

# Microbial Biogeochemistry, Biogeochemical Processes, and Microbial Ecology

## Introduction

Biogeochemistry is a research area whose rapid emergence in recent decades around the world has been fueled by recognition of the interlocking roles of biological, chemical and physical forces in controlling processes vital to sustaining life. The chemical balance of the ocean and of the atmosphere, and the habitability of Earth, is largely maintained by the activities of microorganisms, the chemical engines of the biosphere. Without microorganisms, essential processes that maintain the habitability of the Earth – organic matter degradation, recycling of CO<sub>2</sub> and other greenhouse gasses, nitrogen fixation from the atmosphere – would cease.

Microorganisms form microbial ecosystems whose functional complexity is mirrored by their evolutionary diversity. Gene-based environmental surveys show that more than 99% of the microorganisms in the marine environment have not yet been cultured in the laboratory, with the consequence that their metabolism, geochemical activities, and application potential are unknown. Despite our ignorance of the roles and responses of specific organisms as part of microbial communities, these communities are largely responsible for determining the gross environmental boundaries within which we exist. Human impact on the natural environment via land-use, changes in water balances, and nutrient additions, all affect microbial communities, whose responses can impact human health, recreation, and agriculture. Our understanding of these specific linkages, however, is still rudimentary.

Given the rapidly emerging fields of microbial ecology and biogeochemistry, multi-disciplinary research groups that combine new approaches in microbial genetics and physiology with expertise in geochemistry, measurements of microbial process rates and chemical fluxes, and analytical chemistry, are essential. The Department of Marine Sciences has been able to assemble just such a group. The goal of this group is a full understanding of biogeochemical processes in key environments and ecosystems, and of the evolutionary and physiological diversity, and functional complexity, of microorganisms in the natural environment. Towards this goal, we are integrating the research perspectives and approaches of microbial ecology and geochemistry, by combining studies of microbial community structure (Teske, MacGregor; also see Noble's and Pearl's research described later in this Chapter) with investigations of microbially catalyzed processes (Martens, Alperin, Albert, and Arnosti).

An essential part of our work involves students, from undergraduates who receive research credit, to Ph.D. students working quasi-independently on basic research

questions. Students with interests in geochemistry and microbiology or related topics benefit from the network of scientific resources and national/international contacts at the Department of Marine Sciences, and have immediate access to expertise and support in a variety of different disciplines and laboratories. Advisors actively promote this opportunity to gain substantial research experience not just in a single focus topic, but to develop a network of mutually complementary skills and expertise that prepares a student for the rapidly evolving, multidisciplinary research reality in the wider world.

### Research Focal Points

#### *Teske Lab:*

The general research challenge for the Teske Lab is to link microbial identity and function, and to understand microbial ecosystem diversity function in correlation with in-situ biogeochemical regimes and processes. The Teske Lab is focusing on microbial community composition and function in extreme marine habitats, using nucleic-acid based approaches. The vast majority of prokaryotic phylogenetic lineages has not been cultured yet, and are accessible only with gene-based methods. The occurrence patterns of uncultured microbial groups are correlated with geochemical and physical characteristics of their habitats, as a first step to infer their physiological preferences and environmental controls. Further, identification of functional genes allows conclusions about the metabolism of extremophilic microbial communities.

#### • **Microbial communities of deep-sea hydrothermal vents**

The study the microbiology of deep-sea hydrothermal vents one of the most extreme microbial habitats on earth is another component of the Teske Lab. A black smoker hydrothermal vent at 21°N on the East Pacific Rise (2600 m depth; see figure 3.1) emits hydro-thermal vent fluid of ca. 300 – 350 °C. The walls of the chimney structure, deposited from metal sulfides dissolved in the vent fluid, are inhabited by extremely thermophilic and metal-resistant microorganisms.

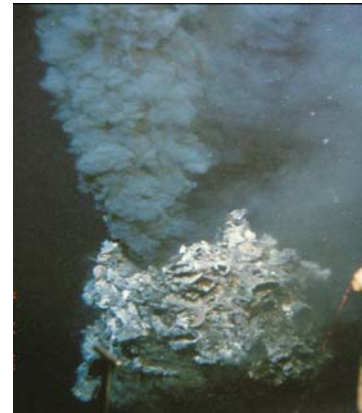


Figure 3.1

#### • **Microbiology of Anaerobic Methane Oxidation (Teske and Martens labs)**

The microbiology of anaerobic methane oxidation in hydrothermal and cold marine sediments constitutes a shared interest of the Teske and

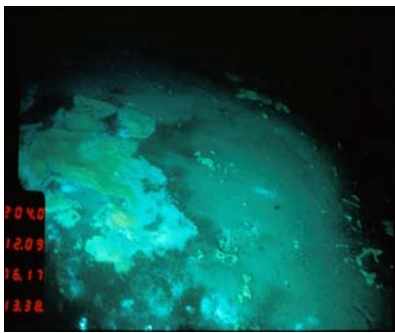


Figure 3.2

Martens labs, and has led to collaborative ties and joint proposals. A focus site of the Teske lab is the Guaymas Basin, a sedimented hydrothermal vent site in the Gulf of California. Here, thermal degradation of sedimentary organic matter produces thermogenic methane that supports anaerobic methane-oxidizing communities. The first occurrence of anaerobic methane oxidation in hydrothermal environments was found by the Teske group at Guaymas. A newly-submitted NSF proposal (Teske, Martens, Albert & MacGregor) focuses on a joint geochemical and

microbiological analysis of this vent system. Graduate students Karen Lloyd (Teske lab) and Laura Lapham (Martens lab) are working on a combined geochemical and biological analysis of a methane-rich sediment community in the Gulf of Mexico.

• **Community Analyses of Deep-Subsurface Sediments**

The sediment cover of the ocean bottom is usually several 100 meters thick and permeated by microbial life top to bottom. This is perhaps the most extensive and least explored microbial habitat on Earth. The structure and metabolic activities of these deep subsurface communities are a current research challenge. The Teske Lab is analyzing the microbial community composition in a range of geochemically divergent subsurface sediments (Nankai Trough; Peru Margin and Equatorial Pacific; Juan de Fuca Ridge flank). This project involved two recent postdocs (Ketil Sørensen and Antje Lauer), and graduate students Mark Lever and Karen Lloyd. Deep subsurface projects also include a recently-submitted joint NSF proposal with the Noble lab that aims at the detection and DNA/RNA analysis of subsurface viruses. Since 2000, the Teske lab has been working with the Ocean Drilling Program and its successor, the International Ocean Drilling Program (IODP). On recent ODP and IODP cruises targeting the deep subsurface biosphere, members of the Teske lab sailed on JOIDES Resolution (Figures 3.3a and 3.3b), the deep-sea drilling vessel for retrieving otherwise inaccessible deep subsurface samples.



Figure 3.3a

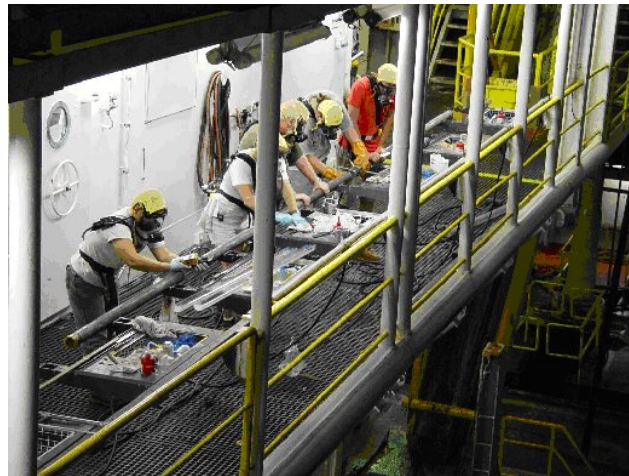


Figure 3.3b

Much of this work is carried out with colleagues at the Univ. Rhode Island and with the Marine Biological Laboratory (Woods Hole, MA).

***Martens Lab:***

Martens' group research focuses primarily on key processes controlling the biogeochemical cycling of carbon and nitrogen in organic-rich marine and tropical ecosystems. The work includes combined studies of microbial transformations and physical transport processes at well-characterized field sites including tropical forest, wetland, estuarine, nearshore and deep-sea environments. Emphasis is placed on

investigating exchange processes and net chemical fluxes between sediments, water column and atmospheric reservoirs.

- **Amazonian tropical forests as CO<sub>2</sub> and trace gas sources and sinks**

The need to understand the role of the Amazon and other tropical forests as globally significant CO<sub>2</sub> sinks and potential sources or sinks for other radiatively important trace gases such as CH<sub>4</sub> and N<sub>2</sub>O, resulted in a major international research program led by Brazil, the Large-scale Biosphere Atmosphere (LBA) Program. The program includes an emphasis on the impact of land-use change on CO<sub>2</sub> and trace gases fluxes. Initial gas flux measurements yielding net CO<sub>2</sub> uptake relied entirely on one-dimensional eddy covariance measurements until the Martens group developed an independent method for quantifying forest canopy-atmosphere gas exchange rates over long time-periods (months to years) using continuous measurements of in- and above-canopy radon-222 activity in combination with determination of the radon flux from underlying soils. We have invented, designed and built a new generation of pulsed ionization continuous radon analyzers at UNC that have functioned in Brazil year-round since April 2000. Using these analyzers, we have been able to demonstrate that the eddy covariance methods were unable to accurately measure nighttime CO<sub>2</sub> losses (Martens et al., 2003), thus producing the net CO<sub>2</sub> sink result. Our data are also proving useful for investigating soil gas release processes and fluxes in Brazilian Amazonia. Our radon array systems are also now in use at one of the DOE-funded FACE (Free Air CO<sub>2</sub> Exchange) sites in Duke Forest and at several other sites along the NC coast where we are experimenting with tracing continental-maritime air exchange processes.



Figure 3.4a



Figure 3.4b

- **The role of marine sponges in the C and N cycles of coral reef and nearshore waters.**

Our NSF and NURC projects are directed at understanding the role of marine sponges in the carbon and nitrogen cycles of coral reef ecosystems. Sponges are an important part of these endangered ecosystems, yet little is known about their role in the transformations and cycling of key nutrient elements, particularly nitrogen. The stable C isotopic composition of sponges along natural environmental gradients in the Florida Keys exhibit significant spatial gradients related to organic carbon sources whereas N isotopes divide sponges into two distinct groups. Sponges hosting large populations of bacteria are more

hypoxic, pump less water and have excurrent waters enriched in nitrate rather than ammonium, signaling microbial nitrification. Hypoxic conditions in bacteriosponges provide potential for other N transformations including N<sub>2</sub> fixation and denitrification. The potential impacts of these processes on the overall nutrient budget of the reef system are large; we are developing collaborative experiments with a fluid dynamics group at Stanford in order to obtain quantitative N flux results. Collaborators include UNC Ph.D. students, Niels Lindquist, Dr. Brian Popp and Jan Riechelderfer (U. Hawaii), and Prof. Dr. Ute Henschel and Dr. Susanne Schmidt (Univ. Würzburg, Germany).

• **Biogeochemical processes controlling formation and decomposition of methane hydrates**

Our NOAA funded research on methane hydrates is focused on novel submersible and ROV deployed probes combined with *in situ* biogeochemical rate measurements to investigate biogeochemical processes controlling their formation and decomposition in Gulf of Mexico margin sediments. The biogeochemical processes data is combined with geophysical data in order to quantify the extent and nature of *in situ* processes controlling the formation and decomposition of exposed and buried gas hydrate deposits. We have

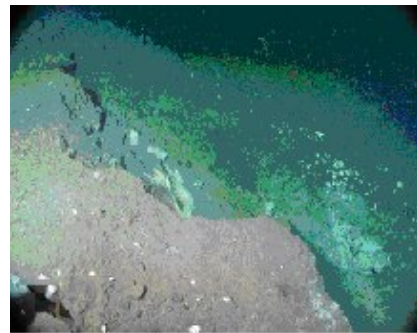


Figure 3.5

successfully deployed methane pore water probes from submersibles and ROVs obtaining the world's first un-decompressed methane gas samples for both chemical and stable isotopic analysis. The results provide information about both methane gas sources and microbial transformations including methane consumption via anaerobic methane oxidation, a process first hypothesized in 1974 (Martens and Berner, *Science*). Work on methane fluxes from sediments at hydrate sites will include continuous remote water column and seafloor measurements with a team of Woods Hole scientists led by Jean Whelan and Rich Camilli, using novel *in situ* mass spectrometer and chemical sensors in the Gulf at approximately 850m depth (See Figure 3.6).

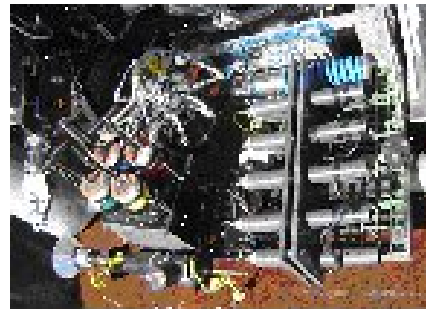


Figure 3.6

***Arnosti Lab:***

The focus of work in the Arnosti lab is to combine chemical approaches with aspects of microbiology to investigate the structure and reactivity of macromolecular organic matter and the role of bacterial communities in organic matter degradation. The rationale behind this research is the fact that although most organic matter in marine systems is initially produced as macromolecules whose production and structure are reasonably well understood, the processes by which these macromolecules are transformed and (for the most part) ultimately remineralized by marine microbes are largely unknown. The identities and specific activities of the vast majority of microorganisms which catalyze these processes are also unknown. Microbes play key roles in the transformations, decomposition, and recycling of marine organic carbon, and are believed to be active in

nearly all known marine environments, ranging from surface waters to sea ice to anoxic sediments, hydrothermal systems, and the deep biosphere. Microbial responses to organic macromolecules in turn help shape the nature, concentration, and characteristics of organic matter which is preserved, as well as the rate, extent, and location of organic matter remineralization in marine systems. Their activities and interactions therefore are major forces shaping the global carbon cycle.

#### • **Carbon Transformations Under Permanently Cold Conditions**

A long-term NSF-funded collaboration with scientists from the Max-Planck Institute for Marine Microbiology (Bremen, Germany) is focused on studying carbon transformations in permanently cold sediments, conditions characterizing ca. 90% of ocean sediments. We seek to determine the means by which sedimentary microbial communities maintain high rates of activity under conditions that inhibit the activities of their more temperate counterparts. Part of this work is a collaboration (Arnosti/Teske labs) to isolate and characterize pure cultures of cold-active, extracellular-enzyme producing bacteria. Fieldwork for this project is conducted on Svalbard, an archipelago in the high Arctic. (Figure 3.7: sampling in Kongsfjorden; Figure 3.8: the Tre Kroner, from the field station in Ny Ålesund, the world's northernmost permanently inhabited settlement)



Figure 3.7



Figure 3.8

#### • **‘Speed Bumps’ in the Carbon Cycle**

Although the importance of microbial communities in the global carbon cycle is undisputed, the rates and specific pathways by which organic carbon is transformed from macromolecules to CO<sub>2</sub> are not well understood. This project (NSF and ACS funding) focuses particularly on identifying potential ‘speed bumps’ in carbon remineralization pathways. We are investigating factors that may help control expression, activities, and ‘lifetimes’ of extracellular enzymes, which initiate the degradation of high molecular weight organic carbon in marine systems. We are also investigating carbon flow—from high molecular weight substrates through fermentation to remineralization—in order to identify other possible locations of ‘speed bumps’. Part of this work is being done in collaboration with Dr. Volker Brüchert at the Max-Planck Institute in Bremen. Other aspects of this project—determining the potential influence of surface sorption on enzyme activities and lifetimes—are being carried out in collaboration with Dr. Neil Blough, at the University of Maryland-College Park.

### •Biocatalytic Filtration and Carbon Cycling in Permeable Shelf Sediments

In collaboration with Drs. Markus Hüttel (Florida State), Joel Koska (FSU), and Peter Berg (UVA), we are investigating the rates and means of carbon cycling and quantifying organic matter flux through permeable sands. The objective is to determine the role of permeable shelf sediments (70% of continental shelf area) in coastal cycling of carbon and nutrients. Fieldwork for this new NSF-funded project begins in the summer of 2005.

### *MacGregor Lab:*

Barbara MacGregor is a microbial ecologist, whose research interests may be described as “Who is there, and what are they doing?” – simple questions, but difficult to answer for the complex mixture of species often found in natural microbial communities. Molecular biological methods based on detection, sequencing, and phylogenetic identification of ribosomal RNA (rRNA) and the genes encoding it (rDNA) now allow description of microbial populations without isolation and culturing. rRNA is found in all species, it shows little evidence of cross-species transfer, and its production is growth-rate regulated, making it a good indicator for the presence of active microbial populations. Specific RNA or DNA sequences are amplified from environmental samples by the polymerase chain reaction (PCR), and compared with known sequences to infer their line of descent. There are a variety of methods for estimating the concentrations of different rRNAs or rDNAs in a sample, giving an idea of the community composition. However, because “functional” (enzyme-encoding) genes can be exchanged among even distantly-related species, rRNA phylogeny is not a reliable guide to physiology: for example, it cannot be assumed that a new rDNA sequence comes from a photosynthetic species, even if its closest known evolutionary relatives are photosynthesizers.

### •New methods to directly link species identity with carbon source utilization

This linkage is being established via carbon isotopic characterization of rRNA, either by taking advantage of natural-abundance differences in  $^{13}\text{C}/^{12}\text{C}$  ratios among substrates, or by addition of  $^{13}\text{C}$ - or  $^{14}\text{C}$ -labeled substrates. In collaboration with Dr. Nicole Dubilier (Max Planck Institute for Marine Microbiology; Bremen, Germany), we are measuring the carbon isotopic composition of host and symbiont rRNAs to determine the contribution of methane-oxidizing symbiotic bacteria to the growth of mussels living near oceanic hydrocarbon seeps and vents. Because methane is naturally depleted in  $^{13}\text{C}$ , methane-derived compounds tend to be isotopically “light” compared to those produced from other carbon sources.

Together with Dr. Dieter Juchelka (Thermo Electron GmbH, Bremen, Germany), we have recently developed a technique to measure the isotopic composition of individual nucleic acids in an RNA sample, using an HPLC/isotope-ratio mass spec approach. This approach will greatly reduce concerns about sample purity, since a single compound is measured at a time. We hope this technique will allow us to work with smaller samples that will be easier to obtain from natural settings. A joint proposal (MacGregor, Martens, Teske & Arnosti) for instrument acquisition has been submitted to NSF in order to purchase an IsoLink interface and to establish the technology at the MASC Department.

### •**Direct profiling of microbial communities without PCR**

We are also developing methods for rRNA-based profiling of microbial communities without a PCR step, to directly identify the species using different carbon substrates. We have found that rRNAs of similar size but different sequences can be separated on non-denaturing polyacrylamide gels. Adam Friedman, a UNC undergraduate, is working with Dr. Stuart Strand (University of Washington – Seattle) to identify bacteria taking up radiolabeled acetate in an activated sludge wastewater treatment plant. A better understanding of microbial processes in treatment plants should help make them more efficient and reliable.

### •**Direct detection of microbial enzymes in environmental samples**

As part of a collaboration (MacGregor/Arnosti/Teske labs), we are exploring the possibility of directly identifying the microbial enzymes involved in degrading high molecular weight carbon compounds. From the amino acid sequences of these enzymes, we hope to be able to work backwards, using RNA- and DNA-targeted probes, to find and identify the species that produced them.

### *Alperin Lab:*

Marc Alperin's work is focused on physical and biogeochemical processes in riverine, estuarine, and coastal marine sediments. The overall strategy is to combine field measurements, laboratory experiments, and numerical models to constrain carbon, nitrogen, sulfur, and metal cycling in a variety of biogeochemically active sedimentary environments. Field approaches used include concentration profiling, rate measurements using isotopic tracers, and natural stable- and radio-isotope distributions. Examples of specific projects are described briefly below:

#### • **Impact of Sediment Processes on Estuarine Water Quality**

Sediment-water interactions play an important role in regulating water quality in many estuaries. In these systems, the ratio of bed area to channel volume is high, the freshwater flushing time and sediment contact time are long, and a large portion of the organic matter produced in the water column settles to the sediment surface. Sediment processes influence the water column in three ways: (1) by serving as a long-term repository of oxygen demand, (2) by recycling fixed nitrogen (primarily ammonium) to the water column, and (3) by serving as a sink for nitrate via denitrification. We are conducting field measurements and long-term laboratory experiments to understand the controls on benthic oxygen and nutrient fluxes, sediment denitrification rates, and the response time of the sediment system to long term changes in nutrient loading.

#### • **Benthic-boundary layer dynamics**

We are developing a sediment process model that simulates interactions between the water column and sediment. The model is designed as a module for coupling with a pelagic water quality model, but can also function in the stand-alone mode. Emphasis has been placed on capturing the details of the sediment-water interface—in particular, the diffusive boundary layer—in order to accurately simulate the impact of bottom water currents and concentrations on sediment processes and benthic fluxes. The sediment process model provides a tool for making quantitative predictions as to how sediment

oxygen demand, benthic ammonium flux, and denitrification rates will respond to a legislated reduction in nitrogen loading. The modeling project involves collaborations with members of the Computer Sciences Department at UNC-Chapel Hill as well as the Engineering Department at UNC-Charlotte.

- **Geomicrobiological Models**

Micro-scale reaction-transport models (also known as “geomicrobiological models”) are ideal for understanding the “thermodynamic ecology” of microbial symbionts. These models are useful for testing specific hypotheses, checking for internal consistency in field data, and can guide the optimization of experimental protocols. We have been working on a micro-scale model of anaerobic methane oxidation. Novel aspects of our high-resolution (~0.1 nanometer) model include: implicit coupling between reaction-transport physics and thermodynamic energy yield; realistic geometry of microbial aggregates; and differential transport kinetics and metabolic functions for microbial cell components such as lipid membrane, cell wall, and cytosol. See Figure 3.9.

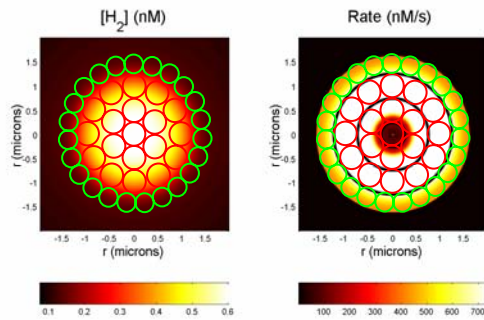


Figure 3.9: Results of micro-scale radial diffusion model simulating archaea-sulfate reducing bacteria interactions in microbial aggregates. The model shows that build-up of molecular hydrogen inhibits methane oxidation by archaea in the interior of the aggregate.

- **Water quality patterns in urban streams**

We are collaborating with faculty from the Geography Department to investigate water quality patterns in the major stream network draining the UNC campus. This area is

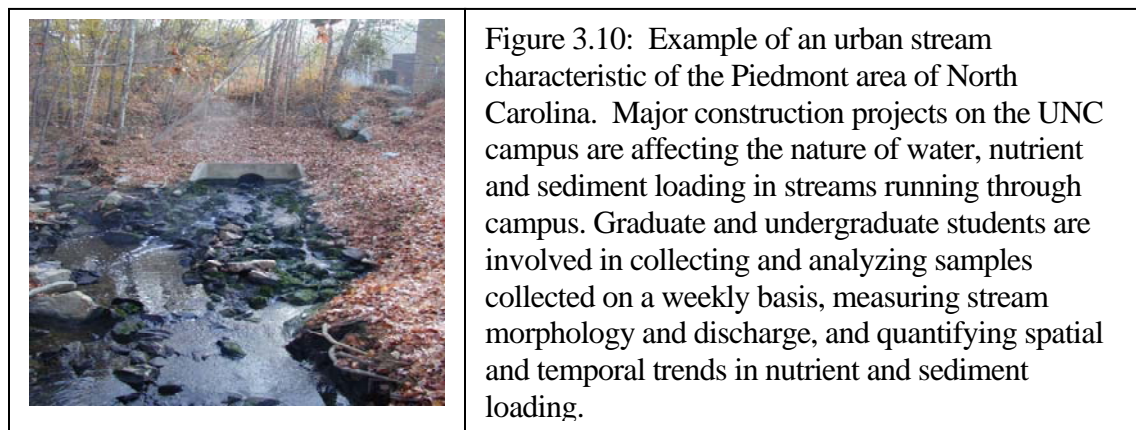


Figure 3.10: Example of an urban stream characteristic of the Piedmont area of North Carolina. Major construction projects on the UNC campus are affecting the nature of water, nutrient and sediment loading in streams running through campus. Graduate and undergraduate students are involved in collecting and analyzing samples collected on a weekly basis, measuring stream morphology and discharge, and quantifying spatial and temporal trends in nutrient and sediment loading.

heavily developed with a dense academic core, major athletic facilities and a large biomedical and hospital complex. We are particularly interested in establishing nutrient source areas as they vary through time and space with wetness conditions and groundwater levels in areas of different ground cover, land management and development

activities, and retention processes resulting from sediment/water interactions. Information generated through the project is being made publicly available through web sites and other outlets, and the project will effectively help to establish the campus as an outdoor laboratory.

***Albert Lab:***

Dan Albert's research is aimed broadly at understanding the cycling of carbon, nitrogen, and sulfur in sedimentary environments and microbial mats. He is interested in the rates and mechanisms of organic matter decomposition in anaerobic systems, particularly the coupling of fermentative and terminal metabolic processes (e.g., sulfate reduction and methanogenesis) and the specific roles of low-molecular weight fermentation products, such as formate, acetate, and propionate, in this coupling. He has studied these processes in coastal sediments for several years, and has also worked on the generation of these organic acids in high-temperature hydrothermal settings, where they are formed abiotically through thermal breakdown of organic materials in sediments (Guaymas Basin) and through presumed reaction of CO<sub>2</sub> with hydrogen in non-sedimented hydrothermal settings (Juan de Fuca ridge sites). He is seeking funding to do further work in Guaymas on sulfur and organic acid cycling in collaboration with the Teske and Martens labs (see above) and independently to pursue work on the Juan de Fuca ridge.

**• C, N, and S-cycling in hypersaline mats**

This study (funded by NASA's Astrobiology program), which involves several researchers from the NASA-Ames lab and other universities, seeks understanding of carbon and energy flow from phototrophs to heterotrophs in two mat types from hypersaline lagoons in Guerrerro Negro, Mexico. Cyanobacteria in the mats store glycogen during daylight hours and ferment this glycogen at night, producing hydrogen, lactate, acetate, formate and propionate. This fermentation is used, in part, to fuel nitrogen fixation by the cyanobacteria. The fermentation products serve as substrates for heterotrophs living in the mat, such as sulfate reducing bacteria, which are also capable of nitrogen fixation. We are investigating this sharing of resources as a syntrophic interaction between the members of this type of primitive community. Phototrophs fermenting glycogen benefit thermodynamically from the removal of their fermentation products, while the heterotrophs receive a steady diet of desirable (albeit low nitrogen) substrates that enables them to grow and fix their own nitrogen, if necessary. The result is greater nitrogen fixation by the community as a whole.