

# Interactive effects of elevated temperature and CO<sub>2</sub> on nitrate, urea, and dissolved inorganic carbon uptake by a coastal California, USA, microbial community

Jenna L. Spackeen<sup>1,\*</sup>, Rachel E. Sipler<sup>1</sup>, Kai Xu<sup>2</sup>, Avery O. Tatters<sup>2</sup>,  
Nathan G. Walworth<sup>2</sup>, Erin M. Bertrand<sup>3,4,5</sup>, Jeffrey B. McQuaid<sup>4,5</sup>,  
David A. Hutchins<sup>2</sup>, Andrew E. Allen<sup>4,5</sup>, Deborah A. Bronk<sup>1</sup>

<sup>1</sup>Virginia Institute of Marine Science, College of William & Mary, PO Box 1346, Gloucester Point, VA 23062, USA

<sup>2</sup>The University of Southern California, Department of Biological Sciences, 3616 Trousdale Parkway, Los Angeles, CA 90089, USA

<sup>3</sup>Department of Biology, Dalhousie University, Halifax, NS B3H 4R2, Canada

<sup>4</sup>Microbial and Environmental Genomics, J. Craig Venter Institute, La Jolla, CA 92037, USA

<sup>5</sup>Integrative Oceanography Division, Scripps Institution of Oceanography, UC San Diego, La Jolla, CA 92037, USA

**ABSTRACT:** Average global temperatures and carbon dioxide (CO<sub>2</sub>) levels are expected to increase in the coming decades. Implications for ocean ecosystems include shifts in microbial community structure and subsequent modifications to nutrient pathways. Studying how predicted future temperature and CO<sub>2</sub> conditions will impact the biogeochemistry of the ocean is important because of the ocean's role in regulating global climate. We determined how elevated temperature and CO<sub>2</sub> affect uptake rates of nitrate, urea, and dissolved inorganic carbon (DIC) by 2 size classes (0.7–5.0 and >5.0 μm) of a microbial assemblage collected from coastal California, USA. This microbial community was incubated for 10 d using an ecostat continuous culture system that supplied the microorganisms with either nitrate or urea as the dominant nitrogen source. Biomass parameters, nutrient concentrations, and uptake rates were measured throughout the experiment. In all treatments, urea uptake rates were greater than nitrate, and larger microorganisms had higher uptake rates than smaller microorganisms. Uptake rates of urea and DIC within both size fractions were higher at elevated temperature, and uptake rates of nitrate by smaller microorganisms increased with elevated CO<sub>2</sub>. These findings suggest that the rate at which nutrients cycle in temperate coastal waters will increase as temperature and CO<sub>2</sub> levels rise and that the effect will vary between nitrogen substrates and different microorganisms.

**KEY WORDS:** Nitrate · Urea · DIC · Uptake · Temperature · CO<sub>2</sub> · Southern California Bight · Microbial communities

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## INTRODUCTION

Global temperatures and atmospheric concentrations of carbon dioxide (CO<sub>2</sub>) are increasing at unprecedented rates compared to Earth's geologic past (Beardall et al. 2009), with the rise predicted to continue in the future (Cubasch & Wuebbles 2014). Depending on the model, average global surface temperature is currently predicted to increase by 1.4 to 5.8°C

over the next century (IPCC 2014), and atmospheric CO<sub>2</sub> levels are projected to reach 750 to 1000 ppm by 2100 (Meehl et al. 2007). Considering the magnitude of the predicted change, it is crucial to understand how marine microorganisms will respond to increasing temperature and CO<sub>2</sub> at both physiological and ecological levels, because they form the base of the marine food web and largely control the cycling of nutrients throughout the ocean (Boyd & Hutchins 2012).

\*Corresponding author: j.spackeen@gmail.com

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Temperature changes will affect the physiology of microorganisms, as well as their global distribution. For example, microorganisms capable of fixing nitrogen typically live in warmer oceans, and their distributions may expand as the temperature of the surface water increases (Boyd & Doney 2002). Additionally, the supply of nutrients may be affected by changes to ocean currents. For example, model studies have found that nitrate ( $\text{NO}_3^-$ ) supply to the coastal California (USA) ecosystem has increased since 1980 (Jacox et al. 2015); in the model,  $\text{NO}_3^-$  input to the euphotic zone was projected to increase by 80% by the year 2100 due to an increased flux of  $\text{NO}_3^-$  from deep waters (Rykaczewski & Dunne 2010). Such a large increase in  $\text{NO}_3^-$  supply has the potential to influence the community composition of microorganisms and their physiology.

In the case of  $\text{CO}_2$ , the large increase in atmospheric  $\text{CO}_2$  concentrations translates to a substantial change in the availability of dissolved inorganic carbon (DIC) in the ocean. The present-day concentration of dissolved  $\text{CO}_2$  in the ocean is  $\sim 13 \mu\text{M}$ , and as the ocean becomes more  $\text{CO}_2$ -saturated, concentrations are expected to more than double, causing the pH of the ocean to drop from 8.07 to 7.77 (Raven et al. 2005, Beardall et al. 2009). We know that microorganisms can have differing levels of sensitivity to  $\text{CO}_2$  and ocean acidification (Riebesell 2004, Doney et al. 2009). It is well documented that calcifying phytoplankton, like coccolithophores, are sensitive to ocean acidification because their liths start dissolving with declining pH (Riebesell et al. 2000, Kroeker et al. 2013), although non-calcifying microorganisms may also be affected by ocean acidification (Tortell et al. 2002, 2008, Rost et al. 2008, Wu et al. 2010). How phytoplankton species respond to ocean acidification is likely affected by whether they have carbon-concentrating mechanisms (CCMs), which help them concentrate inorganic carbon into their cells. As the partial pressure of ambient  $\text{CO}_2$  increases, it has been suggested that there will be less of a need for CCMs (Tortell et al. 2008) and that microorganisms will be able to allocate the energy and resources required to maintain CCMs toward other physiological processes (Raven 1991). Likewise, microorganisms that obtain  $\text{CO}_2$  solely through passive diffusion may also benefit as DIC concentrations increase (Beardall et al. 2009).

If microorganisms are able to take up DIC more effectively, growth rates and the demand for other nutrients, such as nitrogen and phosphorus, will likely increase. The source of these nutrients varies

from system to system. In coastal systems, nutrient supply is influenced by terrigenous sources, including riverine discharge or runoff that contains nutrients from wastewater treatment plants and agricultural production. In the past few decades, the use of fertilizers made with organic forms of nitrogen, primarily urea, has increased more than 100-fold (Glibert et al. 2006). The delivery of excess nutrients to the coastal environment is one factor that has the potential to increase the prevalence of harmful algal blooms (HABs).

In the last few decades, HABs have become a recurring issue off the coast of California (Schnitzer et al. 2007, Lewitus et al. 2012), and within the Southern California Bight region, nutrients supplied from natural sources (upwelling) and anthropogenic sources, namely treated wastewater effluent, can be on the same order of magnitude (Howard et al. 2014). One frequent bloomer in the Southern California Bight is *Pseudo-nitzschia* spp., a genus capable of producing domoic acid, the toxin responsible for amnesic shellfish poisoning (Bates et al. 1989, Bates & Trainer 2006). The response of *Pseudo-nitzschia* spp. to organic forms of nitrogen, such as urea, is particularly important to consider, as urea can sustain blooms of this species when other sources of nitrogen are in low supply (Cochlan et al. 2008), and certain strains produce more domoic acid when grown on urea than when grown on other nitrogen sources (Howard et al. 2007, Auro & Cochlan 2013, Martin-Jézéquel et al. 2015). Additional and different nutrient substrates, coupled with high temperatures, may create ideal conditions for blooms of *Pseudo-nitzschia*. For example, in 2014 and 2015, the North Pacific, including waters off the coast of southern California, was characterized by unusually warm sea surface temperatures that were as much as  $3^\circ\text{C}$  higher than average. The anomalously warm water moved into coastal waters, resulting in the largest and most prolonged bloom of *Pseudo-nitzschia* spp. along the US west coast within the past 15 yr (Bond et al. 2015, Du et al. 2016, McCabe et al. 2016).

As sea surface temperatures and  $\text{CO}_2$  concentrations continue to rise, it is likely that nutrient regimes will shift, new microbial communities will become established (Sarmiento et al. 2010), and rates of nitrogen and carbon uptake will be altered. In this study, a factorial design was used to assess the individual and combined effects of increases in temperature and  $\text{CO}_2$  on the rate of  $\text{NO}_3^-$ , urea, and DIC uptake by a Southern California Bight microbial community.

## MATERIALS AND METHODS

### Sample collection

Seawater was collected from Fish Harbor at the Southern California Marine Institute's (SCMI) dock in Terminal Island, California (33° 44' 59" N, 118° 12' 54" W) on 10 May 2012. Seawater was drawn from just below the surface using a manual suction pump and collected in acid washed (10% HCl) cubitainers that were pre-rinsed with seawater. Collected seawater was transported directly to the University of Southern California (USC), where it was immediately dispensed into 2.7 l polycarbonate bottles and placed within 2 ecostat continuous culture systems. Diluent (i.e. seawater amended with either NO<sub>3</sub><sup>-</sup> or urea) was continually added to the ecostats over the duration of the 10 d experiment. Samples for this study were collected at 48 h intervals to determine the rate of uptake of 2 stable isotopically labeled nitrogen substrates, NO<sub>3</sub><sup>-</sup> and urea, as well as labeled HCO<sub>3</sub><sup>-</sup> (referred to as DIC uptake henceforth) by the microbial community. Additional samples were collected at the start and end of the experiment so that changes in nutrient pools could be assessed.

### Ecostat design

Ecostats are continuous culture systems that create a controlled experimental setting to culture microorganisms over extended periods of time (Hutchins et al. 2003, Hare et al. 2007, Feng et al. 2009, 2010). Continuous culturing is advantageous because nutrients are delivered at a constant rate, there is quantitative removal of biomass, and the response of microbial communities to changing variables can be examined over extended periods of time (i.e. weeks to months; Hutchins et al. 2003, Pickell et al. 2009). As a result, the microbial community has sufficient time to acclimate to experimental conditions and competitive displacement will occur, meaning that microorganisms better suited to the experimental conditions will thrive, while those less adapted will have a lower abundance or be lost. One difference between nature and ecostats, however, is that loss processes, such as grazing, can be selective for certain species while cells lost from the ecostat are removed indiscriminately.

In this experiment, temperature, CO<sub>2</sub> level, and nutrient inputs were manipulated using 2 ecostat systems, 1 for each temperature level, each of which contained 12 individual bottles. The ecostats were set to

test present-day (19°C and 380 ppm CO<sub>2</sub>) and potential future temperature (23°C) and CO<sub>2</sub> (800 ppm) conditions. Control (19°C, 380 ppm CO<sub>2</sub>), +CO<sub>2</sub> (19°C, 800 ppm CO<sub>2</sub>), +Temp (23°C, 380 ppm CO<sub>2</sub>), and Combined (23°C, 800 ppm CO<sub>2</sub>) are the terms that will be used to describe the treatments throughout the manuscript.

Each of the treatment bottles in the Control, +CO<sub>2</sub>, +Temp, and Combined factorial matrix received either NO<sub>3</sub><sup>-</sup> or urea as the primary nitrogen source. Seawater used for diluent was collected from Redondo Beach Harbor on 9 May 2012, filtered using an acid-washed, in-line 0.2 µm cartridge filter, and placed in two 50 l reservoirs. The second collection of water that occurred on 10 May 2012 was necessary because biomass was low in the Redondo Beach Harbor water and could not be used as the inoculant. NO<sub>3</sub><sup>-</sup> was added to 1 reservoir to a final concentration of 15.1 ± 0.6 (SD) µmol N l<sup>-1</sup>, and urea was added to the other to a final concentration of 11.1 ± 0.4 µmol N l<sup>-1</sup> (Table 1). We note that, while the diluent contained these elevated concentrations of NO<sub>3</sub><sup>-</sup> and urea, the cells within the ecostats were never exposed to concentrations this high, because the diluent was added slowly over time and the cells were taking the substrates up as the diluent was added. Both diluent reservoirs were amended with silicic acid (H<sub>4</sub>SiO<sub>4</sub>) and phosphate (PO<sub>4</sub><sup>3-</sup>) to a final concentration of ~42 µmol Si l<sup>-1</sup> and ~2 µmol P l<sup>-1</sup> (Table 1). The concentration of ammonium (NH<sub>4</sub><sup>+</sup>) in both diluent types was ~2 µmol N l<sup>-1</sup>; we note that this concentration is ~1 µmol N l<sup>-1</sup> higher than the site water (Table 1), and may be due to contamination that occurred during the experimental set-up. Diluent from each reservoir was supplied to triplicate incubation bottles for each treatment using Teflon lines and adjustable peristaltic pumps. The Teflon lines entered the incubation bottles through the top and extended to the bottom where the diluent was released. Temperature levels were maintained using thermostatically controlled, recirculating heater/chiller systems set to either 19 or 23°C (Hare et al. 2007, Feng et al. 2009, 2010). The CO<sub>2</sub> concentrations within the +CO<sub>2</sub> and Combined treatments were manipulated using commercially prepared CO<sub>2</sub>/air mixtures (Praxair) bubbled into the incubation bottles through Teflon tubes.

The ecostats were positioned on the roof of the Allan Hancock Foundation building so that they would be exposed to ambient light conditions. The incubation bottles were secured to a plexiglass rack designed to automatically flip on its side every 5 min. This motion re-suspends the cells that may have set-

Table 1. Nutrient concentrations of the seawater collected off the Southern California Marine Institute (SCMI, the starting community) dock and in Redondo Harbor (diluent-base), as well as the 2 diluent types ( $\text{NO}_3^-$ - and urea-based). Nutrient concentrations measured on the final day of the experiment for each treatment (Treatment Tfinals) are displayed below the respective diluent type that the treatment received. Treatment details are provided in the 'Materials and methods'. Concentrations are in units of  $\mu\text{mol C, N, P, or Si l}^{-1} \pm 1 \text{ SD}$  ( $n = 3$ ). DIC (DOC): dissolved inorganic (organic) carbon; DON: dissolved organic carbon; DPA: dissolved organic nitrogen; NM: not measured; BD: below detection

Source	DIC	DOC	$\text{NH}_4^+$	$\text{NO}_2^-$	$\text{NO}_3^-$	DON	Urea	DPA	$\text{PO}_4^{3-}$	$\text{H}_4\text{SiO}_4$	$\text{NH}_4^+$	$\text{NO}_3^-$	% Urea	% DON
SCMI	NM	129 ± 4	0.93 ± 0.0	0.04 ± 0.0	0.51 ± 0.0	6.37 ± 0.6	0.84 ± 0.5	0.21 ± 0.0	0.41 ± 0.0	3.0 ± 0.0	11.9	6.5	10.7	81.2
Redondo	NM	115 ± 2	0.85 ± 0.1	0.19 ± 0.0	4.30 ± 0.0	5.81 ± 0.2	1.44 ± 0.0	0.10 ± 0.0	0.83 ± 0.0	10.4 ± 0.0	7.6	38.6	12.9	52.1
$\text{NO}_3^-$ diluent	NM	116 ± 1	1.89 ± 0.9	0.19 ± 0.0	15.10 ± 0.6	5.67 ± 2.3	1.22 ± 0.0	0.09 ± 0.0	2.17 ± 0.2	41.9 ± 1.7	8.3	66.0	5.3	24.9
Treatment Tfinals														
Control	2056 ± 100	117 ± 10	0.12 ± 0.0	BD	0.03 ± 0.0	5.56 ± 0.2	0.27 ± 0.1	0.13 ± 0.0	BD	9.0 ± 2.1	2.1	0.5	4.8	97.2
+Temp	2095 ± 67	135 ± 5	0.14 ± 0.0	BD	0.06 ± 0.0	5.76 ± 0.3	0.21 ± 0.0	0.15 ± 0.0	BD	27.2 ± 8.8 <sup>a</sup>	2.3	1.0	3.6	96.3
+CO <sub>2</sub>	2053 ± 52	113 ± 1	0.16 ± 0.0	BD	0.06 ± 0.0	5.43 ± 0.1	0.36 ± 0.0	0.14 ± 0.0	BD	7.4 ± 0.8	2.8	1.1	6.4	96.1
Combined	2188 ± 64	156 ± 11	0.15 ± 0.0	BD	0.05 ± 0.0	6.17 ± 0.3	0.18 ± 0.0	0.16 ± 0.0	BD	20.4 ± 8.2	2.4	0.8	2.9	96.9
Urea diluent	NM	126 ± 10	2.10 ± 0.8	0.2 ± 0.0	4.30 ± 0.1	16.50 ± 2.1	11.10 ± 0.4	0.11 ± 0.0	2.17 ± 0.1	41.5 ± 0.8	9.1	18.6	48.1	71.4
Treatment Tfinals														
Control	2133 ± 42	117 ± 11	0.15 ± 0.0	BD	0.05 ± 0.0	5.90 ± 0.4	0.25 ± 0.1	0.13 ± 0.0	BD	17.4 ± 14.2	2.5	0.8	4.1	96.9
+Temp	2052 ± 49	132 ± 6	0.18 ± 0.1	BD	0.04 ± 0.0	5.40 ± 0.2	0.28 ± 0.0	0.12 ± 0.0	BD	8.6 ± 0.4	3.2	0.7	5.0	95.9
+CO <sub>2</sub>	2156 ± 139	112 ± 7	0.10 ± 0.0	BD	0.04 ± 0.0	5.40 ± 0.2	0.23 ± 0.1	0.13 ± 0.0	BD	8.0 ± 6.1	1.8	0.7	4.2	97.7
Combined	2162 ± 18	150 ± 22	0.21 ± 0.1	BD	BD	6.30 ± 0.2	0.28 ± 0.0	0.12 ± 0.0	BD	17.4 ± 11.8	3.1	0.3	4.2	96.5

<sup>a</sup>Indicates  $n = 2$  and error is half the range

tled on the bottom, and allows the biomass to be homogeneously removed through the outflow lines, which are situated on the upper shoulder of the incubation bottles. Consistent, quantitative removal of biomass is key to the ecostat design, as it is this mechanism that allows growth rates of the community to equal loss rates, which enables the conditions within the ecostat to become and remain relatively stable over time. We note that although conditions were generally uniform during the experiment, changes were continuously occurring, which is typical of continuous culture experiments looking at natural microbial assemblages (MacIntyre & Cullen 2005, Pickell et al. 2009). The dilution rate was set to  $0.3 \text{ d}^{-1}$ . This rate was selected based on typical specific growth rates of phytoplankton communities in California coastal waters (Landry et al. 2009, Li et al. 2010). Within the ranges reported in those studies ( $\sim 0.3\text{--}0.6$ ), we conservatively established our dilution rate so we would not risk washing out microorganisms with slower growth rates.

The SCMI community incubated for 24 h in the ecostat bottles before any diluent ( $\text{NO}_3^-$  or urea-based media) was added. After dilutions began, the first sampling event occurred 24 h later. These steps were completed in sequence to ensure that cells within the ecostats were growing and biomass was increasing before we began measuring rates.

### Nutrient uptake experiments

To determine rates of nitrogen and DIC uptake in the ecostat treatments, an acid-washed syringe was used to collect 250 ml from each bottle. Care was taken to only draw off approximately 10% of the bottle volume to avoid substantial disruptions to the continuous culture nutrient inflow/biomass accumulation balance (Hutchins et al. 2003). Sub-samples were collected to measure concentrations of either  $\text{NO}_3^-$  or urea, corresponding to the diluent type that the treatment received. The remaining volume was used to measure uptake rates in acid-washed (10% HCl with multiple rinses) 230 ml polycarbonate conical bottles. Uptake experiments were performed using 2 different  $^{15}\text{N}$ -labeled substrates: potassium nitrate ( $\text{K}^{15}\text{NO}_3$ ; 98%) and  $^{15}\text{N}$ -urea (98%; Cambridge Isotope Laboratories). To measure DIC uptake,  $^{13}\text{C}$ -labeled  $\text{NaH}^{13}\text{CO}_3^-$  (99%; Cambridge Isotope Laboratories) was used and added to all treatments (Hama et al. 1983). We note that the use of  $\text{NaH}^{13}\text{CO}_3^-$  prevented the use of dual  $^{15}\text{N}$  and  $^{13}\text{C}$ -labeled urea, and so uptake of urea-C was not measured; generally

uptake of urea-C is very low (Bradley et al. 2010a, Bronk et al. 2014) or below detection (Bradley et al. 2010b). Uptake of NO<sub>3</sub><sup>-</sup> was measured in the treatments that received NO<sub>3</sub><sup>-</sup>-based diluent, while uptake of urea was measured in the treatments that were supported by urea-based diluent. Concentrations of NO<sub>3</sub><sup>-</sup> and urea were not known at the start of the <sup>15</sup>N incubations, so additions of 1 μmol N l<sup>-1</sup> as <sup>15</sup>NO<sub>3</sub><sup>-</sup> or <sup>15</sup>N-labeled urea were used. These additions were above tracer level for NO<sub>3</sub><sup>-</sup> and urea throughout the experiment. The choice to use a larger addition was made to increase the likelihood that substrate concentrations would be similar across all treatments during the <sup>15</sup>N incubations.

The bottles with added tracer were incubated within the ecostats, under the same temperature and light conditions as the larger ecostat experimental bottles. During the incubations, CO<sub>2</sub> was not bubbled into the uptake bottles; however, the uptake bottles were filled to capacity, which minimized head space and CO<sub>2</sub> exchange. Bottles were incubated for approximately 3 to 4 h and then filtered sequentially to collect 2 size fractions (0.7–5.0 and >5.0 μm). The larger size fraction (>5.0 μm) was collected on a Sterlitech silver membrane filter. The filtrate was then passed through a Whatman glass fiber filter (GF/F; nominal pore size of 0.7 μm) to collect the smaller size fraction (0.7–5.0 μm). Uptake experiments were conducted every other day throughout the duration of the experiment for a total of 5 time points over 10 d.

### Dissolved and particulate nutrient analyses

Concentrations of NO<sub>3</sub><sup>-</sup> and urea were measured on the same days that uptake experiments were performed. Additional nutrient samples (NH<sub>4</sub><sup>+</sup>, nitrite [NO<sub>2</sub><sup>-</sup>], PO<sub>4</sub><sup>3-</sup>, H<sub>4</sub>SiO<sub>4</sub>, dissolved primary amines [DPA], total dissolved nitrogen [TDN], and dissolved organic carbon [DOC]) were collected at the beginning and end of the experiment so that net changes in specific nutrient pools could be assessed. All nutrient samples were filtered through Whatman GF/F filters that had been combusted for 2 h at 450°C. Samples were stored at -20°C until analysis.

Concentrations of NH<sub>4</sub><sup>+</sup> were measured in triplicate using the phenol-hypochlorite method (detection limit [DL] 0.05 μmol N l<sup>-1</sup>, Koroleff 1983) on a Shimadzu UV-1601 spectrophotometer. Concentrations of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup>, and H<sub>4</sub>SiO<sub>4</sub> were measured in duplicate on a Lachat QuickChem 8500 autoanalyzer (DL 0.03 μmol N l<sup>-1</sup>, DL 0.03 μmol P

l<sup>-1</sup>, DL 0.05 μmol Si l<sup>-1</sup>; Parsons et al. 1984). The manual diacetyl monoxime thiosemicarbazide method was used to analyze the concentration of urea in duplicate (DL 0.025 μmol N l<sup>-1</sup>; Price & Harrison 1987). DPA samples were measured in triplicate using the *o*-phthaldialdehyde method followed by analysis on a Shimadzu RF-1501 spectrofluorometer (DL 0.025 μmol N l<sup>-1</sup>; Parsons et al. 1984). TDN and DOC were measured on a Shimadzu 5000A TOC-V/TNM (DL 2 μmol N l<sup>-1</sup>, 5 μmol C l<sup>-1</sup>; Sharp et al. 2004); deep-sea reference water samples from the University of Miami consensus reference material program were included to ensure analytical accuracy (Hansell 2005). Dissolved organic nitrogen (DON) was calculated by taking the difference between TDN and the sum of the inorganic nitrogen species (NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>2</sub><sup>-</sup>); standard deviations for the final DON concentrations were calculated using propagation of error. DIC was measured using coulometry (CM5230, UIC) following King et al. (2011); CO2SYS software (Lewis & Wallace 1998) was used to calculate pCO<sub>2</sub> (in μatm). A Europa 20/20 isotope ratio mass spectrometer was used to analyze particulate nitrogen (PN) and particulate carbon (PC) concentrations and to measure sample isotopic enrichment of <sup>15</sup>N and <sup>13</sup>C.

Samples were collected at the start of the experiment to determine the initial community composition. Cells were preserved using an acidified Lugol's solution, identified and enumerated according to Tomas (1997) and the Utermöhl method (Utermöhl 1931), respectively, using an inverted compound light-microscope with an Accu0Scope 3032.

### Uptake calculations

Specific (*V*) and absolute (*ρ*) uptake rates of NO<sub>3</sub><sup>-</sup> and urea were calculated using Eqs. (1) and (2), respectively (Dugdale & Wilkerson 1986):

$$V = \frac{\text{PN at\%xs}}{\text{SP at\%xs} \times \text{Time}} \quad (1)$$

$$\rho = \frac{\text{PN at\%xs}}{\text{SP at\%xs} \times \text{Time}} \times [\text{PN}] \quad (2)$$

where *V* is specific uptake rate (h<sup>-1</sup>), and *ρ* is absolute uptake rate (μmol N l<sup>-1</sup> h<sup>-1</sup>); PN at%xs is the <sup>15</sup>N atom% enrichment of PN minus 0.366, which is the typical <sup>15</sup>N atom% enrichment of an atmospheric N<sub>2</sub> gas standard. SP at%xs is the initial enrichment of the nitrogen source pool minus <sup>15</sup>N atom% enrichment of the atmospheric standard; in

this study the source pool was either  $\text{NO}_3^-$  or urea. The ambient concentration for  $\text{NO}_3^-$  and urea were measured each time an uptake experiment was conducted, and these values were used to calculate the initial enrichment of the nitrogen source pool.  $[\text{PN}]$  is the concentration of PN at the end of the incubation ( $\mu\text{mol N l}^{-1}$ ).  $V$  expresses the physiological response of the community, and  $\rho$  normalizes for differences in biomass between treatments. Specific and absolute uptake rates of DIC were also calculated using the same equations; however, the nitrogen parameters were substituted with carbon, and PC at%xs is the  $^{13}\text{C}$  atom% enrichment of PC minus 1.08, which is the natural  $^{13}\text{C}$  enrichment of phytoplankton (Slawyk et al. 1977, Lefebvre et al. 2012). DIC concentrations were measured at 2 time points during the experiment, and the average DIC concentration for each experimental condition (Control, +Temp, + $\text{CO}_2$ , Combined;  $n = 12$ ) was used in the calculation of uptake rates.

### Statistical analyses

To calculate average uptake rates and average stoichiometric uptake ratios for the 4 treatments (Control, +Temp, + $\text{CO}_2$ , and Combined), all replicates within each treatment were combined and averaged ( $n = 15$ ; 3 replicates of each treatment  $\times$  5 time points). While the magnitude of  $V$  and  $\rho$  varied throughout the experiment, the trends were generally the same across time points. Expressing uptake rates as a combined average of the time points increased variability, but was done to produce greater statistical power.

Data were analyzed using the R statistical program (R Development Core Team 2010). Data were checked for normality and homogeneity of variance, and those that were not normally distributed were log transformed prior to statistical analysis. A 1-way repeated measures analysis of variance (ANOVA) was used to determine if there were significant differences in uptake rates between treatments, nitrogen source ( $\text{NO}_3^-$  and urea), and size fractions. A post hoc Tukey's test was run in order to locate the uptake means that were significantly different from one another. Means were considered to be significantly different at  $p \leq 0.05$ .

## RESULTS

### Source water characterization and nutrient concentrations

Concentrations of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , and urea were higher in Redondo Harbor relative to the SCMI dock. Concentrations of  $\text{NO}_3^-$  and urea, respectively, were 4.30 and 1.44  $\mu\text{mol N l}^{-1}$  in Redondo Harbor compared to 0.51 and 0.84  $\mu\text{mol N l}^{-1}$  at the SCMI dock. At both locations, DON made up  $>50\%$  of the TDN pool, with urea accounting for 25% of the DON pool in Redondo Harbor and 13% of the DON pool at the SCMI dock. The concentration of  $\text{PO}_4^{3-}$  at Redondo Harbor was double the concentration at the SCMI dock, and the concentration of  $\text{H}_4\text{SiO}_4$  was more than triple that seen off the SCMI dock (Table 1).

Every other day during the experiment, concentrations of  $\text{NO}_3^-$  were measured in the treatments that received  $\text{NO}_3^-$  diluent and urea in the treatments that received urea diluent (Table 2). Concentrations of  $\text{NO}_3^-$  were  $<1 \mu\text{mol N l}^{-1}$  at all time points, with the exception of the + $\text{CO}_2$  treatment on Day 4. Concentrations of urea were consistently higher than concentrations of  $\text{NO}_3^-$  during the experiment. The 2 treatments exposed to elevated temperatures were more efficient at drawing down the available urea in the ecostats (Table 2).

The full suite of nutrients was measured in the ecostat bottles on the final day of the experiment. Nutrient concentrations were generally similar between treatments (Table 1). The concentration of TDN was 5.5–6.5  $\mu\text{mol N l}^{-1}$  in all treatments, with  $\text{NH}_4^+$  comprising the largest portion of the inorganic nitrogen

Table 2.  $\text{NO}_3^-$  and urea concentrations measured on Days 2, 4, 6, 8, and 10 for the different treatments.  $\text{NO}_3^-$  concentrations are from the treatments that received  $\text{NO}_3^-$  diluent, and urea concentrations are from the treatments that received urea diluent. Treatment details are provided in 'Materials and methods'

	Day 2	Day 4	Day 6	Day 8	Day 10
<b><math>\text{NO}_3^-</math> concentrations</b>					
Control	0.75 $\pm$ 0.3	0.22 $\pm$ 0.1	0.09 $\pm$ 0.0	0.09 $\pm$ 0.1	0.03 $\pm$ 0.0
+Temp	0.17 $\pm$ 0.1	0.39 $\pm$ 0.1	0.07 $\pm$ 0.0	0.12 $\pm$ 0.1	0.06 $\pm$ 0.0
+ $\text{CO}_2$	0.25 $\pm$ 0.1	2.74 $\pm$ 3.1 <sup>a</sup>	0.27 $\pm$ 0.1	0.21 $\pm$ 0.1	0.06 $\pm$ 0.0
Combined	0.18 $\pm$ 0.1	0.35 $\pm$ 0.1	0.08 $\pm$ 0.0	0.14 $\pm$ 0.1	0.05 $\pm$ 0.0
<b>Urea concentrations</b>					
Control	5.08 $\pm$ 0.5	4.51 $\pm$ 3.2	3.05 $\pm$ 2.3	0.36 $\pm$ 0.1	0.25 $\pm$ 0.1
+Temp	0.51 $\pm$ 0.1	1.15 $\pm$ 0.9	0.45 $\pm$ 0.1	0.42 $\pm$ 0.0	0.28 $\pm$ 0.0
+ $\text{CO}_2$	4.91 $\pm$ 0.7	4.60 $\pm$ 2.5	1.25 $\pm$ 0.7	0.37 $\pm$ 0.1	0.23 $\pm$ 0.1
Combined	0.43 $\pm$ 0.2	0.52 $\pm$ 0.1	0.42 $\pm$ 0.1	0.44 $\pm$ 0.2	0.28 $\pm$ 0.0

<sup>a</sup>There was an issue with the diluent pump prior to sampling

fraction. NO<sub>3</sub><sup>-</sup> concentrations were low, measuring approximately 0.05 μmol N l<sup>-1</sup> in all treatments, and concentrations of PO<sub>4</sub><sup>3-</sup> were below the limit of detection (Table 1). Although NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup> concentrations were low or below detection on the final day, both were continually supplied in the seawater diluent at a constant rate throughout the experiment. Concentrations of H<sub>4</sub>SiO<sub>4</sub> and DOC varied between treatments at the end of the experiment. For both diluent types, DOC concentrations in the +Temp and Combined treatments were approximately 20 μmol C l<sup>-1</sup> higher in concentration than the Control and +CO<sub>2</sub> treatments.

### Initial community and biomass

Diatoms dominated the initial community that was collected off of the SCMI dock, accounting for 99% of the taxa that were present. Approximately half of the diatom community was comprised of *Pseudo-nitzschia* species, including both *P. multiseriis* and *P. hasleana*, whose relative abundances were 48 and 4%, respectively. Other dominant diatoms included *Leptocylindrus danicus* (39%) and *Chaetoceros* spp. (5%).

PC and PN concentrations of the whole community (>0.7 μm) were more constant in the treatments receiving NO<sub>3</sub><sup>-</sup> diluent, but more variable and higher in the treatments receiving urea diluent (Fig. 1).

### Nitrogen uptake

Results from the community incubated in the ecostats had 2 general trends. Uptake rates of NO<sub>3</sub><sup>-</sup> (both  $V_{NO_3}$  and  $\rho_{NO_3}$ ) were generally lower than uptake rates of urea (both  $V_{UREA}$  and  $\rho_{UREA}$ ), and uptake rates were higher in the larger size fraction (>5.0 μm) relative to the smaller size fraction (0.7–5.0 μm; Fig. 2). Elevated CO<sub>2</sub> resulted in higher NO<sub>3</sub><sup>-</sup> uptake rates by smaller microorganisms, and elevated temperature resulted in higher urea uptake rates by both large and small microorganisms. These general trends were

also observed over the course of the experiment (Fig. 3). Uptake rates changed the most within the first 2 to 4 d of the experiment, particularly in the case of urea. After the fourth day, uptakes rates remained relatively stable in most treatments (Fig. 3).

NO<sub>3</sub><sup>-</sup> uptake rates ( $V_{NO_3}$  and  $\rho_{NO_3}$ ) in the larger size fraction did not significantly differ between any of the treatments (Fig. 2,  $p > 0.05$ ). In the +CO<sub>2</sub> treatment, however,  $V_{NO_3}$  and  $\rho_{NO_3}$  within the smaller size fraction were significantly higher (~50%) than in the Control (Fig. 2,  $p < 0.007$ ). Although CO<sub>2</sub> was also manipulated in the Combined treatment, no significant increase in NO<sub>3</sub><sup>-</sup> uptake was observed in that treatment compared to the Control.

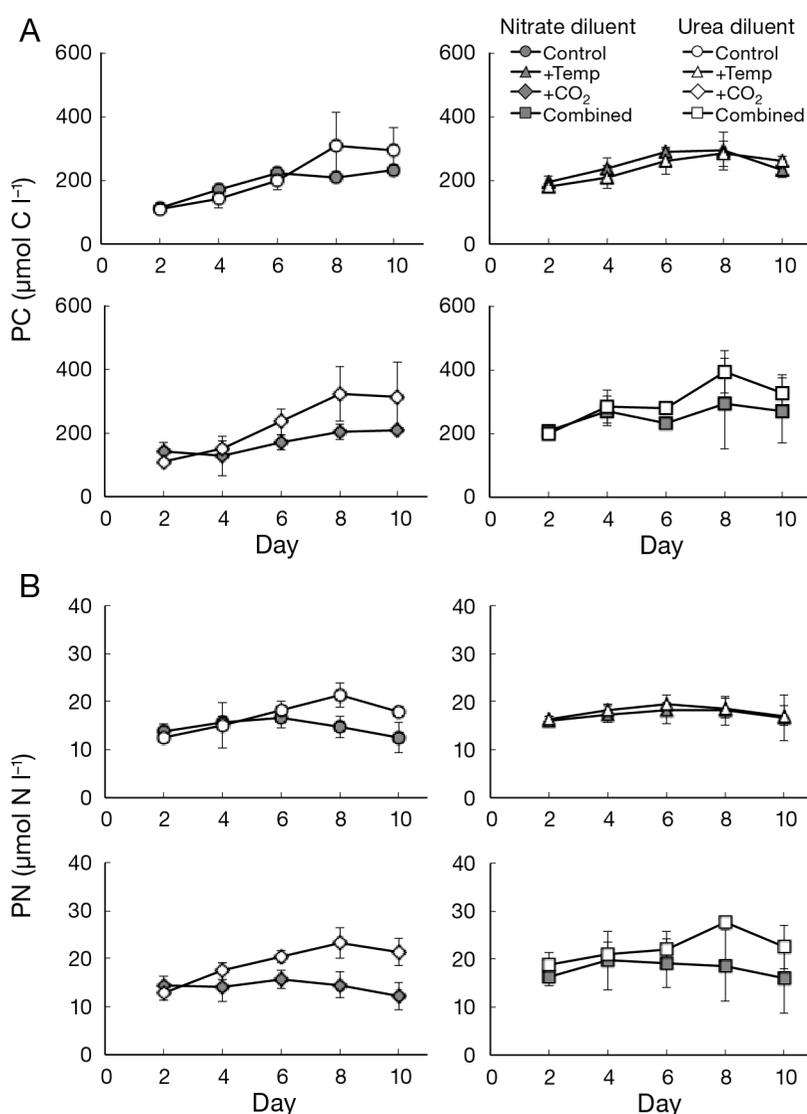


Fig. 1. Mean particulate (A) carbon (PC) and (B) nitrogen (PN) concentrations  $\pm$  1 SD ( $n = 3$ ) of NO<sub>3</sub><sup>-</sup> diluent treatments (shaded markers) and urea diluent treatments (white markers) of the whole community (>0.7 μm) over the course of the experiment. Treatment details are provided in 'Materials and methods'

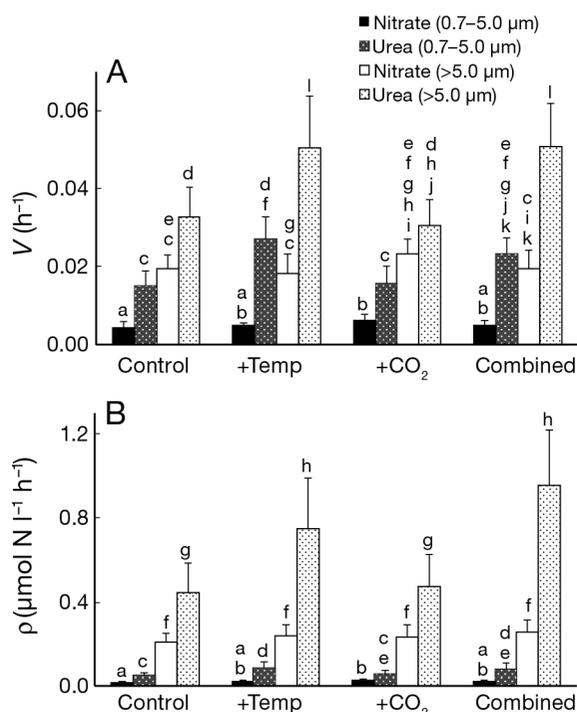


Fig. 2. Mean (A) specific ( $V$ ) and (B) absolute ( $\rho$ ) uptake rates  $\pm$  1 SD ( $n = 15$ ) of  $\text{NO}_3^-$  (solid bars) and urea (dotted bars) by small (0.7–5.0  $\mu\text{m}$ , black bars) and large (>5.0  $\mu\text{m}$ , white bars) microorganisms in the incubated community. Bars are an average of all treatment replicates across 5 time points. Bars that have different letters are significantly different from one another ( $p \leq 0.05$ ). Treatment details are provided in 'Materials and methods'

Urea uptake rates ( $V_{\text{UREA}}$  and  $\rho_{\text{UREA}}$ ) showed a distinctly different pattern. For  $V_{\text{UREA}}$  and  $\rho_{\text{UREA}}$  in the large size fraction, the +Temp treatment and the Combined treatment were significantly higher than both the Control and the +CO<sub>2</sub> treatment (Fig. 2,  $p < 0.01$ ). In the smaller size fraction, the +Temp and Combined treatments had significantly higher  $V_{\text{UREA}}$  than the Control and +CO<sub>2</sub> treatment ( $p < 0.001$ ). The same results were seen for  $\rho_{\text{UREA}}$  ( $p < 0.01$ ), except that the Combined treatment did not have significantly higher uptake rates than the +CO<sub>2</sub> treatment.

### DIC uptake

DIC uptake rates in the larger size fraction were significantly greater than uptake rates in the smaller size fraction for both  $V_{\text{DIC}}$  and  $\rho_{\text{DIC}}$ . This pattern occurred in all experimental treatments (Fig. 4,  $p < 0.001$ ). Higher DIC uptake rates were observed in the +Temp and Combined treatments, following the same general trends observed for urea. In the smaller size fraction, this trend was significant for both  $V_{\text{DIC}}$

and  $\rho_{\text{DIC}}$  when comparing the +Temp treatment to the Control ( $p < 0.006$ ). The trend was also significant in the smaller size fraction when comparing  $V_{\text{DIC}}$  and  $\rho_{\text{DIC}}$  of the Combined treatment grown on  $\text{NO}_3^-$  to the Control ( $p < 0.001$ ). In the larger size fraction,  $\rho_{\text{DIC}}$  of the +Temp treatment grown on  $\text{NO}_3^-$  had significantly higher rates than the Control, and  $\rho_{\text{DIC}}$  of the Combined treatment grown on urea was significantly higher than the Control ( $p < 0.03$ ). None of the +CO<sub>2</sub> treatments was significantly different than the Controls; however,  $V_{\text{DIC}}$  and  $\rho_{\text{DIC}}$  of the larger size fraction in the +CO<sub>2</sub> treatments were significantly lower than the +Temp treatments ( $p < 0.02$ ).

### Stoichiometry

Particulate concentrations and C:N ratios were impacted by elevated temperature, although the trends were not statistically significant ( $p > 0.05$ ). Concentrations of PC for the +Temp and Combined treatments were higher than the Control and +CO<sub>2</sub> treatments for both size fractions and both diluent types (Table 3). This trend was also found for PN concentrations in the treatments that received  $\text{NO}_3^-$  diluent, but not in the treatments that received urea diluent. For all treatments, PC:PN ratios increased in the +Temp and Combined treatments, while the +CO<sub>2</sub> treatment had similar ratios to the Control (Table 3).

Temperature also impacted the stoichiometric uptake ratio of DIC to  $\text{NO}_3^-$ . In the larger size fraction, the ratio of  $\rho_{\text{DIC}}:\rho_{\text{NO}_3}$  for the +Temp treatment was significantly higher than the Control (Fig. 5,  $p < 0.05$ ). In the smaller size fraction, the Combined treatment had a significantly higher  $\rho_{\text{DIC}}:\rho_{\text{NO}_3}$  ratio than the Control (Fig. 5,  $p < 0.02$ ). In contrast, the ratios of  $\rho_{\text{DIC}}:\rho_{\text{UREA}}$  in all treatments were not significantly different from the Control.

### DISCUSSION

The goal of this study was to determine how uptake of  $\text{NO}_3^-$ , urea, and DIC by Southern California Bight microorganisms will be affected by elevated temperature and CO<sub>2</sub> conditions that will likely develop over the next century. The SCMI microbial community used to set up the ecostats was dominated by diatom species that are commonly found in coastal southern California waters (Cupp 1943, Reid et al. 1970, 1978, Cullen et al. 1982, Venrick 2015), including *Pseudo-nitzschia* spp., *Leptocylindrus danicus*, and *Chaetoceros* spp. Both diluent types used in this experiment

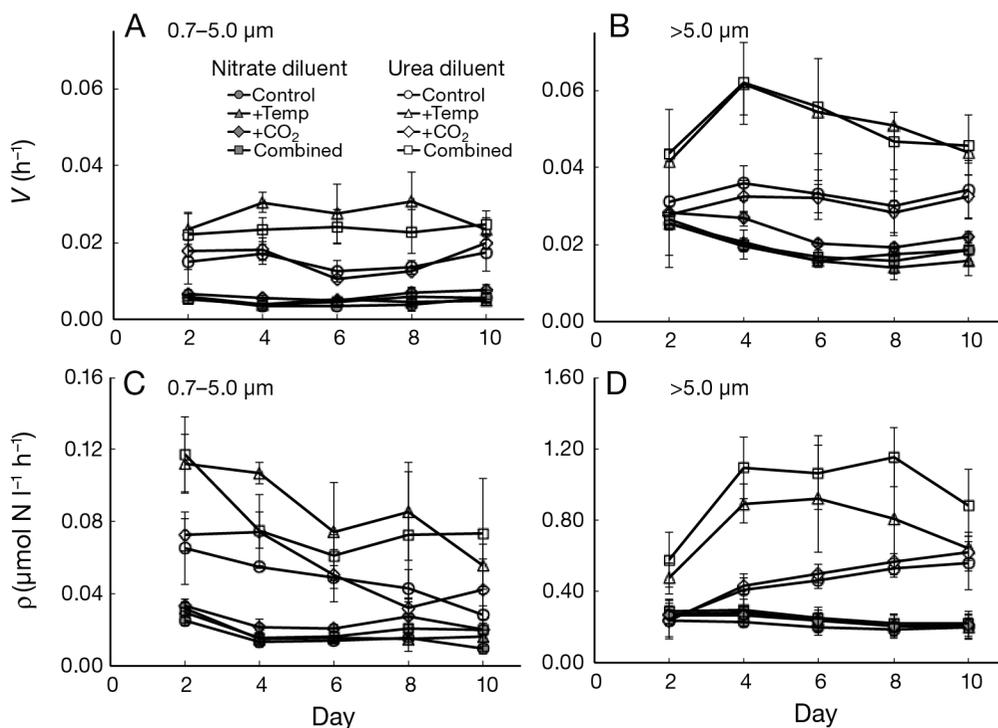


Fig. 3. Mean (A,B) specific ( $V$ ) and (C,D) absolute ( $\rho$ ) uptake rates  $\pm$  1 SD ( $n = 3$ ) of  $\text{NO}_3^-$  (shaded markers) and urea (white markers) by small (0.7–5.0  $\mu\text{m}$ , A and C) and large (>5.0  $\mu\text{m}$ , B and D) microorganisms in the incubated community over the course of the experiment. Panels C and D have a different y-axis scale due to large differences in uptake rates between the small and large size fraction. Treatment details are provided in 'Materials and methods'

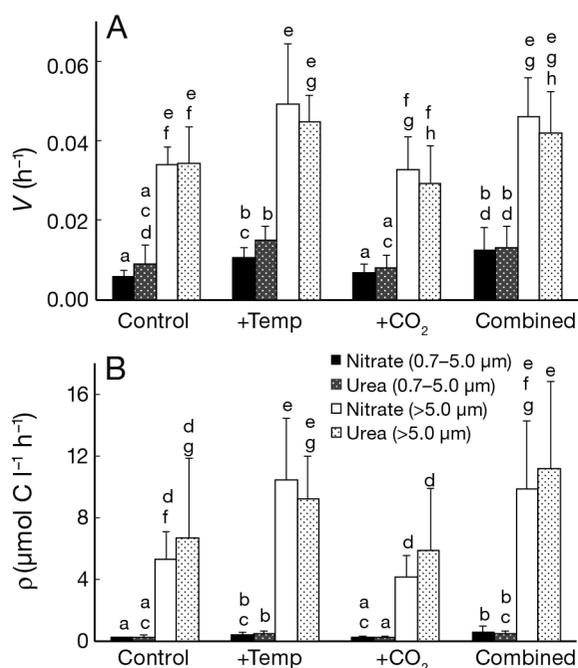


Fig. 4. Mean (A) specific ( $V$ ) and (B) absolute ( $\rho$ ) uptake rates  $\pm$  1 SD ( $n = 15$ ) of dissolved inorganic carbon (DIC) by microorganisms sustained on  $\text{NO}_3^-$ -based diluent (solid bars) and urea-based diluent (dotted bars) by small (0.7–5.0  $\mu\text{m}$ , black bars) and large (>5.0  $\mu\text{m}$ , white bars) microorganisms in the incubated community. Bars are an average of all treatment replicates across 5 time points. Bars that have different letters are significantly different from one another ( $p \leq 0.05$ ). Treatment details are provided in 'Materials and methods'

were supplemented with  $\text{H}_4\text{SiO}_4$ , ensuring that the diatom community would not become  $\text{H}_4\text{SiO}_4$  limited. Thus, our study demonstrates how a coastal California microbial assemblage primarily composed of diatoms would be affected by climate change parameters and changes to the dominant nitrogen substrate. At the end of the experiment, *L. danicus* remained dominant in all treatments, and the relative abundance of other diatom species varied significantly between treatments (A. O. Tatters et al. unpubl.).

#### Nutrient uptake in the +Temp treatment

Many studies have documented that a temperature increase of a few degrees increases microbial growth rates and photosynthesis (Eppley 1972, Raven & Geider 1988, Boyd et al. 2013). Studies using both cultured phytoplankton and field communities found that the relationship between temperature and nitrogen uptake varies between species (Goldman 1977, Kristiansen 1983, Glibert et al. 1995, Lomas & Glibert 1999). Of these studies, that of Lomas & Glibert (1999) is the most similar to our study, as it used <sup>15</sup>N incorporation to measure both  $\text{NO}_3^-$  and urea uptake by diatom-dominated field communities subjected to temperature manipulations that were similar to the temperatures used in our study. In that study, for every 1°C change in temperature,  $\text{NO}_3^-$  uptake decreased by 2.9% on average. We found that temper-

Table 3. Average particulate carbon (PC) and particulate nitrogen (PN) concentrations and PC:PN ratios for 2 size classes of microorganisms (0.7–5.0 and >5.0  $\mu\text{m}$ ) at the Southern California Marine Institute (SCMI, the starting community) dock and in the different treatments (treatment details are provided in 'Materials and methods'). PC and PN concentrations are in units of  $\mu\text{mol C or N l}^{-1} \pm 1 \text{ SD}$  ( $n = 15$ ; 3 reps  $\times 5$  time points)

Source	0.7–5.0 $\mu\text{m}$			>5.0 $\mu\text{m}$		
	PC	PN	PC:PN	PC	PN	PC:PN
<b>SCMI</b>	29.0 $\pm$ 1.1	3.38 $\pm$ 0.2	8.60 $\pm$ 0.6	36.6 $\pm$ 1.6	3.97 $\pm$ 0.1	9.18 $\pm$ 0.2
<b>NO<sub>3</sub><sup>-</sup> diluent</b>						
Control	35.6 $\pm$ 6.1	3.72 $\pm$ 1.1	10.2 $\pm$ 2.3	153.6 $\pm$ 50	11.0 $\pm$ 2.3	14.2 $\pm$ 4.7
+Temp	39.9 $\pm$ 4.8	3.81 $\pm$ 0.9	10.8 $\pm$ 1.7	208.2 $\pm$ 50	13.5 $\pm$ 3.1	15.8 $\pm$ 3.5
+CO <sub>2</sub>	37.4 $\pm$ 7.6	3.96 $\pm$ 0.8	9.6 $\pm$ 1.4	132.5 $\pm$ 46	10.2 $\pm$ 2.4	13.3 $\pm$ 5.2
Combined	45.4 $\pm$ 9.4	4.09 $\pm$ 1.1	11.3 $\pm$ 1.5	210.2 $\pm$ 73	13.9 $\pm$ 5.0	15.6 $\pm$ 3.2
<b>Urea diluent</b>						
Control	28.7 $\pm$ 7.3	3.32 $\pm$ 1.1	9.0 $\pm$ 1.6	181.0 $\pm$ 100	13.7 $\pm$ 4.2	12.5 $\pm$ 3.8
+Temp	32.6 $\pm$ 7.2	3.23 $\pm$ 1.0	10.3 $\pm$ 1.3	205.3 $\pm$ 51	14.7 $\pm$ 2.1	13.9 $\pm$ 2.7
+CO <sub>2</sub>	29.6 $\pm$ 6.9	3.54 $\pm$ 1.2	8.8 $\pm$ 1.9	196.3 $\pm$ 106	15.5 $\pm$ 4.7	12.0 $\pm$ 3.7
Combined	35.9 $\pm$ 9.6	3.43 $\pm$ 1.2	10.7 $\pm$ 1.6	260.6 $\pm$ 78	19.0 $\pm$ 4.7	13.9 $\pm$ 3.9

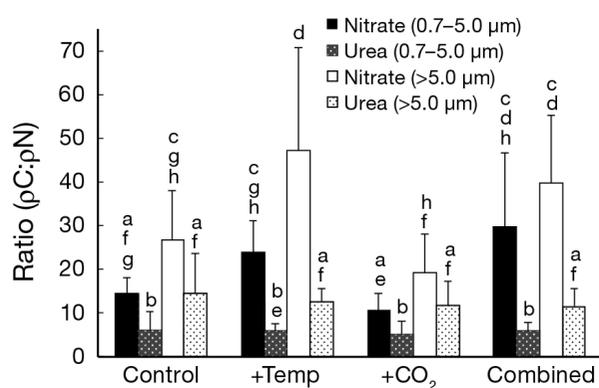


Fig. 5. Stoichiometric ratios of  $p_{\text{DIC}}$  to  $p_{\text{NO}_3}$  (solid bars) and  $p_{\text{DIC}}$  to  $p_{\text{UREA}}$  (dotted bars)  $\pm 1 \text{ SD}$  ( $n = 15$ ) for small (0.7–5.0  $\mu\text{m}$ , black bars) and large (>5.0  $\mu\text{m}$ , white bars) microorganisms in the incubated community, where  $p$  is the absolute uptake rate and DIC is dissolved inorganic carbon. Bars that have different letters are significantly different from one another ( $p \leq 0.05$ ). Treatment details are provided in 'Materials and methods'

ature did not impact  $\text{NO}_3^-$  uptake, but consistent with Lomas & Glibert (1999), temperature did significantly increase uptake of urea (Fig. 2).

There are a few possible explanations for why temperature did not significantly affect  $\text{NO}_3^-$  uptake in our study. Seawater was collected from the surface close to shore, so microorganisms in this region may have already been accustomed to temperature fluctuations of several degrees. The amplitude of sea surface seasonal variation off the coast of Southern California can reach 3.1°C (Nezlin et al. 2004). Thus, our temperature increase of 4°C is close to the realm of nat-

ural seasonal variation. It is also possible that the ambient (19°C) and elevated (23°C) temperatures within this study were above the optimal physiological range for diatom  $\text{NO}_3^-$  uptake. In the diatom *Skeletonema costatum* that typically inhabits seawater that is 12–15°C, the activity of nitrate reductase (NR) is unstable above ~16°C (Gao et al. 2000). Likewise, Berges et al. (2002) showed that  $^{15}\text{N}$  uptake and NR activity of the temperate diatom *Thalassiosira pseudonana* declines between 17 and 25°C. The optimal temperature range for growth of *L. danicus*, a dominant phytoplankton species in our study, is between 15 and 20°C (Verity 1982), so it is possible that  $\text{NO}_3^-$  uptake physi-

ology falls in this range as well.

In the case of urea, our data show a positive relationship between temperature and urea uptake. The majority of studies investigating temperature effects on urea uptake measured the activity and expression of enzymes, like urease, involved in urea metabolism rather than directly quantifying urea uptake. These studies showed that urease activity (Fan et al. 2003) and urea uptake (Lomas & Glibert 1999) are positively correlated with temperature. In our study, raising the temperature by 4°C increased uptake rates of urea ( $V_{\text{UREA}}$ ) by 80% for small microorganisms and 54% for large microorganisms. The substantial increase in urea uptake rates suggests that as sea surface temperatures continue to rise, organisms capable of using urea may have an advantage, especially in temperate coastal systems that receive wastewater effluent or agricultural runoff, both of which contain urea (Bronk et al. 2010, Howard et al. 2014). Urea is also the most common form of nitrogen used in fertilizers for agricultural production (Glibert et al. 2006, Mérigout et al. 2008), and California is the top agricultural center in the US (California Department of Food and Agriculture 2015). With respect to the Southern California Bight, ~60% of the total nitrogen in riverine runoff is comprised of organic forms, including urea, and riverine runoff has been identified as the primary source of urea to this system (Howard et al. 2014). Urea is a small component, however, of the total nitrogen load delivered to the Southern California Bight when both natural and anthropogenic sources are included.

Similar to urea, there was also an increase in DIC uptake with increasing temperature (Fig. 4). This trend occurred regardless of the nitrogen substrate added to the diluent. The general model is that increased temperature elevates carbon fixation and respiration up to a certain threshold that is species-specific and contingent upon the organism's geographic location and inherent ability to acclimate (Eppley 1972, Goldman 1977, Verity 1982, Thomas et al. 2012, Boyd et al. 2013). In the past decade, more work has been done on communities rather than cultures, and the relationship between temperature and carbon consumption is proving to be more complex. A few studies even show that elevated temperature decreases carbon drawdown (Wohlers et al. 2009, Kim et al. 2011), which is contrary to our results. Not only did DIC uptake rates increase as a function of temperature, but concentrations of PC and DOC also increased in the +Temp treatments (Tables 1 & 3). Our findings resemble the results reported by Taucher et al. (2012), where a Baltic Sea microbial community was grown for a month at a range of temperatures resulting in a net increase of particulate and dissolved carbon. The authors hypothesized that increased temperatures may lead to carbon overconsumption and a decoupling between C:N ratios in both the particulate and dissolved form, leading to a build up of particulate and dissolved carbon and an increase in C:N ratios. This hypothesis is also supported by our study, where the PC:PN ratios (Table 3) and the ratio of absolute DIC uptake to absolute NO<sub>3</sub><sup>-</sup> uptake were both elevated in the +Temp treatment (Fig. 5). The average ratio of  $\rho_{\text{DIC}}:\rho_{\text{NO}_3^-}$  was significantly higher in the +Temp treatment compared to the Control for the larger size fraction. Higher DIC to NO<sub>3</sub><sup>-</sup> uptake ratios at elevated temperature may be linked to the presence of diatoms. In a culture experiment, the diatom *Thalassiosira weissflogii* had higher DIC to NO<sub>3</sub><sup>-</sup> uptake ratios when temperature was elevated by 5°C (Taucher et al. 2015). In this same study, however, the opposite pattern occurred for the diatom *Dactyliosolen fragilissimus*. This finding warrants further investigation because of the implications for shaping ocean carbon biogeochemistry and C:N stoichiometry in the future.

### Nutrient uptake in the +CO<sub>2</sub> treatment

The physiological effects that CO<sub>2</sub> has on the uptake of nutrients are complex. Elevated CO<sub>2</sub> can significantly impact enzyme activity and subsequent metabolic processes such as photosynthesis (Mercado et al. 1999, Zou et al. 2011), calcification (Doney et al.

2009 and references therein), and nutrient uptake and assimilation (Magnusson et al. 1996, Zou 2005, Hofmann et al. 2013). Uptake of nitrogen is intrinsically linked to carbon utilization, and the mechanism by which microorganisms use carbon varies between species. Some microorganisms contain CCMs, and those that do not, rely on passive diffusion to acquire CO<sub>2</sub> (Raven 1991, Kaplan & Reinhold 1999, Giordano et al. 2005). Cells that actively use CCMs to acquire CO<sub>2</sub>/and or HCO<sub>3</sub><sup>-</sup> are rendered less 'leaky' when surrounded by seawater with higher CO<sub>2</sub> concentrations, causing the regulatory enzymes that operate the CCM to be down-regulated resulting in more energy that can be allocated to support physiological processes such as growth and nutrient assimilation (Burkhardt et al. 2001, Rost et al. 2003). Production and growth of species that rely on passive diffusion should be enhanced by higher ambient CO<sub>2</sub> concentrations. The metabolic processes of microorganisms without CCMs are more likely to be enhanced when CO<sub>2</sub> is increased than those microorganisms with CCMs (Giordano et al. 2005). Under elevated CO<sub>2</sub> conditions, community shifts can occur depending on the effectiveness of the mechanism that microorganisms use to acquire inorganic carbon (Tortell et al. 2002, 2008, Hare et al. 2007, Feng et al. 2009, 2010), translating to differences in uptake rates of other nutrients. In our study, elevated CO<sub>2</sub> concentrations influenced the uptake of NO<sub>3</sub><sup>-</sup>, while urea uptake was unaffected (Fig. 2).

Although few studies have examined how NO<sub>3</sub><sup>-</sup> uptake by microalgae is affected by CO<sub>2</sub> concentration, the response appears to differ between microbial groups. We found this to be true in our study, given that uptake rates of NO<sub>3</sub><sup>-</sup> by smaller microorganisms were significantly higher at elevated CO<sub>2</sub>, while uptake rates of NO<sub>3</sub><sup>-</sup> by larger microorganisms increased, but not significantly. Consistent with our results for smaller microorganisms, Beardall & Koss (unpubl. data described by Beardall et al. 2009) found that uptake of nitrogen was enhanced by elevated CO<sub>2</sub> concentrations in species of phytoplankton; however, the nitrogen substrate and phytoplankton group(s) that they used were not identified. Other mesocosm studies, however, have shown that uptake of NO<sub>3</sub><sup>-</sup> is unaffected by varying levels of CO<sub>2</sub> in communities where diatoms were present (Riebesell et al. 2007) and absent (Engel et al. 2005).

Like urea, the uptake of DIC in both nitrogen diluent types and both size fractions was unaffected under high CO<sub>2</sub>, contrary to several studies (Hutchins et al. 2007, Trimborn et al. 2009). The coastal California ecosystem is an upwelling system charac-

terized by high levels of DIC and low pH (Hauri et al. 2009) with a high degree of temporal variability (Carpone & Hutchins 2013, Leinweber & Gruber 2013) relative to other oceanic regions, making it likely that the microorganisms in our study have already optimized for high CO<sub>2</sub> conditions. Diatoms in particular may be less susceptible to high CO<sub>2</sub> conditions than other microalgae groups because of their efficient CCMs (Tortell et al. 2002, Fabry et al. 2008).

### Nutrient uptake in the Combined treatment

We did not detect a significant additive effect of temperature combined with CO<sub>2</sub> on the uptake of NO<sub>3</sub><sup>-</sup>, urea, or DIC. Even though  $V_{\text{UREA}}$  and  $\rho_{\text{UREA}}$  were significantly higher than the Control in the Combined treatment, this was likely a function of temperature, as the combined effect was not significantly greater than the impact of temperature alone, and urea uptake was unaffected by elevated CO<sub>2</sub>. Our results for DIC uptake in the Combined treatment were also likely driven by temperature. It is interesting that NO<sub>3</sub><sup>-</sup> uptake rates by smaller microorganisms in the Combined treatment were not significantly higher than in the Control, since rates significantly increased when CO<sub>2</sub> alone was manipulated. The smaller microorganisms in the Combined treatment may have preferred other nitrogen substrates (i.e. urea) when both temperature and CO<sub>2</sub> were elevated.

The results for nitrogen and carbon uptake in the Combined treatment indicate that temperature, more so than CO<sub>2</sub>, has the greater potential to change the cycling of urea and DIC in California coastal waters in the future. Uptake of NO<sub>3</sub><sup>-</sup> may increase as a function of elevated CO<sub>2</sub>; however, this relationship may only be important during the winter, when temperatures are cooler. Our results for dissolved and particulate carbon as well as  $\rho_{\text{DIC}}:\rho_{\text{NO}_3}$  ratios in the Combined treatment further suggest that C:N ratios are likely to be different under future climate scenarios.

### Nitrate versus urea

Within the same experimental conditions, uptake rates for urea were significantly greater than uptake rates for NO<sub>3</sub><sup>-</sup> (Figs. 2 & 3). This result was somewhat counterintuitive, given that dissolved concentrations of NO<sub>3</sub><sup>-</sup> were consistently lower than urea (Tables 1 & 2). One possible explanation is active urea regeneration, which would result in higher urea concentrations than if just uptake was taking place. Regenera-

tion of urea can occur on the order of minutes to days (Bronk et al. 2007), and rates of regeneration can be high in natural waters (Bronk et al. 1998). Sources of water column urea regeneration include zooplankton, bacteria, and phytoplankton (reviewed by Solomon et al. 2010). With respect to phytoplankton, some diatoms possess the genes that are necessary to produce urea within their cells (Armbrust et al. 2004, Bowler et al. 2008). It is definitely possible that urea was being rapidly used and regenerated within the ecostats.

Kinetics and different half saturating constants ( $K_s$ ) for NO<sub>3</sub><sup>-</sup> and urea uptake might also explain why urea uptake was higher than NO<sub>3</sub><sup>-</sup> uptake even though dissolved concentrations of NO<sub>3</sub><sup>-</sup> were lower than urea. For genera that were present in this study, including *Leptocylindrus* spp. and *Pseudo-nitzschia* spp.,  $K_s$  values for NO<sub>3</sub><sup>-</sup> uptake typically fall between 1 and 3  $\mu\text{mol N l}^{-1}$  (Eppley et al. 1969, Cochlan et al. 2008, Seeyave et al. 2009). Less is known about urea uptake kinetics, but  $K_s$  values for diatom urea uptake can range considerably (Bronk & Flynn 2006, Solomon et al. 2010 and references therein), falling lower and higher than  $K_s$  values for NO<sub>3</sub><sup>-</sup> uptake. Cochlan et al. (2008) found that  $K_s$  values for NO<sub>3</sub><sup>-</sup> uptake by *P. australis* were lower than  $K_s$  values for uptake of other nitrogen substrates (NH<sub>4</sub><sup>+</sup> and glutamine), and that urea uptake did not follow typical Michaelis-Menten kinetics because uptake was non-saturating. They suggested that some species of diatoms have a relatively higher affinity for NO<sub>3</sub><sup>-</sup>, and are therefore able to more effectively take it up at low concentrations. In our study, it is possible that the microorganisms were taking up urea more quickly, and yet were not as efficient at drawing urea down to the very low concentrations observed for NO<sub>3</sub><sup>-</sup>.

We chose to assess NO<sub>3</sub><sup>-</sup> and urea utilization in this study; however, another substrate of interest is NH<sub>4</sub><sup>+</sup>. Although the microorganisms in the ecostat bottles received considerably less NH<sub>4</sub><sup>+</sup> than either NO<sub>3</sub><sup>-</sup> or urea, which were added to the diluent, concentrations of NH<sub>4</sub><sup>+</sup> were higher than concentrations of both NO<sub>3</sub><sup>-</sup> and urea at the SCMI site (Table 1). Given the reduced nature of NH<sub>4</sub><sup>+</sup>, this substrate is often thought of as the preferred substrate for phytoplankton nitrogen uptake (McCarthy 1981, Glibert et al. 2016 and references therein), and it can inhibit and/or reduce NO<sub>3</sub><sup>-</sup> uptake by phytoplankton depending on a variety of factors including ambient concentrations, environmental conditions, and species composition (Dortch 1990). In our study, it is possible that NO<sub>3</sub><sup>-</sup> uptake rates were reduced due to the presence of NH<sub>4</sub><sup>+</sup>; however, any inhibitory effects were likely

minimal. First, concentrations of NH<sub>4</sub><sup>+</sup> would be low in the ecostats themselves as the diluent was added slowly. Lack of NH<sub>4</sub><sup>+</sup> inhibition can also be inferred by the continually low NO<sub>3</sub><sup>-</sup> concentrations observed throughout the experiment (Table 2), indicating that NO<sub>3</sub><sup>-</sup> was readily being used. It would be useful to include NH<sub>4</sub><sup>+</sup> as a substrate in future ecostats, because of the dynamic interactions that occur between NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, and urea with respect to microbial utilization and availability. For example, concentrations of NH<sub>4</sub><sup>+</sup> can also increase as urea undergoes hydrolysis (Solomon et al. 2010). It would also be worthwhile to repeat the study and measure urea uptake in the treatments that received NO<sub>3</sub><sup>-</sup> diluent and vice versa. We were unable to do this due to water limitations; however, background concentrations of both NO<sub>3</sub><sup>-</sup> and urea were present in the diluent, and the low concentrations of both NO<sub>3</sub><sup>-</sup> and urea measured at the end of the experiment in all treatments, regardless of the diluent type, indicate that the microorganisms in our study were using all of these nutrients.

In the Southern California Bight, it is particularly important to consider possible future changes in the nature and magnitude of nitrogen utilization by diatoms. The production of toxins by HAB species, such as the *Pseudo-nitzschia* diatoms that frequently bloom off the coast of California, is influenced by nutrient availability. *Pseudo-nitzschia* are capable of utilizing both inorganic and organic forms of nitrogen, including urea, as a source for growth (Cochlan et al. 2008, Loureiro et al. 2009, Auro & Cochlan 2013), and several factors including nutrient limitation, nitrogen source, and CO<sub>2</sub> can influence domoic acid production (Bates et al. 1998, Pan et al. 1998, Bates & Trainer 2006 and references therein, Sun et al. 2011, Sahraoui et al. 2012, Tatters et al. 2012). In this study, *Pseudo-nitzschia* spp. were present throughout the experiment, and domoic acid was detected in all treatments, with varying cellular toxin levels (A. O. Tatters et al. unpubl.).

## CONCLUSIONS

Here we provide evidence that uptake of NO<sub>3</sub><sup>-</sup>, urea, and DIC is enhanced by changes in temperature and/or CO<sub>2</sub>. Our results suggest that uptake of urea and DIC by phytoplankton communities dominated by diatoms will increase as sea surface temperatures rise in temperate nearshore environments. Although smaller microorganisms were a minor component of the community that we studied, we found that rates of NO<sub>3</sub><sup>-</sup> uptake by smaller microorganisms

increased as a function of elevated CO<sub>2</sub> concentration. This relationship may be even more important during the winter, or if elevations in temperature and CO<sub>2</sub> are decoupled in the future, or when smaller microorganisms are more dominant. The relationship that we found between elevated temperature and urea uptake rates indicate that urea will be more rapidly utilized if warming continues. Additionally, the increased DIC to NO<sub>3</sub><sup>-</sup> uptake ratios that occurred at elevated temperature indicate that C:N stoichiometry may also be altered in the future. Finally, this study demonstrates that uptake trends of nitrogen substrates under climate change conditions cannot be generalized, and it is important to study multiple substrates to gain a more comprehensive view of potential change.

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