Triple oxygen isotopes of respiration and photo-oxidation of DOC

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Project
» Clumped Oxygen Isotope Signature of Marine Dissolved Oxygen (Microbial isotope effects)

Contributors	Affiliation	Role
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Abstract
The biogeochemical fluxes that cycle oxygen (O2) play a critical role in regulating Earth's climate and habitability. Triple-oxygen isotope (TOI) compositions of marine dissolved O2 are considered a robust tool for tracing oxygen cycling and quantifying gross photosynthetic O2 production. This method assumes that photosynthesis, microbial respiration, and gas exchange with the atmosphere are the primary influences on dissolved O2 content, and that they have predictable, fixed isotope effects. Despite its widespread use, there are major elements of this approach that remain uncharacterized, including the TOI dynamics of respiration by marine heterotrophic bacteria and abiotic O2 sinks such as the photochemical oxidation of dissolved organic carbon (DOC). Here, we report the TOI fractionation for O2 utilization by two model marine heterotrophs (Vibrio harveyi and Ruegeria pomeroyi DSS-3) and by abiotic photo-oxidation of representative terrestrial and coastal marine DOC. These data are described further in the related publication, Sutherland et al., 2022 (doi: 10.1093/pnasnexus/pgac233).

Table of Contents

Methods & Sampling

Microbial cultures:
Cultures of *Vibrio harveyi* were grown in Tibbles-Rawlings minimal media and cultures of *Ruegeria pomeroyi* DSS-3 were grown in K media. Agar plates of each axenic culture were inoculated with a freezer stock and visually inspected for culture purity. A single colony from each plate was used to inoculate 50 milliliters (mL) of liquid media in Erlenmeyer flasks, which were incubated in the dark on an orbital shaker at 100 rotations per minute (rpm). Cells were transferred fresh media during mid exponential growth phase (as determined by optical density at 600 nanometers (nm)) for a minimum of two generations prior to incubation for analysis. For O2 utilization experiments, several mL of mid exponential cell culture was aseptically added to several 500 mL serum vials, which were filled with fresh media (filter sterilized to avoid the production of O2-consuming byproducts, equilibrated overnight at room temperature on a stir plate to ensure oxygen isotopic equilibrium with lab air) then sealed with no headspace. The incubations were conducted at room temperatures, which range from 21 to 23 degrees Celsius. A typical incubation included between 4 and 6 bottles, which were sacrificially sampled at each time point. At the desired sampling time, sample liquid containing dissolved O2 was transferred to mercury-poisoned bottle.

DOC experiments:
Dissolved organic carbon (DOC) used in this study was sourced from the Suwannee River, Georgia and Martha's Vineyard Sound, MA, USA. Suwannee River DOC was prepared from a freeze-dried reverse osmosis isolate provided by the International Humic Substance Society (#2R101N; 84% recovery). The isolate was reconstituted in ultra-pure water in a 4-liter (L) precombusted amber glass jug at a concentration of 20 milligrams per liter (mg L-1) (9.7 ± 0.2 mg-C L-1; n = 3). The solutions were adjusted to pH 7.0 ± 0.1 and allowed to equilibrate with the atmosphere on a stir plate for 24 hours prior to filtration with a 0.2 micrometer (µm) Sterivex filter (Milli- pore) and use in photochemical incubations.

Martha's Vineyard Sound seawater was pumped from ~300 meters (m) offshore (41.530668, -70.645629) at a depth of ~4 meters into the Environmental Systems Laboratory (ESL) at Woods Hole Oceanographic Institution (WHOI, Woods Hole, MA, USA). At high tide in June of 2021, ~210 L of seawater was filtered through precleaned (200 L of RO and 100 L of DI water) 0.2 µm ultra-pleat in-line filters (Big Brand Water Filter, Inc.). The seawater DOC was collected in 12 five-gallon, acid-rinsed (10% trace metal grade hydrochloric acid; Fisher Scientific), and precleaned (10x rinses with RO and MQ water) polypropylene buckets. The DOC was isolated using six 5 g PPL cartridges (Agilent Technologies). The methanol eluent from the six cartridges was pooled into one sample. DOC recovery was measured by spiking 100 microliters (µL) of the pooled eluent into a precombusted TOC vial, drying over high-purity N2 (Airgas, Inc.), reconstituting in DI water, and quantifying using a Shimadzu 5000A TOC analyzer. DOC recovery was 44% ± 3% (n = 3). This recovery is ~15% lower than reported for Brazilian coastal waters. This discrepancy was expected provided the 10× higher C-loading rate used in this study compared to Dittmar et al. (0.4 vs. 0.04 mmol-C g−1 PPL resin), and the well-documented decline in DOC recovery with increased C-loading rates. Martha's Vineyard Sound DOC SPE eluent was subsequently used to create concentrated solutions of coastal DOC (14.6 ± 0.2 mg-C L−1; n = 3). The eluent was added to a 4 L pre-combusted amber glass jug, dried over high-purity N2, and reconstituted in 0.2 µm filtered Martha’s Vineyard Sound seawater. The coastal DOC solutions were allowed to equilibrate with the atmosphere on a stir plate for 24 hours prior to refiltration with a 0.2 µm Sterivex filter and use in photochemical incubations.

The working solutions of terrestrial and coastal DOC were chemically characterized using optical spectroscopy. Specifically, optical proxies for molecular weight and aromaticity were measured [E2:E3; slope ratio (SR); specific UV absorbance at 254 nm (SUVA254)]. UV-visible light absorbance was determined using a Perkin Elmer Lambda 650 s spectrophotometer, whereas DOC concentration was determined as previously described. The optical proxy values determined in this study for terrestrial and coastal marine DOC strongly aligned with those reported in previous studies. The DOC sources were chemically distinct; terrestrial DOC exhibited notably higher molecular weight than coastal DOC.

TOI analysis:
After each incubation (either microbial or photochemical), the seal on each closed bottle was opened and an O2 electrode was inserted for 15 seconds to get an approximate dissolved O2 concentration for later comparison with O2: Ar ratio to ensure no atmospheric contamination. Roughly half of sample liquid was then siphoned out from the bottom of the sample bottle into a preevacuated, prepoisoned (with 500 uL saturated HgCl2 solution) 1 L custom-built glass bottle affixed with a high-vacuum stopcock, similar to previous studies. Method blanks were conducted with O2-free water (bubbled vigorously with helium for 1 hour) to ensure the above methods did not introduce atmospheric contamination. We found the method blank to be sufficiently low as to not influence oxygen isotope measurements within the precision reported in this study (n = 2). The sample bottle was vigorously shaken and allowed to equilibrate overnight. Following equilibration, the sample bottle was inverted, and the degassed sample liquid was evacuated, save 1 to 2 mL of residual liquid to ensure no sample gas was removed. The sample bottle was submerged in a slurry of dry ice and ethanol to freeze all remaining liquid, and the sample gas was introduced to a vacuum line for purification.

Oxygen gas was separated from N2 and Ar using a 3 m gas chromatography (GC) column packed with 5 Å molecular sieve held at -80 degrees C. The integrated peak area (measured by thermal conductivity detector) of the separated O2 and Ar peaks was used to determine the fraction of the starting O2 that remained at each time point. The effluent O2 was collected on silica gel held at at -196 degrees C and passed to one final silica gel cryofocus. The sample was then transferred to a Thermo Scientific MAT 253 Plus isotope ratio mass spectrometer (IRMS).

Each reported analysis is the average value of four acquisition blocks, each consisting of 20 cycles between the reference gas and sample gas (total counting time on sample gas was 400 seconds per acquisition). Measurements were typically analyzed at 5000 mV signal on the m/z 32 Faraday cup (3 × 10^8 resistor). Total acquisition time for a single analysis is roughly 2 hours. All values of δ17O and δ18O were converted to a VSMOW2-SLAP2 scale using a two point calibration of O2 liberated form VSMOW2 and SLAP2 standards using a CoF3 reactor. All slopes (i.e. λ values) and slope uncertainties were calculated as linear fits to plots of δ17O

vs. δ18O using the polyfit function in MATLAB (linear least-squares). Typical reproducibility of Ar-free lab air is 0.005‰ for D17O and 0.02 ‰ for δ18O(1σ, n = 10).

Data Processing Description

All isotope data was normalized to international standards as noted above.

BCO-DMO Processing Description

- Imported original file "Sutherland PNAS Nexus.xlsx" into the BCO-DMO system.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved final file as "923821_v1_toi_resp_and_photo-oxidation_doc.csv".

[table of contents | back to top]

Related Publications

Dittmar, T., Koch, B., Hertkorn, N., & Kattner, G. (2008). A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater. Limnology and Oceanography: Methods, 6(6), 230–235. doi:10.4319/lom.2008.6.230
Methods

Sutherland, K. M., Johnston, D. T., Hemingway, J. D., Wankel, S. D., & Ward, C. P. (2022). Revised microbial and photochemical triple-oxygen isotope effects improve marine gross oxygen production estimates. PNAS Nexus, 1(5). https://doi.org/10.1093/pnasnexus/pgac233
Results

[table of contents | back to top]

Parameters

Parameter	Description	Units
Sample_ID	Experimental label	unitless
d18O_smowslap	The 18O/16O ratio on a SMOW scale	permil
d17O_smowslap	The 17O/16O ratio on a SMOW scale	permil
d18O_smowslap_log	The 18O/16O ratio on a SMOW scale, log form	unitless
d17O_smowslap_log	The 17O/16O ratio on a SMOW scale, log form	unitless
D17O	The capital delta value	permil
O2_Ar	The oxygen to argon ratio	unitless
lnO2_Ar	The log of the oxygen to argon ratio	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	gas chromatography
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Oxygen gas was separated from N2 and Ar using a 3 m gas chromatography (GC) column.
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Thermo Scientific MAT 253 Plus isotope ratio mass spectrometer
Generic Instrument Name	Isotope-ratio Mass Spectrometer
Generic Instrument Description	The Isotope-ratio Mass Spectrometer is a particular type of mass spectrometer used to measure the relative abundance of isotopes in a given sample (e.g. VG Prism II Isotope Ratio Mass-Spectrometer).

[table of contents | back to top]

Project Information

Clumped Oxygen Isotope Signature of Marine Dissolved Oxygen (Microbial isotope effects)

NSF Award Abstract:
The balance between photosynthetic production of oxygen and biological consumption of oxygen in the marine environment plays a critical role in regulating the composition of Earth's atmosphere and the long-term stability of Earth's climate. The ability to accurately measure the production and consumption of oxygen in the marine environment is central to building an informed understanding of the past, present, and future of oxygen and Earth's climate. Measurement of the abundance of different oxygen isotopes (i.e. oxygen-16, oxygen-17, and oxygen-18) in dissolved oxygen in seawater is a powerful analytical tool that can be used to determine the magnitude of photosynthesis and biological oxygen consumption. This is possible because biological reactions that produce or consume oxygen tend to preferentially utilize different isotopes of oxygen. This analytical tool has been used for two decades to investigate ocean primary productivity, however, there is still significant uncertainty in how biogeochemical processes such as respiration preferentially select and utilize different oxygen isotopes. To remedy this uncertainty, researchers at Harvard University will perform a lab-based calibration and sea-going field deployment of emerging oxygen isotope analyses that target molecules of oxygen that contain two rare oxygen isotopes inside of one molecule (a.k.a. "clumps", i.e. 17O18O and 18O18O). This work will quantify the clumped oxygen isotope signatures of several of the most consequential oxygen-involving reactions that occur in the marine ecosystem. These oxygen isotope signatures will be used to refine current methods and assumptions for the measurement of gross oxygen production in the global ocean. This research will also help train the next generation of Earth scientists through the mentorship of one graduate student and two undergraduate students. This project will also facilitate the participation of researchers in content creation for a national science competition for middle and high school students that reaches thousands of students annually.

The application of clumped O2 isotope measurements to dissolved oxygen in seawater is poised to give a greater mechanistic view of gross primary productivity and marine oxygen cycling than previously attainable. To realize the analytical potential of clumped oxygen isotope methods, these researchers will characterize the clumped oxygen isotope effects associated with enzyme-level reactions, whole organisms, and the marine water column. Enzyme-level studies will include isotope characterization of a terminal-O2 reductase and enzymes that metabolize reactive oxygen species such as superoxide and hydrogen peroxide. Organism studies will target common and numerically abundant phototrophs and heterotrophs to explore the expected breadth of isotope signatures and fractionation factors in the marine water column. Marine water column dissolved oxygen samples will be collected during one of multiple candidate cruises and analyzed using both traditional triple oxygen isotope techniques and newly developed clumped oxygen isotope methods. Lastly, they will employ the results of lab-based study of clumped O2 to build a model for the interpretation of environmental data with the aim of improving the accuracy and precision of field measurements of gross primary production.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2049298

[table of contents | back to top]