

Carbonate removal from coastal sediments for the determination of organic carbon and its isotopic signatures, $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$: comparison of fumigation and direct acidification by hydrochloric acid

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Abstract

Acidification of a solid sample to separate inorganic carbon (IC) from organic carbon (OC) is a widely encountered procedure in limnology and oceanography. Traditionally, OC was isolated to determine the weight-% of OC (%OC), but it is becoming increasingly common to determine the isotopic signatures of the OC ($\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$). This raises a need for a closer scrutiny of the currently used acidification methods. First, because IC and OC typically have distinct carbon-isotopic signatures, $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values can be compromised if IC is not completely removed. Second, it is possible to isotopically fractionate a sample if a small portion of OC is lost during acidification, because OC itself is both chemically and isotopically heterogeneous. This study evaluated two acidification methods by HCl—in the vaporous (HCl_{vap}) and aqueous (HCl_{aq}) phases—to determine %OC, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$ in coastal sediments. Each method was assessed according to the criteria that it (1) has low blank levels, (2) is able to remove dolomite, (3) yields accurate %OC, and (4) yields accurate $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values. HCl_{vap} fulfilled all criteria, given that the samples were not overexposed to acid. Overexposure led to underestimation of $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values. HCl_{aq} gave similar results but was less reliable in that it consistently underestimated %OC and yielded inaccurate $\delta^{13}\text{C}_{\text{OC}}$ value for one test sample. It is recommended that an optimal acid exposure is carefully determined for each sample type to obtain most accurate $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values.

Introduction

Separation of organic carbon (OC) from total carbon (TC; the sum of OC and inorganic carbon, IC) is a common procedure in oceanographic and limnological research. In addition to the determination of the weight-% of OC and IC in the sample (%OC and %IC), OC is frequently isolated for the determination of its natural carbon-isotopic signatures ($\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$). The $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values are used for various purposes, such as to identify food web structure, assess turnover times of carbon pools, and to identify carbon sources to a given system (e.g., Trumbore 2000; Raymond and Bauer

2001; Cloern et al. 2002; McNichol and Aluwihare 2007). To obtain accurate %OC, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$ values, it is imperative that IC is removed quantitatively without loss of OC. The $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values will be compromised if IC is not completely removed, because IC and OC commonly have distinct isotopic signatures. In addition, partial loss of OC could also affect $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values if OC itself is chemically and isotopically heterogeneous. While the accuracy of %OC obtained by different IC removal methods has been a topic of considerable discussion (e.g., review by Bisutti et al. 2004), much less is known about the corresponding accuracy of the isotopic signatures of the isolated OC. This is problematic, because $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values may change without measurable influence on %OC as determined by elemental analysis (Kennedy et al. 2005). With increasing interest in natural carbon-isotopic ratios, there is a need for a better evaluation of the effect of existing treatments to remove IC from natural sample matrices for $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ determinations.

The most popular approach to removing IC from TC is to expel IC as CO_2 by acidification, usually with hydrochloric (HCl), sulfurous (H_2SO_3), or phosphoric (H_3PO_4) acid. In most

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cases, acidification is carried out in one of three ways: samples are acidified, then rinsed with water ("rinse method"); samples that are preweighed in capsules are acidified and then dried ("aqueous method"; Verardo et al. 1990); samples that are preweighed in capsules are exposed to vaporous HCl ("vaporous method"; Hedges and Stern 1984). The rinse method is advantageous for high-carbonate samples because it circumvents the formation of hygroscopic salts (McNichol et al. 1994; Batista et al. 2004). However, it suffers from the loss of OC into the dissolved phase (Roberts et al. 1973; Froelich 1980). Both the vaporous and the aqueous methods should be free from dissolved OC loss, but salt accumulation can cause problems in sealed tube combustions (McNichol et al. 1994; Batista et al. 2004). The vaporous method is arguably the least labor intensive, but there are mixed reports in the literature on its ability to thoroughly remove carbonates (e.g., Yamamuro and Kayanne 1995; Schubert and Nielsen 2000; Ryba and Burgess 2002). The aqueous method ensures thorough contact between sample and acid but is relatively labor intensive, prone to sample spills, and the acid titration endpoint can be ambiguous (Lukasewycz and Burkhard 2005).

The objective of this study was to determine the most suitable method for removing IC from coastal sediments for the determination of %OC, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$. Reports of $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values of marine organic matter at compound-class and compound-specific levels highlight significant isotopic heterogeneity within a single OC sample (e.g., Eglinton et al. 1997; Hwang and Druffel 2003; Loh et al. 2004; Repeta and Aluwihare 2007). OC moieties that are susceptible to hydrolysis are typically enriched in ^{13}C and ^{14}C relative to the bulk pool (e.g., Trumbore and Zheng 1996; Hwang and Druffel 2003; Loh et al. 2004). Therefore, exposure to acid during IC removal may result in alteration of $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values. Such an artifact may be pronounced in coastal and freshwater environments, particularly in regions that receive OC from both terrigenous and marine OC of disparate $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ signatures (Pearson and Eglinton 2000; Blair et al. 2003; Komada et al. 2005). Furthermore, coastal sediments can contain poorly soluble carbonates such as dolomite which, if not removed completely, can significantly compromise carbon-isotopic ratios. Therefore, these samples require acids that are strong enough to remove all carbonates while leaving OC uncompromised.

In the present study, the vaporous method (HCl_{vap}) and the aqueous method using HCl (HCl_{aq}) were tested on coastal and freshwater sediments, as well as on an organic-rich shale. The rinse method was not tested because of the known loss of OC into the dissolved phase (Froelich 1980). HCl was chosen to carry out the aqueous method, because it is able to dissolve dolomite (Chang et al. 1996). Concentrated phosphoric acid can also dissolve dolomite (e.g., Walters et al. 1972), but is problematic because it produces highly viscous and hygroscopic condensation products upon drying. Sulfurous acid (6%) is too weak for efficient removal of dolomite (Nelson and

Sommers 1982; McNichol et al. 1994; Kennedy et al. 2005), and commercially available solutions are a source of carbon contamination (McNichol et al. 1994; Caughey et al. 1995). The drawbacks to using HCl are the production of hygroscopic salts (e.g., CaCl_2 , MgCl_2) which increases the sample drying time, and the introduction of chloride into the sample which could cause difficulties with instrumental analyses. Finally, it should be noted that for all acids mentioned above, there are reports of organic matter loss during carbonate removal (Heron et al. 1997; King et al. 1998; Lohse et al. 2000; Bisutti et al. 2004). Although the mechanisms for organic matter loss are unclear, they may include loss of acid volatiles, decarboxylation, oxidation, and spills. For HCl and H_2SO_3 , heat may exacerbate organic matter loss (Bremner 1949; Bisutti et al. 2004).

The efficacy of HCl_{aq} and HCl_{vap} in isolating OC from sediments was evaluated according to the following criteria: (1) low blank, (2) efficient dolomite removal, (3) accurate %OC values, and (4) accurate $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ values. Criterion (1) is largely set by the blank requirements for $\delta^{13}\text{C}$ and $\Delta^{14}\text{C}$ analyses by isotope ratio mass spectrometry (IRMS) and accelerator mass spectrometry (AMS), respectively. Given that the sample sizes for IRMS and AMS are typically $\sim 300 \mu\text{gC}$ or even less, blank carbon larger than a few micrograms greatly increases the uncertainty of the final isotopic signatures. Criterion (2) applies only to HCl_{vap} , because HCl_{aq} is considered invasive enough to efficiently decarbonate the sample. Neither %N nor $\delta^{15}\text{N}$ were examined in this study, because we are of the opinion that these values should be determined on untreated samples (Cutter and Radford-Knoery 1991; Lohse et al. 2000; Ryba and Burgess 2002). The conclusions of the present study corroborate this suggestion. All samples tested were dried and powdered prior to analyses; suspended solids collected on filters that are not amenable to powdering were not tested. Such samples may require additional acid exposure, and hence should be tested separately. Finally, the highest IC content tested was equivalent to $\sim 33\%$ CaCO_3 . Pelagic sediments with higher carbonate and lower OC contents pose additional challenges (McNichol et al. 1994; Batista et al. 2004) and are beyond the scope of this study.

Materials and procedures

Both methods considered in this text were originally described by other researchers. HCl_{aq} follows that described by Verardo et al. (1990) but using HCl instead of H_2SO_3 (Cutter and Radford-Knoery 1991; Nieuwenhuize et al. 1994). HCl_{vap} is based on Hedges and Stern (1984). HCl_{aq} was carried out using 1N HCl to avoid inefficient carbonate removal that has been reported for dilute (0.1 N) solutions, and potential alteration of $\delta^{13}\text{C}_{\text{OC}}$ that has been reported for concentrated (6 N) solutions (Midwood and Boutton 1998). Bosley and Wainright (1999) found no change in $\delta^{13}\text{C}$ signatures of two species of marine animals when treated with 1N HCl. Van Iperen and Helder (1985) obtained %OC values of calcareous marine

sediments that agreed best with theoretical values when treated with 1 N HCl.

In both methods, all glassware were washed in warm soapy water, acid rinsed, and fired at 550°C for 4 h prior to use. Ag capsules and stainless steel tools were also fired in the same manner. Although firing Ag capsules renders them brittle (Cutter and Radford-Knoery 1991), firing did not affect the outcome of the HCl_{vap} samples as long as the capsules were fired only once. For HCl_{aq} , it was impossible to prevent loss of liquid regardless of whether or not the capsules were fired, possibly due to capillary action up and over the capsule wall.

Aqueous method using 1N HCl (HCl_{aq})—Samples (12–20 mg) were weighed accurately into 5 mm dia, 12.5 mm tall Ag capsules (Elementar Americas Inc.). To minimize sample loss, capsules were filled to no more than about a third full. The capsules were held in twenty-position sample trays fabricated by drilling 6 mm diameter, 7 mm deep wells into 50 mm × 60 mm × 12.5 mm borosilicate glass blocks. Watch glasses were used as covers to protect the samples from dust. To minimize effervescence, UV-irradiated deionized water was first added just enough to evenly moisten the sample. The acidification was started with a small volume of acid (5–10 μL 1N HCl prepared from concentrated HCl, Fisher NF/FCC) even if the sample was not vigorously reacting. To prevent sample loss from heat-induced effervescence, the samples were allowed to react at room temperature for 5–10 min before placing in a 40°C oven to dry (~20 min). Over the course of several acidifying-drying cycles, the amount of acid added per cycle was gradually increased (up to 50 μL), as was the oven temperature to speed drying (up to 60°C). The acid titration was deemed complete when effervescence was no longer detected by visual inspection. Because the titration endpoint can be highly subjective (Lukasewycz and Burkhard 2005), the samples were acidified until effervescence was not detected in two consecutive cycles. Once complete, the samples were dried either in a 50°C oven for a minimum of 3 h to overnight, or under vacuum to drive off excess moisture and HCl.

Vaporous HCl method (HCl_{vap})—Samples (12–20 mg) were weighed accurately into 5 mm dia, 9 mm tall Ag capsules (Costech) held in glass sample trays described above. UV-irradiated deionized water was added just enough to moisten the sample to promote acidification (Yamamuro and Kayanne 1995; Harris et al. 2001). The tray was then placed in a 250 mm ID glass desiccator along with about 25 mL concentrated HCl (Fisher NF/FCC) in a precombusted beaker. The desiccator was scrupulously soaped and preleached with HCl for several days. Grease should never be applied to the desiccator seal as it leads to significant isotopic contamination (Schubert and Nielsen 2000).

The desired time for fumigation will most likely depend on the sample composition, size, as well as physical configuration of the sample and its container. Hence, the desired fumigation time should be determined independently for each type of analysis. The recommended exposure time for the samples

analyzed in this study following the method described above is 6 h, and never over 24 h. The 24 to 48 h exposure recommended by Hedges and Stern (1984) led to frequent disintegration of Ag capsules (Komada, pers. obs.), most likely due to enhanced acid permeation from the added moisture. As discussed below, exposure times of more than 24 h also resulted in increased blanks, and possible alteration of OC isotopic signatures. Once the acidification was complete, the samples were dried as described for HCl_{aq} .

Assessment

Experiment-1: Determination of blank carbon (Criterion 1)—To determine the amount of extraneous carbon that is introduced into the sample during acidification, low-carbon clay was prepared and acidified by HCl_{aq} and HCl_{vap} . Low-carbon clay was prepared by repeatedly washing pre-fired (850°C, 8 h) and powdered intertidal sediment with 1N HCl, followed by repeated rinsing with deionized water. The residue was dried, powdered again, and fired again at 850°C for 4 h. Low-carbon clay (12 to 13 mg) was accurately weighed into Ag capsules. Samples processed by HCl_{aq} received 100, 200, or 500 μL of 1N HCl. Those processed by HCl_{vap} were fumigated for 6, 24, or 48 h. To determine the amount of blank carbon introduced by the acid treatments, four Ag capsules (together containing approximately 50 mg of low-carbon clay) were dried under vacuum, and oxidized together by sealed tube combustion (Druffel et al. 1992). The evolved CO_2 was cryogenically purified and quantified with a capacitance manometer (MKS Baratron). CO_2 yields are reported in μgC normalized to 50 mg sample after subtracting the blank carbon introduced by the sealed tube combustion process (1.0 μgC).

The blank values for HCl_{aq} ranged from 1 to 4 μgC (50 mg clay)⁻¹ for all treatments and overlapped with values of untreated samples (Fig. 1a). The blank values were higher for HCl_{vap} and increased with increasing exposure time, reaching as high as ~10 μgC (50 mg clay)⁻¹ after 48 h (Fig. 1b). The source of this blank carbon is unclear but may be from condensation that develops inside the desiccator during the course of fumigation. Although the desiccator was precleaned,

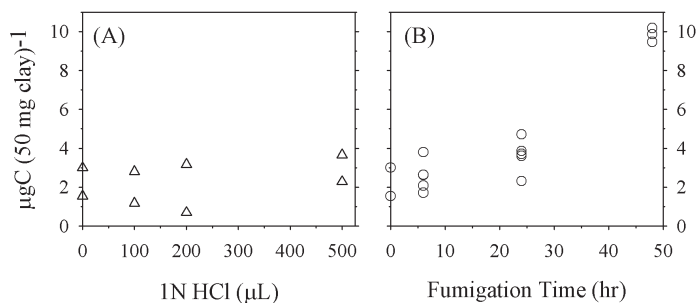


Fig. 1. Mass of carbon recovered from pre-cleaned clay treated by HCl_{aq} (a) and by HCl_{vap} (b) normalized to 50 mg clay. Each data point represents CO_2 recovered from 4 acidified samples that were oxidized together by sealed tube combustion. Results from two to five replicate CO_2 yields are shown for each treatment.

it is also possible that traces of vacuum grease contributed to the blank.

In conclusion, it was possible to obtain low blank values by HCl_{aq} with the addition of as much as 2 mL acid to 50 mg of low-carbon clay. Acidification by HCl_{vap} resulted in higher blanks, especially after 48 h of fumigation. If the HCl_{vap} method is to be employed, the fumigation time should not exceed 24 h to minimize blank carbon incorporation.

Experiment-2: Efficiency of dolomite removal by HCl_{vap} (Criterion 2)—There are mixed reports on the efficiency of HCl_{vap} in removing carbonates (e.g., Yamamuro and Kayanne 1995; Schubert and Nielsen 2000). This is of particular concern if poorly soluble carbonates are present in the sample. Hence, the efficiency with which HCl_{vap} removes dolomite was evaluated using an artificial sediment mixture consisting of 33.5 wt% dolomitic limestone (NIST SRM88b; $12.65 \pm 0.03\%$ C) in low-carbon clay described in Experiment-1. If the carbon in SRM88b is present solely as carbonates, the clay-dolomite mixture is 4.2% IC, which is near the upper limit of carbonate content encountered in the coastal ocean (Kennett 1982). Approximately 15 or 20 mg aliquots of the clay-dolomite mixtures were weighed accurately into Ag capsules and acidified by HCl_{vap} for a period of 6 h. Control samples were acidified by HCl_{aq} until the visual titration endpoint. After thorough drying under vacuum, carbon yield from each capsule was determined by sealed tube combustion as described under Experiment-1.

After correction for the carbon contributed by the sealed tube combustion process and that associated with the low-carbon clay, 3 to 5 μgC were recovered from the acidified samples (Table 1). If one assumes that the carbon in SRM88b is present solely as IC, these carbon recoveries correspond to an average of $99.4 \pm 0.2\%$ and $99.5 \pm 0.1\%$ removal of IC by HCl_{aq} and by the 6-h HCl_{vap} treatments, respectively (Table 1). These results suggest equally efficient dolomitic limestone removal by the two treatments. However, acidification by HCl_{aq} took 1-2 d to reach the visual endpoint and was highly labor intensive. Furthermore, 20 mg samples proved too large even for the 12.5 mm tall Ag capsules and suffered sample spills despite precautions to control effervescence. It is possible that carbon loss from spillage was fortuitously balanced by blank carbon incorporation from excess sample handling.

In conclusion, 6-h HCl_{vap} was equally efficient as HCl_{aq} at expelling IC from as much as 22 mg of sediment that is 33.5 wt% dolomitic limestone. HCl_{vap} was significantly less labor intensive, and did not lead to obvious sample loss which plagued the 20 mg samples treated by HCl_{aq} .

Experiment-3: Accuracy and precision of %OC, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$ determinations (Criteria 3 & 4)

Approach—To assess the accuracy of the %OC, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$ values obtained by HCl_{aq} and HCl_{vap} , it is necessary that the true values are known independently. Standard reference materials consisting of natural sediment containing both IC and OC are available, but to the best of our knowledge, none

are certified for %OC, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$ values. Therefore, the accuracy of the measured $\delta^{13}\text{C}_{\text{OC}}$ and %OC values were evaluated indirectly through comparison to those calculated from independent analyses of %TC, %IC, and associated $\delta^{13}\text{C}$ signatures using the following relationships:

$$\%OC = \%TC - \%IC \quad (\text{Eq. 1})$$

$$\delta^{13}\text{C}_{\text{OC}} = \frac{\delta^{13}\text{C}_{\text{TC}} - \delta^{13}\text{C}_{\text{IC}} \cdot f_{\text{IC}}}{1 - f_{\text{IC}}} \quad (\text{Eq. 2})$$

where f_{IC} is the fraction of TC that is IC, and $\delta^{13}\text{C}_{\text{TC}}$ and $\delta^{13}\text{C}_{\text{IC}}$ are $\delta^{13}\text{C}$ values of TC and IC, respectively. The premise is that %TC, %IC, and associated $\delta^{13}\text{C}$ signatures can be determined reliably. Accurate determination of %TC and $\delta^{13}\text{C}_{\text{TC}}$ is possible by elemental analyzer-IRMS (EA-IRMS). For %IC and $\delta^{13}\text{C}_{\text{IC}}$, the accepted method is IC extraction with 100% H_3PO_4 , followed by analysis of the evolved CO_2 by IRMS (Hoefs 2004). In this study, a modified version using readily available 85% H_3PO_4 was employed to extract IC as CO_2 . The %OC and $\delta^{13}\text{C}_{\text{OC}}$ values were monitored as a function of acidification, and compared with values calculated using Eq. 1 and 2; deviation of measured values from the calculated values was considered an artifact of acidification. Samples were purposefully overexposed to acid to detect the onset of any artifacts (at least 100 μL beyond the visual endpoint for HCl_{aq} and up to 48 h fumigation for HCl_{vap}).

This approach was not taken for the evaluation of $\Delta^{14}\text{C}_{\text{OC}}$ values, because of the high cost of $\Delta^{14}\text{C}$ determinations by

Table 1. Carbon removal from a mixture of 33.5 wt% dolomitic limestone (NIST SRM88b) in precleaned clay.

Treatment	Initial mixture (mg)	C recovered (μg) ¹	C removed (%) ²
HCl_{aq}	15.1	5.1	99.2
	15.2	4.3	99.3
	19.7	3.7	99.6
	22.1	4.0	99.6
	Average		
HCl_{vap} (6 h)	14.5	3.5	99.4
	15.0	3.8	99.4
	15.6	3.8	99.4
	20.6	3.1	99.6
	20.8	3.6	99.6
	22.1	3.4	99.6
Average			99.5 ± 0.1

¹Corrected for carbon from the sealed tube combustion process (1.0 μg) and precleaned clay (0.5 to 0.8 μg ; Fig. 1).

²Fraction of total carbon originally present in the limestone-clay mixture that was removed by the treatment. These values are calculated by assuming that the IC content of the mixture is 4.2 wt%. The assumption is met if all carbon in dolomitic limestone is IC.

Table 2. List of samples tested in Experiment-3

Sample	TC (wt %) ¹	IC (wt %) ²	$\delta^{13}\text{C}_{\text{TC}}$ (‰) ¹	$\delta^{13}\text{C}_{\text{IC}}$ (‰) ³	Calculated $\delta^{13}\text{C}_{\text{OC}}$ (‰) ⁴	Calculated %OC ⁵
MB4 (shale)	6.4 ± 0.1 (n = 4)	2.90 ± 0.08 (n = 5)	-10.5 ± 0.1 (n = 4)	+ 3.17 ± 0.03 (n = 3)	-21.9 ± 0.6	3.5 ± 0.2
SRM8704 ⁶	3.34 ± 0.07 (n = 6)	0.875 ± 0.008 (n = 3)	-19.6 ± 0.2 (n = 7)	-0.64 ± 0.02 (n = 5)	-26.7 ± 0.3	2.43 ± 0.07
MB5 (marsh)	2.5 ± 0.1 (n = 4)	(2.4 ± 0.1)10 ⁻² (n = 4)	-20.2 ± 0.1 (n = 5)	-8.9 ± 0.6 (n = 4)	-20.3 ± 0.1	2.5 ± 0.1
MB21 (estuary)	1.25 ± 0.02 (n = 4)	(3.86 ± 0.05)10 ⁻² (n = 4)	-22.76 ± 0.06 (n = 4)	-3.6 ± 0.1 (n = 4)	-23.3 ± 0.1	1.21 ± 0.02

¹Determined by EA-IRMS²Determined by vacuum distillation (Griffin and Druffel 1998)³Determined by dual inlet IRMS⁴Calculated using values given in columns 2 to 5⁵Calculated using values given in columns 2 and 3⁶NIST Standard Reference Material Buffalo River Sediment. Certified TC is 3.351 ± 0.017%.

AMS. Instead, the accuracy of the $\Delta^{14}\text{C}_{\text{OC}}$ values was assessed by monitoring the $\Delta^{14}\text{C}$ signature of a low-carbonate estuarine sediment (sample MB21; Table 2) as a function of acid exposure. Estuarine sediment is well-suited for this assessment, because it is likely to contain OC having a range of $\Delta^{14}\text{C}$ signatures from modern (e.g., local primary production) to ^{14}C -dead (e.g., petroleum). Deviation from the initial $\Delta^{14}\text{C}$ value was considered an artifact of acidification.

Samples analyzed—Moderately organic-rich (1.2% to 3.5% OC) samples with varying carbonate content (<0.1% to 2.9% IC) and diagenetic history were selected for analyses (Table 2). MB4 is an organic-rich shale collected from a Monterey Shale outcrop in the Santa Clara watershed (Komada et al. 2005). SRM8704 is a river sediment certified for %TC by NIST. MB5 was collected from the uppermost 5 cm of an intertidal sediment in San Francisco Bay in June 2005. MB21 was collected from the uppermost 3 cm of a piston core recovered from 2 m of water in a small embayment in San Francisco Bay in August 2006.

Analytical methods—Carbon content of untreated and acidified samples, and their $\delta^{13}\text{C}$ values (per mil deviation of $^{13}\text{C}/^{12}\text{C}$ ratio from PDB) were determined by EA-IRMS either at the Stable Isotope Facility, Department of Plant Sciences, University of California Davis (UCD), or at the W. M. Keck C Cycle AMS Facility, University of California Irvine (UCI). Analysis of reference materials for %TC (NIST SRM8704, 3.351 ± 0.017% C) and $\delta^{13}\text{C}$ (USGS24 graphite, -15.9 ± 0.25‰) yielded values that agreed within error of, or were close to, certified values: 3.34 ± 0.07% C ($n = 6$), and -15.92 ± 0.07‰ ($n = 16$) at UCD; and 3.31 and 3.23% C, ($n = 2$) and -15.90 ± 0.02‰ ($n = 11$) at UCI.

The %IC values were determined by vacuum distillation (Griffin and Druffel 1998) using 85% phosphoric acid (Fisher, HPLC), with a reaction time of 3 h at 70°C, followed by overnight at room temperature. The evolved CO_2 was purified and quantified as described in Experiment-1. CO_2 yields from blank vessels processed in parallel (1-2 μgC) were subtracted

from all sample yields. Using this method, an average yield of 99.9 ± 0.2% was obtained from triplicate analysis of NIST SRM88b dolomitic limestone. The $\delta^{13}\text{C}_{\text{IC}}$ signature of the evolved CO_2 was determined by dual inlet IRMS at the Stable Isotope Laboratory, Department of Geology, University of California Davis, with a precision of ± 0.05‰ or better.

For the analysis of $\Delta^{14}\text{C}$ (per mil deviation of $^{14}\text{C}/^{12}\text{C}$ ratio from a 19th century wood standard normalized to $\delta^{13}\text{C}$ of -25‰; Stuiver and Polach 1977), four Ag capsules each containing 12 to 13 mg of sample were oxidized together by sealed tube combustion (Druffel et al. 1992) as described in Experiment-1. The evolved CO_2 was sent to the W. M. Keck C Cycle AMS Laboratory at the University of California Irvine for graphitization and analysis by AMS (Southon et al. 2004).

The uncertainties of %OC and $\delta^{13}\text{C}_{\text{OC}}$ values calculated from Eq. 1 and 2 were estimated by propagating the errors of the terms that appear on the righthand side of the equations as random errors. This gave ± 0.1 to 0.6‰ for $\delta^{13}\text{C}_{\text{OC}}$ and ± 0.02 to 0.2 for %OC (Table 2). Due to lower IC content, MB5 and MB21 have smaller uncertainties associated with their calculated %OC and $\delta^{13}\text{C}_{\text{OC}}$ values than MB4 and SRM8704 (Table 2). Therefore, deviations between calculated and measured values are more readily detected in MB5 and MB21.

Results and Interpretation—(a) %OC: For all four samples analyzed, HCl_{vap} yielded %OC values that agreed closely with those calculated using Eq. 1, whereas HCl_{aq} produced %OC values that were consistently low by 5-15% (Table 3). The cause of OC loss during HCl_{aq} treatment is unclear, but as discussed below, appears to be dominated by processes that do not lead to significant isotopic fractionation, such as leaks and fine effervescence. It should be noted that OC loss shows no obvious correlation with the extent of sample handling, which can be estimated from %IC and volume of HCl dispensed (Table 2; also see Fig. 2). Despite our protocol for careful sample handling, the extent of OC loss during HCl_{aq} treat-

Table 3. OC yields from HCl_{aq} - and HCl_{vap} -treated samples relative to calculated values given in Table 2

Sample	HCl_{aq} (%)	HCl_{vap} (%)
MB4 (shale)	90 ± 6 ($n = 7$)	104 ± 7 ($n = 5$)
SRM8704	93 ± 5 ($n = 8$)	102 ± 4 ($n = 6$)
MB5 (marsh)	85 ± 4 ($n = 10$)	102 ± 4 ($n = 8$)
MB21 (estuary)	95 ± 4 ($n = 10$)	103 ± 2 ($n = 7$)

For samples with non-negligible IC content, OC yields were calculated using carbon values obtained after the drop observed during the initial stages of the acidification. The number of data points n used to compute the average is given in parentheses.

ment appears to be correlated most strongly with the analyst who carried out the treatment (MB4 and MB5 by analyst-1; SRM8704 by analyst-2; MB21 by analyst-3). In contrast, HCl_{vap} provided consistently accurate %OC values independent of the analyst.

(b): $\delta^{13}\text{C}$ —MB4 and SRM8704 yielded $\delta^{13}\text{C}$ values that agreed within error of the expected values regardless of treatment (Fig. 2a-d). With the onset of acidification, $\delta^{13}\text{C}$ values fell rapidly and were stable throughout the remainder of the experiments. In agreement with the findings from Experiment-2, a fumigation period of 6 h was sufficient for $\delta^{13}\text{C}$ signatures to approach expected values for these samples. In contrast, MB5 and MB21 showed greater disagreement between measured and expected values (Fig. 2e-h). After 48 h fumigation, MB5 samples displayed a drop in $\delta^{13}\text{C}$ value by approximately 0.4‰ (Fig. 2f). MB21 samples treated by HCl_{aq} gave $\delta^{13}\text{C}$ signatures that were consistently lower than the expected value by approximately 0.2‰ to 0.5‰ (Fig. 2g).

The drop in the $\delta^{13}\text{C}$ values observed for MB5 and MB21 (Fig. 2f,g) may be due to addition of blank carbon having low $\delta^{13}\text{C}$ signatures and/or selective loss of OC having high $\delta^{13}\text{C}$ signatures. For MB21 treated by HCl_{aq} (Fig. 4g), it is unlikely that extraneous carbon was important given the low blank carbon levels associated with the HCl_{aq} technique (Fig. 1a). A more plausible explanation is the loss of ^{13}C -enriched OC into the aqueous phase, with subsequent partial loss of liquid out of the capsule. The fact that significant deviation was observed for HCl_{aq} but not for HCl_{vap} (Fig. 2h) suggests that, for this particular sample, repeated application of heat may have enhanced the loss of ^{13}C -rich moieties.

The cause for the deviation observed for MB5 after 48 h fumigation (Fig. 2f) is less clear but is likely caused by a combination of blank carbon and selective OC loss. If HCl_{vap} treatment introduced 0.2 μg of blank carbon per mg of sample after 48 h fumigation (Fig. 1b), and that vacuum grease having a $\delta^{13}\text{C}$ value of -50 ‰ (Schubert and Nielsen 2000) was the sole source of blank carbon, then the $\delta^{13}\text{C}$ values of MB5 and MB21 should have shifted by -0.24 ‰ and -0.43 ‰, respectively; i.e., a greater shift is expected for MB21 than MB5. The fact that no corresponding drop is observed for MB21 after 48 h (Fig. 2h) suggests that the deviation observed for MB5 was

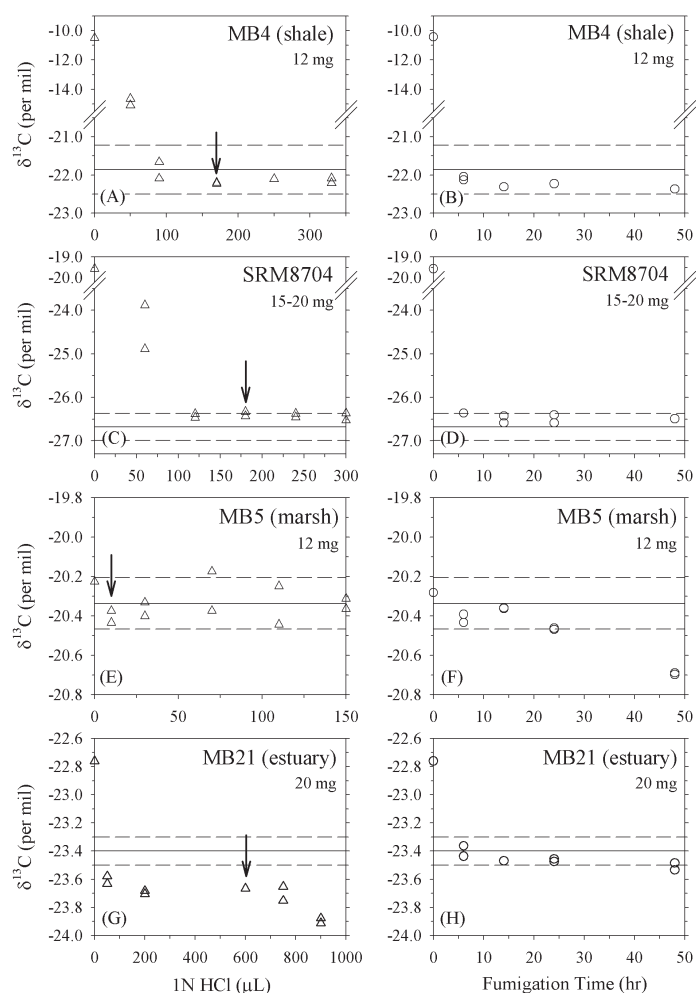


Fig. 2. $\delta^{13}\text{C}$ values of MB4 (a,b), SRM8704 (c,d), MB5 (e,f), and MB21 (g,h) acidified to varying degrees by HCl_{aq} (left column) and HCl_{vap} (right column). The approximate mass of sample placed in each Ag capsule is indicated in each panel. Solid and dashed lines are the $\delta^{13}\text{C}_{\text{OC}}$ value calculated from Eq. 2, and its estimated error, respectively (Table 2). Arrows represent the visual endpoint determined during the HCl_{aq} treatment. Results from duplicate samples are shown as separate data points.

likely caused by a combination of both blank carbon and selective loss of ^{13}C -enriched OC.

(c): $\Delta^{14}\text{C}$ —The $\Delta^{14}\text{C}$ values of MB21 samples decreased with increasing acid exposure during treatment by both HCl_{aq} and HCl_{vap} (Fig. 3). As stated above, a rigorous isotope mass balance was not carried out for $\Delta^{14}\text{C}$, because of the high cost associated with AMS analysis. Therefore, the $\Delta^{14}\text{C}$ value of OC cannot be calculated, but it is possible to estimate a lower limit for $\Delta^{14}\text{C}_{\text{OC}}$ by assigning an upper limit for the $\Delta^{14}\text{C}$ value of IC. Given that neither sedimentation nor mixing rates are available for this site, we estimated the lower limit as follows. The $\Delta^{14}\text{C}$ of dissolved IC extracted from overlying waters collected at the MB21 site in August 2006 is -1 ‰ (Dorfmeier unpubl. data). If sedimentary particulate IC had the same $\Delta^{14}\text{C}$ value, then a mass balance analogous to Eq. 2, along with the known

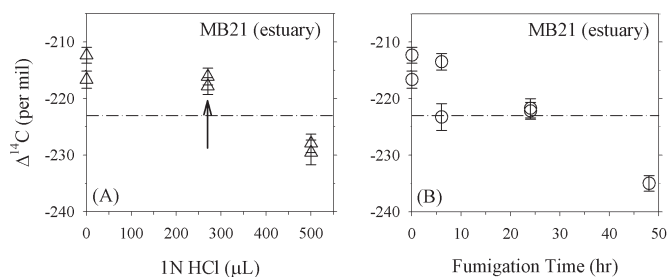


Fig. 3. $\Delta^{14}\text{C}$ values of MB21 samples acidified by varying degrees by HCl_{aq} (a) and HCl_{vap} (b). Four Ag capsules each containing 12–13 mg sample were combusted together to obtain each $\Delta^{14}\text{C}$ value. The dash-dot line is the estimated lower limit for $\Delta^{14}\text{C}_{\text{OC}}$ (see text for details). The arrow in (a) is the visual endpoint. Note that this is different from Fig. 2g because of the difference in sample mass per capsule. Error bars represent uncertainty in the AMS measurement only.

values for f_{OC} and f_{IC} (Table 2) gives an expected $\Delta^{14}\text{C}_{\text{OC}}$ value of -221‰ . If we take a value of $+76\text{‰}$, which is the $\Delta^{14}\text{C}$ of atmospheric CO_2 in 2002 at Jungfrauoch, a station representative of free tropospheric conditions between 40–50°N (Levin and Kromer 2004), the corresponding value for $\Delta^{14}\text{C}_{\text{OC}}$ is lowered by 2‰ to -223‰ . Given the prevalence of carbonate-containing bedrock that is undergoing erosion in the vicinity, it is unlikely that the particulate IC pool in MB21 consists entirely of modern carbon. Therefore, the $\Delta^{14}\text{C}_{\text{OC}}$ value of MB21 is not expected to fall significantly below $\sim -220\text{‰}$.

The results of the HCl_{aq} treatment show that the $\Delta^{14}\text{C}$ value varied little between the HCl_{aq} untreated samples and those at the visual endpoint, but dropped by approximately 12‰ after addition of 500 μL acid to values well below the estimated threshold (Fig. 3a). Blank carbon incorporation after addition of 500 μL of acid is about $1 \mu\text{g}$ (50 mg) $^{-1}$ (Fig. 1a), which is negligible relative to $\sim 600 \mu\text{g}$ sample carbon. The observed drop in $\Delta^{14}\text{C}$ signature is therefore likely caused by loss of ^{14}C -enriched OC from the sample matrix.

Similar to HCl_{aq} , the HCl_{vap} treatment yielded $\Delta^{14}\text{C}$ values that were $\sim 20\text{‰}$ lower relative to untreated samples after 48 h exposure (Fig. 3b). Although the blank carbon values are higher for HCl_{vap} than HCl_{aq} (Fig. 1), incorporation of $10 \mu\text{g}$ blank carbon alone cannot explain the 20‰ drop in $\Delta^{14}\text{C}$ values (the blank would need to be -1523‰). Therefore, it appears that prolonged fumigation results in preferential loss of ^{14}C -enriched carbon.

Discussion

The results of Experiment-3 show that in both treatments, $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ can be compromised if the samples are overexposed to HCl. Although contribution from blank carbon cannot be ruled out for HCl_{vap} , the decreasing trend in $\delta^{13}\text{C}_{\text{OC}}$ and $\Delta^{14}\text{C}_{\text{OC}}$ with increasing acid exposure (Fig. 2 and 3) suggests preferential loss of ^{13}C - and ^{14}C -enriched OC. These results are consistent with the observation that when marine and terrestrial OC is subjected to acid hydrolysis, the acid-resistant residue is

often depleted in ^{13}C and ^{14}C relative to the parent pool (e.g., Trumbore and Zheng 1996; Wang et al. 1998; Hwang and Druffel 2003; Loh et al. 2004; Komada et al. 2005). This is because acid-labile moieties such as carbohydrates and amino acids are relatively enriched in ^{13}C compared with lipids and the nonhydrolyzable residue (Degens et al. 1968; DeNiro and Epstein 1977; Wang et al. 1998; Hwang and Druffel 2003). Given that hydrolysable OC is enriched in N relative to the bulk (Hwang et al. 2006), the results of this experiment argue against the determination of $\%N$ and $\delta^{15}N$ on acidified samples.

Overall, HCl_{vap} was more reliable than HCl_{aq} . Barring overexposure to acid, HCl_{vap} yielded consistently accurate $\%OC$, $\delta^{13}\text{C}_{\text{OC}}$, and $\Delta^{14}\text{C}_{\text{OC}}$ values. Incomplete removal of IC was not an issue (Table 1) likely because sample sizes were kept small ($\leq 22 \text{ mg}$ per Ag capsule) and wetted to promote acid permeation (Yamamuro and Kayanne 1995; Harris et al. 2001). In contrast, HCl_{aq} consistently underestimated $\%OC$ and gave inaccurate $\delta^{13}\text{C}_{\text{OC}}$ value for MB21.

When isotopic results are viewed together with the $\%OC$ results, it is apparent that there is no obvious correlation between OC loss (Table 3) and alteration in $\delta^{13}\text{C}_{\text{OC}}$ (Fig. 2). This is best gleaned from the fact that MB21 and MB5, the two samples that exhibited detectable deviation in $\delta^{13}\text{C}_{\text{OC}}$, showed the smallest (5%) and greatest (15%) deviation from the calculated $\%OC$ values, respectively. Therefore, it appears that small, selective losses of acid-labile OC that cause shifts in isotopic values are superimposed on large OC losses that are largely non-selective.

Comments and recommendations

The following recommendations apply to samples that are similar in chemical composition and physical configuration to those analyzed in this study. For the determination of $\%OC$, HCl_{vap} is recommended over HCl_{aq} on the basis of superior accuracy, precision, and reduced labor. For the samples analyzed here, HCl_{vap} also yielded more accurate $\delta^{13}\text{C}_{\text{OC}}$ values than HCl_{aq} as long as the samples were not overexposed to acid. Fumigation time of 6 h was found to be adequate; it should never exceed 24 h. The findings of this study are consistent with preferential loss of ^{13}C - and ^{14}C -enriched moieties during acidification. Because such moieties may be enriched in N, determination of $\%N$ and $\delta^{15}N$ on acidified samples is not recommended.

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