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Multifaceted impacts of the stony coral *Porites astreoides* on picoplankton abundance and community composition

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Abstract

Picoplankton foster essential recycling of nutrients in the oligotrophic waters sustaining coral reef ecosystems. Despite this fact, there is a paucity of data on how the specific interactions between corals and planktonic bacteria and archaea (picoplankton) contribute to nutrient dynamics and reef productivity. Here, we utilized mesocosm experiments to investigate how corals and coral mucus influence picoplankton and nutrients in reef waters. Over 12 days, we tracked nutrient concentrations, picoplankton abundances and taxonomic composition of picoplankton using direct cell-counts, sequencing of SSU rRNA genes and fluorescent in situ hybridization-based abundances of dominant lineages in the presence or absence of *Porites astreoides* corals and with mucus additions. Our results demonstrate that when corals are present, *Synechococcus*, SAR11 and *Rhodobacteraceae* cells are preferentially removed. When corals were removed, their exudates enhanced the growth of diverse picoplankton, including SAR11 and *Rhodobacteraceae*. A seven-fold increase in nitrate concentration, possibly caused by nitrogen remineralization (ammonification coupled to nitrification) within the coral holobiont, may have further facilitated the growth of these taxa. In contrast, the addition of mucus resulted in rapid initial growth of total picoplankton and *Rhodobacteraceae*, but no measurable change in overall community structure. This study presents evidence of the multifaceted influences of corals on picoplankton, in which the coral holobiont selectively removes and promotes the growth of diverse picoplankton and remineralizes nitrogen.

Coral reefs support diverse communities of fish and invertebrates as well as rich communities of microorganisms (Knowlton 2001; Rohwer et al. 2002; Dinsdale et al. 2008). While reef habitats subsist at tropical latitudes generally containing oligotrophic waters, they are one of the most productive marine ecosystems (Crossland et al. 1991). Reef-building corals form the structural basis for this ecosystem, and they rely on a partnership with endosymbiotic dinoflagellates (*Symbiodinium* spp.) for significant energy supplementation (Muscatine et al. 1981). Corals and reef habitats are threatened by a number of climate-related and anthropogenic impacts, including ocean warming, ocean acidification, eutrophication and sedimentation (Szmant 2002; Fabricius 2005; Carpenter et al. 2008; Doney et al. 2009). As a result, abundances of coral have decreased substantially on reefs worldwide (Carpenter et al. 2008), resulting in major shifts in the balance and stability of reef ecosystems (Mumby et al. 2007). Thus, there is significant motivation to better understand the fundamental processes that corals provide to sustain reef ecosystems.

The microorganisms in reef waters form the basis of the coral reef food web, and their activities are central to the biogeochemistry and productivity of reefs (Ducklow 1990; Sorokin 1995). Reef water picoplankton (planktonic bacteria and archaea) are responsible for recycling more than half of the net productivity on reefs (Ducklow 1990). The microbial biogeochemistry of the waters surrounding coral-rich reefs appear unique, with reef waters depleted in dissolved organic carbon (DOC) and picoplankton compared to adjacent

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coastal waters (Apprill and Rappé 2011; Nelson et al. 2011), despite the fact that corals excrete substantial DOC and dissolved organic nitrogen (DON), as well as particulate mucus (Bythell 1988; Wild et al. 2004; Tanaka et al. 2011). Waters with longer residence time on the reef contain more productive microorganisms (Apprill and Rappé 2011), further suggesting that the presence of corals stimulates reef water microbial dynamics and selects for a distinct picoplankton community.

While corals do appear to influence reef picoplankton, the specific microbial lineages influenced by corals are not well resolved. Some bacterioplankton have been shown to respond to mucus additions from corals, including *Alteromonadaceae* and *Vibrionaceae* growth from *Fungia* mucus (Allers et al. 2008) as well as *Rhodobacterales*, *Oceanospirillales*, *Vibrionales*, *Alteromonadales* and *Bacteroidetes* growth on *Acropora* mucus (Taniguchi et al. 2015). In the case of coral excretion, it appears that certain members of the picoplankton community respond to coral exudates, especially the Alphaproteobacteria families *Erythrobacteraceae*, *Kordiimonadaceae*, *Hyphomonadaceae* and *Sneathiellaceae*, the *Bacteriovoraceae* family of the Deltaproteobacteria and the Planctomycetes clade OM190 (Nelson et al. 2013). In field surveys, *Synechococcus* positively correlated with hard coral cover while *Altermononadales* negatively correlated with hard coral cover and was associated with algae-dominated reefs (Kelly et al. 2014). In both of the previously mentioned studies, it appears that the heterotrophic bacteria associated with reefs are generally members of faster-growing or copiotrophic lineages (Brown et al. 2014). Corals can also influence the picoplankton community by actively feeding on these cells (Sorokin 1973). Although the specificity of feeding on picoplankton has not yet been addressed, it is possible that corals have a finely-tuned, positive feedback-type influence on the reef water microbial community. In fact, studies have shown that bacterioplankton were depleted over reef systems in Moorea (Nelson et al. 2011) and directly over corals and within reef crevices (Gast et al. 1998; Seymour et al. 2005). In this scenario, coral exudates preferentially support the growth of nutrient-rich, fast-growing copiotrophic-type cells, which they in turn feed upon. Uncovering these covert interactions in field settings is challenging because natural reefs harbor diverse and abundant types of life, each with unique influences on the picoplankton community.

The aim of this study was to examine if the presence of *Porites astreoides* corals or mucus originating from *P. astreoides* results in measurable alterations in the picoplankton community composition and nutrient dynamics of reef waters. *P. astreoides* is an ideal coral for this study because its high recruitment success has increased its relative abundance on Caribbean reefs (Green et al. 2008). The bacterial community associated with both the mucus and tissues of *P. astreoides* is relatively well-described (Rowher et al. 2002; Morrow et al. 2012; Rodriguez-Lanetty et al. 2013). Lastly, corals belonging to the *Porites* genus are dominant and long-lived species among diverse habitats in worldwide reefs (Stoddart 1969), and these results may therefore be informative for other reef environments.

In this study, we used mesocosm-based experiments to investigate dynamics in total picoplankton abundance and specific taxonomic groups of cells, as well as nutrients, when exposed to colonies of *P. astreoides* or *P. astreoides* mucus, compared to control conditions. Our results reveal that the immediate presence of *P. astreoides* influences the abundance and composition of reef water picoplankton, which is partially attributed to holobiont grazing, and fuels a surprisingly high rate of nitrogen remineralization. These findings highlight the significant, multifaceted influences that corals have on their surrounding picoplankton community.

Methods

Experimental overview

Colonies of *P. astreoides* (5–20 cm diameter, 30–90 g wet weight) were removed from 3 m to 9 m depth on the Bermudian reefs Hog Breaker (N 32° 27.5′ W 64° 49.8′), an unnamed reef (N 32° 26.042′ W 64° 49.248′), and Three Hills Shoal (N 32° 41′ W 64° 73.3′) in July 2013. Ten colonies were taken from each site in compliance with the Bermuda Institute of Ocean Sciences (BIOS) Collection and Experimental Ethics Policy and were considered Limited Impact Research and as such a collection permit was not required. The colonies were immediately placed in collection bags at depth, sealed and transported in a large collection cooler (<1 h) to the laboratory. At BIOS, the colonies were allowed to acclimate to a mesocosm housing and held in aerated, outdoor fiberglass mesocosms with a flow-through seawater system fed with reef water for two (Three Hills Shoal Reef colonies) or 15 days (all other colonies) prior to the start of the experimental period.

For the experiment, the mesocosms consisted of nine, 30 L static aquaria that were set up as previously described (de Putron et al. 2010). Inshore water from 20 m off shore and one meter in depth was used to fill the aquaria and was pumped through a BIOS flow-through sample system that consisted of a coarse mesh filter and holding tanks followed by a step filtration system of 50 μm, then 5 μm, to remove larger organisms including some planktonic grazers. Mesocosms were then left static for the 12-day experiment. Mesocosms were randomly arranged to consist of three control, three mucus addition, and three coral mesocosms, with artificial lights providing 61 μmol quanta m^{-2} s^{-1} photosynthetically active radiation during sunlight hours consistent with the low end of known compensation ranges (3–233 μmol quanta m^{-2} s^{-1} according to Mass and colleagues (2007)) to minimize bleaching. Mesocosms were covered with a transparent plastic film cover and aerated with an air

wand bubbler (de Putron et al. 2010). Water was collected from the mesocosms via a syringe-siphon system using silicon tubing. Inline, combusted GF/F filters (Whatman, Maidstone, UK) were attached for samples intended for dissolved organic carbon (DOC) analysis. The reef water temperature, salinity, and conductivity in all mesocosms was monitored daily throughout the experiment using a YSI Professional Plus probe (Yellow Springs, Ohio).

Over the course of 12 days, the mesocosms were subjected to one of three conditions: coral (presence/absence of colonies), mucus additions or control (no additions) (Table 1). For the coral mesocosms, the experimental corals (four colonies per mesocosm, three mesocosms) were placed within the mesocosms on days 0 through 4 and removed after water sampling on day 4. Corals were removed for 48-h until after the sampling procedure on day 6, where they were held in running seawater similarly filtered as in the mesocosms, and reintroduced back into their respective mesocosms from days 6 to 10 and removed again after the sampling procedure on day 10. *P. astreoides* colonies were not fed over the course of the 12-day experimental period. For the mucus addition mesocosms, mucus was extracted from *P. astreoides* colonies ($n = 18$) that were not utilized in the experiment and were maintained in a separate, flow-through seawater tank. These corals ($n = 18$) were taken into the lab and inverted on a funnel to collect mucus for 2 h, a timeframe determined from a preliminary experiment, and the corals returned to the holding aquaria with flow through seawater. Mucus from the additional colonies was pooled and 5 mL was added to each mucus addition mesocosm on days 0.2, 2, 6, and 8 (Table 1). Mucus from the experimental corals ($n = 12$; named corals A-L from coral tanks 4, 5, and 6) was sampled for community composition prior to day 0, as well as at the end of day 4 and at the end of day 10 of the experiment. For the latter two timepoints, the corals were removed from the experimental mesocosms for 48 hs.

Picoplankton abundances and specific lineage abundance as determined by FISH

Seawater samples (50 mL) for cell counts and fluorescence *in situ* hybridization (FISH) analyses were taken daily from all mesocosms, fixed with formalin to a final concentration of 10% in the dark for 20 min, and stored at −80°C. Samples were thawed and 3–5 mL were filtered onto Irgalan Black stained 25 mm, 0.2 μm polycarbonate filters (Nucleopore, Whatman) under gentle vacuum (~100 mm Hg) and stained with 1 mL of 6, 6-diamidino-2-phenyl dihydrochloride (5 μg mL^{-1}, DAPI, SIGMA-Aldrich, St. Louis, MO) (Porter and Feig 1980). The filters were mounted onto slides with Resolve immersion oil (high viscosity) (Resolve, Richard-Allan Scientific, Kalamazoo, MI) and stored at −20°C. Slides were then enumerated using an AX70 epifluorescent microscope (Olympus, Tokyo, Japan) under ultraviolet excitation at 100x magnification. At least 500 cells (10 fields) were counted for picoplankton abundance.

FISH was utilized to quantify the abundance of the major picoplankton phylotypes present in the seawater and mucus, and was conducted using previously published protocols and Cy3 labeled probes (Parsons et al. 2014). The bacterial and archaeal groups quantified included the SAR11 clade (152R, 441R, 542R, 732R probes), *Alteromonas* spp. (AC137R), *Vibrio* spp. (127R), *Rhodobacteraceae* (536R), Euryarchaeota (Eury806), and Thaumarchaeota (Cren537). Fixed seawater samples (3–5 mL) were filtered onto 25 mm, 0.2 μm polycarbonate filters and stored at −20°C with desiccant. Quarter filters were washed in 95% ethanol and then probed according to previous protocols (Morris et al. 2002; Parsons et al. 2011, 2014). The cell abundances of the picoplankton phylotypes mentioned above were then determined using image analysis (Parsons et al. 2011, 2014). Detection of Cy3-positive cells and their ratio to DAPI-positive cells was aided by image analysis using an Olympus AX70 microscope (Olympus, Japan) equipped with a Toshiba 3CCD video camera (IK-TU40A Toshiba, Japan), a computer assisted frame grabber and appropriate dichroic filters (Morris et al. 2002; Carlson et al. 2010). Brief exposure times of 1 and 5 s were used for DAPI and Cy3 image channels, respectively. Cy3 images were segmented with Image Pro Plus software (Media Cybernetics, Bethesda, MD) and overlaid onto corresponding segmented DAPI images (Parsons et al. 2014). Objects with overlapping signals in both Cy3 and DAPI images were counted as probe positive. The negative control was determined similarly and subtracted from the positive probe counts to correct for autofluorescence and non-specific binding.

Apparent growth and grazing rates

Here, we use the term 'grazing' to refer to the combination of direct ingestion by *P. astreoides* and/or grazing by coral-associated protista. Specific rates (units of d^{-1}) were calculated for total picoplankton cells (from DAPI counts), *Synechococcus* (from flow cytometry), and the SAR11 clade and *Rhodobacteraceae* (from FISH counts). Grazing rates were calculated using the standard equations of Frost (1972). The growth constant (k), or net apparent growth rate for each microbial group was calculated from each mesocosm as:

$$C_2 = C_1 e^{k(t_2 - t_1)} \quad (1)$$

where C_1 and C_2 are the cell concentrations (cells mL^{-1}) at t_1 and t_2. Grazing coefficients (g) were then calculated as the difference in k between the control and the coral addition mesocosms. Uncertainty in g was calculated using uncertainty propagation (Kline and McClintock 1953). The ANOVA and Tukey ad hoc statistics were done using the aov and tukeyHSD functions in the stats package within the R programming language. The Honesty Significant Difference is

Table 1. Overview of the mesocosm treatment conditions ($n = 3$ per treatment; bolded text indicates when corals were removed).

	Treatment			Analyses			
Time (days)	Corals	Mucus	Control	Nut*	DOC	Cell counts	FISH & sequencing
0	**Corals out**	**NA**[†]	**NA**	**x**[‡]	**x**	**x**	**x**
0.2	Corals in[§]	Mucus added[§]	NA	x		x	
1	Corals in (24 h total)	NA	NA			x	
2	Corals in (48 h total)	Mucus added[§]	NA			x	x
3	Corals in (72 h total)	NA	NA			x	
4	Corals in (96 h total)	NA	NA	x	x	x	x
5	**Corals out (24 h total)**	**NA**	**NA**			**x**	
6	**Corals out (48 h total)**[§]	**Mucus added**[§]	**NA**	**x**	x	**x**	**x**
7	Corals in (24 h total)	NA	NA			x	
8	Corals in (48 h total)	Mucus added[§]	NA			x	x
9	Corals in (72 h total)	NA	NA			x	
10	Corals in (96 h total)	NA	NA	x	x	x	x
11	**Corals out (24 h total)**	**NA**	**NA**			x	
12	**Corals out (48 h total)**	**NA**	**NA**	**x**	**x**	**x**	**x**

*Nut, Nutrients.
[†]NA, not applicable.
[‡]x, analysis was performed.
[§]Corals and mucus added immediately after sampling.

tabled as the mean HSD value with its associated p value for significance at 95% confidence for all time points ($n = 3$) and for the four groups—picoplankton, *Synechococcus*, SAR11 and *Rhodobacteraceae*.

Taxonomic composition of picoplankton using amplicon sequencing

Seawater picoplankton biomass for nucleic acids was taken from all experimental mesocosms on days 0, 2, 4, 6, 8, 10, and 12 of the experiment, and from coral mucus extracted from the experimental corals on days 2, 4, and 10 and processing followed a method modified from Giovannoni and colleagues (1990, 1996). 500 mL of water or 1 mL of coral mucus was filtered through a 47 mm, 0.2 μm pore filter under gentle vacuum (~100 mm Hg), placed into a 4 mL cryovial and stored in 1 mL of sterile sucrose lysis buffer (20 mM EDTA, 400 mM NaCl, 0.75 M sucrose, 50 mM Tris.HCl) at −80°C. For DNA extractions of the tissue, mucus and coral samples, sodium dodecyl sulfate to 1% and proteinase K to 200 μg mL^{-1} were added to the sample and incubated at 37°C for 30 min and then at 55°C for 30 min. The lysates were extracted with an equal volume of phenol:isoamylalcohol:chloroform (25:1:24) followed by two subsequent equal volumes of isoamylalcohol:chloroform (1:24). The DNA was purified by precipitation using sodium acetate (3M) and isopropanol (100%) for at least 1 h at −20°C and centrifuged at room temperature for 30 min at 20,000 × g. The resulting pellet was washed with 80% ethanol, vortexed for 30 s and centrifuged at 16,000 × g for 10 min. The pellet was dried and stored at −20°C.

The microbial composition of the DNA was assessed by targeting the V4 region of the SSU rRNA gene using modified primers, 515F and 806RB, as outlined by Apprill and colleagues (2015). Triplicate 25 μL PCR reactions were conducted per sample, and contained 1.25 U of GoTaq Flexi DNA Polymerase (Promega Cooperation, Madison, WI), 5X Colorless GoTaq Flexi Buffer, 2.5 mM $MgCl_2$, 200 μM dNTP mix, 200 nM of each barcoded primer, and 1–4 ng of genomic template. The reaction conditions consisted of an initial denaturation step at 95°C for 2 min, followed by 27–34 cycles of 95°C for 20 s, 55°C for 15 s, and 72°C for 5 min, concluding with an extension at 72°C for 10 min. Reactions were carried out on a Bio-Rad thermocycler (Bio-Rad Laboratories, Inc., Hercules, CA). Reaction products (5 μL) were screened on a 1% agarose/TBE gel using a HyperLadder 50 bp standard (generally 5 ng ul^{-1}) (Bioline, London, UK). Samples were optimized for the lowest number of cycles that resulted in an amplified PCR product detected on a gel. The three replicate reactions were purified using the QIAquick Purification Kit (Qiagen, Valencia, CA), and quantified using the Qubit fluorescent broad range dsDNA assay (Life Technologies, Grand Island, NY).

Amplicons were pooled in equimolar ratios and shipped to the University of Illinois W.M. Keck Center for Comparative and Functional Genomics and sequenced using 2x250 bp paired-end MiSeq (Illumina, San Diego, CA), as detailed

previously (Kozich et al. 2013). Sequence analyses were conducted using mothur v.3.3.3 (Schloss et al. 2009) and included assembly of the paired ends, amplicon size selection (253 bp median size) and alignment to the SSU rRNA gene. Chimera detection was also conducted via UCHIME (Edgar et al. 2011) using mothur, and chimeric sequences (7–18% of sequences per sample) were removed. Taxonomic classification of sequences was conducted with the SILVA SSU Ref database (release 119) using the k-nearest neighbor algorithm. Sequences were grouped into operational taxonomic units (OTUs) using minimum entropy decomposition (MED) (Eren et al. 2015). Statistical analysis of these data was conducted using Primer (v.7, Primer-E Ltd, Ivybridge, UK). Bray Curtis similarity data was used to create Non-metric Multidimensional Scaling (NMDS) and Principle Coordinate (PCO) analyses, ANOSIMS and PERMANOVA tests with the OTU data. Significant differences between sequence percentages were compared using two-way ANOVA with Tukey's post hoc tests per time point using Prism 7 (GraphPad Software, Inc., La Jolla, CA). Additionally, the data were tested to determine if PCR cycle number impacted the representation of any OTUs using the BEST analysis with Primer, and did not identify any OTUs correlated with PCR cycles. Sequence data are available at NCBI's SRA under accession PRJNA 312300.

Identification of putatively ammonia-oxidizing bacteria and archaea

Quantitative PCR (qPCR) was used to identify betaproteobacterial and archaeal ammonia monooxygenase subunit *a* genes (*amoA*) in seawater samples from the inflow, control and coral mesocosms ($n = 39$), and combined coral tissue plus mucus extracts from a subset of coral colonies in the coral mesocosms ($n = 6$). qPCR protocols were as described previously (Santoro et al. 2010), with minor modifications. Briefly, 20 μL reactions contained 10 μL mastermix (Bio-Rad ssoAdvanced SYBR), 200 nM of each primer, 1 μL of genomic DNA template, and 8.4 μL of nuclease- free water. Samples and no template controls were analyzed in triplicate along with duplicate sets of standards containing 10 to 10^5 templates, on a CFX96 real-time PCR machine (Bio-Rad Laboratories). The betaproteobacterial *amoA* qPCR assay used the 1F/2R primer set (Rotthauwe et al. 1997). The archaeal *amoA* assay used the Arch-amoAF/Arch-amoAR set (Francis et al. 2005) with an additional 2 mM $MgCl_2$. The detection limit for each assay was approximately 10 genes per reaction, corresponding to a detection limit of 2 genes mL^{-1} seawater. *amoA* detection from coral tissue samples was analyzed in undiluted and 1:10 diluted samples; detection is reported as presence/absence.

Nutrient, DOC and flow cytometry analyses

Seawater samples were analyzed for macronutrients (nitrate + nitrite, nitrite, ammonium, ortho phosphate, and silicic acid) were measured at Oregon State University using a continuous segmented flow system consisting of a Technicon AutoAnalyzer II (SEAL Analytical) and an Alpkem RFA 300 Rapid Flow Analyzer (Alpkem) as conducted previously (Apprill and Rappé 2011). DOC was determined via high temperature combustion on a modified Shimadzu TOC-V (Shimadzu Scientific Instruments, Columbia, MD) (Carlson et al. 2010). Flow cytometry was performed on 1 mL seawater preserved to a final concentration of 4% paraformaldehyde to enumerate pigmented picoeukaryotes, *Synechococcus*, and non-pigmented picoplankton using methods described in Apprill and Rappé (2011). High and low DNA-containing cells were enumerated following SybrGreen staining. The ANOVA and Tukey's HSD statistics were conducted as described above. Project data are available through the Biological and Chemical Oceanography Data Management Office (BCO-DMO; http://www.bco-dmo.org/dataset/652849).

Results

Nitrate production associated with corals

Inorganic nitrogen concentrations varied substantially over the course of the experiment, particularly in the coral-inhabited mesocosms (Fig. 1). NO_3^- and NO_2^- concentrations increased seven- and three-fold in the mesocosms containing *P. astreoides*, with NO_3^- as high as 10 μM at T12 (Fig. 1a,b; Supporting Information Table 1). The largest increase in NO_3^- occurred at T10 when the corals were placed back into the tank after being absent for 48 h, and this was significantly higher than the controls and the mucus addition (Fig. 1a, ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 2). Compared to controls, no significant differences in NO_3^- or NO_2^- were observed for the mucus addition treatments (Fig. 1a,b, Supporting Information Table 2). NH_4^+ also increased in the mesocosm experiments at the end of the experiments, but this was not significant compared to controls (Fig. 1c, Supporting Information Table 2). In all mesocosms, PO_4^{3-} decreased over the course of the experiment, from 0.14 to 0.04–0.06 μM, and PO_4^{3-} was significantly higher than controls in the coral mesocosms after the corals were removed for 48-h (T6; Fig. 1d, ANOVA and Tukey HSD $p < 0.05$, Supporting Information Table 2). DOC was variable between the replicates of each treatment, but concentrations in most mesocosms, regardless of treatment, increased 10–20 μM over the 12 days and there was no significant increases in DOC in the coral or mucus treatment compared to controls (Fig. 1d, Supporting Information Table 2). The mesocosms were generally consistent at 25.8°C with ca. 1°C variation over the experiment, and salinity was similarly consistent at 37.2 ppt with a slight increase towards the end of the experimental period due to evaporation (Supporting Information Fig. 1).

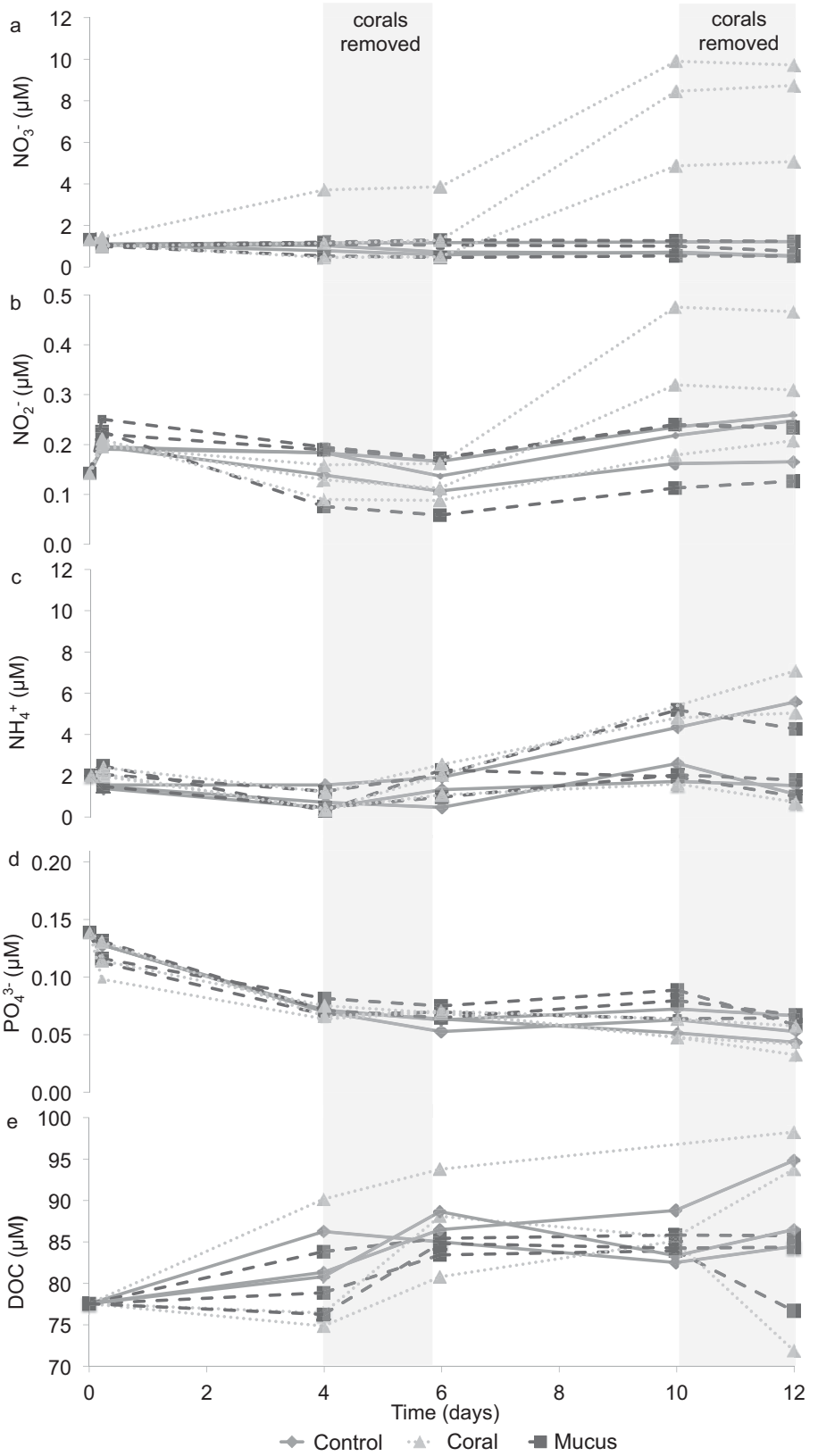

Fig. 1. Concentrations of the dissolved inorganic nutrients (**a**) NO_3^-, (**b**) NO_2^-, (**c**) NH_4^+, (**d**) PO_4^{3-}, and (**e**) dissolved organic carbon (DOC) in the control (solid dark grey line), mucus addition (dashed black line) and coral (dotted light grey lines) mesocosms. Mucus was added to the mucus addition mesocosms (and not the other treatments) after seawater sampling on days 0.2, 2, 6, and 8 of the experiment; shading indicates when corals were removed from the mesocosms and when mucus was not added to the mesocosms.

Corals cause a decrease in picoplankton cells and preferentially graze on *Synechococcus* and SAR11 clade bacteria

Over the course of the experiment, picoplankton abundances repeatedly declined in the presence of corals. Over T0-2 total picoplankton (pigmented and non-pigmented cells) in the coral mesocosms were significantly reduced in abundance compared to the controls (4.9×10^5 compared to 1.3×10^6 cells mL^{-1}; Fig. 2a; ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 3), with a grazing constant of -0.50 ± 0.12 d^{-1} during this period (Fig. 3). In particular, *Synechococcus* declined 87% over T0-2, and were significantly different compared to controls (Fig. 2b, Tukey HSD $p < 0.01$, Supporting Information Table 3), and grazing on *Synechococcus* occurred at the highest rate of any picoplankton groups measured, -1.16 ± 0.31 d^{-1} (Fig. 3). Similarly, SAR11 clade bacteria declined in the presence of corals over T0-2, and were significantly lower than controls (Fig. 2b, Tukey HSD $p < 0.01$, Supporting Information Table 3), and with a specific grazing constant of -0.90 ± 0.20 d^{-1} (Fig. 3). There was a smaller decline in *Rhodobacteraceae* (a family encompassing the Roseobacter clade) and abundances were significantly lower than controls at T2, T4, and T6 (Tukey HSD $p < 0.05$, Supporting Information Table 3).

At T6 and following a 48-h period when corals were removed from the coral mesocosms, total picoplankton increased in abundance (Fig. 2a). Picoplankton growth (0.50 d^{-1}) was significantly greater over T4-T6 than observed in the controls over the same time period (Fig. 3, ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 4). SAR11 were one of the fastest growing cells during this period, with a growth rate of 1.2 d^{-1}, and this was significantly higher than growth in the controls (Fig. 2c, ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 4). *Rhodobacteraceae* cells also grew during this period (T4-T6) when the corals were removed from the aquaria at a rate significantly greater than the controls (0.86 d^{-1}; ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 4).

Re-addition of the corals to the mesocosms during T6-8 again resulted in reduction of picoplankton ($g = -0.60 \pm 0.09$ over T6-8; Fig. 3); grazing was preferential towards *Rhodobacteraceae* and SAR11 (ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 4). As seen earlier in the experiment, subsequent removal of the corals from the mesocosms on T10-12 again resulted in a significant increase in total picoplankton, compared to controls (Fig. 2, ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 3). Total picoplankton cells exhibited a slower growth rate of 0.34 d^{-1} during this period compared to the previous removal period (0.50 d^{-1}; T5-6) (Table 3; Fig. 2b). Only *Rhodobacteraceae* exhibited significantly higher growth rates in the coral mesocosms compared to the controls (1.3 d^{-1}; Fig. 3; ANOVA and HSD $p < 0.01$, Supporting Information Table 4). Photosynthetic picoeukaryotes were also examined throughout the experiment and their abundance was very dynamic within the coral mesocosms, and displayed inconsistent trends between the mesocosms (Supporting Information Fig. 2a).

In the mucus addition mesocosms, abundances of picoplankton were significantly elevated compared to the control mesocosms at T2 and T4 (Fig. 2a, ANOVA and Tukey HSD $p < 0.05$) with significantly higher growth rates of 0.33 d^{-1} compared to 0.11 d^{-1} in the controls (Table 2, ANOVA and Tukey HSD $p < 0.01$, Supporting Information Table 4). This growth was in primarily higher DNA containing cells (Supporting Information Fig. 2b). Also in the mucus addition mesocosms, abundances of picoplankton were significantly elevated when compared to the coral mesocosms for all timepoints but especially when corals and mucus were added to the aquaria (Fig. 2a, ANOVA and Tukey HSD $p < 0.05$). *Rhodobacteraceae* were preferentially stimulated by mucus addition during T0-T2 (Fig. 2d; ANOVA and Tukey HSD $p < 0.05$, Supporting Information Table 3), with growth rates of 0.57 ± 0.15 d^{-1} (Table 2). Growth of SAR11 was significantly enhanced in mucus mesocosms compared to controls and coral mesocosms over T4-T6, a period without any mucus additions (Table 2; ANOVA and Tukey HSD $p < 0.05$, Supporting Information Table 4).

Corals alter the composition of the picoplankton community

Non-metric multidimensional scaling analysis of partial SSU rRNA gene sequences of picoplankton from the mesocosms revealed consistency among the control and mucus-addition communities and high variation among the communities in the coral mesocosms (Fig. 4a). Overall, picoplankton communities did not differ significantly between the control and mucus additions mesocosms (ANOSIM, $p = 0.27$, $r = 0.016$), and there was also no significant difference between these treatments when experimental day was considered (PERMANOVA by treatment(day), $t = 0.73$–0.95, $p = 0.50$–0.70; Fig. 4b). However, the coral mesocosms were significantly different from the control treatments (ANOSIM, $p = 0.001$, $r = 0.59$–0.62), and there was also a significant difference between the treatments by experimental day (PERMANOVA by treatment(day), $t = 1.83$–2.99, $p = 0.017$–0.042; Fig. 4c). The picoplankton community was most distinct between the coral mesocosms at the start of the experiment (T0 through T6) and the latter part of the experiment (T8 through T12) (ANOSIM, $p = 0.001$, $r = 0.87$). As previously mentioned, $NO_3^- + NO_2^-$ concentrations were elevated during T10–T12 compared to T0–T6, and PCO analysis of the picoplankton communities with environmental parameters indicates that these latter timepoints corresponded to concentrations of NO_3^-, NO_2^-, and NH_4^+ (Fig. 5).

The amplicon data demonstrated that picoplankton lineages in these control mesocosms exhibited relatively small alterations in relative abundance over the course of the experiment, with the exception of SAR11 and *Rhodobacteraceae* which decreased and increased, respectively, over T0-T2

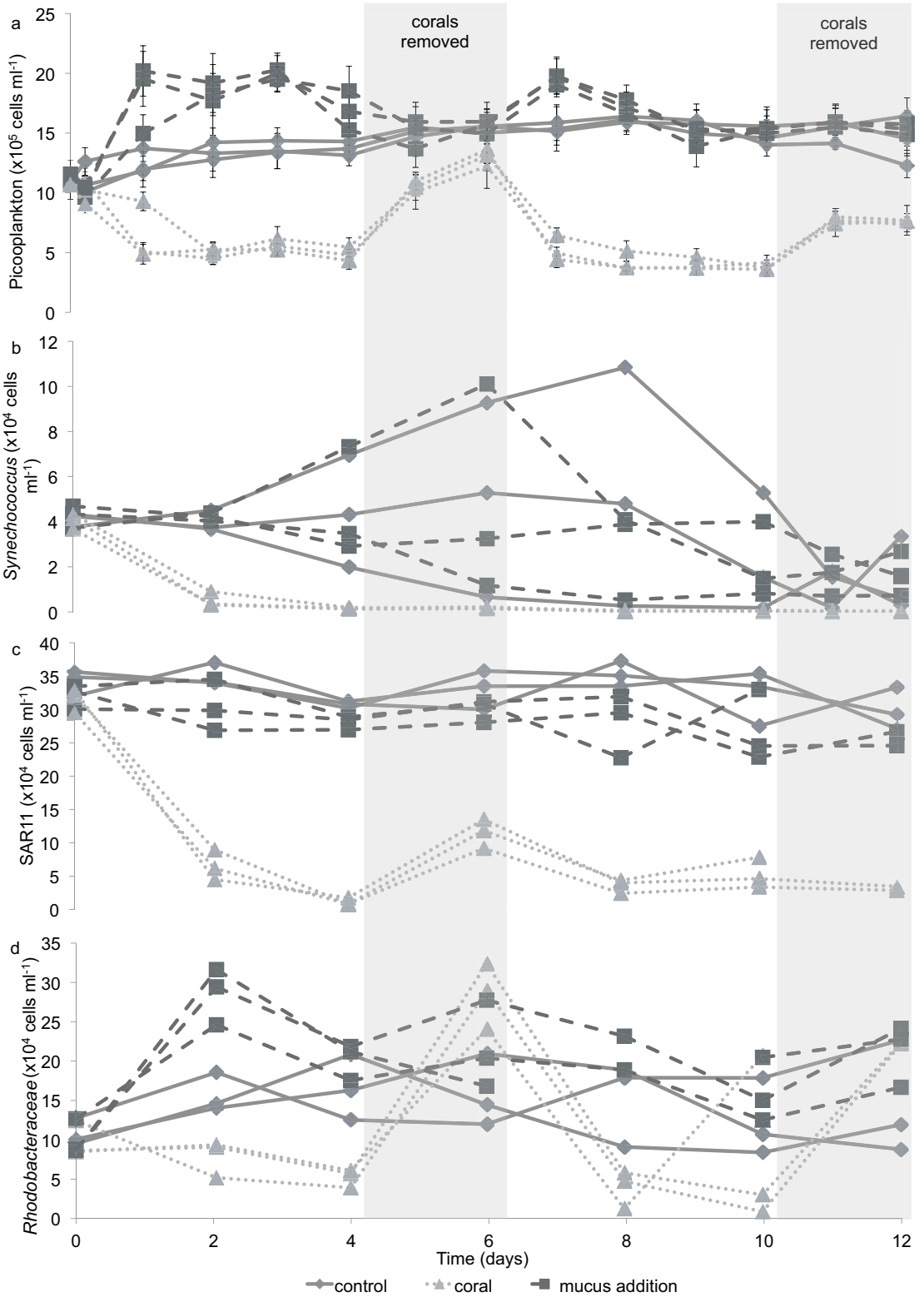

Fig. 2. Abundances of (**a**) total picoplankton (planktonic bacteria and archaea), (**b**) *Synechococcus*, (**c**) SAR11 and (**d**) *Rhodobacteraceae* cells in the control (solid dark grey line), mucus addition (dashed black line) and coral (dotted light grey lines) mesocosms. Mucus was added to the mucus addition mesocosms after seawater sampling on days 0.2, 2, 6, and 8 of the experiment; shading indicates when corals were removed from the mesocosms and when mucus was not added to the mesocosms. Abundances of total picoplankton were from DAPI counts, *Rhodobacteraceae* and SAR11 clades were derived from FISH counts, and *Synechococcus* from flow cytometry.

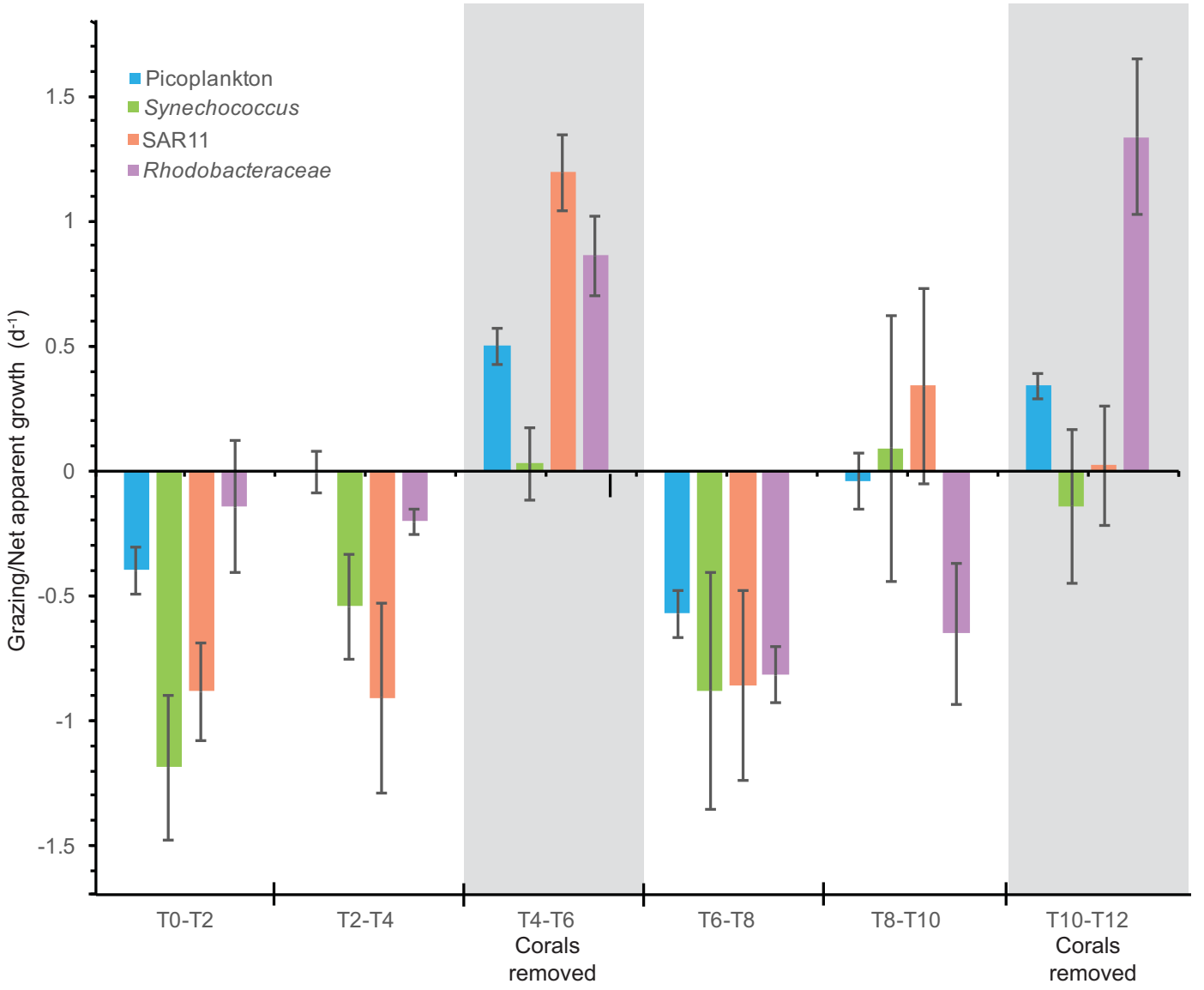

Fig. 3. Comparison of grazing (*g*) in the coral mesocosms over the course of the experiment for total picoplankton (planktonic bacteria and archaea) and select taxa, with the negative values indicating grazing and positive values net apparent growth. Error bars are 95% confidence intervals.

(Fig. 6, Supporting Information Fig. 3). For the coral mesocosms, variation was observed in relation to the presence or absence of corals, and the sequence data generally followed the major patterns detected by the microscopy analyses for SAR11 and *Synechococcus*; however, the amplicon data tended to underestimate *Rhodobacteraceae* and the other taxa (Fig. 6a, b; Supporting Information Figs. 3–5). Significant decreases in SAR11 compared to the controls were observed on days 8 and 10, with day 12 corresponding to corals being removed (two-way ANOVA with Tukey post hoc test, $p < 0.05$; Fig. 6). *Rhodobacteraceae* significantly increased to 30 – 40% of the community, compared to controls, when corals were removed from the mesocosms on T6 and T12 (Fig. 6b). Over the course of the experiment *Alteromonadaceae* and the OM60 (NOR5) clade of Gammaproteobacteria increased in the coral mesocosms (Figs. 6c, d). In the latter part of the experiment after the corals were reintroduced into the mesocosm (day 8) and nitrogen levels were elevated, a decrease in abundances of these same groups was observed, and *Oxalobacteraceae* significantly increased compared to controls and made up ~26% of the community (two-way ANOVA with Tukey post hoc test, $p < 0.05$ on T8 and T10) (Fig. 6e). Some taxa remained steady throughout the experiment. Relative abundances of *Bacteroidetes* (primarily *Flavobacteria* and *Cyptophaga*) were at 8–12%, regardless of whether the corals inhabited the mesocosms (Fig. 6f).

Compared to the coral mesocosms, the addition of coral mucus had less impact on the picoplankton community

Table 2. Net apparent growth rate (k; units of d^{-1}) with standard deviation (SD) for select picoplankton groups in control and mucus addition mesocosms, with periods of no mucus addition shaded.

	T0–T2		T2–T4		T4–T6		T6–T8		T8–T10		T10–T12	
	Control	Mucus	Control	Mucus	Control	Mucus	Control	Mucus	Control	Mucus	Control	Mucus
Picoplankton	0.11	**0.33***	0.01	−0.05	0.06	−0.04	0.02	0.05	−0.05	−0.06	−0.01	0.00
	(0.07)	**(0.04)**	(0.02)	(0.07)	(0.03)	(0.06)	(0.01)	(0.01)	(0.04)	(0.02)	(0.07)	(0.00)
Synechococcus	−0.02	0.00	−0.01	0.00	−0.10	−0.11	−0.14	−0.25	−0.37	−0.09	−0.15	−0.07
	(0.10)	(0.08)	(0.28)	(0.24)	(0.40)	(0.38)	(0.27)	(0.30)	(0.19)	(0.37)	(0.81)	(0.38)
SAR11	0.01	−0.03	−0.07	−0.51	0.09	**0.50***	−0.02	0.02	0.02	0.15	−0.11	−0.21
	(0.06)	(0.07)	(0.02)	(0.22)	(0.06)	**(0.20)**	(0.03)	(0.01)	(0.05)	(0.11)	(0.04)	(0.15)
Rhodobacteraceae	0.21	0.57	0.02	−0.12	−0.03	−0.03	−0.03	−0.10	−0.11	−0.06	0.07	0.14
	(0.03)	(0.15)	(0.20)	(0.04)	(0.16)	(0.11)	(0.22)	(0.06)	(0.15)	(0.27)	(0.14)	(0.09)

*$p < 0.05$, based on ANOVA and Tukey Honest Significant Difference when compared to controls (see Supporting Information Table 4).

composition, and did not demonstrate a significant increase in *Rhodobacteraceae* following mucus addition, as was seen by the cell counts (Fig. 4, Supporting Information Fig. 3). Mucus samples taken from the experimental corals during the experiment were primarily dominated by bacteria belonging to the *Endozoicomonas* lineage of Gammaproteobacteria (Supporting Information Fig. 6). Interestingly, *Oxalobacteraceae* were present in the coral mucus, at overall similar community composition levels found in the seawater during the latter days of the experiment (Fig. 5e, Supporting Information Fig. 6). *Endozoicomonas* also comprised 75–90% of microbial community in the coral tissues at the conclusion of the experiment (Supporting Information Fig. 7).

Low abundance of planktonic and coral-associated ammonia-oxidizing organisms

In order to investigate nitrification as a potential source of the NO_2^- and NO_3^- increase in the coral mesocosms, we examined the SSU rRNA gene sequences for known ammonia oxidizers. Within the picoplankton, ammonia-oxidizing bacteria (AOB; *Nitrosomonas, Nitrosospira*, and *Nitrosococcus*) were absent and putatively ammonia-oxidizing archaea (AOA; Thaumarchaeota) were present in very low abundances (<1% of community composition). qPCR was then used to further explore the abundance of AOA and betaproteobacterial AOB (β-AOB) by quantifying the *amoA* gene. The inflow seawater had the highest abundance of both AOA and β-AOB *amoA* genes, with a range of 317–434 and 6–15 genes mL^{-1}, respectively (Supporting Information Table 5). AOA *amoA* and β-AOB *amoA* genes decreased after 2 d and remained below detection limits for the remainder of the experimental period, regardless of treatment (Supporting Information Table 5). AOA and β-AOB directly associated with the corals were also examined at the end of the experimental period in tissue samples. β-AOB *amoA* genes were not detected, and AOA *amoA* genes were only detected in one coral (Supporting Information Table 6). The coral SSU rRNA gene data also detected low abundances of ammonia-oxidizing archaea (<0.5% of community composition), no AOB, and no nitrite oxidizers (*Nitrospira, Nitrospina, Nitrococcus*, or *Nitrobacter*).

Discussion

This study demonstrated that corals cause declines in the surrounding picoplankton, particularly *Synechococcus*, SAR11 and *Rhodobacteraceae*, which are likely grazed upon by corals or protistan grazers residing within the holobiont. Although many corals, including *P. astreoides*, have obligate symbiotic relationships with *Symbiodinium* spp., heterotrophic feeding on phytoplankton (Yahel et al. 1998) and zooplankton (Sebens et al. 1996; Wijgerde et al. 2011) are thought to be important supplementary energy sources. Depletion of picoplankton and *Synechococcus*, from grazing or other processes, have been previously seen in

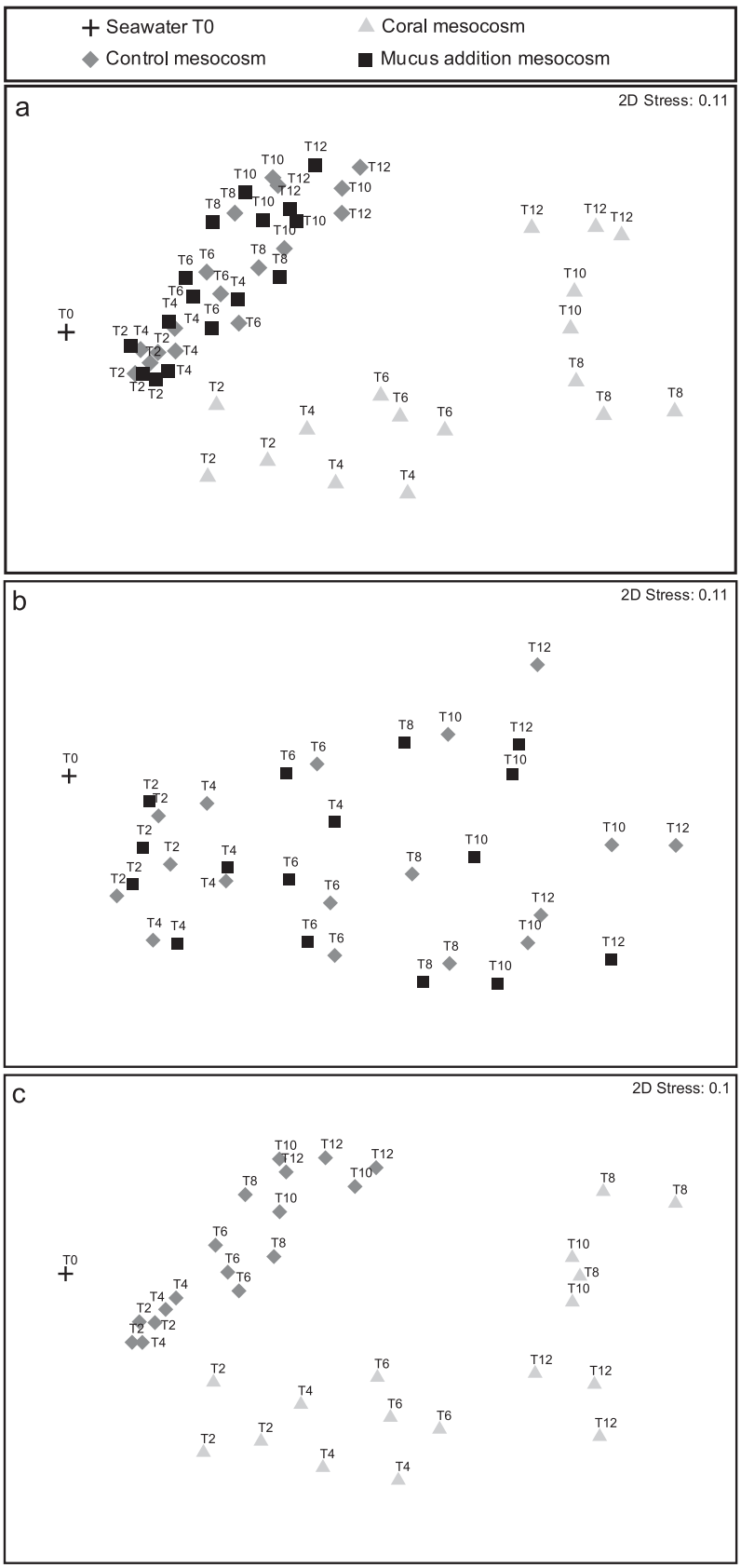

Fig. 4. Nonmetric multidimensional scaling analysis of picoplankton community composition based on Bray-Curtis similarity from SSU rRNA gene amplicon data in all the mesocosm treatments (**a**), the mucus and control mesocosms (**b**) and coral and control mesocosms (**c**) over the timeframe of the experiment. All mesocosms are numbered by timepoint.

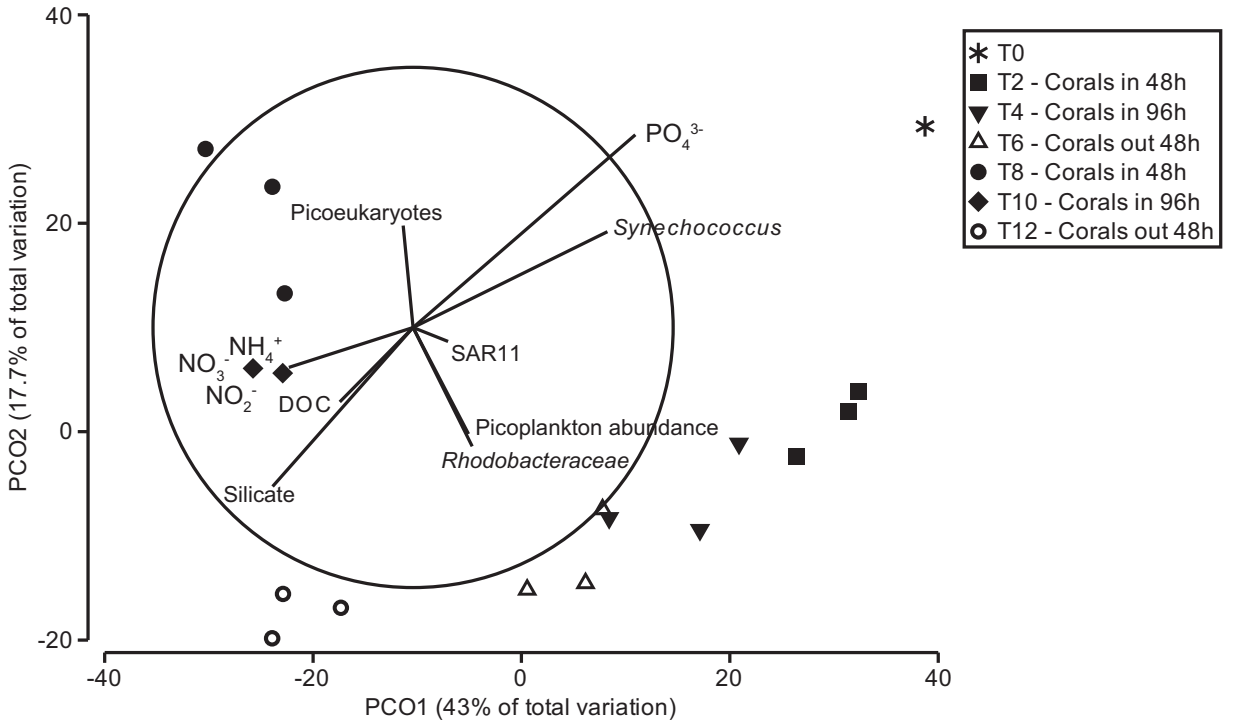

Fig. 5. Principal component ordination based on Bray-Curtis similarity, describing the variation of the picoplankton community (from SSU rRNA gene amplicon data of bacteria and archaea) in the coral mesocosms attributed to the environmental and cell abundance data. Vectors display the ordination of the variables, with a smaller angle representing more tightly coupled variables. Variables that significantly contribute to the ordination have vectors outside of the equilibrium circle.

assemblages of coral, sponge and other reef organisms (Ribes et al. 2003; Genin et al. 2009; Monismith et al. 2010). To our knowledge, this is the first report of putative coral grazing on other picoplankton taxa. It is surprising that removal of SAR11 and *Synechococcus* were preferential because of their large differences in cell size. SAR11 are among the smallest of the free-living picoplankton (0.01 μm^3; Rappé et al. 2002) and nearly two orders of magnitude smaller than *Synechococcus* (1.3 μm^3; Verity et al. 1992). These data suggest either that corals have selective prey acquisition strategies that are not defined by size, and/or other protistan members of the holobiont also play an active role. Overall, grazing on picoplankton may be an overlooked nutritional process supporting the coral holobiont (Sorokin 1973; Ferrier-Pagès et al. 1998).

The data presented here suggest that SAR11 and *Rhodobacteraceae* selectively grow on exudates released from corals. It was surprising to find that SAR11 had one of the highest growth rates measured in the experiment following the first period of coral removal from the mesocosm. SAR11 is a dominant cell in oligotrophic oceans and has been described as an "extreme oligotroph" (Morris et al. 2002; Carini et al. 2012) requiring a fraction of the nutrients needed by other cells. In this study, SAR11 appeared to initially flourish on *Porites* exudates, nearly doubling previously reported growth rates from the field and coastal isolates (Malstrom et al. 2005; Carini et al. 2012, 2014; Lankiewicz et al. 2016). In further support of their growth, SAR11 enrichment has been seen previously on Pacific reefs compared to offshore waters (Nelson et al. 2011). However, SAR11 growth was not sustained throughout the coral treatments with much lower growth rates seen following the second period of coral removal (Fig. 3). SAR11 growth may have ultimately been limited by other unaccounted for, yet essential, growth factors. *Rhodobacteraceae* are generally referred to as copiotrophs (reviewed in Brown et al. 2014), and growth on coral exudates have been previously detected (Nelson et al. 2013). In the present experiment, *Rhodobacteraceae* growth was greatest when NO_3^-, a source of N assimilated by some cells in this family (Moran et al. 2007) was abundant in the mesocosms. *Rhodobacteraceae* can utilize organic sulfur compounds including dimethylsulfoniopropionate (DMSP) for energy (Gonzalez et al. 2003; Buchan et al. 2005; Brinkhoff et al. 2008), which is produced by the corals and their algal endosymbionts (Raina et al. 2013), and therefore may have a nutritional advantage in these coral inhabited waters. *Rhodobacteraceae* are clearly an important group of bacteria on reefs, as studies have confirmed their presence in coral-dominated reef waters (Dinsdale et al. 2008; Kelly et al. 2014), and their intimate association with corals (Apprill et al. 2012; Sharp et al. 2012; Thompson et al. 2015), including those examined here.

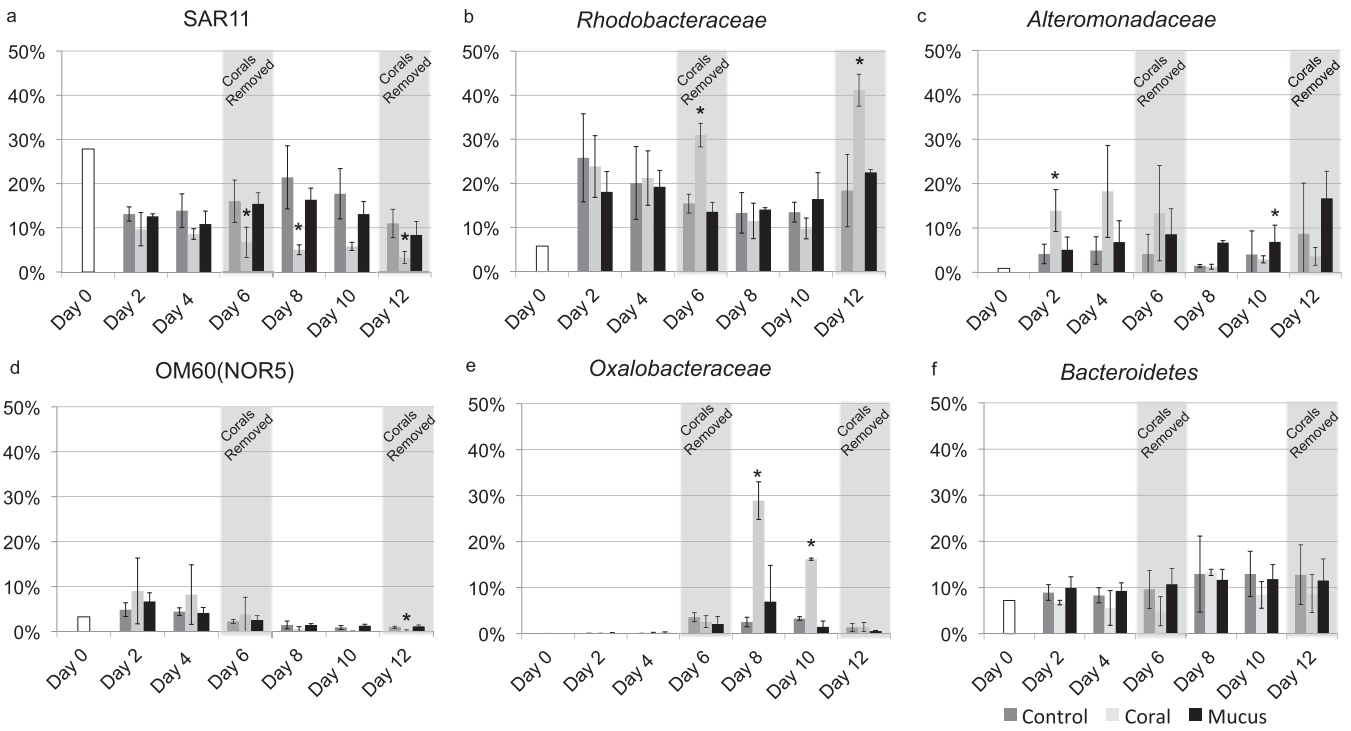

Fig. 6. Taxonomic overview of the percentage contribution of select picoplankton lineages (**a**) SAR11, (**b**) *Rhodobacteraceae*, (**c**) *Alteromonadaceae*, (**d**) OM60(NOR5), (**e**) *Oxalobacteraceae* and (**f**) *Bacteroidetes* to all the picoplankton lineages within initial (white) and control (dark grey), coral (light grey), and mucus (black) addition mesocosms over the course of the experiment from SSU rRNA gene amplicon data. Shaded regions represent when experimental corals were removed from coral mesocosms and when mucus was not added to the mesocosms, the error bars are standard deviations ($n = 3$), and stars represent significant differences compared to controls ($p < 0.05$) based on two-way ANOVA with Tukey's post-hoc tests.

Alteromonadaceae, OM60/NO5 and *Oxalobacteraceae* were identified using amplicon data as growing on *Porites* exudates. *Alteromonadaceae* are fast-growing, high carbon consuming copiotrophs (Pedler et al. 2014), and while less is known about OM60/NOR5, these cells are abundant at the interface of sediments (Yan et al. 2009) and thus may also take advantage of coral-produced substrates. One unexpected result was the increase in the *Oxalobacteraceae* following re-introduction of the corals to the mesocosms, as well as the presence of these bacteria in coral mucus. A recent study demonstrated that *Oxalobacteraceae* were normal inhabitants of new (non-aged) *P. astreoides* mucus (Glasl et al. 2016), and therefore these cells likely originated from the mucus and may have especially proliferated when the corals were dripped of mucus and then presumably produced new mucus. A genome of a marine *Oxalobacteraceae* isolate indicates that they possess a C_1 metabolism (Oh et al. 2011), similar to members of the abundant coastal OM43 clade (Giovannoni et al. 2008; Huggett et al. 2012). These bacteria may grow in the presence of a coral-produced C_1 compound, such as methanol or formaldehyde, or possess a competitive metabolism in the presence of corals. Our results corroborate similar total picoplankton growth rates on coral exudates (0.35 d^{-1}) (Nelson et al. 2013), but provide the first evidence that coral exudates can alter the abundances of specific members of the reef water picoplankton community.

Here we demonstrate that mucus does stimulate the growth of high DNA containing picoplankton at the initial start of the experiment with an almost doubling of *Rhodobacteraceae* cells when compared to controls on Day 2, but this trend was not maintained upon subsequent mucus additions. Surprisingly, the amplicon data did not show this same trend, probably because *Rhodobacteraceae* cells were underestimated in these data compared to the cell counts (Supporting Information Fig. 5). More frequent samplings following mucus addition may have revealed more substantial picoplankton dynamics. Additionally, the composition of the coral mucus may have altered over the course of the experiment. Mucus is composed of mucin glycoprotein, which is biochemically variable among coral species (reviewed in Jatkar et al. 2010) and microbial growth on this substrate is generally not consistent (Taniguchi et al. 2014). Additionally, substrates supporting the growth of the predominantly high DNA containing bacteria may have become unavailable during the latter experimental days. In particular, PO_4^{3-} and NO_3^- were lowered in the mucus mesocosms. Lastly, larger and more frequent

mucus additions may be required to more consistently replicate the reef environment. Overall, conducting mucus addition experiments under nutrient-replete conditions may aid in teasing apart why limited picoplankton growth occurs in the presence of coral mucus, and ultimately allow us to better understand the contribution of mucus to picoplankton growth in reef waters.

Nitrogen budgets within the coral holobiont remain poorly resolved (Fiore et al. 2010; Pernice et al. 2012; Rädecker et al. 2015). While NH_4^+ and dissolved organic nitrogen fluxes to and from the holobiont are frequently observed (e.g., Muscatine and D'Elia 1978; Ferrier-Pagès et al. 1998; Grover et al. 2008), NO_3^- fluxes have not been examined as thoroughly. Nitrate levels in the cavities and interstitial spaces of coral reefs are elevated relative to the surrounding water (Andrews and Muller 1983; Schiller and Herndl 1989; Scheffers et al. 2004). In the present study, significant NO_3^- production was observed in the coral mesocosms following the removal and subsequent re-introduction of the corals. Potential sources of this NO_3^- are nitrogen remineralization (ammonification coupled to nitrification) in the mesocosm seawater or within the coral holobiont. The low abundance of *amoA* genes (<2 mL^{-1}) detected in the mesocosm seawater suggests against planktonic nitrification as the source of the observed nitrate. Archaeal *amoA* genes have, however, been documented as associated with some corals (Beman et al. 2007; Siboni et al. 2008, 2012), and nitrifying activity has been demonstrated in coral mucus (Siboni et al. 2008), suggesting the potential for coral-associated nitrification. Here, we detected archaeal *amoA* genes in only one coral sample, and did not find evidence of known ammonia-oxidizing bacteria or archaea in the SSU rRNA community sequencing. Our recent *in silico* analysis of a *P. astreoides* metageome (Wegley et al. 2007) indicates a single base pair mismatch (Santoro and Apprill, unpublished data) to the archaeal *amoA* forward primer used in this and previous studies, thus we and others may be underestimating the contribution of ammonia oxidation by coral-associated archaea.

Because ammonium consumption in the mesocosms cannot account for the observed nitrate increases, to examine other potential sources of nitrogen for remineralization in the coral mesocosms, we estimated the contribution of *Synechococcus* and picoplankton to the overall nitrogen budget in the experiments based on cell losses between the T0 and T10 timepoints, similar to an analysis used by Scheffers and colleagues (2004) to construct an *in situ* nitrogen budget in coral reef cavities. Even assuming high-end estimates of *Synechococcus* nitrogen content (65 fg N cell^{-1}; Fu et al. 2007) and heterotrophic picoplankton nitrogen content (5 fg N cell^{-1}; Fukuda et al. 1998), these sources would contribute only 0.19 μM and 0.23 μM N, respectively, or approximately 7% of the observed NO_3^- increase. Alternative sources for the NH_4^+ fueling the observed NO_3^- production could be uptake of dissolved organic nitrogen, the breakdown of amino acids within the coral (Szmant et al. 1990), or N_2 fixation by coral-associated cyanobacteria (Lesser et al. 2007). While many previous studies have shown that sponge-hosted microbial communities are important sites of nitrogen remineralization on reefs (e.g., Corredor et al. 1988; Bayer et al. 2008; Southwell et al. 2008), our results suggest that the coral holobiont may also contribute to nitrogen remineralization at a rate of over 1 μM d^{-1}, with particulate N at least partially contributing to the holobiont N budget (Ribes et al. 2003; Genin et al. 2009).

Conclusions

This novel study suggests that the coral holobiont has multifaceted influences on the reef water picoplankton via processes not previously well recognized, including selective grazing on picoplankton taxa, nitrogen remineralization, and supporting the growth of metabolically distinct picoplankton. It is possible that these multifaceted influences have been finely synchronized over the course of the coral holobiont's evolution to enhance their survival in oligotrophic waters. *Porites* exudates appear to contribute to the growth of SAR11, *Rhodobacteraceae*, *Synechococcus*, OM60/NOR5, *Alteromonadaceae* and *Oxobacteraceae* cells on reefs. Grazing on select taxa aids in sustaining the coral or holobiont-associated protists, and might additionally supply NH_4^+ for nitrification. This 'coral-influenced microbial loop' scenario is likely more complex when other benthic and pelagic organisms, including zooplankton, are present on the reef and with flushing from the open ocean. However, the simplified, mesocosm study presented here is a first step in understanding these important coral-picoplankton interactions. Scaling these interactions up to natural reef environments will require tracer-type studies applied to more complex mesocosm communities, as well as simplified field scenarios. Corals are declining from reefs globally at unprecedented rates (e.g., Hoegh-Guldberg et al. 2007; Ruzicka et al. 2013), and there is an urgent need to uncover how specific coral-picoplankton interactions contribute to these threatened ecosystems.

References

Allers, E., C. Niesner, C. Wild, and J. Pernthaler. 2008. Microbes enriched in seawater after the addition of coral mucus. Appl. Environ. Microb. **74**: 3274–3278. doi:10.1128/AEM.01870-07

Andrews, J. C., and H. R. Mueller. 1983. Space-time variability of nutrients in a Lagoonal patch reef. Limnol. Oceanogr. **28**: 215–227. doi:10.4319/lo.1983.28.2.0215

Apprill, A., and M. S. Rappé. 2011. Response of the microbial community to coral spawning in lagoon and reef flat environments of Hawaii, USA. Aquat. Microb. Ecol. **62**: 251–266. doi:10.3354/ame01471

Apprill, A., H. Marlow, M. Martindale, and M. Rappé. 2012. Specificity of bacteria associated with the coral *Pocillopora meandrina* during early development. Appl. Environl. Microb. **78**: 7467–7475. doi:10.1128/AEM.01232-12

Apprill, A., S. McNally, R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 picoplankton. Aquat. Microb. Ecol. **75**: 129–137. doi:10.3354/ame01753

Bayer, K., S. Schmitt, and U. Hentschel. 2008. Physiology, phylogeny and *in situ* evidence for bacterial and archaeal nitrifiers in the marine sponge *Aplysina aerophoba*. Environ. Microbiol. **10**: 2942–2955. doi:10.1111/j.1462-2920.2008.01582.x

Beman, J. M., K. J. Roberts, L. Wegley, F. Rohwer, and C. A. Francis. 2007. Distribution and diversity of Archaeal ammonia monooxygenase genes associated with corals. Environ. Microbiol. **73**: 5642–5647. doi:10.1128/AEM.00461-07

Brinkhoff, T., H. A. Giebel, and M. Simon. 2008. Diversity, ecology, and genomics of the Roseobacter clade: A short overview. Arch. Microbiol. **189**: 531–539. doi:10.1007/s00203-008-0353-y

Brown, M. V., M. Ostrowski, J. J. Grzymski, and F. M. Lauro. 2014. A trait based perspective on the biogeography of common and abundant marine bacterioplankton clades. Marine Genom. **15**: 17–28. doi:10.1016/j.margen.2014.03.002

Buchan, A., J. M. Gonzalez M., and A. Moran. 2005. Overview of the marine Roseobacter lineage. Appl. Environ. Microb. **71**: 5665–5677. doi:10.1128/AEM.71.10.5665-5677.2005

Bythell, J. C. 1988. A total nitrogen and carbon budget for the elkhorn coral *Acropora palmata* (Lamarck). Proceedings of the 6th International Coral Reef Symposium.

Carini, P., L. Steindler, S. Beszteri, and S. J. Giovannoni. 2012. Nutrient requirements for growth of the extreme oligotroph '*Candidatus* Pelagibacter ubique' HTCC1062 on a defined medium. ISME J. **7**: 592–602. doi:10.1038/ismej.2012.122

Carini, P., and others. 2014. Discovery of a SAR11 growth requirement for thiamin/'s pyrimidine precursor and its distribution in the Sargasso Sea. ISME J. **8**: 1727–1738. doi:10.1038/ismej.2014.61

Carlson, C. A., D. A. Hansell, N. B. Nelson, D. A. Siegel, W. M. Smethie, S. Khatiwala, M. M. Meyers, and E. Halewood. 2010. Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. Deep-Sea Res II. **57**: 1433–1445. doi:10.1016/j.dsr2.2010.02.013

Carpenter, K. E., and others. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science. **321**: 560–563. doi:10.1126/science.1159196

Corredor, J. E., C. R. Wilkinson, V. P. Vicente, J. M. Morell, and E. Otero. 1988. Nitrate release by Caribbean reef sponges. Limnol. Oceanogr. **33**: 114–120. doi:10.4319/lo.1988.33.1.0114

Crossland, C. J., B. J. Hatcher, and S. V. Smith. 1991. Role of coral reefs in global ocean production. Coral Reefs. **10**: 55–64. doi:10.1007/BF00571824

de Putron, S. J., D. C. McCorkle, A. L. Cohen, and A. B. Dillon. 2010. The impact of seawater saturation state and bicarbonate ion concentration on calcification by new recruits of two Atlantic corals. Coral Reefs. **30**: 321–328.doi:10.1007/s00338-010-0697-z

Dinsdale, E. A., and others. 2008. Microbial ecology of four coral atolls in the Northern Line Islands. PLoS One. **3**: e1584.doi:10.1371/journal.pone.0001584

Doney, S. C., V. J. Fabry, R. A. Feely, and J. A. Kleypas. 2009. Ocean acidification: The other CO_2 problem. Annu. Rev. Marine Sci. **1**: 169–192. doi:10.1146/annurev.marine.010908.163834

Ducklow, H. W. 1990. The biomass, production and fate of bacteria in coral reefs, p. 265–289 *In* Z. Dubinsky, [ed.], Coral reefs, ecosystems of the world. Elsevier Publ.

Edgar, R., B. Haas, J. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. **27**: 2194–2200. doi:10.1093/bioinformatics/btr381

Eren, A. M., H. G. Morrison, P. J. Lescault, J. Reveillaud, J. H. Vineis, and M. L. Sogin. 2015. Minimum entropy decomposition: Unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME J. **9**: 968–979. doi:10.1038/ismej.2014.195

Fabricius, K. E. 2005. Effects of terrestrial sedimentation on the ecology of corals and coral reefs: Review and synthesis. Marine Pollut. Bull. **50**: 125–146. doi:10.1016/j.marpolbul.2004.11.028

Ferrier-Pagés, C., J. P. Gattuso, G. Cauwet, J. Jaubert, and D. Allemand. 1998. Release of dissolved organic carbon and nitrogen by the zooxanthellae coral *Galaxea fascicularis*. Mar. Ecol. Progr. Ser. **172**: 265–274. doi:10.3354/meps172265

Fiore, C. L., J. K. Jarett, N. D. Olson, and M. P. Lesser. 2010. Nitrogen fixation and nitrogen transformations in marine symbioses. Trends Microbiol. **18**: 455–463. doi:10.1016/j.tim.2010.07.001

Francis, C. A., K. J. Roberts, J. M. Beman, A. E. Santoro, and B. B. Oakley. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc. Natl. Acad. Sci. USA **102**: 14683–14688. doi:10.1073/pnas.0506625102

Frost, B. W. 1972. Effects of size and concentration of food particles on the feeding behavior of the marine planktonic copepod *Calanus pacificus*. Limnol. Oceanogr. **6**: 805–815.doi:10.4319/lo.1972.17.6.0805

Fu, F. X., M. E. Warner, Y. Zhang, Y. Feng, and D. A. Hutchins. 2007. Effects of increased temperature and CO2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria). J. Phycol. **43**: 485–496. doi:10.1111/j.1529-8817.2007.00355.x

Fukuda, R., H. Ogawa, T. Nagata, and I. Koike. 1998. Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Appl. Environ. Microbiol. **64**: 3352–3358.

Gast, G. J., S. Wiegman, E. Wieringa, F. C. van Duyl, and R. P. M. Bak. 1998. Bacteria in coral reef water types: removal of cells, stimulation of growth and mineralization. Marine Ecology Progress Series. **167**: 37–45.

Genin, A., S. G. Monismith, M. A. Reidenbach, G. Yahel, and J. R. Koseff. 2009. Intense benthic grazing of phytoplankton in a coral reef. Limnol. Oceanogr. **54**: 938–951. doi:10.4319/lo.2009.54.3.0938

Giovannoni, S. J., E. F. DeLong, T. M. Schmidt, and N. R. Pace. 1990. Tangential flow filtration and preliminary phylogenetic analysis of marine picoplankton. Appl. Environ. Microbiol. **56**: 2572–2575.

Giovannoni, S. J., M. S. Rappé, K. Vergin, and N. Adair. 1996. 16S rRNA genes reveal stratified open ocean bacterioplankton populations related to the green non-sulfur bacteria phylum. Proc. Natl. Acad. Sci. USA. **93**: 7979–7984. doi:10.1073/pnas.93.15.7979

Giovannoni, S. J., and others. 2008. The small genome of an abundant coastal ocean methylotroph. Environ. Microbiol. **10**: 1771–1782. doi:10.1111/j.1462-2920.2008.01598.x

Glasl, B., G. J. Herndl, and P. R. Frade. 2016. The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. ISME J. doi: 10.1038/ismej.2016.9

Gonzalez, J. M., R. Simo, F. Mayer, M. A. Moran, R. E. Hodson, and W. B. Whitman. 2003. *Silicabacter pomeroyi* sp. nov. and *Roseovarius nubinhibens* sp. nov., dimethylsulfoniopropionate-demethylating bacteria from marine environments. Int. J. Syst. Evol. Micr. **53**: 1261–1269. doi:10.1099/ijs.0.02491-0

Green, D. H., P. J. Edmunds, and R. C. Carpenter. 2008. Increasing relative abundance of *Porities astreoides* on Caribbean reefs mediated by an overall decline in coral cover. Mar. Ecol. Prog. Ser. **359**: 1–10. doi:10.3354/meps07454

Grover, R., J. F. Maguer, D. Allemand, and C. Ferrier-Pagès. 2008. Uptake of dissolved free amino acids by the scleractinian coral Stylophora pistillata. J. Exp. Bio. **211**: 860–865. doi:10.1242/jeb.012807

Hoegh-Guldberg, O., and others. 2007. Coral reefs under rapid climate change and ocean acidification. Science. **318**: 1737–1742. doi: 10.1126/science.1152509

Huggett, M. J., D. H. Hayakawa, and M. S. Rappé. 2012. Genome sequence of strain HIMB624, a cultured representative from the OM43 clade of marine Betaproteobacteria. Standards in Genomic Sciences. **6**: 11–20. doi:10.4056/sigs.2305090

Jatkar, A. A., B. E. Brown, J. C. Bythell, R. Guppy, N. J. Morris, and J. P. Pearson. 2010. Coral mucus: The properties of its constituent mucins. Biomacromolecules. **11**: 883–888. doi:10.1021/bm9012106

Kelly, L. W., and others. 2014. Local genomic adaptation of coral reef-associated microbiomes to gradients of natural variability and anthropogenic stressors. Proc. Natl. Acad. Sci. USA. **111**: 10227–10232. doi:10.1021/bm9012106

Kline, S. J., and F. A. McClintock. 1953. Describing uncertainties in single-sample experiments. Mech. Eng. **75**: 3–8.

Knowlton, N. 2001. The future of coral reefs. Proc. Natl. Acad. Sci. USA. **98**: 5419–5425. doi:10.1073/pnas.091092998

Kozich, J. J., S. L. Westcott, N. T. Baxter, S. Highlander, and P. D. Schloss. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Appl. Environ. Microb. **79**: 5112–5120. doi: 10.1128/AEM.01043-13

Lankiewicz, T. S., M. T. Cottrell, and D. L. Kirchman. 2016. Growth rates and rRNA content of four marine bacteria in pure cultures and in the Delaware estuary. ISME J **10**: 823–832.

Lesser, M. P., L. I. Falcon, A. Rodriguez-Roman, S. Enriquez, O. Hoegh-Guldberg, and R. Iglesias-Prieto. 2007. Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*. Mar. Ecol. Progr. Ser. **346**: 143–152. doi:10.3354/meps07008

Malmstrom, R. R., M. T. Cottrell, H. Elifantz, and D. L. Kirchman. 2005. Biomass production and assimilation of dissolved organic matter by SAR11 bacteria in the Northwest Atlantic Ocean. Applied and environmental microbiology **71**: 2979–2986.

Mass, T., S. Einbinder, E. Brokovich, N. Shashar, R. Vago, J. Erez, and Z. Dubinsky. 2007. Photoacclimation of Stylophora pistillata to light extremes: Metabolism and calcification. Mar. Ecol. Prog. Ser. **334**: 93–102. doi:10.3354/meps334093

Monismith, S. G., and others. 2010. Flow effects on benthic grazing on phytoplankton by a Caribbean reef. Limnol. Oceanogr. **55**: 1881–1892. doi:10.4319/lo.2010.55.5.1881

Moran, M. A., and others. 2007. Ecological genomics of marine Roseobacters. Appl. Environ. Microb. **73**: 4559–4569. doi: 10.1128/AEM.02580-06

Morris, R. M., S. A. Connon, M. Rappé, K. L. Vergin, W. A. Siebold, C. A. Carlson, and S. J. Giovannoni. 2002. High cellular abunbundance of the SAR11 picoplankton clade in seawater. Nature. **420**: 806–809. doi:10.1038/nature01240

Morrow, K. M., A. G. Moss, N. E. Chadwick, and M. R. Liles. 2012. Bacterial associates of two Caribbean coral species reveal species-specific distribution and geographic variability. Appl. Environ. Microb. **78**: 6438–6449. doi: 10.1128/AEM.01162-12

Mumby, P. J., A. Hastings, and H. J. Edwards. 2007. Thresholds and the resilience of Caribbean coral reefs. Nature. **450**: 98–101. doi:10.1038/nature06252

Muscatine, L., and C. F. D'Elia. 1978. The uptake, retention, and release of ammonium by reef corals. Limnol. Oceanogr. **23**: 725–734. doi:10.4319/lo.1978.23.4.0725

Muscatine, L., L. R. McCloskey, and R. E. Marian. 1981. Estimating the daily contribution of carbon from zooxanthellae to coral animal respiration. Limnol. Oceanogr. **26**: 601–611. doi:10.4319/lo.1981.26.4.0601

Nelson, C. E., A. L. Alldredge, E. A. McCliment, L. A. Amaral-Zettler, and C. A. Carlson. 2011. Depleted dissolved organic carbon and distinct bacterial communities in the water column of a rapid-flushing coral reef ecosystem. ISME J. **5**: 1374–1387. doi: 10.1038/ismej.2011.12

Nelson, C. E., S. J. Goldberg, K. L. Wegley, A. F. Haas, J. E. Smith, F. Rohwer, and C. A. Carlson. 2013. Coral and macroalgal exudates vary in neutral sugar composition and differentially enrich reef picoplankton lineages. ISME J. **7**: 962–979. doi:10.1038/ismej.2012.161

Oh, H. M., K. Lee, Y. Jang, I. Kang, H. J. Kim, T. W. Kang, S. Y. Kim, and J. C. Cho. 2011. Genome sequence of strain IMCC9480, a xanthorhodopsin-bearing Betaproteobacterium isolated from the Arctic Ocean. J. Bacteriol. **193**: 3421. doi:10.1128/JB.05088-11

Parsons, R. J., M. Breitbart, M. W. Lomas, and C. A. Carlson. 2011. Ocean time-series reveals recurring seasonal patterns of virioplankton dynamics in the northwestern Sargasso Sea. ISME J. **6**: 273–284. doi:10.1038/ismej.2011.101

Parsons, R. J., and others. 2014. Marine bacterioplankton community turnover within seasonally hypoxic waters of a sub-tropical sound: Devil1s Hole, Bermuda. Environ. Microbiol. **17**: 3481–3499. doi:10.1111/1462-2920.12445

Pedler, B. E., L. I. Aluwihare, and F. Azam. 2014. Single bacterial strain capable of significant contribution to carbon cycling in the surface ocean. Proc. Natl. Acad. Sci. USA. **20**: 7202–7207. doi:10.1073/pnas.1401887111

Pernice, M., A. Meibom, A. Van Den Heuvel, C. Kopp, I. Domart-Coulon, O. Hoegh-Guldberg, and S. Dove. 2012. A single cell view of ammonium assimilation in coral-dinoflagellate symbiosis. ISME J. **6**: 1314. doi:10.1038/ismej.2011.196

Porter, K. G., and Y. S. Feig. 1980. The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr. **25**: 943–948. doi:10.4319/lo.1980.25.5.0943

Rädecker, N., C. Pogoreutz, C. R. Voolstra, J. Wiedenmann, and C. Wild. 2015. Nitrogen cycling in corals: the key to understanding holobiont functioning? Trends Microbiol. **23**: 490–497. doi:10.1016/j.tim.2015.03.008

Raina, J. B., and others. 2013. DMSP biosynthesis by an animal and its role in coral thermal stress response. Nature. **502**: 677–680. doi:10.1038/nature12677

Rappé, M. S., S. A. Connon, K. L. Vergin, and S. J. Giovannoni. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. Nature. **418**: 630–633. doi:10.1038/nature00917

Ribes, M., R. Coma, M. J. Atkinson, and R. A. I. Kinzie. 2003. Particle removal by coral reef communities: picoplankton is a major source of nitrogen. Mar. Ecol. Prog. Ser. **257**: 13–23. doi:10.3354/meps257013

Rodriguez-Lanetty, M., C. Granados-Cifuentes, A. Barberan, A. J. Bellantuono, and C. Bastidas. 2013. Ecological inferences from a deep screening of the complex bacterial consortia associated with the coral, *Porites astreoides*. Mol. Ecol. **22**: 4349–4362. doi:10.1111/mec.12392

Rohwer, F., V. Seguritan, F. Azam, and N. Knowlton. 2002. Diversity and distribution of coral-associated bacteria. Mar Ecol Prog Ser. **243**: 1–10. doi:10.3354/meps243001

Rotthauwe, J. H., K. P. Witzel, and W. Liesack. 1997. The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microb. **63**: 4704–4712.

Ruzicka, R., and others. 2013. Temporal changes in benthic assemblages on Florida Keys reefs 11 years after the 1997/1998 El Nino. Mar. Ecol. Prog. Ser. **489**: 125–141. doi:10.3354/meps10427

Santoro, A. E., K. L. Casciotti, and C. A. Francis. 2010. Activity, abundance and diversity of nitrifying archaea and bacteria in the central California Current. Environ. Microbiol. **12**: 1989–2006. doi: 10.1111/j.1462-2920.2010.02205.x

Scheffers, S., G. Nieuwland, R. Bak, and F. Van Duyl. 2004. Removal of bacteria and nutrient dynamics within the coral reef framework of Curacao (Netherlands Antilles). Coral Reefs. **23**: 413–422. doi:10.1007/s00338-004-0400-3

Schiller, C., and G. J. Herndl. 1989. Evidence of enhanced microbial activity in the interstitial space of branched corals: possible implications for coral metabolism. Coral reefs **7**: 179–184.

Schloss, P. D., and others. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. **75**: 7537–7541. doi:10.1128/AEM.01541-09

Sebens, K. P., K. S. Vandersall, L. A. Savina, and K. R. Graham. 1996. Zooplankton capture by two scleractinian corals, *Madracis mirabilis* and *Montastrea cavernosa*, in a field enclosure. Mar. Biol. **127**: 303–317. doi: 10.1007/BF00942116

Seymour, J. R., N. Patten, D. G. Bourne, and J. G. Mitchell. 2005. Spatial dynamics of virus-like particles and heterotrophic bacteria within a shallow coral reef system. Mar. Ecol. Prog. Ser. **288**: 1–8. doi: 10.3354/meps288001

Sharp, K. H., D. Distel, and V. J. Paul. 2012. Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral *Porites astreoides*. ISME J. **6**: 790–801. doi: 10.1038/ismej.2011.144

Siboni, N., E. Ben-Dov, A. Sivan, and A. Kushmaro. 2008. Global distribution and diversity of coral-associated *Archaea* and their possible role in the coral holobiont nitrogen cycle. Environ. Microbiol. **10**: 2979–2990. doi: 10.1111/j.1462-2920.2008.01718.x

Siboni, N., E. Ben-Dov, A. Sivan, and A. Kushmaro. 2012. Geographic specific coral-associated ammonia-oxidizing Archaea in the Northern Gulf of Eilat (Red Sea). Microb. Ecol. **64**: 18–24. doi: 10.1007/s00248-011-0006-6

Sorokin, Y. I. 1973. Trophical role of bacteria in the ecosystem of the coral reef. Nature. **242**: 415–417. doi:10.1038/242415a0

Sorokin, Y. I. 1995. Role of plankton in the turnover of organic matter on the Great Barrier Reef, Australia. Hydrobiologia. **308**: 35–44. doi:10.1007/BF00037785

Southwell, M. W., J. B. Weisz, C. S. Martens, and N. Lindquist. 2008. In situ fluxes of dissolved inorganic nitrogen from the sponge community on Conch Reef, Key Largo, Florida. Limnol. Oceanogr. **53**: 986–996. doi: 10.4319/lo.2008.53.3.0986

Stoddart, D. R. 1969. Ecology and morphology of recent coral reefs. Biol. Rev. **44**: 433–498. doi: 10.1111/j.1469-185X.1969.tb00609.x

Szmant, A. M. 2002. Nutrient enrichment on coral reefs: Is it a major cause of coral reef decline? Estuaries. **25**: 743–766. doi: 10.1007/BF02804903

Szmant, A. M., L. M. Ferrer, and L. M. FitzGerald. 1990. Nitrogen excretion and O:N ratios in reef corals: Evidence for conservation of nitrogen. Mar. Biol. **104**: 119–127. doi: 10.1007/BF01313165

Tanaka, Y., T. Miyajima, I. Koike, T. Hayashibara, and H. Ogawa. 2008. Production of dissolved and particulate organic matter by the reef-building corals *Porites cylindrical* and *Acropora pulchra*. Bull. Mar. Sci. **82**: 237–245.

Tanaka, Y., H. Ogawa, and T. Miyajima. 2011. Bacterial decomposition of coral mucus as evaluated by long-term and quantitative observation. Coral Reefs. **30**: 443–449. doi: 10.1007/s00338-011-0729-3

Taniguchi, A., T. Yoshida, and M. Eguchi. 2014. Bacterial production is enhanced by coral mucus in reef systems. J. Exp. Mar. Biol. Ecol. **461**: 331–336. doi:10.1016/j.jembe.2014.09.004

Taniguchi, A., T. Yoshida, K. Hibino, and M. Eguchi. 2015. Community structures of actively growing bacteria stimulated by coral mucus. J. Expr. Mar. Biol. Ecol. **469**: 105–112. doi: 10.1016/j.jembe.2015.04.020

Thompson, J. R., H. E. Rivera, C. J. Closek, and M. Medina. 2015. Microbes in the coral holobiont: Partners through evolution, development, and ecological interactions. Front. Cell. Infect. Microbiol. **4**: 1–20. doi: 10.3389/fcimb.2014.00176

Verity, P. G., C. Y. Robertson, C. R. Tronzo, M. G. Andrews, J. R. Nelson, and M. E. Sieracki. 1992. Relationships between cell volume and the carbon and nitrogen content of marine photosynthetic nanoplankton. Limnol. Oceanogr. **37**: 1434–1446. doi: 10.4319/lo.1992.37.7.1434

Wegley, L., R. Edwards, B. Rodriguez-Brito, H. Liu, and F. Rohwer. 2007. Metagenomic analysis of the microbial community associated with the coral *Porites astreoides*. Environ. Microbiol. **9**: 2707–2719.doi:10.1111/j.1462-2920.2007.01383.x

Wijgerde, T., R. Diantari, M. W. Lewaru, J. A. J. Verreth, and R. Osinga. 2011. Extracoelenteric zooplankton feeding is a key mechanism of nutrient acquisition for the scleractinian coral *Galaxea fascicularis*. J. Exp. Mar. Biol. **214**: 3351–3357. doi: 10.1242/jeb.058354

Wild, C., M. Huettel, A. Klueter, S. G. Kremb, M. Y. M. Rasheed, and B. B. Jorgensen. 2004. Coral mucus functions as an energy carrier and particle trap in the reef ecosystem. Nature. **428**: 66–70. doi: 10.1038/nature02344

Yahel, G., A. F. Post, K. Fabricius, D. Marie, D. Vaulot, and A. Genin. 1998. Phytoplankton distribution and grazing near coral reefs. Limnol. Oceanogr. **43**: 551–563. doi: 10.4319/lo.1998.43.4.0551

Yan, S., B. M. Fuchs, S. Lenk, J. Harder, J. Wulf, N. Z. Jiao, and R. Aman. 2009. Biogeography and phylogeny of the NOR5/OM60 clade of Gammaproteobacteria. Syst. Appl. Microb. **32**: 124–139. doi: 10.1016/j.syapm.2008.12.001

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Conflict of Interest

None declared.

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