

Agriculture and the Microbiome



By understanding and harnessing the power of positive-impacting microbes, researchers in both academia and industry can develop new products that will further enhance plant health and crop yields. These products will allow farmers and producers to both increase yields and lessen the reliance on traditional pesticides. (Photo from Lloyd/Shutterstock.)

INTRODUCTION

Agriculture is one of the keystones of human civilization, providing a reliable, stationary source of food that allowed ancient populations to grow and eventually build cities. However, over the course of human history, increases in population have required contemporaneous improvements in agricultural advancements. Modern agriculture is successful today because of advances in mechanization, breeding, nutrients

(e.g., fertilization), and pest and disease management, all of which enhance crop productivity and provide greater food security. Yet even with this progress, the amount of cropland per capita has declined (Figure 1), available farmland is being consumed by urban development at unprecedented rates, and crop yields are plateauing (Grassini, Eskridge, and Cassman 2013). These data are particularly alarming in light of the world's expected population growth

and corresponding global demands for food, feed, and fiber. Crop yields must continue to increase and the gap between plant productivity and consumption must be bridged. But how? What is the next technological wave that will improve plant productivity on already stretched resources and in the face of a changing climate? Here we argue that expanding the use of crop microbiomes to improve plant production is that next agricultural revolution.

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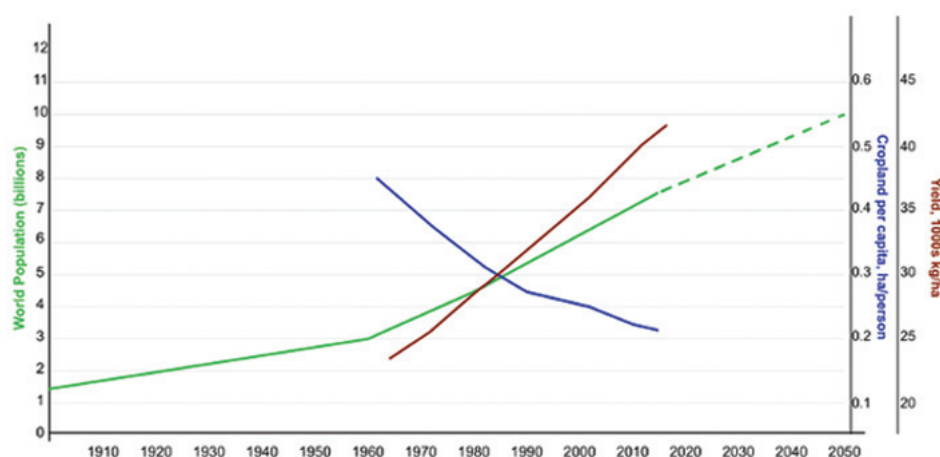


Figure 1. Amount of cropland, in hectares per person, plotted against yield in 1,000s of kilograms and world population (projected population dashed line.) Crops included in yields are: cereals, coarse grain, roots and tubers, primary vegetables, and pulses. Data from FAO 2019; FAO 2020.

et al. 2018), fungi (Rodriguez et al. 2009), and animals (Castillo, Vivanco, and Manter 2017) living on and around the plant can support—and be supported by—the plant. This community of plants, microbes, and animals, plus the geophysical environment, is together called the phytobiome—“phyto” meaning plant and “biome” meaning the distinct environment (Leach et al. 2017, Phytobiomes Roadmap 2016). The set of networks that comprise the phytobiome, especially the microorganisms that comprise both the plant microbiomes (in and on leaves and other plant cells) and soil microbiomes, profoundly influence plant and agroecosystem health and productivity. When all growth conditions are optimal, plants can be abundant producers. But the smallest change can tip the balance towards poor growth, with extreme perturbations resulting in crop failure.

One of the challenges researchers face in this area is trying to understand the complex interactions occurring within the phytobiome. Plant-associated organisms can have direct or indirect, beneficial, or deleterious impacts on plant health through interactions with other phytobiome members or changes to the environment. Our initial understanding of the complex phytobiome has come from focusing on interactions between one plant species, one pathogen, and/or one

MICROBIOMES ARE FASCINATING BUT COMPLEX

The term “microbiome” has become part of our modern vocabulary and refers to a community of microorganisms in a particular environment and includes the biological, chemical, and physical characteristics associated with both the microorganisms and their specific environment (Whipps, Lewis, and Cooke 1988). Humans all have a microbiome—the collection of all microorganisms living

on or within our bodies. An example of this is our gut microbiome, which refers to all of the microbes—the microbial community—in the human gut. Maintaining a healthy microbiome keeps our gut happy, our skin soft, and our mind clear. But what about plants? Plants must successfully gather nutrients, maintain hydration, and protect themselves from disease, all without moving. Fortunately, the plant is not alone in its mission to survive and thrive: bacteria (Bulgarelli et al. 2013), archaea (Moissl-Eichinger

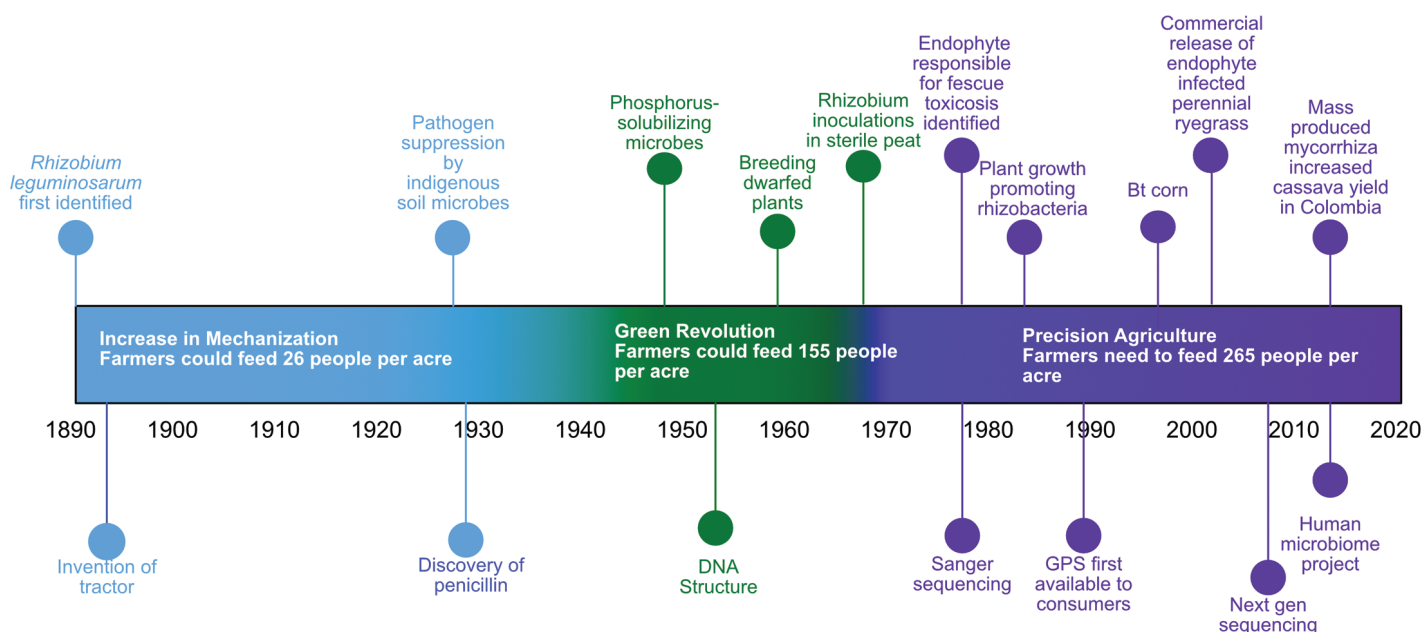


Figure 2. Timeline of agricultural revolutions and scientific advances. Some major advances have seen farmers increase their productivity. In the late 1800s, mechanization with the invention of the tractor each farmer could produce enough food to feed 26 people (world population 1.65 billion). In the 1960s, the advances due to the green revolution meant each farmer could produce enough food to feed 155 people (world population 3 billion). In the 1990s introduction of precision agriculture meant each farmer could produce enough food to feed 265 people (world population 5.3 billion). By 2050, farmers will need to produce enough food to feed a world containing 9.7 billion people.

plant-beneficial microorganism.

However, technology and analytical approaches have now advanced such that scientists are able to study increasingly more complex systems making understanding phytobiome dynamics a real possibility. Researchers from multiple disciplines are beginning to work together to ask broader questions, employ new methods and analytical approaches, and integrate existing knowledge into a more unified understanding of phytobiome system, structure, and function. The prize will be the development of applications and management practices that more effectively employ microbiomes for sustainable enhancements in plant productivity and food and fiber quality.

Science is Ready to Take Advantage of Microbial Research to Advance Agriculture

Over time, scientific and technological advances have sped up our capabilities to make great leaps in shorter time frames (Figure 2). The Green Revolution in the 1950s and 1960s vastly improved plant productivity by applying mul-

ti-ple technologies as a package. These technologies simultaneously included plant varieties bred for specific traits (for example, increased yield or dwarfed stature) herbicides and pesticides for controlling pests and pathogens, application of synthetic fertilizers, controlled irrigation and greater mechanization. The result was a dramatic increase in agricultural productivity. Farmers were suddenly able to produce substantially more with less land. However, intensive management without good stewardship can impose costs to the environment in the form of degradation of soils, and pollution of waterways, ground water, and surrounding wildlife habitat.

Application of microbes to improve crop yields has a long history. For example, nitrogen-fixing bacteria collectively known as rhizobia have been added to soils for plant growth promotion since the early 1910s (Denton et al. 2013). These free-living bacteria are capable of forming nodules on legumes, such as beans, peas, and peanuts. When nodules are formed, the bacteria fix nitrogen into a plant-available form, providing plants with a soil-independent source of

nitrogen. In the 1960s, the use of rhizobia as soil inoculants was accelerated when sterilized peat was introduced as a carrier to improve survivability of rhizobia in soils, allowing for more effective nodulation and increased plant productivity. Another example is the use of fungi that live inside of grass plants (Johnson et al. 2013), which provide their forage grass hosts with protection from insects, and provide resistance to some nematodes and pathogens, tolerance to drought, and overall greater field persistence. There are many other agricultural products derived from microbes that are intended to help crop plants take up nutrients or reduce troublesome pests and pathogens. These examples highlight the positive impact of past microbial research on agricultural products. However, there are still many areas to explore and challenges to overcome; we need to improve efficacy to reduce year-to-year and field-to-field variation, to scale up from small plot studies to getting products in the hands of farmers, and to improve formulations to reduce production costs and overall costs to the farmer. Therefore, a better understanding of the interactions

of the phytobiome, along with current technological advances, should result in products with more consistent results that could provide a farmer a good return on investment.

Although products derived from microbes are not new, recent advances in scientific analytical methods and new agricultural technologies create the ability to develop more advanced agricultural products based on single microbes, soil, or plant microbiomes, or the phytobiome as a whole. High-throughput DNA sequencing (first developed in 2005; see Reuter, Spacek, and Snyder 2015) combined with tremendous computing capacities were key technological advances that first allowed scientists to better grasp the complexity of microbial communities associated with plants. These tools enabled the characterization of not only the genome of a host organism, but also its associated microbiome, with the human microbiome project having led this field (Human Microbiome Project Consortium 2012; Turnbaugh et al. 2007). Microbes identified in the Global Ocean Sampling expedition (Sunagawa et al. 2015) and the Earth Microbiome Project (Gilbert, Jansson, and Knight 2014) have similarly expanded the data available about which microbes are associated with vastly different environments.

Now that sequencing can help resolve which particular microbes might be present within the soil, on a leaf, or associated with a root at any particular time point it allows us to evaluate samples from different environmental conditions, to identify important functional capabilities, and to compare and contrast plants in productive versus unproductive environments. Identifying and understanding the microbiomes of many diverse crops from the numerous environments they can inhabit is still a daunting task. Yet, microbiome science is feeding our knowledge of how plants and microbes form associations and what functional interactions drive successful (or unsuccessful) associations. Considering this question in terms of the complete phytobiome system will ultimately help us solve the questions of, “What is a healthy soil?” and “What are positive microbial associations that will improve plant productivity, reduce the need for harmful chemical inputs,

and lessen agricultural contributions to pollution?”

Now Is the Time to Harness the Power of Microbes

This report will highlight the recent progress made in our understanding of plant microbiomes, as well as the remaining gaps in knowledge of how the exploitation of microbes in agriculture can positively impact crop production and food security. We provide examples of the intricacy of the phytobiome system including the microbiomes therein, of how advancing technologies can provide insight into microbiome function, of the microbial products most recently utilized in agriculture and their benefits, of the training requirements for interdisciplinary research that can merge academic questions with industry outcomes, and of the importance of communication for the continued acceptance of these approaches.

THE COMPLEX PLANT-SOIL MICROBIOME TEEMS WITH MICROORGANISMS

Soil acts as a rich repository of bacteria, fungi, and other organisms that colonize plant roots, shoots, and other organs (Wagner et al. 2016). Fungi comprise much of the microbial biomass in the soil around the world (Fierer 2017), while, impressively, a gram of soil is estimated to contain up to 10 billion bacterial cells and may hold as many as 10,000 bacterial species (Raynaud and Nunan 2014). The number and diversity of microbes found in different soils depends on the composition, acidity, moisture content, and other physical or chemical properties of this complicated substrate. These soil properties can also impact how bacteria, archaea, and fungi associate with each other. Bacteria often live in multispecies communities, where their localization and population structure can be affected by the different types of soil particles available (Stubbendieck, Vargas-Baitista, and Straight 2016). In addition, while soil structure affects microbial composition and species interactions, soil characteristics can also be changed by the activities

of micro- and macroorganisms living in the soil. As an example, fungal hyphae, the threadlike filaments that comprise fungi, can extend up to four square miles (Ferguson et al. 2003), and are critical to the formation of soil aggregates and the stabilization of organic matter in soil (Guhr et al. 2015; Lehmann, Zheng, and Rillig 2017). Fungi may also enhance the movement of bacteria via “fungal hyphal highways” (Simon et al. 2017). Just as soils in different regions can vary in color, texture, density, and nutrient levels, the microbial communities that make up the soil microbiome in a particular field may also vary depending on the soil itself, the plants that are growing at that particular point in time, and even the land management history (e.g., whether the fields are tilled, whether cover crops are planted, and what types of nutrients or pesticides are applied).

If the soil microbiome consists of the microbes living in the soil, then the microbes living in and on the plants make up the plant microbiome. There are multiple mechanisms by which soil microbes can be introduced to plant tissues for subsequent colonization and maintenance in the plant microbiome. Water may carry microbes to the phytobiome (Bai et al. 2015). Microbes can survive in the upper atmosphere and be transported great distances (Jaenicke 2005), and falling rain droplets can release bacteria from the soil upon which they land (Joung, Ge, and Buie 2017), and clouds can harbor diverse microbes and transport them around the world (Fierer 2017).

Although soils harbor great microbial diversity, only four groups of bacteria are generally enriched in the plant microbiome: Proteobacteria, Firmicutes, Actinobacteria, and Bacteroidetes (Bulgarelli et al. 2013). Why are these particular groups of bacteria found at such high abundance on and in plant tissues? Plants can have direct effects on microbiome composition. Specificity is apparent for plants and their associated microbes, as shown in microbiome analyses of various plant species (Agler et al. 2016). This suggests that plant hosts may reproducibly select for a core microbiome, a consistent set of microbes that are always present on certain plants. In addition to abiotic factors such as soil type, weather, and nu-

trient conditions, plant growth stage and immunocompetence (Lebeis et al. 2015) can also influence microbiome composition in the rhizosphere (soil very near to and affected by plant roots) and the root endosphere (tissue within the plant where microbes may reside), generating functional changes in the microbiome that are likely to influence plant fitness. For example, in the rhizosphere the growth of soil microorganisms is enhanced resulting from chemical and physical alterations of the soil by the root including root respiration, exudations, water and nutrient uptake, and even soil pore size (Bakker et al. 2013).

Microbiomes for some crops are consistent over generations or across different fields, even in the absence of vertical (seed) transmission (Berg 2009), suggesting that some plants attract or select for specific microbes. However, plant microbiomes may also vary substantially over a single growing season (Wagner et al. 2016), suggesting that the phytobiome is also a dynamic ecological assemblage. Understanding what determines the stable or dynamic nature of soil and plant microbiomes with different crops under different conditions is an important step in determining how to best use microbes to improve the yield, nutrient content, and overall success of agricultural crops.

Soil Microbes Can Help Crops Grow but Can Also Make Them Sick

Microbes as a whole are not inherently “good” or “bad” when it comes to their effect on organisms like humans and plants. But certain microbes act as pathogens and make people and plants ill, while other microbes are crucial for keeping us healthy. It is the same for plants: microbes and microbiomes can have both positive and negative effects on plants, depending on the specific microbes present as well as other factors, including environmental stressors. Researchers are discovering that many microbes and microbial communities can have a positive effect on plants by warding off pests, reducing the impact of pathogens, increasing a plant’s ability to access nutrients, or providing assistance in surviving stressors such as drought or salt. Some

microbes are pathogens, causing plant diseases—fungi and bacteria damage an estimated US\$10 billion of agricultural products per year (Syed Ab Rahman et al. 2018). Research focusing on how different microbes or microbiomes may help or hinder plant productivity provides insight into the mechanisms through which plants and microbes interact and provides avenues to develop both beneficial products and disease-mitigating solutions. For example, an enhanced understanding of the ways that plant microbiomes may mediate the likelihood and severity of fungal or nematode infections could reduce global losses due to these pathogens.

Studies over the past century show that microbes growing in, on, or directly around the roots can positively affect the growth of plants under a multitude of conditions (Zamioudis and Pieterse 2012). For example, some bacteria and fungi can produce siderophores, which are small organic molecules that can harvest iron from soils and make it accessible to the plant (Harrington, Duckworth, and Haselwandter 2015). Bacteria can help plants resist infection through inhibiting pathogens or by stimulating the plant immune response (Ritpitakphong et al. 2016). Archaea have recently been shown to stimulate plant growth through production of the plant growth hormone auxin (Taffner et al. 2018). Arbuscular mycorrhizal fungi (AMF), well-studied fungi that can increase plant growth and are associated with more than 80% of terrestrial plants (Lee et al. 2013), penetrate the root cortical cells of vascular plants, stimulating their immune system and providing nutrients (Howard and Valent 1996). Other beneficial fungi include members of the ectomycorrhiza or ericoid mycorrhiza, which can be found in the plant rhizosphere or free-living in the nearby soil (Fierer 2017). Fungi have been shown to stabilize soil hydration levels by collecting water in their matrix of hyphae (Gühr et al. 2015). It is likely that these examples only scratch the surface when it comes to ways microbes or microbiomes may benefit crop plants.

Interestingly, interactions between beneficial microbes and the plant appear to occur through similar chemical and physical mechanisms as those observed in plant-pathogen interactions.

For example, root colonization by the bacteria *Bacillus subtilis* leads to a priming of the plant’s immune response to subsequent foliar infection through a mechanism called induced systemic resistance (Chen et al. 2013). Conversely, systemic acquired resistance occurs when a sub-lethal infection by a pathogenic microbe stimulates the plant to change its hormone production in a manner that increases the plant’s survival when faced with another pathogen later on (Durrant and Dong 2004). However, how the plant distinguishes between the types of microbes it encounters and the mechanisms it uses to respond to them have not yet been clearly delineated.

Growth of the plant host and its microbes can also be affected by the partitioning and exchange of nutrients and minerals (Sasse, Martinoia, and Northen 2018). Interdependence of community members’ metabolic processes may stabilize the ecosystem (Leach et al. 2017). Plants provide sugars through root exudation while alive and through recycling of plant matter upon death; an estimated 5 to 21% of a plant’s fixed carbon is released into the soil (Marschner 1995), and these exudates can diffuse centimeters away from plant roots (Luster et al. 2009). On the other hand, bacteria and fungi provide trace minerals by converting less bioavailable forms of minerals into forms useable for plants. The most widely studied example of this is the legume-rhizobium symbiosis where atmospheric N_2 is “fixed” by *Rhizobium* spp. to the bioavailable form of nitrogen, NH_3 , so that it can be absorbed and used by the plant (Fierer 2017). In this mutually beneficial relationship, the rhizobia obtain carbon sugars from the plant and thrive within protective structures on the root called nodules (Remigi et al. 2016). Other examples include bacteria and fungi that can dissolve phosphate minerals to provide this nutrient to the plant (Goswami et al. 2019).

Understanding the Interactions of Soil Microbes and Plants Is Critical for Protecting Crop and Ecosystem Health

The intricate plant-microbe relationships are important for the survival of

the ecosystem as a whole. The balance or failings of interkingdom interactions may be the difference between a plant growing to its full potential and its destruction by fungal or bacterial pathogens (Wei et al. 2015). Historically, in spite of the incredible diversity found within native soils, plant-microbe interactions have been largely studied by examining one species of plant with a single species of bacteria or fungi. The challenge we currently face is that the emergent properties of phytobiome systems are not predictable from the data we have traditionally gathered from these simplified, single plant species-single microbe species interaction systems. With recent advances in analytical methods, it is now possible for researchers to study increasingly complex systems and glean the information necessary to understand the dynamic interactions occurring within the plant and soil microbiomes.

Multispecies models of plant-microbe interactions promise to provide deeper insights into agriculturally relevant plant-soil interactions (van der Heijden and Horton 2009). However, the enormous complexity of plant and soil microbiomes provides challenges to data collection and analysis (Leach et al. 2017). In particular, while we are adept at characterizing the chemistry of soil or the biological composition of plant microbiomes, and metagenomic studies can identify the clades of microbes present along with their metabolic potential (Finkel et al. 2017), such information still gives only limited insight into the complex interactions that generate the collective functional capacity of the plant microbiome. For example, antagonistic and synergistic effects have been demonstrated for phenomena such as bacterial biofilms (Powers et al. 2015; Ren et al. 2015) fungal sporulation (Salvioli et al. 2016), and plant hormonal signaling (Hacquard et al. 2017), all of which can have crucial impacts on microbial community composition and the ability of microbes to associate with plants.

In addition, the soil itself can affect these microbial interactions, with characteristics such as nutrient availability and the size of air pockets changing which chemicals are produced and the distances they can travel (Aksoy et al. 2017). This is important because numerous chemi-

cal compounds such as small molecule (secondary, or specialized) metabolites are known to mediate intra- and interspecies cell-cell communication between microbes (Leach et al. 2017; Little et al. 2008). For example, researchers have found that the plant chemical rosmarinic acid acts as a disruptor of bacterial quorum sensing, affecting the bacteria's ability to colonize the plant (Corral-Lugo et al. 2016). Conversely, volatile organic compounds produced by microbes may stimulate plant immune responses (Zamioudis and Pieterse 2012). These interactions are important to prevent disruption of the microbiome that may result in a decrease in plant health (Chapelle et al. 2016).

Understanding the complex relationships of the phytobiome will by necessity inform future decisions about agricultural practices and crop management systems (Busby et al. 2017; Schlaeppi and Bulgarelli 2015). New findings are forcing us to reconsider our foundational assumptions about the breadth and depth of microbial life in the soil (Haney et al. 2015) and the phytobiome (Baltrus 2017). Going forward, it is therefore essential that, as a field, we develop new strategies for studying and understanding the complexities of plant-soil-microbiome systems and their interactions with the environment in order to advance our capacity to manage important ecological and agricultural phytobiome components for long-term sustainability and productivity. For now, we must recognize that we have only begun to scratch the surface of the exquisite sophistication of the above- and below-ground interactions between microbes and the plants living amongst them.

CUTTING EDGE TECHNOLOGIES MAKE MICROBIAL DATA USEFUL

New Technologies Illuminate the Who, What, and How of the Microbiome

The microbiome of a plant is interwoven and composed of many organisms that change abundance and activity depending on the environmental conditions. How do you identify which components

of the microbiome are important for plant health? How can you improve the overall plant or soil microbiome to improve plant health? The first step in tackling these questions is to determine the composition of the microbiome (the who) and identify which members are likely to have an important function in the community (the what and how).

The “Who”

Advances in DNA sequencing technologies have dramatically increased our ability to identify the organisms present in a microbiome. When next-generation sequencing first became available and, importantly, affordable, it was possible to analyze the bacterial component of microbiomes by sequencing a short portion of a single gene (the 16S ribosomal RNA (rRNA)) that is present in all bacterial genomes (Case et al. 2007; Woese and Fox 1977). Very similar 16S rRNA sequences were correlated with closely related bacterial species, and members of the bacterial community could be estimated by grouping similar sequences. These groupings consisted of operational taxonomic units (OTUs), or more recently, amplicon sequence variants (ASVs) (Callahan, McMurdie, and Holmes 2017) that are a rough proxy for a bacterial species based on comparison to limited reference databases of known bacteria. These classifications could provide relative, but not quantitative, abundances of these bacteria. As sequencing technology and data storage became less expensive, researchers were able to sequence regions of key bacterial genes, improving reference databases for sample identification. This, combined with new techniques for identifying other microbes, like fungi and archaea, vastly improved the breadth and resolution of microbiome knowledge (Amaral-Zettler et al. 2009; Walters et al. 2016). Identified OTUs based on these specific genes are still compared to a reference database of known organisms to make taxonomic calls and functional inferences; the accuracy of the match can only be as good as the database to which it was compared. This remains a challenge when trying to match totally new sequences that are not closely related to known species. New methodologies are now beginning to provide a way to place

sequences into taxonomic categories even without the sequence having a match in reference databases (Carbone et al. 2017; Carbone et al. 2019); this will allow researchers to identify a larger proportion of the microbiome and thus generate a more complete picture of who is present in a microbiome sample.

Eventually, with automation of sequencing technologies it became possible to analyze whole genomes rather than just single genes (Alberts et al. 2008), providing a wealth of genetic information. Initially this genome sequencing was expensive and limited to microbes that could be cultured, but it is now possible to realistically use metagenomic shotgun sequencing data to enumerate and examine a wide array of microbes without needing to isolate or culture them. In metagenomic shotgun sequencing, ideally, all the DNA in a sample is sequenced (not just one gene or one organism). Deciphering which DNA fragment belongs to which organism and making complete genomes from lots of bits of many different genomes is still a tough challenge, but ongoing research is advancing our ability to assemble these genome puzzles (reviewed in Vollmers, Wiegand, and Kaster 2017). This metagenomic sequencing data allows researchers to have a more comprehensive knowledge of the whole plant or soil microbiome in a particular sample at a particular time.

The “What” and “How”

Now that it is no longer necessary to infer genomes solely from cultured reference organisms, scientists can determine more accurately what microbial genes are present and what functions they encode in these microbial communities. This will lead towards understanding how the microbial community may impact

plant health and yield. Other methods aim to detect not just which genes are present, but what the microbes in these communities are actually doing. Many studies have used 16S rRNA sequencing technologies and analyses to predict functional roles of metagenomic samples (Langille et al. 2013). 16S rRNA sequencing data has advantages of cost-effectiveness, established bioinformatic pipelines, and a large resource of archived data;

disadvantages include the need to make broad assumptions about the activities of bacteria possessing a specific 16S rRNA gene.

In metatranscriptomics, all the messenger RNAs in a sample are sequenced to get a snapshot of what genes are being expressed (Turner et al. 2013). In metaproteomics, protein fragments are examined to see what proteins have been produced (Heyer et al. 2015). In metabolomics, the small molecules that are being produced are characterized (Chong and Xia 2017). Methods have been developed to extract samples for multiple types of analyses simultaneously (Nakayasu et al. 2016) and to visualize and analyze such data (Eren et al. 2015). Using combinations of all these technologies allow researchers to get a more complete snapshot of what is happening within a soil- or plant-associated microbiome, information which then can be used to focus efforts on improving plant productivity. The next steps will be to efficiently and accurately connect molecules to function to organism and to map out and understand the dynamic processes and feedback loops that control how a particular community of organisms interact with the environment and each other. This level of understanding will allow us to predict how the systems will function when certain organisms are removed or added with the goal of increasing plant health and yield through management of the microbiome.

Occasionally Only a Handful of Microbes in a Microbiome are Important

Comparing the membership of organisms in different samples can provide an idea of which are “core” members (taxa that are always present on a certain plant or in a certain condition) and which are accessory members (taxa only present under certain conditions) (Bulgarelli et al. 2012; Lundberg et al. 2012). A similar analysis can be done to find the functional core microbiome or the core activities being performed by members of the microbiome (Vandenkoornhuys et al. 2015); in this case the actual organisms present may differ but the same functions are still being performed. Depending

on the questions being asked, researchers may be interested in the members/functions that are present or missing in a sample. For example, it is useful to know if a particular microbe or microbial function is always present or absent in a healthy field—this could indicate whether a specific organism or a specific function is correlated to plant health.

A complementary way to examine the importance or utility of members/functions of a microbiome is by using synthetic communities (Busby et al. 2017). In these experiments, a simple version of a microbiome is reconstituted using bacterial isolates in the same proportions and similar taxonomic classifications/functional annotations as the original community. These synthetic communities can then be used to test their effects on plant phenotypes or to assess the importance and/or function of specific isolates within the synthetic community by adding or subtracting an isolate and watching how the phenotype changes (Herrera Paredes et al. 2018). This same approach can be used with other members of the microbiome as well, such as fungi.

Data-driven Techniques Empower Researchers to Focus on the Most Relevant Information

Precision agriculture provides critical context for microbiome research

New technologies continue to improve our ability to more specifically describe phenotypes in the field that can then be used to correlate to members of the plant microbiome community. Smart tractors enable precise spacing of plants and collect data on inputs such as fertilizers and pesticides. Drones can take multispectral pictures of plants growing in a field over time, allowing growers to monitor plant growth and health within and between fields (Figure 3). Microsensors can collect information on weather, water availability, and nutrition status of the soil, giving growers necessary information to make targeted interventions to improve plant health. All of these advances provide better visualization and contextualization at the macroscopic level—seeing a field through sensors from drones and

satellite imagery or following a specific path with accuracy using GPS. Collectively, this technology provides greater accuracy than manual assessments by the human eye. For example, a plant that has succumbed to fungal infestation may show variation in leaf chlorophyll content or leaf discoloration as a result of disease and accompanying changes in moisture content, which may also be influenced by soil type (sandy versus clay soil) and environmental conditions (fertilizer, irrigation and pesticide regiments). Digital phenotyping provides an objective assessment of all the potentially interacting factors allowing farmers to pinpoint not only the individual plant or variety that is more susceptible to disease but also evaluate what other factors are contributing to the disease. For example, aerial drone images can be used to assess plant health (Figure 3), correlate differences in soil within a field and plant health (Figure 4), and even measure biomass of plants as they grow during a season (Figure 5). What is currently missing from the suite of precision agriculture technologies is the ability to meaningfully connect microbiome data to the plant, soil, climate, weather, and land management data. Correlating macroscopic observations to the members and/or functions of the soil and plant microbiomes will pave the way for researchers and companies to apply this knowledge to improving agriculture through microbe-based solutions.

Big Data approaches and Machine Learning tools can, together, unearth useful patterns for interpreting data

Currently, precision ag data is not directly coupled with microbial data; however, given the rise of precision-farming techniques coupled with the increasing cost-effectiveness of genomic technologies such as metagenomic and whole genome sequencing and the advancement of metaproteomics, metatranscriptomics, and metabolomics, there would be an unprecedented volume of data currently being produced that can advance phytobiome research. Advanced statistical methods and modern machine-learning methods are now being used to enable trait associations and predictions of the functional roles of microbial communi-

ties, but several key challenges remain to usefully link these new technologies and precision-farming techniques. There is a need for a direct link to be made between genomic and microbial scale technologies and farm-scale agricultural techniques in order to effectively determine appropriate precision agriculture treatments under a variety of environmental and pest conditions. Ideally, assessing the relationships among (1) plant genomes of different plant varieties, (2) the plant phenotypic traits, (3) microbial community identity and functions in both plant and soil microbiomes, (4) soil characteristics, and (5) environmental stressors, whether abiotic (e.g., flood, drought, salt) or biotic (e.g., insect pests, fungal pathogens,

bacterial diseases), will provide dynamic information on how best to manage a specific farm or field. However, while generating each of these very large indi-

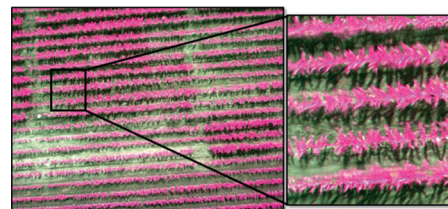


Figure 3. False color infrared image captured from UAV-mounted multispectral camera. Healthier vegetation appears darker red. Photos from R. Austin and J. Taylor, N.C. State University.



Figure 4. Vegetative health of sweet potato fields as measured from UAV-based imagery one week before harvest (left). A strong spatial correlation exists with soil color as observed in the image on the right. Photos from R. Austin N.C. State University.

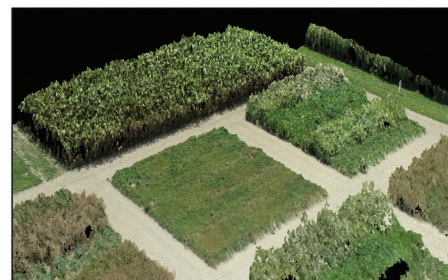


Figure 5. UAV-collected aerial survey of bioenergy crops in Williamsdale, North Carolina (left) and UAV-derived three-dimensional surface model of various bioenergy crops. In-season biomass is estimated using the remotely measured canopy heights. Photos from R. Austin and C. Crozier, N.C. State University.

vidual data sets is now possible, it is still a daunting task to establish statistically and practically meaningful associations between them that would lead to better management strategies. Nevertheless, now is the time to focus data and analytical resources to understand the microbiome aspects of the phytobiomes system and how they relate to precision agriculture practices.

Traditional statistical methods are not enough to tackle the massive data sets that are being generated by these new analyses and technologies. The ability to generate vast amounts of data is useful when trying to understand complex systems, but often the environmental or ecological interpretation of these datasets can be quite challenging. Metagenomic data is high-dimensional (for example, microarray data from tens of thousands of sample wells), often incomplete, and typically has skewed, non-normal or unknown distributions. Many traditional statistical algorithms, such as analysis of variance (ANOVA), are not valid for these types of data. Additionally, data analysis decisions regarding these large datasets often take into account different factors than those most relevant to the scientific question at hand. For example, a research question may require comparing the relative abundance of a certain microorganism under different experimental or field conditions. However, if the data are being analyzed as compositional data, where the total must always add up to 100%, the presence or increase in abundance of a different species could translate to an apparent reduction in the abundance of the species of interest even when there is no actual change in the species in the samples (Weiss et al. 2017). To reduce reliance on these potentially problematic compositional analyses, researchers have developed methods to determine quantitative abundances. One method currently being developed uses total microbial biomass to normalize relative abundances into real abundances and shows promise. More research needs to be done to find both direct and indirect ways to determine absolute microbial cell abundances related to the agricultural microbiome.

As the type and scale of plant and soil microbiome data increases, more

advanced methods must be developed and used to analyze and interpret these increasingly immense data sets. One method researchers are using is machine learning. Machine-learning methods refer to a class of statistical algorithms that iteratively update tuning parameters, or “self-learn,” from each prediction step to fit better models in subsequent predictions. Machine learning has great potential to increase our ability to effectively handle the types of data collected from the plant and soil microbiomes, as well as the phytobiome.

Using machine learning can provide superior results. In a review of application methods, Wassan and colleagues (2018) reviewed applications of machine learning methods to this type of data and showed that there are machine learning methods that were better for predicting environmental roles and outcomes from microbiome profiles compared to older approaches (Chen and Guestrin 2016). This area of machine-learning research lends itself well to incomplete, high-dimensional data, making it an ideal fit for metagenomic research. One of the most exciting aspects of such research is the promise of the development of “biologically smart” machine-learning algorithms, where biological knowledge—such as pathway perturbation, phylogeny, or other biological mechanisms of the microorganism—are built into the statistical methodology. Ranjan and colleagues (2016) highlight the multiple benefits that whole genome sequencing technology can provide to this research, which includes increased detection of species and diversity profiles and, most importantly, enables individual gene-level analysis. The wealth of data now available at the gene, metagenomic, metatranscriptomic, and metaproteomic levels provide unique opportunities to perform integrative analyses to understand relationship patterns not only for microbiomes in isolation but also as a critical component of the entire phytobiome.

Consistency in data collection and standardization is also critical when tackling complex systems (Dundore-Arias et al. 2020). There was an initial crisis of reproducibility in the early years of genomic experiments, and scientists subsequently realized the importance

of ensuring that data collection is well designed to avoid confounding factors and to accurately match the functional roles of the microbiome to the traits of interest. This not only includes standardization and collaboration with projects like the Earth Microbiome Project mentioned previously but also standards that require collecting metadata such as environmental factors like seasonality, climate, soil sample depth, etc. (Knight et al. 2018); this technique is codified in a variety of “Minimal Information” Standards created by the Genomic Standards Consortium (see <https://press3.mcs.anl.gov/genesc/>).

THERE ARE MYRIAD OPPORTUNITIES AND CHALLENGES FOR COMMERCIALIZATION OF MICROBIAL PRODUCTS

Research Is Needed to Ensure Products Can Be Scaled Up and Useful to Growers

There is no shortage of preliminary research findings supporting the idea of using biologicals in agriculture. From 2015 to 2018, more than 2,000 peer-reviewed scientific publications relating to agriculture and microbiome research were published worldwide (source: Scopus). Nearly all of these publications suggest at least one relationship between a particular microbe or microbial community and an important agricultural trait. Additionally, many large agriculture biotechnology companies have very large strain collections (some in excess of 100,000 strains) that are actively being evaluated for beneficial agricultural properties. The challenge for product development comes in the transferability of those early laboratory results to meaningful replicated field trials. Parnell and colleagues (2016) described four critical factors—efficacy, versatility, practicality, and delivery—required to successfully bring a biological product to market. Efficacy is the ability to increase or protect yield; versatility is the ability to function across different environments, stress conditions, and crop varieties; practicality is the ability to be compatible with agricultural practices

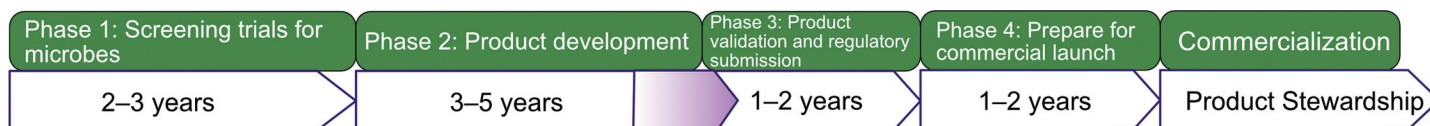


Figure 6. Timeline of product development.

and equipment; and delivery is the ability to logistically deliver a functioning product to the crop when needed. All of these challenges must be met to develop new biological products for conventional and organic agricultural markets.

The use of biological products (microbes or their derivatives) in agriculture, whether as a biocontrol or a biostimulant, has surged in recent years. Biocontrol products, also known as biopesticides, are those that use biologicals to control pests, e.g., insects and plant pathogens. Biostimulants are biologicals that stimulates natural processes to enhance or benefit nutrient uptake, nutrient use efficiency, tolerance to abiotic stress, or crop quality and yield.

The global commercialization of biologicals highlights the desire by both farmers and the general populace for more natural and sustainable agricultural practices, without sacrificing crop yields and by providing alternative modes of action (MOA) for resistance management. For example FMC Corporation's First Quarter 2020 revenue was \$1.25 billion, up 5% from Q1 2019. (FMC 2020). The growing revenue can indicate a surge in interest and investment in biological products.

Other companies are also seeing large investments in their products. Pivot Bio, which uses nitrogen-fixing microbes as an alternative to synthetic fertilizers, has been backed with an \$100 million investment from Breakthrough Energy Ventures and Temasek (Pivot Bio 2020). Bayer has partnered with Gingko Bio-works to create Joyn Bio, which aims to "develop a new class of biologicals that are sustainable and reach unprecedented levels of performance and reliability (Joyn Bio 2020). Like Pivot Bio, Joyn Bio plans to create a product using microbes to fix nitrogen in the soil. Both these products hope to lessen the reliance on nitrogen fertilizer, which will reduce

water pollution and greenhouse gas emissions.

Generating a commercially viable agricultural product

The process to produce a new biological product can be long (Figure 6), with a lot of regulations that must be met. A company may start with thousands of biological candidates, and ultimately end up with only a few as potentially viable products. This screening process reflects the many considerations and challenges that a company faces while developing a product. For a product to be commercially viable, these minimum criteria must be met: it must be effective in the environment in which it will be used, manufactured reasonably at commercial scale, be robust and of good/consistent quality, and the company must have the legal right/authority to commercialize the product. To ensure manufacturability of a biological product, one must first demonstrate the ability to produce at laboratory scale, then pilot scale, and finally to commercial scale production. On top of these criteria are complexities added by the diversity of farming practices (specialty versus row crops), application requirements (seed treatment, in-furrow, or foliar), available library of microorganisms, and regional considerations, such as agricultural-use practice and regulatory requirements (i.e., product registrations).

Novel product concept

There are many products for sale for every type of crop and market imaginable, and this can add difficulty in marketing a new product that will grab enough market share and acreage penetration. Therefore, the novelty of a product concept is crucial to building a solid business case. A product can differentiate itself by target crop, target pest, target geography, mode of action, application method, product format, cost, registration

status, etc. A new product ideally has an edge over all other products in its class, and that edge needs to be apparent to the grower or end-user.

Another consideration to vet a product concept is if a strong business case can be made to support the development investment. An assessment should be made to determine if there is strong demand for the product, market share potential, estimated acreage penetration potential, projected sales, profit, costs, and other factors. The team will also need to consider if the product can be taken to market solely by the company itself or if a partner is needed. There are instances when a partnership is beneficial or even necessary for R&D, production, distribution, sales, and/or marketing. Project timeline also factors heavily into a business case. For instance, if the product takes six years to launch, then the team must predict if there will still be enough demand at the time of launch.

Intellectual property and freedom to operate

Once a product concept has been identified, it is prudent to research the current intellectual property (IP) landscape around the concept before jumping into R&D activities. Larger companies may have a legal team or hire a contractor to investigate the patent landscape and existing technology. This is a good starting point to assess if a company will have the freedom to commercialize the product or if it will require some adjustments to differentiate it from existing IP. An alternative option that is becoming more popular is to license a patent or technology from another entity to gain freedom to operate with a product concept. One also needs to consider how to maintain exclusivity further down the road. Relevant questions companies ask during this stage include: "Is the product concept one that can be patented; Can parts of the production

process be patented or kept as an industry secret? And can a strong barrier to entry for competitors be established?”

Efficacy and performance consistency

Once a novel product concept has been identified, efficacy needs to be validated. In some cases, a single active ingredient (microbe, molecule, etc.) may already be available to move into efficacy trials. On the other hand, a company may have to screen thousands of microorganisms to identify ones that fit the product concept. Those possible hits may enter greenhouse trials, which would bridge efficacy from *in vitro* (outside a living organism, i.e., in a petri/culture dish) to *in planta* (i.e., within the plant) activity. If some of the *in vitro* hits perform well in the greenhouse trial, it provides justification for these strains to move forward into field trials. To truly have confidence in efficacy, trials should span multiple geographies, environments, and crop germplasm (seeds or other tissues that contain a plant’s genetic material and can be used to grow a whole plant), and ideally testing different rates of application of the microbe(s) of interest. It’s not expected that a winning microbe(s) will perform in all these situations every time, but it is expected to perform well in most with an acceptable win rate (the percentage of sites in which a positive effect was observed).

Cost-Effective Screening of Potential Candidates

Obviously, doing field trials of this magnitude are very expensive. For this reason, it is practical to first pass potential microbe-based products through other less expensive and more rapid screens during product development. For example, screening microbes for their ability to grow at large scale is a good way to weed out poor-growing microbial candidates from a product pipeline and ensure that only the candidates with the best chance of commercial success are used in the broad field trials.

When performing growth condition screening and optimization of candidate strains, the scientist must consider medium components that are easily sourced and cost effective at production scale, in

addition to the health of the microorganism. If a strain cannot grow in a cost-effective medium, then it will be difficult to produce on a commercial scale. Likewise, a strain also needs to grow well under select conditions. Yields and microbial characteristics can be heavily influenced by the growth conditions, such as media components, pH, and temperature and it is crucial that the microbes of interest both grow and perform as expected in conditions necessary for commercial production.

Formulation, stability, and compatibility

Different formulations (e.g., liquid suspension, wettable power, or dispersible granules) help preserve the microbe, optimize product robustness and shelf life, and ensure its ease of use. The formulation format chosen depends on the viability of a microbe when taken out of its native environment, along with the targeted application (seed coat, in furrow, foliar, irrigated). In addition, the compatibility of the biological product with other agrochemical inputs, both physically and biologically, should be considered. For example, a biological seed treatment product will likely be added into a slurry tank with agrochemical inputs, which requires the microbe to remain viable during the residence time in a homogenous slurry in the tank, the treatment process, and upon desiccation on the seed. One must also consider that the packaging design can be critical in guaranteeing a consistent and efficacious product from the time of packaging until the end of the product’s shelf-life, as these products may be exposed to variable storage environments.

EPA, USDA-APHIS, and FDA regulate microbial products used in agriculture

The Environmental Protection Agency (EPA) and the USDA’s Animal and Plant Health Inspection Service (USDA-APHIS) are the two agencies responsible for overseeing most in-field crop treatments in the United States.

The EPA regulates the distribution, sale, and use of all pesticides, including biopesticides. Microbial products, that function as biocontrol products or plant

growth regulators fall under the EPA’s purview whereas the definition and regulation of biostimulants is currently being decided.

Currently there is no legal definition of a biostimulant and all microbial products that make claims that they are plant regulators are currently defined by FIFRA and EPA regulations as biopesticides. All biological control agents that claim they kill pathogens or manage disease are also classified as biopesticides. The EPA is aware of these gaps in overseeing new microbial innovations and has signaled its interest in further defining its role in addressing them (McNally 2018).

The USDA-APHIS regulates the importation, intra- and interstate movement, and environmental release of organisms used to control plant pests, diseases, and weeds and their environmental impact (NEPA 2020; U.S. Congress 2000). Classified as biological control organisms, microbial pathogens and non-pathogenic microorganisms used to control plant pests or weeds are regulated by the USDA-APHIS Plant Protection and Quarantine. Even sometimes when the microbe in question already exists in the United States, the USDA-APHIS may regulate organisms if there are potential negative effects on plant health or on other beneficial organisms (although it waives its permit requirements for pesticides already approved by the EPA). Additionally, any genetically modified biological control organisms, specifically those deployed to control plant pests, require permits from APHIS Biotechnology Regulatory Services.

Lastly, the Food and Drug Administration (FDA) is another agency with the potential to regulate microorganisms used on farms through the Federal Food Drug and Cosmetic Act, though its purview is primarily food safety as opposed to environmental or plant health (FSMA 2011). The FDA has not historically exercised oversight of crop treatments, microbial or otherwise, but it may step in and require premarket approval if a novel treatment results in a “food additive” and becomes part of the food for sale (FFDCA 1958), for example if there are residual microbes on food that would be eaten by consumers. However, the FDA may not have reason to oversee a treatment if there are

no such residues. One could imagine, for example, microbial treatments that extend the shelf life of produce.

Product safety will be assessed during the EPA review, after which the registrant may need to generate additional data, e.g., toxicology tests. The outcomes of these tests must be favorable for registration of the product to be granted in its current form and at the recommended label rate. A responsible company will consider product safety a top priority and may perform additional tests (internally or externally) regardless of the EPA mandate, to assure the safety of its employees, the end-user, and the environment. Additionally, products that are making certain biocontrol claims must undergo regulatory approval which varies by region, although there are groups such as The International Organisation for Biological Control (IOBC; www.iobc-global.org) and the Biological Products Industry Alliance (BPIA; www.bpia.org) that are working to make this process more unified and transparent.

Once all the criteria described above are met, the company must then have a distribution plan in place, as well as sales and marketing strategies. Meeting all these criteria assures high-quality, and consistently high-quality biologicals are the best way to gain consumer confidence in this class of products.

An Educated Public Is Essential for Successful Commercialization

A decrease in the growth rate of U.S. agricultural productivity (USDA 2017) and an increase in consumer pressures for agricultural products produced with reduced chemical inputs has driven increased development of biological products for agriculture. In recent years, many new biological products consisting of living microbes, microbial extracts, or compounds derived from microbes have entered the market. Many established agriculture companies and new start-up companies are aggressively developing new products in this area, and global sales of biologicals are expected to reach \$10.7 billion by 2025 (Bomgardner 2018).

Regulations of new technologies can

launch or stymie an industry, but public policy is not created by a scientific algorithm. The regulations that federal agencies use to govern agricultural practices are human interpretations of laws, based on intersecting priorities, from science and knowledge to economics and politics. For example, Congress passed a law in 2016 to nationalize mandatory labeling of genetically modified food products, a law driven to enactment through passionate public advocacy tempered by economics, science, and political interests. The USDA agency in charge of interpreting this law and specifying how the food industry needed to comply asked the public for answers to thirty questions and, after issuing a proposed rule, also accepted public comments. More than 112,000 comments were received in response to the questions and 14,000 in response to the proposed rule. Public input does not necessarily take a scientific view of risk, but such comments informed the USDA's ultimate decisions on the content and application of the rule in places where the law itself was not specific. This will have a direct impact on what the public sees in grocery stores and, likely, a subsequent impact on how people spend their money and how farmers choose their crops.

Even in less controversial cases, public opinion can play an outsized role in policy. If political currency is short, often only the most visible or popular policies benefit from agency attention. In this case and others, the way one chooses to communicate the benefits and risks of new technologies can have an enduring effect on the very utility and promise of those technologies for decades to come.

Microbial Products Must Be Effectively Communicated to Public Audiences

Advancement depends on adoption, and adoption depends on effective communication of potential benefits to growers and consumers. Once the *how the products work* is figured out, it is time to communicate the *why these products are beneficial*. Clearly communicated benefits are a necessary first step towards general acceptance, but the performance of biological treatments will vary on a given farmer's field in a given

year. Without clear communication from researchers and industry about what to expect, stakeholders may be caught off guard when effects assumed to be guaranteed are not realized. This could engender mistrust, with microbial treatments considered “snake oil,” and the expansion of the use of microbes in agriculture would be severely curtailed. Researchers need to convey how microbial products work and interact with fields and the environment so that full information about the economic and environmental benefits is provided, and stakeholders can make a fully informed decision about adoption.

Those who study science communication research focus primarily on three interrelated areas: (1) beliefs, attitudes, knowledge, and behaviors; (2) acceptance of or resistance to adoption of a novel technology; and (3) the social communication surrounding risks and benefits. Research has demonstrated that, rather than a simple yes/no dichotomy between the options of acceptance or resistance, adults in the United States enter into a complicated calculus that integrates deeply held beliefs (worldviews or values), social relationships (including trust in key actors), and past knowledge or experience. Effective science communication builds upon a framework that describes and explains how and why citizens feel the way they do, and meaningful ways to engage all parties (e.g., scientists, policymakers, and citizens) on topics that are important. Understanding this framework and using appropriate engagement mechanisms will provide tools to more effectively communicate the benefits of using microbes to improve crops and better feed our world.

Importantly, research has consistently shown that public acceptance or rejection of a technology is not necessarily because of a lack of knowledge about that technology (Allum et al. 2008); public opinion on such issues can be based upon a range of factors, especially trust and values (Priest, Bonfadelli, and Rusanen 2003). Even the evaluation of how risky or beneficial people see a technology can be tightly related to the ties that person has to others in their family, among friends, and in their broader community (Binder et al. 2011). Public perception is important because it can significantly impact public policy, especially around

an issue as important and personal as food (Frewer et al. 2011), which then translates to the availability of that new technology to farmers and, depending on the technology in question, to eaters as well.

Given the many challenges that confront the widespread adoption of a technology, even one that scientific evidence has found to be safe, it seems fair to ask how best to communicate the potential risks and benefits to the public. Why are some new technologies adopted without much fuss, for example rhizobia inoculants and hybrid seeds, while others, including genetic manipulation of crops, meet significant challenges in the realm of public opinion? The answer to this question is complicated, and much of the scholarship in risk communication tackles different possible answers (Cho, Reimer, and McComas 2014).

One area of research that can inform such questions is the study of public engagement in science and technology, which outlines how communication can and should unfold surrounding a technology. According to this perspective, there are three main types of public engagement activities (Rowe and Frewer 2005). First, public communication encompasses the typical approaches to the communication of science, where researchers: take on the role of expert, identify information they believe to be relevant to a target audience, decide how to present that information, and attempt to deliver it to the audience. Second, public consultation often takes the form of surveying individuals' knowledge, attitudes, and behaviors in an effort to inform problem formulation for research. When carried out with appropriate methodological care, public consultation can provide sound insights into the research process, particularly when executed during the problem-formulation stage. Third, public participation occurs when researchers and non-researchers share an equitable stake in the decision-making process. Whereas the first two forms of engagement have single-direction flows of information, this third form takes on an explicitly bidirectional information flow. The advantage of a participatory framework on a decision-making process is that is more transparent and agreeable for all relevant

stakeholders (Rowe and Frewer 2005). Which of the three approaches is best depends on the circumstances, and while none guarantees a positive *outcome* (i.e., general acceptance) each represents a different way of ensuring a positive *process* (i.e., the opportunity for various parties voice their opinions and hear from others in an open communication environment). Full public participation is often viewed as the option with the highest risk as well as the highest reward.

By communicating directly with growers, buyers, and consumers, it is possible to accelerate the adoption process of new technologies. For example, Indigo is a leading ag-biotech company focused on improving crop yields for growers and buyers and provides tailored recommendations and solutions for each stakeholder. Indigo uses catch phrases such as “food whose origin you trust” when communicating to consumers and “rethinking ag from soil to sale” when interacting with growers. Adoption is further bolstered by highlighting user testimonials and success stories. In addition, Indigo provides the tools and services to manage market value and volatility to ensure that crops receive the best pricing and value. Indigo is just one example of the types of effective communication strategies that can be employed to lure both growers and buyers to their products.

Another facet of positive science communication is through University Extension services. These services bring an increased focus on the microbiome and microbial products in agricultural settings. This allows many universities to increase the positive and effective communication of the benefits of microbiome-based products to a range of stakeholders.

ADVANCING MICROBIOME RESEARCH AND ITS APPLICATION REQUIRES EDUCATION AND PARTNERSHIPS

The dynamic interactions occurring among plants and microbes can be viewed as a human engineered system where carefully selected plants with specific genes interact with soil—the most complex material on earth (Masoom

et al. 2016)—under increasingly intricate physical and chemical management, all intersecting with the microbial communities associated with the soil and the plants growing in it. The goal is to harness the microbiome to maximize yields while reducing use of water and chemicals in a holistic approach that views the whole phytobiome system through the perspective of multiple disciplines simultaneously (Kropff et al. 2001).

Understanding these systems and eventually using them to improve agricultural performance requires a broad understanding of the phytobiome. The current generation of researchers is rapidly adjusting the multidisciplinary challenges in the plant-microbe-environment field. However, in order to successfully develop the non-traditional research framework necessary to make advances in this field, existing university infrastructure and university partnerships with industry and government must continue to adapt. More importantly, the training of the next generation of scientists must focus on fostering interdisciplinary perspectives and collaborative team research to understand the complexity of the agricultural microbiome.

For example, to understand how water perturbations like drought or flooding ultimately impact crop yield, it is necessary to understand how too little or too much water affects soil chemistry, soil aeration, soil microbial community composition and function, nutrient availability, plant physiology, plant-microbe interactions, plant growth and development, and crop yield and quality. With a sufficiently detailed understanding of the dynamic interactions among all of the components of a phytobiome, it will be possible to increase a crop plant's ability to tolerate or minimize the stress induced by these environmental perturbations. Furthermore, translating all of this knowledge into mathematical models could provide the ability to predict the negative impacts of flood/drought and the positive impacts of microbiome-based solutions that may be applied. If this all sounds like a lot to expect one researcher to handle, it is. However, while one person cannot be an expert in all relevant fields, researchers must be well-versed enough in multiple disciplines to effectively communicate

with scientists in disparate fields to plan experiments, analyze data, and develop applications collaboratively.

Researchers in a variety of settings can take concrete steps to promote interdisciplinary projects, in addition to generally supporting research on agriculturally relevant microbiomes. For example, steps for universities can be: recognition for promotion and tenure for faculty engaging in multidisciplinary research, establishing collaborative research centers, providing cross-disciplinary seed grants, and engaging in “cluster hires” of faculty that cross departmental boundaries but are linked to a specific complex research area (Harris 2010; Holley 2009; Sá 2008). Similar standards and incentives should be implemented for graduate students. As the range of disciplines required to holistically address problems of the agricultural microbiome continues to increase (Textbox 1), efforts to hire, reward, and promote collaboration between faculty in disparate fields will become even more essential.

Support New Models for Training Young Scientists

Universities need to train students to conduct interdisciplinary research. Employers in the agrosocieties consistently indicate that broad knowledge and interdisciplinary training (especially social science training) are needed but often lacking in new graduates (Scanlon, Bruening, and Cordero 1996; Schneider et al. 2005). Institutional support for the training needed may range from relaxing traditional, strict, discipline-based course credit structures (e.g. giving computer science, social science, or and especially traditional biology majors credit for taking agriculture courses) to supporting the creation of completely new courses in cross-disciplinary subjects (e.g. multidisciplinary communication and experimental design or microbial product impacts on farmer economics). A survey of U.S. universities showed that many food-related interdisciplinary programs exist, but most were rooted in a single discipline and lacked true educational integration, such as interdisciplinary courses (Hartle et al. 2017). In general, few existing models truly present interdisciplinary

Textbox 1.

A breadth of research expertise is needed to attack modern agricultural problems, such as those presented by the phytobiome. Students must be trained to collaborate with scientists from innumerable fields of science to lead the next era of transformative research (Duckworth et al. 2017).

Analytical Chemistry	Environmental Science
Bioinformatics/Statistics	Mathematics/Modeling
Communications	Microbiology
Economics	Plant Biology
Engineering	Soil Science

curricula that stress multidisciplinary perceptive and team research, further hindering education (Duckworth et al. 2017; Lunde et al. 1995; Miller 2016).

To take graduate student training to the next level, a new paradigm is need. This new cohort model as described by Duckworth and colleagues (Figure 7D in Textbox 2) takes the interdisciplinary committee model a step further and requires a team of faculty to collaboratively train and mentor cohort-based teams of graduate students from different disciplines. These cohorts would design experiments so that research groups from multiple disciplines would gather data from each experiment. For example, plants from a greenhouse experiment could be analyzed by a plant scientist, soil scientist, microbial ecologist, and environmental scientist. A mathematician could incorporate these data into robust models, and economists could predict appropriate land management applications. The resulting team of graduate researchers could communicate across disciplines and generate theses that are distinct parts of a much greater whole.

Funding, predominantly from federal sources, will ultimately drive changes in graduate educational models. The recent emphasis by National Science Foundation (NSF) and National Academies of Sciences on “Convergence Research”, which is defined as problem driven and transdisciplinary, interdisciplinary, and

multidisciplinary, is recognition of the realigned science funding geared at societal problems, including those of food and agriculture (NASEM 2019). However, to meaningfully facilitate interdisciplinary education, joint funding between agencies, such as the NSF and the USDA, is essential. The NSF could strive for more collaboration across directorates (such as engineering, geosciences, biology, physical sciences, and education), and interdisciplinary “training grants” must be created to support students pursuing these studies (Van Hartesvelt and Giordan 2008). Industry partnerships supporting education could be encouraged by innovative programs such as the NSF’s new Non-Academic Research Internships for Graduate Students (INTERN) Supplemental Funding Opportunity. Interestingly, these partnerships are more common in Europe than in the United States (Spiertz and Kropff 2011); their expansion in the United States may improve education, research, and employment outcomes here.

Increase Crop-Microbiome Focused Academic and Ag-biotech Industry Partnerships

The previous discussion the challenges in getting microbial products from lab research to marketable products highlights why it is important for academia and industry to form partnerships to advance

Textbox 2.

To illustrate how graduate-training models can impact inter- and multi-disciplinary research, imagine a graduate student, Emma, who is studying the interactions between a specific beneficial microbe and a crop plant. Under the traditional single-investigator model, Emma would have a primary advisor whose expertise aligns with her specific research interest (Figure 7A) and a committee that would include faculty from outside the primary department, but may or may not be from different disciplines. She would receive her degree from a single department, and largely be exposed only to classes and expertise housed in that department. Her resulting dissertation would illustrate a very deep, specific expertise within a single field of research.

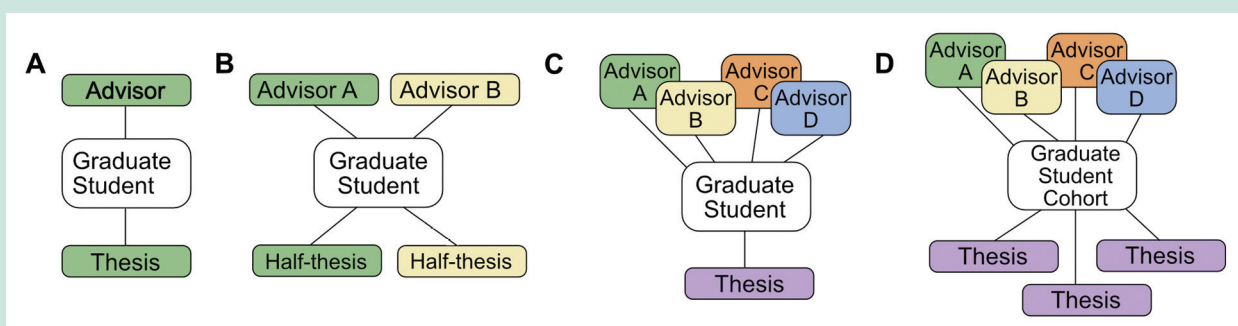


Figure 7. Schematics describing the (A) traditional, (B) multi-disciplinary, (C) interdisciplinary committee, and (D) interdisciplinary cohort models of graduate student training.

In comparison, Emma could be trained using an interdisciplinary model of graduate student education. Two such interdisciplinary models include a co-advisor scenario (Figure 7B) and an interdisciplinary committee model (Figure 7C). Under the co-advisor model, Emma would have two primary advisors, potentially one in microbiology and one in plant science. This could give Emma access to the courses and expertise of two departments, as well as provide a broader network for her to gain broad expertise in two fields. The downside of this model is that both advisors may retain single-investigator mindsets, thus mentoring Emma using two separate approaches to the same research problem and thus placing her in a position of doing more or conflicting work trying to please both advisors. The third model (Figure 7C) requires more collaborative work for faculty because they need to function as a single, interdisciplinary advising entity but provides a more cohesive interdisciplinary research experience for Emma; in this case Emma's thesis exploring microbe-plant interaction might take into account factors outside of any one advisor's expertise, such as microbial diversity and soil physical and chemical parameters.

microbiome- and phytobiome-based solutions for agriculture. Academic researchers can make discoveries that have potential to become industrial products, or make advances that provide more detailed insight into existing products (see Case Study inset on page 18); however, these discoveries alone are not sufficient to create marketable products. Most academic laboratories are not equipped to scale-up potential microbial products or to develop formulations that are practical, stable, and easy for growers to use. Taking a successful laboratory-based idea through the necessary large-scale field trials, market research, regulatory hurdles, and the challenges of production typi-

cally would require a partnership with a company. In addition, research supported by industry has a greater likelihood of being translated into a patent, license, or commercial product than research funded solely through federal funds (Wright, Drivas, and Merrill 2014). These partnerships provide a mechanism for the translation of important academic microbial or plant discoveries into tangible solutions with the potential to impact modern agriculture.

The complementary skill sets and perspectives that are required for and fostered by these relationships have a powerful potential to accelerate the knowledge gained from such collabora-

tions. While there are a number of real and perceived challenges to fostering effective microbiome focused academic-industry partnerships, these obstacles are not insurmountable. For each potential stumbling block, there are a number of solutions that can be implemented to smooth the way to successful research endeavors. These concerns include issues regarding disclosure of research, specific objectives, time scales, and IP.

Constraints on disclosure of research results are necessary to protect potential IP at both the industry and university levels. However, presenting and publishing the results of research are both significant academic career metrics, and restrictions

and delays of these activities can have a negative impact on faculty tenure and promotion or on student graduation. Although industry-academic research may result in a patent, patents often carry little weight in academic evaluations (Zappe 2013). University technology transfer offices can function as a critical bridge between industry and academics in this realm (Debackere and Veugelers 2005; Perkmann and Walsh 2007; Reingand 2011). Industry direct-sponsored projects are negotiated directly with the university, either for each project or as part of larger master research agreements, and this provides consistency across multiple projects by streamlining contract negotiation (Taylor 2018). Another option is a pre-competitive research consortium, where the IP concerns are spelled out in the by-laws, with a format that encourages researchers to explore new areas, test high-risk ideas, and develop methods and tools (Boardman et al. 2013; Rivers 2009). Regardless of the partnership type and specific rules, it is important to keep in mind that a patent is not the same as—nor does it guarantee—a product, and the risks and expense of taking a technology to fruition often fall to industry (Lutchen 2018; Zappe 2013).

Incentive structures and time-scales

Incentive structures need to be aligned with both academic and industry scientist priorities (Perkmann and Walsh 2007). A critical incentive for academics is to have industry research and patents sufficiently credited and valued in tenure promotion guidelines, graduate student dissertation requirements, and other academic evaluations (Debackere and Veugelers 2005; Zappe 2013). Industry internships and fellowships that allow graduate students to gain experience in industry labs during their graduate work (Taylor 2018) can, simultaneously, further graduate training, increase graduate employment, reduce hiring costs for industry, and result in scientists who can bridge the two research cultures. For example, having first-hand experience with the challenges of scaling up production and formulation of a microbial product can provide an invaluable reference framework for evaluating future microbial discoveries, regardless of what

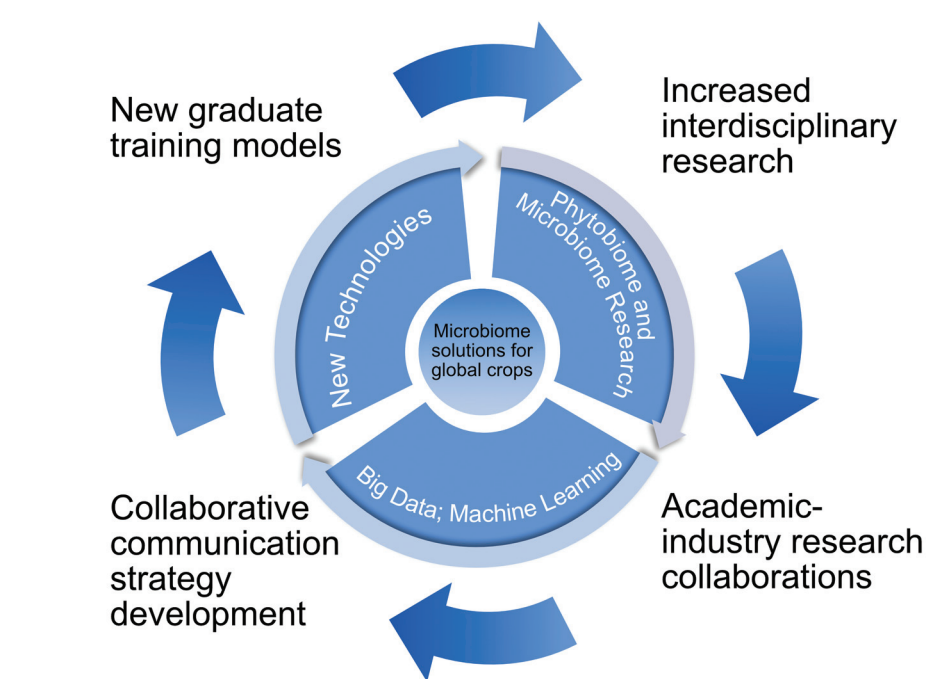


Figure 8. Developing and implementing microbiome solutions to solve the problems surrounding feeding an increasing population with fewer land and input resources will require an integrated approach that encompasses training and education, interdisciplinary research, research collaborations, and collaborative communication. Each of these components drives and improves the others, providing advances in technology, data analysis, and research that can synergistically create microbiome solutions for agriculture.

career path the researcher ultimately pursues. Frequent communication and interactions may result in synergistic research outcomes; realization of value of existing research or tools being developed through the course of academic research (Carbone et al. 2017, Carbone et al. 2019) broader training for students, post-docs, and early career researchers; and organic networking opportunities for young scientists and potential industry employers. In addition, many successful collaborations provide opportunities for students, postdocs, and young faculty to take on leadership roles and obtain valuable project management training that can be applied to future activities in academia or industry. To create a successful collaboration, it is important to connect early, understand and respect different research cultures, find a middle ground when it comes to publishing, determine IP rights and value fairly and transparently, and above all, communicate clearly and frequently (Taylor 2018). These strong partnerships enable

synergistic science that leads to useful, marketable products that improve lives around the globe.

HARNESS THE POWER OF MICROBES NOW WITH INVESTMENTS IN INTERDISCIPLINARY EDUCATION, RESEARCH, AND TECHNOLOGIES

Current population growth, land use, and environmental and management challenges combine to make increasing crop yields a priority for society. The past 20 years have shown a significant shift in the adoption of biotech crops, with subsequent benefits to plant productivity (James 2014). Technologies that use microbes either as a biological treatment or for trait modification can increase yields and farmer profits but also reduce traditional pesticide use and enable the

better use of limited environmental resources. Recent major technological advances now make it possible to tackle the immensity of the phytobiome, leading to the potential for microbiomes to be a solution to improving crop health and yields. Microbiomes and their interactions with crops and the environment are multifaceted, but more knowledge about them has already reduced risks of pest and pathogens for farmers. Further research will continue to yield benefits. We are now capable of generating vast amounts of data from plants, microbes, and soils. New technologies will additionally enable scientists to see not just which microbes are present in soils, but also what their functional potential is, maximizing the possibility of meaningful interventions and successful products. Developing “biologically smart” machine learning methods will help us to unravel the critical plant-microbe interactions that provide plants with protection from pathogens and abiotic stressors and increase crop yields in the face of environmental perturbations.

It will take interdisciplinary training, collaborative team structures, and a full range of advancing technologies to match the complexity of microbiomes and the phytobiome (Figure 8). Only by understanding the dynamic and synergistic effects of plant and soil microbiomes can we use beneficial microbes to break through the current crop-yield plateau. To ensure success, it is imperative to increase training, incentives, and support for interdisciplinary research and academic-industry partnerships in order to create a multi-faceted path forward. Interdisciplinary research is key, and we won’t succeed without it. The research field is now uniquely poised to combine these new scientific and analytic technologies with insights stemming from a mix of academic, federal, and industry personnel to generate tangible management solutions for crop growth. Finally, scientific innovation can create a beneficial product, but it requires the multi-disciplinary efforts of social scientists and policy-makers to ensure public engagement, adoption of new technologies by growers, and effective and safe product regulation.

TASK FORCE RECOMMENDATIONS

- Commit resources to understanding the dynamic complexity of the phytobiome, through both research funding and infrastructure development.
 - Building infrastructure to support research into the phytobiome is necessary to provide the shared analytical instrumentation, interdisciplinary center programs, and new cross-disciplinary educational courses that will aid the field in moving forward.
- Fund the development and application of cutting edge technologies (machine learning, precision agriculture, sensors, etc.) relevant to agricultural microbiome research
 - Often, funding this type of research requires cooperation between different directorates within a single funding agency or even multiple agencies to bring together the computer scientists, engineers, plant biologists, soil scientists, crop scientists, and microbiologists that are needed to create the technologies required to advance agriculture.
- Create initiatives to bring scientists, social scientists, and stakeholders together to develop strategies and best practices for effective science communication, public engagement, product regulation, and policy development for microbiome-based solutions.
 - Cutting-edge science alone is not sufficient to create global solutions. Input from social scientists and stakeholders is required to generate effective practices to disseminate information about microbiome-based products to growers, regulators, policy-makers, and consumers.
- Increase funding and incentives for interdisciplinary research, education, and training in the agricultural and microbiome fields.
 - Ultimately, funding drives research, and it is critical that diverse funding sources (federal, private, non-profit, internal university) provide incentive to develop truly interdisciplinary research programs, specifically

in the fields that impact agricultural technologies.

- Encourage new models for training graduate students to increase cross-disciplinary literacy, ability to work as part of a team, and communication skills.
 - In order to tackle the immense complexity of the phytobiome, scientists from diverse disciplines need to be able to communicate with each other, design and interpret research as a team, and then communicate the impact of their results to a broader audience. Programs and funding that creates both the requirement and opportunity for graduate students to develop these skills in addition to their specific expertise are needed.
- Encourage crop microbe focused industry-academic partnerships and provide programs and funds to help bridge the gap between promising laboratory results and successful field trials.
 - Partnerships between academia and industry provide the opportunity to explore the agricultural potential of a broader range of microbiome research, increasing the probability of finding viable solutions. Programs specifically aimed at developing these partnerships in the agriculture arena can reduce barriers, bridge research gaps, and create lasting collaborations to produce effective, and safe microbe-based solutions to feeding the world.

CASE STUDY:

BASIC FUNGAL BIOLOGY RESEARCH PROVIDES A WAY TO IMPROVE EXISTING BIOCONTROL METHODS

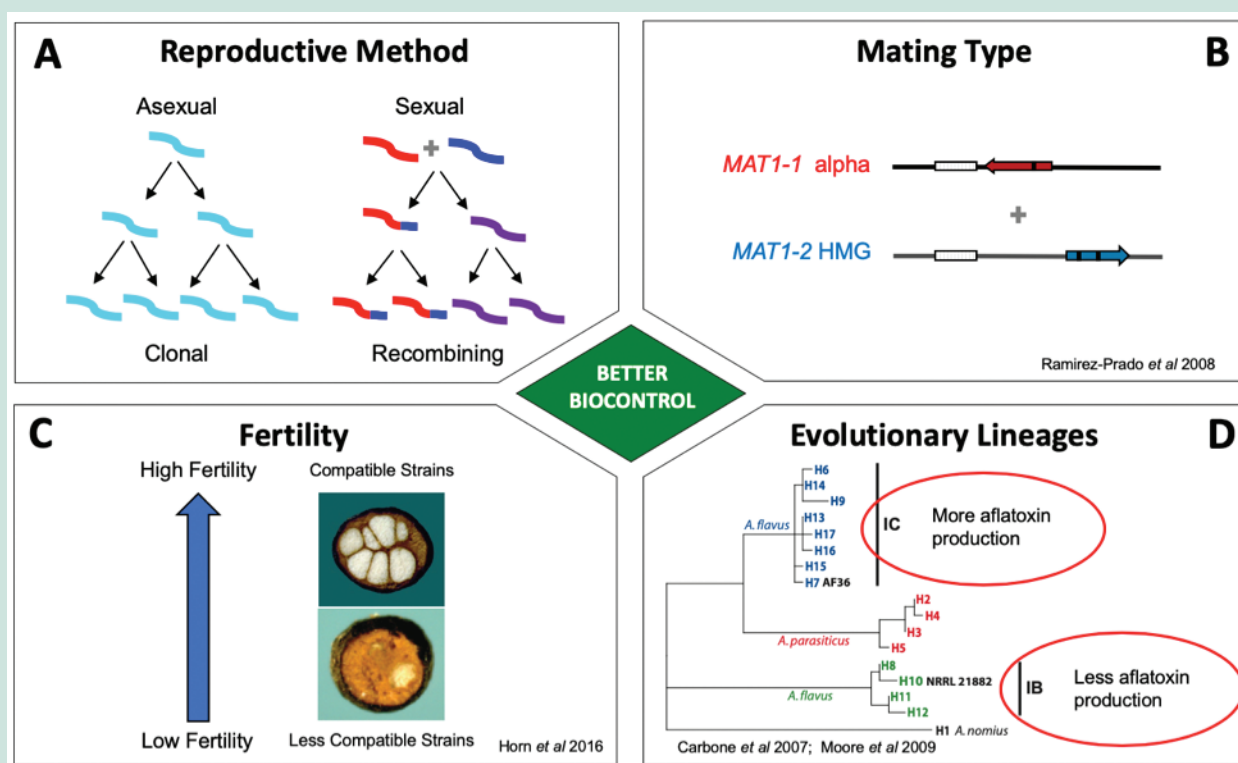
Disease-management practices have traditionally focused on breeding crops for resistance to pathogens and pests. Very little attention has been given to microbes as a way to keep pests in check and to reduce toxin contamination. Biological control (or biocontrol) is an effective strategy for controlling pests through the release of natural biological agents that compete with targeted pathogens and reduce their population size (van Lenteren 2012; van Lenteren et al. 2018). This can result in the mitigation of disease and mycotoxins (toxins produced by fungi) (CAST 2003; Kagot et al. 2019), increased plant yields (Molo et al. 2019), and improved food safety (Bale, van Lenteren, and Bigler 2008). However, developing a successful biocontrol agent can be quite challenging. First, an organism that has the ability to safely control pathogen populations in some way must be identified, either through laboratory studies or field observations. Next, this organism must be tested in the laboratory, greenhouses, and field trials to ascertain how effective it is in controlling pathogens under different environmental conditions, in different geographies, and with different crops. Ultimately, the success of a biocontrol agent requires intimate knowledge of the problem microbe as well as the biocontrol agent that will be used.

Aflatoxins are carcinogenic mycotoxins produced by certain fungi belonging to *Aspergillus* section *Flavi* (*Aspergillus flavus* and *A. parasiticus*) that contaminate maize and other oil-seed crops (Horn

2007), tree nuts (Bayman, Baker, and Mahoney 2002), and spices (Makhlouf et al. 2019). Aflatoxins can also accumulate in the milk of animals that are reared on contaminated feed (Serraino et al. 2019) and adversely affect infants' health and development (Awaisheh et al. 2019). Increasing concentrations of aflatoxin in agricultural commodities result in significant health impacts to humans and animals, and economic losses worldwide (Bennett and Klich 2003; Ojiambo et al. 2018; Wu 2004). In the United States alone, the losses to the corn industry due to aflatoxins can exceed \$1 billion in years where environmental conditions (high soil temperature, drought) are favorable for aflatoxin production (Mitchell et al. 2016). The amount of aflatoxin produced by different, but closely related, strains of *A. flavus* fungi is highly variable (Moore et al. 2013; Moore et al. 2017; Olarte et al. 2012; Olarte et al. 2015;) and not all *A. flavus* strains make the toxin (Carbone et al. 2007b; Moore et al. 2013; Moore et al. 2017). The strains that do not make the toxin are key to current biological control strategies in the continental United States that mitigate the impact of the pathogen. These biocontrol methods apply the EPA-approved non-aflatoxigenic *A. flavus* strains, AF36 (*A. flavus* strain NRRL 18543) and AFLA-GUARD® (strain NRRL 21882), to transiently increase the proportion of the *A. flavus* population that does not make the toxin, thus decreasing the overall amount of aflatoxin present in the field during that season (Cotty 1990; Dorner 2004; Dorner 2005). This strategy has proven to be very effective in reducing aflatoxin con-

tamination and has been successfully deployed with non-aflatoxin producing fungal strains in maize-production regions of Italy (Mauro et al. 2018), Africa (Atehnkeng et al. 2016; Bandyopadhyay et al. 2016), China (Zhou et al. 2015), Thailand (Pitt et al. 2015) and Argentina (Alaniz Zanon, Barros, and Chulze 2016; Camiletti et al. 2018). Biocontrol using atoxigenic *A. flavus* strains is effective not only in the short term (i.e., a single growing season) but in reducing in aflatoxin levels over to multiple years (Cotty 2006). However, there is an opportunity to improve on the persistence of these strains in fields to provide controlled reductions in aflatoxin levels by selecting strains that work in concert with the reproductive and mating biology of this fungus.

This means that researchers need to understand the reproductive and mating biology of *A. flavus* in order to use these characteristics to improve existing biocontrol methods. For example, until quite recently, *A. flavus* was assumed to reproduce only clonally (asexually). This meant that any *A. flavus* biocontrol applied to a field would not be able to mate with other *A. flavus* present in the soil, and would only make exact copies of itself in any subsequent generations. However, in 2009, the discovery of sexual reproduction in *A. flavus* (Horn, Moore, and Carbone 2009) meant that this assumption was not valid and that, in fact, there was potential for the applied biocontrol to mate with native strains in the field. This discovery provided new perspectives on how the genetics and genomic composition of this fungus can influence its aflatoxin-producing



A population genetics approach to biological control of mycotoxin production that incorporates different components of *Aspergillus flavus* biology: (A) reproductive method, (B) mating type, (C) fertility, and (D) evolutionary lineage.

potential (Box 1). This also suggests that by introducing atoxigenic biocontrol strains that are able to mate with the native population, the sexual biology of *A. flavus* can be used to reduce the overall population's toxin production.

Researchers can now begin to better understand how different biocontrol strains may persist in fields, and thus consistently reduce fungal toxin levels over multiple seasons, by understanding key aspects of fungal biology. These areas include population genetics (i.e., how genetic variation within populations changes over space and time [e.g. see Lewis et al 2019]) and reproductive methods, mating types, and fertility (Box 1). Using fundamental science to assess the underlying genetic, fertility,

and reproductive characteristics that play a role in governing a particular *A. flavus* strain's potential to make or not make aflatoxin, as well as that strain's ability to create progeny, improves our ability to create biocontrol strategies that will persist and reduce toxin contamination in crops. This basic research will also allow us to evaluate the potential role aflatoxin may play in fungal fitness and assess potential ecological effects of reducing aflatoxin production in a population (Carbone et al. 2007a; Drott et al. 2017; Moore et al. 2009).

This biocontrol example illustrates how we can use basic knowledge of fungal biology to better design biocontrol agents. This is also an example of how focusing on only one aspect of the microbiome rather than

the microbiome as a whole can still lead to important advances. More research needs to be done to assess how these biocontrol strains impact the overall function of the phytobiome. However, even once we have a thorough understanding of both the problem and the solution, there are still more challenges to overcome to create microbial formulations that are easily used by growers, can successfully navigate the regulatory system, can be scaled up in production, and actually be sold as a product. Translating this exciting new research into a marketable product often takes partnerships that connect academic and industry scientists to tackle many facets of biocontrol development.

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