B52C MCC: 3002 Friday 1340h

Microscopic and Spectroscopic Analysis of Microbes, Minerals, and Their Interactions *(joint with A, H, OS, P, V)*

Presiding: **D Stoner,** Idaho National Engineering and Environmental Laboratory**; J R Scott,** Idaho National Engineering and Environmental Laboratory

B52C-01 1340h INVITED

Use of MALDI Mass Spectrometry for Identification of Microbes

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Recently, it has been demonstrated that bacteria can be characterized using whole cells and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). However, identification of specific bacterial proteins usually requires analysis of cellular fractions or purified extracts. This presentation will discuss the first application of Fourier transform mass spectrometry (FTMS) to analysis of bacterial proteins directly from whole cells. In this research it is seen that accurate mass MALDI-FTMS can be used to characterize specific ribosomal proteins directly from Escherichia coli cells. Using the high-accuracy mass measurements and high resolution isotope profile data thus available it is possible to confirm posttranslational modifications proposed previously on the basis of low resolution mass measurements. In our initial work, ribosomal proteins from E. coli whole cells were observed with errors of less than 27 ppm. This was accomplished directly from whole cells without fractionation, concentration, or overt overexpression of characteristic cellular proteins. More recently, by use of carbon and nitrogen isotopically-depleted growth media additional E. coli proteins have been identified with even smaller mass measurement errors. MALDI FTMS also provided information regarding E. coli lipids in the low-mass region. Although ions with m/z values below 1000 were previously observed by FTMS of whole cells, the work to be presented was the first report of detection of ions in the 5000 to 10 000 m/z range by MALDI-FTMS using whole cells. The implications of these results for genus, species, and strain assignments of such organisms will be discussed.

B52C-02 1405h INVITED

3-D Raman Imagery and Atomic Force Microscopy of Ancient Microscopic Fossils

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Investigations of the Precambrian (540- to 3,500-Ma-old) fossil record depend critically on identification of authentic microbial fossils. Combined with standard paleontologic studies (e.g., of paleoecologic setting, population structure, cellular morphology, preservational variants), two techniques recently introduced to such studies – Raman imagery and atomic force microscopy – can help meet this need. Laser-Raman imagery is a non-intrusive, non-destructive technique that can be used to demonstrate a micron-scale one-to-one correlation between optically discernable morphology and the organic (kerogenous) composition of individual microbial fossils(1,2), a prime indicator of biogencity. Such analyses can be used to characterize the molecular-structural makeup of organic-walled microscopic fossils both in acid-resistant residues and in petrographic thin sections, and whether the fossils analyzed are exposed at the upper surface of, or are embedded within (to depths >65 microns), the section studied. By providing means to map chemically, in three dimensions, whole fossils or parts of such fossils(3), Raman imagery can also show the presence of cell lumina, interior cellular cavities, another prime indicator of biogenicity. Atomic force microscopy (AFM) has been used to visualize the nanometer-scale structure of the kerogenous components of single Precambrian microscopic fossils(4). Capable of analyzing minute fragments of ancient organic matter exposed at the upper surface of thin sections (or of kerogen particles deposited on flat surfaces), such analyses hold promise not only for discriminating between biotic and abiotic micro-objects but for elucidation of the domain size – and, thus, the degree of graphitization – of the graphene subunits of the carbonaceous matter analyzed. These techniques – both new to paleobiology – can provide useful insight into the biogenicity and geochemical maturity of ancient organic matter. References: (1) Kudryavtsev, A.B. et al. (2001) Proc. Nat. Acad. Sci. USA 98: 823-826. (2) Schopf, J.W. et al. (2002) Nature 416:73-76. (3) Schopf, J.W. (2003) 7th Exobiol. PI Symp. (NASA Ames): 57. (4) Kempe, A. et al. (2002) Proc. Nat. Acad. Sci. USA 99: 9117-9120.

B52C-03 1430h INVITED

X-ray Microprobe Investigations of Elemental Distributions and Concentrations at Mineral-Microbe Interfaces

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Understanding the fate of heavy-metal contaminants in the environment is of fundamental importance in the development and evaluation of effective remediation and sequestration strategies. Bacteria and the extracellular material associated with them are thought to play a key role in determining a contaminant's speciation and thus its mobility in the environment. Additionally, the metabolism and surface properties of bacteria can be quite different depending upon whether the bacteria exhibit a planktonic (free-floating) or biofilm (surface adhered) habit. The microenvironment at and adjacent to actively metabolizing cells also can be significantly different from the bulk environment. Thus, to understand the microscopic physical, geological, chemical, and biological interfaces that determine a contaminant's macroscopic fate, the spatial distribution and chemical speciation of contaminants and elements that are key to biological processes must be characterized at micron and submicron lengthscales for bacteria in both planktonic and adhered states. Hard x-ray microimaging is a powerful technique for the element-specific investigation of complex environmental samples at the needed micron and submicron resolution. An important advantage of these techniques results from the large penetration depth of hard x-rays in water. This advantage minimizes the requirements for sample preparation and allows the detailed study of hydrated samples. The objectives of the studies to be presented are (1) to determine the spatial distribution, concentration, and chemical speciation of metals at, in, and near bacteria and bacteria-geosurface interfaces, (2) to use this information to identify the metabolic processes occurring within the microbes, and (3) to identify the interactions occurring near these interfaces among the metals, mineral surfaces, and bacteria under a variety of conditions. We have used x-ray fluorescence microscopy to investigate the spatial distribution of 3d elements in Pseudomonas fluorescens cells in both planktonic and surface-adhered states. We have used x-ray fluorescence spectromicroscopy to investigate the chemical speciation and distribution of Cr that was introduced to these cells as Cr(VI). Additionally, we have used these techniques to identify the distribution of an over expressed cytochrome c7 in individual E. coli. Finally, we have used x-ray fluorescence microscopy to investigate Shewanella oneidensis MR-1 cells adhered to iron oxyhydroxide thin films. The zone plate used in these microscopy experiments produced a focused beam with a cross section (and hence spatial resolution) of 100-300 nanometers. Results from x-ray fluorescence imaging experiments indicate that the distribution of P, S, Cl, Ca, Fe, Ni, Cu, and Zn can define the location of the microbe. Additionally, quantitative elemental analysis of individual microbes identified significant changes in concentration of 3d transition elements depending on the age of the culture and the type of electron acceptor presented to the microbes. These results and a discussion of the use of this technique for identifying metabolic states of individual microbes within communities and the chemical speciation of metal contaminants at the mineral-microbe interface will be presented.

B52C-04 1455h

Evaluation of Electron Microscopic Techniques to Observe Microbe-Mineral Interactions in Anaerobic Marine Sediments

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Marine sediments contain a variety of microorganisms that help determine the local ecosystems and biogeochemistry through their metabolic activities. In anaerobic marine sediments, bacteria use inorganic acceptors to catalyze the oxidation of organic matter. In this study we discuss the advantages and disadvantages of electron microscopic techniques that we have used to study spatial relationships of the microorganisms as well as the location of the minerals within anaerobic sediments from the Santa Barbara Basin, California. Different microscopic techniques have been used to examine the spatial relationships of microbes in marine sediments (1,2,3). Epifluorescence and scanning confocal laser microscopy are useful for the identification and quantification of the living component. However, the study of microbe-mineral interactions needs more resolution to describe the contact between minerals and bacterial cells. To overcome such limitations, we used scanning electron microscopy with back-scattered electron imaging (SEM-BSE). This technique involves the sample preparation to increase the ability of the microorganisms to emit electrons when they are excited by a electron beam and the sedimentological method of embedded the sediments in a resin. The simultaneous application of the X-ray microanalytical system (EDS) permits undisturbed in situ analysis within the marine sediments of the interaction between the microorganisms and the different minerals. The final purpose is the integration a whole set of microscopies to provide an insight into the structure, composition and physical properties of the anaerobic marine sediments at a scale important for microbial ecology. 1 Bernhard JM, Bowser SS. (1996) Novel Epifluorescence Microscopy Method to Determine Life Position of Foraminifera in Sediments. Journal of Micropalaeontology 15:68 2 Bernhard JM, Buck KR, Farmer MA, Bowser SS. (2000) The Santa Barbara Basin is a Symbiosis Oasis. Nature 403:77-80 3 Bernhard JM, Visscher PT, Bowser SS. (2003) Sub-millimiter Life Positions of Bacteria, Protist,and Metazoans in Laminated Sediments of the Santa Barbara Basin. Limnology and Oceanography 48: 813-828.

B52C-05 1510h

High Spatial Resolution Study of Microbe-Carbonate-Silicate Interfaces by FIB and TEM

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High resolution transmission electron microscopy (HRTEM), chemical micro-analysis (EDX) and electron energy loss spectroscopy (EELS) are among the most powerful analytical techniques for studying microbe-mineral interactions, allowing to observe the microbe-mineral interface at almost the angstrom scale, to evidence transformations of the mineral structure and chemical alterations at the nanometer scale. However, the samples must be very thin and only a small area can be investigated. A key limitation for using this technique is thus to prepare natural geomicrobiological samples which combine hard minerals, preventing use of ultramicrotomy, with soft organic matter inadequate to ion milling procedures. Additonaly the areas of interest are usually restricted to few micrometer large areas which have to be selected from macroscopic

samples. In this study we present two procedures : micromanipulation and FIB (Focused Ion Beam) which allow the study of microbe-mineral interfaces with TEM. The micromanipulation procedure has been presented in Benzerara et al (2003, PNAS). We have evidenced nannobacteria-like objects at the surface of the Tatahouine orthopyroxenite meteorite fallen in the tunisian desert in 1931. SEM observations suggest a complex interaction pattern between the nannobacteria-like objects, the pyroxene and microorganisms which have colonized the surface of the meteorite during its seventy years of residence on Earth. The TEM study on the very same area shows that the nannobacteria-like rods are actually well-crystallized nanometric calcite single crystals surrounded by an amorphous layer of carbonate composition. Those morphologies and structures are unusual for calcite single crystals. We discuss these observations in regard to the criteria of biogenicity i.e. biosignatures. Moreover, we examine the implications for carbonate production associated to silicate bio-weathering under aridic conditions. This work is relevant both to astrobiological and geomicrobiological issues showing that it is possible to select a microbe-mineral interface of interest at the micrometer scale, and then to prepare it for TEM observation.

B52C-06 1525h

Mapping Mineral and Microbe Interactions using Laser-based Optical and Chemical Imager (LOCI)

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Our primary focus has been development of the laser-based optical and chemical imager (LOCI) that provides optical and chemical imaging of complex, heterogeneous sample surfaces. LOCI combines a Fourier transform mass spectrometer (FTMS), unique laser-scanning system, custom optics for surface fluorescence detection, and software for automated data acquisition and analysis. A key feature of LOCI is the unique laser-scanning device that provides high spatial reproducibility true depth-profiling studies and has been automated to provide imaging capabilities. The excellent reproducibility and wavelength independence of the laser-scanning device affords the opportunity to interrogate the same sample surface via optical spectroscopy prior to obtaining mass spectra. Interpretation of mass spectra has been automated using a Fuzzy-logic inference engine, which also produces the surface maps. Our current imaging interests include characterizing heterogeneous minerals, such as basalt, with and without microbe present to determine microbial affinities for specific mineral phases. Additionally, we are interested in characterizing surface contaminant speciation.

B52D MCC: 3014 Friday 1340h

Estimating Terrestrial Carbon, Water, and Energy Fluxes From Site to Region V *(joint with A, H)*

Presiding: **B Law,** Oregon State University**; D Baldocchi,** University of California, Berkeley

B52D-01 1340h

Recent Advances in Ecosystem-Atmosphere Interactions: an Ecological Perspective

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The atmosphere and terrestrial ecosystems are fundamentally coupled on a variety of time-scales. On short time-scales, this bi-directional interaction is dominated by the rapid exchange of CO_2, water and energy between the atmosphere and the land surface; on long time-scales, the interaction involves changes in ecosystem structure and composition in response to changes in climate that feed back through biophysical and biogeochemical mechanisms to influence climate over decades and centuries. Here, I explore some of the recent advances in our understanding of long-term ecosystem-atmosphere interactions then examine how efforts to assess the stability and resilience of ecosystem-atmosphere interactions over these long time-scales using Dynamic Global Vegetation Models are hampered by the presence of important functional diversity and heterogeneity within plant communities. Recent work illustrates how this issue can be addressed through the use of Structured Ecosystem Models that more accurately scale between the short-term physiological responses of individual plants and the long-term, large-scale dynamics of heterogeneous, functionally diverse ecosystems.

B52D-02 1400h

Partitioning Direct and Indirect Human-induced Carbon Sequestration in Managed Conifer Forests in Central Europe Using Model Simulations and Forest Inventories.

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Forest ecosystems have long been known to be an important sink in the global carbon budget. The factors responsible for the strength of the sinks and their permanence, however, are not so clear. To distinguish between direct and indirect impact on the carbon sequestration we have to work on spatial and temporal scales where humans have a major impact on the ecosystem. In this paper we contrast the effects of indirect human induced environmental changes and forest age on carbon sequestration as well as direct human induced effects on carbon accumulation such as forest management of coniferous forests in central Europe from the end of 19-th century until present. Ecosystem process model BIOME-BGC has been used to study these effects and results have been corroborated with forest inventories. We focused on conifer forests of Thuringia as a study case, because these forests are representative of central European forests and good forest inventories were available. Grouping the forests in three different strata differing in annual average temperatures and precipitation allowed us also to study the effect of elevation on carbon sequestration. Forests in all elevational strata showed an increase in vegetation carbon accumulation in all age classes as a result of environmental changes in the last 20 years (1982-2001). Young and old trees had the highest annual changes in the vegetation carbon during this period. Under pre-industrial conditions, trees older than 80 years showed almost no annual increase in vegetation C carbon accumulation. With industrial climate scenario those trees were still carbon sinks. Trees older than 100 years in the low elevations were an atmospheric carbon source in the pre-industrial case, but carbon sinks in the industrial case. Nitrogen-deposition had the highest impact on the net ecosystem production (NEP) for the high and middle elevations, whereas CO_2 fertilization was most responsible for NEP changes in the low elevations. Under current environmental conditions, the total modeled carbon accumulation in the coniferous forests of Thuringia was estimated at 2.09 t C $ha^{-1}y^{-1}$ with an averaged annual NEP of 1.78 t C $ha^{-1}y-1$ and net biome production (NBP) of 1.12 t C $ha^{-1}y^{-1}$. Once corrected with the actual forest harvest statistics, the modeled NBP estimate was reduced to 0.83 t C $ha^{-1}y^{-1}$. Under pre-industrial conditions the NBP estimates were 0.43 and 0.15 t C ha-1 y-1 respectively. The environmental changes led to the sink strength of 62% or 82% depending on how the harvest was estimated. Based on the estimates of annual accumulated vegetation carbon (NBP_{VEG}), the forest inventory data gave NBP_{VEG} varying from 0.45 to 2.6 t C $ha^{-1}y^{-1}$ depending on the method used for estimating the wood increments. The modeled NBP_{VEG} was estimated at 1.42 t C $ha^{-1}y^{-1}$. Environmental changes or indirect human impact resulted in 44% increase in modeled vegetation carbon accumulation of conifer forest in Thuringia. From the forest inventory data we estimated the effect of changing area of the forest age classes, or the direct human impact on the carbon sequestration, to be approximately 37%.

B52D-03 1420h

The Effects of Shading and Diffuse Radiation in Estimating Carbon and Water Fluxes in Tropical and Midlatitude Sites

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The interactions between radiation, water, and carbon are a crucial component in determining terrestrial carbon and water fluxes. In this paper, we will present evidence to demonstrate the close relationship between these processes in an integrated climate system using the Simplified Simple Biosphere Model (SSiB, Xue et al., 1991). The SSiB implemented Collatz et al' (1991, 1992) parameterizations of plant photosynthesis and stomatal conductance to consider CO2 assimilation of vegetation. Quasi-analytical solutions of these parameterizations were developed (Zhan et al., 2003) to improve the computational efficiency and to produce stable solutions for long-term simulations in GCMs and regional models. We tested this enhanced SSiB using observational data from LBA, Boreal, and AmeriFux sites. The results indicated that the model in general produced a higher than normal rate of photosynthesis which led to an overly large transpiration. We examined model performance and found that this was mainly caused by the scaling methodology. In our earlier approach described above, the sunlit and shaded leaf areas were not considered. Furthermore, only direct radiation effect was included in the scaling equation, which was adapted from SiB2. Diffuse radiation, which arises from atmospheric scattering and from scattering within the canopy, has been shown to have a crucial role in the photosynthetic process (e.g., Norman, 1982; Baldocchi, 1997). Therefore, we developed a new parameterization for shading and scaling to more realistically simulate the land/atmosphere interaction processes. The shading parameterization is based on Norman's approach (mainly relies on solar zenith angle), but we further take vegetation properties and solar radiative transfer property within canopy into consideration. The scaling method considers the effects of both direct and diffuse radiations. We have tested the new method using the observational data from the LBA experiment (2000) and the Boreal experiments (1996). This new method substantially improved the simulations of daily mean carbon and water fluxes and their diurnal variations, especially in the tropical area. For example, in the LBA experiment, the root-mean-square (RMS) error for the latent heat flux and the carbon flux are 15.1 w m-2 and 2.9 mmol m-2 s-1, respectively, which are much smaller than the RMS error 45.4 w m-2 and 12.7 m mol m-2 s-1 in the simulation with the old method. Experiments are also conducted to comprehensively test the effects of soil moisture, diffuse radiation, and vegetation properties in estimating the variability of carbon and water fluxes.

B52D-04 1440h

Old and Not-So-Old: Examining Changes in Forest Ecosystem Carbon Exchange With Stand Age in the Upper Midwest U.S.

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Forest stand age is an important determinant of ecosystem carbon uptake. Though there are biometric measurements and ecological models for forests of all ages, there are few stand-scale eddy-flux measurements of net carbon exchange in older forests, though the number is increasing. In order to scale carbon fluxes from sites to regions, where stands of multiple ages may exist, it is necessary to measure to the effect of stand age on carbon exchange. Measuring the effect of stand age on carbon exchange is also necessary when trying to predict future or past carbon exchange (scaling across time). Many researchers have noted that site disturbance history is the fundamental factor in determining carbon uptake by forests over time scales of