

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- ☐ ☒ An indication of 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 statistics 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
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

PeakSimple 2000 chromatography integration software (SRI instrument, CA, USA)
IsodatTM 2 (Thermo Fisher Scientific, USA)
Pyro Oxygen Logger (PyroScience, Germany)

Data analysis

BamM v1.7.3
BBTools v36.92 (<https://sourceforge.net/projects/bbmap/>)
CheckM v1.0.11
ClustalX
DAS_Tool v1.1.1
deblur
Diamond
dRep v2.0.5
GraftM
GroopM v0.3.0
GTDB-TK pipeline
hmmer v3.1b2
IQ-Tree
LSU v132 database
MaxBin v2.2.4

MEGA7
 MetaBat v2.12.1
 mother v.132 database
 Panther database
 PeakSimple 2000
 Pfam v31.0 database
 Prodigal v2.6.3
 QIIME 2 platform version 2018.4 (<https://qiime2.org/>)
 RefineM
 RNAmmer v1.2
 SILVA SSU database
 SINA web aligner
 SPAdes v.3.11.1
 TIGRfam v15.0 database
 v132 database

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Community data based on shotgun metagenome sequencing and amplicon sequencing are provided as supporting information (Table S1 & S2). In addition, the metagenomes and metagenome-assembled genomes have been deposited on to public servers and accession codes provided. All other data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Ecological, evolutionary & environmental sciences study design

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

Study description

This study describes the metabolic cycling of H₂ in permeable sediments. In particular, we show through a combination of in-situ measurements and flow-through reactor and slurry experiments that H₂ accumulates in sandy sediments due to decoupling of production by fermentative bacteria at the onset of anoxia and aerobic respiratory H₂ consumption. Community and metagenomic profiling of the sediments reveal that abundant [NiFe]-hydrogenases among facultatively fermentative bacteria facilitate this H₂ cycling in an environment with varying oxygen availability.

$\delta^2\text{H-H}_2$ of seawater was determined with 10 single measurements ($n=1$) during one day. Pore water H₂ concentrations were determined from the average of three separate sediment cores ($n=3$). Flow-through reactor ($n=3$) and slurry ($n=3-6$) experiments were prepared using subsamples of homogenised surface sediment collected on the corresponding sampling days. Metagenomes ($n=5$) were prepared using samples collected from intertidal samples (2-5 cm, 15-18 cm), subtidal samples (2-5 cm, 15-18 cm) flow-through reactor samples.

Research sample

Seawater and sediment samples collected from Port Phillip Bay, Melbourne, Australia were used in this study. All microbial and biogeochemical work was performed on these samples. This site is chosen because it has previously been shown that the sediments from here produce large amounts of H₂ gas (Bourke et al., Nat. Geo., 2017). We do not propose that this site is representative of any larger system, and use this site as a model to showcase this novel metabolism.

Sampling strategy

For seawater samples, 500 mL water samples were drawn up from approximately 50 cm below the surface using a 1.6 L polycarbonate syringe. The remainder of the syringe was filled with ambient air. The syringe was then sealed and shaken vigorously for 2 minutes to transfer the H₂ to the gas phase. The headspace was transferred to an evacuated 1 L glass flask for later analysis.

Due to the expensive and time-consuming nature of $\delta^2\text{H-H}_2$ analysis, no replicates were performed for this work (i.e. $n=1$). For sediment collection, samples were collected from well-mixed silicate sands from both the subtidal zone (~1 m deep at low tide) and intertidal zone (~1 m deep at high tide) using polyacrylamide cores (20 cm depth, 6.7 cm ID). These samples were then transferred to flow-through reactors ($n=3$) and/or slurry reactors ($n=3-6$, see Materials and Methods), or preserved for microbial analysis.

For sediment depth profiles, three sediment cores of 20 cm depth were collected from subtidal sands. Cores were carefully extruded and segmented into 2 cm slices. They were immediately transferred to beakers containing 30 mL Ar-purged carbonate-free artificial seawater supplemented with 2 % ZnCl₂. The slices were gently stirred to homogenize the porewater with the artificial seawater. 3 mL of the solution was transferred to a gas tight vial and 1.5 mL of the liquid was replaced with a He headspace.

For flow-through reactor, slurry and sediment depth profile experiments, at least three replicates were performed so that the uncertainty in our measurements could be determined. This is the standard sample size for geomicrobiological studies, and the results show that the treatments vary to such an extent that this replication is sufficient. No statistical methods were used to pre-determine sample size.

Data collection

Samples were collected by the authors as described above, and all data were recorded digitally or analysed computationally as follows.

- H₂ was measured by an SRI Model 8610 Gas Chromatograph or a Valco TGA-6791-W-4U-2 Trace Gas Analyzer.
- δ²H-H₂ was measured using a ThermoFinnigan Delta Plus XL.
- δ²H-H₂O was measured using a Thermo Finnigan H-Device coupled to a Thermo Finnigan Delta Plus Advantage gas source mass spectrometer.
- Shotgun metagenomic sequencing was performed on an Illumina NextSeq500 platform and analysed using BBDuk, GratM, mothur, hmmer, DIAMOND, and MEGA7.
- Metagenome-assembled genomes were assembled and analyzed using SPAdes, BamM, MetaBAT, MaxBin, GroopM, DAS_Tool, dRep, CheckM, RefineM, RNAmmer, GTDB-TK, and Prodigal.
- 16 rRNA gene amplicons were sequenced using an Illumina MiSeq and analysed using the deblur pipeline on the QIIME 2 platform.
- O₂ was measured using a Pyroscience Firesting optical probe.
- H₂S was measured using a GBC UV/Visible Spectrophotometer using the methylene blue method.
- 15N₂ production was analyzed using a Sercon isotope ratio mass spectrometer coupled to a gas chromatograph.

Different authors were responsible for in situ measurements (Y.J.C., A.J.K., S.K., P.L.M.C., T.H., C.G.), isotope mass spectrometry analysis (M.E.P., A.J.K., P.L.M.C.), diatom experiments (Y.J.C., J.B., C.G., P.L.M.C.), flow-through reactor experiments (A.J.K., S.K., P.L.M.C.), slurry experiments (T.H., Y.J.C., A.J.K., P.L.M.C., C.G.), community analysis (Y.J.C., D.W., C.G., P.H.), and functional gene analysis (D.W., C.G., P.H., Y.J.C.). C.G., A.J.K., Y.-J.C., P.L.M.C., and D.W. analyzed the data.

Timing and spatial scale

All sample collections were conducted at Middle Park Beach, Melbourne, Australia (37.852425 S, 144.957519 E).

Seawater samples were collected from beyond the wash zone (~1 m deep) on October 28, 2016. Samples were collected every hour over an 11-hour period that encompassed low tide and high tide. This timing was chosen in order to observe any changes with tide.

Sediment samples for metagenome sequencing, and flow-through reactor experiments were collected on October 28, 2016. Samples for successive slurry experiments were collected on April 10, 2018 (experiment 1), April 30, 2018 (experiment 2), June 20, 2018 (experiment 3), or October 22, 2018 (experiment 4). Sediment samples for depth profiles were collected on February 28, 2018.

Flow-through reactor experiments were performed until H₂ was no longer observed and H₂S production dominated. Slurry experiments were performed until H₂ concentrations were stable at equilibrium concentration.

Data exclusions

No data were excluded from the manuscript. For the Keeling plot on Figure 1c, an outlying value (9 am) time point was excluded that is shown in Figure 1b.

Reproducibility

Experiments were performed periodically from October 2016 to October 2018, and similar results are obtained throughout this period. All attempts to repeat aspects of experiments were successful, and are included herein.

Randomization

Where samples were collected on site, we returned to the same site throughout the period described. Where samples were returned to the laboratory for flow-through reactor or slurry experiments, samples were homogenized and sub-sampled for the various treatments.

Blinding

As our study describes microbial processes and all data were collected using automated, digital instruments, blinding was not necessary in our experimental design.

Did the study involve field work? ☒ Yes ☐ No

Field work, collection and transport

Field conditions

Middle Park Beach is situated in Port Phillip Bay, ~ 5 km from the central business district of Melbourne, Australia. The site is accessible to general public. Port Phillip Bay is a relatively large, shallow embayment (1930km², mostly < 8 m deep, max depth 24 m) which provides protection to its beaches from severe weather. Seawater temperature ranged from 13.6 to 20.3°C based on historical record (<https://www.seatemperature.org/australia-pacific/australia/port-phillip.htm>).

Location

All sample collections were conducted at Middle Park Beach, Melbourne, Australia (37.852425 S, 144.957519 E). All samples were collected manually in fine weather from shallow (~ 1m deep) water less than 30 m from shore.

Access and import/export

Suitable footwear, gloves and equipments were used during the sampling process to minimize anthropogenic effects.

Disturbance

In total, <10 L of porewater and <10 kg of sediment samples were collected for this from the nearshore sampling. Disturbance was negligible as small volumes of samples were collected from a large beach in an urbanised area and any sediment taken will be replaced during tidal flows. In addition, appropriate cores and syringes were used.

Reporting for specific materials, systems and methods

Materials & experimental systems		Methods	
n/a	Involved in the study	n/a	Involved in the study
☒	☐ Unique biological materials	☒	☐ ChIP-seq
☒	☐ Antibodies	☒	☐ Flow cytometry
☒	☐ Eukaryotic cell lines	☒	☐ MRI-based neuroimaging
☒	☐ Palaeontology		
☒	☐ Animals and other organisms		
☒	☐ Human research participants		