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Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used to collect data.
Data analysis	Net reaction rates of sulfate were estimated based on measured sulfate concentration profiles in the sediment cores using open source MATLAB script published in Wang et al., 2008, which can be downloaded from online supplementary materials: https://www.sciencedirect.com/science/article/pii/S0016703708002287?casa_token=L43vab1nnkMAAAAA:THuRvBJ3DI_l4NJ_Y2SOZxsXsRtciX-IK0HqT7owlkcHF_FKhHdFbr_sBc5XWaaOSoZDrtVWo8Zc#app1. The MATLAB software used in this study was version MATLAB 2021b. All the statistical analyses were carried out in R (version 4.1.3). The raw reads of 16S rRNA gene of both cores QDN-G1 and QDN-14B were processed and analyzed using the QIIME 2 platform (version 2020.11). The age model of QDN-G1 was established using the software QAnalySeries (v. 1.4.2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw Illumina sequence data of the 16S rRNA gene generated for cores QDN-14B and QDN-G1 in this study has been deposited in the NODE (the National Omics Data Encyclopedia, https://www.biosino.org/node/) database under the project number OEP004264 and OEP004265, respectively. All other data discussed in the paper are available in the Supplementary Data.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☒ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description

Reactive iron (FeR) serves as an important sink of organic carbon (OC) in marine surface sediments, where approximately 20% of total OC (TOC) is bound to FeR (FeR-OC). However, the fate of FeR-OC in subseafloor sediments and its availability to microorganisms, remains undetermined. Here, we reconstructed continuous FeR-OC records in two sediment cores of the northern South China Sea encompassing the suboxic to methanic biogeochemical zones and reaching a maximum age of ~100 kyr.

To quantify the amount of FeR-OC, the citrate-bicarbonate-dithionite (CBD) method was applied in this study. This method targets only reactive iron (oxyhydr)oxides, which are presumably accessible for microorganisms, and leaves unreactive phases such as iron-containing silicates untouched. By incorporating analyses of pore water geochemistry, including the concentrations of ferrous iron, sulfate, dissolved inorganic carbon (DIC), and the carbon isotope ratio of DIC (δ13CDIC), all of which are tied to microbially mediated processes, our study takes a critical step in assessing the stability of sedimentary FeR-OC in response to post-depositional microbial activities and sheds lights on its fate in subseafloor sediments.

Research sample

To disentangle the effect of FeR-OC supply and early diagenetic reworking on FeR-OC, downcore FeR-OC records need to be established and related to both geochemical zonation and sediment chronology. In this study, we analyzed two gravity cores (QDN-G1 and QDN-14B) from the northern South China Sea in order to determine the fate of the sedimentary FeR-OC via its quantitative and isotopic analysis. The core QDN-G1 represents typical continental slope sediments (1478 m water depth), while core QDN-14B (1370 m water depth), around 35 km southwest from core QDN-G1, was influenced by nearby cold seeps expelling methane-rich fluids. Consequently, QDN-14B can be used to comparatively evaluate the influence of microbial activities on the potential remobilization of FeR-OC in the diagenetically active zones where sulfate reduction coupled to either organic matter remineralization or methane oxidation occurs. Meanwhile, the core QDN-G1 consisting of sediments with relatively low microbial activity is used for exploring the long-term preservation of FeR-OC on glacial-interglacial timescale with well established age model covering the past 97 kyr .

Sampling strategy

Sediment samples were subsampled every 40 cm and preserved at -80 ?. Known volumes of sediments were taken using tip cut-off

Sampling strategy

syringes and sealed in glass vials for further measurements of density and porosity. Porewater samples were extracted immediately onboard using Rhizon samplers (0.22 um filter) before the core was cut open for sediment subsampling. To prevent oxidation of Fe(II), an aliquot of porewater was added to the ferrozine solution. Porewater samples for DIC measurements were preserved in pre-vacuumed glass vials. Porewater samples for ion measurements were acidified with concentrated HNO_3. The rest of the porewater samples were preserved in pre-combusted amber glass vials at -20°C for dissolved organic carbon measurements. The sediment cores were sub-sampled at an interval of 30-50 cm, which is generally enough to observe the changes in microbial community structures and sedimentary OC records on the glacial-interglacial timescale in continental slope environments.

Data collection

Yunru Chen collected all pore water geochemistry data. The Fe(II) concentration was determined by ferrozine assay using a spectrophotometer (Hach DR5000). The concentrations of major cations and anions were determined by ion chromatography (Dionex ICS-5000+). Dissolved inorganic carbon (DIC) was measured using a total carbon analyzer (Multi 3100, Jena).

Yunru Chen measured the physical properties of bulk sediments. Porosity was calculated by the volume ratio of water and wet sediment, where the volume of water was calculated from the difference between the wet and dry sample weight. The dry bulk density was calculated by dividing the dry sample weight by the original wet sample volume.

Yunru Chen extracted the FeR and FeR-OC. FeR and FeR-OC were extracted using the citrate-bicarbonate-dithionite (CBD) method. Samples were freeze-dried and homogenized using an agate mortar and pestle. Samples (0.5 g) were weighed carefully into 40 mL Teflon tubes and extracted in a 30 mL solution of sodium dithionite and trisodium citrate buffered with sodium bicarbonate at 80°C for 15 min in a water bath. After the extraction, the suspensions were centrifuged for 10 min at 4000×g and then rinsed 5 times with artificial seawater. To evaluate the amount of OC remobilized during the extraction that was not bound to FeR, another sample aliquot was extracted as a control under the same experimental conditions, but replacing sodium dithionite and trisodium citrate with sodium chloride with equivalent ion strength. The residuals were dried overnight in a 50°C oven, carefully weighed and manually ground. The supernatant and rinse water were combined, acidified to pH<2 and filtered through 0.22 m filters. The dissolved iron was determined using a ferrozine assay.

Yunru Chen finished FeR-OC quantification and carbon isotope analyses. The OC content and carbon isotope ratio were determined for both untreated and treated samples using an elemental analyser (Vario EL III, Elementar) coupled to an isotope ratio mass spectrometer (Isoprime, Elementar) at the instrumental analysis centre, Shanghai Jiao Tong University. The inorganic carbon was removed by acid fumigation before the analysis. The samples were measured in triplicate, and the standard deviation was <0.05% for TOC and <0.2‰ for 13C. The analytical precision was <0.06% for TOC (standard deviation for repeated measurements of the low organic content soil standard; n=3) and <0.09‰ for $\delta^{13}C$ (standard deviation for repeated measurements of the USGS40 standard, n=3).

Yunru Chen modelled the net reaction rates of sulfate using the MATLAB script published in Wang et al., 2008.

Weikang Sui conducted DNA extraction, qPCR and amplicon sequencing. The DNA for qPCR and V4 region of 16S rRNA gene sequencing was extracted from ~0.25 g of sediments using DNeasy® PowerSoil® Pro Kit (Qiagen), according to the manufacturer's instructions. The extracted DNA was used as template for qPCR to determine the abundance of bacterial 16S rRNA gene with the primer set 331F/797R. Standard curves were constructed using a 10-fold series dilution of the plasmids for six gradients carrying the bacteria 16S rRNA gene. qPCR was carried out in a volume of 20 μL, including 10 μL 2 × PowerUp™ SYBR™ Green Master Mix (Thermo Fisher), 1.6 μL each primer (10 μM), 2 μL template DNA and 4.8 μL steriled deionized water. The qPCR program consisted of an initial cycle of 95 °C for 5 min; 40 cycles of 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, 80 °C for 10 s, and the data was collected at the final step of each cycle. The melting curve was generated using default program. All samples were subject to qPCR measurement with three technical replicates. The bacterial cell number was evaluated based on the abundance of 16S rRNA gene, applying the average copy number of the 16S rRNA gene on genomes of Bacteria (5.3 copies/genome, rrnDB version 5.8). The V4 region of 16S rRNA gene was amplified using the primer set 515F/806R. DNA was amplified using the following cycling conditions: 95°C, 5 min; 30 cycles (95°C, 30 s; 50°C, 30 s; 72°C, 30 s); 72°C, 7 min. The PCR products of samples were sent to Shanghai Personal Biotechnology Co., Ltd. (Shanghai, China) for high-throughput sequencing of the 16S rRNA gene using the Illumina Novaseq PE250 platform.

Weikang Sui and Yunru Chen conducted Sequence analysis. The raw reads of 16S rRNA gene of both cores QDN-G1 and QDN-14B were processed and analyzed using the QIIME 2 platform (version 2020.11). The primers and adaptors were first trimmed out using Cutadapt (version 3.1). Raw sequences were then processed using DADA2, including quality filtering, denoising, paired-end sequence merging, chimera filtering and producing amplicon sequence variants (ASVs) and ASV Table. Taxonomy was assigned using q2-feature-classifier (a scikit-learn naive Bayes machine-learning classifier) with Silva database release 138. Multiple sequence alignment and phylogenetic tree construction were performed using the QIIME 2 plugin q2-phylogeny (align-to-tree-mafft-iqtree). Unassigned sequences, singletions and sequences affiliated with eukaryotes were discarded. Eventually, to eliminate uneven sequencing depths, the ASV table was rarefied to 14935 and 71773 sequences per sample for QDN-14B and QDN-G1, respectively, determined by the sample with the fewest sequences.

Liang Dong established the age model of core QDN-G1 using the software QAnalySeries (v. 1.4.2).

Yunru Chen did the statistical analysis. Statistical analyses were carried out in R (version 4.1.3). Significance tests were conducted when comparing the difference in TOC and FeR-OC records between SMTZ and non-SMTZ sediments in two sediment cores studied, including TOC content, FeR-OC content, FeR-OC/TOC ratio, 13CTOC , and 13CFeR-OC. In detail, the normality and homogeneity of variance were checked using Shapiro-Wilk normality test and F-test, respectively. For data that were normally distributed and had similar variances, a classical two-sided Student's t-test was computed. If the data had different variances, a Welch t-test was applied instead. For data that were not normally distributed, the significance level was first calculated with classical two-sided Student's t-test and rechecked with Wilcoxon test. One-way analysis of variance (ANOVA) was used to determine if there are significant differences in the FeR-OC/TOC ratio in surface sediments of different marine environments. Tukey Honest Significant Difference (HSD) test was used for performing multiple pairwise-comparison between the environments to determine which among them were significantly different.

Timing and spatial scale

All data were collected from December 10th, 2019 to October 18th, 2021. The measurements on pore water samples were finished as soon as possible after being transported to home lab on land. All the measurements on solid sediment samples were scheduled

based on the availability of the instruments.

The two sediment cores studied covers both typical continental slope sediments and continental slope sediments influenced by methane-rich fluids from a nearby cold seep.

Data exclusions	No data were excluded for analyses.
Reproducibility	The samples were pretreated following the reported methods and were measured in replicates or triplicates if possible, with standard controls measured in between to monitor the reproducibility of the measurements. The results were reproducible across replicates.
Randomization	Randomization is not relevant to our study as the all the samples collected from different sediment depths were treated in the exactly the same way.
Blinding	Blinding is not applicable in our study as all data were collected using objective pretreatment and measurement methods like citrate-bicarbonate-dithionite extraction, ferrozine assay, elemental analysis-coupled isotope ratio mass spectrometry, qPCR and amplicon sequencing. While invetigators were not blinded during sample collection, sediment samples were collected at certain depth intervals from sediment cores and used for further studies based on availability.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions	Core QDN-14B was collected during the cruise '20150402' (R/V Haiyang IV) organized by the the Guangzhou Marine Geological Survey in April 2015. Core QDN-Q1 was collected during the 'Hydrothermal vents-cold seeps' cruise (R/V KEXUE) organized by the Institute of oceanology, Chinese Academy of Sciences in July 2018. Both cores were collected from the Qiongdongnan Basin in the northern South China Sea.
Location	Core QDN-14B was recovered from the Qiongdongdan basin in the northern South China Sea at a water depth of 1370 m in 2015, ~600 m east of ROV1, which is an active seep site of "Haima" cold seeps. Core QDN-G1 (110.497°E, 17.054°N) was recovered outside the area of "Haima" cold seeps around 35 km away from QDN-14B at a water depth of 1478 m in 2018.
Access & import/export	The samples were collected from the South China Sea during two cruises organized by Chinese institutes. No import and export related.
Disturbance	Sediments were sub-sampled on the research vessel at required depth intervals before transportation to avoid disturbance.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*