Biological and Environmental Research

Oak Ridge National Laboratory

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OAK RIDGE
National Laboratory

Annual Report FY 2010

Oak Ridge National Laboratory (ORNL) conducts basic and applied research and development to create scientific knowledge and technological solutions that strengthen the nation's leadership in key areas of science; increase the availability of clean, abundant energy; restore and protect the environment; and contribute to national security.

OAK RIDGE
National Laboratory

U.S. DEPARTMENT OF ENERGY | Office of Science

Foreword

Martin Keller
Associate Laboratory Director
Biological and Environmental Sciences
Oak Ridge National Laboratory

Fiscal year 2009 was a year filled with change and amazing progress for the Biological and Environmental Sciences (BES) Directorate. Between Dr. Reinhold Mann's departure in November 2008 until my arrival in June 2009, Dr. Gary Jacobs served as our interim Associate Laboratory Director. I know I speak for everyone in BES and across ORNL when I thank Gary for all his hard work and the invaluable support he provided during this transition period.

A collaborative and interdisciplinary approach to biological research—a New Biology—is recommended in *A New Biology for the 21st Century*, a report recently published by the national academies. Prepared by a committee of representatives from the National Academy of Sciences, the National Academy of Engineering, the Institute of Medicine, and the National Research Council, the report examines the current state of biological research and recommends how the United States might best capitalize on recent technological and scientific advances. Several multidisciplinary projects of key societal importance are suggested that include adaptation of food plants to local growing conditions, ecosystem monitoring and restoration, combining crop research and microbial engineering to make biofuels a viable alternative to fossil fuels, and personalized medicine to improve health care. This new approach requires greater integration within areas of biological research, closer collaboration with scientists of different disciplines, and increased leveraging of resources across federal, private, and academic sectors. Although our directorate is already actively engaged in many of these recommendations and suggested multidisciplinary projects, we, and all of ORNL, continue to expand our cross-disciplinary approach among our many areas of research in an effort to provide solutions to the world's major technological challenges.

In this spirit, we continued to support cross-cutting research teams and projects at ORNL to further advance science and contribute to the missions of the Department of Energy. The multidisciplinary BioEnergy Science Center (BESC) has already made substantial progress toward the goal of overcoming biomass recalcitrance. Using an integrated approach to solving the challenge of producing biofuel from cellulosic materials, the center will not only provide the scientific basis for affordable and sustainable biofuel but will also advance our understanding in areas of fundamental biological science. After 2 years of operation, BESC has already achieved scientific breakthroughs that demonstrate proof of concept and has accelerated the initial 5-year timeline by more than 1 year. The center has also managed to integrate its scientific activities and demonstrate considerable synergy beyond individual research projects. BESC researchers have published over 100 scientific articles, 60% of which represent multi-institution collaborations both within BESC and throughout the scientific community.

ORNL recently initiated a cross-cutting Climate Change Science Institute (CCSI) with the goal

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of further aligning climate change science across the Laboratory. Under the leadership of Dr. Jim Hack, staff from various directorates will focus their attention on four thrust areas: Carbon Cycle and Ecosystem Science; Earth System Modeling; Climate Data Integration, Dissemination, and Informatics; and Climate Impacts, Vulnerabilities, and Adaptation Science. I look forward to their future contributions in this area of research.

ORNL also established the Center for BioEnergy Sustainability (CBES) to bring together the unique skills and technical expertise of the ORNL staff in addressing sustainability issues of national and global concern related to environmental impact and biomass production, and the conversion of biomass to biofuels and bio-based products. The mission of CBES is to better understand the sustainability (environmental, economic, and social) of current and future bioenergy production and distribution; to identify approaches that enhance bioenergy sustainability; and to serve as an independent source of the highest quality data and analyses available to bioenergy stakeholders and decision-makers. The CBES provides opportunities for ORNL scientists and other stakeholders to work collaboratively and develop strategies that best address these challenges.

Another area of focus this year was the implementation of the new BER Office of Science funding models. Scientific projects will now be divided into Science Focus Areas (SFA). We have established SFAs in Fundamental Science, Bioenergy, Climate Change Research, Subsurface Science, Ethical, Legal and Societal Implications, Low-Dose Radiation, and Radiochemical Imaging. These SFAs will provide us additional opportunities to create research programs spanning various scientific disciplines.

Our Directorate, consisting of the Biosciences Division and the Environmental Sciences Division, covers a wide span of business lines that includes bioenergy, climate and environmental change, stewardship science, data and bionformatics, biomedical science, and sensor technology. Our research involves scientists from across ORNL as well as other institutions as we move more and more toward a multidisciplinary culture.

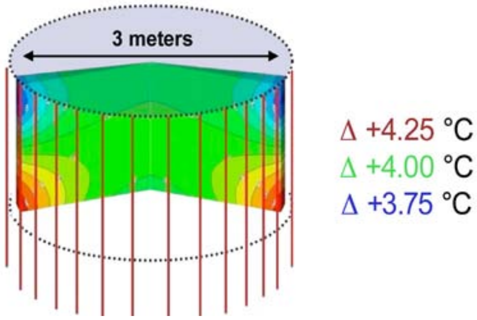
I hope you will enjoy reading about our recent research and the inspiring work being performed by our staff as we continue to collaborate with other scientists across the nation and around the world in an effort to provide viable and timely solutions to the serious scientific and technological challenges facing our world today.

Research Highlights

CLIMATE

A method for experimental heating of intact soil profiles for climate change experiments

A multidisciplinary group of ORNL scientists conceived, developed, simulated, constructed, and tested a new experimental system for simulating future belowground temperature increases in a temperate deciduous forest in Oak Ridge. The new system uses low-wattage, 3-m-long deep heaters installed around the circumference of a defined soil volume. The heaters provide the energy necessary to achieve a set soil temperature differential within the treatment area and soil volume and additional energy equal to that which might be lost as a result of lateral heat conduction away from the treatment area. The new method, which was designed to work in conjunction with aboveground heated chambers, requires only two control sensor positions—one for aboveground air temperatures at 1 m and another for belowground temperatures at a defined soil depth beneath the biologically active layers of the soil. The method was demonstrated to be capable of achieving target temperature differentials throughout an annual cycle while maintaining both diurnal and seasonal cycles at all soil depths. This new method is being applied at larger spatial scales to in situ studies of a broad range of temperature differentials to understand plant and ecosystem process-level changes leading to altered organism vulnerability to climate change or greatly modified net carbon exchange rates. Understudied systems at high latitudes, characterized as having high amounts of stored soil carbon, are one logical target for next-generation studies.



Prototype warming chamber and belowground performance simulations for deep belowground warming

P. J. Hanson, K. W. Childs, S. D. Wullschleger, J. S. Riggs, W. K. Thomas, D. E. Todd, and J. M. Warren. "A method for experimental heating of intact soil profiles for application to climate change experiments," **Global Change Biol.** (2010), DOI:10.1111/j.1365-2486.2010.02221.x.

Forty-year record of tree growth and succession clarifies climate sensitivity of eastern forests

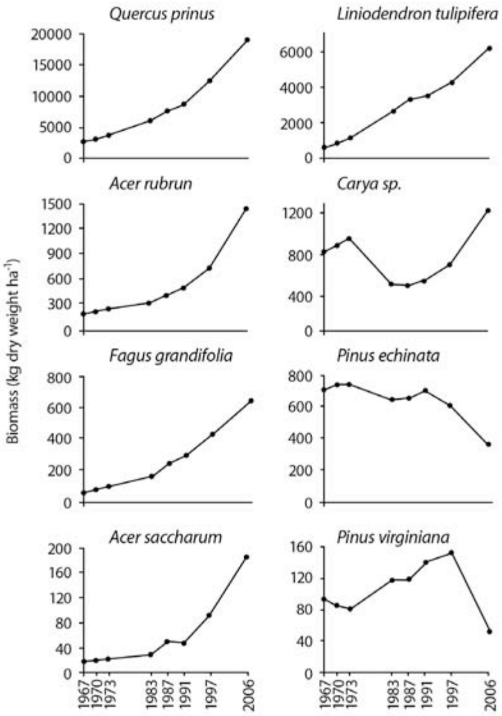
The importance of species, succession, and climate on forest composition and biomass accumulation was investigated by ORNL scientists using a long-term data set (1967–2006) of tree diameter growth and survival taken at a mixed young forest at the Walker Branch

Research Highlights

Watershed near Oak Ridge. Over the period of study, forest communities underwent successional change and substantially increased in biomass.

Possible differences in the sensitivity of various tree species to variations in climate and initial stand composition were analyzed. Summer temperatures and drought were found to affect biomass accumulation in some species, and *Pinus echinata*, the dominant species in pine stands, decreased over time due to periodic outbreaks of the pine bark beetle (*Dendroctonus frontalis*).

The results of this study indicated that the direct effects of climate variability on the biomass accumulation and composition of eastern hardwood forests were small in comparison to changes resulting from natural succession or insect outbreaks. The sensitivity of the developing forest to changes in climate was found to be a function of species composition. However, in the long term, the direct effect of climatic variability on forest dynamics may be small relative to successional processes or stand-modifying insect outbreaks. Empirical studies that examine the interactions between forest succession and climatic variability are needed.

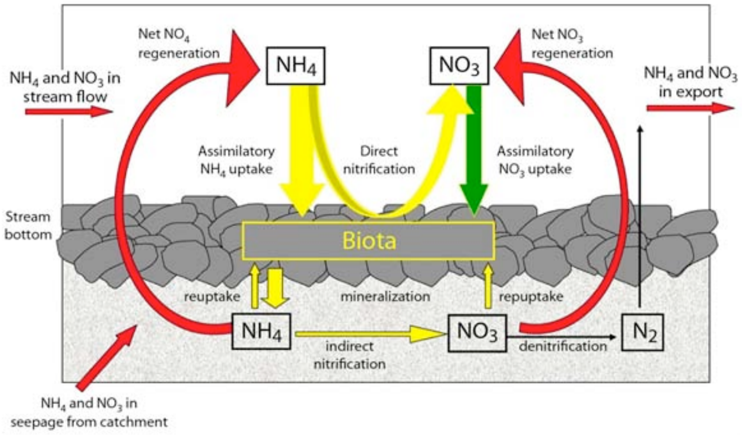


Biomass accumulation at a mixed young forest at Walker Branch Watershed

P. Kardol, D. E. Todd Jr., P. J. Hanson, and P. J. Mulholland. “Long-term successional forest dynamics: species and community responses to climatic variability,” **J. Veg. Sci.** 21(4): 627–642 (August 2010).

Nutrient dynamics in streams

Nutrient dynamics in streams has been an important topic of research since the 1960s. Early studies of nutrient cycling in streams demonstrated (1) nutrient limitations, (2) a commonly observed downstream decrease in nutrient concentrations, and (3) the importance of experimental radioisotope additions and budget studies as tools for determining cycling rates and controls. Subsequent advances in our understanding of nutrient cycling in streams over the past 20 years include the roles of microbial communities associated with decomposing



Stream nitrogen cycle

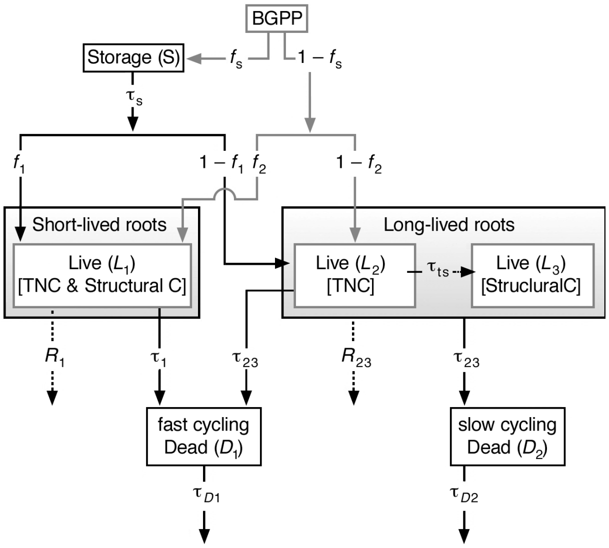
organic matter, stream sediments and the hyporheic zone, adjacent terrestrial biota, and spawning fish in the modification of nutrient concentrations and exports from catchments.

Field ^{15}N-addition experiments conducted by ORNL researchers have demonstrated high rates of nitrogen uptake, nitrification, and denitrification in streams, even those in high-impact agricultural and urban areas. This improved understanding of nutrient cycling in streams has led to better models of nutrient transport, transformation, and export from streams as well as the river networks they comprise. Topics needing further study include nutrient dynamics in larger streams and rivers, the ultimate fate of nutrients taken up by plants and microbes in streams, the effects of climate change on stream ecosystems and river networks, and the role of streams and rivers in nutrient transformation and retention at the landscape scale.

P. J. Mulholland and J. R. Webster. "Nutrient dynamics in streams and the role of J-NABS," **J. N. Am. Benthol. Soc.** 29(1): 100–117 (2010), DOI: 10.1899/08-035.1.

Unique radiocarbon data allow new understanding and models of root growth

To uncover the true nature of root growth, staff from Lawrence Berkeley National Laboratory, Lawrence Livermore National Laboratory, and Argonne National Laboratory, in collaboration with ORNL researchers, developed Radix 1.0, a model of fine-root dynamics, using an inadvertent whole-ecosystem carbon-14 label at a temperate forest in Oak Ridge. Radix simulates two live-root pools, two dead-root pools, non-normally distributed root mortality turnover times, a stored carbon pool, and seasonal growth and respiration patterns and was used to analyze measurements from two root size classes and three soil-depth increments. Predicted live-root turnover times were less than 1 year and approximately 10 years for short- and long-lived carbon pools, respectively. A root population with decadal turnover times implies a lower amount of belowground net primary production used to grow fine-root tissue than is currently predicted by models with a single annual turnover pool.

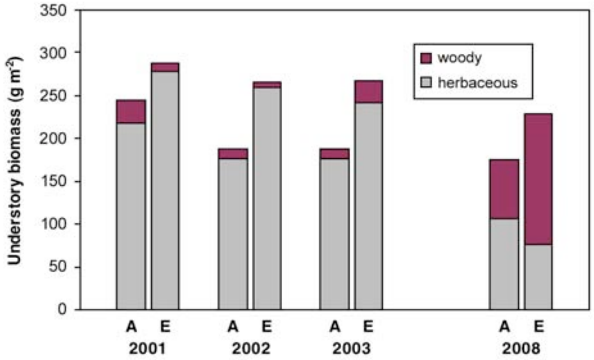


Requisite root carbon pools needed to reflect belowground processes for upland forests

W. J. Riley, J. B. Gaudinski, M. S. Torn, J. D. Joslin, and P. J. Hanson. "Fine-root mortality rates in a temperate forest: estimates using radiocarbon data and numerical modeling," **New Phytol.** 184: 387–398 (2009).

Research Highlights

CO_2 enrichment accelerates ecosystem succession in an understory plant community



The effect of elevated levels of atmospheric CO_2 on the relative abundance of herbaceous and woody species in a total understory biomass

The long-term impact of rising concentrations of CO_2 on forest dynamics and ecosystems has been the subject of much investigation at ORNL. For the past 12 years, researchers have studied the effects of elevated levels of CO_2 on a deciduous forest at the ORNL Free-Air Carbon Dioxide Enrichment (FACE) facility, and as part of that study they investigated changes in the aboveground biomass of the understory community. The objective of their study was to determine (1) whether CO_2 enrichment affected total understory biomass and (2) whether total biomass responses could be explained by changes in understory species composition or changes in relative abundance of functional groups over time. Results showed that the understory community aboveground biomass was on average 25% greater in elevated CO_2 plots than in ambient CO_2 plots. Elevated CO_2 also caused a shift in the relative abundance of plant functional groups, which reflected important structural changes in the understory community. Observations made from 2001 to 2003 indicated that 94% of the total understory biomass was made up of herbaceous species, with little woody species present. However in 2008 the herbaceous species accounted for 61% of the total understory biomass in ambient CO_2 concentrations and only 33% in elevated CO_2 concentrations. The increasing dominance of woody species in the CO_2-enriched plots suggests that rising concentrations of atmospheric CO_2 affect forest dynamics and could accelerate successional development.

L. Souza, R. T. Belote, P. Kardol, J. F. Weltzin, and R. J. Norby. "CO_2 enrichment accelerates successional development of an understory plant community," **J. Plant Ecol.** 3: 33–39 (2010).

Scientific American article highlights global change experiments to a worldwide audience

An article on the serious global consequences of climate change written by Stan Wullschleger of the Environmental Sciences Division appeared in the March 2010 issue of *Scientific American*. In the article, Stan explains, to a lay audience, the science that underlies climate change and rising greenhouse gas concentrations. Much of the information available to the public is based on observed changes in nature that have already occurred, such as the amount a glacier has receded. Rather than waiting to see how climate change will affect the biosphere, Stan and other scientists at ORNL, Los Alamos National Laboratory, Brookhaven National Laboratory, and several universities are conducting large-scale field experiments to improve models that predict how ecosystems will respond in the future to more or less precipitation, increasing concentrations of

CO_2, and higher temperatures. Enough data from long-term experiments conducted at ORNL have now been generated to better predict how woodlands, prairies, and agricultural crops may be affected, but new experiments in more remote regions are needed to better understand how boreal, tundra, and tropical plants will respond.

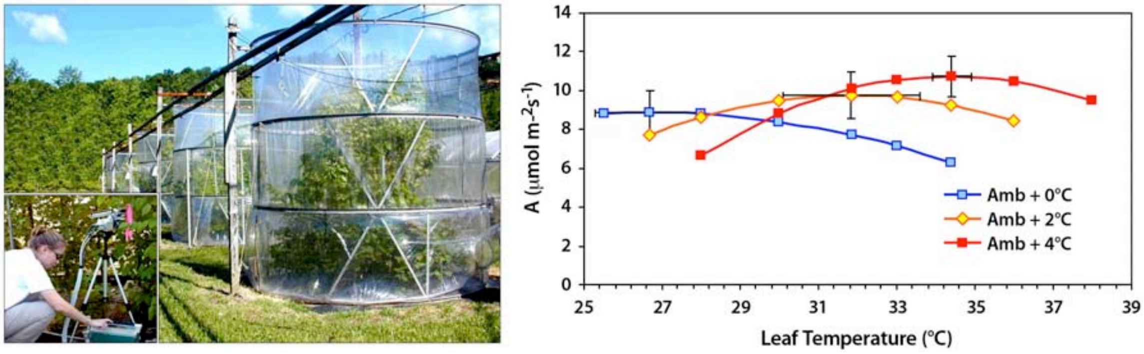
Experimentation has shown that plants and ecosystems are remarkably capable of adjusting to new conditions, but scientists believe that thresholds exist beyond which serious consequences could occur. Higher concentrations of CO_2 have been shown to enhance yields of wheat, rice, barley, soybeans, and cotton, but simultaneous warming could negate this effect. Deep soils hold plenty of water and support the growth of mature deciduous trees in the eastern United States, but surface water holds little water and dries out quickly, leaving seedlings and saplings more vulnerable. A CO_2-enriched environment has been found to promote greater root growth and thus could provide more nutrients and increased access to soil water in dryland ecosystems.

Stan concludes that future experiments are needed in under-studied ecosystems including boreal and arctic tundra ecosystems at high latitudes. Soils in the ecosystems contain large amounts of carbon that could be vulnerable to loss as CO_2 and CH_4. In a warmer world, this could represent significant positive feedback to climate change.

S. D. Wullschleger and M. Strahl. "Climate Change: A Controlled Experiment," **Sci. Am.** 302(3): 78–83 (March 2010).

Forest responses to climatic change: photosynthetic temperature optima adjust to environmental warming

Increasing air temperatures associated with global climate change have the potential to alter forest ecosystems by exceeding the optimal growth temperatures. The survival of sensitive species can thus be threatened, leading to local extinctions, range migrations, and altered forest composition. The goal of researchers at ORNL was to quantify the responsiveness of



Effect of growth and measured temperatures on rates of CO_2 assimilation photosynthesis (net CO_2 assimilation, A) to temperature and to quantify the potential for photosynthetic acclimation to a warmer environment. Five species of deciduous trees were used—sweetgum (*Liquidambar styraciflua*), northern red oak (*Quercus rubra*), southern red oak (*Q. falcata*, mature trees only), yellow birch (*Betula alleghaniensis*), and bigtooth aspen (*Populus grandidentata*). The species chosen represent a broad continuum of habitats and north–south distribution limits across North America.

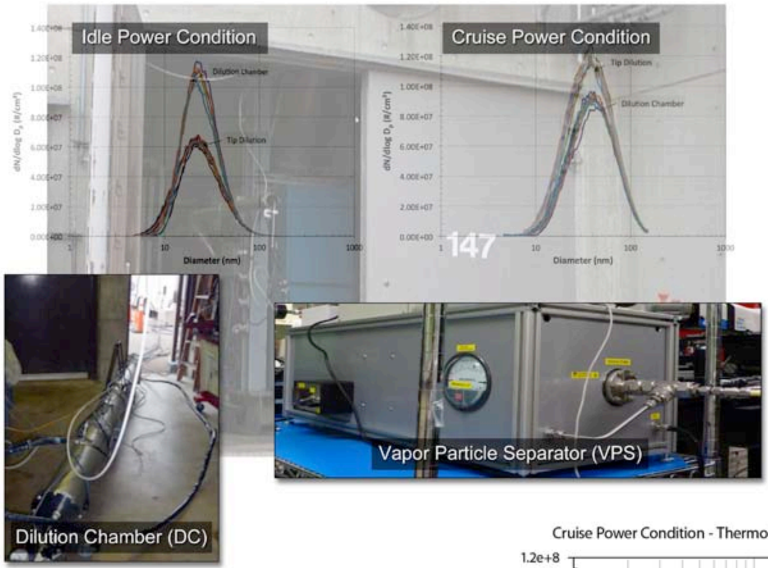
Open-top chambers supplied three levels of warming (+0, +2, and +4°C above ambient) to the trees over a 4 year period, tracking natural temperature variability and plasticity. Optimal temperatures for CO_2 assimilation were strongly correlated with recent daytime temperatures, the treatment chambers, or outdoors, in mature trees. Rates of A at the respective optimal temperatures were comparable. Adjustment of thermal optima was confirmed in all species, whether temperatures varied with season or warming treatment, and regardless of the climate of the species' natural range or the site where seedlings were collected. Temperature optima from 17 to 34°C were observed over the course of the experiment. Across species, acclimation potentials varied from 0.55 to 1.07°C per degree change in daytime temperature. Responses of seedlings to the temperature manipulation did not differ from the seasonal acclimation of mature indigenous trees, suggesting that photosynthetic responses to warming should be modeled not with a static temperature function but with a degree of temperature adjustment. The high degree of homeostasis observed indicates that the direct impacts of climatic warming on forest productivity, species survival, and range limits may be less than predicted by previous models, although temperature-related changes in soil water availability will generate interactions.

C. A. Gunderson, K. H. O'Hara, C. M. Campion, A. V. Walker, and N. T. Edwards. "Thermal plasticity of photosynthesis: the role of acclimation in forest responses to a warming climate," **Global Change Biol.**16: 2272–2286 (2010), DOI: 10.1111/j.1365-2486.2009.02090.x.

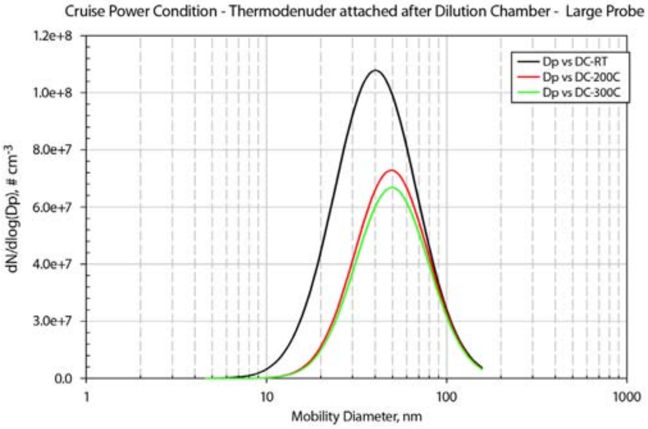
SUBSURFACE

Thermal separation and aerosol dynamics of volatile engine particles

Toxic materials exist on ultrafine particles as a result of a series of complex chemical reactions that take place within an internal combustion engine and in the exhaust plume. These ultrafine particles are present in significant quantities at all engine power conditions, but at idle or low- engine power conditions where combustion efficiency is low a significant fraction of the engine particles is volatile. Up to now, there has been no satisfactory technology for sampling and measuring these volatile engine particles from aircraft emissions. Under the auspices of the Strategic Environmental Research and Development Program (SERDP), researchers at ORNL have developed a new volatile particle separator (VPS) and simulated dilution chamber (DC) system that could provide reliable data for improved understanding of the formation and transport of ultrafine particulate species in engine plumes and the atmosphere. Using an ORNL specialty material, the VPS enables scientists to thermodynamically partition the engine particles



Background figure shows the T63 engine test cell at the Wright-Patterson Air Force Research Laboratory. Two overlaid particle-size distribution plots show the ability of DC-VPS to detect volatile particles through condensation, simulating the process occurring in the exhaust plume in the near field.



by the volatility of the particulate materials. Integrated with the DC, this new sampling and measurement system allows controlled mixing of the engine plume, simulated particle condensation and nucleation processes, and dynamic partitioning of vapors and particles. High-speed particle sizing, online laser spectroscopy, and mass spectrometry permit the quantitative determination of the size distribution and size-dependent chemical concentration of the particles and the composition of the vapor phase. Laboratory tests using synthetic particles at ORNL and engine particles emitted from a turboshaft military engine operated with JP-8 (jet fuel) and alternative fuels at the Wright-Patterson Air Force Research Laboratory show the system performed successfully as designed. Comparison with the results from current techniques such as a tip-dilution probe suggests that only this new SERDP technology could accurately generate volatile particle data and that conventional tip-dilution technology cannot be used for particle volatility work. Further field study is scheduled for calendar year 2011 in a large campaign coordinated by NASA, the Navy, EPA, and several academic and industrial institutions. Additional field tests using helicopter engines are also planned in FY 2011.

M.-D. Cheng. "Development and Application of a Membrane-Based Thermodenuder for Measurement of Volatile Particles Emitted by a Jet Turbine Engine," ASME International Gas Turbine Institute, GT2010-22175, 2010.

E. Corporan, M. J. DeWitt, C. D. Klingshirn, R. Striebich, and M.-D. Cheng. "Emissions Characteristics of Military Helicopter Engines with JP-8 and Fischer-Tropsch Fuels," **J. Propul. Power** 26(2): 317–324 (2010).

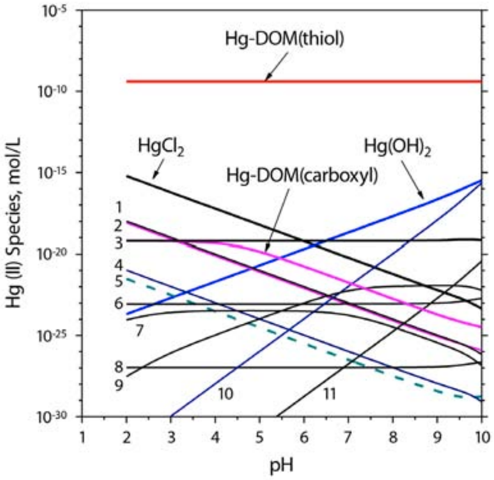
Research Highlights

Roles of dissolved organic matter in the speciation of mercury and methylmercury in a contaminated ecosystem

Mercury presents an environmental concern due to its ability to be converted to the potent neurotoxin methylmercury (CH_3Hg^+). Complexation of the mercuric ion (Hg^{2+}) and methylmercury with organic and inorganic ligands influences transformation and bioaccumulation of mercury in aquatic environments. In this study, scientists at ORNL conducted thermodynamic modeling studies to evaluate the influence of metal–ligand complexation on dominant mercury species of Hg^{2+} and CH_3Hg^+ in a contaminated creek on the Oak Ridge Reservation.

Results showed that even at low concentrations (~3 mg/L), naturally dissolved organic matter (DOM) controls mercury speciation by forming strong complexes with Hg^{2+} and CH_3Hg^+ through the reactive sulfur or thiol-like ligands in the natural organic matter at a pH of about 7.8. In the presence of DOM, concentrations of neutral species $Hg(OH)_2$, Hg(OH)Cl, CH_3HgCl, and CH_3HgOH are negligible. Among various metal ions that coexist in the creek water (including calcium, magnesium, zinc, copper, nickel, cadmium, lead, iron, and uranyl ions), only the zinc ion, in 0.16–0.26 μM concentrations, was found to compete with Hg^{2+} for binding with DOM. Zinc ions caused a decrease in Hg–DOM complexation but had little impact on CH_3Hg^+–DOM complexation.

This study concluded that DOM contains important functional groups that act as strong ligands for mercury complexation; thus, DOM plays a dominant role in the speciation of Hg^{2+} and CH_3Hg^+ and potentially controls the biological uptake and methylation of mercury in this contaminated ecosystem.

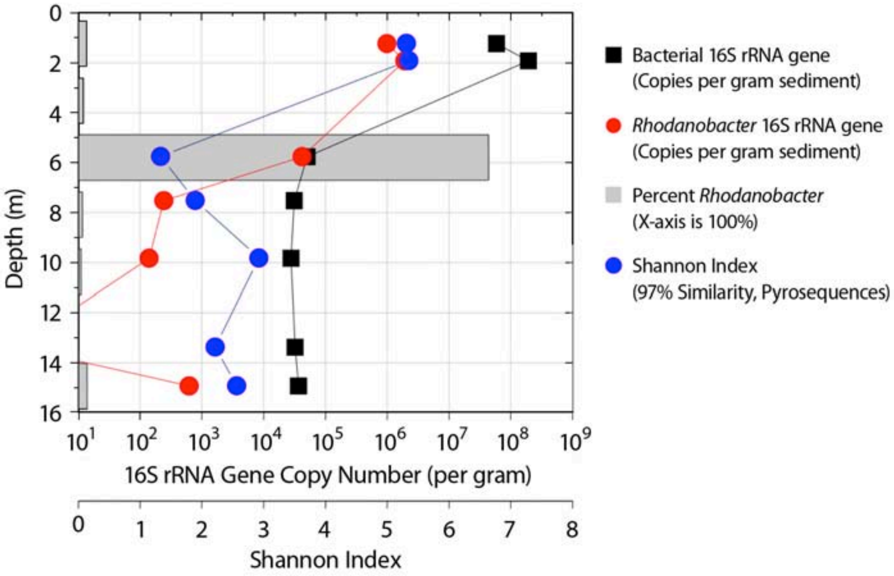


Calculated aqueous species of Hg(II) as a function of pH based on geochemical parameters and reactions. The total [Hg(II)] concentration of 0.4 nM and an ionic strength of 0.01 mol/L in the EFPC (East Fork Poplar Creek) water. Model parameters were used in calculations, including log K = 28.7 and $pK_a = 10$ *for the 1:2 Hg(II):thiol complexes, and log K = 10 and* $pK_a = 4.5$ *for the 1:1 Hg(II):carboxyl complexes with dissolved organic matter (DOM). Numbers in the figure: 1 =* $HgCl_3^-$, *2 =* $HgCl^+$, *3 = Hg(OH)Cl, 4 =* $HgCl4^{2-}$, *5 =* Hg^{2+}, *6 =* $Hg(OH)^+$, *7 =* $HgHPO_4$, *8 =* $HgPO_4^-$, *9 =* $HgCO_3$, *10 =* $Hg(OH)CO_3^-$, *and 11 =* $Hg(OH)_3^-$. *The estimated reactive thiol concentration* (RS^-) *in DOM in EFPC is 4 nM, and the carboxyl content is 16 μM.*

W. Dong, L. Liang, S. Brooks, G. Southworth, and B. Gu. "Roles of Dissolved Organic Matter in the Speciation of Mercury and Methylmercury in a Contaminated Ecosystem in Oak Ridge, Tennessee," **Environ. Chem.** 7: 94–102 (2009).

Genome-enabled investigations of denitrifying bacteria in a uranium-contaminated subsurface

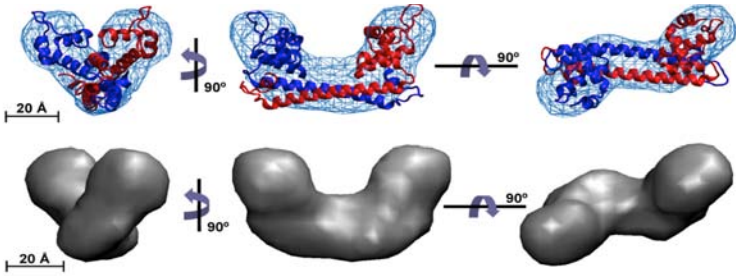
Members of the ORNL Integrated Field-Research Challenge (IFRC) team have shown that commonly used molecular biology techniques that target denitrifying bacteria systematically underestimate their diversity and abundance and that a combination of geochemical, cultivation, genomic, and metagenomic mining approaches is necessary to characterize denitrifying microbial communities. They isolated several new strains of previously unknown denitrifying bacteria and detected genetic sequences of these organisms in the terrestrial subsurface where the organisms were isolated. Denitrification capability was verified in situ using isotopic techniques in laboratory cultures at FSU and in draft genomes sequenced at ORNL for at least two of the isolates (*Rhodanobacter* and *Intrasporangium*). Comparison of genome sequences from known denitrifiers to primer sequences revealed a remarkable sequence divergence in *nirK* and *nosZ* targets, indicating that key denitrifying taxa would not be detected in environmental samples using current methods. Using newly designed primers that take advantage of genome and metagenome data-mining efforts, the team demonstrated that members of the Gammaproteobacterial genus *Rhodanobacter*, in particular, were abundant and broadly distributed in acidic groundwaters and sediments from the Oak Ridge IFRC site. These results indicate that current molecular methods used for determining the abundance and diversity of denitrifiers should be reevaluated. Because nitrate is a key contaminant of aquifers used for drinking water in agricultural and municipal watersheds worldwide, these results are likely to have an impact on sites outside DOE.



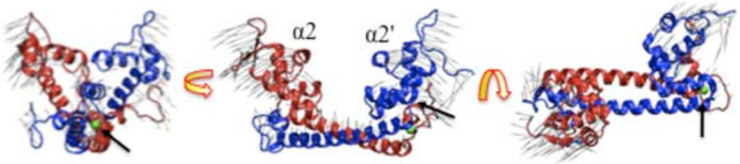
S. J. Green, O. Prakash, T. M. Gihring, D. M. Akob, P. Jasrotia, P. M. Jardine, D. B. Watson, S. D. Brown, A. V. Palumbo, and J. E. Kostka. "Denitrifying bacteria from the terrestrial subsurface exposed to mixed waste contamination," **Appl. Environ. Microbiol.** (2010), DOI:10.1128/AEM.03069-09.

Research Highlights

Structure and conformational dynamics of the metalloregulator MerR upon binding of Hg(II)



The SAXS envelope shape of Hg(II)-bound form of MerR (top) superimposes well with the atomistic model from the molecular dynamics simulation (bottom)



Largest-amplitude modes from principal component analysis of the molecular dynamics trajectories of Hg(II)–MerR

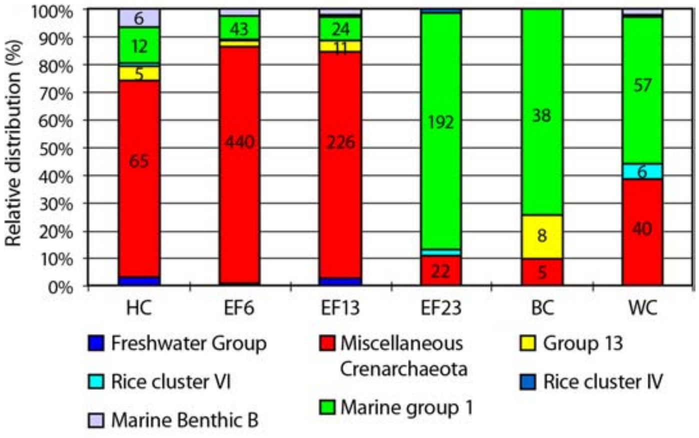
Many bacteria living in contaminated environments possess a mercury resistance (mer) system, which is regulated by the metalloregulator protein MerR. The homodimeric MerR protein represses transcription in the absence of mercury and activates transcription upon Hg(II) binding through its structural change, which turns on DNA transcription of the genes for several other proteins and enzymes and ultimately rids the microbes of toxic mercury species. Scientists at ORNL have used a combined experimental and computational approach to determine the structure and molecular dynamics of the MerR protein when mercury binds to it. Experimental small-angle x-ray scattering (SAXS) studies and computational simulations (molecular dynamics) were used to provide clues as to how a single mercury ion can induce a significant change in the structure of MerR. Results indicated an extended conformation of the Hg(II)-bound protein and revealed the existence of a novel compact conformation in the absence of Hg(II). The molecular dynamics studies agreed well with SAXS results and provided additional details as to the atomic structure of MerR. Simulations revealed large-amplitude domain opening and closing of Hg(II)-bound MerR. Domain motions in MerR are thought to play an important role in initiating DNA transcription of mercury-resistant genes in the presence of nanomolar levels of mercury in bacterial cells.

H.-B. Guo, A. Johs, J. M Parks, L. Olliff, S. M. Miller, A. O. Summers, L. Liang, and J. C. Smith. "Structure and conformational dynamics of the metalloregulator MerR upon binding of Hg(II)," **J. Mol. Biol.** 398: 555–568 (2010), DOI:10.1016/j.jmb.2010.03.020

Archaeal communities in surface stream sediments

How do anthropogenic sources of heavy metal contamination impact the diversity of archaeal communities in surface streams? A team of scientists from ORNL has characterized archaeal communities from mercury- and uranium-contaminated freshwater stream sediments and compared them to those present in an uncontaminated stream site located on the Oak Ridge Reservation. They determined the distribution of the archaea by pyrosequencing analysis of the V4 region of 16S rRNA amplified from 12 sediment samples. Crenarchaeota composed

76% of the 1,670 archaeal sequences, and the remaining 24% were from Euryarchaeota. Phylogenetic analysis further classified the Crenarchaeota as a Freshwater Group, Miscellaneous Crenarchaeota group, Group I3, Rice Cluster VI and IV, Marine Group I, and Marine Benthic Group B and the Euryarchaeota as Methanomicrobiales, Methanosarcinales, Methanobacteriales, Rice Cluster III, Marine Benthic Group D, Deep Sea Hydrothermal Vent Euryarchaeota 1, and Eury 5. Both hydrogen- and acetate-dependent methanogens were found in all samples. Most of the groups (with 60% of the sequences) described in this study were not similar to any cultivated isolates, making it difficult to discern their function in the freshwater microbial community. A significant decrease in the number of sequences, as well as in the diversity of archaeal communities, was found in the contaminated sites. The Marine Group I, including the ammonia oxidizer *Nitrosopumilus maritimus*, was the dominant group in both mercury- and uranium-nitrate-contaminated sites. The uranium-contaminated site also contained a high concentration of nitrate; thus, Marine Group I may play a role in the nitrogen cycle.



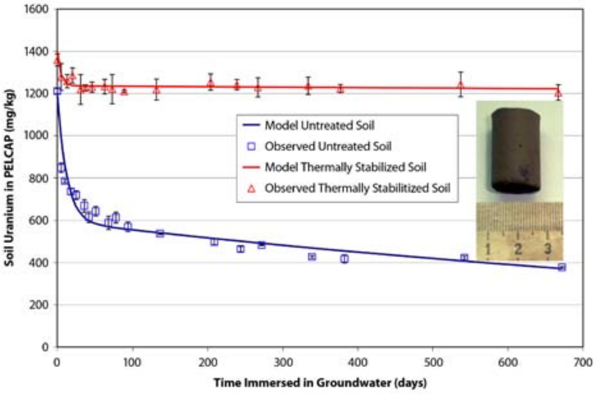
Crenarchaeota community structures within each sampling site. The sequences from samples collected at mid-channel and stream bank were combined for each location. Sampling locations in creeks near Oak Ridge, TN: Hinds Creek (HC), East Fork Poplar Creek (EF, 3 sites), Bear Creek (BC), and White Oak Creek (WC).

I. Porat, T. A. Vishnivetskaya, J. J. Mosher, C. C. Brandt, Z. Yang, S. C. Brooks, L. Liang, M. M. Drake, M. Podar, S. D. Brown, and A. V. Palumbo. "Characterization of archaeal community in contaminated and uncontaminated surface stream sediments," **Microb. Ecol.** 60: 784–795 (2010).

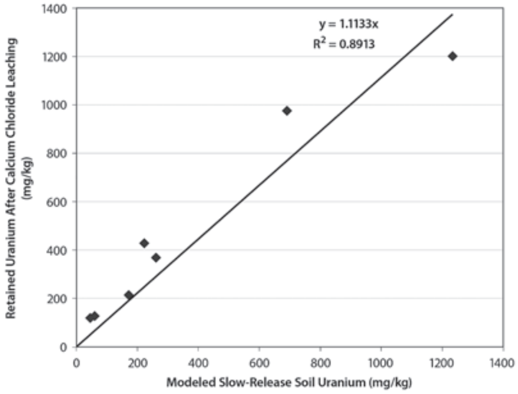
Hydrogel-encapsulated soil: a tool to measure contaminant attenuation in situ

Development of protocols to measure contaminant attenuation in situ, whether natural or manipulated, particularly for toxic metals and long-lived radionuclides, remains an important and difficult challenge for environmental scientists. Hydrogel encapsulation of soil offers an approach to study contaminant adsorption and desorption onto and from soil in groundwater in the field at lower cost and with significantly less sampling variance.

Hydrogel encapsulation allows many water–solid contaminant interactions to be observed in situ in a variety of aqueous media (including groundwater) and solids (including contaminated soil) using various nondestructive analytical methods. Retention of uranium by a variety of materials in permeable environmental leaching capsules (PELCAPs) was successfully monitored using nondestructive analysis over more than 3 years of in situ leaching. After intervals of groundwater immersion, polyacrylamide hydrogel–encapsulated solid specimens were retrieved, assayed nondestructively for uranium and other elements using x-ray fluorescence spectroscopy, and replaced in groundwater for continued reaction. Desorption dynamics of uranium from contaminated soils and other solids, when moved to uncontaminated groundwater, were fit to a general two-component kinetic retention model with slow-release and fast-release fractions for the total uranium. In a group of Oak Ridge soils with varying ambient uranium contamination (169–1,360 mg/kg), the uranium fraction retained under long-term in situ kinetic behavior was strongly correlated ($r^2 = 0.89$) with residual uranium after laboratory sequential extraction of water-soluble and cation-exchangeable fractions of the soils. To illustrate how potential remedial techniques can be compared to natural attenuation, thermal stabilization of one soil increased the size of its long-term in situ retained fraction from 50% to 88% of the total uranium and increased the half-life of that long-term retained fraction from 990 to 40,000 days.



In situ retention of uranium by untreated (naturally attenuated) soil and by thermally stabilized soil in PELCAPs during 2 years of in situ leaching in non-impacted or end-state groundwater at the DOE Field Research Center, Oak Ridge, Tennessee. Inset is a typical uncontained soil-encapsulating PELCAP cylinder 1.5 cm in diameter and 3 cm in height

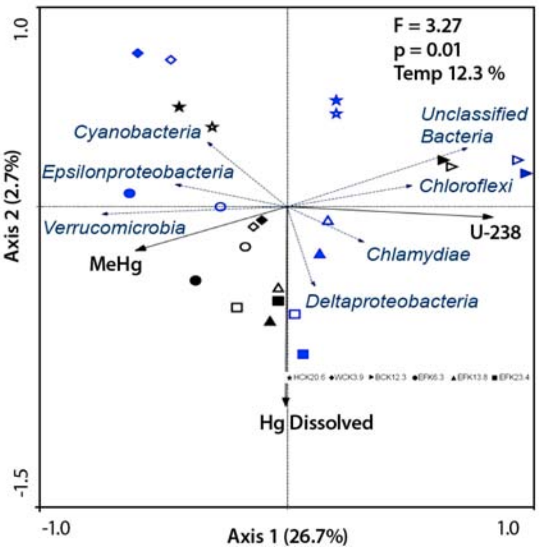


Relation between modeled uranium concentrations in the slow-release fraction and the concentrations of uranium remaining in the soil after laboratory sequential extractions with water and 0.1 N $CaCl_2$ for seven uranium contaminated hydrogel-encapsulated soils

B. P. Spalding, S. C. Brooks, and D. B. Watson. "Hydrogel-encapsulated soil: a tool to measure contaminant attenuation in situ," **Environ. Sci. Technol.** 44(8): 3047–3051 (2010), DOI: 10.1021/es903983f.

Mercury and other heavy metals influence bacterial community structure in contaminated Tennessee streams

High concentrations of uranium, inorganic mercury Hg(II), and methylmercury (MeHg) have been detected in streams located on the Oak Ridge Reservation. To determine the potential effects of surface water contamination on the microbial community composition and the subsequent potential for MeHg generation, surface stream sediments were collected seven times during the past year from five contaminated locations and one control stream. Fifty-nine samples were analyzed for bacterial community composition and geochemical parameters across a range of mercury, MeHg, and U(VI) concentrations. Community characterization and coordination with measured geochemical parameter concentrations revealed that the Verrucomicrobia and the ε-Proteobacteria were most closely correlated with MeHg, while the δ-Proteobacteria were more closely associated with mercury concentrations. The latter includes the sulfate-reducing bacteria and *Geobacter* spp., both of which are known to methylate mercury.



Redundancy analysis of bacterial phyla from 24 stream sediments (six sites). Solid arrows represent geochemical parameters significantly associated ($p < 0.05$) with the variation in bacterial community structure and account for 29.4% of the variability. The remaining 70.6% of community variability between samples was not explained by measured parameters. Dashed arrows represent individual phyla (|r|>0.6) significantly associated with mercury, MeHg, or U(VI). Arrow length correlates the relationship strength. Open symbols represent mid-channel; closed symbols represent near bank. Color coding: May—black; July—blue.

However, no reports demonstrated that either the Verrucomicrobia or ε-Proteobacteria can generate or degrade MeHg. These data suggest that an as yet unknown function for either group in mercury biotransformations may exist, and this is currently being investigated.

This study is the first to indicate an effect of MeHg on the in situ microbial community and suggests possible roles of the various bacteria in the overall Hg/MeHg cycle.

T. A. Vishnivetskaya, J. J. Mosher, A. V. Palumbo, Z. K. Yang, M. Podar, S. D. Brown, S. C. Brooks, B. Gu, G. R. Southworth, M. M. Drake, C. C. Brandt, and D. A. Elias. "Mercury and other heavy metals influence bacterial community structure in contaminated Tennessee streams," **Appl. Environ. Microbio.** 77(1), 302–311 (January 2011), DOI: 10.1128/AEM.01715-10.

Improving contaminant transport experiments data analysis with the CXTFIT/Excel code

Increasingly complex transport experiments are conducted to investigate the coupled hydrological, geochemical, and biological processes that control the fate and transport of contaminants at many U.S. Department of Energy sites. Interpretation of these experimental data is challenging due to uncertainty in experimental conditions and correlations among multiple mechanisms. Despite widespread use of analytical and numerical solutions for analyzing subsurface nutrient and contaminant migration, little guidance exists for discriminating simple versus complex models and for reliably separating and quantifying multiple processes in complex systems.

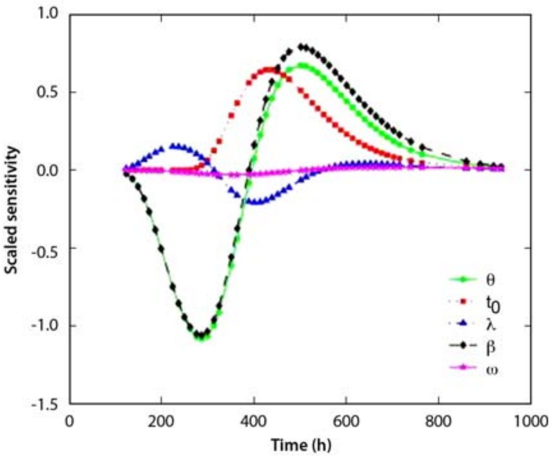
Scientists at Oak Ridge National Laboratory implemented many analytical and numerical solutions for groundwater flow and contaminant transport models in Excel and added comprehensive sensitivity and uncertainty analysis functions with user-friendly interfaces. Their work provides flexibilities and functions that are important to identifying and quantifying multiple processes from complex experiments.

Field tracer injection and monitoring

The code was used to analyze lab and field experiments, and simulate groundwater flow and contaminant transport in the field. Application of this code has led to new insights, improved understanding, and model predictability. It was also used to conduct confirmatory analysis for the construction of new nuclear plants. Professors have found it to be a good teaching tool for graduate courses. The code was downloaded ~50 times within 1 month of its release on the internet.

G. Tang, M. A. Mayes, J. C. Parker, and P. M. Jardine. “CXTFIT/Excel: A modular adaptable code for parameter estimation, sensitivity analysis and uncertainty analysis for lab and field tracer experiments,” **Comput. Geosci.** 36(9): 1200–1209 (2010), DOI:10.1016/j.cageo.2010.01.013.

G. Tang, M. A. Mayes, J. C. Parker, X. L. Yin, D. B. Watson, and P. M. Jardine. “Improving parameter estimation for column experiments by multi-model evaluation and comparison,” **J. Hydrol.** 376: 567–578 (2009), DOI: 10.1016/j.jhydrol.2009.07.063.



Parameter sensitivity

CXTFIT/Excel
www.ornl.gov/~t6g/cxtfit

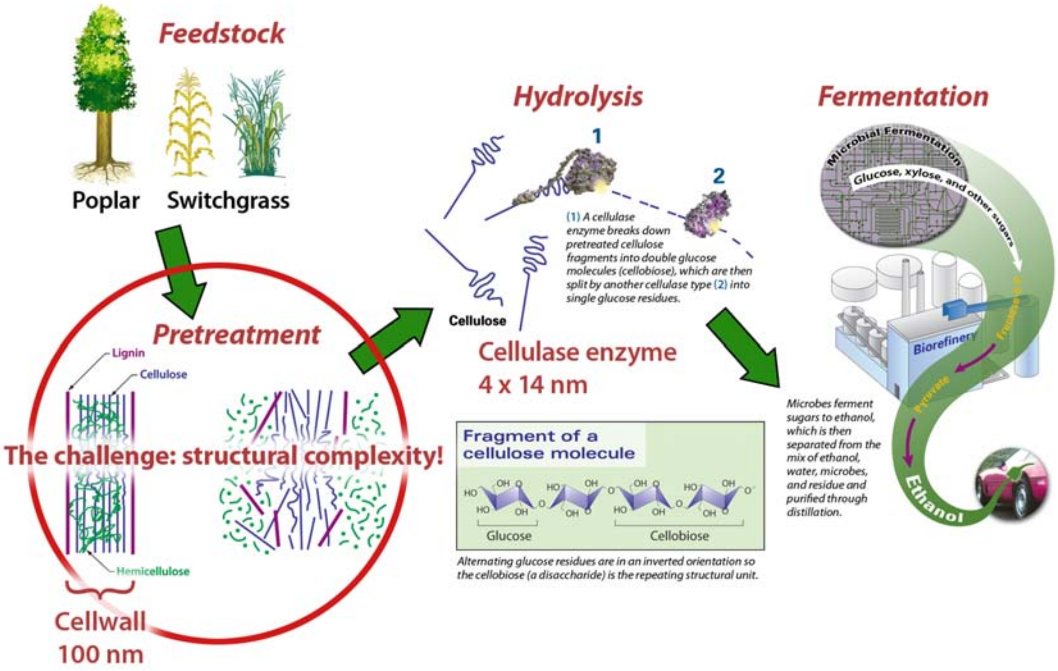
BIOLOGICAL SYSTEMS

Neutron scattering study of dilute acid pretreatment of switchgrass shows changes in cellulose structure

The generation of bioethanol from lignocellulosic biomass holds great promise for renewable and clean energy production, but a better understanding of the complex mechanisms involved in lignocellulose breakdown during various pretreatment methods is needed to realize this potential in a cost-effective and energy efficient way. Scientists at ORNL used small-angle neutron scattering to characterize multiscale structural changes in switchgrass lignocellulose during dilute acid pretreatment and component extraction of biomass. Pretreatment is an essential step in cellulose extraction and is intended to facilitate the enzymatic hydrolysis and conversion of cellulose into sugars and then ethanol; however, current pretreatment methods fall short of their potential. Therefore, better understanding of the nanoscale structural features such as a cellulose fibril crystallinity, lignin redistribution, and surface morphology holds the key to improving the efficiency of biofuel production.

A major finding of this study was that native switchgrass pretreated with hot dilute sulfuric acid undergoes significant morphological changes. While the data collected demonstrated that switchgrass materials are very similar at length scales greater than 1,000 Å, the materials are profoundly different at shorter lengths. The dilute acid pretreatment was found to cause an increase in crystalline cellulose fibril diameter from 21 to 42 Å and lignin redistribution into

100 to 200 Å aggregates. No significant changes to the smooth surface morphology of the micron-sized structures were detected even though significant nanoscale structural changes were observed. Interestingly, it was determined that the apparent increase in cellulose microfibril diameter may also indicate a cellulose re-annealing, which would be counterproductive and may limit the efficiency of the dilute sulfuric acid pretreatment process.



(1) A cellulase enzyme breaks down pretreated cellulose fragments into double glucose molecules (cellobiose), which are then split by another cellulase type (2) into single glucose residues.

Alternating glucose residues are in an inverted orientation so the cellobiose (a disaccharide) is the repeating structural unit.

Microbes ferment sugars to ethanol, which is then separated from the mix of ethanol, water, microbes, and residue and purified through distillation.

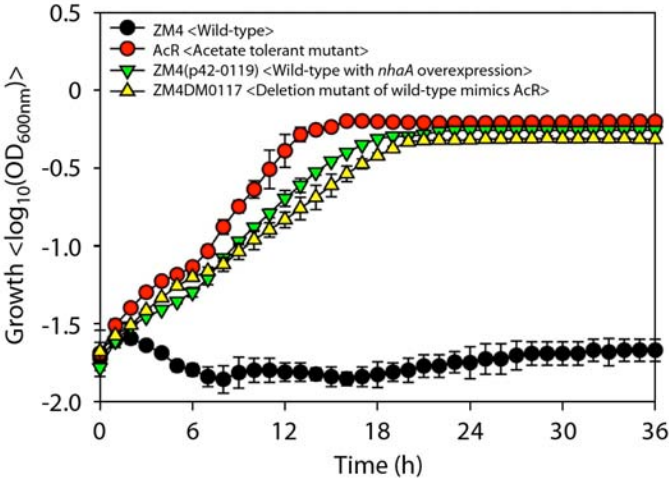
Biomass to cellulosic ethanol (adapted from DOE Genomics Image Gallery at http://genomics.energy.gov)

According to lead scientist Volker Urban, "Our study suggests that hot dilute sulfuric acid pretreatment effectively decreases recalcitrance by making cellulose more accessible to enzymes through lignin redistribution and hemicellulose removal." New insights regarding the interplay of different biomolecular components during the breakdown process of switchgrass by dilute acid pretreatment provided by this study could help scientists identify the most effective pretreatment strategy and lower the cost of the biomass conversion process.

S. V. Pingali, V. S. Urban, W. T. Heller, J. McGaughey, H. M. O'Neill, M. Foston, D. A. Myles, A. J. Ragauskas, and B. R. Evans. "Breakdown of cell wall nanostructure in dilute acid pretreated biomass," **Biomacromolecules** 11: 2329–2335 (2010).

New paradigm for accelerated improvement of microbial strains with potential applications to biofuels industry

Systems biology tools are promising for characterization and rational development of industrial microorganisms such as those needed for the economic production of biofuels. A core challenge in converting cellulosic material to biofuels cost-effectively is the recalcitrance of biomass to breakdown. Severe biomass pretreatments are required to release the sugars from the cellulose for fermentation to cellulosic ethanol by microorganisms. Pretreatment processes also generate a range of chemicals that inhibits the growth of the microorganisms, such as



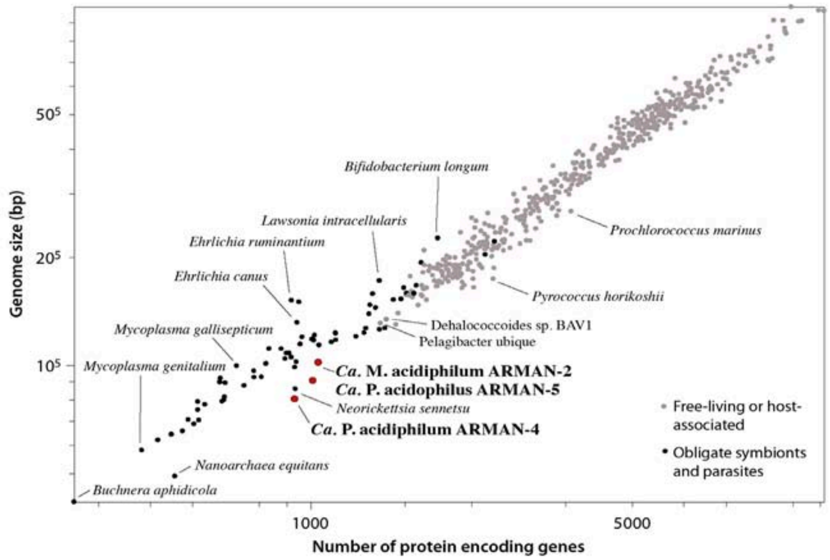
Overexpression of the nhaA gene confers tolerance to inhibitor acetate

acetate from the deacetylation of hemicellulose, potentially increasing biofuel costs. In this study, scientists at ORNL identified transport genes in two microorganisms that confer greater tolerance to inhibitory acetate salts. They characterized a *Zymomonas mobilis* mutant (AcR) that demonstrates tolerance to sodium acetate (NaAc). The genome changes associated with AcR were determined using microarray comparative genome sequencing (CGS) and 454-pyrosequencing. Transcriptomics, genetic data, and growth studies indicate that overexpression of the *Z. mobilis* sodium–proton (Na^+/H^+) antiporter gene *nhaA* confers the elevated sodium acetate tolerance phenotype. Overexpression of *nhaA* mostly confers enhanced tolerance of sodium (Na^+) but not of acetate (Ac^-), unless both ions are present in sufficient quantities. NaAc is more inhibitory for *Z. mobilis* than both potassium acetate and ammonium acetate, and the combination of elevated Na^+ and Ac^- ions exerts a synergistic inhibitory effect for the ZM4 wild-type strain. The scientists then constructed a structural model for the *NhaA* sodium–proton antiporter to provide mechanistic insights into the protein and used Sanger sequencing analysis to validate genomic differences between the ZM4 and AcR strains and to investigate CGS and 454-pyrosequencing limitations that have not been widely reported previously. They demonstrated that *Saccharomyces cerevisiae* sodium–proton antiporter genes also contribute to tolerances of sodium, potassium, and ammonium acetate. The present combination of classical and systems biology tools is a paradigm for accelerated improvement of microbial strains with industrial applications. This approach combines the benefits of a few a priori assumptions with detailed, rapid, mechanistic studies. The work has great impact on basic microbial physiology research, functional genomics techniques, and practical microbial strain development.

S. Yang, M. L. Land, D. M. Klingeman, D. A. Pelletier, T.-Y. S. Lu, Stanton L. Martin, H.-B. Guo , J. C. Smith, and S. D. Brown. "Paradigm for industrial strain improvement identifies sodium acetate tolerance loci in *Zymomonas mobilis and Saccharomyces cerevisiae*," **Proc Natl Acad Sci USA** 107(23): 10395–10400 (June 2010).

Enigmatic, ultrasmall, uncultivated Archaea

Metagenomics is providing new insights into the physiology and evolutionary histories of microorganisms from a wide range of environments. Entire communities of microbes, rather than individual microbial species, are being examined via genomic analysis. In this study, a team of scientists explored the biology of three unique, uncultivated lineages of ultrasmall Archaea (<500 nm in diameter), referred to as ARMAN (archaeal Richmond Mine acidophilic nanoorganisms), that branch near the crenarchaeal/euryarchaeal divide in a variety of acidic ecosystems. Divergent lineages of microbes are the subject of interest because they provide



insight into the form and pattern of biological evolution. By combining metagenomics, community proteomics, and three-dimensional tomographic analysis of cells and cell-to-cell interaction in natural biofilms, they reported several unexpected metabolic features that illustrate unique facets of microbial biology and ecology. They also reconstructed composite, near-complete ~1 Mb genomes for the three lineages from environmental samples and a biofilm filtrate. Genes of two of the lineages are among the smallest yet described, enabling a 10% higher coding density than that found for genomes of the same size, and there are noncontiguous genes. This study expanded the number and variety of known genes, some of which are believed to play a role in newly described interorganism interactions and the formation of large internal organelles in these microorganisms.

B. J. Baker, L. R. Comolli, G. J. Dick, L. J. Hauser, D. Hyatt, B. D. Dill, M. L. Land, N. C. VerBerkmoes, R. L. Hettich, and J. F. Banfield. "Enigmatic, ultrasmall, uncultivated Archaea," **PNAS** 107: 8806–8811 (2010).

Shotgun proteome profile of *Populus* developing xylem

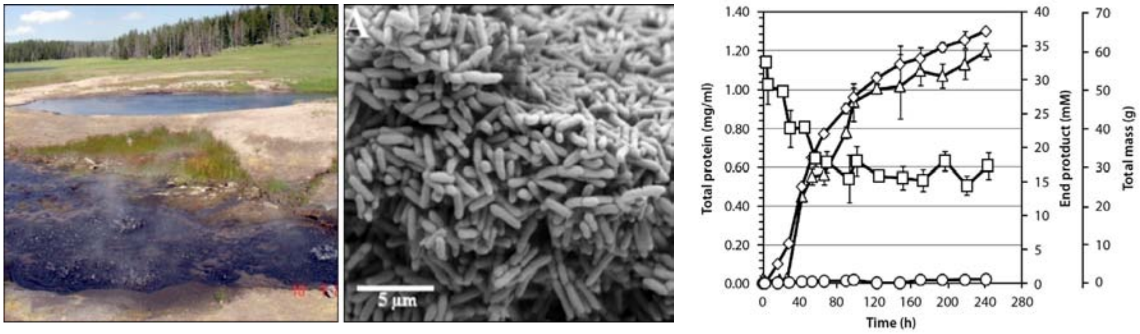
Proteomics Journal cover. Top image: Populus *xylem tissue (autofluorescence) overlaid with tandem mass spectrum from a xylem protein*

Understanding the molecular pathways of plant cell wall biosynthesis and remodeling is central to interpreting biological mechanisms underlying plant growth and adaptation as well as leveraging that knowledge towards development of improved bioenergy feedstocks. In this study, scientists at ORNL applied shotgun tandem mass spectrometry profiling to the proteome of *Populus* developing xylem. Nearly 6,000 different proteins were identified from the xylem proteome. To identify low-abundance DNA-regulatory proteins from the developing xylem, a selective nuclear proteome profiling method was devised. Several putative transcription factors and chromatin remodeling proteins were identified using this method, such as NAC domain, CtCP-like and CHB3-SWI/SNF-related proteins. Public databases were mined to obtain information in support of subcellular localization, transcript-level expression, and functional categorization of identified proteins. In addition to finding protein-level evidence of candidate cell wall biosynthesis genes from xylem (wood) tissue such as cellulose synthase, sucrose synthase, and polygalacturonase, several other potentially new candidate genes in the cell wall biosynthesis pathway were discovered. Further application of such proteomics methods will aid in plant system biology modeling efforts by enhancing the understanding not only of cell wall biosynthesis but also of other plant developmental and physiological pathways.

U. C. Kalluri, G. B. Hurst, P. K. Lankford, P. Ranjan, and D. A. Pelletier. "Shotgun profiling of *Populus* developing xylem proteome," **Proteomics** 9(21): 4871–4880 (2009).

New isolate from Yellowstone National Park displays rapid hydrolysis of plant biomass at high temperatures

Overcoming the recalcitrance of lignocellulosic materials to enzymatic hydrolysis is critical for economical conversion of biomass to fuels. Recent research has focused on the use of extreme thermophiles and their heat-stable cellulases for breaking down plant material. Members of the genus *Caldicellulosiruptor* possess a suite of multifunctional enzymes that are capable of hydrolyzing both cellulose and hemicellulose. These thermophilic bacteria grow in neutral to slightly alkaline environments and at temperatures ranging from 60 to 83°C.



Obisidian Pool located in the Mud Volcano area of Yellowstone National Park (left). Scanning electron micrograph of C. obsidiansis *(middle). Plot showing growth of* C. obsidiansis *on dilute acid-pretreated switchgrass (right). Growth is expressed as total protein (triangles) with acetate as the major end product (diamonds). The cellulosic fraction of the switchgrass is consumed in ~72 hours (squares). Lactate production was not observed during growth on biomass (circles)*

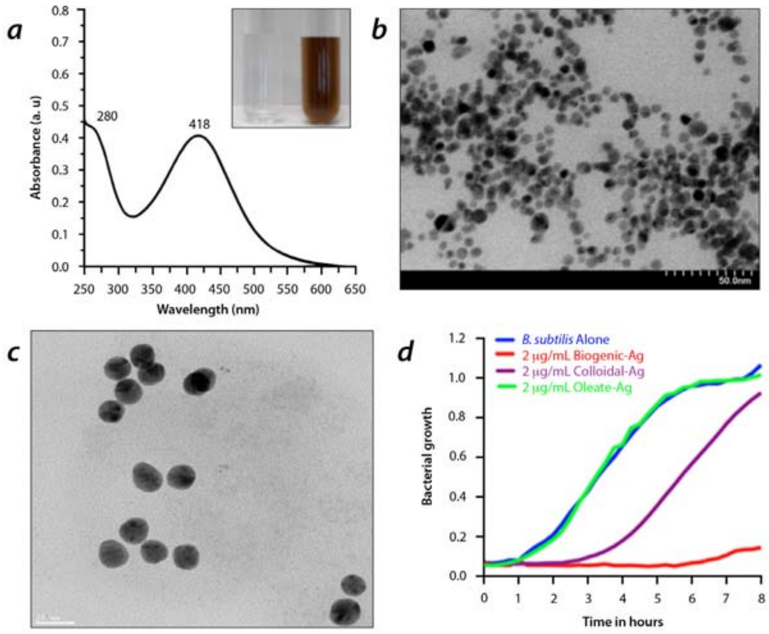
Scientists from ORNL sampled thermally active sites within Yellowstone National Park in an attempt to identify novel microorganisms involved in lignocellulosic biomass degradation at elevated temperatures (60–90°C). More than 200 samples were screened and 135 putative isolates were recovered from growth on switchgrass and *Populus*. They discovered a new isolate from the genus *Caldicellulosiruptor* that displayed rapid growth on crystalline cellulose and pretreated switchgrass at 80°C. The organism, named *C. obsidiansis* in reference to Obsidian Pool, metabolizes a wide range of monomeric and polymeric carbohydrates and produces acetate, lactate, hydrogen, carbon dioxide, and ethanol. The genome sequence for *C. obsidiansis* has been finished by the Joint Genome Institute. The genome is rich in genes for carbohydrate-active enzymes, with 104 hits to known glycoside hydrolase gene families. Organisms such as *C. obsidiansis* that can efficiently hydrolyze recalcitrant biomass, ferment both hexose and pentose sugars, and produce ethanol are of great interest for both basic and applied studies regarding biomass conversion to fuels and chemicals.

S. D. Hamilton-Brehm, J. J. Mosher, T. Vishnivetskaya, M. Podar, S. Carroll, S. Allman, T. J. Phelps, M. Keller, and J. G. Elkins. "*Caldicellulosiruptor obsidiansis* sp. nov., an anaerobic, extremely thermophilic, cellulolytic bacterium isolated from Obsidian Pool, Yellowstone National Park," **Appl. Environ. Microbiol.** 76(4): 1014–1020 (February 2010).

J. G. Elkins, A. Lochner, S. D. Hamilton-Brehm, K. Walston Davenport, M. Podar, S. D. Brown, M. L. Land, L. J. Hauser, D. M. Klingeman, B. Raman, L. A. Goodwin, R. Tapia, L. J. Meincke, J. C. Detter, D. C. Bruce, C. S. Han, A. V. Palumbo, R. W. Cottingham, M. Keller, and D.E. Graham. "Complete Genome sequence of the cellulolytic thermophile *Caldicellulosiruptor obsidiansis* OB47T," **J. Bacteriol.** 192(22): 6099–6100 (November 2010).

Biofabrication of silver nanocrystallites using *Shewanella oneidensis* and an evaluation of toxicity

The intrinsic properties of nanoparticles make them highly suitable for applications in biology, medicine, and electronics. Usually chemical methods are used to synthesize nanoparticles; however, these methods are complex and often lead to variable results. Other means by which nanoparticles can be produced are being pursued. Scientists at ORNL have investigated biofabrication as an alternative approach to synthesizing nanoparticles.



Uv-vis spectroscopy (a) and transmission electron microscopy (TEM) (b–c) measurements of the biogenic-Ag nanoparticles produced by Shewanella oneidensis *and their comparative bactericidal assessment (d).*

When microorganisms are incubated with silver ions, extracellular silver nanoparticles can be generated as a defense mechanism against the toxicity of the metal. ORNL scientists have found that when silver nitrate is added to the actively growing bacterium *Shewanella oneidensis*, silver nanocrystallites are produced. It is thought that the bacteria convert toxic Ag^+ to stable silver at room temperature and pressure. The results of this investigation showed for the first time that biological synthesis can be used to produce small, spherical, reasonably monodispersed nanoparticles (biogenic-Ag) that range in size from 2–11 nm. Surprisingly, the bacterially derived silver nanoparticles possess better antibacterial properties than those that are chemically synthesized.

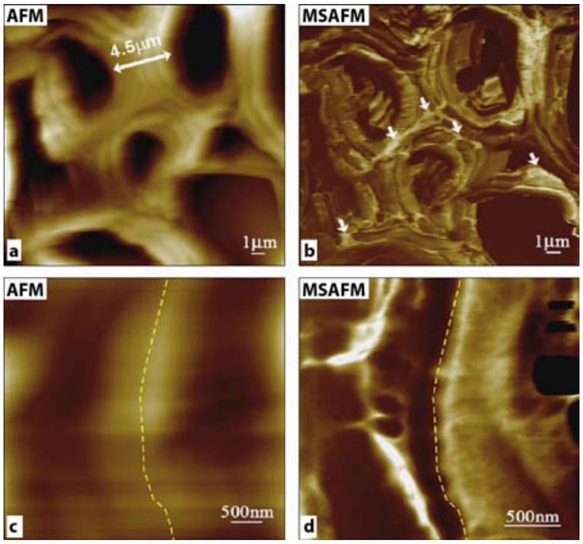
Though the mechanism of toxicity is not completely understood, it is believed the coating on the surface of the nanoparticles plays a key role. Hence this coating may provide a means by which nanoparticles can be adapted and designed for different applications. Determining the chemical makeup of the surface coating, as well as understanding the mechanisms involved in biogenic-Ag synthesis and toxicity, will be the subject of future study.

A. K. Suresh, D. A. Pelletier, W. Wang, J. W. Moon, B. Gu, N. P. Mortensen, D. P. Allison, D. C. Joy, T. J. Phelps, and M. J. Doktycz. "Silver nanocrystallites: Biofabrication using *Shewanella oneidensis*, and an evaluation of their comparative toxicity on Gram-negative and Gram-positive bacteria," **Environ. Sci. & Technol.** 44(13): 5210–5215 (2010), DOI: 10.1021/es903684r.

New mode of atomic force microscopy to characterize lignocellulosic biomass

Scanning probe microscopy has emerged as a powerful nondestructive nanoscale characterization technique that can be used to better understand the molecular architecture of cell walls. The adaptation of a new imaging technology to study lignocellulosic biomass materials at the nanoscale level has been reported by scientists at ORNL. Using a technique known as mode-synthesizing atomic force microscopy (MSAFM), they have shown that an atomic force microscope can be used to obtain a wider range of surface and subsurface information about a sample. Atomic force microscopy (AFM) uses a force-sensing probe with a sharp tip to measure the topography and other surface properties of a sample. In general AFM operation is limited to surfaces of a material. However, MSAFM breaks this barrier by using the nanomechanical coupling between the probe and the sample. In this study, results obtained using MSAFM revealed a rich spectrum of first- and higher-order couplings, thus providing a multitude of new operational modes for force microscopy.

Preliminary images of both *Populus* and switchgrass samples using AFM showed distinctive features that are shared by switchgrass and *Populus*. These features may be attributable to the lignocellulosic cell wall composition. The images obtained using both AFM and a single mode of MSAFM to characterize *Populus* clearly showed the structure of the cell wall. The single mode of MSAFM revealed variations in the mechanical properties of the different layers of the cell walls and differences in composition within the tissue sample.



AFM (a and c) and MSAFM (b and d) images of a cross section of Populus *wood. MSAFM images exhibit more detailed information than regular topography.*

The results of this study provide a better understanding of the characteristic features of both mature cells and developing plant cells. MSAFM was also shown to be a suitable technique in nanoscale topography and subsurface imaging of *Populus* and switchgrass samples. In the future, cross-linking of information obtained from various technological platforms will be needed to gain a greater understanding of plant cell wall architecture in order to improve lignocellulosic feedstock properties for bioenergy production.

L. Tetard, A. Passian, R. H. Farahi, U. C. Kalluri, B. H. Davison, and T. Thundat. "Spectroscopy and atomic force microscopy of biomass," **Ultramicroscopy** 110: 701–707, DOI: 10.1016/j.ultramic.2010.02.035.

L. Tetard, A. Passian, and T. Thundat. "New modes for subsurface atomic force microscopy through nanomechanical coupling," **Nature Nanotech.** published online December 20, 2009, DOI: 10.1038/NNANO.2009.454.

Prodigal: prokaryotic gene recognition and translation initiation site identification

A new gene prediction algorithm called Prodigal (PROkaryotic DYnamic programming Gene-finding ALgorithm) was developed by scientists at ORNL that focused specifically on the objectives of

- improved gene structure prediction,
- improved translation initiation site recognition, and
- reduced number of false positives.

Prodigal (http://compbio.ornl.gov/prodigal/) met these objectives and is considered to be a valuable asset to automated microbial annotation pipelines. The results of Prodigal were compared to existing methods for both purely experimentally verified genes as well as curated Genbank files for a number of genomes. When tested, this fast, lightweight, open-source gene prediction program proved equal to or better than other existing gene-finding methods at locating genes, while predicting fewer overall genes, and when used in translation initiation site prediction in almost every case.

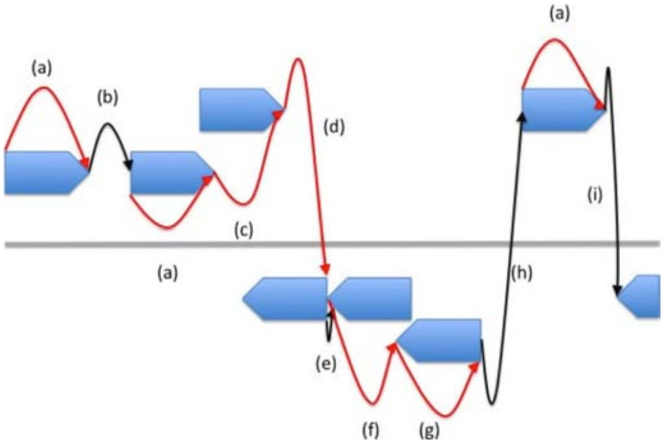
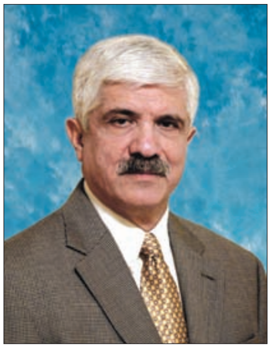


Illustration of the dynamic programming connections in Prodigal. The blue boxes represent genes, red arrows represent gene connections, and black arrows represent intergenic connections.

Prodigal is now used as the primary gene prediction algorithm for the Joint Genome Institute–ORNL microbial annotation pipeline.

D. Hyatt, G.-L. Chen, P. F. LoCascio, M. L. Land, F. W. Larimer, and L. J. Hauser. "Prodigal: prokaryotic gene recognition and translation initiation site identification," **BMC Bioinformatics** 11:119 (2010), DOI:10.1186/1471-2105-11-119.

Awards & Achievements



Sokhansanj

Shahab Sokhansanj receives citations from the American Society of Agricultural and Biological Engineers

Shahab Sokhansanj of the Environmental Sciences Division received two citations from the American Society of Agricultural and Biological Engineers (ASABE). He was awarded the President's Citation for his work with industry and government agencies in addressing bioenergy engineering issues and the Leadership Citation for serving as cochair of the first Bioenergy Engineering Conference held in October 2009 in Bellevue, Washington. These citations were presented to Shahab during ASABE's annual international meeting in Pittsburgh, Pennsylvania, in June 2010.

The ASABE is an educational and scientific organization dedicated to the advancement of engineering research applicable to agricultural, food, and biological systems. Founded in 1907, ASABE comprises 9,000 members in more than 100 countries. Its membership includes agricultural, food, and biological engineers working to develop efficient and environmentally sensitive methods of producing food, fiber, timber, and renewable energy sources for an ever-increasing world population. As the President of the Canadian Society for Bioengineering, Shahab also served on ASABE's Board of Trustees from 2007 to 2008.

ORNL Sustainable Campus Initiative receives two EStar awards and a Federal Energy and Waste Management award

The DOE EStar Awards honor achievements in the development of environmental sustainability practices and technologies and are conferred on projects that demonstrate excellence in pollution prevention and sustainable environmental stewardship. This year the ORNL Sustainable Campus Initiative received "best in class" and "noteworthy practice" awards for its Net-Zero Energy Building and Sustainable Campus Initiative programs, respectively. The Sustainable Campus Initiative team, which includes Environmental Sciences Division staff members Amy Wolfe, James Saulsbury, Kathy Gant, and Marti Salk, was recognized for its efforts to accelerate environmental sustainability. The team identified current ORNL sustainability efforts and projected sustainability needs 10 years into the future. By implementing the recommendations of this initiative, ORNL increased its energy consumption by only 6% while increasing its space by 35%.

Team members also received a Federal Energy and Waste Management award from the Office of Energy Efficiency and Renewable Energy Federal Energy Management Program in honor of its accomplishments and for "outstanding contributions in the areas of energy efficiency, water conservation, and the use of advanced and renewable energy technologies at federal facilities."

Dale

Virginia Dale serves on the National Research Council Committee on Environmental and Economic Impacts of Increasing Biofuels Production

Virginia H. Dale, a leading landscape ecologist and Corporate Fellow in the Environmental Sciences Division, was asked to serve on the National Research Council (NRC) Committee on Environmental and Economic Impacts of Increasing Biofuels Production. At the request of the U.S. Congress, the committee conducted an intense analysis of the impacts of increased biofuel production and the Renewable Fuels Standard (RFS) in the United States on the environment, availability and cost of animal feed, forest products, food, and more, as well as on policy options to alleviate the potential adverse impacts of the RFS. As part of the analysis, the committee sought the input of feed grain producers, food animal producers, energy producers (renewable and petroleum based), forest owners and forest product manufacturers and users, those interested in nutrition or in the relationship of the environment to energy production, producers and users of renewable fuel feedstocks, users of renewable fuel, and experts in agricultural economics from land grant universities. Upon completion of the analysis, the committee submitted a report of its findings, including recommendations for future research.

Watson

David Watson receives the DOE Office of Science Outstanding Mentor Award

David Watson, a hydrogeologist in the Environmental Sciences Division specializing in remediation of contaminants in the subsurface, received the DOE Office of Science Outstanding Mentor Award in recognition of his dedication as a mentor for the Science Undergraduate Laboratory Internship Program. He was selected for this honor based on his willingness to share knowledge and to inspire and instill confidence in the next generation of scientists and engineers by setting high expectations, seeking creative solutions, and immersing inquisitive minds in the world of science.

Awards & Achievements

Hanson

Thornton

Paul Hanson and Peter Thornton serve on the North American Carbon Program Science Steering Group

The North American Carbon Program (NACP) is a multidisciplinary research program established to enhance scientific understanding of North America's carbon sources and sinks and of changes in carbon stocks needed to meet societal concerns and to provide tools for decision makers. Paul Hanson and Peter Thornton of the Environmental Sciences Division serve on the NACP Science Steering Group (SSG), which provides scientific leadership for the program and develops direction and strategies to optimize scientific synergy with other organizations, projects, and networks. The NACPSSG interacts closely with the Carbon Cycle Interagency Working Group and the NACP Office and, as needed, with the Carbon Cycle Science Steering Group and the Ocean Carbon and Climate Change Science Steering Group. SSG also assists in implementing the NACP Science Plan and works to ensure that scientific returns are maximized.

Mulholland

Pat Mulholland receives the 2011 Award of Excellence from the North American Benthological Society

Pat Mulholland of the Environmental Sciences Division was named the recipient of the 2011 Award of Excellence by the North American Benthological Society, a scientific organization of about 2,000 biologists, ecologists, and biogeochemists who study streams, lakes, and wetlands. This award is the society's highest honor and is given annually in recognition of the career accomplishments of one if its members. Pat was chosen for this award based on his research and publications on the ecology and biogeochemistry of stream ecosystems over the past 30 years. The award will be presented to him at the next annual meeting of the North American Benthological Society in Providence, Rhode Island, in May 2011, where he will give a plenary talk on his research.

Awards & Achievements

Wilbanks

Preston

Three ORNL researchers selected as authors for IPCC's Fifth Assessment Report

Tom Wilbanks, Peter Thornton, and Ben Preston of the Environmental Sciences Division have been selected by the Intergovernmental Panel for Climate Change (IPCC) as chapter authors for its Fifth Assessment Report. Tom will be coordinating lead author of Chapter 20, "Climate-Resilient Pathways: Adaptation, Mitigation, and Sustainable Development," of the Working Group II report (on *Impacts, Adaptation, and Vulnerabilities*), and Ben will be a lead author of Chapter 16, "Adaptation Opportunities, Constraints, and Limits" of the Working Group II report. Peter will be a lead author of Chapter 6, "Carbon and Other Biogeochemical Cycles," of the Working Group I report (on *The Physical Science Basis*).

IPCC assessment reports are undertaken periodically to summarize the current state of global knowledge about climate change and its potential consequences. Authors are nominated by their respective governments and selected by IPCC leaders as recognized global leaders in their subject areas. The IPCC Fifth Assessment Report process will be completed in 2014.The major assessment reports are supported by a number of special reports and expert meetings on topics of particular interest. Tom is currently serving as a lead author of a special report on *Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation* and as a contributing author to a special report on *Renewable Energy Sources and Climate Change Mitigation*. He is also a member of the Scientific Steering Group for Expert Meetings on Socioeconomic Scenarios (November 2010) and Human Settlements and Infrastructures (March 2011).

Awards & Achievements

Mielenz

Jonathan Mielenz cochairs biotechnology symposium

Jonathan Mielenz of the Biosciences Division served as the lead cochair for the 32nd Symposium on Biotechnology for Fuels and Chemicals at Clearwater Beach, Florida. His responsibilities included the selection of 12 session topics and 24 session chairs, a third of whom were from outside the United States. Despite the loss of 24 speakers due to cancelled flights as a result of the Icelandic volcano, Jonathan and his staff managed to replace every missing speaker and session chair. Among the highlights of the meeting were presentations by the DOE Office of Science Bioenergy Research Center directors and representatives of international centers from The Netherlands and Japan. The symposium was hosted by ORNL and the National Renewable Energy Laboratory.

Brooks

Scott Brooks to serve on the National Research Council Committee on Uranium Mining in Virginia

Scott Brooks of the Environmental Sciences Division has been named to a 13-member provisional committee of the National Research Council to conduct a study of uranium mining in Virginia. The committee will examine the scientific, technical, environmental, human health, safety, and regulatory aspects of uranium mining, milling, and processing in the state. Virginia Tech, under a contract with the National Research Council, will serve as a conduit for funding from Virginia Uranium Inc., which has agreed to pay for the $1.4 million study. Virginia Uranium announced plans 3 years ago to explore mining at the Coles Hill uranium deposit, near the town of Chatham. Discovered in the early 1980s, this uranium deposit is one of the largest in the United States and is worth an estimated $7 billion. The committee will submit a final report of its findings to the Uranium Mining Subcommittee of the Virginia Commission on Coal and Energy in December 2011. The commission will then present the findings to the General Assembly, which will reconsider a moratorium that has been in place on uranium mining in Virginia since 1982.

Awards & Achievements

Center for BioEnergy Sustainability

The Center for BioEnergy Sustainability (CBES) was established by ORNL to bring unique skills and technical expertise to sustainability problems of national and global concern regarding the environmental impacts and sustainability of biomass production and its conversion to biofuels and bio-based products. The mission of this center is to use science and analysis to understand the sustainability (environmental, economic, and social) of current and potential future bioenergy production and distribution; to identify approaches to enhance bioenergy sustainability; and to serve as an independent source of the highest quality data and analyses for bioenergy stakeholders and decision makers. Highlights this year included "A Watershed Perspective on Bioenergy Sustainability" workshop held at ORNL to address water-related sustainability issues (particularly hydrology and water quality) associated with intensive cellulosic bioenergy feedstock production. A second workshop, "China–U.S. Workshop on the Climate–Energy Nexus," jointly organized by ORNL and the University of Tennessee, brought together leading scientists in climate, ecology, energy, and green technology from China and the United States to exchange perspectives and findings, identify opportunities, and make action plans for promoting bilateral research collaboration in climate and energy. Other symposia organized and chaired by CBES staff included the 32nd Symposium on Biotechnology for Fuels and Chemicals, two symposia at the annual meeting of the International Association of Landscape Ecology, and a symposium on policy needs of bioenergy at the 2010 American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America Meeting. More than 25 presentations were given and 6 new papers were published by CBES staff on a variety of subjects related to bioenergy, land use, sustainability, ecosystems, biofuels, climate, and environmental changes. Monthly forums continued to be hosted by CBES as a means of sharing information on bioenergy-related topics. Virginia Dale, Director of CBES, continued to serve on the National Academy of Sciences Committee on Economic and Environmental Impacts of Increasing Biofuel Production. Other ongoing activities included participation in the Global Bioenergy Sustainability Project by Keith Kline and Martin Keller. Keith is also serving on the California Low Carbon Fuel Standard Expert Work Group and was nominated and approved by the U.S. Advisory Group for the ISO Standard on Sustainability Criteria for Bioenergy to serve on work groups addressing greenhouse gas methods.

Additional information regarding recent CBES activities is available on the web at http://www.ornl.gov/sci/besd/cbes/.

Awards & Achievements

BioEnergy Science Center

The BioEnergy Science Center (BESC) is a multidisciplinary research center focused on achieving the breakthroughs in basic science needed to make production cellulosic biofuels—biofuels from nonfood plant fiber—cost-effective on a national scale. Headquartered at ORNL, BESC includes 19 institutional partners, comprised of national laboratories, universities, and private firms.

At the ORNL BESC, scientists are developing methods of producing biofuels from a variety of nonfood plants rich in cellulose such as switchgrass and poplar trees. Their research involves seeking ways to modify plant cell walls, which could reduce their resistance to breakdown and thus decrease or eliminate the need for costly chemical pretreatments that are currently being used. Consolidated bioprocessing, another area of their research, involves the use of a single microorganism or group of organisms to break down plant matter, which would allow biomass to be converted to biofuels through a one-step process.

Climate Change Science Institute

The Climate Change Science Institute (CCSI) was formed to coordinate and develop ORNL's efforts in climate change science. At the core of CCSI is an Earth System Modeling (ESM) enterprise, which will use the scientific capabilities of its staff and the ORNL leadership-class computational facilities to project future climates and provide the basis for assessing likely impacts, how we will adapt to changes in the environment, and the effectiveness of various mitigation options. ORNL data capabilities are a key enabling technology to enhance ESM efforts. ORNL's carbon cycle and ecosystem science will combine the development and improvement of terrestrial land surface models with the deployment of new measurements and experiments to focus on the critical scientific gaps and uncertainties needed to improve the quality of global climate projections by using the most complete formulations for the terrestrial carbon cycle.

The CCSI will focus on three areas of research:

- Integration of modeling, observations, and experimentation—process studies, climate data analysis, attribution, uncertainty quantification
- Knowledge for consequences, mitigation, and adaptation—includes policy and impacts analysis
- Earth system modeling—global atmospheres, oceans, land, ice, and ecosystems with biogeochemical cycles

Awards & Achievements

ORNL staff chair sessions at the Goldschmidt Conference

The 20th annual Goldschmidt Conference was jointly hosted by the University of Tennessee and ORNL in Knoxville June 13–18. Sponsored by the Geochemical Society and the European Association of Geochemistry, the Goldschmidt Conference is the premier forum for the international geochemical community. The theme of this year's conference was earth, energy, and the environment, blending areas of active research with global science policy.

Environmental Sciences Division staff Scott Brooks, Baohua Gu, Alex Johs, Liyuan Liang, and David Watson cochaired several sessions on the hydrogeochemistry of surface Earth processes, with an emphasis on the transformation of mercury and uranium, and the application of X-ray and neutron methods to geochemistry. The conference included more than 1,500 presentations and was attended by about 2,000 delegates.

Ali Passian and Laurene Tetard receive R&D 100 award for mode-synthesizing atomic force microscope

Ali Passian and Laurene Tetard were the recipients of a 2010 R&D 100 award for their development of the Mode-Synthesizing Atomic Force Microscope (MSAFM). The MSAFM provides a noninvasive and nondestructive means of detecting multiple surface and subsurface properties of materials at the nanoscale level. By combining existing and new imaging modalities in a single platform and using nonlinear nanomechanical interactions at ultrasonic frequencies, the MSAFM is able to characterize such properties as porosity, granularity, elasticity, density, and morphology. Modeled based on the microcantilever probe of an atomic force microscope, the MSAFM allows the probe and sample to engage in nonlinear mechanical interaction, which generates information gathered by a patented microelectricalmechanical–based mode synthesizing sensor. The capability of MSAFM to nondestructively characterize nanoscale features, or inhomogeneities, at high resolution is critical to understanding biological processes that lead to cell signaling, protein folding, and gene expression. Equally important is its usefulness in microelectronics and solid state applications such as detecting dopants and defects in silicon chips.

Awards & Achievements

Graham

Robin Graham serves on U.S. National Committee for Soil Science

Robin Graham of the Environmental Sciences Division is a member of the U.S. National Committee for Soil Science, which represents the interests of the community of soil scientists in the United States in the International Union of Soil Sciences and provides leadership in the advancement of soil science nationally and internationally. The committee also advises The National Academies in all matters pertaining to the International Union of Soil Sciences and supports U.S. participation in the international arena.

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