MICROENERGY 2015

3rd International Workshop on

Microbial Life under Extreme Energy Limitation

Sandbjerg Manor, Denmark

September 21-25, 2015

Program, Abstracts, and Information

Convened by

Bo Barker Jørgensen, Center for Geomicrobiology, Aarhus University, Denmark

Jan P. Amend, University of Southern California, California, USA

Tori M. Hoehler, NASA Ames Research Center, California, USA

Center for Dark Energy Biosphere Investigations C-DEBI

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Background for the workshop

A growing body of work on microbial life in deep subsurface environments has altered our perspective on the limits of living organisms and challenged our understanding of their need for nutrients and energy. Microbial cells in these very stable and oligotrophic settings apparently catabolize 10^4 to 10^6 fold more slowly than organisms in nutrient-rich cultures and thereby subsist with energy fluxes orders of magnitude below what are considered to be "maintenance" levels. Such organisms may in fact represent a truly basal state of metabolism, with a corresponding basal power requirement, that is not easily reproduced in culture. Do these organisms have extraordinary properties that are beyond our current understanding of microbial energy metabolism and not represented in cultured organisms, or are the capability to subsist at extremely low energy fluxes an inherent property of many microorganisms? What are the energetic requirements and limits to life, how are they affected by the environment, and how can we characterize them more fully in both natural and culture-based systems? These questions bear on a physiological state that is pervasive and meaningful in the environment. Prokaryotic cells in the terrestrial or marine sub-surface comprise a significant fraction of all living microorganisms on Earth and, at the interface between the inhabited and uninhabited realms of our planet, they represent the ultimate biological arbiters of chemical exchange between the biosphere and the geosphere. Much remains to be done to understand this physiological state and its consequences and implications for microbial ecology and biogeochemistry.

To address these and many other challenging questions, we organize the 3rd International Workshop on Microbial Life under Extreme Energy Limitation to explore the biological demand for energy, with a specific focus on microorganisms.

This workshop follows up on the outcome of an earlier 2nd International Workshop on the same theme which was organized 2012 in Aarhus, Denmark, by Tori Hoehler and Bo Barker Jørgensen. The new understanding of slow microbial life that emerged from that workshop was recently published in a comprehensive review authored by the rapporteurs of the working groups and the organizers of the workshop. (Lever, M.A., K. Rogers, K.G. Lloyd, J. Overmann, B. Schink, R. Thauer, T.M. Hoehler, B.B. Jørgensen (2015) Microbial life under extreme energy limitation: a synthesis of laboratory- and field-based investigations. FEMS Microbiology Reviews, doi: 10.1093/femsre/fuv020).

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Program

Monday, Sept. 21:

15:00 – 18:00 **Registration and mounting of posters**

18:00 – 18:30 Opening address and statement of goals and charter (**Bo Barker Jørgensen, Tori Hoehler and Jan Amend**)

18:30 – 19:15 **Keynote Lecture:** Far, fast, and surprising: length, time, and energy scales of microbial electron transport (**Moh El-Naggar** Sahand Pirbadian, Yamini Jangir, Hye Suk Byun, Benjamin J. Gross, Shuai Xu)

19:15 – ? **Mixer with buffet dinner**

Tuesday, Sept. 22:

08:30 – 10:10 ***Energy sources in nature***

- Controls on organic matter degradation rates (**Jack Middelburg**) *[Invited]*
- Generation of energy sources in rock-hosted ecosystems (**Alexis Templeton**) *[Invited]*
- Boosting the deep biosphere: subseafloor sediment a natural catalyst for radiolytic hydrogen production (**Justine Sauvage** Arthur Spivack, Ann Dunlea, Richard Murray, Steven D'Hondt)
- How geochemistry provides habitability: a case study of iron oxidation (**Brian St Clair**, Everett Shock)

10:10 – 10:40 **Break**

10:40 – 12:00 ***Energy requirements***

- Microbial maintenance energy as linked to microbial ecological strategies and ecosystem fluxes (**Peter van Bodegom**) *[Invited]*
- Minimum energy quantum of anaerobic microbial catabolism: Hydrogen-formate interconversion (**Caroline M. Plugge,** João A.B. Sousa, Alfons J.M. Stams) *[Invited]*
- Volatile fatty acids as substrates for sulfate reduction in sediments from Southwest Greenland (**Clemens Glombitza,** Marion Jaussi, Hans Røy, Bo Barker Jørgensen)

12:00 – 13:30 **Lunch**

13:30 - 15:40 ***Energy requirements and process rates***

- The energetics of anabolism in natural environments (**Doug LaRowe**, Jan Amend) *[Invited]*

- Biogeochemical metabolic modeling: Linking thermodynamics and kinetics of subsurface microbial processes (**Qusheng Jin**, Benjamin M. Shapiro, Zena D. Jensvold, Shannon E. McKernan) *[Invited]*
- Bulk microbial respiration in the buried biosphere (**Hans Røy**, Marion Jaussi, Kasper Urup Kjeldsen, Bo Barker Jørgensen) *[Invited]*
- Sulfate reduction near the energetic limit: Implications for sulfur isotope fractionation in marine sediments (**Itay Halevy** Boswell A. Wing, Christine Wenk, Claire Guimond, Andre Pellerin)
- Slow, deep phototrophy in conspicuous pinnacle mats from magical blue hole (**Jennifer Macalady** J.L., Haas, S., Hamilton, T. L., Kakuk, B., Fink, A, Meyer, V, Rench, R.M., de Beer, D.)

15:40 – 16:00 **Break**

16:00 - 17:30 **Poster session - I**

17:30 – ? **Cultural evening and dinner at Sønderborg Castle**

Wednesday, Sept. 23:

08:30 – 10:00 ***Metabolic states in culture***

- Mechanisms of long-term survival and evolution in laboratory microcosms (**Steven** Finkel Karin Kram, Nicole Ratib, Christopher Geiger, Lacey Westphal, Christopher Corzett, Christina Ferraro, Fabian Seidl, Ian Ehrenreich) *[Invited]*
- Near-zero growth of microorganisms under deep nutrient limitation (**Nicolai S. Panikov**) *[Invited]*
- Life signs in apparently dead bacteria (**Charles William Keevil**) *[Invited]*

10:00 – 10:20 **Break**

10:20 – 12:00 ***Metabolic states and turnover in nature***

- Abundance and functions of archaea and bacteria in the seabed (**Karen G. Lloyd** Jordan Bird, Shawn Campagna, Eric Tague, Hector Castro, Ian Marshall, Brandi Reese, Gordon Webster, Andrew Weightman) *[Invited]*
- D:L-amino acids and the turnover of microbial biomass (**Bente Aa. Lomstein**) *[Invited]*
- Ecological and evolutionary insight into bacterial persistence during starvation (**Jay Lennon**)
- Viral lysis plays a minor role in controlling microbial populations in oligotrophic cold saline sediments (**Jesse Colangelo-Lillis**, L.G. Whyte, B.A. Wing)

12:00 – 13:30 **Lunch**

13:30 – 15:30 **1st Working Group Session**

15:30 – 16:00 **Break**

16:00 – 18:00 **Poster session II**

18:00 **Dinner; evening free**

Thursday, Sept. 24:

08:30 – 09:50 ***Strategies***

- Microbial life cycle: differentiation in growth and de-programming in dormancy (**Slava Epstein**) *[Invited]*
- Optimizing substrate utilization on a mixed diet (**Mark A. Lever**) *[Invited]*
- Life strategies of bathyarchaeota in the subsurface (**Fengping Wang**, Ying He, Meng Li, Stefan M Sievert,Vengatesha Perumal)

09:50 – 10:20 **Break**

10:20 – 12:00 ***Adaptations***

- Archaeal adaptations to extreme energy limitation (**Volker Müller**) *[Invited]*
- A single cell perspective of cooperation between methanotrophic archaea and their sulfate-reducing bacterial partners (**Victoria Orphan** Shawn McGlynn, Grayson Chadwick, Chris Kempes, Roland Hatzenpichler, Connor Skennerton) *[Invited]*
- Energy limits of electrical cable bacteria in the subsurface (**Lars Peter Nielsen**)
- Peeling the onion of life's anaerobic and low energy origin (**Filipa Sousa**, William F. Martin)

12:00 – 13:30 **Lunch**

13:30 – 14:40 ***Evolution***

- Evolution in the deep biosphere (**Andreas Schramm**) *[Invited]*
- Adaptive microbial evolution in deepsea sediments (**Alfred M. Spormann**)
- The dominant constraints motivating evolutionary transitions and limiting body size (**Chris Kempes**, Tori Hoehler, Jan Amend, John Doyle, Michael Follows, Stephanie Dutkiewicz)

14:40 – 16:30 **2nd Working Group Session**

16:30 – 17:00 **Break**

17:00 – 18:30 **Plenary Session: Synthesis of workshop results**

19:00 **Workshop dinner**

Friday, Sept. 25:

06:30 - 9:00 **Breakfast, departure**

Invited talks = 30 minutes, including time for questions & changeover
Contributed talks = 20 minutes, including time for questions & changeover

Social events

Mixer, Magasinet, Sandbjerg Manor – Monday, September 21 at 19:15
The mixer will take place after the opening lecture by Prof. Moh El-Naggar. The mixer is with buffet dinner and beverages will be served. The mixer is included in the registration fee.

Cultural evening: Sonderborg Castle – Tuesday, September 22 at 17:30
Museum Inspector René Rasmussen will give an introduction to Sonderborg Castle and its history followed by a guided tour. After the tour participants will be served dinner (including beverages) in the Great Hall's eastern antechamber. Bus transportation will be arranged for all participants. The Cultural evening and dinner is included in the registration fee.

Workshop dinner – Thursday, September 24 at 19:00
Sandbjerg Manor, Magasinet (B), will serve a three-course dinner with beverages followed by coffee and sweets in the Manor House. The workshop dinner is included in the registration fee.

Posters

Name	Title
Gilad Antler, Alexandra V. Turchyn, Jennifer V. Mills, Serena Ppovia, Kelly Redeker	The Sulfur-Iron Interplay and its Role in the Fate of Carbon in Salt Marsh Sediments
Dimitra Atri	Can Galactic Cosmic Ray-Induced Radiolysis Power a Subsurface Biosphere?
Itay Bar-Or, Eitan Ben-Dov, Eckert Werner, Ariel Kushmaro and Orit Sivan	Exploring the Mechanisms of Anaerobic Oxidation of Methane in Deep Sediments of Lake Kinneret (Israel)
Felix Beulig, Bo B. Jørgensen	Biotic and Abiotic Controls of Methane Production and Turnover in Marine Sediments
Jennifer G. Blank and the BRAILLE Team	Microenergy in the Subsurface: The Case for Microbial Communities in Lava Tube Caves
Stefan Braun, Yuki Morono, Kevin Becker, Kai-U. Hinrichs, Bo B. Jørgensen, Bente Aa. Lomstein	Analysis of Biomolecules of Sub-Seafloor Microbial Cells Seperated from the Sediment Matrix
Brandon R. Briggs	Metagenomic Insights Into Altered Genomes of Firmicutes from the Deep Biosphere
Håkon Dahle, Ingeborg Økland, Ingunn H. Thorseth, Rolf B. Pedersen, Ida H. Steen	Energy Landscapes Shape Microbial Communities in Deep-Sea Hydrothermal Systems along the Artic Mid-Ocean Ridge
Steven D'Hondt, Guizhi Wang, Arthur J. Spivack	The Underground Economy (Energetic Constraints and Survavial Strategies of Subseafloor Sedimentary Life)
Clemens Glombitza Florian Schwarz, Kai Mangelsdorf	Feeding Potential for Deep Microbial Ecosystems of Free and Macromolecular-Bound Formate and Acetate in 2 km Deeply Buried Coalbeds Offshore Shimokita Penninsula (Japan)
Merja Itävaara	Studies of Fennoscandian Shield Fractures Reveal Rock Inhabited Deep Life
Marion Jaussi, Kasper U. Kjeldsen, Marit-Solveig Seidenkrantz, Bente Aa. Lomstein, Bo B. Jørgensen, Hans Røy	What is the Lower Limit for Cellular Metabolic Rates?

Steffen Leth Jørgensen, Rui Zhao, Rolf Birger Pedersen, Tamara Baumberger, Ingeborg Økland, Desiree Roerdink, Ingunn Thorseth	Increased Energy Supply in Transition Zones Support Enhanced Nitrogen Cycling in Deep-Sea Sediments
John B. Kirkpatrick, Emily A. Walsh, Mitchell Sogin, Robert Pockalny, and Steven D'Hondt	Whole Community Richness, Diversity Loss, and Selection in an Energy-Limited Deep Biosphere
Lara M. Jochum, Caitlin Petro, Piotr Starnawski, Lars Schreiber, Alexander Loy, Bo B. Jørgensen, Andreas Schramm, **Kasper U. Kjeldsen**	Assembly of Sulfate-Reducing Microbial Communities in Marine Subsurface Sediments
Ajinkya Kulkarni, Ines Hesse, **Oluwatobi E. Oni**, Yin Xiuran, Sabine Kasten, **Michael W. Friedrich**	Methane-Cycling Archaeal Populations in Sub-Surface Sediments of The Helgoland Mud Area, North Sea
Shawn E. McGlynn, Grayson L. Chadwick, Mason Mackey, Andrea Thor, Thomas J. Deerinck, Mark H. Ellisman, Victoria J. Orphan	Invaginated Cytoplasmic Membranes in Low Energy Syntrophic Sulfate Reducing Bacteria
Snehit S. Mhatre, Bo B. Jørgensen, Bente Aagaard Lomstein	Study of Microbial Activity in Marine Sediments of the Island Basin Using D:L Amino Acid Model
Alexander B. Michaud, Amanda A Achberger, Brent C. Christner, John E. Dore, Mark L. Skidmore, Trista J. Vick-Majors, John C. Priscu	Microbial Methane Cycling in Subglacial Lake Whillans, West Antarctica
Heath J. Mills, Brandi Kiel Reese	Metabolically Active Microbial Populations within North Pond Subsurface Crustal Basalts Expands the Biosphere and the Limits of Life
Ali Nawaz, Tesfaye Wubet, Francois Buscot	First Insights into the Fungal Communities of Subsurface Karstic Aquifers in the Earth's Critical Zone
Hideshi Ooka (*), Kazuhito Hashimoto (*), Ryuhei Nakamura (**)	The Asymmetry of Multi-Electron Transfer Processes at the Enzyme Gene Structure Level
Shane S. O'Reilly, Sharon A. Newman, Frank McDermott, Roger E. Summons	Lipid Biomarkers Preserved within Oman Ophiolite Carbonates: Insights into a Subsurface Serpentinite-Hosted Ecosystem

Magdalena R. Osburn, Doug E. LaRowe, Lily M. Momper, Jan P. Amend	The Energetic Landscape of Chemolithotrophy in the Continentanl Deep Subsurface: Sanford Underground Research Facility (SURF), USA
Marco Blöthe, Anna Wegorzewski, Cornelia Müller, Frank Simon, Thomas Kuhn, **Axel Schippers**	Manganese Cycling Microbial Communities Inside Deep-Sea Manganese Nodules
Orit Sivan, Itay Bar-Or, Gilad Antler, Alexandra V. Turchyn, Victoria J. Orphan, Werner Eckert	Iron Driven Anaerobic Methane Oxidation in Marine and Freshwater Sediments
Alfred M. Spormann	Extracellular Enzymes Facilitate Electron Uptake in Biocorrosion and Bioelectrosynthesis
Piotr Starnawski (*), Kasper U. Kjeldsen, Andreas Schramm, Thomas Bataillon	Accelerated Microbial Mutation Rates in a Marine Subsurface Sediment
Stephanie Turner, Marco Blöthe, Robert Mikutta, Sandra Meyer-Stüve, Georg Guggenberger, Reiner Dohrmann, Axel Schippers	Temporal and Depth-Related Variability of Microbial Communities in Soils along an Ecosystem Development Gradient
Verona Vandieken, Oscar Chiang, Bert Engelen, Heribert Cypionka	Potential Impact of Salinity Changes on Bacterial Isolates from the Deep Biosphere of the Baltic Sea
Trista J. Vick-Majors, Alexander B. Michaud, Amanda Achberger, Brent Christner, Mark Skidmore, Jill Mikucki, Andrew C. Mitchell, John C. Priscu	Limitations on Heterotrophic Activity in Subglacial Lake Whillans, West Antartica
Boswell A. Wing, Itay Halevy,Jyotsana Singh, André Pellerin, Christine Wenk	Isotope Fractionation Informs the Respiratory Proteome of Sulfate Reducing Microbes
Katinka Wouters, Mohamed Mysara, Hugo Moors, Natalie Leys	Variations in Microbial Community Composition in Deep Subsurface Piezometer Installations
Tingting Yang , Lars Peter Nielsen, Nils Risgaard-Petersen	Observation of Polyphosphate Granules in Cable Bacteria
Rui Zhao, Steffen L. Jørgensen, Ingeborg Økland, Tamara Baumberger, Desiree Roerdink, Rolf B. Pedersen, Ingunn Thorseth	Energetics of Nitrifiers in Oxygenated Deep-Sea Sediments

Abstracts

THE SULFUR-IRON INTERPLAY AND ITS ROLE IN THE FATE OF CARBON IN SALT MARSH SEDIMENTS

Gilad Antler (*), Alexandra V. Turchyn (*), Jennifer V. Mills (*), Serena Ppovia, Kelly Redeker (**)

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(**) *Department of Biology, University of York, Heslington, York, UK.*

Salt marshes are highly productive coastal wetlands that serve a critical role in carbon sequestration and nutrient trapping. In contrast to marine sediments that accumulate slowly over many thousands or millions of years, salt marshes are highly dynamic transitional environments between the terrestrial and the marine realms. Because salt marshes are flushed daily or monthly with seawater (with high concentrations of sulfate), our understanding is that the oxidation of organic carbon in salt marsh sediments is dominated by microbial sulfate reduction, similar to deeper marine sediments. These high sulfate concentrations either inhibit methanogenesis or anaerobically oxidize any methane produced; thus salt marshes are not currently a large source of methane to the atmosphere, unlike terrestrial wetlands.

We present pore fluid geochemical results from salt marsh sediments in eastern England. The subsurface geochemistry can be divided into two types; ferruginous-sediments with very high ferrous iron concentrations (up to 2.5mM) and sulfidic-sediments with high dissolved sulfide concentration (up to 8mM) and methane. These two types of sediment are found as close as a few meters apart and are remarkably different in both geochemistry and sediment type and texture. We suggest that spatial variation in the ferrous-iron-rich saline groundwater creates two geochemically distinguishable sediments within the salt marsh. Where this saline water body is close to the surface, the supply of ferrous iron allows bioturbation by scavenging sulfide that would otherwise be toxic. This bioturbation enhances bioirrigation through which oxygen is supplied from the overlying oxygenated water mixing with iron from the saline water body below. In the aftermath of the bioturbation, the sediment becomes organic-matter poor but sulfate- and iron-oxide rich. In contrast, in locations where the ferrous iron is depleted, the sediment becomes toxic with excess dissolved sulfide and bioturbation is prevented. The end result in this case is sediment that is methane and sulfide rich and iron and sulfate poor.

CAN GALACTIC COSMIC RAY-INDUCED RADIOLYSIS POWER A SUBSURFACE BIOSPHERE?

Dimitra Atri (*)

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The discovery of *Desulforudis audaxviator* in a 3.2 km deep South African mine has forced us to think beyond the geochemical and geothermal sources of energy available in the subsurface environments. The organism utilizes the alpha, beta and gamma radiation emitted by radioactive U, Th and K present in rock. This energy source accompanied by small amount of water and nutrients from rock has enabled *Desulforudis audaxviator* to lead an independent lifestyle completely cutoff from the photosphere. Another source of subsurface radiation are Galactic Cosmic Ray induced secondary particles, especially muons, which can penetrate several kilometers depending on their energy. We show that muon-induced radiolysis can also produce beta and gamma-radiation in subsurface environments and can potentially power a subsurface biosphere. Quantitative analysis of this radiation source is described and compared with the radiation environment of *Desulforudis audaxviator*.

EXPLORING THE MECHANISMS OF ANAEROBIC OXIDATION OF METHANE IN DEEP SEDIMENTS OF LAKE KINNERET (ISRAEL)

Itay Bar-Or (*), Eitan Ben-Dov (*), Eckert Werner (**), Ariel Kushmaro (*) and Orit Sivan (*)

(*) *Ben-Gurion University of the Negev, P.O. BOX 653, Beer Sheva, Israel.*

(**) *Israel Oceanographic and Limnological Research, The Yigal Allon Laboratory, Tiberias , Israel.*

Recently, we showed geochemical evidence for anaerobic oxidation of methane (AOM) driven by iron reduction in Lake Kinneret (LK) (Israel) sediments, and suggested that this process can be an important global sink for methane. This AOM via iron reduction can occur by two main potential pathways: 1) Direct mechanism where Fe(III) is reduced directly by methane 2) Indirect mechanism which shows complex coupling between Fe, S and CH_4 in AOM or other couplings. In this study we investigate the possibility for complex coupling with sulfate, which could generate sulfate and be similar to methanotrophic process performed at sea via sulfate reduction. Geochemical analyses of slurry experiments with different manipulations and inhibitors (such as molibdate) show that the mechanism in LK is different than in the marine environment. In addition 16S rRNA gene sequencing shows no similarity to microorganisms involve in the AOM at sea. Therefore, sulfate reduction via AOM is revoked, however the mechanism of AOM is still not discovered.

BIOTIC AND ABIOTIC CONTROLS OF METHANE PRODUCTION AND TURNOVER IN MARINE SEDIMENTS

Felix Beulig (*,)**, Bo Barker Jørgensen (**)

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The fate of elements in marine sediments is determined by microbially mediated electron accepting processes (EAP). Substrate competition and differences in the 'potency' of these EAP (as determined by the associated Gibbs free energy) result in the formation of distinct redox zones and dominance of associated microbial communities. Therefore, methanogenesis typically becomes the main terminal process of organic matter (OM) mineralization in anoxic sediments, when sulphate is depleted. However, the traditional view of a distinct spatial separation of these EAP, has frequently being challenged by molecular evidence of eg, methanogenic activity in the sulphate zone and sulphate reducing activity in the methanogenic zone. The presented project aims at a better understanding of how biotic and abiotic parameters interactively and differentially impact *in situ* methanogenic activity and energetics along marine sediment columns. Together with a concurrent project studying rates of methane production and turnover, involved organisms will be characterized and quantified to resolve how mean cell-specific activities are regulated in the different geochemical zones and depths. Due to the apparent difficulty to accurately detect the diversity of methane cycling communities and pathways in the marine environment, the suitability of different molecular marker and 'metaomics' approaches will be assessed. As methanogens ultimately rely on the capacity of other organisms to supply viable substrates (ie. hydrogen, acetate or methylated compounds) from the decomposition of complex organic matter, a further focus of this project lies on the importance of the 'background' functional diversity of the sediment biome.

MICROENERGY IN THE SUBSURFACE: THE CASE FOR MICROBIAL COMMUNITIES IN LAVA TUBE CAVES

**Jennifer G Blank (***) and the BRAILLE Team (**)

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We are beginning a study of the microbial diversity and its relation to secondary mineralogy in basaltic lava tube caves. Our field site is the Lava Beds National Monument ("LABE") in N California, situated on the lower, northern flank of the Medicine Lake Volcano. More than 780 caves have been identified at LABE, corresponding to >50 km of total length, and almost all are basaltic in composition. The caves formed from multiple eruptions over a period of ~500,000 years, though most are related to flows dated at 30-35 Ka or 11 Ka. They are relatively shallow, in the vadose zone, reaching ~20-45 m below land surface at their maximum.

The presence of abundant fractures makes the basalt at LABE highly permeable to infiltrating ground water, and the distribution of fractures in the lava tube caves serves as the primary control over the influx and removal of water into and from cave interiors. Water in the caves comes from rain and snow melt, and the LABE landscape is so porous that it does not support standing water. Dissolved constituents in the waters are incorporated during transport from the surface to the cave interiors, through porous soil, fractured lava, and fractures in cave walls; our initial water chemistry measurements are consistent with the hypothesis that the composition of the inorganic constituents in water in the caves reflects the maturity of the soil and solubility of the constituents; older flows lie under a more mature and thicker soil horizon, resulting in water with higher TDS. Deep caves are often characterized by stable environmental conditions that reflect the mean annual surface temperatures in the region, typically 10-13°C at LABE. In some caves, cold air sinks and becomes trapped, resulting in colder temperatures and the persistence of perennial ice. Away from their entrances and skylights, the caves are in complete darkness, and thus autotrophs must subsist through utilization of allochthanous carbon and chemosynthesis.

We will use TOUGHREACT, a numerical simulation program for chemically reactive, non-isothermal flows of multiphase fluids in porous and fractured media, to constrain the compositions of fluids (water and gases) and secondary mineral phases that would develop from reactions with primary mineral phases in LABE basalts. Through this approach, we can consider interactions between mineral assemblages and fluids under local equilibrium or constrained by kinetic rates. We can also model the degree to which precipitation and dissolution reactions affect porosity and permeability and modify the unsaturated flow properties of the rock. We will use our results from geochemical simulations to constrain the chemical energy resources available to support microbial life in the caves. Human visitation at LABE has provided empirical evidence, through graffiti dating back to 1892 (!), of re-growth rates of the abundant yellow mat, dominated by actinomycetes, that coats many of the caves walls. The example of microbial communities in lava tube caves will be a useful comparison for studies of deeper, more extreme subsurface microbial communities.

ANALYSIS OF BIOMOLECULES OF SUB-SEAFLOOR MICROBIAL CELLS SEPARATED FROM THE SEDIMENT MATRIX

Stefan Braun (*), Yuki Morono (***), Kevin Becker (****), Kai-U. Hinrichs (****), Bo B. Jørgensen (*), Bente Aa. Lomstein (*,**)

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Microbial biomolecules, typically from the cell envelope, can provide crucial information about distribution, activity, and adaptations of sub-seafloor microbial communities. However, these molecules can be preserved in the sediment on timescales that are likely longer than the lifetime of their microbial sources. We separated microbial cells from 4 marine sediment samples, including shallow, deep, organic-rich, and organic-lean sediment. Amino acids, amino sugars, muramic acid, and intact polar lipids were analyzed in both bulk sediment and cell extract for each sample, and cell separation was optimized and evaluated in terms of purity, separation efficiency, and compatibility to high-performance liquid chromatography and mass spectrometry. Cell extracts from density centrifugation contained only low to moderate amounts of detrital particles and non-cellular biomolecules as judged from electron microscopy and concentrations of amino acid degradation products, respectively, and were representative of the indigenous sediment community at the phylum level with a dominance of *Proteobacteria*, as revealed from 16S rRNA sequencing. Moreover, taxa in cell extracts and sediment were still in good agreement at the genus level. Total cellular amino acid concentrations ranged from 111 to 152 fg $cell^{-1}$, and those of intact polar lipids from 1 to 6 fg $cell^{-1}$. D:L-amino acid ratios were lower in cell extracts than in sediment, and muramic acid concentrations indicated a contribution of mostly gram-negative bacteria, consistent with DNA sequencing data. Interestingly, cellular amino acid-carbon was in the higher range of what has been reported for subsurface ecosystems, and cellular lipid composition was different from that of sediment with a dominance of hexose-diacylglycerols and betaine lipids. Cells from a highly purified cell extract obtained from multilayer density centrifugation and fluorescence activated cell sorting had average cellular contents of amino acids and lipids of 18 fg $cell^{-1}$ and 2.5 fg $cell^{-1}$, respectively, with a calculated carbon content of 14 fg $cell^{-1}$. We provide for the first time cellular concentrations of biomolecules from sedimentary microbial cells. Cellular concentrations of these molecules will improve their use as proxies for living cells in subsurface environments and will help improving global biomass estimates.

METAGENOMIC INSIGHTS INTO ALTERED GENOMES OF *FIRMICUTES* FROM THE DEEP BIOSPHERE

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The ability of a microbe to persist in low-nutrient environments requires adaptive mechanisms to survive. These microorganisms must reduce metabolic energy and increase catabolic efficiency. For example, *Escherichia coli* surviving in low-nutrient extended stationary phase have mutations that confer a growth advantage in stationary phase (GASP) phenotype, thus allowing for persistence for years in low-nutrient environments. Based on the fact that subseafloor environments are characterized by energy flux decrease with time of burial we hypothesize that cells from older (deeper) sediment layers will have more altered genomes compared to sequenced surface relatives and that these differences reflect adaptations to a low-energy flux environment. To test this hypothesis, sediment samples were collected from the Andaman Sea from the depth of 21, 40 and 554 meters below seafloor, with the ages of 0.34, 0.66, and 8.76 million years, respectively. A single operational taxonomic unit within *Firmicutes*, based on full-length 16S rDNA, dominated these low diversity samples. This unique feature allowed for metagenomic sequencing using the Illumina HiSeq to identify nucleotide variations (NV) between the subsurface *Firmicutes* and the closest sequenced representative, *Bacillus subtilis* BEST7613. NVs were present at all depths in genes that code for proteins used in energy-dependent proteolysis, cell division, sporulation, and (similar to the GASP mutants) biosynthetic pathways for amino acids, nucleotides, and fatty acids. Conserved genes such as 16S rDNA did not contain NVs. More NVs were found in genes from deeper depths. Furthermore, there was a decrease high metabolic cost amino acids (i.e. asparagine and leucine) and an increase in metabolically cheap amino acids (i.e., methionine and phenylalanine) compared to *B. subtilis*. These NV may be beneficial allowing them to survive for millions of years in the deep biosphere or may be latent deleterious gene alterations that are masked by the minimal-growth status of these deep microbes. Either way these results show that microbes present in the deep biosphere experience environmental forcing that alters the genome.

VIRAL LYSIS PLAYS A MINOR ROLE IN CONTROLLING MICROBIAL POPULATIONS IN OLIGOTROPHIC COLD SALINE SEDIMENTS

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Viruses are ubiquitous with microbial life and, in the global ocean, viruses can regulate microbial growth, influence genetic facility and mediate carbon transfer between particulate and dissolved pools. The impact of viruses on the starving microbial majority of the deep biosphere is much less clear. Viruses have been found to extreme depths (marine sediments, vents, and deep mine groundwater), leading to inferences of viral production and attenuated decay in the subsurface. In order to evaluate these suggestions, we characterized the abundance, diversity and activity of viruses from the sediments of a cold oligotrophic hypersaline spring in the Canadian High Arctic. This model system shares many characters in common with deep subsurface sediments, including low temperature, salinity, anoxia and low biomass, and has the advnatange of being relatively accessible. Virus and microbe abundances from the sediments in the anoxic spring outlet were comparable to those found in marine sediments >100 mbsf while abundances in sediments from the spring channel increased with distance from the anoxic outlet. Low virus-bacteria ratios (≈1), an absence of visibly infected cells, and limited contact between viruses and bacteria suggest that viruses are unlikely to persist by lytic replication in this energy limited ecosystem. An alternative means of replication - lysogeny - may be the dominant replication strategy in these and other oligotrophic sediments. While a lysogenic replication strategy is less likely to play a role in controlling microbial diversity or influencing biogeochemical cycling, it is more likely to influence the genetic potential of infected hosts. We will discuss this possibility and how it may inform the current debate about the tempo and mode of microbial evolution in the deep biosphere.

ENERGY LANDSCAPES SHAPE MICROBIAL COMMUNITIES IN DEEP-SEA HYDROTHERMAL SYSTEMS ALONG THE ARCTIC MID-OCEAN RIDGE

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Clearly, there are thermodynamic constraints on the distribution of metabolic functional groups in any community. Yet, analyses as to what extent chemical energy availability determines the structure of chemotrophic microbial communities, has received little attention. We used available geochemical mixing models followed by calculation of Gibbs free energy from selected redox reactions to model energy landscapes in two geochemically different hydrothermal systems on the Arctic Mid Ocean Ridge: the Soria Moria Vent field (SMVF) and the Loki's Castle Vent Field (LCVF). Both of these vent fields are basalt hosted, but the sedimentary influence on LCVF is responsible for elevated concentrations of methane, ammonium, and hydrogen in the venting fluids. Modelled energy landscapes were used to develop community structure models using the assumption that relative energy availabilities are proportional to relative abundances of functional groups of microorganisms. In order to evaluate the performance of the community structure models, we used pyrosequencing of 16S rRNA gene sequences to analyse microbial communities in natural samples. A dominance of aerobic methane oxidizers of Methylococcales or aerobic sulfur oxidizers of Epsilonproteobacteria dominated on chimney walls at LCVF whereas putative thermophilic anaerobic methane oxidizers from the ANME clade or aerobic sulfur oxidizers of Aquificales occurred in high numbers inside SMVF chimney walls. Metatranscriptomic analysis revealed a versatile *in situ* energy metabolism among Epsilonproteobacteria and confirmed the *in situ* methane oxidation capabilities of Methylococcales. Analyses of Bray-Curtis dissimilarities between observed and modelled communities indicate that the models have a high predictive power, further indicating that energy availability to a large extent shape microbial communities in hydrothermal systems. Our study demonstrates how the combination of modelled and observed communities provides a framework for the generation of hypothesis about abiotic geochemical processes occurring in hydrothermal systems, which energy sources that are most efficiently utilized by microorganisms, and the overall distribution of microorganisms in specific hydrothermal systems.

THE UNDERGROUND ECONOMY (ENERGETIC CONSTRAINTS AND SURVIVAL STRATEGIES OF SUBSEAFLOOR SEDIMENTARY LIFE)

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Subseafloor sedimentary communities provide an extraordinary opportunity to examine the nature of microbial survival under extreme energy limitation. Studies of subseafloor sediment have consistently shown that mean per-cell rates of catabolic activity, energy flux, and biomass turnover are orders of magnitude slower in subseafloor sediment than in the surface world (e.g., D'Hondt et al., 2002; Jørgensen and D'Hondt, 2006; Lomstein et al., 2012; Roy et al., 2012; Hoehler & Jørgensen, 2013). They have also shown that potentially competing metabolic pathways co-occur for hundreds of meters deep in subseafloor sediment deposited over millions of years (Mitterer et al., 2001; Bralower et al., 2002; D'Hondt et al., 2002, 2004; Wang et al., 2010; Holmqvist et al., 2011).

Our study of an example site (eastern equatorial Pacific ODP Site 1226) indicates that the energy yields of these competing reactions are pinned to a thermodynamic minimum (Wang et al., 2010). The simplest explanation of this long-term co-existence is thermodynamic cooperation, where microorganisms utilize different but co-existing pathways that remove each other's reaction products (e.g., Bethke et al., 2008; Wang et al., 2010).

Our Site 1226 results indicate that the energy flux to subseafloor sedimentary microbes is extremely low (D'Hondt et al., 2014). Comparison of this energy flux to the energy cost (actually power, since it is energy per time) of adding amino acids to growing peptide chains suggests that most of the energy flux may be used for building biomolecules from existing components (e.g., amino acids in the surrounding sediment), rather than for de novo biosynthesis from inorganic chemicals (D'Hondt et al., 2014).

This apparent balance between the energy flux to the community and the energy cost of amino acid recycling has intriguing implications for understanding of the mechanisms that may allow subseafloor cells to survive at such extraordinarily slow energy fluxes. It underscores the possibility that subseafloor sedimentary microbes utilize a "Frankencell" strategy, in which they incorporate organic molecules from the surrounding sediment. Such a strategy might allow cells to build or repair biomass more cheaply than cells that build biomass de novo. Although use of this strategy by subseafloor microbes has not yet been directly demonstrated, Takano et al. (2010) showed use of this strategy by archaea in nearshore marine sediment, which incorporate membrane lipids from the surrounding sediment.

This near balance also underscores the prospect that individual cells may survive in subseafloor sediment for extraordinarily long periods of time. Because molecular repair, or even replacement of single molecules, is energetically much cheaper than wholesale synthesis of new cells, the ongoing birth and death of cells is energetically much costlier than ongoing repair of existing cells (e.g., Hoehler and Jørgensen, 2013). Taken to an extreme, energy-starved cells in subseafloor sediment may survive for many millions of years without reproducing, but simply repairing or replacing their molecular machinery as their energy flux allows.

FAR, FAST, AND SURPRISING: LENGTH, TIME, AND ENERGY SCALES OF MICROBIAL ELECTRON TRANSPORT

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Electron Transfer is the stuff of life. The stepwise movement of electrons within and between molecules dictates all biological energy conversion strategies, including respiration and photosynthesis. With such a universal role across all domains of life, the fundamentals of ET and its precise impact on bioenergetics have received considerable attention, and the broad mechanisms allowing ET over small length scales in biomolecules are now well appreciated. Coherent tunneling is a critical mechanism that allows ET between cofactors separated by nanometer length scales, while incoherent hopping describes transport across multiple cofactors distributed within membranes.

In what has become an established pattern, however, our planet's oldest and most versatile organisms are now challenging our current state of knowledge. With the discovery of bacterial nanowires and multicellular bacterial cables, the length scales of microbial ET observations have jumped by 7 orders of magnitude, from nanometers to centimeters, during the last decade alone! This talk will take stock of where we are and where we are heading as we come to grips with the basic mechanisms and immense implications of microbial long-distance electron transport. We will focus on the biophysical and structural basis of long-distance, fast, extracellular electron transport by metal-reducing bacteria. These remarkable organisms have evolved direct charge transfer mechanisms to solid surfaces outside the cells, allowing them to use abundant minerals as electron acceptors for respiration, instead of oxygen or other soluble oxidants that would normally diffuse inside cells. From an environmental perspective, these microbes are major players in global elemental cycles. From a technological perspective, microbial extracellular electron transport is heavily pursued for interfacing redox reactions to electrodes in multiple renewable energy technologies.

But how can an organism transfer electrons to a surface many cell lengths away? What molecules mediate this transport? And, from a physics standpoint, what are the relevant length, time, and energy scales? We will describe new experimental and computational approaches that revealed how bacteria organize heme networks on outer cell membranes, and along the quasi-one-dimensional filaments known as bacterial nanowires, to facilitate long-range charge transport. In addition, we will examine the fundamental limits of extracellular electron transport, down to microbial energy acquisition by single cells. These findings are shedding light on one of the earliest forms of respiration on Earth while unraveling surprising biotic-abiotic interactions.

MICROBIAL LIFE CYCLE: DIFFERENTIATION IN GROWTH AND DE-PROGRAMMING IN DORMANCY

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Microbial "uncultivability", or the Great Plate Count Anomaly, is arguably the oldest unresolved microbiological phenomenon. Most microbial species from the environment and human body are yet to be cultivated, which hampers progress in microbial biology and biotechnology. The current explanations of the nature of the phenomenon have not led to bridging the gap between the large number of species in nature and the limited richness of microbial collections. This suggests these explanations may not be general enough. Here we propose a new hypothesis on why so many species resist cultivation in the lab, which we term The Four Seasons of Microbial Life. The new model consists of three main postulates.

First is that microbial species, both spore- and non spore-forming, exit the state of dormancy as a result of stochastic fluctuations in gene regulatory networks. The low-frequency random events of awakening ("Spring") produce scout cells whose function is to explore the environment and proliferate if the conditions are growth-permissive (or die otherwise). Similarly to the well-known bistability, this effectively divides the isogenic population into two phenotypes, dormant and active. We postulated the stochastic transition from dormancy to activity in the "scout" hypothesis several years ago, and have since obtained a significant amount of supporting empirical evidence in all species we studied, from environmental strains to *E. coli.*

Second, the successful scout forms a growing population ("Summer") that becomes progressively better and better adapted to the specific set of environmental conditions into which it awoke. The pattern of gene expression of growing cells will become ever more specific to these conditions, and epigenetically inherited by the progeny. This should lead to a significant differentiation of the population, possibly to the point of it being seemingly incapable of utilizing other conditions. This may be analogous to differentiation of a pluripotent mammalian stem cell into a particular – yet isogenic - cell type. If such a differentiated microbial population is transferred from its natural habitat to a different environment, for example Petri dish, it may not be able to grow – unless de-differentiation takes place. This proposal finds support in the phenomenon of diauxic shift discovered by J. Monod in 1941: a population of *E. coli* that utilized glucose as the single carbon source is, for a period of time, incapable of utilizing lactose. In our terms, this population differentiated to the glucose environment and became "uncultivable" in the lactose habitat.

Third, once the resources have been exhausted ("Fall"), the population returns to its original dormant state, completing the cycle. We propose that the purpose of inactivity is not only to survive the unfavorable conditions but to also de-program differentiated cells ("Winter") - by the virtue of "forgetting" the former gene expression pattern formed during growth. If so, dormancy returns the cells to their pluripotent state, from which they can wake up and successfully proliferate under a number of different environmental scenarios, followed by a new differentiation event. If the range of such in-principle growth-permissive scenarios includes artificial media in the lab, the population will appear perfectly cultivable on such media, whereas the phenotype differentiated in the environment will not. This third component of the overall hypothesis is novel and, albeit logical, presently does not have empirical support.

We argue that the Four Seasons of Microbial Life hypothesis is consistent with most, if not all, empirical observations related to the Great Plate Count Anomaly. It helps explaining the nature of VBNCs, persister cells, and a range of other microbiological facts and phenomena. It also suggests

new and simple ways to cultivate species that so far have escaped the efforts of cultivation microbiologists.

MECHANISMS OF LONG-TERM SURVIVAL AND EVOLUTION IN LABORATORY MICROCOSMS

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Bacteria such as *Escherichia coli* can survive for long periods of time in batch culture without the addition of nutrients, experiencing what have traditionally been referred to as the five phases of the bacterial life cycle in the laboratory: (1) lag phase, (2) exponential or logarithmic phase, (3) stationary phase, (4) death phase, and (5) long-term stationary phase. During incubation in long-term stationary phase, mutants are frequently identified expressing the Growth Advantage in Stationary Phase, or GASP, phenotype. GASP mutants are descended from the original parental population, but express phenotypes that confer a competitive advantage. Using a variety of genomic and genetic techniques we have shown that a single culture may contain many mutants with novel genotypes at any time and that parallel cultures, initiated from identical clones and grown under identical initial conditions, can possess populations with different GASP mutant populations. Analysis of *E. coli* GASP mutants has shown that many novel alleles alter the ability of cells to catabolize a broad variety of biomolecules. We have shown that the GASP phenomenon occurs in many bacterial species, including members of the genera *Shewanella, Marinobacter, Vibrio*, and *Pseudomonas*. Further, we have shown that per cell oxygen consumption rates vary with the growth phase in species of *E. coli, Shewanella,* and *Marinobacter*. The sources of the genetic variation leading to novel mutant phenotypes is likely due to a combination of factors including both the effect of exogenous and endogenously produced DNA-damaging agents (including reactive oxygen species generated as a part of normal metabolism), as well as errors due to the repair of DNA damage. We have recently shown that the expression patterns in *E. coli* of the error-prone "SOS" DNA polymerases Pol II, Pol IV, and Pol V (encoded by *polB*, *dinB,* and *umuDC*, respectively) change as cells transition through the five growth phases, which likely alters the basal mutation frequency and spectrum of potential mutations as cells experience nutrient stress. Current efforts are focused on further elucidation of the mechanisms that generate genotypic diversity during long-term stationary phase, using a combination of genomic tools. We are performing whole-genome sequencing of both individual mutants and population samples isolated from long-term cultures. These analyses are allowing us to identify common variants or targets of selection in these cultures. In addition, we are studying the long-term growth, survival and adaptive evolution characteristics of bacteria incubated in long-term stationary phase in a variety of different growth media. This is allowing us to identify medium-specific patterns of mutation. These analyses should allow us to begin to make connections between the novel genotypes observed in long-term cultures with specific genetic changes. Further, these experiments may shed light on the adaptive forces at work in naturally occurring energy-limited environments, allowing us to develop new models of the processes adaptive evolutionary and/or ecological selection.

VOLATILE FATTY ACIDS AS SUBSTRATES FOR SULFATE REDUCTION IN SEDIMENTS FROM SOUTHWESTERN GREENLAND

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Volatile fatty acids (VFAs) are key intermediates in the anaerobic mineralization of organic matter in marine sediments. We studied the role of VFAs for the carbon and energy turnover in the sulfate reduction zone in sediments from the sub-arctic Godthåbsfjord (Greenland) and the adjacent continental shelf in the Davis Strait. VFA porewater concentrations were measured by a new two-dimensional ion chromatography-mass spectrometry method that enabled the direct analysis of VFAs without sample pretreatment (Glombitza et al., 2014). VFA concentrations were low and surprisingly constant (4-6 µM for formate and acetate, 0.5 µM for propionate) throughout the sulfate reduction zone. Hence, VFAs are turned over while maintaining a stable concentration that is obviously under a strong control. Estimated mean diffusion times of acetate between neighboring cells were below 1 s whereas VFA turnover times increased from several hours at the sediment surface to 4 years at the bottom of the sulfate zone. Thus, diffusion was not limiting the VFA turnover. Measured in-situ pore water concentrations of VFAs and inorganic ions, i.e. sulfate, bicarbonate (dissolved inorganic carbon) and sulfide were used to calculate the Gibbs free energies (ΔG_r) of VFA-dependent sulfate reduction. Standard Gibbs free energies were calculated for approximated in-situ temperature and pressure with the software package SUPCRT92 (Johnson et al., 1992). Despite constant substrate concentrations, the Gibbs free energies decreased downcore, -28 to -16 kJ (mol formate)$^{-1}$, -68 to -31 kJ (mol acetate)$^{-1}$ and -124 to -65 kJ (mol propionate)$^{-1}$ and thus ΔG_r is not determining the in-situ VFA concentrations. It is not clear what controls VFA concentrations in the porewater but cell physiological constraints such as VFA activation or uptake could be important. We speculate that such constraints limit the substrate turnover and result in a minimum ΔG_r that consequently depends on the cell physiology and is different for individual substrates. Chemostat experiments with pure cultures under energy depleted conditions are required to further address this assumption. However, in batch reactor experiments with *Desulfobacter hydrogenophilus* (DSM3380) growing on acetate and sulfate, Jin and Bethke (2009) observed that sulfate reduction ceased when ΔG_r was decreasing to ~-33 kJ (mol acetate)$^{-1}$ which is similar to what we observe at the bottom of the sulfate zone at the continental shelf in the Davis Strait. This is in accordance with calculations of Rabus et al. (2006) who estimated that 2/3 mole sulfate (respectively acetate) are required to synthesize 1 mole of ATP with an estimated energy requirement of approximately 50 kJ (mol ATP)$^{-1}$ (Schink, 1997).

FEEDING POTENTIAL FOR DEEP MICROBIAL ECOSYSTEMS OF FREE AND MACROMOLECULAR-BOUND FORMATE AND ACETATE IN 2 KM DEEPLY BURIED COALBEDS OFFSHORE SHIMOKITA PENINSULA (JAPAN)

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In summer 2012, the Integrated Ocean Drilling Program (IODP) Expedition 337 retrieved the deepest core sample in the history of scientific ocean drilling from a depth of 2466 meters below sea floor and pioneered the riser drilling technique in scientific ocean drilling. Main target of the IODP Expedition 337 was a coal bearing horizon at approximately 2 km below the seafloor, 80 km off the coast of the Japanese peninsula Shimokita in the Pacific Ocean. The objective was to explore the deep subsurface biosphere in the coalbeds. Scientific investigations of subseafloor hydrocarbon reservoirs require the use of a riser-drilling system that only recently became available with the implementation of the Japanese drilling vessel Chikyu. First results of Exp. 337 prove the existence of a methanogenic microbial community of forest soil deriving organisms that have survived in the coalbeds over > 20 Ma (Inagaki et al., 2015). Goal of this study was to assess the potential of the macromolecular organic matter (MOM) of the coals to provide formate and acetate, which are prime substrates for microbial metabolism. We aimed to find evidence of their metabolic turnover and to estimate the energy stored by these acids in the coal MOM that is available to the deep coalbed biosphere by a continuous release during ongiong geological maturation. A large pool of free formate and acetate was extracted from the coals. Calculation of Gibbs energy reveals that organoclastic methanogenesis is energetically feasible even at 1000-fold lower acid concentrations. A large amount of energy for the methanogens is stored by ester-bound acids in the kerogen [~4 kJ $g(Sed)^{-1}$ by formate, ~0.8 kJ $g(Sed)^{-1}$ by acetate]. Based on previously found release rates (Glombitza et al., 2009) the bound acid pool will be depleted within 3 - 10 million years. Differences in the acetate to formate ratio in the free and kerogen-bound pools suggest the presence of acetogenesis which is energetically feasible in the Shimokita coalbeds due to high H_2 concentrations.

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SULFATE REDUCTION NEAR THE ENERGETIC LIMIT: IMPLICATIONS FOR SULFUR ISOTOPE FRACTIONATION IN MARINE SEDIMENTS

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The fractionation of sulfur isotopes associated with microbial sulfate reduction has been best studied in laboratory cultures with relatively rapid sulfate reduction rates. These studies repeatedly show an inverse relationship between isotopic fractionation and sulfate reduction rate, with maximal fractionations of approximately 70‰ that are close to the thermodynamic fractionation between the sulfate substrate and the sulfide product [1,2]. Such high fractionations are encountered only at the slowest laboratory rates of sulfate reduction. In turn, the slowest reduction rates in the laboratory overlap with only the highest values of cell-specific sulfate reduction rates measured in marine sediments [3]. This chain-of-logic implies that sulfate-reducing microbes in the marine sedimentary biosphere are expected to fractionate sulfur isotopes at magnitudes close to the thermodynamic limit. Isotope fractionation close to the thermodynamic limit is possible only when all of the reactions in the sulfate reduction pathway are almost completely reversible (i.e., near thermodynamic equilibrium [4]), suggesting that energy limitation is characteristic of populations of sulfate-reducing microbes in marine sediments.

Despite the expectation that the intrinsic microbial sulfur isotope fractionation should be near the thermodynamic limit, a range of fractionations between 0 and ~70‰ is observed in marine sediments [2]. Sulfate limitation has been suggested as a first-order control on the magnitude of cell-specific isotope fractionation [5]. However, as modern marine sediments are in communication with a water column containing ~28 mM sulfate, this range cannot be simply a function of the concentration of sulfate in the microbes' local environment. Furthermore, recent observations from sulfate-poor environments [6] indicate that large fractionations are possible even at micromolar concentrations of sulfate, in agreement with predictions of near-thermodynamic sulfur isotope fractionation at these sulfate concentrations as long as cell-specific sulfate reduction rates are low [4].

We suggest that the observed range of sulfur isotope fractionations in marine sediments does not reflect variability in intrinsic microbial fractionation in response to environmental parameters, such as sulfate concentration, organic substrate availability or temperature. Instead we suggest that it results from an early diagenetic modulation of large near-constant microbial fractionations, which are pinned to the thermodynamic limit by the low sulfate reduction rates of natural sulfate reducers. In this presentation we will discuss these ideas and present model and observational results in their support.

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STUDIES OF FENNOSCANDIAN SHIELD FRACTURES REVEAL ROCK INHABITED DEEP LIFE

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Radioactive wastes are planned to be disposed in the bedrock of Finland at 400 m depth. Crystalline rock fractures form the space for microbial life where minerals in water and gases can fuel microbiological activity which is still considered to be very slow. Deep groundwaters may contain variable quantities of sulphur compounds which may be oxidized to sulphates near the surface and reduced to sulphides at deeper depths. Formation of hydrogensulphides is considered as a major threat which may cause hazard and corrosion of copper canisters used to protect high radioactive wastes in the repositories. Methane gases may locally evolve from the deep earth crust and hydrogen gases be formed by several abiotic and biotic processes generating energy for microorganisms. Metagenomics and metatranscriptomics research is ongoing to provide deeper insight into metabolic processes of sulphur cycling microbial communities present at the sulphate and sulphide rich goundwaters and energy mechanisms. This presentation aims to give an overview of research done during the recent past years in several research projects connected to this topic.

WHAT IS THE LOWER LIMIT FOR CELLULAR METABOLIC RATES?

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One fundamental requirement for life is the repair of the continuous damages due to environmental stresses (e.g. heat, radiation). Microbial cells need energy for coping with them. The rates of energy uptake from the environment must be sufficiently high to counterbalance the rate of the damages. What is this minimum power required to sustain an active individual cell in natural environments? This question can be addressed by considering the energy needed to react against those damages, or the mean cell-specific metabolic rates in energy-limited environments. While the former is problematic to estimate due to the complexity and variety of stresses, the latter can be quantified directly from the environment by combining volume-specific metabolic rates and cell density. In marine sediments, most of energy available for microorganisms is contained in a finite amount of organic molecules. Microbial communities, living in the deeper parts of the sediments, get only access to reduced energy fluxes, remained from above layers. Previous studies revealed that metabolic rates and cell abundance decrease with depth, but at different paces. On average, individual cells had lower metabolic rates in deep than in the surface sediment. Interestingly, at depth, the decline of the rates slowed down and converged to a similar value, although the community size, volume-specific metabolic rates, pathways, timescales and methods were vastly different in those studies. Are these extremely low rates reflecting the basal power needed to counterbalance molecular damages?

In this study, we associated carbon oxidation rates with cell abundance from various marine sediments, all within the sulfate reduction zone. The six analysed sediments cores were located in a broad range of marine environments (deep sea and coastal sediments, temperate and subarctic sediments). To calculate the volume-specific carbon oxidation rate, we combined $^{35}SO_4^{2-}$ incubation and modelling of rates from pore water solute profile. Microbial cells were quantified by fluorescence microscopy. We used this information to constrain experimentally mean cell-specific metabolic rates and explore the link between the total community size and the carbon oxidation rates. Our results show a tight coupling between those two parameters; however the mean cell-specific metabolic rates progressively drop without reaching any clear minimum. This raises the question if we are missing a part of the full picture with microorganisms, whose physiologies remain still unidentified.

BIOGEOCHEMICAL METABOLIC MODELING: LINKING THERMODYNAMICS AND KINETICS OF SUBSURFACE MICROBIAL PROCESSES

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Subsurface microbes account for a significant portion of live carbon and play a central role in global cycling of elements. But subsurface microbial metabolism has remained inaccessible to direct laboratory investigation, because of relatively sluggish activities and fastidious growth requirements of subsurface microbes. As a result, it is still elusive how natural microbes make a living and cope with their environments. Here we propose to investigate subsurface metabolism by simulating metabolic reaction networks of natural microbes.

We have developed a new approach – biogeochemical metabolic modeling – to simulate enzyme reaction networks. This approach takes advantage of recent development in biogeochemical reaction modeling and genome-scale metabolic modeling, and describes enzyme reaction networks according to the kinetics and thermodynamics of enzyme reactions. It simulates biomolecular attrition, membrane electrical potential, and energy transduction and conservation, and predicts the abundance and reaction rates of enzymes under the constraints of cellular physiology and environmental conditions. The new modeling approach differs from flux balance analysis of system biology in that it accounts for the thermodynamics and kinetics of enzymatic reactions. It builds on subcellular metabolic reaction networks, and hence also differs from classical biogeochemical reaction modeling.

We applied the new approach to *Methanosarcina acetivorans*, an anaerobic, marine methanogen capable of disproportionating acetate to carbon dioxide and methane. The input of the new model includes (1) enzyme reaction network, (2) kinetics and thermodynamics of enzyme reactions, and (3) representative conditions of laboratory and natural environments. The output of the simulation includes the proteomics, metabolomics, and energy and matter fluxes of the methanogenesis network. The simulation results match well with the results of laboratory experiments, including microbial physiology and kinetics. The results also demonstrate the predictive power of the new modeling approach and its potential in studying subsurface microbial processes. Specifically, the results illustrate how *M. acetivorans* regulates its enzyme expression and how methanogenesis rates vary in response to environmental changes.

INCREASED ENERGY SUPPLY IN TRANSITION ZONES SUPPORT ENHANCED NITROGEN CYCLING IN DEEP-SEA SEDIMENTS

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Microorganisms involved in nitrogen transformation processes, such as nitrification, denitrification, and anaerobic ammonium oxidation (anammox), serve as a major control on the nitrogen flux between seawater and sediments. However, their distribution, reaction rates and interactions in deep-sea sediments are not well studied. We investigate the vertical distribution of nitrifiers, denitrifiers and anammox bacteria in marine sediments, focusing on five gravity cores retrieved from the Arctic Mid-Ocean Ridge at water depths exceeding 2000 meters. Fluxes and reaction rates of nitrate and ammonium were calculated based on pore water profiles. Further, enumeration of functional groups related to the nitrogen cycle was estimated throughout the cores by quantitative PCR, targeting their respective functional genes. Abundances of archaeal and bacterial ammonia oxidizers, nitrite oxidizers and denitrifiers generally decrease with depth, but local peaks were observed at the oxic-anoxic transition zone in all investigated cores. Likewise, we find that the nitrate-ammonium transition zone (NATZ), where ammonium defusing upward meet the downward flux of nitrate, are intervals with increased abundance of microbial nitrogen transformation. In particular we see a marked increase in functional genes related to anammox (*hzo* gene), indicating that this group is important in regulating nitrogen fluxes in these sediments. Cell-specific rates of nitrification and denitrification, calculated as the bulk reaction rates divided by functional group abundances, suggest that although the targeted organisms are maintaining a very low metabolic activity it is sufficient to keep them in a state of maintenance.

LIFE SIGNS IN APPARENTLY DEAD BACTERIA

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For over 100 years microbiology and the public health have largely relied on the detection of microorganisms by culture using rich agar and broth media. Nevertheless, microorganisms exist mostly in polymicrobial communities and/or association with eukaryotes, making isolation of pure cultures problematic without knowledge of their actual nutrient requirements (many species are secondary feeders from primary metabolism) and concentration range, and physico-chemical parameters such as O_2, CO_2, E_h and pH. Moreover, many species may have experienced stress e.g. iron sequestration in the host or environment, exposure to host defences, solar irradiation, desiccation, oligotrophic environments, disinfectants *in vitro* and antibiotics *in vivo*. Some Gram-positive, aerobic or anaerobic bacteria respond to environmental stressors through the process of sporulation, producing near dormant, robust spores that can survive for thousands of years and can still be recovered using conventional culture techniques. Non-spore formers must adapt their physiology to one of several increasingly severe stress states, requiring careful resuscitation. Importantly, many have also evolved an alternative strategy of becoming viable but non-culturable (VBNC) where resuscitation proves difficult although species remain metabolically active and capable of infection in amoebae or higher organisms. So, how dead is dead? New physiological and molecular biology tools have been developed to monitor the transition into the VBNC state and track the location and persistence of these "dormant" bacteria. Consequently, they may remain undetected using classical culture recovery techniques, or even qPCR techniques which rely on pre-enrichment culture, giving a false impression of their absence and whether antimicrobial treatment or cleaning strategies actually work. This may be despite strong epidemiological evidence implicating point sources of a disease outbreak, such as food- or water-borne, which remains undetectable. Moreover, it is now recognized that during the biofilm mode of growth a sub population of cells become quiescent and resilient to antimicrobial treatment. They spontaneously enter a reversible dormant state which is refractory to antibiotic treatment that usually relies on active growth of bacteria to be effective. The consequence is that chronic biofilm infections of either Gram-negative or Gram-positive bacteria, estimated to cause 70-80% of human infections, are very difficult to treat with antibiotics. This "persistence" occurs through toxin-antitoxin modules and other dormancy pathways which are now proving amenable to the development of new classes of antibiotics. This presentation describes recent microscopy and biochemical techniques measuring cell membrane integrity, respiration, energy generation and cell growth, as well as eukaryote infectivity, that shed new insight into the importance of the VBNC state in monoculture and polymicrobial biofilm communities. It is clear that there are frequent false negative culture detection reports which have profound implications for waterborne or foodborne pathogen control and epidemiology, and prophylactic or therapeutic disease management.

THE DOMINANT CONSTRAINTS MOTIVATING EVOLUTIONARY TRANSITIONS AND LIMITING BODY SIZE

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A central question in understanding the energetic limitations of organisms is what are the fundamental tradeoffs in cellular processes that determine the allowable combinations of physiological and metabolic processes. Furthermore, if we understand these tradeoffs can we use them to determine the fundamental limitations and extreme bounds for the largest and smallest biological energy expenditure, body size, and metabolic complexity? Our focus is on under- standing cross-species trends as connected with basic energetic, informational, and physical limitations of cells. Using fundamental physical theories and cross-species data analysis we are able to predict a wide variety of organism tradeoffs and fundamental limitations. Most notably we can predict the size of the largest and smallest bacteria from several independent perspec- tives: energetic partitioning, tradeoffs in cellular composition, and basic rate processes. Notably we predict that the smallest bacteria are limited by fundamental maintenance metabolism along with general space requirements. We predict that cells below a certain limit are not possible because of an inability to meet metabolic requirements or contain enough cellular components (e.g. proteins) to survive.

WHOLE COMMUNITY RICHNESS, DIVERSITY LOSS, AND SELECTION IN AN ENERGY-LIMITED DEEP BIOSPHERE

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Energy limitation in the subsurface can put tremendous selective pressure on the microbial community. The overall impact of energy limitation on patterns of community diversity and natural selection is not well understood. How much selection occurs, what kinds of organisms are selected for, and where do they come from? To address these questions, we have examined whole community 16S sequence data from sites in the Bering Sea, equatorial Pacific, and Indian Ocean. In near-seafloor marine sediment, diversity varies from site to site. However, at each site, diversity declines exponentially with sediment age. The majority of operational taxonomic units (OTUs) disappear in the first few hundred kiloyears (kyr), regardless of sedimentation rate and predominant terminal electron acceptor.

Comparison of OTUs in deep sediment to OTUs in the overlying near-seafloor sediment and OTUs in the overlying water column yields several patterns that shed light on the nature of selection of these communities. First, relatively abundant sequences in surface sediment are often found as trace constituents of the 16S communities in the overlying waters; young sediment appears to be colonized from the water column. Second, most taxa abundant in the water column are absent from the sediment. Third, the community in our deep subseafloor sediment samples are predominantly a subset of the community in near-seafloor sediment. Fourth, overall culling during the transition from shallow to deep subseafloor sediment appears fairly non-discriminatory; the sequences most abundant in shallow sediment are most likely to persist at depth. It is possible that the energy constraints in this ecosystem restrict both mobility and replication so severely that they also generally prevent the formation of distinct layers of unique communities. We discuss this hypothesis in relation to the observed patterns of overall microbial diversity and community composition in deep sediments relative to those nearer to the surface world.

ASSEMBLY OF SULFATE-REDUCING MICROBIAL COMMUNITIES IN MARINE SUBSURFACE SEDIMENTS

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Dissimilatory sulfate-reducing microorganisms (SRM) are key drivers of anaerobic organic matter mineralization in marine coastal sediments. Here the rate of sulfate-reduction decreases rapidly with sediment depth as the availability of reactive detrital organic matter and pore water sulfate is exhausted. Yet sulfate-reduction is known to take place even in deep subsurface sulfate-depleted sediments where SRM subsist despite the extreme energy limitation of this environment. Using qPCR and high-throughput sequencing of *dsrB* (a functional marker gene for SRM) PCR amplicons, we aimed to identify the SRM populating deep subsurface sediments and determine where in the sediment column the community of these putative "low-energy specialists" is assembled.

We analyzed samples from the sediment surface, the underlying sulfate-rich zone, the narrow sulfate-methane transition-zone (SMTZ: the depth at which sulfate becomes depleted and methane begins to accumulate) and the underlying methanogenic zone from 4 sampling stations in Aarhus Bay, Denmark. The deepest samples from the methanogenic zones represented Holocene sediment with an age of 5000-9000 years, depending on the station. According to qPCR enumeration of *dsrB* and 16S rRNA gene copies the relative abundance of SRM as compared to the total prokaryotic community size decreased from 10-30% in surface sediments to 1-2% in deep methanogenic sediments. In agreement with depth profiles of sulfate reduction rates a single pronounced decrease in SRM abundance occurred within the uppermost 10 cm of the sediments. The taxonomic diversity of the SRM community decreased continuously with sediment depth with a 5-10 fold reduction in the number of observed *dsrB*-phylotypes between the surface and the deepest sediment samples. SRM community composition changed dramatically with sediment depth. Surface sediment and the underlying sulfate-rich sediment zone both harbored SRM communities with a distinct composition whereas the SMTZ and the methanogenic zone shared more similar communities. Predominant SRM phylotypes in the deepest sampled methanogenic parts of the sediments were only distantly related to known SRM, and likely represented novel families of sulfate reducers. These phylotypes were also present throughout the overlying sediment where they however constituted SRM community members of minor relative abundance. This pattern was consistent for all 4 sampling stations.

In conclusion, our data suggest that SRM communities in marine subsurface sediments assemble by selective survival of members of surface sediment SRM communities which become buried over time, and that this selection gradually takes place within the uppermost sulfate-rich part of the sediments.

METHANE-CYCLING ARCHAEAL POPULATIONS IN SUB-SURFACE SEDIMENTS OF THE HELGOLAND MUD AREA, NORTH SEA

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The Helgoland mud area is one of the few depocenters of fine-grained sediments in the North Sea having a high sedimentation rate recorded in the past (up to 13 mm $year^{-1}$). A shallow sulfate-methane transition located at approx. 50 cm sediment depth, organic matter content in the order of 0.6-2.2 wt%, and elevated dissolved iron concentrations (up to 350 µM) in the pore water of the methanic zone of the Helgoland mud area sediment provide an avenue to investigate the relationships between the methanogens and depth-wise profiles of dissolved iron and methane. Recently, we could show that 16S rRNA gene copy numbers of bacteria and archaea were specifically higher around the peak of dissolved pore-water iron in the methanic zone (250-350 cm bsf) in a 5 m-long sediment core from the Helgoland mud area (Oni et al. 2015). The higher copy numbers at these depths were also reflected by the relative sequence abundances of members of the candidate division JS1 bacteria (SB45 lineage), methanogens and *Methanohalobium*/ANME-3 related archaea suggesting their co-involvement in iron and methane cycling.

With this in view, our present study focused on the distribution of potential methane-cycling archaea by pyrosequencing of the *mcrA* gene, which encodes the alpha subunit of the key enzyme methyl co-enzyme M reductase, at selected depths of the Helgoland mud area sediment. Sequences of anaerobic methanotrophic archaea (ANME) members (ANME-1 and 2) were highly abundant throughout the sub-surface sediment contributing to up to 44-47% of the sequences at some depths, whereas ANME-3 sequences were only abundant in the methanic zone (9.5-11.2%). Relative abundances of unidentified *Euryarchaeota* sequences increased with depth, reaching up to 37% in the methanic zone. Sequences related to the members of *Methanosarcina* (14.5%) and *Methanomicrobiales* (13.1%) were distinctly found at the depth of 275-300 cm bsf whereas those related to *Methanosaetaceae*, *Methanococcales*, and *Methanobacteriales* were sparsely present throughout the sediment (0.1-6.8%). Our study suggests that the process of methane formation in the Helgoland mud area could be linked to diverse methanogenesis pathways, in which unidentified *Euryarchaeota* might be playing an important role. The elevated concentrations of dissolved iron in the methanic zone along with a high abundance of various ANME members suggest their involvement in anaerobic methane oxidation (AOM) coupled to iron reduction. This study provides further insight into the role of methanogenic archaea in iron and methane cycling in the Helgoland mud area.

Oni O, Miyatake T, Kasten S, Richter-Heitmann T, Fischer D, Wagenknecht L, Kulkarni A, Blumers M, Shylin SI, Ksenofontov V, Costa BFO, Klingelhöfer G and Friedrich MW (2015) Distinct microbial populations are tightly linked to the profile of dissolved iron in the methanic sediments of the Helgoland mud area, North Sea. Front. Microbiol. 6:365. doi: 10.3389/fmicb.2015.00365

THE ENERGETICS OF ANABOLISM IN NATURAL ENVIRONMENTS

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The environmental conditions that describe an ecosystem define the amount of energy available to the organisms that live in it. Similarly, the energetic cost of producing biomass – either new cells or new cellular components – is also a function of the prevailing chemical and physical variables that define a habitat. As a result, the number of microorganisms living in a particular setting can be understood by quantifying the amount of energy that is available to them as well as the cost of biosynthesis. In this presentation, we will demonstrate the range of Gibbs energies associated with biomass synthesis as a function of environmental variables such as temperature, pressure, redox state, sources of C, N and S, cell mass and the amount of time that an organism requires to double or replace all of its biomass. Specifically, the energetics of biomass synthesis per dry gram of biomass are calculated from 0 – 125 ^{o}C, 0.1 – 500 MPa and -0.38 to +0.86 V using various combinations of CO_2, acetate and CH_4 for C, NO_3^- and NH_4^+ for N and SO_4^{2-} for HS^- for S. Results show that the amount of energy associated with synthesizing all of the biomolecules that make up a cell, in the proportions that they exist in a model organism, varies over a range of nearly 40 kJ (g cell)$^{-1}$ The amounts of energy required to make cellular material are used to compute energy-based yield coefficients for a vast range of environmental conditions that are compatible with a recently published bioenergetic model. Taken together, environmental variables and the range of cell sizes leads to a nearly 4 order of magnitude difference between the number of microbial cells that can be made from a Joule of Gibbs energy , 5.21 x 10^7 cell J^{-1} and 5.06 x 10^{11} cell J^{-1}, under the most and least ideal conditions. Finally, when doubling or cell replacement time is taken into account, the range of energies associated with making a microbial cell can expand even further. For example, the maintenance power required by some aerobic heterotrophs doubling once a month reaches 1.3 x 10^{-7} J cell^{-1}, exceeding the amount of energy required to synthesize all of its biomolecules from CO_2 or acetate serving as the carbon source. On the other end of the spectrum, an organism existing at a low basal maintenance power state would only use 6 x 10^{-9} J cell^{-1} over the course of 1,000 years. By considering such a wide range of environmental conditions, the results presented in this study are applicable to just about any type of environment in which life is found.

ECOLOGICAL AND EVOLUTIONARY INSIGHT INTO BACTERIAL PERSISTENCE DURING STARVATION

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Microorganisms typically experience conditions that are suboptimal for growth and reproduction. Despite this, many populations of bacteria survive by entering a reversible state of reduced metabolic activity, or dormancy. Our previous work has demonstrated that persistence is an important trait that contributes to the maintenance of microbial diversity. However, a variety of persistence strategies are thought to have evolved among bacteria, which might have different costs and trade-offs. In this presentation, first, I will outline the theoretical frameworks and expectations for bacterial persistence under starvation. Second, I will describe results from a long-term experiment that quantified persistence for a phylogenetically diverse collection of soil bacteria. Results from these multi-year experiments suggest there is upwards of four-orders-of-magnitude of the variation in bacterial death rate under starvation. Some strains persisted with almost no loss of viability while others rapidly succumbed to energy limitation. Interestingly, for the majority of our strains, the decay rate of viability significantly deviated from first-order expectations suggesting that bacterial death rates declined over time. Simulation models and follow-up experiments indicate that this type of functional response may arise from cannibalism, evolution, or possibly both. The findings have implications for understanding ecological and evolutionary of persistence in both natural and managed ecosystems.

OPTIMIZING SUBSTRATE UTILIZATION ON A MIXED DIET

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Studies on the energetics of microbial substrate utilization frequently concentrate on the role of enzyme kinetics and Gibbs free energy yields per reaction in determining microbial fitness and competitive outcomes between different microorganisms and metabolic guilds. Models that have resulted from these studies are often based on incubation experiments with single energy substrates. Yet, most natural environments host a highly diverse range of microbial energy substrates, and many microorganisms in nature are capable of utilizing multiple energy substrates at the same time. This raises the question whether traits that are advantageous to catabolically specialized microbes in incubations with single substrates, e.g. use of only one or a few energy substrates for which these microbes have evolved high enzymatic specificities and affinities, are always advantageous in the natural setting, where energy substrate availability is typically limiting microbial biomass and yet diverse ranges of energy substrates are present. Using published data from starvation experiments, comparing substrate spectra within and across different metabolic guilds, and taking into account energetic costs of genome maintenance and protein synthesis, I will argue that the ability to live on a mixed diet is not only essential to the survival of many microorganisms, but that generalist adaptations that are opposite to those that confer advantages under single substrate conditions enhance the fitness of many microbes in natural environments.

ABUNDANCE AND FUNCTIONS OF ARCHAEA AND BACTERIA IN THE SEABED

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Microorganisms in the deep marine subsurface have extremely low activity and very slow growth. These factors, combined with the fact that none of the abundant clades have been cultured, makes it difficult to identify the physiological functions that are active in situ and to determine which uncultured clades perform them. We have addressed this issue by combining single cell genomics, which gives the potential physiological functions of uncultured clades with meta-metabolomics, which demonstrates the in situ activity of those functions. Our samples were obtained during the IODP Leg 347: Baltic Sea Paleoenvironment expedition, and allow us to compare high organic matter (~6% total organic carbon, TOC) and low organic matter (<0.5% TOC) sites. Although 16S rRNA gene surveys indicated very little taxonomic difference at the phylum level between these different samples, the metabolite profiles were quite different. This indicates that the same phyla may be performing different survival functions depending on the environmental conditions. Genomic evidence for the enzymatic pathways responsible for the metabolite profiles were readily apparent in the single cell amplified genomes of abundant uncultured clades. From this collection of data, a complex picture begins to emerge of how uncultured microorganisms survive in the deep subsurface.

D:L-AMINO ACIDS AND THE TURNOVER OF MICROBIAL BIOMASS

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Decades of ocean drilling have demonstrated wide spread microbial life in deep sub-seafloor sediment, and surprisingly high microbial cell numbers. Despite the ubiquity of life in the deep biosphere, the large community sizes and the low energy fluxes in the vast buried ecosystem are still poorly understood. It is not know whether organisms of the deep biosphere are specifically adapted to extremely low energy fluxes or whether most of the observed cells are in a maintenance state.

Recently we developed and applied a new culture independent approach – the D:L-amino acid model – to quantify the turnover times of living microbial biomass, microbial necromass and mean metabolic rates. This approach is based on the built-in molecular clock in amino acids that very slowly undergo chemical racemization until they reach an even mixture of L- and D- forms, unless microorganisms spend energy to keep them in the L-form that dominates in living organisms. The approach combines sensitive analyses of amino acids, the unique bacterial endospore marker (dipicolinic acid) with racemization dynamics of stereo-isomeric amino acids.

Based on a heating experiment, we recently reported kinetic parameters for racemization of aspartic acid, glutamic acid, serine and alanine in bulk sediment from Aarhus Bay, Denmark. The obtained racemization rate constants were faster than the racemization rate constants of free amino acids, which we have previously applied in Holocene sediment from Aarhus Bay and in up to 10 mio yr old sediment from ODP Leg 201. Another important input parameter for the D:L-amino acid model is the cellular carbon content. It has recently been suggested that the cellular carbon content most likely is lower than previously thought. In recognition of these new findings, previously published data based on the D:L-amino acid model were recalculated and will be presented together with new data from an Arctic Holocene setting with constant sub-zero temperatures.

SLOW, DEEP PHOTOTROPHY IN CONSPICUOUS PINNACLE MATS FROM MAGICAL BLUE HOLE

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Magical Blue Hole is a vertical, water-filled limestone cave with an anoxic and slightly sulfidic water column below 25 meters (ca. 5 μM total dissolved sulfide). The anoxic deep waters are separated from a freshwater lens by a halo-chemocline. Conspicuous biofilms are found on the cave walls in the upper part of the anoxic water column where light availability is below 0.1 μmol photons m^{-2} s^{-1}. The biofilms consist of several centimeters of orange layers with a very thin veil of green on top. Pinnacles formed by filamentous organisms in the orange layer extend 1-2 cm into the water above. Phototrophic Chlorobiaceae (Green Sulfur Bacteria) and sulfate-reducing bacteria within the Deltaproteobacteria are the most abundant taxa in the biofilm. Planctomycetes, nonphototrophic Chloroflexi, and populations affiliated with candidate bacterial divisions having no cultivated representatives (OP1, OP3, OP8, OP11, OD1, WS3, TM6, and BRC1) are also significant. Consistent with very low light and bulk aqueous geochemical measurements indicating low energy fluxes available for microbial metabolism, none of the cells in the biofilm could be visualized using traditional Fluorescence *In Situ* Hybridization with a suite of domain- and phylum-level probes. Using microsensors, isotope tracing techniques (^{13}C, ^{35}S), highly sensitive light sensors and HPLC pigment analysis along with *in situ* measurements of geochemical parameters, we aimed to illuminate the ecophysiology of the biofilm and to estimate the rates of anoxygenic phototrophy and sulfate reduction.

Low amounts of light (ca. 0.27 μmol m^{-2} s^{-1}) significantly increased the uptake of DIC (dissolved inorganic carbon) and acetate compared to dark controls. The light-driven uptake rates were 0.0482 nmol DIC (μg C)$^{-1}$ h^{-1} and 0.007 nmol acetate (μg C)$^{-1}$ h^{-1}. Photopigments in the biofilm appear to be primarily isorenieratene and bacteriochlorophyll *e*. These are typical of brown-colored species of Green Sulfur Bacteria which are known as low-light specialists. Isorenieratene was present in all layers of the biofilm while bacteriochlorophyll *e* was found in upper layers only. Remarkably, we found that the light arriving at the biofilms was mainly in wavelengths <400 nm and 475 – 530 nm. The light quality coincided with the 505 nm in-cell absorption maximum of isorenieratene. We concluded that isorenieratene is used in combination with bacteriochlorophyll *e* for light-harvesting in autotrophic, anoxygenic photosynthesis.

Large amounts of chromium reducible sulfur (includes FeS_2, S°, S8, Sx) in biofilm pore water as well as sulfide depletion in the upper part of the biofilms suggested that the biofilms are net sulfide sinks. Independent evidence from sulfide-trapping films inserted vertically into biofilms confirms this view and suggests that sulfide does not accumulate even in deep layers of the biofilm after days or weeks of *in situ* incubation. Sulfate reduction rates in the biofilms were found to be low and heterogeneous (0 - 99 nmol cm^{-3} d^{-1}). The quantitative role of anoxygenic photosynthesis in sulfide oxidation remains unknown and further investigations are necessary to improve our understanding of both phototrophic and sulfide oxidizing processes in the biofilm. Potential roles for abiotic sulfide oxidation by iron crusts and/or sulfur disproportionation by Deltaproteobacteria are suggested by our data but remain to be investigated in future work.

INVAGINATED CYTOPLASMIC MEMBRANES IN LOW ENERGY SYNTROPHIC SULFATE REDUCING BACTERIA

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Usually we can think of cells as roughly globular and can approximate the ratio or surface area to volume easily. However, some gram negative cells exhibit an invaginated inner membrane which complicates the globular perspective. Recently we found invaginated membranes in sulfate reducing bacteria which associate with ANME archaea during syntrophic anaerobic methane oxidation. These cells exist in a low energy regime, and these membrane features may somehow relate to this. Detailed ultra-structural investigations showed that these membrane structures are highly reminiscent of those observed in mitochondria – they connect through a narrow tubular opening similar to the cristae junction and were found to harbor substantial re-dox activity as observed by a new TEM based redox assay. These observations suggest that these cells may enjoy an enhanced respiratory capacity, but it is unclear how this may be related to their physiology. Current work and experimental design is focused on quantifying membrane bound redox activity and cell surface area, and relating these parameters to growth rates, yields. In this way we are trying to connect cellular architecture to energy flux and nutrient acquisition.

STUDY OF MICROBIAL ACTIVITY IN MARINE SEDIMENTS OF THE ISLAND BASIN USING D:L AMINO ACID MODEL

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Subseafloor sediment harbors a large proportion of microorganisms- about 3 X 10^{29} according to the recent census. Microbial cells in these very stable and oligotrophic settings catabolize at a much slower rate than model organisms in nutrient rich cultures. Microbial metabolic activity depends upon various factors like pH, temperature, pressure, sedimentation rates and distance from the land. In order to increase our understanding of the life in deep biosphere, we carried out a study on bacterial activity, and turnover times of bacterial necromass and biomass using newly developed D:L-amino acid racemization model in marine sediments from the Island basin. Sediment cores were up to 5 meters long and covered a time scale from present to ~□□□□□□□□years. Sediment was analyzed for total hydrolysable amino acids (THAA), the bacterial endospore marker dipicolinic acid (DPA), and amino acid enantiomers (L- and D-form) of aspartic acid. The amino acid carbon content, and the ratio between the protein amino acids and their respective non-protein degradation products were used for determining the degradation state of the organic matter with the sediment depth and age. Endospores quantified using DPA quantification method were found to be as abundant as vegetative cells. The microbial necromass was estimated to be recycled over the range of thousand years, while turnover times for the vegetative cells were observed to be in the range of tens to hundred years. Studies with deeper sediment cores will further improve our understanding of the energetic limits of life in the deep biosphere.

MICROBIAL METHANE CYCLING IN SUBGLACIAL LAKE WHILLANS, WEST ANTARCTICA

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Subglacial environments beneath the Antarctic ice sheets have been hypothesized to contain a significant pool of biomass and biogeochemically and climatically relevant gases, such as methane (CH_4). Recently, direct sampling of Subglacial Lake Whillans (SLW), which lies 800 m beneath the West Antarctic ice sheet, revealed that this environment harbors a metabolically active microbial ecosystem. The specific microbial metabolisms of SLW, their energy requirements and their contributions to global biogeochemical cycles remain unknown.

Past interglacial periods exposed much of the West Antarctic basin to open ocean conditions; hence, ancient marine sediments may provide the primary carbon and energy sources for contemporary microbial metabolism beneath the ice sheet. The subglacial environment is sealed from above, but there is a sustained release of atmospheric O_2 from overlying glacial ice melt that can act as the major metabolic electron acceptor for reductants supplied by relict marine sediments.

SLW sediment porewater had high concentrations of dissolved methane (100-300 µM). Methane $\delta^{13}C$ values ranged from -77‰ to -70‰, indicating a biological origin. This biological methane is diffusing upward and may be produced at depth from the breakdown of relict marine carbon. The vertical CH_4 gradient could support an estimated flux of 0.32 mg CH_4 m^{-2} d^{-1} to the overlying water column. The high flux of methane to the sediment-water interface may explain the abundance of methane-oxidizing bacteria. Methane-oxidizing bacteria were abundant (2.5% of 16S rRNA gene sequence libraries) in the surficial sediments of SLW, suggesting that CH_4 may be an important carbon and energy source for aerobic heterotrophic microbial activity. The metabolic potential for energy generation and biomass incorporation from methane oxidation will be assessed using metagenomics data from SLW sediment. These data are being processed. Methane monooxygenase, an enzyme that catalyzes the first step of aerobic CH_4 oxidation, was detected in the top 10 cm of sediment through molecular analysis of the pmoA gene sequence. These pmoA sequences were closely (>95%) related to *Methylobacter tundripaludum*, which possess a pmoA gene sequence related to that of the type I methanotrophs. Type I methanotrophs have a low-affinity methane monooxygenase and are characteristically found in CH_4-rich environments, such as SLW sediments.

Our data represent the first direct evidence of a microbial methane reservoir beneath the West Antarctic ice sheet and lead us to infer that CH_4 supports an active methanotrophic bacterial assemblage. Our data also indicate that a large fraction of the CH_4 from the subglacial sediments may be converted to CO_2 via methane oxidation before it can be released to the atmosphere through subglacial water outflow or ice sheet collapse.

CONTROLS ON ORGANIC MATTER DEGRADATION RATES

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Transformation and mineralization of organic matter fuels most benthic life and it is therefore essential to understand the factors governing processing of organic matter. Sedimentary organic matter is a complex mixture of thousands of different compounds. This heterogeneity frustrates chemists that are incapable to characterize more than 40% of the material at the compound level while consumers eventually consume more than 90% of the material delivered to sediments. Without proper characterization of sedimentary organic matter (in terms of composition, linkage with mineral phases and spatial context) it is difficult to design tracer experiments to identify organic matter transformation pathways and to accurately quantify organic matter degradation beyond the use of oxidants or accumulation of metabolites. This heterogeneity is also underlying the apparent continuum of reactivities towards degradation. Overall organic matter reactivity is not only a function of the organic matter composition, but also depends on the consumer community and environmental context.

In this presentation I will discuss the various angles, strength and weakness of organic-matter-degradation conceptual models. Organic geochemists appear to underappreciate secondary production and the consequences for subsequent organic matter degradation. Their preferential degradation and preservation model needs to be modified for organic matter produced by consumers. Biogeochemists focus on the transformation of organic carbon to inorganic carbon and on the use of oxidants or production of metabolites, but largely ignore information on organic matter composition and down-core changes therein. Microbial ecologists focus on the identity and growth of consumers involved, in particular the ones utilizing small organic compounds, nutrients or oxidants. The relation between microbial diversity and organic chemical diversity is poorly known and microbial mortality and longevity are understudied. A porous medium such as sediment provides another level of heterogeneity in terms of small-scale organic matter distribution, space for movement and presence of organisms, and transport of solutes.

METABOLICALLY ACTIVE MICROBIAL POPULATIONS WITHIN NORTH POND SUBSURFACE CRUSTAL BASALTS EXPANDS THE BIOSPHERE AND THE LIMITS OF LIFE

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The detection and characterization of active microbial populations within subsurface crustal basalts represents an expansion of Earth's biosphere and provides a new environment to test the limits of life. Over the past several decades, microbial populations have been qualitatively and quantitatively characterized in marine sediments from near shore locations to gyre centers, and from the sediment surface to two kilometers below the surface. Recent exploration of the crustal material has targeted exposed basalts and free-living, interstitial water populations. Limited access to subsurface basalt samples has inhibited biological characterization of microbial communities within this unique, isolated, potentially habitable environment. Basalt contains redox active iron and additional metals available as energy sources for microbial populations (Canfield et al., 2006). Basalt has been shown to geochemically oxidize over millions of years, thus remaining biologically redox active (Bach and Edwards, 2004). Basalt is also porous allowing fluid exchange and providing single-celled life with dissolved metabolites and nutrients required for cell maintenance and growth. Initial cultivation-based and in situ analysis of subsurface basalt has produced some structural identification of populations that have the potential to alter the crust. The study presented here represents the first to directly sample and identify metabolically active microbial populations within subsurface crustal material. Drilling during Integrated Ocean Drilling Program Expedition 336 in the 'North Pond' sediment pond feature on the western flank of the Mid-Atlantic Ridge (Expedition 336 Scientists, 2012. IODP Preliminary Report 336) provided basalt samples from 144-219 meters below seafloor. Samples were collected using standard biological sampling protocols to prevent operational contamination. Each sample passed drilling contamination quality control standards for microbial analysis. Descriptions of each sample were recorded shipboard and then visually confirmed prior to RNA extraction. The samples from drill site 1382 were aphyric with glassy margins (7R-2B) and a sedimentary breccia with basalt clasts and a rust color (8R-4D). Site 1383 samples included two highly altered phyric basalts (10R-1B and 11R-1B) and a basalt glass with rust alterations (20R-1A). Total RNA was extracted from all samples and multiple controls following methods described in Mills et al. (2012). The targeted 16S rRNA V1-V3 region was sequenced using 454 pyrosequencing and analyzed following a standardized Mills Lab work flow. Sequencing efforts produced 2539 sequences; with each sample library containing 124 to 1323 sequences phylogenetically related to 4 to 10 phyla. These lineages were not observed in any of the control samples, thus represent metabolically active populations only found within the crustal material. Distinct communities were observed in each of the five samples with few lineages shared. Analysis of these lineages suggested both anaerobic and aerobic metabolisms with populations capable of utilizing the available carbonate present within the basalt samples. Additional characteristics suggested metabolisms capable of taking advantage of substrates within the basalt. As seen in the overlying sediments, many lineages were capable of using nitrogen species in redox reactions, supporting the importance of this pathway in energy-limited environments. This analysis will support further targeted approaches for enhanced molecular characterizations and culture-based analysis to enhance understanding of the metabolic processes and potential biomarkers available for detection of life within basalts on Earth and potentially in the Martian subsurface.

ARCHAEAL ADAPTATIONS TO EXTREME ENERGY LIMITATION

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Some anaerobic archaea live on substrates that do not allow the synthesis of one mol of ATP per mol of substrate. Energy conservation in these cases is only possible by a chemiosmotic mechanism that involves the generation of an electrochemical ion gradient across the cytoplasmatic membrane that then drives ATP synthesis *via* an A_1A_O ATP synthase. The minimal amount of energy required is thus depending on the magnitude of the electrochemical ion gradient, the phosphorylation potential and the ion/ATP ratio of the ATP synthase. Methanogens, *Thermocccus*, *Pyrococcus* and *Ignicoccus* have evolved different ways to energize their membranes, such as methyltransferases, H^+ or NAD^+ reducing electron transport systems fueled by reduced ferredoxin or H_2-dependent sulfur reduction that all operate at the thermodynamic limit of life. The structure and function of the enzymes involved is discussed. Despite the differences in membrane energization they have in common an A_1A_O ATP synthase that shows an extraordinary divergence in rotor composition and structural adaptations to life under these conditions. Adaptation to energy limited substrates involves chemiosmotic energy coupling with Na^+ as coupling ion and a structurally and functionally highly adapted ATP synthase.

FIRST INSIGHTS INTO THE FUNGAL COMMUNITIES OF SUBSURFACE KARSTIC AQUIFERS IN THE EARTH'S CRITICAL ZONE

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The presence of different fungal groups have been reported in a number of studies from terrestrial to aquatic habitats. But there are still some habitats in which fungi are either understudied or uncharacterized. One of such habitats is the Earth's Critical Zone (CZ). CZ is the thin veneer of our planet from the top of the tree canopy to the bottom of drinking water aquifers, upon which humanity is utterly dependent for life support. This is "heterogeneous, subsurface environment in which complex interactions between rock, soil, water, air, and microorganisms regulate the natural habitat and determine the availability of life-sustaining resources. Although our understanding about life in the subsurface has increased since previous years but the knowledge of microbial biodiversity especially fungal biodiversity and their mode of living in shallow and deep aquifers (which are major source of drinking water), is still poor. It is known that the flow rates of infiltrating water along with organic matter from the surface to the subsurface is very slow so microbes living in such environments must have evolved their own self-sustaining mechanism. This mechanism of getting energy in the CZ is a "BLACKBOX" at the moment. To extract the required information from this "BLACKBOX", the first step is to find out who is living there?

To take this first step towards the broader understating of Earth's CZ energy flow, we conducted a study in the newly established Critical Zone Exploratory (CZE) located in the Hainich region of Germany which is characterized by two superimposed limestone aquifers named as upper (oxygen deficient) and lower (oxygen rich) aquifer. The objectives of this study were (i) to make an inventory of different fungal OTUs in the aquifers, and (ii) to find if there are any differences in the fungal OTUs in upper and lower aquifers. DNA extracts of groundwater samples of upper and lower aquifers from seven different wells were subjected to massive parallel pyrosequecning. Results of 454-sequencing revealed that fungal OTUs affiliated to phylum Ascomycota and Basidiomycota are highly represented in all the wells of upper and lower aquifers whereas members of the phylum Zygomycota and Chytridiomycota are least represented. Non metric multidimensional scaling suggested that the fungal communities from upper and lower aquifers are well separated in 2-dimensional ordination. This was further confirmed by SIMPER overall dissimilarity value of 82.5 % based on Bray-Curtis dissimilarity index. Our data not only revealed the presence of fungi in the earth's critical zone but it also showed that the fungal communities were different in upper and lower aquifers. These initial findings laid down the foundation for further experiments to study the temporal shifts in the fungal communities in aquifers, the impact of above ground surface conditions on the subsurface fungal communities, their functional diversity and eventually tracking the flow of energy in the CZ.

ENERGY LIMITS OF ELECTRICAL CABLE BACTERIA IN THE SUBSURFACE

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Cable bacteria are multicellular filamentous bacteria that can conduct electrons from one end to the other, thereby coupling oxidation and reduction processes centimeters apart. So far only sulfide has been confirmed as electron donor while both oxygen and nitrate/nitrite can serve as electron donors for the anodic and cathodic processes, respectively. At present the electron carriers through cable bacteria are not identified but several models have been developed to simulate present observations in marine sediment and direct further studies of cable bacteria electron transport.

The charge flow by electrons in cable bacteria is balanced by ion migration in the sediment porewater, and the associated electric fields have recently been mapped with microsensors. The total voltage difference along the field expresses the energy cost of transporting one charge unit between the most distant active cells, and correspondingly less energy is therefore available for cell metabolism. Recently cable bacteria have been reported from the fringe of groundwater contamination plumes in the subsurface, readily suggesting that cable bacteria might be the "biogeobatteries" previously proposed as an explanation for the electric field anomalies commonly registered above such plumes. The anomalies may amount to several hundred mV which means that for some cells a major share of the roughly 1000 mV provided by aerobic sulfide oxidation is not available. In the presentation I will discuss to what extend the large anomalies may represent situations, where cable bacteria through competition have increased the distance between oxygen and sulfide until the energy loss in ion conduction have become unaffordable.

In marine sediment the high salinity ensures high ionic conductivity, and there it is more likely resistance in the bacterial electron transport than resistance in the porewater ion transport that poses limits to how far the cable bacteria may spread.

THE ASYMMETRY OF MULTI-ELECTRON TRANSFER PROCESSES AT THE ENZYME GENE STRUCTURE LEVEL

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The redox conversion between water and dioxygen represents a key component in biological energy processes. The reduction reaction ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$) is the terminal step in aerobic respiration mediated by Cytochrome C oxidase (COX), and is responsible for generating the membrane potential necessary for ATP production. The reverse reaction ($2H_2O \rightarrow O_2 + 4H^+ + 4e^-$) is equally as important, and is found in the initial step of oxygenic photosynthesis, which takes place at Photosystem II (PSII). The hole generated by excitation at the light harvesting center provides the oxidizing energy necessary to oxidize water, generating the proton motive force and ultimately driving ATP synthesis, much like the chemical reaction at COX.

Interestingly, although the 2 redox reactions share many common features such as the thermodynamic potential (E = 1.23 V for both reactions), the enzymes responsible for each redox reaction have obvious differences such as the structure of the metal center and surrounding ligands.[1,2] These differences illustrate the irreversibility of each redox reaction and show how Nature tailored each enzyme towards a specific function. For example, the D1 subunit of PSII is in closest proximity of the reaction center, and is constantly exposed to oxidative decomposition. To compensate, it is well known that cyanobacteria have the ability to switch between multiple isoforms of the D1 protein, including one that is tolerant even under oxidative stress.[3]

In this study, we hypothesized that specialization of each enzyme would occur not only at the protein level, but also at the gene level as well. The gene structure of the subunits closest to the reaction center of COX and PSII were evaluated across 70 different cyanobacteria and plant strains, based on the hypothesis that a shorter gene would need less energy to express. We found that in PSII, 58 out of the 70 strains investigated had a gene encoding only the D1 protein (Table 1). In contrast, all species investigated expresses subunit I, II, III of COX with other protein subunits inside the supercomplex. The distinct difference in the gene structure of PSII and COX suggests that a smaller gene structure is bioenergetically favorable for enzymes with high rates of turnover.

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PSII	(A) Within PSII	(B) Outside PSII	(C) Single
D1	1	52	58
D2	59	14	48
CP43	59	6	6
CP47	7	56	4
B559	66	10	0

COX	(A) Within COX	(B) Outside COX	(C) Single
subunit I	70	0	0
subunit II	70	3	0
subunit III	70	4	1

Table 1. The number of strains out of the 70 investigated which can (A) co-express another gene within PSII or COX, (B) co-express another gene outside PSII or COX, and (C) express the gene without co-expression of other genes. The subunit in question is indicated on the left. Note that many strains have multiple copies which encode the same subunit.

LIPID BIOMARKERS PRESERVED WITHIN OMAN OPHIOLITE CARBONATES: INSIGHTS INTO A SUBSURFACE SERPENTINITE-HOSTED ECOSYSTEM

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Serpentinization-based ecosystems, such as described at the Lost City Hydrothermal Field [1], are some of the most extreme settings where life is known to exist. Diverse microbial communities are sustained by serpentinization reactions [2] when obducted ophiolites react exothermically with seawater to produce large quantities of hydrogen, methane and other hydrocarbons. These ecosystems may be analogous to some of the oldest hydrothermal systems on Earth, and are potentially pervasive in the Earth's subsurface today. The Semail Ophiolite complex formed during the Late Cretaceous probably as a result of arc-continent collision following closure of the Tethys Ocean, and is a site of potentially active subsurface serpentinization [3]. Large areas of ophiolite are exposed in the Hajar Mountains of Oman, providing a unique opportunity to measure preserved biosignatures of microbial communities associated with past, and potentially ongoing, serpentinite-hosted ecosystems. This study, combined with recent work [4], reports the occurrence of a suite of lipid biomarkers preserved within carbonate veins sampled from the Semail Ophiolite. The distributions of diagnostic bacterial and archaeal lipids, combined with compound specific isotope ratio analysis are described, and compared with reports from the Lost City Hydrothermal Field [5], to gain an improved appreciation for community diversity and function in these systems. Studies such as these are important for our understanding of the limits and origins of microbial life.

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A SINGLE CELL PERSPECTIVE OF COOPERATION BETWEEN METHANOTROPHIC ARCHAEA AND THEIR SULFATE-REDUCING BACTERIAL PARTNERS

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Sulfate-coupled methane oxidation is a microbially-mediated process of global importance in the marine carbon cycle. Uncultured methanotrophic archaea (ANME) and sulfate-reducing bacteria (SRB) are the primary organisms driving this process in anoxic marine sediments through a syntrophic coupling that is not well understood. In deep-sea methane seeps, these syntrophic microorganisms form highly structured, multi-celled consortia that often consist of one dominant ANME and SRB lineage. The spatial arrangement of partners and specific species membership among individual sediment-hosted consortia in the environment is highly diverse. This inherent environmental complexity, combined with slow rates of growth and difficulty with cultivation, has slowed progress in understanding ANME ecophysiology and the metabolic interactions between these methanotrophic archaea and their syntrophic partners using conventional bulk geochemical and molecular techniques. Here we are applying single-cell targeted stable isotope analyses and click-chemistry enabled methods to learn about the interactions occurring within consortia. With this high-resolution approach, we studied the influence of spatial proximity between co-associated syntrophic partners on their anabolic activity within diverse methanotrophic consortia from environmental samples. Activity patterns across multiple ANME-SRB consortia are inconsistent with intermediate exchange based on molecular diffusion and instead point to the potential for alternative mechanisms of electron transfer. Combined with data from genomic analysis, we outline new ideas about energy conservation and interactions within the AOM symbiosis.

THE ENERGETIC LANDSCAPE OF CHEMOLITHOTROPHY IN THE CONTINENTAL DEEP SUBSURFACE: SANFORD UNDERGROUND RESEARCH FACILITY (SURF), USA

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The deep subsurface has the potential to harbor a vast array of microbial life, subsisting in relative degrees of isolation from the surface, on the mineralogical offerings of host rocks. Assessing the deep subsurface biosphere without introducing surficial nutrients is an experimental challenge. Here we exploit a gradient of surficial impact offered by the Sanford Underground Research Facility (SURF), a former gold mine in South Dakota, USA. Sites range from flowing bore holes and pools open to the mine atmosphere to capped manifolds that have been isolated for decades. SURF is situated in a geologically complex region of Paleoproterozoic deposits including basalts, marginal siliciclastic sediments, iron formation, and carbonate-rich sediments. This package has been affected strongly by metamorphism and ore-mineralizing fluids, resulting in complex spatial heterogeneity of potential nutrient sources.

Detailed geochemical measurements (redox sensitive *in situ* analyses, anions, cations, and dissolved gases) and samples (for culturing, DNA and RNA sequencing, lipid compositions, and mineralogy) were collected during a number of field excursions beginning in September of 2013. These aggregate data represents the greater work of the NASA Astrobiology Institute *Life Underground*. Geochemical measurements reflect the vast chemical diversity encountered in these samples, ranging from fully oxic to very reducing (ORP 330 to -328 mV), with iron and sulfide measurements varying from saturated to below the level of detection, all at circumneutral pH (6.55-8.46) and a limited range of temperatures (10 – 32.9 °C). At each study site, we calculated Gibbs energies using these data for 144 inorganic reactions using O_2, NO_3^-, MnO_2, Fe_3O_4, SO_4^{2-}, S^0, HCO_3^-, and CO as electron acceptors and Fe^{++}, CH_4, H_2, S^0, NH_4^+, HS^-, CO, and Mn^{++} as electron donors.

When viewed in units of kilojoules per mole of electrons transferred, the patterns in our Gibbs energy calculations progress in a fairly typical way with the most exergonic reactions being those that use O_2 and NO_3^- as electron acceptors, regardless of the electron donor. Given the scarcity of oxygen in most of these sites, this analysis does not facilitate understanding the *in situ* microbial ecology. To overcome this problem we recast the Gibbs energy calculations into energy density units (in joules per kilogram of water) for each reaction, taking into account the concentrations of limiting reactants. In this view the electron donors dictate the most exergonic processes with sulfur and sulfide oxidation yielding the most energy, followed closely by ammonium, iron, and manganese oxidation. Interestingly, these calculations also showed dramatic changes between sites with the most energy in mine atmosphere-impacted sites coming from oxidation of elemental sulfur with oxygen and nitrate, whereas the more isolated sites revealed more sulfide oxidation with manganese oxides and nitrate. These results agree well with predicted physiologies of microbes present at each site and have been useful predictors to guide culturing efforts. Upcoming work targeting currently sealed boreholes that capture the full range of available host lithologies will further elucidate the connection between the geochemistry, microbiology, and energetics to the underlying geology.

NEAR-ZERO GROWTH OF MICROORGANISMS UNDER DEEP NUTRIENT LIMITATION

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Introduction. Specific growth rate of microorganisms μ varies from the maximum μ_m to zero as dependent on nutrients availability, temperature and other factors. Laboratory cultivation mainly occurs under optimal conditions with μ approaching μ_m. In majority of natural habitats (soils, subsurface sediments, oligotrophic lakes, etc.) microorganisms grow continuously and very slowly ($\mu << \mu_m$), typically with one cell division per month or even longer period [2]. Such a ***continuously sustained near-zero growth*** **(NZG)** should be distinguished from a trivial growth deceleration and cessation observed ***transiently*** in a post-exponential batch culture. An authentic reproduction of the natural NZG in laboratory requires a long-term uninterrupted continuous cultivation bringing microbial population to equilibrium with extremely low but stable flux of deficient nutrients.

Methods and organisms. Our own and published studies on NZG were performed by using chemostat with cell retention (CCR) or dialysis culture operated for as long as 60-200 days under full environmental control. Diverse bacteria and fungi were examined but illustrations will be restricted to *Pseudomonas putida, Arthrobacter globiformis* and *Mycobacterium smegmatis.*

Principal conclusions:

Physiological state of cells. A long-standing interpretive controversy about the NZG is whether cells in mature CCR enter a non-growing maintenance state (μ=0, the energy supply matches the maintenance requirements) [1] or there is a minimum growth rate μ_{min}>0 below which cells die [4]. We resolve this controversy [3] by showing that under NZG-conditions bacteria differentiate into growing and VBNC (viable-but-not-nonculturable) forms, the latter preserving measurable catabolic activity. The proliferating cells attained a steady state, their slow growth balanced by VBNC production. The popular concept of the non-growing maintenance state could not be applied to non-differentiated cells (spores, cysts, VBNC) that are able to metabolically adjust their maintenance requirements and therefore keep their growth balance positive.

The reproduction minimum μ_{min} for the active subpopulation was not experimentally confirmed.

Proteomic and transcriptomic data revealed that under NZG-conditions cells change their expression profile at least by 40% with upregulated (transporters, stress-response, self-degrading enzymes and extracellular polymers) and downregulated (ribosomal, chemotactic and primary biosynthetic enzymes) proteins vs intensive growth. Based on these profiles, we identified intracellular processes associated with NZG and generated a mechanistic mathematical model that adequately simulated all available observation data. We conclude that NZG requires controlled partial self-digestion and deep reconfiguration of the metabolic machinery that results in the biosynthesis of new products and development of broad stress-resistance.

Spontaneous mutations were shown to be enhanced under NZG-conditions accelerating microevolution of chronically starving culture in favor of the GASP-phenotype. Thus both epigenetic and genetic changes are essential for adequate understanding of NZG.

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MINIMUM ENERGY QUANTUM OF ANAEROBIC MICROBIAL CATABOLISM: HYDROGEN-FORMATE INTERCONVERSION

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Short chain fatty acids such as acetate, propionate and butyrate are characteristic products of fermentation in the anaerobic food chain**.** They can serve as substrates for secondary fermentations in bacteria generating acetate, H_2 and formate that serve as substrates for methanogenic archaea. This process is thermodynamically unfavorable under standard conditions but may become favorable by the instant consumption of H_2, formate or acetate by methanogenic archaea. The required interaction between producers and consumers is commonly termed as syntrophy. Syntrophy is the heart of how methanogenic and other anaerobic microbial communities function. The astonishing characteristic of syntrophic communities is that they are able to operate at the limits of what is thermodynamically possible. The syntrophic microorganism and its partner have contrary requirements with respect to the formate and H_2 concentrations. A niche for microorganisms growing on the conversion of formate to H_2 (or vice versa) in anoxic communities seems therefore unlikely. Yet, growth on formate by a syntrophic consortium of *Desulfovibrio* G11 with *Methanobrevibacter arboriphilus* has been reported (Dolfing et al., 2008). *Desulfovibrio* G11 most probably conserves energy from the conversion of formate *via* a formate-hydrogen-lyase system containing an energy conserving hydrogenase. Growth on the conversion of formate to H_2 has also been demonstrated for a thermophilic archaeon (Kim et al., 2010). Recently, two *Tindallia* sp. strains were isolated from extreme haloalkaline sediments (Sorokin et al., 2011). They differed from previously described acetogenic *Tindallia* species, as both grew lithoautotrophically with H_2 and formate. Interestingly, formate was detected as a product of anaerobic H_2 metabolism instead of acetate, which is expected for an acetogen. The formation of formate was additionally detected in a hydrogen fed bioreactor operated at haloalkaline conditions (Sousa et al., 2015). It can be speculated that formate is the product of a *reversed* formate lyase reaction.

The presentation will focus on the energetic challenges that microbes encounter while converting formate to H_2 or vice versa.

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BULK MICROBIAL RESPIRATION IN THE BURIED BIOSPHERE

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In situ catabolic activity of prokaryotes in buried sediment can only be quantified on community level, and only via geochemical methods. Such measurements of depletion of electron acceptors or accumulation of waste products have been performed on surficial sediments for decades, but little data exists from the deep biosphere. Most of this data come from atypical geochemical settings because rate measurements in the more typical sulfate reducing or methanogenic sediments are very difficult. We illustrate the limitations, but also the possibilities for determination of sulfate reduction rates via both reaction-transport modeling and via incubation with radioactive $^{35}SO_4^{2-}$. We show that if the methods are used uncritically they can easily lead to conflicting and artifactual results. But also that that the two methods can complement each other to cover a sulfate reduction rates that span at least 8 orders of magnitude and can pick up metabolic rates in ancient sediments reliably. We present a comprehensive combined data set of community level catabolic activity and community size from sediments that have been isolated from the surface for up to 15 million years. We show that community size is closely tied to community activity. But that it is difficult to determine what constitutes the link.

BOOSTING THE DEEP BIOSPHERE: SUBSEAFLOOR SEDIMENT A NATURAL CATALYST FOR RADIOLYTIC HYDROGEN PRODUCTION

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Production of molecular hydrogen (H_2) by radiolysis of water naturally occurs in subseafloor sediment as a result of radiation from decay of the U, Th, and K intrinsic to the sediment. This process has been hypothesized to be a significant source of electron donors for the deep biosphere, especially in environments with very low organic content, such as deep continental rock and very slowly accumulating deep-sea sediment (Lin et al. 2005; Blair, et al. 2007).

Two lines of previous research indicate that potential catalytic effects of solid and dissolved compounds need to be carefully considered when evaluating the total subsurface radiolytic H_2 budget and its potential importance to subsurface life. First, studies on radiolysis of water mixed with some compounds (e.g. ZrO_2, SiO_2, Al_2O_3, synthetic zeolites) have reported relatively high H_2 production compared to H_2 production from water in the absence of solid phases (LaVerne and Tandon, 2002; Cecal et al. 2004; Yamada et al. 2008). Second, a theoretical study predicted enhanced H_2 production by radiolysis of seawater compared to pure water, due to the interaction of anions with hydroxyl radicals (Bjergbakke, et al. 1989).

To constrain these effects in subseafloor sedimentary environments, we experimentally quantified H_2 yields from gamma radiation of pure water, seawater, and slurries ($\varphi = 0.85$) of seawater with representative marine sediment (abyssal clays, carbonate oozes, siliceous oozes, coastal sediment), ZrO_2, and natural smectite and zeolite powders.

Our pure water experiments indicate that the ratio of H_2 production to gamma radiation rate is linear over six orders of magnitude (Crumière, et al. 2013). Our seawater experiments show that there is no statistically significant difference (within the 90% confidence limit) between the radiolytic H_2 yields of pure water and seawater. Our experiments with marine sediment show that specific lithologies increase radiolytic H_2 yields by up to a factor of 8, compared to pure seawater.

These results have significant implications for understanding subseafloor microbial activity in organic-poor subsurface environments. For example, at IODP Expedition 329 sites in the South Pacific gyre (U1365 through U1370), dissolved H_2 abundance is generally below detection, despite the relatively high in-situ production rates of radiolytic H_2 predicted using our newly constrained yields. Hydrogen from in-situ water radiolysis may be the principal electron donor for microbes at depths greater than a few meters in this subseafloor ecosystem.

MANGANESE CYCLING MICROBIAL COMMUNITIES INSIDE DEEP-SEA MANGANESE NODULES

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Polymetallic nodules (manganese nodules) have been formed on deep sea sediments over millions of years and are currently explored for their economic potential, particularly for cobalt, nickel, copper and manganese. Here we explored microbial communities inside nodules from the northeastern equatorial Pacific. The nodules have a large connected pore space with a huge inner surface of 120 m^2/g as analyzed by computer tomography and BET measurements. X-ray photoelectron spectroscopy (XPS) and electron microprobe analysis revealed a complex chemical fine structure. This consisted of layers with highly variable Mn/Fe ratios (< 1 - > 500) and mainly of turbostratic phyllomanganates such as 7 and 10 Å vernadites alternating with layers of Fe-bearing vernadite (δ-MnO_2) epitaxially intergrown with amorphous feroxyhyte (δ-FeOOH). Using molecular 16S rRNA gene techniques (clone libraries, pyrosequencing, real-time PCR) we show that polymetallic nodules provide a suitable habitat for prokaryotes with an abundant and diverse prokaryotic community dominated by nodule-specific Mn(IV)-reducing and Mn(II)-oxidizing bacteria. These bacteria were not detected in the nodule-surrounding sediment. The high abundance and dominance of Mn-cycling bacteria in the manganese nodules argue for a biologically driven closed manganese cycle inside the nodules relevant for their formation and potentially degradation.

EVOLUTION IN THE DEEP BIOSPHERE

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Marine sediments cover more than two-thirds of the Earth's surface and harbor the largest reservoir of organic carbon on the planet. Diverse communities of Bacteria and Archaea inhabit these vast subsurface habitats, with viable cells detectable down to > 2 km below seafloor. These communities are fueled by the deposition and burial of particulate organic carbon from the water column, and thus characterized by a rapidly decreasing energy flux with depth, as the main energy source, buried detrital organic matter, is gradually degraded. Even though microbial community size typically decreases by about 3-4 orders of magnitude from the surface to a depth of a few hundred meters, the number of cells is still large compared to the minute energy flux available at depth. It is unknown how cells can subsist at energy fluxes 1000-fold lower than the typical maintenance energy of pure cultures: did these deep subsurface populations acquire special adaptations after millions of years of evolution under severe energy-limitation? Or, are they selected as most persistent but ultimately doomed survivors of surface populations? In other words, did they evolve *in* the deep biosphere or *for* the deep biosphere?

We followed the transition of the microbial community in Aarhus Bay (Baltic Sea, Denmark) from the bioturbated surface sediment through several distinct biogeochemical zones down to a depth of 10 m. In addition, we quantified the fraction of motile microbes across depth, and determined sulfate reduction rates, which can, together with a sediment age model, give an estimate of the maximum number of generations that have passed during burial of the microbial populations. From these combined data, we infer the relative importance of the main drivers for microbial community assembly, i.e. diversification, selection, and dispersal, in marine sediments.

The data suggest a transition of assembly and evolutionary processes in marine sediments: in the upper, bioturbated layers, diversification and dispersal can be significant: adaptive mutations can thus spread through the population while deleterious mutations will be effectively removed by purifying selection, leading to highly adapted populations but not necessarily low energy specialists. Below a critical depth, a combination of exceedingly long generation times and absence of motility and transport dramatically restricts diversification and dispersal. Selection of taxa pre-adapted to the low energy flux of the deep biosphere becomes the major driver for community assembly, and these "persisters" gradually come to dominate the subsurface community. Fine-scale analysis of the uppermost 50 cm revealed that the greatest change in community composition, cell numbers, metabolic rates, and generation times occurred within the first 10 cm, i.e. at the transition from the bioturbated zone to the subsurface zones, and that dominance of typical "deep biosphere clades" like the archaeal Miscellaneous Crenarchaeotal Group and Marine Benthic Group D, or the bacterial Candidate division OP8, Planctomycetes, and Chloroflexi, already starts at that depth. Single cell genome comparisons of dominant persisters suggest that they in fact are relics selected from the surface layers; once buried, they remain over thousands of years, with little indication of genetic change or further diversification.

IRON DRIVEN ANAEROBIC METHANE OXIDATION IN MARINE AND FRESHWATER SEDIMENTS.

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Microbial dissimilatory processes in sediments generate energy through the decomposition of substrates. Under anoxic conditions, below the depth of sulfate depletion, traditionally the only presumed process is methanogenesis (methane production). In marine sediments anaerobic oxidation of methane (AOM) coupled to sulfate reduction has been shown to consume above 90% of the upward methane flux. We have shown recently also in the deep methanogenesis zone of freshwater sediments the occurrence of AOM that is coupled to iron reduction.

We present here a geochemical approach based on carbon, iron, sulfur and oxygen isotopes to investigate the mechanism of AOM process in marine and freshwater sediments. This is through geochemical profiles, their modeling and incubation experiments. The isotope analyses indicate that AOM by sulfate reduction in methane seep habitats differs than that in diffusive profiles in and above the sulfate-methane transition zone. The results suggest also the involvement of iron oxides in sulfate-driven AOM in methane seeps. It seems that beyond the function of iron as nutrient, the presence of iron oxides stimulates sulfate-driven AOM to a greater extent than in sediments with low concentrations of iron oxides. On the contrary, the results from fresh water sediments indicate that the iron driven AOM there is not enhanced by sulfur cycling but rather competes with sulfur on the reduction of inhibited.

PEELING THE ONION OF LIFE'S ANAEROBIC AND LOW ENERGY ORIGIN

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During microbial evolution, organisms learned to explore the environment, taking advantage of a myriad of substrates that allow them to grow[1,2] at the expense of various energy-releasing redox reactions that allow metabolism to move forward according to the second law of thermodynamics[3]. At life's start, there was also a main energy-releasing reaction that gave rise to the first cells. In an early life context, identifying the primordial energy and carbon metabolism can not be dissociated from how life originated and evolved. And since life arose in a world without oxygen[4], the protein families that anaerobes share might hold important clues.

Here we wish to investigate the nature of the prokaryote ancestor, specifically which genes it contained and to which lineages of modern prokaryotes it was most closely related to, as inferred from the gene collection present in extant genomes. Previously we clustered 134 archaeal genomes into 25,762 protein-families and looked to their corresponding homologous in 1,847 bacterial genomes[5]. Phylogenetic analysis showed that in 4,397 cases, the prokaryotic domains were monophyletic. From those, 3347 correspond to protein-families where either only one archaeal or one bacterial group is represented, these likely betray lateral gene transfers (LGT) that occurred at a time where the prokaryotic domains had already started their diversification, and thus do not contain clues about the early metabolism and were excluded from our present analysis.

To assess the oxygen-tolerance of the organisms present in our dataset we looked for the presence of heme-copper oxygen reductases (HCOs) in their genome. This allowed us to distinguish (facultative) aerobes from anaerobic organisms and moreover, to classify protein-families as aerobic or anaerobic. In aerobic families, 90% of their members are encoded in genomes of aerotolerant organisms (they co-occur with HCOs). Conversely, the occurrence of anaerobic protein-families is restricted, at the 90% threshold, to anaerobic organisms. If we now consider the genomic (taxonomic) distribution of the families that are present in archaea and bacteria, with archaea and bacterial branches both being monophyletic, we see that aerotolerant and anaerobic proteins have very different distributions. Aerotolerant familes are shared between sulfolobales, halobacteriales, actinobacteria and proteobacteria. Functionally, these protein-families are mainly cytochrome and copper-containing proteins, dioxygenases, some NADH- and FAD-dependent oxidoreductases, whose invention and spread occurred most likely after the global oxygenation of earth. These are thus probably not proteins of Luca, rather they are yet another class of genes spread by LGT.

However, the anaerobic protein-families are specifically distributed among methanogens and clostridia, organisms that live from the low free energy changes, at the thermodynamic limit of life[6]. The anaerobic families encompass genes related with the Wood-Ljungdahl pathway such as two subunits of the bifunctional acetyl-CoA-synthase/CO-dehydrogenase complex, the soluble heterodisulfide reductase subunits C and A, ferredoxins, and several subunits of the H^+/Na^+-antiporter-Mrp or related complexes, in addition to numerous SAM-methyltransferases. This points to a methyl-dependent metabolism of Luca, and a cytochrome- and quinone-free start to life[7-9]. In this scenario, chemiosmotic coupling via the universal ATP synthase would occur possible with the help of a primitive Mrp-antiporter complex or any of its related complexes.

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ADAPTIVE MICROBIAL EVOLUTION IN DEEPSEA SEDIMENTS

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Microbial organisms are found in million year old deepsea sediments and believed to be metabolically active based on biogeochemical data. One favored hypothesis to explain the presence of these microbes is that the extant microbes are genetically adapted in deepsea environments to new energy source(s), to life at low growth rates, and to enhanced maintenance metabolism. An alternative hypothesis is that their presence is not due genetic adaptation, but simply to an ecological selection for microbes with decelerated death. To provide a conceptual framework for designing experiments to distinguish between these hypotheses, I present a theoretical paper on fundamental potentials and constraints of adaptive evolution in deepsea sediments. Drivers for generation of genetic variation as well as selection and population genetic aspects will be discussed.

EXTRACELLULAR ENZYMES FACILITATE ELECTRON UPTAKE IN BIOCORROSION AND BIOELECTROSYNTHESIS

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Direct, mediator-free transfer of electrons between a microbial cell and a solid phase in its surrounding environment has been suggested to be a widespread and ecologically significant process. The high rates of microbial electron uptake observed during microbially influenced corrosion of iron [Fe(0)] and during microbial electrosynthesis have been considered support for a direct electron uptake in these microbial processes. However, the underlying molecular mechanisms of direct electron uptake are unknown. We investigated the electron uptake characteristics of the Fe(0)-corroding and electromethanogenic archaeon *Methanococcus maripaludis* and discovered that free, surface-associated redox enzymes, such as hydrogenases and presumably formate dehydrogenases, are sufficient to mediate an apparent direct electron uptake. In genetic and biochemical experiments, we showed that these enzymes, which are released from cells during routine culturing, catalyze the formation of H_2 or formate when sorbed to an appropriate redox-active surface. These low-molecular-weight products are rapidly consumed by *M. maripaludis* cells when present, thereby preventing their accumulation to any appreciable or even detectable level. Rates of H2 and formate formation by cell-free spent culture medium were sufficient to explain the observed rates of methane formation from Fe(0) and cathode-derived electrons by wild-type *M. maripaludis* as well as by a mutant strain carrying deletions in all catabolic hydrogenases.

Our data collectively show that cell-derived free enzymes can mimic direct extracellular electron transfer during Fe(0) corrosion and microbial electrosynthesis and may represent an ecologically important but so far overlooked mechanism in biological electron transfer.

ACCELERATED MICROBIAL MUTATION RATES IN A MARINE SUBSURFACE SEDIMENT

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The seabed harbors diverse microbial communities that live off detrital organic matter. The degradability of the organic matter decreases rapidly with sediment depth and consequently less and less energy is available for division and maintenance of individual microbial cells in subsurface sediments. Upon burial into the sediment cells experience a drastic environmental change compared to the surface layers. The bacterial mutation rate (presented in number of single nucleotide polymorphisms [#SNP's]/genome per replication) has been reported to be very conserved in stable environments. The factors influencing this rate are the ratio between adaptive and deleterious mutations, the viral load and the fidelity of DNA repair. Theory predicts that everything else being equal higher mutation rates are favored in populations evolving in a new environment. We hypothesize that environmental change during sediment burial would select for increased mutation rates and tested this hypothesis in subsurface sediment populations.

To do so, we used single cell genomics approaches to quantify the amount of #SNPs present in genomes of members of the same lineage present in at two different sediment depth (25 and 175 cm) separated by 1800 years of sedimentation.

Sediment samples were obtained by gravity coring at station M5 in Aarhus Bay, Denmark. For each sample, cells were extracted, sorted individually and their genomes amplified. Based on 16S rRNA gene screening of the Single Amplified Genomes (SAGs) 4 Atribacterial SAGs (2 from 25 cm and 2 from 175 cm) were shot gun sequenced on a MiSeq instrument using 2x300bp chemistry. Genomes were assembled in SPAdes, read coverage was calculated by bbmap and the SNPs were called using samtools package on regions with coverage above 10-fold. The amount of pairwise diversity at a given sediment depth and between depths was calculated by mapping raw reads of one SAG on the assembled fragments of the other SAG from the same horizon.

The pairwise diversity between SAGs showed a 2-fold decline from the upper to the lower sediment depth (based on between 57 and 450 coding sequences alignments of an average length of 588 bp compared between and within the 2 horizons). Using these data combined with a generation estimate of 170 generations separating the layers (derived from average cell-specific growth rate estimates based on measurements of sulfate reducing activity, Jørgensen and Marshall, 2015) the mutation rate for the time separating the two sediment depths was estimated to be on the order of 0.38 SNPs/genome per replication. This is 100-fold higher than observed in stable environments, and in par with mutator-phenotypes observed in pure cultures evolving in novel/changing environments.

HOW GEOCHEMISTRY PROVIDES HABITABILITY: A CASE STUDY OF IRON OXIDATION

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Two things have to be true for chemotrophic microbes to gain chemical energy from their environment. First, there must be a source of energy, provided by compounds in differing oxidation states that are out of thermodynamic equilibrium with one another. Second, there must be mechanistic difficulties that are keeping those compounds from reacting, which means that the chemical energy cannot dissipate on its own. Using this energetic reference frame, geochemical habitability requires the combined presence of energy sources and kinetic barriers. Here we present habitable geochemical space visually as a habitability diagram. The habitability diagram maps the pH and temperature ranges that can sustain life for a specific reaction, bounded by the aforementioned kinetic and energetic boundaries and the commonly attainable pH / temperatures of aqueous environments at Earth's surface. Using pH and temperature as master variables to construct the diagrams provides the most utility to geo- and astrobiologists. Secondary habitability diagrams, however, can be constructed for any combination of relevant geochemical variables to better illustrate the inherently multidimensional problem. We have chosen iron oxidation reactions to illustrate this point, as kinetic and energetic boundaries can be found at conditions readily attainable in natural systems.

Construction of the habitability diagrams for iron oxidation reactions requires quantification of both the energetic and kinetic boundaries, and as such requires complete geochemical characterization of field sites and *in situ* assays of iron metabolism rates. The energetic availability (affinity) in each system is readily assessed from compositional data where concentrations of all reactants and products are known, and is used to define the energy boundary. The absolute boundary is then determined by the regression of these values on plots of affinity and the desired variable (usually pH) to affinity = 0. The true energetic boundary for any reaction, however, is the microbial maintenance energy. This is the minimum energy required to sustain a microbial cell in all processes other than active growth. This is estimated for microbial iron oxidation in literature reports, so another regression can be performed using these values. The result is presented on our diagram as the *de facto* energy boundary. Evaluating the kinetic boundary means measuring the relative rates of the biotic and abiotic processes. We have made *in situ* field assays of microcosms containing sediment and water. These measurements were made in systems ranging in temperature from hot springs to acid mine drainage. Locations were chosen based on either apparent iron metabolism (iron staining), abundant substrate, or calculations showing high potential energy yields. Many systems have yielded measureable biological rates. All have yielded abiotic rates, which range from inconsequential, to rates too rapid for biology to compete (ferrous iron half-life < 10 seconds). These sites encompass both sides of the kinetic boundary, defining its trajectory. Abiotic iron oxidation laboratory experiments performed through 90°C further refine the abiotic boundary at all ranges of temperature where the biological process can occur. The final construction of the habitability diagram includes the two boundaries plotted in pH / temperature space, with sample sites plotted to show context. Literature reports that contain sufficient accompanying compositional data are also incorporated to provide context. Secondary habitability diagrams of ferrous iron and oxygen vs pH have also been constructed to illustrate how the concentrations of reactants influence the rate and energy yield of iron oxidation reactions. This tool for visualizing habitability helps redefine extreme environments as those near the kinetic or thermodynamic limits.

GENERATION OF ENERGY SOURCES IN ROCK-HOSTED ECOSYSTEMS

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Long title: GENERATION OF ENERGY SOURCES DURING WATER/ROCK REACTIONS: IS FE(II)-DRIVEN ELECTRON TRANSFER IMPORTANT TO SUSTAIN LIFE IN ROCK-HOSTED SYSTEMS?

Typically, the energy availability in rock-hosted ecosystems is poorly known, particularly in environments where Fe-bearing primary minerals are the source of reducing power, rather than organic carbon. To date, it has been difficult to predict or calculate the natural energy fluxes generated as fluids react with rocks within a temperature regime suitable to host in-situ life. To assess the energy availability of a rock-hosted biome, not only do the fluxes of fluid and delivery of oxidants need to be known, but the reactions that give rise to the release of bioavailable electron carriers (e.g. Fe(II), H2 or formate) also need to be effectively quantified under the prevailing environmental conditions.

How, when and where do water/rock reactions release energy sources within the subsurface? And are there critical electron-transfer mechanisms that should be considered when estimating reaction rates?

In this talk, I will discuss a spectrum of water/rock reaction experiments, field observations, and thermodynamic, spectroscopic and mechanistic studies, that all provide conflicting views on the rates at which H2 is generated in modern geological systems. There are several orders of magnitude differences in the rate of low-temperature hydrogen production that are predicted from theoretical, experimental and field studies, which directly impacts our estimates of the potential to sustain H2-driven microbial metabolisms within basalts, gabbros and peridotites. The accuracy of these estimates is highly dependent upon identifying the operative mechanisms for H2 generation (including source of the electrons) and rate-determining steps for electron-transfer.

A key question to address is the reactivity of Fe in geobiological systems. Often, the solubility and reactivity of Fe(II) within both the fluid phase and the mineral matrix is a critical factor for the generation of energy sources such as H2. However, the factors that control the release of Fe from primary minerals under in-situ conditions, and the trapping of Fe(II) into secondary phases, are not well constrained. Similarly, there is a lack of data on rate and mechanisms of electron-transfer from Fe-bearing minerals in reactions with H2O or stronger oxidants or when in direct contact with microbial cells.

Therefore, I will also present spectroscopic characterizations of experimental and subsurface peridotites that have undergone extensive low-temperature alteration -- accompanied by changes in Fe chemistry and H2 generation -- and use these observations to infer the energy-releasing reactions that commonly occur in hard-rock systems undergoing fluid flow and mineral replacement. Detailed characterization of changes in the Fe mineralogy in rock-hosted systems can have direct implications for our predictions about potential metabolisms that could be sustained during water/rock reactions, such as methanogenesis, sulfate reduction and iron reduction, and understanding the coupling between mineralogical changes and habitability.

TEMPORAL AND DEPTH-RELATED VARIABILITY OF MICROBIAL COMMUNITIES IN SOILS ALONG AN ECOSYSTEM DEVELOPMENT GRADIENT

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During long-term ecosystem development, soil nutrient contents and mineralogical properties change, therefore probably altering microbial community composition. Vice versa, microbial communities play a key role in soil formation processes and nutrient cycling. While many studies focus on topsoil environments, patterns in communities of the nutrient-poor subsoil remain poorly understood despite the importance of subsoils in soil ecosystem functioning, e.g. as carbon storage. The aim of this study was to analyze the variability of microbial communities along soil development and depth gradients and further to identify the physicochemical and mineralogical factors that shape the community composition.

To study microbial long-term patterns we sampled whole soil profiles up to one meter depth along the 120,000 year old Franz Josef chronosequence in New Zealand. This soil chronosequence developed from initial states with sparse vegetation to a rainforest with high productivity and then with ongoing retrogression to an extreme phosphorus-limited stage. Microbial community composition was determined for selected samples from mainly subsoil horizons by tag-encoded pyrosequencing of bacterial and archaeal 16S rRNA genes. Further, the abundance of archaea and bacteria was estimated by quantitative PCR.

Along the ecosystem development gradient, microbial diversity was highest at young to intermediate-aged soils (500 - 12,000 years) and declined at the oldest stage (120,000 years). We found distinct depth-related archaeal communities in organic and mineral horizons and a clear shift in community composition with soil age with a dominance of the Miscellaneous Crenarchaeotic Group (MCG) at the oldest, phosphorus-limited site. This compositional shift over time was accompanied by an increasing archaea to bacteria ratio in subsoils pointing to a better adaptation of archaea to nutrient-limited conditions. Temporal changes were mainly linked to factors associated with soil development such as nitrogen and phosphorus content as well as mineralogical properties. In contrast to the archaea, bacterial community profiling revealed no overall consistent trend along the ecosystem development gradient and showed only shifts for particular taxa. However, changes in bacterial community composition seem to be related to soil horizons.

Our results indicate that also subsoil microbial communities, especially archaeal ones, show a compositional shift along an ecosystem development gradient, analogously to previously described changes for the vegetation and topsoil microbial communities. The dominance of the archaea, especially the MCG, at the extreme phosphorus limited site is in line with the detection of this group in other nutrient-poor environments e.g. the deep marine biosphere.

MICROBIAL MAINTENANCE ENERGY AS LINKED TO MICROBIAL ECOLOGICAL STRATEGIES AND ECOYSTEM FLUXES

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Microorganisms have been shown to prosper at extreme environments. They can only survive in these environments by having very low doubling rates and hence long turnover times in order to reduce their biosynthesis costs. This is only feasible by decreasing the maintenance costs to major extents. These findings once more stress the importance of considering maintenance energy a variable rather than a constant and of understanding the drivers of this variation. Previous studies that teased apart the various components contributing to maintenance energy showed that several components that will likely contribute to maintenance at high energy conditions will not be required for minimal maintenance. Dynamics in growth-related processes may explain apparent discrepancies found between the dynamics of microbial fluxes vs. microbial communities. Unfortunately, despite awareness on these issues, a fundamental understanding on which components can be downregulated or shut down is lacking. It seems clear though that the extent to which downregulation is feasible will likely relate to the ecological strategies of the species involved. Like any ecological strategy, microbial strategies will contain trade-offs and will know alternative solutions to face environmental challenges. Traits-based approaches may facilitate gaining comprehension on these strategic choices in general and on maintenance demands across species and across different environments in particular. From the limited information currently available, it is apparent that a trade-off of maintenance energy with growth yield may be a fundamental feature of microbial strategies in general. The impacts of these trade-offs on microbial kinetics are large and may fundamentally change our understanding of microbial kinetics. For instance, accounting for such trade-offs was shown to be of critical importance when quantitatively modelling soil carbon dynamics. By combining ecological concepts, kinetic models and physiological knowledge, an improved understanding may be obtained in the near future.

POTENTIAL IMPACT OF SALINITY CHANGES ON BACTERIAL ISOLATES FROM THE DEEP BIOSPHERE OF THE BALTIC SEA

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Marine subsurface sediments are inhabited by an incredibly diverse prokaryotic community (Lever, 2013). Adaptations to specific environmental conditions often only emerge by cultivation-based approaches. We have isolated new bacterial strains from sediments of the Baltic Sea sampled during IODP Exp. 347 with the aim to investigate the potential impact of salinity changes on prokaryotic and viral communities within the deep subsurface. Sediments from the Baltic Sea have undergone alterations between limnic, brackish and marine conditions due to repeated glaciations. The new isolates belong to, e.g., gammaproteobacterial *Vibrio*, *Aeromonas* and *Shewanella*, deltaproteobacterial *Desulfovibrio*, *Marinifilum* within the *Bacteriodetes* and Gram-positive *Firmicutes* including genera of *Sporomusa* and *Desulfosporosinus*. Albeit isolation of new strains was carried out by anoxic deep-agar dilution series, many strains are facultative anaerobes, similar to other cultivated bacteria from subsurface sediments (Batzke *et al.*, 2007). Most isolates grow by glucose fermentation. Some strains have been identified to possess temperate phages. The new isolates show considerable differences in their salinity tolerance with optimum growth at freshwater, brackish or marine conditions as well as narrow or broad salinity tolerances. More investigations of their physiology and temperate phages are underway. We hypothesize that salinity changes during the paleoenvironmental history of the Baltic Sea had major impacts on the structure of the microbial communities by i) influencing their energy metabolism, ii) resulting in induction of temperate phages and finally lysis of infected cells and iii) the release of labile organic compounds by the lysis of cells and thus stimulation of the remaining cells via the viral shunt.

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Lever M A, 2013. Functional gene surveys from ocean drilling expeditions – a review and perspective. FEMS Microbiology Ecology 84, 1-23.

LIMITATIONS ON HETEROTROPHIC ACTIVITY IN SUBGLACIAL LAKE WHILLANS, WEST ANTARCTICA

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Subglacial Lake Whillans (SLW) is one of more than 400 subglacial lakes that have been discovered beneath the Antarctic ice sheet over the past two decades. Taken together, these subglacial environments comprise an estimated 10^4 km^3 of liquid water, making them one of the largest unexplored habitats for life on Earth. The lakes and water saturated sediments beneath the East and West Antarctic ice sheets have been isolated from the atmosphere and from sunlight for many thousands of years. As such, organisms living in these environments must rely on inorganic substrates as energy sources, or relict organic matter. SLW lies beneath the West Antarctic Ice Sheet, in a region that has been inundated with seawater during past periods of ice sheet retreat. We used clean hot water drilling to penetrate 800 m of ice overlying SLW in 2013, retrieving the first discrete samples of water and sediment from a subglacial lake. The ~2 m deep SLW water column was characterized by temperature at the pressure freezing point (-0.49^oC), low oxygen (71 □M, ~16% of air saturation) and moderate conductivity (720 □S cm^{-1}). Inorganic N (3.3 µM), soluble reactive P (3.1 µM) and dissolved organic C (221 □M) concentrations were adequate to support microbial growth. The water contained ~10^5 cells ml^{-1}, with diverse cell morphologies present. In spite of relatively abundant nutrients, turnover times, calculated from cell-specific carbon turnover rates determined via ^{3}H-thymidine and ^{3}H-leucine incubations, were on the order of hundreds of years. Growth rates (average ~0.0043 d^{-1}) obtained from the same incubations, were at least an order of magnitude lower than those measured in Antarctic surface lakes in the McMurdo Dry Valleys and in oligotrophic areas of the ocean; coupled with low bacterial growth efficiency (8%), these data indicate that microbial populations in SLW partition a majority of their carbon demand to activities other than cellular growth. The water column and surficial sediments contained degraded, nitrogen-poor particulate organic matter (PC:PN = 65.4 and 17.9 [molar ratio], respectively; water column PN:PP = 0.78). The □^{15}N of particulate organic matter in SLW (9.9‰) was consistent with that of organic matter that has undergone diagenesis in the presence of oxygen, supporting the contention that particulate organic matter in SLW is a relict of marine productivity during past seawater incursions into the region. The high DOC:DON molar ratio (95.2) suggested that the pool of bulk dissolved organic matter may also be of low nutritional quality. Chemolithoautotrophic productivity was 32.9 ng C L^{-1} d^{-1}, indicating that contemporaneous new C production via this pathway may support a portion of the heterotrophic activity in SLW. Given the low quality of the standing pools of organic matter in SLW, the rate of organic C production by chemolithoautotrophs may limit heterotrophic growth rates. In addition to regulation by chemolithoautotrophic carbon production, experimental additions of inorganic N and P and increased temperatures stimulated heterotrophic microbial productivity (N and P = 36% increase versus control; temperature = 5% increase per ^oC increase). Collectively, our data indicate that microorganisms in SLW persist in a low-energy environment that is limited by the availability of high quality substrates for growth and

by temperature. Subglacial lakes, such as SLW, serve as important natural laboratories for physiological and biogeochemical studies of microorganisms in energy limited environments.

LIFE STRATEGIES OF BATHYARCHAEOTA IN THE SUBSURFACE

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Archaea belonging to the Miscellaneous Crenarchaeota Group (MCG) are widespread and abundant in the subsurface marine sediments, yet their phylogenetic position, life strategies and ecological roles remain speculative. MCG was recently proposed as a novel archaeal Phylum "Candidatus Bathyarchaeota", while current database for this assignment is relatively small and further conformation is still needed. Here, a total of 6 MCG genomic bins were obtained from a metagenome analysis, which provides an unprecedented opportunity to revisit the phylogeny, metabolic capabilities of this group of uncultivated archaea. A phylogenomic analyses based on 116 concatenated conserved archaeal single copy genes confirmed the placement of MCG as a distinct archaeal Phylum. Bathyarchaeota is shown to be a substrate generalist, has the capability to gain energy from a wide range of substrates such as protein, chitin, and aromatic compounds. This remarkable metabolic versatility, together with the energy saving pathways for energy production and biosynthesis, could be the successful life strategies of Bathyarchaeota in the energy starving subsurface sediments.

ISOTOPE FRACTIONATION INFORMS THE RESPIRATORY PROTEOME OF SULFATE REDUCING MICROBES

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Dissimilatory sulfate reduction is a respiratory process used by some bacteria and archaea to generate energy under anaerobic conditions. Aqueous sulfate serves as the terminal electron acceptor in this process, leading to the oxidation of organic carbon compounds and sometimes hydrogen. The abundance and stability of aqueous sulfate on modern Earth drives widespread anoxic respiratory carbon cycling by sulfate reducers throughout the marine sedimentary column, accounting for the remineralization of much of the organic carbon that reaches the seafloor. From the standpoints of both potential influence and available information dissimilatory sulfate reduction is a valuable model system for understanding microbial catabolic functioning under energy limitation.

The preferential consumption of ^{32}S-bearing sulfate during dissimilatory sulfate reduction leads to sulfate in sedimentary porewaters that is enriched in ^{34}S, and aqueous sulfide that is ^{34}S-depleted. As a result, isotopic fractionation provides a rational framework for understanding how sulfate reduction operates in energy-limited marine sediments. Through careful regulation of the environmental controls on cell-specific sulfate reduction rate (csSRR), recent culture experiments have shown a non-linear response between sulfur isotope fractionation and csSRR, with the fractionation increasing rapidly with decreasing rate. At the low-rate limit, fractionation appears to approach levels defined by thermodynamic equilibrium between aqueous sulfate and sulfide.

Structural reasons are partly behind the non-linear trajectory away from near thermodynamic fractionation as csSRRs increase. In a metabolic pathway consisting of multiple linked enzymatic reactions, the dependence of upstream reaction reversibility on downstream rates means that isotope fractionation ends up being a polynomial function of overall metabolic rate. However, even in simple single-step catabolic pathways (e.g., polysulfide reduction), experiments have shown that sulfur isotope fractionation decreases non-linearly with reduction rate. We hypothesize that a direct correlation between the abundance of respiratory enzymes and csSRR may influence the fractionation-rate relationship as well, leading to rate-independent fractionation when csSRR is high.

Incorporation of this hypothesis into a quantitative model for sulfur isotope fractionation during dissimilatory sulfate reduction leads to testable predictions. For example, the increase in respiratory enzymes with csSRR appears to be strain-specific, with thermophilic sulfate reducers requiring more enzymes to sustain a unit increase in csSRR. When the predicted enzyme-rate relationships are extrapolated to the slow csSRRs estimated from natural sediments, however, they all converge on a similar value, suggesting that there may be a base respiratory proteome that is required to sustain maintenance metabolism in energy-limited environments. Finally, calculation of the respiratory fitness landscape at low csSRR illustrates that microbial fitness in energy-limited environments is largely independent of the kinetic efficiency of key enzymes (e.g., dissimilatory sulfite reductase), implying that molecular evolution of certain functional genes may be selectively neutral.

VARIATIONS IN MICROBIAL COMMUNITY COMPOSITION IN DEEP SUBSURFACE PIEZOMETER INSTALLATIONS

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The Boom Clay layer is presently investigated as a potential host rock for geodisposal of nuclear waste in Belgium. The HADES underground research facility (EIG Euridice c/o SCK•CEN), located at 230 m depth under the site of SCK•CEN (Mol, Belgium), provides access to this clay layer for *in situ* geological, geochemical and geomicrobiological testing. In order to predict how microbiology will affect the biogeochemical processes in a disposal scenario, the resident microbial communities of Boom Clay and the man-made structures within this clay are being characterised.

In this study, water samples were collected from Boom Clay via various existing HADES piezometers. Microbial cells from these samples were concentrated on a 0.45 µm filter membrane, followed by DNA extraction, PCR amplification of the V1-V3 region of bacterial 16S rDNA, automated sequencing and an in-house developed bio-informatics pipeline. The aim was to assess differences or shared features of the microbial communities residing in piezometer boreholes, supplementary to the previous screening of a single, vertical piezometer [1], and to correlate variations to geochemical analyses.

While the boreholes of two piezometers seem highly enriched in one family of *Betaproteobacteria* (*Rhodocyclaceae*), the other piezometers seem to balance dominance between members of the *Chloroflexi, Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes* and *Chlorobi*, although in different ratios and with variations in overall diversity. Along the five piezometers, bacterial communities of the filters within one piezometer seem more similar to each other compared to those in other piezometers, despite a variety of filter materials or Boom Clay layers sampled within one piezometer.

The observed variability in bacterial community composition suggests enrichment of certain members of the community according to the engineering properties of the piezometer installation. Although such locally enriched microbial community might not be representative for the total (engineered) clay environment, it shows that **technical installations (such as piezometers) can introduce and promote local variations in the otherwise oligotrophic clay environment and the associated bioprocesses**.

Further studies of other piezometers and of clay samples are needed, to pinpoint the source bacterial community underlying *in situ* enrichment, to unravel the mechanism that shapes such microbial community in different repository conditions and to outline the relevance of the (dominant) microbial classes in defining borehole water (and gas) chemistry.

[1] Wouters K, Moors H, Boven P & Leys N (2013) Evidence and characteristics of a diverse and metabolically active microbial community in deep subsurface clay borehole water. *FEMS Microbiol Ecol*. 86: 458-473.

OBSERVATION OF POLYPHOSPHATE GRANULES IN CABLE BACTERIA

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Cable bacteria are long filamentous bacteria that capable for long distance electron transport: transporting electrons derived from oxidizing sulfide in anoxic layers, to oxygen at the sediment surface, over a distance of centimeters. Cable bacteria are found in many types of freshwater and marine sediment, including seafloor, all over the world, with density of approximately thousands of kilometers per square meter. These long filaments are composed by individual cells closely related to Desulfobulbaceae, connected with a shared outer membrane inside which the strings structure are presumed to be highly conductive. The observed doubling time of cells within the filament is about 20 hours, which is among the shortest compare to other bacteria. The amount of energy associated with the fast growth of the cables is still unknown. However, we constantly observed polyphosphate granules (poly-P), a potential energy source, in cable bacterial cells, regardless of cell dimension and shape. This is very interesting since it has long been recognized that the microbial polyP content is low during rapid growth and increases under unfavorable conditions, for example, increasing sulfide concentration and anoxia resulted in a decomposition of poly-P in Beggiatoa. Here, we investigated marine cable bacteria from Netherland and Aarhus Bay, focusing on the poly-P dynamics under various redox conditions. In toluidine blue stained cells, typically there are two big poly-P granules locate at each polar. In dividing cells, however, the morphology of poly-P changed to six small granules precisely arranged to two row. Moreover, the cells seem be able to continuously divide more than one time without elongating themselves. These varied poly-P morphologies demonstrate that poly-P is closely related to the cell growth and cell division, by an unknown mechanism. Individual cable filaments were picked up and were exposed to different redox conditions; our primary data indicated the cable cells could suffer anoxic condition better than oxic condition. We also detected decomposition of poly-P under anoxia. These results call for an in-depth examination for the function of the poly-P granules inside cable bacteria, and indicate poly-P may reflect different physiological and environmental conditions rather than genetic differences.

ENERGETICS OF NITRIFIERS IN OXYGENATED DEEP-SEA SEDIMENTS

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Nitrifiers are omnipresent in marine sediments where they play an important role in regulating the global nitrogen cycling; however, the bioenergetics and physiological status of this functional group are not well constrained. We calculated the catabolic power production and estimated the single-cell maintenance power requirements of nitrifiers in oxygenated subseafloor sediments based on two oligotrophic sediment columns from the Mid-Atlantic Ridge (North Pond, IODP Expedition 336), and two mesotrophic sediment cores from the Arctic Mid-Ocean Ridge (AMOR). Geochemical profiles of oxygen, ammonium, and nitrate were used to calculate the reaction rates and Gibbs free energy of nitrification. Further, nitrifier abundances were estimated by quantitative PCR based on their diagnostic functional genes (archaeal and bacterial *amoA* genes). Our results shows that rates of nitrification and abundances of nitrifiers are two to three orders of magnitude lower in North Pond than AMOR, consistent with the deeper oxygen penetration for North Pond (22- 30 meters below seafloor, mbsf) than AMOR (1 -2 mbsf), due to the lower organic content. Similarly, the catabolic power produced by nitrifiers, given as the product of reaction rate and Gibbs free energy per reaction (LaRowe and Amend, 2015), varied between 3 – 100 fW cm^{-3} for North Pond, and 910 – 5600 fW cm^{-3} for AMOR. Previous estimations have identified the nitrifying population at both sites as in maintenance state. This catabolic power combined with the abundances of nitrifiers (assuming all nitrifiers are equally active), suggests that the single-cell maintenance energy requirements of nitrifiers in the oligotrophic North Pond and the mesotrophic AMOR overlap, varying in the range of 10^{-5} – 10^{-2} fW $cell^{-1}$. Importantly, these values are three to five orders of magnitude lower than the requirements obtained for nitrifiers under laboratory condition [28 fW $cell^{-1}$, (Tijhuis et al., 1993)]. These findings suggest that nitrifiers in deep subseafloor sediments at different regions probably are in the same basal energetic state and sustained by a much lower power supply than previously appreciated.

LaRowe DE, Amend JP (2015). *Am. J. Sci.* **315**:167-203.

Tijhuis L, van Loosdrecht MCM, Heijnen JJ (1993). *Biotechnol. Bioeng.* **42**: 509-519.

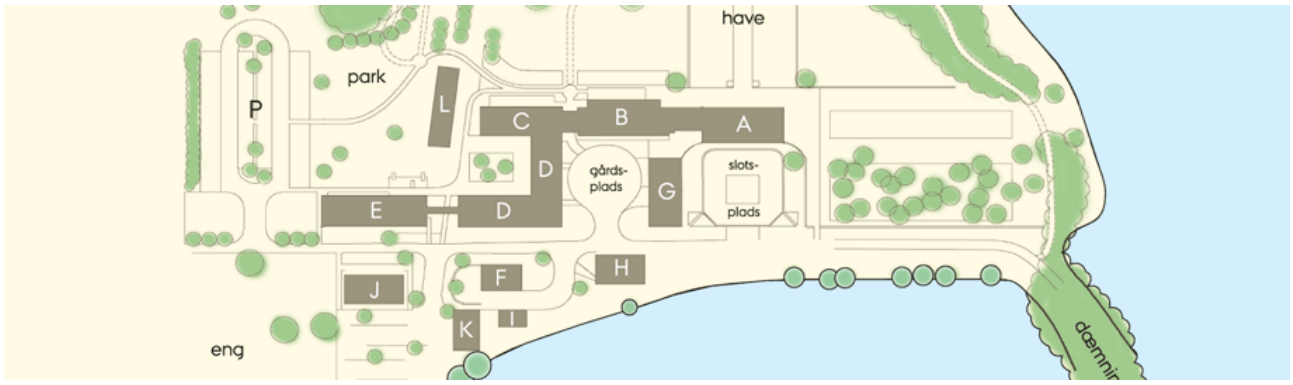
Welcome to the workshop

Registration and information during the workshop

Registration, will take place in the reception which is situated between the Stable (C) and the restaurant Magasinet (B), lectures will take place in the Auditorium in the Stable (C) and poster sessions will take place in the Distillery (H). The registration desk is open Monday 15:00-18:00.

Breakfasts, lunches, dinners, mixer and Workshop dinner will all be served in buffet-style in Magasinet (B). Participants are expected to find their own way to the dining room. Cultural evening dinner will be served at Sonderborg Castle in the Great Hall's eastern antechamber, Sønderbro 1, 6400 Sønderborg. Participants will be transported by bus to the Castle.

Working group sessions will take place in the meeting rooms at the Manor House (A), The Tenants farmer's House (G) and the Medow House (J). Participants will be guided to these rooms.



Overview of Meeting Rooms & Conference Rooms at Sandbjerg Manor

Information for speakers

There will be 30 minutes available for invited speakers and 20 minutes for other speakers. Please note that this allotted time encompasses presentation, questions and time for change-over between speakers. It is therefore recommended that speakers target their presentations to be 25 minutes for invited presentations and 15 minutes for contributed presentations. Please give your PowerPoint presentation on USB memory stick to André Pellerin (PC-users) or Angeliki Marietou (Mac-users) at the latest in the morning before your presentation. Angeliki and André will be ready in the lecture room from 8:00 a.m. and also during the last half hour of the lunch break. A PC and a Mac laptop will be available. You may also connect your own laptop to the projector via a serial interface. If you have a video presentation, please inform Angeliki or André well ahead of time.

Information for poster presentations

Poster sessions will take place in The Distillery (H). Posters should be mounted as soon as possible after arrival. There is no particular order or numbering of the posters. Poster pins are available in

the poster room. All posters will be displayed throughout the workshop and poster sessions will take place in The Distillery (H) where the presenters should be available at the posters.

WiFi

There is free WiFi access at Sandbjerg Manor. Please note there is limited WiFi access in the basement of the Manor House.

Banks

Banks are open from 09:30 to 16:00 on weekdays with late hours until 18:00 on Thursdays (closed Saturdays and Sundays). Most banks have ATMs outside their building. There are bank ATMs at Copenhagen and Billund Airports and in Sonderborg. There is no ATM at Sandbjerg Manor.

Currency

The currency in Denmark is the Danish Krone (DKK). One Krone is divided into 100 Øre.
Rates 1 September, 2015: 100.00 USD = 667.06 DKK. 100.00 EUR = 746.11 DKK.

Credit Cards

In most places VISA, Eurocard and Mastercard are accepted. If you got American Express we recommend that you withdraw cash as only a few accept this credit card. E.g. the Danish Railway systems and most restaurants do not accept it.

Emergency phone number

(+45 only when calling from outside Denmark)
Police, fire, ambulance: 112
Falck rescue services: (+45) 70 10 20 30
Police: (+45) 114
Doctor, outside normal working hours: (+45) 70 11 07 07.
Emergency room at Sonderborg Hospital: (+45) 7418 2500 Sydvang 1, 6400 Sonderborg. To get treatment you need referral from a Doctor (+45) 70 11 07 07.
Dentist, outside normal working hours: (+45) 6541 4551.
Pharmacy, outside normal hours: (+45) 7442 3502 (Jernbane Apoteket: Jernbanegade 10, 6400 Sønderborg) or 7442 2000 (Løve Apoteket, Grundtvigs Alle 179, 6400 Sønderborg).

Electricity

Denmark, like most other European countries, has 220-volt AC, 50Hz current and uses two-pin continental plugs. If you visit from the UK and Ireland, you will need an adaptor for electric

appliances, whereas North Americans need a transformer in order to use their 110/125V appliances. Plug and socket types.

Language

The mother tongue in Denmark is Danish, which is closely related to both Swedish and Norwegian. In general Danes speak English very well, and some also speak German and/or French.

Time Zone
Denmark follows Central European Time (CET) which is one hour ahead of Greenwich Mean Time (GMT) and six hours ahead of US Eastern Standard Time (EST).

Tipping

Tipping is appreciated, but not expected, and you should only do so if you feel you are getting exceptionally good service. If you do so 10 percent of the bill is sufficient. Tipping is included in taxi fares.

Drinking water

In Denmark you can drink the water straight from the tap.

Beverages, tobacco and kiosk items

Beverages, tobacco and kiosk items can be purchased at the Sandbjerg Manor. Purchase of beverages is self service and can be paid directly upon purchace (cash only) or upon check out (credit card or Danish Kroner). This means participants are to register their every purchase on a list and note wheter they have paid up front or wish to bill it. Beverages are in a fridge in Magasinet (B).

Sandbjerg only accepts Danish Kroner and credit cards.

Printing

Laser printing is available in the lobby at Sandbjerg Manor for a minor fee (2 DKK).

Sponsors

The conveners express their gratitude to the following sponsors:

Aarhus University Research Foundation: The foundation's objective is to support scientific research at Aarhus University. The foundation awards grants to concrete research projects and larger multi-year projects and initiatives that strengthen research at Aarhus University

AARHUS UNIVERSITY RESEARCH FOUNDATION
AARHUS UNIVERSITETS FORSKNINGSFOND

The Center for Dark Energy Biosphere Investigations (C-DEBI) is a National Science Foundation-funded Science and Technology Center focused on the deep subseafloor biosphere. C-DEBI's mission is to explore life beneath the seafloor and make transformative discoveries that advance science, benefit society, and inspire people of all ages and origins

Center for Dark Energy Biosphere Investigations
C-DEBI

The European Research Council (ERC). The ERC is a public body for funding of scientific and technological research conducted within the European Union (EU).The main goal of the ERC is to encourage high quality research in Europe through competitive funding.

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European Research Council
Established by the European Commission
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The Danish National Research Foundation (DNRF). The DNRF is an independent organization with the objective to promote and stimulate basic research. The Center of Excellence program is the main funding objective.

Danmarks Grundforskningsfond
Danish National Research Foundation

The Center for Geomicrobiology (CfG). The CfG at Aarhus University is co-financed by the DNRF and the ERC. The Center is the main organizer of the workshop and is also an important sponsor.

Center for Geomicrobiology

Welcome to Sandbjerg Manor

Sandbjerg Castle is situated 5 kilometres north of present day Sonderborg and can be traced back to the 16th century. Around 1500, Sandbjerg Manor is mentioned for the first time, and in 1564 King Frederik II transferred one third of the royal share of the duchies to his brother Duke Hans the Younger (1545–1622), who thereby came into the possession of the islands of Ærø and Als and the Sundeved peninsula in the duchy of Schleswig. Towards Alssund the duke had a dike built that still exists.

The Sandbjerg Estate is situated in a scenic area directly to Alssund (Als Sound) about 7 km northwest of Sonderborg

In 1788, Conrad Georg Reventlow (1644–1708) Prefect – later Chancellor in Haderslev, had a mansion built on the headland facing Alssund. The builder was Christian August Bohlsmann from Sønderborg. The Mansion together with the Tenant's Farmer House, which was erected in 1783, the other utility buildings and the park constitute a total complex between Møllesøen and Alssund – the present-day Sandbjerg Estate.

The Manor House

The Reventlow family owned Sandbjerg right up until 1930. For a number of years in the 1850s, the Mansion was the honorary residence of General Frederik Bülow, victorious at the Battle of Fredericia in 1849. He died at Sandbjerg and lies buried at Dybbøl Cemetery.

During the war of 1864, the Germans bivouacked in Storskoven wood – the Danish army was on the other side of Alssund. On the morning of 28 June, the great clock at Sandbjerg gave the signal at 2 o'clock for the invasion. Conservation boat runways and canon emplacements can still be seen along the coast, and there are also remains of column markers in the oldest trees.

Sandbjerg Manor today

After the death of the last Reventlow in 1929, Sandbjerg was sold to the Copenhagen barrister Knud Dahl and his wife, Ellen Dahl, née Dinesen - sister to the Danish author Karen Blixen. In 1954 she donated the estate to Aarhus University and after her death in 1959, Aarhus University took over the full rights of the entire Sandbjerg Estate.

The Manor House today

Sønderborg

Sonderborg (in Danish Sønderborg) is an old city with a population of around 30.000. It is located in Southern Jutland close to the German border and it has been at the center of many important events in Denmark’s history.

Old Sonderborg

The city of Sonderborg was founded in 1256 and is situated on both sides of Alssund (Als Sound). Sonderborg developed around Søndre Castle, whose name was later changed to Sønderborg Castle. Sønderborg Castle was founded a few years before 1200 and became one of the kingdom's strongest castles.

Sonderborg Castle and the royal Danish ship "Dannebrog"

One of the most famous battles of Danish history is the Battle of Dybbøl, which took place in 1864 just outside Sonderborg. In the aftermath of the war, Denmark lost Southern Jutland to Germany, who occupied the area for 56 years. After the 1st World War it was decided that the occupied territory of Southern Jutland should be returned to the Danish Kingdom. This happened in June 1920.

Sønderborg today

Nowadays the Sonderborg area houses a dynamic business environment within the field of high technology, machinery, food and textile industries and has many educational institutions including the University of Southern Denmark, Sonderborg Sports College, School of Fine Arts and the School of Nursing. The city is also home to the Southern Jutland Symphony Orchestra. Sonderborg is well-known for the castle and the recurring "Tilting at the Ring" – a peerless summer feast that lasts for several days. To many visitors it is a green holiday island, with a coastline perfect for swimming, sailing and fishing.

Sonderborg Marina

Historical Tilting at the Ring at Sonderborg Castle

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