Solution 31P NMR spectra files from sediment samples collected during R/V JOIDES Resolution cruise JRES-336 (IODP336, North Pond) to the western flank of the mid-Atlantic Ridge in November of 2011

Website: https://www.bco-dmo.org/dataset/816623
Data Type: Cruise Results
Version: 1
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Project
» Potential phosphorus uptake mechanisms of the deep sedimentary biosphere (Deep sea sediments)

Program
» Center for Dark Energy Biosphere Investigations (C-DEBI)

Contributors	Affiliation	Role
Paytan, Adina	University of California-Santa Cruz (UC Santa Cruz)	Principal Investigator
Defforey, Delphine	University of California-Santa Cruz (UC Santa Cruz)	Co-Principal Investigator
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Abstract
Solution 31P nuclear magnetic resonance (NMR) spectra files from sediment samples collected during R/V JOIDES Resolution cruise JRES-336 (IODP336, North Pond) to the western flank of the mid-Atlantic Ridge in November of 2011. Samples were analyzed in 2016.

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Coverage

Spatial Extent: **Lat**:22.75589 **Lon**:-46.08125
Temporal Extent: 2011 - 2016

Dataset Description

Solution 31P nuclear magnetic resonance (NMR) spectra files from sediment samples collected during R/V JOIDES Resolution cruise JRES-336 (IODP336, North Pond) to the western flank of the mid-Atlantic Ridge in November of 2011. Samples were analyzed in 2016.

These data were published in Defforey et al. (2016) and Defforey et al. (2017). See the related-resource page https://www.bco-dmo.org/project/664073 for other datasets related to this publication.

Additional award information:

* NSF C-DEBI subaward # 156246 to Adina Paytan
* NSF C-DEBI subaward # 157598 to Delphine Defforey

Acquisition Description

Location: North Atlantic, western flank of the mid-Atlantic Ridge 22.75589 N 46.08125 W

Methodology:

Prior to the extraction, we freeze-dried, ground and sieved sediment samples to less than 125 µm (Ruttenberg 1992). For a given sample, we weighed four sample replicates (2 g) and placed each in 250 mL HDPE bottles. Sodium dithionite (F.W. 147.12 g/mol; 7.4 g) was added to each sample split, followed by 200 mL of citrate-bicarbonate solution (pH 7.6). This step produces effervescence, so the solution should be added slowly to the sample. We shook samples for 8 h and then centrifuged them at 3,700 rpm for 15 min. We filtered the supernatants with a 0.4 µm polycarbonate filter. We took 20 mL aliquots from the filtrate for each sample split for MRP and total P analyses, and kept them refrigerated until analysis within 24 h. We added 200 mL of ultrapure water to the solid residue for each sample split as a wash step after the above reductive step, shook samples for 2 h, and then centrifuged them at 3,700 rpm for 15 min. We filtered the supernatants with 0.4 µm polycarbonate filters and set aside 20 mL of filtrate from each sample split for MRP and total P analyses. We then extracted the solid sample residues in 200 mL of sodium acetate buffer (pH 4.0) for 6 h. At the end of this extraction step, we centrifuged the bottles at 3,700 rpm for 15 min, filtered the supernatants with 0.4 µm polycarbonate filters and took a 20 mL aliquot of filtrate from each sample split for MRP and total P analyses. We added 200 mL of ultrapure water to the solid residue for each sample split as a wash step, shook samples for 2 h, and then centrifuged them at 3,700 rpm for 15 min. We filtered the supernatants with 0.4 µm polycarbonate filters and set aside 20 mL of filtrate from each sample split for MRP and total P analyses. We repeated the water rinse step, and collected aliquots for MRP and total P analyses as in the previous steps. The concentrations of TP were determined as described below.

Solid sediment sample residues following the pretreatment described above were transferred to two 50 mL centrifuge tubes (2 sample replicates combined per tube). We added 20 mL of 0.25 M NaOH + 0.05 M Na_2EDTA solution to each tube, vortexed until all sediment was resuspended and then shook samples for 6 h at room temperature (Cade-Menun et al. 2005). We used a solid to solution ratio of 1:5 for this step to minimize the amount of freeze-dried material that will need to be dissolved for the ^{31}P NMR experiments. Large amounts of salts from the NaOH-EDTA concentrated in NMR samples lead to higher viscosity and increase line broadening on NMR spectra (Cade-Menun and Liu 2014). We chose an extraction time of 6 h to improve total P recovery while limiting the degradation of natural P compounds in the sample. At the end of the extraction, samples were centrifuged at 3,700 rpm for 15 min and supernatants decanted into 50 mL centrifuge tubes. We collected a 500 µL aliquot from each sample, which we diluted with 4.5 mL of ultrapure water. These were refrigerated until analysis for total P content on the ICP-OES. The sample residues and supernatants were frozen on a slant to maximize the exposed surface area during the lyophilization step; this was done immediately after the removal of the 500 µL aliquot. Once completely frozen, the uncapped tubes containing supernatants and residues were freeze-dried over the course of 48 h. Each tube was covered with parafilm with small holes from a tack to minimize contamination. Freeze-dried supernatants from identical sample splits were combined and dissolved in 500 µL each of ultrapure water, D_2O, NaOH-EDTA and 10 M NaOH prior to ^{31}P NMR analysis. The D_2O is required as signal lock in the spectrometer (Cade-Menun and Liu 2014). Sample pH was maintained at a pH > 12 to optimize peak separation (Cade-Menun 2005; Cade-Menun and Liu 2014). Sample pH was assessed with a glass electrode, and verified with pH paper to account for the alkaline error caused by the high salt content of our samples (Covington 1985).

Spectra were acquired immediately following sample preparation on a Varian Unity INOVA 600 MHz spectrometer equipped with a 10-mm broadband probe [operated by the Stanford Magnetic Resonance Laboratory at Stanford University]. We used a 10-mm rather than a 5-mm probe because larger tubes contain a greater concentration of P and thus require fewer scans to achieve similar signal to noise ratios (Cade-Menun and Liu 2014). The analytical parameters used were: 20^oC, 90^o pulse, 0.48 s acquisition time, 4.52 s delay time, 5600 scans (8 h experiments), no spin and an external H_3PO_4 standard. We maintained samples at a temperature of 20^oC during experiments to achieve optimal spectral resolution (Crouse et al. 2000) and to minimize sample degradation. No proton decoupling was used out of concern for sample degradation (Cade-Menun and Liu 2014). The ratio of P to Fe and manganese (Mn) was used as a proxy for spin-lattice relaxation times (T_1) to ensure adequate delays between pulses and thus quantitative spectra (McDowell et al. 2006). We used 5 s recycle delays, which correspond to three to five times the calculated T_1 values, as recommended by McDowell et al. (2006). Peak identification was based on literature (Turner et al. 2003; Cade-Menun 2015).

^{31}P NMR data were processed using the NMR Utility Transform software (NUTS, Acorn NMR). Peak areas were calculated by integration of spectra processed with a 7 Hz line broadening following baseline correction, peak picking and phasing. We accepted peaks that (1) represented at least 1% of the tallest peak in the total integrated area, (2) were identified by the NUTS software and (3) were confirmed as signal by visual inspection.

Freeze-dried sample residues were ashed in crucibles at 550 ^oC for 2 h and then extracted in 25 mL of 0.5 M sulfuric acid for 16 h (Olsen and Sommers 1982; Cade-Menun and Lavkulich 1997). We centrifuged samples at 3,700 rpm for 15 min, filtered supernatants with 0.4 µm polycarbonate filters, and measured P content on an ICP-OES.

Total P concentrations in sediment extracts were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). Standards were prepared with the same solutions as those used for the extraction procedure in order to minimize matrix effects on P measurements. Sediment extracts and standards (0 µM, 3.2 µM, 32 µM and 320 µM) were diluted to lower salt content to prevent salt buildup on the nebulizer (1:20 dilution for step 1, 1:10 for steps 2 – 4). Concentration data from both wavelengths (213 nm and 214 nm) were averaged to obtain extract concentrations for each sample. The detection limit for P on this instrument for both wavelengths is 0.4 µM. The MRP concentrations were measured on a QuikChem 8000 automated ion analyzer. Standards were prepared with the same solutions used for the extraction step to minimize matrix effects on P measurements. Sediment extracts and standards (0 – 30 µM PO_4) were diluted ten-fold to prevent matrix interference with color development. The detection limit for P on this instrument is 0.2 µM. We derived MUP concentrations by subtracting MRP from total P concentrations.

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Data Files

File	Version
NMR FID files filename: NMR_FID_files.zip (ZIP Archive (ZIP), 137.78 KB) MD5:c28cbf2af346a6d430259034956aa11d *A .zip file package containing NMR FID files. The contents of the individual folders are standard outputs from the Varian Unity INOVA 600 MHz spectrometer software. The spectra are in the fid (free induction decay) files.* *The folder names in this package include the following sample names:* *1382B1H-3.fid* *1382B7H-3.fid* *A file named "text" within each folder contains additional notes.*	original

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Related Publications

Cade-Menun, B. (2005). Characterizing phosphorus in environmental and agricultural samples by 31P nuclear magnetic resonance spectroscopy. Talanta, 66(2), 359–371. doi:10.1016/j.talanta.2004.12.024
Methods

Cade-Menun, B., & Liu, C. W. (2013). Solution Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy of Soils from 2005 to 2013: A Review of Sample Preparation and Experimental Parameters. Soil Science Society of America Journal, 78(1), 19–37. doi:10.2136/sssaj2013.05.0187dgs
Methods

Cade-Menun, B. J., & Lavkulich, L. M. (1997). A comparison of methods to determine total, organic, and available phosphorus in forest soils. Communications in Soil Science and Plant Analysis, 28(9-10), 651–663. doi:10.1080/00103629709369818
Methods

Covington, A. K. (1985). Procedures for testing pH responsive glass electrodes at 25, 37, 65 and 85 C and determination of alkaline errors up to 1 mol dm-3 Na+, K+, Li+. Pure and Applied Chemistry, 57(6), 887–898. doi:10.1351/pac198557060887
Methods

Crouse, D. A., Sierzputowska-Gracz, H., & Mikkelsen, R. L. (2000). Optimization of sample ph and temperature for phosphorus-31 nuclear magnetic resonance spectroscopy of poultry manure extracts. Communications in Soil Science and Plant Analysis, 31(1-2), 229–240. doi:10.1080/00103620009370432
Methods

Defforey, D. (2016). Phosphorus cycling in the deep sedimentary subseafloor environment. PhD Thesis, UC Santa Cruz. https://escholarship.org/uc/item/85p2s7dx
Results

Defforey, D., Cade-Menun, B. J., & Paytan, A. (2017). A new solution 31 P NMR sample preparation scheme for marine sediments. Limnology and Oceanography: Methods, 15(4), 381–393. doi:10.1002/lom3.10166
Results

McDowell, R. W., Stewart, I., & Cade-Menun, B. J. (2006). An Examination of Spin-Lattice Relaxation Times for Analysis of Soil and Manure Extracts by Liquid State Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy. Journal of Environmental Quality, 35(1), 293–302. doi:10.2134/jeq2005.0285
Methods

Olsen, S. R., & Sommers, L. E. (1982). Phosphorus. p. 403–430. In A.L. Page, R.H. Miller, and D.R. Keeney [eds.], Methods of Soil Analysis. Soil Science Society of America.
Methods

Ruttenberg, K. C. (1992). Development of a sequential extraction method for different forms of phosphorus in marine sediments. Limnology and Oceanography, 37(7), 1460–1482.

doi:10.4319/lo.1992.37.7.1460
Methods

Turner, B. L., Mahieu, N., & Condron, L. M. (2003). The phosphorus composition of temperate pasture soils determined by NaOH–EDTA extraction and solution 31 P NMR spectroscopy. Organic Geochemistry, 34(8), 1199–1210. doi:10.1016/s0146-6380(03)00061-5 https://doi.org/10.1016/S0146-6380(03)00061-5
Methods

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	QuikChem 8000 automated ion analyzer
Generic Instrument Name	Flow Injection Analyzer
Generic Instrument Description	An instrument that performs flow injection analysis. Flow injection analysis (FIA) is an approach to chemical analysis that is accomplished by injecting a plug of sample into a flowing carrier stream. FIA is an automated method in which a sample is injected into a continuous flow of a carrier solution that mixes with other continuously flowing solutions before reaching a detector. Precision is dramatically increased when FIA is used instead of manual injections and as a result very specific FIA systems have been developed for a wide array of analytical techniques.

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Deployments

JRES-336

Website	https://www.bco-dmo.org/deployment/628214
Platform	R/V JOIDES Resolution
Report	http://dmoserv3.whoi.edu/data_docs/C-DEBI/cruise_reports/336PR.pdf
Start Date	2011-09-16
End Date	2011-11-16
Description	More information is available from the IODP website: http://iodp.tamu.edu/scienceops/expeditions/midatlantic_ridge_microbio.html

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Project Information

Potential phosphorus uptake mechanisms of the deep sedimentary biosphere (Deep sea sediments)

Coverage: Mid-Atlantic Ridge flank

The goal of this project is to explore potential microbial P uptake mechanisms in marine sediments beneath the North Atlantic Gyre and their effects on the relative distribution of organic P compounds as a function of burial depth and changing redox conditions. We use a combination of metagenomic analyses and solution 31P nuclear magnetic resonance spectroscopy (31P NMR) to investigate (1) the presence of microbial functional genes pertaining to P uptake and metabolism and (2) the possible P substrates for the deep biosphere in these oligotrophic sediments. NSF C-DEBI Award #156246 to Dr. Adina Paytan NSF C-DEBI Award #157598 to Dr. Delphine Defforey

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Program Information

Center for Dark Energy Biosphere Investigations (C-DEBI)

Website: http://www.darkenergybiosphere.org

Coverage: Global

The mission of the Center for Dark Energy Biosphere Investigations (C-DEBI) is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins. C-DEBI provides a framework for a large, multi-disciplinary group of scientists to pursue fundamental questions about life deep in the sub-surface environment of Earth. The fundamental science questions of C-DEBI involve exploration and discovery, uncovering the processes that constrain the sub-surface biosphere below the oceans, and implications to the Earth system. What type of life exists in this deep biosphere, how much, and how is it distributed and dispersed? What are the physical-chemical conditions that promote or limit life? What are the important oxidation-reduction processes and are they unique or important to humankind? How does this biosphere influence global energy and material cycles, particularly the carbon cycle? Finally, can we discern how such life evolved in geological settings beneath the ocean floor, and how this might relate to ideas about the origin of life on our planet? C-DEBI's scientific goals are pursued with a combination of approaches: (1) coordinate, integrate, support, and extend the research associated with four major programs—Juan de Fuca Ridge flank (JdF), South Pacific Gyre (SPG), North Pond (NP), and Dorado Outcrop (DO)—and other field sites; (2) make substantial investments of resources to support field, laboratory, analytical, and modeling studies of the deep subseafloor ecosystems; (3) facilitate and encourage synthesis and thematic understanding of submarine microbiological processes, through funding of scientific and technical activities, coordination and hosting of meetings and workshops, and support of (mostly junior) researchers and graduate students; and (4) entrain, educate, inspire, and mentor an interdisciplinary community of researchers and educators, with an emphasis on undergraduate and graduate students and early-career scientists. Note: Katrina Edwards was a former PI of C-DEBI; James Cowen is a former co-PI. Data Management: C-DEBI is committed to ensuring all the data generated are publically available and deposited in a data repository for long-term storage as stated in their Data Management Plan (PDF) and in compliance with the NSF Ocean Sciences Sample and Data Policy. The data types and products resulting from C-DEBI-supported research include a wide variety of geophysical, geological, geochemical, and biological information, in addition to education and outreach materials, technical documents, and samples. All data and information generated by C-DEBI-supported research projects are required to be made publically available either following publication of research results or within two (2) years of data generation. To ensure preservation and dissemination of the diverse data-types generated, C-DEBI researchers are working with BCO-DMO Data Managers make data publicly available online. The partnership with BCO-DMO helps ensure that the C-DEBI data are discoverable and available for reuse. Some C-DEBI data is better served by specialized repositories (NCBI's GenBank for sequence data, for example) and, in those cases, BCO-DMO provides dataset documentation (metadata) that includes links to those external repositories.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-0939564

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