Geobiology (2006), **4**, 191–201

Oxygen isotopic composition of sulfate in deep sea pore fluid: evidence for rapid sulfur cycling

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ABSTRACT

We present new data of oxygen isotopes in marine sulfate ($\delta^{18}O_{SO4}$) in pore fluid profiles through organic-rich deep-sea sediments from 11 ODP sites around the world. In almost all sites studied sulfate is depleted with depth, through both organic matter oxidation and anaerobic methane oxidation. The $\delta^{18}O_{SO4}$ increases rapidly near the top of the sediments, from seawater values of 9‰ to maxima between 22 and 25‰, and remains isotopically heavy and constant at these values with depth. The $\delta^{18}O_{SO4}$ in these pore fluid profiles is decoupled from variations in sulfur isotopes measured on the same sulfate samples ($\delta^{34}S_{SO4}$); the $\delta^{34}S_{SO4}$ increases continuously with depth and exhibits a shallower isotopic increase. This isotopic decoupling between the $\delta^{34}S_{SO4}$ and the $\delta^{18}O_{SO4}$ is hard to reconcile with the traditional understanding of bacterial sulfate reduction in sediments. Our data support the idea that sulfate or sulfite and water isotopically exchange during sulfate reduction and that some of the isotopically altered sulfur pool returns to the environment. We calculate that the rapid increase in the $\delta^{18}O_{SO4}$ in the upper part of these sediments requires rates of this oxygen isotope exchange that are several orders of magnitude higher than the rates of net sulfate reduction calculated from the sulfate concentration profiles and supported by the $\delta^{34}S_{SO4}$. We suggest several mechanisms by which this may occur, including 'net-zero' sulfur cycling, as well as further experiments through which we can test and resolve these processes.

Received 2 February 2006; accepted 16 June 2006

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INTRODUCTION

Changes in the profiles of redox-sensitive elements in pore fluids through organic-rich sediments suggest that various microbes use a succession of electron acceptors during the remineralization of organic matter (Froelich *et al.*, 1979). These electron acceptors are used to depletion in order of their decreasing free energy, beginning with oxygen, which is often exhausted in the uppermost centimeters, proceeding through nitrate, iron and manganese oxides, sulfate, and finally methanogenesis (e.g. Martens & Berner, 1974; Belyaev *et al.*, 1977; Froelich *et al.*, 1979; Whiticar *et al.*, 1986). Of these electron acceptors, sulfate is by far the most abundant and bacterial sulfate reduction (BSR) is responsible for over half the organic matter remineralization in sediments (Kasten & Jørgensen, 2000). In addition, sulfate typically oxidizes nearly all of the methane produced in sediments through a process known as anaerobic methane oxidation (AMO) – Sansone & Martens (1981), Borowski *et al.* (1996), Niewöhner *et al.* (1998), Davie & Buffett (2003). The shape of the sulfate concentration profile through pore fluids provides a considerable amount of information about the carbon donor for bacterial sulfate reduction in sediments (Kasten & Jørgensen, 2000; Hensen *et al.*, 2003). When sulfate is consumed by organic matter oxidation (OMO), the sulfate concentration profile is typically concave down, reflecting the largely continuous consumption of sulfate with depth in the core. In sites where sulfate is consumed largely through AMO, sulfate is reduced at a single zone, which can be tens of meters below the sediment–water interface (Sansone & Martens, 1981; Borowski *et al.*, 1996; Sivan *et al.* in review). In this case, the sulfate concentration profiles exhibit linear diffusion from seawater concentrations at the sediment–water interface to consumption in the zone of AMO (Niewöhner *et al.*, 1998).

Isotope profiles through pore fluids of organic-rich sediments have been used to confirm these microbial processes (e.g. Martens & Berner, 1974; Martens *et al.*, 1999; Borowski *et al.*, 2000; Moore *et al.*, 2004). For example, carbon isotopes in dissolved inorganic carbon ($\delta^{13}C_{DIC}$) can record a decrease in $\delta^{13}C_{DIC}$ from seawater values of 0‰ (PDB) to $\delta^{13}C_{DIC}$

values as light as –38‰ with depth resulting from the oxidation of organic matter and methane (methane has a $\delta^{13}C$ of –50 to –100‰, e.g. Whiticar *et al.*, 1986; Alperin *et al.*, 1994; Martens *et al.*, 1999; Borowski *et al.*, 2000). This isotopic decrease can be followed by an isotopic increase in the zone of methanogenesis, when present, because of the preferential reduction of isotopically light DIC to form methane; the $\delta^{13}C_{DIC}$ in the zone of methanogenesis can therefore reach values of more than +10‰ (Whiticar & Faber, 1986; Whiticar, 1999; Moore *et al.*, 2004). 'Isotopically light' refers to an enrichment in the light carbon isotope ($\delta^{13}C < 0$) and 'isotopically heavy' refers to an enrichment in the heavy carbon isotope ($\delta^{13}C > 0$) and we will follow this convention throughout the paper.

Another example of the use of isotopes in elucidating microbial processes in organic-rich sediments is sulfur isotopes measured in pore fluid sulfate ($\delta^{34}S_{SO4}$), which typically show a trend towards isotopically enriched values with depth in the core, from seawater sulfate at +20‰ to values as high as +60‰ (Jørgensen, 1982; Brüchert & Pratt, 1999; Brüchert *et al.*, 2003; Jørgensen *et al.*, 2004). This reflects the reduction of isotopically light sulfate through OMO and AMO, leaving a pool of residual sulfate that is increasingly isotopically heavy. The average fractionation during sulfate reduction (ε_S) in pure cultures ranges from 10 to 45‰ (e.g. Kaplan & Rittenberg, 1962; Chambers *et al.*, 1975; Canfield, 2001) although fractionations as high as 70‰ have been reported in nature (Werne *et al.*, 2003).

Taken together, the redox changes and the isotope measurements suggest a similar story; organic matter is oxidized at the top of the sediments by microbes using oxygen, nitrate, Fe and Mn oxides and potentially sulfate as their terminal electron acceptor. Once organic matter is buried below the zone of sulfate depletion, it undergoes methanogenesis. This methane diffuses upward and is consumed by sulfate that diffuses downward into the zone of AMO.

If the only process affecting the oxygen isotopic composition of sulfate ($\delta^{18}O_{SO4}$) in pore fluid profiles through organic-rich sediments was a kinetic isotope fractionation during sulfate reduction, then the $\delta^{18}O_{SO4}$ should track the $\delta^{34}S_{SO4}$ and exhibit a continuous increase with depth in the core. Studies of the isotopic fractionation for oxygen isotopes during sulfate reduction (ε_O) indicate that sulfate with light oxygen isotopes is preferentially reduced, leaving a residual pool that is increasingly isotopically heavy (Bottrell *et al.*, 2000). The magnitude of ε_O ranges between 0 and 10‰ (Fritz *et al.*, 1989; Aharon & Fu, 2000; Brunner *et al.*, 2005), which is much smaller than ε_S. We therefore might expect the slope of a linear $\delta^{18}O_{SO4}$ profile to be shallower than the corresponding slope in $\delta^{34}S_{SO4}$. However, in several pure culture experiments, it has been demonstrated that the $\delta^{18}O_{SO4}$ is decoupled from the $\delta^{34}S_{SO4}$ during sulfate reduction, a result that has been interpreted as reflecting oxygen isotope exchange between sulfate or sulfite and water during BSR (Fritz *et al.*, 1989; Brunner *et al.*, 2005). In this paper we present profiles of the $\delta^{18}O_{SO4}$ through organic-rich sediments from 11 ODP cores from around the world. Similar to the work done in pure culture experiments, our measured $\delta^{18}O_{SO4}$ is decoupled from the $\delta^{34}S_{SO4}$ in the same pore fluid profiles. We will explore a range of possible explanations for our data, including oxygen isotope exchange between sulfate and/or sulfur intermediates and water during BSR. Our data suggest that the microbial processes occurring during organic-matter remineralization involving sulfate must be more complicated than previously thought. Our results emphasize the use of $\delta^{18}O_{SO4}$ as a tool to better understand the sulfur cycle in organic-rich sediments.

METHODS

Sites description

We used pore fluid samples from sediments collected during three Ocean Drilling Program (ODP) legs (Fig. 1); Leg 175 along the West African Margin (Wefer *et al.*, 1998), Leg 181 in the SW Pacific (Carter *et al.*, 1999), and Leg 201 in the Eastern Equatorial Pacific and Peru Margin (D'Hondt *et al.*, 2002). Leg 175 sampled the Angola-Benguela Current system off the coast of West Africa. The sediments in Leg 175 are largely diatomaceous and carbonate-rich clays with variable high organic carbon content (3–8%). The sites were drilled at water depth ranging from 400 to 2200 m. Methane is found at almost all sites, but below saturation, so no methane gas or gas hydrates exist. We will present data from seven sites from Leg 175 (Sites 1077, 1079, 1081, 1082, 1083, 1085, and 1086). Leg 181 sampled the South-west Pacific, east of New Zealand, with the primary objective of assessing palaeocenographic currents from the outflow of the Antarctic bottom water over the past 30 million years. Most sites in Leg 181 consist of carbonate-rich fine-grained sediments deposited in deep water (3000–4000 m water depth) with low organic content (0.1 to 0.3% – Carter *et al.*, 1999). We present data from one site in Leg 181 (Site 1123). Leg 201 sampled the Eastern Equatorial Pacific and the Peru Margin, and was dedicated to studying microbial life beneath the seafloor. Water depth in sites from Leg 201 ranged from 150 to 5300 m (D'Hondt *et al.*, 2002). Sites in Leg 201 range from carbonate and silicate oozes in the Eastern Equatorial Pacific to clays and organic-rich silts on the Peru Shelf. We present data from three sites from Leg 201 (Sites 1225, 1229, and 1230). Organic matter concentrations are very low at site 1225 (~0.1%), and high at Sites 1229 and 1230 (3–5%) (D'Hondt *et al.*, 2002).

Analytical methods

Major ions and methane concentrations in the pore fluid were measured during the ODP cruise using standard procedures and the headspace method for methane (Murray *et al.*, 1998). Pore fluid samples for $\delta^{18}O_{SO4}$ analysis were treated and analysed at the Laboratory for Geochemical Oceanography at

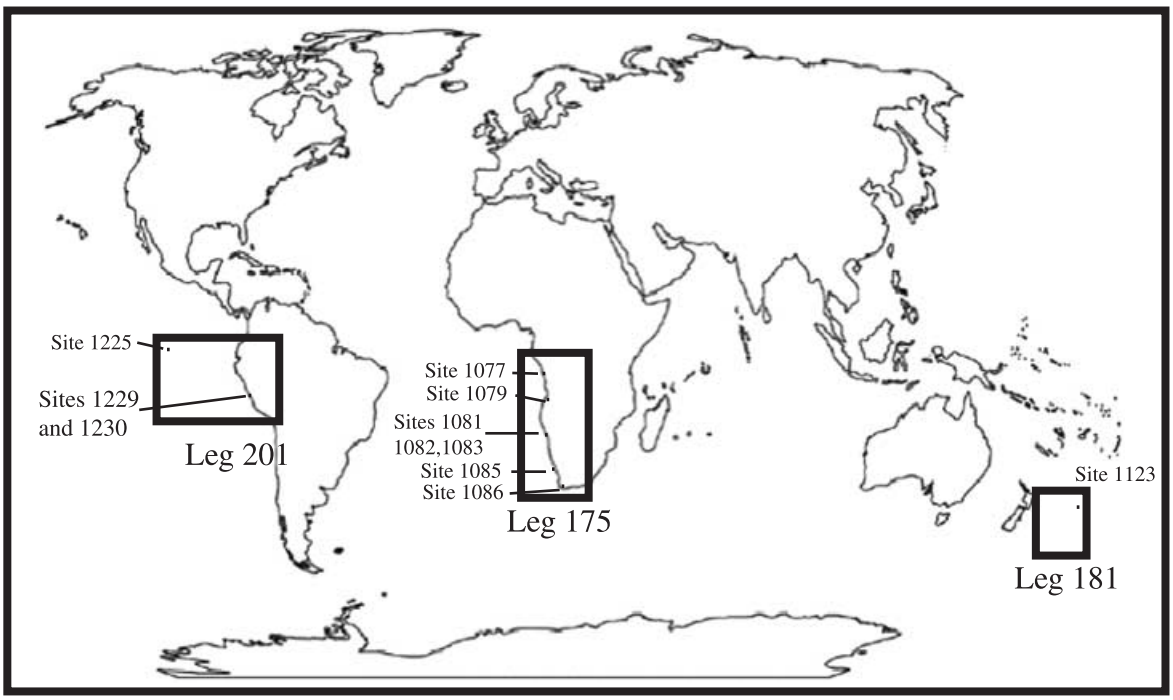

Fig. 1 Map of world with locations of sites marked.

Harvard University. Pore fluid sulfate was precipitated as barium sulfate (barite) using a supersaturated barium chloride solution. Barite was analysed for its $\delta^{18}O_{SO4}$ through pyrolysis in a graphite crucible in a Temperature Conversion Element Analyser (TC/EA) at 1450 °C coupled by continuous He flow to a Delta Plus mass spectrometer (Turchyn & Schrag, 2006). All barite measurements were corrected to NIST-127 value of 9.3‰, and $\delta^{18}O_{SO4}$ values are presented in parts per thousand (permil or ‰ – vs. Vienna Standard Mean Ocean Water – VSMOW). Sulfur isotopes in pore fluid sulfate ($\delta^{34}S_{SO4}$) were measured at the Stable Isotope Research Facility at Indiana University and are presented in permil (‰ – vs. the Cannon Diablo Troilite – CDT).

RESULTS

Sulfate concentrations (previously measured) and $\delta^{18}O_{SO4}$ profiles (our data) are shown in Figs 2, 3, and 4. Site 1225 (Leg 201) represents a deep-water area (water depth 3760 m), where the sediments are fully oxidized and there is no appreciable sulfate reduction (Fig. 2). This site serves as a 'control' $\delta^{18}O_{SO4}$ profile, showing little isotopic change (<1‰) in the $\delta^{18}O_{SO4}$ with depth, in the core.

Site 1086 and Site 1123, from Legs 175 and 181, respectively, are sites where there is no methane and sulfate is reduced entirely through OMO (Fig. 3). Sulfate concentrations (in grey) decrease with depth, showing a concave down shape typical of pore fluid profiles controlled mainly by OMO (Fig. 3). In these sites the $\delta^{18}O_{SO4}$ increases rapidly at the top of the core to around +22

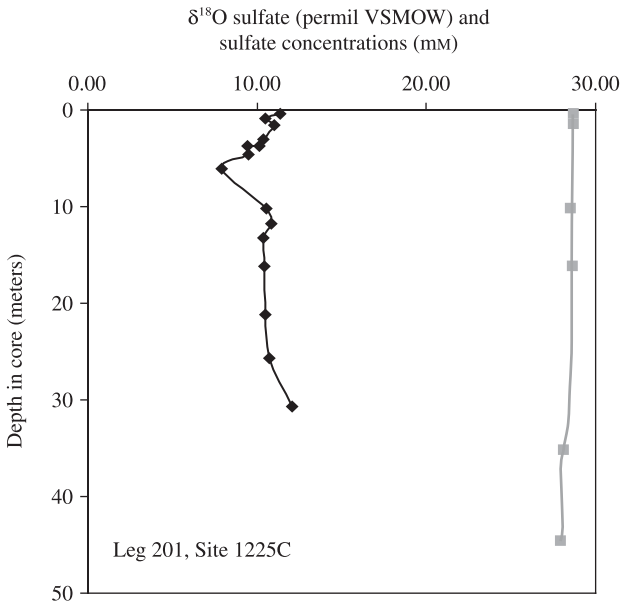

Fig. 2 The $\delta^{18}O_{SO4}$ profile from a pelagic core with no appreciable rates of sulfate reduction. Sulfate concentrations are in grey and $\delta^{18}O_{SO4}$ is in black. The $\delta^{18}O_{SO4}$ does not change significantly with depth in the core from seawater values of 9.3‰.

to +25‰, and remains isotopically heavy through the rest of the core. In Site 1123 (Fig. 3B) sulfate concentrations are not fully depleted with depth in the core, yet the same isotopic increase is observed. For Site 1086 (Fig. 3A), the $\delta^{34}S_{SO4}$ profile is also presented (open circles with dashed line). The

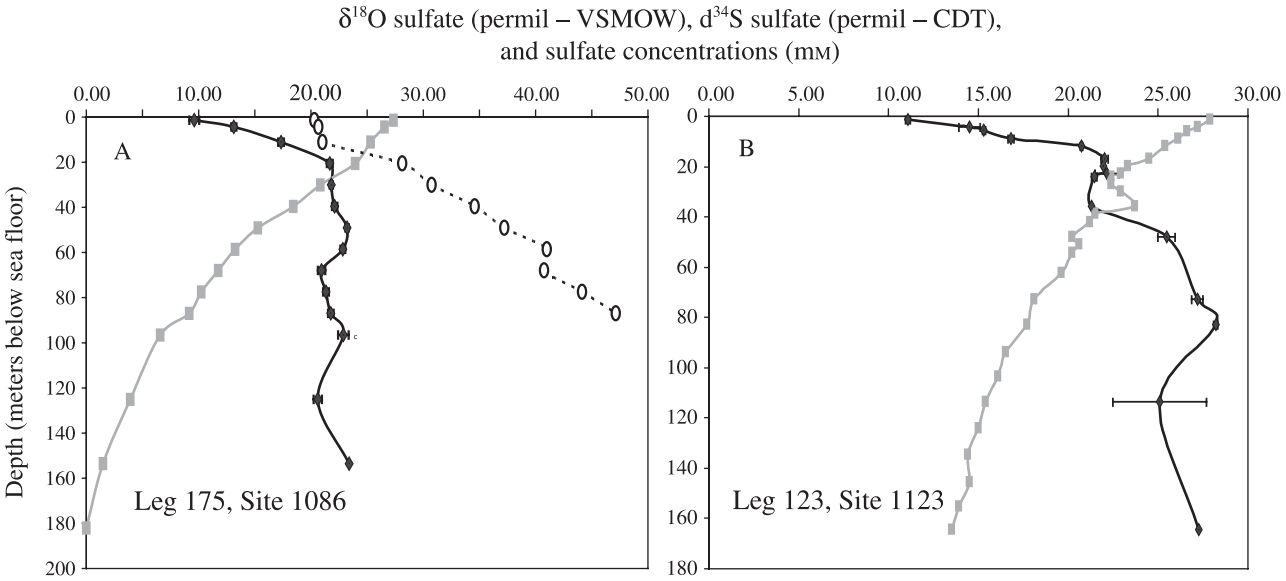

Fig. 3 The $\delta^{18}O_{SO4}$ profiles from two sites where sulfate is only reduced through organic matter oxidation. In both plots, sulfate concentrations are in grey and $\delta^{18}O_{SO4}$ is in black. In Fig. 3A, the $\delta^{34}S_{SO4}$ profile is also shown, in open circles with a dashed line. Error bars represent 2σ standard deviation based on three to six replicate measurements.

$\delta^{34}S_{SO4}$, which increases monotonically with depth in the core, is decoupled from the pore fluid $\delta^{18}O_{SO4}$.

Eight sites from Legs 175 and 201 where sulfate is reduced mainly through AMO are presented in Fig. 4. Sulfate concentrations in these cores largely exhibit a linear diffusion profile from seawater values to depletion, consistent with consumption in a single zone. The $\delta^{18}O_{SO4}$ profiles in Fig. 4A–F (Sites 1081, 1082, 1083, 1085 and 1079 from Leg 175 and Site 1229 from Leg 201) show a rapid increase at the top of the profile to values of about +22–25‰, similar to our observation in the sites without AMO (Fig. 3). In Fig. 4(A,B), from Sites 1081 and 1082 (Leg 175) we also present $\delta^{34}S_{SO4}$ data from pore fluid sulfate. As shown, the $\delta^{34}S_{SO4}$ increases monotonically with depth in the core and does not covary with the $\delta^{18}O_{SO4}$. The $\delta^{18}O_{SO4}$ levels off at isotopically heavy values and remains heavy until right above the zone of AMO, where in some sites there is a 5–8‰ isotopic decline into the sulfate minimum zone. This is particularly apparent in Site 1082 from Leg 175 (Fig. 4B). It should be noted that while the profiles from Leg 175 (Fig. 4A–E) show the isotopic increase in the $\delta^{18}O_{SO4}$ at the top of the pore fluid profiles, the isotopic increase is not apparent in the profile from Leg 201 (Site 1229, Fig. 4F). In this site, when the first sample was obtained at 1.5 m, the sulfate concentration is depleted by over 10 mM and the $\delta^{18}O_{SO4}$ is already over 20‰. The isotopic increase in the $\delta^{18}O_{SO4}$ likely occurred during the upper 1.5 m, above the depth where the first pore fluid samples were obtained.

Sites 1077 and 1230 from Legs 175 and 201, respectively, have the highest rates of sulfate reduction (via methane) and therefore the shallowest depletion depth for sulfate (Fig. 4G,H). In both of these sites we observe a rapid increase in the $\delta^{18}O_{SO4}$ of pore fluid sulfate with no subsequent leveling off of $\delta^{18}O_{SO4}$ or isotopic decline into the zone of AMO.

DISCUSSION

Lloyd (1968) performed some of the first experiments on oxygen isotopes in sulfate and demonstrated that sulfate and water do not readily exchange isotopes above pH of around 3. In this study, he estimated that at ocean pH, it would take over 20 million years for sulfate and water to isotopically equilibrate (Lloyd, 1968). The residence time of sulfate in the ocean with respect to its oxygen isotopic composition is believed to be closer to 1 million years, indicating that sulfate and water should not isotopically equilibrate over the lifetime of sulfate in the ocean (Turchyn & Schrag, 2006). Marine sulfate is relatively well mixed and understood to have a homogenous $\delta^{18}O_{SO4}$ of 9.3‰ (Longinelli & Craig, 1968). Previous measurements of the $\delta^{18}O_{SO4}$ during sulfate reduction suggest that there is isotopic selection for light oxygen isotopes, leaving a residual pool that is isotopically heavy (e.g. Bottrell *et al.*, 2000). The magnitude of the isotopic selection (ε_O) is between 2 and 10‰ and studies suggest it may depend on the carbon substrate and sulfate concentrations, similar to the environmental controls on ε_S (Aharon & Fu, 2000; Brunner *et al.*, 2005). If kinetic isotope fractionation during sulfate reduction were the only process modifying the $\delta^{18}O_{SO4}$ of pore fluid sulfate, we would expect the $\delta^{18}O_{SO4}$ to increase continuously with depth through organic-rich sediments, tracking the sulfur isotope increase, although perhaps with a shallower trajectory due to the fact that ε_O is smaller than ε_S. However, as shown in Figs 3A and 4(A,B), the trajectories are decoupled, indicating that the

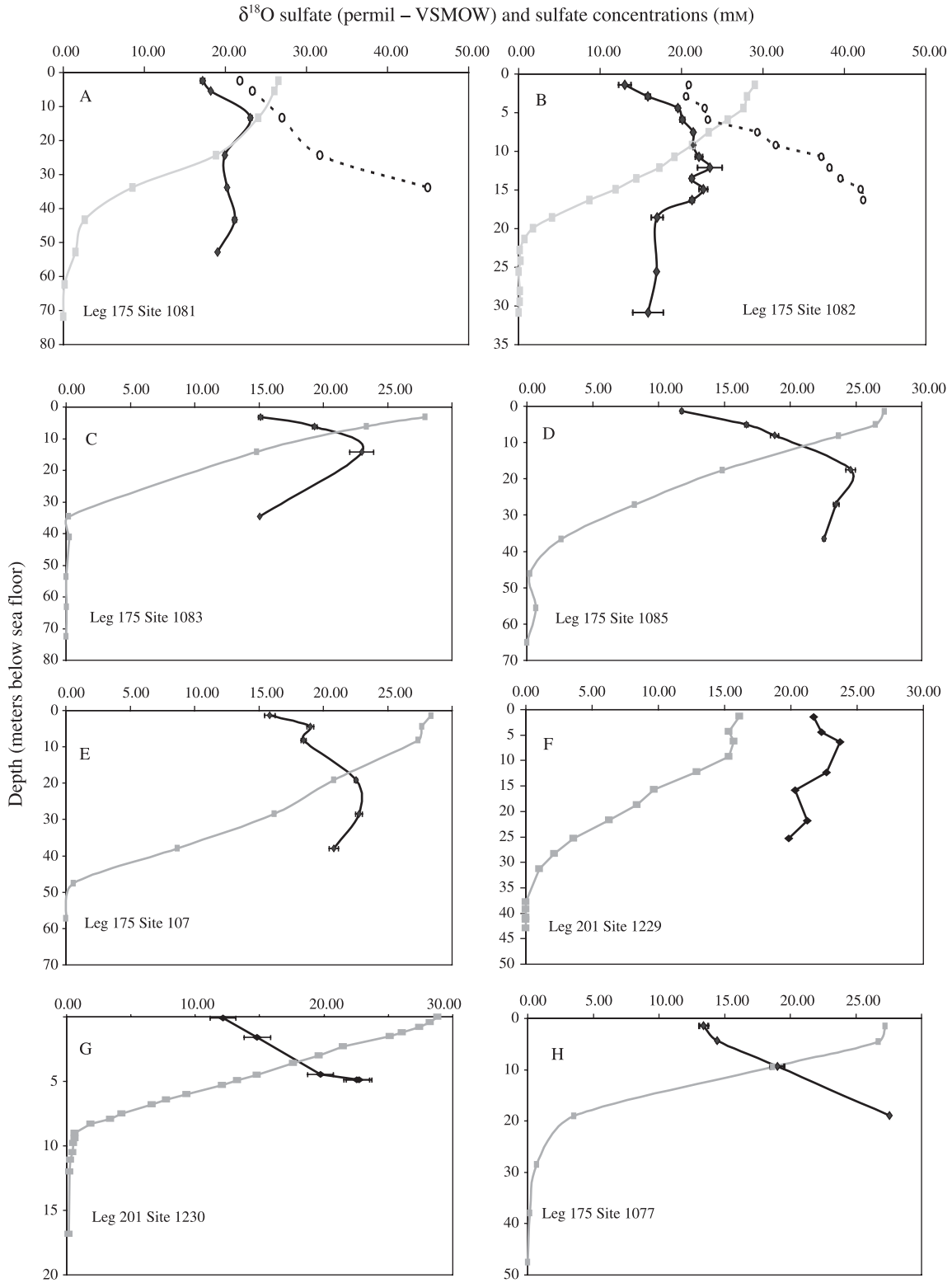

Fig. 4 (A–H) The $\delta^{18}O_{SO4}$ from 8 sites with anaerobic methane oxidation. Sulfate concentrations are in grey and $\delta^{18}O_{SO4}$ is in black. In Fig. 4(A,B) the $\delta^{34}S_{SO4}$ profile is also shown, in open circles with a dashed line. Error bars represent 2σ standard deviation based on three to six replicate measurements. Figure 4(A–E) show $\delta^{18}O_{SO4}$ from sites within Leg 175 while Fig. 4F is from a site in Leg 201. All six sites (Fig. 4A–F) show the rapid isotopic increase in the $\delta^{18}O_{SO4}$ and the leveling off at 22–25‰. Figure 4(G,H) show data from sites with the highest rates of sulfate reduction and do not exhibit the established profiles seen in the other sites. Sulfur isotopes in pore fluid sulfate ($\delta^{34}S_{SO4}$) are also shown (open circles with a dashed line) for sites 1081 and 1082 from Leg 175 (Fig. 4A,B).

processes that involve sulfate could be more complicated than previously thought based on pore fluid sulfate concentrations and sulfur isotope profiles.

This decoupling of the $\delta^{18}O_{SO4}$ and $\delta^{34}S_{SO4}$ in pore fluid profiles appears to conflict with our understanding of the fractionations associated with the microbial processes involving sulfate reduction occurring in organic-rich sediments. One possible way to explain our data is to conclude that the isotopic variability we measure is an artifact of sampling. However, we observe the same trends at sites located around the world, making it hard to argue that they all result from sampling effects. The changes in both sulfate concentrations and the $\delta^{34}S_{SO4}$ of the residual sulfate pool appear internally consistent; sulfate reduction rates can be calculated from changes in pore fluid sulfate concentrations and, with the continuous increase in the $\delta^{34}S_{SO4}$, we can calculate the kinetic fractionation factor for sulfur isotopes during sulfate reduction. The rapid increase in the $\delta^{18}O_{SO4}$ and the fact that it levels off, however, suggest at least qualitatively that if kinetic isotope effects during sulfate reduction were the only process affecting the $\delta^{18}O_{SO4}$ (e.g. Bottrell *et al.*, 2000), much higher rates of sulfate reduction would be needed to increase the oxygen isotopic composition of the residual sulfate pool so dramatically, and theoretically no leveling off should occur. This isotope decoupling requires that different processes are affecting the $\delta^{18}O_{SO4}$ and the $\delta^{34}S_{SO4}$ of pore fluid sulfate. The 'leveling off' of $\delta^{18}O_{SO4}$ at values between 22 and 25‰ suggests that the processes that led to the rapid isotopic increase in $\delta^{18}O_{SO4}$ either cease or reach some steady state.

Because the isotope fractionation during sulfate reduction is lower for oxygen isotopes (ε_O) than for sulfur isotopes (ε_S), the rapid rise in the $\delta^{18}O_{SO4}$ seen in these profiles cannot be driven by a kinetic isotope fractionation during sulfate reduction alone, which would suggest a faster rise in the $\delta^{34}S_{SO4}$. The decoupling of the $\delta^{18}O_{SO4}$ from the $\delta^{34}S_{SO4}$ highlights the possibility of oxygen isotope exchange between sulfate and water during sulfate reduction, which has been suggested from isotopic results in previous studies of pure culture sulfate-reducing bacteria (e.g. Fritz *et al.*, 1989; Brunner *et al.*, 2005). For example, Brunner *et al.* (2005) grew sulfate-reducing bacteria in waters with $\delta^{18}O$ ranging from –20‰ to +80‰ and demonstrated that the $\delta^{18}O_{SO4}$ of the residual sulfate pool was strongly affected by the isotopic composition of the water. In another study, Fritz *et al.* (1989) grew sulfate-reducing bacteria in waters with initial sulfate of differing $\delta^{18}O_{SO4}$ and demonstrated that the residual sulfate pool approached ~+20‰ regardless of the initial $\delta^{18}O_{SO4}$, although this was dependent on both the temperature of the experiment and the $\delta^{18}O$ of the water in which the experiment was performed. Both of these studies concluded that there must be isotopic exchange between sulfate and water during sulfate reduction, allowing the residual $\delta^{18}O_{SO4}$ to evolve differently than the $\delta^{34}S_{SO4}$.

Oxygen isotope exchange between sulfate and water during sulfate reduction suggests that bacteria import sulfate into their cells, facilitate isotope exchange, then release the isotopically modified sulfate back into the environment (Fritz *et al.*, 1989; Canfield, 2001; Brunner *et al.*, 2005). In this way the bacteria would modify the environmental $\delta^{18}O_{SO4}$ without impacting the $\delta^{34}S_{SO4}$. It has been suggested that the high energetic costs of importing sulfate into the bacterial cell render it unlikely that incorporated sulfate would 'leak' back into the environment (Detmers *et al.*, 2001; Brüchert, 2004). However, studies performed with labelled ^{35}S have shown that reverse transport of sulfate across the cell membrane is possible (Warthmann & Cypionka, 1990; Stahlmann *et al.*, 1991). In addition, most quantitative treatment of sulfur isotopic fractionation during sulfate reduction assume that some sulfate must be transported out of the cell in order to observe the large range of isotopic fractionations associated with sulfate-reducing bacteria (low isotope fractionation is thus associated with little reverse transport and sulfate-limiting conditions and high isotope fractionations are associated with higher reverse transport and a more isotopic expression of the sulfate-reducing enzymes – see Farquhar *et al.*, 2003 and Canfield *et al.*, 2006 for more complete discussions).

If our data do represent oxygen isotope exchange between sulfate and water, we could postulate that this could take place either extracellularly or intercellularly. If, for example, there were membrane-bound enzymes that bind sulfate from the environment, facilitate oxygen isotope exchange between this bound-sulfate and water, then release the modified sulfate to the pore fluids, this would alter the oxygen isotopic composition of the extracellular sulfate pool at little energetic cost to the sulfate reducer and with little impact on the sulfur isotopic composition. Alternatively, sulfate could be imported into the cell, isotopically modified and then washed back out of the cell. In either case, we feel that it is unlikely that isotopic equilibration between sulfate and water is the mechanism by which our pore fluid $\delta^{18}O_{SO4}$ is being modified, because if this were the case we would expect the residual sulfate pool to approach 34–38‰, the equilibrium fractionation factor between sulfate and water (Mizutani & Rafter, 1969; Lloyd, 1968). Instead our data suggest the residual sulfate pool approaches 22–25‰ over the $\delta^{18}O$ of the water in which the reduction occurs. This value is very close to the equilibrium fractionation between sulfite (SO_3^{2-}), an intermediate valence state sulfur species, and water (~22‰, Van Stempvoort & Krouse, 1994).

Our data therefore imply that sulfate-water isotope exchange during sulfate reduction must occur through sulfite. The sequence of events would include sulfate incorporation into the bacterial cell, partial reduction to sulfite, equilibration between sulfite and water, and reoxidation to sulfate and release to the environment (e.g. Fritz *et al.*, 1989; Brunner *et al.*, 2005). Biochemically, sulfate is brought into the cell of the bacterial sulfate reducer, activated to APS-sulfate and then, in a two electron reduction, reduced to sulfite (Canfield, 2001). It has been suggested that there could be a short-lived intercellular pool of sulfite awaiting the six electron reduction to H_2S (Canfield, 2001). This intercellular sulfite pool could isotopically

equilibrate with water and instead be reoxidized back to sulfate, either intercellularly or extracellularly, ultimately modifying the oxygen isotopic composition of the extracellular sulfate pool. The energetic investment required for incorporation of sulfate into the bacterial cell is recovered during the two-electron reduction to sulfite. It has been suggested, however, that bacteria may be at a disadvantage by allowing part of any intercellular sulfur pool to 'leak' back to the environment rather than reducing it further to sulfide, because the reduction of sulfite to sulfide has a high-energy yield for the bacteria (e.g. Brüchert, 2004). Ultimately, the oxygen isotopic exchange between an intercellular sulfite pool and an extracellular sulfate pool may gain no net-energy for the bacteria, and they would get the bulk of their energy from the final six-electron reduction of sulfite to sulfide.

It would be helpful to understand the rate of this partial sulfate reduction and cellular 'leakage' required to observe the rapid increase in the $\delta^{18}O_{SO4}$ in our pore fluid profiles. To estimate this we use a numerical model that describes transport and reaction in the sediment and pore fluid system similar to the approach based on the seminal work by Berner (1980). The conservation equation for the concentration of a chemical species i (in this case sulfate) in porewater has a general form that includes terms for diffusion, sedimentation advection and reactions, respectively:

$$\frac{\partial C_i}{\partial t} = \frac{\partial}{\partial z}\left(Ds_i \frac{\partial C_i}{\partial z}\right) - (U + \omega)\frac{\partial C_i}{\partial z} + \sum \mathrm{Re}\, actions_i \quad (1)$$

where z is depth within the sediment column; t is time; ϕ is porosity; D_s is the diffusion coefficient of sulfate in sediments, where we assume that $D_{s(i)} \sim D_{0(i)} \cdot \phi^2$ with D_0 being the diffusion coefficient of sulfate in seawater; U is the term for pore fluid advection; ω is the sedimentation rate; and ΣReactions is the sum of the production/consumption rates of sulfate. Since the variation in the porosity is small, we assumed a constant porosity along the profiles, which simplifies equation 1. In addition, similar to previous treatments, we assume that diffusion dominates and advection is negligible (Richter & DePaolo, 1987). We can thus simplify equation 1 to:

$$\frac{\partial C_i}{\partial t} = DS_i \frac{\partial^2 C_i}{\partial z^2} - \omega \frac{\partial C_i}{\partial z} + \sum \mathrm{Re}\, actions_i \quad (2)$$

Equation 2 was solved numerically through finite difference with 1-m boxes and a time step of 10 years. We simulated the reactions for both sulfate concentrations and the $\delta^{18}O_{SO4}$ (by calculating separately the reactions for end members $S16O_4$ and $S^{18}O_4$ then calculating the $\delta^{18}O_{SO4}$).

The reactions that affect the $\delta^{18}O_{SO4}$ at any given depth are sulfate incorporation into the cell, oxygen isotope exchange between sulfite and water, and the reoxidation and export of either sulfite or sulfate from the cell. For simplicity we assume that sulfite is the primary intermediate valence state sulfur species, although other intermediate sulfur species, such as thiosulfate or tetrathionate, could be present. The first reaction that can affect the $\delta^{18}O_{SO4}$, sulfate reduction, exerts a kinetic isotope fractionation (ε_O is between 0‰ to +10‰ Aharon & Fu, 2000), preferentially selecting isotopically light sulfate and leaving the extracellular sulfate pool isotopically heavy. The second reaction is oxygen isotope exchange between sulfite and water, with a fractionation factor ($\alpha_{SO3\text{-}H2O}$) of ~1.025 (Van Stempvoort & Krouse, 1994). The isotopic equilibration between sulfite and water is rapid at neutral or slightly acidic pH and at ocean temperature; for example experiments performed with sulfite and water at a pH of 8.7 suggested a half-life for oxygen isotope exchange of 1.3 min (Betts & Voss, 1970). This fractionation factor and timescale for equilibration was confirmed in experiments with SO_2 and water vapor conducted in closed containers at 20–25 °C, where the SO_2 presumably dissolves in water (producing sulfite) prior to exchange (Holt *et al.*, 1983). The timescale for equilibration increases dramatically above pH of 9, however, this is an unlikely condition in our ODP cores. We therefore assume that the entire intracellular sulfite pool equilibrates with water and is reset to 25‰ heavier than the $\delta^{18}O$ of the pore fluid. The $\delta^{18}O$ of the pore fluid becomes progressively isotopically lighter with burial depth due to low temperature weathering reactions with the underlying basalt (Lawrence & Gieskes, 1981). For simplicity, we use a value of −1‰ for the $\delta^{18}O$ of the pore fluid at the site of isotopic exchange. The isotopic fractionation for oxygen isotopes during the reoxidation of sulfite to sulfate (ε_{ROX}) depends on where the oxygen atoms come from and the pathway through which they are incorporated into the sulfate molecule (Van Stempvoort & Krouse, 1994). In anoxic sediments the final oxygen atom for the sulfate molecule derives from water with little isotopic fractionation during its incorporation (ε_{ROX} 0‰ Van Stempvoort & Krouse, 1994). Theoretically, there could also be a kinetic isotope fractionation during reoxidation or during export from the cell, although this has not directly been measured. We neglect the effect of Rayleigh distillation on the isotopic evolution of the intercellular sulfite pool, either from oxidation or from export from the cell, since our calculations suggest that nearly all sulfite must be reoxidized and return to the pore fluid (below). This is consistent with no long-lived sulfite pool, similar to other studies (cf. Brüchert, 2004).

We can solve our conservation equation 2 for steady state, using the measured sulfate concentrations to calculate rates of net sulfate reduction, that is, the rate at which sulfate is reduced to sulfide and, since there is little measurable sulfide concentrations in these sites, scavenged (likely as pyrite). We can then constrain our modelled rates by the measured $\delta^{18}O_{SO4}$ profiles to calculate the likely fractionation factor if kinetic isotope fractionation during sulfate reduction was the only process impacting the $\delta^{18}O_{SO4}$ of pore fluid sulfate. We can also solve our equations 'in reverse', that is, if we assume a kinetic isotope fractionation factor associated with sulfate reduction for oxygen isotopes (ε_O), what would the implied rates of sulfate reduction need to be to fit our observations?

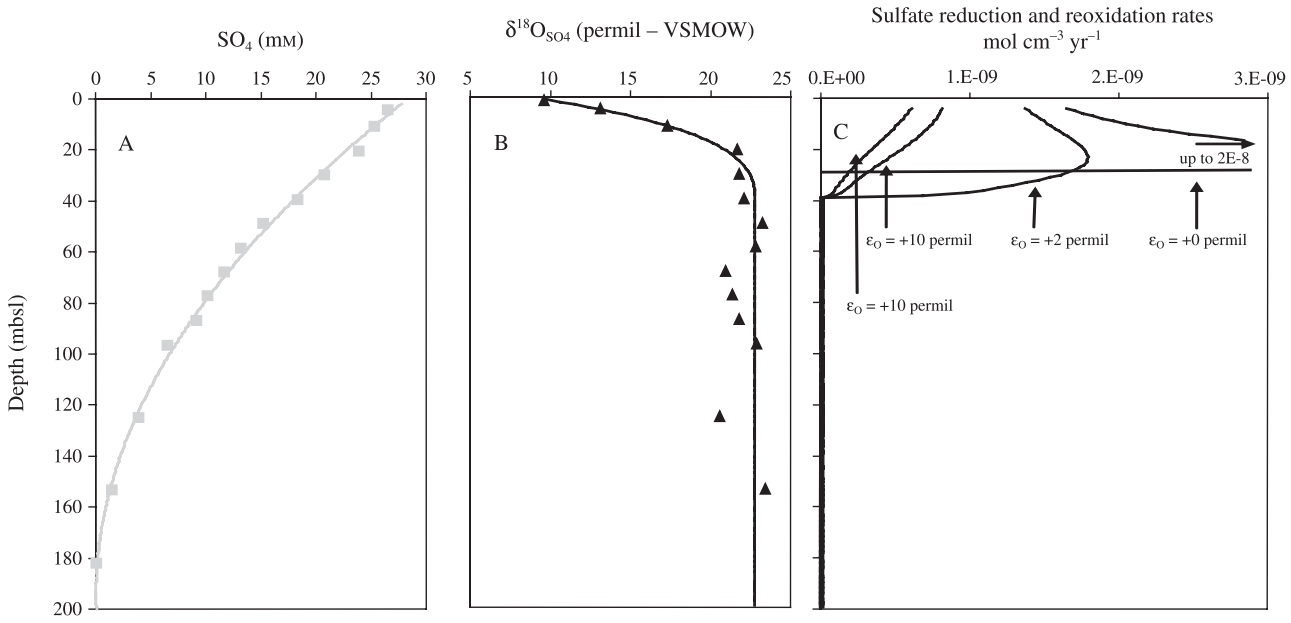

Fig. 5 Model results with data from Leg 175, Site 1086, a site with no AMO (sulfate is consumed entirely through OMO). Figure 5A – Sulfate concentration profile from Site 1086 with the model curve. Figure 5B – $\delta^{18}O_{SO4}$ profile through the core with model profile in dark line. Figure 5C – model results for sulfate reduction and reoxidation rates for a series of oxygen isotopic fractionation factors for sulfate reduction (ε_O). Reoxidation rates are nearly identical to reduction rates implying sulfur cycling. See Discussion part for details.

To demonstrate our calculations we present results from Site 1086 (Figs 3A and 5). At this site, sulfate reduction rates calculated from a best-fit curve of sulfate concentrations alone are ~1×10^{-11} mol cm^{-3} yr^{-1} (Fig. 5A) and are constant with depth in the core until sulfate is fully consumed at 180 m. However, sulfate reduction rates suggested by the increase in the $\delta^{18}O_{SO4}$ (Fig. 5B) must be at least 6×10^{-10} and perhaps as high as 3×10^{-8} mol/cm^{3}yr (depending on the value used for ε_O: the lower ε_O, the higher the rates of sulfate reduction must be to match the rapid increase in the $\delta^{18}O_{SO4}$ profiles). These rates are between a factor of 6 and 300 higher than the calculated rates of net sulfate reduction calculated from the sulfate concentrations. When using the $\delta^{18}O_{SO4}$ to estimate sulfate reduction rates, in order to model sulfate concentrations profiles that match the observations, nearly 100% of the sulfite must be reoxidized to sulfate, with a small portion going permanently to sulfide and, presumably being buried as pyrite. We call this cycling of sulfate to sulfite and back 'net-zero' sulfur cycling, which we suggest might be a separate process from net sulfate reduction. This would imply that the $\delta^{18}O_{SO4}$ levels off at 22–25‰ because the sulfur cycling is fast enough to create complete isotopic exchange. More rapid rates of sulfur cycling below the depth where the $\delta^{18}O_{SO4}$ levels off are possible but cannot be constrained by our measurements.

We can describe these results in more familiar terms used in recent models exploring sulfate-reducing metabolism with respect to sulfur isotope fractionation, such as developed by Farquhar *et al.* (2003) and used by Canfield *et al.* (2006). In their approach sulfate reduction is described by a series of reactions as follows:

$$SO_{4(extracellular)} \overset{1,\varphi_1}{\underset{2,\varphi_2}{\Leftrightarrow}} SO_{4(\text{int}\,ercellular)} \overset{4a}{\underset{5a}{\Leftrightarrow}} APS \overset{4b,\varphi_{4b}}{\underset{5b,\varphi_{5b}}{\Leftrightarrow}} SO_3 \overset{3,\varphi_3}{\rightarrow} H_2S \quad (3)$$

In this reaction network φ refers to the amount of sulfur that flows through any particular numbered reaction. Many of the steps in equation 3 would have associated kinetic isotope fractionations for both sulfur isotopes and oxygen isotopes. As defined by Farquhar *et al.* (2003) and Canfield *et al.* (2006), the fraction of sulfate that ultimately exits the cell as sulfide is defined as:

$$f_3 = \frac{\varphi_3}{\varphi_2 + \varphi_3} \quad (4)$$

Thus when f_3 is small, most sulfate imported into the cell for sulfate reduction never arrives at H_2S, but rather is cycled through reactions 1, 4a, 4b, 5b, 5a and 2 (in that order) in equation 3. Our calculations suggest that in the ODP environments we studied, f_3 approaches zero, although theoretically it could be as high as 0.15. As discussed above, we feel that the sulfate is reduced to sulfite (reactions 1, 4a, and 4b), isotopically equilibrates with water, then returns to the porefluid as either sulfite or sulfate (via reactions 5b, 5a, and 2) rather than isotopic exchange as an intercellular pool of sulfate (reactions 1 and 2 only) because our data show an asymptotic approach of the $\delta^{18}O_{SO4}$ to the equilibrium fractionation factor between sulfite and water. The fact that f_3 is so small would suggest that the overall fractionation for sulfur isotopes in this system would be large since the sulfate-reducing metabolism is not limited by transport of sulfate into the cell but rather by the isotopic fractionation associated with the enzymatic pathways of intercellular sulfate reduction.

Similar to the studies by Brunner *et al.* (2005) and Fritz *et al.* (1989), we suggest that the rapid increase in the $\delta^{18}O_{SO4}$ of pore fluid profiles represents sulfate–water oxygen isotope exchange through sulfite. However, our data require that the vast majority of sulfate that is imported into the cells is returned to the environment and only a small fraction proceeds via reaction 3 in equation 3. This leads us to suggest that sulfate is actively being cycled through the cells as sulfite, with only a small fraction proceeding to sulfide. If the rates required by our data were only one or two times higher than the net rate of sulfate reduction suggested by sulfate concentrations, then we might conclude that this isotopic decoupling was an effect of cellular 'leakage'. Because the rates calculated for our data are so much higher than those calculated from sulfate concentrations, we suggest that sulfur cycling, or rapid isotopic exchange between the internal and external sulfate pools, must occur. Other studies have suggested similar sulfur cycling in hypersaline lagoons off the coast of Brazil (Moreira *et al.*, 2004) and in Florida Bay (Ku *et al.*, 1999), although these are both very different environmental settings than the ODP cores we studied.

The geochemical data presented here and in previous work provide evidence that sulfur cycling is occurring in anoxic sediments in the ocean, or an active exchange between the external sulfate pool and the internal sulfite pool that is 'independent' from net sulfate reduction. A fundamental question is what might be driving this cycle of sulfur through oxidized and reduced states when there is no energetic sense to the bacteria in doing so, and the largest energy gain for the bacteria would be to take sulfate and simply reduce it to sulfide. Although this is highly speculative, one possibility is that the cycling of sulfur allows these organisms to make use of an external electron acceptor, such as iron, that would otherwise be unavailable. For example, studies in terrestrial environments suggest that ferrihydrite can be reduced to ferrous iron through sulfur cycling with intermediate sulfur compounds like thiosulfate and elemental sulfur as the primary reductant (Straub & Schink, 2004a, 2004b). These authors also suggested that ferric iron minerals that are extremely insoluble at ocean pH could be reduced through similar electron shuttling by intermediate valence state sulfur species. In anoxic ocean sediments, microbially available Fe(III) is depleted through organic matter oxidation in the uppermost part of the sediment column (Froelich *et al.*, 1979). Other Fe(III) minerals may be present in the sediments, although not available for use as an electron acceptor by microbes. These minerals would be highly reactive to sulfide (forming pyrite, e.g. Canfield *et al.*, 1992), suggesting that sulfide is not the primary reductant in any sulfur cycling and rapid exchange between the internal and external sulfate and sulfite pool must play a pivotal role. One way to test this hypothesis would be to measure changes in the iron isotope profile with depth through organic-rich sediments; iron isotopes should be fractionated during Fe-related redox processes (Malinovsky *et al.*, 2005). Another would be to culture bacteria in the presence of insoluble iron minerals using a knock-out for the sulfite reductase gene, making it impossible for them to fully reduce sulfate and observe both if growth occurs and if the external sulfate pool is isotopically modified as we would anticipate.

In the two sites where sulfate reduction rates are the highest and sulfate is consumed within the top 10 or 20 m, we do not see the leveling off of the $\delta^{18}O_{SO4}$ as in all the other sites studied (Fig. 4G,H). These sites, 1230 and 1077, from Legs 201 and 175, respectively, exhibit a monotonic increase in the $\delta^{18}O_{SO4}$. This monotonic increase is similar to a previous study of $\delta^{18}O_{SO4}$ in ODP pore fluid profiles from the Cascadia Margin where the authors concluded that the kinetic isotope fractionation during sulfate reduction was the only process modifying the pore fluid $\delta^{18}O_{SO4}$ (Bottrell *et al.*, 2000). The ODP sites studied by Bottrell *et al.* (2000) had similarly high rates of sulfate reduction, with sulfate consumed within 10 m of the sediment–water interface. This may indicate that when sulfate reduction rates are high, we do not have the sulfur cycling or isotopic exchange between sulfur intermediates and water as suggested by the other sites in this study, or that the isotopic exchange between the internal sulfite pool and external sulfate pool is much slower. Alternatively, the sampling resolution may not be high enough to capture the leveling off. Further measurements should be made in organic-rich sediments with high rates of sulfate reduction to ascertain if this is a global processes or one confined to deeper sediments with lower overall net rates of sulfate reduction.

Another observation in our data is that the $\delta^{18}O_{SO4}$ appears to decline into the zone of AMO (Fig. 4). This is only clear at one site (1082, Fig. 4B), and potentially present at two more (1081 and 1083, Fig. 4A,C, respectively). If it is correct, then it presents another dilemma; it implies that, at least at this site, sulfate with heavy oxygen isotopes is being preferentially reduced during AMO, leaving a residual pool of isotopically light sulfate. This is counterintuitive to the basic principles governing kinetic isotope fractionation, by which light isotopes should be concentrated in the product. Whether this is a real or an artifact of sampling at these sites should be confirmed by more high-resolution measurements of the $\delta^{18}O_{SO4}$ through the zone of AMO. If it is confirmed, then oxygen isotopes in sulfate may help elucidate the biochemical pathway of sulfate reduction during methane oxidation and could help ascertain whether it is different from bacterial sulfate reduction during OMO. For example, if there is no selection for oxygen isotopes during sulfate reduction through AMO ($\varepsilon_O = 0‰$) while strong selection for sulfur isotopes ($\varepsilon_S = 10–45‰$), this might imply that the enzyme that binds to sulfate during reduction by methane may bind solely to the sulfur atom in the sulfate molecule and care little for what oxygen isotopes may be attached to the sulfur atom. If there is a negative selection for oxygen isotopes during sulfate reduction ($\varepsilon_O < 0‰$), there could be some isotopic equilibration between sulfate and some enzyme complex that binds oxygen more tightly than sulfur does (e.g. C = O). When sulfate is attached to this enzyme, the sulfur oxygen

bond is broken and the heavy oxygen atoms are exchanged with the oxygen atoms in the enzyme, releasing sulfate that is isotopically lighter than it began.

CONCLUSIONS

Oxygen isotopes in sulfate of pore fluid profiles through organic-rich sediments were presented from 11 ODP sites located around the world. The $\delta^{18}O_{SO4}$ exhibits a rapid increase from seawater values to +22 to +25‰ and remains at these values until the zone of anaerobic methane oxidation (where present), where we occasionally observe a small isotopic decrease. This decoupling of the $\delta^{18}O_{SO4}$ from the $\delta^{34}S_{SO4}$ supports the idea that there is oxygen isotope exchange between sulfate and water during sulfate reduction, allowing modification of the oxygen isotopic composition of the residual sulfate pool without modifying the sulfur isotopic composition. The oxygen isotopic exchange between sulfate and water that occurs during sulfate reduction probably occurs through an intercellular sulfite pool. We use our data to calculate the rates of sulfate reduction needed to model the rapid rise in the $\delta^{18}O_{SO4}$. The calculated rates were several orders of magnitude higher than those suggested from sulfate concentration profiles, and suggest 100% reoxidation. Rather than the occasional 'leaking' of an isotopically modified sulfite pool back to the environment, our data suggest an active cycling of sulfate through this intermediate sulfite pool, which we termed 'net-zero' sulfur cycling. One possibility is that sulfite serves as an electron shuttle to an electron acceptor that is otherwise unavailable to microbial metabolism (e.g. insoluble Fe(III) minerals), thereby coupling the sulfur and iron redox cycles in organic-rich sediments. We suggest that further studies of oxygen isotopes in pore fluid sulfate may be useful in elucidating biogeochemical processes and pathways.

ACKNOWLEDGEMENTS

We thank two anonymous reviewers and D.E. Canfield for comments that greatly improved this manuscript. Discussions with Francois Morel, Ann Pearson and Dianne Newman were invaluble to the development of many ideas in this paper. Sulfur isotope analyses were performed at the Stable Isotope facility at the University of Indiana. This work was supported by a Schlanger Ocean Drilling Program Graduate Fellowship and the Miller Institute for Basic Research Postdoctoral Fellowship to A.V. Turchyn, the Bikura Fellowship to O. Sivan, and NSF Grant OCE-0452329 to D.P. Schrag.

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