Marine anoxia initiates giant sulfur-bacteria mat proliferation and associated changes in benthic nitrogen, sulfur, and iron cycling in the Santa Barbara Basin, California

Borderland

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Abstract

The Santa Barbara Basin naturally experiences transient deoxygenation due to its unique geological setting in the Southern California Borderland and seasonal changes in ocean currents. Long-term measurements of the basin showed that anoxic events and subsequent nitrate exhaustion in the bottom waters have been occurring more frequently and lasting longer over the past decade. One characteristic of the Santa Barbara Basin is the seasonal development of extensive mats of benthic nitrate-reducing sulfur-oxidizing bacteria, which are found at the sediment-water interface when the basin’s bottom waters reach anoxia but still provide some nitrate. To assess the mat’s impact on the benthic and pelagic redox environment, we collected biogeochemical sediment and benthic flux data in November 2019, after anoxia developed in the deepest waters of the basin and dissolved nitrate was depleted (down to 9.9 µM). We found that the presence of mats was associated with a shift from denitrification to dissimilatory nitrate reduction to ammonium. The zone of sulfate reduction appeared near the sediment-water interface in sediment hosting these ephemeral white mats, but that alone seems insufficient to spur their growth. We found that a high sediment TOC content (>5%) and an exhaustion of iron oxides in the surface sediment were additional prerequisites for mat proliferation. Our research further suggests that cycles of deoxygenation and reoxygenation of the benthic environment result in extremely high benthic fluxes of dissolved iron from the basin’s sediment. This work expands our understanding of nitrate-reducing sulfur-oxidizing mats and their role in sustaining and potentially expanding marine anoxia.
Introduction

Naturally occurring low-oxygen waters in the ocean are commonly observed below the ocean’s mixed layer where respiration consumes oxygen faster than it is produced or ventilated. When low oxygen conditions occur along the western continental shelf in regions susceptible to upwelling events and/or undergoing eutrophication, organic matter remineralization can frequently drive oxygen concentrations to hypoxic ($O_2 < 63 \mu M$) (Middelburg and Levin, 2009) and/or anoxic levels ($O_2 < 3 \mu M$) (Fossing et al., 1995b; Canfield et al., 2010). These areas usually referred to as Oxygen Minimum Zones (OMZs). In the water column of OMZs, nitrogen transformation through canonical denitrification or dissimilatory nitrate reduction to ammonium (DNRA) becomes the dominant organic matter remineralization mechanism (Ward et al., 2009). OMZs within coastal basins that experience seasonal changes in upwelling can experience anoxic and denitrifying conditions that extend to the benthic environment, especially when high productivity (and associated organic matter export fluxes) coincide with seasonal patterns in physical mixing. This fundamental change in the redox conditions at the sediment-water interface encourages elevated rates of anaerobic microbial processes and promotes organic matter preservation in the sediments (Middelburg and Levin, 2009; Treude, 2011). Persistent anoxia in these coastal OMZ can lead to huge releases of sulfide (up to 13.7 mmol m$^{-2}$ d$^{-1}$) and ammonium (up to 21.2 mmol m$^{-2}$ d$^{-1}$) into the water column (Sommer et al., 2016).

The Santa Barbara Basin (SBB) is a coastal basin in the California Borderland with an approximate maximum depth of 600 m characterized by a seasonally anoxic water column (Sverdrup and Allen, 1939; Sholkovitz and Gieskes, 1971). The transform boundary along the
California Borderland heavily affects the geomorphology of basins in this region; these basins become twisted as the plates rub against each other and form a series of “bathtubs” blocked by sills and seamounts off the coast of California. The SBB is bordered by the California coast in the north, the Channel Islands in the south, the Santa Monica basin to the east, and the Arguello Canyon to the west. A sill to the west of the basin at around 475 m depth (Fig. 1) prohibits most water transfer between the Santa Lucia Slope and the deeper waters of the SBB (Sholkovitz and Gieskes, 1971). The highly productive surface waters in the basin provide ample organic matter to the basin’s water column, encouraging strong remineralization processes below the euphotic zone, which can induce anoxia below the sill depth, with typically less than 1 µmol O$_2$ L$^{-1}$ (Sholkovitz, 1973; Emery et al., 1962; Thunell, 1998; Emmer and Thunell, 2000). During upwelling events (usually in Spring), oxygenated waters from the California Current spill over the western sill and ventilate the SBB, increasing oxygen concentrations to approximately 20 µmol O$_2$ L$^{-1}$ (Goericke et al., 2015). SBB water-column oxygen and nitrogen concentrations have been evaluated through a longitudinal survey by the California Cooperative Oceanic Fisheries Investigations (Calcofi) with data starting in the 1950’s. The data collected by this survey shows increasingly ubiquitous anoxia and denitrification in the basin with the SBB becoming completely nitrate-depleted below the sill at least three times between 2012 and 2017 (https://calcofi.org/data/).

These drastic changes in SBB oxygenation and seafloor redox conditions drive complex changes in benthic biogeochemistry and microbiology, evidenced most clearly by the development of thick, expansive mats of giant sulfur-oxidizing bacteria (GSOB) mats at the seafloor (Bernhard et al., 2003; Prokopenko et al., 2006; Valentine et al., 2016; Kuwabara et al., 1999). A 2016 survey
of the basin seafloor identified a vast GSOB mat spread over 1.6 contiguous km, confined between 487 and 523 km in the SBB depocenter where conditions were anoxic but not depleted of NO$_3^-$ (Valentine et al., 2016). Similar GSOB mats have been identified in several other transiently deoxygenated OMZs including the Peruvian/Chilean coast (Sommer et al., 2016; Schulz et al., 1996; Zopfi et al., 2001; Høgslund et al., 2009). These chemoautotrophic bacteria (typically *Thioploca* and/or *Beggiatoa*) utilize sulfide as an electron donor and O$_2$ or NO$_3^-$ as a terminal electron acceptor (Jørgensen and Nelson, 2004). Some GSOB can hyperaccumulate NO$_3^-$ in cell vacuoles up to 500 mM (Fossing et al., 1995a) and use this NO$_3^-$ reserve to oxidize sulfide that diffuses from the underlying sediment to perform their metabolism. (Huettel et al., 1996; Mußmann et al., 2003; Sayama, 2001).

![Figure 1. Maps of sampling locations in the Santa Barbara Basin and photographs of deployed equipment: (A) bathymetric map of the Santa Barbara Basin with locations of all sampled stations; (B) cross-section of the Santa Barbara Basin with locations of all sampled station; (C) sediment push coring with ROV arm; (D) sediment microprofiler; (E) benthic flux chamber; (F) closeup of a syringe system from a benthic flux chamber. The map in (A) was generated using the Bathymetric Data Viewer provided by the National Centers for Environmental Information.](https://doi.org/10.5194/egusphere-2023-1198)
These GSOB mats are ephemeral in the SBB, appearing to proliferate and potentially migrate depending on bottom water oxidant concentrations (Kuwabara et al., 1999). The activity of GSOB mats in turn contribute significantly to element cycling in the SBB with large effects on biogeochemical conditions in the bottom water. Isotopic measurements of $^{15}$N/$^{14}$N and $^{18}$O/$^{16}$O from NO$_3^-$ suggest that sedimentary organisms are responsible for approximately 75% of the total NO$_3^-$ uptake in the SBB (Sigman et al., 2003). Other studies found that GSOB mats inhibit the diffusion of NO$_3^-$ into sediments via hyper-accumulation in vacuoles thereby creating conditions ideal for bacterial organoclastic sulfate reduction beneath them (Fossing et al., 1995b; Zopfi et al., 2001). These studies suggest that GSOB mats in the SBB may be responsible for the majority of NO$_3^-$ consumption in the basin rather than water-column microbes. Additionally, GSOB mats deplete NO$_3^-$ via Dissimilatory Nitrate Reduction to Ammonium (DNRA) in the anoxic bottom water of the Peruvian OMZ (Dale et al., 2016). By contrast, benthic microbial communities in the hypoxic (42 µM) Mauritanian OMZ perform canonical denitrification instead (Dale et al., 2014). The contrast between the Peruvian and Mauritanian OMZ suggests that anoxia triggers the appearance of GSOB mats, and that DNRA is more prevalent where GSOB mats are present.

The rapid accumulation and consumption of NO$_3^-$ by GSOB mats has ramifications for the redox conditions in the sediment underneath. The depletion of NO$_3^-$ and shallowing of the nitracline could promote high rates of sulfate reduction in the sediment underneath the GSOB mat. In return, the sulfate reduction zone exists close to the sediment-water interface, providing the GSOB mat with readily accessible sulfide. If a metabolic feedback loop is then established between sulfur-oxidizing bacteria at the sediment-water interface and sulfate-reducing bacteria in the sediment, increased NO$_3^-$ loss from the water column and spreading of sulfidic conditions in...
SBB sediment is expected. With these mats being potentially crucial to nitrogen and sulfur cycling in sediments underlying OMZ, their biogeochemical transformations and ergo effect upon basin redox conditions are critically important to understanding element cycling in the SBB. Such gained knowledge would have additional benefits for predicting biogeochemical feedbacks to the projected expansion of oceanic oxygen deficiency, in the SBB and in OMZs more general, as a result of global change (Stramma et al., 2008).

Utilizing in-situ technologies, sediment porewater extraction, solid phase analyses, and radiotracer techniques, this study aims to answer the following overarching questions: (1) Which environmental conditions initiate and sustain the proliferation of GSOB mats? (2) Which biogeochemical transformations occur in the sediment underneath these mats? (3) What role do the mats play in the increasingly prevalent anoxic and nitrate-depleted condition found in the SBB?
2. Materials and Methods

2.1 Benthic sediment sampling and instrument deployment

Sediment samples were taken between 30 October and 11 November 2019 during an expedition aboard the research vessel (R/V) Atlantis equipped with the remote operated vehicle (ROV) Jason. Samples were taken at stations along a bimodal, north-south transect through the depocenter of the SBB, as well as one station on a separate transect. Details of sampling stations can be seen in Fig. 1A and 1B. Briefly, depocenter stations are labeled as NDRO and SDRO (northern and southern depocenter radial origin, respectively). The remaining stations are named for the cardinal direction (north vs. south) and the transect number (e.g., SDT1-A is on transect 1 while SDT3-A is on transect 3). As station depth decreases, the alpha suffix increases (e.g., NDT3-A is deeper than NDT3-B, etc.). ROV Jason conducted sediment push coring and deployed automated benthic flux chambers (BFC) and microprofilers at each station. Station depth, latitude, and longitude were automatically generated by the Jason data processor using navigation data derived from the Doppler Velocity Log system and the ultrashort baseline positioning system. Bottom water oxygen concentration was determined using an Aanderaa 4831 oxygen optode (Aanderaa Instruments, Bergen, Norway) installed on the ROV. Optical modems (Luma 250LP, Hydromea, Renens, Switzerland) were used to transmit deployment settings and initiate/terminate communications between ROV Jason and the BFC/microprofilers. Multiple push cores (polycarbonate, 30.5 cm length, 6.35 cm inner diameter) per sampling station were retrieved during ROV Jason deployments (Fig. 1C). These cores were inserted into the sediment and retrieved using Jason’s manipulator arm. Cores were then stored in a 6-core capacity basket and transported to the surface using a free-ascending underwater elevator. Replicate cores from each station were immediately transferred to a 6°C cold room (representing average in-situ
temperature) and subsampled for either solid phase analyses, porewater geochemistry, or radiotracer experiments.

### 2.2 Sediment Core Sub-Sampling

For sediment porewater geochemistry analyses, two replicate ROV push cores that were collected near each other were processed under a constant argon flow to protect redox-sensitive species. Cores were sectioned in 1-cm increments up to 10 cm followed by 2-cm increments below 10 cm. Note, sediments from the NDT3-B station were sliced in 2-cm increments. Sediment subsections were transferred into argon-filled 50-mL conical centrifuge tubes.

Porewater was retrieved after sediment samples were centrifuged at 4255 x g for 20 minutes. Solid phase cores were sectioned similar to porewater cores and sub-sampled for sediment density and porosity and organic matter analyses. A cut-off plastic syringe was used to collect 6 mL of sediment into pre-weighed plastic vials (15 mL snap-cap vials) and stored in the dark at 4°C for sediment porosity and density analysis. Two-mL microcentrifuge tubes were filled with sediment from each depth interval and stored at -30°C for sediment organic matter analyses. One ROV push core per station was sub-sampled with a miniaturized push core (length 20 cm, inner diameter 2.6 cm) and taken to the shipboard radioisotope van for radiotracer experiments (see section 2.5).

### 2.3 Sediment Porewater Geochemistry

Geochemical analyses were performed to provide context for electron donors and acceptors of benthic microbial metabolisms, sediment redox states, and organic matter degradation.

Concentrations of sulfide (Cline, 1969), NH₄⁺, PO₄³⁻, and Fe²⁺ (Grasshoff et al., 1999) were
determined shipboard with a Shimadzu UV-Spectrophotometer (UV-1800). Detection limits for sulfide, \( \text{NH}_4^+ \), \( \text{PO}_4^{3-} \), and \( \text{Fe}^{2+} \) were 1 \( \mu \text{M} \). Subsamples (2 mL) for porewater \( \text{NO}_3^- \) and \( \text{NO}_2^- \) concentrations were stored in 2-mL plastic vials with an O-ring, frozen shipboard at -30°C and analyzed in the lab on the same spectrophotometer using the method following (García-Robledo et al., 2014). The detection limit for \( \text{NO}_3^- \) and \( \text{NO}_2^- \) was 0.5 \( \mu \text{M} \). Samples for porewater DIC were preserved shipboard with 5 \( \mu \text{L} \) saturated \( \text{HgCl}_2 \) in headspace free glass vials and stored at 4°C for later analysis following (Hall and Aller, 1992). DIC detection limit was 0.5 mM. Total alkalinity was determined shipboard using direct titration of 500 \( \mu \text{L} \) of pore water with 0.01M Titrisol® HCl (Pavlova et al., 2008). The analysis was calibrated using IAPSO seawater standard, with a precision and detection limit of 0.05 meq L\(^{-1}\). Subsamples (1 mL) for sulfate and chlorinity were stored in 2-mL plastic vials with an o-ring, frozen shipboard at -30°C and later measured in the lab using a Metrohm 761 ion chromatograph with a methodological detection limit of 30 µM (Dale et al., 2015).

### 2.4 Solid Phase Analyses

Plastic vials for sediment porosity and density measurements were weighed prior to the expedition. Samples collected in the vials were dried at 50°C for up to 96 hr until the dry weight was stable. Sediment porosity was determined by calculating the difference between wet and dry sediment weight divided by the volume of the wet sediment. Sediment density was determined by dividing the wet sediment weight by its volume. Analyses for sediment total organic carbon (TOC), total organic nitrogen (TON), and organic carbon isotope composition (\( \delta^{13}\text{C} \)) were modified from (Harris et al., 2001). Briefly, samples were dried up to 48 hours at 50°C until the dry weight was stable and then treated with direct addition of 1 mL of 6N HCl to dissolve...
carbonate minerals. These samples were then washed in triplicate with 1-mL of ultrapure water or until a neutral pH was re-established. Samples were centrifuged at 4255 x g for 20 minutes, the supernatant was decanted, and vials were re-dried at 50°C. A subsample (approximately 10-15 mg of sediment) was then packed into individual 8x5 mm pressed tin capsules and sent to the University of California Davis Stable Isotope Facility for analysis using Elemental Analyzer – Isotope Ratio Mass Spectrometry. TOC and TON were calculated based on the sample peak area corrected against a reference material (alfalfa flour). Limit of quantification based on peak area was 100 μg C with an uncertainty of ± 0.2 ‰ for δ¹³C.

2.5 Sulfate Reduction

To determine ex-situ microbial sulfate reduction rates, whole round sub-cores were injected with 10 μL carrier-free ³⁵S-Sulfate radiotracer (dissolved in water, 200 kBq, specific activity 37 TBq mmol⁻¹) into pre-drilled, silicon-filled holes at 1-cm increments according to (Jørgensen, 1978). These sub-cores were incubated at 6°C in the dark for 6-8 hours. Incubations were stopped by slicing sediment cores in 1-cm increments into 50-mL centrifuge tubes filled with 20-mL zinc acetate (20% w/w) and frozen at -20°C until analysis at the land-based laboratory. Triplicate “killed” controls were produced using homogenized sediment from the same ROV push core.

Microbial activity in controls was terminated with zinc acetate (20 mL of 20% w/w) before the addition of radiotracer and subsequent freezing. Lab-based analysis of sulfate reduction rates were determined following the cold-chromium distillation procedure (Kallmeyer et al., 2004).

Sulfate reduction rates were calculated per volume of wet sediment (cm³) following equation:

\[
SRR = [SO₄] \times P_{SED} \times \frac{\text{aTRIS}}{\text{aTOT}} \times \frac{1}{t} \times 1.06 \times 1000
\]  
(EQ # 1)
Where SRR is sulfate reduction rate (nmol cm$^{-3}$ d$^{-1}$); [SO$_4$] is porewater sulfate concentration; $P_{sed}$ is sediment porosity; $a_{TRIS}$ is radioactivity of the total reducible inorganic sulfur (counts per minute); $a_{TOT}$ is total radioactivity used in incubation (counts per minute); $t$ is incubation time in days; 1.06 is the correction factor for expected isotope fractionation; and 1000 is the factor to convert from mmol to nmol.

2.6 Benthic In-Situ Investigations

Per station, one to three microprofiler (Fig. 1D) and three BFC (Fig. 1E) deployments were carried out by the ROV Jason at the seafloor. Construction, deployment and operation of automated microprofilers and BFCs followed those described in (Treude et al., 2009). The microprofiler deployed in this study represents a modified, miniaturized version of the instrument described in (Gundersen and Jørgensen, 1990) that was constructed specifically for use by ROV. Microprofilers were outfitted with three O$_2$-microelectrodes (Glud et al., 2000), two pH-microelectrodes (Revsbech and Jørgensen, 1986), two H$_2$S-microelectrodes (Jeroschewsky et al., 1996), and one conductivity sensor to determine the position of the sediment-water interface relative to the tips of the microelectrodes. Concentrations of oxygen and sulfide, as well as pH were each calculated from microelectrode readings as an average per site where replicates existed. Microprofiler frames (and benthic flux chamber frames) were outfitted with syntactic foam to reduce the negative buoyancy of the instruments in water and prevent them from sinking into the extremely soft sediments in the SBB.
The BFC consisted of a frame equipped with a cylindrical polycarbonate chamber (inner diameter = 19 cm) with its lower portion sticking out of the frame. The upper side of the chamber was closed by a lid containing a stirrer (Type K/MT 11, K.U.M., Kiel, Germany), oxygen optodes (Type 4330, Aanderaa Data Instruments, Bergen Norway and Hydroflash, Contros/Kongsberg Maritime, Kongsberg, Norway), a conductivity sensor (type 5860, Aanderaa Data Instruments), and a valve. Prior to insertion into the sediments, the chambers were held upside down by the ROV manipulating arms within approximately 10 m of the seafloor and moved back and forth to make sure that water from shallower depth that may have been trapped was replaced by bottom water. Chamber incubations lasted between 240 and 390 minutes. Each BFC was outfitted with a custom-built syringe sampler containing seven syringes that were connected by tubes to sampling ports in the upper wall of the chambers (Fig. 1F): one injection syringe and six sampling syringes that were fired at regular time intervals over the time course of the deployment. The injection syringe contained de-ionized water and the reduction in salinity in the overlaying water after salinity readings stabilized (i.e., full mixing was achieved) 10-30 min after injection was used to determine BFC volumes (Kononets et al., 2021). Samples obtained from the overlaying water of the BFC were examined for the same geochemical constituents as described above (section 2.4). Benthic fluxes of \( \text{NO}_3^- \), \( \text{NH}_4^+ \), \( \text{PO}_4^{3-} \), and \( \text{Fe}^{2+} \) were calculated as follows:

\[
J = \frac{4c}{\Delta t} \cdot \frac{V}{A}
\]  

(EQ # 2)

Where \( J \) is the diffusive flux in mmol m\(^{-2}\) d\(^{-1}\), \( \Delta C \) is the concentration change in mmol m\(^{-3}\), \( \Delta t \) is the time interval in d, \( V \) is the overlying water volume in m\(^3\), and \( A \) is the surface area of the
sediment covered by the benthic flux chamber in m². Final diffusive flux in BFC was averaged for stations of similar depth (e.g., we averaged calculated diffusive flux from NDT3-A and SDT3-A and report as “A stations”). One chamber per site contained $^{15}N$-NO$_3^-$ in the injection syringe for in-situ nitrogen cycling experiments. Results are reported from two of these chambers (SDRO and NDT3-D) and all $^{15}N$-NO$_3^-$ chambers were excluded from benthic flux calculations (see next section).

### 2.7.1 In Situ $^{15}N$ Incubations

Two hundred $\mu$mol of $^{15}N$-labeled potassium nitrate (99% $^{15}N$; Cambridge Isotopes) was injected into the $^{15}N$ incubation chamber at each site to obtain a final concentration of $\sim$50 – 100 $\mu$M $^{15}N$-labeled nitrate. Nitrate was amended at this level to prevent its depletion before the last sampling time point (Valentine et al., 2016). Samples for $\delta^{15}$N analysis were preserved by filling a pre-vacuumed 12-ml exetainer vial with 0.1 ml 7M zinc chloride as a preservative. Another aliquot (~12 ml) of seawater for ammonium isotope analysis (see section 2.7.2) was filtered through 0.2 $\mu$m syringe filters and stored frozen. Prior to analyzing the samples in 12-ml exetainer vials, 5 mL of sample was replaced with ultra-high purity helium to create a headspace. The concentration and $\delta^{15}$N of dissolved N$_2$ and N$_2$O was determined using a Sercon CryoPrep gas concentration system interfaced to a Sercon 20-20 isotope-ratio mass spectrometer (IRMS) at the University of California Davis Stable Isotope Facility.

### 2.7.2 Ammonium Isotope Analyses

The production of $^{15}$NH$_4^+$ in seawater samples was measured using a method adapted from (Zhang et al., 2007) and described previously by (Peng et al., 2016). In brief, NH$_4^+$ was first
oxidized to NO$_2^-$ using hypobromite (BrO$^-$) and then reduced to N$_2$O using an acetic acid-azide working solution (Zhang et al., 2007). The $\delta^{15}$N of the produced N$_2$O was determined using an Elementar Americas PrecisION continuous flow, multicollector, isotope-ratio mass spectrometer coupled to an automated gas extraction system as described in (Charoenpong et al., 2014). Calibration and correction were performed as described in (Bourbonnais et al., 2017). The measurement precision was ±0.2 ‰ for $\delta^{15}$N. Depending on the in-situ ammonium concentration, the detection limit for total NH$_4^+$ production rates ranged between 0.006 and 0.0685 mmol m$^{-2}$ d$^{-1}$. 
3. Results

3.1 Bottom water conditions

Oxygen and nitrate concentrations in the bottom water, i.e., in the water surrounding the ROV during its operations at the seafloor and in the benthic flux chambers at $T_0$, respectively, along the transects can be seen in Table 1. $O_2$ concentration decreased with basin depth, with average concentrations of 9 and 10 µM at the shallowest stations (NDT3-D and SDT3-D, respectively) and concentrations below detection (<3 µM) at the deepest stations (NDRO and SDRO), which were confirmed to represent anoxia by different methods (see discussion section 4.1). Bottom water solute concentrations (as defined by the average time 0 concentration in BFC at each site) can be seen in Suppl. Figs. 1-4. Bottom water NO$_3^-$ concentrations decreased with station depth; NO$_3^-$ was 27 and 28 µM at NDT3-D and SDT3-D, respectively, and decreased to 19 and 10 µM at NDRO and SDRO, respectively. Bottom water NO$_2^-$ concentrations were below detection at all stations. Bottom water NH$_4^+$ concentrations were 9 µM at NDRO and 13 µM at SDRO and below detection in shallower stations. Bottom water PO$_4^{3-}$ concentrations showed similar trends to NH$_4^+$, with an average concentration of 1 and 2 µM at NDT3-D and SDT3-D stations, respectively, and 5 and 7 µM at NDRO and SDRO stations, respectively. Finally, Fe$^{2+}$ was 2 and 5 µM at the NDRO and SDRO stations, respectively. Bottom water Fe$^{2+}$ was below detection at all shallower stations.

3.2 Sediment characteristics

Photographs of sediment cores with a depth scale are shown below Table 1. Sediment colors were classified according to (Hossain et al., 2014). Cores from the shallowest (D) stations were uniformly reddish in color with small pockets of black. The sediment color changed with station
depth, transitioning from a reddish color in the shallowest stations to predominantly black with reddish lamination at the depocenter stations. The band of black sediment appeared at approx. 8 cm sediment depth in the C-station cores and became progressively more ubiquitous with station depth. Sediment cores from shallower stations (D and C stations) contained signs of bioturbation and, in some cases, contained visible macrofauna, such as polychaetes and mollusks. Deeper in

Table 1. Station details and photos of representative ROV push cores taken at each station. Mat presence (Y = yes, N = no) was determined visually. Station water depth and oxygen concentration were determined by sensors attached to ROV Jason. Anoxia was confirmed by additional methods (see discussion section 4.1). Latitude and longitude were determined by triangulation between the ROV and the ship. Bottom water nitrate concentration was derived from an average of benthic flux chamber nitrate measurements at time 0 for each station (chambers with no calculatable flux and 15N-nitrate addition excluded). Note, benthic flux chambers were not deployed at SDT1-A. Photographs show the sediment-water interface (SWI; top part) and each sediment core in full length (lower part).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NDT3-D</th>
<th>NDT3-C</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDRO</th>
<th>SDRO</th>
<th>SDT1-A</th>
<th>SDT3-A</th>
<th>SDT3-B</th>
<th>SDT3-C</th>
<th>SDT3-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mat Present</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>447</td>
<td>498</td>
<td>537</td>
<td>572</td>
<td>580</td>
<td>586</td>
<td>573</td>
<td>571</td>
<td>536</td>
<td>494</td>
<td>447</td>
</tr>
<tr>
<td>Longitude (°)</td>
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<td>-120.016</td>
<td>-120.019</td>
<td>-120.026</td>
<td>-120.031</td>
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<td>-120.116</td>
<td>-120.047</td>
<td>-120.053</td>
<td>-120.050</td>
<td>-120.052</td>
</tr>
<tr>
<td>Oxygen (µM)</td>
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<td>5.2</td>
<td>12.2</td>
<td>9.2</td>
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<td>0.0</td>
<td>0.0</td>
<td>1.8</td>
<td>3.1</td>
<td>9.6</td>
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<tr>
<td>Nitrate (µM)</td>
<td>27.3</td>
<td>26.0</td>
<td>11.5</td>
<td>24.4</td>
<td>18.5</td>
<td>9.9</td>
<td>20.4</td>
<td>20.6</td>
<td>16.3</td>
<td>28.0</td>
<td></td>
</tr>
</tbody>
</table>

the basin (A and depocenter stations) no signs of bioturbation were detected, and the sediment-water interface was colonized by patches of white GSOB mats. Spherical cells (given the
moniker ‘ghost balls’) were found mixed amongst giant sulfur bacteria filaments within the top 0-1 cm of sediment at NDRO (Suppl. Fig. 5). These unknown species had similar morphological characteristics to the species *Thiomargarita namibiensis* (Schulz et al., 1999; Schulz and Schulz, 2005) containing a translucent cell with sulfur granules giving them a ghostly white appearance. A small sample of cells were measured, with a diameter that ranged from 48.0 – 99.6 µm (n = 8), amounting to an average biovolume of 2.5 x 10^5 µm^3, compared to *T. namibiensis* with a cell diameter up to 700 µm (Schulz et al., 1999).

### Table 2. Sediment solid phase data: porosity, density, total organic carbon (TOC), total organic nitrogen (TON), C:N ratio, and δ^{13}C. All data were averaged for the top 0-19 cm sediment, except NDT3-C (17 cm), NDT3-A (11 cm) and SDRO (7 cm), where the core length was shorter. Integrated sulfate reduction rates (iSRR) were integrated over 0-14 cm sediment depth. No sulfate reduction rates are available for NDT3-B, SDT3-A, and SDT3-B; rates were not integrated for SDRO due to missing surface samples.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NDT3-D</th>
<th>NDT3-C</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
<th>NDT3-B</th>
<th>NDT3-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porosity</td>
<td>0.79 ± 0.03</td>
<td>0.81 ± 0.04</td>
<td>0.86 ± 0.04</td>
<td>0.88 ± 0.03</td>
<td>0.88 ± 0.04</td>
<td>0.87 ± 0.03</td>
<td>0.88 ± 0.03</td>
<td>0.86 ± 0.04</td>
<td>0.85 ± 0.04</td>
<td>0.82 ± 0.04</td>
<td>0.78 ± 0.04</td>
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</tr>
<tr>
<td>Density (g cm^-3)</td>
<td>1.21 ± 0.07</td>
<td>1.16 ± 0.08</td>
<td>1.06 ± 0.08</td>
<td>1.05 ± 0.04</td>
<td>1.06 ± 0.03</td>
<td>1.04 ± 0.03</td>
<td>1.11 ± 0.23</td>
<td>1.05 ± 0.05</td>
<td>1.12 ± 0.06</td>
<td>1.22 ± 0.05</td>
<td>1.22 ± 0.03</td>
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</tr>
<tr>
<td>TOC (%)</td>
<td>3.67 ± 0.00</td>
<td>3.89 ± 0.01</td>
<td>4.65 ± 0.01</td>
<td>5.23 ± 0.01</td>
<td>5.05 ± 0.01</td>
<td>5.34 ± 0.01</td>
<td>5.23 ± 0.01</td>
<td>5.83 ± 0.01</td>
<td>5.05 ± 0.01</td>
<td>4.53 ± 0.01</td>
<td>4.13 ± 0.00</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TON (%)</td>
<td>0.41 ± 0.00</td>
<td>0.45 ± 0.00</td>
<td>0.55 ± 0.00</td>
<td>0.62 ± 0.00</td>
<td>0.62 ± 0.00</td>
<td>0.66 ± 0.00</td>
<td>0.61 ± 0.00</td>
<td>0.71 ± 0.00</td>
<td>0.61 ± 0.00</td>
<td>0.52 ± 0.00</td>
<td>0.49 ± 0.00</td>
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</tr>
<tr>
<td>C:N Ratio</td>
<td>8.9 ± 0.2</td>
<td>8.7 ± 0.5</td>
<td>8.5 ± 0.5</td>
<td>8.2 ± 0.2</td>
<td>8.2 ± 0.4</td>
<td>8.0 ± 0.2</td>
<td>8.6 ± 0.8</td>
<td>8.3 ± 0.6</td>
<td>8.3 ± 0.3</td>
<td>8.7 ± 0.3</td>
<td>8.5 ± 0.2</td>
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<tr>
<td>δ^{13}C (%)</td>
<td>-22.4 ± 0.3</td>
<td>-22.4 ± 0.4</td>
<td>-22.2 ± 0.4</td>
<td>-22.1 ± 0.2</td>
<td>-22.1 ± 0.2</td>
<td>-22.0 ± 0.3</td>
<td>-21.3 ± 0.7</td>
<td>-22.1 ± 0.4</td>
<td>-22.0 ± 0.2</td>
<td>-21.9 ± 0.2</td>
<td>-22.0 ± 0.1</td>
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</tr>
<tr>
<td>iSRR (mmol m^-2 d^-1)</td>
<td>2.9</td>
<td>3.8</td>
<td>2.7</td>
<td>4.1</td>
<td>2.9</td>
<td>1.7</td>
<td>1.9</td>
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</table>

B station cores contained sporadic filaments slightly deeper in the sediment (approx.. 2-4 cm sediment depth). Depocenter and A-station sediment was laminated throughout the entire core length, B-station sediment showed some lamination below the first 4 cm, but sediment from shallower stations had little (C station) to no signs of lamination (D station). Sediment solid phase parameters (averaged over the entire sediment core depth) can be seen in Table 2. Average sediment porosity increased with basin depth (e.g., from 0.79 at NDT3-D to 0.88 at NDRO), TOC and TON increased slightly with station depth (from 3.67% TOC and 0.41 TON at NDT3-D to 5.34% and 0.66% TON at SDRO) while the C/N ratio and the δ^{13}C isotopic signature of
organic carbon remained relatively constant (8.0 – 8.7 and 21.3 – 22.4 ‰, respectively) over all stations.

3.3 Sediment porewater geochemistry

Geochemical parameters of sediment porewater are shown in Fig. 2 (northern stations) and 3 (southern stations). Total alkalinity (Figs. 2 A-E & 3 A-F) increased steadily with sediment depth at all stations starting with, on average, 2.4 mM in the core supernatant reaching a maximum at the respective deepest sediment sample (20 cm). Total alkalinity also increased with basin depth; cores in the depocenter exhibited higher total alkalinity (max. 9.6 mM at NDRO) than D-stations (max. 3.4 mM at NDT3-D). Porewater DIC (Figs. 2 A-E & 3 A-F) showed patterns very similar to total alkalinity, indicating that total alkalinity was dominated by the carbonate system.

Porewater DIC was, on average, 2.2 mM in the core supernatant and reached maximum concentrations at the deepest sediment depth (20 cm) at most stations. Porewater DIC also increased with basin depth; depocenter sediment had higher DIC concentrations (max. 8.3 mM at NDRO) than D-station (max. 2.8 mM at NDT3-D).

Porewater PO$_4^{3-}$ profiles (Figs. 2 A-E & 3 A-F) were markedly different between the depocenter and shallower C and D stations. PO$_4^{3-}$ concentration increased by approx. one order of magnitude between the core supernatant and the 0-1 cm section at most stations (e.g., 7 and 48 µM at NDRO, respectively). Porewater PO$_4^{3-}$ concentrations in the depocenter and A stations generally increased with sediment depth but several profiles (NDT3-C, NDT3-A, SDRO, SDT1-A) remained unchanged or decreased deeper in the sediment (starting at approx. 10 cm). The profiles in C and D stations showed a peak in PO$_4^{3-}$ concentrations near the sediment-water
interface, particularly in the northern basin. Below 2 cm, PO$_4^{3-}$ decreased with sediment depth, but sometimes showed a second small peak deeper in the sediment (12-14 cm at NDT3-D and 10-12 cm at SDT3-D).

Porewater NH$_4^+$ concentrations (Figs 2 & 3 A-E) showed trends often similar to alkalinity and DIC; NH$_4^+$ concentrations increased downcore and were higher at depocenter than at D stations (e.g., 370 and 91 µM at 20 cm for SDRO and SDT3-D, respectively). NH$_4^+$ concentration increased by approx. one order of magnitude between the core supernatant and the 0-1 cm section at most stations (e.g., 16 µM and 139 µM at NDRO, respectively). Supernatant NH$_4^+$ was below detection at D and C stations (and SDT3-B) and above 10 µM at the deeper A (except SDT1-A) and depocenter stations. NO$_2^-$ and NO$_3^-$ were at or near zero concentration below 2 cm at every station. NO$_3^-$ concentration decreased substantially in the transition from core supernatant to the 0-1 cm section at most stations (e.g., 24 µM and 0 µM at NDT3-D, respectively). Sediment cores from SDRO and NDT3-A stations exhibited strong peaks in NO$_3^-$ (376 and 81 µM, respectively) and NO$_2^-$ (37 and 5 µM, respectively) in the top 1 cm.

Porewater Fe$^{2+}$ concentrations (Figs. 2 F-J & 3 G-L) were several orders of magnitude higher at shallow D stations (max. 722 and 395 µM at NDT3-D and SDT3-D, respectively) compared to depocenter stations (max. 13 and 51 µM at NDRO and SDRO, respectively). Fe$^{2+}$ concentration increased by approx. one order of magnitude between the core supernatant and the 0-1 cm section at all stations (e.g., 16 µM and 139 µM at NDRO, respectively). Concentrations reached a max. at 0-2 cm and declined sharply with depth in depocenter and A-station sediment. Northern basin sediment was similar, but the decline in Fe$^{2+}$ below 0-2 cm was less pronounced.
Max. porewater sulfide concentrations (Figs. 2 F-J & 3 G-L) were several orders of magnitude lower at shallow D stations (5 and 4 µM at NDT3-D and SDT3-D, respectively) compared to A stations (350 and 148 µM at NDT3-A and SDT1-A, respectively). Unlike Fe$^{2+}$, peaks in sulfide concentration occurred deeper in the sediment (e.g., below 5 cm depth at A stations). Porewater sulfate concentrations (Figs. 2 K-O & 3 M-R) decreased slightly with depth, but never reached values below 20 mM at any station.

### 3.4 In-situ microprofiling

Microprofiler O$_2$ and sulfide measurements are shown in Fig. 4. Oxygen was rapidly consumed within the first 0-1 cm of sediment at every station where O$_2$ was detected in the bottom water (i.e., at all stations except NDRO, SDRO, and SDT3-A, which were anoxic). Sulfide concentrations from microsensors showed similar trends to spectrophotometric measurements, albeit with different absolute values (e.g., 196 and 808 µM sulfide at the 7 cm depth at NDT3-A using the cline method and microprofilers, respectively). Sulfide was below detection of the microprofilers in shallower stations (B, C, and D), all of which lacked GSOB mats. The A and depocenter stations had high sulfide concentrations (>1,000 µM) in the sediment underlying GSOB mats, which rapidly decreased towards zero near the sediment-water interface.

Microprofiler pH (Fig. 4) was near 7.5 in the bottom water at all stations, and slowly decreased...
Figure 2. Biogeochemical data from ROV sediment push cores collected at stations on the northern transect (NDT3) and in the northern depocenter (NDRO): total alkalinity (TA), dissolved inorganic carbon (DIC), ammonium (NH$_4^+$), phosphate (PO$_4^{3-}$) in the first row; nitrate (NO$_3^-$), total sulfide (sulfide), and iron (II) (Fe$^{2+}$) in the second row; sulfate (SO$_4^{2-}$) and bacterial sulfate reduction rate (SRR) in the third row. Data analyzed from sediment core supernatant are plotted at -1 cm sediment depth; the dotted line connotes the sediment-water interface. Note the change in scale on the primary x-axis in panel I and the change in scale of the secondary x-axis in panels F and I. No sulfide and SRR data are available for NDT3-B. For station details see Fig. 1 and Table 1.
Figure 3. Biogeochemical data from ROV sediment push cores collected at stations on the two southern transects (SDT1 and SDT3) and the southern depocenter (SDRO): total alkalinity (TA), dissolved inorganic carbon (DIC), ammonium (NH₄⁺), phosphate (PO₄³⁻) in the first row; nitrate (NO₃⁻), total sulfide (sulfide), and iron (II) (Fe²⁺) in the second row; sulfate (SO₄²⁻) and bacterial sulfate reduction rate (SRR) in the third row. Data analyzed from sediment core supernatant are plotted at -1 cm sediment depth; the dotted line connotes the sediment-water interface. Note the change in scale on the primary x-axis in panel L and the change in scale of the secondary x-axis in panel G. No sulfide and SRR data are available for SDT3-B and -A; at SDRO, the top 0-4 cm of sulfate reduction data are not available. For station details see Fig. 1 and Table 1.
to near 7.0 within the top 20 cm of sediment at most stations. At NDT3-C and SDT3-D, pH exhibited no discernable trend over depth with values remaining around pH 7.5.

Figure 4. In-situ sediment microprofiler results for all stations (except SDT1-A): oxygen (O$_2$) and total sulfide (sulfide) concentration in the first row; pH profiles in the second row. Note the change in scale on the secondary x-axis for NDRO sulfide. Values determined in the overlying water are plotted at negative sediment depths; the dotted line connotes the sediment-water interface.

3.5 In-situ fluxes of benthic solutes

NO$_3^-$, NH$_4^+$, PO$_4^{3-}$, and Fe$^{2+}$ flux measured in the BFC revealed different patterns of uptake and release from the sediment throughout the basin (Fig. 5 and Suppl. Figs. 1-4). BFC O$_2$ concentrations were compromised by O$_2$ release from the chamber’s polycarbonate walls, which prevented an accurate calculation of O$_2$ fluxes from sensor data. NO$_3^-$ was consumed at all stations as indicated by a negative flux (i.e., a flux into the sediment). On the contrary, benthic release (i.e., a flux out of the sediment) was observed for all other analyzed solutes (NH$_4^+$, PO$_4^{3-}$, Fe$^{2+}$).
25 and Fe$^{2+}$), with the lowest fluxes in the shallow D and C-stations and highest fluxes in the depocenter. Ammonium fluxes were the highest of all the determined solutes and showed the largest difference between deep and shallow stations, with a flux of 1.6 mmol m$^{-2}$ d$^{-1}$ at NDT3-C (there were no measurable NH$_4^+$ fluxes in D-station chambers) and reaching 11.1 ± 3.1 mmol m$^{-2}$ d$^{-1}$ (n = 3) at the two depocenter stations. The depocenter ammonium flux far-outpaced the concomitant flux of nitrate into depocenter sediments (3.2 ± 0.7 mmol m$^{-2}$ d$^{-1}$, n = 3). Iron and phosphate fluxes were similar at depocenter stations (4.1 ± 0.7, n = 3, and 3.2 ± 0.7, n = 3, mmol m$^{-2}$ d$^{-1}$, respectively) but there was no discernable trend between the two solutes at shallower stations. Results from BFCs injected with $^{15}$N-NO$_3^-$ at the SDRO and NDT3-D station are shown in Fig. 6. The rates of denitrification, anammox, and N$_2$O production were higher at SDRO compared to NDT3-D. $^{15}$NH$_4^+$ production (DNRA) was one order of magnitude higher at the SDRO station (2.674 mmol m$^{-2}$ d$^{-1}$) compared to the NDT3-D station (0.140 mmol m$^{-2}$ d$^{-1}$). DNRA accounted for a much higher percentage of NO$_3^-$ reduction at SDRO (54.1%) than NDT3-D (13.3%).

### 3.6 Sulfate reduction rates

Vertical profiles of bacterial sulfate reduction as determined by the radioisotope technique differed throughout the basin (Figs. 2 & 3). Peaks in sulfate reduction (120.2, 151.0, 85.3 nmol cm$^{-3}$ d$^{-1}$) were seen in the top 0-1 cm of sediment at stations with a visible GSOB mat on the surface (NDRO, SDT1-A, NDT3-A, respectively). Sediments at most shallower basin depths (SDT3-C, NDT3-D, and SDT3-D) exhibited peaks slightly deeper in the sediment and of lower magnitude (25.5, 44.5, 22.5 nmol cm$^{-3}$ d$^{-1}$ respectively). NDT3-C had no visible GSOB mats present but also exhibited a peak (133.7 nmol cm$^{-3}$ d$^{-1}$) in sulfate reduction at 0-1 cm depth.
Figure 5. Benthic fluxes of solutes (positive flux = release from the seafloor; negative flux = uptake by the seafloor) determined with in-situ benthic flux chambers. Rates were averaged for stations of same depth from the northern and southern transect and the depocenter (NDRO and SDRO). Error bars represent standard errors.

Figure 6. Areal rates of total N2 production, denitrification, anammox, NH4+ production (DNRA), and N2O production.

Sulfate reduction rates at depocenter and A stations decreased sharply below 0-1 cm sediment depth (up to an order of magnitude decrease at NDRO and SDT1-A), while remaining low and
steady over depth in C and D station sediment. Integrated sulfate reduction rate (0-14 cm depth) at NDRO (4.1 mmol m$^{-2}$ d$^{-1}$) was noticeably higher than most other stations with the exception of NDT3-C (3.8 mmol m$^{-2}$ d$^{-1}$) (Table 2). NDT3-D and NDT3-C exhibited higher integrated rates (2.9 and 3.8 mmol m$^{-2}$ d$^{-1}$) than their southern station counterparts SDT3-D and SDT3-C (1.9 and 1.7 mmol m$^{-2}$ d$^{-1}$).
4. Discussion

4.1 Giant sulfur-oxidizing bacterial mats proliferated in response to deoxygenation in the Santa Barbara Basin

The SBB is an ideal environment to study the effect of transient deoxygenation on benthic biogeochemistry. In November 2019, the SBB was undergoing a transition from oxygenated to anoxic conditions (Qin et al., 2022). When the AT42-19 cruise occurred, most of the bottom water in the basin was hypoxic (B, C, and D stations), except for the depositional center and the A stations. Importantly, separate O\textsubscript{2} measurements from the ROV sensor (Table 1), microprofiler (Fig. 4), and Winkler titration from CTD/rosette casts (Qin et al., 2022) indicated full anoxia in the bottom water at these deeper stations. Notably, bottom water conditions revealed a slight asymmetry between the basin transects (Fig. 1); bottom water along the northern transect had generally more O\textsubscript{2} and NO\textsubscript{3}\textsuperscript{-} than the southern transect (e.g., 9 µM at NDT3-A and 0 µM at SDT3-A). This asymmetry indicated differences in the circulation and/or microbial communities between the northern and southern portions of the basin. Whether this asymmetry is a permanent feature of the basin or symptomatic of the specific conditions in November 2019 is unclear; previous studies in the SBB have been restricted to the depocenter or one side of the basin (Sholkovitz, 1973; Reimers et al., 1996; Kuwabara et al., 1999). Regardless of bottom water oxidant concentration, the energetically most powerful terminal electron acceptors (O\textsubscript{2} and NO\textsubscript{3}\textsuperscript{-}) disappeared in a very narrow zone below the sediment-water interface (Fig. 4 and Figs. 2 and 3, respectively), consistent with their expected rapid consumption by the benthic microbial community.
The sediment-water interface represents the front-line in a battle between microbes for powerful electron acceptors. In the present study, benthic GSOB mats were primarily limited to the anoxic depocenter of the SBB. Similarly, such mats were replete in the core of the anoxic Peruvian OMZ (Levin et al., 2002; Sommer et al., 2016; Mosch et al., 2012), but absent from the seafloor below the hypoxic, i.e., slightly oxygenated, Mauritanian OMZ (Schroller-Lomnitz et al., 2019).

GSOB mats in November 2019 were observed deeper in the basin than in October 2013 (Valentine et al., 2016) but in a similar location to June 1988 (Reimers et al., 1996) and April 1997 (Kuwabara et al., 1999). During the 2013 sampling, dense GSOB mats were confined to depths between approx. 500-570 m (equivalent to the B stations from this expedition), corresponding with anoxic conditions in the bottom water. This habitat was sandwiched between an anoxic, anitric (i.e., nitrate-free) deep and a hypoxic, nitrogenated (i.e., nitrate-rich) shallower water layer. The difference in depth distribution between the 2013 and 2019 expedition provides evidence that GSOB mats in the SBB are ephemeral and proliferate where the bottom water is anoxic but not anitric.

As our study represents only a snapshot of an oxygen- and nitrate-driven mat dynamic, we can only speculate how areas of the basin that did not contain GSOB mats in November 2019 fit into this dynamic. For example, mat-forming sulfur bacteria found slightly deeper in the sediment at B stations (see section 3.2) could be progenitors to surface sediment colonization of thick GSOB mats, as it has been recorded in other transiently deoxygenated environments (Jørgensen, 1977).

Alternatively, these subsurface colonies could also be remnants of a former surface GSOB mat that retreated under changing redox conditions. Oxygenated conditions in the water preceding the 2019 expedition would, in this context, mean the mats migrated following a previous anoxic...
event (Qin et al., 2022). If deoxygenation persisted in the SBB after the AT42-19 cruise, then
anitria (i.e., anitric conditions - similar to anoxia) would likely follow in the deepest basin water.
These conditions would be similar to those seen during the 2013 expedition by D. Valentine and
co-workers (Valentine et al., 2016), where GSOB mats formed a contiguous “donut ring” at
shallower basin depths.

4.2 Shift from benthic denitrification to dissimilatory nitrate reduction to ammonium in
response to complete deoxygenation in the Santa Barbara Basin

Benthic release and uptake of nitrogen species by SBB sediment appeared to be affected by the
presence of GSOB mats. While benthic nitrate consumption was evident at all stations based on
porewater and in-situ flux measurements, NH$_4^+$ release from the sediment into the water column
increased with station depth (Fig. 5). This trend is supported by the porewater profiles of NH$_4^+$,
which tend to increase in steepness with station depth (Figs. 2 & 3). Incubations with $^{15}$N-NO$_3^-$
revealed that N$_2$ production via denitrification and anammox accounted for 86% of NO$_3^-$ removal
in the shallow basin (NDT3-D, Fig. 6). Conversely 54% of NO$_3^-$ removal at the depositional
center (SDRO) occurred via DNRA (X. Peng et al., to be submitted to this special issue). This
data suggests a transition from denitrification-dominated sediment in the oxygenated basin to an
increasing influence of DNRA on N-cycling in the deeper, anoxic basin. Placed in the context of
other OMZs, nitrate consumption in the shallow SBB sediment (below hypoxic water) was
similar to Mauritanian shelf sediments (Dale et al., 2014), while nitrate consumption in the
sediment of the deeper SBB (below anoxic water) was similar to anoxic Baltic Sea basins (Dale
et al., 2011) and the core Peruvian OMZ (Sommer et al., 2016).
Declining nitrate concentrations may be as important as anoxia itself to GSOB mat proliferation. High ratios of electron donor (organic matter or sulfide) to electron acceptor (nitrate) encourage DNRA over denitrification (Marchant et al., 2014). In these nitrate-deficient areas in the ocean, DNRA linked to anaerobic ammonium oxidation (anammox) can be more thermodynamically favorable and thus organisms that participate in this consortium could have a competitive advantage over other denitrifying organisms (Jensen et al., 2011). NO$_2^-$ was at or near-zero concentrations in the sediment porewater of all stations, except in the 0-1 cm depth interval of two stations (SDRO and NDT3-A) featuring GSOB mats (Suppl. Table 1). These spikes in NO$_2^-$ (and more pronounced, NO$_3^-$) are likely caused by the bursting of GSOB vacuoles during centrifugation (Reimers et al., 1996). GSOB mats can be associated with symbiotic anaerobic ammonium-oxidizing (annamox) bacteria (Prokopenko et al., 2006). Since the annamox process utilizes NO$_3^-$ as the electron acceptor and NH$_4^+$ as the electron donor, a consortium of GSOB mats and annamox bacteria were possible given the right conditions for proliferation in the SBB depocenter. Anoxia could trigger the uptake of NO$_3^-$ into bacterial vacuoles, creating declining NO$_3^-$ concentrations that are more favorable to DNRA in the bottom water. In these conditions, GSOB in the SBB benthic environment could utilize their ability to perform DNRA to out-compete similar denitrifying taxa, and proliferate into thick, contiguous mats. While low-nitrate conditions could benefit GSOB mats, the mats do not persist once bottom water reaches anitria, as evidenced by GSOB mat distribution during the 2013 expedition (Valentine et al., 2016).
4.3 Microbial mat proliferation and benthic phosphate remineralization dependent on high rates of organic matter degradation in the Santa Barbara Basin

Organic carbon delivery to the benthic environment appears to be a key control on sulfate reduction rates near the sediment-water interface as well as microbial mat proliferation. Sulfate reduction rates in the SBB depocenter are most similar in magnitude and profile (i.e., highest rates found at the sediment-water interface and decline drastically thereafter) to those found in sediments below the transiently deoxygenated portion of the Peruvian shelf (e.g., 4.1 mmol m$^{-2}$ d$^{-1}$ at the SBB NDRO station vs. 2.5-3.8 mmol m$^{-2}$ d$^{-1}$ at 128-144 m water depth on the Peruvian margin (Gier et al., 2016; Treude et al., 2021)). The TOC content of surface sediments in these two regions are both high and within the same order of magnitude; 5.3% in SBB depocenter compared with 7.6% in the Peruvian margin 145 m depth (Noffke et al., 2012). In comparison, sulfate reduction rates in the SBB were at least one order of magnitude lower than found in sediment below the OMZ on the Namibian Shelf, which has much higher TOC contents of >10% (Brüchert et al., 2003; Bremner, 1981). Sulfate reduction rates in the shelf sediments below the Eastern Arabian OMZ were an order of magnitude lower (0.18 – 1.27 mmol m$^{-2}$ d$^{-1}$) than rates in the SBB depocenter (Naik et al., 2017) despite similar hypoxic to anoxic bottom water conditions. These lower rates were attributed to the relatively low benthic organic matter delivery compared to other transiently deoxygenated systems (surface sediment TOC content approx. 2 – 3%) (Naik et al., 2017). Sedimentary organic matter content appears to be important in the proliferation of GSOB mats; too much TOC could result in toxic levels of sulfide at the sediment-water interface (*Beggiatoa* exhibit an aversion to sulfidic sediments but toxicity has not...
been quantified, (Preisler et al., 2007)) whereas too little sulfide would not provide enough electron donor for the GSOB’s chemoautotrophic metabolism.

The profiles of several indicators for benthic anaerobic organic matter remineralization (total alkalinity, DIC, PO$_4^{3-}$, NH$_4^+$) increased in steepness with increasing water depth (Figs. 2 A-E & 3A-F). One divergence from this trend can be seen in PO$_4^{3-}$ profiles from the shallow C and D stations, which also featured low rates of sulfate reduction. PO$_4^{3-}$ profiles in these sediment track closely to Fe$^{2+}$ profiles; both solutes dip in concentration in areas with visible iron sulfide formation (e.g., 5-11 cm in NDT3-D as seen in Fig. 2A). Additionally, several stations that exhibited high sulfate reduction rates in surface sediment (e.g., SDT1-A) showed almost no change in PO$_4^{3-}$ at depths below 5 cm (e.g., Fig. 2 K-O compared to Fig. 2 A-E). This phenomenon has been previously documented in SBB sediment and is attributed to the precipitation of carbonate fluorapatite (Reimers et al., 1996). The confinement of these flat PO$_4^{3-}$ profiles to stations with >100 nmol cm$^{-3}$ d$^{-1}$ sulfate reduction in surface sediment suggests that this mineralogical sink of PO$_4^{3-}$ in SBB sediment may be dependent on high sulfate reduction rates, owing to the bicarbonate produced by sulfate reduction (Reimers et al., 1996), and is not found throughout the basin. Flat PO$_4^{3-}$ profiles were also reported from the transiently deoxygenated portion of the Peruvian OMZ, where phosphate mineral precipitation has been documented (Noffke et al., 2012). Similar to the shallow margins of the SBB, PO$_4^{3-}$ in Mauritanian OMZ porewater tracks closely with changes in porewater Fe$^{2+}$ (Schroller-Lomnitz et al., 2019), indicating that iron mineralization/dissolution mechanics hold a greater influence on PO$_4^{3-}$ concentrations under hypoxic bottom waters.
4.3 Iron cycling dictates the location of sulfate reduction in Santa Barbara Basin sediment.

While low or absent oxygen concentrations in the bottom water appeared to facilitate sulfate reduction close to the sediment-water interface in the SBB (e.g., NDRO and NDT3-A as seen in fig. 2N and 2O), iron sulfide formation in deeper sediment layers may also play a crucial role in controlling the distribution of sulfate reduction. SBB sediments showed a wide vertical and horizontal heterogeneity of redox states based on visual appearance (Fig. 1A-K). Sediment beneath the hypoxic bottom water at the shallowest D stations was reddish, consistent with a high content of iron oxides. Interestingly, porewater Fe\(^{2+}\) concentrations in shallower parts of the basin (e.g., NDT3-D, max. \(~700 \mu M\) Fe\(^{2+}\)) were an order of magnitude larger than those found anywhere in both the Peruvian (max. \(~60\) and \(~30 \mu M\) Fe\(^{2+}\), respectively; Noffke et al., 2012; Plass et al., 2020) and Mauritanian (max. \(~50 \mu M\) Fe\(^{2+}\); Schroller-Lomnitz et al. 2019) OMZ.

Deeper in the basin, bands of black sediment that appear mid-core at NDT3-C (6-14 cm) and SDT3-C (6-10 cm) indicate the formation of iron sulfides as a result of sulfide produced by sulfate reduction (Canfield, 1989). At the C stations, peaks in sulfate reduction were located in the surface sediment, above the iron sulfide layers, and declined below approx. 4 cm, indicating a discrepancy between observed peak sulfate reduction activity and the mineralogical clues left behind by the process.

Comparing data from shallow stations at the same depth (e.g., NDT3-C vs. SDT3-C) revealed differences in sediment sulfate reduction in the SBB, potentially due to changes in iron mineralogy. Both D-stations had similar bottom water conditions (Table 1), sulfate reduction rates (Fig. 3W-AG), porewater concentrations of solutes (Figs. 2 and 3), and visual sediment
characteristics (Section 3.1). On the contrary, there are some noticeable differences in the porewater geochemistry between the two C-stations, which in contrast to D-stations are below the SBB sill depth, irrespective of similar bottom water oxygen conditions. NDT3-C porewater Fe$^{2+}$ concentration (Fig. 2G) peaked in the top 1 cm of sediment (similar to deeper stations) while SDT3-C porewater Fe$^{2+}$ concentration (Fig. 3H) peaked around 5-cm sediment depth. NDT3-C sediment (Table 1B) exhibited iron sulfide formation from approx. 6-14 cm sediment depth, while SDT3-C sediment (Table 1J) had a much narrower band around 8-10 cm (the same depth at which Fe$^{2+}$ concentrations decrease in the porewater). Finally, the sulfate reduction rate at NDT3-C (Fig. 2L) mirrored deeper station profiles (e.g., NDRO in Fig. 2O) rather than other shallow station profiles (e.g., SDT3-C in Fig. 3N). While sulfate reduction rates for B-stations are absent, porewater Fe$^{2+}$ profiles show a similar difference between the north and south basin (Fig. 2H compared to Fig. 3I) as did visual sediment characteristics (Table 1C compared to 1I). This difference in biogeochemical profiles and apparent minerology between the north and south C and B stations could be a result of hydrographic and/or bathymetric differences in the basin (Sholkovitz and Gieskes, 1971; Bograd et al., 2002), but a discernable link between the differences in sediment biogeochemistry and the differences in bottom water oxygen (Table 1) need to be further explored.

Deeper in the basin (depocenter and A-stations), porewater Fe$^{2+}$ concentrations in sediment beneath anoxic bottom water (max. 84 µM Fe$^{2+}$) were similar to concentrations found below the Peruvian OMZ in 2008 under anoxic bottom water conditions (78 m water depth, max. 80 µM Fe$^{2+}$) (Noffke et al., 2012). SBB porewater Fe$^{2+}$ concentrations were an order of magnitude larger than those found at a similar site on the Peruvian shelf (75 m water depth, max. 1 µM
Fe$^{2+}$ in 2017 during a kelvin-wave-associated “Coastal El Niño” event that created oxygenated bottom waters during the sampling and the disappearance of previously observed dense GSOB mats (Plass et al., 2020). As the SBB water column was undergoing rapid deoxygenation in the weeks preceding this study (Qin et al., 2022), the sediments below the sill appeared to be actively shifting from a ferruginous state to a sulfidic state, with this change starting around the C stations and being complete in the depocenter. Comparing apparent iron sulfide formation with dips in porewater Fe$^{2+}$ concentrations in C-station profiles (Fig. 1B compared to Fig. 2G and Fig. 1J compared to Fig. 3H) signals a shift away from a ferruginous state occurring just below the SBB sill.

C-station porewater Fe$^{2+}$ concentrations and sulfate reduction rates indicate that migration of the sulfate reduction zone towards the sediment-water interface is associated with iron sulfide formation deeper in the sediment. The activity of cable bacteria, which are able to bridge the gap between the oxidized sediment-water interface and reduced sediment below using a biofilament (Pfeffer et al., 2012), could explain the interplay between sulfate reduction and iron cycling in SBB sediments. Cable bacteria contain the genes required for DNRA (Kjeldsen et al., 2019) and can perform nitrate reduction in incubation experiments (Marzocchi et al., 2014), but their direct transformation of NO$_3^-$ in the environment appears limited (Kessler et al., 2019) and they appear to be inactive in anoxic aquatic environments (Seitaj et al., 2015; Marzocchi et al., 2018). Cable bacteria primarily conduct aerobic sulfide oxidation (Pfeffer et al., 2012), though they can also utilize Fe$^{2+}$ as an electron donor (Seitaj et al., 2015). The maximum recorded filament length of cable bacteria is 7 cm (Van De Velde et al., 2016), roughly in line with the appearance of black sediment in the SBB C-station sediments. Further, cable bacteria have been found to directly
compete with GSOB in transiently deoxygenated systems, with cable bacteria active under oxygenated conditions and GSOB active in anoxic conditions (Seitaj et al., 2015). Cable bacteria can also prevent the formation of euxinic conditions at the sediment-water interface via the creation of an iron-oxide buffer (formed through Fe$^{2+}$ oxidation) in near-surface sediments (Seitaj et al., 2015). Therefore, if cable bacteria activity in the SBB decreased with declining oxygen concentrations below the sill, their iron oxide buffer could have been reduced, encouraging the sulfate reduction zone to migrate towards the sediment surface (as seen at NDT3-C). The presence of cable bacteria can be detected by a slight increase in pH below the sediment-water interface (typically pH > 8) (Schauer et al., 2014), which was not reflected in our pH results, but forthcoming DNA analyses from these sediments should elucidate the role cable bacteria play in the SBB. Additionally, forthcoming sequential iron and sulfur extractions from SBB sediments should provide more information about the differences in early diagenesis throughout the basin.

4.4 Iron and phosphate flux into SBB bottom water is a feature of transient deoxygenation.

As postulated previously (Kuwabara et al., 1999), oxidation of iron sulfides at the sediment-water interface upon basin flushing with oxygen likely encourages microbial iron reduction following later deoxygenation. This iron reduction causes strong Fe$^{2+}$ and PO$_4^{3-}$ fluxes out of SBB depocenter sediment. High porewater Fe$^{2+}$ concentration (>100 µM) in the SBB D stations (Figs. 3L and V) indicate regions of prolific bacterial iron reduction in the sediment. As basin depth increases and oxygen concentration in the water decreases, the zone of iron reduction thins and is found closer to the sediment-water interface (Figs. 2 & 3). Further, high benthic fluxes of
Fe²⁺ and PO₄³⁻ were observed in the transiently anoxic depocenter (Fig. 5). High Fe²⁺ and PO₄³⁻ fluxes were also seen on the Peruvian shelf during transient anoxia (Noffke et al., 2012). The release of these solutes was interpreted to be sourced from a layer of reactive iron hydroxides existing near the sediment surface, likely established during a recent oxygenation event. Similar conditions, i.e., visibly oxidized (reddish) sediment laminae and a thin zone of iron reduction apparent from a peak in Fe²⁺ at the sediment-water interface, were found in sediment from the SBB depocenter. These analogous observations highlight the importance of alternating redox conditions to establish high benthic iron fluxes. On the contrary, the persistently anoxic core of the Peruvian OMZ appears to have little to no benthic flux of Fe²⁺ and PO₄³⁻ into the bottom water (Noffke et al., 2012). Here, iron at the sediment-water interface is hypothesized to be locked up in iron sulfides, which are rarely re-oxidized due to persistent anoxia.

It is further notable that benthic fluxes of Fe²⁺ and PO₄³⁻ from the SBB depocenter were approx. five times higher (Fig. 5) compared to the Peruvian shelf (4.9 vs. 0.9 mmol Fe²⁺ m⁻² d⁻¹ and 3.6 vs. 0.8 mmol PO₄³⁻ m⁻² d⁻¹, respectively) (Noffke et al., 2012). Based on Fe²⁺ profiles, the zone of iron reduction in Peruvian shelf sediments extended down to approx. 10 cm, while the zone appeared to be much shallower and narrower (less than the top 5 cm) in the SBB depocenter. These differences in magnitude of Fe²⁺ concentration and Fe²⁺ and PO₄³⁻ flux between the SBB depocenter and the Peruvian shelf could be attributed to differences in the recency and magnitude of reoxygenation events. The release of Fe²⁺ from sediment into the bottom water could create a buffer against reoxygenation in transiently deoxygenated systems, giving a competitive advantage to anaerobic benthic metabolisms (Dale et al., 2013; Wallmann et al., 2022). Additionally, both Fe²⁺ and PO₄³⁻ release from the SBB sediment could allow for higher
rates of primary productivity if those constituents diffused into the photic zone (Robinson et al., 2022). The fate of Fe$^{2+}$ and PO$_4^{3-}$ diffusing into SBB waters from the sediment-water interface is a focus of ongoing work within the basin.

5 Conclusions

This research expands upon the wealth of science already conducted in the SBB and other transiently deoxygenated environments by examining changes in benthic biogeochemistry promoted by the onset of anoxia. We found that GSOB mats proliferate in the SBB where the bottom water is anoxic and nitrate concentrations are declining, initiating a shift from primarily denitrification to an increase in DNRA. We conclude that GSOB mat proliferation in the SBB is confined to areas of the benthic environment with anoxic (though not completely anitric) bottom water. The sulfate reduction zone is elevated to the surface sediments underneath theses mats, but mat presence alone seems insufficient to change the depth of sulfate reduction. We conclude that changes in iron minerology, specifically the formation of an iron sulfide layer deeper in sediments, encourages the elevation of the sulfate reduction zone. If anoxic events become longer and more frequent in the SBB because of global warming (Qin et al., 2022; Stramma et al., 2008), the iron oxide buffer built up in shallower depths (e.g., NDT3-B) could be exhausted, allowing for surface sulfate reduction and the proliferation of GSOB mats in shallower margins of the basin than currently seen. Further, the same transient deoxygenation that allows for these mats to flourish also allows for a high Fe$^{2+}$ and PO$_4^{3-}$ flux into the SBB water column. In order to fully understand the complex changes in the benthic environment in response to deoxygenation, genomic and molecular work of the upper sediment community needs to be characterized.
Overall, the insights gleaned from this research will aid in the understanding of fundamental biogeochemical changes that occur when marine environments become anoxic.
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Data availability.

Biogeochemical data presented in this manuscript are accessible through the Biological & Chemical Oceanography Data Management Office (BCO-DMO) at the following landing pages:

Author contributions.

TT, DV, FK, NL, and JT designed the project. DJY, SK, JT, DR, and TT processed sediment cores at sea. DJY conducted geochemical analyses of sediment porewater and benthic flux chamber water. DJY prepared TOC and TON samples. DR and SK analyzed sediment porosity and density. TT and SK performed shipboard sulfate reduction incubations. DJY and DR conducted sulfate reduction analyses. DJY, NL, and JT transformed and interpreted ROV Jason data. FJ and FW operated BFC and microprofilers and analyzed associated data. XP conducted $^{15}$N experiments and analyses.

Competing interests.

At least one of the (co-)authors is a member of the editorial board of Biogeosciences.
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California Cooperative Oceanic Fisheries Investigations: https://www.calcofi.org/ccdata.html, last


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