The Roots of the Matter

By Lori Dajose (BS '15)

Caltech faculty are working to make farming practices more sustainable.

Off the coast of Peru, a group of small islands in the Pacific Ocean sit encrusted with layers of unusual riches: bird dung.

Bird dung, or guano, contains nitrates and ammonia—chemical ingredients that nourish plants—making it a valuable natural fertilizer to help crops grow. In the mid-1800s, countries with burgeoning populations required an ever-increasing supply of crops to feed their people, leading to international conflict over mining rights to the so-called Guano Islands off the Peruvian coast.

The guano gold rush subsided in the early 20th century thanks to a scientific feat that revolutionized the nature of food production: Scientists Fritz Haber and Carl Bosch developed a method now known as the Haber–Bosch process to synthesize ammonia and thereby create synthetic fertilizers. Both researchers won a Nobel Prize in Chemistry for their efforts (Haber in 1918, Bosch in 1931). These synthetic fertilizers greatly improved crop yields, reducing famines and enabling population growth around the world.

While this technological innovation changed the planet and saved many lives, the Haber–Bosch process produces a significant carbon footprint. And with the world's population projected to hit 10 billion by the end of the century, agriculture is at yet another crossroads: Can humanity grow enough food for the growing population in a way that doesn't hasten the planet's demise?

A group of Caltech faculty members, with help from Caltech's own Resnick Sustainability Institute (RSI), have taken on this challenge by tackling innovative projects in the hope of making agriculture more sustainable and revolutionizing the farming practices of the future. At the forefront of these efforts are the quest for a cleaner way to synthesize ammonia for use in fertilizer, a "communication" system that would allow farmers to monitor nutrient levels in plants and soil as a way to make fertilizer application less wasteful, and a genetically engineered plant that can uncover the workings of the underground root environment.

Did you know?

Global fertilizer consumption was expected to grow from 177.2 million metric tons in 2011–12 to 195.4 million metric tons in 2023–24, a 10.3 percent increase over 12 years, per the data firm Statista.

'Clean' Fertilizer?

All crops, from wheat to blueberries to lettuce, need the same basic chemical building blocks to grow. While plants can get certain elements from their surroundings—such as oxygen from the air and phosphorous from the soil—they need nitrogen in a specific form that does not exist in the atmosphere. Analogous to how humans cannot digest uncooked potatoes, plants cannot utilize N_2, the common form of nitrogen found in air. Nitrogen gas needs to be "fixed," or transformed into a usable form to make it edible for plants. Soil bacteria naturally fix N_2 using nitrogenase, an enzyme that transforms the nitrogen into NH_3, or ammonia, which plants then absorb through their roots. But this natural process cannot keep up with the massive scale of agricultural food production required to feed the global population. So, farmers apply fertilizer, be it dung or synthetic ammonia made through the Haber–Bosch process, which helps farmers keep up with demand for their crops.

But fertilizer produced by the Haber–Bosch process has the largest carbon footprint of any human-made chemical product; carbon dioxide emissions from its production make up 1 to 2 percent of the global total. That is because the Haber–Bosch chemical reaction combines hydrogen from fossil fuels like oil or natural gas, with nitrogen gas. Under high pressures and temperatures, hydrogen gas extracted from the fossil fuels is combined with nitrogen to create the usable form of ammonia. However, the process creates the greenhouse gas carbon dioxide. It also requires costly infrastructure, which is why it is performed at only a few hundred industrial sites worldwide. This means transporting the fertilizer to rural farmers is expensive and results in ammonia-based fertilizers like urea costing two to three times more in sub-Saharan Africa than in the United States, says Caltech chemist and chemical engineer Karthish Manthiram.

To address these issues, Caltech researchers, led by Manthiram and Jonas Peters, a Bren Professor of Chemistry and director of the RSI, are working on a project to synthesize ammonia in a clean, more sustainable way. Instead of extracting hydrogen molecules from fossil fuels, the team seeks to use the hydrogen in water: H_2O. Oxygen is the only by-product of this process, which can occur at room temperature and pressure.

"My parents grew up involved in agriculture in India," Manthiram says. "The ability to feed yourself and your family depends deeply on the productivity of the soil and the animals that live off of it, and it gives you a deep respect and attachment to the earth. Enabling farmers to produce their own fertilizer—in a cheap, clean, local manner—is a major motivation for our project."

However, this cleaner chemical reaction between air and water does not occur spontaneously: It needs a catalyst to start. That is why researchers in the Manthiram lab are developing an electrochemical cell that contains a recyclable catalyst to enable the process. The device at present is a lab-scale prototype and uses lithium to drive the reaction forward.

"With distributed ammonia production, a farmer could synthesize their fertilizer right where they need it instead of importing it from far away," Manthiram says. "This helps food production to be resilient against geopolitical strife as well. For example, fertilizer prices quadrupled at their peak since the war in Ukraine began, as natural gas from Russia was a major source of the hydrogen required to synthesize ammonia."

Meanwhile, chemist Doug Rees and Steve Mayo (PhD '87), the Bren Professor of Biology and Chemistry, are looking at nitrogen fixation through a biological lens, examining how bacteria use the enzyme nitrogenase to transform N_2 into NH_3 at normal temperatures and pressures. The triple bond between N_2's two nitrogen atoms is one of the strongest bonds in nature, hence why the Haber–Bosch process requires such high temperatures and pressures to break it. But nitrogen-fixing bacteria, including those that have symbiotic relationships with plants, can cleave this bond using only the enzyme nitrogenase.

For decades, Rees has been a leading expert on deciphering the complex structure and workings of the enzyme. With Mayo's protein engineering expertise, the two are attempting to engineer a version of the nitrogenase enzyme in the lab that is more effective and efficient at fixing nitrogen. The ultimate goal would be to develop biologically inspired methods of nitrogen fixation that do not require the extreme temperatures and pressures of current technologies. "Nitrogenase is essential for life," Rees says. "The goal is to learn from nature's own nitrogen-fixation methods to possibly develop our own."

Karthish Manthiram holds a prototype of his lab's electrochemical cell for cleaner ammonia production. Behind him, from left to right, are graduate students Channing Klein, Michael Yusov, and Anukta Jain.

The Overfertilization Problem

When farmers apply too much fertilizer to their soil, ecosystems can become damaged. Eutrophication is a phenomenon that occurs when runoff from agricultural systems enters nearby water environments, causing an excess of nutrients to inundate the aquatic ecosystem. This creates a sudden proliferation of plant life, like algae, that suffocates other life-forms and throws off the delicate ecosystem balance.

Eutrophication has been present in California's Salton Sea for decades, killing fish and birds, including endangered species.

Runoff is also an issue because fertilizer is not an infinite resource. In addition to nitrogen and potassium, one of fertilizer's three major components is phosphorous, which must be mined from ever-shrinking deposits around the world. Experts estimate that farms waste 9–14 million tons of phosphorous each year through agricultural runoff, so the judicious application of fertilizer is crucial both for protecting ecosystems and for managing the globe's limited phosphorous supplies.

To help farmers know exactly when and where to fertilize their crops, researchers are developing methods to monitor the complex underground ecosystem of the rhizosphere, the place not far below ground where plant roots, soil, and microbes converge. On average, there are a billion bacterial cells per gram of soil in the rhizosphere all battling one another for coveted niches near a plant's roots. In addition, plants secrete chemical signals and nutrients into the soil, hoping to attract beneficial microbes and repel harmful ones. With such a complex environment, there are still many unanswered questions about this underground frontier.

Now Caltech researchers are beginning to uncover some of those answers. "The study of the rhizosphere was lacking real-time methods for data acquisition," says microbiologist Dianne Newman. "There was a large unmet need in the field where Caltech expertise could really make a difference."

If Plants Could Talk

Newman is combining her microbiology expertise with the skills of Azita Emami, the Andrew and Peggy Cherng Professor of Electrical Engineering and Medical Engineering, and director of the Center for Sensing to Intelligence; Changhuei Yang, the Thomas G. Myers Professor of Electrical Engineering, Bioengineering, and Medical Engineering; and Julia Kornfield (BS '83), the Elizabeth W. Gilloon Professor of Chemical Engineering, to develop technology that can measure phosphorous levels in soil in real time. In a project led by postdoc Reinaldo Alcalde (who works in Newman's lab), the interdisciplinary team has created a biosensor roughly the size of a quarter that can be buried underground and contains soil bacteria genetically engineered to produce fluorescent proteins when the bacteria experience certain conditions, such as low phosphorous levels. When the device detects the fluorescent glow, it sends a wireless message above ground to a receiver that alerts the farmers, who can then make informed decisions about whether to apply additional fertilizer.

The device is currently in the assembly stage: The team has generated fluorescent biosensors and is assessing their activity when contained in polymers, as they will be in the finished product. In parallel, a complementary metal-oxide semiconductor device has been designed to interface with the bacteria in order to translate their fluorescent signal into one that can be received by Bluetooth. By next fall, the team hopes to bring the two components together and begin testing the performance of the device in soils.

"Our long-term goal is to plant these biosensors throughout a field and get information on when and where you need fertilization," Alcalde says. "That would be the dream. But there are many fundamental scientific and engineering challenges we are currently working on: For instance, how long can engineered bacteria encased within a polymer remain metabolically active without growing? There are all sorts of interesting questions."

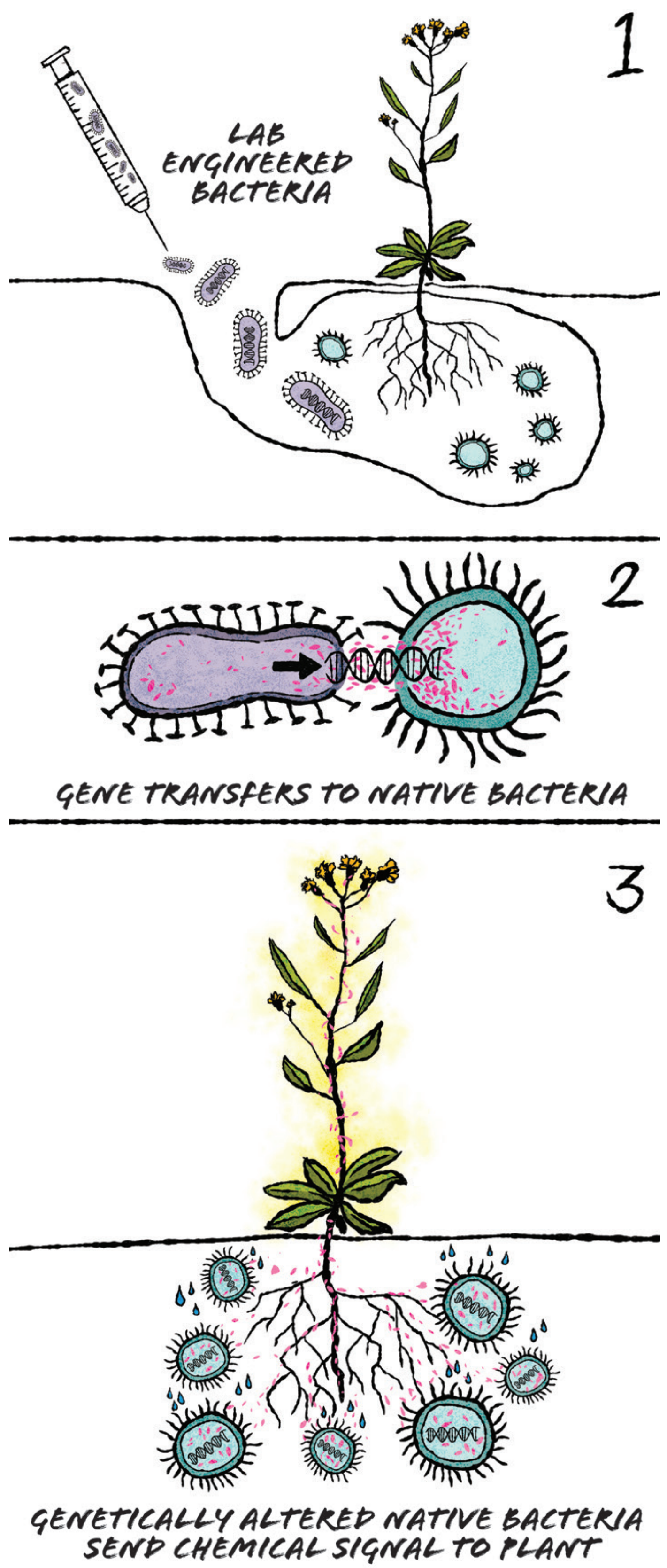
Meanwhile, postdoc John Marken and his colleagues at Caltech are designing a fluorescent system to observe conditions in the rhizosphere, but their version is geared toward scientists rather than farmers. Utilizing genetic engineering expertise from the labs of Niles Pierce, the John D. and Catherine T. MacArthur Professor of Applied and Computational Mathematics and Bioengineering, and Gözde Demirer, the Clare Boothe Luce Assistant Professor of Chemical Engineering, along with plant and microbial biology knowledge from the labs of Bruce Hay, professor of biology (in which Marken is a postdoc), and Elliot Meyerowitz, the George W. Beadle Professor of Biology, the team is developing a noninvasive system that will allow researchers to study the basic biology of root systems and the complicated relationships between microbes in the rhizosphere.

"The challenge with studying the rhizosphere is that it's all underground," Marken says. "You can't see what's happening inside, but you want to monitor what's happening in real time without having to disturb the system."

One way to do that is to monitor the natural signals a plant's leaves convey about the plant's underground conditions. For example, a houseplant's leaves may turn yellow, indicating that its roots are receiving too much water. In this project, the team took inspiration from such natural processes to design genetically engineered "sentinel" plants that can keep watch over the rhizosphere, delivering signals to researchers through their leaves in the form of a fluorescent glow that occurs when the microbes in the rhizosphere experience specific conditions.

The system is crafted so that the fluorescence can be coupled with the exact traits a researcher would like to study. For example, a sentinel plant could be designed such that its leaves will glow when rhizosphere bacteria nearby are expressing genes involved in nitrogen metabolism.

The *Science* of Sentinel Plants



1. Laboratory bacteria are engineered with a genetic program that causes them to secrete a signal compound (such as a plant hormone or a bacterial communication compound) if a specific target gene (such as *nosZ*) is being expressed. These bacteria are inserted into the soil, where the sentinel plant has already been planted.

2. Once in the soil, the lab bacteria pass on this ability to the native root bacteria through a natural process known as horizontal gene transfer.

3. When the native bacteria secrete the chemical signifying that the gene of interest is being expressed, the chemical is taken up by the sentinel plant's roots and travels to the leaves. There, the signal compound activates an engineered genetic reporter in the sentinel plant that causes its leaves to glow. The system can be designed to detect any gene of interest, enabling researchers to learn about multiple bacterial processes in the rhizosphere.

Postdocs Reinaldo Alcalde, front, and Hannah Jeckel inspect a plant and soil sample in the lab of Dianne Newman.

Pinpointing the conditions under which bacteria switch on their ability to metabolize nitrogen is particularly useful in the case of nitrous oxide (N_2O), a potent greenhouse gas. N_2O is produced as a by-product of applying nitrogenous fertilizers to cropland. Some microbes in the soil can convert it into harmless nitrogen gas—provided those microbes are expressing the gene known as *nosZ*. The conditions that drive these microbes to express *nosZ* are not completely understood. If sentinel plants can fluorescently indicate aboveground that *nosZ* is being expressed by root bacteria, then scientists can develop experiments to tweak various rhizosphere conditions and study how they affect *nosZ* expression. Answering basic biological questions like these could ultimately lead to more sustainable farming practices.

"This technology isn't necessarily going into a farmer's field; it's letting us conduct experiments to answer questions about the workings of complex root ecosystems," Marken says. "Just as basic scientific advances in our understanding of cellular biology and human physiology allow us to develop medical treatments to promote health and treat disease, the insights from technologies that let us understand the rhizosphere will empower farmers and policymakers to make informed sustainable agricultural decisions."

While the complex sentinel plant system has many scientific and engineering challenges, its potential rewards are profound. "Once we understand the causal underpinnings of microbial behaviors in the rhizosphere, we can develop new technologies and land-management strategies to promote a more sustainable stewardship of our natural and agricultural soils, simultaneously promoting both human benefits and ecosystem health," Marken says.

Newman says Caltech's multifaceted approach is well designed to redefine the nature of farming in the future. "It's important to have methods for both lab work and field research," she explains. "These high-risk, high-reward projects are pushing the frontiers of certain technologies in the lab. Meaningful research happens all along the continuum from the lab to real-world applications."

Reinaldo Alcalde is a postdoctoral scholar fellowship trainee in biology and biological engineering.

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