

# Stable sulfur isotopes in the water column of the Cariaco Basin

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## Abstract

Previous geochemical and microbiological studies in the Cariaco Basin indicate intense elemental cycling and a dynamic microbial loop near the oxic–anoxic interface. We obtained detailed distributions of sulfur isotopes of total dissolved sulfide and sulfate as part of the on-going CARIACO time series project to explore the critical pathways at the level of individual sulfur species. Isotopic patterns of sulfate ( $\delta^{34}\text{S}_{\text{SO}_4}$ ) and sulfide ( $\delta^{34}\text{S}_{\text{H}_2\text{S}}$ ) were similar to trends observed in the Black Sea water column:  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  and  $\delta^{34}\text{S}_{\text{SO}_4}$  were constant in the deep anoxic water (varying within 0.6‰ for sulfide and 0.3‰ for sulfate), with sulfide roughly 54‰ depleted in  $^{34}\text{S}$  relative to sulfate. Near the oxic–anoxic interface, however, the  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  value was  $\sim 3$ ‰ heavier than that in the deep water, which may reflect sulfide oxidation and/or a change in fractionation during *in situ* sulfide production through sulfate reduction (SR).  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  and  $\Delta^{33}\text{S}_{\text{H}_2\text{S}}$  data near the oxic–anoxic interface did not provide unequivocal evidence to support the important role of sulfur-intermediate disproportionation suggested by previous studies. Repeated observation of minimum  $\delta^{34}\text{S}_{\text{SO}_4}$  values near the interface suggests ‘readdition’ of  $^{34}\text{S}$ -depleted sulfate during sulfide oxidation. A slight increase in  $\delta^{34}\text{S}_{\text{SO}_4}$  values with depth extended over the water column may indicate a reservoir effect associated with removal of  $^{34}\text{S}$ -depleted sulfur during sulfide production through SR. Our  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  and  $\Delta^{33}\text{S}_{\text{H}_2\text{S}}$  data also do not show a clear role for sulfur-intermediate disproportionation in the deep anoxic water column. We interpret the large difference in  $\delta^{34}\text{S}$  between sulfate and sulfide as reflecting fractionations during SR in the Cariaco deep waters that are larger than those generally observed in culturing studies.

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## 1. INTRODUCTION

The isotopic composition of sulfur compounds has been used to explore the biogeochemical cycling of sulfur in both modern and ancient marine environments (e.g., Canfield and

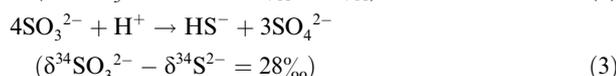
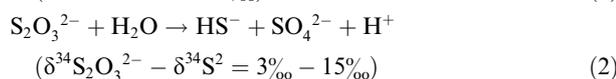
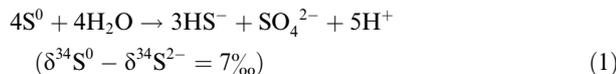
Teske, 1996; Lyons, 1997). Sulfate reducing prokaryotes (SRP) produce sulfide depleted in  $^{34}\text{S}$  compared to the initial sulfate (Kaplan and Rittenberg, 1964), and  $^{34}\text{S}$  depletion of sulfide in sediments and anoxic water in natural systems is commonly 45–70‰ relative to coexisting sulfate. Prior to Canfield et al. (2010), no experiments with pure cultures or natural populations of SRP had yielded fractionations greater than 46‰ (Kemp and Thode, 1968; Habicht and Canfield, 1997; Canfield, 2001; Detmers et al., 2001), although some studies (Goldhaber and Kaplan, 1980; Rudnicki et al.,

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2001; Wortmann et al., 2001; Werne et al., 2003) have suggested that large fractionations can be produced by sulfate reduction (SR) acting alone. Recent models suggest that the metabolic pathways of SR can produce fractionations as large as 75‰ (Brunner and Bernasconi, 2005; Johnston et al., 2007; Farquhar et al., 2008). Much of the current literature, however, has invoked additional fractionations associated with oxidative sulfur cycling to explain the large sulfur isotope offsets between coeval sulfide and sulfate observed in nature.

Canfield and Thamdrup (1994) noted that sulfide produced via SR can be oxidized to elemental sulfur, which can subsequently undergo disproportionation to yield  $^{34}\text{S}$ -depleted sulfide and  $^{34}\text{S}$ -enriched sulfate. Repeated cycles of oxidation and disproportionation can theoretically produce progressively larger sulfur isotope offsets between the sulfide and sulfate pools. In this model, fractionation during sulfide oxidation is assumed to be very small, as supported by previous observations of natural and experimental systems (Fry et al., 1986, 1988; Zerkle et al., 2009), and the isotopic composition of sulfide and sulfate records the initial fractionation during SR plus the additional fractionations generated through repeated cycles of sulfide oxidation to sulfur intermediates (e.g., elemental S, thiosulfate, and sulfite) and disproportionation of these compounds (Canfield and Thamdrup, 1994; Habicht et al., 1998; Böttcher et al., 2001, 2005). Typical fractionations during a single-cycle of disproportionation of sulfur intermediates in bacterial cultures are shown in Eqs. (1)–(3) (Habicht and Canfield, 2001):



Disproportionation of sulfur intermediates can generate sulfide highly depleted in  $^{34}\text{S}$  via reactions above. In contrast, the isotope effects associated with chemical and chemoautotrophic sulfide oxidation differ from disproportionation in direction and magnitude. For example, Fry et al. (1988) reported a fractionation factor during chemical (abiotic) sulfide oxidation by  $\text{O}_2$  of  $-5.2\text{‰}$ . Kaplan and Rittenberg (1964) reported that sulfide oxidation mediated by chemoautotrophs would also generate  $^{34}\text{S}$  enrichments in the sulfide, with fractionations ranging from  $-18\text{‰}$  to  $-1\text{‰}$  (the large value of  $-18\text{‰}$  was questioned by Fry et al. (1986), since it was only measured in one experiment that produced significant amounts of minor reaction products in the form of polythionates and has not been confirmed since). This contrary effect in sulfide isotope fractionation between oxidation of sulfide and sulfur-intermediate disproportionation is potentially useful for investigation of the comparative importance of these two processes.

Large isotopic offsets between sulfide and sulfate, about  $52\text{‰}$ , were reported in the deep Cariaco water column by Fry et al. (1991). A gap in that study, however, is that data were only reported for depths below 400 m, where sulfide

concentrations were higher than  $10 \mu\text{mol/L}$  and the isotopic compositions of sulfide and sulfate were relatively constant with depth. Therefore, the mechanistic controls on sulfur isotope compositions near the Cariaco oxic–anoxic interface remain elusive because of the small size of the sulfur pool and rapid turnover of sulfur intermediates. Werne et al. (2003) analyzed the sulfur isotope compositions of sulfur species in the Cariaco sediment pore water and found that the  $\delta^{34}\text{S}$  difference between pore water sulfate and sulfide generally increased from  $53\text{‰}$  near the sediment water interface to  $60\text{--}63\text{‰}$  at 4 m depth.

As part of the on-going CARIACO (CARbon Retention in a Colored Ocean) time series project, Li et al. (2008) found maxima in concentrations of  $\text{S}_2\text{O}_3^{2-}$ ,  $\text{SO}_3^{2-}$ , particulate  $\text{S}^0$ , and total zero-valent  $\text{S}^0$  near the oxic–anoxic interface. They hypothesized that disproportionation of elemental sulfur and thiosulfate could play an important role near the oxic–anoxic interface in supporting dark carbon fixation of chemoautotrophic bacteria. In the present study, we tested this hypothesis by investigating the sulfur cycle from the perspective of stable isotope compositions of sulfate and sulfide in the water column. We focused specifically on sulfur chemistry near the oxic–anoxic interface where chemoautotrophic production intensifies, and sulfur-intermediate disproportionation is suggested to be significant (Taylor et al., 2001; Ho et al., 2004; Li et al., 2008). In addition, we examined temporal variability in the sulfur isotope signal, related to the physical forcing of oxygen intrusions associated with basin-scale water mass circulation.

We also present data from an emerging sulfur isotope technique that involves analysis of  $^{33}\text{S}/^{32}\text{S}$  and  $^{36}\text{S}/^{32}\text{S}$  isotope ratios in addition to the more conventional  $^{34}\text{S}/^{32}\text{S}$  ratios. These data shed further light on the relative fractionation imprints of SR, disproportionation of sulfur intermediates, and sulfide oxidation metabolisms. Variations among  $^{33}\text{S}/^{32}\text{S}$  and  $^{36}\text{S}/^{32}\text{S}$  ratios can provide information that is independent but complementary to that obtained from typical  $^{34}\text{S}/^{32}\text{S}$  measurements (Farquhar et al., 2003, 2007, 2008; Johnston et al., 2005, 2007; Ono et al., 2006; Zerkle et al., 2009, 2010).

## 2. MATERIALS AND METHODS

### 2.1. Field sites

The Cariaco Basin is a deep, relatively isolated basin located on the continental shelf of northeastern Venezuela (Fig. 1). Station A ( $10^\circ 30' \text{ N } 64^\circ 40' \text{ W}$ ) was sampled on 30 November 2007 (non-upwelling) and 20 May 2008 (upwelling) aboard the R/V *Hermano Ginés*. Water samples were collected in 8-L Teflon-lined Niskin bottles deployed on a Seabird rosette system equipped with a CTD, a YSI oxygen probe, a SeaTec c-beam transmissometer (660 nm), and a Chelsea profiling fluorometer for chlorophyll-a. The Niskin bottles were pressurized slightly with  $\text{N}_2$  during subsampling to preclude chemical oxidation. Peaks in the transmissometer beam attenuation, which were found to be reliable proxies for bacterial maxima near the oxic–anoxic interface, were used to target the interface.

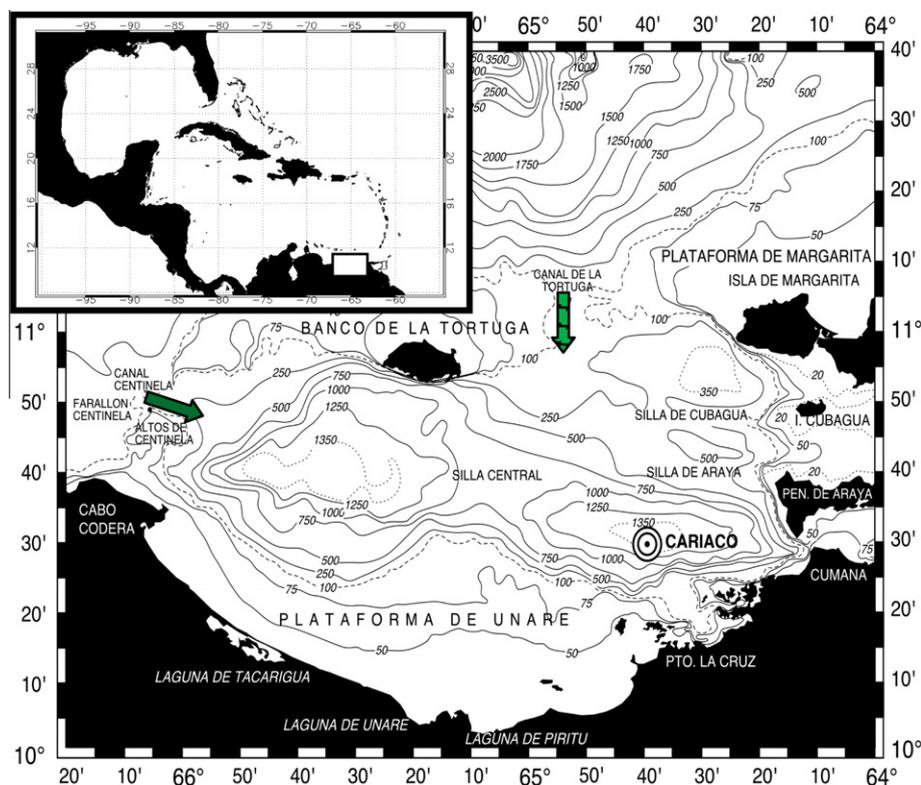


Fig. 1. Map of the Cariaco Basin with the Cariaco time series station indicated (circle). Isobaths are in meters. Arrows indicate pathways for water intrusion from the Caribbean Sea.

## 2.2. Methods

### 2.2.1. Oxygen and sulfur species analysis

Detailed methods for measurement of oxygen, sulfide, and sulfur intermediates species have been reported in Li et al. (2008). Briefly, oxygen was measured *in situ* with an oxygen probe attached to the CTD and confirmed by Winkler titration. Dissolved sulfide was measured by the methylene blue method (Cline, 1969). Thiosulfate and sulfite were analyzed with the method of Vairavamurthy and Mopper (1990) as modified by Hayes et al. (2006). Total zero-valent sulfur was analyzed with UV–vis by HPLC (Li et al., 2008). Sulfate concentrations were measured from aliquots of samples collected for sulfur isotope analysis on an Agilent 7400 Quadrupole ICP-MS system using Xe as the collision cell gas at the University of California, Riverside (UCR). Replicate analyses of  $\text{SO}_4\text{-S}$  concentration agreed within 5% at the 100 ppb range.

### 2.2.2. Sulfur isotope measurement

Samples for isotope analysis were collected during both cruises and analyzed by two methods (continuous flow for the November 2007 cruise and a dual-inlet approach for the May 2008 cruise). We first describe sampling technique and then the specific analysis methodologies for each cruise. Inter-calibration between labs for the  $\delta^{34}\text{S}$  isotope composition shows strong agreement within analytical error (0.2‰; data not shown).

**2.2.2.1. Sample collection.** Ten ml of seawater were collected from discrete depths in the water column for sulfate-S isotope analysis. Exposure of dissolved sulfide to atmospheric oxygen was minimized by sampling water column splits with a 10 ml Hamilton Gas-Tight glass syringe (Li et al., 2008). The sample was immediately injected into a glass serum vial containing 0.55 ml buffered formalin (final conc. 2%) and 1.25 ml 0.05 M zinc acetate (final conc. 5 mM) and stored frozen until analysis. Zinc acetate-fixed water samples were filtered with a 0.2  $\mu\text{m}$  membrane filter to remove sulfides precipitated as ZnS. The sulfate in the filtrate was then precipitated with 4 ml 25% (w/v)  $\text{BaCl}_2$ . Ten ml of 4 M HCl were used to rinse the filter for 30 s to remove any co-precipitated  $\text{BaCO}_3$ .

Large volumes of water (1–10 L) were collected in collapsible LDPE cubitainers for sulfide isotopic analysis. Each container was allowed to overflow at least half of the volume before 8 ml 0.05 M zinc acetate was added. Care was taken to mix the zinc solution throughout the cubitainer to ensure quantitative precipitation of dissolved sulfide as ZnS. Samples were shaken and left for one day to form a coarse ZnS precipitate at the bottom of the vessel before filtration with 0.45  $\mu\text{m}$  HA Millipore filters in the lab at Estación de Investigaciones Marinas de Margarita (EDIMAR). The filters were frozen immediately and stored at  $-20^\circ\text{C}$  until analysis.

Sulfur distillations were performed at UCR. Filters were first acidified with 6 N HCl under  $\text{N}_2$ , and the released sulfide was precipitated in traps containing 30 ml aqueous silver nitrate (3% w/v) with 10%  $\text{NH}_4\text{OH}$ . Precipitated

Ag<sub>2</sub>S was collected by filtration on a 0.45 μm nitrocellulose filter. Sulfide precipitation also collects metal monosulfides present in the water column, and these will dissolve during our acidification step. Independent measurements confirm that concentrations of metal monosulfide in the water column are at least three orders of magnitude lower than those of dissolved sulfide (Li et al., 2010). Furthermore, the small fractionation (−0.5‰ to 1.2‰) observed during metal monosulfide formation (Böttcher et al., 1998) suggests minimal isotopic separation between these reduced sulfur pools. As such, sulfur liberated during acidification is herein referred to as dissolved sulfide.

**2.2.2.2. Sulfate and sulfide isotopes (November 2007).** Precipitates of sulfate (BaSO<sub>4</sub>) and sulfide (Ag<sub>2</sub>S) were dried at room temperature, homogenized, and weighed with excess V<sub>2</sub>O<sub>5</sub> in tin capsules for continuous flow <sup>34</sup>S/<sup>32</sup>S isotopic analysis. Sulfur isotope ratios of samples were determined using a Thermo Finnigan Delta V Advantage stable isotope ratio mass spectrometer coupled with a Costech ECS elemental analyzer for online sample combustion and analysis at UCR. The sulfur isotope results are expressed in ‰ relative to Vienna Canyon Diablo Troilite (VCDT) using the standard δ notation:

$$\delta^{34}\text{S}_{\text{sample}} = \left[ \frac{{}^{34}\text{S}/{}^{32}\text{S}_{\text{sample}}}{{}^{34}\text{S}/{}^{32}\text{S}_{\text{VCDT}}} - 1 \right] \times 1000 \quad (4)$$

Sulfate isotope measurements were normalized to international standards NBS-127 (+21.1‰), IAEA SO-5 (+0.49‰), and SO-6 (−34.05‰). Sulfide isotopes were calibrated against IAEA S1 (−0.3‰), S2 (+22.65‰), and S3 (−32.5‰). Reproducibility of standard reference materials and replicate sample analyses were better than ±0.2‰.

**2.2.2.3. Multiple sulfur isotopes for sulfate and sulfide (May 2008).** For samples collected in May 2008, <sup>33</sup>S/<sup>32</sup>S, <sup>34</sup>S/<sup>32</sup>S, and <sup>36</sup>S/<sup>32</sup>S ratios were determined at University of Maryland (UM). For sulfide sample processing, a similar approach as described above was used to convert ZnS to Ag<sub>2</sub>S. For sulfate isotope analysis, BaSO<sub>4</sub> was chemically reduced to H<sub>2</sub>S using a reducing solution (125 ml concentrated HI, 205 ml concentrated HCl, and 61 ml concentrated H<sub>3</sub>PO<sub>2</sub>) heated to boiling under a N<sub>2</sub> atmosphere for 3 h (Forrest and Newman, 1977). The evolved sulfide was sparged with nitrogen and captured as ZnS using a zinc acetate trapping solution. The ZnS precipitates were converted to Ag<sub>2</sub>S by addition of 1 M AgNO<sub>3</sub> and rinsed with 250 ml MilliQ water and 50 ml concentrated NH<sub>4</sub>OH.

Details of the sulfur isotope fluorination methods have been described previously (Farquhar et al., 2008; Zerkle et al., 2009). Briefly, a 1–3 mg Ag<sub>2</sub>S sample (for S in sulfate and sulfide) were wrapped in aluminum foil and pumped under vacuum (10<sup>−3</sup> torr) in a Ni reaction vessel. A ten-times excess of pure F<sub>2</sub> was added at 250 °C for 8 h to produce sulfur hexafluoride (SF<sub>6</sub>). The fluorinated gas mixture was purified cryogenically (distilled at −110 °C) and by TCD gas chromatography (on a 12' molecular sieve 5 Å/Haysep Q column). SF<sub>6</sub> gas was measured as SF<sub>5</sub><sup>+</sup> (m/e of 127, 128, 129, 131 for <sup>32</sup>SF<sub>5</sub><sup>+</sup>, <sup>33</sup>SF<sub>5</sub><sup>+</sup>, <sup>34</sup>SF<sub>5</sub><sup>+</sup>, and <sup>36</sup>SF<sub>5</sub><sup>+</sup>) on a Thermo Finnigan MAT 253 gas source mass spectrometer.

As for the November 2007 cruise, we report <sup>34</sup>S/<sup>32</sup>S data using standard delta notation (δ) relative to VCDT for this cruise. The less abundant sulfur isotopes (<sup>33</sup>S/<sup>32</sup>S and <sup>36</sup>S/<sup>32</sup>S) are reported using the capital delta notation (Δ), where

$$\Delta^{33}\text{S} = \left[ \left( \frac{{}^{33}\text{S}/{}^{32}\text{S}_{\text{sample}}}{{}^{33}\text{S}/{}^{32}\text{S}_{\text{VCDT}}} \right) - \left( \frac{{}^{34}\text{S}/{}^{32}\text{S}_{\text{sample}}}{{}^{34}\text{S}/{}^{32}\text{S}_{\text{VCDT}}} \right)^{0.515} \right] \times 1000 \quad (5)$$

$$\Delta^{36}\text{S} = \left[ \left( \frac{{}^{36}\text{S}/{}^{32}\text{S}_{\text{sample}}}{{}^{36}\text{S}/{}^{32}\text{S}_{\text{VCDT}}} \right) - \left( \frac{{}^{34}\text{S}/{}^{32}\text{S}_{\text{sample}}}{{}^{34}\text{S}/{}^{32}\text{S}_{\text{VCDT}}} \right)^{1.90} \right] \times 1000 \quad (6)$$

The exponents in these relationships (0.515 and 1.90) define the reference fractionation line (RFL) and approximate single-step thermodynamic equilibrium isotope exchange effects (Hulston and Thode, 1965). An estimate of the uncertainties is provided by the current long-term reproducibility for fluorinations and isotopic analyses of reference materials in the UM lab (past 2 years), which are (2σ) 0.28, 0.016, and 0.4‰ for δ<sup>34</sup>S, Δ<sup>33</sup>S, and Δ<sup>36</sup>S, respectively. Measurements of IAEA S1 at UM presently yield δ<sup>34</sup>S = −0.30‰, Δ<sup>33</sup>S = +0.0944‰, and Δ<sup>36</sup>S = 0.69‰. Measurements of δ<sup>34</sup>S for IAEA S2 and S3 undertaken at UM are reported in Ono et al. (2006) as 22.31‰ and −32.51‰, respectively (note these are direct measurements and no scale compression corrections were applied).

### 3. RESULTS

#### 3.1. Oxygen and sulfur compounds

Water column oxygen concentration decreased abruptly to below the detection limit (2 μM) at 230 and 260 m for November 2007 and May 2008, respectively. The concentration of H<sub>2</sub>S increased below 260 m (November 2007) and 270 m (May 2008), reaching nearly 63 μM at 1300 m (Fig. 2A and C). During the November 2007 cruise, a comparatively narrow peak for total zero-valent S<sup>0</sup> was found near the oxic–anoxic interface, with a maximum of 1.45 μM at a depth of 260 m (Fig. 2B). A broader yet smaller peak for total S<sup>0</sup> was observed in May 2008 (Fig. 2D). Thiosulfate and sulfite concentrations were only slightly above their detection limits (0.3 and 0.1 μM, respectively) at all depths. Compared to seawater (SO<sub>4</sub>/Cl = 0.14; g/g), sulfate concentration normalized to chloride in the Cariaco water column was constant within the precision of the method (Fig. 3).

#### 3.2. Sulfur isotope composition of sulfide and sulfate

The sulfur isotope composition of dissolved sulfide in the Cariaco water column varied from −29.9‰ to −32.6‰ and from −28.9‰ to −32.8‰ for November 2007 and May 2008, respectively (Table 1 and Fig. 4). In the vertical distribution of δ<sup>34</sup>S<sub>H<sub>2</sub>S</sub>, <sup>34</sup>S enrichments are detected in the uppermost and lowest parts of the anoxic water column on both cruises. <sup>34</sup>S enrichment in sulfide

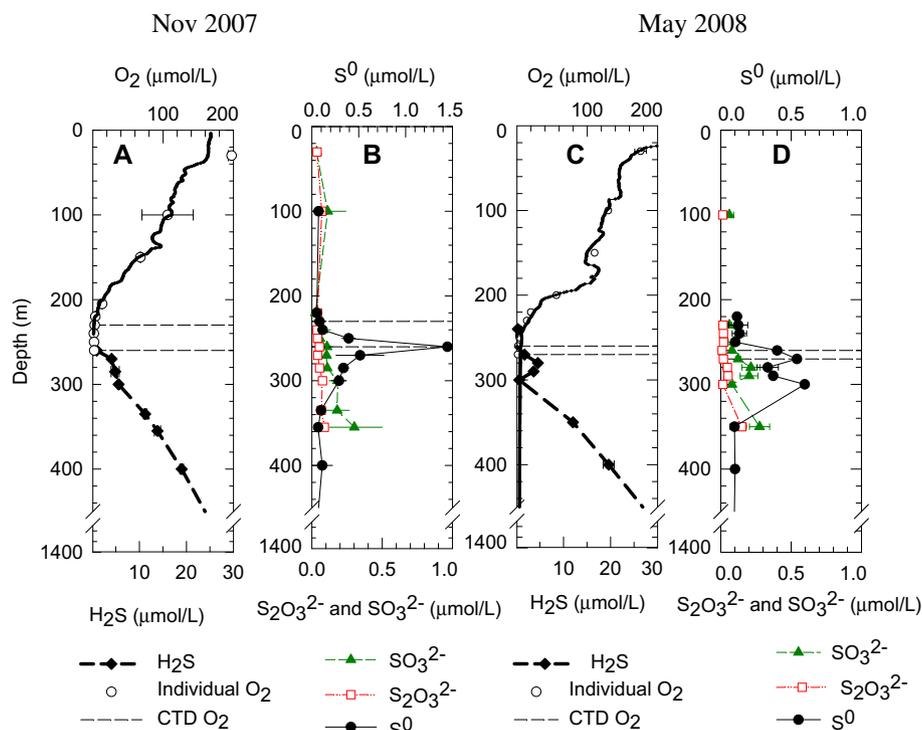


Fig. 2. Chemical gradients in the water column. (A) Concentrations of oxygen and hydrogen sulfide for November 2007. (B) Concentrations of thiosulfate, sulfite, and total zero-valent sulfur by depth for November 2007. (C) Concentrations of oxygen and hydrogen sulfide for May 2008. (D) Concentrations of thiosulfate, sulfite, and total zero-valent sulfur by depth for May 2008.

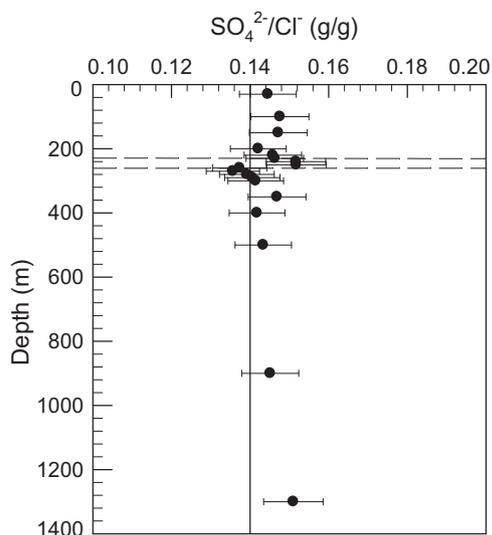


Fig. 3. Water-column profile of sulfate normalized to chloride in November 2007. Error bars reflecting  $\pm 5\%$  analytical uncertainty are included. The vertical line delineates the normal seawater  $\text{SO}_4/\text{Cl}$  ratio of 0.14 (g/g). The chemocline is marked by horizontal dashed line.

from the upper anoxic zone was seen in Cariaco samples as deep as 400 m (sulfide concentration  $19 \mu\text{mol/L}$ ), which is coincident with the maximum depth at which chemoautotrophic  $\text{CO}_2$  fixation was consistently detected (Taylor

et al., 2001, 2006). This pattern has also been observed in the Black Sea (Sweeney and Kaplan, 1980; Fry et al., 1991; Neretin et al., 2003).

$\delta^{34}\text{S}_{\text{SO}_4}$  ranged from  $21.0\text{‰}$  to  $21.3\text{‰}$  (average  $21.1\text{‰}$ ) in November 2007 and from  $21.2\text{‰}$  to  $21.7\text{‰}$  (average  $21.4\text{‰}$ ) in May 2008 (Table 2 and Fig. 5). Similar variations near the oxic–anoxic interface were observed during January 2005 and May 2005 (Table 2; Percy, 2006). The extent of variation in  $\delta^{34}\text{S}_{\text{SO}_4}$  value was only slightly greater than analytical precision ( $\pm 0.2\text{‰}$ ); an isotopic minimum was observed repeatedly near the oxic–anoxic interface during all four cruises, suggestive of a real and persistent environmental pattern.

We complemented the May 2008  $\delta^{34}\text{S}$  data set with multiple sulfur isotope measurements ( $\Delta^{33}\text{S}$  and  $\Delta^{36}\text{S}$ ) in an attempt to elucidate the effects of sulfide oxidation and sulfur-intermediate disproportionation (Fig. 6). We observed  $^{34}\text{S}$  sulfide enrichments near the oxic–anoxic interface and relatively constant values with greater depth, similar to the water column patterns observed during November 2007 (Fig. 4). A minimum  $\Delta^{33}\text{S}_{\text{SO}_4}$  was detected at 280 m ( $0.027\text{‰}$ ), while the  $\Delta^{33}\text{S}$  of sulfate did not change significantly elsewhere in the oxic and anoxic water column, varying from  $0.034\text{‰}$  to  $0.048\text{‰}$ . The  $\Delta^{33}\text{S}$  of sulfide was lowest near the oxic–anoxic interface, increasing from 0.123 to  $0.155\text{‰}$  at depth. The  $\Delta^{36}\text{S}$  was more negative for sulfide than for sulfate. No depth-dependent variation of  $\Delta^{36}\text{S}$  was observed for sulfide or sulfate outside the estimates of analytical uncertainty.

Table 1  
Sulfide concentration and sulfide isotope composition in the Cariaco water column in November 2007 and May 2008.

Cruise time	Depth (m)	Sulfide ( $\mu\text{mol/L}$ )	$\delta^{34}\text{S}_{\text{H}_2\text{S}}$ (‰)	$\Delta^{33}\text{S}_{\text{H}_2\text{S}}$ (‰)	$\Delta^{36}\text{S}_{\text{H}_2\text{S}}$ (‰)
November-07	260	0.9	-31.1		
November-07	265	2.2	-29.9		
November-07	270	4.1	-30.8		
November-07	280	4.1	-30.8		
November-07	290	5.4	-31.1		
November-07	400	19.0	-31.3		
November-07	500	29.0	-32.6		
November-07	900	55.6	-32.5		
November-07	1300	64.2	-32.4		
May-08	290	3.7	-28.9	0.123	-1.207
May-08	320	8.6	-31.1	0.143	-1.058
May-08	400	19.6	-32.3	0.156	-1.258
May-08	500	34.1	-32.8	0.155	-1.407
May-08	900	56.8	-32.4	0.146	-1.420
May-08	1300	62.2	-32.2	0.147	-1.369

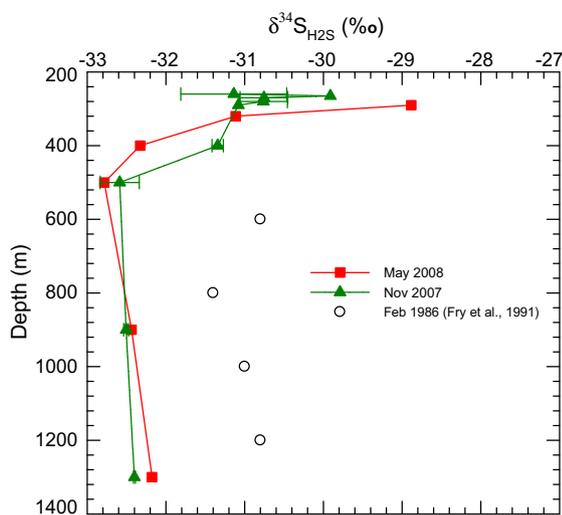


Fig. 4. The vertical distribution of  $\delta^{34}\text{S}$  (dissolved  $\text{H}_2\text{S}$ ; VCDT) in the Cariaco water column during November 2007 and May 2008. Plotted together are 1986 data collected from the Cariaco station reported by Fry et al. (1991). Note that the Fry data were reported using CDT scale rather than VCDT.

## 4. DISCUSSION

### 4.1. Sulfate $\delta^{34}\text{S}$

The mean  $\delta^{34}\text{S}_{\text{SO}_4}$  (21.2‰) in the Cariaco Basin is similar to isotopic values of sulfate found in coastal waters and the modern open ocean (Hoefs, 2004), and the observed variation only slightly exceeded the precision of the method. This relationship reflects the very small fraction of sulfate converted to sulfide relative to renewal rates. For the observed sulfate concentration of 28 mM, a change of  $\delta^{34}\text{S}_{\text{SO}_4}$  values by 0.1‰ over the whole Cariaco water column would require addition or removal of 63  $\mu\text{mol/L}$  sulfide given the 54‰ isotope offset between sulfide and sulfate. Measurements of sulfate concentration in the water column were not sensitive enough to show a change with depth

(Fig. 3). Even so, we consistently observed small excursions in the sulfate-S isotope composition near the oxic–anoxic interface (Fig. 5), suggesting (re)addition of  $^{32}\text{S}$  during sulfide oxidation. Throughout the water column, the slight increase of  $\delta^{34}\text{S}$  for sulfate with water depth (especially in May 2008, 0.4‰ increase) is consistent with SR in the water, during which preferential reduction of the lighter sulfate produces a  $^{34}\text{S}$ -enriched sulfate residue in the deep anoxic water. This detectable  $\delta^{34}\text{S}_{\text{SO}_4}$  increase with depth may imply a small preferential sink for  $^{32}\text{S}$  associated with settling of sulfide minerals from the water column, consistent with syngenetic pyrite formation in the water column (Lyons et al., 2003; Li et al., 2010).

### 4.2. Temporal shift in $^{34}\text{S}_{\text{H}_2\text{S}}$

$\delta^{34}\text{S}$  values for dissolved sulfide in the upper water column varied with time in contrast to invariant values in the deep anoxic water (below 500 m).  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  near the oxic–anoxic interface was 1‰ heavier in May 2008 than in November 2007 (Fig. 4). This difference is likely associated with an oxygen intrusion event observed in May 2008 (Figs. 2C and D, 7 and 8). The temperature–salinity diagram indicates that the water mass during our May 2008 cruise was different than that present in April 2008 and returned to approximately its original state in June 2008, probably due to lateral mixing of water within the basin (Fig. 7). We detected free sulfide (based on the Cline, 1969 method) from 260 to 290 m water depth when the station was first sampled. However, we did not observe ZnS precipitation in our samples between 260 and 290 m collected 12 h later (confirmed by acid distillation), implying that most of the sulfide above 290 m had been scavenged by intruding oxygenated water within  $\sim 12$  h. Unfortunately, the transient nature of the intrusion event precluded the collection of a second sulfide profile to verify this suggestion.

There is considerable evidence for periodic ventilation of Cariaco Basin deep water. For example, Holmen and Rooth (1990) argued convincingly based on tritium data that relatively young water must reach the deep waters of

Table 2  
Sulfate isotope composition in the Cariaco water column.

January-05			May-05		
Depth (m)	$\delta^{34}\text{S}_{\text{SO}_4}$ (‰)	Stdev (‰)	Depth (m)	$\delta^{34}\text{S}_{\text{SO}_4}$ (‰)	Stdev (‰)
200	21.2	0.0	201	21.2	0.0
220	21.1	N.D.	220	21.2	0.1
235	21.1	0.0	236	21.2	0.0
250	21.1	0.0	250	21.3	0.0
260	21.1	0.1	260	21.2	0.0
270	21.2	0.1	270	21.2	0.1
280	21.2	0.0	280	21.1	0.1
290	21.2	0.1	291	21.2	N.D.
300	21.2	0.0	301	21.2	0.1
310	21.1	0.0	310	21.3	0.0
340	21.2	0.1	340	21.3	0.0
400	21.2	N.D.	401	21.3	0.0
39,387.0			39,569.0		
Depth (m)	$\delta^{34}\text{S}_{\text{SO}_4}$ (‰)	Stdev (‰)	Depth (m)	$\delta^{34}\text{S}_{\text{SO}_4}$ (‰)	
30	21.0	0.0	100	21.4	N.D.
100	21.1	0.1	270	21.3	N.D.
150	21.0	0.1	280	21.2	N.D.
205	21.1	N.D.	290	21.3	N.D.
220	21.1	0.1	300	21.4	N.D.
230	21.0	0.0	900	21.7	N.D.
240	21.1	0.1			
250	21.1	0.1			
260	21.1	0.1			
270	21.0	0.0			
285	21.1	0.1			
300	21.0	0.0			
335	21.1	0.0			
355	21.1	N.D.			
400	21.0	0.0			
500	21.3	0.1			
900	21.2	0.0			
1300	21.2	N.D.			

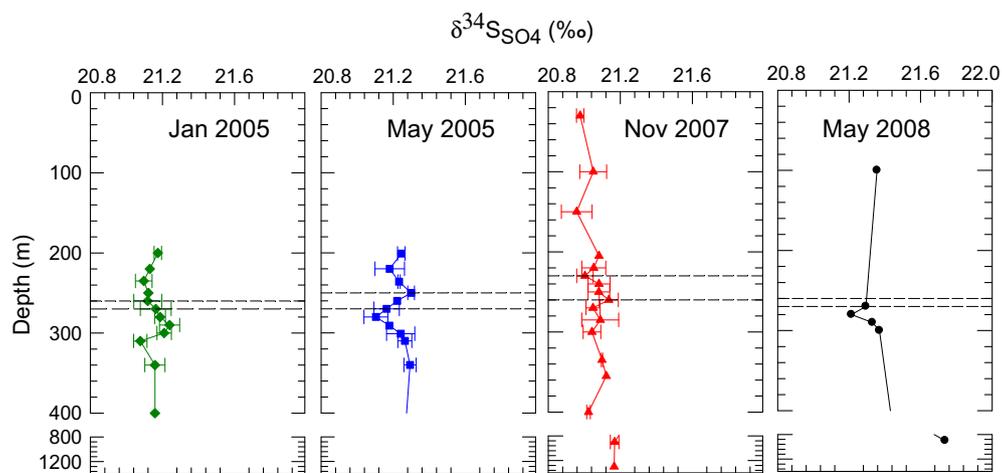


Fig. 5. Vertical distribution of  $\delta^{34}\text{S}$  for water column sulfate in November 2007 and May 2008 (this study) and January 2005 and May 2005 (Percy, 2006). Note the small scale changes across the oxic–anoxic interface (horizontal dashed line). Although the variability in the sulfate isotope data approaches analytical resolution ( $\pm 0.2\text{‰}$ ), the consistent pattern of an isotopic minimum near the oxic–anoxic interface suggests active sulfur cycling.

the basin on a decadal time scale. They proposed two distinct sources: a high-salinity but low-volume source, origi-

inating along the Venezuela coast sinking to the bottom, and a larger source from water injected at the sills,

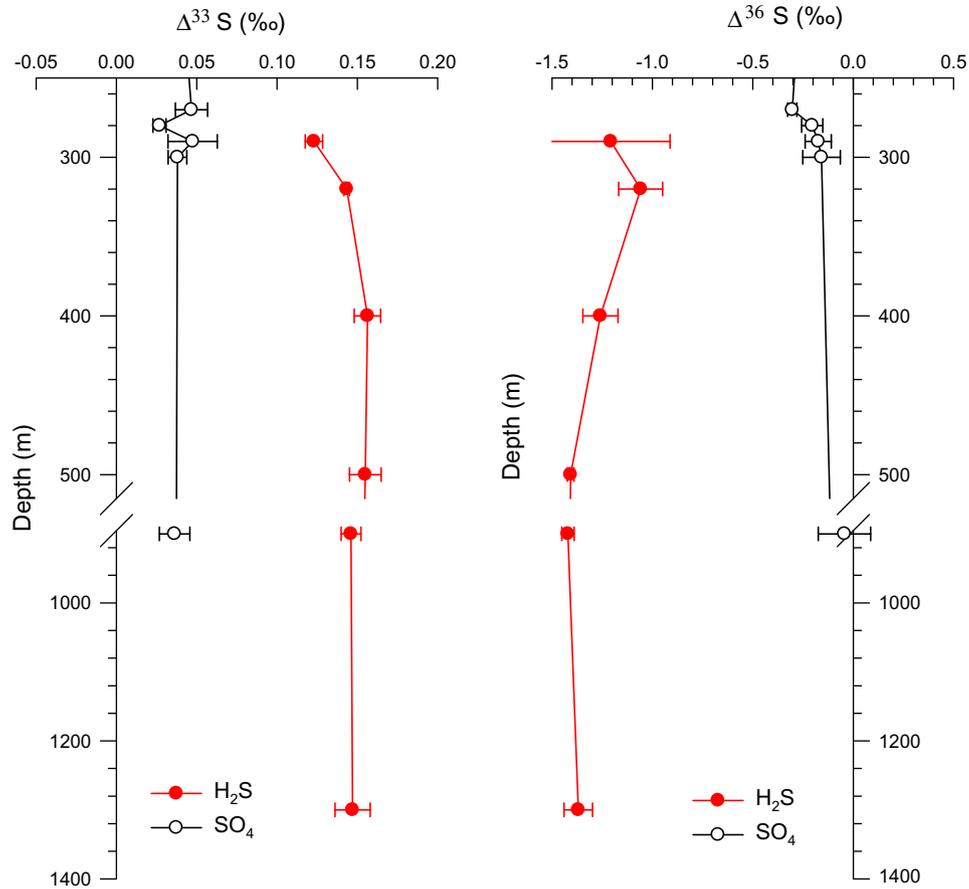


Fig. 6. Water profiles for  $\Delta^{33}\text{S}$  and  $\Delta^{36}\text{S}$  for sulfide and sulfate with depth in May 2008. The oxic–anoxic interface was located at about 270 m water depth.

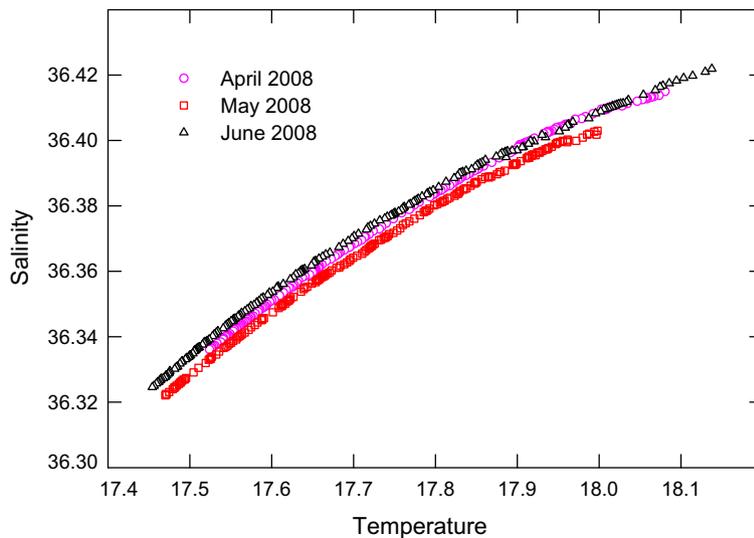


Fig. 7. Temperature–Salinity diagram during May 2008. Also included for comparison are T–S data from April 2008 and June 2008.

providing water that sinks to mid-water depths. Since 1995, repeated observations of small maxima in dissolved oxygen near the oxic–anoxic interface have suggested the occurrence of intrusions of oxygenated water into the upper part

of the anoxic water column (Scranton et al., 2001). Ventilation events occurring in 1997 and 1998 were associated with eddies near the shelf in the southeastern Caribbean Sea. It was hypothesized that these eddies would transport colder

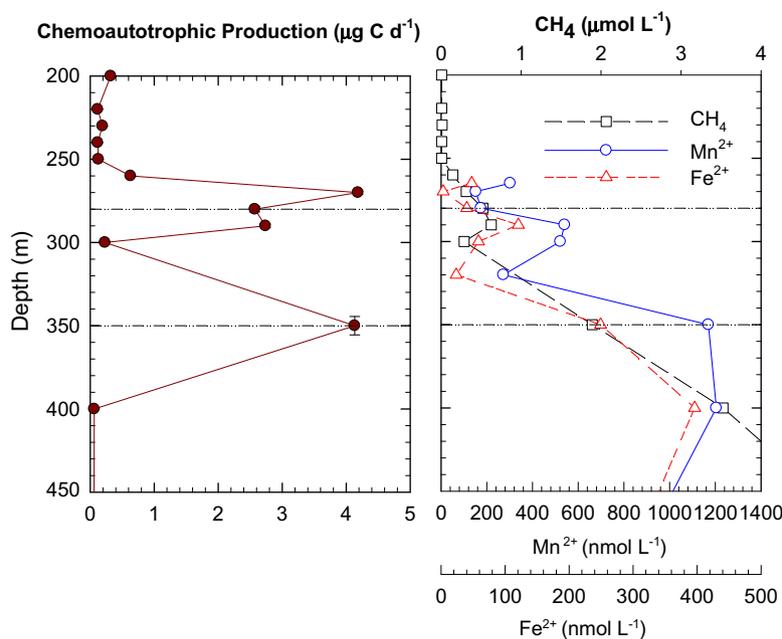


Fig. 8. Minima for microbial activity and  $\text{CH}_4$ , dissolved  $\text{Fe}^{2+}$ , and  $\text{Mn}^{2+}$  concentrations near the interface support the idea that relatively large volumes of oxygenated water were recently intruded to depths of at least 300 m over a short period of time. Dashed lines mark the zone that is mostly like to be influenced by oxygen intrusion.

and denser Caribbean Sea water into the basin, which subsequently sinks and spreads along isopycnals (Astor et al., 2003). The most  $^{34}\text{S}$ -enriched sulfide isotope composition in May 2008 was observed at the same depth as the sulfide minimum (Fig. 2C), consistent with isotopic fractionation during oxidation processes. Sulfide oxidation is also suggested by an increase in total zero-valent sulfur concentration (Fig. 2D) and minima for chemoautotrophic production, dissolved  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ , and methane (Fig. 8).

Our deep-water values for  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  were 1.4‰ lighter than those reported by Fry et al. (1991). This difference suggests temporal variation in the Cariaco Basin from 1986 to 2007/8. Over this period, sulfide concentration varied from about 47  $\mu\text{mol/L}$  in 1982 (Hastings and Emerson, 1988) to 75  $\mu\text{mol/L}$  (Scranton et al., 2001) in 1995, 35  $\mu\text{mol/L}$  in 1999 (Scranton et al., 2001), and 63  $\mu\text{mol/L}$  in 2008 (this study). A portion of the isotope difference between our study and Fry et al. (1991) may also be due to a shift in the preferred standardization of stable sulfur isotope ratios (Coplen and Krouse, 1998; Ding et al., 2001) from CDT (used in Fry et al., 1991) to VCDT (used here). Differences stemming from use of the two approaches can be as large as 0.7‰ (Sweeney and Kaplan, 1980; Böttcher et al., 1997), and the inhomogeneity of the CDT material itself can be as much as 0.4‰ (Coplen and Krouse, 1998). As a result, the estimated uncertainty between these two standards could be as large as 1.1‰.

#### 4.3. Relative enrichment of $^{34}\text{S}$ in sulfide near the oxic–anoxic interface

Our sulfide isotope data consistently show an increase in  $\delta^{34}\text{S}$  values near the oxic–anoxic interface (Fig. 4). A similar

trend has been observed near the Black Sea interface, where sulfide is enriched in  $^{34}\text{S}$  by 3–8‰ compared to dissolved sulfide in the deeper anoxic water (Fry et al., 1991; Neretin et al., 2003). This trend in both basins is presumably related to either the production (from SR) or the consumption (oxidation) of sulfide. We explore these possibilities below.

Sulfur isotope fractionations associated with SR may be smaller near the interface compared to deeper waters if there is, for instance, a difference in the quality of organic matter, the pathway by which SRP metabolize sulfur in different parts of the water column, or a difference in the community structure of SRP as a function of depth (Detmers et al., 2001). An inverse relationship between sulfate reduction rate (SRR) and isotope fractionation has been observed within bacterial cultures and in natural populations of sulfate reducing bacteria (Kaplan and Rittenberg, 1964; Canfield, 2001; Aharon and Fu, 2003). Differences in rates and fractionations have also been observed as a consequence of differences in organic substrate quantity and/or quality, with more abundant or more labile organic matter associated with higher SRR and smaller  $\delta^{34}\text{S}$  fractionations (Canfield, 2001; Detmers et al., 2001). Carbon fixation by chemoautotrophic bacteria is higher near the oxic–anoxic interface (Taylor et al., 2001), and the resulting compounds could be used as organic substrates by SRP, perhaps enhancing reduction rates and resulting in smaller fractionations associated with SR. This speculation is consistent with what has been found in the Black Sea water column where direct measurements using the radiotracer technique found higher SRR near the interface relative to the deeper waters (Albert et al., 1995). We do not have data to delineate changes in the structure of the community of SRP with depth, but such population changes, if present,

could also contribute to smaller fractionations associated with sulfide production near the interface.

Another way to explain greater  $^{34}\text{S}$  enrichment in sulfide near the interface is sulfide loss via oxidation. There are several different pathways for sulfide oxidation, and the direction and magnitude of the associated isotope effect depend on which pathway(s) prevail(s). Fry et al. (1988) and Zerkle et al. (2009) reported isotope fractionations from 0 to +3‰ for the oxidation of  $\text{H}_2\text{S}$  to  $\text{S}^0$  by anoxygenic phototrophic sulfur oxidizing organisms. Oxidation with this fractionation would yield sulfide that is depleted in  $^{34}\text{S}$  rather than the enrichments we observed at the Cariaco interface. Furthermore, phototrophic oxidation is not expected here because light levels are extremely low (Taylor et al., 2001). Other oxidation pathways, however, could produce the  $^{34}\text{S}$  enrichments seen at the Cariaco oxic–anoxic interface. These possibilities include chemical (abiotic) and chemoautotrophic sulfide oxidation, as discussed above.

We can evaluate these two possibilities (i.e., smaller fractionation during SR vs. sulfide oxidation) for  $^{34}\text{S}$  enrichment in sulfide near the interface using reasoning similar to that presented by Fry et al. (1991) and Mariotti et al. (1981). Fry et al. (1991) explored the possibility of mixing of two sources: a small standing pool of sulfide with isotope value of  $\delta^{34}\text{S}_\text{I}$  and sulfide added at each depth with isotopic value of  $\delta^{34}\text{S}_\text{A}$ . The mass balance relationship follows:

$$\delta^{34}\text{S}_\text{D} * c_\text{D} = \delta^{34}\text{S}_\text{I}c_\text{I} + \delta^{34}\text{S}_\text{A} * (c_\text{D} - c_\text{I}) \quad (7)$$

where  $c$  and  $\delta^{34}\text{S}$  are the concentration and isotope composition of sulfide, the subscript D refers to the sulfide at a given depth, I refers to sulfide present near the oxic–anoxic interface, and A denotes sulfide added at each depth. Rearranging this equation gives the linear relationship (Fig. 9A):

$$\delta^{34}\text{S}_\text{D} = (\delta^{34}\text{S}_\text{I} - \delta^{34}\text{S}_\text{A}) * c_\text{I}/c_\text{D} + \delta^{34}\text{S}_\text{A} \quad (8)$$

The second approach, described by Mariotti et al. (1981), evaluates the possibility that the relationship between  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  and concentration can be described by a Rayleigh process with constant fractionation:

$$\delta_t - \delta_0 = 10^3 * (\alpha - 1) * \ln f \quad (9)$$

where  $\delta_t$  is the  $\delta^{34}\text{S}$  value of the residual fraction ( $f$ ) of  $\text{H}_2\text{S}$ , and  $\delta_0$  is the  $\delta^{34}\text{S}$  value of the original  $\text{H}_2\text{S}$ . Rayleigh fractionation does not strictly apply to the water column of the Cariaco Basin since it is not a closed system. However, this approach provides a minimum estimate of the fractionation factor and serves as an end-member for comparison with the results derived from the Black Sea (Fry et al., 1991). The fractionation factor,  $\epsilon$ , is given by  $10^3 * (\alpha - 1)$ . Using this approach, the  $\epsilon$  value during sulfide oxidation near the interface in November 2007 is  $-0.69\text{‰}$  (excluding the 260 m data, since the  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  value at this depth is off the  $^{34}\text{S}$  enrichment trend) and  $-1.15\text{‰}$  in May 2008 (Fig. 9B). This result is similar to fractionation near the interface of the Black Sea, where  $\epsilon = -1.6\text{‰}$  (Fry et al., 1991). It is also similar to an experimental value measured for chemical and chemoautotrophic sulfide oxidation (Fry et al., 1988). Because SRR have not been measured directly in the Cariaco water column, it is difficult to unravel the specific role of SR-dependent fractionation effects near the interface of the water column. However, maxima of sulfur intermediates,

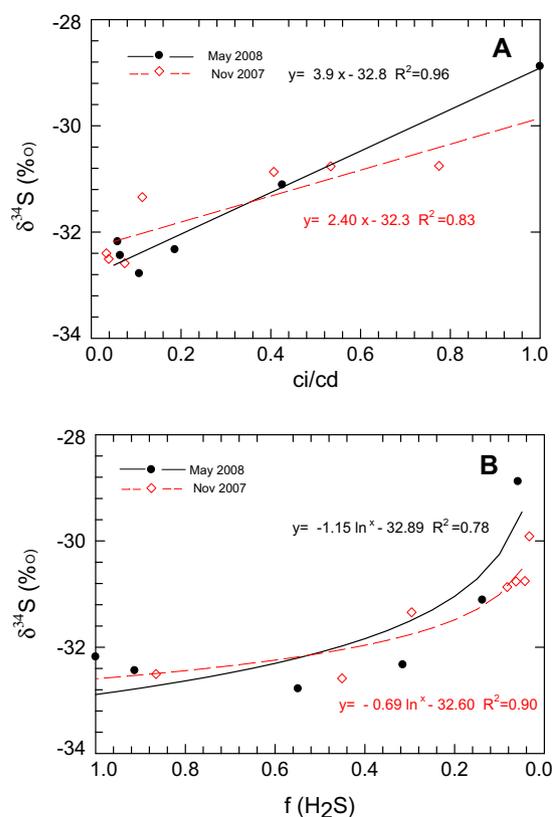


Fig. 9. Modeling of  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  based on (A) mixing with sulfide produced at the interface and sulfide produced in the deep anoxic water and (B) isotopic fractionation during sulfide oxidation.

indicative of sulfide oxidation, are always observed near the Cariaco oxic–anoxic interface (Hayes et al., 2006; Li et al., 2008; Percy et al., 2008; Fig. 2, herein). We therefore favor an explanation that attributes the change in  $^{34}\text{S}/^{32}\text{S}$  of sulfide in the upper parts of the water column to sulfide oxidation via chemical or chemoautotrophic pathways.

#### 4.4. Variation of $^{34}\text{S}_{\text{H}_2\text{S}}$ in the deep water

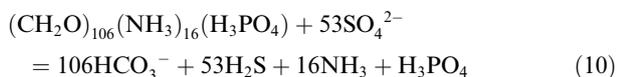
A slight  $^{34}\text{S}$  enrichment in dissolved sulfide was also observed at the bottom water column (900 and 1300 m, Fig. 4). The sulfide at 500 m ( $\delta^{34}\text{S}_{\text{H}_2\text{S}} = -33\text{‰}$ ) is isotopically lighter than Cariaco surface sediments ( $\delta^{34}\text{S}_{\text{H}_2\text{S}} = -29\text{‰}$ , Werne et al., 2003). A mixture of sulfide produced within the anoxic water column and that produced in the sediment may explain bottom-water  $\text{H}_2\text{S}$  of intermediate isotopic values ( $\delta^{34}\text{S}_{\text{H}_2\text{S}} = -32\text{‰}$ ).

There is only indirect information on organic matter decomposition by SR in the water column of the Cariaco Basin. Fanning and Pilson (1972) and Scranton et al. (1987) argued that the sulfide profiles in the Cariaco water column could be explained if SR only took place in the sediment. Based on molybdate inhibition of acetate uptake, Ho et al. (2004) suggested that SR was not a dominant pathway for organic carbon decomposition in the Cariaco water column near the interface. However, other studies have posited SR in the water column. For example,

Hastings and Emerson (1988) argued for SR on the basis of alkalinity and total inorganic carbon measurements. Lin et al. (2006) identified an enrichment of sulfate-reducing proteobacterial cells (SRB385 positive) near the oxic/anoxic interface. Based on both the concentration profiles of sulfide reported here, which do not show a simple monotonic increase with depth, and by the minimum in  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  in the middle of the water column (500 m, Fig. 4), we argue that SR is occurring in the Cariaco water column.

Because the rate of SR in the Cariaco water column and in the sediment has never been determined directly using the  $^{35}\text{S}$  radiotracer technique, we estimated a plausible upper limit for these rates based on the particulate organic carbon (POC) flux measured at the same station. Sediment trap data show a sharp decrease in POC flux from 410 to 1210 m (Thunell et al., 2000). The most likely pathway for organic matter remineralization between these depths is through SR, since other oxidants that can yield energy more efficiently have already been depleted. In the Black Sea, the integrated rates of SR obtained using radiotracer techniques and those estimated from particulate carbon flux are in reasonable agreement (Albert et al., 1995).

Thus, we can estimate the amount of sulfate that is reduced for a given POC flux loss based on the following reaction stoichiometry (Froelich et al., 1979):



This equation yields a C:S ratio of 2:1. Using the sediment trap data from 1995 to 2006 between 410 and 1210 m (<http://www.imars.usf.edu/CAR>), the mean decrease in carbon flux is  $2.4 \pm 2.4 \text{ mmol C m}^{-2} \text{ d}^{-1}$  ( $n = 199$ ), and therefore an upper limit on the mean areal rate for SR is  $1.2 \pm 1.2 \text{ mmol S m}^{-2} \text{ d}^{-1}$ . If we assume that the SRR from 410 to 1210 m is constant, the volumetric SRR would be  $1.6 \pm 1.5 \text{ nmol L}^{-1} \text{ d}^{-1}$ . Dohahue et al. (2008) estimated the SRR in the Cariaco sediment to be 100–1000  $\text{nmol L}^{-1} \text{ d}^{-1}$ . Therefore, the SRR in the deep anoxic water column (well below the interface) is 2–3 orders of magnitude lower than that of the sediment. This result is consistent with

the scenario described in the Black Sea, with a narrow band of intense SR near the oxic–anoxic interface, a constant and low rate in the deep anoxic waters, and a sub-maximum at the sediment–water interface (Albert et al., 1995). SRR in the Black Sea surface sediments was also one to three orders of magnitude higher than the rates in the deep anoxic water column (Albert et al., 1995; Weber et al., 2001). Therefore, consistent with the inverse relationship between SRR and isotope fractionation during SR (Canfield, 2001), the mid-depth minimum of  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  in Cariaco water column is likely a reflection of SRR lower than those at the oxic–anoxic interface in the water column and at the sediment–water interface.

#### 4.5. Evaluating SR and disproportionation in the deep water

The sulfur isotope offset between dissolved sulfide and sulfate in the deep Cariaco anoxic water column is large ( $-54\text{‰}$ ) and comparable to fractionations seen in many other euxinic basins (Table 3). For the Black Sea water column ( $\delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4} = -62\text{‰}$ ), Sweeney and Kaplan (1980) argued that isotopic fractionation resulting from biological SR controlled the  $\delta^{34}\text{S}$  of dissolved sulfide, while Neretin et al. (2003) attributed the large fractionation to a combination of SR and biological disproportionation of sulfur intermediates. Neretin et al. (2003) argued that either sulfur intermediates formed near the interface were transported to the deep water where they undergo disproportionation or that sulfur intermediates were formed *in situ* by oxygen intrusion to the deep anoxic water. The role of disproportionation was demonstrated for the Mariager Fjord ( $\delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4} = -42\text{‰}$ ) using a combination of incubations and *in situ* measurements (Sorensen and Canfield, 2004). Based on modeling of  $\delta^{34}\text{S}$  and  $\Delta^{33}\text{S}$  values in Fayetteville Green Lake ( $\delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4} = -58\text{‰}$ ), Zerkle et al. (2010) concluded that the large fractionations were a consequence of sulfur-intermediate disproportionation in addition to large fractionations during SR. In Framvaren Fjord, on the other hand, Mandernack et al. (2003) argued that large fractionations ( $\delta^{34}\text{S}_{\text{H}_2\text{S}-\text{SO}_4} = -42\text{‰}$ ) reflected slow SRR and low quality organic matter. Other researchers have highlighted the problems associated with anaerobic sulfide oxidation

Table 3  
Sulfur isotope measurements in euxinic water bodies.

Site	Sulfide sulfur isotopes	Sulfate sulfur isotopes	Reference
Black Sea	$-38\text{‰}$ to $-40\text{‰}$ (1)	N.D.	Neretin et al. (2003)
	$-35\text{‰}$ to $-40\text{‰}$ (1)	N.D.	Fry et al. (1991)
	$-38.7\text{‰}$ to $-40.9\text{‰}$ (1)	$18.2\text{‰}$ to $20.2\text{‰}$ (2)	Sweeney and Kaplan (1980)
Cariaco Basin	$-30.2\text{‰}$ to $-32.5\text{‰}$ (1)	$21.0\text{‰}$ to $21.7\text{‰}$ (4)	This study
	Average $-31.2\text{‰}$ (3) <sup>a</sup>		Fry et al. (1991)
Framvaren Fjord	$-33\text{‰}$ to $-10\text{‰}$ (2)	$20.1\text{‰}$ to $42.5\text{‰}$ (2)	Mandernack et al. (2003)
Mariager Fjord	$-14\text{‰}$ to $-22\text{‰}$ <sup>b</sup> (4)	$20.5 \pm 0.2\text{‰}$ (3)	Sorensen and Canfield (2004)
Orca Basin		$19.3\text{‰}$ (3)	Sheu et al. (1988)
Lake Cadagno	$-7.3\text{‰}$ to $-22.2\text{‰}$ <sup>b</sup> (2)	$13.1\text{‰}$ to $26.7\text{‰}$ <sup>b</sup> (2)	Canfield et al. (2010)
Fayetteville Green Lake, NY	$-24.1\text{‰}$ to $-31.0\text{‰}$ (2)	$21.9\text{‰}$ to $31.4\text{‰}$ (2)	Zerkle et al. (2010)

1, decrease with depth; 2, increase with depth; 3, constant; 4, irrespective of depth.

<sup>a</sup> Below 400 m.

<sup>b</sup> The range shows annual cycle instead of change with depth.

and bacterial disproportionation of resulting sulfur intermediates under hypersulfidic conditions (Wortmann et al., 2001; Werne et al., 2003; Brunner and Bernasconi, 2005). These studies suggest that the role of sulfur-intermediate disproportionation in environmental settings is variable and likely depends on a number of factors, including sulfide concentrations and the availability of sulfur intermediates.

It is possible for SR alone to produce large isotope fractionations without invoking additional oxidative sulfur cycling. Brunner and Bernasconi (2005) expanded upon existing models for the SR metabolism (Rees, 1973) using larger fractionation factors for steps in the reduction of sulfite to sulfide and through incorporation of additional steps involving other sulfur intermediates during the reduction of sulfite to sulfide. This revised model allowed larger maximum sulfur isotope fractionation by SRP (up to  $\sim 70\%$ ), and support for this model comes from recent incubations with natural populations of sulfate reducers (Canfield et al., 2010).

One constraint is provided by considering the limits (fractionation of sulfide relative to sulfate expressed in  $\delta^{34}\text{S}$  vs.  $\Delta^{33}\text{S}$ ) allowed by metabolic models of SR (Farquhar et al., 2003, 2008; Johnston et al., 2005). We compare our multiple S isotope measurements with experimental data for pure culture and natural population studies of SRP (Fig. 10). Below 320 m in the Cariaco Basin,  $\Delta^{33}\text{S}$  values fall within the range observed for sulfate reducers in pure culture and natural environmental samples. However, for similar  $\Delta^{33}\text{S}$ , larger  $\delta^{34}\text{S}$  fractionations were observed in the Cariaco Basin, which may reflect the relative deficiency of the natural substrate used as an electron donor compared to the transport of sulfate into and out of the cell (Canfield, 2001).

The minor isotope fractionation factor ( $^{33}\lambda$ ) offers an additional constraint on the isotopic offset between sulfate and sulfide in the Cariaco water column. We have used cur-

rent convention (Farquhar et al., 2003, 2008; Johnston et al., 2005) to describe the relationship between fractionations for  $^{33}\text{S}/^{32}\text{S}$  and  $^{34}\text{S}/^{32}\text{S}$  using the exponent  $^{33}\lambda$  in the deeper parts of the water column. Using an approach as described in Eq. (9) (Fry et al., 1991; Farquhar et al., 2003) yields  $^{33}\lambda$  values of  $0.5127 \pm 0.0002$  for the fractionation between sulfate and sulfide in the deep water (from 320 to 1300 m). The experimental range in  $^{33}\lambda$  observed in pure cultures of SRP varies from 0.5077 to 0.5125, and from 0.5145 to 0.5187 for disproportionation of elemental sulfur and sulfite (Johnston et al., 2005, 2007). The  $^{33}\lambda$  exponent of 0.5127 calculated from multi-sulfur isotope measurements of coeval sulfate and sulfide in the anoxic deep waters of the Cariaco Basin is at the upper range observed for SR. Although such a  $^{33}\lambda$  value remains to be demonstrated in experiments with pure cultures and natural populations of SRP, the collective data point to disproportionation as being unimportant in the anoxic deep water column (below 400 m) of the Cariaco Basin. Therefore, we suggest that low SRR is the main factor influencing the large fractionations between sulfate and sulfide in the deep water column.

#### 4.6. Sulfur-intermediate disproportionation near the oxic–anoxic interface

A pronounced peak in the rate of dark  $\text{CO}_2$  fixation in the Cariaco Basin has been observed repeatedly near the oxic–anoxic interface of the water column (Tuttle and Jannasch, 1979; Taylor et al., 2001). However, the presence of a large chemoautotrophic community is hard to explain, since vertical fluxes of reductants ( $\text{H}_2\text{S}$ ,  $\text{NH}_4^+$ ,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ ) and oxidants ( $\text{O}_2$ ,  $\text{MnO}_2$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{NO}_3^-$ ) can only support a few percent of the measured chemoautotrophic production at the Cariaco station (Taylor et al., 2001). Imbalance between chemoautotrophic production and calculated vertical diffusive fluxes of reductants and oxidants has also been

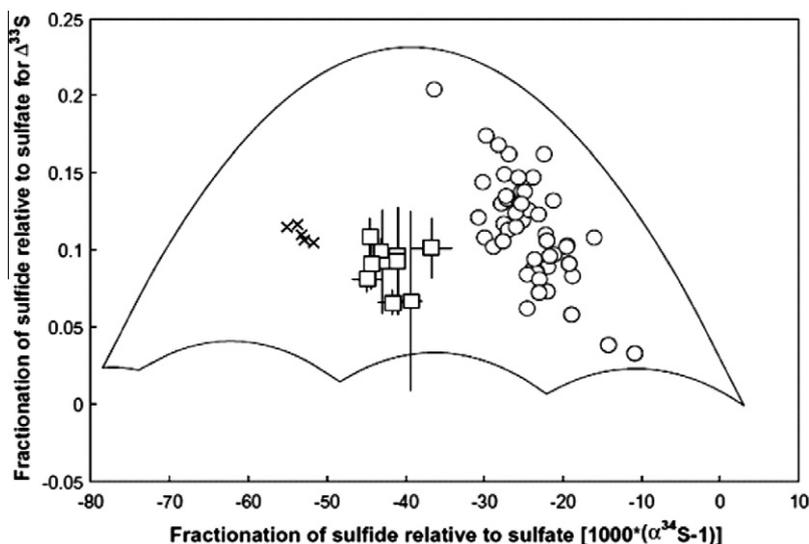


Fig. 10. Plot of the fractionation for  $\Delta^{33}\text{S}$  [ $1000 * (\alpha^{33}\text{S} - \alpha^{34}\text{S}^{0.515})$ ] vs. fractionation for  $\delta^{34}\text{S}$  [ $1000 * (\alpha^{34}\text{S} - 1)$ ] of sulfide normalized to sulfate for dissimilatory sulfate reduction (modified from Farquhar et al., 2008). Crosses are from depths  $>300$  m in the Cariaco Basin (this study). Unfilled circles represent pure cultures of sulfate reducers from Farquhar et al. (2003) and Johnston et al. (2005, 2007). Unfilled squares are natural populations of sulfate reducers from Farquhar et al. (2008). (The error bars are estimates for  $2\sigma$  uncertainty.)

observed in other anoxic water bodies, such as the Black Sea (Jørgensen et al., 1991; Murray et al., 1995), the Mariager Fjord (Fenchel et al., 1995; Zopfi et al., 2001) and the Baltic Sea (Jost et al., 2008). Efficient sulfur cycling, especially through disproportionation of sulfur intermediates, has been proposed to explain the high biological production in the Cariaco (Taylor et al., 2001; Ho et al., 2004; Hayes et al., 2006; Li et al., 2008). One of our original goals in this study was to look for isotope signals from sulfur-intermediate disproportionation near the interface. If this process contributed significantly to the  $\delta^{34}\text{S}$  and  $\Delta^{33}\text{S}$  signatures of sulfur species near the interface, direct measurement of sulfur isotope composition and modeling work should help quantify its role. However, as argued above, disproportionation of sulfur intermediates does not appear to be the dominant process influencing sulfur isotope composition near the oxic–anoxic interface in this system.

If not dominant, the question remains as to whether the process of sulfur disproportionation is occurring near the interface. We observed one very light value for  $\delta^{34}\text{S}_{\text{H}_2\text{S}}$  ( $-31.1\text{‰}$ , Fig. 4) at 260 m in November 2007, consistent with an isotope effect associated with disproportionation. The values of  $^{33}\lambda$  near the interface (290 m) from our analysis of samples collected in May 2008 are  $0.506 \pm 0.002$ . Although fractionations of the minor S isotopes during abiotic and chemoautotrophic sulfide oxidation have not been measured directly, our value for  $^{33}\lambda$  is significantly lower than that anticipated from sulfide oxidation alone (Farquhar et al., 2003). Therefore, the value of  $\lambda$  at the interface is not easily reconciled with sulfide oxidation acting as the sole control. While this discrepancy may imply a role for disproportionation, validation awaits a larger and higher resolution data set.

The detection of an isotopic signal tied to disproportionation of sulfur intermediates can be complicated by a number of factors. Zerkle et al. (2009) examined the isotopic consequence of including sulfide oxidation in an ecosystem-scale, multi-sulfur isotope model. Also included in that model are SR and sulfur-intermediate disproportionation. This work suggested that during oxidation of sulfide compounds, redistribution of sulfur mass down the oxidative pathway (despite the small fractionation effect) diminishes the larger  $\delta^{34}\text{S}$  and  $\Delta^{33}\text{S}$  isotope signals from SR plus sulfur-intermediate disproportionation, potentially masking the isotopic signal of disproportionation. Furthermore, sulfur isotope effects during disproportionation are much smaller when  $\text{MnO}_2$  is present due to the superimposed chemical reoxidation of the resulting  $\text{H}_2\text{S}$  (Böttcher and Thamdrup, 2001). Future studies employing fine-scale sampling at the very top of the sulfide zone using multiple sulfur isotope analysis of sulfide, sulfate, and even sulfur intermediate pools in the Cariaco Basin have the potential to further distinguish the relative roles of sulfide oxidation, SR, and disproportionation.

## 5. CONCLUSIONS

Small magnitude variations in  $\delta^{34}\text{S}_{\text{SO}_4}$  values in the water column were observed repeatedly in the Cariaco Basin. These variations are broadly consistent with a reservoir effect asso-

ciated with sulfide production via SR and may be slightly larger than that allowed by the standing pool of dissolved sulfide alone (e.g., they predict a sink for  $^{34}\text{S}$ -depleted sulfur such as iron sulfide that settles from the water column). Changes in the isotopic composition and concentrations of dissolved sulfate and sulfide appear to reflect temporal variability associated with carbon supply and recirculation of oxygenated water throughout the basin.

Sulfur isotope variations ( $\delta^{34}\text{S}$ ) and concentration data for the upper 400 m of the water column appear to be consistent with fractionation related to sulfide loss tied to chemoautotrophic and/or chemical sulfide oxidation, but  $^{33}\text{S}/^{32}\text{S}$  relationships ( $\lambda$ ) imply that additional processes have also contributed to the observed isotopic variations. These processes may reflect changes in the magnitude of the fractionation associated with SR within the water column, perhaps as a result of variation in the quantity and quality of organic matter present and acting as electron donors. The sulfur isotope ( $\delta^{34}\text{S}$  and  $^{33}\lambda$ ) and concentration data for the water column below 500 m are consistent with a significant contribution of sulfate reducers to the sulfur cycle, and we interpret the large fractionations as solely controlled by the slow rates for SR. The fractionations also appear to decrease toward the sediment–water interface, suggesting a contribution of sulfide from the sediment and/or a water column source that is more  $^{34}\text{S}$ -depleted. Although our isotope data cannot rule out disproportionation of sulfur intermediate in the Cariaco water column, they do point to a more important role played by sulfide oxidation and SR.

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## REFERENCES

- Aharon P. and Fu B. (2003) Sulfur and oxygen isotopes of coeval sulfate-sulfide in pore fluids of cold seep sediments within sharp redox gradients. *Chem. Geol.* **195**, 201–218.
- Albert D. B., Taylor C. and Martens C. S. (1995) Sulfate reduction rates and low molecular weight fatty acid concentrations in the water column and surficial sediments of the Black Sea. *Deep-Sea Res.* **42**, 1239–1260.
- Astor Y., Muller-Karger F. E. and Scranton M. I. (2003) Seasonal and interannual variation in the hydrography of the Cariaco Basin: implications for basin ventilation. *Cont. Shelf Res.* **23**, 125–144.

- Böttcher M. E., Thamdrup B., Gehre M. and Theune A. (2005)  $^{34}\text{S}/^{32}\text{S}$  and  $^{18}\text{O}/^{16}\text{O}$  fractionation during sulfur disproportionation by *Desulfobulbus propionicus*. *Geomicrobiol. J.* **22**, 219–226.
- Böttcher M. E. and Thamdrup B. (2001) Anaerobic sulfide oxidation and stable isotope fractionation associated with bacterial sulfur disproportionation in the presence of  $\text{MnO}_2$ . *Geochim. Cosmochim. Acta* **65**, 1573–1581.
- Böttcher M. E., Thamdrup B. and Vennemann T. W. (2001) Oxygen and sulfur isotope fractionation during anaerobic bacterial disproportionation of elemental sulfur. *Geochim. Cosmochim. Acta* **65**, 1601–1609.
- Böttcher M. E., Smock A. M. and Cypionka H. (1998) Sulfur isotope fractionation during experimental precipitation of iron (II) and manganese (II) sulfide at room temperature. *Chem. Geol.* **146**, 127–134.
- Böttcher M. E., Rusch A., Hopner T. and Brumsack H. J. (1997) Stable sulfur isotope effects related to local intense sulfate reduction in a tidal sandflat (southern North Sea): results from a loading experiment. *Isot. Environ. Health Stud.* **33**, 109–129.
- Brunner B. and Bernasconi S. M. (2005) A revised isotope fractionation model for dissimilatory sulfate reduction in sulfate reduction bacteria. *Geochim. Cosmochim. Acta* **69**, 4759–4771.
- Canfield D. E., Farquhar J. and Zerkle A. L. (2010) High isotope fractionations during sulfate reduction in a low-sulfate euxinic ocean analog. *Geology* **38**, 415–418.
- Canfield D. E. (2001) Isotope fractionation by natural populations of sulfate-reducing bacteria. *Geochim. Cosmochim. Acta* **65**, 1117–1124.
- Canfield D. E. and Teske A. (1996) Late Proterozoic rise in atmospheric oxygen concentration inferred from phylogenetic and sulfur isotope studies. *Nature* **382**, 127–132.
- Canfield D. E. and Thamdrup B. (1994) The production of  $^{34}\text{S}$ -depleted sulfide during bacterial  $\text{S}^0$  disproportionation. *Science* **266**, 1973–1975.
- Cline J. D. (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.* **14**, 454–458.
- Coplen T. B. and Krouse H. R. (1998) Sulfur isotope data consistency improved. *Nature* **392**, 32.
- Detmers J., Brüchert V., Habicht K. S. and Kuever J. (2001) Diversity of sulfur isotope fractionations by sulfate-reducing prokaryotes. *Appl. Environ. Microbiol.* **67**, 888–894.
- Ding T., Valkier S., Kipphardt H., De Bievre P., Taylor P. D. P., Gonfiantini R. and Krouse R. (2001) Calibrated sulfur isotope abundance ratios of three IAEA sulfur isotope reference materials and V-CDT with a reassessment of the atomic weight of sulfur. *Geochim. Cosmochim. Acta* **65**, 2433–2437.
- Dohahue M. A., Werne J. P., Meile C. and Lyons T. W. (2008) Modeling sulfur isotope fractionation and differential diffusion during sulfate reduction in sediments of the Cariaco Basin. *Geochim. Cosmochim. Acta* **72**, 2287–2297.
- Fanning K. A. and Pilson M. E. (1972) A model for the anoxic zone of the Cariaco Trench. *Deep-Sea Res.* **19**, 847–863.
- Farquhar J., Canfield D. E., Masterson A., Bao H. and Johnston D. (2008) Sulfur and oxygen isotope study of sulfate reduction in experiments with natural populations from Fællestrand, Denmark. *Geochim. Cosmochim. Acta* **72**, 2805–2821.
- Farquhar J., Johnston D. T. and Wing B. A. (2007) Implications of conservation of mass effects on mass-dependent isotope fractionations: influence of network structure on sulfur isotope phase space of dissimilatory sulfate reduction. *Geochim. Cosmochim. Acta* **71**, 5862–5875.
- Farquhar J., Johnston D. T., Wing B. A., Habicht K. S., Canfield D. E., Airieau S. A. and Thiemens M. H. (2003) Multiple sulfur isotopic interpretations of biosynthetic pathways: implications for biological signatures in the sulfur isotope record. *Geobiology* **1**, 27–36.
- Fenchel T., Bernard C., Esteban G., Finlay B. J., Hansen P. J. and Iversen N. (1995) Microbial diversity and activity in a Danish fjord with anoxic deep water. *Ophelia* **43**, 45–100.
- Froelich P. N., Klinkhammer G. P., Bender M. L., Luedtke N. A., Heath G. R., Cullen D., Dauphin I., Hammond D., Hartman B. and Maynard V. (1979) Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. *Geochim. Cosmochim. Acta* **43**, 1075–1090.
- Forrest J. and Newman L. (1977) Ag-110 microgram sulfate analysis for short time resolution of ambient levels of sulfur aerosol. *Anal. Chem.* **49**, 1579–1584.
- Fry B., Jannasch H. W., Molyneus S. J., Wirsén C. O., Muramoto J. A. and King S. (1991) Stable isotope studies of the carbon, nitrogen and sulfur cycles in the Black Sea and the Cariaco Trench. *Deep-Sea Res.* **38**, 1003–1019.
- Fry B., Ruf W., Gest H. and Hayes J. M. (1988) Sulfur isotope effects associated with oxidation of sulfide by  $\text{O}_2$  in aqueous solution. *Chem. Geol.* **73**, 205–210.
- Fry B., Cox J., Gest H. and Hayes J. M. (1986) Discrimination between  $^{34}\text{S}$  and  $^{32}\text{S}$  during bacterial metabolism of inorganic sulfur compounds. *J. Bacteriol.* **165**, 328–330.
- Goldhaber M. B. and Kaplan I. R. (1980) Mechanisms of sulfur incorporation and isotope fractionation during early diagenesis in sediments of the Gulf of California. *Mar. Chem.* **9**, 95–143.
- Habicht K. S. and Canfield D. E. (2001) Isotope fractionation by sulfate-reducing natural populations and the isotopic composition of sulfide in marine sediments. *Geology* **29**, 555–558.
- Habicht K. S., Canfield D. E. and Rethmeier J. (1998) Sulfur isotope fractionation during bacterial reduction and disproportionation of thiosulfate and sulfite. *Geochim. Cosmochim. Acta* **62**, 2585–2595.
- Habicht K. S. and Canfield D. E. (1997) Sulfur isotope fractionation during bacterial sulfate reduction in organic rich sediments. *Geochim. Cosmochim. Acta* **61**, 5351–5361.
- Hastings D. and Emerson S. (1988) Sulfate reduction in the presence of low oxygen levels in the water column of the Cariaco Trench. *Limnol. Oceanogr.* **33**, 1113–1119.
- Hayes M. K., Taylor G. T., Astor Y. and Scranton M. I. (2006) Vertical distributions of thiosulfate and sulfite in the Cariaco Basin. *Limnol. Oceanogr.* **51**, 280–287.
- Ho T. Y., Taylor G. T., Astor Y., Varela R., Muller-Karger F. E. and Scranton M. I. (2004) Vertical and temporal variability of redox zonation in the water column of the Cariaco Basin: implications for organic carbon oxidation pathways. *Mar. Chem.* **86**, 89–104.
- Hoefs J. (2004). In *Stable Isotope Geochemistry*, vol. 5, p. 244. Stable Isotope Geochemistry. Springer Press, New York.
- Holmen K. J. and Rooth C. G. H. (1990) Ventilation of the Cariaco Trench, a case of multiple source competition? *Deep-Sea Res.* **37**, 203–225.
- Hulston J. R. and Thode H. G. (1965) Cosmic ray produced  $^{36}\text{S}$  and  $^{33}\text{S}$  in metallic phase of iron meteorites. *J. Geophys. Res.* **70**, 4435–4442.
- Johnston D. T., Farquhar J. and Canfield D. E. (2007) Sulfur isotope insights into microbial sulfate reduction: when microbes meet models. *Geochim. Cosmochim. Acta* **71**, 3929–3947.
- Johnston D. T., Farquhar J., Wing B. A., Kaufman A., Canfield D. E. and Habicht K. S. (2005) Multiple sulfur isotope fractionations in biological systems: a case study with sulfate reducers and sulfur disproportionators. *Am. J. Sci.* **305**, 645–660.
- Jørgensen B. B., Fosing H., Wirsén C. O. and Jannasch H. W. (1991) Sulfide oxidation in the anoxic Black Sea chemocline. *Deep-Sea Res.* **38**, 1083–1103.

- Just G., Zubkov M. V., Yakushev E., Labrenz M. and Jurgens K. (2008) High abundance and dark CO<sub>2</sub> fixation of chemolithoautotrophic prokaryotes in anoxic waters of the Baltic Sea. *Limnol. Oceanogr.* **53**, 14–22.
- Kaplan I. R. and Rittenberg S. C. (1964) Microbiological fractionation of sulfur isotopes. *J. Gen. Microbiol.*, 195–212.
- Kemp A. W. and Thode H. G. (1968) Mechanism of bacterial reduction of sulfate and of sulfite from isotope fractionation studies. *Geochim. Cosmochim. Acta* **32**, 71–91.
- Li X. N., Taylor G. T., Yrene A. and Scranton M. I. (2008) Relationship of sulfur speciation to hydrographic conditions and chemoautotrophic production in the Cariaco Basin. *Mar. Chem.* **112**, 53–64.
- Li X. N., Cutter G. A., Thunell R. C., Tappa E., Gilhooly III W., Lyons T. W., Astor Y. and Scranton M. I. (2010) Particulate sulfur species in the water column of the Cariaco Basin. *Geochim. Cosmochim. Acta*. doi:10.1016/j.gca.2010.09.039.
- Lin X., Wakeham S. G., Putnam I. F., Astor Y., Scranton M. I., Chistoserdov A. Y. and Taylor G. T. (2006) Comparison of vertical distributions of prokaryotic assemblages in the anoxic Cariaco Basin and Black Sea by use of fluorescence *in situ* hybridization. *Appl. Environ. Microbiol.* **72**, 1–12.
- Lyons T. W. (1997) Sulfur isotopic trends and pathways of iron sulfide formation in upper Holocene sediments of the anoxic Black Sea. *Geochim. Cosmochim. Acta* **61**, 3367–3382.
- Lyons T. W., Werne J. P. and Hollander D. J. (2003) Contrasting sulfur geochemistry and Fe/Al and Mo/Al ratios across the last oxic-to-anoxic transition in the Cariaco Basin, Venezuela. *Chem. Geol.* **195**, 131–157.
- Mandernack K. W., Krouse H. R. and Skei J. M. (2003) A stable sulfur and oxygen isotopic investigation of sulfur cycling in an anoxic marine basin, Framvaren Fjord, Norway. *Chem. Geol.* **195**, 181–200.
- Mariotti A., Germon J. C., Hubert P., Kaiser P., Letolle R., Tardieux A. and Tardieux P. (1981) Experimental determination of nitrogen kinetic isotope fractionation: some principles, illustration for the denitrification and nitrification process. *Plant Soil* **62**, 413–430.
- Murray J. W., Codispoti L. A. and Friederich G. E. (1995) Oxidation–reduction environments: the suboxic zone in the Black Sea. In *Aquatic Chemistry: Interfacial and Interspecies Processes. ACS Advances in Chemistry Series*, vol. 244 (eds. C. P. Huang, C. R. O'Melia and J. J. Morgan). Oxford Univ. Press, pp. 157–176.
- Neretin L. N., Böttcher M. E. and Grinenko V. A. (2003) Sulfur isotope geochemistry of the Black Sea water column. *Chem. Geol.* **200**, 59–69.
- Ono S., Wing B., Johnston D., Farquhar J. and Rumble D. (2006) Mass-dependent fractionation of quadruple stable sulfur isotope system as a new tracer of sulfur biogeochemical cycles. *Geochim. Cosmochim. Acta* **70**, 2238–2252.
- Percy D., Li X. N., Taylor G. T., Astor Y. and Scranton M. I. (2008) Controls on iron, manganese and intermediate oxidation state sulfur compounds in the Cariaco Basin. *Mar. Chem.* **111**, 47–62.
- Percy D. (2006) Temporal and spatial investigations of geochemical variability in the Cariaco Basin. Master thesis, Stony Brook University.
- Rees C. E. (1973) A steady state model for sulfur isotope fractionation in bacterial reaction reduction processes. *Geochim. Cosmochim. Acta* **37**, 1141–1162.
- Rudnicki M. D., Elderfield H. and Spiro B. (2001) Fractionation of sulfur isotopes during bacterial sulfate reduction in deep ocean sediments at elevated temperatures. *Geochim. Cosmochim. Acta* **65**, 777–789.
- Scranton M. I., Astor Y., Bohrer M., Ho T. Y. and Muller-Karger F. E. (2001) Controls on temporal variability of the geochemistry of the deep Cariaco Basin. *Deep-Sea Res.* **48**, 1605–1625.
- Scranton M. I., Sayles F. L., Bacon M. P. and Brewer P. G. (1987) Temporal changes in the hydrography and chemistry of the Cariaco Trench. *Deep-Sea Res.* **34**, 945–963.
- Sheu Der-Due, Shakur A., Pigott J. D., Wiesenburg D. A., Brooks J. M. and Krouse H. R. (1988) Sulfur and oxygen isotopic composition of dissolved sulfate in the Orca Basin: implications for origin of the high salinity brine and oxidation of sulfides at the Brine–seawater interface. *Mar. Geol.* **78**, 303–310.
- Sorensen K. B. and Canfield D. E. (2004) Annual fluctuations in sulfur isotope fractionation in the water column of an euxinic marine basin. *Geochim. Cosmochim. Acta* **68**, 503–515.
- Sweeney R. E. and Kaplan I. R. (1980) Stable isotope composition of dissolved sulfate and hydrogen sulfide in the Black Sea. *Mar. Chem.* **9**, 145–152.
- Taylor G. T., Iabichella-Armas M., Varela R., Muller-Karger F., Lin X. and Scranton M. I. (2006) Microbial ecology of the Cariaco Basin's oxic/anoxic interface. the U.S.–Venezuelan CARIACO times series program. In *Past and Present Water Column Anoxia* (ed. L. N. Neretin), pp. 473–499. NATO Sci. Ser., Springer, Netherlands.
- Taylor G. T., Iabichell M., Ho T. Y. and Scranton M. I. (2001) Chemoautotrophy in the redox transition zone of the Cariaco Basin: a significant midwater source of organic production. *Limnol. Oceanogr.* **46**, 148–163.
- Thunell R. C., Varela R., Llano M., Collister J., Muller-Karger F. and Bohrer R. (2000) Organic carbon fluxes, degradation, and accumulation in an anoxic basin: sediment trap results from the Cariaco Basin. *Limnol. Oceanogr.* **45**, 300–308.
- Tuttle J. H. and Jannasch H. W. (1979) Microbial dark assimilation of CO<sub>2</sub> in the Cariaco Trench. *Limnol. Oceanogr.* **24**, 746–753.
- Vairavamurthy A. and Mopper K. (1990) Determination of sulfite and thiosulfate in aqueous samples including anoxic seawater by liquid chromatography after derivatization with 2,2'-dithiobis(5-nitropyridine). *Environ. Sci. Technol.* **24**, 333–337.
- Weber A., Riess W., Wenzhoefer F. and Jørgensen B. B. (2001) Sulfate reduction in Black Sea sediments: *in situ* and laboratory radiotracer measurements from the shelf to 2000 m depth. *Deep-Sea Res.* **48**, 2073–2096.
- Werne J. P., Lyons T. W., Hollander D. J., Formolo M. J. and Damste J. S. (2003) Reduced sulfur in euxinic sediments of the Cariaco Basin: sulfur isotope constraints on organic sulfur formation. *Chem. Geol.* **195**, 159–179.
- Wortmann U. G., Bernasconi S. M. and Böttcher M. E. (2001) Hypersulfidic deep biosphere indicates extreme sulfur isotope fractionation during single-step microbial sulfate reduction. *Geology* **29**, 647–650.
- Zerkle A. L., Kamyschny A., Kump L. R., Farquhar J., Oduro H. and Arthur M. A. (2010) Sulfur cycling in a stratified euxinic lake with moderately high sulfate: constraints from quadruple S isotopes. *Geochim. Cosmochim. Acta* **74**, 4953–4970.
- Zerkle A. L., Farquhar J., Johnston D. T., Cox R. P. and Canfield D. E. (2009) Fractionation of multiple sulfur isotopes during phototrophic oxidation of sulfide and elemental sulfur by a green sulfur bacterium. *Geochim. Cosmochim. Acta* **73**, 291–306.
- Zopfi J., Ferdelman T. G., Jørgensen B. B., Teske A. and Thamdrup B. (2001) Influence of water column dynamics on sulfide oxidation and other major biogeochemical processes in the chemocline of Mariager Fjord (Denmark). *Mar. Chem.* **74**, 29–51.