

Heather Akau¹ (530-666-8848; heather.akau@yolocounty.org)

Don Augenstein² (650-856-2850; iemdon@aol.com)

¹Yolo County Central Landfill, 292 West Beamer Street, Woodland, CA 95695, United States

²Institute for Environmental Management, 4277 Pomona Avenue, Palo Alto, CA 94306, United States

Sanitary landfilling is the dominant method of solid waste disposal in the United States, accounting for about 217 million tons of waste annually (U.S. EPA, 1997) and has more than doubled since 1960. In spite of increasing rates of reuse and recycling, population and economic growth will continue to render landfilling as an important and necessary component of solid waste management. Yolo County Department of Planning and Public Works, Division of Integrated Waste Management is demonstrating a new landfill technology called Bioreactor Landfill to better manage solid waste. In a Bioreactor Landfill, controlled quantities of liquid (leachate, groundwater, gray-water, etc.) are added and recirculated to increase the moisture content of the waste and improve waste decomposition. As demonstrated in a small-scale demonstration project at the Yolo County Central Landfill in 1995, this process significantly increases the biodegradation rate of waste and thus decreases the waste stabilization and composting time (5 to 10 years) relative to what would occur within a conventional landfill (30 to 50 years or more). When waste decomposes anaerobically (in absence of oxygen), it produces landfill gas (biogas). Biogas is primarily a mixture of methane, a potent greenhouse gas, carbon dioxide, and small amounts of Volatile Organic Compounds (VOC's) which can be recovered for electricity or other uses. Other benefits of a bioreactor landfill composting operation include increased landfill waste settlement which increases in landfill capacity and life, improved leachate chemistry, possible reduction of landfill post-closure management time, opportunity to explore decomposed waste for landfill mining, and abatement of greenhouse gases through highly efficient methane capture over a much shorter period of time than is typical of waste management through conventional landfilling. This project also investigates the aerobic decomposition of waste of 13,000 tons of waste (2.5 acre) for elimination of methane production and acceleration of waste decomposition. In the first phase of this project a 12-acre module that contains a 9.5-acre anaerobic cell and a 2.5-acre aerobic cell has been constructed and filled with over 220,000 tons of municipal solid waste. Water and leachate addition began in April 2002 and to date less than 200,000 gallons of liquid has been added to the 3.5-acre anaerobic cell. The waste filling phase of the aerobic cell was completed in June of 2002 and a 12-inch soil cover and 12-inch of greenwaste compost cover was placed on top of the cell. A vacuum will be applied to the piping within the waste to draw air through the landfill. Instrumentations have been installed to monitor the following parameters: waste temperature, moisture, leachate volumes, leachate hydraulic head over the primary liner, leachate composition, gas volumes and composition. A supervisory Control and Data Acquisition (SCADA) system has been installed to monitor and control the operation of the bioreactor cells. Waste samples were taken from each cell for laboratory testing in early June 2002.

URL: <http://www.yolocounty.org/org/PPW/diwm/bioreactor.htm>

B51A-0710 0830h INVITED POSTER

Engineered Municipal Waste Landfills: Climate Significance, Benefits, and some Landfill Geophysics

Don Augenstein¹ (650-856-2850; iemdon@aol.com)

Ramin Yazdani² (530-666-8848; Ramin.Yazdani@ccm.Yolocounty.org)

John R Benemann (925-352-3352; jbenemann@aol.com)

¹Institute for Environmental Management, 4277 Pomona Avenue, Palo Alto, CA 94306

²Yolo County Planning and Public Works, 292 West Beamer, Woodland, CA 95695

Municipal Solid Waste (MSW) landfills have unique features: Wastes worldwide emit biogenic methane to the atmosphere of magnitude comparable to the total atmospheric buildup between 1980 and 1990. Carbon sequestered in landfills is large in geologic terms

Management of decomposition in landfilled waste is desirable: (a) Control of waste decomposition and methane promises over tenfold cheaper greenhouse gas abatement compared to most other greenhouse gas abatement strategies. This is due in part to carbon sequestration and landfill gas energy offset of fossil fuel consumption (b) Landfill gas energy potential worldwide, is up to 1% of world energy. Use of landfill gas conserves a resource otherwise wasted (c) Monetary benefits of landfill life extension from decomposition and rapid volume reduction can be quite attractive This is a benefit for the US, where landfills are increasingly difficult and expensive to site. (d) Landfills

containing mixed waste can be significant sources of atmospheric and groundwater pollutants needing control. Control is possible from advancing landfill management approaches (e) The stabilization of waste lessens pollutant risk and needs for costly long-term landfill after-care.

Greater control of landfill decomposition has been advocated in the form of controlled or bioreactor landfills. (SWANA, 1999; Reinhart and Townsend, 1996). Field trials are encouraging by several environmental/monetary criteria. Control of moisture and temperature have given fivefold or more acceleration of methane generation (Augenstein et al, 1998, 2000). There has been rapid volume loss of the landfilled waste as well, with conversion of waste organics to gas. Many trials over years have shown potential for abatement of pollutants in landfill leachate. Demonstration work by the solid waste management community attests to the benefits potential.

Increasing field demonstrations, have been accompanied by observation and/or solution of several issues. As noted the heat generation in landfills may become controlling. Heat can be dissipated, but at energy and monetary cost. Increased waste liquid content, required for biological activity has been a concern. Offsetting risk is the accelerated treatment of many dissolved contaminants in landfill liquid with time. It has proven possible to manage liquid flows within environmental and regulatory constraints. There have been concerns about containment by chemosynthetic lining of leachate liquids draining from landfills. Yet molecular bonds of lining under anaerobic conditions could be expected to last for centuries (and in fact up to millennia). There is of course no landfill experience over millennia but analogous compounds of geologic relevance have shown very desirable long term stability. Two other areas being investigated are waste slope stability and the precipitation of carbonate salts

The climate significance and geophysical issues with landfills will be discussed, and some experimental findings leading to conclusions will be reviewed

B51A-0711 0830h POSTER

Two Bioreactors for Removing Methyl Bromide Following Contained Fumigations

Laurence G. Miller¹ (650-329-4475; lgmiller@usgs.gov)

Shaun M. Baesman² (650-329-4459; sbaesman@usgs.gov)

Ronald S. Oremland² (650-329-4482; roremilan@usgs.gov)

¹U.S. Geological Survey, M/S 465 345 Middlefield Rd., Menlo Park, CA 94025, United States

²U.S. Geological Survey, M/S 480 345 Middlefield Rd., Menlo Park, CA 94025, United States

The continued use of methyl bromide (MeBr) as a quarantine, commodity or structural fumigant is in question because its release to the atmosphere contributes to depletion of stratospheric ozone. However, no single alternative to the use of MeBr as a fumigant has been identified. Nonetheless, future regulation of the amount of MeBr released by structural and commodity fumigations is likely. Hence, if MeBr use is to continue, it is imperative to lower the amount released to the atmosphere by collecting the gas following fumigation for eventual recycling or destruction. We report here on two bioreactors that remove MeBr from waste air streams. The bioreactors utilize the enzymatic activity of a previously described, methylothrophic bacterium, strain IMB-1, to oxidize MeBr directly during growth. The first bioreactor, operated as a closed system, consisted of 0.5 L of growing culture of strain IMB-1 which removed MeBr (>2,500 ppm) from recirculating air. Strain IMB-1 grew to high cell densities in this bioreactor by using pulsed additions of MeBr as its sole carbon and energy source. Bacterial oxidation of MeBr produced CO₂ and hydrobromic acid (HBr) which required continuous neutralization with NaOH for the system to operate effectively. Addition of oxygen was required for long-term (>30 days) operation of the closed-system bioreactor. Strain IMB-1 was capable of oxidizing large amounts of MeBr (170 mmol in 46 days). The second bioreactor, operated as an open system, consisted of a 10-L flow-through fermenter, in which strain IMB-1 oxidized a continuous supply of MeBr (5,000 ppm in air). NaOH was added by pH stat to maintain neutrality. Growth was continuous and 500 mmol (46 g) of MeBr was removed from the air supply in 14 days. Bioreactors using strain IMB-1 can therefore be used to remove large quantities of contaminant MeBr. Considerable range in the inlet concentration of MeBr can be tolerated, however very high concentrations of MeBr (>10,000 ppm) are toxic to the organisms comprising the bioreactor. Strategies for limiting the range of inlet concentrations may include load dampening by adsorption of MeBr on solids such as activated charcoal or zeolite, followed by desorption and subsequent controlled introduction of MeBr, along with a supply of air, into the bioreactor.

B51B MCC: Hall C Friday 0830h

Merging Molecular Techniques and Genomics With Biogeochemistry I Posters (joint with H, OS)

Presiding: Y Fujita, Idaho National Energy and Environmental Laboratory;
E Shock, Arizona State University

B51B-0712 0830h POSTER

Biomarkers of Microbial Metabolism for Monitoring in-situ Anaerobic PAH Degradation

L.Y. Young¹ (732-932-8165, x312; Lyoung@aesop.rutgers.edu)

Craig Phelps¹ (phelps@aesop.rutgers.edu)

Joseph Battistelli¹ (jmb4tm@virginia.edu)

¹Biotech Center, Foran Hall, Rutgers University 59 Dudley Rd., New Brunswick, NJ 08901, United States

Monoaromatic and polycyclic aromatic compounds found in petroleum and its products are subject to biodegradation in the absence of oxygen. These anaerobic pathways reveal novel mechanism of microbial transformation through a series of metabolites and intermediates which are unique to the anaerobic degradation process. The presence of these compounds in-situ, then conceptually can serve as indicators that anaerobic degradation is taking place. We have laboratory studies and field samples which support this concept for BTX and PAH compounds.

Environments in which these anaerobic degradation processes have been observed include freshwater and estuarine sediments, groundwater from impacted aquifers at a former manufactured gas plant and gasoline station, and a creosote-contaminated aquifer. Analytical protocols were developed to detect nanomolar concentrations from soil slurries and groundwater samples and microcosm studies verified their formation from field samples and use as biomarkers of activity.

Recent studies on the mechanisms of anaerobic naphthalene and methyl-naphthalene metabolism have identified several unusual compounds that can serve as biomarkers for monitoring in situ PAH biodegradation. For naphthalene these include 2-naphthoic acid (2-NA), tetrahydro-2-naphthoic acid (TH-2-NA), hexahydro-2-naphthoic acid (HH-2-NA) and methyl-naphthoic acid (MNA) generated by sulfate-reducing bacteria degrading naphthalene or methyl-naphthalene. Groundwater samples were analyzed from wells distributed throughout an anaerobic, creosote-contaminated aquifer and also from a leaking underground storage site. Samples were extracted, derivatized and analyzed by GC/MS. The concentration of 2-NA at each monitoring well was quantified and correlated to the zones of naphthalene contamination. Taken together with measurements of the aquifer's physical characteristics, these biomarker data can be used to describe the extent of naphthalene biodegradation at these site.

B51B-0713 0830h POSTER

Potential for Methanotroph-Mediated Natural Attenuation of TCE in a Basalt Aquifer

Frederick S Colwell¹ (208-526-0097; fxc@inel.gov);

Deborah T Newby¹ (208-526-7779; newbdt@inel.gov); David W Reed¹ (208-526-7788; reeddw@inel.gov); Amber Igoe¹

(aigoe@albertson.edu); Lynn Petzke¹ (208-526-0479; petzlm@inel.gov); Mark E

Delwiche¹ (208-526-1870; mdel@inel.gov); James P McKinley² (509-376-6573;

james.mckinley@pnl.gov); Francisco F Roberto¹ (208-526-1096; ffr@inel.gov); Michael J Whiticar³ (250-721-6514; whiticar@uvic.ca)

¹Biotechnology, Idaho National Engineering and Environmental Laboratory, P.O. Box 1625, Idaho Falls, ID 83415-2203, United States

²Pacific Northwest National Laboratory, P.O. Box 999, Richland, WA 99352, United States

³School of Earth and Ocean Sciences, University of Victoria, P.O. Box 3050, Victoria, BC K1A0E8, Canada

Methanotrophic bacteria are one of the microbial communities believed to be responsible for natural attenuation of a trichloroethylene (TCE) plume in the Snake River Plain Aquifer (SRPA). To better understand the role that indigenous methanotrophs may have in TCE degradation in the aquifer, groundwater was

collected from four SRPA wells and analyzed for geochemical properties and methanotroph diversity. Dissolved methane concentrations in the aquifer ranged from 1 to >1000 nM. Stable carbon isotope ratios for dissolved methane suggest a microbial source for the methane (del 13C values of ca. -61 per mil in three wells). The combination of 13C enriched methane and 13C depleted-dissolved inorganic carbon in one of the wells suggests that microbial oxidation of methane occurs. Filtered groundwater yielded microorganisms that were used as inocula for enrichments or were frozen and subsequently extracted for DNA. Primers that target taxonomic (type I and type II 16S rDNA) or functional (mmoX and pmoA methane monooxygenase subunits) genes were used to characterize the indigenous methanotrophs via PCR, cloning, and sequencing. DNA sequencing and alignment results suggest that clones with sequences most similar to *Methylocystis* sp. (a type II methanotroph) and *Methylobacter* sp. (a type I methanotroph) are frequently present in filtered groundwater with the former often represented in enrichment cultures as well. Methanotroph genes are detected in the aquifer even in wells having methane concentrations as low as 1 nM. Methanotroph presence and a microbial origin for the dissolved methane indicate that microbial cycling of this key gas may play a role in the destruction of TCE in the aquifer.

B51B-0714 0830h POSTER

Stable Carbon Isotope Evidence and Quantification of Reductive Dechlorination of Chlorinated Ethenes at Kelly AFB, TX

Penny Morrill¹ ((416) 978-6807; morrill@geology.utoronto.ca); Georges Lacrampe-Couloume¹ (glc@geology.utoronto.ca); Gregory Slater¹ (gslater@whoi.edu); Brent Sleep² (sleep@civ.utoronto.ca); Elizabeth Edwards³ (edwards@chem-eng.utoronto.ca); Michaye McMaster⁴ (MMcMaster@geosyntec.com); Dave Major⁴ (dmajor@geosyntec.com); Barbara Sherwood Lollar¹ (bsl@geology.utoronto.ca)

¹Stable Isotope Laboratory University of Toronto, 22 Russell Street, Toronto, Ont M5S 3B1, Canada

²Department of Civil Engineering University of Toronto, 35 St. George Street, Toronto, Ont M5S 1A4, Canada

³Department of Chemical Engineering and Applied Chemistry University of Toronto, 200 College Street, Toronto, Ont M5S 3E5, Canada

⁴Geosyntec Consultants, 160 Research Lane, Suite 206, Guelph, Ont N1G 5B2, Canada

Cis-1, 2-dichloroethene (cDCE) was the primary volatile organic compound (VOC) after biostimulation of a perchloroethene (PCE) plume in a pilot test at Kelly Air Force Base (AFB) in San Antonio Texas. A stable natural microbial consortium, KB-1, shown in laboratory experiments to reduce chlorinated ethenes to non-toxic ethene was added in a pilot test area (PTA). After the addition of KB-1 stable carbon isotope values were measured for each chlorinated ethene to verify the occurrence of reductive dechlorination and quantify the extent of cDCE degradation. After bioaugmentation with KB-1, PCE, TCE and cDCE concentrations declined, while VC concentrations increased and subsequently decreased, as ethene became the dominant transformation product measured. Shifts in carbon isotopic values up to 2.7 permil, 6.4 permil, 10.9 permil and 10.6 permil were observed for PCE, TCE, cDCE and VC respectively. These isotopic shifts are consistent with the effects of biodegradation observed during laboratory and field studies. Most notably, isotopic enrichment trends characteristic of reductive dechlorination were detectable in the parent compounds before measurable concentrations of daughter products VC and ethene were produced. These results illustrate the advantage of using the more sensitive compound specific isotope analysis to confirm degradation in addition to the traditional method of monitoring the appearance of degradation products. Fractionation factors obtained from laboratory studies were used in conjunction with isotope data measured in the field to estimate the extent of cDCE degraded. It is estimated that within a 44 day period, 37 to 48 percent of the cDCE was reductively dechlorinated. Independent biodegradation estimates using data from a bromide tracer test, a groundwater flow model, and concentration analyses were all in good agreement with the isotope degradation estimate.

B51B-0715 0830h POSTER

Mechanism of Uranium Sorption by Apatite Materials from a Permeable Reactive Barrier Demonstration at Fry Canyon, Utah

John R Bargar¹ (650-926-4949; bargar@ssrl.slac.stanford.edu)

Chris C Fuller² (650-329-4479; ccfuller@usgs.gov)

James A Davis² (650-329-4494; jadavis@usgs.gov)

¹Stanford Synchrotron Radiation Laboratory, 2575 Sand Hill, Menlo Park, CA 94025, United States

²US Geological Survey, Water Resources Division, 345 Middlefield Rd, MS 465, Menlo Park, CA 94025, United States

Ground water at the Fry Canyon, Utah site (pH 7; 4.8 mM alkalinity) is contaminated with uranium (to 20 mg/L) leached from tailings at a now-abandoned ore upgrader facility. An apatite-based chemical reactive barrier (PRB) was installed in the hydrologic flow path at Fry Canyon in 1997 to determine its technological and economic feasibility for ground water remediation. As part of this study, uranium (U(VI)) was reacted with apatite materials, evaluated for use in the PRB, under laboratory conditions. The speciation of U(VI) was subsequently characterized by EXAFS spectroscopy. U(VI) speciation in pelletized bone charcoal apatite recovered from the PRB was also characterized. In carbonate-containing solutions and in ground water, uranium was found to be adsorbed to the apatite materials as apatite-uranyl-carbonate (i.e., ternary) surface complexes. In carbonate-free solutions, apatite-uranyl-phosphate ternary complexes were found to predominate. At high total uranium concentrations, uranium solubility was limited by precipitation of the uranyl phosphate phase, chernikovite. Uranium solubility was significantly enhanced in the presence of dissolved carbonate. In an apatite-based PRB application, close monitoring will be required to ensure that U(VI) breakthrough does not occur when adsorption equilibrium is reached. The sorptive capacity of the PRB will be affected by the dissolved carbonate concentration of the ground water.

B51B-0716 0830h INVITED POSTER

Biostimulation of Metal-Reducing Microbes at a Former Uranium Mill Tailings Site

Aaron D Peacock¹ ((865) 974-8014; apeacock@utk.edu)

R. Todd Anderson² (rtanders@microbio.umass.edu)

Janet Chang¹ (yjc@utkux.utcc.utk.edu)

Phillip E. Long³ (philip.long@pnl.gov)

David C. White¹ (dwhite1@utk.edu)

¹The University of Tennessee Center for Biomarker Analysis, 10515 Research Dr. Ste. 300, Knoxville, TN 37932, United States

²University of Massachusetts, Department of Microbiology, Amherst, MA 01003, United States

³Pacific Northwest Laboratory, P.O. Box 999, Richland, WA 99352, United States

In situ biological treatment strategies are currently being used or considered to address groundwater contamination at hundreds and perhaps thousands of sites in the United States. A key to demonstrating the effectiveness of biological treatment strategies at a site is establishing cause and effect relationships, which provide evidence that the desired bioprocesses are occurring, or are likely to occur. These methods involve directly measuring various biochemical constituents of the bacteria themselves (i.e. "biomarkers"), which are indicators of their metabolic processes, and therefore provide direct, relevant information regarding the environment in which they are growing. These biomarkers include the presence and viability of biomass, the ability of the organisms to degrade or transform target contaminant(s), the presence of nutrients to promote bacterial growth and activity, and the oxidation/reduction (redox) status of the system. Using these tools we monitored an in situ biostimulation test at the field scale at the Old Rifle Uranium Mill Tailings Remedial Action (UMTRA) Project site, a former uranium ore processing facility located approximately 0.3 mile east of the city of Rifle in Garfield County, Colorado. The purpose of the study was to investigate if the addition of low concentrations of acetate (approx. 1 millimolar) as an electron donor into the subsurface would create anaerobic conditions that would stimulate growth of metal reducing bacteria capable of reducing soluble U(VI) to insoluble U(IV). Phospholipid fatty acid (PLFA), respiratory quinone, and DNA data showed that addition of acetate into the subsurface increased the microbial biomass and altered the microbial community structure to one that contained more anaerobic microorganisms (i.e. *Geobacter* sp.) capable of the reduction of U(VI).

B51B-0717 0830h POSTER

Assessment of Anaerobic Metabolic Activity and Microbial Diversity in a Petroleum-Contaminated Aquifer Using Push-Pull Tests in Combination With Molecular Tools and Stable Isotopes

Martin H Schroth¹ (+41-1-633-6039; schroth@ito.umnw.ethz.ch)

Jutta Kleikemper¹ (kleikemper@ito.umnw.ethz.ch)

Silvina A Pombo¹ (pombo@ito.umnw.ethz.ch)

Josef Zeyer¹ (zeyer@ito.umnw.ethz.ch)

¹Institute of Terrestrial Ecology - Soil Biology, Swiss Federal Institute of Technology (ETH) Zurich, Grabenstrasse 3, Schlieren CH-8952, Switzerland

In the past, studies on microbial communities in natural environments have typically focused on either their structure or on their metabolic function. However, linking structure and function is important for understanding microbial community dynamics, in particular in contaminated environments. We will present results of a novel combination of a hydrogeological field method (push-pull tests) with molecular tools and stable isotope analysis, which was employed to quantify anaerobic activities and associated microbial diversity in a petroleum-contaminated aquifer in Studen, Switzerland. Push-pull tests consisted of the injection of test solution containing a conservative tracer and reactants (electron acceptors, 13C-labeled carbon sources) into the aquifer anoxic zone. Following an incubation period, the test solution/groundwater mixture was extracted from the same location. Metabolic activities were computed from solute concentrations measured during extraction. Simultaneously, microbial diversity in sediment and groundwater was characterized by using fluorescence in situ hybridization (FISH), denaturing gradient gel electrophoresis (DGGE), as well as phospholipids fatty acid (PLFA) analysis in combination with 13C isotopic measurements. Results from DGGE analyses provided information on the general community structure before, during and after the tests, while FISH yielded information on active populations. Moreover, using 13C-labeling of microbial PLFA we were able to directly link carbon source assimilation in an aquifer to indigenous microorganisms while providing quantitative information on respective carbon source consumption.

B51B-0718 0830h POSTER

Single-Well-Gas-Sparging Tests for Assessing the Feasibility of In-situ Aerobic Treatment of CAH Mixtures

Young Kim¹ (541-737-8870; kimyo@engr.orst.edu)

Jonathan Istok¹ (541-737-8547; jack.istok@orst.edu)

Lewis Semprini¹ (541-737-6895; lewis.semprini@orst.edu)

¹Lewis Semprini, Department of Civil, Construction, and Environmental Engineering, Corvallis, OR 97331, United States

Single-well-gas-sparge tests were performed to assess the feasibility of in-situ aerobic cometabolism of chlorinated aliphatic hydrocarbons (CAHs), such as trichloroethylene (TCE) and cis-1,2-dichloroethylene (c-DCE), using propane and methane as growth substrates. The tests were performed in the saturate zone at the McClellan Air Force Base, CA. The effectiveness of gas sparging to stimulate indigenous propane-utilizers or methane-utilizers was evaluated in standard monitoring wells. Transport characteristics of dissolved solutes [sulfur hexafluoride (SF₆) or bromide (tracer), propane or methane (growth substrate), ethylene, propylene (nontoxic surrogates to probe for CAH transformation activity), and dissolved oxygen], were evaluated by push-pull transport tests. Mass balance showed about 90% of the injected bromide and about 80% of the injected SF₆ were recovered, and the recoveries of other solutes were comparable with bromide and SF₆. The transport tests demonstrated that bromide and SF₆ could be used as conservative tracers for biological activity tests and that little loss of the dissolved gaseous substrates prior to biostimulation occurred. The dissolved gases were also conservatively transported indicating negligible trapped gas was present in the aquifer prior to sparging.

A series of gas-sparging biostimulation tests were performed by sparging propane-(or methane)-oxygen-argon-SF₆ gas mixture at specific depth intervals using a straddle packer. Temporal groundwater samples were obtained from the injection well under natural gradient drift conditions. Biostimulation was demonstrated with repeated gas sparging tests where the time to deplete methane and propane concentrations decreased compared to SF₆. Gas sparging activity tests were performed using the same procedures as the gas-sparging biostimulation tests, except that ethylene and

propylene were included in the sparging gas mixtures. Propane (or methane) utilization, DO consumption, and ethylene and propylene cometabolism were well demonstrated. The stimulated propane- and methane-utilizers cometabolized ethylene and propylene to produce ethylene oxide and propylene oxide as cometabolic by-products. The results confirmed the biostimulation of indigenous microorganisms with cometabolism ability. When acetylene was included in the sparge gas mixture, propane and methane utilization and ethylene and propylene transformation were effectively blocked, indicating monooxygenase enzymes were involved

B51B-0719 0830h POSTER

Bioaugmentation of an Aerobic Culture Capable of Chlorinated Solvent Cometabolism to a Subsurface Test Zone

Mark E Dolan¹ (541 737-3862; Mark.Dolan@orst.edu)

Lewis Semprini¹ (541 737-6895; Lewis.Semprini@orst.edu)

Perry L McCarty² (650 723-4131; mccarty@cive.stanford.edu)

Gary Hopkins² (408 262-2070; hopkins@cive.stanford.edu)

¹Oregon State University, Department of Civil, Construction, and Environmental Engineering, Apperson Hall Rm 202, Corvallis, OR 97331

²Stanford University, Department of Civil and Environmental Engineering, Stanford, CA 94305

A butane-utilizing culture able to cometabolize chlorinated aliphatic hydrocarbons (CAHs) was bioaugmented into an aquifer test zone at Moffett Federal Airfield, CA. Microcosm bioaugmentation tests conducted with groundwater and aquifer solids collected from the test site indicated a strong potential for viability of the bioaugmented culture in the site subsurface. Microcosms bioaugmented with the butane-utilizing culture were able to degrade aqueous concentrations of 1,1-dichloroethylene (1,1-DCE) up to 1 mg/L and could successfully transform mixtures of 1,1-DCE, 1,1,1-trichloroethane (TCA) and 1,1-dichloroethane (DCA) when fed butane. T-RFLP analyses showed the presence of bioaugmented organisms within the microcosms throughout the 10-month test period. An isolate from the butane-utilizing culture was grown in batch bottles containing mineral media and a butane-in-air headspace. Approximately 4 g dry weight of culture was harvested and bioaugmented to the field site. The site consisted of two parallel well legs, each with an injection well, two fully penetrating monitoring wells containing solid support media, three groundwater monitoring wells and an extraction well. One well leg was bioaugmented with the isolate and the other was used as an indigenous control leg. A mixture of 1,1-DCE, TCA and DCA (50 ug/L, 135 ug/L and 150 ug/L respectively) was continuously pumped through both well legs with alternate pulses of dissolved oxygen and butane. Fifty percent removal of 1,1-DCE occurred within one day in the bioaugmented leg; however, it took about 6 days to achieve complete butane utilization and 1,1-DCE removal to below 2 ug/L. During this period DCA and TCA were reduced by 70-90 percent and 30-50 percent respectively. When the butane/oxygen pulses were changed from a 1-hr cycle to a 24-hr cycle 1,1-DCE removal fell to 50 percent and DCA and TCA concentrations increased to influent levels. Upon returning to short pulse cycles, 1,1-DCE removal efficiency returned to 95 percent while DCA and TCA were not effectively transformed. Groundwater microbial samples obtained 1 m from the injection well did not show the presence of the bioaugmented organism. Butane uptake was observed in the non-bioaugmented leg after about 14 days of butane addition, but no CAH transformation was observed. Butane oxidation without CAH transformation continued through the period of longer pulse cycles; however, upon return to the shorter pulse cycles 1,1-DCE removal efficiencies similar to those obtained in the bioaugmented leg were achieved without significant removal of DCA or TCA. Comparison of initial groundwater microbial samples showed no significant differences between the two legs. Microbial analyses are ongoing and a second field season will begin in September 2002 to assess TCA transformation ability upon re-bioaugmentation of the site.

B51B-0720 0830h POSTER

Culture-independent Community Genomics Study of Microorganisms Associated with Acid Mine Drainage

Gene W Tyson¹ ((510) 642 9690; gtyson@nature.berkeley.edu)

Philip Hugenholtz¹ ((510) 623 2155; philiph@nature.berkeley.edu)

Christopher Detter² ((925) 296 5846; detter2@lbl.gov)

Paul M Richardson² (pmrichardson@lbl.gov)

Jillian F Banfield¹ ((510) 642 9488; jill@eps.berkeley.edu)

¹University of California, Berkeley, Department of Environmental Science, Policy and Management, Berkeley, CA 94720, United States

²DOE Joint Genome Institute, JGI Production Genomic Facility, 2800 Mitchell Drive, Walnut Creek, CA 94598, United States

Acid mine drainage (AMD) is a serious environmental problem that occurs when pyrite (FeS₂)-rich rocks are exposed to air, water, and oxidizing agents. Pyrite dissolution is exothermic, yielding hot, metal-rich, extremely acidic solutions. Despite the hostile nature of AMD environments, microbial communities thrive, and are believed to control the rates of pyrite dissolution and acid generation. Previous studies of microbial communities within the Richmond Mine at Iron Mt., Redding, CA, revealed low species-level diversity and established close connections between metabolism and geochemistry. Thus, these communities are ideal candidates for culture-independent genomics-enabled analyses. Samples collected from the Richmond Mine in March 2002 were screened using quantitative fluorescence *in situ* hybridization to choose the most suitable target community for a genomics-enabled ecological study. The community selected (a pink subaerial biofilm) has low species complexity but high phylogenetic diversity, containing ~75% *Leptospirillum* group II (closely related to *Leptospirillum ferriphilum*), ~10% *Leptospirillum* group III, and ~10% archaea that fall into three distinct groups within the *Thermoplasmatales* order (*Ferroplasma* sp., for which genome data is already available, A-plasma and D-plasma). High throughput sequencing of four 16S rDNA clone libraries confirmed the low species-level diversity in this community and suggested that the extent of microheterogeneity within each of the five populations was limited. 17 unique *Leptospirillum* group II 16S rDNA phylotypes (defined by one or more base change) were resolved, with ~85% belonging to one phylotype. The remaining ~15% were very similar in sequence identity, only diverging by 1.2% in total. Similarly, 14 unique *Leptospirillum* group III phylotypes formed a tight cluster with < 0.8% sequence divergence. The limited inter-species, and significant intra-species variation in the populations should allow determination of the gene content and metabolic pathways of the majority of community members (especially *Leptospirillum* spp.), permit evaluation of the genomic heterogeneity within each species population, and potentially enable reconstruction of genome fragments and whole genomes. We have constructed small (3-4 Kb; shotgun) and large-insert (>40 Kb; fosmid, BAC) libraries from community DNA. We will use sequencing results from the small insert library to determine whether it will be possible to reassemble parts of genomes using shotgun data. The large insert libraries will ensure links between genes and host organisms and provide information about gene order. Using the first 2 Mb of small insert library data we have established reasonable coverage of the major microbial groups but more data is required before the overall sequencing strategy can be established. Organism-resolved metabolic pathway information will be used to develop methods to monitor microbial activity in the environment. Ultimately, our goal is to develop a predictive analytical ecological model that resolves the linkages, feedbacks, and controlling variables that underpin acid mine drainage generation.

B51B-0721 0830h POSTER

Connecting genomics and biogeochemistry via the carbon-isotopic composition of ribosomal RNA

Alex L Sessions¹ (508-289-2570; asessions@whoi.edu)

Ann Pearson² (pearson@eps.harvard.edu)

John M Hayes¹ (jhayes@whoi.edu)

Edward F DeLong³ (delong@mbari.org)

Peter Girguis³ (girguis@mbari.org)

¹Woods Hole Oceanographic Institution, Department of Geology and Geophysics, 266 Woods Hole Road, Woods Hole, MA 02543, United States

²Harvard University, Department of Earth and Planetary Science, 20 Oxford St., Cambridge, MA 02138, United States

³Monterey Bay Aquarium Research Institute, 7700 Sandholt Road, Moss Landing, CA 95039, United States

A significant shortcoming of genomic methods is that the metabolic functions of organisms cannot be determined directly. In many cases this is because the organism cannot be cultured, but even many culturable microbes possess multiple, inducible metabolic pathways so that doubt can remain about their role

in a specific environment. We are pursuing the measurement of ¹³C in ribosomal RNA purified directly from environmental samples as a means of identifying metabolic activities. RNA is purified using newly-developed probe-capture techniques, with the specificity of the capture process depending on probe design and potentially ranging from the domain level to very specific subgroups. Initial efforts have focused on capturing Bacterial and Archaeal small-subunit rRNA at the domain level. ¹³C content is measured using a moving-wire combustion system which provides accurate $\delta^{13}C$ values from as little as 25 ng of RNA. For all organisms studied thus far, from all domains, RNA is enriched in ¹³C by 1-2 permil relative to biomass. Two cultures of *E. coli* produced 16S rRNA enriched in ¹³C by 1 permil relative to total RNA. Bacterial RNA captured from eukaryotes hosting bacterial symbionts indicates varying isotopic relationships between host and symbiont, probably depending on details of the symbiotic relationship. The approach is also suitable for use with labeling experiments. As an example, salt-marsh sediments containing natural organic matter with $\delta^{13}C = -12\text{‰}$ were incubated with diesel fuel ($\delta^{13}C = -25\text{‰}$). Analyses of captured bacterial rRNA show rapid incorporation of petroleum carbon into biomass, accounting for 14% of bacterial RNA after one week of incubation. No incorporation of petroleum into eukaryotic biomass was detected.

B51B-0722 0830h POSTER

Microbial and Geochemical Zoning of the Middendorf Aquifer, South Carolina

Qusheng Jin¹ (217-244-8337; qjin@aquifer.geology.uiuc.edu); Jungho Park (217-244-8767; j-park16@uiuc.edu); George Bonheyo (217-333-0672; bonheyo@life.uiuc.edu); George Roadcap (217-333-7951; roadcap@uiuc.edu); Jorge Frias-Lopez (217-333-0672; friaslop@uiuc.edu); Bruce W Fouke (217-244-5431; fouke@uiuc.edu); Craig M Bethke (217-333-3369; c-bethke@uiuc.edu)

¹Department of Geology, University of Illinois, 245 Natural History Building, 1301 West Green Street, Urbana, IL 61801, United States

The Cretaceous Middendorf aquifer in South Carolina, which extends from the Fall Line to the offshore reaches of the Atlantic Coastal plain, is distinctly zoned in both the chemical composition of the groundwater it contains and its microbial community. Groundwater flowing along the aquifer, which is confined over much of its extent, passes through a series of redox zones that have been inferred to represent segregation of the aquifer microbes according to terminal electron accepting process. The zones include ecological niches supporting microbial aerobic respiration, denitrification, iron reduction, sulfate reduction, and methanogenesis. We analyzed groundwater from water supply wells across the aquifer along the direction of groundwater flow for chemical species that might serve as electron donor or acceptor for chemolithoautotrophic and acetoclastic organisms in the aquifer. These chemical species included acetate, dihydrogen, dioxygen, ferrous iron, sulfate, sulfide, nitrate, nitrite, ammonia, bicarbonate, and methane. We filtered microorganisms from water produced from these wells and then amplified their 16S rDNA genes by polymerase chain reaction (PCR) using universal Eubacterium- and Archaea-specific primers. To characterize the microbial community at each sampling location, we analyzed the amplified genes by terminal restriction fragment polymorphism (TRFP). Sequencing of cloned genes is currently in progress. We find that along the groundwater flow path the microbial population shifts from a community dominated by bacteria to one dominated by Archaea, and that the community structure is indeed zoned by the predominant terminal electron accepting process. This zoning, furthermore, closely reflects variation along the aquifer in the thermodynamic energy available for inferred metabolisms of the observed microbes, as calculated from the results of the chemical analyses. These results provide the first direct confirmation that the chemical zoning of groundwater flows on a regional scale arises from the metabolisms of microbes residing in aquifers.

B51B-0723 0830h POSTER

Molecular Signatures of Methanogens in Cultures and Environmental Samples

Roger E Summons¹ (617 452 2791; rsummons@mit.edu)

Tsegereda Embaye² (tembaye@mail.arc.nasa.gov)

Linda L Jahnke² (ljahnke@mail.arc.nasa.gov)

Manuela Baumgartner³ (manuela.baumgartner@biologie.uni-regensburg.de)

¹Dept Earth, Atmospheric and Planetary Sciences, Massachusetts Ave, Cambridge, MA 02139, United States

²NASA, Ames Research Center, Moffett Field, CA 94035, United States

³LS für Mikrobiologie, Universität Regensburg, Regensburg D-93053, Germany

The core lipids of methanogens comprise C₂₀ and C₄₀ isoprenoid chains, linked through ether bonds to glycerol. Additional structural diversity is encoded into the polar head groups that are attached to the glycerol ether cores. These compounds are potentially very useful as taxonomic markers in microbial mats and other environmental samples while the nature of the hydrocarbon chains provide a means to identify methanogenic inputs to ancient sediments. The structural diversity of methanogen polar lipids is most valuable when it can be directly correlated to 16S rRNA phylogeny. On the other hand, this diversity can also lead to analytical challenges because there is no single approach that works for all structural types.

While some intact methanogen lipids have been identified using mass spectrometry and NMR spectroscopy, the most common means of analysing the lipid cores involves cleavage of the ether bonds using HI and subsequent reduction of the alkyl iodides to hydrocarbons with LiAlH₄. One class of methanogenic lipids, the 3 β -hydroxyarchaeols, escaped detection for some years because strong acid treatments in the analysis protocols destroyed hydroxyl-containing isoprenoid chains. We have been systematically re-examining the lipids of methanogens, using milder procedures involving weak acid hydrolysis of polar head groups, derivatisation to form trimethylsilyl ethers and analysis by GC-MS. As well as archaeol, sn-2- and sn-3-hydroxyarchaeol, we have tentatively identified a dihydroxyarchaeol in several *Methanococcus* sp. For *Methanococcus thermolithotrophicus* an analysis of the total lipid extracts using BB₃ as an ether cleavage reagent followed by LiBET₃H, reduction revealed a very complex mixture consisting of phytane, phytene, biphytane, biphytene and a suite of related alcohols. The latter compounds were analysed by GC-MS as their trimethylsilyl ethers and found to comprise a mixture tentatively identified as phytan-N-ol and biphytan-N-ol where N = 3 or 7 or 11 or 15. Apart from phytan-3-ol, these compounds appear to have been overlooked in previous analyses of methanogen lipids. *Methanopyrus kandleri*, a hyperthermophile that is distinguished from other methanogens by some unusual biochemical features, is also differentiated by having biphytanyl chains with cyclopentane rings. This is a structural feature more commonly found in the Crenarchaeota. We are applying this biomarker methodology to environmental samples in studies of the spatial distribution of methanogenesis relative to other physiologies.

B51B-0724 0830h POSTER

Genomic Analysis of Uncultured Marine Viral Communities

Mya Breitbart¹ (619-594-1336;

mya@sunstroke.sdsu.edu); Peter Salamon²; Bjarne Andresen³; Joseph M Mahaffy²; Anca M Segall¹; David Mead⁴; Farooq Azam⁵; Forest Rohwer¹

¹Department of Biology, San Diego State University 5500 Campanile Drive, San Diego, CA 92182-4614, United States

²Department of Mathematical Sciences, San Diego State University 5300 Campanile Drive, San Diego, CA 92182-7720, United States

³Orsted Laboratory, University of Copenhagen Universitetsparken 5, Copenhagen, 0 DK-2100, Denmark

⁴Lucigen Corporation, 2120 W. Greenview Drive, Middleton, WI 53562, United States

⁵Marine Biology Division, Scripps Institution of Oceanography, La Jolla, CA 92093, United States

Viruses are the most common biological entities in the oceans by an order of magnitude. Diversity of these viruses undoubtedly plays an important role in controlling bacterial populations and biogeochemical cycles in the marine environment. However, very little is known about the diversity of marine viral communities. Here we report the first genomic analysis of uncultured viral communities from two nearshore marine water samples and one marine sediment sample. In all three marine libraries, over 65% of the sequences were not significantly similar to previously reported sequences, suggesting that much of the diversity is novel. The most common significant hits amongst the known sequences were to viruses. The viral hits included sequences from all the major families of dsDNA tailed phage, as well as some algal viruses. BLAST analysis of the sequence data suggested fundamental differences between the viral communities. Several independent mathematical models based on the observed number of contigs predicted that the most abundant viral genome comprised 2-3% of the total population in the water communities, which were estimated to contain between 374 and 7114 viral types. Diversity of the sediment community was significantly higher. The results also showed that it would be possible to sequence the entire genome of an uncultured marine viral community.

B51B-0725 0830h POSTER

Why Microbial Ecology and Ecogenomics Needs Geochemistry: An Illustrated Example

Carrine E Blank (314-935-4456; blank@levee.wustl.edu)

Washington University, Department of Earth and Planetary Sciences Campus Box 1169 One Brookings Drive, St. Louis, MO 63130-4899, United States

Yellowstone National Park's near-boiling silica-depositing thermal springs have been the subject of a handful of molecular microbial ecological studies. They are potentially an ideal model system for studying natural microbial populations for two reasons: the populations are dominated by uncultured lineages and the communities are very simple. In a recent comprehensive study of a large number of silica-depositing springs distributed throughout Yellowstone (Blank, Cady, and Pace, in press), only 11 different types of organisms were found in these environments (as measured by small subunit ribosomal RNA analyses). Diversity was very low: some springs only contain one organism, while other contain several (but never more than 5). All springs contained *Thermocrinis ruber*, also known as the pink filamentous streamer bacterium.

When one looks at population-level diversity within the *Thermocrinis ruber* species at a finer scale, on the level of the hypervariable internal transcribed spacer region, one finds a great deal more diversity. Some springs contain low diversity within this clade (and only have one ITS sequence type), while other springs show more diversity within this clade (and have several different ITS sequence types). Interestingly, diversity within this clade is also observed on the morphological level using fluorescence *in situ* hybridization and scanning electron microscopy. It appears that the diversity within ITS sequences may correlate with morphological diversity, although more work will need to be done to firmly establish these correlations.

Why are some microbial communities in these springs diverse and others not? What does heterogeneity on a population level really mean? How diverse are these populations with respect to metabolic characteristics and fitness? How do each of these different lineages or sub-species contribute to geochemical cycles in the environment? There are many factors that can control population diversity. The most important is likely to be geochemistry and the availability of energy sources and nutrients, however others could include geography, hydrodynamics, and trophic interactions. Obviously, in order to answer fundamental questions regarding the nature of microbial populations, we must take a comprehensive, interdisciplinary approach that includes geochemistry as an essential component.

Similarly, as microbiologists start moving in the direction of ecogenomics, where the whole genomes of novel uncultured prokaryotic lineages are sequenced directly from the natural environment, we will need to deal with this issue of population-level diversity. Traditionally, microbiologists have studied individual isolates in the laboratory and these have served as our knowledge base for environmental processes. However, just as molecular microbiological studies have been showing a great deal more uncultured diversity in the natural microbial world, they are showing just how little we understand about diversity on all levels. In addition to sequencing the whole genomes of single isolates, ecogenomics need to quantify population-level diversity within the whole genome and correlate this variation with environmental parameters (including geochemistry) that may be controlling the patterns of observed diversity.

B51B-0726 0830h POSTER

Microbial ecology of -Proteobacteria ammonia-oxidizers along a concentration gradient of dry atmospheric nitrogen deposition in the San Bernadino Mountain Range.

Fiona L Jordan¹ (909-787-3698; fiona@mail.ucr.edu)

Mark E Fenn² (909-680-1565; mfenn@fs.fed.us)

Lisa Y Stein¹ (909-787-2704; steinl@citrus.ucr.edu)

¹Department of Environmental Sciences, Geology 2217 University of California, Riverside, Riverside, CA 92521, United States

²USDA Forest Service, Pacific Southwest Research Station, 4955 Canyon Crest Dr., Riverside, CA 92507, United States

The fate of atmospherically-deposited nitrogen from industrial pollution is of major concern in the montane ecosystems bordering the South Coast California Air Basin. Nitrogen deposition rates in the more exposed regions of the San Bernardino Mountains (SBM) are among the highest in North America often exceeding 40 kg ha⁻¹ year⁻¹ in throughfall deposition of nitrate and ammonium (Fenn and Poth, 1999). Forest ecosystems with elevated N deposition generally exhibit elevated accumulation of soil nitrate, leaching

and runoff, elevated emissions of nitrogenous gases, increased nitrification, and decreased litter decomposition rates. The role of nitrifying microbial populations, especially those taxonomically associated with the beta-Proteobacteria ammonia-oxidizers (AOB), will provide insight into nitrogen-cycling in these extremely N-saturated environments. Using 16S ribosomal DNA-based molecular techniques (16S rDNA clone library construction and Restriction Fragment Length Polymorphism), we are comparing AOB community diversity at 3 different locations along a natural atmospheric N-deposition concentration gradient in the SBM: from high at Camp Paviaka (CP), medium at Strawberry Peak (SP) to low at Dogwood (DW). As observed for wet N-deposition systems on the east coast, we hypothesized a negative correlation between AOB community diversity, abundance and function with nitrogen loading in the dry N deposition system of SBM. Nitrification potentials determined for the 3 sites along the N-deposition gradient were in the order of CP less than SP less than DW. Preliminary results indicate no correlation between diversity of AOB and increased nitrogen loading. Shannon-Weiner diversity indices calculated for ammonia-oxidizer RFLP group units were 2.22, 2.66 and 1.80 for CP, SP and DW, respectively.

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B51B-0727 0830h POSTER

Linking Microbial and Biogeochemical Studies: Biological Controls of Methane Release from an Acidic Natural Wetland in Central Pennsylvania

Jennifer F Biddle^{1,4} (jfg150@psu.edu)

Courtney Turich^{2,4} (cturich@geosc.psu.edu)

Susan Brantley^{2,4} (brantley@essc.psu.edu)

Mary Ann Bruns^{3,4} (mvb10@psu.edu)

¹Dept. of Biochemistry and Molecular Biology, S. Frear Penn State University, University Park, PA 16802

²Dept. of Geosciences, Deike Bldg. Penn State University, University Park, PA 16802

³Dept. of Crop and Soil Sciences, Agricultural Sciences and Industries, University Park, PA 16802

⁴Biogeochemical Research Initiatives for Education, Penn State University, University Park, PA 16802

Wetlands produce between 55 and 150 Tg of methane per year, or 70% of all natural methane, and 20% of total methane (natural and anthropogenic). Understanding inputs to the global methane cycle depends on integrated *in situ* study of the sources and sinks of methane, as well as the rate and magnitude of methane production and consumption. Bear Meadows Natural Area in central Pennsylvania (N 40° 43.796 W 077° 45.310; 554 m elevation) contains an acidic, methane-producing, peaty bog with vegetation that is typical of wetlands at higher latitudes. In this four year study conducted within a cross-disciplinary training course offered by the NSF-IGERT Biogeochemical Research Initiative in Education (BRIE) program at Penn State University, graduate students applied a combination of geochemical and microbiological techniques to explore microbial diversity and activity in Bear Meadows sediments. The methane flux at the peat:water interface was highly variable, from 0.01 to over 3000 $\mu\text{mol}/\text{m}^2/\text{min}$ in both sphagnum and sedge vegetation. The methane released from the bog had a carbon isotopic composition of -60 ‰, typical of biogenic methane. Analysis of peat pore waters showed that the most methane was produced 30 cm below the peat:water interface, with a broad peak of methane in pore waters from 20-40 cm. At 21 cm below the peat:water interface, profiles of Archaeal 16S-23S ribosomal RNA spacer regions revealed the presence of populations having 92% similarity to 16S rRNA sequences of *Methanoculleus marisnigri*. Phospholipid fatty acids (PLFA) and compound specific isotope analysis revealed other biological controls on the methane cycle. PLFAs typical of methanotrophic bacteria were also present within peat cores from 20-30 cm below the water interface. The depleted carbon isotopic composition of these biomarkers (C16:1 and C18:1 fatty acids) was -31.4 ‰ and 33.8 ‰, indicative of methane oxidation. The presence of biomarkers of methane oxidizing bacteria within the zone of methane production may indicate that there is temporal or spatial heterogeneity in oxygen concentration within the peat. This interdisciplinary approach helped define specific ecological niches where novel methanogens and methane oxidizers may be active in a typical northern wetland. Through BRIE, on-going studies of the Bear Meadows wetland will focus on detecting other potentially novel aerobic and anaerobic microbes, and determining the biological influence on methane release to the atmosphere.

URL: <http://www.essc.psu.edu/BRIE>

B51B-0728 0830h POSTER

Incorporation of arsenic in mammal bone: X-ray absorption spectroscopy

Xiomara Kretschmer¹ (9157475754; xkretschmer@utep.edu)

Nicholas E. Pingitore¹ (9157475754; nick@geo.utep.edu)

Gustavo Cruz-Jimenez¹ (9157475754; gustavoc@utep.edu)

¹University of Texas at El Paso, Dept. of Geological Sciences, El Paso, TX 79968-0555, United States

X-ray absorption spectroscopy (XAS) of the distal tibia of a modern deer, *Odocoileus virginianus*, revealed that the energy position of the As K edge matched that of a reference arsenic(V) model compound. Comparison of the x-ray absorption near edge structure (XANES) of the deer spectrum to the spectra of model As compounds indicated a close match to arsenate(V), e.g., zinc orthoarsenate(5). This indicates that the nearest-neighbor shell of the arsenic in the bone consists of four oxygens in the tetrahedral arrangement typical of arsenic(V) oxyanions.

The XANES analysis demonstrates that the arsenic in the deer bone is not associated with an organic compound as a result of methylation. This suggests that the arsenic is associated with the mineral fraction of the bone, most likely with As substituting for P at the latter's structural site in the hydroxyapatite. The XAS data for the deer bone were very noisy due to the low level of arsenic present, just over 1 ppm. A total of 18 scans, taking nearly a full 8-hour beam shift, were averaged to obtain the spectrum studied. It is not clear that the second neighbor shell can be characterized sufficiently from these data to confirm that As substitutes for P in hydroxyapatite.

We conducted our XAS experiments on beam line 4-3 at the Stanford Synchrotron Radiation Laboratory. Data were collected in the fluorescence mode, using a solid state, 13-element Ge-detector. The energy reference was As(0) metal foil run parasitically in transmission mode during collection of the bone spectra. The edge shift seen in the experimental and As(V) model compound relative to the energy position of the arsenic(0) foil is consistent with the additional energy required to photoeject the 1-s electron of As(V), relative to that required for As(0). Arsenic content of the deer bone was determined by inductively coupled plasma mass spectrometry.

B51B-0729 0830h POSTER

Use of Synchrotron X-ray Fluorescence to Measure Trace Metal Distribution in the Brain

David Linkous¹ (703 993 1358; dlinkous@gmu.edu); Jane M. Flinn¹ (703 993 4107; jflinn@gmu.edu); Antonio Lanzirotti² (631 344 5626; lanzirotti@bnl.gov); Christopher Frederickson³ (409 762 0678; cjfreder9ickson@hotmail.com); Blair E. Jones⁴ (703 648 5835); Paul M. Bertsch⁵ (803 725 5637; bertsch@srel.edu)

¹George Mason University, 4400 University Blvd., Fairfax, VA 22030, United States

²University of Chicago, Dept. of Geological Sciences, Chicago, IL 60637, United States

³University of Texas, Medical Branch, Galveston, TX 77550, United States

⁴U.S. Geological Survey, National Center, ms 432, Reston, VA 20192, United States

⁵Savannah River Ecology Lab, University of Georgia, SREL station, Aiken, SC 20892, United States

X26A, National Synchrotron Light Source, was used to quantitatively evaluate the spatial distribution of trace metals, such as Zn and Cu, in brain tissue. X-ray microprobe techniques offer distinct advantages over other analytical methods by allowing analysis to be done in-situ with little or no chemical pretreatment and low detection limits (about 1 ppm). In the context of neuroscience, SXRF can provide non-destructive measurements of specific metal concentrations and distribution within nerve (brain) tissue. Neuronal tissue from organisms having undergone different normal or experimental conditions may be compared, with analytical capacities not limited by binding states of the metal (i.e., vesicular or enzymatic), as is the case with staining techniques. Whole regions of tissue may be

scanned for detectable trace metals at spatial resolutions of 10um or less using focused monochromatic x-ray beams. Here special attention has been given to zinc because it is the most common trace metal in the brain, and levels have been increasing in the environment. In this investigation, zinc concentrations present within the hilus of a rat hippocampus, and to a lesser extent in the cortex, have been shown to increase following long-term ingestion of zinc-enhanced drinking water that was associated with deficits in spatial memory. Concomitantly, copper concentrations in the internal capsule were comparatively lower. Other first order transition metals, Cr, V, Mn, and Co were not detected. In contrast, elevated levels of Zn, Cu, and Fe have been seen in amyloid plaques associated with Alzheimer's disease.

B51C MCC: 132 Friday 0830h

Mechanisms of Carbon Stabilization and Loss in Soils I (joint with GC)

Presiding: J Harden, U.S. Geological Survey; K O'Neill, USDA Forest Service

B51C-01 0840h INVITED

Thermally Altered Biomass (Black Carbon) in Soils: Formation, Analysis, Distribution, and Implications

Michael W. I. Schmidt (+41 1 635 5140; michael.schmidt@geo.unizh.ch)

Univ. Zurich, Dept. Geography, Soil Biogeochemistry, Wintherturstr. 190, Zurich 8057, Switzerland

Black Carbon (BC), formed during biomass burning, is a chemically heterogeneous, biologically refractory class of carbon compounds (1, 5). BC is purely terrestrial in origin and occurs ubiquitously in soils and terrestrial sediments and is coupled to a common marine fate via atmospheric and fluvial transport, potentially representing a significant reservoir of extremely slowly cycling carbon (1). However, because of its physicochemical heterogeneity and a lack of established analytical techniques, the geochemistry and quantitative importance of BC in the global carbon cycle remains largely undescribed. Existing methods rely on operational definitions with clear-cut but different boundaries inherently designed to analytically determine different parts of the BC continuum (1, 2, 3).

In a set of German chernozem soils, BC from biomass burning makes up 15 to 45 percent of the soil organic carbon (SOC), as determined via UV-high energy photooxidation combined with ¹³C NMR (4, 6). High resolution microscopy and spectroscopy unambiguously confirmed the presence of submicron BC particles with short-range variability in elemental composition, and two sometimes coexisting modifications, i. e. amorphous char-BC from pyrolyzed cellulose and graphitic soot-BC. BC, up to 3990 years older than bulk SOC, is 1160 to 5040 carbon-14 years old, indicating significant residence times of BC in soils.

These results suggest three major implications: First, it seems that besides climate, vegetation and ioturbation, fire also plays an important role in the pedogenesis of Chernozems (4, 5). Second, BC can be a useful tracer for prehistoric human slash-and-burn activities, and thus represent a novel type of archaeological evidence (7). Third, the concept that BC from biomass burning is the source of the chemically stable aromatic components of soil organic matter, and point toward a different understanding of the large quantitative importance and longevity of BC in the terrestrial system (3, 4). BC provides a final sink for terrestrial organic carbon, removing substantial amounts of carbon from rapid circulation between the atmosphere, terrestrial and marine biosphere, and thus could be important to close the carbon budget in global biogeochemical models.

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B51C-02 0855h

The Sequestration and Protection of Organic Matter Within Mineral Mesopores

Andrew R Zimmerman¹ (1-814-865-9384; azimmer@geosc.psu.edu)

Jon D Chorover² (1-520-626-5635; chorover@ag.arizona.edu)

Sridhar Komarneni³ (1-814-865-1542; komarneni@psu.edu)

Susan L Brantley¹ (1-814-863-1739; brantley@essc.psu.edu)

¹Penn State University, Department of Geosciences, University Park, PA 16802

²University of Arizona, Department of Soil, Water and Environmental Science, Tucson, AZ 85721-0038

³Penn State University, Department of Crop and Soil Science, University Park, PA 16802

Organic matter-mineral interactions may explain diverse phenomena such as sequestration of pollutants and preservation of organic matter in soils and sediments. Mineral mesopores (2-50 nm diameter) may sequester organic matter (natural and pollutant) and protect it from microbial and fungal enzymatic degradation in soils and sediments. To test this idea, we carried out batch aqueous experiments to examine adsorption of amino acid monomers and polymers onto synthetic mesoporous and nonporous alumina and silica with controlled intraparticle porosity with similar surface chemistry. All amino acid monomers and polymers smaller than about one-third the pore diameter exhibited significantly greater adsorption (on a surface area normalized basis) to mesoporous alumina (8.2 nm mean pore diameter) and silica (3.4 nm mean pore diameter) versus nonporous phases. Amino acid polymers (lysozyme, albumin, g-globulin) of sizes approaching and larger than the mesopores, however, exhibited greater adsorption to the nonporous phases indicating their exclusion from the internal surfaces of the mesoporous minerals. Further, we observed a sharp decrease in the surface area of the mesoporous materials after adsorption of these proteins indicating blockage of pore openings. These results provide a potential mechanism for the selective sequestration of sedimentary organic matter.

To test whether these pore-sequestered materials are likely to be preserved, we incubate mesoporous and nonporous minerals and sorbed organic matter (model amino acid compounds and natural organic matter extracts) in solutions containing bacterial or fungal derived enzymes. The amount of degradation of sorbed organic matter is detected by measuring sediment TOC before and after the experiment while qualitative changes are indicated by diffuse reflectance infrared Fourier transform (DRIFT) spectra. We expect that small organic compounds sorbed within pores will be protected from degradation while organic matter sorbed to nonporous mineral will be minimally protected. An overall bias toward the preservation of lower molecular weight organic matter is predicted. These results highlight the importance of particle surface morphology for organic matter preservation in soils and sediments.

B51C-03 0910h INVITED

Interactions between carbon and nitrogen mineralization and soil organic matter chemistry in Arctic tundra soils

Michael Weintraub¹ (805-893-5226; weintrau@lifesci.ucsb.edu)

Joshua P Schimel¹ (schimel@lifesci.ucsb.edu)

¹University of California, Santa Barbara, Dept. EEMB, Santa Barbara, CA 93106, United States

We used long term long-term lab incubations and chemical fractionation to characterize the mineralization dynamics of organic soils from tussock, shrub, and wet meadow tundra communities, to determine the relationship between soil organic matter (SOM) decomposition and chemistry, and to quantify the relative proportions of carbon (C) and nitrogen (N) in tundra SOM that are biologically available for decomposition. Despite large losses of soil C, respiration rates generally did not decline, and SOM chemistry was relatively unchanged after the incubation. The decomposition dynamics we observed suggest that tundra SOM, which is largely plant detritus, fits within existing concepts of the litter decay continuum. The lack of changes in organic matter chemistry indicates that this material had already decomposed to the point where the breakdown