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THE LITTLEST FARMHANDS

Scientists are discovering thousands of microbes that help plants survive and thrive. Could these symbionts help farmers as well?

By Jop de Vrieze in Palencia, Spain

“Look. Nothing flourishes here. Not even weeds.” Pius Floris picks up one of the dozens of stones scattered around him on the degraded, barren-looking soil. Decades of drought, monoculture, overuse of fertilizer, and excessive plowing have taken their toll on this field in the Spanish region of

Castilla y León. As a result, wind and rain have washed away all but 25 centimeters of the fertile topsoil that used to nourish the grain here. For centuries, this area was a bread basket; today, yields are so low that farmers work the area only because of subsidies from the European Union.

Floris, a Dutch entrepreneur in plant health, wants to turn that situation around.

Mycorrhiza fungi (yellow) help the roots of this soybean plant absorb nutrients and water; to return the favor, the plant excretes nutrients for the fungi.

With researchers at the University of Valladolid and a team of local farmers, he participates in an E.U.-funded pilot project that aims to make profitable agriculture possible again on such damaged soil, without irriga-

PHOTO: MERTON BROWN/VISUALS UNLIMITED INC.

tion. His key collaborators: microbes.

One reason the soil here has gone to waste, Floris says, is that farming has destroyed its microbial ecosystem, which can help plants survive and thrive. His team has recently applied beneficial microbes—in particular fungi that live around plant roots—to this degraded area, in addition to organic fertilizer. “Farmers have ignored these symbionts for decades,” Floris says. “We are bringing them back into the game.”

He's not the only one trying to do that. Scientists have recently discovered a dizzying diversity of bacteria, viruses, and fungi that live in, on, and around plant roots in the soil. Many of these microbes, together called the rhizobiome, help plants one way or another, from providing nutrients to warding off crop pests and diseases. Small biotechnology and major plant science companies think they have huge potential benefits in agriculture and have recently begun a spate of new field trials. A 2013 report published by the American Academy of Microbiology (AAM), with the optimistic title *How Microbes Can Help Feed the World*, concluded that microbes have the potential to increase harvests while allowing farmers to use less fertilizer and pesticides; certain microbes can even enable plants to grow in very dry or salty places, which could help the world adapt to climate change.

A few denizens of the soil have been on the market for decades, such as *Trichoderma* fungi that suppress pathogenic fungi, and the now well-known caterpillar killer *Bacillus thuringiensis*, or *Bt*. (The gene for the bacterium's toxin has also been introduced into some crops' genomes.) Recently, major agrochemical companies such as Bayer have jumped on the biologicals bandwagon. “It's a revolution of microbiology,” says Thomas Schäfer, vice president of microbial R&D at Novozymes, a company developing microbial fertilizers and pesticides that recently struck up an alliance with agriculture giant Monsanto. Schäfer believes farmers are headed for “precision agriculture,” in which they would add beneficial microbes, or support existing ones, after an in-depth analysis of a field's microbiological makeup.

Whether these high expectations can be met by the budding research field is anyone's guess. The diversity in the soil microbiome is so staggering that finding out which organisms benefit plants most, how they do it, or what combinations work best is a gargantuan task. Also unclear is whether microbes can dramatically curb the use of pesticides and chemical fertilizers, and whether conventional farmers will trust these new options. The central question, some scientists say, is: How much can microbiology replace chemistry in agriculture?

SCIENTISTS AND FARMERS have long seen microbes primarily as problems. A fungus-like unicellular organism named *Phytophthora infestans*, responsible for potato blight and other crop diseases, has caused famines throughout history and is still a major problem. A variety of other fungi and bacteria cause the decay of roots and leaves.

To be sure, farmers know that some microbes are helpful: The group of bacteria called rhizobia, which live inside the roots of legumes and fix nitrogen from the air into a biologically useful form for the plants, are a textbook example. But recently, new methods of DNA sequencing and analysis have brought a vast, complex web of mutually beneficial interactions into view, comparable to the symbiotic roles researchers now believe are played by the thousands of bacterial

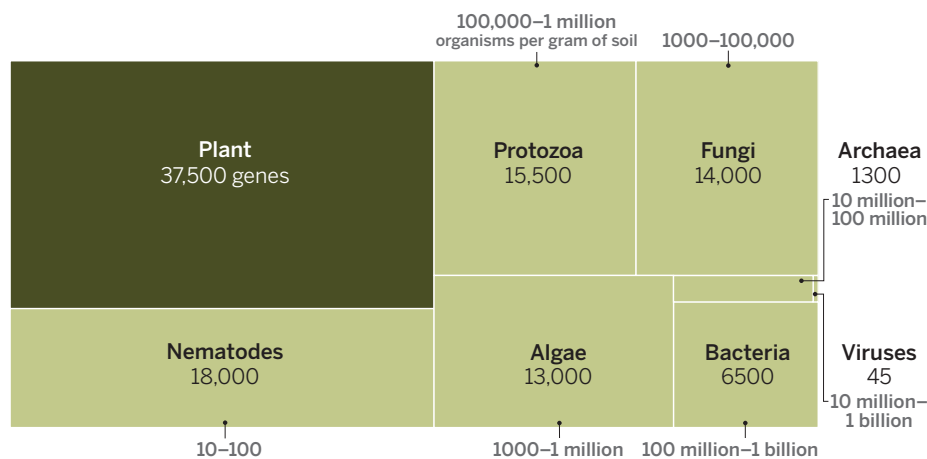
plants, produce hormones that spur growth, stimulate the plant immune system, and trigger or dampen its stress responses. “In general, we can say that a more diverse soil microbiome results in fewer plant diseases and higher yield,” Raaijmakers says.

Among the most helpful microbes are so-called mycorrhizae or root fungi, which form a dense network of thin filaments reaching far into the soil, acting as extensions of the plant roots they live on or in. These fungi facilitate the uptake of water and a wide range of nutrients—Floris calls them “the plant's shopping carts.”

Microbes can also help plants survive extreme conditions. A 2007 study showed that a complex symbiosis with fungi and viruses makes it possible for a grass called *Dichanthelium lanuginosum* to thrive in

Underground world

Vast numbers of organisms—presented here along with their average number of genes and their numbers per gram of soil—live in and around a plant's root system.



and viral species that inhabit the human gut, skin, and other tissues.

Studies have shown that there are up to 10 billion bacterial cells per gram of soil in and around plant roots, a region known as the rhizosphere. This domain is tremendously diverse; in 2011, a team led by soil microbiologist Jos Raaijmakers of the Netherlands Institute of Ecology in Wageningen detected more than 33,000 bacterial and archaeal species on sugar beet roots. Dozens of species appear to suppress plant disease by excreting substances that ward off pathogenic microbes or occupying niches otherwise taken up by the pathogens. A study of vineyards in New York, published in March, showed that the composition of the rhizobiome depends heavily on the soil type.

Lab and greenhouse experiments have also shown that microbes make a variety of nutrients and minerals in the soil available to

geothermal soils in Yellowstone National Park, where temperatures reach 60°C. The fungus, now thoroughly studied and introduced in the U.S. market in 2014 for application on corn and rice, triggers a stress response that the plants can't switch on themselves.

Similarly, a bacterium called *Stenotrophomonas rhizophila* has been shown to strongly increase drought tolerance in crops like sugar beets and maize. A 2013 study offered an explanation: The microbe excretes a variety of molecules that help plants withstand stress, including so-called osmoprotectants, which prevent the catastrophic outflux of water from plants in very salty environments. Microbes can even affect the flavor of food plants: A bacterium called *Methylobacterium extorquens* increases the production of furanones, a group of molecules that gives strawberries their characteristic flavor.

The services provided by microbes are apparently hugely important to plants, as they put in a lot of energy to return favors. Studies have shown that up to 30% of the carbon fixed by plants is excreted from the roots as so-called exudates—including sugars, amino acids, flavonoids, aliphatic acids, and fatty acids—that attract and feed beneficial microbial species while repelling and killing harmful ones.

THE GROWING ACADEMIC understanding of the rhizobiome has increasingly made its

way into the corporate world and onto farmers' fields. One early example was Serenade, a biopesticide containing a *Bacillus subtilis* strain that has antifungal and antibacterial properties and promotes plant growth. It was discovered by AgraQuest, a biotech in Davis, California. "So many pharmaceutical products were extracted from the soil, but for agriculture, this potential was hardly exploited," recalls Denise Manker, who co-founded the company in 1995. Serenade, registered by the U.S. Department of Agriculture in 2001, can be applied in a liquid form on

the plants and in the soil to fight a range of pathogens. "Initially, most of our customers were organic farmers," Manker says. Soon, she says, innovative conventional farmers started experimenting with the product as well, and some became converts.

So far, the market for such products has been modest. Almost all of the registered ones are biopesticides; the AAM report estimated that they bring in about \$1 billion annually, which pales compared with the global markets for chemical pesticides and fertilizer, estimated at \$50 billion and \$60 billion annually, respectively. But big agrochemical companies see the potential of microbial alternatives. "It took us 17 years to get the big companies interested, but we made it," Manker says: In 2012, German agro giant Bayer bought AgraQuest for \$425 million. Manker became Bayer's director of global agronomic development of biologicals, a job that comes with a €10 million annual research budget. She's using it to field-test dozens of new fungi and bacteria to replace chemical pesticides or serve as biostimulants, which promote the health and growth of crops.

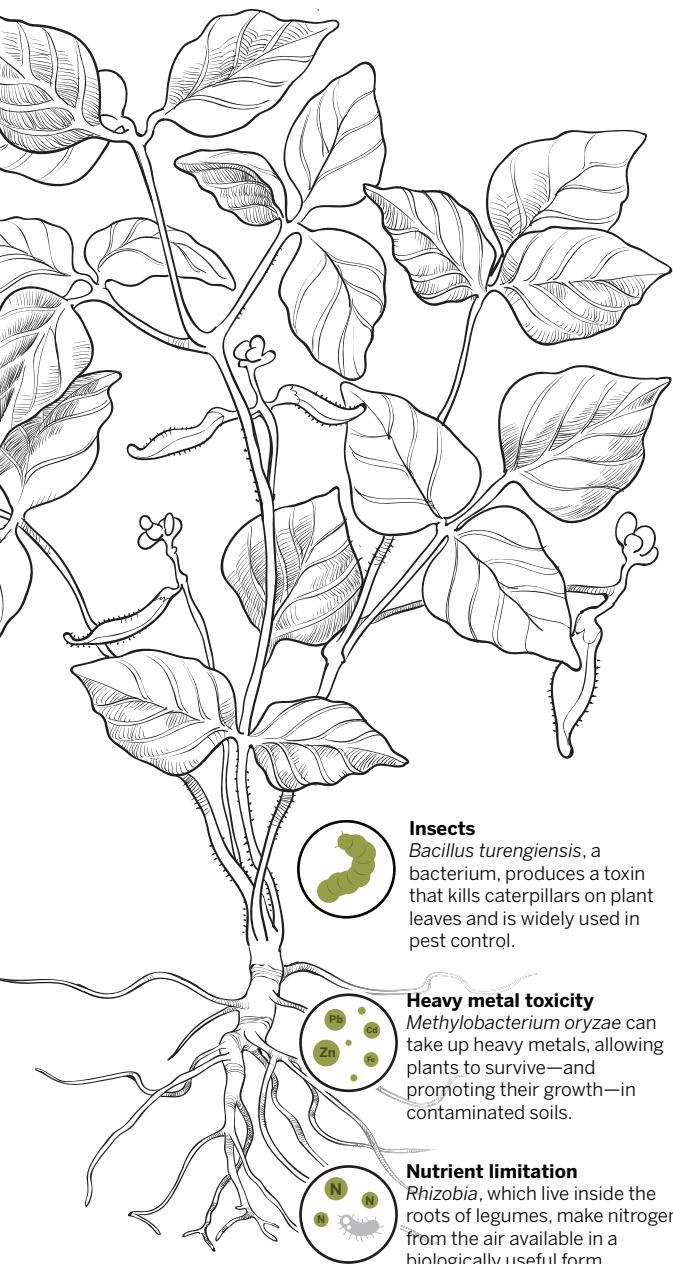
One explanation for Bayer's interest: Growing public resistance against chemical pesticides and a 2009 European directive aiming to reduce their use caused the market for chemical crop protection to stagnate, whereas the demand for biologicals was growing close to 10% per year. Given that, it's not surprising that Bayer's competitors have made similar moves. Syngenta and BASF acquired smaller companies developing microbial products last year; so did Dupont in April of this year. Monsanto's new partner, Novozymes, has invested heavily in a biofertilizer containing the soil fungus *Penicillium bilaii*, and a bioinsecticide that contains the fungus *Metarhizium anisopliae*.

The list of potentially suitable microbes is endless, says Matteo Lorito, a plant pathologist at the University of Naples Federico II in Italy, and that poses a daunting task for companies. "The challenge they are facing is selection of the ones that are commercially viable and effective," Lorito says, especially because many microbes are plant-specific and the composition of the rhizobiome can change rapidly.

Traditionally, selected microbes were first tested and investigated extensively in labs and greenhouses. But promising strains often failed to prove effective in the field, because of soil, climate, and ecosystem effects. Today, most companies use a "field-first approach," in which hundreds or even thousands of microbial strains are tested on field plots. If one proves successful, the mechanism of action is unraveled in the lab later. But even a promising field

How microbes help plants

Microorganisms living in roots and the soil can shield plants from a wide variety of threats. Here are some examples.



Heat

A symbiosis of *Curvularia* fungi and the *Curvularia* thermal tolerance virus living in the roots can increase plants' heat tolerance by more than 20°C.



Cold

A cold-tolerant *Pseudomonas* bacterium helps plants grow by fixing nitrogen from the atmosphere at temperatures as low as 4°C.



Drought

Mycorrhiza fungi such as *Glomus deserticola* extend the root system and can provide water from deeper soil layers to the plant.



Flooding

Enterobacter cloacae, a bacterium, protects plants from the destructive impact of flooding by reducing levels of a stress hormone that impairs root growth.



Osmotic stress

A bacterium called *Stenotrophomonas rhizophila* can excrete osmoprotectants, which prevent the catastrophic outflow of water from plants in very salty environments.



Pathogens

Soil fungi named *Trichoderma* can kill and outcompete pathogenic fungi and can activate the plant's own immune system.

Insects

Bacillus turengiensis, a bacterium, produces a toxin that kills caterpillars on plant leaves and is widely used in pest control.

Heavy metal toxicity

Methylobacterium oryzae can take up heavy metals, allowing plants to survive—and promoting their growth—in contaminated soils.

Nutrient limitation

Rhizobia, which live inside the roots of legumes, make nitrogen from the air available in a biologically useful form.

study doesn't guarantee success on the farm. "People are using microbial products on a variety of crops, with different application methods and in different soils and climates," says Matthew Wallenstein, an ecosystem scientist at Colorado State University (CSU), Fort Collins. "That will make the results a lot more variable. It's very hard to make a miracle product that works everywhere."

That biologicals are living things is part of the problem; to work best, they need to become established and thrive on their own. One way to give them an edge is to apply them on plant seeds instead of into the soil; that way, they can enter the plant's rhizosphere early on as the first roots form and have a better shot at dominating the space.

Populations of beneficial microbes also dwindle over time. Spraying Serenade, for instance, results in a high *B. subtilis* density in the soil initially, but levels rapidly decrease during a farming season as the bacterium fails to obtain a permanent niche. That may be because it's outcompeted by the existing community of microbes. "Applying just that one strain is often not enough," Raaijmakers says. "You need a consortium of two, three, or even five or more collaborating strains that can withstand the ecological forces." To find successful combos, scientists have recently begun selecting these combinations in a systematic way, by identifying naturally occurring microbial networks in the field and studying their interactions down to the molecular level in the lab. "Scientifically, that's the way to go," Raaijmakers says.

Registering cocktails as biopesticides is a challenge, however, he says. Both in the United States and Europe, companies have to provide regulatory authorities with evidence that both the individual strains and the product as a whole are safe for consumers and the environment. "This is a laborious and expensive process," Raaijmakers says. That's why many of the existing products are not labeled "biopesticides," but "biostimulants"; the latter category is easier to get registered, but the market is less lucrative.

Because centuries of breeding may have robbed crop plants of an ability to attract beneficial microbes themselves, Raaijmakers also leads a project to study ancient crops and their microbes in their natural environment, such as wild beans in Colombia and the wheat ancestor *Triticum tauschii* (Tausch's goatgrass). The hope is to identify plant traits and symbiotic microbes that could benefit modern crops. The effort could yield compounds that can be applied to plants or soil,

but in the long run, scientists hope to find the genes encoding the exudate molecules that attract microbes and reintroduce them into modern crops. "For the first time, breeders and biocontrol people are talking with each other," Lorito says. "They are now starting to include the interaction with microbes into the breeding."

FLORIS, THE DRIVING FORCE behind the pioneering project to restore the soil in Spain, has helped various factions speak with one another. Decades ago, while working for



Pius Floris is using a mix of microbes and organic pesticides to help restore a depleted field in Spain.

a Dutch tree care company—and studying with a famed plant pathologist of the U.S. Forest Service—he realized that roots do much more than anchor trees and suck up water. Today, he owns a company that offers advice and biological products to improve soil life to farmers, gardeners, and horticulturists; Raaijmakers and several other scientists also tap his practical knowledge.

Floris's vision is more radical than most: a "cure" for degraded farmland, based on a holistic set of measures that puts microbes front and center. The hope is that this will even do away with the need for artificial fertilizer, "the mother of all evil," according to Floris, because it's disastrous for microbial life. Fertilizers deplete soil of organic matter and trace elements, cause salination, and suppress mycorrhizae; studies have shown that they can also turn symbiotic bacteria into competitors that kill each other.

The pilot project, now in its third year and as-yet unpublished, suggests that Floris's approach may work. The team borrowed an ancient Roman plow from a local museum to loosen the soil just slightly and made ridges to retain rainwater. They seeded oat plants along with vetch, which attracts bacteria that fix nitrogen and leave it in the vetch's roots after the harvest. They planted small olive trees to boost microbial diversity. Then they split

the 100-hectare field into three zones. Zone A was treated with chemical fertilizer and pesticides; zones B and C were supplied with different amounts of an organic biofertilizer, consisting of fermented grape leftovers containing a variety of bacteria and fungi, and a dose of four different types of mycorrhiza spores. None of the areas were irrigated.

In May of this year, Floris strode through hip-high oats and vetch fields, bending over to inspect the crops every 2 meters. The crops in zone B, which received the most organic fertilizer, had reached nearly twice the height of those in zone A and were inches taller than zone C. "This really turns me on!" Floris shouted. (The yield of zone B, it would turn out after the harvest, equaled that of irrigated crops, whereas that of the conventional zone was negligible.)

Why this was possible became clearer when Floris's colleague Pedro Alonso dug a deep hole in zone B. Plant roots had found their way almost 2 meters into the rocky soil, deep enough to reach the groundwater. This could not have happened without the mycorrhiza, which penetrated the rock by excreting acids, Floris says: "These fungi enable the crops to survive without irrigation, even through the driest parts of the year."

Others are following the experiment with interest, but not everyone is convinced that Floris's recipe is the solution for agriculture as a whole. "If you want to make a difference, you need to fit into the existing system," CSU's Wallenstein says. "It is unrealistic to expect a sudden radical change. For farmers, it would just be too much of a financial risk to abandon fertilizers and pesticides and invest in new equipment to apply biologicals."

Prem Bindran, executive director of the industry-backed Virtual Fertilizer Research Center in Washington D.C., acknowledges that chemical fertilizers can harm beneficial microbes. "But in my view, chemical fertilizer is still essential to maintain the yield," he says. "We need to find a solution to combine the benefits of fertilizer and symbiotic microbes." Novozymes's vice president Schäfer agrees. "Biologicals can help reduce the amount of fertilizer and water used, but there will still be a role for chemistry," Schäfer says.

But Floris wants to see just how far he can push his microbial helpers. On that May day, as he leaves the Spanish acre and returns to his car, a tractor passes in an adjacent field, leaving a cloud of organic dust. "Plowing, at 30°, midseason? A disaster for the soil and its inhabitants." He sighs. "Well, we shouldn't be too pessimistic. A lot of change is happening already, and more rapidly than ever." ■