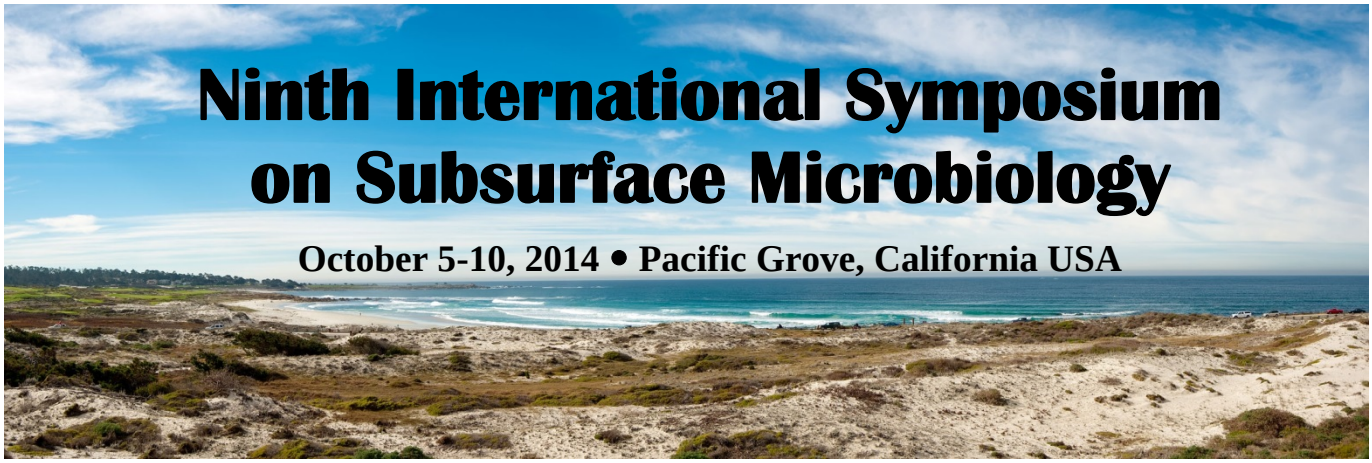


# Friday, October 10, 2014

## ORAL PRESENTATION ABSTRACTS

### ORAL PRESENTATION SCHEDULE

| | | |
|---|---|---|
| 8:30 am – 10:00 am | **Plenary Session #4** | Merrill Hall |
| 10:20 am – 12:00 pm | **Session #10** | |
| | A10. Ecology | Merrill Hall |
| | B10. Contaminants | Fred Farr Forum |
| | C10. Methods | Nautilus Room |
| 1:00 pm – 1:30 pm | **Closing Presentation** | Merrill Hall |

**8:30 AM – 10:00 AM**
**MERRILL HALL**

**PLENARY SESSION**
Moderated by **Menu Leddy,** Principal Scientist, Orange County Water District, USA

**Cultivable and 16S rDNA Diversity of Archaea and Bacteria over Depth, Sample Site, and Time in Hard Rock Aquifers**
Presented by Karsten Pedersen, Ph.D., Professor, Microbial Analytics Sweden AB, Sweden
Co-Authors: K. Pedersen, A. Bengtsson, J. Edlund, L. Eriksson, L. Hallbeck, L. Johansson, and L. Rabe

Microbiology cultivation, biomass and 16S rDNA data of planktonic and biofilm communities were assembled since 1999 until present from >200 discrete aquifers distributed over 42 to 1116 m depth in Sweden and Finland. Biomass was determined by counting total numbers of microbial cells (TNC) and concentrations of ATP and aerobic cultivation comprised aerobic plate counts. Anaerobic most probable number cultivation was used to determine nitrate-, iron-, manganese-, and sulfate-reducing bacteria (SRB), acetogenic bacteria, and methanogens. Cloning followed by Sanger sequencing and high throughput sequencing platforms 454 pyrotag and Illumina paired end sequencing explored 16S rDNA. These methods focus on different characters of microbial communities; TNC analyses whole cells with a microscope, ATP analyses a cell component with a biochemical method, MPN is based on cultivation and DNA sequencing shows community composition. Diversity expressed as cultivability and 16S rDNA generally varied over depth and sample sites. Repeated sampling over time showed site-dependent, reproducible diversity that for some sampled sites was sustained for more than 10 years. In Finland, it was found that cultivability was positively correlated with the presence of methane and sulfate, possibly with dissolved hydrogen gas nested within methane; in the absence of one of these groundwater components, numbers of cultivable microorganisms, in particular SRB, diminished. However, molecular diversity did not diminish, pointing at the need for other cultivation procedures at depth where sulfate concentration is below detection. Archaea sequences, representative for methane producing microorganisms were detected and sequences that have been identified as belonging to anaerobic methane-oxidizing consortia were also found.

**Scale Challenges in Utilization of Microbial Metabolic Network Models in Application-Scale Simulations**
Presented by Timothy D. Scheibe, Ph.D., Staff Scientist, Pacific Northwest National Laboratory, USA
Co-Authors: T.D. Scheibe, G.D. Tartakovsky, A.M. Tartakovsky, Y. Fang, R. Mahadevan, and D.R. Lovely

Advances in microbial metabolic network models enable high fidelity representation of functional responses of microbial communities to environmental stressors in process-based simulators. However, a critical challenge is the fact that the environment that microorganisms sense is very small (and heterogeneous) relative to the typical resolution of applied simulations. We have performed pore-scale simulations of fluid flow and solute transport to define dynamic local environmental conditions,

coupled to a genome-scale metabolic model of an iron-reducing bacterium based on solid-phase iron reduction coupled to oxidation of a soluble electron donor. We have used our pore-scale model to explore the relationship between genome-scale metabolic models and conventional Monod-type formulations, and to assess the manifestation of pore-scale variability (microenvironments) in terms of apparent Darcy-scale microbial reaction rates. When the fundamental metabolic network model was applied to averaged concentrations, the model over-predicted the rates of biomass growth and iron and acetate utilization. This discrepancy is caused by an inherent assumption of complete mixing over the Darcy-scale domain, which is clearly violated in the pore-scale models. These results help to explain the need to modify flux constraint parameters in order to match observations in previous applications of the genome-scale model at larger scales. These results also motivate further investigation of quantitative multi-scale relationships between fundamental behavior at the pore scale (where genome-scale models are appropriately applied) and observed behavior at larger scales (where predictions of reactive transport phenomena are needed).

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**10:20 AM – 12:00 PM**
**MERRILL HALL**

**SESSION A10 ECOLOGY: AQUIFER ECOLOGY**
Moderated by:

- **Murray Close,** Principal Scientist, Institute of Environmental Science and Research, Ltd. (ESR), New Zealand
- **Dr. Christian Griebler,** Institute of Groundwater Ecology, Helmholtz Zentrum München, Germany

**Groundwater Biofilm Resilience to Desiccation in an Alluvial Gravel Aquifer**
Presented by Murray Close, Principal Scientist, Institute of Environmental Science and Research, Ltd. (ESR), New Zealand
Co-Authors: M. Close, L. Weaver, A. Hickson, J. Webber, and P. Abraham

Biofilms are the foundation for groundwater ecosystems. This study investigates the effects of a dry period on biofilm biomass and activity, to mimic the effects of fluctuating groundwater levels. Groundwater levels fluctuate naturally due to the seasonal variation of recharge (which occurs mostly during winter), but the range of fluctuations is likely to increase with the increasing development of irrigation from groundwater sources. Gravel samples were placed in wells in an oligotrophic alluvial gravel aquifer in Central Canterbury, New Zealand and in laboratory biofilm growth chambers and left to allow biofilms to establish for at least 5 months. Some of the samples were then lifted and suspended above the water table (in the field) or the growth chambers were drained (in the laboratory). The dry period chosen was 4 months; at the end of this period the samples were rehydrated. Samples were analysed before desiccation, after 4 months' desiccation, 1 month following rehydration, and 3 months following rehydration. Biofilm responses were assessed using biomass assays and enzyme assays for carbon turnover, phosphate metabolism, generic esterase activity and bacterial respiration. Analysis of the biomass and activity data indicates that the biofilm communities are fairly resilient to periods of 4 months' desiccation. Some of this resilience would be due to the relatively high and stable humidity just above the groundwater table, which is in marked contrast to the low humidity, high sunlight environment that often characterises a dry period for a surface water environment.

**The Distributional Structure of Microbial Communities in a South Australian Aquifer**
Presented by James G. Mitchell, Ph.D., Professor, School of Biological Sciences, Flinders University, Australia
Co-Authors: J.G. Mitchell, B. Roudnew, R. Smith, T. Lavery, T. Jeffries, and J. Seymour

Subsurface geological structures can create hydrologically distinct aquifer layers with different water exchange and community compositions. This heterogeneity likely drives changes in groundwater microbiology. Here we used flow cytometry to examine the microbial diversity in vertically stratified aquifer layers consisting of an unconfined aquifer, a confining layer and a confined aquifer. Microbial sup-populations were cytometrically defined with 1 to 4 bacterial and 1 to 3 viral sub-populations. Despite the total microbial abundances remaining constant, the viral community composition varied among aquifer layers. Aquitard viral communities were particularly abundant, which may reflect bacterial exclusion. The results suggest that aquitards may act as viral refugia. In general, the results indicate complexity and segregation in microbial communities in discrete aquifer layers that may be overlooked when reporting general abundances. Groundwater bacteria and viruses appear to be sensitive indicators of the biological dynamics of aquifer systems.

**Ammonia-Oxidizing Thaumarchaeota Form a Large Fraction of the Archaeal Community in Deep Pristine Limestone Aquifers**
Presented by Dr. Cassandre Lazar, Aquatic Geomicrobiology Department, Institute of Ecology, Friedrich Schiller University Jena, Germany
Co-Authors: C. Lazar, M. Herrmann, S. Opitz, P. Lange, K.-U. Totsche, and K. Küsel

Archaea constitute a considerable fraction of the microbial communities in subsurface ecosystems, however, their role in biogeochemical cycles especially in aquifers is still poorly understood. In this study we investigated archaeal communities in two superimposed aquifers which differed strongly in oxygen availability. Samples were obtained from eight groundwater wells ranging from 12 to 88 m depth in the Hainich-Dün region (Thuringia, Germany). The aims of this study were (i) to compare the structure of total and metabolically active archaeal communities between the upper, oxygen-deficient aquifer and the lower, oxygen-rich aquifer, (ii) to follow fluctuations in the abundance of archaea over a period of three years, and (iii) to assess the relevance of ammonia-oxidizing archaea targeting the amoA gene encoding ammonia mono-oxygenase. Quantitative PCR of bacterial and archaeal 16S rRNA genes revealed that archaea accounted for up to 9 % of the groundwater microbiota. In a DNA-based analysis, 66 to 99 % of the archaeal 16S rRNA gene sequence reads were related to the ammonia oxidizing thaumarchaeon Candidatus Nitrosopumilus koreensis, which was supported by high archaeal amoA/16S rRNA gene ratios. However, analysis of the metabolically active archaeal communities on the RNA level showed equal fractions of these Thaumarchaeota and of crenarchaeotal and euryarchaeotal groups only distantly related to cultured representatives. Our results clearly demonstrated that ammonia oxidation could be an important metabolism of Archaea in deep limestone aquifers and could also make a considerable contribution to autotrophic archaeal CO2-fixation. The key metabolisms of the observed uncultured Archaea remain to be elucidated.

**Effect of Aquifer Recharge on the Taxonomic Composition of Endemic Microbial Communities**
Presented by James S. Paterson, Postdoctoral Research Fellow, School of Biological Sciences, Flinders University, Australia
Co-Authors: J.S. Paterson, R.J. Smith, C.A. Sibley, J.L. Hutson, and J.G. Mitchell

Drought events and the overexploitation of freshwater resources have led to the increased need to manage groundwater reserves. Aquifer storage and recovery (ASR), whereby artificial water is injected into aquifers for storage is one of the proposed methods by which fresh water supplies can be increased. Microbial clogging following injection however is a major issue. Here, we utilised modern sequencing protocols to investigate microbial community dynamics in laboratory experiments before and after the addition of synthetic wastewater. A fundamental shift in taxa was seen with an overrepresentation of Sphingomonadales, Sphingobacteriales, Rhodospirillales, Caulobacterales, Legionellales, Bacillales, Fusobacteriales and Verrucomicrobiales in groundwater prior to the addition of synthetic wastewater. Following the addition of synthetic wastewater Burkholderiales, Actinomycetales, Pseudomonadales, Xanthomonadales, Rhodobacterales, Thizobiales and Thiotrichales were overrepresented in the groundwater. Furthermore, a significant difference between the groundwater samples before and after the addition of synthetic wastewater was observed, with water samples exhibiting more similarity to sediment samples. Together, these results suggest that the introduction of wastewater in ASR practices can fundamentally alter the taxonomic composition of the endemic microbial communities

**In Situ Extraction of Microorganisms from Aquifer Sediments**

Presented by Martin H. Schroth, Ph.D., Professor, Institute of Biogeochemistry and Pollutant Dynamics, ETH Zürich, Switzerland
Co-Authors: M.H. Schroth, F. Ugolini, R. Henneberger, H. Bürgmann, and J. Zeyer

Sampling methods for characterization of microbial communities in aquifers should target both suspended and attached microorganisms (biofilms). We conducted laboratory and field experiments to develop an alternative sampling method to obtain more representative samples of biofilms in groundwater, reducing the need for coring/drilling, which is often time-consuming and expensive. We initially assessed the feasibility of chemical (using different detachment-promoting agents as eluent) and physical (using shear, sonication, and heat) extraction of microorganisms from water-saturated, packed sediment columns containing established biofilms. Effluent cell concentrations were used as a measure of extraction efficiency; extraction quality was determined by comparing T-RFLP profiles of extracted bacterial communities with destructively sampled sediment community profiles. Results indicated that physical extraction was far more efficient in extracting microorganisms from aquifer sediments compared with chemical extraction. Specifically, sonication and heat increased the extraction efficiency up to 200-fold and yielded communities comparable to the original sediment community. In subsequent field experiments, we investigated the effectiveness and reproducibility of low-frequency (200 Hz) sonication pulses on improving in-situ extraction efficiency and quality of microorganisms in a petroleum-contaminated aquifer. Sonication pulses at different power levels (0.65, 0.9 and 1.1 kW) were applied to three different groundwater monitoring wells. Sonication enhanced the extraction efficiency up to 13-fold, with most of the biomass being associated with sediment fines extracted with groundwater. Our results indicate that low-frequency sonication may be a viable and cost-effective tool to improve the extraction of microorganisms from aquifers, taking advantage of existing groundwater monitoring wells.

**10:20 AM – 12:00 PM**
**FRED FARR FORUM**

**SESSION B10 CONTAMINANTS: COAL AND BITUMEN**
Moderated by:

- **Tamara N. Nazina, Ph.D.,** Head of Laboratory, Winogradsky Institute of Microbiology, Russian Academy of Sciences, Russia
- **Joseph M. Suflita, Ph.D.,** Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA

**Stimulation of Methane Production in a Gas-Free Coal Seam**
Presented by Mike Manefield, Ph.D., Associate Professor, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Australia
Co-Authors: S. Beckmann, L. Koop, T. Thomas, H. Hazrin-Chong, J. Webster, M. Lee, and M. Manefield

Coal seam gas represents a valuable energy source globally and substantial portions of existing coal seam gas resources are of biogenic origin. We have tested biostimulation and bioaugmentation options for accelerating methane production in situ in a non-gassy subbituminous coal seam.
Five wells extending 80 m below ground into a 3 m thick coal seam were used to assess three treatment strategies. Firstly, ammonium chloride and potassium phosphate were applied to formation water in contact with the seam. Secondly, calcium peroxide (slow oxygen release) was provided in addition to the mineral nutrient formulation. Thirdly, a coal fed methanogenic enrichment culture was added. A negative control well was left untreated and a positive control well was amended with sodium acetate. Methane production was monitored monthly for one year along with microbial community composition, cell abundance, anions, cations, VFAs, pH and ORP.
Despite methanogens being present in formation water, methane production was not observed in the untreated well. Methane production was observed in the nutrient amended wells with calcium peroxide, bioaugmentation and acetate amendments increasing methane yield by 1, 2 and 3 orders of magnitude respectively, despite high background sulfate concentrations. Methane production was acetoclastic based isotope ratios. Methanosarcina lineages are likely largely responsible for the methane production observed. Desulfovibrio, Geobacter, Clostridium and Ignavibacterium lineages were also enriched.

**Enhanced In Situ Biogas Production from Coal Using Semi-Conductive Neutral Red Crystals**
Presented by Sabrina Beckmann, Ph.D., Research Fellow, School of Biotechnology and Biomolecular Sciences, University of New South Wales, Australia
Co-Authors: S. Beckmann, C. Welte, and M. Manefield

Biologically produced combustible gas (biogas) has a large role to play in Australia's future energy security. Australian coal deposits have a large potential for methane generation via microbial biogasification. In our project we enhanced microbial methane formation from coal-associated ground waters through the application of the synthetic phenazine neutral red. The amendment was conducted in 80 m deep wells penetrating a coal seam in Australia and performed in triplicate. The synthetic phenazine neutral red (2-amino-8dimethylamino-3-methylphenazine) is a redox active dye shown to

stimulate methane production in bioelectrochemical systems. Recently, we discovered that 250-500 μM neutral red substantially increases methane production from anaerobic coal-fed cultures through the formation of self-assembling neutral-red needle like structures. Our studies proved that these flexible neutral red structures are crystalline with a length of 100-1500 μm and a width of 1-5 μm. Furthermore, we showed that they act as organic semiconductors mediating the electron transfer and enhancing the conversion of carbonaceous material to methane by rewiring the electron flow in favor of biogas producing microbial communities. In vitro membrane test showed that reduced neutral red donates electrons into the respiratory chain of Methanosarcina mazei. In situ methanogenesis outperformed other enhancement methods shown in the past as well as the stimulation of biomass production of methanogenic archaea. Furthermore, bacterial representatives of the coal-associated groundwater community related to Rhizobium, Shewanella, Hydrogenophaga, Clostridium, Geosporobacter, and Geobacter species were stimulated whilst the growth of sulfate-reducing bacteria was inhibited favoring methane production.

**Mining of Coal and Methane: Integration of Chemistry and Microbiology**

Presented by Joseph M. Suflita, Ph.D., Professor, Department of Microbiology and Plant Biology, University of Oklahoma, USA
Co-Authors: J.M. Suflita, C. Lyles, and M. Mendivelso Castro

Economic prosperity and national security depend on how societies manage a myriad of energy challenges. Minimally, societies will strive to exploit subsurface energy reserves in a more environmentally conservative manner. Coalbed methane is an increasingly important global energy resource, but the catalytic mechanisms involved in this bioconversion, the responsible microorganisms and the environmental conditions governing this process are poorly understood. The potential for modern coalfield methanogenesis was assessed using formation water from a variety of basins as inocula for nutrient-replete incubations amended with C1-C5 fatty acids as presumed intermediates of anaerobic coal biodegradation. Instead of the expected rapid mineralization of these substrates, methanogenesis was inordinately slow (~1 μmol•d-1), following long lag periods (>100d), and methane yields typically did not reach stoichiometrically expected levels. We hypothesized that the major factor limiting the conversion of coal to methane is the recalcitrant nature of coal organic matter. When ozone was used to partially oxidize coal, it resulted in the formation of an acidic aqueous effluent that was far more amenable to methanogenesis than the particulate coal – either before or after ozonolysis. The measured rates were at least 2 orders of magnitude greater than solid coal samples incubated with the same inocula and far greater than coalbed methane production rates previously reported for bituminous coal formations. These findings suggest that major advances in energy recovery processes will involve an integration of chemical oxidation mechanisms followed by anaerobic digestion of the resulting products

**Shifts in Native Powder River Basin Microbial Communities Associated with Recharge Nutrients and Stimulated Coalbed Methane Production**

Presented by Elliott P. Barnhart, Ph.D., Microbiologist, Wyoming-Montana Water Science Center, U.S. Geological Survey, USA
Co-Authors: E.P. Barnhart, B.D. Ramsay, K. Bowen De León, K.A. Brileya, D.M. Akob, R.E. Macur, A.B. Cunningham, and M.W. Fields

Understanding in situ microbial responses to nutrient additions within coal beds has become increasingly important with the growing interest in enhancing coalbed methane (CBM) production. Nutrient additions in the form of yeast extract and several individual components of yeast extract

(proteins and amino acids) were added to microcosms containing native microorganisms from Powder River Basin (PRB) coal beds. CBM production more than doubled when these components were added to coal-containing microcosms. Microbial populations capable of hydrogen production/utilization were detected in situ and under non-stimulated conditions in microcosms. Stimulation with yeast extract caused a shift in the detectible community to a higher percentage of acetoclastic methanogens and acetogenic bacteria. Methane isotope analysis from CBM production wells has indicated a similar microbial community shift as observed in stimulation experiments. Hydrogenotrophic methanogenesis signatures dominated most PRB coalbeds but the signature shifted to acetoclastic methanogenesis near major groundwater recharge areas. In conjunction, a high proportion of cyanobacterial and algal SSU rRNA gene sequences were detected in a CBM well within a major recharge area, suggesting that these phototrophic organisms naturally stimulate acetoclastic methane production. In laboratory studies, adding phototrophic (algal) biomass stimulated CBM production by PRB microorganisms similarly to yeast extract (~30 µmol methane increase per gram of coal). These results provide insight into the microbial community shifts that occur when nutrients are added to coal beds, as well as the processes that may naturally stimulate CBM production in the PRB.

**Anaerobic Microbial Communities and Their Potential for Bioenergy Production in Heavily Biodegraded Crude Oil Reservoirs**

Presented by Julia Rosa de Rezende, Ph.D., Research Associate, School of Civil Engineering and Geosciences, Newcastle University, United Kingdom
Co-Authors: J.R. de Rezende, A. Sherry, T. Korin, I.M. Head, A. Grigoryan, G. Voordouw, and C.R.J. Hubert

Subsurface oil reservoirs are exceptional deep biosphere environments in terms of organic matter content and composition. Most of the oil in low temperature, non-uplifted reservoirs is biodegraded due to millions of years of microbial activity. To assess the potential of heavy oil for bioenergy production through methanogenesis, we combined basal water and surface-mined bitumen from an Athabasca oil sands reservoir (Alberta, Canada) in microcosms that were incubated for 3000 days under different redox conditions, with bitumen as the only organic substrate. Maximal rates of methanogenesis were below 15 nmol/day/g oil sands. This is 10 to 1000x lower than other published reports of methanogenesis from lighter crude oils. Methanogenesis was eventually observed in microcosms originally set up under sulfate- or oxygen-reducing conditions suggesting that these electron acceptors were consumed first. Interestingly, in sulfate-reducing microcosms methanogenesis took place in the presence of >20 mM sulfate that had not been removed. No methanogenesis was observed in nitrate-reducing microcosms. Ion torrent sequencing of 16S rRNA genes in the 3000-day microcosms revealed significant differences in community composition between methanogenic and non-methanogenic microcosms. The presence and high relative abundance of Methanosarcinaceae, Syntrophaceae, Desulfobulbaceae, Desulfovibrionaceae and Geobacteraceae in libraries from methanogenic microcosms point to possible syntrophic partnerships involved in methane generation from bitumen. These results demonstrate that microbial communities in Athabasca oil sands are capable of accessing a limited pool of organic carbon present in severely biodegraded heavy oil as substrates for further biodegradation resulting in methanogenesis, but that rates are relatively low.

**10:20 AM – 12:00 PM**
**NAUTILUS ROOM**

**SESSION C10 METHODS: METAGENOMICS**
Moderated by:

- **Tillmann Lueders, Ph.D.,** Group Leader, Institute of Groundwater Ecology, Helmholtz Zentrum München, Germany
- **Wilfred F.M. Röling, Ph.D**., Associate Professor of Geomicrobiology, Molecular Cell Physiology Research Group, VU University Amsterdam, The Netherlands

**Metagenomic Analysis of the Deep Subseafloor Bacterial Lineage JS1 of the Candidate Phylum "Atribacteria"**
Presented by Gordon Webster, Ph.D., Research Associate, Cardiff School of Biosciences, Cardiff University, United Kingdom
Co-Authors: G. Webster, J.A. Dodsworth, S.K. Murugapiran, N. Masaru, C. Rinke, P. Schwientek, E.A. Gies, G. Tsiamis, B.B. Jørgensen, R. Stepanauskas, W.-T. Liu, S.J. Hallam, T. Woyke, B.P. Hedlund, H. Sass, R.J. Parkes, P. Kille, and A.J. Weightman

The deep sub-seafloor biosphere is one of the largest habitats on Earth and contains a large microbial biomass. In this challenging and extreme environment, microorganisms are thought to contribute to fundamental biogeochemical processes driving organic matter and mineral transformations, elemental cycles, oceanic crust weathering and methane hydrate formation. However, very little is known about the many novel bacterial groups present due to a lack of cultivated representatives. The recently proposed candidate phylum "Atribacteria" comprises the JS1 and OP9 lineages, members of which are widely distributed in anoxic habitats. On the basis of current data, the phylotypes designated JS1 constitute the majority of atribacterial sequences and are particularly abundant in deep subsurface sediments. Using next generation sequencing we have investigated the metabolic diversity, physiology and ecology of JS1 using a combination of data sets obtained from published and recently obtained JS1 and OP9 single-cell genomes, as well as metagenomic analysis of mud volcano sediment enrichment culturess maintained on acetate and hydrogen. We have also undertaken assessment of published and newly obtained bacterial 16S rRNA gene amplicon data from a range of sedimentary habitats. Phylogenomic analyses of these combined datasets confirm the monophyly of the "Atribacteria", and 16S rRNA gene amplicon data supports previous findings that JS1 are globally distributed within marine sediments. In addition, statistical analysis (PCA) demonstrated that the abundance of JS1 correlated with methane-associated sediments. Comparison of metabolic potential, inferred from genomic datasets, indicates that some members of JS1 and OP9 are heterotrophic anaerobes, with a potential to catabolise carbohydrates, but lacking respiratory capacity.

**Assessing the Diversity of Groundwater Viral Community through Metagenomics**
Presented by Li Deng, Ph.D., Postdoctoral Researcher, Institute of Groundwater Ecology, Helmholtz Zentrum München, Germany
Co-Authors: L. Deng, R. Kallies, L. Pei, R. Niessner, C. Drosten, M. Seidel, and C. Griebler

Microbes are fundamental drivers in myriad ecosystem processes, and their viruses modulate such microbial-driven processes through mortality, horizontal gene transfer and more recently recognized broad-scale metabolic reprogramming by auxiliary metabolic genes (AMGs). However, our understanding of viral-host interactions is severely data limited and dominated by the 'unknown' as 90% of the average virome is completely novel. We adopted the recently developed protein clusters (PCs) analysis for the 1st groundwater viral metagenome. Here, we constructed 15 K high confidence PCs from 18 freshwater and hypersaline viral metagenomes. Together with 456K PCs previously generated from marine viral metagenomes, we performed large-scale comparative analyses of these PCs and two major results revealed. (i) 94% PCs in our metagenome represented dsDNA bacteriophage families of Myoviridae, Podoviridae and Siphoviridae which is contrast to the previous description of the viral composition of two groundwater microbial metagenomes (generated by particles caught on 0.22 μm filters) which were dominant by ssDNA viruses (72% and 21%). Such shift of viral taxonomy is probably due to the multiple displacement amplification (MDA) bias which was used by the latter study. (ii) Despite geographical proximity, no significant difference between freshwater (including groundwater) and marine viral assemblies were observed. The cluster richness of each viral assembly, however, was significantly different between each us with the highest diversity being observed in our groundwater sample.

**Evidence for Oxygen Ingress in Hydrocarbon Resource Environments from Metagenomic Analyses**

Presented by Gerrit Voordouw, Ph.D., Professor, Department of Biological Sciences, University of Calgary, Canada
Author: G. Voordouw

Subsurface hydrocarbon resource environments (HREs; coal beds, bitumen deposits, oil and gas reservoirs) are characterized by excess electron donors and absence of electron acceptors. As a result microbial communities in HREs are thought to be strictly anaerobic, including syntrophic bacteria and methanogenic archaea, which catalyze the water-mediated conversion of hydrocarbons to methane and CO2. If oil is produced by injection of seawater with a high sulfate concentration (~25 mM), then growth of anaerobic sulfate-reducing bacteria is also stimulated. A large 16S rRNA gene survey of microbial communities in HREs has indicated that fractions of methanogens varied from 0% in some coal bed methane samples to 69% in produced water samples from an oil reservoir. A more refined index, the R-score, which has a value R=1 for strictly anaerobic and R=-1 for strictly aerobic genera, has been defined and calculated from microbial community compositions. R-scores varied from R = -0.32 for coal bed methane to R = 0.74 for oil reservoir communities, indicating that the former were dominated by microbes that can respire air, whereas the latter were dominated by strictly anaerobic taxa. However, even the oil reservoir communities can have high fractions of Epsilonproteobacterial genera, which grow chemolithotrophically by oxidation of sulfide or sulfur with oxygen or nitrate. These bacteria use CO2 as the carbon source and their presence in oilfield environments is an enigma, unless we assume that the oil fields where these microbes are found are also subject to oxygen ingress.

**Metagenomics of Deep Terrestrial Subsurface Microbiomes: How Depth and Geological Conditions Affect the Species Diversity and Their Functions**

Presented by Merja Itävaara, Ph.D., Principal Scientist, VTT Technical Research Centre of Finland, Finland
Author: M. Itävaara

The continental earth crust contains regions of very different age and composition including crystalline rocks, metamorphic systems, sedimentary basins and organic deposits, and magmatic intrusions. Throughout the crust, fluids are the principal agents in transporting and focusing Earth's energy and mineral resources. In Finland the earth crust is around 50km deep and the bedrock is very stable crystalline bedrock which is also supposed to be safe for long term storage of nuclear wastes. Crystalline rock hosted biosphere containing bacteria, archaea, fungi and viruses is now known to extend several kilometers deep in terrestrial fracture zones in the bedrock of Finland. Comparison of several sites in the middle and western Finland demonstrate that the biodiversity vary considerably at different sites and depths of the earth crust. Hydrogen has been found at several sites and is considered as major energy source. In addition deep groundwaters may also contain considerable quantities of methane which also supports deep life by providing carbon and energy source. Our studies based on metagenomics have revealed several ongoing processes such as deep nitrogen fixation, carbon cycling and other geobiological processes occurring in deep terrestrial habitats.

**The Effects of Long-Term Exposure of Different Nitrogen Contaminants on Community Structure and Metabolism within a Cape Cod Aquifer**

Presented by Bongkeun Song, Ph.D., Assistant Professor, Virginia Institute of Marine Science, College of William & Mary, USA
Co-Authors: C. Buckner, B. Song, R. Smith, and D. Repert

Beginning in 1936, more than 8 billion gallons of treated sewage was disposed of through rapid infiltration over the course of 60 years by the Massachusetts Military Reservation (MMR). This created a contamination plume within the Cape Cod aquifer consisting of a shallow, suboxic, nitrate-containing zone and a deeper, anoxic, ammonium-containing zone. We conducted metagenomic shotgun pyrosequencing to examine the structure of two microbial communities that saw long-term exposure to either NH4+ or NO3- contaminants and to compare carbon and nitrogen metabolisms within the two communities. Metagenomic analysis revealed that both communities contained a greater abundance of Geobacter spp., Burkholderia spp., Pseudomonas spp., and "Candidatus Solibacter spp.". The NO3- community also contained greater abundance of Nitrobacter spp., while Nitrosococcus spp. and "Candidatus Kuenenia spp." were greater in the NH4+ community. Autotrophic and heterotrophic carbon metabolisms including serine-glyoxylate cycle, glycolysis, gluconeogenesis, TCA cycle, pentose phosphate pathway, and Calvin Benson cycle were in similar abundance within the two communities. For nitrogen metabolism, genes involved in NH4+ assimilation were more abundant in the NH4+ community while the genes involved in NO3- assimilation and DNRA were more abundant in the NO3- community. However, the genes involved in denitrification and nitrogen fixation were equally present within the two communities. This suggests that the long-term exposure of different N contaminants has a greater impact on nitrogen metabolisms than carbon metabolisms in aquifer microbial communities.

**1:00 PM – 2:00 PM**
**MERRILL HALL**

**CLOSING CEREMONY**

**Closing Presentation on "Subsurface Microbiology: Then and Now"**
1:00 pm – 1:30 pm
Presented by **William C. Ghiorse, Ph.D.,** Professor, Department of Microbiology, Cornell University, USA

Subsurface Microbiology has roots in shadowy human concepts of the self-purifying abilities of groundwater aquifers, which for many centuries were based on traditional assumptions with little or no knowledge of the subsurface microbes controlling them. The traditional attitudes about groundwater purity endured well into the 20$^{th}$ century. Improved sampling and microbial detection methods, introduced in the 1980s, turned a spotlight on subsurface microbes and their activities. Since then, subsurface microbiological research has been driven largely by our need to understand the microbial ecology of subsurface habitats, and the biogeochemical processes occurring in them. Our knowledge has expanded steadily for the past 35 years, prodded by environmental pollution problems associated with drinking water aquifers, the search for new sources of petroleum, exploration of subterranean ecosystems, underground nuclear waste disposal, and for life in remote and extreme environments on Earth and other worlds. The variety of specific topics presented at this symposium is testament to the continued expansion of subsurface microbiological research well into the future.