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Elevated pCO_2 enhances bacterioplankton removal of organic carbon

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Abstract

Factors that affect the removal of organic carbon by heterotrophic bacterioplankton can impact the rate and magnitude of organic carbon loss in the ocean through the conversion of a portion of consumed organic carbon to CO_2. Through enhanced rates of consumption, surface bacterioplankton communities can also reduce the amount of dissolved organic carbon (DOC) available for export from the surface ocean. The present study investigated the direct effects of elevated pCO_2 on bacterioplankton removal of several forms of DOC ranging from glucose to complex phytoplankton exudate and lysate, and naturally occurring DOC. Elevated pCO_2 (1000–1500 ppm) enhanced both the rate and magnitude of organic carbon removal by bacterioplankton communities compared to low (pre-industrial and ambient) pCO_2 (250 –~400 ppm). The increased removal was largely due to enhanced respiration, rather than enhanced production of bacterioplankton biomass. The results suggest that elevated pCO_2 can increase DOC consumption and decrease bacterioplankton growth efficiency, ultimately decreasing the amount of DOC available for vertical export and increasing the production of CO_2 in the surface ocean.

Introduction

Marine heterotrophic bacterioplankton play a key role in the biogeochemical cycling of carbon through their use of dissolved organic carbon (DOC) [1]. These communities convert a portion of consumed DOC into biomass and respire the remainder to CO_2, thereby, decreasing total organic carbon concentrations in the ocean through the production of CO_2. Via air—sea exchange, the production of CO_2 in the surface ocean could lead to the loss of carbon from the ocean [2]. DOC that escapes bacterioplankton consumption and persists on the timescales of months to years has the potential to be exported out of the surface ocean by physical processes and represents a major export pathway in the biological pump [3, 4, 5]. Thus, factors that affect bacterioplankton consumption of DOC impact surface-ocean carbon inventories and the amount of organic carbon available for export as either DOC or organic carbon associated with bacterioplankton biomass.

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How ocean acidification affects marine bacterioplankton directly or indirectly remains a topic of interest [6]. Recent mesocosm and culture experiments comprised of photoautotrophs, heterotrophic bacterioplankton, and grazers have demonstrated clear effects of elevated pCO_2 on bacterioplankton growth [7–9] and extracellular enzyme activities [9–13]. Bacterioplankton protein production [9] and abundance [10] were shown to increase as a function of elevated pCO_2, while Motegi et al. [8] observed no effect of pCO_2 on other aspects of bacterioplankton activity (i.e. respiration, growth efficiency, and carbon demand). Multiple studies observed enhanced rates of extracellular enzyme activity such as leucine-aminopeptidase [10, 12], protease [9], and glucosidase [9, 11–13], suggesting accelerated hydrolysis of various components of dissolved organic material as a function of increasing pCO_2. These studies provide valuable insight to the net community effects of pCO_2 on organic carbon cycling. However, the effects of pCO_2 on heterotrophic bacterioplankton for these studies were evaluated during nutrient-induced phytoplankton blooms (mesocosm studies) [7–10, 12, 13] or by inoculating pH-manipulated phytoplankton cultures with natural bacterioplankton communities (culture study) [11]. Both of these experimental designs make it difficult to differentiate the direct effects of pCO_2 on heterotrophic bacterioplankton physiology and organic carbon removal from the indirect effects that pCO_2 may have had on the quantity and quality of the organic carbon produced by the phytoplankton [14, 15], which could potentially also alter microbial organic carbon removal.

Studies conducted in the absence of phytoplankton showed somewhat different results: Yamada and Suzumura [16] observed no effect of elevated pCO_2 on extracellular glucosidase activity of the free-living bacterioplankton communities. They did, however, observe an increase in extracellular leucine-aminopeptidase and lipase activity, suggesting an increase in the processing of protein and lipid substrates and no effect on polysaccharide use. Siu et al. [17] observed a decrease in bacterioplankton respiration and bacterioplankton production, as measured via ^3H –thymidine incorporation, and a clear shift from more diverse bacterioplankton communities at ambient pH to less diverse communities under elevated pCO_2 conditions. These studies provide insight to the direct effects of elevated pCO_2 on bacterioplankton extracellular enzyme activity and community structure but did not address the effects of pCO_2 on bacterioplankton removal of organic carbon.

Here we present results from seawater culture experiments that were designed to examine the direct effect of pCO_2 on net removal of organic carbon by bacterioplankton and discuss resulting implications for bacterioplankton respiration and biomass production. Laboratory perturbation experiments using natural bacterioplankton communities were conducted at three contrasting sites: the Sargasso Sea, the Santa Barbara Channel, and the South Pacific Subtropical Gyre. In the oligotrophic Sargasso Sea, deeper waters with elevated pCO_2 are brought to the surface through convective mixing annually [18, 19]. Coastal upwelling off the west coast of North America can induce higher-frequency variability in pCO_2 [20] and result in strong shifts in pH on timescales of days in places like the Santa Barbara Channel, CA [21]. In contrast, seasonal or higher-frequency pulses of elevated pCO_2 are less likely in the more permanently stratified oligotrophic systems such as the waters surrounding the islands of Moorea and Tahiti, within the South Pacific Subtropical Gyre [21]. All ocean surface waters are also subject to rising atmospheric CO_2 concentrations and are expected to be impacted gradually. How this broad range in the frequency and magnitude of elevated pCO_2 exposure impacts DOC processing by bacterioplankton remains largely unknown. The objective of this study was to assess the direct effect of short-term exposure to elevated pCO_2 on bacterioplankton organic carbon removal, across a variety of ocean sites.

Our results show that short-term perturbations of elevated pCO_2 can result in an increased rate and magnitude of organic carbon removal by bacterioplankton, independent of the

environmental origin of the bacterioplankton communities. These results suggest that short-term increases in pCO_2 can lead to an increased loss of organic carbon due to respiration, which may ultimately reduce surface ocean carbon inventories and the amount of DOC available for vertical export from the surface ocean.

Materials and methods

General experimental set-up and design

The same general approach was used for all three study sites. Experiments consisted of 0.2 μm-filtered (0.2 μm GSWP, Millipore, Billerica, MA) seawater or 0.2 μm-filtered phytoplankton exudate that was inoculated with natural bacterial communities. The inoculum of natural bacterial communities consisted of either unfiltered whole seawater (Sargasso Sea and South Pacific Subtropical Gyre experiments) or 1.2 μm filtrate (Santa Barbara Channel experiments; 1.2 μm RAWP, Millipore, Billerica, MA). Particulate organic carbon concentration in oligotrophic gyres is low (1–3 μmol C L^{-1}) so to avoid filtration artifacts such as reduced bacterial production (unpublished data) and contamination of DOC due to handling, the inoculum was not pre-filtered for the experiments conducted in oligotrophic waters. Because particulate organic carbon concentration can be much greater in coastal upwelling systems it was necessary to remove large particles and organisms from the inoculum. Inoculum was added at 25–30% of final volume (Table 1), effectively diluting grazer concentrations and grazing pressure. All filters were pre-rinsed with ~2 L of deionized distilled water and sample water prior to use in order to remove organic contaminants from the filters.

Treatments. The four types of DOC treatments used in the experiments included either: (1) unamended seawater, which provided naturally occurring DOC, (2) naturally occurring DOC amended with glucose (~10 μM C), NH_4 Cl (1μM) and K_2HPO_4 (0.1μM) (CNP) [22], or one of two phytoplankton-derived DOC mixtures: (3) phytoplankton exudate or (4) naturally occurring DOC amended with phytoplankton lysate (~10 μM C). There was no attempt to standardize the initial concentration of DOC in the phytoplankton exudate treatments; thus, those treatments contained total organic carbon concentrations that were 2–3 fold greater than those in the unamended seawater at the beginning of the experiments (Table 1).

The various treatments were generated by inoculating the 0.2 μm pre-filtered seawater or exudate with the microbial community; this solution was then divided into two polycarbonate (PC) containers to adjust pCO_2 (Table 1). pCO_2 levels were adjusted via chemical additions (Sargasso Sea experiment) or by bubbling with CO_2-mixed air (Santa Barbara Channel and South Pacific Subtropical Gyre experiments). Adjusted seawater incubations were then transferred into new PC carboys and CNP or lysate was added, if appropriate. A very small volume of lysate (1.2 mL to 11.5 L of experimental volume) or CNP (12 mL to 10 L of experimental water for the Sargasso Sea experiment; 0.28 mL to 10 L of experimental volume for the Santa Barbara Channel experiment) was added to minimize perturbing the carbonate chemistry. All experiments were conducted in duplicate, at in situ temperatures, and in the dark to eliminate photoautotrophic production (Table 1). All PC bottles had been acid-washed (5% or 10% HCL) and rinsed with deionized distilled water and sample water before use.

***pCO_2* adjustments.** Except in the Sargasso Sea, the pCO_2 was adjusted by bubbling with CO_2-mixed air (Scott Marrin Inc.) at ~100 mL min^{-1} through an air stone to produce fine bubbles for 45 min– 4 hours depending on volume. The pCO_2 adjustment via bubbling was conducted only once, prior to the start of the experiment, to eliminate the possibility of abiotic removal of organic matter as a result of continued bubbling. The air in the headspace of each sample bottle was exchanged with target CO_2-mixed air every 24 hours to minimize the change in pCO_2 levels throughout the experiment. Bubbling was conducted at experimental

Table 1. Summary of experimental conditions and results.

		Conditions at the Start of the Experiment								Incubation Times		Results		
Exp #	Exp	DOC Source	Volume of Incubation (L)	Ratio of 0.2μm: InoculumSW	T (˚C)	TargetpCO_2 (ppm)	Actual pCO_2 (ppm)	In situ TOC (μmol C L^{-1})	InitialTOC (μmol C L^{-1})	Time from T_0 to Stationary-Growth (Days)	Durationof Exp (Days)	TOC Removal (μmol C L^{-1}) From T_0 to Stationary-Growth	TOC Removal (μmol C L^{-1}) Over Duration of Exp	BGE (%)
1A	SS Sept. 2012	Natural DOC	2	3:7	26.5	400	~389	73.1 ± 1.3	76.0 ± 0.2	2.13	6.7	ND	ND	ND
1A	–	–	–	–	–	800	~760	–	77.9 ± 0.8	2.13	–	ND	ND	ND
1B	SS Sept. 2012	CNP	2	3:7	26.5	400	~389	73.1 ± 1.3	87.3 ± 1.3	2.13	6.7	-6.7 ± 2.6	-10.2 ± 2.3	11.9 ± 4.9
1B	–	–	–	–	–	800	~760	–	89.5 ± 1.5	2.13	–	-9.2 ± 1.3	-10.6 ± 1.1	9.1 ± 1.3
2A	SBC Dec. 2012	Natural DOC	2	3:7	14.5	250	349 ± 10	85.4 ± 2.0	86.4 ± 0.1	1.34	3.6	-1.7 ± 0.7	-2.0 ± 1.9	40.5 ± 9.4
2A	–	–	–	–	–	1000	1064 ± 10	–	86.1 ± 0.3	1.34	–	-3.8 ± 1.2	-4.7 ± 0.1	30.3 ± 7.2
2B	SBC Dec. 2012	CNP	2	3:7	14.5	250	349 ± 10	85.5 ± 0.5	93.8 ± 0.8	1.34	3.6	-5.8 ± 1.5	-12.0 ± 1.1	35.7 ± 6.0
2B	–	–	–	–	–	1000	1064 ± 10	–	96.5 ± 0.3	1.34	–	-10.7 ± 0.8	-15.1 ± 0.3	26.4 ± 2.1
3	SBC May 2013	*D. frag.* Exudate	0.5	1:3	12	250	238 ± 2	88.0 ± 0.1	398.6 ± 3.4	2.75	8	-65.5 ± 1.6	-115.6 ± 3.9	9.1 ± 2.5
3	–	–	–	–	–	1500	1376 ± 45	–	389.6 ± 9.8	2.75	–	-69.9 ± 10.3	-119.2 ± 12.4	6.8 ± 2.8
4	SBC August 2013	*T. weiss.* Exudate	2	1:3	14	250	132 ±3	64.2 ± 1.6	157.4 ± 0.9	5.11	6.8	-3.2 ± 0.8	-2.4 ± 0.5	47.0 ± 2.3
4	–	–	–	–	–	1500	1262 ± 6	–	158.2 ± 0.1	5.11	–	-4.9 ± 0.6	-6.52 ± 1.0	36.4 ± 0.3
5A	SBC Oct. 2013	*T. weiss.* Exudate	0.5	1:3	16	250	233 ± 3	73.1	155.9 ± 0.3	4.42	12.9	-10.5 ± 0.3	-22.9 ± 3.7	35.6 ± 0.3
5A	–	–	–	–	–	1500	1563 ± 74	–	156.3 ± 0.1	4.42	–	-17.2 ± 0.5	-32.5 ± 13.9	27.1 ± 5.5
5B	SBC Oct. 2013	*C. soc.* Exudate	0.5	1:3	16	250	244 ± 5	73.1	141.2 ± 3.2	3.0	12.9	-4.0 ± 0.1	-24.1 ± 4.4	26.1 ± 1.1
5B	–	–	–	–	–	1500	1551 ± 10	–	140.0 ± 2.3	3.0	–	-8.0 ± 4.0	-29.9 ± 4.8	15.7 ± 5.8
5C	SBC Oct. 2013	*A. glac.* Exudate	0.5	1:3	16	250	194 ± 10	73.1	142.9 ± 1.5	4.42	12.9	-2.7 ± 2.3	-21.5 ± 3.1	38.4
5C	–	–	–	–	–	1500	1363 ± 5	–	143.5 ± 0.9	4.42	–	-10.2 ± 0.5	-25.2 ± 0.7	31.3 ± 2.9
6	SBC May 2014	*E. hux.* Exudate	2	1:3	14.5	250	325 ± 23	ND	103.8 ± 0.1	4.92	14.7	-2.1 ± 1.7	-7.4 ± 1.3	58.4 ± 22.9
6	–	–	–	–	–	1500	1454 ± 14	–	106.9 ± 0.1	5.75	–	-7.5 ± 0.2	-13.5 ± 0.9	28.6 ± 0.6
7A	SPSG July 2014	Natural DOC	2.8	1:3	22	250	ND	70.6	75.4 ± 1.0	ND	10	ND	ND	ND
7A	–	–	–	–	–	1000	ND	–	73.8 ± 0.5	ND	–	ND	ND	ND
7B	SPSG July 2014	*E. hux.* Lysate	2.8	1:3	22	250	ND	70.6	85.8 ± 0.6	1.81	10	-4.8 ± 2.3	-11.1 ± 1.1	11.9 ± 5.2
7B	–	–	–	–	–	1000	ND	–	84.4 ± 1.3	1.81	–	-7.0 ± 0.7	-12.4 ± 0.5	8.4 ± 0.7
8A	SPSG July 2014	Natural DOC	2.8	1:3	22	250	ND	76.7	75.2 ± 0.4	ND	10.5	ND	ND	ND
8A	–	–	–	–	–	1000	ND	–	73.7 ± 0.9	ND	–	ND	ND	ND
8B	SPSG July 2014	*E. hux.* Lysate	2.8	1:3	22	250	ND	76.7	87.2 ± 0.4	0.92	10.5	-4.7 ± 1.0	-8.0 ± 2.1	24.7 ± 3.0
8B	–	–	–	–	–	1000	ND	–	84.8 ± 0.6	1.84	–	-7.0 ± 0.8	-8.8 ± 2.3	8.5 ± 1.8
9A	SPSG July 2014	Natural DOC	2.8	1:3	22	250	ND	70.9	75.0 ± 2.0	ND	9	ND	ND	ND
9A	–	–	–	–	–	1000	ND	–	75.0 ± 3.6	ND	–	ND	ND	ND
9B	SPSG July 2014	*E. hux.* Lysate	2.8	1:3	22	250	ND	70.9	83.7 ± 0.9	1.79	9	-6.7 ± 1.3	-8.9 ± 2.1	8.9 ± 0.7
9B	–	–	–	–	–	1000	ND	–	85.6 ± 0.7	1.79	–	-7.9 ± 0.6	-12.3 ± 0.6	7.3 ± 0.3

(*Continued*)

Table 1. (*Continued*)

		Conditions at the Start of the Experiment								Incubation Times		Results		
Exp #	Exp	DOC Source	Volume of Incubation (L)	Ratio of 0.2μm: InoculumSW	T (˚C)	TargetpCO_2 (ppm)	Actual pCO_2 (ppm)	In situ TOC (μmol C L^{-1})	InitialTOC (μmol C L^{-1})	Time from T_0 to Stationary-Growth (Days)	Durationof Exp (Days)	TOC Removal (μmol C L^{-1}) From T_0 to Stationary-Growth	TOC Removal (μmol C L^{-1}) Over Duration of Exp	BGE (%)
10	SBC BC Dec 2015	*T. weiss.* Lysate	5	3:7	14	250	ND	69.6	86.4 ± 2.8	2.8	17.5	-15.6 ± 0.1	-19.7 ± 3.0	36.4 ± 0.2
10	–	–	–	–	–	1500	ND	–	1 ± 0.5	2.8	–	-18.0 ± 0.6	-23.2 ± 0.5	34.1 ± 3.7

Conditions at the start of the seawater culture experiments, total organic carbon (TOC) removal from T_0 to stationary phase, TOC removal over the duration of the incubations, and bacterioplankton growth efficiency (BGE). Mean BGE was calculated as the ratio of the change in the carbon content of bacterioplankton biomass (BC) to dissolved organic carbon (DOC) removal from T_0 to stationary phase. DOC was calculated as TOC—BC. Bacterioplankton cell abundance was converted to BC carbon using a carbon conversion factor of 30 fg C $cell^{-1}$ for the Santa Barbara Channel (SBC) experiments and 10 fg C $cell^{-1}$ for the experiments conducted in the Sargasso Sea (SS) and South Pacific Subtropical Gyre (SPSG). Error refers to mean ± standard deviation and 'ND' refers to not determined. Only one value is recorded in the table when only one valid result was obtained. Dashes refer to the information in the cell above the dash.

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temperatures, which were identical to the in situ temperatures of the inoculums. In the Sargasso Sea experiment, pCO_2 was adjusted through the addition of 0.206 g $CaCO_3$, 0.032 g $NaHCO_3$, and 29 mL of 0.1 N HCL according to the best practices guide [23]. This closed system approach results in an inorganic carbon perturbation that is chemically identical to bubbling [24].

To maintain pCO_2 over the course of the experiments, the incubation bottle lids used for the Santa Barbara Channel and South Pacific Subtropical Gyre experiments were equipped with built-in gas and sampling ports to allow for sampling via positive pressure displacement in which the treatment pCO_2 gas was used to pressurize the head space (< 3 PSI); thus displacing the sample volume from the incubation vessel through the sample line directly into a collection vessel. In the Sargasso Sea, samples were collected by decanting directly from the incubation bottles into sample bottles.

To assess the potential change in pH over the course of an incubation we measured pH as described below for experiments # 1–4. We found that pH changes, within a given incubation, were minimal (0–0.04 pH units) over the course of the experiments. These pH changes correspond to changes in pCO_2 of ~ 1–60 ppm, which is much less than the differences in pCO_2 between low and elevated-pCO_2 treatments (~370 –~1100 ppm). Thus, we concluded that pH adjustments were maintained throughout experiments with very little drift in pCO_2.

Sargasso Sea experiment. Water was collected from Hydrostation S (32˚ 10'N, 64˚ 30'W) in the northwestern Sargasso Sea at a depth of 10 m using a conductivity, temperature, depth (CTD) rosette. Sampling met the limited impact research requirements under the Bermuda Institute of Sciences Collection and Experimental Ethics Policy. As a result, collection and export permits were not required from the Bermuda Government at the time of this study. Seawater culture experiments were set-up in the lab at the Bermuda Institute of Ocean Science within hours of collection. The experiment consisted of four treatments, each in duplicate: two pCO_2 treatments (ambient and elevated) combined with two DOC treatments (CNP amended and unamended) as a full factorial design (experiment # 1, Table 1). The inoculated seawater was partitioned into two 10 L PC carboys, one remaining unadjusted (ambient pCO_2 treatment); the pCO_2 of the second carboy was adjusted to ~760 ppm using the closed system approach (23). Duplicate 2 L PC bottles were filled with each pCO_2 treatment for the unamended DOC treatments. CNP was added to the seawater remaining in the 10 L carboys to a final concentration of 10 μM C, 1 μM N, and 0.1 μM P (as described above) and each was then split into duplicate 2 L PC bottles, representing the amended DOC treatments. All eight bottles were incubated within a temperature controlled upright incubator (Table 1).

Santa Barbara Channel experiments. Five experiments were conducted with surface seawater collected from the Santa Barbara Channel between 2012 and 2014 (experiments # 2–6, Table 1). No specific permissions were required for near-shore seawater collection in the Santa Barbara Channel. Each time, surface seawater was collected using a PC carboy from a near-shore site along the coast of Santa Barbara, CA (34˚ 24'N, 119˚ 50'W). The DOC source and starting pCO_2 conditions, as well as the bacterioplankton inoculum varied between experiments (Table 1). pCO_2 of seawater incubations were adjusted to pre-industrial (250 ppm) and elevated (1000 ppm or 1500 ppm) levels by bubbling with CO_2-mixed air in two 8 L or 20 L PC carboys (see above) depending on experimental water requirements (Table 1). pCO_2 adjusted seawater was split into duplicate 0.5 L or 2 L PC bottles and placed within a temperature controlled upright incubator (Table 1).

South Pacific Subtropical Gyre experiments. Three separate experiments were conducted in July 2014 with water collected near French Polynesia in the South Pacific Subtropical Gyre (17˚ 36'S, 149˚ 43'W) (experiments # 7–9, Table 1). All research was performed under annual research permits (permit no. 438/AME) issued by the French Polynesian Ministry of

Foreign Affairs of International Development of the French Republic, Americans and Caribbean Islands Division. Seawater was collected from a depth of 25 m using a CTD rosette and seawater cultures were prepared in two 20 L PC carboys. Treatments for each of the three experiments included unamended and amended DOC treatments and pre-industrial (250 ppm) and elevated (1000 ppm) pCO_2 in a full factorial design. One of two 20 L carboys was bubbled with pre-industrial (250 ppm) CO_2-mixed air and the other with elevated (1000 ppm) CO_2-mixed air as described below. pCO_2 adjusted seawater was then amended with phytoplankton-lysate to a final addition of ~10 μM C and placed within an upright incubator (Table 1). We were unable to measure pCO_2 at sea but applied the same flow rate and bubbling time that resulted in effective pCO_2 adjustment previously.

Production of phytoplankton-derived DOC. Phytoplankton exudates and lysates were derived from phytoplankton cultures in order to assess the impact of varying pCO_2 on the microbial consumption of complex DOC. Exudates from five different diatom cultures (*Dactyliosolen fragilissimus*, *Thalassiosira weissflogii*, *Chaetoceros socialis*, *Asterionellopsis glacialis*) and one coccolithophore (*Emiliana huxleyi*) were used in the Santa Barbara Channel experiments where diatoms are often abundant. In addition, phytoplankton lysate was produced from *Emiliana huxleyi* and used for the experiments conducted in the South Pacific Subtropical Gyre where coccolithophores are often present [25].

Phytoplankton cultures were grown in sterilized (double 0.2 μm filtered) seawater collected from the Santa Barbara Channel and enriched with inorganic nutrients in a modified version of f/2 medium [26]. Preliminary growth experiments were used to determine the ratio of N:P:Si nutrient additions needed to induce nitrate limitation within each culture. This was done to enhance the production of DOC in the phytoplankton cultures and to minimize the addition of nitrate to the culture experiments. A light/ dark cycle of 14/10 hours, a photon flux density of ~100 μmol m^{-2} s^{-1}, and a temperature of 18˚C was maintained for all cultures throughout the growth period.

Nitrate concentration was monitored over the course of phytoplankton growth via UV detection using an HP452A spectrophotometer (Hewlett Packard 8452A) [27] and calibrated against a series of chemical nitrate standards (4 point curve; 0–100 μmol N L^{-1}). Exudate and lysate was harvested from the cultures two days after nitrate concentration fell below 2.4 ± 1 μmol N L^{-1} according to Nelson and Carlson [28]. Briefly, exudate was harvested via filtration (0.2 μm GSWP, Millipore, Billerica, MA), whereas lysate was harvested through a series of steps including cell concentration via centrifugation (10,000 rpm) and freeze-thaw cycles. After final centrifugation the cell pellet was abraded with a pre-combusted glass rod to generate cell lysate. Final lysate volume was 0.2 μm filtered and then acidified (4 M HCl) to a pH of ~3 and stored at -20˚C for one week prior to use in the South Pacific Subtropical Gyre experiments.

Experimental samples. Experimental samples were not filtered upon removal from experimental incubations in order to minimize contamination due to transfer and handling. Samples for total organic carbon (TOC; carbon content of bacterioplankton biomass plus DOC) and bacterioplankton abundance were collected throughout the incubations. pH was monitored over regular intervals for the Sargasso Sea experiments and experiments # 2–5 in the Santa Barbara Channel. pCO_2 was measured at the start of the incubations for Santa Barbara Channel experiments # 2 and 4–6. Measurements of pCO_2 and pH were not made for the South Pacific Subtropical Gyre experiments or the Santa Barbara Channel #10 experiment due to logistical constraints. However, our ability to effectively alter the pCO_2 from ambient to target levels was demonstrated numerous times prior to these experiments (Table 1, experiments # 2–6). Thus, we used previously determined bubbling rates and duration to achieve target

pCO_2 levels. The direct effect of pCO_2 on carbon content of bacterioplankton cells was determined during the Santa Barbara Channel follow-up experiment # 10.

Sample processing

TOC measurements—The procedures used to set up each experiment (inoculum filtration and dilution with 0.2 μm filtrate) removed the majority of particulate organic carbon such that changes in bacterioplankton carbon production and DOC removal were mainly a function of the growth of the bacterioplankton. Ideally, samples collected for organic carbon would be filtered in order to directly assess DOC removal independently from bacterioplankton carbon production. However, sample handling during filtration can result in contamination that obscures changes in DOC on the scale of a few micro-molar C. To avoid contamination, seawater samples from the incubation experiments were not filtered. Therefore, measured values of organic carbon include both DOC and bacterioplankton carbon and are considered TOC.

TOC samples were collected into 60 mL high-density polyethylene bottles (Sargasso Sea and South Pacific Subtropical Gyre) or in combusted 40 mL glass EPA vials with Teflon coated silicone septa (Santa Barbara Channel). All TOC samples were frozen at -20˚C until analysis. Samples were analyzed via high temperature combustion method on a modified Shimadzu TOC-V or Shimadzu TOC-L using the standardization and referencing approaches described in Carlson et al. [29].

Bacterioplankton abundance measurement—Samples for bacterioplankton abundance were analyzed by epifluorescence microscopy with 0, 6-diamidino -2-phenyl dihydrochloride (5μg mL^{-1}, DAPI, SIGMA-Aldrich, St. Louis, MO, USA) according to Porter and Feig [30] (experiments # 1 and 10), or by Flow Cytometry (FCM) on an LSR II with SYBR Green I according to Nelson et al. [31] (experiments # 2–9). See Parsons et al. [32] and Nelson et al. [31] regarding sample preparation and instrument settings for epifluorescence microscopy and FCM analyses, respectively. DAPI direct counts and FCM analysis enumerate total prokaryotic abundance. We were not able to differentiate between bacterial and archaeal domains and refer to the combined cell densities as bacterioplankton abundance [33].

Bacterioplankton carbon measurement—To assess the direct effect of pCO_2 on the carbon content of bacterioplankton cells a follow-up experiment was conducted with water collected from the Santa Barbara Channel during December 2015 (experiment # 10, Table 1). The experimental procedure used was identical to that for all other experiments. Surface seawater was collected using a PC carboy from a near-shore site along the coast of Santa Barbara, CA (34˚ 24'N, 119˚ 50'W). One of two 20 L carboys filled with 0.2 μm- and 1.2 μm-filtered seawater was bubbled with pre-industrial (250 ppm) CO_2-mixed air and the other with elevated (1500 ppm) CO_2-mixed air. pCO_2 adjusted seawater was then amended with phytoplankton-lysate to a final addition of ~20 μmol C L^{-1} and placed within a temperature controlled incubator in the dark (Table 1).

Samples for determining the carbon content of bacterioplankton cells were collected at the beginning of stationary phase to maximize bacterioplankton abundance and to minimize the contribution of nanoflagellate grazers to carbon estimates. A one-liter sample was filtered onto combusted GF75 filters (Advantec®; Dublin CA). GF75 filters were used because they have a nominal pore size of 0.3 μm and demonstrated 92 ± 2% (range = 3.7%; n = 5) cell retention. DOC-blanks were collected simultaneously to account for adsorption of organic carbon to the filters and were collected by filtering ~1 L of pre-filtered (0.2 μm) experimental volume onto a 0.3 μm GF75. Filters were placed into pre-combusted (4 hr at 400˚C) glass vials, and frozen at -40˚C. Filters were shipped on dry ice to Bigelow Analytics (Bigelow Laboratories for Ocean Sciences) and particulate organic carbon was determined using a Costech ECS 4010 elemental

analyzer (980˚ combustion). Filters were analyzed within two weeks of collection and DOC-blank values were subtracted from experimental particulate organic carbon values. Carbon content of bacterioplankton cells (i.e. the carbon conversion factor) was calculated as particulate organic carbon concentration divided by bacterioplankton abundance.

Carbonate parameters. pH- Spectrophotometric pH measurements were made via absorbance of seawater sample at 25˚C with m-cresol purple indicator dye (unpurified) following Dickson et al. [34] for the Santa Barbara Channel experiments. The dye was frequently calibrated against certified reference material (Batch # 10, Dickson, La Jolla, California). pH was measured using a Corning 430 pH meter for the Sargasso Sea experiments.

pCO_2 –Samples for pCO_2 analysis were collected into ~400 mL borosilicate bottles according to Dickson et al. [34]. The bottles were capped immediately upon collection and pCO_2 analysis was conducted within 24 hrs of sampling on a custom pCO_2 system according to Hales et al. [35]. All pCO_2 samples were systematically referenced against standard quality gasses (Scott Marrin Inc.).

Measurements of carbonate parameters were not made for the South Pacific Subtropical Gyre and Santa Barbara Channel # 10 experiments due to logistical constraints. We used bubbling flow rates and duration determined from previous experimental volumes to achieve target elevated and pre-industrial pCO_2 levels for these experiments. For all other experiments where pCO_2 was not directly measured (experiments # 1 and 3), it was calculated based on pH and a recent local in situ measurement of total alkalinity using CO2SYS [36] and the dissociation constants for carbonic acid as refitted by Dickson and Millero [37].

Calculations

Cell-specific TOC removal—Cell-specific TOC removal was calculated as the difference in TOC concentration (ΔTOC) from the start of the experiment to the beginning of stationary phase divided by the concomitant change in bacterioplankton abundance.

Bacterioplankton carbon demand—Bacterioplankton carbon demand (BCD) is equivalent to the carbon required for bacterioplankton biomass production (ΔBC) plus bacterioplankton respiration (BR; BCD = ΔBC + BR), over a given period of time. BCD is also equivalent to the total amount of DOC consumed by heterotrophic microbial communities. Therefore, bacterioplankton carbon demand was calculated as the change in the concentration of DOC from the start of the incubations to stationary phase. DOC concentration was calculated as the difference between the concentrations of TOC and BC (i.e. DOC = TOC—BC).

Bacterioplankton growth efficiency—Bacterioplankton growth efficiency, which provides an estimate of how much of the consumed organic carbon is partitioned into cellular biomass (BC) versus respiration [38, 39], was calculated as the increase in BC from the start of the incubations to stationary growth phase divided by bacterioplankton carbon demand. For the Santa Barbara Channel experiments, bacterioplankton abundance was converted to BC using the carbon conversion factor of 30 fgC $cell^{-1}$ measured during Santa Barbara Channel experiment # 10. This is comparable to other published carbon conversion factors ([40], A Cano, unpublished). A lower conversion factor of 10 fgC $cell^{-1}$ was used for communities from the Sargasso Sea and the South Pacific Subtropical Gyre to reflect smaller cells in oligotrophic regions [41, 42].

Bacterioplankton respiration—Bacterioplankton respiration can be equated to the change in ΔTOC by the following argument. The change in TOC between time 1 and time 2 is given by:

$$\Delta TOC = (DOC_1 + BC_1) - (DOC_2 + BC_2) \quad (1)$$

Then because bacterioplankton grow by consuming DOC, converting a portion of consumed DOC into BC and respiring the remainder to CO_2, the change in DOC between two time points (ΔDOC) is given by:

$$\Delta DOC = (BC_2 - BC_1) + BR \quad (2)$$

Note that in this formulation ΔDOC is a positive value such that when Eq 1 is rearranged as:

$$\Delta TOC = (DOC_1 - DOC_2) + (BC_1 - BC_2) \quad (3)$$

the right hand side of Eq 2 can be substituted for (DOC_1 –DOC_2) in Eq 3 yielding:

$$\Delta TOC = (BC_2 - BC_1) + BR + (BC_1 - BC_2) = BR \quad (4)$$

Results

Growth patterns of the natural bacterioplankton communities within all experiments demonstrated typical batch culture patterns of lag, exponential, and stationary growth (Figs 1, 2, and 3). However, the magnitude of the response differed between sites and treatments as described below.

Experiments with naturally occurring DOC

Bacterioplankton communities in both elevated and pre-industrial pCO_2 treatments, grown on naturally occurring DOC in the Sargasso Sea, demonstrated a less than two-fold change in bacterioplankton abundance and no resolvable removal of TOC over the course of the incubations (Fig 1A). pCO_2 treatments showed no difference in cell yield ($4.8 \pm 0.8 \times 10^5$ cells mL^{-1}; elevated pCO_2 vs. $4.8 \pm 0.3 \times 10^5$ cells mL^{-1}; ambient pCO_2) or production rate ($6.0 \pm 2.2 \times 10^4$ cells mL^{-1} d^{-1}; elevated pCO_2 vs. $7.0 \pm 1.4 \times 10^4$ cells mL^{-1} d^{-1}; ambient pCO_2) (Fig 1A). A similar pattern was detected in the South Pacific Subtropical Gyre for the unamended experimental treatments (Fig 1B).

In contrast, the experiment with naturally occurring DOC from the Santa Barbara Channel demonstrated significant increases in bacterioplankton abundance and measurable removal of TOC in both pre-industrial and elevated pCO_2 treatments (Fig 1C). Although the initial rate of increase in bacterioplankton abundance was similar for both pCO_2 treatments, greater maximum abundance was obtained by stationary phase with elevated pCO_2 ($1.0 \pm 0.02 \times 10^6$ cells mL^{-1}), compared with pre-industrial (250 ppm) pCO_2 levels ($0.9 \pm 0.01 \times 10^6$ cells mL^{-1}) (Fig 1C). The rate of TOC removal was also greater in the elevated pCO_2 treatment (-2.8 ± 0.9 μmol C L^{-1} d^{-1}) relative to the pre-industrial pCO_2 treatment (-1.3 ± 0.5 μmol C L^{-1} d^{-1}) (Fig 1C). The enhanced TOC removal relative to the corresponding change in bacterioplankton abundance led to greater cell-specific consumption of TOC at elevated pCO_2 (71.9 ± 24.4 fg C $cell^{-1}$) relative to pre-industrial pCO_2 (46.1 ± 17.7 fg C $cell^{-1}$; Fig 4).

Experiments with CNP

The addition of glucose in the CNP- Sargasso Sea experiment raised TOC concentration by 11.2 and 11.6 μmol C L^{-1} over the unamended treatment for the ambient and elevated pCO_2 treatments, respectively. This additional labile organic carbon (in combination with ammonium and phosphate) enhanced overall bacterioplankton yield by greater than an order of magnitude (Fig 1D) compared to the unamended treatment (Fig 1A). The bacterioplankton

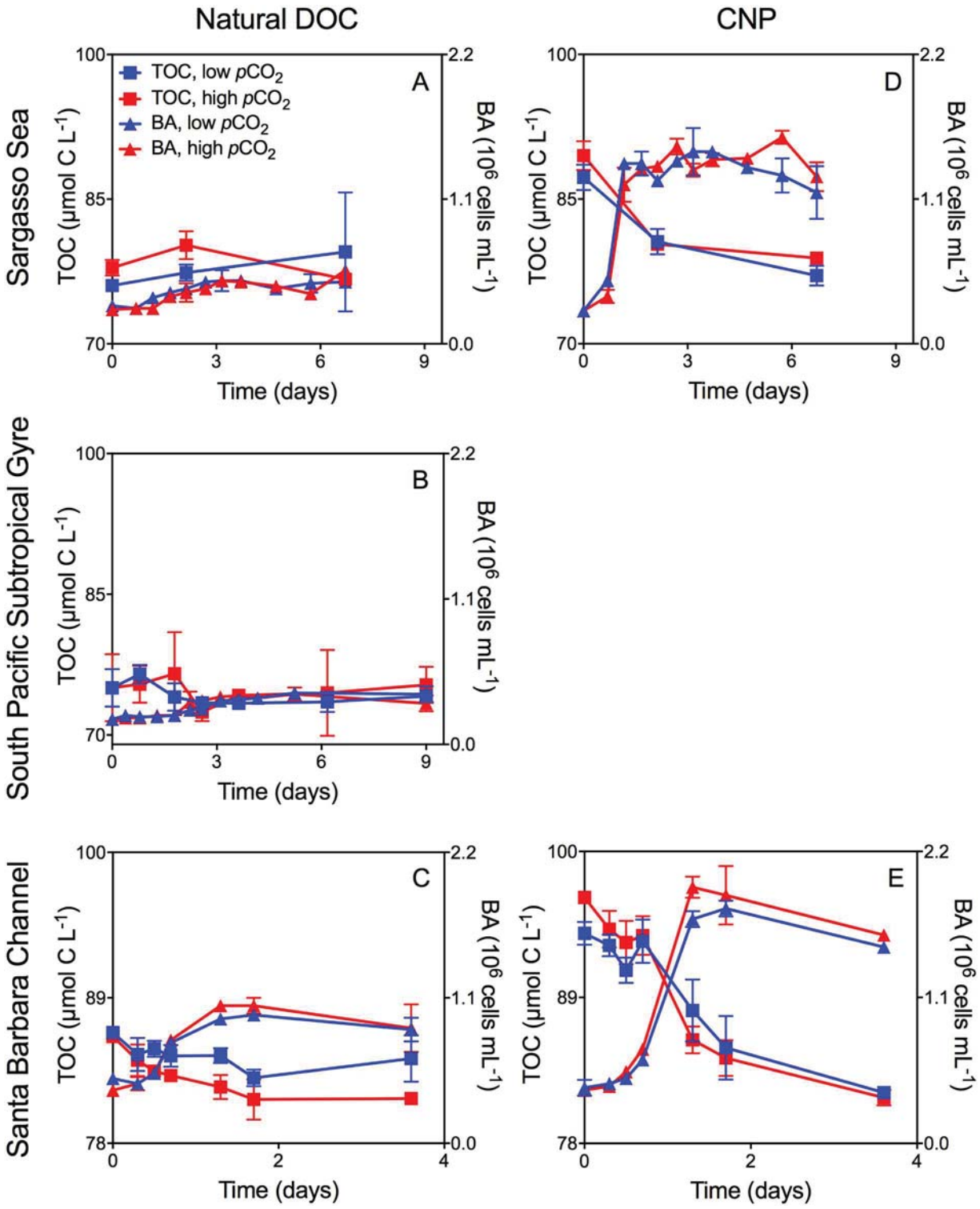

Fig 1. Mean TOC concentration and mean bacterioplankton abundance for Sargasso Sea and Santa Barbara Channel CNP- and natural-DOC experiments. Mean TOC concentration (± SD) and mean bacterioplankton abundance (BA; ± SD) averaged across duplicate incubations through time and as a function of pCO_2 for natural bacterioplankton assemblages incubated with: A: Natural DOC, Sargasso Sea Exp. #1A; B: Natural DOC, South Pacific Subtropical Gyre Exp. #7; C: Natural DOC, Santa Barbara Channel Exp. #2A; D. CNP, Sargasso Sea Exp. #1B; E. CNP, Santa Barbara Channel Exp. #2B. For treatments at all sites, red denotes elevated pCO_2, while blue denotes ambient pCO_2 in the Sargasso Sea experiment and pre-industrial pCO_2 in the Santa Barbara Channel and South Pacific Subtropical Gyre experiments. Squares represent TOC (µmol C L^{-1}) while triangles denote BA (10^6 cells mL^{-1}) over the course of the incubation.

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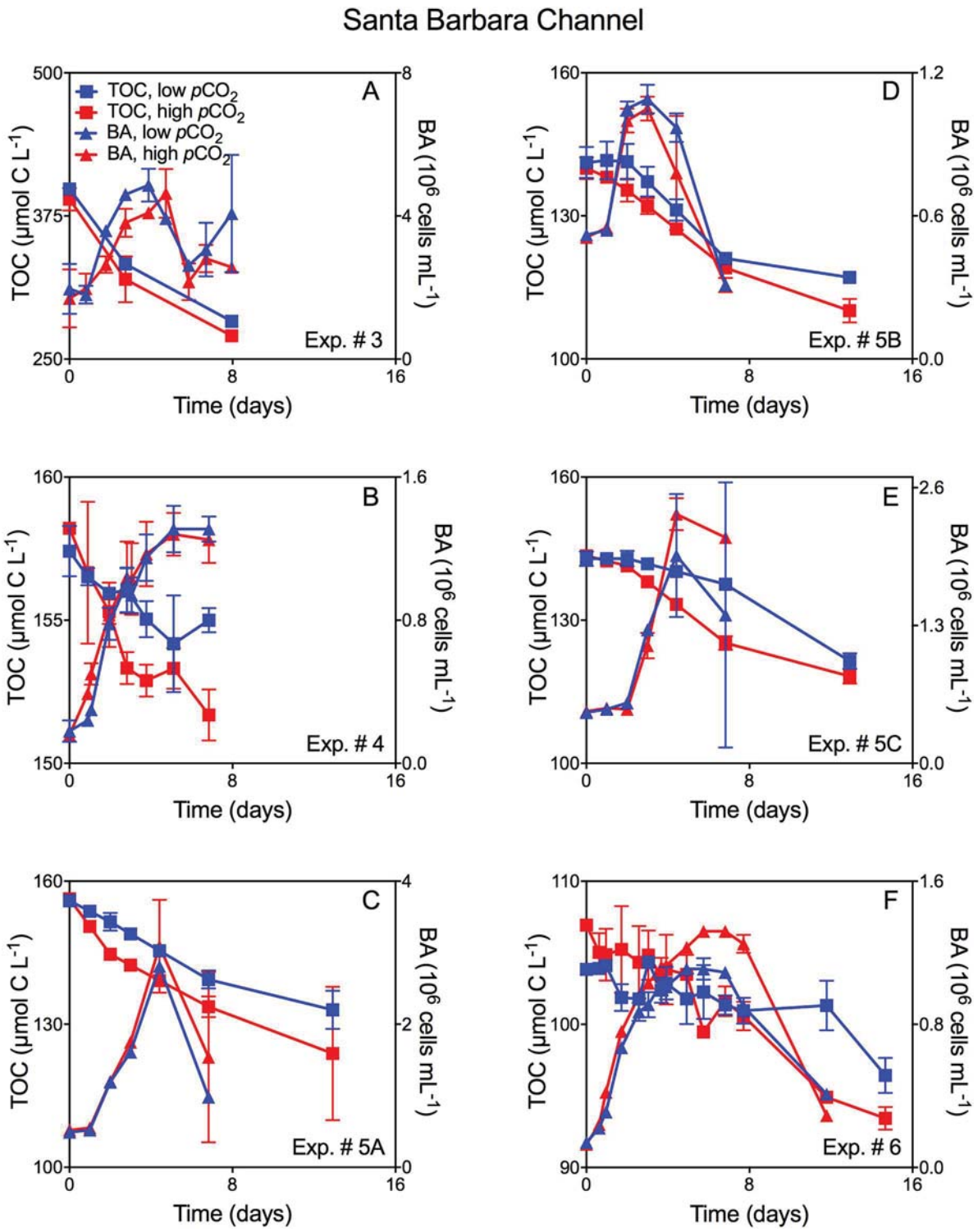

Fig 2. Mean TOC concentration and mean bacterioplankton abundance for Santa Barbara Channel phytoplankton-DOC experiments. Mean TOC concentration (± SD) and mean bacterioplankton abundance (BA; ± SD) averaged across duplicate incubations through time and as a function of pCO_2 for natural bacterioplankton assemblages in the Santa Barbara Channel experiments incubated with: A: *D. fragilissimus*-derived DOC, Exp. #3; B: *T. weissflogii*-derived DOC, Exp. #4; C: *T. weissflogii*-derived DOC, Exp. #5A; D: *C. socialis*-derived DOC, Exp. #5B; E: *A. glacialis*-derived DOC, Exp. #5C; F: *E. huxleyi*-derived DOC, Exp. #6. Red denotes elevated pCO_2, while blue denotes low (250 ppm) pCO_2. Squares represent TOC (µmol C L^{-1}) while triangles denote bacterioplankton abundance (10^6 cells mL^{-1}) over the course of the incubation.

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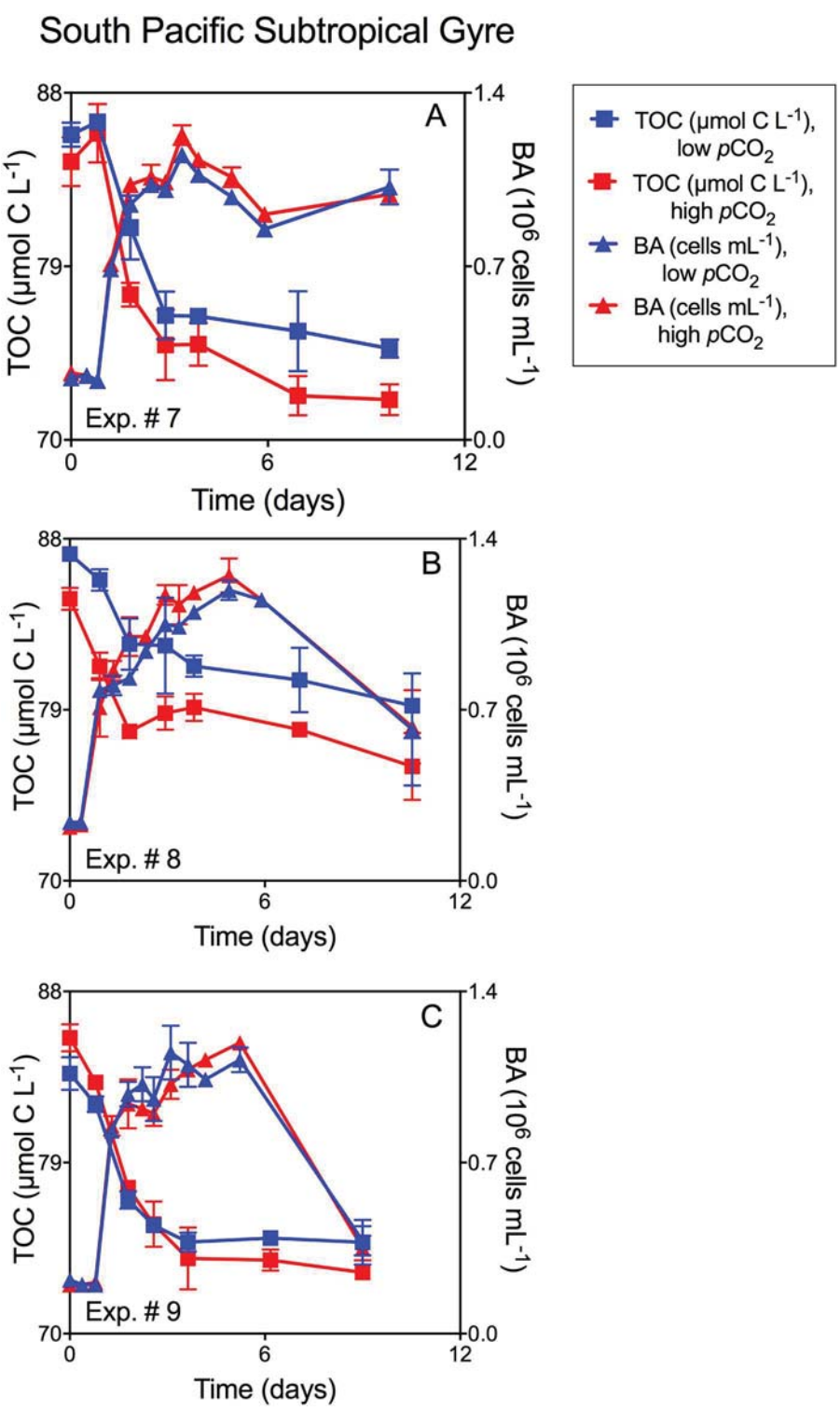

Fig 3. Mean TOC concentration and mean bacterioplankton abundance for phytoplankton-DOC South Pacific Subtropical Gyre experiments. Mean TOC concentration (± SD) and mean bacterial abundance (BA; ± SD) averaged across duplicate incubations through time and as a function of pCO_2 for natural bacterioplankton assemblages in the South Pacific Subtropical Gyre experiments incubated with *E. huxleyi*-derived DOC: A: Exp. #7B; B: Exp. #8B; C: Exp. #9B. The same experimental design was used for these experiments (note that only the amended treatments are shown). Red denotes elevated pCO_2, while blue denotes low (250 ppm) pCO_2. Squares represent TOC (µmol C L^{-1}) while triangles denote BA (10^6 cells mL^{-1}) over the course of the incubation.

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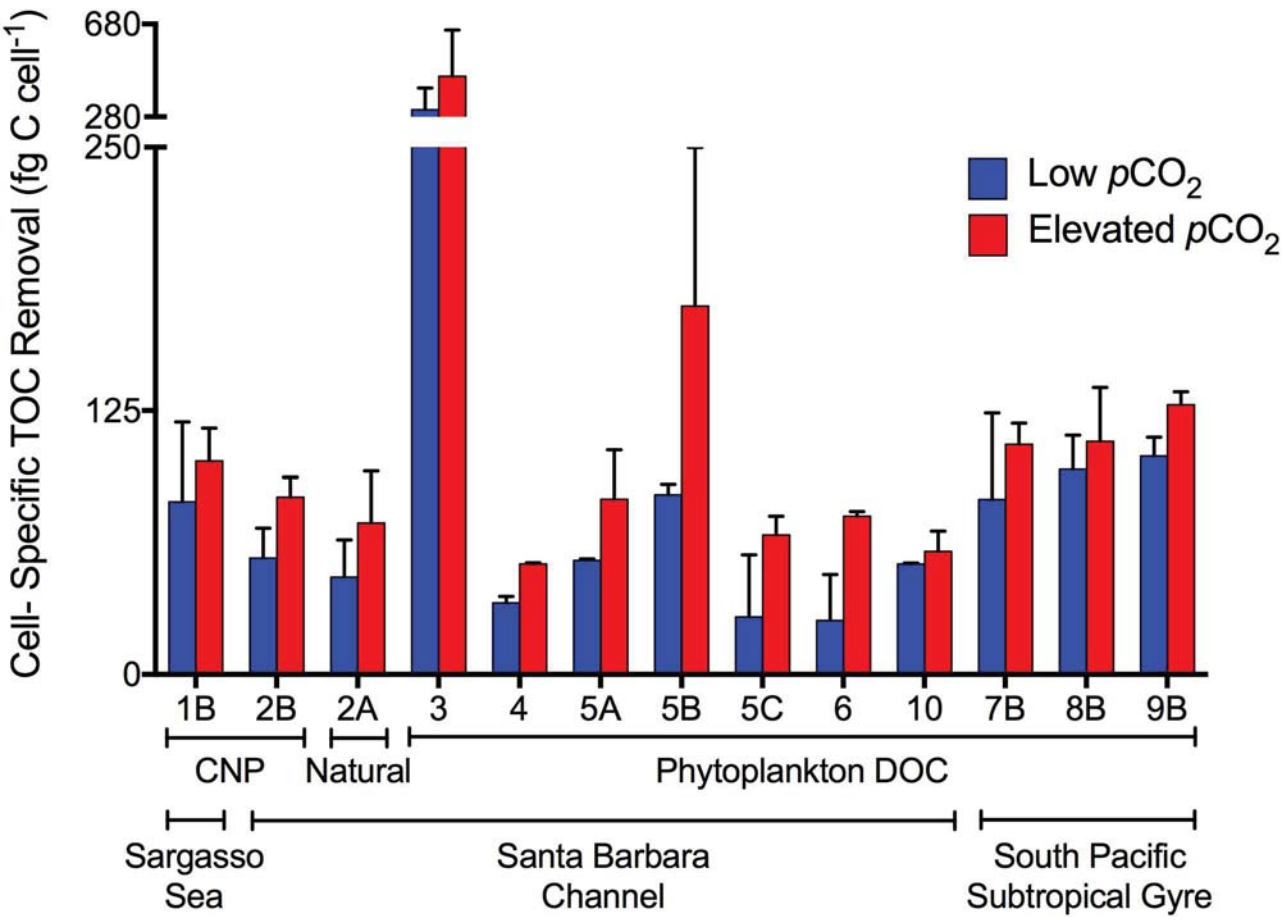

Fig 4. Cell-specific TOC removal. Mean (± SD) cell-specific TOC removal averaged across duplicate incubations and calculated as the change in TOC divided by the concurrent change in bacterioplankton abundance (BA) from the start of the incubations to stationary phase. Numbers on X-axis label refer to the experiment number (Table 1). Colors denote cell-specific TOC removal at elevated pCO_2 (red) and low (ambient or pre-industrial) pCO_2 (blue). Only experiments where a change in TOC was resolvable are shown.

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communities removed an amount of TOC equal to those carbon amendments in both elevated and ambient pCO_2 treatments implying that throughout the incubation little, if any, of the naturally occurring DOC was consumed, e.g. no priming effect was observed. However, TOC removal rates during exponential growth were greater under elevated pCO_2 compared to ambient pCO_2 (-4.3 ± 0.5 μmol C L^{-1} d^{-1} vs. -3.1 ± 1.2 μmol C L^{-1} d^{-1}, respectively; Fig 1D). Greater bacterioplankton abundance at elevated pCO_2 (1.4 ± 0.02 x10^6 cells mL^{-1}) relative to ambient pCO_2 (1.2 ± 0.04 x10^6 cells mL^{-1}) was proportionately smaller than enhanced TOC removal during the same period, resulting in greater cell-specific consumption of TOC at elevated pCO_2 (101.1 ± 15.6 fg C $cell^{-1}$) relative to ambient pCO_2 (81.9 ± 37.7 fg C $cell^{-1}$; Fig 4).

The addition of glucose in the CNP- Santa Barbara Channel experiment raised TOC concentration by 7.5 and 10.5 μmol C L^{-1} over the unamended treatment for the pre-industrial (250 ppm) and elevated (1000 ppm) pCO_2 treatments, respectively. Bacterioplankton abundance increased by over an order of magnitude (Fig 1E) compared to the unamended treatment (Fig 1C). An amount of TOC greater than the amendments was removed in both pCO_2 treatments over the course of the experiment (compare initial TOC in Fig 1C with the final TOC in Fig 1E); however, both total TOC drawdown and the rate of TOC removal during

exponential phase were greater under elevated pCO_2 (-8.0 ± 0.6 μmol C L^{-1} d^{-1}) relative to the pre-industrial pCO_2 treatment (-4.3 ± 1.1 μmol C L^{-1} d^{-1}). The bacterioplankton abundance in stationary phase was also greater at elevated pCO_2 (1.9 ± 0.1 x10^6 cells mL^{-1}) than at pre-industrial pCO_2 (1.7 ± 0.06 x10^6 cells mL^{-1}); however, the enhanced TOC removal relative to the increase in bacterioplankton, again resulted in greater cell-specific TOC consumption at elevated pCO_2 (84.1 ± 9.3 fg C $cell^{-1}$) relative to ambient pCO_2 (55.2 ± 14.2 fg C $cell^{-1}$; Fig 4).

Final TOC concentration in the Santa Barbara Channel experiment was drawn down to the same final concentration with and without CNP in elevated pCO_2 incubations (compare Fig 1C and 1E) indicating that CNP addition did not enhance the consumption of naturally occurring DOC under elevated pCO_2. In contrast, final TOC concentration in the pre-industrial pCO_2 CNP incubations was ~3 μmol C L^{-1} lower than in the unamended incubations (compare Fig 1C and 1E), indicating that the addition of CNP allowed for greater consumption of the naturally occurring DOC (a priming effect) under pre-industrial pCO_2.

Experiments with phytoplankton-derived DOC

TOC removal rates and the magnitude of TOC removal through stationary growth phase were enhanced in elevated pCO_2 treatments for all experiments with phytoplankton-derived DOC (Figs 2 and 3), indicating greater consumption of the exudates and lysates at elevated pCO_2 compared to pre-industrial levels. While trends in TOC removal were consistent across experiments and DOC sources, the bacterioplankton abundance yield was variable with some treatments having elevated yield in high pCO_2 treatments (Fig 2E and 2F; Fig 3B), and others showing no difference (Fig 1A, 1B, 1C and 1D; Fig 3A and 3C) between pCO_2 treatments. Despite variability in bacterioplankton abundance yield, elevated pCO_2 resulted in TOC removal by bacterioplankton that was always proportionately greater than the corresponding increases in abundance, leading to greater cell-specific removal at elevated pCO_2 (Fig 4).

Carbon content of bacterioplankton cells

Trends in TOC removal and bacterioplankton abundance in the Santa Barbara Channel experiment # 10 were consistent with all previous Santa Barbara Channel experiments: Greater TOC removal through stationary growth phase was observed in elevated pCO_2 incubations (-18.0 ± 0.6 μmol C L^{-1}) relative to pre-industrial pCO_2 incubations (-15.6 ± 0.1 μmol C L^{-1}), while bacterioplankton abundance yield was similar across pCO_2 treatments (4.0 ± 0.5 x10^6 cells mL^{-1}; elevated pCO_2 vs. 3.8 ± 0.1 x10^6 cells mL^{-1}; pre-industrial pCO_2). No resolvable difference in the carbon content of bacterioplankton cells was observed between cells as a function of pCO_2 (31.4 ± 1.2 fg C $cell^{-1}$; pre-industrial pCO_2 vs. 31.8 ± 1.8 fg C $cell^{-1}$; elevated pCO_2; p-value $>$ 0.66, t-test).

Bacterioplankton carbon removal and growth dynamics

Bacterioplankton carbon removal and growth dynamics were evaluated for experiments in which TOC removal was measureable. Effects of pCO_2 on carbon removal and bacterioplankton growth dynamics within experiments were tested for significance by the Alexander-Govern test. This first approximation, unequal variance test was used to account for small sample sizes (n = 2 for each pCO_2 level) and, therefore, an inability to test for normality and homoscedasticity with substantial power (p-values are reported in Table 2). Despite the fact that few within experiment comparisons resulted in significant effects of pCO_2, patterns in carbon removal and bacterioplankton growth dynamics were consistent across all experiments and locations, and are highly significant: cell-specific TOC removal (Fig 4) and bacterioplankton carbon demand (Fig 5) were consistently enhanced at elevated pCO_2 (p-value = 0.0003,

Table 2. P values for bacterioplankton carbon removal and growth dynamics.

Experiment #	Cell-specific TOC removal p-value	BCD p-value	BGE p-value
1B	0.62	0.39	0.57
2A	0.40	0.17	0.40
2B	0.18	0.07	0.25
3	0.52	0.80	0.51
4	0.05	0.33	0.08
5A	0.33	0.03*	0.27
5B	0.34	0.41	0.23
5C	0.30	0.03*	NA
6	0.19	0.12	0.32
7B	0.54	0.41	0.52
8B	0.64	0.02*	0.04*
9B	0.12	0.45	0.15
10	0.54	0.05	0.54

Alexander-Govern approximate p-values, calculated for cell-specific TOC removal, bacterioplankton carbon demand (BCD) and bacterioplankton growth efficiency (BGE). Asterisks denote p-values < 0.05 and 'ND' refers to not determined, due to lack of replication.

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Fisher's sign test; p-value < 0.0001, t-test, respectively), while bacterioplankton growth efficiency (Fig 6) was significantly reduced at elevated pCO_2 (p-value = 0.0003, Fisher's sign test). Significance of these consistent patterns was tested using the consensus t-test or the Fisher's sign-test, when tests for normality and homoscedasticity failed. Thus, while our measurement precision was inadequate to demonstrate statistically significant differences at any given location, the ecological and biogeochemical significance across all sites is statistically clear in that the consistency of the response shows that an enhancement of cell-specific TOC removal and carbon demand, and a reduction in bacterioplankton growth efficiency, under elevated pCO_2 is a common feature across the wide range of ocean habitats examined.

Discussion

The results indicate that elevated pCO_2 can directly influence bacterioplankton removal of organic carbon. Elevated pCO_2 led to greater TOC removal by bacterioplankton communities growing on a range of DOC from glucose to more complex DOC, consisting of phytoplankton products or naturally occurring DOC. This result was observed across all ocean regions for experiments where DOC was amended. Similar results were observed in the unamended Santa Barbara Channel incubations at elevated pCO_2 but it was not possible to discern whether pCO_2 affected consumption of naturally occurring DOC in the South Pacific Subtropical Gyre and Sargasso Sea, as changes in TOC were below analytical detection limits in these more oligotrophic environments.

Our results suggest that short-term increases in pCO_2 will lead to enhanced removal of bioavailable surface organic carbon by heterotrophic bacterioplankton communities. Through observations of enhanced extracellular glucosidase activity, previous mesocosm and culture studies suggested that elevated pCO_2 conditions might lead to greater removal of organic carbon [9, 11–13]. Here we show a clear, direct effect of elevated pCO_2 on bacterioplankton-mediated organic carbon removal. Both our work and that of the mesocosm and culture studies [9, 11–13] suggest that short-term exposure to elevated pCO_2 will enhance the bacterioplankton removal of bioavailable DOC in the surface ocean.

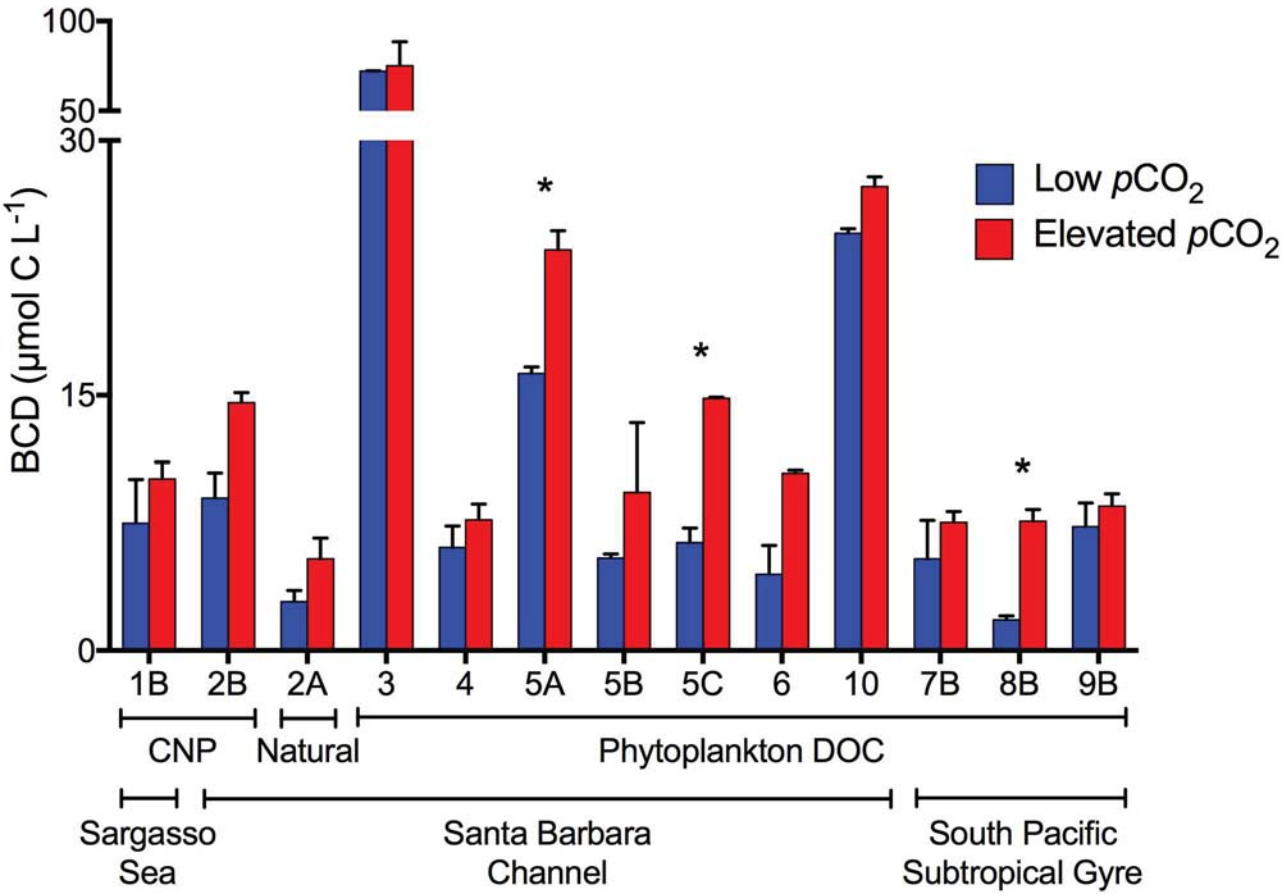

Fig 5. Bacterioplankton Carbon Demand. Mean (± SD) bacterioplankton carbon demand (BCD) averaged across duplicate incubations and calculated as the change in DOC from T_0 to stationary phase. Change in DOC was calculated as TOC less the carbon content of bacterioplankton biomass (using a carbon conversion factor of 30 fg C $cell^{-1}$ for bacterioplankton in the Santa Barbara Channel and 10 fg C $cell^{-1}$ for bacterioplankton in the Sargasso Sea and South Pacific Subtropical Gyre). Colors denote BCD at elevated pCO_2 (red) and low (ambient or pre-industrial) pCO_2 (blue). Numbers refer to the experiment number (Table 1). Only experiments where a change in TOC was resolvable are shown.

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Experimental design considerations

In order to investigate the direct effect of pCO_2 on organic carbon consumption by natural bacterioplankton communities, we employed the following in our experimental design: (1) Experiments were conducted in the dark to eliminate photoautotrophic organic carbon production; (2) To ensure that the majority of organic carbon removal could be attributed to heterotrophic bacteria, we used seawater culture dilution methods [43] to dilute and minimize the effects of protistan grazer populations [44, 45, 46]; (3) To further minimize the potential effects of grazers on organic carbon consumption, calculations were made from the start of the incubations to stationary growth phase, ensuring low grazer densities and high bacterial densities during the period over which bacterioplankton physiological parameters were estimated. However, we cannot rule out the possibility that groups other than heterotrophic bacteria (e.g. archaea and marine protists) contributed to organic carbon removal in these experiments because we were unable to resolve changes in their abundance. The consumption of specific organic compounds (i.e. amino acids) by other members of marine plankton has been

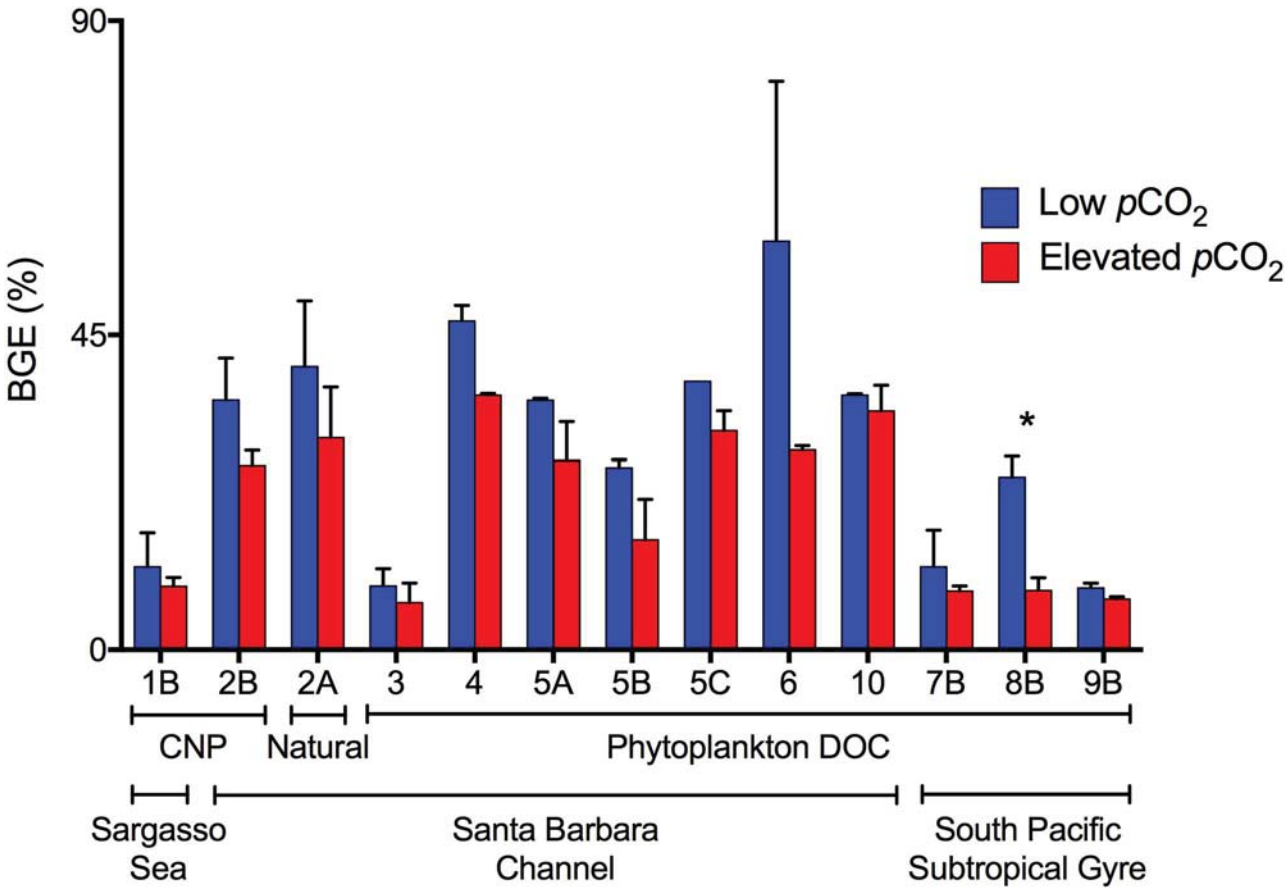

Fig 6. Bacterioplankton growth efficiency. Mean (± SD) Bacterioplankton growth efficiency averaged across duplicate incubations and calculated as the change in bacterioplankton biomass carbon (using a carbon conversion factor of 30 fg C $cell^{-1}$ for bacterioplankton in the Santa Barbara Channel and 10 fg C $cell^{-1}$ for bacterioplankton in the Sargasso Sea and South Pacific Subtropical Gyre) divided by the concurrent change in DOC from T_0 to stationary phase. Colors denote bacterioplankton growth efficiency at elevated pCO_2 (red) and low (ambient or pre-industrial) pCO_2 (blue). Numbers refer to the experiment number (Table 1). Only experiments where a change in TOC was resolvable are shown.

doi:10.1371/journal.pone.0173145.g006

observed [47–49] but their effect on removal of bulk dissolved organic material remains largely unquantified [50].

To minimize the potential for abiotic removal of organic carbon (e.g. via adhesion to the incubation-bottle walls or accumulation at the air-water interface) our seawater cultures were only bubbled before the start of the experiments (before T_0). pCO_2 levels were maintained over the course of the experiments through the use of gas-tight sampling ports (see methods) and by replacing the headspace with target pCO_2 –gas, daily. We cannot rule out the possibility that abiotic removal of organic carbon occurred during bubbling; however, continued abiotic removal over the course of the experiment did not occur as is evidenced by the fact that no resolvable removal of TOC was detected in experiments in which bacterioplankton growth was minimal (Fig 1A and 1B, Table 1: experiments #1A, and 7A, 8A, and 9A).

We attribute organic carbon removal (ΔTOC) to consumption by heterotrophic bacterioplankton in our experiments. The filtration and dilution steps used in our experimental set-up were designed to minimize or eliminate the contribution of non-living particulate organic carbon in experimental incubations. That means that a measurement of TOC represents the sum of DOC and the carbon content of bacterioplankton biomass. It also means that the change in

TOC (ΔTOC) is a measure of the amount of carbon lost to bacterioplankton respiration by the argument described in the materials and methods section (see *bacterioplankton respiration* calculation). This tight coupling between the direct measurement of ΔTOC and the production of CO_2 (i.e. bacterioplankton respiration) was shown empirically in similar seawater culture experiments, supporting the use of ΔTOC as a proxy for heterotrophic bacterioplankton respiration in this type of experiment [45].

Bacterioplankton removal of organic carbon

Highly productive systems like the coastal upwelling regions of the Santa Barbara Channel can create food-webs that allow for greater DOC production than heterotrophic bacterioplankton consumption, resulting in the accumulation of bioavailable DOC [51, 52, 40]. In the present study we show enhanced removal of naturally occurring organic carbon by Santa Barbara Channel heterotrophic bacterioplankton communities under increased pCO_2, suggesting that rapid increases in pCO_2, common in upwelling systems, can accelerate the consumption of DOC in the surface ocean.

In contrast, removal of TOC in the naturally occurring DOC treatments conducted in the Sargasso Sea and the South Pacific Subtropical Gyre was below limits of detection, indicating that only a very small fraction of the naturally occurring DOC was available to the extant bacterioplankton on the time-scale of these experiments. This result is consistent with previous observations indicating that because DOC production and consumption processes in the oligotrophic gyres are so tightly coupled little, if any, of the DOC that accumulates in these regions is bioavailable to surface bacterioplankton communities on the time scale of hours to days [44, 53].

To assess the effect of pCO_2 on the removal of recently produced DOC in systems where DOC use is difficult to resolve, we simulated food-web production of complex DOC by adding phytoplankton-derived DOC to seawater culture experiments. These amendment experiments were conducted in both coastal (Santa Barbara Channel) and open ocean systems (South Pacific Subtropical Gyre) and show a consistent pattern of an increased magnitude of TOC removal with elevated pCO_2. While these results show clear short-term trends regarding the removal of phytoplankton-derived DOC, longer-term experiments (weeks to months) must be conducted to properly evaluate whether exposure to elevated pCO_2 sustains an offset in the magnitude of TOC removal compared to ambient and pre-industrial pCO_2 conditions.

Collectively, our experiments indicate that short-term increases in pCO_2 directly influence bacterioplankton removal of organic carbon. Elevated pCO_2 led to greater TOC removal by natural bacterioplankton communities growing on a range of organic carbon compounds from glucose, to more complex phytoplankton products, to naturally occurring DOC. Assuming that heterotrophic bacterioplankton are driving the removal of TOC, then these results suggest that short-term exposure to elevated pCO_2 leads to enhanced bacterioplankton respiration relative to pre-industrial pCO_2. Enhanced respiration under elevated pCO_2 coupled with minimal change in bacterioplankton abundance yielded systematically greater cell-specific respiration (i.e. cell-specific TOC removal) in elevated pCO_2 treatments (Fig 4). Even in cases where bacterioplankton yield was greater under elevated pCO_2, that effect was outweighed by enhanced respiration leading to greater amounts of carbon respired per cell. This result suggests that during periods of acidified conditions, more bioavailable organic carbon in surface waters will be converted to CO_2, decreasing the amount of organic carbon available for potential export and increasing the likelihood that carbon will be lost from the surface ocean to the atmosphere through outgassing of CO_2.

Bacterioplankton growth dynamics

Organic carbon removal was tracked by measuring TOC; as a result, these seawater experiments do not directly address the effect of pCO_2 on BC production or DOC consumption. However, these components were evaluated using the direct estimates of the carbon content of bacterioplankton cells from the December 2015 Santa Barbara Channel experiment. Knowledge of the carbon content of bacterioplankton cells enables the calculation of BC to be easily extrapolated from cell abundance data and also enables DOC to be calculated as the difference between TOC and BC. Estimates of BC and DOC removal can then be used to inform aspects of bacterioplankton physiology by providing estimates of bacterioplankton carbon demand (i.e. DOC consumption) and the fraction of consumed DOC that is converted to BC (i.e. bacterioplankton growth efficiency). Thus, understanding how pCO_2 affects the carbon content of bacterioplankton cells enabled us to extend our results beyond carbon loss via respiration, to the partitioning of surface organic carbon between dissolved (that which escapes or resists bacterioplankton consumption) and particulate (as BC) organic carbon.

We observed no direct effect of pCO_2 on the carbon content of bacterioplankton cells. Assuming this equivalency across all experiments, we calculated bacterioplankton carbon demand for all of the experiments where a change in TOC was resolvable. Bacterioplankton carbon demand was estimated as the change in DOC from the start of the incubations to stationary growth phase and DOC was estimated as TOC—BC. These calculations revealed a consistent pattern across DOC amendments and experimental location: bacterioplankton communities grown at elevated pCO_2 exhibited enhanced carbon demand relative to communities at pre-industrial and ambient pCO_2 (Fig 5). These results suggest that bacterioplankton communities exposed to short-term increases in pCO_2 reduce the amount of DOC available for vertical export through enhanced consumption of recently produced DOC (Fig 7).

Estimates of bacterioplankton growth efficiency revealed a consistent pattern across DOC amendment and experimental location: growth efficiencies were consistently lower at elevated pCO_2 than at pre-industrial or ambient pCO_2 (Fig 6). This implies that a greater portion of consumed DOC is converted to CO_2 by bacterioplankton communities exposed to elevated pCO_2 (Fig 7). It is important to note that variable initial bacterioplankton communities likely resulted in variable BC and that our estimate of cellular carbon conversion factor may not accurately represent communities across experiments; however, only the absolute magnitude of the growth efficiency values will be affected if, as our results suggest, BC is similar across pCO_2 levels. Enhanced bacterioplankton respiration at elevated pCO_2 will consistently result in lower growth efficiencies, regardless of the exact carbon conversion factor used.

Our estimates of bacterioplankton carbon demand and growth efficiency demonstrate a direct effect of pCO_2 on bacterioplankton processing of organic carbon in the surface ocean. While elevated pCO_2 had little impact on BC production, enhanced DOC removal through accelerated bacterioplankton consumption and reduced growth efficiencies reduced the amount of DOC available for export, relative to pre-industrial pCO_2 conditions (Fig 7). Enhanced removal of DOC could also lead to increased production of recalcitrant organic compounds via the microbial carbon pump [54]. Further experiments and measurements of long-term DOC removal and DOC characterization are required to evaluate this possibility.

Possible mechanisms

Consumption of organic carbon is often a function of bacterioplankton community structure and a number of studies have demonstrated that slight variations in DOC may select for specific bacterioplankton populations over timescales of hours to days [28, 40, 53, 55]. It is likely that bacterioplankton community composition shifted over time in our experiments and it is

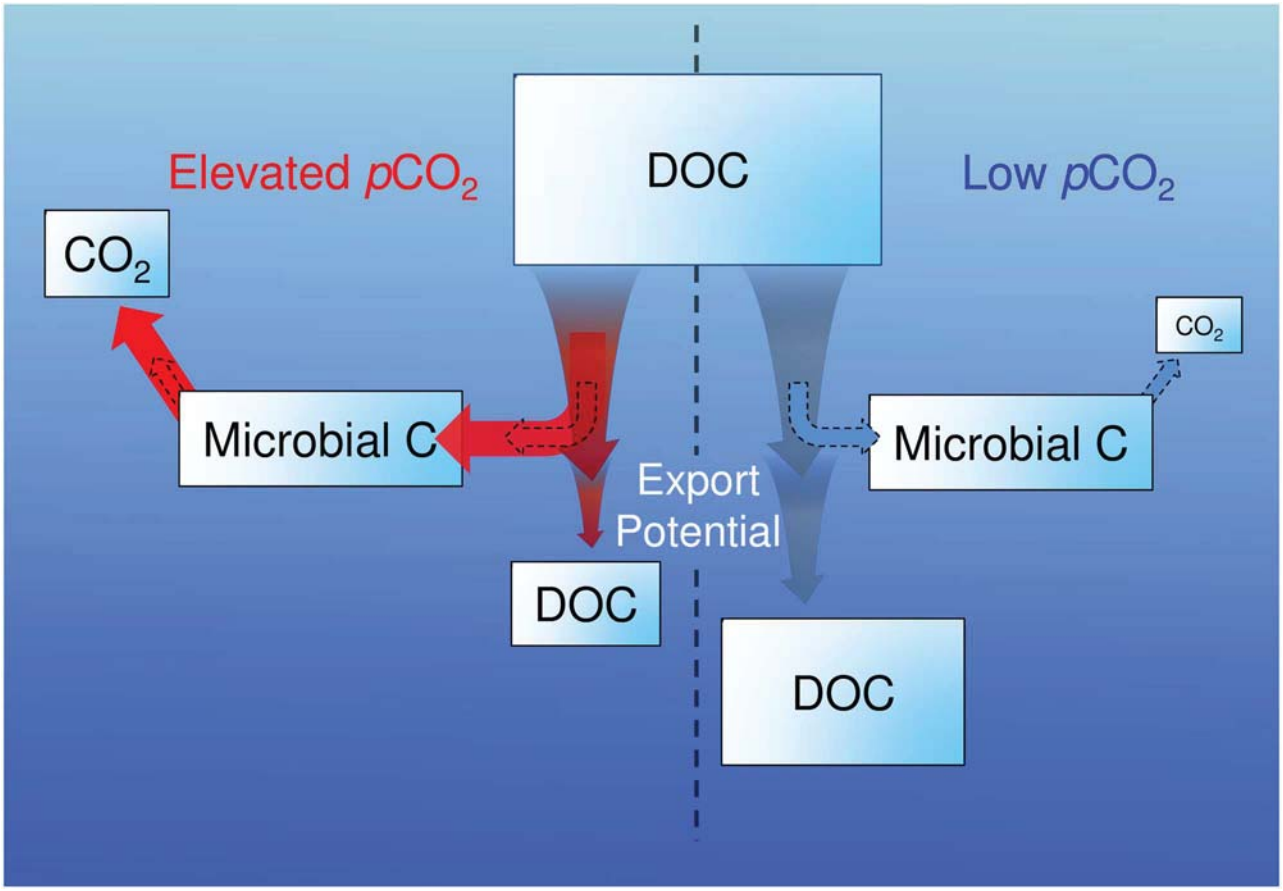

Fig 7. Effects of pCO_2 on bacterioplankton removal of organic carbon. A depiction of the short-term effects of pCO_2 on the use of DOC by bacterioplankton communities in ocean surface waters. Elevated pCO_2 may increase the use of DOC at lowered bacterioplankton growth efficiencies by natural bacterioplankton communities, increasing the respiration of DOC and decreasing the magnitude of accumulated DOC in these regions, ultimately decreasing the amount of DOC available for vertical export (i.e. export potential). Arrows represent the flux of carbon between identified pools. Red represents processes occurring under elevated (> ambient) pCO_2 conditions while blue represents processes occurring under low (ambient or pre-industrial) pCO_2 conditions. Dotted black arrows denote the flux of carbon between identified pools at low pCO_2 to enable an easy comparison between elevated and low pCO_2 fluxes.

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also possible that a shift in community composition between pCO_2 treatments contributed to the consistent differences in TOC removal. A recent study by Siu et al. [17] indicated that elevated pCO_2 (~1050 ppm) could induce shifts in bacterioplankton composition. However, pCO_2 manipulations in mesocosm experiments in Bergen [56, 57] and Svalbard, Norway [58–60] showed that the free-living bacterioplankton community structure was mostly unaffected by elevated pCO_2, which ranged from ~750–1085 ppm, depending on the experiment. In the present study the trend of enhanced bacterioplankton respiration in the presence of elevated pCO_2 was observed across DOC experiments for which water was collected at various times of the year and presumably contained different initial bacterioplankton communities. While it is possible that the bacterioplankton community structure shifted under elevated pCO_2 conditions, the response of enhanced respiration at elevated pCO_2 appeared universal despite likely differences in initial microbial composition sampled across sites and time.

Alternatively, enhanced bacterioplankton respiration combined with low bacterioplankton growth efficiency in elevated pCO_2 treatments may be a physiological response to decreased pH. A recent study examined the expression of bacterioplankton transcripts in response to

elevated pCO_2 and showed that transcripts associated with respiration were significantly enhanced in elevated pCO_2 mesocosms [61]. The authors specifically showed upregulation of respiratory proton pumps that aid in translocating protons across the cell membrane. These results suggest that as environmental pH decreases, heterotrophic bacterioplankton upregulate respiratory proton pumps to export protons that invade the cell as a result of low external pH. Consistent with our observations, Bunse et al. [61] suggested that upregulating proton pumps under low pH would be energetically costly to heterotrophic bacterioplankton and thus reduce their overall efficiency.

There is also evidence to suggest that increasing pCO_2 may lead to increased removal of organic matter in the ocean through up-regulation and/or enhanced efficiencies of extracellular enzymes [9–12]. Extracellular enzymes convert high molecular weight organic compounds to low molecular weight compounds that can be used by heterotrophic bacterioplankton [62–64]. These enzymes are not buffered by the cell's cytoplasm and are directly impacted by external changes in pH. An increase in the concentration of hydrogen ions in an enzyme's environment, as a result of declining pH, may alter the three-dimensional protein structure of the enzyme's active site and thus affect enzymatic activity [65, 66]. Recent studies report that extracellular α and β-glucosidase activity increased in response to elevated hydrogen ion concentration, suggesting that a decline in pH of 0.2–0.3 pH units was closer to the pH optimum of glucosidase activity than ambient pH [11, 12]. It may be that up-regulation of enzymes like α and β-glucosidase by marine heterotrophic bacterioplankton in conjunction with altered enzymatic active sites led to the increased removal of DOC measured within elevated pCO_2 treatments. Further experimentation is required to explore these mechanisms.

Thus, while we do not exclude the possibility of a shift in the bacterioplankton community under elevated pCO_2 conditions, we suggest that the increased respiration at elevated pCO_2 is likely due to up-regulation of proton pumps and possibly the enhancement of extracellular enzyme activity (via up-regulation and/or enhanced efficiencies).

Conclusions

This study reveals a direct impact of pCO_2 on bacterioplankton removal of organic carbon. In all experiments for which TOC removal was measurable, enhanced bacterioplankton respiration under elevated pCO_2 coupled with minimal change in bacterioplankton biomass yield resulted in systematically greater cell-specific respiration in elevated pCO_2 treatments (i.e. cell-specific TOC removal; Fig 4). Even in cases where bacterioplankton abundance yield was greater under elevated pCO_2, enhanced respiration led to greater amounts of carbon respired per cell, increasing the likelihood that surface organic carbon will be lost to the atmosphere through outgassing of CO_2. Estimates of bacterioplankton carbon demand and growth efficiency suggest that acidified conditions may also increase the ability of bacterioplankton to consume DOC but with reduced growth efficiencies. The cumulative effect of enhanced consumption and enhanced respiration under elevated pCO_2 would increase the fraction of surface-ocean DOC respired to CO_2, ultimately decreasing the effectiveness of DOC as a sink of carbon in the ocean (Fig 7). Incorporation of these results into numerical models will enable more accurate understanding of current air-sea carbon exchange and the impact of elevated pCO_2 in surface waters on biogeochemical cycles.

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References

1. Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, and Thingstad F. The ecological role of water-column microbes in the sea. Mar. Ecol. Prog. Ser. 1983; 10: 257–263.
2. Zeebe RE and Wolf-Gladgrow D. CO2 in seawater: Equilibrium, kinetics, isotopes. Elsevier Oceanography Series, 2001; 65, pp. 346. Amsterdam, Book.
3. Carlson CA, Ducklow HW, and Michaels AF. Annual flux of dissolved organic carbon from the euphotic zone in the northwestern Sargasso Sea. Nature 1994; 371: 405–408.
4. Hansell DA, Carlson CA, Repeta DJ, and Schlitzer R. Dissolved organic matter in the ocean: a controversy stimulates new insights. Oceanogr. 2009; 22: 202–211.
5. Passow U and Carlson CA. The biological pump in a high CO_2 world. Mar. Ecol. Prog. Ser. 2012;
6. Joint I, Doney SC, and Karl DM. Will ocean acidification affect marine microbes? ISME J 2009; 5: 1–7.
7. Riebessell U, Gattusho J-P, Thingstad TF, and Middelburg JJ. Arctic ocean acidification: pelagic ecosystem and biogeochemical responses during a mescocosm study. Biogeosciences 2013;
8. Motegi C, Tanaka T, Piontek J, Brussard CPD, Gatusso JP, and Weinbauer MG. Effect of CO_2 enrichment on bacterial metabolism in an Arctic fjord. Biogeosciences 2013; 10: 3285–3296.
9. Grossart H-P, Allgaier M, Passow U, and Riebesell U. Testing the effect of CO_2 concentration on the dynamics of marine heterotrophic bacterioplankton. Limnol. Oceanogr. 2006; 51(1): 1–11.
10. Endres S, Galgani L, Riebesell U, Schulz K-G, and Engel A. Stimulated bacterial growth under elevated pCO_2: Results from an off-shore mesocosm study. PLoS ONE 2014; 9(6): e99228. doi: 10.1371/journal.pone.0099228 PMID: 24941307
11. Piontek J, Lunau M, Handel N, Borchard C, Wurst M, Engel A. Acidification increases microbial polysaccharide degradation in the ocean. Biogeosciences 2010; 7: 1615–1624.
12. Piontek J, Borchard C, Sperling M, Schulz KG, Riebesell U, and Engel A. Response of bacterioplankton activity in an Arctic fjord system to elevated pCO_2: results from a mesocosm perturbation study. Biogeosciences, 2013; 10: 297–314.
13. Mass EW, Law CS, Hall JA, Pickmere S, Currie KI, Chang FH, Voyles KM, and Caird D. Effect of ocean acidification on bacterial abundance, activity, and diversity in the Ross Sea, Antarctica. Aquat. Microb. Ecol. 2013;

14. Engel A, Delille B, Jacquet S, Riebesell U, Rochelle-Newall E, Terbrüggen A, and Zondervan I. Transparent exopolymer particles and dissolved organic carbon production by *Emiliana huxleyi* exposed to different CO_2 concentrations: a mesocosm experiment. Aquat. Microb. Ecol. 2004;
15. Taucher J, Jones J, James A, Brzezinski MA, Carlson CA, Riebesell U, and Passow U. Combined effects of CO_2 and temperature on carbon uptake and partitioning by the marine diatoms *Thalassiosira weissflogii* and *Dactyliosolen fragilissimus*. Limnol. Oceanogr. 2015; Yamada N and Suzumura M. Effects of seawater acidification on hydrolytic enzyme activities. J. of Oceanogr. 2010;66: 233–241.
16. Yamada N and Suzumura M. Effects of seawater acification on hydrolytic enzyme activities. J of Oceanogr. 2010; 66: 233–241.
17. Siu N, Apple JK, and Moyer CL. The effects of ocean acidity and elevated temperature on bacterioplankton community structure and metabolism. Open J. of Ecol. 2014; 4: 434–455.
18. Bates N, Best M, Neely K, Garley R, Dickson A, and Johnson R. Detecting anthropogenic carbon dioxide uptake and ocean acidification in the North Atlantic Ocean. Biogeosciences Disc. 2012; 9: 989–1019.
19. Lomas M, Bates N, Johnson R, Knap A, Steinberg D, and Carlson C. Two decades and counting: 24-years of sustained open ocean biogeochemical measurements in the Sargasso Sea. Deep Sea Res. Part 2. 2013; 93: 16–32.
20. Feely RA, Sabine CL, Hernandez-Ayon M, Ianson D, Hales B. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science 2008; 320: 1490–1492. doi: 10.1126/science.1155676 PMID: 18497259
21. Hofmann GE, Smith JE, Johnson KS, et al. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. PLoS ONE 2011; 6: e28983–e28983. doi: 10.1371/journal.pone.0028983 PMID: 22205986
22. Carlson CA, Giovannoni SJ, Hansell DA, Goldberg SJ, Parsons R, Otero MP, Vergin K, and Wheeler BR. The effect of nutrient amendments on bacterioplankton growth, DOC utilization, and community structure in the Northwestern Sargasso Sea. Aquat. Microb. Ecol. 2002; 30: 19–36.
23. Gattuso J-P, Gao K, Lee K, Rost B, and Schulz KG. Approaches and tools to manipulate the carbonate chemistry. Guide to best practices for ocean acidification research and data reporting, eds. Riebesell U, Fabry VJ, Hansson L, and Gattuso JP (Luxembourg: Publications Office of the European Union). 2010; pp 41–52.
24. Rost B, Zondervan I, and Wolf-Gladrow D. Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. Mar. Ecol. Prog. Ser. 2008; 373: 227–237.
25. Legendre L, Demers S, Delesalle B, and Harnois C. Biomass and photosynthetic activity of phototrophic picoplankton in coral reef waters (Moorea Island, French Polynesia). Mar. Ecol. Prog. Ser. 1988; 47: 153–160.
26. Guillard RRL and Ryther JH. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt and Detonula confervacea Cleve. Can. J. Microbiol. 1962; 8: 229–239. PMID: 13902807
27. Johnson KS and Coletti LJ. In site ultraviolet spectrophotometry for high resolution and long-term monitoring of nitrate, bromide and bisulfide in the ocean. Deep Sea Res. Part 1 2002; 49: 1291–1305.
28. Nelson C and Carlson C. Tracking differential incorporation of dissolved organic carbon types among diverse lineages of Sargasso Sea bacterioplankton. Environ. Microbiol. 2012; 14: 1500–1516. doi: 10.1111/j.1462-2920.2012.02738.x PMID: 22507662
29. Carlson CA, Hansell D, Nelson N, Siegel D, Smethie W, Khatiwala S, Meyers MM, and Halewood E. Dissolved organic carbon export and subsequent remineralization in the mesopelagic and bathypelagic realms of the North Atlantic basin. Deep Sea Res. Part 2 2010; 57(16): 1433–1445.
30. Porter KG and Feig YS. The use of DAPI for identifying and counting aquatic microflora. Limnol. Oceanogr. 1980; 25: 943–948.
31. Nelson CE, Alldredge AL, McCliment EA, Amaral-Zettler LA, Carlson CA. Depleted dissolved organic carbon and distinct bacterial communities in the water column of a rapid-flushing coral reef ecosystem. ISME J. 2011; 5: 1374–1387. doi: 10.1038/ismej.2011.12 PMID: 21390080
32. Parsons RJ, Nelson CE, Carlson CA, et al. Marine bacterioplankton community turnover within seasonally hypoxic waters of a subtropical sound: Devils Hole, Bermuda. Environ. Microbiol., 2014;
33. Glockner FO, Fuchs BM, & Amann R. Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. Appl. Environ. Microbiol. 1999; 65: 3721–3726. PMID: 10427073
34. Dickson AG, Sabine CL, Christian JR. Guide to best practices for ocean CO2 measurements, PICES Special Publication, 2007; 3.

35. Hales B, Chipman D, Takahashi T. High-frequency measurement of partial pressure and total concentration of carbon dioxide in seawater using microporous hydrophobic membrane contactors. Limnol. Oceanogr. Methods 2004; 2: 356–364.
36. Robbins LL, Hansen ME, Kleypas JA, Meylan SC. CO2calc—A user-friendly seawater carbon calculator for Windows, Max OS X, and iOS (iPhone): U.S. Geological Survey Open-File Report 2010; 2010–1280, 17 p. 13.
37. Dickson AG and Millero FJ. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media, Deep-Sea Res. Pt. A. 1987; 34(10): 1733–1743.
38. del Giorgio PA and Cole JJ. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 1998; 29: 503–541.
39. del Giorgio PA and Cole JJ. Bacterial growth energetics and growth efficiency, in Microbial Ecology of the Oceans, ed. Kirchman DL, pp. 289–325, John Wiley, NY. 2000.
40. Wear EK, Carlson CA, James AK, Brzezinski MA, Windecker LA, and Nelson CE. Synchronous shifts in dissolved organic carbon bioavailability and bacterial community responses over the course of an upwelling phytoplankton bloom. Limnol. Oceanogr. 2015; 00: 1–32.
41. Fukuda R, Ogawa H, Nagata T, Koike I. Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. Appl. Environ. Microbiol. 1998; 64: 3352–3358. PMID: 9726882
42. Gundersen K, Heldal M, Purdie DA, Knap AH. Elemental C, N, and P cell content of individual bacteria collected at the Bermuda Atlantic Time-series Study (BATS) site. Limnol. Oceanogr. 2002; 47: 1525–1530.
43. Ammerman JW, Fuhrman JA, Hagstrom A, and Azam F. Bacterioplankton growth in seawater: I. Growth kinetics and cellular characteristics in seawater cultures. Mar. Ecol. Prog. Ser. 1984; 18: 31–39.
44. Cherrier J, Bauer JE, Druffel ERM. Utilization and turnover of labile dissolved organic matter by bacterial heterotrophs in eastern North Pacific surface waters. Mar. Ecol. Prog. Ser. 1996; 139: 267–279.
45. Carlson CA, Bates NR, Ducklow HW, and Hansell DA. Estimation of bacterial respiration and growth efficiency in the Ross Sea, Antarctica. Aquat Microb Ecol 1999; 19: 229–244.
46. Letscher RT, Knapp AN, James AK, Carlson CA, Santoro AE, and Hansell DA. Microbial community composition and nitrogen availability influence DOC remineralization in the South Pacific Gyre. Mar. Chem. 2015;
47. Sherr E and Sherr B. Roles of microbes in pelagic food webs: a revised concept. Limnol. Oceanogr. 1988; 33(5): 1225–1257.
48. Tranvik L J, Sherr E, and Sherr BF. Uptake and utilization of'colloidal DOM by heterotrophic flagellates in seawater. Collections. 1993.
49. First M R and Hollibaugh JT. The model high molecular weight DOC compound, dextran, is ingested by the benthic ciliate *Uronema marinum* but does not supplement ciliate growth. Aquat. Microb. Ecol. 2009; 57(1): 79.
50. Hansell DA and Carlson CA (Eds.) Biogeochemistry of marine dissolved organic matter, Second Edition. Academic Press. 2015; 693 pp.
51. Sintes E, Witte H, Stodderegger K, Steiner P, and Herndl GJ. Temporal dynamics in the free-living bacterial community composition in the coastal North Sea. FEMS Microbiol. Ecol. 2010; 83: 413–424.
52. Halewood E, Carlson C, Brzezinski MA, Reed DC, Goodman J. Annual cycle of organic matter partitioning and its availability to bacteria across the Santa Barbara Channel continental shelf. Aquat. Microb. Ecol. 2012; 67: 189–209.
53. Carlson CA, Giovannoni SJ, Hansell DA, Goldberg SJ, Parsons R, and Vergin K. Interactions among dissolved organic carbon, microbial processes, and community structure in the mesopelagic zone of the northwestern Sargasso Sea. Limnol. Oceanogr. 2004; 49: 1073–1083.
54. Jiao N and Zheng Q. The microbial carbon pump: from genes to ecosystems. AEM. 2011;
55. Cotrell MT and Kirchman DL. Natural assemblages of marine proteobacteria and members of *Cytophaga-Flavobacteri* cluster consuming low- and high- molecular-weight dissolved organic matter. Aquatic. Microb. Ecol. 2000; 66: 1692–1697.
56. Newbold L, Oliver A, Booth T, Thwarl B, DeSantis R, Maguire M, Anderson G, van der Gast CJ, and Whiteley AS. The response of marine picoplankton to ocean acidification. Environ. Microbiol. 2012; 14: 2293–2307. doi: 10.1111/j.1462-2920.2012.02762.x PMID: 22591022
57. Oliver AE, Newbold LK, Whiteley AS, van der Gast CJ. Marine bacterial communities are resistant to elevated carbon dioxide levels. Environ. Microbiol. Rep. 2014; 6(6): 574–582. PMID: 25756110
58. Zhang R, Xia X, Lau SCK, Weinbauer MG, and Jiao N. Response of bacterioplankton community structure to an artificial gradient of pCO_2 in the Arctic Ocean. Biogeosciences 2013; 6: 3679–3689.

59. Sperling M, Piontek J, Gerdts G, et al. Effect of elevated CO_2 on the dynamics of particle-attached and free-living bacterioplankton communities in an Arctic fjord. Biogeosciences 2013; 10: 181–191.

60. Roy AS, Gibbons SM, Schunk H, et al. Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms. Biogeosciences 2013; 10: 555–566.

61. Bunse C, Lundin D, Karlsson CMG, Akram N, Vila-Costa M, Palovaara J, et al. Response of marine bacterioplankton pH homeostasis gene expression to elevated CO_2. Nature Clim. Change. 2016;

62. Hoppe HG. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. Mar. Ecol. Prog. Ser. 1983;

63. Chróst R. Environmental control for the synthesis and activity of aquatic microbial ectoenzymes. In Microbial enzymes in aquatic environments, edited by: Chróst R, Springer, Heidelberg 1991;29–59.

64. Arnosti C. Microbial extracellular enzymes and the marine carbon cycle. Annu. Rev. Mar. Sci. 2011;

65. King GM. Characterization of ß-glucosidase activity in intertidal marine sediments. Appl. Environ. Microbiol. 1986; 51: 373–380. PMID: 16346994

66. Münster U. Extracellular enzyme activity in eutrophic and polyhumic lakes. In Microbial enzymes in aquatic environments, edited by: Chróst R, Springer, Heidelberg 1991:96–122.