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EFFECT OF ACIDIFIED VERSUS FROZEN STORAGE ON MARINE DISSOLVED ORGANIC CARBON CONCENTRATION AND ISOTOPIC COMPOSITION

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ABSTRACT. The standard procedure for storing/preserving seawater dissolved organic carbon (DOC) samples after field collection is by freezing (−20°C) until future analysis can be made. However, shipping and receiving large numbers of these samples without thawing presents a significant logistical problem and large monetary expense. Access to freezers can also be limited in remote field locations. We therefore test an alternative method of preserving and storing samples for the measurement of DOC concentrations ([DOC]), stable carbon (δ¹³C), and radiocarbon (δ¹⁴C) isotopic values via UV photooxidation (UVox). We report a total analytical reproducibility of frozen DOC samples to be [DOC] ± 1.3 μM, Δ¹⁴C ± 9.4‰, and δ¹³C ± 0.1‰, comparable to previously reported results (Druffel et al. 2013). Open Ocean DOC frozen versus acidified duplicates were on average offset by ΔDOC ± 1.1 μM, ΔΔ¹⁴C ± −1.3‰, and Δδ¹³C ± −0.1‰. Coastal Ocean frozen vs. acidified sample replicates, collected as part of a long-term (380-day) storage experiment, had larger, albeit consistent offsets of ΔDOC ± 2.2 μM, ΔΔ¹⁴C ± 1.5‰, and Δδ¹³C ± −0.2‰. A simple isotopic mass balance of changes in [DOC], Δ¹⁴C, and δ¹³C values reveals loss of semi-labile DOC (2.2 ± 0.6 μM, Δ¹⁴C = −94 ± 105‰, δ¹³C = −27 ± 10‰; n = 4) and semi-recalcitrant DOC (2.4 ± 0.7 μM, Δ¹⁴C = −478 ± 116‰, δ¹³C = −23.4 ± 5.0‰; n = 3) in Coastal and Open Ocean acidified samples, respectively.

KEYWORDS: Dissolved organic carbon, chemical analysis, radiocarbon dating, stable isotopes, seawater.

INTRODUCTION

Dissolved organic carbon (DOC) is the largest organic carbon reservoir in the ocean and plays a central role in the marine carbon cycle. Increasing numbers of DOC Δ¹⁴C measurements have been made in the past few years, providing new insight into the sources and cycling of DOC molecules. Measurements of DOC isotopic (Δ¹⁴C and δ¹³C) composition, in addition to DOC concentrations ([DOC]), have allowed for an unprecedented view of the biogeochemical cycling of DOC.

Ultraviolet photooxidation (UVox) of seawater DOC for ¹⁴C analysis has been used for decades; Williams et al. (1969) were the first to report oceanic DOC ¹⁴C results. Recently, there has been renewed interest in improving upon the UVox technique and several different UVox systems have been developed to address the oceanographic community’s need for more DOC Δ¹⁴C measurements with small C blanks, small sample volumes, and high (>1 × per day) sample throughput (Beaupre et al. 2007; Xue et al. 2015). These improvements necessitate continuous evaluation of sample preservation techniques, UVox methodologies, and isotopic measurements, such that [DOC], Δ¹⁴C, and δ¹³C isolated from each UVox system can be considered intercomparable.

A primary logistical challenge for all DOC studies is freezing samples in the field and shipping frozen samples quickly to the laboratory such that DOC molecules are not sufficiently altered (either by compositional changes or respiration by residual microbial communities) prior to analysis. To overcome the challenges associated with storing and shipping large volumes of frozen DOC samples, a few studies have preserved DOC samples via acidification (Gasol et al. 2009; Griffith et al. 2012; Calleja et al. 2013; Ruiz-Halpern et al. 2014). The National Science Foundation–sponsored DOC Consensus Reference Materials (CRMs) program also preserves reference DOC waters with hydrochloric acid. These CRMs are verified to provide consistent [DOC] for up to 2 years (http://yyy.rsmas.miami.edu/groups/biogeochem/CRM.html). However, the addition of hydrochloric acid (HCl) can be limiting for DOC molecular level and Δ¹⁴C
analyses. HCl is a strong acid that can hydrolyze and/or induce compositional changes to ambient DOC molecules. The addition of excess Cl− is also undesirable for 14C studies since it may affect the reduction of sample CO2 to graphite (Vogel et al. 1984, 1987). Very few studies have reported [DOC] and/or Δ14C measurements from samples preserved with a small aliquot of 85% phosphoric acid (H3PO4), which is much weaker and should not present as many problems for Δ14C analysis (Sharp et al. 2002; Griffith et al. 2012).

The first study to evaluate freezing vs. acidiﬁed storage for seawater [DOC] measurements was Sugimura and Suzuki (1988). However, this early work was later retracted (Suzuki 1993). A subsequent study by Tupas et al. (1994) represents the ﬁrst rigorous evaluation of sample containers and preservation techniques for DOC analysis—including acidification with H3PO4 and dark, cold storage. Tupas et al. found that acidification (50 µL of 50% H3PO4) and cold (4°C) storage of ﬁltered seawater, collected into precombusted 10-mL ampoules, gave statistically identical results to samples collected in acid-cleaned (10% HCl), high-density polyethylene (HDPE) bottles that were flash-frozen using liquid nitrogen and stored at −20°C. It was perhaps this ﬁrst rigorous evaluation that proposed H3PO4 acidification as a viable alternative to frozen DOC sample storage. It should be noted, however, that high-temperature combustion (HTC) instruments at the time of the Tupas et al. study had relatively low measurement precision (±3 µM) and high analytical C blanks (~19 µM). These large measurement errors and analytical blanks likely precluded the observance of small (<3 µM) differences in frozen vs. acidiﬁed seawater [DOC].

To the best of our knowledge, the effect of long-term storage of DOC samples preserved by freezing versus H3PO4 acidification has not been re-evaluated since these initial studies. These early comparisons also reported lower instrument precision and higher C blanks than UVOx and HTC systems currently used by the community. The effect of long-term acidified storage on the carbon isotopic (Δ14C, δ13C) composition of DOC has also never been directly tested. Our current instrument precision for individual UVOx DOC measurements is 0.2–0.5 µM, and the total analytical uncertainty of multiple sample replicates is ~1.3 µM. This improved instrument precision and analytical uncertainty allows for a more detailed study of the effect of acidified sample storage on seawater [DOC], Δ14C, and δ13C values.

In this study, we ﬁrst revisit our reproducibility of UVOx [DOC], Δ14C, and δ13C on frozen seawater samples. Second, we evaluate the changes in open ocean (Open Ocean samples in this study) [DOC], Δ14C, and δ13C values as preserved with H3PO4 and stored in the dark at room temperature. We compare these H3PO4-treated samples to frozen replicates stored for 59 to 286 days. Third, we compare [DOC], Δ14C, and δ13C values from acidiﬁed vs. frozen sample replicates as part of a long-term (380-day) coastal water (Coastal Ocean samples) storage experiment. Finally, we discuss these results in the context of mechanisms of DOC loss via potential biological reactivity, humic acid precipitation, and/or decarboxylation in the Coastal vs. Open Ocean samples.

METHODS

Frozen Open Ocean sample duplicates were collected from the Gulf of Mexico, Station ALOHA, and CLIVAR/GO-SHIP line P16N (Table 1). Acidified and frozen sample replicate pairs were collected from the South Paciﬁc and North Atlantic as part of CLIVAR/GO-SHIP lines P16N and A16N (Table 2). Coastal sample replicates (n = 10) for the long-term storage test were collected from Newport Beach Pier (NBP) in Newport Beach, California, on 16 September 2014 (Table 3).
Table 1 Frozen replicate sample [DOC], Δ¹⁴C, and δ¹³C values. In the case of Newport Beach Pier (NBP) samples, determined ΔDOC, ΔΔ¹⁴C, and Δδ¹³C values (italics) represent the standard deviation of n = 5 frozen replicates. All others are subtracted duplicate values. Δdays is the time between sample collection and UVox measurement in days. [DOC] errors listed above (±a) represent the propagated errors of individual [DOC] measurements. Similarly, Δ¹⁴C (±b) represent the propagated Δ¹⁴C errors for either individual AMS measurements, or the total reproducibility of primary standards (OX-I), whichever was highest. These [DOC] and Δ¹⁴C errors should not be confused with our total analytical reproducibility for DOC and Δ¹⁴C measurements, which are higher (~1 µM and ~4‰). The average (avg), 1σ standard deviation (±), and standard error of the mean (SEM) for ΔDOC, ΔΔ¹⁴C, and Δδ¹³C offsets are reported in bold italics.

| Location or Cruise | UCIAMS# | UCID# | Depth (m) | Filtered? (y/n) | UVox date (mm/dd/yy) | Δdays | Treatment | DOC [µM] | Δ¹⁴C (‰) ±a | δ¹³C (‰) ±b | ΔDOC [µM] | ΔΔ¹⁴C (‰) | Δδ¹³C (‰) |
|-------------------|---------|-------|-----------|-----------------|----------------------|-------|-----------|----------|--------------|-------------|----------|----------|-----------|
| NBP, CA 150739    | 18694   | 1     | 10/29/14  | 43 frozen       | 74.1 0.5 ~233.3 1.5 ~21.4 |
| NBP, CA 150759    | 18893   | 1     | 12/3/14   | 78 frozen       | 73.6 0.5 ~227.4 1.5 ~21.3 |
| NBP, CA 158125    | 19067   | 1     | 4/9/15    | 205 frozen      | 76.1 0.5 ~219.5 3.5 ~20.9 |
| NBP, CA 164221    | 19237   | 1     | 6/24/15   | 281 frozen      | 74.3 0.5 ~227.3 1.6 ~21.1 |
| NBP, CA 164610    | 19436   | 1     | 9/30/15   | 379 frozen      | 74.5 0.5 ~227.4 2.6 ~21.3 0.9 4.9 0.2 |
| Gulf of Mexico    | 158132  | 19071 | 1500 n    | 4/16/15         | 38.4 0.3 ~452.1 3.5 ~21.5 |
| Gulf of Mexico    | 158136  | 19075 | 1500 n    | 4/21/15         | 40.0 0.3 ~456.9 3.5 ~21.7 1.6 4.8 0.2 |
| ALOHA 164244      | 19260   | 3500 n| 8/17/15   | 101 frozen      | 36.2 0.3 ~548.0 1.3 ~22.3 |
| ALOHA 164245      | 19261   | 3501 n| 8/18/15   | 102 frozen      | 37.7 0.3 ~536.4 1.2 ~22.3 1.5 11.6 0.0 |
| ALOHA 164250      | 19419   | 201 y | 8/21/15   | 105 frozen      | 59.5 0.4 ~279.1 1.3 ~21.1 |
| ALOHA 164251      | 19420   | 201 y | 8/22/15   | 106 frozen      | 62.3 0.4 ~302.9 1.2 ~21.5 2.8 23.8 0.3 |
| CLIVAR P16N 164252| 19421   | 2900 n| 8/25/15   | 117 frozen      | 34.0 0.2 ~535.5 1.3 n.d. |
| CLIVAR P16N 164253| 19422   | 2900 n| 8/26/15   | 118 frozen      | 32.6 0.2 ~540.9 1.7 ~21.4 ~1.4 5.4 n.d. |
| CLIVAR P16N 164605| 19425   | 2400 n| 9/3/15    | 117 frozen      | 33.0 0.2 ~547.7 2.6 ~21.8 |
| CLIVAR P16N 164261| 19430   | 2400 n| 9/14/15   | 128 frozen      | 35.4 0.3 ~553.7 1.4 ~21.8 2.4 6.0 0.0 |

avg  1.3  9.4  0.1
±   1.5  7.5  0.1
SEM  0.6  3.1  0.1
Table 2: Open Ocean acid vs. frozen replicate sample [DOC], $\Delta^{14}$C and $\delta^{13}$C values. [DOC] errors listed above (±a) represent the propagated errors of individual [DOC] measurements. Similarly, $\Delta^{14}$C (±b) represent the propagated $\Delta^{14}$C errors for either individual AMS measurements, or the total reproducibility of primary standards (OX-I), whichever was highest. These [DOC] and $\Delta^{14}$C errors should not be confused with our total analytical reproducibility for DOC and $\Delta^{14}$C measurements, which are higher (~1 µM and ~4‰). Individual $\Delta$DOC, $\Delta\Delta^{14}$C, and $\Delta\delta^{13}$C offset values were determined by subtraction (frozen - acid). The average (avg), 1σ standard deviation (±) and standard error of the mean (SEM) for $\Delta$DOC, $\Delta\Delta^{14}$C and $\Delta\delta^{13}$C offsets are reported in bold italics. Measurements that were not determined are indicated (n.d.).

| Location | Cruise | UCIAMS# | UCID# | Depth (m) | Filtered? (y/n) | UVox date (mm/dd/yy) | Δdays | Treatment | DOC [µM] ± a | $\Delta^{14}$C (‰) ± b | $\delta^{13}$C (‰) | $\Delta$DOC [µM] | $\Delta\Delta^{14}$C (‰) | $\Delta\delta^{13}$C (‰) |
|----------|--------|---------|-------|-----------|-----------------|---------------------|-------|----------|-------------|-----------------|----------------|----------------|----------------|----------------|----------------|
| CLIVAR   | P16N   | 164224  | 19241 | 2934      | n               | 7/9/15              | 89    | acid     | 33.6 0.2  | -540.4 1.3    | -21.8         |                |                |                |                |
| CLIVAR   | P16N   | 164225  | 19242 | 2934      | n               | 7/10/15             | 90    | frozen   | 36.7 0.2  | -542.8 1.2    | -22.1         | 3.1         | -2.4         | -0.3          |
| CLIVAR   | P16N   | 164237  | 19255 | 3197      | n               | 8/4/15              | 114   | acid     | 33.1 0.2  | -540.8 1.2    | -21.9         |                |                |                |                |
| CLIVAR   | P16N   | 164238  | 19256 | 3197      | n               | 8/5/15              | 115   | frozen   | 34.9 0.2  | -530.8 1.1    | -21.8         | 1.8         | 10.0         | 0.1           |
| CLIVAR   | P16N   | 164257  | 19426 | 10 y      | y               | 9/8/15              | 142   | acid     | 61.3 0.5  | -262.8 1.5    | -21.2         |                |                |                |                |
| CLIVAR   | P16N   | 164258  | 19427 | 10 y      | y               | 9/9/15              | 143   | frozen   | 63.5 0.5  | -271.6 1.4    | -21.3         | 2.2         | -8.8         | -0.1          |
| CLIVAR   | P16N   | 164242  | 19258 | 33 y      | y               | 8/11/15             | 109   | acid     | 61.6 0.4  | -272.0 1.3    | -21.3         |                |                |                |                |
| CLIVAR   | P16N   | 164246  | 19262 | 33 y      | y               | 8/19/15             | 117   | frozen   | 61.4 0.4  | -275.3 1.5    | n.d.          | -0.2        | -3.3         | n.d.          |
| CLIVAR   | P16N   | 164222  | 19239 | 1999      | n               | 7/7/15              | 59    | acid     | 33.5 0.2  | -549.7 1.4    | -21.8         |                |                |                |                |
| CLIVAR   | P16N   | 164223  | 19240 | 1999      | n               | 7/8/15              | 60    | frozen   | 33.4 0.2  | -548.3 1.5    | -21.7         | -0.1        | 1.4          | 0.1           |
| CLIVAR   | A16N   | 139440  | 17979 | 1525      | n               | 1/15/14             | 157   | acid     | 43.8 0.3  | -375.3 2.0    | -21.7         |                |                |                |                |
| CLIVAR   | A16N   | 141179  | 18294 | 1525      | n               | 5/7/14              | 269   | frozen   | 43.8 0.3  | -380.3 2.4    | -21.8         | 0.0         | -5.0         | -0.1          |

| avg      | 1.1    | -1.3   | -0.1  |
| SEM      | 0.6    | 2.6    | 0.1   |
Table 3  Coastal Ocean acid vs. frozen replicate sample [DOC], $\Delta^{14}$C, and $\delta^{13}$C values. [DOC] errors listed above ($\pm^a$) represent the propagated errors of *individual* [DOC] measurements. Similarly, $\Delta^{14}$C ($\pm^b$) represent the propagated $\Delta^{14}$C errors for either *individual* AMS measurements, or the total reproducibility of primary standards (OX-I), whichever was highest. These [DOC] and $\Delta^{14}$C errors should not be confused with our *total analytical reproducibility* for DOC and $\Delta^{14}$C measurements, which are higher (~1 $\mu$M and ~4‰). Newport Beach Pier (NBP) individual $\Delta$DOC, $\Delta\Delta^{14}$C and $\Delta\delta^{13}$C offset values were determined by subtraction (frozen - acid). The average (avg), 1σ standard deviation ($\pm$), and standard error of the mean (SEM) for $\Delta$DOC, $\Delta\Delta^{14}$C, and $\Delta\delta^{13}$C offsets are reported in bold italics. Measurements that were not determined are indicated (n.d.).

| Location or Cruise | UCIAMS# | UCID# | Depth (m) | Filtered? (y/n) | UVox date (mm/dd/yy) | Δdays | Treatment | DOC [µM] | $\Delta^{14}$C [‰] $\pm^a$ | $\delta^{13}$C [‰] $\pm^b$ | $\Delta$DOC [µM] | $\Delta\Delta^{14}$C [‰] | $\Delta\delta^{13}$C [‰] |
|------------------|---------|-------|-----------|------------------|----------------------|--------|-----------|---------|-----------------------|------------------------|-----------------|----------------------|-----------------------|----------------------|
| NBP, CA 150738   | 150738  | 18693 | 1         | y                | 10/28/14             | 42     | acid      | 72.2    | 0.5                   | -224.7                 | 1.6              | -20.9               |
| NBP, CA 150739   | 150739  | 18694 | 1         | y                | 10/29/14             | 43     | frozen    | 74.1    | 0.5                   | -233.3                 | 1.5              | -21.4               |
|                  |         |       |           |                  |                      |        |           | 74.1    | 0.5                   | -233.3                 | 1.5              | -21.4               |
| NBP, CA 150758   | 150758  | 18892 | 1         | y                | 12/2/14              | 77     | acid      | 71.3    | 0.5                   | -229.7                 | 1.5              | -21.2               |
| NBP, CA 150759   | 150759  | 18893 | 1         | y                | 12/3/14              | 78     | frozen    | 73.6    | 0.5                   | -227.4                 | 1.5              | -21.3               |
|                  |         |       |           |                  |                      |        |           | 73.6    | 0.5                   | -227.4                 | 1.5              | -21.3               |
| NBP, CA 158124   | 158124  | 19066 | 1         | y                | 4/8/15               | 204    | acid      | 73.0    | 0.5                   | -222.2                 | 3.5              | -20.7               |
| NBP, CA 158125   | 158125  | 19067 | 1         | y                | 4/9/15               | 205    | frozen    | 76.1    | 0.5                   | -219.5                 | 3.5              | -20.9               |
|                  |         |       |           |                  |                      |        |           | 76.1    | 0.5                   | -219.5                 | 3.5              | -20.9               |
| NBP, CA 164220   | 164220  | 19236 | 1         | y                | 6/23/15              | 280    | acid      | 72.1    | 0.5                   | -230.5                 | 1.2              | -20.9               |
| NBP, CA 164221   | 164221  | 19237 | 1         | y                | 6/24/15              | 281    | frozen    | 74.3    | 0.5                   | -227.3                 | 1.6              | -21.1               |
|                  |         |       |           |                  |                      |        |           | 74.3    | 0.5                   | -227.3                 | 1.6              | -21.1               |
| NBP, CA 164610   | 164610  | 19437 | 1         | y                | 10/1/15              | 380    | acid      | 72.5    | 0.5                   | -235.1                 | 2.6              | -21.1               |
| NBP, CA 164611   | 164611  | 19438 | 1         | y                | 9/30/15              | 379    | frozen    | 74.5    | 0.5                   | -227.4                 | 2.6              | -21.3               |
|                  |         |       |           |                  |                      |        |           | 74.5    | 0.5                   | -227.4                 | 2.6              | -21.3               |
|                  |         |       |           |                  |                      |        |           | avg 2.2 | 1.5                   | -0.2                   |                 |                     |
|                  |         |       |           |                  |                      |        |           | ± 0.2  | 6.0                   | 0.2                    |                 |                     |
|                  |         |       |           |                  |                      |        |           | SEM 0.1 | 2.7                   | 0.1                    |                 |                     |
In the field, DOC samples were collected into precombusted (540°C/2 hr) 1-L amber Boston round bottles with acid cleaned PTFE caps. An additional PTFE sheet, cleaned by soaking in concentrated chromic-sulfuric acid (Fisher Scientific Chromerge™; CAS# 1333-82-0), was also placed between the cap and bottle. Duplicate samples designated for acidified or frozen storage were either immediately acidified with 1 mL 85% w/w phosphoric acid (H₃PO₄; Fisher Scientific; ACS grade; CAS#7664-38-2) and stored in the dark at ambient temperature or frozen at −20°C until analysis. All Coastal samples and Open Ocean samples collected shallower than 400 m depth were filtered via gravity using precombusted (540°C/2 hr) Whatman™ 70-mm glass-fiber filters (GF/F; 0.7 µm) using acid-cleaned stainless steel in-line filter manifolds and silicone tubing attached directly to the Niskin, or in the case of Coastal samples, an HDPE bucket with stainless steel spigot.

Seawater DOC was oxidized to CO₂ using a high-energy (1200 W), ultraviolet Hg-arc light source modified for an 800-mL sample volume and low blanks (Beaupre et al. 2007; Griffin et al. 2010). Frozen samples were thawed, homogenized by shaking, and decanted into a quartz reaction vessel. The sample was then acidified to pH ~2 with 1 mL 85% H₃PO₄. No additional acid was added to samples already pretreated for acidified storage. Dissolved inorganic carbon (DIC) stripped with ultra-high-purity helium to remove inorganic C, irradiated for 4 hr, and the resultant CO₂ purified and collected for Δ¹⁴C and δ¹³C analysis. Procedural blanks were small (2–3 µg C), and measurement uncertainties for Δ¹⁴C and δ¹³C were less than ±4‰ and ±0.2‰, respectively. One DOC sample was prepared per day; a modern standard (NBS oxalic acid 1, HOx1; NIST-SRM-4990B, Fm = 1.040), a dead standard (ACROS Organics #220911000, Glycine 99+%, Fm = 0.0010 ± 0.0005), or a Milli-Q™ blank are run for every 6–10 samples. All isotope ratios were blank corrected with error propagation following previously described methods for DOC Δ¹⁴C measurement correction (Beaupre et al. 2007; Griffin et al. 2010).

Equilibrated sample CO₂ gas was split and isolated for zinc method graphitization (Xu et al. 2007) and δ¹³C analysis. For δ¹³C analysis, equilibrated splits of DOC CO₂ were cryogenically transferred into 3-mm-diameter, 60-mm-length Pyrex® tubes and sealed under vacuum. These tubes were then scored with a glass cutter, placed into Exetainer® vials with two 8-mm solid glass marbles, inverted and flushed with ultra-high-purity He gas for 20 s in a glove bag, and capped. CO₂ in the scored Pyrex tube was released into the Exetainer vial when the tube was broken by gently shaking the marbles. DOC δ¹³C values were measured using a Gas Bench II and a Finnigan Delta Plus isotope ratio mass spectrometer (GB-IRMS). All Δ¹⁴C and δ¹³C isotopic analyses were performed at the University of California, Irvine Keck Carbon Cycle Accelerator Mass Spectrometry (KCCAMS) Laboratory. Statistics reported herein were determined using JMP® version 12.0 (SAS Institute Inc., Cary, NC, 1989-2007).

RESULTS AND DISCUSSION

Reproducibility of Frozen Seawater [DOC] and Δ¹⁴C and δ¹³C Values

Prior to addressing potential changes in measured [DOC], Δ¹⁴C, and δ¹³C values based on sample storage treatments, we first revisit the reproducibility of our UVox measurements on frozen sample replicates. A summary of [DOC], Δ¹⁴C, and δ¹³C values are shown for these frozen seawater DOC samples in Table 1. Duplicate frozen seawater samples comprised a wide range of sample depths (1–3500 m), [DOC] (32.6 to 76.1 µM), Δ¹⁴C (~220 to ~553‰) values, and a smaller range in δ¹³C (~20.9 to ~22.3‰). Here, we assess measurement reproducibility by subtracting measured initial vs. later [DOC] (µM), Δ¹⁴C (%), and δ¹³C (%) values based on UVox date and storage time (days). These positive and/or negative subtracted differences are
reported as \( \Delta \text{DOC} \) (µM), \( \Delta \Delta^{14}\text{C} \) (‰), and \( \Delta \delta^{13}\text{C} \) (‰). In the case of \( n = 5 \) frozen sample replicates from Newport Beach, \( \Delta \text{DOC} \), \( \Delta \Delta^{14}\text{C} \), and \( \Delta \delta^{13}\text{C} \) values represent the standard deviation of all \([\text{DOC}], \Delta^{14}\text{C}, \) and \( \delta^{13}\text{C} \) values.

The absolute differences between each pair of duplicates ranged from \( |\Delta \text{DOC}| = 0.9–2.8 \) µM, \( |\Delta \Delta^{14}\text{C}| = 4.8–23.8 \) ‰, and \( |\Delta \delta^{13}\text{C}| = 0.0–0.3 \) ‰. By averaging both positive and negative subtracted differences, we determine an overall UVox measurement reproducibility of \( \Delta \text{DOC} = 1.3 \pm 1.5 \) µM, \( \Delta \Delta^{14}\text{C} = 9.4 \pm 7.5 \) ‰, and \( \Delta \delta^{13}\text{C} = 0.1 \pm 0.1 \) ‰ (Table 1). Both absolute and average differences are similar to those reported previously for frozen duplicates (Druffel et al. 2013), where average uncertainties were \( \Delta \text{DOC} = 0.2 \pm 2.2 \) µM, \( \Delta \Delta^{14}\text{C} = 2.2 \pm 7.8 \) ‰, and \( \Delta \delta^{13}\text{C} = -0.3 \pm 0.3 \) ‰. However, if we instead average the absolute difference between frozen duplicates, the Druffel et al. (2013) study had \( |\Delta \text{DOC}| = 1.7 \pm 1.3 \) µM, \( |\Delta \Delta^{14}\text{C}| = 6.8 \pm 3.9 \) ‰, and \( |\Delta \delta^{13}\text{C}| = 0.3 \pm 0.3 \) ‰ (Supplementary Material Table 1). Our results also do not show clear changes in \([\text{DOC}], \Delta^{14}\text{C}, \) and \( \delta^{13}\text{C} \) values as a function of storage time (days), as was observed by Druffel et al. (2013) where samples stored <20 days had lower uncertainties. This is likely due to longer storage times of frozen samples measured in the present study (>43 days). Our measurements also did not reveal systematic \([\text{DOC}] \) or isotopic offsets (i.e. later duplicates were not always high or low) as was the case in the previous study.

While the ranges in \( \Delta \text{DOC}, \Delta \Delta^{14}\text{C}, \) and \( \Delta \delta^{13}\text{C} \) are generally consistent with those previously reported by Druffel et al. (2013), there are a few slight improvements. For example, our \( \Delta \text{DOC}, \Delta \Delta^{14}\text{C}, \) and \( \Delta \delta^{13}\text{C} \) values have slightly smaller standard deviations (1σ). This suggests that our UVox measurement precision has improved slightly. This is especially true for \( \delta^{13}\text{C} \) values, which now have a 1σ standard deviation of 0.1‰—lower than our measurement error (0.2‰). We believe this improvement in \( \delta^{13}\text{C} \) can be attributed to additional equilibration and sample \( \text{CO}_2 \) freeze-down time prior to isolation into 3-mm Pyrex tubes for GB-IRMS analysis.

**Open Ocean Acidified vs. Frozen Sample Storage Comparison**

Results from our acidified vs. frozen storage comparison are summarized in Table 2 and Figure 1. Here we compare and discuss \( n = 6 \) frozen and acidified sample duplicates that were collected from the South Pacific and North Atlantic as part of CLIVAR lines P16N and A16N. To first order, we find \([\text{DOC}], \Delta^{14}\text{C}, \) and \( \delta^{13}\text{C} \) values to be similar between acidified and frozen storage treatments (Figure 1A–C). However, upon closer examination and by subtracting frozen and acidified \([\text{DOC}], \Delta^{14}\text{C}, \) and \( \delta^{13}\text{C} \) values, several offsets are observed (Table 2 and Figure 2D–F). Half of our acidified replicates (\( n = 3 \)) had \([\text{DOC}] \) values that fell outside of our measurement uncertainty of \( \pm 1.3 \) µM (Figure 1D). Of these three samples, only two showed \( \Delta \text{DOC} \) offsets (±8–10‰) outside our measurement uncertainty (±2–3‰) and no significant offsets were observed in DOC \( \delta^{13}\text{C} \) (Figure 1E–F). A closer examination of these samples suggests that these three offset duplicates were from the South Pacific (3–15°S, 150°W) while the other three duplicates with no offset were from the North Pacific (0–14°N and 150–152°W) and North Atlantic (47°N, 19°W).

The absolute offset of each duplicate ranged from \( |\Delta \text{DOC}| = 0.0–3.1 \) µM, \( |\Delta \Delta^{14}\text{C}| = 1.4–10.0 \) ‰, and \( |\Delta \delta^{13}\text{C}| = 0.1–0.3 \) ‰. Despite the large range in these offsets, by averaging both positive and negative offset values and assuming the frozen values represent the true \([\text{DOC}] \) and isotopic values, we determine an average acidified sample offset of \( \Delta \text{DOC} = 1.1 \pm 1.4 \) µM, \( \Delta \Delta^{14}\text{C} = -1.3 \pm 6.5 \) ‰, and \( \Delta \delta^{13}\text{C} = -0.1 \pm 0.2 \) ‰ (Table 2). Somewhat surprisingly, these average offsets are not significantly different than those determined for our frozen
Figure 1  Open Ocean time series [DOC], $\Delta^{14}$C, and $\delta^{13}$C values. In plots A–C, frozen and acidified duplicate sample measurements are indicated by blue circles and red diamonds, respectively. Error bars represent the 1σ standard deviations of individual sample measurements (smaller than symbols for plot A/B and ± 0.2‰ for plot C). The dashed ovals represent a duplicate sample from the CLIVAR A16N cruise in which the frozen and acidified sample were measured >100 days apart. In plots D–F, black diamonds represent $\Delta$DOC, $\Delta\Delta^{14}$C, and $\Delta\delta^{13}$C offsets (frozen - acid) of duplicate samples. Error bars represent the propagated errors of determined $\Delta$DOC, $\Delta\Delta^{14}$C, and $\Delta\delta^{13}$C values.
sample replicates. However, this does not necessarily mean that acidified samples will always result in [DOC] and isotopic values comparable to frozen samples. As mentioned above, approximately half of the acidified samples did not fall within measurement error of frozen duplicates. For example, acidified samples with significantly different [DOC] and $\Delta^{14}C$ values were not always from similar depth, water mass, or [DOC] ranges, but with a possible effect seen in sample latitude. We later discuss how the role of external factors such as decarboxylation, humic acid precipitation, nutrient limitation, DOM quality (composition), and residual microbial community composition may determine the effectiveness of the acid storage treatment.

**Coastal Ocean Acidified vs. Frozen Samples: A Long-Term Storage Experiment**

At present, the determination of [DOC], $\Delta^{14}C$ and $\delta^{13}C$ values by UVox is a lengthy endeavor. The majority of UVox systems can isolate ~1–4 samples per 24-hr period (Beaupre et al. 2007; Xue et al. 2015). However, when considering the many standards and total C blanks required for correcting and reporting high-precision DOC $\Delta^{14}C$ and $\delta^{13}C$ values, the net rate of sample throughput is smaller. Because of this low sample throughput, most labs will have to store DOC samples for significant periods of time (months to years) prior to analysis. In order to evaluate the long-term effects of storing acidified DOC samples, we conducted a long-term storage experiment of many replicate Coastal Ocean DOC samples collected from Newport Beach, California. Here, $n = 5$ acidified and $n = 5$ frozen sample replicates were measured on back to back days, periodically over a period of 380 days. The results from this long-term storage experiment are summarized in Table 3 and Figure 2.

The average offset between acidified vs. frozen DOC samples was $\Delta$DOC = 2.2 ± 0.2 µM, $\Delta\Delta^{14}C = 1.5 \pm 6.0 \text{‰}$, and $\Delta\delta^{13}C = -0.2 \pm 0.2 \text{‰}$ (Table 3). In contrast to the Open Ocean results, we find that acidified [DOC] values from the Coastal Ocean were consistently ~2 µM lower than those from frozen replicates throughout the storage experiment (Figure 2A,D). Several two-sample F tests were used to test the null hypothesis that acidified vs. frozen [DOC], $\Delta^{14}C$, and $\delta^{13}C$ values from each treatment had the same variance. Computed F values for [DOC], $\Delta^{14}C$, and $\delta^{13}C$ ($F(1) = 1.29, 1.07, and 1.10$, respectively) were within the 95% confidence limits of the means ($F$-Critical = 6.39; $\alpha = 0.05$), suggesting acidified and frozen populations had the same variance. Since the variance was not significantly different, we applied two-tailed Student’s t tests assuming equal variances to test whether acidified vs. frozen [DOC], $\Delta^{14}C$, and $\delta^{13}C$ values had equal means. For [DOC], the t-Stat value (–3.87) was less than $-t$-Critical (–2.31), and t-Critical (2.31) values, indicating the [DOC] offset between treatments is statistically significant (frozen DOCmean = 74.5 ± 1.0 µM and acidified DOCmean = 72.3 ± 0.8 µM; $p = 0.0049$, df = 8, $\alpha = 0.05$). This result suggests the ~2-µM [DOC] loss we observe was, in fact, lost during the first month of the experiment.

With the exception of the first sample time point in the storage time series, acidified samples were consistently lower in $\Delta^{14}C$ (by 4.0 ± 2.5‰) throughout the experiment (Figure 2B,E). Also, acidified sample $\delta^{13}C$ values were slightly more positive (0.2 ± 0.2‰) than their frozen replicates (Figure 2C,F). However, t-Stat values for $\Delta^{14}C$ and $\delta^{13}C$ (–0.46 and 1.98, respectively) fell between $-t$-Critical (–2.31) and t-Critical (2.31) values, suggesting these isotopic offsets were not statistically significant ($p = 0.66$ and $p = 0.08$ for $\Delta^{14}C$ and $\delta^{13}C$, respectively; df = 8, $\alpha = 0.05$), with the $\delta^{13}C$ offset falling just outside the 95% confidence limits.

**Loss of DOC during Storage: Open vs. Coastal Ocean Isotopic Mass Balance**

To first order (±10%), it appears that acidified DOC samples generally reproduce the bulk $\Delta^{14}C$ and $\delta^{13}C$ isotopic signatures of frozen DOC samples—albeit more often than not, there were
Figure 2 Coastal Ocean time series [DOC], Δ\(^{14}\)C, and Δ\(^{13}\)C values. All samples measured from Newport Beach were collected on 16 September 2014. In plots A–C, frozen and acidified replicate sample measurements are indicated by blue circles and red diamonds, respectively. Error bars represent the 1σ standard deviations of individual sample measurements. In plots D–F, black diamonds represent ΔDOC, ΔΔ\(^{14}\)C, and ΔΔ\(^{13}\)C offsets (frozen - acid) of duplicate samples. Error bars represent the propagated errors of determined ΔDOC, ΔΔ\(^{14}\)C, and ΔΔ\(^{13}\)C values.
ΔΔ^{14}C differences (4–10‰) that fell outside our total uncertainty (<4‰). This was not the case for [DOC], which was significantly different for all Coastal samples (ΔDOC = 2.2 ± 0.6 µM) and half of the Open Ocean samples (ΔDOC = 2.4 ± 0.7 µM). The loss of DOC we observe during acidified storage likely precludes this storage method for accurate determination of [DOC]. This is especially true for UVoX [DOC] measurements, since UV photooxidation kinetics result in non-recovery of some (~2%) residual DOC (Beaupré et al. 2007). This is one reason that DOC measurements via UVox often report slightly lower [DOC] (~1–2 µM) than high-temperature combustion (HTC) measurements—another being a comparatively larger and more variable HTC total C blanks (Sharp et al. 2002; Beaupré et al. 2007).

The fact that 8 of 11 acidified duplicates had lower [DOC] than frozen samples (Figure 3A,B) poses the question: What DOC was lost during acidified storage? DOC is operationally defined as all organic carbon smaller than a bacterial cell (<0.1 µm). However, the majority of DOC studies rely on glass-fiber filters (GF/F; 0.7 µm) because they are readily available and easy to clean via combustion. GF/F filters may allow some small bacterial cells (0.1–0.7 µm) to enter the sample. The aqueous dissociation of H$_3$PO$_4$ into H$_2$PO$_4^-$ (and to a lesser extent, HPO$_4^{2-}$ and PO$_4^{3-}$) may also stimulate residual bacterial community growth and DOC remineralization. Abiotic processes could also be responsible for DOC loss. For example, acidification to pH <2 can result in humic acid precipitation, which could result in a slightly lower recovery of DOC. However, we note that visible precipitation or flocculation of humics was not observed in our acidified samples, which were also thoroughly mixed prior to loading and continuously stirred during UVox. If a major cause of DOC loss, humic acids would have to strongly adhere to the walls of the glass sample bottle and not be transferred to the reaction vessel. Acidification and long-term storage at room temperature could also result in decarboxylation reactions (i.e. malonic ester synthesis) and a loss of DOC to CO$_2$. However, decarboxylation reactions typically require heat and effect only substituted malonic esters and β-keto acids (i.e. molecules with two carbonyl groups, two atoms away from the COOH group) (McMurry 2011). In order to understand more about resulting ΔDOC, ΔΔ$^{14}$C, and Δδ$^{13}$C values, we use a simple isotopic mass balance to estimate the Δ$^{14}$C and δ$^{13}$C values of DOC lost during Open and Coastal Ocean acidified sample storage.

Only three of six acidified Open Ocean samples showed significant [DOC] offsets (ΔDOC = 2.4 ± 0.7 µM; Figure 3A). Two of these samples are from ~3000 m depth and one is from 10 m depth. These samples also had ΔΔ$^{14}$C values that fell outside our measurement precision for these samples (<1.5‰; Table 2). An isotopic mass balance of the Open Ocean frozen vs. acidified sample populations suggests DOC lost during acidified storage had an isotopic composition of Δ$^{14}$C = −478 ± 116‰ and δ$^{13}$C = −23.4‰ ± 3.0‰ (n = 3). Previous work has shown that labile DOC is generally more nitrogen rich, and has higher Δ$^{14}$C values, while recalcitrant DOC generally is more carbon rich and has lower Δ$^{14}$C values (Guo et al. 1996; Walker et al. 2011, 2014). However, this is not precisely what we observe for DOC lost during acidified storage. One possibility is that residual bacterial communities in these three samples subsisted on POC (since we generally do not filter DOC samples below 400 m depth). However, suspended POC in the deep ocean has very low concentrations (<1 µM), and Δ$^{14}$C values that can be as low as ~200‰ (Hwang et al. 2010), would be inconsistent with our isotopic mass balance. If microbial respiration of DOC occurred, then either recalcitrant DOC with low Δ$^{14}$C signatures was made bioavailable during the acidified storage, or semi-labile DOC in the Open Ocean has low Δ$^{14}$C values. If decarboxylation or humic acid precipitation occurred, then acidification removed a small portion of recalcitrant DOC with low Δ$^{14}$C values.
Figure 3  Comparison of Open vs. Coastal Ocean lost [DOC], $\Delta^{14}C$, and $\delta^{13}C$ values. For all plots, error bars represent the propagated uncertainties of lost $\Delta$DOC, $\Delta^{14}C$, and $\delta^{13}C$ values, determined via isotopic mass balance. Here, individual measurement uncertainties were used during error propagation (see Supplementary Material). In plots A–B, black diamonds represent $\Delta$DOC, offsets (frozen - acid) as in Figures 1–2. Open diamonds indicate acid/frozen duplicates with identical [DOC]. In plots C–F, squares represent determined $\Delta^{14}C$ and $\delta^{13}C$ values of DOC lost during acidified storage. In plots C and E, only half of the samples had $\Delta$DOC significantly different than zero; thus, we only report $\Delta^{14}C$ and $\delta^{13}C$ isotopic mass balance values for these $n = 3$ samples.
Surface vs. deep ocean DOM elemental and chemical composition are considerably different and could affect the loss of DOC we observe. It is well known that the surface ocean generally has a higher proportion of labile DOC biomolecules (i.e. carbohydrates, amino acids, and amino sugars), whereas the deep ocean has a higher proportion of recalcitrant and degraded molecules, such as carboxyl-rich alicyclic molecules (CRAM) (Hertkorn et al. 2006; Benner and Amon 2015). Abiotic decarboxylation or humic acid precipitation during acidified storage may also be a function of CRAM abundance in DOC. Finally, previous work has shown that recalcitrant DOC can be made bioavailable through photooxidation and/or hydrolysis (Cherrier et al. 1999). It could be that for these deep Open Ocean samples, DOC was made bioavailable via molecular-level changes induced by the addition of H$_3$PO$_4$ and decrease in sample pH.

In contrast to the Open Ocean, an isotopic mass balance of the Coastal frozen vs. acidified sample population suggests on average (with the exception of $n=1$ sample on day 43), DOC with $\Delta^{14}$C = $-94 \pm 105\%$ and $\delta^{13}$C = $-27 \pm 10\%$ ($n=4$) was lost during acidified sample storage (Figure 3D,F). These Coastal results suggest the majority of this DOC was lost in the first few weeks of collection. The fact that DOC loss did not continue throughout the experiment suggests either that complete decarboxylation or humic acid precipitation of DOC was relatively fast, or that residual bacterial growth (and organic matter respiration) eventually ceased due to prolonged exposure to pH <2, exhaustion of labile DOC or O$_2$, or external factors leading to population collapse (i.e. viral lysis).

Overall, it appears that there is some differential loss of Open vs. Coastal Ocean acidified DOC that is not simply a function of storage time or sample depth, but is instead likely affected by (1) dissolved organic matter chemical composition, elemental stoichiometry, and/or bioavailability; (2) the presence of macronutrient (phosphate) limitation; (3) potential weak acid hydrolysis of DOC acting to increase bioavailability, i.e. possible loss of carboxyl (COOH) functional groups; (4) the microbial community composition within the sample; (5) physical and chemical water mass properties (pH, alkalinity, etc.); and/or (6) humic acid precipitation.

**SUMMARY AND METHODOLOGICAL RECOMMENDATIONS**

Analysis of open ocean frozen duplicates resulted in similar procedural reproducibility to that reported previously (Druffel et al. 2013). Acidification with H$_3$PO$_4$ results in generally similar DOC $\Delta^{14}$C and $\delta^{13}$C values, but often lower [DOC] in comparison to frozen samples. An isotopic mass balance of acidified samples revealed differential remineralization of Open vs. Coastal Ocean DOC constituents. In the open ocean, DOC with low $\Delta^{14}$C signatures in the Open Ocean (irrespective of sample location or depth) and in the Coastal Ocean semi-labile DOC with high $\Delta^{14}$C signatures was lost. Possible causes of DOC loss during pH <2 storage include abiotic decarboxylation (and loss to CO$_2$), humic acid precipitation, or residual microbial population DOC remineralization via the addition of phosphate (via H$_3$PO$_4$) or storage at ambient temperatures. Using H$_3$PO$_4$ as a preservative, and storing samples in the dark at room temperature, up to ~3.5% and 8% of total DOC was removed in Coastal and Open Ocean samples, respectively.

We currently do not recommend the application of acidified (H$_3$PO$_4$) sample storage when high-precision [DOC], $\Delta^{14}$C, and $\delta^{13}$C values are desired. On one hand, it is possible that other acids (e.g. HCl) or lowering the pH < < 1 may inhibit DOC utilization by more rapidly crashing residual microbial populations. On the other hand, care not to decrease the sample pH <3 would likely avoid problems of DOC loss via decarboxylation and/or humic acid precipitation.
Analysis of changes in DOC molecular composition, bacterial abundance, carbon demand, and apparent oxygen utilization would also help elucidate the mechanisms of DOC loss we observe. More work is clearly needed to resolve these observations. Following the above hypothesized mechanisms, it is possible that analogous acidification/storage approaches could possibly be used as a tool for studying the source and cycling of either bioavailable (semi-labile) DOC, humic acids, or carboxylated DOC in the ocean.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Beaupre SR, Druffel ERM, Griffin S. 2007. A low-blank photochemical extraction system for concentration and isotopic analyses of marine dissolved organic carbon. Limnology and Oceanography-Methods 5(6):174–84.

Benner R, Amon RMW. 2015. The size-reactivity continuum of major bioelements in the ocean. Annual Review of Marine Science 7(1):185–205.

Calleja ML, Batista F, Peacock M, Kudela R, McCarthy MD. 2013. Changes in compound specific delta N-15 amino acid signatures and D/L ratios in marine dissolved organic matter induced by heterotrophic bacterial reworking. Marine Chemistry 149:32–44.

Cherrier J, Bauer JE, Druffel ERM, Coffin RB, Chanton JP. 1999. Radiocarbon in marine bacteria: evidence for the ages of assimilated carbon. Limnology and Oceanography 44(3):730–6.

Druffel ERM, Griffin S, Walker BD, Coppola AI, Glynn DS. 2013. Total uncertainty of radiocarbon measurements of marine dissolved organic carbon and methodological recommendations. Radiocarbon 55(2–3):1135–41.

Gasol JM, Alonso-Saez L, Vaque D, Baltar F, Calleja ML, Duarte CM, Aristegui J. 2009. Mesopelagic prokaryotic bulk and single-cell heterotrophic activity and community composition in the NW Africa-Canary Islands coastal-transition zone. Progress in Oceanography 83(1-4):189–96.

Griffin S, Beaupre SR, Druffel ERM. 2010. An alternate method of diluting dissolved organic carbon seawater samples for 14C analysis. Radiocarbon 52(2–3):1224–9.

Griffith DR, McNichol AP, Xu L, McLaughlin FA, Macdonald RW, Brown KA, Eglinton TI. 2012. Carbon dynamics in the western Arctic Ocean: insights from full-depth carbon isotope profiles of DIC, DOC, and POC. Biogeosciences 9(3):1217–24.

Guo LD, Santschi PH, Cifuentes LA, Trumbore SE, Southon J. 1996. Cycling of high-molecular-weight dissolved organic matter in the middle Atlantic bight as revealed by carbon isotopic (13C and 14C) signatures. Limnology and Oceanography 41(6):1242–52.

Hertkorn N, Benner R, Frommberger M, Schmitt-Kopplin P, Witt M, Kaiser K, Kettrup A, Hedges JI. 2006. Characterization of a major refractory component of marine dissolved organic matter. Geochimica et Cosmochimica Acta 70(12):2990–3010.

Hwang J, Druffel ERM, Eglinton TI. 2010. Wide-spread influence of resuspended sediments on oceanic particulate organic carbon: insights from radiocarbon and aluminum contents in sinking particles. Global Biogeochemical Cycles 24(4):GB4016.

McMurry J. 2011. Organic Chemistry. Belmont: Cengage Learning. 1376 p.

Ruiz-Halpern S, Calleja ML, Dachs J, Del Vento S, Pastor M, Palmer M, Agusti S, Duarte CM. 2014. Ocean-atmosphere exchange of organic carbon
and CO₂ surrounding the Antarctic Peninsula. *Biogeosciences* 11(10):2755–70.

Sharp JH, Carlson CA, Peltzer ET, Castle-Ward DM, Savidge KB, Rinker KR. 2002. Final dissolved organic carbon broad community intercalibration and preliminary use of DOC reference materials. *Marine Chemistry* 77(4):239–53.

Sugimura Y, Suzuki Y. 1988. A high-temperature catalytic-oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. *Marine Chemistry* 24(2):105–31.

Suzuki Y. 1993. On the measurement of DOC and DON in seawater. *Marine Chemistry* 41(1–3):287–8.

Tupas LM, Popp BN, Karl DM. 1994. Dissolved organic carbon in oligotrophic waters – experiments on sample preservation, storage and analysis. *Marine Chemistry* 45(3):207–16.

Vogel JS, Southon JR, Nelson DE, Brown TA. 1984. Performance of catalytically condensed carbon for use in accelerator mass spectrometry. *Nuclear Instruments and Methods in Physics Research B* 5(2):289–93.

Vogel JS, Southon JR, Nelson DE. 1987. Catalyst and binder effects in the use of filamentous graphite for AMS. *Nuclear Instruments and Methods in Physics Research B* 29(1–2):50–6.

Walker BD, Beaupre SR, Guilderson TP, Druffel ERM, McCarthy MD. 2011. Large-volume ultrafiltration for the study of radiocarbon signatures and size vs. age relationships in marine dissolved organic matter. *Geochimica Cosmochimica Acta* 75(18):5187–202.

Walker BD, Guilderson T, Okimura KM, Peacock M, McCarthy M. 2014. Radiocarbon signatures and size-age-composition relationships of major organic matter pools within a unique California upwelling system. *Geochimica et Cosmochimica Acta* 126:1–17.

Williams PM, Oeschger H, Kinney P. 1969. Natural radiocarbon activity of dissolved organic carbon in north-east Pacific Ocean. *Nature* 224(5216):256–8.

Xu XM, Trumbore SE, Zheng SH, Southon JR, McDuffee KE, Lutgen M, Liu JC. 2007. Modifying a sealed tube zinc reduction method for preparation of AMS graphite targets: reducing background and attaining high precision. *Nuclear Instruments and Methods in Physics Research B* 259(1):320–9.

Xue Y, Ge T, Wang X. 2015. An effective method of UV-oxidation of dissolved organic carbon in natural waters for radiocarbon analysis by accelerator mass spectrometry. *Journal of Ocean University of China* 14(6):989–93.