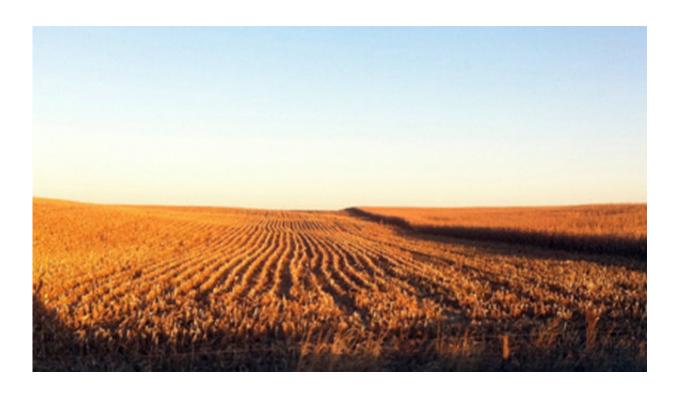


Sequencing the genomes of a microbial ecosystem

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One of the Iowa corn field soil sampling sites used to generate data for the Great Prairie Metagenome Grand Challenge. Credit: US Department of Energy

Corn stalks rustle and tractors trundle along, their wheels grinding into the Kansas soil. While it seems like farmers and machinery conduct most of the work on a modern farm, much of the heavy lifting goes on below the surface. The American Midwest's Great Prairie is home to one of the planet's most complex systems of organisms that can't be seen



with the naked eye. Researchers supported by the Department of Energy's Office of Science are charting the genes of this community of microorganisms, an effort that could unlock new approaches to genetics, ecology, and agriculture.

Once called America's Breadbasket, the Great Prairie's productive soil locks in more carbon than any other place in the country. Storing carbon is essential to limiting climate change and maintaining agricultural productivity. However, due to agricultural practices like tillage, the soil has lost 20 to 60 percent of the carbon it once stored.

"[The Great Prairie] is one of the great resources of the world," said James Tiedje, a microbiologist at Michigan State University.

To better understand the tiny organisms that support this ecosystem's fertility, researchers working with the Joint Genome Institute (JGI), an Office of Science user facility, are tackling the Great Prairie Metagenome Grand Challenge. Just a half a teaspoon of soil houses billions of microbial cells, which carry out essential processes such as carbon and nitrogen cycling. But scientists don't fully understand how they function or react to environmental changes.

"In the past, microbes have only been considered secondary because they're small and can't be seen," said Tiedje. "But their biochemical capabilities are tremendous. And they essentially work for free for us."

Under the Ground and Into the Lab

Because most of these microbes are difficult to cultivate in the lab, researchers went straight to the source – the fields and prairies of Iowa, Wisconsin, and Kansas. To investigate the difference in microbial diversity and abundance between farmed land and <u>native prairie</u>, they compared eight sites: three that had never been cultivated, three that had



corn grown on them for more than 50 years, one site that had switchgrass (a native plant that can be used to make biofuels) grown on it, and one site that had been restored to prairie after long-term cultivation.

The scientists' task began in the mid-summer heat, when plant growth was at its peak. After clearing away grasses, wildflowers, and corn stalks, researchers plunged a soil corer five inches into the ground. For all of this effort, they extracted just 1 cm2 worth of soil and its associated microbes – a little less than the size of a sugar cube – per location. To preserve the precious microbial cargo, they shipped it on ice overnight to the lab.

That's where the real challenge began. Metagenomic studies assemble the genomes of all of the individual microbes within a community, collectively called the microbiome. To sequence a single organism's genome, scientists must identify the order of all of the nucleotides – the four base pairs that serve as genetic building blocks – within its DNA. For the Human Genome Project, scientists isolated and studied human DNA, which has about three billion nucleotides. In comparison, a single gram of Great Prairie soil – about a small pinch of salt – contains more than one trillion bases of DNA (300 times more than the human genome), from more than 100,000 different species.

Taking a Risk on Research

In fact, the Great Prairie project generated so much complex data that when it started in 2009, JGI devoted more sequencing resources to it than it ever had to a single project.

"It was daring at the time," said Janet Jansson, division director of biological sciences at Pacific Northwest National Laboratory. While JGI provides the resources, researchers from a number of DOE national laboratories and universities are collaborating on the project.



Before the Challenge, scientists thought it might be impossible to piece together the genomes of individual microbes. But these investments resulted in tools and platforms that opened up new areas of research.

The scientists began with a common process called shotgun sequencing. This process cuts long stretches of DNA into fragments of the same size, analyzes the order of the base pairs that make them up, and then puts the pieces back together. It's like shattering a stained glass window, looking at the pieces under a microscope, and then reassembling them. However, in the case of genomes, the researchers had no idea what the original "picture" was. While this technique inevitably leaves gaps, it's the best one that researchers have to analyze this type of genomic information.

Normally, powerful software searches databases to see if the microbial genes being studied resemble those that researchers have previously sequenced. But scientists could match fewer than half of the Great Prairie microbial proteins to existing information.

So they started from scratch. After filtering the data to make it simpler but still representative, they pieced together sequences of the genome by comparing different sections in the sample to each other.

"If you can achieve genome binning in Kansas native prairie, you can do it anywhere," said Jansson. From an analysis point of view, she says, "This is the worst case scenario in terms of high diversity and complexity."

The Language of Biology

Previously, studying prairie microbiomes was a lot like watching a movie in a foreign language – you could see that people were doing things, but not know why or how. Sequencing these microbes' genes provides a dictionary. Scientists still don't have the full script, but it's the first step



towards a broader understanding.

Scientists from universities around the country are already using these data to examine how land management practices influence microbial communities. They found that the native prairie had twice as much microbial biomass, a key indicator of its quality and health, than the farmed areas.

In addition, they found that cultivation seems to diminish not just quantity but also much of the natural diversity across soil types and geography. The microbiome in the Iowa native prairie was far different from the microbiome of the Wisconsin native prairie. In contrast, cultivated fields had similar sets of microbes no matter where they were located.

But the scientists also found something surprising – the cultivated areas had a higher total number of microbial species on average than the native ones. Perhaps fertilizer use that provides more nitrogen and simulates plant growth provides more food for a number of different microbes. In native <u>prairie</u>, microbes need to compete for key nutrients, including nitrogen and phosphorus. They also have less carbon from dead and dying plants available. Not needing to compete as much for nutrients could lead to more microbial diversity in farmed areas.

"Life on our planet is dependent on microorganisms that we know very little about," said Jansson.

To enable future studies that can further explore the connections between genes, proteins, and microbial functions, the <u>Great Prairie data</u> is <u>publically available</u> in the JGI's Genome Portal. The Great Prairie Metagenome Grand Challenge is only one of thousands of complex projects on microbial genomics that JGI has supported.



Through the Great Prairie project, scientists are learning more every day about the <u>microbes</u> that are so fundamental to life in Kansas and beyond.

Provided by US Department of Energy

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