Dr. Kuske in one of her microbiology labs. The image on the microscope screen is a photosynthetic cyanobacteria, *Microcoleus vaginatus*, which is a major constituent of biological soil crusts in the arid Southwest.
CREDIT: Michael Pierce/LANL

IN THEIR OWN WORDS

CHERYL KUSKE explains how to better understand the environment by examining its tiniest inhabitants.

SOMETIMES I GET ASKED WHAT IT'S LIKE to be a scientist and have a career in research. I've found the best way to answer this is to share my favorite M.C. Escher drawing, one that I keep framed on the window ledge in my office. The drawing depicts the surface of a person's desk upon which a sketchbook is laid open, surrounded by various accoutrements such as a bottle, a plant, and a book. On the page of the sketchbook, and—in true Escher fashion—there is a melee of abstract shapes from which the form of a reptile emerges on one side, climbing up off the page, walking across the various desk accessories, stopping once to blow steam from its nostrils (Yay! Success!), and then climbing back down into the chaos of shapes on the page.

I have always enjoyed working on complex biological systems where easy answers are rare. The answers I seek require understanding the intricate and elusive interactions of the millions of microorganisms found in soils, sediments, and water. Escher's melee of shapes and future reptile parts represents most of my time spent as a research scientist—swimming in data that doesn't always make sense or have a direction, trying to prove one hypothesis or another (and most of the time disproving it) and then going on to another hypothesis. Sometimes, I find that pieces come together as a cohesive idea or discovery—like the reptile—and it takes me somewhere out of the melee for a while, allowing me to enjoy the success and teaching me something before I go back into the pool of data to start building on what I've just learned. The key to being a scientist is understanding how to recognize a success when you're down in that melee; you have to figure out what information is useful and what is not. Fortunately, through the course of my career as a microbiologist, my incredible team and I have found a few of these "reptiles," and they have taught us a great deal about the tiny microbial world that surrounds us and interacts with our planet and its inhabitants.

Out of the tide pools

Growing up in coastal North Carolina, I was fortunate to spend my early years exploring firsthand the natural world in the estuaries and wetlands near my house. In a canoe with muddy sneakers, I learned about frogs, turtles, water lilies, and algae.

I was and still am in awe of biochemistry, biodiversity, and how plants, fungi, and bacteria live and interact with each other. For instance, studying what an organism metabolizes—what it eats and excretes—gives us clues about its impact on its surroundings, such as cycling carbon or nitrogen. Another example is that as organisms interact with other living things, some of them can cause devastating disease, which we want to know about. Finally, we have also learned that the compounds some bacteria or fungi produce in order to protect themselves from each other can in fact be used by humans as life-saving pharmaceuticals and antibiotics. Today we are discovering more and more evidence about how these microbes impact everything from the balance within our bodies to that of the entire global environment. It's the interactions and the interfaces between participants that hold the keys.

THE KEY TO BEING A SCIENTIST IS FIGURING OUT WHAT INFORMATION IS USEFUL AND WHAT IS NOT.

A metaphor for the scientific process?

My graduate work and first job were in plant pathology and the biochemistry of plant disease: studying which organisms cause disease in crop plants like rhododendron, soybeans, and tree fruits. I became interested in the study of uncultured bacterial pathogens—bacteria that have not been grown in a laboratory and therefore are not well-studied—being discovered by microscopy and DNA detection. Little did I know that later in my career I would have the molecular tools to thoroughly examine these finicky organisms and their elusive lifestyles without having to isolate and grow them on a Petri dish.

After earning my Ph.D., I came to Los Alamos and began a postdoc in the then Life Sciences Division (now Bioscience Division). My postdoctoral work was to investigate the enzymology of heavy-metal resistance (primarily cadmium) and sequestration in the jimsonweed plant. I also began a project to pioneer new techniques for isolating fragments of DNA directly from soil and aerosol samples to look for "select agent" pathogens and their close genetic relatives that may share common DNA traits. Select agents are specific pathogens that have the potential to pose a severe threat to animals, plants, or the public.

As my career took shape in the mid-1990s, I began to focus on two major aims, supported by different agencies. The first was to document the "background" of microorganisms related to the current suite of select bacterial agents present in the air and soil of major U.S. cities. The second was to identify and track the metabolic responses of soil bacterial and fungal communities to increases in temperature, changes in precipitation patterns, and increases in nitrogen deposition

(from industrial agriculture and power plants) in arid grasslands, cyanobacterial biocrusts, and pine forests. Both of these projects have been ongoing for many years and have led to a variety of important discoveries.

One of our Escher "reptiles" for the first aim came when we discovered that for some pathogens, such as *Francisella tularensis* (which causes tularemia, or rabbit fever), many of the pathogen's near relatives that had not previously been identified by culturing, in fact, occur naturally in the environment—particularly in soil or coastal marine areas. Early surveillance schemes did not take into account the natural presence of these close relatives, which could hamper detection scenarios by generating false positives. We have worked on this issue for a couple of decades now and have identified many new *Francisella* species present in the environment, ones that can

WE HAVE BEEN EXAMINING HOW CHANGES IN THE ENVIRONMENT IMPACT MICROBIAL COMMUNITIES IN THE SOIL.

be pathogens and that are closely related to the select agents. Sequencing and comparing all of these genomes was one of the eureka moments in my career because we could now define specific DNA fragments and begin to understand the purpose of these organisms—such as what roles do they have in the environment (e.g., cycling carbon) or do they cause disease (making it vital to be able to detect them). Although at the time it was conventional to grow pure, isolated cultures of target organisms to sequence their genomes, we were able to develop culture-independent methods of isolating fragments of DNA or RNA that could be amplified and sequenced to identify the organisms in environmental samples.

Building on the early success of the environmental DNA and select-agent pathogen work, I began to further investigate ways of using DNA-based approaches to identify and characterize other kinds of microorganism communities—including ones that are not pathogenic. Through an environmental study at Sunset Crater near Flagstaff, Arizona, we discovered another one of our "reptiles": a whole new kingdom of bacteria called *Acidobacteria*. This was a great discovery, but the finding raised more questions than it answered. Members of this kingdom are ubiquitous in soils and sediments around the world. They are very diverse and the handful of cultured members that we and others have obtained displays very different lifestyles. We have sequenced many of their genomes; however, we still don't know what they are doing in soils. For example, the most abundant *Acidobacteria* members in the Los Alamos area are highly diverse, and although they are likely stuck to the soles of all of our shoes, they have not been cultured or sequenced and their functions in the ecosystem remain unexplored.

Genomics and sequencing advances

Our DNA-based approach was proving to be a good technique, but the timing of it all—in the mid-2000s—coincided with extensive advances in genomic sequencing that would lead us to even more breakthroughs in soil ecology. Sequencing machines became more automated, making the process exponentially less expensive and much faster, but often resulting in more data than we knew what to do with. Fortunately, cutting-edge bioinformatics tools help scientists like me sort through this "big data" to make sense of it all by comparing new sequences with validated ones and helping us recognize relationships among organisms and their traits.

The most significant part of this advancement for my research purposes was that we could now sequence an entire microbial community together, as one complex sample. By sequencing all the DNA in a community at once, a process called metagenomics, we can learn about all the types of organisms present and therefore the potential metabolic activities of the community as a whole. Alternately, a related process called meta-transcriptomics is a way to understand what genes are currently being used in the community by sequencing the actively transcribed messenger RNA in the sample. (Messenger RNA molecules are only made when a cell needs to produce a certain protein or enzyme for a specific function.)

The tools had arrived. These advances in genomics improved our ability to identify the composition of microbial populations—which microbes live in which environments—as well as what roles they have in different ecosystems. We anticipate this work will continue to give rise to a better

Samples from a soil microcosm experiment. Soils from an eastern pine forest (darker soil) and Utah grassland (lighter soil) are incubated in sealed bottles and exposed to various environmental conditions. For example, some are inoculated with target fungal and bacterial communities and then assessed over time for carbon dioxide production, dissolved organic carbon, and the organismal makeup of the community.

understanding of the complex interactions between microorganisms. The relationships within these soil communities are the "reptiles," and little that is useful will come from examining them in isolation.

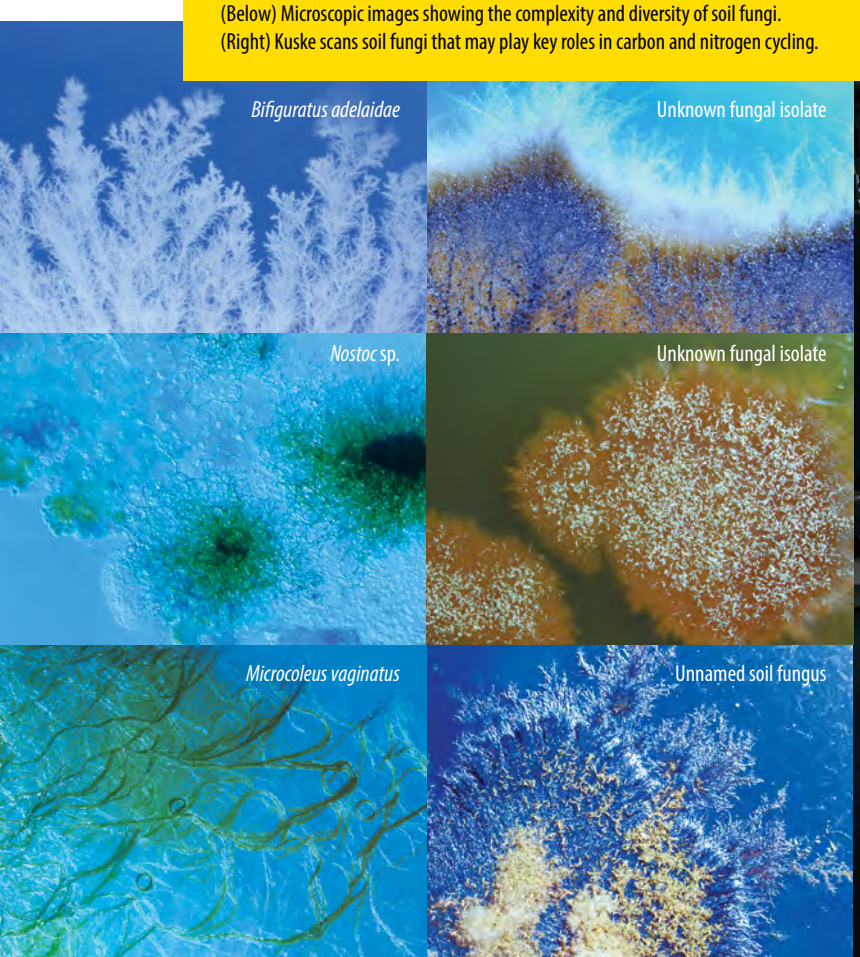
Relationships matter

So now I get to spend my time truly investigating interactions in the microbial world: the thing that inspired me long ago. One of the dominant interactions among soil microorganisms is to decompose organic matter and recycle nutrients in the ecosystem. Dead plants and animals are deposited in the ground, and as the fungal and bacterial communities break down their tissues, their components are recycled into other living things. Soil fungi are experts at this. Some nitrogen and carbon atoms go back into the soil to feed new plant growth, while others are released into the atmosphere in the form of carbon dioxide or nitrogen gas. This nitrogen and carbon cycling is critical to achieving balance in our global ecosystem.

In 2007, I secured long-term funding for a Department of Energy "Science Focus Area (SFA)" in Soil Metagenomics and Carbon Cycling in Terrestrial Ecosystems. Through this program, my team has been able to examine how changes in the environment impact microbial communities in the soil in arid grasslands and pine forests, as well as how examining these changes can help us model what the environment might be like in the future. For instance, as the modern, industrial world releases more carbon and nitrogen into the air and the soil, we want to understand what is happening to the microbial communities. Are they feeding more carbon and nitrogen back into the air where it can exacerbate warming? Or are they sequestering this new biomass in the soil? Furthermore, we need to ask how these populations respond to increases in air temperature or changes in the pattern and timing of regional precipitation.

To do this work, we set up various types of experiments, all involving the collection of soil samples for metagenomic sequencing and analysis. For the first phase of our project, we collected samples from DOE sites called Free Air Carbon Dioxide Enrichment (FACE) field experiments. These sites were replicated free-standing enclosures, placed in multiple biomes across the United States, where carbon dioxide was pumped into sectioned-off areas of vegetation for a sustained, long period of time, usually many years. By 2012, our team was able to draw a number of conclusions about the soil microbes and how the elevated carbon dioxide affected them. For instance, we observed that elevated carbon dioxide impacts were identifiable but were often strongly influenced by other local variables like soil depth or plant type. We also learned that decomposition is due to the interplay of the fungal and bacterial communities, each having hundreds to thousands of species in a single gram of soil. This is indeed a complex system. The fungal and bacterial communities are structured based on different soil features, and we were surprised to find a much broader taxonomy of nitrogen-fixing bacteria than expected.

(Below) Microscopic images showing the complexity and diversity of soil fungi.
(Right) Kuske scans soil fungi that may play key roles in carbon and nitrogen cycling.




CREDIT: Michael Pierce/LANL

For the second phase of our project we collected soil from temperate ecosystems in the pine forests of North Carolina and in the arid grasslands of Utah. We also refined our genomics strategy—based on our experience in the first phase—and decided to do much more meta-transcriptomics so that we could really identify the metabolic activities relating to changes in temperature, precipitation, and nitrogen deposition that are dominant in each ecosystem.

By sequencing and analyzing the mRNA in soils, we have begun to pinpoint which specific enzymes are responsible for impacting the fate of carbon in the soil. For instance, some microbial processes use enzymes that will create dissolved organic carbon (DOC) as a byproduct, while other processes and enzymes will create carbon dioxide. DOC will ultimately remain in the soil and likely be incorporated into new plant material, while carbon dioxide could end up back in the atmosphere. When we sequence mRNA, we can identify statistical information about the prevalence of each enzyme under different treatment conditions, giving us an idea of the overall carbon balance in a specific area of soil.

THE LAB HAS FACILITATED STUDIES I WOULD NEVER HAVE DREAMED OF DOING AT A UNIVERSITY.

Another way my team is examining the terrestrial world is to create test systems in the laboratory. Here we are taking samples from the abovementioned sites and creating microcosms in the laboratory, so we can artificially alter the conditions in a controlled environment, such as by adding nitrogen or water to the soil. By comparing transcriptomic data from the microcosms with those from the field, we can begin to identify trends that we hope will help us model future outcomes, such as: Will an increase of certain microbial species translate into an increase of carbon dioxide released back into the atmosphere? Which are the more important contributors to this process, fungi or bacteria? And how can we manage these populations to track or manage carbon sequestration in soils?

Through this research, our team hopes not only to provide better input variables for process models of what the terrestrial environment will be like in the future, but also perhaps to help mitigate the impacts of excess carbon. For instance, by identifying the types of microbes that contribute to keeping carbon in the soil (such as those that produce DOC), we are investigating the idea of "inoculating" or seeding soil with the right mix of microbes to alter the carbon flow towards increased sequestration.

Over the years, we have emerged from the Escher-sheet to make some striking discoveries. We have found that the environment is not a clean slate upon which we detect pathogens but instead harbors a complex microbiome that is required for elemental cycling and response to environmental changes—and most of this microbiome remains uncultured and is therefore inaccessible except by DNA- or RNA-based surveys. We have also observed that the surface soils in forests and grasslands are highly stratified and contribute significantly to carbon and nitrogen cycling. The soil fungal and bacterial communities are intimately associated with almost all plant life where, through root interactions, they control plant growth, survival, and resistance to pathogens. Although unseen by the naked eye, these organisms control many functions in terrestrial ecosystems.

It's not just the reptiles

I remember the moment I realized I wanted to be a scientist. I was in a horticultural science class as an undergraduate, hearing a woman speak about a career in research. At that moment, I realized that's what I wanted to do—it seemed like a career that had no rules. I've since amended this impression, based on my experience, and one of the rules I've learned is that you have to get out of the Escher melee as much as possible. I tell my students to think outside the box and to be brave enough to discuss ideas with others from different scientific backgrounds because their perspective can help you find answers. I also tell them to take advantage of the diversity of their colleagues, hone their communication skills, and find common dialog with others to discuss their science. Finally, I recommend they have fun in research by looking for novel hypotheses and unusual phenomena, but also that they be ready to drop a topic and explore a new one every year.

Coming to New Mexico from North Carolina, I found Los Alamos to be an unplanned surprise. I knew little about the Manhattan Project or the history of World War II, and I wasn't sure I would fit in well. But it has been an enormously rewarding and successful adventure. Every year that I'm here I learn more about other scientific disciplines, such as physics, engineering, chemistry, and especially computation. This diversity enables us scientists the flexibility to craft a research team appropriate for each scientific grant, and being at the Lab has facilitated studies I could never have dreamed of doing at a university and could not have done as a solo investigator. Los Alamos has exceptional scientific, technical, and support staff all in one place. By working in this complex, interdisciplinary environment, I have also come to appreciate more about the Lab's history in World War II and the Manhattan Project—how innovation was fueled by allowing scientists of varied backgrounds to come together and push the boundaries of knowledge.

—Cheryl Kuske