Coupled sulfur and oxygen isotope insight into bacterial sulfate reduction in the natural environment

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Abstract

We present new sulfur and oxygen isotope data in sulfate (δ 34S SO 4 and δ 18O SO 4 , respectively), from globally distributed marine and estuary pore fluids. We use this data with a model of the biochemical steps involved in bacterial sulfate reduction (BSR) to explore how the slope on a δ 18O SO 4 vs. δ 34S SO 4 plot relates to the net sulfate reduction rate (nSRR) across a diverse range of natural environments. Our data demonstrate a correlation between the nSRR and the slope of the relative evolution of oxygen and sulfur isotopes (δ 18O SO 4 vs. δ 34S SO 4 ) in the residual sulfate pool, such that higher nSRR results in a lower slope (sulfur isotopes increase faster relative to oxygen isotopes). We combine these results with previously published literature data to show that this correlation scales over many orders of magnitude of nSRR. Our model of the mechanism of BSR indicates that the critical parameter for the relative evolution of oxygen and sulfur isotopes in sulfate during BSR in natural environments is the rate of intracellular sulfite oxidation. In environments where sulfate reduction is fast, such as estuaries and marginal marine environments, this sulfite reoxidation is minimal, and the δ 18O SO 4 increases more slowly relative to the δ 34S SO 4 . In contrast, in environments where sulfate reduction is very slow, such as deep sea sediments, our model suggests sulfite reoxidation is far more extensive, with as much as 99% of the sulfate being thus recycled; in these environments the δ 18O SO 4 increases much more rapidly relative to the δ 34S SO 4 . We speculate that the recycling of sulfite plays a physiological role during BSR, helping maintain microbial activity where the availability of the electron donor (e.g. available organic matter) is low.

1. INTRODUCTION

1.1. General

During the anaerobic oxidation of organic matter, bacteria respire a variety of electron acceptors, reflecting both the relative availability of these electron acceptors in the natural environment, as well as the decrease in the free energy yield associated with their reduction (Froelich et al., 1979). The largest energy yield is associated with aerobic respiration (O 2 ), then denitrification (NO 3 − ), then manganese and iron reduction, followed by sulfate reduction (SO 4 2− ) and finally fermentation of organic matter into methane through methanogenesis (Froelich et al., 1979; Berner, 1980). Due to the high concentration of sulfate in the ocean (at least two orders of magnitude more abundant than oxygen at the sea surface), dissimilatory bacterial sulfate reduction (BSR) is responsible for the majority of oxidation of organic matter in marine sediments (Kasten and Jorgensen, 2000). In addition, the majority of the methane produced during methanogenesis in marine sediments is oxidized anaerobically by sulfate reduction (e.g. Niewöhner et al., 1998; Reeburgh, 2007). The microbial utilization of
sulfur in marine sediments is thus critical to the oxidation of carbon in the subsurface.

At a cellular level, the biochemical steps during BSR have been well studied over the past 50 years (Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Rees, 1973; Farquhar et al., 2003; Brunner and Bernasconi, 2005; Wortmann et al., 2007; Eckert et al., 2011; Holler et al., 2011). During BSR, bacteria respire sulfate and produce sulfide as an end product. This process consists of at least four major intracellular steps (e.g. Rees, 1973; Canfield, 2001a and Fig. 1): during step 1, the extracellular sulfite enters the cell; in step 2, the sulfite is activated with adenosine triphosphate (ATP) to form Adenosine 5’ Phosphosulfate (APS); in step 3, the APS is reduced to sulfite (SO$_3^{2-}$); and in step 4 the sulfite is reduced to sulfide. It is generally assumed that all four steps are reversible (e.g. Brunner and Bernasconi, 2005; Eckert et al., 2011). The reduction of sulfite to sulfide (step 4) remains the most enigmatic, and may occur in one step with the enzyme dissimilatory sulfite reductase or through the multi-step trithionite pathway producing several other intermediates (e.g. thiosulfate (S$_2$O$_3^{2-}$) and thiosulfate (S$_2$O$_3^{3-}$) – Kobayashi et al., 1969; Brunner et al., 2005; Sim et al., 2011a; Bradley et al., 2011); although there is evidence that whatever pathway step 4 occurs through, it is also reversible (Trudinger and Chambers, 1973; Trudinger and Chambers, 1973; Eckert et al., 2011; Holler et al., 2011; Tapgaar et al., 2011).

Given that each of the four steps is reversible, understanding the relative forward and backward fluxes at each step and how these fluxes relate to the overall rate of sulfate reduction, is critical for understanding the link between the BSR and the rate of organic matter oxidation. Changes in environmental conditions (e.g. temperature, carbon substrate, pressure) likely impact the relative forward and backward fluxes at each step within the cell as well as the overall rate of BSR, but the relative role of these factors with respect to one another in the natural environment remains elusive. Within the marine subsurface, measurements of sulfate concentrations in sedimentary pore fluids and subsequent diffusion-consumption modeling of the rate of sulfate depletion with depth can be used for calculating the overall rate of sulfate reduction below the ocean floor (e.g. Aller and Blair, 1996; Berner, 1980; D’Hondt et al., 2004; Wortmann, 2006; Wortmann et al., 2007). These sulfate concentration profiles alone, however, cannot provide details about how the individual biochemical steps at a cellular or community level may vary with depth or under different environmental conditions.

A particularly powerful tool for studying these biochemical steps during BSR (hereafter termed the ‘mechanism’ of BSR) is sulfur and oxygen isotope ratios measured in the residual sulfate pool while sulfate reduction progresses (Mizutani and Rafter, 1973; Fritz et al., 1989; Böttcher et al., 1998; Aharon and Fu, 2000, 2003; Brunner et al., 2005; Turchyn et al., 2006, 2010; Wortmann et al., 2007; Farquhar et al., 2008; Aller et al., 2010). With respect to isotopes, we refer to the ratio of the heavier isotope of sulfur or oxygen ($^{34}$S or $^{18}$O) to the lighter isotope ($^{32}$S or $^{16}$O), reported in delta notation relative to a standard (VCDT for sulfur and VSMOW for oxygen) in parts per thousand or permil ($\%_{oo}$).

Although both sulfur and oxygen isotopes are partitioned during each intracellular step, their relative behavior (e.g. $\delta^{34}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$) in the natural environment is not fully understood. The sulfur isotope composition of sulfate ($\delta^{34}$S$_{SO_4}$) typically increases monotonically as BSR progresses (e.g. Harrison and Thode, 1958; Kaplan and Rittenberg, 1964; Rees, 1973). This occurs because most of the enzymatic steps during BSR preferentially select the lighter sulfur isotope ($^{32}$S), slowly distilling it into the produced sulfide pool and leaving $^{34}$S behind. The magnitude of the sulfur isotope fractionation (fractionation) during the overall process of BSR can be as high as 72$\%_{oo}$ (Wortmann et al., 2001; Brunner and Bernasconi, 2005; Sim et al., 2011a). Theoretical and experimental studies have suggested that this magnitude is a function of microbial metabolism and carbon source (e.g. Brüchert, 2004; Sim et al., 2011b), amount of sulfate available (e.g. Canfield, 2001b; Habicht et al., 2002), and temperature (e.g. Brüchert et al., 2001; Canfield et al., 2006). In addition, previous studies also noted a relationship between the magnitude of the sulfur isotope fractionation and the sulfate reduction rate (Kaplan and Rittenberg, 1964; Rees, 1973; Chambers et al., 1975). This relationship has been shown in pure culture experiments (e.g. Canfield et al., 2006), batch culture experiments using natural populations (e.g. Stam et al., 2011) and

![Fig. 1. The steps of bacterial sulfate reduction and the potential of oxygen and sulfur isotopic fractionations.](Image)

$X_k = b_k / l_k$ (where $k = 1, 2, 3$) is the ratio between the backward and forward fluxes.
calculated in situ using pore fluids profiles (e.g. Aharon and Fu, 2000; Wortmann et al., 2001); in all these studies, higher sulfur isotope fractionation corresponded to slower sulfate reduction rates.

On the other hand, the $\delta^{18}O_{SO_4}$ has shown variable behavior during BSR in natural environments. In some cases, the $\delta^{34}S_{SO_4}$ exhibits a linear relationship with $\delta^{34}S_{SO_4}$, also suggesting a distillation of the light isotope from the reactant sulfate. The magnitude of the oxygen isotope fractionation during this distillation was suggested to be 25% of the magnitude for sulfur isotopes (Mizutani and Rafter, 1969), although it has been observed to range between 22% (Mandernack et al., 2003) and 71% (Aharon and Fu, 2000). In most measurements of $\delta^{18}O_{SO_4}$ during BSR in the natural environment, however, the $\delta^{34}S_{SO_4}$ increases initially until it reaches a constant value and does not increase further, while the $\delta^{34}S_{SO_4}$ may continue to increase (e.g. Fritz et al., 1989; Böttcher et al., 1998, 1999; Turchyn et al., 2006; Wortmann et al., 2007; Aller et al., 2010; Zeebe, 2010). This ‘oxygen isotope equilibrium’ value (usually between 22% and 30% in most natural environments) has been shown to depend on the $\delta^{18}O$ of the ambient water (Mizutani and Rafter, 1973; Fritz et al., 1989; Brunner et al., 2005; Mangalo et al., 2007, 2008). Because the timescale for oxygen isotope exchange between sulfate and water is exceptionally slow (e.g. Lloyd, 1968; Chiba and Sakai, 1985; Zak et al., 1980), it has been suggested that, during BSR, oxygen isotopes of sulfate intermediate species such as APS and $\text{SO}_3$ exchange oxygen atoms with water (Mizutani and Rafter, 1973; Fritz et al., 1989).

Recent studies have suggested that it is more likely sulfate when bound in the AMP-sulfite complex facilitates this oxygen isotopic exchange (Kohl and Bao, 2006; Wortmann et al., 2007; Brunner et al., 2012; Kohl et al., 2012). This requires that some percentage of the sulfate that is brought into the cell does not get reduced all the way to sulfide but undergoes oxygen isotope exchange with water, reoxidation to sulfate, and release back to the extracellular sulfate pool (Mizutani and Rafter, 1973; Fritz et al., 1989; Brunner et al., 2005, 2012; Mangalo et al., 2007, 2008; Wortmann et al., 2007; Farquhar et al., 2008; Turchyn et al., 2010).

Interpreting the relative evolution of the $\delta^{18}O_{SO_4}$ and the $\delta^{34}S_{SO_4}$, the processes that control BSR in natural environments when the coupled sulfur and oxygen isotopes increase linearly (Trend A), compared to when they are decoupled and oxygen isotopes are seen to plateau (Trend B)? A second problem is that the majority of our understanding of the biochemical steps during BSR comes from pure culture studies; how does this understanding translate, if at all, to the study of BSR in the natural environment?

In this paper we will forward this discussion by presenting a compilation of sulfur and oxygen isotopes in pore fluids, including seven new sites collected over a range of different subsurface marine and near-marine environments, covering a broad range of sulfate reduction rates. This will allow us to investigate how the relative behavior of the sulfur and oxygen isotopes varies in these different environments. We will begin with a discussion of modeling sulfur and oxygen isotope evolution during BSR, most of which is a review of a previous seminal work. We will then discuss how these models for the biochemical steps during BSR can be applied to pore fluids in the natural environment. Finally, we will present our results, along with a compilation of previously published data into the context of our model.

1.2. Kinetic and equilibrium isotope effects on sulfur and oxygen isotopes during dissimilatory bacterial sulfate reduction (BSR)

The overall sulfur and oxygen isotope fractionation during BSR should be the integration of the various forward and backward fluxes at each step with any corresponding isotope fractionation at each step, be it kinetic or equilibrium (Fig. 1 and Rees, 1973). In this section we will outline the previous modeling efforts and the related equations, upon which our model (Section 2) is based. We begin with sulfur isotopes, which have been more extensively studied than oxygen isotopes. The total sulfur isotope fractionation was first calculated by Rees (1973):

$$
\varepsilon^{34}S_{\text{total}} = \varepsilon^{34}S_{f} + X_1 \cdot (\varepsilon^{34}S_{f} - \varepsilon^{34}S_{b}) + \ldots + X_1 \cdot X_2 \cdot (\varepsilon^{34}S_{f} - \varepsilon^{34}S_{b}) + X_1 \cdot X_2 \cdot X_3 \cdot (\varepsilon^{34}S_{f} - \varepsilon^{34}S_{b})
$$

(1)

where $\varepsilon^{34}S_{\text{total}}$ is the total expressed sulfur isotope fractionation, $\varepsilon^{34}S_{f,j}$ is the sulfur isotope fractionation during the forward ($i=f$) and backward ($i=b$) reaction $j$ (where $j=1,\ldots,4$) and $X_k$ (where $k=1, 2, 3$) is the ratio between the backward and forward fluxes of the respective intracellular steps (Fig. 1). The overall expressed sulfur isotope fractionation in the residual sulfate pool, according to this model, is always dependent on the isotope fractionation in the first step (the entrance of sulfate into the cell). The fractionation during the subsequent steps can be expressed in the residual sulfate pool only if there is a backward reaction at each step and a flux of sulfate back out of the cell. The overall expressed sulfur isotope fractionation has been linked to various environmental factors that must result in changes in the relative forward and backward fluxes at each step (Rees, 1973; Farquhar et al., 2003, 2007; Brunner and Bernasconi, 2005; Canfield et al., 2006; Johnston et al., 2007).

The sulfur isotope fractionation for the forward reaction at steps 1, 3 and 4 (Fig. 1), that is, sulfate incorporation into the cell, the reduction of APS to sulfide, and the reduction of sulfite to sulfide, are understood to be $-3\%_\text{oo}$, 25%_oo and 25%_oo respectively (all other steps are assumed to have no sulfur isotope fractionation, Rees, 1973). Therefore, Eq. (1) can be written as:

$$
\varepsilon^{34}S_{\text{total}} = -3\%_\text{oo} + X_1 \cdot X_2 \cdot 25\%_\text{oo} + X_1 \cdot X_2 \cdot X_3 \cdot 25\%_\text{oo}
$$

(2)
In order to generate an expressed sulfur isotope fractionation larger than ~3%\textsubscript{iso}, there must be back reactions during at least the first three steps. It has also been observed that the total expressed sulfur isotope fractionation during BSR decreases with increased sulfate reduction rates (e.g. Aharon and Fu, 2000; Canfield et al., 2006; Stam et al., 2011; Sim et al., 2011b). This suggests, as previous research has concluded, that as the sulfate reduction rate increases, backward reactions become less significant relative to forward reactions, and the total sulfur isotope fractionation approaches the fractionation associated with transfer of sulfate through the cell wall (Canfield, 2001a,b).

Eq. (2) predicts a maximum possible expressed sulfur isotope fractionation during BSR of 47%\textsubscript{iso}. However, particularly in natural environments, the measured sulfur isotope fractionation can often exceed these values, reaching up to 72%\textsubscript{iso} (Habicht and Canfield, 1996; Wortmann et al., 2001). Such large offsets are often attributed to repeated redox cycles of sulfur in the subsurface: the initial reduction of sulfate through BSR, the subsequent reoxidation of sulfide to elemental sulfur, followed by sulfur disproportionation to sulfate and sulfide, which produces more sulfate for BSR (Canfield and Thamdrup, 1994). These repeated cycles allow for a larger overall expressed sulfur isotope fractionation. Another explanation for the large sulfur isotope fractionations observed in nature is the trithionite pathway, in which the reduction of sulfite to sulfide (step 4) proceeds through multiple steps rather than one (Kobayashi et al., 1969; Brunner and Bernasconi, 2005; Johnston et al., 2007; Bradley et al., 2011; Sim et al., 2011a). This could induce additional sulfur isotope fractionation and result in expressed sulfur isotope fractionation as large as 72%\textsubscript{iso} (Brunner and Bernasconi, 2005; Sim et al., 2011a).

Defining a relationship like Eq. (1) for oxygen isotopes is somewhat more difficult because both kinetic oxygen isotope fractionation and equilibrium oxygen isotope fractionation need to be considered. If we first consider the case where kinetic oxygen isotope fractionation is the only process affecting δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}} during BSR, then the overall oxygen isotope fractionation can be formulated similar to Eq. (1) (Brunner et al., 2005):

\[ \delta^{18}O_{\text{total}} = \epsilon^{18}O_{\text{H}_{2}O} + \frac{1}{X} \epsilon^{18}O_{\text{f,2}} \]  

(4)

In this case, the δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}} also exhibits equilibrium oxygen isotope fractionation during BSR, often linked to the isotopic composition of the ambient water (Mizutani and Rafter, 1973; Fritz et al., 1989; Brunner et al., 2005, 2012; Mangalo et al., 2007, 2008; Farquhar et al., 2008; Turchyn et al., 2010; Zeebe, 2010). Field studies have found that this ‘equilibrium isotope exchange’ results in the δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}} in the residual sulfate pool evolving to a value between 22%\textsubscript{iso} and 30%\textsubscript{iso} across a range of natural environments (Böttcher et al., 1998, 1999; Turchyn et al., 2006; Wortmann et al., 2007; Aller et al., 2010). The fact that the δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}} reaches a constant value is interpreted as oxygen isotope exchange between intracellular sulfur intermediates and water. The measured oxygen isotope equilibrium value therefore includes the kinetic oxygen isotope fractionation associated with each step, the equilibrium partitioning of oxygen isotopes between intracellular water and the intermediate sulfur species, and any oxygen isotope fractionation associated with the assimilation of oxygen atoms from water during reoxidation. Because of the myriad of factors impacting the observed equilibrium value of δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}}, the measured value in the residual sulfate δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}} is termed the ‘apparent equilibrium’ (Wortmann et al., 2007). Turchyn et al. (2010) formulated a mathematical term for the apparent equilibrium of δ\textsuperscript{18}O\textsubscript{SO\textsubscript{4}}, assuming full isotope equilibrium between intra-cellular intermediates and water, and kinetic oxygen isotope fractionation only during the reduction of APS to sulfite (step 3):

\[ \delta^{18}O_{\text{SO\textsubscript{4}}(A, E)} = \delta^{18}O_{\text{H}_{2}O} + \epsilon^{18}O_{\text{exchange}} + \frac{1}{X} \epsilon^{18}O_{\text{f,2}} \]  

(3)
where $\delta^{18}$O$_{SO_4}$ is the isotopic composition of sulfate at ‘apparent equilibrium’, $\delta^{18}$O$_{H_2O}$ is the isotopic composition of the ambient water, $\varepsilon^{18}$O$_{\text{exchange}}$ is the oxygen isotope fractionation between sulfate in the AMP-sulfite complex and ambient water, $X_2$ is the ratio between the backward and forward fluxes at Step 3 as in Eq. (1) (Fig. 1) and $\varepsilon^{18}$O$_{3,3}$ is the kinetic oxygen isotope fractionation associated with APS reduction to sulfite.

In summary, current models for BSR suggest that sulfur and oxygen isotopes in the residual sulfate pool respond to changes in the relative forward and backward rates of reaction, and isotope fractionation associated with each step during BSR. The relative contribution of these various forward and backward fluxes and their individual isotope fractionation should be expressed by different relationships between $\delta^{18}$O$_{SO_4}$ and $\delta^{34}$S$_{SO_4}$ in sulfate as BSR progresses. When the kinetic oxygen isotope fractionation outcompetes the equilibrium oxygen isotope fractionation, the plot of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ should exhibit a linear relationship (‘Trend A’ in Fig. 2 – e.g. Mizutani and Rafter, 1969; Aharon and Fu, 2000, 2003; Mandernack et al., 2003). When the equilibrium isotope effect dominates, a plot of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ will tend concavely towards the ‘apparent equilibrium’ (‘Trend B’ in Fig. 2 – e.g. Böttcher et al., 1998, 1999; Turchyn et al., 2006; Aller et al., 2010). In between these two extremes, the relative intensity of the kinetic and equilibrium isotopic effects will determine the moderation of the curve and how quickly it reaches equilibrium, if at all.

It has been suggested that this relative evolution of the $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ during BSR should be connected to the overall sulfate reduction rate (Böttcher et al., 1998, 1999; Aharon and Fu, 2000; Brunner et al., 2005) where the steeper the slope on a plot of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$, the slower the sulfate reduction rate. This suggestion was elaborated upon by Brunner et al. (2005), who formulated a model for mass flow during BSR. In this work, Brunner et al. (2005) deduced that the overall SRR is important for the relative evolution of $\delta^{18}$O$_{SO_4}$ and $\delta^{34}$S$_{SO_4}$, but that the rate of oxygen isotope exchange between sulfur intermediates and water, and the relative forward and backward fluxes at each step further modifies the evolution of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$.

The above models as developed previously have applied largely to understanding the relative forward and backwards steps during BSR in pure culture. We hypothesize that we can investigate a wider range of sulfate reduction rates in the natural environment, and thus are poised to be able to address this relationship more completely. This is a particularly good juncture to investigate this further as the models for BSR and the relationship between the mechanism and the couple sulfate isotopes have experienced several significant advances in recent years (e.g. Brunner et al., 2005, 2012; Wortmann et al., 2007). Although there are potentially other processes in natural environments that may impact the measured $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ – for example anaerobic pyrite oxidation (e.g. Balci et al., 2007; Brunner et al., 2008; Heidel and Tichomirova, 2011; Kohl and Bao, 2011), or sulfur disproportionation (Cypionka et al., 1998; Böttcher et al., 2001, 2005; Böttcher and Thamdrup, 2001; Aharon and Fu, 2003; Blake et al., 2006; Aller et al., 2010), we feel there is significant knowledge to be gained by revisiting the mechanism of BSR as deduced from geochemical analysis of pore fluids.

The use of the evolution of the $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ to inform the biochemical steps during BSR has been applied to two previous studies. Wortmann et al. (2007) produced a detailed study of an ODP site off the coast of southern Australia and Turchyn et al. (2006) studied 11 ODP sites off the coasts of Peru, Western Africa and New Zealand. Both studies found a rapid increase in the $\delta^{34}$S$_{SO_4}$, while the $\delta^{18}$O$_{SO_4}$ increased and then leveled off (similar to ‘Trend B’ in Fig. 2). Both Wortmann et al. (2007) and Turchyn et al. (2006) used their data with reactive transport models to calculate the relative forward and backward fluxes through bacterial cells during BSR. These studies, which greatly advanced our understanding of in situ BSR, focused on deep-sea sediments, with necessarily slow sulfate reduction rates. Furthermore, both of these studies considered only one branching point within the microbial cell, whereas more recent models of the mechanism of BSR have invoked the importance of at least two branching points to help explain the decoupled sulfur and oxygen isotopes during BSR (Brunner et al., 2005, 2012).

In this paper, we will present sulfur and oxygen isotopes of pore fluid sulfate from seven new sites with sulfate reduction rates that span many orders of magnitude. We will combine our new data with previously published results of subsurface environments where sulfur and oxygen isotopes in sulfate have been reported. We will use a model derived from the equations above, to understand how the relative evolution of sulfur versus oxygen isotopes in pore fluid sulfate inform us about the intracellular pathways and rates involved in BSR.

2. MODEL FOR OXYGEN ISOTOPE DURING BSR

2.1. The proposed model for oxygen isotopes in sulfate

Our model for oxygen isotopes in sulfate is derived from the work of Brunner et al. (2005, 2012). In order to understand the relative evolution of sulfur and oxygen isotopes in sulfate during BSR in pure culture, Brunner et al. (2005, 2012) solved a time dependent equation in which the oxygen isotope exchange between sulfur intermediates and ambient water and the cell specific sulfate reduction rates are the ultimate factors controlling the slope of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ during the onset of BSR. For the purpose of this study (as applied to natural environments rather than pure cultures) we reconsider this model in three ways. First, the cell specific sulfate reduction rate varies over orders of magnitudes in different natural environments, yet the relative evolution of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ plot versus depth may exhibit the same pattern. Therefore, we suggest that any time dependent process related to the isotope evolution (e.g. the rate of the oxygen isotopic exchange between ambient water and sulfur intermediate such as sulfite) is faster than the other biochemical steps during BSR. Second, in the
models of Brunner et al. (2005, 2012) the equilibrium value for the δ\(^{18}\)O, depended critically on the value of δ\(^{18}\)O of the ambient water. However, the equilibrium value for δ\(^{34}\)S, in natural environments shows a range (22–30\(^{0}\)) that cannot be explained only by the variation in δ\(^{18}\)O of the ambient water (which ranges from 0\(^{0}\) to \(-4\^{0}\)). It was initially suggested that these equilibrium values may reflect oxygen isotope equilibrium at different temperatures (Fritz et al., 1989) although more recent studies have shown that the temperature effect is small (∼2\(^{0}\), between 23 and 4\(^{0}\)C – Brunner et al., 2006; Zeebe, 2010). Temperature may impact the relative intracellular fluxes during BSR (Canfield et al., 2006), and this will change the apparent equilibrium value (Turchyn et al., 2010). For our model, therefore, we attribute the change in the δ\(^{18}\)O to change in the mechanism of the BSR and not to changes in the δ\(^{18}\)O of the water. Third, the model of Brunner et al. (2005, 2012) ruled out a linear relationship between δ\(^{18}\)O and δ\(^{34}\)S, which has not been observed in pure culture. Our model will need to account for a linear relationship, which has been observed in natural environments.

To address these issues, we remove the characteristic timescale used by Brunner et al. (2005, 2012) for the cell-specific sulfate reduction rate and focus instead on how the different fluxes at each step impact the evolution of δ\(^{18}\)O, δ\(^{34}\)S. We further allow changes in the equilibrium values of the δ\(^{18}\)O, due to a combination of equilibrium and kinetic oxygen isotope effects (apparent equilibrium) rather than through a change in the δ\(^{18}\)O of the ambient water.

The assumptions in our model include:

- The system is in steady state. This means SRR = \(f_i - b_i\) (where \(i = 1, 2, 3\) — Fig. 1).
- We model oxygen isotopic exchange between ambient water and the sulfite (Betts and Voss, 1970; Horner and Connick, 2003), recognizing that this exchange may occur when sulfite is already bound in the AMP-sulfite complex. This oxygen isotope exchange contributes three oxygen atoms to the sulfite that will ultimately be produced during reoxidation, while the fourth oxygen atom is gained during the reoxidation of the AMP-sulfite complex to sulfate (Wortmann et al., 2007; Brunner et al., 2012).
- Oxygen isotopic exchange was considered to be much faster with respect to other biochemical steps, which means, that for any practical purpose, the sulfite is constantly in isotopic equilibrium with the ambient water. This results in a solution that is independent of the timescale of the problem. This is because the timescale for this isotope exchange, given intracellular pH (6.5–7—Booth, 1985), should be shorter than minutes (Betts and Voss, 1970).
- The kinetic oxygen isotope fractionation during the reduction of APS to sulfite (\(f_3\)) is equal to 25\% of the sulfur isotope fractionation (\(\varepsilon^{18}O_{2} : \varepsilon^{34}S_{2} = 1:4\)) (Mizutani and Rafter, 1969). This value for the kinetic oxygen isotope fractionation is the lowest value that was found in lab experiments, and therefore we consider it to be the closest to the real ratio between \(\varepsilon^{18}O_{2} \) and \(\varepsilon^{34}S_{2}\). This is assumption has not been made by Brunner et al. (2005, 2012) and allows our model to simulate a linear relationship between δ\(^{18}\)O and δ\(^{34}\)S.
- Any kinetic oxygen isotope fractionation in step 4 (the reduction of sulfite to sulfide) is not significant for oxygen isotopes, since oxygen isotope exchange during the back reaction (step 3) resets the δ\(^{18}\)O of the sulfite.
- We simplified step 4 by making it unidirectional. We are able to do this because recent work has suggested that even if sulfide concentrations are high (>20 mM), only ∼10\% of the sulfide is re-oxidized (Eckert et al., 2011) which is insignificant with respect to the overall recycling of other sulfur intermediates (Turchyn et al., 2006; Wortmann et al., 2007).

The full derivation of the model equations using these assumptions, and similar to the derivation in Brunner et al., 2012, is in Appendix A and yields the following continuous solution for δ\(^{18}\)O\(_{SO_4}\) as function of δ\(^{34}\)S\(_{SO_4}\):

\[
\delta^{18}O_{SO_4(t_0)} = \left\{ \begin{array}{ll}
\frac{\delta^{18}O_{SO_4; \Delta}}{\delta^{34}S_{SO_4; \Delta}} (\delta^{34}S_{SO_4; 0} - \delta^{34}S_{SO_4(t_0)}); & + \delta^{18}O_{SO_4; 0; 0} \\
X_1 \cdot X_2 \cdot X_3 = 0 \\
\delta^{18}O_{SO_4(A,E)} - \exp(-\theta_0 \frac{\delta^{18}O_{SO_4; 0} - \delta^{34}S_{SO_4; 0}}{\epsilon^{18}O_{SO_4}}); & 0 < X_1 \cdot X_2 \cdot X_3 < 1
\end{array} \right.
\] (5)

where δ\(^{18}\)O\(_{SO_4(t_0)}\) is the oxygen isotopic composition of the residual sulfite at time \(t\), δ\(^{18}\)O\(_{SO_4(A,E)}\) is the oxygen isotopic composition of the initial sulfite. The δ\(^{34}\)S\(_{SO_4(0)}\) is the sulfur isotope composition of the residual sulfite at apparent equilibrium (see Section 1.2 above) and δ\(^{18}\)O\(_{SO_4(0)}\) is the oxygen isotope composition of the initial sulfite. The δ\(^{34}\)S\(_{SO_4(t_0)}\) is the initial sulfur isotope composition of the residual sulfite, \(\epsilon^{18}O_{SO_4\text{total}}\) \(\epsilon^{34}S_{SO_4\text{total}}\) are the overall expressed sulfur and oxygen isotope fractionation, respectively, and \(\theta_0\) is a parameter initially formulated by Brunner et al. (2005, 2012). This parameter (\(\theta_0\)) measures the ratio between the apparent oxygen isotope exchange and sulfate reduction rate. However, since we assumed constantly full oxygen isotopic equilibrium between sulfite and ambient water, in our case this parameter should only be a function of the ratio between the backward and forward fluxes, and is less impacted by changes in the initial isotopic composition of the sulfite, the isotopic composition of the water, the kinetic isotope fractionation factor for step 3, or the magnitude of the fractionation factor during oxygen isotope exchange (see Appendix A).

The solution to our model (Eq. (5)) suggests two distinct phases for the relative evolution of δ\(^{18}\)O\(_{SO_4}\) vs. δ\(^{34}\)S\(_{SO_4}\) during BSR:

1. **Apparent linear phase.** This phase refers to the initial stage of BSR, where the sulfur and oxygen isotopic compositions increase in the residual sulfite pool at a constant ratio (see also Trend b in Fig. 2b). The first-order Taylor series expansion around the point (δ\(^{34}\)S\(_{SO_4}\), δ\(^{18}\)O\(_{SO_4}\)) = (δ\(^{34}\)S\(_{SO_4; 0}\), δ\(^{18}\)O\(_{SO_4; 0}\)) of
Eq. (5) provides information about the behavior of $\delta^{18}O_{\text{SO}_4}$ vs. $\delta^{34}S_{\text{SO}_4}$ at the onset of the BSR and is equal to:

$$
\delta^{18}O_{\text{SO}_4(0)} = \delta^{18}O_{\text{SO}_4(0)} + (\delta^{18}O_{\text{SO}_4(A:E)} - \delta^{18}O_{\text{SO}_4(0)}) \cdot \theta_0
$$

$$
\frac{\delta^{34}S_{\text{SO}_4(0)} - \delta^{34}S_{\text{SO}_4(0)}}{\varepsilon^{34}S_{\text{total}}} \cdot \theta_0
$$

(6)

We term this the slope of the apparent linear phase (SALP) in $\delta^{18}O_{\text{SO}_4}$ vs. $\delta^{34}S_{\text{SO}_4}$ space:

$$
\text{SALP} = \theta_0 \cdot \frac{\delta^{34}S_{\text{SO}_4(A:E)} - \delta^{18}O_{\text{SO}_4(0)}}{\varepsilon^{34}S_{\text{total}}} \cdot \theta_0
$$

(7)

This equation suggests that the SALP is directly proportional to $\theta_0$. SALP is also inversely proportional to $\varepsilon^{34}S_{\text{total}}$.

1. **Apparent equilibrium phase.** This phase refers to the later phase of BSR where the oxygen isotope composition of the residual sulfate pool reaches a constant value, while the sulfur isotope composition continues to increase (Wortmann et al., 2007 and Turchyn et al., 2010, see also ‘Trend b’ in Fig. 2b). Here we modified the term for the apparent equilibrium of $\delta^{18}O_{\text{SO}_4}$ that was given by Turchyn et al. (2010), and also presented in Eq. (4). This is because the term that was formulated by Turchyn et al. (2010) assumed that the uptake of sulfate into the cell (step 1) involves no kinetic isotope effect for oxygen, although a kinetic isotope effect for sulfur does exist. If there is a kinetic oxygen isotope fractionation during sulfate uptake, (step 1) and during the reduction of APS to sulfite (step 3), then the apparent equilibrium value of $\delta^{18}O_{\text{SO}_4}$ ($\delta^{18}O_{\text{SO}_4(A:E)}$) is given by (see Appendix B for the full derivation):

$$
\frac{\delta^{18}O_{\text{SO}_4(A:E)}}{\varepsilon^{34}S_{\text{total}}} = \frac{\delta^{18}O_{\text{H}_2\text{O}} + \delta^{18}O_{\text{exchange}} + \frac{\varepsilon^{18}O_{S}X_1}{X_1}}{X_1}
$$

(8)

Previous studies have used plots of $\theta_0$ vs. $\varepsilon^{34}S_{\text{total}}$ to investigate the mechanism of BSR (Turchyn et al., 2010; Brunner et al., 2012). There is an ambiguity with calculating $X_1$ and $X_2$ separately using isotopes since there is understood to be no isotopic fractionation at step 2 (e.g. Rees (1973)). Therefore, if we consider the two main intracellular branching points in the schematic in Fig. 1 (similar to Farquhar et al., 2003; Canfield et al., 2006), we can rethink the reaction schematic in Fig. 1 without the APS intermediate as shown in Fig. 3 (another way to work around this ambiguity is by merging steps 1 and 2 into one single step. This choice would also have no impact on the calculation). In this case, $\theta_0$ is equal to (after Brunner et al., 2012):

$$
\theta_0 = \frac{X_1 \cdot X_3}{1 - X_1 \cdot X_3}
$$

(9)

and the $\varepsilon^{34}S_{\text{total}}$ according to Rees (1973) is:

$$
\varepsilon^{34}S_{\text{total}} = -3 + 25 \cdot X_1 + 25 \cdot X_1 \cdot X_3
$$

(10)

We acknowledge the fact that recent studies have found sulfur fractionation much higher than $47\%_{\text{o}}$ (e.g. Habicht and Canfield, 1996; Wortmann et al., 2001; Sim et al., 2011a), which is the maximum fractionation that Eq. (10) predicts. This however, can be solved by adding another branching point and not simply adding the additional fractionation (about $50\%_{\text{o}}$) to step 3 (Brunner et al., 2012). Since it is not clear what are the exact environmental constraints activate the trithionate pathway, at this point, we stick to the traditional pathway and will examine if it can simulate pore fluid $\delta^{18}O_{\text{SO}_4}$ and $\delta^{34}S_{\text{SO}_4}$.

These equations provide unique solutions for $X_1$ (the ratio between sulfate being brought in and out of the cell) and $X_3$ (the ratio between the forward and backward fluxes at step 3). Because $\theta_0$ and $\varepsilon^{34}S_{\text{total}}$ can be written in terms of $X_1$ and $X_3$, we can calculate $\varepsilon^{34}S_{\text{total}}$ and $\theta_0$ for a range of $X_1$ and $X_3$ values and contour them on a $\theta_0$ vs. $\varepsilon^{34}S_{\text{total}}$ diagram (Fig. 4). This allows us to depict variations in $\theta_0$ vs. $\varepsilon^{34}S_{\text{total}}$ in terms of variations in $X_1$ and $X_3$ during BSR. $X_1$ provides nearly vertical contours in $\theta_0$ vs. $\varepsilon^{34}S_{\text{total}}$ space, suggesting that variations in the flux at step 1 are the main cause for changes in the expressed sulfur isotope fractionation ($\varepsilon^{34}S_{\text{total}}$), especially at lower values of $X_1$. On the other hand, $X_3$ contours horizontally, suggesting that changes in this step cause the most significant impact on $\theta_0$. The plot of $\theta_0$ vs. $\varepsilon^{34}S_{\text{total}}$ (Fig. 4) has similarities with the theoretical $\lambda_{\text{H}_2\text{SO}_4}$ vs. 1000 ln($r^{34}S_{\text{SO}_4}/r^{34}S_{\text{SO}_4}$) diagram designed by Farquhar et al. (2003). Both diagrams are based on multiple reaction pathways for sulfate within the bacterial cell. The rate and direction of these reactions control the sulfur and oxygen isotope evolution of sulfate. We can use the $\theta_0$ vs. $\varepsilon^{34}S_{\text{total}}$ to interpret the mechanism of BSR for our data and previously published work. An extension would be to investigate the mechanism using a $\lambda_{\text{H}_2\text{SO}_4}$ vs. 1000 ln($r^{34}S_{\text{SO}_4}/r^{34}S_{\text{SO}_4}$) diagram as more $r^{33}S_{\text{SO}_4}$ data becomes available.

2.2. Testing the proposed model

Our changes to the existing models of bacterial sulfate reduction now allow it to be applied to a wider range of timescales and parameter space observed in natural environments. We will apply it now to a pure culture study to show its applicability. Mangalo et al. (2008) carried out five pure culture experiments, with Desulfovibrio desulfuricans and $^{18}O$ enriched water (about $700\%_{\text{o}}$) and varied the nitrite concentration. Nitrite is an inhibitor for the enzyme dissimilatory sulfite reductase used in Step 4 (Greene et al., 2003). Increased nitrite concentrations should, therefore, lead to less reduction of sulfite to sulfide and potentially more recycling of sulfite back to sulfate (Fig. 1). In other words, the higher the nitrite concentration, the higher the backward flux at step 3 (the reoxidation of sulfite to APS), and $\theta_0$ should increase.

The $\delta^{18}O_{\text{H}_2\text{O}}$ in these experiments was strongly enriched in $^{18}O$ ($700\%_{\text{o}}$, Mangalo et al., 2008). This allows us to investigate the contribution of each step during BSR to the evolution of $\delta^{18}O_{\text{SO}_4}$ vs. $\delta^{34}S_{\text{SO}_4}$, since it significantly reduces
the uncertainty on the expected $\delta^{34} \text{SO}_4$ value. We calculated the $\theta_0$ for each experiment in Mangalo et al. (2008) using Eq. (7). The SALP was obtained from a linear regression of $\delta^{34} \text{SO}_4$ vs. $\delta^{34} \text{SSO}_4$ presented in Mangalo et al. (2008) and the sulfur isotope fractionation ($\epsilon^{34} \text{Stotal}$) was taken from their calculation. The Mangalo et al. (2008) data is presented on the $\theta_0$ vs. $\epsilon^{34} \text{Stotal}$ diagram (Fig. 4).

By changing the nitrite concentration, Mangalo et al. (2008) were indeed able to affect the value of $X_3$, the ratio of the forward and backward fluxes at step 3. Our analysis shows that the SALP of each experiment shows a strong correlation to the nitrite concentration (Fig. 5a) and with $X_3$ (Fig. 5b) ($R^2 = 0.9987$). However, it seems that there is a poor correlation between $X_1$ and the SALP (Fig. 5b) ($R^2 = 0.3002$). This suggests that $X_3$ is directly responding to nitrite concentration, confirming that nitrite was inhibiting sulfite reduction at step 4 ($f_4$ decreases) and resulting in more sulfite being reoxidized to APS ($b_3$ increases). In addition, these results suggest that $X_3$ is the dominant factor controlling the SALP in these experiments.

Analysis of the Mangalo et al. (2008) data shows that the model may help calculate $X_1$ and $X_3$ during BSR in pure culture. Application to the natural environment still requires consideration of how the expression of the mechanism of BSR will be seen within pore fluid profiles, which we will consider in Section 5. First we will present our analytical methods and results.

3. METHODS

3.1. Study sites

We present pore fluid profiles from seven new sites (see Map, Fig. 6). The first two sites, Y1 and Y2 are in the Yarqon Stream estuary, Israel (Fig. 6b), with a water depth of ~2 m. Cores were taken using a gravity corer, total core lengths were 29 and 9 cm, for Y1 and Y2 respectively. The Yarqon estuary sediments have a very high organic carbon content of 2.5% and are in contact with brackish...
bottom waters ($\sim 19 \text{ g Cl}^{-1}$), due to seawater penetration into the estuary.

Cores were collected at three sites on the shallow shelf of the Eastern Mediterranean Sea off the Israeli coast; Sites HU, 130 and BA1 (Fig. 6b), with water depths of 66, 58 and 693 m, respectively. Total core lengths for the three sites were 234, 254 and 30 cm, respectively. The sediment from site BA1 was collected using a box corer, while a piston corer was used for sites 130 and HU. The organic carbon content at these sites ranges from $\sim 0.5-1.0\%$. Finally, pore fluid profiles are also presented from advanced piston cores collected by the Ocean Drilling Program (ODP) at ODP Sites 1052 and 807. Site 1052 (Leg 171B), is located on Blake Nose (NW Atlantic Ocean) at a water depth of 1345 m, with a total sediment penetration of 684.8 m (60.2% recovery). Site 807 (Leg 130) (Fig. 6a), is located on the Ontong-Java Plateau (tropical NW Pacific) at a water depth of 2805 m with a total sediment penetration of 822.9 m (87.1% recovery). The organic carbon content at Site 1052 it is below 1%, while at Site 807 ranges between 0.02% and 0.6%.

---

Fig. 5. The SALP vs. nitrite concentration (a) and $X_1$ (grey squares) and $X_3$ (black squares) vs. the SALP from pure culture *D. desulfuricans* (modified after Mangalo et al., 2008) (b). Error bars for the SALP are calculated by the difference between two parallel growth experiments, and the error bars for $X_1$ and $X_3$ indicate the maximum and minimum values calculated using Eqs. (9) and (10). The lines in panel (b) are the best-fit curves of the linear regression.

Fig. 6. Maps of the study area in a map of the world (a), and a map of the Eastern Mediterranean region (b). The dots and the corresponding labels indicate the site locations and names, respectively.
3.2. Analytical methods

The samples from the Yarqon estuary and the Eastern Mediterranean sites were processed at Ben Gurion University of the Negev, Israel, usually on the same day as coring. The cores were split into 1 cm slices under an argon purge. The pore fluids were extracted from each cm slice by centrifuging under an argon atmosphere to avoid oxygen contamination. The samples were acidified and purged with argon to remove sulfides and prevent their oxidation to sulfate. The sulfate concentration in the pore fluids from the Yarqon estuary was measured by high performance liquid chromatography (HPLC, Dionex DX500) with a precision of 3%. The total sulfur (assumed to be only sulfate) concentrations from the Eastern Mediterranean were measured by inductively coupled plasma-atomic emission (ICP-AES, P-E optima 3300) with a precision of 2%.

The ODP sediments were handled using standard shipboard procedures. Sulfate concentrations of the pore fluids from the ODP Sites were measured by Dionex ion chromatography onboard the ship. Pore fluid sulfate from the Yarqon estuary, the Eastern Mediterranean and the ODP sites were then precipitated as barium sulfate (barite) by adding a saturated barium chloride solution. The barite was subsequently rinsed with acid and deionized water and set to dry in a 50 °C oven.

The sulfur and oxygen isotope composition of the pore fluid sulfate was analyzed in the Godwin Laboratory at the University of Cambridge. The barite precipitate was pyrolyzed at 1450 °C in a Temperature Conversion Element Analyzer (TC/EA), and the resulting carbon monoxide (CO) was measured by continuous flow GS-IRMS (Delta V Plus) for its δ18OSO4. For the δ34SSO4 analysis the barite was combusted at 1030 °C in a Flash Element Analyzer (EA), and resulting sulfur dioxide (SO2) was measured by continuous flow GS-IRMS (Thermo, Delta V Plus). Samples for δ34SSO4 were run in replicate and the standard deviation of these replicate analyses was used (0.4‰). The error for δ34SSO4 was determined using the standard deviation of the standard NBS 127 at the beginning and the end of each run (∼ 0.2‰). Samples for both δ18OSO4 and δ34SSO4 were corrected to NBS 127 (8.6‰ for δ18OSO4 and 20.3‰ for δ34SSO4). A second laboratory derived barite standard was run for δ18OSO4, (16‰) to correct for linear changes during continuous flow over a range of δ18OSO4 values and to map our measurements more accurately in isotope space. Since the bulk of our δ18OSO4 data falls between 8‰ and 21‰, these standards were appropriate for the isotope range of interest.

4. FIELD RESULTS

The pore fluid sulfate concentrations and oxygen and sulfur isotope compositions for the seven new sites are shown in Fig. 7. The cores from the Yarqon estuary (Y1, 29 cm and Y2, 9 cm, Fig. 7a–c) are similar and show almost total depletion in pore fluid sulfate (site Y1, Fig. 7c). As sulfate concentrations decrease, both the δ18OSO4 and δ34SSO4 of the sulfate increase. At the greater depths, δ34SSO4 continues to increase, while δ18OSO4 reaches a constant value of 23–24‰ (site Y1, Fig. 7c).

The results from sites BA1 (30 cm) HU (234 cm) and P130 (254 cm) are shown in Fig. 7e–f. There is a maximum of 40% consumption of sulfate, within the upper 234 cm at Site HU, and within 250 cm at Site P130. Both the δ18OSO4 and δ34SSO4 increase with depth at both sites: the δ34SSO4 increases to 30.3‰ and the δ18OSO4 increases to 19.0‰ at site HU, while at site P130 the δ34SSO4 increases to 38.8‰ and the δ18OSO4 increases to 24.0‰. At site BA1, δ18OSO4 and δ34SSO4 both increase while the pore fluid sulfate concentration decreases (Fig. 7d–f).

In ODP Sites 807 and 1052, pore fluid sulfate concentrations remain constant in the upper 30 m, and then decrease over the next ~200 m by 25% and 50%, respectively (Fig. 7g–i). At both sites, the δ34SSO4 increases with decreasing sulfate concentrations, to values of 28–29‰, at ~300 m. The δ18OSO4 also increases to 22–23‰ at both Sites.

5. DISCUSSION

5.1. Applying our time-dependent closed system model to pore fluid profiles

In this section we discuss the use of our model of BSR (Sections 2.1 and 2.2) to understand what controls the relative evolution of δ18OSO4 vs. δ34SSO4 in the natural environment. Applying what is effectively a “closed system” model to an “open system” (environmental pore fluids) requires understanding the physical parameters that control each of the sulfate species concentrations (in our case 34S, 16O4−, 32S, 18O4− and 32S, 18O4−) within the fluids in the sediment column (Jorgensen, 1979; Chernyavsky and Wortmann, 2007; Donahue et al., 2008; Wortmann and Chernyavsky, 2011).

In this study we utilize SALP, that is the relative change of δ18OSO4 vs. δ34SSO4, rather than the δ18OSO4 value during apparent equilibrium although both hold information about the mechanism of the BSR (see Eqs. (7) and (8)). Focusing on SALP enables investigating the mechanism of BSR from sites that were not cored deep enough to observe apparent equilibrium (e.g. Mediterranean Sea sediments from this study, Fig. 7d–f). Also, it is not clear whether the δ18OSO4 really reaches equilibrium values at some sites (e.g. ODP Sites, Fig. 7g–i).

The outstanding question is how can we apply SALP as observed in the relative evolution of the δ18OSO4 and δ34SSO4 in the pore fluids to the model for the biochemical steps during BSR as derived for pure cultures? How do you bridge the gap between the “closed system” equations and the application to the “open system”? To explore this, we will briefly explore how SALP changes between closed and open systems in two extreme cases: (a) Deep-sea temperature (2 °C), low sedimentation rate (10−3 cm year−1) and low net sulfate reduction rate (low as 10−12 mol cm−3 year−1), typical of deep-sea environments versus (b) Surface temperature (25 °C), high sedimentation rate (10−1 cm year−1) and high net sulfate reduction rate (5 × 10−4 mol cm−3 year−1) conditions similar to shallow marginal-marine
environments. In each case we have calculated the “closed system” solution for a given mechanism, or intracellular fluxes during BSR, and then separately calculated the “open system” for the same mechanism give the natural conditions described above. For the entire model description see Appendix C.

Fig. 8 presents the calculated open system versus closed system SALP for the two extreme environments, as function of the change in $X_3$ (where $X_1$ is fixed and equal to 0.99). It can be seen that in applying the close system solution to the open system can lead to underestimation of as much as 10% in the value of $X_3$ (For changes in $X_1$, the
misestimate will be similar in magnitude). Although there are vastly different physical parameters between these two synthetic sites, the resulting calculated SALPs are not significantly different. This similarity in calculated SALP is because the main difference moving to an open system from a closed system is the change the relative diffusion flux of any of the isotopologues. We conclude that we can read the SALP from $d^{18}$OSO$_4$ and $d^{34}$SSO$_4$ pore fluid profiles (e.g. Fig. 2) and apply our closed system model to understand the mechanism, with the caveat that we have error bars on our resulting interpretation.

5.2. What controls the relative evolution of $d^{18}$OSO$_4$ vs. $d^{34}$SSO$_4$ in marine sediments during BSR

It has been suggested that in the natural environment as well as in pore fluids, the relative evolution of $d^{18}$OSO$_4$ vs. $d^{34}$SSO$_4$ (SALP) is connected to the overall sulfate reduction rate (Böttcher et al., 1998, 1999; Aharon and Fu, 2000; Brunner et al., 2005). We further suspect that the relative evolution provides information about the mechanism, or individual intracellular steps, during BSR. A plot of our data in $d^{18}$OSO$_4$ vs. $d^{34}$SSO$_4$ space displays a close-to-linear relationship between $d^{18}$OSO$_4$ and $d^{34}$SSO$_4$ (Fig. 9). The slope, however, varies greatly among the different sites (Fig. 9). In general, the sites from the shallower estuary environments have a more moderate slope (0.35–0.44), meaning the sulfur isotopes increase rapidly relative to the oxygen isotopes, while the shallow marine sediments have steeper slopes (0.99–1.1), and the deep-sea sediments have the steepest slopes (1.7 and 1.4, respectively). The ODP Sites thus show the fastest increase in the $d^{18}$OSO$_4$ relative to the $d^{34}$SSO$_4$ compared with the shallower sites. The changes in the slope among the different sites correlates with the depth dependent sulfate concentration profiles, where the higher the rate of change in the sulfate concentration with depth below the sediment–water interface, the lower the slope, or the more quickly the sulfur isotopes evolve relative to the oxygen isotopes. Site P130 (Mediterranean) is the exception and does not show a linear relationship between $d^{18}$OSO$_4$ and $d^{34}$SSO$_4$, likely due to poor sampling resolution.

Previous studies have shown a similar initial linear relationship between $d^{18}$OSO$_4$ and $d^{34}$SSO$_4$, with the slope ranging between 1:1.4 (=0.71 compared to our cross plots, Aharon and Fu, 2000) to 1:4.4 (=0.22, Mandernack et al., 2003). Our data (Fig. 9) displays a wider variation in slope than previously reported, as anticipated in this study. Most authors have attributed the linear evolution of sulfur versus oxygen isotopes in sulfate during BSR to a fully kinetic isotope effect in a closed system under ‘Rayleigh distillation’, neglecting equilibrium oxygen isotope fractionation. The SALP, however, includes the equilibrium oxygen isotope effect during initial BSR prior to reaching apparent equilibrium.

We calculated the net sulfate reduction rate (nSRR) from each site from a curve fit of the sulfate concentration profiles in the pore fluids using the general diagenetic equation (Berner, 1980). As sulfate from the ocean diffuses into the sediments to be reduced to sulfide, the length, or depth, scale over which sulfate concentrations decrease relates to the overall rate of sulfate reduction. We assume the sulfate concentration is in steady state (this is based on the fact that the age of the sediments at all the sites in this study is much higher than the characteristic timescale of diffusion) and no advection. However, we acknowledge that these assumptions may be wrong in some of our sites. To augment our data we also present nSRR from pore fluids profiles in previously published studies, where sulfate concentrations and sulfur and oxygen isotopes in sulfate were published. This allows us to scale our results and model to an even wider range of environments than those we directly measured. Table EA.1 in the Electronic Annex summarizes data from the literature and the location for each site.

In this larger dataset, the inverse of the slope between $d^{18}$OSO$_4$ vs. $d^{34}$SSO$_4$ is positively correlated with the logarithm of the nSRR (Fig. 10). This observation confirms...
the hypothesis of Böttcher et al. (1998, 1999), who suggested that increases in overall nSRR, would result in decreases in the expressed sulfur and oxygen isotope fractionation, and thus the shape of $\delta^{18}O_{SO_4}$ vs. $\delta^{34}S_{SO_4}$ in sedimentary pore fluids.

5.3. The mechanism of BSR in marine sediments

Our compilation from pore fluids in a diverse range of natural environments suggests a correlation between the SALP and the nSRR (Fig. 10). This association may provide further understanding about the mechanism of BSR in the natural environment. Combining the first order approximation for the SALP (Eq. (7)) together with Eqs. (8)–(10) yields:

$$\text{SALP} = \frac{1}{1 - X_1 \cdot X_3} \left( \frac{\epsilon^{18}O_{SO_4} + \delta^{18}O_{H_2O} + \epsilon^{18}O_{\text{exchange}} - \delta^{18}O_{SO_4}}{\frac{\epsilon^{34}S_{SO_4}}{X_1} + \frac{\epsilon^{34}S_{H_2O}}{X_1} + \epsilon^{34}S_{\text{exchange}}} \right).$$

Eq. (11) shows that the SALP is a function of both $X_1$ and $X_3$ and does not depend on one more than the other. Hence, a change in the SALP does not necessarily tell us which one of the above ($X_1$ or $X_3$) plays more important role in the relative evolution of $\delta^{18}O_{SO_4}$ vs. $\delta^{34}S_{SO_4}$.

In order to address the question of the relative importance of $X_1$ vs. $X_3$ in the natural environment, we solved Eq. (5) for three different cases:

$$\text{SALP}^{-1} = \frac{1}{1 - X_1 \cdot X_3} \left( \frac{\epsilon^{18}O_{SO_4} + \delta^{18}O_{H_2O} + \epsilon^{18}O_{\text{exchange}} - \delta^{18}O_{SO_4}}{\frac{\epsilon^{34}S_{SO_4}}{X_1} + \frac{\epsilon^{34}S_{H_2O}}{X_1} + \epsilon^{34}S_{\text{exchange}}} \right).$$

Fig. 9. $\delta^{18}O_{SO_4}$ vs. $\delta^{34}S_{SO_4}$ data in pore fluid sulfate of all studied sites. The lines are the linear regressions for Sites Y1, HU and 807.

Fig. 10. The slope of $\delta^{34}S_{SO_4}$ vs. $\delta^{18}O_{SO_4}$ in the apparent linear phase of BSR vs. the average nSRR, as deduced from our data and worldwide pore fluid profiles. Data are presented from this study (open circles) and from other references (close circles). The labels of each point indicate the site’s name (the corresponding references for each site are given in Table EA.1 in the Electronic Annex).
(1) $X_1$ varies and $X_2$ is fixed (close to unity) – that is, the flow of sulfate in and out of the cell varies but the recycling of sulfite is fixed such that nearly all the sulfite is reoxidized back to the internal sulfate pool.

(2) $X_3$ varies and $X_4$ is fixed (close to unity) – that is the percentage of the recycling of the sulfite varied but the flow of sulfate in and out of the cell is fixed such that nearly all the sulfate that is brought into the cell exit the cell eventually.

(3) Both $X_1$ and $X_2$ vary simultaneously.

The initial condition for this calculation is set by the isotopic composition of surface seawater sulfate (roughly 10$^{0}_{\text{so}}$ and 20$^{0}_{\text{so}}$ for oxygen and sulfur isotopes, respectively). The kinetic sulfur isotope effect for each step is similar to the values previously described (Rees, 1973). The kinetic oxygen isotope fractionation is taken to be 1/4 of the fractionation of the sulfur isotope (Mizutani and Rafter, 1969). The total equilibrium oxygen isotope fractionation between sulfite and the AMP-sulfite complex and ambient water is taken as 17$^{0}_{\text{so}}$ which produces an apparent equilibrium of about 22$^{0}_{\text{so}}$ in the case where $X_1$ and $X_3$ equal 1 (Eq. (8)). As discussed in the introduction, it is enigmatic what impact temperature has on the $\delta^{18}O_{\text{SO}_4}(A_{eq})$. We therefore consider equilibrium oxygen isotope fractionation between sulfite and the AMP-sulfite complex and ambient water as constant among the different environments (Eq. (8)). The results from this calculation are shown in Fig. 11a–c, with the measured data included for comparison in Fig. 11d.

The model solution for $\delta^{34}S_{\text{SO}_4}$ and $\delta^{18}O_{\text{SO}_4}$, when varying $X_3$ only (Fig. 11b) fits the general behavior of pore fluid sulfur and oxygen isotopes (Fig. 11d) highlighting the importance of $X_3$ on the relative evolution of $\delta^{34}S_{\text{SO}_4}$ and $\delta^{18}O_{\text{SO}_4}$ in the natural environment. The best-fit curves for the pore fluids in this study are presented as the solid lines in Fig. 11d. This calculation suggests values for $X_1$ near unity (ranging between 0.96 and 0.99 – indicating up to 99% of the sulfate brought into the cell is ultimately recycled back out the cell). However, we suggest that this kind of forward modeling is not accurate enough to estimate the real values for $X_1$ and $X_4$ in natural environments due to the uncertainty with the values in our model as well as the application of a closed system model to pore fluids. Therefore, changes in $X_1$ may be more important to the relative evolution of $\delta^{18}O_{\text{SO}_4}$ vs. $\delta^{34}S_{\text{SO}_4}$ than our calculation suggests. In addition, our solution is valid only if BSR is the only process that affects sulfur and oxygen isotopes in sulfate – which may not be the case. Other subsurface processes can also affect this evolution, such as pyrite oxidation (e.g. Balci et al., 2007; Brunner et al., 2008; Heidel and Tichomirowa, 2011; Kohl and Bao, 2011) or sulfur disproportionation (Cypionka et al., 1998; Böttcher et al., 2001, 2005; Böttcher and Thamdrup, 2001).

Although most of the sites with $\delta^{18}O_{\text{SO}_4}$ and $\delta^{34}S_{\text{SO}_4}$ data seem to fit our model, our closed system model cannot replicate scenarios where the apparent equilibrium values are relatively high (26–30$^{0}_{\text{so}}$) together with a steep SALP (higher than ~1) in the uppermost sediments. As a result, by applying the closed system model, we cannot simulate data from Sites like ODP Site 1225 (Blake et al., 2006; Böttcher et al., 2006) and ODP Site 1130 (Wortmann et al., 2007). We suggest that this may be an artifact of the uncertainty in the values of the oxygen isotopic fractionation during various intracellular processes or erroneous model assumptions; these include the possible importance of temperature on oxygen exchange with ambient water (e.g. Fritz et al., 1989; Zeebe, 2010) or our assumption that this isotope exchange is complete, which it may not be (Brunner et al., 2012). The high sulfur isotope fractionation (>40$^{0}_{\text{so}}$) at these sites is consistent with the occurrence other complicating factors, such as activation of the trithionate pathway or subsurface sulfur disproportionation (Canfield and Thamdrup, 1994; Brunner and Bernasconi, 2005) that may skew the SALP, but which our model does not take into account.

5.4. The role of sulfite reoxidation in marine sediments

Our model suggests that $X_3$ varies between 0.4 and ~1 in the natural environments we studied (Fig. 11), and is inversely correlated with nSRR. This hints that the reduction of sulfite to sulfide (Step 4) is connected to nSRR in marine sediments and may be the “bottleneck reaction”, or significant branching point, for overall BSR. The faster the reduction of sulfite to sulfide, and therefore faster overall SRR, less sulfite is being reoxidized back to the outer sulfate pool. But what environmental or natural parameters control the functioning of this bottleneck?

We attribute secondary importance to pressure differences (also Vossmeier et al., 2012) among natural environments, since we found similar isotopic behavior among sites that varied in water depth (i.e. pressure). Similar to Kaplan and Rittenberg (1964) and Bradley et al. (2011), we speculate that one of the major environmental factors that could impact the different behavior of the communities of sulfate reducing bacteria might be related to the supply of the electron from the electron donor or carbon source. It has been shown that the nature and concentration of different electron donors is connected to the dynamics of each step during BSR (Brüchert, 2004; Sim et al., 2011b), and the overall nSRR (e.g. Westrich and Berner, 1984). Our data suggest that the higher the nSRR, the lower the sulfite reoxidation (over step 4, sulfite reduction). This recycling of sulfite likely plays a critical role during BSR in marine sediments. One possibility is that where the availability of the electron donor is low (less organic matter availability), such as in deep marine sediments, sulfate reducing bacteria might maintain high intracellular concentrations of sulfite, which is manifest geochemically as the rapid change in $\delta^{18}O_{\text{SO}_4}$ relative to the slower change in $\delta^{34}S_{\text{SO}_4}$. This could be contrasted with environments where there is high organic matter availability (for example marginal and shallow marine environments) where significant concentrations of intracellular sulfite would be unnecessary. Although highly speculative, we suggest there is a relationship between the concentration of intracellular sulfite and the availability of the electron donor in the natural environment. Our data suggests that this relationship may impact the relative fluxes within the bacterial sulfate reducing community.
Although this paper deals specifically with BSR in the marine environment, it is likely that our results are applicable to BSR in other systems including freshwater and groundwater systems. In these environments the hydrology is much more poorly constrained and the effects of advection and dispersion must be considered (Kno¨ller et al., 2007). While we have taken the first steps towards expanding the applicability of this isotope approach to resolving mechanism, the next logical steps would be to extend the approach to the terrestrial environment where BSR can play a critical role in water quality.

6. SUMMARY AND CONCLUSIONS

In this study we presented pore fluid measurements of $\delta^{34}$S$_{SO_4}$ and $\delta^{18}$O$_{SO_4}$, from seven new sites spanning a shallow estuary to a deep-sea sediment. These pore fluid profiles exhibited behavior similar to previously published pore fluid profiles; the $\delta^{34}$S$_{SO_4}$ increases monotonically during bacterial sulfate reduction, while the $\delta^{18}$O$_{SO_4}$ increased and at some point levels off, when it has reached apparent equilibrium. When we plot the $\delta^{34}$S$_{SO_4}$ vs $\delta^{18}$O$_{SO_4}$ in this large range of natural environments we explored the reason behind the change in slope of $\delta^{34}$S$_{SO_4}$ vs $\delta^{18}$O$_{SO_4}$. Combining our results with literature data, we demonstrated that the slope of this line correlated to the net sulfate reduction rate, as has been suggested in previous studies. At sites with high sulfate reduction rates, the $\delta^{18}$O$_{SO_4}$ increases more slowly relative to the $\delta^{34}$S$_{SO_4}$, while at sites with lower sulfate reduction rates, the $\delta^{18}$O$_{SO_4}$ increases more quickly relative to the $\delta^{34}$S$_{SO_4}$. We reformulated the widely used model for the relative evolution of sulfur and oxygen isotopes in sulfate during BSR. We used this new model with our data to explore how the intracellular fluxes impact the evolution of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ during bacterial sulfate reduction.

Our new data, together with our new model, suggested that the most significant factor controlling the evolution of $\delta^{18}$O$_{SO_4}$ vs. $\delta^{34}$S$_{SO_4}$ in the natural environment is the ratio between the fluxes of intracellular sulfite oxidation and APS reduction ($X_3$). The variation in the ratio and its correlation to the nSRR implies that sulfite reduction may be the bottleneck reaction during BSR. We suggested that this recycling allows sulfate reduction to proceed even when the organic matter availability is low.
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APPENDIX A. COMPUTING $\theta_{\text{e}}$ DIAGRAM

First, we consider the following reaction:

\[
\text{SO}_4^{2-} \rightarrow \text{SO}_4^{2-} + \text{H}_2\text{S}
\]

At steady flow the mass balance equation for the sulfate can be written as:

\[
\frac{d[\text{SO}_4^{2-}]}{dt} = b_1 - f_1 \tag{A.1}
\]

And therefore:

\[
dt = \frac{d[\text{SO}_4^{2-}]}{b_1 - f_1} \tag{A.2}
\]

If the oxygen isotopic exchange between the sulfite and the ambient water >> than $\varphi_1$, $\varphi_2$, $\varphi_3$, the isotopic mass balance equation for $\delta^{18}\text{SO}_4$ can be written as:

\[
dt \left[\text{SO}_4^{2-} \right] \cdot \delta^{18}\text{SO}_4 = b_1 \cdot \left(\delta^{18}\text{H}_{2}\text{O} + \varepsilon^{18}\text{O}_{\text{exchange}} \right) - f_1 \cdot \left(\delta^{18}\text{O}_{\text{SO}_4} - \varepsilon^{18}\text{O}_{\text{SO}_4}\right) \tag{A.3}
\]

According to the derivative's chain rule and Eq. (A.1):

\[
\frac{d[\text{SO}_4^{2-}]}{dt} = \text{SO}_4^{2-} \cdot \frac{d(\delta^{18}\text{SO}_4)}{dt} + \delta^{18}\text{SO}_4 \cdot (b_1 - f_1) \tag{A.4}
\]

And the combination between Eqs. (A.3) and (A.4) gives:

\[
\frac{\frac{d(\delta^{18}\text{SO}_4)}{dt}}{\left(\frac{\delta^{18}\text{SO}_4}{\left[\text{SO}_4^{2-}\right]}\right)} = b_1 \cdot (\delta^{18}\text{H}_{2}\text{O} + \varepsilon^{18}\text{O}_{\text{exchange}} - \delta^{18}\text{SO}_4) + f_1 \cdot (\varepsilon^{18}\text{O}_{\text{SO}_4}) \tag{A.5}
\]

Rearranging Eq. (A.5) results with:

\[
\frac{dt}{\left(\frac{\delta^{18}\text{SO}_4}{\left[\text{SO}_4^{2-}\right]}\right)} = b_1 \cdot (\delta^{18}\text{H}_{2}\text{O} + \varepsilon^{18}\text{O}_{\text{exchange}} - \delta^{18}\text{SO}_4) + f_1 \cdot (\varepsilon^{18}\text{O}_{\text{SO}_4}) \tag{A.6}
\]

The combination between Eqs. (A.6) and (A.2) yields:

\[
\frac{1}{b_1 - f_1} \cdot \frac{d[\text{SO}_4^{2-}]}{dt} = b_1 \cdot (\delta^{18}\text{H}_{2}\text{O} + \varepsilon^{18}\text{O}_{\text{exchange}} - \delta^{18}\text{SO}_4) + f_1 \cdot (\varepsilon^{18}\text{O}_{\text{SO}_4}) \tag{A.7}
\]

The solution of Eq. (A.7) with the initial conditions of $\delta^{18}\text{O}_{\text{SO}_4(0)}$ and $[\text{SO}_4^{2-}(0)]$ at $t = 0$.

\[
\ln \left(\frac{b_1}{b_1 - f_1} \cdot \frac{\delta^{18}\text{SO}_4}{[\text{SO}_4^{2-}]}(t)\right) = \frac{X_1}{1 - X_1} \cdot \ln \left(\frac{\delta^{18}\text{O}_{\text{SO}_4(0)}}{\delta^{18}\text{O}_{\text{SO}_4(0)}}\right) \tag{A.8}
\]

According to Turchyn et al. (2010):

\[
\delta^{18}\text{O}_{\text{SO}_4(0)} = \delta^{18}\text{O}_{\text{H}_{2}\text{O}} + \varepsilon^{18}\text{O}_{\text{exchange}} + \frac{1}{X_1} \cdot (\varepsilon^{18}\text{O}_{\text{SO}_4}) \tag{A.9}
\]

Embedding Eqs. (A.10) into (A.9):

\[
\ln \left(\frac{\delta^{18}\text{O}_{\text{SO}_4(0)}}{\delta^{18}\text{O}_{\text{SO}_4(0)}}\right) = -\frac{X_1}{1 - X_1} \cdot \ln \left(\frac{\delta^{18}\text{SO}_4}{[\text{SO}_4^{2-}]}(0)\right) \tag{A.11}
\]

and can be written in more general form:

\[
\ln \left(\frac{\delta^{18}\text{O}_{\text{SO}_4(0)}}{\delta^{18}\text{O}_{\text{SO}_4(0)}}\right) = -\theta_0 \cdot \ln \left(\frac{\delta^{18}\text{SO}_4}{[\text{SO}_4^{2-}]}(0)\right) \tag{A.12}
\]

where $\theta_0$ is only a function of the ratio between the backward and forward fluxes.

According to Rayleigh distillation:

\[
\frac{\delta^{18}\text{SO}_4 - \delta^{18}\text{SO}_4(0)}}{\varepsilon^{18}\text{O}_{\text{Total}}} = \ln \left(\frac{\delta^{18}\text{SO}_4}{[\text{SO}_4^{2-}] (0)}\right) \tag{A.13}
\]
\[ \delta^{18}O_{SO_4(t)} = \left\{ \begin{array}{l} \frac{\partial^{34}SSO_4}{\partial t_{\text{total}}} \cdot (\delta^{34}SSO_4 - \delta^{34}SSO_4(0)) + \delta^{18}O_{SO_4(0)}; \\ \delta^{18}O_{SO_4(A,E)} - \exp \left(-\theta_0 \cdot \frac{\delta^{34}SSO_4 - \delta^{34}SSO_4(0)}{e^{34}S_{\text{total}}} \right) \cdot (\delta^{18}O_{SO_4(A,E)} - \delta^{18}O_{SO_4(0)}); \\ \end{array} \right. \]

And ultimately:

\[
\ln \left( \frac{\delta^{18}O_{SO_{4,A,E}} - \delta^{18}O_{SO_4}}{\delta^{18}O_{SO_{4,A,E}} - \delta^{18}O_{SO_4(0)}} \right) = -\theta_0 \cdot \frac{\delta^{34}SSO_4 - \delta^{34}SSO_4(0)}{e^{34}S_{\text{total}}} \]  

(A.14)

This relationship should be conserved at higher reaction complexity (e.g. the reaction presented in Fig. 1—Brunner et al., 2005, 2012 and according to a numerical solution in those works that is not presented here).

Based on these assumptions, the solution for \( \delta^{18}O_{SO_4} \) with initial conditions at \( t = 0 \), \( \delta^{18}O_{SO_{4,0}} = \delta^{18}O_{SO_{4,0,0}} \) and \( \delta^{34}S_{SO_{4,0,0}} = \delta^{34}S_{SO_{4,0}} \) for \( \delta^{18}O_{SO_{4,0}} \) and \( \delta^{34}S_{SO_{4,0}} \) yields the continuous solution for \( \delta^{18}O_{SO_{4,0,0}} \) (After Brunner et al., 2012):

where \( \delta^{18}O_{SO_{4,0,0}} \) is the oxygen isotopic composition of the residual sulfate at time \( t \), \( \delta^{18}O_{SO_{4,A,E}} \) is the oxygen isotopic composition of the residual sulfate at apparent equilibrium (see Section 1.2 above) and \( \delta^{18}O_{SO_{4,0}} \) is the oxygen isotopic composition of the initial sulfate. \( \delta^{34}S_{SO_{4,0,0}} \) is the sulfur isotopic composition of the residual sulfate at time \( t \), \( \delta^{34}S_{SO_{4,0}} \) is the initial sulfur isotopic composition of the residual sulfate, \( e^{34}S_{\text{total}} \) are the overall expressed sulfur and oxygen isotope fractionation, respectively, and \( \theta_0 \) is a parameter initially formulated by Brunner et al. (2005). According to Brunner et al. (2005), this parameter (\( \theta_0 \)) measures the ratio between the apparent oxygen isotope exchange and sulfate reduction rate. However, since we assumed instantaneous isotopic equilibrium with the ambient water, in our case this parameter should only be a function of the ratio between the backward and forward fluxes, and is less impacted by changes in the initial isotopic composition of the sulfate, the isotopic composition of the water, the kinetic isotope fractionation factor for step 3, or the magnitude of the fractionation factor during oxygen isotopic exchange (Brunner et al. (2012)).

**APPENDIX B. MODIFICATION OF THE APPARENT EQUILIBRIUM**

Considering the reaction as in Fig. 3:

At steady state:

\[
\frac{d}{dt}(SO_{4(0)}^{2-}) = -f_1 + b_1
\]

\[
\frac{d}{dt}(SO_{4(0)}^{2-}) = 0
\]

\[-f_1 + b_1 = -f_3 + b_3\]

The mass balance equation for \( \delta^{18}O_{SO_{4,0,0}} \):

\[
\frac{d}{dt}(SO_{4(0)}^{2-} \cdot \delta^{18}O_{SO_{4,0,0}}) = -f_1 \cdot (\delta^{18}O_{SO_{4,0,0}} - \epsilon^{18}O_{O_2}) + b_2 \cdot (\delta^{18}O_{SO_{4,0,0}}) \]  

(B.1)

The mass balance equation for \( \delta^{18}O_{SO_{4,0}} \):

\[
\frac{d}{dt} \left( \frac{SO_{4(0)}^{2-} \cdot \delta^{18}O_{SO_{4,0}}}{X_1 \cdot X_2 \cdot X_3} \right) = 0 \]  

X_1 \cdot X_2 \cdot X_3 < 1

APPENDIX C. OPEN SYSTEM MODEL DESCRIPTION

In order to investigate to what extent the closed system model can be apply to an open system (such as natural environmental pore fluids), we generated sulfate, \( \delta^{18}O_{SO_4} \) and \( \delta^{34}S_{SO_4} \) synthetic profiles for two extreme cases in this study, deep sea sedimentary pore fluids and estuarine sedimentary pore fluids. The profiles were generated using the general diagenetic equation (Berner, 1980):

\[
\frac{\partial \rho f_c}{\partial t} = \rho \frac{\partial}{\partial z} \left( D_i \frac{\partial C_i}{\partial z} \right) - \rho (U + \omega) \frac{\partial C_i}{\partial z} - \Sigma R_i \]  

(C.1)

where \( C_i \) represents each of the different sulfate isotopologues (in our case \( ^{32}S^{16}O_2^+, ^{34}S^{16}O_2^+, ^{32}S^{18}O_2^+, ^{34}S^{18}O_2^+ \) as we considered all other species as much less abundant) concentrations in mass per unit volume of total sediment, \( t \) is time, \( Z \) is the depth in unit length, \( \rho \) is the porosity , \( D_i \) is the diffusion coefficient in unit of length square per time, \( U \) is the velocity of flow relative to the sediment-water interface in units of length per time, \( \omega \) is the rate of burial of the layer below the sediment-water interface in units of length per time and \( R_i \) is the reaction rate in units of mass.
per volume per time. For our purpose we assume no advection and uniform porosity throughout the sediment column. In addition the sulfate reduction rates were considered as constant in space and time (the SRR of each sulfate species was calculated using the overall SRR and the expected change in $\delta^{18}O_{SO_4}$ and $\delta^{34}S_{SO_4}$ according to our close system model—see Section 4). Therefore Eq. (C.1) can be written as:

$$\frac{\partial C_i}{\partial t} = \varphi \cdot D_i \frac{\partial^2 C_i}{\partial X^2} - \varphi \cdot \frac{\partial C_i}{\partial X} - \text{SRR}_{(C_i)}$$

We solved Eq. (C.2) using finite difference. The concentrations of the sulfate species isotopologues were initiated from seawater values throughout the entire sediment. The concentrations at the top and the bottom of the sediment were fixed to the initial concentration as boundary conditions. We let the profiles reach steady state (we define steady state when the maximum different between the concentrations two time intervals at given depth is smaller than 10 orders of magnitude than the concentration at this depth). The parameters that been used for each on of the cases in order to solved Eq. (C.2) are given in Table EA.1.

**APPENDIX D. SUPPLEMENTARY DATA**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2013.05.005.

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