Dataset: Removal of organic carbon by natural bacterioplankton communities as a function of pCO2 from laboratory experiments between 2012 and 2016

Project(s): Will high CO2 conditions affect production, partitioning and fate of organic matter? (OA - Effects of High CO2)

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 CO2. 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 pCO2 on bacterioplankton removal of several forms of DOC ranging from glucose to complex phytoplankton exudate and lysate, and naturally occurring DOC. Elevated pCO2 (1000 – 1500 ppm) enhanced both the rate and magnitude of organic carbon removal by bacterioplankton communities compared to low (pre-industrial and ambient) pCO2 (250 – ~400 ppm). The increased removal was largely due to enhanced respiration, rather than enhanced production of bacterioplankton biomass. For a complete list of measurements, refer to the supplemental document 'Field_names.pdf', and a full dataset description is included in the supplemental file 'Dataset_description.pdf'. The most current version of this dataset is available at: http://www.bco-dmo.org/dataset/472032

Description: Data Set #3A: Utilization of dissolved organic carbon by a natural bacterial community as a function of pCO2

This dataset includes results of laboratory experiments which measured dissolved organic carbon (DOC) usage by natural bacteria in seawater at different pCO2 levels. Included in this dataset are; bacterial abundance, total organic carbon (TOC), what DOC was added to the experiment, target pCO2 level. The experiments were conducted between 2012 and 2016 during the R/V Kilo Moana cruise KM1416, at the Bermuda Institute for Ocean Sciences (BIOS), and the University of Santa Barbara.

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 CO2. 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 pCO2 on bacterioplankton removal of several forms of DOC ranging from glucose to complex phytoplankton exudate and lysate, and naturally occurring DOC.

Elevated pCO2 (1000 – 1500 ppm) enhanced both the rate and magnitude of organic carbon removal by bacterioplankton communities compared to low (pre-industrial and ambient) pCO2 (250 – ~400 ppm). The increased removal was largely due to enhanced respiration, rather than enhanced production of bacterioplankton biomass.

Acquisition Description: **TOC measurements:**

The procedures used to set up each experiment (inoculum filtration and dilution with 0.2 um 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 inoculum. Ideally, samples collected for organic carbon would be filtered in order to directly assess DOC removal separate from bacterioplankton carbon production over the course of the incubations. 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 total organic carbon (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. 2010.

Bacterioplankton abundance measurement – Samples for bacterioplankton abundance were analyzed by epifluorescence microscopy with 0, 6-diamidino -2-phenyl dihydrochloride (5ug/mL, DAPI, SIGMA-Aldrich, St. Louis, MO, USA) according to Porter and Feig 1980, or by Flow Cytometry (FCM) on an LSR II with SYBR Green I according to Nelson et al. 2011. See Parsons et al. 2014 and Nelson et al. 2011 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 (Glockner et al. 1999).

Water sources:

Experiment OA11 was conducted on board a research cruise R/V Kilo Moana KM1416. The Sargasso Sea experiments were conducted at the Bermuda Institute

for Ocean Sciences (BIOS) with water was collected via the R/V Atlantic Explorer. The Santa Barbara Channel experiments were conducted with water collected near-shore via a pier near the UCSB campus.

Experimental design:

At all three study sites, experiments consisted of 0.2 um-filtered (0.2 um GSWP, Millipore, Billerica, MA) seawater or 0.2 um-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 um filtrate (Santa Barbara Channel experiments; 1.2 um RAWP, Millipore, Billerica, MA). Particulate organic carbon concentration in oligotrophic gyres is low (1-3 umol 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, 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.

Four types of DOC treatments were used and are described in the data as "doc_additions":

1. None: unamended seawater, which provided naturally occurring DOC.
2. CNP: Naturally occurring DOC amended with glucose (~10 uM C) plus NH4 Cl (1uM) and K2HPO4 (0.1uM) (CNP)
3. Species name + " exudate": phytoplankton exudate
4. Species name + " lysate": naturally occurring DOC amended with phytoplankton lysate (~10 uM C L-1; labeled by phytoplankton species used).

The various treatments were generated by inoculating the 0.2 um pre-filtered seawater or exudate with the microbial community; this solution was then divided into two polycarbonate (PC) containers to adjust pCO2. pCO2 levels were adjusted via chemical additions (Sargasso Sea experiment) or by bubbling with CO2-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. All PC bottles had been acid-washed (5 % or 10 % HCL) and rinsed with deionized distilled water and sample water before use.

Processing Description: Experiment refers to the experiment name; sites refer to the Sargasso Sea, the Santa Barbara Channel (SBC) and the South Pacific Subtropical Gyre (SPSG); bacterial abundance; standard error and standard deviation. Toc refers to measurements of total organic carbon, for which the units are uM C.

DMO Processing Notes:

* New data version 28 Nov 2016 replaces previous data version from 21 Nov 2013. This version includes more exprimental runs. Data parameter names vary between the two data versions. This version also added lat/lon locations for sample sites.
* New data version 5 Nov 2016 which includes updated data for experiment O15.
* added a conventional header with dataset name, PI name, version date
* modified parameter names to conform with BCO-DMO naming conventions
* Data values of "None" replaced with "nd" meaning no data.
* Date format changed from mm.dd.yyyy to ISO date format yyyy-mm-dd
* More exact lat/lon value of 34.4070,-119.8433 for SBC, supplied by Anna James.

Deployment Information

Deployment description for UCSB MSI Passow UCSB_Passow_OA

Samples were collected on a pier with a bucket.

Instrument Information

Instrument	modified Shimadzu TOC-L
Description	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. 2010.
Generic	

Instrument Name	Shimadzu TOC-V Analyzer
Generic Instrument Description	A Shimadzu TOC-V Analyzer measures DOC by high temperature combustion method.

Instrument	Flow Cytometry (FCM)
Description	Flow Cytometry (FCM) on an LSR II with SYBR Green I according to Nelson et al. 2011.
Generic Instrument Name	Flow Cytometer
Generic Instrument Description	Flow cytometers (FC or FCM) are automated instruments that quantitate properties of single cells, one cell at a time. They can measure cell size, cell granularity, the amounts of cell components such as total DNA, newly synthesized DNA, gene expression as the amount messenger RNA for a particular gene, amounts of specific surface receptors, amounts of intracellular proteins, or transient signalling events in living cells. (from: http://www.bio.umass.edu/micro/immunology/facs542/facswhat.htm)

Instrument	Epifluorescence microscopy
Description	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 1980.
Generic Instrument Name	Microscope-Fluorescence
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of fluorescence and phosphorescence instead of, or in addition to, reflection and absorption of visible light. Includes conventional and inverted instruments. From: http://vocab-mappings.whoi.edu/taxonomy/term/109

Instrument	modified Shimazdu TOC-L

Description	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. 2010.
Generic Instrument Name	Shimadzu TOC-L Analyzer
Generic Instrument Description	A Shimadzu TOC-L Analyzer measures DOC by high temperature combustion method. Developed by Shimadzu, the 680 degree C combustion catalytic oxidation method is now used worldwide. One of its most important features is the capacity to efficiently oxidize hard-to-decompose organic compounds, including insoluble and macromolecular organic compounds. The 680 degree C combustion catalytic oxidation method has been adopted for the TOC-L series. http://www.shimadzu.com/an/toc/lab/toc-l2.html