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Sediment mineralogy influences the rate of microbial sulfate reduction in marine sediments

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A B S T R A C T

Sedimentary microbial communities play a critical role in the global carbon cycle, oxidizing deposited organic carbon and thus influencing the type of carbon buried from Earth’s surface. The rate of microbial metabolism within sedimentary microbial communities is often linked to the liability and amount of organic carbon deposited. Here we show that, in pure culture, for sulfate-reducing bacteria (\textit{Desulfovibrio bizzertensis}) the rate of microbial sulfate reduction is a function of the proportion of clay minerals present in the incubation vials. We argue that the presence of clay minerals stimulates the growth of the sulfate-reducing bacteria and the rate at which sulfate is consumed; we conclude that this is not linked to nutrients and carbon on the clay minerals but rather is a function of the high specific surface area of clay minerals. We further use a global compilation of sedimentary pore fluid data to demonstrate that these observations can be seen in marine sediments, that is pore fluid sulfate concentration gradients in marine sediments correlate with the percentage of clay minerals in the sediment. Our findings suggest that sediment mineralogy influences the rate of microbial activity in marine sediments, which has heretofore not been considered.

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1. Introduction

The majority of marine sediments are suboxic or anoxic, comprising detrital and authigenic minerals including carbonate, siliceous, and clay minerals. Marine sediments host the ‘deep biosphere’: bacteria and archaea that live at the low-energy limit of life (D’Hondt et al., 2009). Many microbial metabolisms are present in anoxic marine sediments: of these, microbial sulfate reduction is ubiquitous and is responsible for the majority of the oxidation of sedimentary organic carbon (Jørgensen et al., 2019). The geochemical manifestation of sedimentary microbial metabolism can be seen in the diminished subsurface pore fluid sulfate concentrations and elevated concentrations of dissolved inorganic carbon (DIC) and methane. Indeed, the concentration of DIC in marine sedimentary pore fluids is often an order of magnitude larger than that in the overlying ocean, while marine sedimentary methane reservoirs are the largest single reservoir of methane on the planet (Boetius and Wenzhöfer, 2013). The overall metabolic activity of these heterotrophic bacteria and archaea influences the global carbon cycle through the conversion of organic carbon to fluid-mobile DIC and methane, but also more broadly through the production of carbonate alkalinity, which impacts the partitioning of carbon dioxide between the atmosphere and ocean (Cavicchioli et al., 2019). Decades of research on sedimentary geochemistry has led to an understanding of the various environmental controls on microbial metabolism in marine sediments: nutrients, sediment pH, temperature, sedimentation rate and presence of suitable electron donors and acceptors (Burdige, 2011; Canfield, 1991; Pallud and Van Cappellen, 2006; Westrich and Berner, 1984). What has previously not been addressed is whether the type of marine sediment

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itself, in terms of sediment mineralogy, might influence microbial metabolic rates in marine sediments.

Marine sediments contain a wide range of detrital and authigenic minerals, but are often broadly categorized into two types: carbonate-rich and clay-rich; this is typically reported as the percentage of carbonate in the sediment, with the assumption that the remaining sediment comprises a range of detrital, authigenic clay minerals, organic material, and/or biogenic silica (Bennett et al., 1989). Organic carbon and biogenic silica in marine sediments are concentrated only in certain regions of the ocean where they can be preserved through the water column and delivered to the sediment-water interface. The distinct physical and chemical characteristics of clay and carbonate minerals create different broad sediment characteristics in sediment columns dominated by one or the other, characteristics including porosity, permeability, and redox geochemistry (Bennett et al., 1989; Lavoie and Bryant, 1993). Furthermore, clay minerals have been shown to stimulate bacterial growth through a larger surface-area-to-volume ratio and a net negative charge which helps neutralize cations from the surrounding solution, promoting surface adhesion and clumping (Cuadros, 2017; Mueller, 2015). Bacterial growth can, in turn, alter the physicochemical characteristics of sediment through mineral dissolution, precipitation, refinement and transformation, and reduction/uptake of trace elements. Specifically, bacteria can influence the surface charge of minerals, the cation exchange capacity (CEC), surface area, swelling, and the rheological properties of sediment via the secretion of exopolymeric substances (Mueller, 2015).

Although changes in sediment mineralogy have been shown to influence microbial activity in *Agrobacterium radiobacter*—a common soil bacterium—(Stotzky, 1966a; Stotzky and Rem, 1966) and fungi (Chae run and Tazaki, 2003; Filip et al., 1972; Stotzky, 1966b), studies specifically involving sulfate-reducing bacteria are limited; this is striking given the global importance of microbial sulfate reduction in modern marine sediments. Studies specifically linking microbial sulfate reduction and sediment mineralogy are limited to a single study from a landfll aquifer where cultures of sulfate-reducing bacteria had reduced growth with increased clay content of the sediment, which the authors concluded was due to aluminum toxicity from clay minerals (Wong et al., 2004). However this finding is not relevant in marine sediments where the pH is significantly higher and aluminum solubility is low; the question of whether, or how, sediment mineralogy influences the rate of sulfate reduction therefore remains unresolved. Here, we ran two experiments with pure-culture sulfate-reducing bacteria (*Desulfovibrio bietertensis*), one with varying proportions of kaolinite (Al₂Si₂O₅(OH)₄—a clay mineral) to calcite (CaCO₃), and a second experiment with a range of different minerals present (calcite, quartz (SiO₂), kaolinite, and montmorillonite (Na₂Ca₀.₃₃(Al₂Mg₂)(Si₄O₁₀)(OH)₂·nH₂O), with several of these minerals ultra-cleaned to remove potential nutrient contaminants. These experiments demonstrate the stimulation of growth in sulfate-reducing bacteria in the presence of clay minerals. We then compile sedimentary pore fluid data from the Ocean Drilling Program, and Integrated Ocean Discovery Program (ODP/IODP) database (shortened to ‘IODP’ hereafter) and show that our laboratory findings may be extrapolated to the natural environment, and a previously unrecognized influence on sedimentary microbial metabolism.

2. Materials and methods

2.1. Incubation experiments

Sulfate-reducing bacteria *Desulfovibrio bietertensis* were grown in 100 mL reaction vessels, tightly sealed with a blue butyl rubber stopper under strictly anoxic conditions. Atlantic seawater (OSIL) was used to make up the culture media and all experiments were performed at 25 ± 1°C in a water bath incubator (Supplementary Material Table S1). The initial pH of the media was slightly lower relative to typical seawater pH values (~6–6.5) in the experiments because the vials were flushed with 90% N₂:10% CO₂ mixed gas. Sodium formate (7.5 g L⁻¹ or 110 mM) was used as the sole electron donor in the incubation experiments.

Two sets of incubation experiments were conducted. In the first experiment (Experiment #1) varying proportions of kaolinite and calcite (0.3 g total) were placed in preprepared vials of media. The kaolinite is commonly used as a standard for X-ray diffraction (mined at Oneal Pit, Macon, Georgia, USA) and the calcite is from Sigma-Aldrich, ACS reagent, >99.0%, powder—Lot #MKBP1684V, CAS: 471-34-1. In this experiment, the proportion of the kaolinite and calcite in the samples were gravimetrically determined. The percentage of calcite that was used were: 100% (n = 2), 99%, 90%, 80%, 75%, 50%, 25%, 10%, 0%, as well as a seedless, mineral-free, control experiment. The experiment was run in duplicate and sampled periodically for pH, alkalinity, OΔSO₄, sulfate, calcium, phosphate, and sulfide concentrations. All control samples were immediately re-autoclaved after inoculation to kill the bacteria, then placed into the water bath and sampled alongside the growing incubations.

A second experiment (Experiment #2) was designed to explore whether surface area or nutrients associated with the minerals themselves influences bacterial growth using a range of different types of minerals present (calcite, quartz, kaolinite, and montmorillonite). A second type of kaolinite (hereafter called Sigma-kaolinite—Sigma-Aldrich reagent-grade—Lot #BCBP9408V, CAS: 1332-59-7) was used in addition to the original kaolinite. A total of 0.3 g of calcite, original kaolinite, cleaned original kaolinite, cleaned Sigma-kaolinite, cleaned quartz, and cleaned montmorillonite were used in six experimental vials. The original kaolinite, Sigma-kaolinite, and montmorillonite (mined at Husband Mine, Polkville, Mississippi, USA) were cleaned by the following procedure. The minerals were first soaked in 0.5 M NaCl in a Falcon tube and sonicated for 20 minutes for disaggregation. Next, the solution was removed and replaced with 5 M H₂O₂ and left overnight on an orbital shaker at 150 rpm. The samples were left to settle and the H₂O₂ solution was decanted and replaced by 0.5 M NaCl to promote cation exchange. The samples were subsequently placed on a shaker for 18 hours. Finally, the samples were repeatedly rinsed with MQ-water and centrifuged three times prior to being dried at 50°C. The goal of the cleaning was to remove organic matter and nutrients (including any labile trace metals) from the clay minerals. The incubation samples in this experiment were run in triplicate.

2.2. Aqueous sample analysis

Unless otherwise stated all sample analysis was done in the Laboratory for Marine Biogeochemistry at the University of Cambridge in the Department of Earth Sciences. Aqueous samples for all experiments were collected using a sterilized needle and syringe without shaking the vials to prevent the re-suspension of the minerals present in the vial. Samples were filtered (0.22 μm) and pH was measured at 25°C on the NBS scale using an Orion 3 Star meter with ROSS micro-electrode (ORION 8220 BNWP PerpHect ROSS—using platinum wire as a reference in iodine/potassium iodide solution, ROSS internal filling solution of 3 M KCI). Alkalinity was titrated potentiometrically with 0.1 M HCl using a Metrohm 848 Titrino plus with an error of 2.5%. The HCl used for titrations was standardized with certified reference material (CRM) 2.2298 mEq L⁻¹. The CRM batch #157 was used was provided by A.G. Dickson of Scripps Institution of Oceanography (Dickson et al., 2003).
An unfiltered sample was centrifuged at 900 rpm to separate any suspended solid in the solution before measurement for OD at 600 nm wavelength (as a proxy for cell concentration) in an Aquamate Plus UV-VIS spectrophotometer. Aqueous sulfide was fixed with 0.05 M zinc acetate and measured spectrophotometrically using the methylene blue method at a wavelength of 670 nm (Cline, 1969). Phosphate concentrations were measured spectrophotometrically using the molybdovanadophosphoric acid method (at 380 nm) described in Kitson and Mellon (1944). Major cations and anions were diluted 20 times before being measured using ion chromatography on a Dionex ICS-5000+ ICP. The cations and anions were analyzed with IonPac CS16 or AS18 column using methanesulfonic acid (MSA 30 mM) or potassium hydroxide (KOH 31 mM) as the eluent, respectively. Diluted OSIL Atlantic Seawater standard (2–10%) solutions were used as calibration standards for all analyses. Sulfate reduction rates were determined based on the rate of change in sulfate concentrations over the initial log-phase of the experiment.

Samples for the analysis of dissolved organic carbon (DOC) were filtered, flame-sealed in glass ampules under N2 gas after collection. Concentrations of DOC were then determined at Old Dominion University by high temperature combustion using a Shimadzu TOC-V total carbon analyzer after being diluted 1:100 with distilled deionized water (Komada et al., 2016).

2.3. Abiotic experiments

Abiotic experiments involving the addition of bicarbonate (HCO$_3^-$) to media were carried out at room temperature. The initial solution chemistry of the media for these experiments was identical to the biotic experiments, including the 10% CO$_2$ 90% N$_2$ degassing procedure. Two different masses of minerals were used, 0.3 g and 3.0 g. The kaolinite-to-calcite ratio was tested at 100%, 50%, and 0% in separate experiments, each at 0.3 g and 3.0 g (six experiments). Sodium bicarbonate (NaHCO$_3$–1 M) was added to artificially raise the alkalinity and pH of the medium (80 mL) to the maximum alkalinity seen in the biotic experiments (approximately 140 mEq L$^{-1}$). The rate of NaHCO$_3$ addition was 1.25 mL h$^{-1}$, and the pH and alkalinity were immediately measured after each addition.

2.4. Solid sample analysis

The minerals used in this study were analyzed for Brunauer-Emmett-Teller (BET) surface area using a Micromeritics (ASAP 2020) surface area and porosity analyzer in the Department of Material Science at the University of Cambridge. The sample was decanted into a 50 mL falcon tube which was centrifuged at 5000 rpm for five minutes and the solution discarded. The remaining solid samples were rinsed twice with MQ-water and oven-dried at 40°C overnight. The samples were degassed at 200°C under vacuum for 2 hours prior to BET analysis. Dried samples were then powdered prior to mineralogical analysis through X-Ray Diffraction (XRD). The dried samples were prepared by pipetting ~0.40 mL of an acetone smear containing the precipitate onto a zero-background holder. Powder X-ray diffraction was collected from Bruker diffractometers. The accuracy is ±1–2% for major phases.

2.5. Quantification of exopolymeric substances (EPS)

For quantification of EPS, we modified a semi-quantitative method for anaerobic samples and the presence of minerals in the samples (O’Toole, 2011). Two milliliters of growing media, including the minerals, were placed in a 10 mL sealed vial, and incubated for 48 hours to allow the formation of EPS. The OD$_{600}$ of the samples was subsequently measured using a spectrophotometer. Then, 2 mL of crystal violet was added to stain the EPS in the samples. The samples were then rinsed with MQ-water three times prior to the addition of 1 mL of ethanol to dissolve the crystal violet. The solution was then measured in the spectrophotometer at a wavelength of 550 nm. A total of five samples and two controls were measured. No bacteria were inoculated in the controls. The absorbance (ABS) value was then subtracted from the ABS of the controls to determine the relative amount of EPS. The EPS index was calculated by normalizing the values to the sample OD$_{600}$ to eliminate differences caused by differences in bacterial growth rate. Further information on the procedure is given in Supplemental Material A.

2.6. Global marine sulfate concentration gradients

The sulfate concentration gradient in sedimentary pore fluids was calculated from the IODP databases (Sites 626–1465, n = 640). The data is freely available in http://iodp.tamu.edu/janusweb/chemistry/chemiw.shtml for OD$_{600}$ cores and http://web.iodp.tamu.edu/lore/ for IODP cores. Detailed IODP data used in the analysis is given in the Supplementary Material Table S2. We did not include Deep-Sea Drilling Project (DSDP) cores due to the low-depth-resolution of pore fluid data. The sulfate concentration gradient was calculated to the depth at which sulfate concentrations went below 1 mM (if sulfate was sufficiently reduced). For sites where the sulfate concentration did not reach <1 mM, the sulfate gradient was calculated to the minimum sulfate concentration if there was a clear inflection point, and over the upper 50 m in the sediment column if the site did not meet the first two criteria. The alkalinity gradient was calculated over the same depth interval as the sulfate gradient at each site to capture the alkalinity produced by organoclastic sulfate reduction (OSR), and not by other processes in the sediment. The occurrence of AOM was assigned by the presence of methane below the sulfate reduction zone (Bradbury and Turchyn, 2019; Turchyn et al., 2021).

3. Results

3.1. Experiments with mineral seeds

In Experiment #1, the proportion of kaolinite and calcite varies, although the total amount of minerals, in terms of weight, remained constant. Significant bacterial growth is observed in all samples over the first 50 hours with OD$_{600}$ ~0.15–0.25, reaching stationary phase approximately 100 hours after inoculation (Fig. 1a). Bacterial growth is not detected in the control samples (see Supplementary Materials B). The pH increases from 6.0 to 8.3 as microbial sulfate reduction progresses. A stark difference among different experiments is the increase in alkalinity (Fig. 1b). In general, the incubation reaches higher maximum alkalinity in experiments with a low proportion of calcite compared to samples with a high proportion of calcite in the minerals present (Fig. 1b). The experiment with only kaolinite reaches an alkalinity of 140 mEq L$^{-1}$ when the electron donor has been consumed. However, in the samples with only calcite the maximum alkalinity is only 84 mEq L$^{-1}$ (Fig. 1b). Over the course of the experiment, sulfate concentrations decrease while concentration of aqueous sulfide increases, and these differ among the vials where there are purely calcite versus mixtures with kaolinite (Fig. 1d, e). We note that less sulfide is consumed in the ‘calcite-only’ and ‘seedless’ experiments than in the ‘kaolinite-only’ experiments (Fig. 1d). A similar trend can be observed in the calcium concentration. Microbially-induced carbonate mineral precipitation, as evidenced by a decrease in calcium concentrations (Bradbury et al., 2020), was observed in all
Fig. 1. Variables in pure culture (Desulfovibrio bizertensis) growth over time. Experimental results with varying proportion of kaolinite-to-calcite presented with a gradient in shading, where the darker lines indicate a higher proportion of CaCO₃ relative to kaolinite seeds. The open circle symbols are the samples without any mineral seeds. Some experiments were terminated early. Data presented include (a) OD₆₀₀; (b) alkalinity; (c) pH; (d) sulfate; (e) sulfide; and (f) calcium. Calcium concentrations decrease when the activity of the bacteria stimulates carbonate mineral precipitation, which does not occur with high percentages of CaCO₃ seeds in the vials. Samples with 100% calcite are presented as two separated lines because they were run in different batches (each batch was in duplicate). We did not observe any change in the control samples (Supplementary Materials B).

Experiments that have <99% CaCO₃. Samples with no clay minerals (including the ‘seedless’ sample) do not show any change in calcium concentration suggesting no microbially-induced carbonate mineral precipitation occurred over the course of the experiment (Fig. 1f).

The second incubation (Experiment #2) tested various minerals (cleaned calcite, quartz, kaolinite, cleaned Sigma-kaolinite, cleaned montmorillonite) to explore the influence that contaminates on the mineral surfaces may have on stimulating microbial growth (Fig. 2a–h). Similar to Experiment 1, the bacteria grow rapidly during the first 50 hours of the incubation. The OD₆₀₀ in most samples increases from 0.07 to 0.25, before experiencing a slight drop at 100 hours, and then stabilizing over the remainder of the experiment (Fig. 2a). The samples incubated with only calcite have an overall lower OD₆₀₀, although changes in pH are similar. The alkalinity, however, differs among the experiments with the different minerals, with the highest rates of alkalinity generation observed in the experiments containing cleaned Sigma-kaolinite or cleaned montmorillonite, slightly lower rates of alkalinity generation in the experiments containing kaolinite or cleaned kaolinite, and the lowest rates of alkalinity generation in the experiments containing quartz or calcite. Sulfide concentrations display a similar pattern to the changes in alkalinity, whereas sulfate concentrations display the reverse (Fig. 2c, d, f). The phosphate concentration in all samples decreases by at least 60% from its original concentration (700–800 μM). A decrease in calcium concentration is noted when the alkalinity of the samples reaches 80 mM L⁻¹ in all samples (Fig. 2g) – with mean saturation state with respect to calcite (Ωcalcite) of 13.53 ± 1.06. Magnesium concentrations decrease slightly over the course of the incubation (Fig. 2h). The rate of sulfate reduction was calculated based on the rate of change in sulfate concentrations during the exponential growth phase—when the bacteria are doubling at a constant rate (0–100 hours in Fig. 2)—for the individual experiments (Widdel, 2007). The incubation with cleaned Sigma kaolinite had the highest rate of sulfate consumption (1.93 mM day⁻¹). This is followed by incubations with cleaned montmorillonite (1.89 mM day⁻¹), uncleaned kaolinite (1.23 mM day⁻¹), cleaned kaolin-
ite (1.21 mM day$^{-1}$), cleaned quartz (1.19 mM day$^{-1}$), and finally calcite (1.08–0.87 mM day$^{-1}$) (Fig. 3).

3.2. Link between mineral surface area and sulfate reduction rate

BET analysis shows, as expected, that the calcite mineral seeds used (0.365 m$^2$ g$^{-1}$) have a very low surface area relative to the kaolinite (14.312 m$^2$ g$^{-1}$), but that Sigma-kaolinite (15.685 m$^2$ g$^{-1}$) has a higher surface area than the original kaolinite used. To compare rates of sulfate consumption among all the incubations in Experiment 2, we have to use the estimated BET surface area for montmorillonite (39.800 m$^2$ g$^{-1}$) and quartz (7.678 m$^2$ g$^{-1}$) reported in Aylmore et al. (1970) and Clouter et al. (2001) respectively, as we did not measure the BET for montmorillonite and quartz used in this study. Our XRD results suggest that the Sigma-kaolinite contains minor amounts of illite, despite being reagent-grade kaolinite (Supplementary Material D); the presence of illite may influence the rate of sulfate reduction if our hypothesis that different clay minerals influence rates of microbial activity is correct. There is a statistically significant correlation between the surface area of the different minerals and the sulfate reduction rate of the individual incubation ($R^2 = 0.59$, p-value = 0.0265—Fig. 3). The results of abiotic experiments are presented in Supplementary Material E.

3.3. Results comparing carbonate content and sulfate consumption in global marine sediments

Our analysis of the IODP dataset shows a significant correlation between the gradient in sulfate concentrations in sedimentary pore fluids (in mM m$^{-1}$) with the percentage of carbonate minerals in the sediments (Fig. 4, $R^2 = 0.95$, p-value < 0.0001). This hints that the rate of sulfate consumption in global marine pore fluids increases with the amount of clay in the sediment, as will be discussed below (Supplementary Material I).
4. Discussion

In growing Desulfovibrio bijetensis with varying proportions of kaolinite and calcite, we observe striking differences in bacterial growth depending on the proportion of kaolinite-to-calcite in the incubation vials. The higher the proportion of kaolinite relative to calcite, the higher the maximum alkalinity reached over the same time period (140 mEq L\(^{-1}\)—100% kaolinite vs. 80 mEq L\(^{-1}\)—100% calcite), the faster the rate that sulfate was consumed in the vials, the higher the overall pH reached during the experiment, and the quicker the vials reach carbonate mineral saturation (as seen by the time during the experiment where there is a decrease in calcium concentrations). When we test a wider range of mineral as seeds in the experimental vials we observe a similar stimulating effect in clay-seeded samples but no difference when the minerals (quartz, calcite, or clay minerals) have been cleaned. These results suggest that, in the presence of clay minerals, the bacteria are growing faster or to higher overall number of cells, and more greatly altering the chemistry in the incubation vials. While a similar result has been reported for Agrobacterium radiobacter—a common soil bacterium—(Stotzky, 1966a; Stotzky and Rem, 1966) and fungi (Chaerun and Tazaki, 2003; Filip et al., 1972; Stotzky, 1966b), this is the first report of clay-mineral microbial stimulation in sulfate-reducing bacteria. It should be noted that the OD\(_{600}\) in our incubation experiments is not a direct proxy for cell count, as some of the sulfate-reducing bacteria grow at the mineral surface, and hence are not measured in the solution OD\(_{600}\) (Lin et al., 2018). We observe this when we compare the kaolinite-seeded experiment to the ‘seedless’ experiment; changes in sulfate, sulfide, pH, and alkalinity all suggest the higher rates of sulfate consumption in the kaolinite-seeded experiment, although the OD\(_{600}\) observed in the ‘seedless’ experiment is higher, which we conclude is because there are more cells in suspension than growing on the mineral surfaces (which are absent in the ‘seedless’ experiment).

As the OD\(_{600}\) may not be representative of the true cell numbers due to the growth of the cells on the mineral surfaces, it is challenging to assess whether the clay minerals are stimulating growth (i.e. higher cell numbers over the course of the experiments) or stimulating cells’ metabolic rate (i.e. the cell-specific Sulfate Reduction Rate—csSRR). Given the relative invariance in the OD\(_{600}\) over the course of the experiments, which suggests the cell numbers in solution are similar among the experiments, we surmise that clay minerals are stimulating cells’ metabolic rate, perhaps through the buffering of the solution around the clay minerals. Alternatively, there may be a higher number of cells growing on the mineral surfaces (due to the high specific surface area of the clay minerals). Future work on the stimulation effect of clay minerals on microbial sulfate reduction could measure the sulfur isotope fractionation during microbial growth in the presence of different minerals to confirm a change in metabolic rate (Wing and Halevy, 2014).
We note that the formate used as an electron donor for the sulfate reducing bacteria in these experiments does not behave like a weak acid (formic acid has a $pK_a$ of 3.76), and therefore does not influence the measured alkalinity. As evidence of this, we demonstrate that the control samples, which had 110 mM formate added to them, have similar alkalinity values to seawater (~$2 \text{ mEq L}^{-1}$—Supplementary Material E). At the final time point, the concentration of dissolved organic carbon (DOC—in the form of formate) remaining in the calcite-seeded sample is higher than the kaolinite-seeded sample. This suggests that in the experiments with kaolinite there is more complete consumption of the electron donor (formate—Supplementary Material Table S3). We find that when we calculate the predicted alkalinity change based on the amount of sulfate reduced—and thus the amount of organic carbon (formate) theoretically oxidized—the predicted alkalinity versus the measured alkalinity are very similar when we account for the removal of calcium and magnesium through microbially-induced precipitation of carbonate minerals (Supplementary Material F).

Our calculations of the inorganic carbon in the system also suggest that some reduced organic carbon compounds in the yeast extract (which was ~38% of DOC in the media) were utilized by the sulfate-reducing bacteria in the kaolinite experiment.

We also note that the bacterial cultures secrete 3–10 times as much exopolymeric substances (EPS) in calcite-seeded samples relative to quartz- and clay-seeded samples (Fig. 6) hinting that the bacteria may be more stressed when grown in the presence of calcite seeds (Costa et al., 2018; Spring et al., 2019). In the calcite-seeded experiment, we speculate that the bacteria secrete a thicker EPS layer at the base of the vial (associated with higher OD due to more free living cells in the medium). The dissolution of calcite seeds (due to the low initial pH and the production of EPS) could have resulted in a microenvironment with higher pH and the break down of surface area that impacts bacterial survival and mobility. Contrary to the experiments with the calcite seeds, the experiments with clay minerals may offer enhanced buffering (see Supplementary Material G) against the alkaline medium and, hence, less EPS was secreted by the bacteria. Given the low EPS index in the clay-seeded experiments, we suspect that the bacteria may exist without EPS or surface-attached aggregates, forming colonies within the clay matrix (pore space) (Decho and Gutiérrez, 2017) (Supplementary Material H). We note that in our abiotic experiments performed with an addition of DIC, changes in pH and alkalinity show the same changes regardless of the minerals present; alkalinity increases in tandem with volume of NaHCO$_3$ added, and not more or less if there is kaolinite or calcite in the vial (Supplementary Material E). Thus we conclude that abiotic reactions including dissolution and re-precipitation of minerals, or ion exchange on mineral surfaces, are not critical in mediating alkalinity and pH in our experimental setup and that the difference in the generation of alkalinity, consumption of DOC, and production of EPS are driven by the metabolic activity of Desulfovibrio bietortensis. These experimental results confirm that an increase in the fraction of clay within the incubation vials impacts the rate of sulfate consumption during microbial sulfate reduction and thus the solution chemistry.

Several properties of clay minerals have been suggested to be responsible for influencing microbial metabolism: the inorganic nutrients and trace metals associated with clay minerals, the pH buffering and ion-exchange capacity of clay minerals, and specific surface area of clay minerals (Gadd, 2010; Matschiavelli et al., 2019; Stotzky, 1966a, 1966b; Stotzky and Rem, 1966). We note that nutrients which may be associated with the mineral substrate are not influencing bacterial metabolism in our experiments as evidenced by similarity in sulfate consumption rates in the experiments using cleaned and uncleaned kaolinite seeds (1.21 and 1.23 mM day$^{-1}$ respectively—Fig. 2). This agrees with previous studies that concluded clay minerals are not a significant source of trace elements or other nutrition to micro-organisms (D’Hondt et al., 2009; Spring et al., 2019; Wong et al., 2004) although studies have shown that clay minerals can be a significant source for Fe$^{3+}$ under reduced conditions (Favre et al., 2002; Pentrakova et al., 2013). It has been suggested that the buffering capacity of clay minerals creates a suitable environment to stimulate sustained bacterial growth, and favorable pH helps the relative basicity of the exchangeable cations on the clays (Stotzky, 1966a). Montmorillonite, in particular, may exert a marked influence on the activity of bacteria due to its greater ion-exchange capacity (17–20 times greater) relative to many other clay minerals (Matschiavelli et al., 2019; Stotzky, 1966a). Our incubation experiments support this, and show that montmorillonite-seeded samples have a greater rate of sulfate consumption compared to other samples (Figs. 3 and 5). We suggest that the buffering capacity (proton exchange) of the seeding material may play an important role in influencing the microbial activity in the system (Supplementary Material G). The correlation between the surface area of the different seeding materials and the sulfate reduction rate of the individual incubation, as shown in Fig. 3, hints that the surface area of the mineral seeds may be influencing the microbial activity in the system.

**4.1. Insights into substrate-influenced metabolism in global marine sediments**

There are inherent challenges when extrapolating laboratory results to the natural environment, and this study is no exception, given the range of conditions that differ between well-constrained pure-culture experiments and sedimentary pore fluids where microbial sulfate reduction is occurring. Laboratory experiments involving sulfate reduction have, in general, been conducted at surface pressures and temperatures to observe the changes on a reasonable experimental timescale. The rates of sulfate reduction that are occurring beneath the seafloor are orders of magnitude slower than would be observable in the laboratory, with even rapid rates of sulfate reduction in marine sediments taking many hundreds of years. There are a multitude of reasons why the laboratory experiments do not reproduce conditions in the natural environment, including the physical conditions of the vials, the presence of only a single strain of bacteria, and the headspace gas composition, among others. Notably, the pure culture experiments are organic-carbon replete, while most marine sediments are organic-
carbon limited (D’Hondt et al., 2009). Furthermore, marine sediments are not a binary mixture between calcite and kaolinite, and contain many other mineral phases including siliceous oozes, recalcitrant organic material, and authigenic silica phases. The utility of our pure-culture laboratory experiments is to mimic behavior that should be observable in the environment; the reduction in the “free parameters” allows for the manipulation of a single aspect of the system in a tightly controlled environment.

Before we can attribute the correlation seen in Fig. 4 between the rate of decrease in pore fluid sulfate concentrations and the amount of carbonate in marine sediments to an increase in microbial sulfate reduction associated with a change in sediment mineralogy, we must consider other factors that may differ between the broad categories of clay-rich and carbonate-rich sediments and might also influence rates of microbial sulfate reduction. These include whether the measured carbonate content of the sediment can be related to clay content, open-versus closed system behavior as a function of sedimentation rate, the reduction of sulfate through the anaerobic oxidation of methane versus organic-matter oxidation, and overall sediment porosity as well as the supply of electron donors (organic carbon) that also differ with sediment type (Brune et al., 2000; Egger et al., 2018; Orcutt et al., 2013).

We first consider whether lower carbonate content reported for IODP sediments means these sediments contain more clay minerals. Natural gamma radiation (NGR) is produced during the breakdown of the radioactive isotopes of potassium, uranium and thorium and has previously been used to characterize clay type and abundance, depositional environment and diagenetic processes in the sediment (De Vleeschouwer et al., 2017; Dunlea et al., 2013). We note a statistically significant negative correlation between the average NGR and the carbonate content in sediment (Fig. 6a), suggesting that, broadly speaking, when there is lower carbonate content in marine sediment there is higher clay content. This allows us to conclude that the inverse correlation between the gradient in sulfate concentrations and carbonate content is due to the difference in sedimentation rate and ‘open’ versus ‘closed’ system dynamics typical of carbonate versus clay-dominated sediments. We note that in marine sediments, advection is typically negligible relative to diffusion (Berner, 1980; Burdige, 2006; Egger et al., 2018). The Peclet number, the ratio of advective transport rate over diffusive transport rate, has been used to determine the relative importance of the two transport phenomena (Berner, 1980; Burdige, 2006). When we calculate Peclet numbers for the IODP sites using porosity, sedimentation rate (Egger et al., 2018) and depth, the frequency distribution of Peclet numbers shows that in ~77–100% of the sites in the compilation (dependent on carbonate content), diffusion dominates sulfate transport in the sediment column (Fig. 6b). This occurs regardless of whether the sites are clay-rich or carbonate-rich, although among clay-rich sites only, advection-dominated sites are more common than in carbonate-rich sites only. The fact that the majority of the sites are diffusion-dominated supports the idea that any difference we observed in sulfate concentration gradients between clay-rich and carbonate-rich sediments is not due to differences in the dynamics of fluid transport between the two different sediment types. We conclude that, overall, the inverse correlation we find between the rate of sulfate consumption and the percentage carbonate in the sediment is not due to the degree of open/closed system behavior.

Previous studies have shown that where anaerobic oxidation of methane (AOM) is the dominant sulfate-reducing process in sediment there is a steeper sulfate concentration gradient than where there is no AOM (Bradbury and Turchyn, 2019; Canfield, 1991; Turchyn et al., 2021). We note that the negative correlation between the gradient in sulfate in the pore fluid and percentage of carbonate minerals in the sediment is apparent regardless of whether there is methane present (Fig. 7a, d).

Sediments that are dominated by clay minerals often have closed system conditions higher in the sediment column, promoting overall higher rates of depletion of sulfate within the pore fluid (Yang and Aplin, 2010). Our analyses suggest no significant correlation between sediment porosity and the percentage of carbonate minerals at sites without methane (Fig. 7f), although a stronger correlation between porosity and the amount of carbonate in the sediment observed is sites with methane (Fig. 7c, 8).
p-values < 0.01). Sediments with a higher percentage of clay minerals appear to have a higher overall porosity (Fig. 7c), although the correlation is weak and based on an exceptionally small number of sites that have both high percentages of carbonate minerals and methane (Fig. 7c). In general, the sulfate pore fluid gradient is significantly higher when methane is present in the sediment column, than when it is not, due to the consumption of sulfate through the anaerobic oxidation of methane (Fig. 7a,d).

Another possibility is that sediments with high clay content are also those with high organic carbon content (Burdige, 2007); high organic carbon delivery would naturally drive higher rates of sedimentary microbial sulfate reduction. However, our analysis suggests that there is no significant correlation between the percentage of carbonate minerals in the sediment (and thus the gradient in the pore fluid sulfate concentration) and the total organic carbon (TOC) in the sediment for sites with or without methane present (Fig. 7b, e, p-values > 0.1). This result suggests that total organic carbon has a less significant influence on the rate of sulfate reduction or there may be inaccuracies in reported measurement of total organic carbon; we favor the latter explanation. Total organic carbon is typically calculated by subtracting the inorganic carbon (e.g. carbonate minerals) from the total carbon content measured. This mechanism for measuring the total organic carbon is potentially not recording the amount of labile carbon available for microbial sulfate reduction or methane production. There is also no significant correlation observed between the TOC in the upper 50 m of the sediment and the sediment mineralogy, where more of the originally deposited, or labile, TOC should be observed (Fig. 7b, e); this further supports that traditional methods for measuring sedimentary organic carbon content may be less informative for understanding the link between TOC and sediment microbial activity. The flux of organic carbon to the sediment is roughly correlated with water depth, with ~40% of organic carbon deposition occurring on continental shelves (Muller-Karger et al., 2005). The average water depth of the binned sites compared to the carbonate percentage displays a strong negative correlation, as would be expected by the decrease in carbonate saturation with increasing water depth, which also suggests that organic carbon content and quality alone does not explain the trends observed (Supplementary Material J).

Although our study shows similar trends of an increase in sulfate consumption with an increase in clay mineral content, both in the laboratory and in the global IODP dataset, it should be emphasized that the rate of sulfate reduction in the natural environment are orders-of-magnitude lower than rates obtained in the laboratory (Jørgensen et al., 2019). Cell-specific sulfate reduction rates would help directly compare the laboratory and IODP data, but cell counts typically are not available for IODP sites. Nevertheless, we note the significance that the same processes can be observed to have a similar effect both at a laboratory and global scale; sediment mineralogy has not previously been shown to influence the rate of sulfate reduction in marine sediments.

Pore fluid alkalinity—as a rough proxy for dissolved inorganic carbon—reaches concentrations nearly two orders of magnitude higher than seawater in sediments on the continental slope due to alkalinity generation during sulfate reduction and silicate mineral weathering caused by methanogenesis in the sediment (Hong et al., 2020; Torres et al., 2020; Turcyn et al., 2021). The maximum alkalinity reached in sediments on the continental slope is often much higher than on the continental shelf, which can be dominated by carbonate sediments (Supplementary Material I). This large alkalinity flux from sediments could match that from rivers, and could explain the long-observed discrepancy between the predicted and observed depths of carbonate mineral dissolution (Berelson et al., 2007; Cao and Dai, 2011). We suggest that increased clay content in sediments on the continental slope may stimulate microbial activity and partially drive the enhanced alkalinity found in these sediments. Furthermore, at its most speculative, our results suggest that geological-time-scale changes in sea level that lead to global changes in the distribution and type of sediment may play a heretofore unrecognized role in the global carbon budget.

Fig. 7. Comparison of sulfate pore fluid gradient, organic carbon percentage and porosity with carbonate percentage in sites with AOM (top) and without AOM (bottom) (n = 640). For organic carbon and porosity, we present both the average over the entire measured profile (Total—blue circles), and over the upper 50 m of the sediment (Upper 50 m—green squares).
5. Conclusions

Our results suggest that there is an independent variable that is globally significant in the overall activity of sulfate-reducing bacteria within the marine sediments: the mineralogy of the sediment itself. The overall rate of microbial sulfate reduction changes as a function of the proportion of clay minerals present in pure culture incubations and in the global marine pore fluid database. A substantial decrease in sulfate reduction rate is particularly noted at above 80% carbonate minerals. We suggest that the preference of the bacteria for clay-dominated substrate is due to the higher surface area of the clays and the buffering capacity this provides in systems with high pH (greater than 8). Combining results from both laboratory and IODP cores, we suggest that the substrate mineralogy impacts the rate of sulfate reduction in more direct ways than previously thought.

CRediT authorship contribution statement

Chin Yik Lin: Conceptualization, Investigation, Methodology.
Writing – original draft. Harold J. Bradbury: Conceptualization, Formal analysis, Methodology, Visualization, Writing – original draft. Glad Antler: Conceptualization, Writing – review & editing. David J. Burdige: Investigation, Writing – review & editing. Thomas D. Bennett: Investigation. Shichun Li: Investigation. Alexandra V. Turchyn: Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

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References


