Friends of the Earth

Genetically Engineered Soil Microbes:
Risks and Concerns

Acknowledgements
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About Friends of the Earth
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I. Introduction

A handful of healthy soil contains more microorganisms than there are people on the planet. These tiny creatures, such as bacteria and fungi, play a massive role in agriculture, from making nutrients in the soil available to crops and providing crops greater immunity to pests and diseases to regulating global carbon and nitrogen cycles. Just as we are beginning to comprehend the fundamental role that the human gut microbiome plays in maintaining our health, scientists are increasingly recognizing the importance of agricultural microbiomes. And yet, soil microbiomes — and their relationship to the crops we grow and the broader ecosystems of which they are a part — are marked by incredible complexity that we are only beginning to understand. Of the billions of species of microbes that make up the living soil, only a few hundred thousand, far less than one percent, have been scientifically characterized in detail.[1]

Despite these unknowns, biotech companies have begun to commercialize genetically engineered (GE) soil microbes for use across millions of acres of farmland. At least two products are currently being used by U.S. farmers — a GE bacteria from Pivot Bio called Proven® and BASF's '2.0' version of its Poncho®/VOTiVO® seed treatment (see page 15 and 16). The U.S. Environmental Protection Agency (EPA) reports that it has registered eight GE microbes that act as pesticides, however, it is all but impossible to determine what they are and whether they have been commercialized given the EPA's extreme lack of transparency (see page 7).[2]

This report provides historical context for this novel technology, insight into future trends, a summary of potential risks and policy recommendations that would ensure robust assessment and oversight as more GE microbes move from the lab to the field.[i]

A quick history of microbial inputs in agriculture

Since ancient times, farmers have worked with microbes — adding organic matter to soil in the form of compost is essentially feeding the soil microbiome. The history of applying specific microbes to achieve specific goals in agriculture dates back to the late 19th century when scientists began isolating soil bacteria and exploring their role in key agricultural activities like nitrogen fixation and control of plant pests.[3,4] In 1938, what is now the most widely used microbial pesticide, *Bacillus thuringiensis (Bt)*, was first sold to farmers in France.[5]

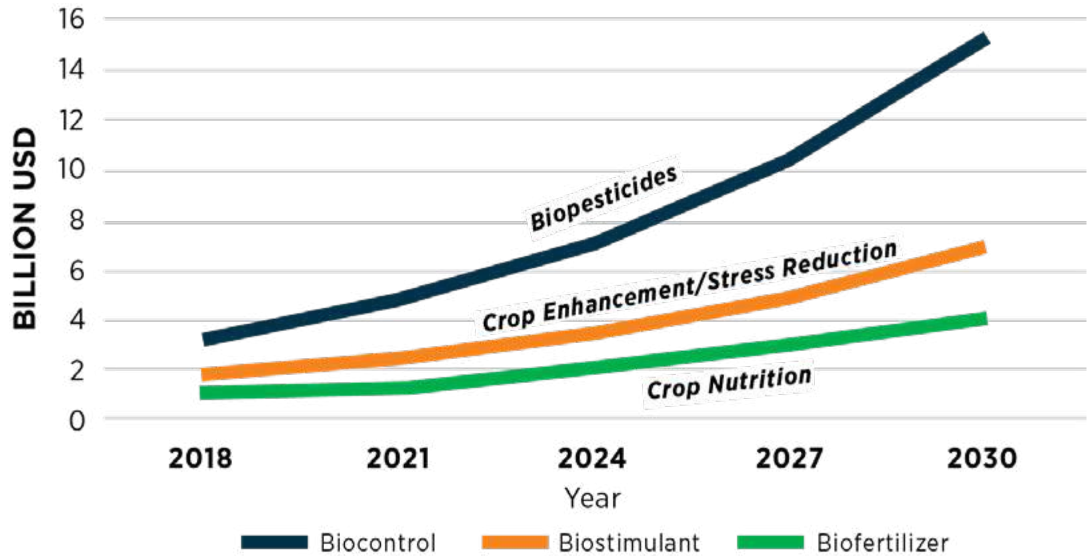
Today, over 1,200 companies worldwide offer agricultural products derived from naturally occurring microbes and plants.[6] In industry terms, these are known as 'biologicals.' Unlike synthetic chemical fertilizers and pesticides, which are derived largely from fossil fuels, biologicals are derived from living microorganisms and plants. Along with microbes, they include naturally-derived compounds like pheromones and biomolecules like amino acids. Biologicals are typically categorized as biostimulants to improve plant growth, biofertilizers to improve crop nutrition, and biocontrols or biopesticides to manage pests and diseases. They can be applied directly to soil, to the leaves of crops or as seed treatments.

Farmers can choose from many hundreds of biological products. Over 600 microbial 'fertilizers' and 490 microbial 'pesticides' are approved for use in organic production in the U.S. according to the Organic Materials Review Institute's catalogue. Biological inputs are often associated with organic and other ecological forms of production because

i It is important to understand that major agrichemical companies, along with a significant number of mid- and small-sized companies, already do — and will continue to — sell biological products that are not genetically engineered. This report aims to explore potential concerns related to GE biologicals. It is also important to understand that this report does not address genetically engineered microbes that are grown indoors in industrial conditions to make chemicals that are then applied to crops. As one example, the company Vestaron has a pesticide product called Spear® which is created by using genetically engineered yeast cells to produce components of spider venom. These GE yeast cells are grown in vats and then killed before being applied as a spray to control crop pests. Rather, this report focuses on current and prospective applications of living GE microbes released into agroecosystems.

Figure 1:

Biologicals market projected growth, 2018-2030



Source: DunhamTrimmer International Bio Intelligence

they are non-toxic, biodegradable, and work with natural systems. However, they are also used in conventional production, for example, 49 percent of respondents to an American Fruit Grower's survey report using biological inputs.[7]

The booming 'biologicals' market

We are at a major turning point in the use of biologicals in agriculture. The global biologicals market is expected to nearly triple in a span of eight years, from $10.25 billion in 2021 to $29.31 billion by 2029.[8] A major driver of the changing landscape is the entry of the largest agrichemical companies — Bayer, Syngenta (ChemChina), Corteva (Dow-Dupont) and BASF. These companies have spent millions acquiring biologicals companies in recent years and now offer a range of biological products. There is also increased interest on the part of the government, as evidenced by the U.S. Department of Agriculture (USDA) including the "development of microbial technologies" in its Science and Research Strategy platform this year.[9] And conventional growers, which, on the whole, have historically dismissed biologicals, are also showing increased interest. For example, the Western Growers Association — one of the largest agricultural trade associations in the U.S. — hosted its first-ever 'Biological Summit' this year, with Syngenta and Bayer among the sponsors.[10]

The pending proliferation of GE microbes in agriculture

While many new biological products will not be engineered, we are on the verge of a proliferation of the use of GE microbes in agriculture, as agrichemical companies and biotech startups are investing heavily in these technologies. The release of GE microbes directly onto millions of acres of farmland is a new chapter in agricultural biotechnology — GE microbes are live organisms that can reproduce and interact with other species. And, unlike plants and animals, microbes are able to share genetic material with each other far more readily, even across completely unrelated organisms in a process known as 'horizontal gene transfer.' As a result, the genetic modifications released inside engineered microbes may move across species boundaries in unpredictable ways.

Releasing GE microbes in agriculture represents an unprecedented open-air experiment that may have irreversible consequences. Once they are released, microbes cannot be 'recalled.' The scale of release is far larger, and the odds of containment are far smaller, than for GE crops. Consider the following: just under 3 trillion corn plants are grown each year in the United States, most of which are genetically engineered. An application of GE bacteria releases the same number of modified organisms about every *half an acre*.

> *The scale of release is far larger and the odds of containment are far smaller than what we have come to know for genetically engineered crops. Consider the following: just under 3 trillion corn plants are grown each year in the United States, most of which are genetically engineered. An application of genetically engineered bacteria releases the same number of modified organisms about every half an acre.*

By their nature, biologicals — engineered or not — seek to change the microbiomes of the fields or plants that they are applied to. Research shows that, while many commercial biologicals have only a short-lived effect on indigenous soil microbes or no effect at all, others may have a long-term impact. Changing the microbiome can result in directly observable changes to plant growth and insect communities, hence the interest in these products in the first place. However, while many of these changes can be beneficial to farmers, there may also be unintended consequences, such as lowering rather than improving crop yield, or increasing rather than decreasing disease suppression.[11]

We urgently need a shift in agriculture from the dominant chemical paradigm to a biological paradigm. Use of toxic synthetic chemical pesticides and fertilizers continues to rise, underpinning industrial agriculture systems that cost the world an estimated $3 trillion annually in environmental damage, according to the UN.[12] Biologicals may be able to play a significant role in helping farmers transition to ecologically regenerative and resilient systems.

At the same time, the entry of massive agrichemical companies into the field of biologicals and their interest in genetically engineering microbes raises critical questions about whether GE microbes will be used in a way that further entrenches industrial approaches to agriculture and unjust relationships of power within the food system or whether they have any potential to become part of ecological farming systems.

The problems that biotech companies are purporting to solve via genetically engineering microbes — such as pest resistance to chemical pesticides and depleted soils lacking fertility — result from monoculture industrial farming systems in the first place. In the long run, we cannot engineer our way out of these problems, we must shift to agroecological approaches that protect and regenerate the natural resources we depend on to grow food, now and for generations to come. We have decades of scientific data and millennia of farmer experience demonstrating that ecological approaches to farming already achieve what GE microbes are being marketed to do, such as provide ample nutrients for plant growth, produce healthy plants that better resist pests and diseases, and achieve greater soil carbon sequestration and improve farmers' resilience to floods and droughts.[13,14,15]

Agrichemical corporations' false marketing claims: From 'feeding the world' to 'regenerative agriculture'

Agrichemical companies are leaning on the debunked trope that we need to increase yield to 'feed the world' in their marketing of GE microbes, as they have with GE crops. Yet, a vast body of data show that hunger is not primarily a problem of overall supply of food but rather of poverty, lack of democracy, and unequal access to land, water, and other resources, especially for women.[16] What's more, these same corporations have a long track record of actively working *against* long-term food security — blocking regulations and manipulating science to keep their toxic pesticides, which harm biodiversity and human health, on the market; designing GE crops only to withstand their proprietary pesticides rather than any humanitarian goal; and leveraging their market power to increase prices of inputs for farmers. Expert consensus around the globe has called for a rapid shift from the type of input-intensive industrial agriculture that these companies profit from to agroecological farming methods in order to address world hunger and the biodiversity and climate crises we are facing.[17,18,19]

Agrichemical companies are also citing their investment in biologicals as evidence of their leadership in 'regenerative agriculture' — a movement focused on improving soil health in order to sequester carbon, restore biodiversity, conserve water and improve farmers' resilience in the face of climate change. Yet, they are primarily selling biologicals as part of 'integrated' platforms, such that the biologicals cannot be obtained separately from their engineered seeds, pesticides, and other proprietary products that are widely associated with significant harm to soil life and other biodiversity. Bayer plainly states this strategy on its website, saying that biologicals "complement traditional crop protection tools as part of an integrated crop management system." The company sells a GE microbe as part of Poncho®/VOTiVO® 2.0 seed treatment, which also contains the neonicotinoid insecticide clothianidin, which is associated with serious harm to soil organisms, pollinators and other beneficial insects, and aquatic ecosystems.

Regulatory agencies’ extreme lack of transparency

The ‘green’ wall of silence

Detailed information about engineered microbes is usually hidden by a deep desire for secrecy on the part of developers and a regulatory system with serious failings in relation to transparency.[20] This report goes deep into scientific research and regulatory filings to uncover details that would be almost impossible to obtain for concerned members of the public. Despite these efforts, we could not even identify the agricultural GE microbes that are in the pipeline of the regulatory process or that are commercialized beyond the two described in this report, which have received press coverage. This highlights the fact that lack of transparency is an overarching, urgent problem for how we evaluate and oversee products of biotechnology, including genetically engineered microbes.[21]

For example, an EPA process known as TERA (TSCA Environmental Release Application) is used to approve field trials of genetically engineered microbes. Someone with prior information about a specific microbe can find brief, generic summaries of these decisions online.[22] Should a member of the public want to see the actual government documents allowing this release, they would be required to telephone the EPA to make a request — and what they receive would be only the information that the developer of the technology deems ‘nonconfidential.’ And that is only for GE microbes that one already knows exist. There is no way to do a general search for agricultural GE microbes that are going through the regulatory process or that are commercialized. Nor is there a way to use the scientific name of genetically engineered microbes as one finds them described in the scientific literature to search for actual agricultural on-the-shelf products.

USDA has historically done better than the EPA at making detailed documents about regulatory decisions available but allows developers of GE crops and microbes to self-designate unlimited amounts of information as ‘Confidential Business Information’ (CBI). And, as of 2021, the process the USDA uses to review exemptions from regulation for GE microbes and crops became significantly more opaque with the establishment of the SECURE Rule under the Trump administration.

Below is an excerpt of a table from a letter sent by Pivot Bio to the USDA regarding its GE microbe, Proven®. This is as it appears in the publicly accessible regulatory system — the entire 4-page table is completely blank because the information has been deemed ‘Confidential Business Information,’ as stated in the margin throughout. Every detail about the nature of the product has been hidden from the public. The ability for developers to do this is at the discretion of the regulatory agencies that currently allow these extreme levels of secrecy.

It is imperative that systems for far greater transparency are implemented by government agencies to allow for robust, informed decision-making processes that include all relevant stakeholders, including independent scientists, farmers, and members of the public.

Table 1. List of the [— CBI-deleted

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List of Common Modifications	Explanation of Modifications	Depiction of the Modified Genome	
[			CBI-deleted

Excerpt from Pivot Bio letter submitted to USDA APHIS June 12, 2020 as it appears in the public record

Key conclusions

This report provides background on the importance of microbes to soil health and agriculture, the history of genetic engineering of microbes, and examples and trends of GE agricultural microbe products reaching the market. We highlight the potential risks and concerns related to genetically engineered microbes intended for use in agriculture and explore the economic and political relationships shaping their development. Finally, we summarize the current U.S. regulatory system and provide recommendations to inform the development of robust, science-based regulatory oversight of this novel technology.

We make the following key conclusions:

- The current regulatory system is confusing, inadequate and lacks transparency and ongoing oversight. Precautionary, science-based regulations that account for the unique features of genetically engineered microbes should be established ahead of further environmental release and commercialization of these novel technologies. A system of monitoring their spread and impact once released into the environment and re-evaluating their safety over time must be established.

- The definition of what constitutes genetic engineering must be defined broadly to include both conventional genetic engineering and gene editing techniques and should be rooted in the definition of modern biotechnology from the Codex Alimentarius of the United Nations Food and Agriculture Organization.[23]

- Lack of transparency is an overarching, urgent problem for how we evaluate and oversee products of biotechnology, including genetically engineered microbes. It is imperative that systems for far greater transparency are implemented by government agencies to allow for robust, informed decision-making processes that include all relevant stakeholders, including independent scientists, farmers and members of the public.

- The companies investing in commercializing genetically engineered microbes include major agrichemical corporations along with startups that have roots in those companies. In this context, there is significant potential for the development and deployment of GE microbes to further entrench an industrial approach to agriculture.

- Companies' ability to patent genetically engineered microbes raises important questions about how the technology may be used to reinforce relationships of power in the food system that benefit corporations at the expense of family-scale farmers, communities and the environment.

- There are massive gaps in our knowledge that impede our ability to thoroughly assess risks and to predict the consequences of releasing GE microbes into the environment.

- Adoption of genetically engineered microbes on an industrial scale will represent an unprecedented open-air experiment in the release of GE living organisms. Any release of GE microbes has the potential to affect the environment in unintended ways.

- Containment of GE microbes applied in agriculture is impossible. Fungal spores, bacterial cells and viruses can drift long distances on air currents, moving across national borders. Releasing genetically engineered microbes into the environment raises serious new questions about containment of genetically engineered sequences.

- We must consider the prospect that in rare cases, genetically engineered microbes could become human or animal pathogens and could impact the human microbiome.

- A moratorium should be placed on applications which seek to create microbes that genetically engineer other microbes, known as 'guided biotics.' Companies are actively working on applications for livestock feed. This system shares properties with gene drives, which have been treated with far more trepidation than other types of genetic engineering; the UN has essentially halted the environmental release of gene drives anywhere in the world.

- Engineered microbes have not yet shown effects that could not have been achieved by non-engineered microbes and agroecological approaches to farming. Decades of scientific data and millennia of farmer experience show that organic and other agroecological approaches to farming already achieve the benefits that proponents of GE microbes claim for the technology.

II. Background

The fundamental role of microbes in the soil and farm ecosystem

When you imagine a thriving ecosystem, you might picture a forest rich with plants, roamed by herbivores that are hunted by carnivores. All of this is biomass, living carbon. But you should know that, as a rule of thumb, there is just as much biomass living in the soil as on it. In fact, soils represent the most biodiverse ecosystems on the planet. Vibrant soil ecosystems are an essential part of the natural environment, and they must be respected and protected as strongly as more familiar targets of conservation. Indeed, without healthy soil neither the wild environment nor human needs can be sustained into the future.

Soil biomass is made up mostly of two things, plant roots and microbes. Microbes are by far the most diverse of these two. Microbes are tiny living things that are found all around us and are often too small to be seen by the naked eye. They live in water, soil, air, on plants as well as in human and other animal bodies.

Viruses, bacteria, and protozoans are all found in the soil microbiome, as are larger and more complex organisms like fungi. Some microbes blur the boundaries of the category. Most people would not consider a mushroom a microbe, but many mushrooms are merely temporary reproductive structures formed by microscopic fungi below the ground. Tiny animals like nematodes and water bears that have bodies with specialized organs and show complex behaviors are also members of the soil microbiome.[24]

Figure 2: Soil microorganisms

What are microbes?

Microbes are tiny living things that are found all around us and are often too small to be seen by the naked eye. They live in water, soil, air on plants, as well as in human and other animal bodies. Viruses, bacteria and protozoans are all found in the soil microbiome, as are larger and more complex organisms like fungi. In recent years, science has come to understand that microbes are responsible for essential functions in ecosystems, human health, and agriculture.

Complex interactions take place among these microbes just as they do between organisms above the ground. There is spatial variation in habitats with different communities of microbes inhabiting subtly different parts of the same patch of soil. The roots of different plant species are a major source of this variation, and many microbes specialize in growing around, on, and in plant roots.

We now know that plants recruit microbes and have a stable consortium that is specific to plant variety. In the short term the microbes in a plant's root zone, or rhizosphere, have a strong influence on how the plant experiences its environment. In the long term, soil microbes shape the environment itself by changing the chemistry of the soil, its structure, and the decomposition of organisms from above the ground.

Agricultural crops are no exception to the effect of the soil microbiome. Some of these effects are obvious. Bacteria that convert nitrogen gas from the air into a form usable by plants have symbiotic relationships with plants like legumes, forming nodule structures that are visible to the naked eye.[25,26] Nitrogen is a nutrient that is critical to crop growth. For legumes like soy and peas, microbially-fixed nitrogen can satisfy the great majority of the plants' needs.[26,27] In agroecological farming systems, crop rotation with plants that host these bacteria is a key source of nitrogen. Most crops also have symbiotic associations with mycorrhizal fungi in their root systems that receive carbon from the plants in exchange for water, phosphorus and other nutrients.[28] In this way, microbes play a central role in soil carbon sequestration as the 'bridge' that moves carbon from the plant into the soil.

Many other effects of the microbiome, however, are just as important but less obvious from the human perspective. Beneficial microbes protect plants from disease by signaling to the plant, secreting protective chemicals in the soil and competing with pathogens.[29] Microbes can also make nutrients more available to plants by releasing them from soil particles or changing their chemical form. Crucially, these functions often depend on the microbial community as a whole, not just a single species, and can vary for different plants. This is one of the reasons that science has been slow to realize the importance of the soil microbiome and is still just beginning to understand how it works.

The importance of organic and agroecological farming for fostering healthy soil microbiomes

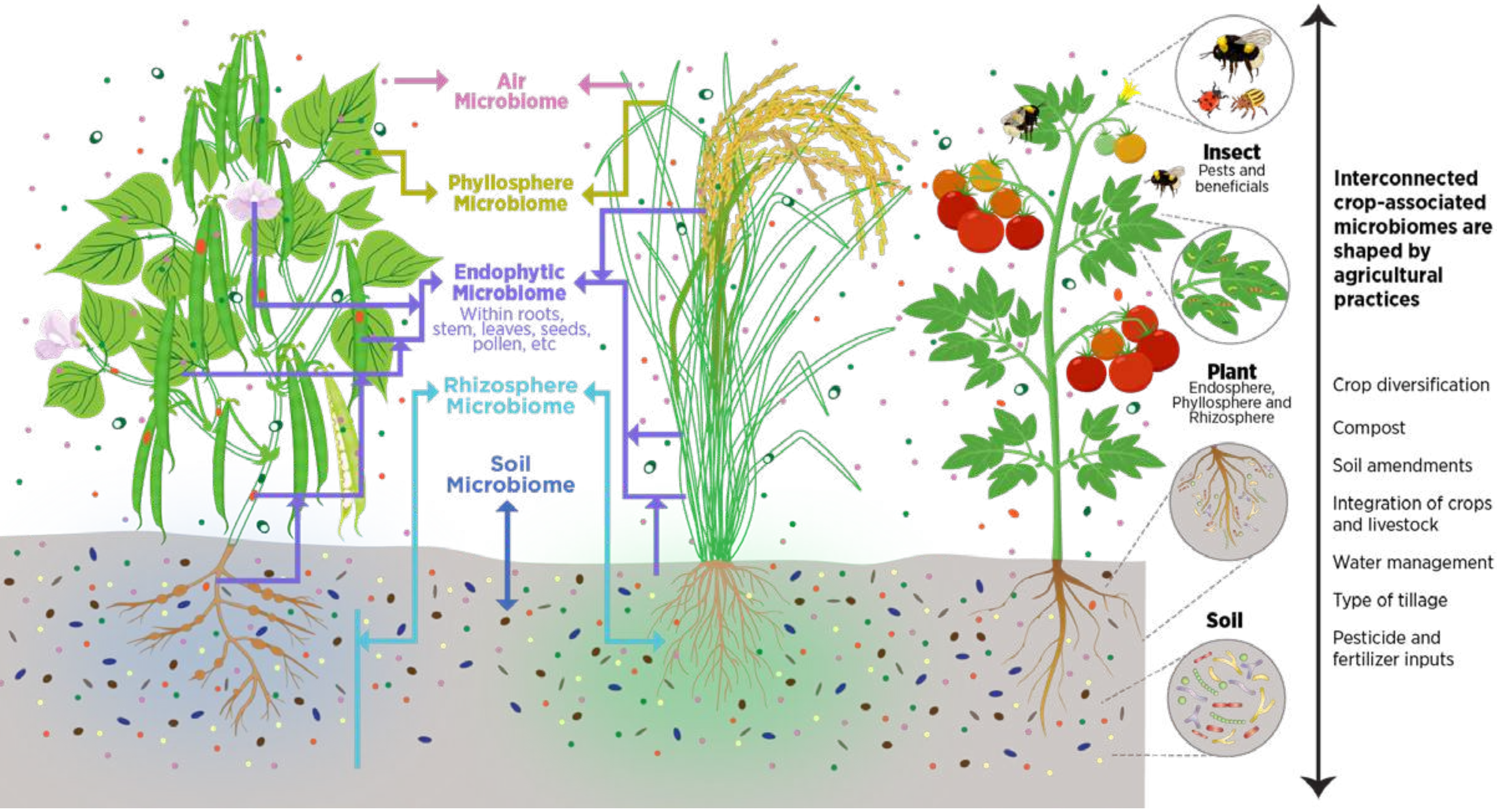
Even when we don't set out specifically to work with microbes, by changing plant species and soil conditions we radically alter the microbiome.[30] The microbes that live in a farmer's field, no matter what approach is used in cultivation, are different from the microbes that live in a nearby wild habitat or the microbes that were there before the field was cleared.

Even when we don't set out to work with microbes, by changing plant species and soil conditions we radically alter the microbiome.

A wide range of choices we make about how to farm dramatically shape the soil microbiome — which crop varieties we use; whether we depend on synthetic fertilizers or instead use compost and integrate legumes and livestock into the system for fertility; whether we grow monocultures versus incorporate diversity through cover cropping, crop diversification, hedgerows, silvopasture or other means; whether we depend on synthetic chemical pesticides or instead holistically manage the system to prevent pests and use least-toxic pesticides as a last resort.

A growing body of data show that it is imperative that we rapidly transform our agricultural systems from a reductionist, industrial paradigm that is dependent on synthetic pesticides and fertilizers to an agroecological paradigm that is rooted in the principles of diversity and that works with natural systems to manage pests and provide fertility. The basis of agroecological farming is the holistic integration of practices like cover cropping, diversified crop rotations, intercropping, inclusion of perennial crops and hedgerows, incorporation of livestock and composting. These practices create high levels of soil organic matter, a carbon and nitrogen-rich substance that is both the natural product and the habitat of microbes.[31,32]

Figure 3: Agricultural microbiomes are shaped by choices about agricultural practices



Agroecology includes a wide range of ecologically restorative food and farming systems, including diversified organic production that meets or exceeds the standards of the U.S. National Organic Program. Research has found that organic farming can significantly enrich soil microbial abundance, diversity and activity.[33,34] Conversely, research shows that conventional agricultural practices result in severe impacts on the microbiome, including reduced diversity, abundance and function.[35,36,37,38]

By fostering healthy, living soils, organic and other agroecological farming systems generate many environmental benefits, including water conservation, decreased soil erosion and greater soil biodiversity.[39,40] They also enhance farmers' resilience in the face of climate change by improving soil structure and water-holding capacity which helps farmers better cope with drought and floods.[13,41] And by sequestering more carbon in the soil than conventional practices, these methods can be an important part of climate change mitigation strategies.[42]

In relation to the use of commercial biological products — engineered or not — two general themes that emerge from the available research show the importance of an ecological versus reductionist paradigm. First, biologicals are more efficacious when used in a holistic farming system. Second, organic and agroecological approaches often achieve the environmental benefits discussed above even without the addition of biologicals. In fact, in healthy, biologically active soils, the existing soil microbiome tends to outcompete and overwhelm commercial biological products.[11,ii]

In other words, we can't just replace a jug of chemicals with a jug of biologicals and think we're going to have a sustainable farming system — a holistic approach achieves better, more consistent and more long-lasting results than "silver bullet" microbes that can be isolated, produced and marketed.

ii Exceptions include rhizobia and mycorrhizal inoculants applied directly to seeds or root balls, disease-suppressive microbes applied to plants, seeds, or starts, and microbes applied to roots or in root zone that induce systemic resistance in the plant.

We can't replace a jug of chemicals with a jug of biologicals and think we're going to have a sustainable farming system — a holistic approach achieves better, more consistent results than "silver bullet" microbes that can be isolated, produced and marketed.

An agroecological approach to nitrogen fixation for corn

Mandaamin Institute research on land races

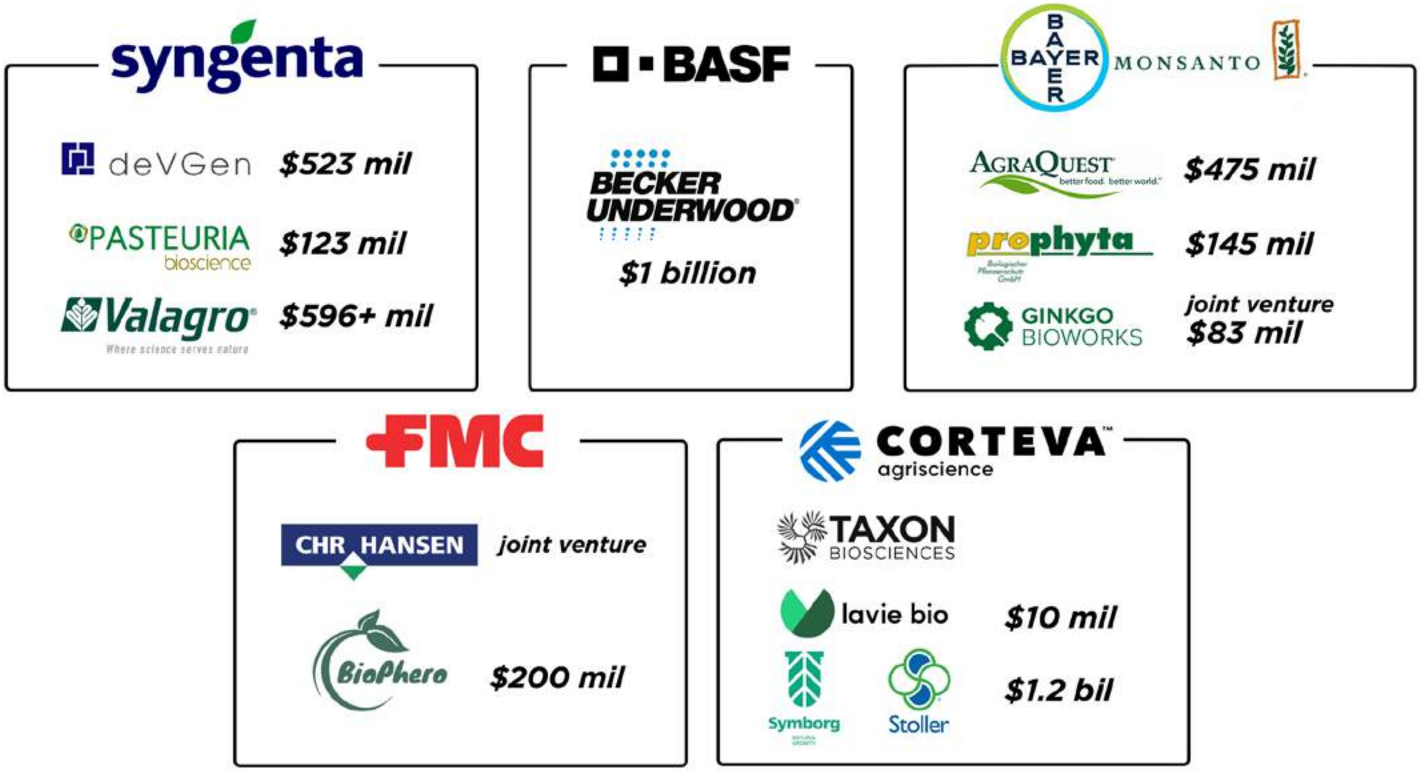
Our choices about farming practices shape the microbiome, which in turn shapes farming practices. Dr. Walter Goldstein at the Mandaamin Institute has published in-depth research finding major differences in the microbiomes of the root zones of traditional land races of maize versus modern Corn Belt hybrids. Land races are crop varieties that are adapted to specific regions and climates and therefore exhibit a lot of genetic diversity. The land races support free-living nitrogen fixing bacteria and can derive up to half of their nitrogen requirement that way, whereas the modern hybrids host Fusarium fungi that help them thrive in the high-input conventional systems for which they were developed. However, those Fusarium inhibit the nitrogen fixing bacteria in the soil, making the modern hybrids more dependent on external inputs of nitrogen.[43] The Mandaamin Institute is using this knowledge to develop non-engineered corn varieties that are extremely efficient at obtaining nitrogen and other nutrients from the soil and even from the air with the help of bacteria.

III. The growing investment of agrichemical companies in biological products

The creation and distribution of genetically engineered crops has infamously been controlled by large agrichemical corporations that have a long track record of disregarding the massive environmental and human health impacts of their products, disenfranchising family-scale farmers, obfuscating the truth about their products and obstructing regulations.[44] These same companies are now rapidly moving into the field of biological products.

Syngenta now claims to be "one of the strongest players in the global biologicals market," and Corteva acquired the leading biologicals and biotechnology company Symborg and another firm in this sector, the Stoller Group, in 2022.[45,46] While biologicals are currently only 5 percent of the global pesticide market, Corteva's CEO has stated that by 2035, "biologicals will make up 25 percent of crop protection revenue."[47] Bayer has amassed a collection of at least 125,000 wild microbial strains and in 2019 created an umbrella branch for related products called 'Biologicals by Bayer.' The company has rapidly expanded their activities in this area via acquisitions.[48] Between 2012 and 2014, Bayer acquired three biologicals companies and in 2022 established a strategic partnership with Ginkgo Bioworks, a startup company which has received $15 billion in investment to develop a platform to automate the genetic engineering of thousands of microbes at once. Bayer also acquires and markets individual microbial products from other companies. The most prominent microbial products released by the company to date are bacteria-based fungicides as well as some plant growth promoting products.

Figure 4: Consolidation in the biologicals industry, 2012-2023



Factors driving agrichemical corporations' investment in biologicals

Several factors are likely driving agrichemical corporations' investment in biologicals. The discovery and commercialization of new chemical pesticides has become increasingly difficult in the past two decades, while the development and launch of biopesticides is much quicker and cheaper. One analysis estimates that it takes more than $280 million to develop one new synthetic pesticide and nearly twelve years to launch it in contrast to $3 to $7 million and approximately four years to get a new biopesticide to market in the U.S.[49]

Some other likely drivers are growing public concern about the enormous harm of synthetic chemical pesticides and fertilizers on human health, biodiversity and the climate. The vast and growing body of science detailing these harms is resulting in bans on hazardous pesticides in countries around the world, lawsuits that are costing agrichemical companies millions — or billions in the case of Bayer and its flagship herbicide Roundup (glyphosate), and consumer demand for organic food grown without toxic synthetic pesticides. Farmers are also interested in alternative approaches as they face increased drought and floods related to climate change, rising costs of inputs like synthetic fertilizers and staggering challenges from weeds and insects that have developed resistance to synthetic pesticides.[50]

Agrichemical companies have been quick to market the sustainability potential of biologicals. Syngenta, for example, stated that its acquisition of one biologicals company was a move to "help farmers deliver a food system working in harmony with nature."[51] And Bayer has claimed that its development of biologicals is part of a plan to "reduce the environmental impact of crop protection by 30 percent without sacrificing yield and the health of the harvest" by 2030.[52]

Reductionism vs ecology

Research and development of GE microbes for agricultural use is often rooted in a reductionist mindset. As one leader in the traditional biologicals field noted, agrichemical companies have been more interested in innovations that are "chemical-like," such as single peptides for crop protection rather than microbial consortiums that contain mixtures of compounds — the complex mixtures are "hard for the big companies to grasp, they like single compounds and narrow approaches."[53]

Will 'squeezing' biological products into a reductionist approach strip away some of the factors that have historically made them environmentally friendly? If companies genetically engineer microbes to survive on a wider range of crops or soils than they would naturally occur on, would it potentially make them more competitive and more likely to overtake naturally occurring microbes in the environment? While naturally occurring microbial products are typically highly biodegradeable, will companies engineer microbes to persist longer in the environment, thereby also increasing this likelihood?

While naturally-occurring microbes produce a cocktail of pesticidal compounds and typically work together in a consortium or 'broth,' agrichemical companies are interested in engineering microbes to express a single mode of pesticidal action (meaning a single way to kill a pest). This will increase the likelihood of pests developing resistance to them. Another factor driving the likelihood of resistance is that, rather than applying microbial pesticides sporadically in relation to actual pest pressures, agrichemical companies are likely to sell them as seed treatments on commodity crops like corn and soy — as we've already seen with Poncho®/VOTiVO® 2.0 — making their presence in the environment ubiquitous across millions of acres. Thus, pesticidal microbes may perpetuate the 'pesticide treadmill' common to industrial agricultural systems rather than offer a true alternative.

Pivot Bio's Proven®

Unproven sustainability claims

A product called Proven®, released in 2019, is the first genetically engineered microbe widely available in agriculture. It's produced by the biotech company Pivot Bio and is backed by hundreds of millions in investment funds including from the Gates Foundation and some of the world's wealthiest individuals. According to Pivot Bio, the product was used on 3 million acres of corn in the U.S. by 2022.[54] In that year, the company released three related products, Proven®40 On-Seed for corn and Return® On-Seed and In-Furrow for barley, millet, oats, sorghum, sunflower and spring wheat. Pivot Bio is also working with Bayer on a similar technology for soybeans.

Proven® consists of live *Kosakonia sacchari* and *Klebsiella variicola* bacteria that have been gene edited to eliminate the 'off switch' on the microbes' process for fixing nitrogen.[55,56] Nitrogen is an element that plants need to grow. They can only access it with the help of bacteria that 'fix' it by turning atmospheric nitrogen into a form that plants can use. Normally, when nitrogen fixing bacteria sense high levels of nitrogen in the soil, they stop converting it. In Proven® the bacteria are prevented from down-regulating their nitrogen fixing activity.

In industrial farming systems, nitrogen is typically applied as synthetic petrochemical fertilizers which are associated with major environmental problems, from run-off that produces 'dead zones' in water bodies around the world to significant greenhouse gas emissions linked to their production and application.[57] Reducing use of synthetic nitrogen fertilizer is a critical environmental goal.

While Pivot Bio's website for the product claims to have solved the environmental problem of "how to replace synthetic nitrogen with something better," peer-reviewed scientific evidence has not yet borne out those claims. In fact, Pivot Bio's field trials of Proven® from 2019 to 2021 published in the scientific literature didn't include any reduction of applications of synthetic nitrogen fertilizer, they only evaluated how crop yield was impacted by adding Proven® in addition to growers' existing use of synthetic fertilizer.[58] In other trial data shared on the company website, the 'replaced' synthetic nitrogen is in fact a minority fraction of what's applied, and there are no negative control fields to see how plants do without Proven® at all. While the internet is full of bold headlines proclaiming the benefits of the product, there is no peer-reviewed science backing those claims.

There is evidence that we can eliminate the use of synthetic nitrogen fertilizer through entirely different means — agroecological farming methods. As discussed in the section above, organic farmers meet their crops' nutrient needs without the use of any synthetic fertilizers, which are prohibited by law in organic systems. Crop rotations using nitrogen-fixing plants like legumes and application of compost and manure are key sources of nitrogen in organic and other ecological farming systems. Also see page 12 on corn varieties that naturally form relationships with nitrogen-fixing bacteria in the soil. What's more, other companies offer naturally occurring microbes that claim the same benefits as Proven® without the use of genetic engineering.

BASF's Poncho®/VOTiVO® 2.0

Engineering false fixes

A seed treatment called Poncho®/VOTiVO®, along with its '2.0' version, are examples of the increasing use of biologicals within monoculture, industrial farming systems.

Poncho®/VOTiVO® was originally developed by Bayer and is now sold by BASF (as a result of divestitures forced by the U.S. government after Bayer acquired Monsanto). According to news reports, it was used on 40 million acres of corn and soy in the U.S. by 2017.[59,60] The treatment combines the neonicotinoid insecticide clothianidin (Poncho®) with a non-engineered *Bacillus firmus* bacteria with nematicidal properties (VOTiVO®).

In 2019, Poncho®/VOTiVO® 2.0 was released by Bayer and is now sold by BASF. It is one of two GE biologicals currently known to be on the market. The '2.0' version added a genetically modified *Bt* bacteria to the seed treatment. It's marketed as a novel form of crop protection, in essence aiming to improve plant health in order to increase plants' resistance to pests.[61] Specifically, the *Bt* bacteria is engineered to express a foreign protein which produces an enzyme that aids in converting dead plant residue in the soil to sugars. This encourages microbial activity in the soil with the aim of increasing nutrient availability and uptake by the crop.

The widespread use of these two microbes in industrial monoculture farming systems raises certain questions. First, agrichemical companies are marketing biological pesticides like VOTiVO® as a solution to a problem they largely created — the resistance of hundreds of pest species to commonly used pesticides. The intensive use of pesticides in industrial farming systems is a perfect breeding ground for resistance — the 'strongest' of a population survive exposure to a pesticide, they reproduce, and a species evolves resistance. As one example, 'superweeds' resistant to glyphosate (aka Bayer-Monsanto's Roundup) now plague over 60 million acres in the U.S.[62] As a result, farmers who don't turn toward ecological pest and weed management must spray ever more, and more toxic, pesticides to kill pests and weeds, a process often referred to as the 'pesticide treadmill.'

While a shift toward biological solutions could be a huge win for the environment and public health, BASF is selling this biological treatment in combination with a highly problematic neonicotinoid insecticide, clothianidin, known for its extreme toxicity to pollinators and other beneficial insects and linked to a growing set of health concerns. What's more, rather than applying the bacteria in response to real-time pest pressures and as part of a holistic system for managing pests, the prophylactic, ubiquitous use of this microbial nematicide across millions of acres annually increases the likelihood that it will only contribute to the pesticide treadmill that it is being marketed as a solution to.

Second, BASF is marketing the '2.0' part of its product — the GE microbe meant to increase nutrient availability for the crop — as a 'next-generation' enhancement in plant health. But it is taking a well-known principle borne out in ecological farming systems — that healthy soils grow healthy plants that better resist pests — and applying it in a profoundly reductionist way that will not resolve the underlying problem. In healthy soils, there is no need for an engineered bacteria to convert plant residue to sugar, that activity is happening in abundance among complex communities of microbes. This technology is an inadequate response to another problem the agrichemical industry has largely created — a farming system marked by depleted soils with vastly diminished microbial communities. What's more, other companies offer naturally-occurring microbes that claim the same benefit that the '2.0' GE microbe promises without the potential risks associated with releasing engineered bacteria into the environment.

Independent biologicals companies and startups

Most biologicals specialists engage in the discovery of naturally occurring microbes rather than in microbe engineering. However, some companies have openly indicated an interest in using genetic engineering techniques. For example, AgBiome, a prominent and recently founded biologicals startup, used its library of bacterial strains to develop its own gene editing platform that it intends to incorporate alongside its non-engineering activities in development of new biologicals. Other companies like BioConsortia, Robigo, Switch Bioworks and Quorum Bio are working on genetically engineered microbes for agriculture. It is important to understand there is a great deal of overlap between large agrichemical corporations and many of these startups. For example, AgBiome was founded by two public university professors alongside three ex-executives from Bayer, including its past CEO. Its first investor was the venture capital arm of Monsanto. Large chemical companies are also increasingly handling the marketing and distribution of biologicals made by smaller companies as they seek to expand their presence in this arena.

If we already know that the people staffing and funding independent companies have been trained inside agribusiness corporations whose track records on the use of genetic engineering is associated with myriad social and environmental harms, we should not take their claims about the potential benefits of their products at face value.

At the same time, the lower cost and technology barriers for development of genetically engineered microbes as compared to engineered crops could enable smaller companies, startups, academics, or nonprofits to make products that reach the market. Some of these new players could have genuine interests in sustainability and in creating products that displace chemical treatments and challenge harmful business practices.

The role of academic research

Academic work on GE microbes in agriculture intersects with major biotechnology corporations. As the trade group Biotechnology Innovation Organization describes, "on the academic side, in addition to maintaining the spirit of research for the common good, there is also a growing trend in support of the commercialization of discoveries. . .and for industry's part, sponsored research with academic partners broadens the search for innovative R&D."[63,iii] For promising commercial technologies, the intellectual property owned by universities is often licensed to established companies like those described above for commercialization. This includes innovations that emerge from research funded by federal grants. These publicly funded developments should be subject to conditions of use for the public good, although they currently are not.

Research by academic labs has contributed to the largest number of environmental releases of engineered microbes so far. Seventy-eight of all 128 environmental release permits ever issued through the EPA's TERA program for genetically engineered microbes were provided to universities. These were for diverse types of work, often in limited areas and intended only for study, not direct application in agriculture.

iii While this report focuses on medical innovation, the intersections between corporate and academic research also reflects the agricultural sphere.

IV. The rise of genetically engineered microbes in agriculture

The concept of genetically engineered microbes as agricultural treatments is not new; such technologies have been proposed and tested at small scale since the 1980s (see call-out box 6 on Ice-minus) but have never gained traction for availability to the public.[64] Since the mid-1990s, the primary focus of genetic engineering in agriculture has been crops. However, microbes are far easier to engineer. And the advent of gene editing techniques — in which DNA is modified but not moved between species — is driving a new set of discoveries as well as new investments in edited microbes just as it is in other types of biotechnology.[65,66]

This has made GE microbes essential tools for basic science — tens of thousands of research studies using engineered microbes are published every year. Whereas the generation of a genetically engineered plant or animal requires specialized equipment, expensive chemicals, and access to sterile conditions and can take years before reaching a point where the effect can be assessed, for tractable organisms like brewer's yeast and *E. coli* bacteria, a gene may be permanently inserted and its effect observed within a single day.[67,68] Tools and reagents for this process are cheap and accessible, to an extent that microbial genetic engineering is readily performed by high school students.[69,70] (Note — this does not mean that the results are predictable or precise, see page 25 for more on unintended outcomes.)

A much wider range of engineered microbes can be found in academic work and field trials than those that have advanced to commercialization. These include more complex engineered systems and use of transgenes from distant sources. Giving microbes completely new traits is the most extreme form of genetic engineering. In general, this is done when a microbe that lives on or in a certain part of a plant or habitat can be used to deliver a useful trait from a different microbe. Industry work for near-future applications appears to emphasize the use of gene editing rather than transgenic engineering (when genes are moved across species) due to the lower regulatory burden for products of those technologies in the U.S., particularly following the adoption of the 2020 SECURE rule at the USDA. It is possible that, in addition to the factors mentioned above, the trend toward deregulation of agricultural biotechnologies in the U.S. is contributing to companies' interest in development of GE microbes.

The first GE microbe to be field tested - 'Ice-minus'

The first engineered microbe to be field tested, in the early 1980s, was 'ice-minus' *Pseudomonas*, any of several strains of engineered bacteria in this genus. They were developed for commercial use under the name Frostban but never made available to farmers.

The presence of wild *Pseudomonas* bacteria on plant surfaces enables damaging ice to form at temperatures about 7 degrees higher than in plants that are completely free of bacteria (30.2 vs. 23 degrees Fahrenheit). The wild bacteria were therefore found to result in frost damage for crops in the field, limiting the growing season.[71,72]

The engineered *Pseudomonas* had an alteration in their surface structure that removes their ability to form ice crystals, and they were able to outcompete the natural ice-forming bacteria for space.[73] In a test with strawberries, the engineered *Pseudomonas* were sprayed on crops where they displaced their wild relatives. As predicted, the majority of plants treated with the engineered bacteria avoided frost damage at temperatures as low as 23 degrees while the majority of plants covered in wild bacteria were frozen by the time temperatures reached 30 degrees.[64,74] Evidence from the field also showed the engineered *Pseudomonas* could protect pears and potatoes from similar damage.[73,75]

Proponents argued that enabling plants to withstand lower temperatures would protect fruit production from weather extremes. Opponents of ice-minus bacteria were concerned that its application could alter rain and snow patterns because bacteria serve as a natural source of ice crystal formation in the atmosphere.[76,77]

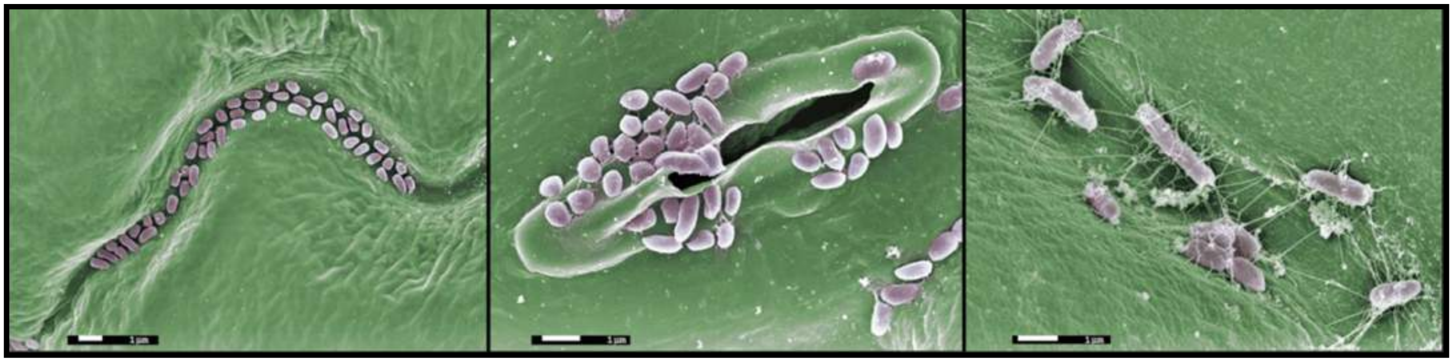
However, within a few years the scientist who originated ice-minus bacteria and the company attempting to commercialize it had both moved on to working with a naturally-occurring mutant strain with identical benefits.[64] The non-engineered microbial products are in use on a variety of crops today. The research with engineered surface proteins continues, however, for different applications such as disease prevention in fruits and tomatoes.

Types of applications of GE microbes in agriculture

Bacteria, viruses and fungi have been targeted for agricultural genetic engineering, with bacteria being the most commonly used.

- **Bacteria**: To many people in the field "microbiome," and especially "microbiome engineering," are synonymous with "bacteria." The GE microbes now available for commercial use are bacteria. Bacteria are prokaryotes, meaning they have no nucleus and a simpler genetic system than eukaryotes—organisms whose cells have a nucleus, including fungi, algae, plants and animals. It can be easier to insert genes and control how they function in prokaryotes. Most beneficial microbes are bacteria that are found to naturally live in a very tight relationship with plants (Figure 6).[78,79] These beneficial bacteria make up most of the species that are targeted for engineering. For example, *Pseudomonas* is a large and diverse family of bacteria which are tractable to work with in the lab and contain members with important roles in plant growth.
- **Fungi**: In general, engineering of fungi for use as live microbes in agriculture lags behind engineering of bacteria because their complex genome structure and biology makes many fungi difficult to apply genetic engineering methods to. It can be harder to insert genes into eukaryotes and to control how they function in a new organism. The technologies described below pertaining to fungi are not currently commercialized or on a clear path to commercialization.
- **Viruses***:* Work with genetically modified viruses has been subject to concern about the persistence and potentially wide host range of viruses, and no engineered virus has neared commercialization for agriculture.

Figure 6:
Most engineered microbes are species that have intimate relationships with plants. The bacteria in this figure are growing between plant cells on the leaf surface as well as on and into the pores that allow the plant to take up CO2 for photosynthesis; *Pseudomonas*, right, and *Burkholderia*, left & center.



Nitrogen fixation

Plants need nitrogen to grow — it is a major component of chlorophyll, the compound by which plants use sunlight energy to produce sugars from water and carbon dioxide (i.e. photosynthesis), and it is a major component of the building blocks of proteins, without which plants wither and die. Some crops, such as lentils and other legumes, naturally form symbiotic relationships with nitrogen-fixing soil bacteria called rhizobia, while others such as corn, wheat and rice do not. Despite years of trying, researchers have failed to successfully engineer crops that do not naturally fix nitrogen to do so, either by engineering nitrogen-fixing bacteria to create relationships with crops or by attempting to put genes for nitrogen fixation directly into the crops.[80,81,82,83]

Over the past decade, a growing number of researchers have taken interest in engineering nitrogen fixation into microbes themselves.[81] Recent work is focused on bacteria that do not form a deep symbiosis with plants but which can be used to make nitrogen available to crops other than legumes.[84,85] Prominent examples of this approve are Pivot Bio's Proven® product (see page 15) and the research of a group at Shangdong Academy of Agricultural Sciences in China.[58,86]

Pest control

Pesticides

The most widely used insecticidal bacteria in agriculture is *Bacillus thuringiensis (Bt)*, which naturally lives in the soil and on plants. It produces proteins that kill insects, including plant-eating crop pests. Non-engineered *Bt* concentrate is allowed in organic production as an insecticide.
For decades, researchers have applied genetic engineering techniques to try to expand the ways *Bt* bacteria can be used as a pesticide. In field trials beginning in the 1980s and continuing to the present, *Bt* proteins have been moved into other types of microbes, including bacteria that live on different plant species and different parts of plants than *Bt*, which would expand the scope of application.[87] Some of these engineered bacteria are more durable than *Bt*, better surviving contact with UV light, temperature and other organisms in the field.[88,89,90,91] In field tests on corn, cabbage, cotton and legumes, the engineered bacteria achieved 40-80 percent control of a wide range of pests that could not effectively be targeted with natural *Bt* treatments.[92,93,94] *Bt* strains were also genetically engineered to have additional toxins from other subspecies of *Bt* in order to target a wider variety of insects, and even to make *Bt* effective against fungi.[95,96,97,98,99] This academic work has been successful in producing functional *Bt* toxins in a variety of organisms. However, there are no documented attempts to commercialize this type of engineered microbe.

Figure 7: Bacteria targeted for genetic engineering

The white mass is the fungus *Beauveria bassiana*, which infects and kills insects such as this stinkbug, and is used as a bioinsecticide in organic and conventional agriculture. Scientists have tested an engineered strain of the fungus with *Bt* toxin to augment its pesticidal effect.

Within a few years of the first experiments with engineered *Bt* microbes, researchers, including those at major pesticide companies, turned toward genetically engineering crops to express Bt toxin throughout their tissues. Genetically engineered *Bt* corn and cotton were introduced in 1996 by Novartis and Monsanto, respectively. Today, 80 percent of corn and 85 percent of cotton grown in the U.S. are genetically engineered to express *Bt*. While these GE crops were associated with an initial decrease in the use of insecticides, data show populations of key pests developed resistance, undercutting the initial success and contributing to the 'pesticide treadmill' discussed above.[100] This result is not surprising given that planting GE *Bt* crops in vast monocultures increases pests' exposure to the toxin, speeding the development of resistance; scientists warned of this outcome at the advent of this technology.[101]

Wild fungi are a common component of biopesticides that do not involve genetic engineering. *Beauveria bassiana* is a fungus which naturally attacks insects and is used as a popular biopesticide in its natural form. Researchers have attempted on many separate occasions to engineer *Beauveria* by adding genes for environmental durability, insect toxicity and degradation of molecules in the insect's shell.[102,103]

There has also been research on engineering viruses to act as delivery systems for proteins or molecules that could kill agricultural pests. The most prominent such example is the use of baculoviruses that specifically infect insects but not vertebrates or crustaceans. Researchers added genes to attempt to make these viruses more toxic to their target pests, such as arachnid venom proteins or genes that affect insect developmental hormones, so that pest insects that feed on a plant treated with the virus quickly die.[104] As mentioned above, concerns have been raised about genetically engineering viruses given their potential persistence and ability to infect a wide range of hosts, and no engineered virus has neared commercialization.

Making pathogens less harmful

Some research focuses on genetically engineering plant pathogens in attempts to make them less harmful or even beneficial. *Pseudomonas syringae*, unlike its beneficial relatives, is a plant pathogen which has been the subject of engineering to produce less-harmful strains that can displace the wild population of *P. syringae*.

Another application sought to address a fruit tree disease called fireblight. Researchers from the University of Pennsylvania produced a less virulent mutant of the bacterium that causes the disease through gene deletion. In 2018, they inquired to the USDA whether coating trees with the mutant to suppress colonization by its pathogenic wild relative would be subject to government regulation.[105,106] The USDA determined that the engineered bacterium was subject to regulation not because of the genetic modification, but because the weakened pathogen was nevertheless still a pathogen. The modified bacterium has been the subject of continued academic work but there have been no further attempts at application.[107,108]

In the early 2000s, researchers made a genetically modified microbe system to control a disease in grapes called Pierce's Disease that occurs when insects transmit a pathogenic bacterium to the vascular system of plants. The researchers added a protein which targets and suppresses the pathogenic bacterium to a different species of benign bacteria that naturally lives inside plant veins. When the insects fed on plants treated with the engineered bacteria, instead of transmitting the disease to the plants, as the engineered bacteria that were inside the plant were eaten, they prevented the pathogen from being transmitted by suppressing it within the insects' digestive systems. Small-scale field studies of this microbe were approved by EPA, but it was not specifically intended for commercialization and has never been publicly available.[109,110,111] Pierce's Disease remains a major problem in grape farming and is controlled primarily by spraying insecticides to kill the insects that carry the disease.

Pathogenic fungi have been engineered to make harmless or less harmful strains that could displace their wild relatives. This has been done, notably, with the chestnut blight fungus through both direct engineering and use of a virus that infects the fungus.[112,113]

In another example, researchers at the University of Kentucky gene-edited *Epichloe coenophiala*, a beneficial fungus that lives inside the tissues of grasses. The fungus protects grass from environmental stress but also produces protective chemicals called ergot alkaloids. These chemicals can damage livestock that eat grass containing the fungus. The engineered fungus cannot create alkaloids, and the goal of the research was to inoculate pasture grass with it in order to gain growth benefits while preventing colonization by the wild, toxic strain that would harm grazing farm animals. USDA determined that the edited fungus was not subject to regulation, but it has not been commercialized.

Making microbes 'easier to handle'

Industry actors have also expressed significant interest in using genetic engineering to make biologicals "easier to handle."[114,115] This means finding ways to produce them in large volumes, package them and add them to tank mixes of pesticides and fertilizers.[116,117,118,119] This can include providing genes that enable microbes to grow on a wider range of feedstocks or tolerate a higher density of culture. This is already commonly done with industrial microbes outside agriculture (e.g. for medicine, biofuels, synthetic chemicals) and in academic research, though no microbe carrying this type of modification has yet reached the public as an agricultural product.[120,121,122]

Table 1: Commercially available GE microbes in agriculture

Developer	Product	Microbe	Goal	Regulation
Pivot Bio	**Proven®** Applied in-furrow during planting. Available for corn, wheat and sorghum. In 2022, three related products were released.	*Kosakonia sacchari* and *Klebsiella variicola*	Nitrogen fixation — the company gene edited soil bacteria to eliminate the 'off switch' on the microbes' process for fixing nitrogen; thus they continue to fix nitrogen even in high-nitrogen soil conditions.	In 2020, the USDA determined that the product is not regulated by the agency because the wild form of the bacterium is not a plant pathogen and the engineered form contains no foreign DNA. The publicly available form of the USDA letter is heavily redacted.
BASF (originally developed by Bayer)	**Poncho® / Votivo® 2.0** Applied as a seed treatment. Available for corn, soybeans, sorghum and cotton	*Bacillus thuringiensis*	Plant protection — the company engineered a bacteria to express a foreign protein which produces an enzyme that aids in converting dead plant residues in the soil to sugars to encourage soil microbial activity with the aim of increasing nutrient availability for plants.	As a 'biostimulant' the genetically engineered component of Poncho® / Votivo® 2.0 faces far less oversight and transparency than a biopesticide. Few documents on the regulatory history are publicly available.

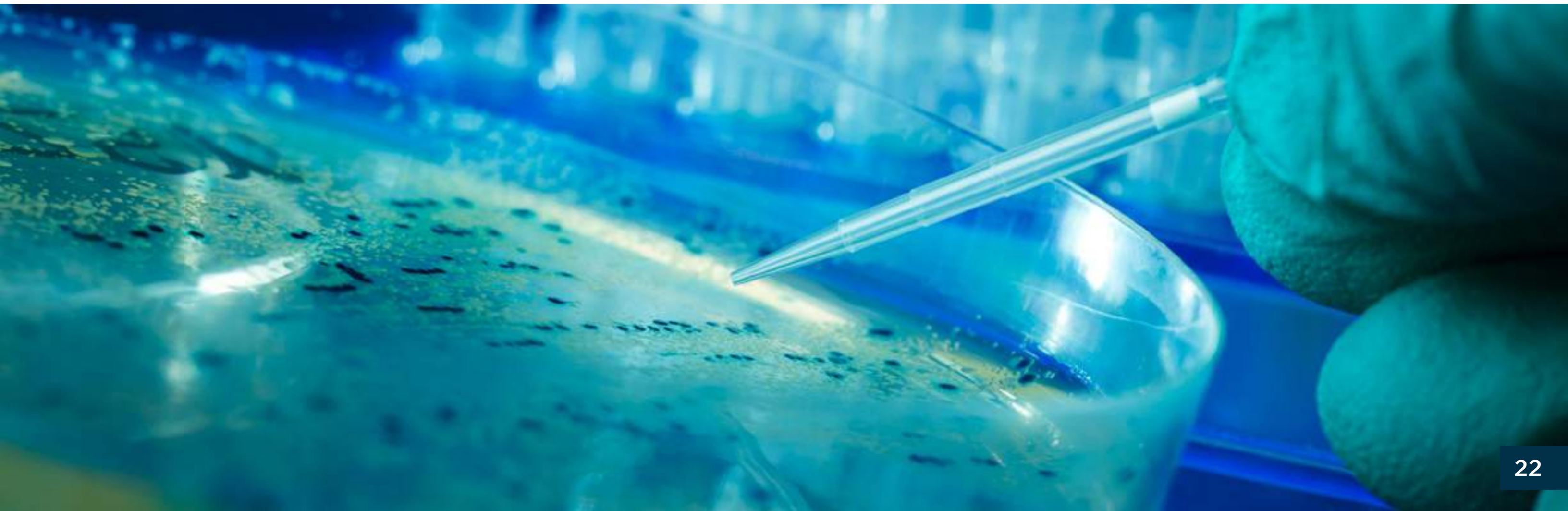


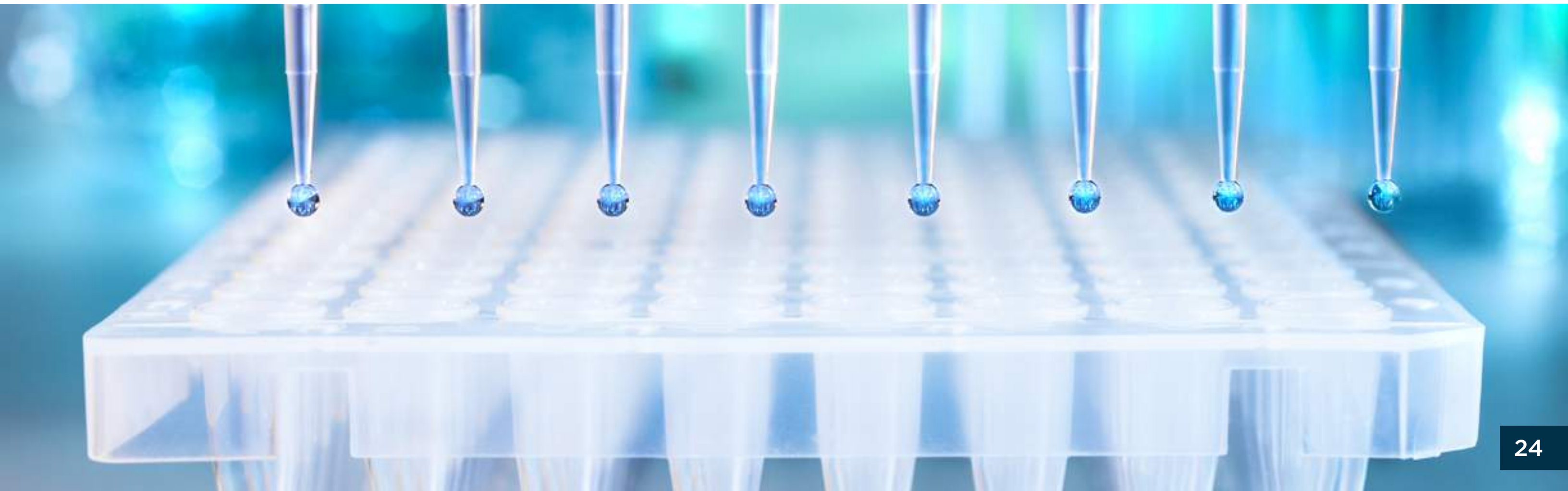
Table 2: Examples of GE microbe research & development in agriculture

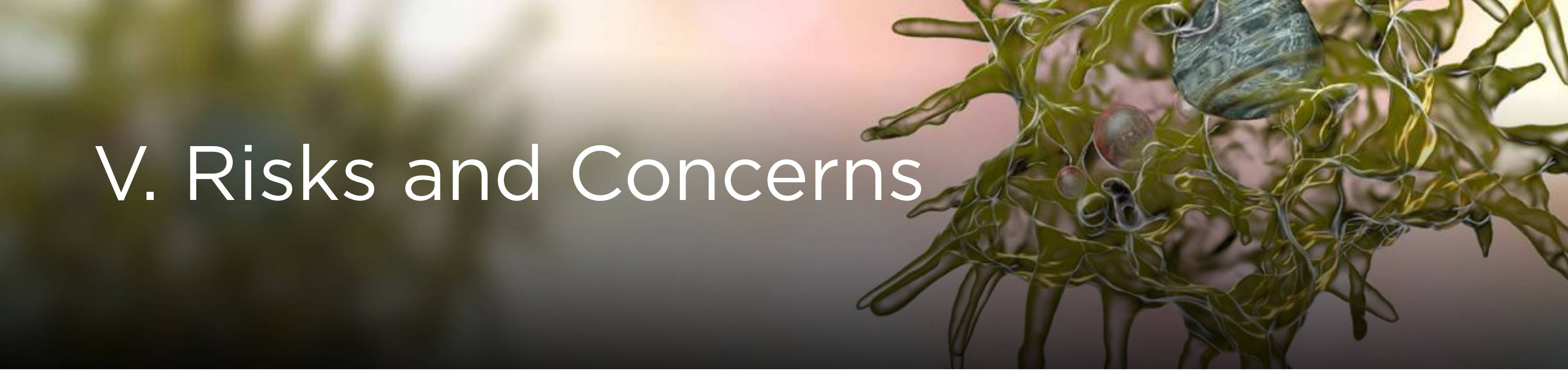
Applications submitted to USDA			
Developer	**Microbe**	**Summary**	**Regulation**
West Virginia University	*Cryphonectria parasitica* – invasive fungal pathogen responsible for chestnut blight	Researchers engineered an invasive chestnut blight pathogen to be less virulent and less harmful to wild native American chestnut trees.	In 2020, the USDA* determined that although the engineered fungus is less pathogenic than its wild parent, it remains a plant pathogen and is therefore subject to regulation.
Folium Science	*E. coli* bacteria	The company engineered *E. coli* to transfer DNA to pathogenic *Salmonella* bacteria containing instructions for CRISPR gene editing that cause the *Salmonella* to destroy its own cells. Chickens fed the engineered *E. coli* were cleared of the pathogen in this way.	In 2020, the USDA determined that this product is not regulated by the agency because it is not a plant pathogen, but the product is subject to regulation by FDA and EPA.
University of Kentucky	*Epichloe coenophiala* – beneficial fungus that lives inside tissues of grasses	Scientists gene-edited a fungus which helps grass grow so that it was unable to produce chemicals that can harm grazing animals that eat the grass.	In 2020, the USDA determined that the edited fungus was not subject to regulation.
Penn State University	*Erwinia amylovora* – pathogenic bacteria responsible for fruit tree disease fireblight	Researchers deleted a gene from a fruit tree pathogen. The engineered bacterium was less pathogenic than its wild relative and was intended to prevent disease by displacing its wild relative.	In 2018, the USDA determined that the engineered bacteria remained a plant pathogen for regulatory purposes and the researchers would be required to submit a full permit application to conduct the field trial.
Penn State University		Researchers used gene editing to create mushrooms that brown less when cut and last longer in storage.	In 2016, the USDA determined that the engineered mushroom was not subject to regulation because its wild form is not a plant pathogen, and although genetic components of plant pests were used transiently in the engineering process, no foreign DNA remained in the final product.

Applications submitted to EPA			
University of Florida, Southern Gardens Citrus	Citrus tristeza virus	Researchers collaborating with a private orange company engineered the Citrus Tristeza Virus to deliver antimicrobial genes to orange trees which they hoped would protect the trees from bacterial diseases.	In 2011, the EPA under FIFRA** Determined that the researchers were permitted to conduct field trials.
University of Maryland	*Metarhizium anisopliae* – fungus that attacks insects	Researchers engineered a fungus which attacks insects and is already used for biocontrol of plant pests to produce a component of scorpion venom.	In 2007, the EPA determined under FIFRA that the researchers were permitted to conduct field trials.
University of California Riverside	*Alcaligenes xlyosoxidans* – bacteria that coexists with plants	Researchers inserted a red fluorescent protein into the bacterium. The goal of the red color was to be able to detect the engineered organism in the environment if it was used in future applications to help control disease.	In 2005, the EPA under TSCA** determined that the researchers were permitted to conduct field trials.
Mycogen	*Pseudomonas fluorescens* – plant bacteria	The company engineered a bacterium to produce insecticidal *Bt* toxins derived from *Bacillus thuringiensis*. Their goal was to use the engineered cells to produce larger amounts of *Bt* toxin in the lab than *B. thuringiensis* can itself produce, then kill the bacteria and apply it to plants as a more convenient and effective form of *Bt*.	In 1992, the EPA, under TSCA determined that the researchers were permitted to conduct field trials with the product containing the dead GE bacteria.

* The U.S. Department of Agriculture (USDA) Am I Regulated (AIR) program which reviewed all of these applications was terminated in 2021 and replaced with the Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient (SECURE) rule.)

** U.S. Environmental Protection Agency (EPA) Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and Toxic Substances Control Act (TSCA)





V. Risks and Concerns

Genetically engineered microbes were one of the earliest topics of debate and risk analysis for engineered organisms before trends in agriculture moved mostly to engineered crops. Some of the risks raised for these products were very specific and based on underlying knowledge of the individual microbes. For example, opponents of ice-minus bacteria were concerned that its application could alter rain and snow patterns because bacteria serve as a natural source of ice crystal formation in the atmosphere (see page 19).[76,77] Engineered bacteria with *Bt* toxin concerned scientists who worried the microbes would affect non-target organisms, such as pollinators, in their new areas of application.[123] Neither of these concerns were borne out, but they illustrate an overarching unique risk in regard to engineered microbes: we fundamentally know less about their biology in the wild than we do about the plants they cohabitate with.

> *When we attempt to intentionally alter the microbiome by applying chemical treatments or inoculating a field with a new strain of microbe, there is no guarantee that things will go as intended. The fine details of the existing microbial community, other treatments, and environmental factors all affect how microbial treatments work, often in ways we still don't understand.*

When we attempt to intentionally alter the microbiome by applying chemical treatments or inoculating a field with a new strain of microbe, there is no guarantee that things will go as intended. The fine details of the existing microbial community, other treatments being applied, and environmental factors all affect how microbial treatments work, often in ways we still don't understand.

It's possible that widely distributing microbes with 'useful' functions could enable new and unintended associations to form with weed or pest species with unintended consequences for agriculture.

Adoption of biologicals on a scale that matches the chemical products they compete with will represent an unprecedented free-air experiment in the release of living organisms. Engineered microbes will not suddenly turn into virulent pathogens because they are engineered, but both engineered and non-engineered microbes may behave in unexpected ways when they encounter new environments. Although it is not likely that such unexpected behavior will be common either in engineered or non-engineered microbes, we must understand that "rare events" are real, and their number is meaningful when we are discussing vast applications.

Limits of our knowledge & unintended consequences

The gaps in our knowledge and limitations of our ability to predict or control the outcomes of this novel technology are profound and varied. The idea that genetic engineering of organisms is 'precise', as manufacturers claim, is not founded in the latest understanding of genes. The genetic structure of organisms is now understood to be considerably more complex than previously thought, and we have only just begun to explore and understand the expanse of relationships that regulate genomic activities.[124] A cascade of activities involving a vast number of cellular processes and multiple stretches of DNA located both near and far from a particular gene may be involved for a particular action to take place or to result in a particular trait.[125] This complexity is reflected in Pivot Bio's patent application for Proven®40, which lists at least 29 different genes and myriad proteins and enzymes that can be manipulated to result in changes to the targeted bacteria's ability to fix nitrogen.[126]

The patent details methods to "disrupt" and "short-circuit" the cellular nitrogen sensing cascade and "trick" the cells into perceiving a nitrogen-limited state. That we can tinker with genetic regulatory processes does not mean we understand the full complexity of the system or what might happen as a result of our intervention. As an example, a study published by the developers of Proven® shows that they were surprised to find that knocking out two genes (*GlnD* and *GlnE*) — which normally play double roles of enhancing nitrogen fixation under starvation (soil conditions with little nitrogen) but repressing it under sufficiency — clearly enhanced nitrogen fixation, as it could just as easily have ended up reducing it due to the genes' dual role.[56] This suggests that there is more going on than they understand.

> *That we can tinker with genetic regulatory processes does not mean we understand the full complexity of the system or what might happen as a result of our intervention. Genetic engineering can result in an array of unintended genetic consequences, including insertions, deletions, inversions and translocations that were not expected.*

Genetic engineering (including gene editing techniques like CRISPR which are often claimed to be more 'precise') can result in an array of unintended genetic consequences, including insertions, deletions, inversions and translocations that were not expected.[127,128,129]

Further, we have only scientifically characterized a small fraction of the microbes living in the soil. Of the billions of species of bacteria, archaea, fungi, viruses and microeukaryotes below ground, only a few hundred thousand have been characterized in detail.[1] We have only scratched the surface of understanding the complexity of how they function, interact with plants and shape agricultural and wild ecosystems.

> *We have only scratched the surface of understanding the complexity of how microbes function, interact with plants and shape agricultural and wild ecosystems. We do not have enough knowledge to meaningfully assess the possible ecological ramifications of releasing GE microbes into the environment.*

In the case of Proven®, what might be the repercussions of releasing billions of microbes that no longer have the ability to down-regulate nitrogen production across millions of acres? Consider that plants and bacteria have sophisticated responses to environmental conditions and to each other — if you swamp a legume with nitrogen, it will down-regulate colonization of bacteria that fix nitrogen so that it can focus energy on other relationships and processes. When an organism is engineered to do something that nature has not designed it to do — and when that ability may spread to other microbes via horizontal gene transfer — we do not have enough knowledge to meaningfully assess the possible ecological ramifications.

Ecological risks

Gene containment and lack thereof

Releasing genetically engineered microbes into the environment raises serious new questions about containment of the engineered sequences. This is for three reasons.

The scale of release

First, the sheer number of organisms released in an application of genetically engineered microbes is orders of magnitude larger than our current experiences with genetically engineered crops. A field of corn contains about 35,000 plants per acre.[130] A typical application of a bacteria-based biological will use 1 to 5 kilograms of material containing a billion cells per gram.[131,132] This equates to the release of up to 5,000,000,000,000 genetically engineered organisms on each acre of cropland, greater than a hundred million times the number we are used to seeing with crops. If every acre of corn on earth was genetically engineered, that would be about 17,143,496,298,999 plants.[133] A typical application of a bacteria-based biological would release the same number of genetically engineered organisms about every four acres. The huge difference in scale means we must treat the probability of rare events differently.

> *Releasing genetically engineered microbes into the environment raises serious new questions about containment of the engineered sequences. The genetic modifications released inside engineered microbes may move across species boundaries in unpredictable ways.*

Containment is impossible

Second, the complete containment of microbes to the area of application is impossible. Fungal spores, bacterial cells and viruses can drift long distances on air currents, moving across national borders in a single year.[134,135,136] Microbes also move in groundwater and are transported by the movement of animals and insects.[137] Unlike plants, microbes are not limited in time or space by sexual reproduction because they can reproduce clonally. The spread of individual microbes also cannot be detected directly even by the most vigilant farmer or scientist, requiring technological methods to spot and confirm their identity.

On one hand, the scale and ability of microbes to spread when applied in agriculture is not because they are genetically engineered — it is because they are microbes, and applications of nonengineered microbes with these properties have long been accepted. On the other hand, genetically engineered microbes will be no easier to contain than their non-engineered relatives, which past experience shows is nearly impossible.

Horizontal gene transfer

Third, microbes are capable of horizontal gene transfer — DNA from one organism can be incorporated into the genome of another organism, sometimes completely unrelated, without sexual reproduction (see Appendix II).[138] As a result, the genetic modifications released inside engineered microbes may move across species boundaries in unpredictable ways. Horizontal gene transfer can enable genes to move between completely unrelated organisms and is more likely to occur in organisms that engage in pathogenic and symbiotic relationships — the same categories that are of highest interest as targets of genetic engineering.[139] The question of horizontal transfer is most pressing for bacteria, which are the greatest target for development of biologicals and very active in horizontal transfer. Microbes have key biological differences that increase the frequency with which they undergo horizontal transfer.

Tan spot fungus – a cautionary tale of horizontal gene transfer

Horizontal gene transfer in microbes has been documented to rapidly change the nature of their interactions with plants. For example, tan spot fungus disease of wheat, one of the most important diseases in this crop, causes yield losses of up to 50 percent but was not a significant problem prior to 1941.[140,141] Naturally occurring transfer of a single gene from the fungus *Pheosphaeria nodorum* to its relative *Pyrenophora tritici-repentis* happened around this time and caused *P. tritici-repentis* to transform overnight from a neutral member of the microbiome to the aggressive pathogen that causes tan spot disease.[142] The pathogenic strain spread rapidly to become a worldwide issue within ten years and remains a serious challenge for wheat agriculture. Cases of horizontal transfer like this are gaining increasing recognition for their role in the evolutionary history of agriculturally important microbes.[143,144,145,146]

Altering microbial communities

The currently available research shows that most microbial treatments in agriculture don't persist long-term because they don't overcome the balance of local factors that shape the existing microbial community composition. However, research on non-GE biologicals has shown the possibility for persistent impacts on microbial communities. Even when microbial inoculants die off relatively quicky, their application can significantly alter the plant and soil microbiome by changing the overall diversity of bacterial and fungal species.[35,147] This inevitably disrupts an existing, complex community.[148] Most experiments with microbial treatments in agriculture fail to assess the impact and length of effect on the native microbiome as a whole.[149] Those that do show that the effect on other microbes can sometimes be long term, taking months or years for the native microbial community to return to its original state after a single treatment.[150,151,152] While this can be desirable for the application, the effect on native microbes cannot be neglected and is not limited to microbes themselves. Changes to the microbiome can snowball into directly observable changes to plant growth and insect communities — and again, these may be different from the intended effects of the inoculum.[153,154]

Effects on plant-microbe-insect relationships are particularly important. Symbiotic microbes enable many plant-eating insects to survive by participating in amino acid synthesis, or enable them to digest difficult molecules like cellulose from plant cell walls.[155] A beneficial microbe in one area of agriculture might function differently if it shows up in the wrong place — for example, bacteria can protect insect pests from fungal disease in the same way they protect plants, ultimately increasing pest populations.[155] Or, for example, altered microbial communities can degrade agricultural chemicals like pesticides, therefore enabling pest insects to survive.[156]

Some of the effects of microbiome changes alter features of plant growth that could have major impacts on wild plants and the communities they support. Microbe treatments can cause *Arabidopsis* and related plants to flower a week sooner in the lab.[157,158] A similar effect was seen in field-grown genetically identical poplar trees, where differences in the microbiome caused flowering up to ten days earlier in response to climate change.[159] In other contexts, earlier flowering time has been identified as a major crisis point for effects of climate change because it can cause a mismatch between when flowers form and when pollinators are available to fertilize them, and consequently when and how much ripe fruits and nuts of wild plants are available. These effects trickle up into how many insects, birds and mammals can be supported by the ecosystem. [160]

Microbiome treatments can affect the growth of trees and other long-lived plants just as they do crops and annuals. Just as in other cases, the effect of microbe treatment can last long after the microbiome has returned to normal, and this can be magnified in trees and perennial ecosystems: an effect that changes which seedlings survive to become established over a few early months of life could be sustained for hundreds of years — the whole lifetime of those trees.

Invasive species provide a cautionary tale

The plant microbiome is already known as a warfront in the establishment of invasive species that should provide cautionary examples for the power of the microbiome to drive environmental harms as well as benefits.[161] The invasive plant garlic mustard (*Alliara petiolata*) secretes chemicals that kill off or repel the mycorrhizal symbionts of native plants.[162,163,164] This not only harms the native plants by reducing their access to nutrients, it increases the availability of soil nutrients to nonmycorrhizal plants like garlic mustard, providing a double competitive advantage in taking over native ecosystems.[165,166,167] Another invasive plant in desert habitats of the Americas and Australia, buffelgrass (*Cenchus ciliaris*) does the opposite: it shapes its own specialized microbiome with many beneficial bacteria and fungi and uses these associations to outperform and outgrow native species.[168] Even the nitrogen-fixing symbiosis of legumes considered so beneficial in sustainable agriculture is, in other contexts, a way for weeds from this family to better invade new areas.[169]

Finally, the wild microbiome itself deserves conservation. Even if there are no 'aboveground' effects, altering the structure of a community with thousands of native, potentially rare or even undiscovered species, should not be taken lightly just because these organisms are small. Even the rare chance of a foreign, agriculturally applied microbe becoming permanently established in the wild environment is a serious concern due to the ways it may then influence other relationships and even the structure of whole ecosystems. While such effects might be counteracted in the heavily managed agricultural context, there will be little ability to do so in wild systems.

The pesticide treadmill

As with chemical pesticides, pest resistance is a critical concern. Since the advent of widespread use of chemical pesticides, hundreds of insect and weed species have developed resistance. In the history of chemical pesticides, social pressure and economic profit-seeking have driven their use in ways that reduce, rather than prolong, their useful lifespan and lead to higher doses, additional toxic pesticides and combinations of toxic pesticides to manage resistant pests, in a process often referred to as the ‘pesticide treadmill’.[50,170] Genetically engineered crops have played a massive role in entrenching the pesticide treadmill. Currently, 98.2 percent of all genetically engineered crop acreage in the U.S. is devoted to herbicide-tolerant crops, primarily Roundup Ready corn and soy that are engineered to withstand the application of Bayer-Monsanto’s Roundup (aka glyphosate) and other harmful herbicides.[171] As noted above, ‘superweeds’ resistant to Roundup now plague over 60 million acres in the U.S.

If microbes are used within industrial monoculture agriculture systems to suppress insects or other plant-pathogenic microbes, there is no reason to think that the same concerns of resistance development that apply to chemical pesticides would not apply (see page 16). Some microbial products with multiple modes of action may be protected from resistance for longer than chemical treatments but are not otherwise unique. If we are driven to adopt engineered microbes as a result of the chemical pesticide treadmill, we should not assume new, biological technology will produce better outcomes unless we learn the lessons of the past and shift toward ecological, systems-based approaches to managing pests.

Action needed – A moratorium on ‘guided biotics’

While there are reasons to be concerned about *un*intended horizontal gene transfer, developers are also exploring technology that *intentionally increases* the horizontal transfer of transgenes. In essence, this constitutes an organism that indiscriminately genetically engineers other organisms.

For example, the ‘guided biotics’ system developed by Folium Bioscience uses bacteria that are designed to widely transmit DNA encoding a CRISPR-Cas system to other bacteria. When the system is transmitted to a specified harmful microbe, the target bacterium expresses a CRISPR construct that destroys an essential piece of its own DNA, killing it. The safety of the technology relies on the assumption that when transmitted to non-target species the system will neither persist nor create gene edits. However, there is a significant history of off-target editing in CRISPR that indicates the specificity of this system is unlikely to be perfect, especially when used at large scale. This system shares properties with gene drives, which have been treated with much more trepidation than other types of genetic engineering. The UN Convention on Biological Diversity has essentially imposed a moratorium on release of any gene drives worldwide.[172] A similar moratorium should be placed on guided biotics.

If we are driven to adopt engineered microbes as a result of the chemical pesticide treadmill, we should not assume new, biological technology will produce better outcomes unless we learn the lessons of the past and shift toward ecological, systems-based approaches to managing pests.

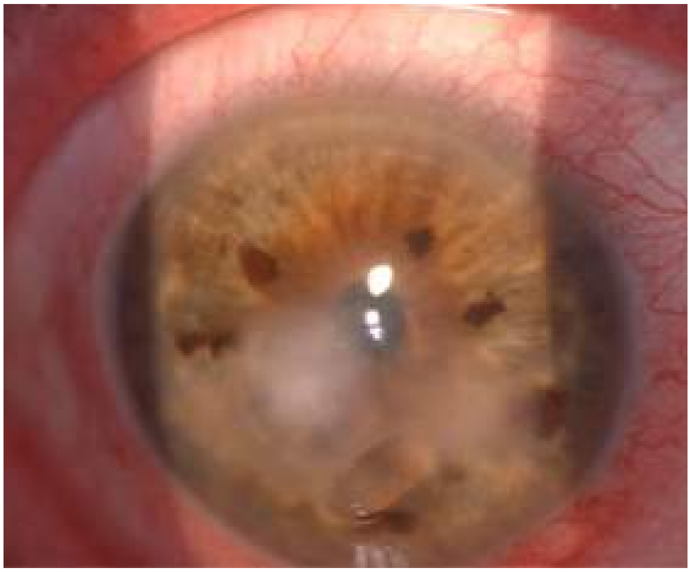
Human health risks

Novel pathogens

Microbes, like sprays of agricultural chemicals, can travel long distances quickly. While agrichemicals raise serious health concerns when they do this, they cannot reproduce or become infectious. We should consider the prospect that in rare cases, genetically engineered microbes could become human or animal pathogens. Opportunistic infections occur from microbes that are not normally considered pathogens when certain conditions are met — such as encountering immunocompromised individuals or co-infection with specific other microbes. The soil microbiome is a known source of some of these pathogens, including members of genera that are targeted for use in agbiotech, such as *Pseudomonas* and *Ochrobactrum*.[173] Engineering these organisms for faster growth may make them more able to grow pathogenically.[174]

Figure 8: A human eye infected with Beauveria bassiana

Beauveria bassiana is the agriculturally beneficial fungus shown attacking a stinkbug in Figure 7. The patient was a 76-year-old farmer with a number of underlying conditions.



Antibiotic resistance

Antibiotic resistance — when germs, bacteria and fungi have developed the ability to withstand the drugs applied to treat them — is an urgent worldwide public health crisis. Antibiotic resistance markers are commonly used in the development of transgenic organisms. Scientists often add genes that code for resistance to antibiotics like tetracycline, ampicillin and others during genetic modification so that the GE organisms and cells can be distinguished from non-GE ones. While the risk of antibiotic resistance from transgenic organisms resulting in resistance in human pathogens has been substantially investigated and found to be low, the use of antibiotic markers in microbes that might directly become human pathogens casts their presence in GE microbes in a new light. Figure 8, for example, shows a rare incidence of infection of a human by *Beauveria bassiana*, a fungus that is used as a biocontrol and has been used for engineering experiments.[175,176,177] This patient's infection was susceptible to only two of nine tested antibiotics.[178] Had this been a genetically engineered fungus expressing an antibiotic resistance marker, the outcome of the case may have been affected. The same would be true if the fungus were engineered to express spider venom to increase its effect against insects, as has been tried before.

Immunosuppression and opportunistic pathogens

Given that the present trend in GE biologicals development involves co-integration with synthetic chemicals rather than complete elimination, the real-world scenario of application includes a potentially grim intersection of factors. First, multiple pesticides that are in active use today have been noted to have immunosuppressive effects on wildlife, livestock and humans near farms.[179,180,181,182,183,184] Thus, we should not rule out the possibility that deployment of biologicals in the same locations as chronic pesticide use could be a very real intersection of microbes that might become opportunistic animal pathogens and the types of individuals that are susceptible to such infections. A special factor for people living in agricultural communities is that, subsequent to the epidemiological incidence of high cancer rates among pesticide applicators, the same people who apply and live near potential sites of microbe application are more likely than the rest of the population to have medically suppressed immune systems due to treatment for cancer.[185,186,187,188,189,190] Evaluation of pathogenicity is not currently an explicit aspect of approval for GE or non-GE biologicals, but should be included in future regulation as microbial products become more common.

Consumer exposure and the human microbiome

Separate from the question of pathogenicity, it should be assumed that while most microbial treatments are transient and die off while crops are still in the field, as use of GE biologicals grow, some amount of engineered bacteria will come into contact with consumers via the food supply. The overall risks of these live bacteria causing health problems is very low relative to food processing and animal factory farming, which are dominant sources of foodborne illness.[191,192] However, the obvious concern is for impacts on the human microbiome. Some companies like Folium and Pebble are developing engineered microbes explicitly intended for animal feed to alter the animal microbiome by producing CRISPR-Cas or RNAi constructs that target harmful bacteria (see page 29). It should be assumed that if they come in contact with humans, these microbes will continue to live and carry out their intended job, but with potentially unknown effects on new species of bacteria or bacteria that play different roles in the human microbiome compared to animals. Evaluation of low-level exposures from food residue in human cell and microbiome models would be an essential consideration to guard against risks from this type of exposure.

Socioeconomic risks

Since the commercial release of genetically engineered microbes in agriculture is so new, we don't yet know how companies' intellectual property rights to these products might impact farmers and other stakeholders in the food system. However, the history of agrichemical giants' use of intellectual property rights related to GE crops to pursue predatory lawsuits against hundreds of small farmers should raise important red flags.[193]

In the U.S., microbes cannot be patented unless they are genetically engineered. Companies can patent products containing non-engineered microbes if they demonstrate that the formulation, as a whole, meets the criteria for a patent (such as novelty, usefulness, and being a composition of matter that does not occur naturally without human intervention). But the microbes in those formulations are not patented.

Thus, companies patenting engineered microbes potentially have different opportunities to enforce their intellectual property rights. In theory, it would be possible for a company to test a farmers' soil for the presence of one of their GE microbes in the same way that they have tested farmers' crops for evidence of genetic engineering. Companies could potentially engineer genetic 'tags' into GE microbes that could allow them to screen for them without trespassing on farmers' fields, for example showing up under UV light. Microbes are prolific and readily transfer genetic material with each other, making the likelihood that they will rapidly spread to neighbor's fields nearly guaranteed. Would a company be able to legally enforce their intellectual property rights to the microbes or genetic constructs that have drifted or that remain in a farmer's field long after application? Although it seems far-fetched, we should not rule it out as a possibility.

Of note, the high likelihood of genetic drift also poses a threat to organic farming systems, which by law prohibit the use of GE organisms. As the organic farming community has argued in the case of genetically engineered crops, the burden for dealing with the outcomes of genetic contamination of organic fields should be on the manufacturers of GE organisms.[194]

Another socioeconomic concern is the way consolidation within the industry may shape innovation.[195] In addition to patenting GE microbes, companies can also patent the methods of producing a genetically engineered organism. For example, Corteva holds a patent for a process modifying the genome of a cell using CRISPR techniques and claims the intellectual property rights to any cells, seeds and plants that include the same genetic information, whether in broccoli, maize, soy, rice, wheat, cotton, barley or sunflower.[196] The already apparent trend of consolidation with the biologicals market, as major agrichemical companies buy up smaller biotech companies and acquire patents on research and discovery processes, may narrow the scope of innovation in this sphere, further entrenching the economic interests of powerful corporations over the public good.

VI. Policy context

The regulations, norms and strategic alignments that we have developed over decades of debate about genetically engineered crops must be reexamined and supplemented for application to genetically engineered microbes. GE microbes do not fit neatly into past experience with genetic engineering. While their usage involves releasing tremendous numbers — trillions — of GE organisms, they are mostly invisible. Unlike engineered crops, they include species that have only recently become known to science. GE microbes are unlikely to be contained in any meaningful sense and cannot be tracked except by methods that require laboratory analysis. In addition to a robust regulatory process governing all GE microbes developed for use in agriculture, case by case consideration of the unique features of each new GE microbe proposed for use in agriculture should be required.

Summary of current regulatory system

The current regulatory system for GE microbes is confusing and inadequate. We must make sure that applications of engineered microbes aren't assumed to be acceptable — let alone beneficial — simply because they are approved faster than we can understand them.

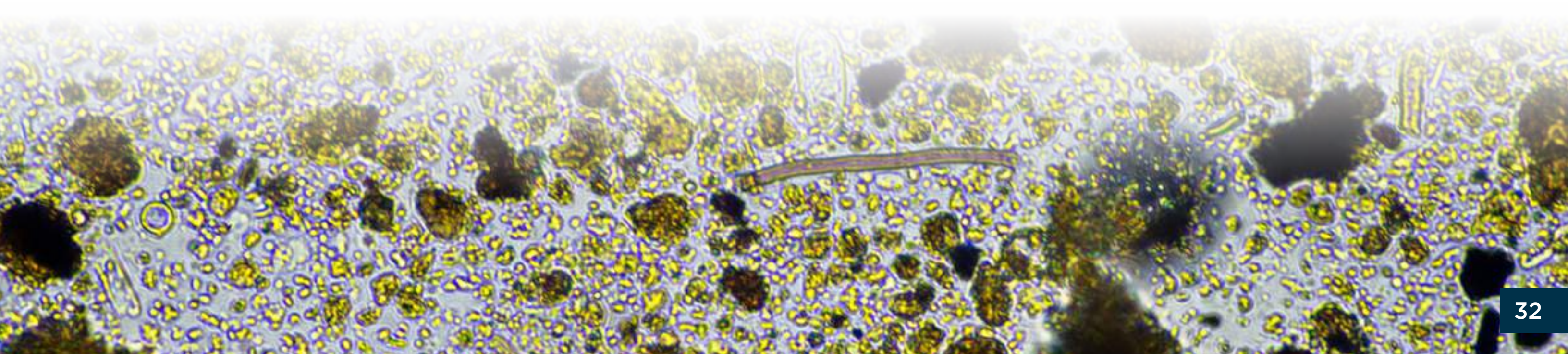
All of the systems described below have extremely poor transparency (see page 7). Submitting entities are able to redact almost all details from public view in most regulatory filings under the self-designation of 'Confidential Business Information.' This may include the engineered gene and even the type of organism. Even these redacted records are difficult to access and not clearly identified with the end products in which they appear.

U.S. Environmental Protection Agency

Pesticidal microbes

Any microbe that acts as a 'biopesticide' must be approved by the U.S. Environmental Protection Agency under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The EPA Office of Pesticides Programs has a specific division that is responsible for all regulatory activities related to biologically-based pesticides, the Biopesticides and Pollution Prevention Division. Of note, the EPA's definition of 'biopesticides' does not align with the common use of it to refer to biologicals, it includes gene-silencing RNAi pesticides and crops that have been genetically engineered to incorporate a 'plant protectant' (the most prominent being *Bt*).

The EPA does not have specific regulations that take into account the unique properties of genetically engineered microbes. First, the same general authority and standards used for chemical pesticides apply to microbes with pesticidal qualities, both GE and non-GE. Second, the EPA makes clear that it does not have specific regulations pertaining to GE microbes. The agency states that, aside from requiring data on the genetic engineering process used and the results of that process, GE microbial pesticides are regulated using "essentially the same data requirements used for naturally occurring microbial pesticides." Under FIFRA, the EPA approves field trials and Experimental Use Permits of any scale for any sort of pesticide, including microbes. Unlike regulators in most countries, the EPA does not ask for field data demonstrating the efficacy of any new pesticides before approving them.



Unlike regulators in most countries, the EPA does not ask for field data demonstrating the efficacy of new pesticides before approving them.

GE microbes that contain foreign DNA (transgenic) and may or may not be biopesticides

The EPA also has authority under the Toxic Substances Control Act (TSCA) to regulate any "new substance" which has been applied to include GE microbes that contain a gene from a different genus of microbe, regardless of their function. GE microbes that function to improve plant growth or perform other non-pesticidal functions are regulated through TSCA. The components of TSCA review, however, are vague and include no specific standards or required information.

U.S. Department of Agriculture

GE microbes that do not contain foreign DNA (i.e. gene-edited) and are not biopesticides

Many microbial products are not used as biopesticides or have a function that is hard to place under a single category. Many such microbes fall through the cracks of current regulation. For gene-edited microbes that are non-pesticidal, neither FIRFA nor TSCA apply.

In the past, some gene-edited microbes were considered by the U.S. Department of Agriculture (USDA) under the Plant Pest Act through a process called "Am I Regulated" (AIR). Those reviews almost always concluded that the edited microbes were exempt from regulation due to the nature of the engineering method.[20] As of 2021, the AIR program was terminated and replaced with the Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient (SECURE) rule, which exemplifies the federal government's deregulatory trend toward biotechnology. SECURE codified several exemptions from regulation that could apply to genetically engineered microbes. One such exemption for certain gene-edited organisms is such that developers can self-determine exempt status and commercialize a microbe without any oversight (though USDA still provides a voluntary exemption-confirmation process).[208] USDA retains the ability to regulate microbes that it deems a plant pest risk, but under SECURE, the agency moves from applying default data-based review of any genetically engineered organisms to making *ad hoc* decisions about the need and scope of examination required for any product.[209]

The need for a system to track the spread and impacts of GE microbes

Each application of genetically engineered microbes in the field essentially constitutes an uncontained environmental release. Once GE microbes are used in field trials or released commercially, there is no program dedicated to surveilling the extent of their use or re-evaluating their safety over time. However, methods to evaluate changes to the microbiome are effective and accessible within the scientific system, including metagenomics and metabolic profiling now offered as off-the-shelf services by universities and commercial scientific providers.[149,197,198,199] Making use of these methods would involve mandating their inclusion in safety studies for regulatory agencies.[200]

In the absence of federal action, scientists, farmers or members of the public may be able to submit samples of soil and track changes caused by new treatments. State-level programs and citizen science studies have already been designed which enable members of the public to collect microbiome samples which are analyzed and made available in published research.[201,202,203] Such programs could analyze DNA sequences against a genetic database containing known genetic engineering tools or screen microbes for inserted genes that are often used in laboratory research, such as antibiotic resistance markers.[204,205] However, the efficacy of such efforts will be severely limited in the absence of formal coordination and transparent standards. Each environmental microbiome sample is a single snapshot of a jumble of all microbial genes present at that place and time; special analysis tools are used to identify the species and genes of interest that are there.[206] Some genes and mutations, like common laboratory markers, are cataloged in public databases and are easy to find, but proprietary changes that developers have not shared are extremely hard to find without advance knowledge of what they are.[207] Even when a particular modified microbe can be confidently identified, actually knowing where it is in the world requires constant sampling of even unexpected locations in order to follow its spread.

A system for monitoring the spread and impact of GE microbes once released into the environment and re-evaluating their safety over time must be established.

Understanding how engineering techniques shape potential risks

Evaluating the potential for horizontal gene transfer

Not every type of genetic engineering will affect whether a microbe is likely to undergo horizontal transfer when it leaves the lab, but some might. Key factors that affect the likelihood of transfer are the method of transformation, the nature of the engineered trait and whether stabilizing chemicals or surfactants are used. Regulators could use these criteria to help determine potential risks on a case-by-case basis.

Methods of transformation

Biotechnology often uses plasmids, small circular molecules of DNA, to move transgenes around. Genes contained on plasmids are more likely to continue undergoing horizontal transfer than those in the main genome.[139,208] Genetic engineering may also make use of *transposons* or *recombinase recognition sites* to assemble and insert DNA as desired. These are short DNA sequences that flank a gene of interest and make it easier to move in and out of the genome. However, via the same mechanisms that make them useful, these sequences can make the genes associated with them more likely to undergo horizontal transfer later on.[209,210,211,212]

Nature of the engineered trait

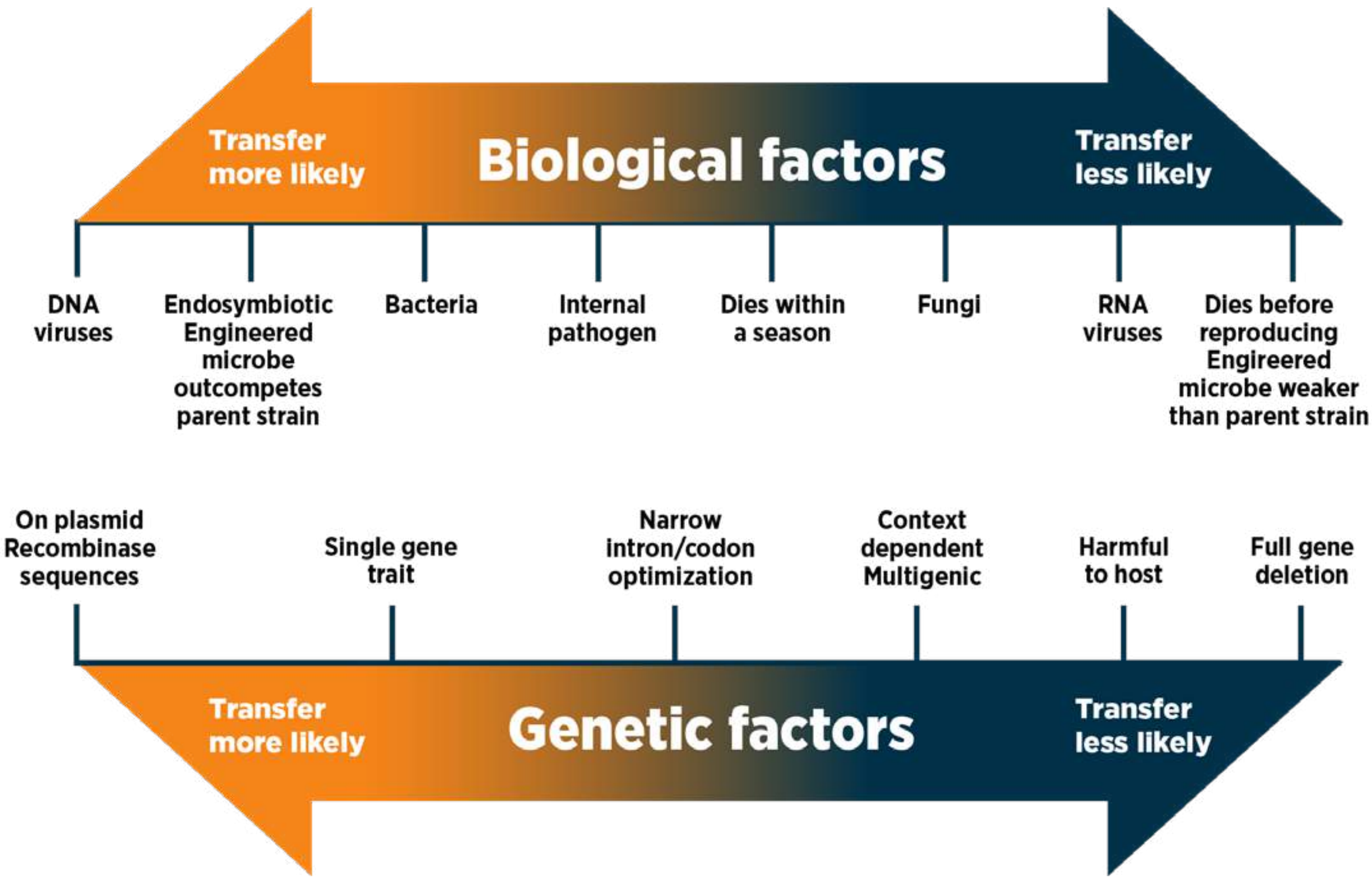
The nature of the engineered trait can also be an important predictor of whether a modified gene is likely to have a biological effect in other species and/or spread through a population following horizontal transfer. If the modification enhances the natural tendencies of a microbe that carries out a rare function, moving any one gene from that microbe into another is not likely to have a meaningful biological effect because the function is dependent on interaction with the rest of the native genetic context. Consider the analogy of taking a spring from a fancy watch and putting it in a toaster; outside its interaction with all the other components of the watch, the spring does not move the toaster towards being able to tell time in any way.

An example of an engineered microbe with multiple features that lessen its risks as a source of horizontal transfer is a gene edited fungus *Epichloë coenophiala* produced by researchers at the University of Kentucky. *Epichloë* naturally lives inside grasses that belong to the fescue family commonly found in pastures and residential lawns. *Epichloë* has many benefits for the grass, making it resistant to pests, drought and stress. However, this beneficial fungus also produces toxic chemicals called ergot alkaloids. In nature the alkaloids probably help protect the grass from herbivores, but in agriculture they can have serious toxic effects on animals that graze on the grass. The researchers gene-edited the fungus to delete two regions of the genome containing several genes that enable it to produce the alkaloids, rendering it and the grass it lives in nontoxic to livestock while keeping the growth benefits. Because the organism is a fungus, it is overall less likely to undergo horizontal transfer. Because the modification made was a complete deletion, there is no new protein-coding sequence that could be transferred to another organism. Even if the developers made an error in characterizing the engineered fungus or if some of the remaining sequence retained an effect related to alkaloids, the transferred gene would be unlikely to be biologically relevant in most of the organisms that could receive it via horizontal transfer, including its host grasses, because they lack the other biological mechanisms of alkaloid synthesis that would put the transferred gene in a functional context.[213]

Use of stabilizing chemicals and surfactants

The use of stabilizing chemicals and surfactants (agents that reduce surface tension) can significantly change the likelihood of horizontal transfer. Biological products that are mixed together before, during, or by sequential application are also prime opportunities for horizontal transfer to occur. For example, in a universally used laboratory method, the plant pathogen *Agrobacterium* is able to transfer its DNA to the ovules of *Arabidopsis* at high levels upon contact with its flowers — but only in the presence of Silwet L-77, a surfactant which is also commonly used as a wetting agent in agricultural sprays.[214]

Figure 9: Factors influencing the likelihood of horizontal gene transfer in genetically modified microbes



VII. Policy Recommendations

The information in this report supports adopting the following principles in policy, advocacy and regulation of genetically engineered microbes intended for use in agriculture.

1: Recognize that engineered microbes are novel

GE microbes for use in agriculture do not fit neatly into past experience with genetic engineering. Unlike GE microbes used in industrial processes, they are not contained. Unlike GE crops, they include species that have only recently become known to science. GE microbes for use in agriculture are unlikely to be contained and cannot be thoroughly tracked except by advanced laboratory methods combined with systematic environmental sampling of public and private land. The norms, regulations and strategic alignments that we have developed over decades of debate about GE crops must be reexamined and supplemented for application to microbes.

2: Distinguish genetic engineering from biologicals in general

GE microbes are a small fraction of microbial applications in agriculture. There is no general requirement to use genetic engineering for microbes to be useful, and some microbial technologies are ancient and grounded in traditional agricultural knowledge. Genetically engineered microbes should be evaluated in a way that assesses the specific contribution of engineering.

3: Define genetically engineered microbes inclusively

In the realm of GE crops, there is a history of exemptions for certain types of genetic engineering, such as gene editing. Even if the reasons given for these exemptions are accepted, they do not apply equally to microbes. Microbes engage in a wide array of genetic functions that are not found in plants and animals. Legalistic exemptions based on whether the outcome of genetic engineering could conceivably be 'natural' should not be permitted. Any microbe that has been subjected to direct manipulation of its DNA should be subject to regulatory review.

4: Initiate a rulemaking on field trials vs. contained testing

The EPA and any other potential regulators should initiate a rulemaking on the extent to which field trials of genetically engineered microbes constitute an irrevocable environmental release. In acknowledging that full containment is unlikely to impossible, agencies should develop protocols for rigorous monitoring of the spread and effects of GE microbes.

5: Prevent containment failure from being used as a weapon of economic coercion

The tendency of genetically engineered crops to escape their fields and show up elsewhere is nothing compared to microbes that can cross national boundaries on an air current. Acknowledging that full containment is essentially impossible, developers and users of GE microbes must not allow escaped self-replicating microbes to harm others' crops or, worse, to force adoption of the technology.

6. Place a moratorium on any field testing of 'guided biotics' applications

Guided biotics refers to the development of GE microbes that are *intended* to propagate and transmit engineered DNA — organisms that indiscriminately genetically engineer other organisms. This system shares properties with gene drives, which have been treated with much more trepidation than other types of genetic engineering and should be subject to similar concern.

7: Require greater transparency

Lack of transparency on the part of federal regulatory agencies is an overarching, urgent problem for how we evaluate and oversee products of biotechnology, including GE microbes. Far greater transparency is fundamental to our ability to grapple, as a society, with the potential risks and benefits of this novel technology.

8: Use a precautionary approach

Regulatory bodies should use the Precautionary Principle to guide action, meaning that precautionary measures to minimize or avoid threats to human health or the environment should be taken based on the weight of the available scientific evidence rather than waiting for full scientific certainty about cause and effect, which can take years or decades while harm accrues.

The Precautionary Principle also elevates the importance of a full evaluation of safer approaches before moving ahead with a potentially risky new technology. Oversight should include independent assessment for public health and environmental safety, and long-term impacts should be assessed before products are released onto the market or into the environment. The Precautionary Principle also guides the incorporation of public input into decision-making processes, as the impacts of new technologies such as GE microbes in agriculture will be borne by society as a whole. In addition, socioeconomic concerns arising from the expansion of corporate property rights over microbes must be incorporated into decision-making before products are commercialized.

Appendices

Appendix I. Methods of genetically engineering microbes

A. Gene insertion

Gene insertion is the process of adding a piece of DNA to a microbe. The genetically engineered herbicide tolerant (e.g. Roundup Ready) and insect resistant (*Bt*) crop plants that make up the majority of corn and soy acreage in the US are mostly the result of gene insertion. Usually, the new DNA contains instructions for one or more proteins. The proteins may have a direct function — for example, the Cry proteins found in *Bacillus thuringiensis* are responsible for its insecticidal activity — or may carry out chemical reactions, such as nitrogen fixation. In microbes, unlike plants, the DNA may be inserted as part of a plasmid, a piece of circular DNA that is permanently incorporated into the microbe but stays separate from its genome. DNA may also be inserted directly into the genome, as has been done with engineered plants.

When DNA is integrated into the genome, processes called site-specific recombination and homology-directed repair are commonly used to try to control the precise location of the transgene. The use of these methods are much more common and effective in microbes than in plants. Genes must also be inserted with (or in a site containing) promoter and terminator DNA sequences; these are responsible for directing when the gene is expressed and are extremely important to successful applications of transgenic microbes. Promoter activity is generally lost or changed across organisms as they become less closely related to the one where the promoter originated.

A former barrier to moving genes across unrelated organisms was access to the donor sequence, as well as something called codon optimization. The version of the genetic code (or 'codon usage') varies across species, and genes may not be made into proteins if there are large differences between the donor and host organisms. In even the recent past, obtaining a specific DNA sequence from an organism, and altering its codon optimization, were both significant undertakings that required access to quality samples of the organism and a lot of time in the lab.

However, gene synthesis has now become widely available. In gene synthesis a DNA sequence is made from raw materials by a lab or company rather than extracted from the donor organism. This effectively allows genetic engineers to order up any gene sequence from any organism on demand, with codon optimization for any target organism. Gene synthesis also makes genetic components more accessible; a piece of DNA encoding the fairly large Cry1a protein from *Bt* that is found in many transgenic plants, can be obtained for about $250 from any of several synthesis companies, with a production time of about 1 week. Gene synthesis is now an integral component of industry R&D programs, where large numbers of gene-promoter combinations go directly from computational design to synthesis and insertion into microbes, without requiring individual design and construction.

B. Gene editing

Gene editing is the process of targeting a sequence of DNA in the microbe and altering or removing it. This process uses CRISPR-Cas complexes or other sequence-specific enzymes that cut DNA. Editing can cause random mutations at the targeted site, or it can lead to a prespecified small change in the DNA there, depending how it is done. Editing is now a prominent method in biotechnology. To edit a gene, the machinery of the editing system is introduced into the microbe as either DNA or protein, then removed once the targeted edit has been achieved.

Editing does not necessarily mean that the targeted gene is removed. A common tactic, for example used in the construction of Pivot Bio's nitrogen-fixing bacteria, is to edit a gene's promoter or proteins that interfere with its expression, rather than the protein-coding sequence itself. The expression of the target gene can be increased by this type of editing as well as decreased. Editing can also change the function of a gene rather than destroying it. For example, it is possible to make glyphosate-resistant corn by editing a single base pair of an enzyme found in the plant, which changes the structure of the protein made by that gene so that it is no longer sensitive to glyphosate.[215]

Appendix II. Horizontal gene transfer

Horizontal transfer does not just mean that foreign DNA is present inside an organism. In fact, all living things interact with foreign DNA all the time. The DNA of any organism you consume will be present in your body for a period of time, as is the DNA of all the microbes in your personal microbiome. A few fragments of foreign DNA from these sources will enter the cells of your body in the course of normal life, and the same is true for microbes and plants in the environment. Most of these events will be quickly eliminated, either because the DNA is degraded by enzymes as soon as it enters the cell, is lost when the cell divides because it does not stably integrate into the cell's genome, or it is lost when the cell that contains the DNA dies without replicating.

Horizontal transfer in the meaningful sense occurs in rare events where foreign DNA becomes a permanent part of the host genome, in a form that is biochemically stable and heritable across generations. Horizontal transfer is not necessarily harmful or helpful to the receiving organism, but sometimes confers important new functions. When the transferred DNA also confers a meaningful biological advantage, it can spread through the real-world population as was the case for tan spot disease (see call-out box 7). Any organism can provide or receive DNA for horizontal transfer, and there are traces of it in the evolutionary history of all species. The human genome contains about 1,500 regions that clearly result from horizontal transfer, and likely many others that have yet to be identified.[216] These events happen on the evolutionary, not day-to-day, timescale — but bear in mind that for microbes whose generation time is between minutes and days, the evolutionary timescale can be as short as a few months.

Microbes have key biological differences that increase the frequency with which they undergo horizontal transfer. For all categories of microbes, a tremendously important difference from transgenic GE crops is that the microbes are able to reproduce asexually. In plants and animals, a horizontally transferred gene will only be heritable if it happens to affect one of the small number of cells in a reproductive organ. In microbes that are unicellular or that can reproduce from any fragment of their bodies, any transformed cell has the potential to initiate the spread of a population-wide transfer event.

Bacteria readily engage in transformation and conjugation and very commonly maintain plasmids, small pieces of DNA that can be physically exported from the cell and exchanged with other organisms.[217]Bacteria are targeted by bacteriophages, a common and rapidly evolving type of virus that are well known to transmit genes across species. The genome of a bacterium is not contained inside a nucleus and is therefore more chemically accessible for integration of foreign DNA. Likely due to this underlying fact, a range of other processes can lead to horizontal transfer into and out of bacteria even when one of the well-known mechanisms is not in place. The presence of these 'non-canonical' mechanisms that have yet to be understood are a further illustration of the fact that complete containment of genetically modified sequences released within engineered microbes is impossible.

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