strongly affected by the introduction of an infectious virus (time × virus, $F_{49,294} = 5.72$, $P < 0.0001$). In the control chemostats (−V), *Synechococcus* obtained an equilibrium density (mean ± SEM) of $1.4 \times 10^7 \pm 4.51 \times 10^6$ cells mL^{-1} (Fig. S1). In contrast, *Synechococcus* densities declined immediately after virus addition (Fig. 1), dropping as low as 140 cell mL^{-1} in one of the replicate chemostats. By day 50, however, *Synechococcus* began to recover in the +V chemostats as indicated by an exponential increase in cell densities (Fig. 1). By day 100, *Synechococcus* densities began a second period of decline in two of the replicate +V chemostats, while host densities appeared to level-off in the other two +V chemostats (Fig. 1).

Virus densities were consistently higher than *Synechococcus* densities in all of the +V chemostats, yielding a virus : bacteria (V : B) ratio of 1525 ± 632.4 (mean ± SEM). Viruses closely tracked the host population dynamics. Cross correlation analyses on the individual chemostats revealed that *Synechococcus* and virus densities positively covaried through time ($P \leq 0.004$). In all four of the +V chemostats, the strongest cross correlation coefficients ($r = 0.71$–0.91) occurred with a zero lag (Fig. 1) suggesting that *Synechococcus* and virus populations were in-phase with one another.

Rapid evolution of virus resistance

In the control (−V) chemostats, all *Synechococcus* were sensitive to the ancestral virus over the duration of the experiment. Similarly, prior to virus addition (day 29), *Synechococcus* was sensitive to the ancestral virus and all virus populations isolated from the same +V chemostat on subsequent sampling dates (Fig. 2). In contrast, *Synechococcus* enriched from +V chemostats *after* virus addition were resistant to the ancestral virus and all virus populations isolated from the same chemostat on subsequent sampling dates. Together, these results suggest that resistance to the ancestral virus arose rapidly in all +V chemostats.

We did not find evidence for the evolution of a virus host-range mutant in the +V chemostats. For example, host populations that evolved resistance to the ancestral virus were not sensitive to virus populations isolated from the same chemostat at later time points in the experiment. However, we were unable to enrich for *Synechococcus* in chemostat $+V_1$ on day 50 or chemostat $+V_2$ on days 88, 102, and 166 (Fig. 2).

Heterotrophic bacteria

Based on 16S rDNA sequencing, the six heterotrophic bacteria isolated from our initial inoculum and chemostats were most closely related to *Bacillus aquaemaris* (Firmicutes, 98% similarity), *Bacillus megaterium* (Firmicutes, 99% similarity), *Dietzia daqingensis* (Actinobacteria, 100% similarity) *Brevibacterium sanguinis* (Actinobacteria 99%, similarity), *Brevundimonas vesicularis* (α-Proteobacteria, 100% similarity), and *Alcanivorax* sp. (γ-Proteobacteria, 99% similarity). These bacteria are commonly found in marine environments. *Alcanivorax* attained relatively high population densities in both the −V and +V chemostats, accounting for 65–100% of the heterotrophic community. Although *Alcanivorax* was able to persist in the absence of *Synechococcus* in replicate chemostats receiving AN, its equilibrium densities were reduced by almost 90% (*Synechococcus* presence-absence, $F_{1,8} = 12.46$, $P = 0.007$; Fig. 3). The other heterotrophic isolates were not capable of growing on AN medium (Fig. S2). Together, these results suggest that *Synechococcus*-derived organic matter was an important source of carbon and energy for the heterotrophic bacteria in our experiments.

Viruses had a weak indirect effect on heterotrophic bacteria. The total abundance of heterotrophic bacteria in the chemostats changed through time (time, $F_{48,288} = 9.78$. $P < 0.0001$), but was not affected by the virus treatment (virus, $F_{1,6} = 0.07$, $P = 0.81$; time × virus, $F_{48,288} = 0.98$, $P = 0.51$). The T-RFLP results suggest there were likely more than six strains of heterotrophic bacteria in our chemostats, because there were a total of 16 unique T-RFLP

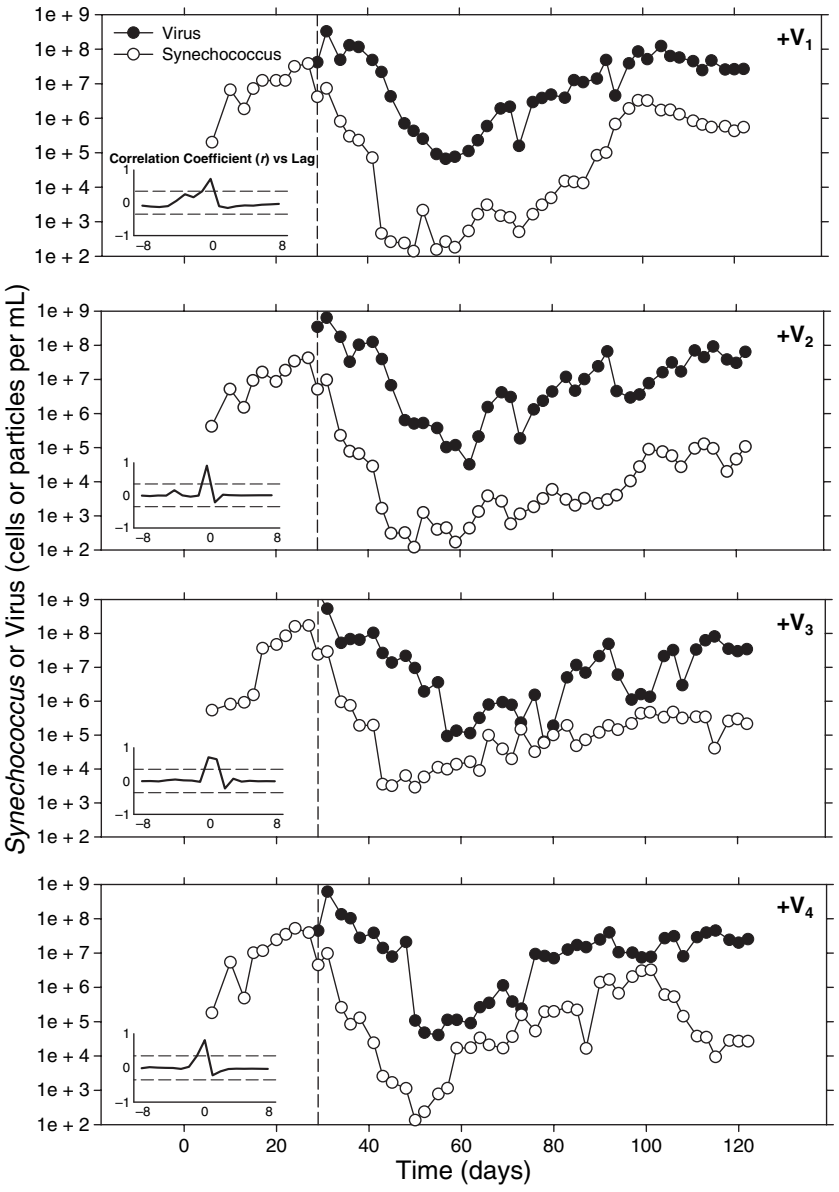

Figure 1 Population dynamics of *Synechococcus* and its infectious virus in four replicate chemostats ($+V_1$ to $+V_4$). The dashed vertical line (day 29) represent the time of virus addition. The inlayed panels reflect the cross correlation coefficients (r) for *Synechococcus* and virus populations at different sampling lags (−8 through +8). The dashed horizontal lines on the inlayed panels represent two standard errors corresponding to the overall cross correlation analysis for a given chemostat. See Fig. S1 for *Synechococcus* population dynamics in the control chemostats ($-V_1$ to $-V_4$).

peaks when pooled across all eight chemostats. However, at the end of the experiment, heterotrophic composition was only marginally affected by the virus treatment (ANOSIM, $R = 0.474$, $P = 0.086$).

Virus impact on nutrient cycling

Viruses significantly altered the concentrations of TDP, the limiting resource in our chemostats (time × virus, $F_{14,84} = 5.60$, $P < 0.0001$). There was a five-fold increase in TDP immediately following virus addition. Although this effect decreased with time, TDP in the +V chemostats did not return to the concentrations that were observed in the −V chemostats or the concentrations observed in the +V chemostats prior to virus addition (Fig. 4). In addition, the among-replicate variability of TDP was higher in the +V treatment than in the −V treatment (Fig. 4).

Stoichiometric ratios of particulate C, N, and P suggest that viruses modified the way in which microbial communities allocated nutrients into biomass. Although the C : P ratios of microbial biomass changed over time (time, $F_{14,84} = 4.72$, $P < 0.001$), they were not affected by virus treatment (virus, $F_{1,6} = 0.62$, $P = 0.46$; time × virus, $F_{14,84} = 0.63$, $P = 0.83$). In contrast, viruses significantly altered C : N ratios (time × virus, $F_{14,84} = 2.48$, $P = 0.005$) and N : P ratios (time × virus, $F_{14,84} = 2.63$, $P = 0.003$) over the course of the experiment. In general, +V

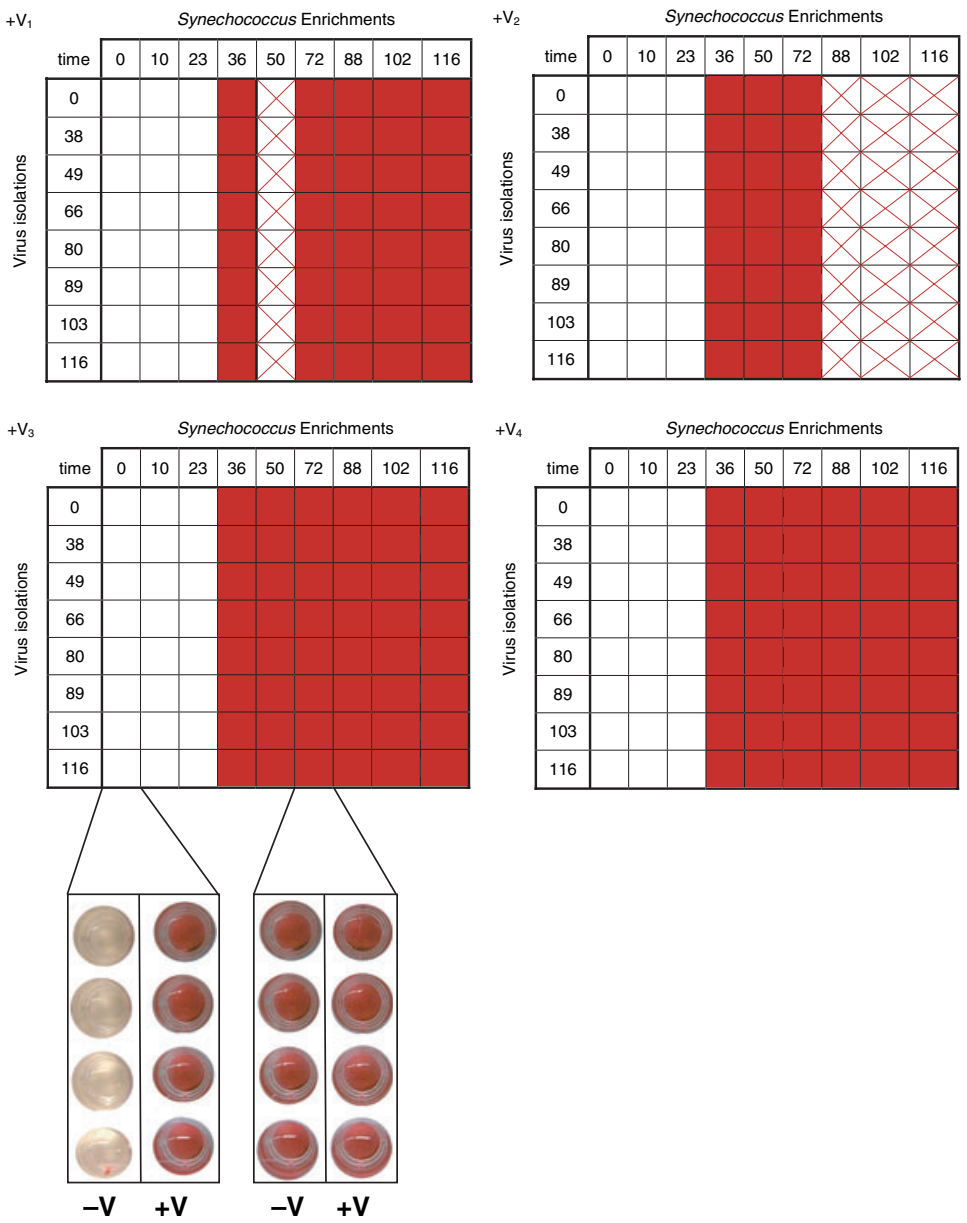

Figure 2 Sensitivity and virus-resistance of *Synechococcus* in four replicate chemostats ($+V_1$ to $+V_4$). At each time point, samples were taken from each chemostat to enrich for *Synechococcus* populations or to isolate virus populations. Populations of *Synechococcus* were then challenged against viruses isolated from different time points. White cells indicate that *Synechococcus* was sensitive and lysed when exposed to particular virus population (lower left photo). Pink-red cells indicate that *Synechococcus* was resistant when challenged by a particular virus population (lower right photo). For chemostats $+V_1$ and $+V_2$, we were unable to evaluate the sensitivity or resistance of the host on sampling dates 50, 88, 102, and 116 (cells with 'X') because *Synechococcus* taken from the chemostats did not grow in the enrichment flasks.

chemostats had lower C : N ratios and higher N : P ratios than −V chemostats indicating that viruses increased the storage and/or allocation of N into biomass. Like TDP, however, the magnitude of this virus effect on C : N and N : P diminished with time (Fig. 4). Together, the attenuation of the virus effect coincided with the recovery of *Synechococcus* (Fig. 1) and the detection of resistant hosts (Fig. 2), suggesting that rapid evolution buffered the impacts of viruses on nutrient cycling.

DISCUSSION

Because they are abundant and parasitic, it is often assumed that viruses have strong top-down effects on microbial and

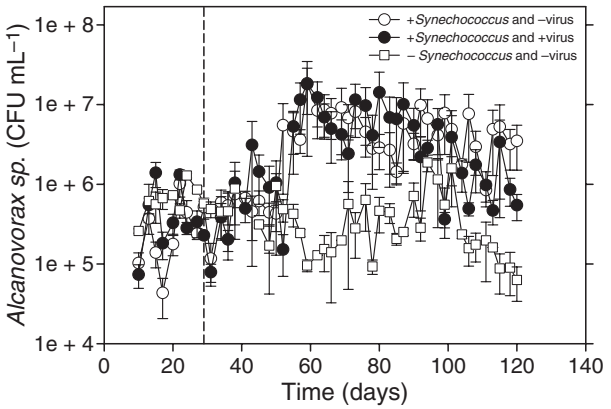

Figure 3 The abundance of *Alcanivorax,* the dominant heterotrophic bacterium, in three replicated chemostat treatments: absence of *Synechococcus*, presence of *Synechococcus* minus virus, presence of *Synechococcus* plus virus. Although its abundance was significantly reduced, *Alcanivorax* was able to persist in chemostats that did not contain *Synechococcus*. Viruses had no affect on the abundance of *Alcanivorax* in chemostats containing *Synechococcus*. The dashed vertical line (day 29) represents the time of virus addition for +V chemostats.

ecosystem processes (Wilhelm & Suttle 1999). In addition, viruses may have strong bottom-effects because lysis alters the quantity and quality of growth-limiting resources (Brussaard *et al.* 2008). Results from our controlled experiments support these general views: viruses altered host population dynamics (Fig. 1), which in turn influenced resource availability and the allocation of nutrients into microbial biomass (Fig. 4). However, these virus-induced effects were somewhat transient and diminished over time. In contrast to our prediction, indirect effects of viruses on non-target microorganisms were relatively weak. Therefore, heterotrophic bacteria did not appear to compensate for the virus impact on nutrient dynamics. Instead, our results suggest that the evolution and subsequent invasion of resistant *Synechococcus* (Fig. 2) modified the top-down effect of viruses on nutrient cycling in our experimental food web. While intraspecific genetic variation and evolutionary processes are often thought to have minimal impacts on ecosystem processes, our results are consistent with theoretical predictions (Loeuille & Loreau 2004; Fussmann *et al.* 2007) that rapid evolution can strongly affect community dynamics and nutrient cycling.

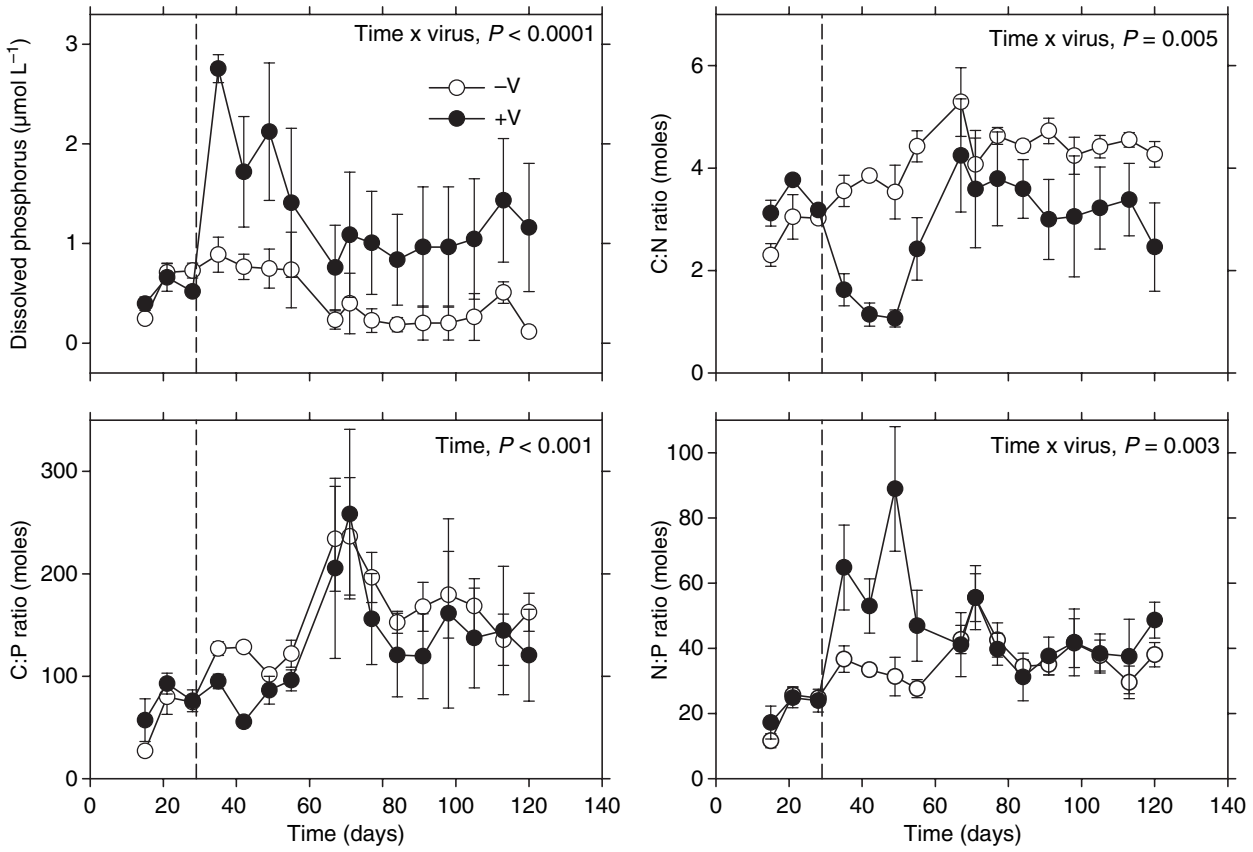

Figure 4 Effects of virus on dissolved nutrients and microbial stoichiometry. The dashed vertical line (day 29) represents the time of virus addition for +V chemostats; −V denotes control chemostats without virus.

Eco-evolutionary effects of viruses on *Synechococcus*

As expected, viruses had a strong impact on *Synechococcus* dynamics in our relatively long-term chemostat experiment. Temporal covariation between bacteria and viruses in aquatic ecosystems has been attributed to seasonal forcing and predator–prey cycling, but may also be influenced by evolutionary processes (Middelboe *et al.* 2001). For example, many strains of marine cyanobacteria are resistant to their co-occurring viruses (Waterbury & Valois 1993) and the composition of infectious viruses has been shown to change over seasonal time scales (Marston & Sallee 2003). Furthermore, studies examining whole genome expression provide evidence for coevolution between marine cyanobacteria and their viruses (Lindell *et al.* 2007).

An increasing number of studies have demonstrated that prey or host evolution can influence population and community dynamics (e.g. Abrams & Matsuda 1997; Fussmann *et al.* 2007). For example, Yoshida *et al.* (2003) explored the interactions between rotifers and algae in chemostats that manipulated the genetic diversity of the prey population. Treatments with low prey genetic-diversity exhibited classic Lotka–Volterra dynamics, characterized by relatively short population cycles that were approximately one-quarter out-of-phase. In contrast, treatments with high prey genetic-diversity had longer cycles that were perfectly out-of-phase. These 'cryptic' population dynamics were attributed to a trade-off between prey competitive ability and antipredator defense traits (Yoshida *et al.* 2003).

Results from our experiment suggest that rapid evolution may have influenced virus and *Synechococcus* dynamics. In each of the +V chemostats, there was a significant positive cross-correlation with a zero-lag between *Synechococcus* and viruses, indicating that these populations were in-phase with one another (Fig. 1). Such patterns are not predicted by traditional ecological theory (Turchin 2003), but are generated in adaptive simulation models where there is directional selection for increased parasite virulence and increased host investment for resistance (Sasaki & Godfray 1999). However, it is possible to simulate in-phase dynamics given our sampling schedule and realistic parameter combinations (Lennon, unpublished data). As such, additional studies are needed to explore the potential for rapid evolution to lead to in-phase virus–bacteria dynamics.

Other lines of evidence provide stronger evidence that rapid evolutionary processes may have influenced community and ecosystem processes in our chemostat experiment. We were able to retrieve resistant *Synechococcus* from all of the +V chemostats in as little as seven days following virus addition (Fig. 2), while resistant populations were never recovered from −V chemostats under our experimental conditions. *Synechococcus* population densities began to recover in the +V chemostats after the resistant phenotypes were detected. In our system, resistance does not seem to be explained by phenotype plasticity (see Thyrhaug *et al.* 2003), since resistant strains of *Synechococcus* rarely reverted to the sensitive phenotype when they were cultured in the absence of the virus for extended periods of time (> 1 year; Lennon *et al.* 2007). Likewise, adsorption kinetic assays with *Synechococcus* WH7803 indicated that resistance most likely arises from the loss or modification of cell receptor sites that are used for virus attachment (Stoddard *et al.* 2007). As theory suggests, we have documented that there is often a fitness cost associated with virus-resistance for *Synechococcus*. When detected, virus-resistance led to an average 20% reduction in relative fitness. However, the exact magnitude of the cost was dependent on the identity of the virus, suggesting that different cell receptors were under selection and that there may be multiple pathways leading to virus-resistance in *Synechococcus* (Lennon *et al.* 2007). Such differences in the cost of resistance are critical for maintaining multiple prey phenotypes (e.g. sensitive and resistant *Synechococcus* strains) and for understanding how rapid evolution influences ecological dynamics (Yoshida *et al.* 2007).

We did not find evidence for coevolution in our experiment. Despite the prevalence of virus-resistant *Synechococcus* in the +V chemostats, we did not detect virus host-range mutants. If a host range mutant evolved, *Synechococcus* would have been resistant when challenged against viruses isolated from early time points in the experiment, but sensitive to viruses isolated from later time points in the experiment. In contrast, our results suggest that resistant *Synechococcus* were present throughout most the experiment following virus addition (Fig. 2). A potential exception may have been in chemostat $+V_4$, where *Synechococcus* enrichments failed to grow into dense cultures for the last three sampling dates (after day 72) and so could not be tested for their infectivity to the virus communities (Fig. 2). Although coevolution is a common outcome of experiments involving bacteria–virus interactions (Buckling & Rainey 2002; Mizoguchi *et al.* 2003), it is not necessarily the rule. For example, some viruses (e.g. T7) evolve host-range mutations that allow them to infect resistant hosts (e.g. *E. coli*), while some viruses do not (e.g. T4) (Bohannan & Lenksi 2000).

Indirect effects of viruses on heterotrophic bacteria

Viruses had strong direct effects on the temporal dynamics and evolutionary trajectory of the autotrophic host population, *Synechococcus* (Figs 1 and 2). We hypothesized that these effects might also indirectly affect other populations (i.e. heterotrophs) and processes (i.e. nutrient cycling) in the experimental food web. For example, viruses are thought to influence the abundance and composition of microbial communities via density-dependent shifts in predation

pressure ('kill the winner' hypothesis, Thingstad & Lignell 1997) and/or through changes in the availability of growth-limiting resources (Middelboe *et al.* 1996). Either of these mechanisms might have allowed heterotrophic bacteria to compensate for the effects of viruses on *Synechococcus* (see Bratbak *et al.* 1998).

Viruses only had weak indirect effects on heterotrophic bacteria in our study. These results were somewhat surprising given that virus-mediated changes in *Synechococcus* abundance drastically altered resource availability for heterotrophic bacteria (Figs 4 and S3). *Alcanivorax* was unaffected by the virus treatment, even though this dominant heterotroph could only attain one-tenth of its equilibrium abundance in chemostats lacking *Synechococcus* (Fig. 3). The remaining heterotrophic populations were unable to grow in AN media without *Synechococcus* (Fig. S2). Together, these results suggest that heterotrophic bacteria relied heavily on photosynthetically derived organic carbon, but at the same time it did not seem to matter whether this resource was supplied continuously as exudates or as lysates following virus infection. Similarly, T-RFLP profiles from the end of the experiment indicate that the virus treatment only had a marginally significant indirect effect on heterotrophic community composition. In other systems, viruses have been shown to influence bacterial community composition (e.g. Schwalbach *et al.* 2004; Bouvier & del Giorgio 2007), but it is important to note that these studies experimentally manipulated the abundance of entire virus communities and thus were not designed to test for the indirect effects of individual viruses on non-target populations. In sum, results from our study suggest that viruses had weak indirect effects on the abundance and composition of heterotrophic bacteria. Therefore, heterotrophic bacteria were not capable of buffering the ecosystem impacts of viruses on an autotrophic host population.

Virus effects on nutrient cycling

Viruses are thought to play an important role in regulating the nutrient cycling of aquatic ecosystems (e.g. Wilhelm & Suttle 1999; Suttle 2007). Results from our study show that viruses had a strong effect on total dissolved phosphorus (TDP), the growth-limiting resource in our chemostats. TDP increased fivefold after virus addition, presumably due to reduced uptake of inorganic P and/or the release of cytoplasmic organic P following host lysis. Although this virus effect diminished with time, concentrations of TDP did not converge upon those found in the controls. At the end of the experiment TDP was still 1 μmol L^{-1} higher in the +V vs. −V chemostats (Fig. 4). The magnitude of this difference is on par with estimates of the phosphate half-saturation constant for *Synechococcus* (Ikeya *et al.* 1997), suggesting that virus-mediated effects on nutrient cycling may influence the outcomes of resource competition.

Recently, it has been hypothesized that viruses may alter microbial stoichiometry via changes in the bioavailability of C, N, and P (Suttle 2007). For example, virus-induced lysis releases labile carbon substrates (e.g. amino acids, dissolved DNA, carbohydrates), which when consumed by some microorganisms can increase the demand for N and P. Results from our study support this general hypothesis. For example, the C : N ratio was significantly lower and the N : P ratio was significantly higher in the +V vs. −V chemostats (Fig. 4), indicating that viruses increased the incorporation of N into microbial biomass. There are at least two potential explanations for why viruses may have affected microbial stoichiometry in our experiment. First, viruses may have altered microbial stoichiometry via their effect on bacterial growth rates. Prior to virus addition, *Synechococcus* was at equilibrium and thus had a constant population growth rate as determined by the dilution rate. After virus addition, however, *Synechococcus* was no longer at equilibrium and therefore could grow faster than the dilution rate. Such changes in growth rate can alter the uptake, storage, and assimilation of nutrients in ways that modify microbial stoichiometry (Sterner & Elser 2002). Second, it still remains unclear whether the stoichiometries of microbial populations are homeostatic (i.e. fixed) or plastic (Makino & Cotner 2004). If the microbes in our study were homeostatic and also had different C : N : P ratios, then virus-induced shifts in microbial composition over time might explain the stoichiometric patterns in our experiment (Fig. 4).

At the same time, our results are consistent with the view that evolutionary processes buffer the effects of viruses on ecosystem processes (Olofsson & Kjelleberg 1991). The treatment effects in our experiments were most pronounced immediately after virus addition and then attenuated with time. This pattern cannot be attributed to compensation by heterotrophic bacteria. Rather, our results suggest that rapid host evolution was responsible for dampening the virus impacts on nutrient cycling. The recovery of *Synechococcus*, which began *c.* 3 weeks after virus addition, coincided with the appearance of a resistant host phenotype and a decrease in the virus effect on nutrient cycling. Although virus-resistant *Synechococcus* experience reduced fitness (Lennon *et al.* 2007), this effect may be negligible in resource-rich environments when sensitive hosts are being maintained at low densities due to virus predation. The potential for rapid evolution to influence host–parasite dynamics has important implications for understanding the 'top-down' role of viruses in controlling phytoplankton and harmful algal blooms (Brussard *et al.* 2005). Taken together, our results add to a growing body of literature demonstrating the importance of evolutionary processes on ecologically

relevant time scales (Hairston *et al.* 2005; Strauss *et al.* 2008), while also confirming theoretical predictions that rapid evolution has the potential to not only influence community dynamics, but also ecosystem processes (Loeuille & Loreau 2004; Fussmann *et al.* 2007).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Figure S1 Population dynamics of *Synechococcus* in four replicate chemostats ($-V_1$, $-V_2$, $-V_3$, $-V_4$).

Figure S2 Growth curves for some of the heterotrophic bacteria that were isolated from the inoculum and chemostats.

Figure S3 Concentrations (mean ± SEM) of particulate (i.e. microbial) carbon, nitrogen, and phosphorus in +V and −V treatments over the duration of the chemostat experiment.

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