Enigmatic Fe-Mn-fueled Anaerobic Oxidation of Methane in sulfidic coastal sediments of the Eastern Arabian Sea

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Abstract

Anaerobic oxidation of methane (AOM) coupled with Fe-Mn reduction (Fe-Mn-AOM) is considered a globally important biogeochemical process in marine sediments in addition to sulfate-driven AOM (SO₄²⁻-AOM) responsible for the consumption of methane, a strong greenhouse gas. Most existing studies have emphasized the significance of Fe-Mn-AOM activities in sediments below the depth of the sulfate methane transition zone (SMTZ) with insignificant dissolved sulfide and sulfate concentrations in the porewaters. Here, we report for the first time enigmatic geochemical evidence of focused Fe-Mn-AOM activity across the SMTZ in the presence of high dissolved sulfide concentrations in a sediment core collected within the seasonal coastal hypoxic zone of the Eastern Arabian Sea (West coast of India (WCI)). The Fe-Mn-AOM activity is evident from the concurrent decrease in CH₄ concentrations, δ¹³C(CH₄) and δ¹³C(DIC) values coupled with the enrichment of porewater Fe²⁺ and
Mn\(^{2+}\) concentrations at multiple depths below the seafloor. Since neither CH\(_4\) nor reactive Fe appears to be the limiting factor controlling the Fe-Mn-AOM activity, we hypothesize that the focused Fe-Mn-AOM at multiple depths is likely fueled by the localization of metal-reducing and methanotrophic microbial communities, leading to biogeochemical heterogeneity in a dynamic seasonally hypoxic coastal environment sensitive to climate change. This study highlights new insight into CH\(_4\)-S-Fe-Mn biogeochemical cycling with far-reaching implications in climate studies linked to the estimation of sedimentary methane production and consumption.

1 Introduction

The metal-driven anaerobic oxidation of methane (AOM) is a vital biogeochemical process and may have a significant impact on global biogeochemical cycles, particularly in relation to coupled CH\(_4\)-Fe-Mn-S cycling (Slomp et al., 2013; Riedinger et al., 2014; Leu et al., 2020). Microbial Fe-Mn-driven AOM contributes to the global consumption of CH\(_4\) in marine sediments in addition to sulfate (SO\(_4^{2-}\)), nitrite (NO\(_2^-\)), and nitrate (NO\(_3^-\))-driven AOM (Raghoebarsing et al., 2006; Beal et al., 2009; Knittel et al., 2009; Ettwig et al., 2010; Riedinger et al., 2014; Xiao et al., 2023). A potential syntrophic coupling of AOM and reduction of Fe-Mn-(oxyhydr)oxides in marine sediments was first inferred from incubation experiments using \(^{13}\)C-labeled CH\(_4\) (Beal et al., 2009). The coupling of AOM and Fe-Mn-reduction is potentially attributed to the activities of methanotrophs (ANME-1, ANME-3) and metal-reducing bacteria (Beal et al., 2009; Oni et al., 2015), while Fe-Mn-(oxyhydr)oxide reduction may also be carried out by ANME-2a, ANME-2c, and ANME-2d using multiheme cytochromes without syntrophic metal-reducing bacterial partners (Ettwig et al., 2016; Scheller et al., 2016; Cai et al., 2018; Leu et al., 2020). Plausible electron transport pathways coupling AOM and metal oxide reduction include (a) direct electron transfer via microbial contact; (b) indirect electron transfer by electron shuttling; (c) indirect electron transfer by a metal chelate; and (d) direct electron
transfer by nanowires (Folgosa et al., 2015; Wegener et al., 2015; He et al., 2018). The biogeochemical reactions during Fe-Mn-AOM (Eq. 1 and 2) result in the mutual consumption of CH$_4$ and Fe-Mn-(oxyhydr)oxides coupled with the enrichment of Fe$^{2+}$ and Mn$^{2+}$ in the interstitial waters (Beal et al., 2009; Riedinger et al., 2014). Although SO$_4^{2-}$-AOM (Eq. 3) is responsible for > 90% of global methane consumption, Fe-Mn-AOM is thermodynamically more favorable than SO$_4^{2-}$-AOM (Beal et al., 2009) as evident from the $\Delta G$ values (Eq. 1, 2 and 3). The global predominance of SO$_4^{2-}$-AOM may be attributed to its kinetics (Lovley and Phillips, 1987).

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\begin{align*}
\text{CH}_4 + 8 \text{Fe(OH)}_3 + 15\text{H}^+ & \rightarrow \text{HCO}_3^- + 8 \text{Fe}^{2+} + 21\text{H}_2\text{O} \quad \Delta G = -270.3 \text{ kJ mol}^{-1} \text{CH}_4 \\
\text{CH}_4 + 4 \text{MnO}_2 + 7\text{H}^+ & \rightarrow \text{HCO}_3^- + 4 \text{Mn}^{2+} + 5\text{H}_2\text{O} \quad \Delta G = -556 \text{ kJ mol}^{-1} \text{CH}_4 \\
\text{CH}_4 + \text{SO}_4^{2-} & \rightarrow \text{HS}^- + \text{HCO}_3^- + \text{H}_2\text{O} \quad \Delta G = -10 \text{ to } -40 \text{ kJ mol}^{-1} \text{CH}_4
\end{align*}
\]

The availability of reactive Fe-Mn-(oxyhydr)oxides and low to undetectable SO$_4^{2-}$ concentrations are considered essential geochemical conditions to drive Fe-Mn-AOM (Beal et al., 2009; Oni et al., 2015; Aromokeye et al., 2019), however, a recent report (Li et al., 2019) has shown the feasibility of Fe-Mn-AOM in SO$_4^{2-}$-rich porewaters. The kinetics of Fe-Mn-AOM depend on the content and composition of particulate Fe-Mn-(oxyhydr)oxides and soluble ferric-organo complexes (e.g., ferric citrate) (Ettwig et al., 2016). It is observed that the soluble metal complexes and ferrihydrite have higher bioavailability and AOM activity than other solid metal-(oxyhydr)oxides (Lovley and Phillips, 1987; Norði et al., 2013; Ettwig et al., 2016; Scheller et al., 2016). However, several incubation experiments and cultures have shown that crystalline goethite, hematite, and magnetite can also serve as significant electron acceptors for Fe-AOM (Bar-Or et al., 2017; Aromokeye et al., 2019; Li et al., 2021).

The global distribution of Fe-Mn-AOM is plotted in Figure 1a. Here, we present the first tell-tale evidence of Fe-Mn-AOM from a seasonally hypoxic shelf zone. The study was carried out
in a sediment core (SSD070/7/GC6) collected off the west coast of India (WCI) at a water depth of 28.5 m (Figure 1b). The seasonal hypoxic zone off the WCI covers an area of 1,80,000 km² and is the largest of all coastal hypoxic systems (Naqvi et al., 2000). The hypoxia is attributed to the upwelling of cold, nutrient-rich waters onto the shelf region during July to September, associated with the southwest monsoon. During the southwest monsoon, the WCI receives substantial terrestrial as well as marine organic matter due to enhanced land runoff and upwelling-driven marine productivity respectively. In contrast, during northeast monsoon, the WCI experiences downwelling from November to April resulting in the prevalence of oxic and oligotrophic conditions in the water column (Naqvi et al., 2000; Schott et al., 2001). Consequently, the region exhibits contrasting seasonal biogeochemical conditions including hydrographic and depositional features (Naqvi et al., 2006; Mazumdar et al., 2012). In the present study, we have investigated possible driving factors fueling Fe-Mn-AOM activity in a seasonally hypoxic shelf zone characterized by contrasting redox and depositional conditions.
Figure 1. a) Global map showing various study locations (red-filled triangles) with reported Fe-Mn-AOM activities. The yellow-filled circle represents the present study location. The references are listed in the supplementary material. (b) Map showing locations of the present study (SSD070/7/GC6) along with those collected in previous studies (Maya et al., 2011; Bardhan et al., 2014; Gomes et al., 2019; Bhattacharya et al., 2021; Naik et al., 2023; Parab et al., bioRxiv) from the inner shelf off the West Coast of India (WCI). The red star represents the present study location (SSD070/7/GC6), while other colored symbols represent locations in the WCI where δ¹³C and δ¹⁵N of suspended particulate organic matter and microbiological studies have been conducted.
2 Methodology

2.1 Onboard Sampling

The studied gravity core (SSD070/7/GC6, 5.25 m long) was collected onboard ORV Sindhu Sadhana in February 2020. The coring operation was carried out off Goa (latitude: 15.50985°N, longitude: 73.65313°E) from the seasonal hypoxic coastal zone of the Eastern Arabian Sea. Immediately after recovery, the core was subsampled onboard for hydrocarbon gas analysis and porewater extraction.

For headspace methane analysis, the sediment was extracted using 50 ml cut syringes at an interval of 10 cm and transferred into 20 ml headspace vials filled with 3 ml of KOH and 3 ml of NaN₃ to trap CO₂ and arrest microbial activities respectively. The vials were flushed with helium, homogenized, and stored at 4°C after sealing with butyl rubber septa. The core was subsampled onboard for porewater extraction at 10 cm intervals and transferred into 50 ml tarsons centrifuge vials using a 50 ml cut syringe under a stream of argon gas to avoid oxidation of dissolved sulfide. The samples were centrifuged at 7000 rpm for 20 minutes in a Remi C-30 centrifuge. The supernatant porewater was filtered through a 0.2 µm Whatman syringe filter.

The filtered porewater was then stored in crimp vials under a helium head and subsequently preserved at 4°C for measurement of various constituents. For dissolved sulfide (ΣHS⁻ = H₂S + HS⁻ + S²⁻) analysis, 1 ml of 1 M CdNO₃ was added to fix all sulfides present as CdS.

Porewater samples for trace metal analysis were acidified with a known amount of concentrated supra-pure HNO₃. All porewater samples were immediately crimp-sealed under a helium head and stored at 4°C until shore-based analysis. The core was subsampled for solid phase analysis at 1 cm resolution and stored at 4°C for further analysis.
2.2 Porewater and headspace gas analysis

The headspace hydrocarbon gas concentrations were determined using a trace GC equipped with a flame ionization detector. Methane concentration measurements were carried out using an external calibration line prepared from six different gas mixtures (CH$_4$, C$_2$H$_6$, C$_3$H$_8$, C$_4$H$_{10}$, iso-C$_4$H$_{10}$, and CO$_2$) standards with concentrations varying from 10-1000 ppm. The carbon isotopic composition of methane was determined using an isotope ratio mass spectrometer (Thermo-Delta V plus) coupled with a gas chromatograph (Thermo-Trace GC Ultra). The carbon isotope ratios of methane ($\delta^{13}$C$_{CH4}$) are reported in delta notation as permil deviation from Vienna Pee Dee Belemnite (VPDB) standard, and the external precision calculated for the measurement is typically 0.07–0.09‰. The concentration of dissolved inorganic carbon (DIC) was measured using a carbon coulometer (UIC-CM5130) with ultrapure CaCO$_3$ as standard. The measurement yielded a sample reproducibility of ± 0.3 %. The carbon isotope ratios of DIC ($\delta^{13}$C$_{DIC}$) were analyzed using a Thermo Delta V continuous flow isotope ratio mass spectrometer coupled with a GASBENCH II equipped with a PAL auto-sampler, following Torres et al. (2005) and Peketi et al. (2020). Reference materials NBS-18, NBS-19, Carrara marble standards, and in-house laboratory standards (MERCK high purity CaCO$_3$ and Carbonate reference standard) were measured multiple times during the analysis with each batch. The carbon isotope composition of DIC is reported in delta notation as permil deviation from the VPDB standard. The measurement yielded a sample reproducibility of ± 0.02 ‰.

Porewater samples were diluted 40-fold for the determination of trace metals using high-resolution inductively coupled plasma–mass spectrometry (Nu-ATTOM ES). NASS-5 (Seawater-certified reference material) and CASS-6 (Near Shore Seawater reference material) were analyzed to check the accuracy and reproducibility of the sample analysis. The analytical precision was monitored by repeated measurements of sample/standard and the calculated RSD was less than 3%.
The porewater sulfate concentration was measured from the ΣHS\(^-\) free supernatant solution using a Metrohm ion chromatograph (Basic IC plus 883) equipped with a suppressed conductivity detector (Metrohm, IC detector 1.850.9010). A mixed solution of 1 mM NaHCO\(_3\) and 3.2 mM Na\(_2\)CO\(_3\) was used as the eluent, and 0.2 N H\(_2\)SO\(_4\) was used as the suppressor regeneration fluid. The samples were diluted 1000-fold with 18 MΩ ultrapure water prior to analysis. The calibration line was prepared using a standard IC sulfate solution from a 100-ppm mixed anion standard (Fluka), and the sample reproducibility was ± 0.1%. Quantification of dissolved sulfide (ΣHS\(^-\)) was carried out following the methylene blue method (Cline et al., 1969). Absorbance was measured at 670 nm on a spectrophotometer (Chemito Spectrascan UV-2700). Sodium sulfide nonahydrate (≥99.99% purity, Merck) was used for the preparation of calibration standards. The analytical error based on replicate standard measurements was <3%.

### 2.3 Solid phase analysis

Iron extractions were conducted under anaerobic conditions using frozen sediment samples following ascorbate and sodium dithionite steps. The leaching solutions were nitrogen-flushed before extraction. The iron concentrations in ascorbate leached fraction (Fe\(_{Asc}\)) were determined following previous studies (Hyacinthe et al., 2006; Raiswell et al., 2010) using Atomic Absorption Spectrometer (Agilent-240AA) using air-acetylene flame. The sample reproducibility for replicate analysis was within 5%. The Fe\(_{Asc}\) free sediment residue was leached for 2 hrs using sodium dithionite buffer for determination of dithionite extractable iron (Fe\(_D\)) content following previous works (Mehra and Jackson, 1960; Canfield et al., 1989; Volvaikar et al., 2020). The Fe\(_D\) content was determined using ferrozine-complexometry technique on a Chemito spectrophotometer (Spectroscan UV 2700). The calibration line was prepared from pure ferrous ammonium sulfate (Merck) and absorbance was measured at 515 nm. The replicate for Fe\(_D\) analysis yielded sample reproducibility within 6%. The total
inorganic carbon content was measured using a UIC carbon coulometer (CM 5130). Ultrapure CaCO$_3$ from Sigma-Aldrich was used as a standard reference material for measurement (Carbon content: 12.0 ± 0.25%). The measurement of total carbon (TC) content was carried out on freeze-dried and desalinated samples using an EA1112 elemental analyzer (Thermo Fisher Scientific, Germany). The total organic carbon (TOC) content (Figure S2; Table S2) was calculated by subtracting TIC from TC. NC soil was used as a calibration standard for TC. The reproducibility for TC in soil standards B2184 and B2152 was found to be 2.11 ± 0.1 % and 1.53 ± 0.07 % respectively. The carbon and nitrogen isotope ratio of TOC (δ$^{13}$C$_{TOC}$) and nitrogen (δ$^{15}$N) were measured on decarbonated samples using a Delta-V-plus isotope ratio mass spectrometer coupled with an elemental analyzer (Thermo Flash EA 2000). The carbon and nitrogen isotope ratios are reported as per mil (‰) deviations from the isotopic composition of VPDB and atmospheric N$_2$ with reproducibility better than ± 0.3‰.

3 Results and Discussion

3.1 Evidence for SO$_4^{2-}$ and Fe-Mn-AOM

Based on the porewater SO$_4^{2-}$ and Fe-Mn concentration profiles (Figures 2a-2e; Table S1), the sediment core can be divided into three distinctive diagenetic regimes (zone-i, zone-ii, and zone-iii). Zone-i (63 to 303 cmbsf) is characterized by Fe$^{2+}$ and Mn$^{2+}$ enrichment (Fe$^{2+}$: 10.75 to 361.3 μM and Mn$^{2+}$: 0.28 to 7.39 μM) at high SO$_4^{2-}$ concentrations (0.5 to 18.2 mM); zone-ii (303 to 463 cmbsf) is characterized by SO$_4^{2-}$ depletion (0.4 to 1.45 μM) coupled with Fe$^{2+}$ and Mn$^{2+}$ enrichment (Fe$^{2+}$: 14.9 to 387.5 μM and Mn$^{2+}$: 0.41 to 10.17 μM); and zone-iii (463-523 cmbsf) shows SO$_4^{2-}$ depletion (below detection limit to 0.8 μM) coupled with depleted Fe$^{2+}$ and Mn$^{2+}$ concentrations (Fe$^{2+}$: 11.85 to 58.2 μM and Mn$^{2+}$: 1.13 to 2.68 μM). The ΣH$^-$ concentrations remain consistently high (163 to 10,385 μM) below 63 cm from the seabed.
The SMTZ is identified within an approximate depth window of 263 to 303 cmbsf (Figures 2a and 2b). Within the SMTZ, AOM activity is identified by simultaneous depletion in CH$_4$ concentrations (188.9 to 503.2 µM) and C-isotope ratios of CH$_4$ ($\delta^{13}$C$_{CH_4}$: -105.6 to -98.6 ‰) and DIC ($\delta^{13}$C$_{DIC}$: -38 to 35.2 ‰). The AOM-driven depletion in $\delta^{13}$C$_{CH_4}$ and $\delta^{13}$C$_{DIC}$ values within the SMTZ may be attributed to (i) DIC back flux during AOM (Yoshinaga et al., 2014); (ii) concurrent methanotrophic and methanogenic activity (Borowski et al., 1997); (iii) the ability of ANMEs to perform as facultative methanogens (Pohlman et al., 2008); and (iv) intracellular reaction reversibilities along enzymatic AOM pathway (Wegener et al., 2021). Above SMTZ, the upward increasing trend in $\delta^{13}$C$_{CH_4}$ values may be attributed to $^{13}$C enrichment (Figure 2b; Alperin et al., 1988; Whiticar et al., 1996; Martens et al., 1999; Whiticar, 1999) in the residual methane (kinetic isotope effect) due to methane oxidation and lack of carbon recycling at high SO$_4^{2-}$ concentrations (SO$_4^{2-}$>0.5 mM; Yoshinaga et al., 2014; Wegener et al., 2021). Below the SMTZ, the methanogenesis trend is superimposed by depths of intermittent AOM activity evident from depletion in CH$_4$ concentrations (1.04 to 4.19 mM) coupled with a sharp decrease in $\delta^{13}$C$_{CH_4}$ (-17.95 to -61 ‰) and $\delta^{13}$C$_{DIC}$ values (-1.8 to -4.1 ‰) and increase in DIC concentrations (1 to 2.7 mM). The cross plot of DIC concentrations vs $\delta^{13}$C$_{DIC}$ values for the overall core and the Fe-Mn-AOM specific points show negative correlations (Figure 3). The intermittent increase in DIC concentrations and decrease in $\delta^{13}$C$_{DIC}$ values can also be identified within the sulfatic zone-i.
Figure 2. Figure showing Fe-Mn-AOM and SO$_4^{2-}$-AOM activities by means of pore fluid chemistry in seasonally hypoxic coastal sediment of the Eastern Arabian Sea. (a) Porewater SO$_4^{2-}$ (mM) and ΣHS$^-$ (µM) concentrations. (b) Dissolved CH$_4$ concentrations (µM) and δ$^{13}$C$_{CH_4}$ values. (c) Dissolved DIC concentrations (mM) and δ$^{13}$C$_{DIC}$ values (d, e) Dissolved Fe and Mn (µM) concentrations. The blue dashed lines represent the depth layers with AOM activities. The red curly bracket depicts SMTZ. The different color shades represent zone-i, zone-ii, and zone-iii.
Figure 3. Cross plot of DIC concentration (mM) vs δ\text{13C}_{DIC} (‰) values in the studied sediment core (represented by black dots with an R² value of 0.82). The inset cross plot of DIC vs. δ\text{13C}_{DIC} (represented by red dots with an R² value of 0.96) corresponds to values specific to Fe-Mn-AOM (marked by blue dashed lines in Figure 2).

The most intriguing observation of the present study is the intermittent increase in porewater Fe\textsuperscript{2+} and Mn\textsuperscript{2+} concentrations (Figures 2d & 2e). Within zone-i and ii, the marked increase in porewater Fe\textsuperscript{2+} and Mn\textsuperscript{2+} concentrations are associated with AOM activities. However, in zone-iii, the AOM activities are not associated with significant porewater Fe\textsuperscript{2+} and Mn\textsuperscript{2+} enrichment.

The porewater SO\textsubscript{4}\textsuperscript{2-}, Fe\textsuperscript{2+}, Mn\textsuperscript{2+}, CH\textsubscript{4}, and DIC concentrations along with δ\text{13C}_{CH\textsubscript{4}} and δ\text{13C}_{DIC} depth profiles provide tell-tale evidence of both SO\textsubscript{4}\textsuperscript{2-}-AOM and Fe-Mn-AOM in the present study. Zonation of the geochemical profiles shows co-occurrence of SO\textsubscript{4}\textsuperscript{2-}-AOM and Fe-Mn-AOM in zone-i, and Fe-Mn-AOM in zone-ii and iii. The occurrence of Fe-Mn-AOM in SO\textsubscript{4}\textsuperscript{2-} rich zone has also been reported in an earlier study from the Dongsha area, South China Sea (Li et al., 2019). These observations are further supported by an incubation study (Segarra et al., 2013) where notable rates of Fe-Mn-AOM coincide with comparatively elevated levels of...
SO$_4^{2-}$-AOM. Within zone-ii, where SO$_4^{2-}$ concentrations are reduced to 0.4 to 1.45 µM, the Fe$^{2+}$ and Mn$^{2+}$ concentration spikes may be attributed primarily to Fe-Mn-(oxyhydr)oxides fueling AOM activity (Riedinger et al., 2014; Egger et al., 2015; Egger et al., 2017).

In most of the previous studies (Crowe et al., 2009; Sivan et al., 2011; Slomp et al., 2013; Riedinger et al., 2014; Egger et al., 2015; Egger et al., 2016a; Egger et al., 2016b; Egger et al., 2017; Aromokeye et al., 2019; Vigderovich et al., 2019; Luo et al., 2020; Xiao et al., 2023), AOM-driven Fe$^{2+}$-Mn$^{2+}$ enrichment has been reported at low to negligible ΣHS$^-$ concentrations (Figure S1). However, the present study demonstrates tell-tale signatures of substantially high porewater Fe$^{2+}$-Mn$^{2+}$ concentrations despite high ΣHS$^-$ concentrations. A similar observation was also reported in previous studies (Ramírez-Pérez et al., 2015; Li et al., 2019). Equation-1 shows that for every mole of DIC produced via Fe-AOM, 8 moles of Fe$^{2+}$ are produced. The DIC concentration perturbations (along the blue dashed lines) may be converted to equivalent amount of Fe$^{2+}$ produced via Fe$^{3+}$ reduction in the pore waters. The stoichiometrically calculated amount of Fe$^{2+}$ produced ranges from 11673 µM to 17163 µM in zone-i-ii and 6192 to 8259 µM in zone-iii. The measured porewater Fe$^{2+}$ concentrations are approximately 1.65-3% of the calculated Fe$^{2+}$ in zone-i-ii and 0.2-0.6 % in zone-iii. Thus, the measured Fe$^{2+}$ concentration is the residual Fe$^{2+}$ left after sulfidization (FeS/FeS$_2$: Hensen et al., 2003; Treude et al., 2014; Peketi et al., 2015). Therefore, the lack of significant Fe$^{2+}$ and Mn$^{2+}$ enrichment in zone-iii may indicate complete consumption of Fe$^{2+}$ and Mn$^{2+}$ via sulfidization.

Additional processes that may enhance the metal concentrations in the sediment pore waters is the presence of metal (Fe-Mn) sulfide collides (Rickard et al., 2007) as nanoparticles. Dissolution of porewater Fe-Mn sulfide nanoparticles (Morse et al., 1999; Olson et al., 2017) in acid during sample preparation results in the simultaneous occurrence of Fe$^{2+}$ and Mn$^{2+}$ spikes in porewater. However, if the observed Fe$^{2+}$-Mn$^{2+}$ concentration spikes in this study are
merely an analytical artifact, then under the presence of such high dissolved sulfide concentrations, Fe$^{2+}$-Mn$^{2+}$ spikes would be expected at all depths. The absence of Fe-Mn spikes corroborated with distinct increase in DIC concentration and decrease in $\delta^{13}$C$_{DIC}$ values rules out such artifact generation.

### 3.2 Possible limiting factors and focused Fe-Mn-AOM

An essential prerequisite for Fe-Mn-AOM is the presence of appreciable quantities of Fe-Mn-(oxyhydroxides) to react with CH$_4$ (Beal et al., 2009) as evident from the stoichiometric ratios (Eq. 1). Results from iron extractions indicate the presence of significant amount of iron in highly reactive/bioavailable (Fe$_{Asc}$) (Figure 4b) and dithionite extractable phases (Fe$_D$) (Figure 4c, Table S2). The Fe$_{Asc}$ and Fe$_D$ contents in the sediment range from 0.01 to 4.27 mg/g (Avg: 1.2±0.86 mg/g) and 0.54 to 7.2 mg/g (Avg:3.03±1.36 mg/g) respectively. The porewater Fe$^{2+}$ concentration spikes are associated with marked depletion in Fe$_{Asc}$ content, indicating consumption of ferrihydrite and other bioavailable iron through Fe-AOM (Figure 4). However, the presence of substantial content of both Fe$_D$ and Fe$_{Asc}$ throughout the core length suggests Fe-AOM is not limited by reactive iron content. In the present study, the high reactive iron flux in the shelf zone may be attributed to rapid sedimentation rates (150 to 1500 cm/ky) and fluvial input from Fe-Mn-rich provenances (Fernandes et al., 2020). Additionally, the presence of CH$_4$ throughout the Fe-Mn-AOM zones suggests that neither CH$_4$ nor reactive Fe are limiting factors responsible for the focused occurrences of Fe-AOM in the sediment core.
Figure 4. Depth profiles of (a) Porewater Fe$^{2+}$ concentrations (µM). (b) solid phase ascorbic acid leachable iron (Fe$_{Asc}$) and (c) sodium dithionite leachable iron (Fe$_D$) contents (in mg/g). The blue dashed lines represent the depth layers with AOM activities. The different color shades represent zone-i, zone-ii, and zone-iii.

One of the plausible factors responsible for the occurrence of focused Fe-Mn-AOM in distinct sediment layers could be the spatiotemporal enrichment of the microbial population driving Fe-Mn-AOM. The latter in turn could be the result of the interplay between several biogeochemical factors. For instance, the shallow shelf off WCI is highly dynamic, experiencing drastic changes in water column redox conditions, marine productivity, fluvial fluxes of organic matter, sediment load, and extensive denitrification (Naqvi et al., 2000; Schott et al., 2001; Naqvi et al., 2006; Maya et al., 2011; Mazumdar et al., 2012; Fernandes et al., 2020). A large range in TOC contents (1.45 to 31.3 mg/g), (TOC/TN)$_{molar}$ ratios (3.47 to 27.32), $\delta^{13}$C$_{TOC}$ (-20.69 to -25.93 ‰), and $\delta^{15}$N values (-3.6 to 7.7 ‰) in the present and previous studies (Mazumdar et al., 2012; Fernandes et al., 2020) indicate marked temporal variation in the fluvial and marine organic matter fluxes and denitrification conditions in WCI (Figure S2, 2020).
Table S2). Previous studies (near SSD070/7/GC6; Figure 1b) investigating carbon and nitrogen stable isotopes of suspended particulate organic matter (SPOM) in the estuary (Bardhan et al., 2014) and shelf zone (Maya et al., 2011) of WCI revealed significant intra-annual variations in $\delta^{15}$N (estuary: 0.69 to 7.26 ‰; shelf: -4.17 to 10.43 ‰) and $\delta^{13}$C (estuary: -30.14 to -19.52 ‰, shelf: -17.64 to -26.74 ‰) throughout the year. These variations reflect the complex and dynamic nature of biogeochemical processes and organic matter sources in the coastal waters of the WCI. Corroboratively, significant variations in the diversity, abundance, and activity of microorganisms, attributable to seasonal differences in nutrient availability, have been recorded in the water column between monsoon and non-monsoon seasons (Gomes et al., 2019; Naik et al., 2024; Parab et al., bioRxiv). Spatiotemporally contrasting biogeochemical conditions of shallow coastal waters have profound influence on the structure and function of underlying sedimentary microbiomes (Bhattacharya et al., 2021). An earlier study (Orsi et al., 2017) from the North Eastern Arabian Sea oxygen minimum zone reported stratigraphic microbial distribution attributed to palaeoceanographic conditions. It was also proposed that focused abundances of microbial communities in sediment layers are expected to be most pronounced in the coastal region due to drastic variations in marine redox conditions and terrestrial fluxes. In the context of the biogeochemical phenomenon revealed here, we hypothesize a dominant role of the localized abundance of metal-reducing bacterial/archaeal communities in restricting Fe-Mn-AOM activities into specific sedimentary strata. The focusing of microbial activity in different sediment layers may be attributed to factors such as past environmental conditions and depositional processes (Parkes et al., 2000; Orsi et al., 2017; Hoshino et al., 2020). Seasonal coastal hypoxia coupled with a wide range of organic matter (marine to terrestrial) fluxes may play important roles in stratigraphic microbial distributions and heterogeneity in biogeochemical processes.
Our hypothesis is supported by metagenomic sequencing (Sarkar et al., in prep; Table S3) carried out in a ~3 m long, sulfide-rich, sediment core (SSK42/9; water depth: 31 m; Figure 1b), collected from a nearby shallow coastal site (Bhattacharya et al., 2021) which shows the presence of two species-level entities affiliated to the uncultivated archaeon Candidatus Methanoperedens (GenBank assembly numbers GCA_003104905.1 and GCA_001317315.1) reported thus far for potential abilities to harness anaerobic methane oxidation to the reduction of Fe (III) (Ettwig et al., 2016; Cai et al., 2018). However, the final microbiological corroboration of the focused Fe-Mn-AOM phenomenon hinges on the future enrichment of the genome sequence database for microorganisms having definite and complete Fe-Mn-AOM attributes.

Being the world's largest coastal hypoxic zone covering an area of 0.18 million sq. km, the net influence of Fe-Mn-AOM could be significant in sedimentary methane consumption. A schematic representation of CH₄-S-Fe-Mn cycling in coastal hypoxic sediments of the Eastern Arabian Sea is presented in Figures 5a, 5b and 5c respectively.
Figure 5. (a) Schematic representation of Fe-Mn-AOM and SO$_4^{2-}$-AOM activities within the sediment column. The figure depicts different zonation within the sediment column, showing the co-occurrence of SO$_4^{2-}$-AOM and Fe-Mn-AOM in zone-i and Fe-Mn-AOM in zone-ii-iii. Schematic representation of
pore fluid vertical profiles in the absence (b) and presence (c) of Fe-Mn-AOM activity. The green-shaded regions in the methanogenic zone represent potential Fe-Mn-AOM depth layers, controlled by the localization of metal-reducing microbial communities.

4 Conclusions

The comprehensive geochemical investigation of the sediment core (5.25 m) retrieved from the seasonally hypoxic shelf sediment off the eastern Arabian Sea (west coast of India) has unveiled compelling evidence of focused Fe-Mn-AOM occurring at multiple depths beneath the seafloor. The accumulation of porewater Fe$^{2+}$ and Mn$^{2+}$ despite elevated concentrations of $\Sigma$HS$^-$, suggests the predominant production of Fe$^{2+}$ and Mn$^{2+}$ rather than consumption through metal sulfidization processes. This study demonstrates that Fe-Mn-AOM remains a significant biogeochemical phenomenon even within SO$_4^{2-}$ and $\Sigma$HS-rich sediments. Our findings are also relevant in assessing the consumption of CH$_4$, a greenhouse gas in organic-rich coastal sediments, thus having implications for climate change research. This study presents a new perspective by documenting the biogeochemical heterogeneity in the occurrence of Fe-Mn-AOM, possibly attributed to deposition-controlled Fe-Mn reducing microbial population distribution in a highly dynamic coastal environment sensitive to climate change. The findings of the present study may have a far-reaching influence on coupled CH$_4$-Mn-Fe-S cycling in expanding hypoxic coastal regions of the global ocean. Our findings lead to a hypothesis that necessitates future examination of microbial communities in seasonally hypoxic sediments and SPOM at high depth and temporal resolution which may uncover the complex interactions between microbial life and their environment.

Appendices: No appendix is there in this manuscript.

Code Availability: No code was used in this manuscript.

Data availability: All the data used in this study is incorporated in the supplementary text as table S1, table S2 and table S3.
Sample availability: Samples are available with the author.

Video supplement: No video component is there in this manuscript.

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References


**Supplementary Data.** For figures (Figures S1 and S2), tables (Tables S1, S2 and S3), and supporting text, please see supplementary data file.