EBRC Engineering Biology Research Consortium

Microbiome Engineering

A Research Roadmap for the Next-Generation Bioeconomy

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Overview

The EBRC roadmap *Microbiome Engineering: A Research Roadmap for the Next-Generation Bioeconomy* is a critical assessment of the current state of microbiome engineering and areas of anticipated research and development in the next twenty years. It also details how those scientific advancements can be applied across different industry sectors. This roadmap aims to serve as a "go-to" resource for scientists working to engineer microbiomes, as well as policymakers interested in advancing microbiome engineering. *Microbiome Engineering* examines technical progress needed to advance microbiome engineering (Technical Themes) and details how these advances can be used to develop engineered microbiomes for specific applications (Application Sectors).

The roadmap is comprised of three Technical Themes: Spatiotemporal Control, Functional Biodiversity, and Distributed Metabolism (Figure 1). Spatiotemporal Control considers how to engineer microbiomes for precise, predictable position and function in space and over time. Functional Biodiversity discusses how to engineer microbiomes based on functionally similar, but taxonomically diverse, collections of organisms to increase the interoperability of engineered microbiomes in different environments. Finally, Distributed Metabolism focuses on designing microbiomes that leverage the unique metabolic capabilities of individual microbial species or taxa to collectively produce and/or degrade specific compounds.

Each of the three Technical Themes is built around Breakthrough Capabilities that will significantly advance microbiome engineering. The Breakthrough Capabilities are further divided into a stepwise path of research milestones anticipated to be reached by 2022, 2025, 2030, and 2040 (2-, 5-, 10-, and 20-years into the future, respectively). The shorter-term milestones for 2022 and 2025 are intended to be achievable with existing programs, infrastructure, and facilities. The longer-term 2030 and 2040 milestones may require significant investments, technically, financially, or otherwise, but will substantially advance microbiome engineering.

During the roadmapping process we identified several technical challenges that were broadly applicable and would be necessary for advancing all three Technical Themes. These challenges were captured under Transformative Tools & Technologies and will be some of the most critical areas of research for advancing microbiome engineering. In particular, substantial efforts will be needed to help advance microbiome models, cellular signaling and communication, and computational methods for predicting microbiome design, growth, and function.

The Application Sectors are derived from *Engineering Biology: A Research Roadmap for the Next Generation Bioeconomy* (Engineering Biology Research Consortium, 2019): Industrial Biotechnology, Health & Medicine, Food & Agriculture, Environmental Biotechnology, and Energy. Within each Sector we examine how advancements in microbiome engineering can help address several broad societal challenges. The societal challenges in *Microbiome Engineering* partially overlap with those in *Engineering Biology*, but some were revised to more effectively capture microbiome engineering research. Specific technical achievements are identified that would contribute to solving these societal challenges, demonstrating the diverse potential applications of microbiome engineering. Individual species of engineered microbes have been used in many of these sectors already, but developing microbiomes for similar

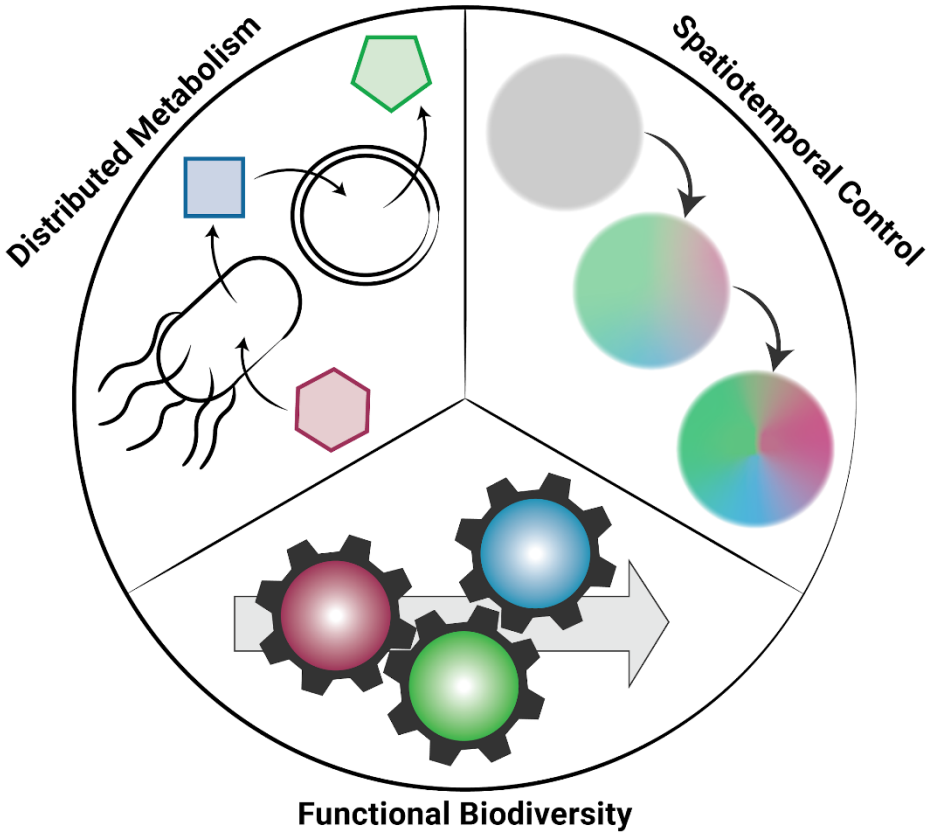


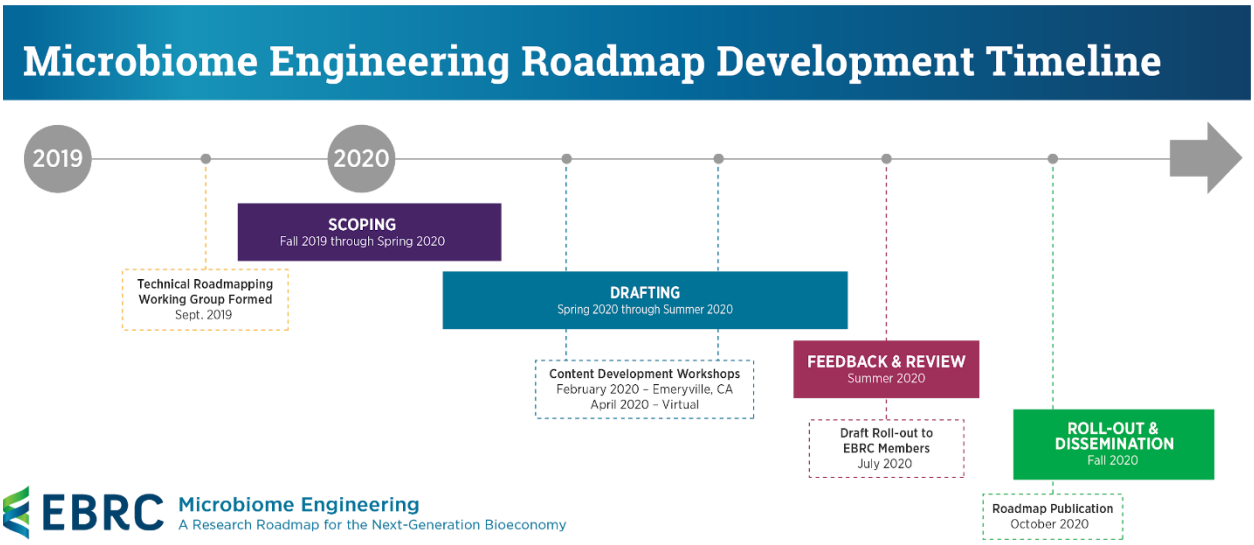
Figure 1. Technical Themes *for Microbiome Engineering*. The roadmap examines the future of microbiome engineering in the context of three different Technical Themes. Each Theme addresses a different, broad aspect of microbiome engineering that will need to be advanced to fully realize the potential of engineered microbiomes. Spatiotemporal Control discusses the advancements needed to control where cells and species localize in a microbiome over time, Functional Biodiversity explores how functional redundancy can be engineered using diverse, genetically unrelated organisms to contribute greater stability in the microbiome as conditions change, and Distributed Metabolism details how to engineer chemical transformations across multiple organisms in a microbiome.

applications will help decrease costs, expand functionality, and enable greater accessibility and usability. In some cases, microbiome engineering will also enable completely new functions that are not feasible with a single microbe. As a result, developments in microbiome engineering will have both short-term impacts on applications of existing technologies and more transformative long-term impacts on humans' interactions with the microbial world.

Topic Selection and Project Development

Microbiome Engineering: A Research Roadmap for the Next-Generation Bioeconomy is a follow-up to the 2019 EBRC Roadmap *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy*. It further elaborates on the role of engineering biology for microbial biomes and consortia. The topic was covered briefly in *Engineering Biology*, but many members of the engineering biology community recognized that microbiome engineering presents unique technical challenges and potential applications to warrant a more in-depth examination.

Over the course of late 2019 and the first half of 2020, the EBRC Technical Roadmapping Working Group helped develop the framework and content of this roadmap. Our work over conference calls was intended to be supplemented by several in-person workshops, but in the wake of the SARS-CoV-2/COVID-19 pandemic we were forced to adjust and adapt. Instead, this roadmap is the product of a two-day writing workshop, supplemented with countless Zoom calls and Slack messages during the spring and summer of 2020. Over forty faculty, industry experts, postdoctoral scholars, and graduate students all contributed their expertise to this roadmap, and this roadmap would not have been possible without them. We intend for this roadmap to provide a comprehensive look into the current state of microbiome engineering and help set long-term goals to advance the field.



Introduction

Microbiomes, or communities of diverse microbes, are ubiquitous in nature and can be found on or in nearly every environment on Earth. Thousands of species of microbes live in the human gut where they help break down food, help train our immune system, and contribute to health in ways that are still not fully understood. They form communities everywhere humans have looked, from hot springs, to glaciers, to the deep ocean. Studies on microbiomes have been ongoing for the past two decades as their impacts on the world have been recognized. Large scientific initiatives like the Human Microbiome Project (The NIH HMP Working Group et al., 2009) sought to document the diversity of human microbiomes, leveraging new advances in DNA sequencing to observe how the microbiome changes from person-to-person and from location-to-location on the body (Proctor, 2011; Turnbaugh et al., 2007). However, even though microbiomes are now recognized to have important functions in many different environments (Eisenstein, 2020; Jansson & Hofmockel, 2018), most research on microbiomes to date has been limited to correlative conclusions rather than mechanistic insights.

Advancing past correlations to a point where different genetic, proteomic, or metabolic processes can be intentionally altered to induce specific microbiome functions will require more fine-tuned control over a microbiome. Over the past decade, scientists have built some tools to facilitate the creation of artificial microbiomes or to help manipulate natural microbiomes (Foo et al., 2017; Pham et al., 2017). As these tools become more reliable, more specific, and higher-throughput, they enable a more mechanistic understanding of natural microbiome function, and make engineering new functions a feasible goal. However, continued advancements will still be needed, from additional tools like programmable bacteriocins and antimicrobial peptides (Inda et al., 2019), to greater coordination of frameworks used to study microbiomes (Lawson et al., 2019). *Microbiome Engineering* aims to provide a forward-looking examination of both the short- and long-term needs for microbiome engineering, to help scientists and policymakers fully utilize microbiome engineering.

Linking to Societal Considerations and Integrating Public Values in Engineering Microbiomes

The challenges and achievements presented in EBRC's roadmaps do not occur in a vacuum. Advancing engineering biology will require significant parallel, non-technical efforts to address societal, legal, and regulatory challenges. In this section, we examine some of the broader considerations that need to be addressed to ensure that new technologies in microbiome engineering work in the best interests of society.

Regulatory Challenges for Microbiome Engineering

A major hurdle in microbiome engineering will be the regulatory barriers that prevent engineered organisms from being used outside the lab - as therapeutics and diagnostics, or in open environments like agricultural tracts. Many intersecting factors contribute to the difficulties that will need to be overcome if engineered microbiomes, or methods for engineering native microbiomes, are to see wider adoption. At a regulatory level in the U.S., multiple federal- and state-level agencies may have jurisdiction over a genetically modified organism, depending on how it is created and how it is used. At the moment, the United States Department of Agriculture (USDA), Food and Drug Administration (FDA), and Environmental Protection Agency (EPA) may all have regulatory authority over an engineered organism (Committee on Future Biotechnology Products and Opportunities to Enhance Capabilities of the Biotechnology Regulatory System et al., 2017). If the same organism is to be used outside the U.S., then additional sets of guidelines need to be followed to become compliant with local regulations. Policymakers and regulators should take an active role to help scientists and biotechnology companies navigate these frameworks and streamline the processes used to vet and approve new technologies.

Microbiome engineering will also require significant collaboration between government, industry, and academia to share risks at multiple steps in the research and development process. Engineered microbiomes are a high-risk area for biotech companies due to additional regulatory hurdles and additional possible points of technical failure. Additionally, there are still many unknown aspects of microbial ecology that make it difficult to predict the impact of adding an engineered organism to a natural environment. Increased collaboration between government, industry, and academia can encourage communication throughout the research and development process so expectations of potential hurdles, such as regulatory requirements, are understood well before any technology comes to market. As microbiome engineering research advances it will be important that sufficient controls are put into place to ensure safety, without creating so many legal and regulatory hurdles that companies are discouraged from developing high-risk, high-reward technologies.

Policies to Support Microbiome Engineering

Even though costs associated with microbiome engineering will decrease on a per-replicate basis, it is important to recognize that the total costs of an experiment may actually increase over time. Comprehensive multi-omic studies will require a sufficient number of samples to assess microbiome dynamics, and a sufficient number of replicates to have practical statistical power. Additionally, a significant technical challenge is improving data reproducibility,

and in particular, better translating data obtained in controlled environments with expected outcomes in natural microbiomes. While scientists have an interest in advancing reproducibility for the sake of their research, institutions like the National Institute of Standards and Technology have started getting involved with microbial measurements (NIST Food Safety Working Group, 2020a, 2020b), and should be encouraged to invest further for microbiome engineering technologies.

Addressing these challenges may require funding agencies to shift support towards centralized facilities that have specialized technical expertise and infrastructure for performing such experiments, but work with and serve all microbiome researchers. The Department of Energy's Joint Genome Institute is one possible model for centralized research centers, but such centralized research infrastructure would broadly be a significant departure from current practices in biology; however, it is reminiscent of the national and international collaborations found in experimental physics, where the scale of facilities required to perform experiments necessitates coordination between many organizations.

Security Considerations of Microbial Misuse

Microbiome engineering will pose novel biosecurity risks for the environment, existing natural microbiomes, animals, plants, and humans. Used properly, engineered microbiomes can help address many societal problems identified in our Application Sectors. However, the same technologies that allow microbiomes to enhance agricultural soils or cure diseases as therapeutics, have the potential for abuse. Despite the ubiquitous synthesis and use of engineered DNA, government guidance on the risk assessment of manufactured DNA sequences has lagged behind technological advances, leaving the decision-making process to industry groups or individual labs and companies. Microbiome engineering will be even more challenging, as a microbiome that is deemed safe in one ecosystem under a given set of environmental conditions may function differently or pathologically in a different ecosystem or under different conditions. Therefore, it is of great interest to invest in tools and methods for estimating, anticipating, and monitoring potentially unintended consequences of microbiome engineering on ecosystems and organisms.

Government agencies, funders, researchers, and industry partners will need to work together to solve this challenge. Scientists will need to demonstrate that a microbiome will not cause harm if accidentally or intentionally released into the wrong environment, or that it does not produce harmful or illegal chemicals if fed a different set of chemical precursors. Additionally, even if released in the correct environment, engineered microbiomes must not detrimentally impact the broader ecosystem (*e.g.,* microbiomes that improve a crop, but harm pollinator species). As a result, additional financial and administrative support for field trials (particularly for agricultural microbiomes) will be important to understand how microbiome engineering can go wrong. These studies will not only be important for gaining a better understanding of how engineered microbiomes function, but will also improve their safety and security. Funding agencies should provide monetary support both to develop mitigating technologies and containment approaches as well as to train scientists to reduce the chances that their science could be misused.

Technical Themes

Engineering Microbe-Microbe Interactions

Understanding microbe-microbe interactions is critical to predict and control microbiome function. These interactions can be incredibly rich and encompass physical linkages, metabolite/nutrient exchange, cell-to-cell signalling, electron/cofactor transfer, and secretion of antimicrobial compounds, over both short and long length scales. The ability to control these interactions will be fundamental to advancing microbiome engineering as they help direct when a cell grows or dies, regulate the metabolites or proteins a cell produces, and dictate where a cell colonizes in the environment. However, the nature and existence of these interactions are poorly understood for all but a few pairs of microbes. Fully engineering a microbiome will require controlling the interactions between cells of the same species, and between different microbial species, over long periods of time and distances.

Several broad aspects of engineering biology research will need to be advanced to achieve fully-programmable cellular interactions in an engineered microbiome. First, highly parallelizable or high throughput technologies that can tease out the positive and negative interactions within the thousands of taxons within a microbiome are needed to establish rules for community design. Emerging advances in microfluidics, microwell design, emulsion nano-scale reactions, high resolution imaging, and single cell -omics now promise to interrogate the rich network of interactions that drive community function in thousands of permutations of microbial consortia simultaneously (Hansen et al., 2016; Kehe et al., 2019; Lambert et al., 2017; Massalha et al., 2017; Niepa et al., 2016). These systems must tease out not only binary pairwise interactions but the higher-order emergent interactions of *n*-member microbial communities required for community resilience and function. Quantification of higher-order interactions is a key challenge for microbiome engineering that is largely unresolved.

At their most basic, microbe-microbe interactions allow for information and energy to be transferred from one cell to another. This can be achieved through different means, such as a signaling molecule that is synthesized and secreted by the host organism, or one or more transmembrane proteins or polymers that interact with the same on another cell. However, additional interactions between a microbiome and its environment, competition for nutrients, and signal molecules acting at a distance are further factors that must be considered when engineering cell-to-cell signaling (Fischbach & Segre, 2016; Foo et al., 2017). While achieving functional biodiversity, spatiotemporal control, and distributed metabolism will require advances in cell-to-cell signaling technologies, cell-to-cell signaling plays a unique role in each one. Engineering functionally and taxonomically diverse microbiomes will require developing interspecies signaling networks that have multiple levels of redundancy to be robust over time. Achieving the necessary specificity and stability across species will likely require building novel orthogonal chemical and genetic signaling pathways due to the challenges that have arisen when engineering endogenous pathways (Kapp et al., 2012; Mandell & Kortemme, 2009; Pai et al., 2009; Young et al., 2018). Further work will be needed to understand the role of microbe-microbe communication over long distances, on the range of millimeter distances or more, and how those interactions change over time.

Models for Microbiome Engineering

Fully detailing the mechanisms of microbe-microbe interactions within a microbiome will require significant advances in experimental microbiome models. Current models for microbiome engineering are still in their infancy. As is the case with every model system, researchers are forced to choose between the system's relative complexity and accuracy, versus the ease of use for addressing a scientific question (Chevrette et al., 2019). Coculture systems (*e.g., Bacillus subtilis* and *Streptomyces* spp. to study secondary metabolites) are easy to create and manipulate, but offer relatively little insight into the actual environmental pressures these bacteria might naturally encounter. On the other side, microbiome enrichment methods may be more representative of a microbiome, but offer relatively little opportunity to perturb or manipulate the system (Zegeye et al., 2019). Experimental model development should occur from both directions, such that models currently focusing on molecular mechanisms can increase in complexity to better reflect natural microbiomes, and ecologically complex models can be controlled more easily. Experimental models should also factor in the environmental surroundings of natural microbiomes as they are improved (*e.g.,* replicate the immune system's influence on human, animal, or plant microbiomes). Computational models of increasing accuracy will depend, and be supported by, more complex and controllable experimental models to feed data back into the model, driving future development.

Generally speaking, we need to improve our ability to model and determine the composition and stability of a microbial community over time in a 3D environment (Wu et al., 2019). Without a robust experimental and computational modeling effort, the prediction and control of temporal and spatial interactions in microbial communities will be limited. Several distinct areas of research will need to be advanced to enable improved microbiome model systems. Microbiome measurements and metrology must be improved, methods for manipulating microbes within a microbiome must be developed, and better predictive models must be designed. Improved models will have an important role for both capturing natural interactions and providing a means of designing engineered microbiomes for new functions. Particularly for complex or hard-to-access microbiomes, such as those in the soil or human gut, better models will capture more of their natural complexity, making it easier to engineer microbiomes with a reasonable expectation that they will work once added to a natural environment.

Advances in the three areas detailed below will be critical for realizing the full potential of engineered microbiomes. Increased speed and ease of experimental measurements or manipulations will be the starting point for improved computational models. Those computational models can in turn be used to perform better-informed experiments, and develop predictive frameworks that will be necessary for engineering spatiotemporal dynamics of a microbiome, distributing metabolism between species, and generating the biodiversity needed for engineered microbiomes to function in any desired environment.

Measurements and Data Generation

Several existing technologies will need to be improved or further developed to facilitate experimental models. Not only will -omics techniques (*e.g.,* genomics, transcriptomics, metabolomics, or proteomics) need to continue decreasing in cost to allow for increased temporal resolution, they will need to become more parallelizable. Significant advances in

technologies will be needed, or orthogonal approaches will need to be developed, to measure microbiomes in their native environments, because these measurements are crucial for benchmarking the utility of experimental or computational models against natural microbiomes. Additional novel techniques such as stable isotope probing, non-canonical amino acid tagging, and various meta -omics methods will also contribute to a better understanding of microbiomes (Gebreselassie & Antoniewicz, 2015; Lawson et al., 2019). Simultaneous measurements of parameters such as transcription, translation, and metabolism will be critical for reducing experimental error and generating robust data sets used to build new experimental and computational models.

Furthermore, improved microbiome engineering will require these technologies to develop increased spatial resolution, as the three-dimensional structure of a microbiome (*i.e.,* both the percentages of individual species and their distribution in space) is critical for a microbial community's function. New imaging techniques are beginning to address this challenge by combining microscopy, imaging mass spectrometry, and other techniques, but there are still severe experimental bottlenecks that make it difficult to increase the number of measurements and scale-down measurements to micron-sized voxels.

As the quantity of data generated increases, further challenges arise to ensure that the data can be used to its fullest potential. The concept of FAIR (Findable, Accessible, Interoperable, and Reusable) data should be applied as much as possible, both to existing data sets and any future pieces of scientific infrastructure (Wilkinson et al., 2016). FAIR data will also facilitate faster development of software tools and application of machine learning techniques to advance computational models. In addition to the existing recommendations for FAIR data, it will be valuable to capture the number of samples taken at any given time and the intervals at which they are taken. In combination with further developments in analysis and modeling methods that can accept non-uniform timepoints, these data will allow for richer analysis of microbiomes at specific times when they may be more dynamic.

Model Manipulation

A key aspect of useful experimental models is how easily or quickly they can be manipulated. For microbiome engineering, many novel approaches will need to be developed to control parameters that stretch across scales. At an organism-level, new techniques must be developed to culture, and then genetically or transcriptionally manipulate, a single microbial species growing within a community (Brophy et al., 2018; G. Wang et al., 2019). Alongside this effort is a need to better understand and annotate gene functions across microbiomes, as a functional understanding is key for targeted engineering approaches. At larger scales, advancements will be needed to control intra- and inter-species cell signaling (as discussed in "Engineering Microbe-Microbe Interactions"), leading to altered metabolism, growth, and spatial distributions.

This will also need to be paired with techniques to spatially control microbes within a microbiome. Many existing techniques either create a homogeneous mixture of microbes within the model system, or enrich existing communities with little to no ability to control the locations of the microbial cells that grow up. Ultimately, *a priori* knowledge of microbiome growth will mean that physical manipulation becomes unnecessary, but the process of generating that knowledge will be greatly assisted by improved manipulation techniques.

Computational Approaches

Computational modeling will be a central tool for engineering microbial interactions and predicting the necessary interaction networks within a community. Models will be helpful for determining how many new pathways will need to be added to achieve a desired outcome in a community. New computational advances will also be needed to determine the minimum network needed to execute a community's function. Minimum networks will be crucial for reducing size of engineered pathways, which is itself important for long-term stability and maintenance of function. Generally, we have a poor understanding of how to represent microbial interactions and at what scale of interaction. Thus, the prediction capabilities of microbial models are limited (S. Wang et al., 2019) and under-informed by experimental data measurements. As experimental models advance, more data will become available to help generate better computational models with increased predictive power.

A central challenge will be understanding the relevant principles that govern interactions and growth within multispecies communities. This will require finding the appropriate level of abstraction to deal with the combinatorial complexity of multi-species interactions, using mechanistic-based modeling or machine learning approaches. It will also require improved models that can analyze chemical interactions and transport within a microbiome in four dimensions, and account for additional boundary layer effects where a microbiome interacts with the environment.

Additionally, mathematical frameworks and computational tools will need to be developed to capture the temporal and spatial dynamics of microbial communities at different scales for the purpose of designing their composition. This will require both top-down and bottom-up modeling efforts, because each approach individually suffers from distinct drawbacks. Top-down modeling efforts often capture thousands of species and a very simplified picture of their interactions (*e.g.,* commensalism is captured only by one or two parameters that have little biological meaning since they are aggregates of many biological quantities). In contrast, bottom-up models (Gorochowski et al., 2012; Heirendt et al., 2019; Jang et al., 2012; Klavins, 2014; Matyjaszkiewicz et al., 2017; Oishi & Klavins, 2014) are computationally expensive and the parameters used are rarely measured (Green et al., 2019; Ren & Murray, 2018). Regardless of approach, models must be capable of simulating microbiomes at time-scales that have practical value (*e.g.,* hours to weeks) due to the temporal variations that microbiomes undergo (Bogart et al., 2019; Bucci et al., 2016), which may require increased computational resources or new mathematical frameworks to reduce computational requirements. They also need to capture ecological and evolutionary timescales, and the feedback mechanisms between evolution and ecology that dictate spatial distributions and functions.

As models are developed, it will be important to consider how an output value could be translated back to experimental procedures. Black box models that produce parameters that are unknown, cannot be measured experimentally, or do not correspond to a determinable biochemical quantity, make it challenging to replicate a model's findings at the bench or in a bioreactor. Increased collaborations between computational biologists and experimental biologists, as well as investments in cross-disciplinary training, will help ensure that computational models are built on a foundational knowledge of biology.

Spatiotemporal Control

Engineered microbiomes need to function predictably within space and over time (Cao et al., 2019). This requires new technical capabilities that control microbiome composition, architecture, and spread across diverse length and timescales (Figure 2). These scales can range from micrometers in human, animal, and plant applications to kilometers in agricultural and environmental applications, and from short – seconds to minutes – timescales, to years-long evolutionary timescales. In some cases, engineered microbiomes may need to be robust to dispersion forces, environmental changes, and introgression from wild organisms to resist changes in structure or composition. In other cases, microbiomes may need to be capable of evolving along predefined routes to adapt to their environment. Engineered microbiomes may need to undergo programmed succession or spatial reorganization. This theme examines how these parameters will need to be measured, manipulated, and controlled in order to engineer reliable and predictable microbiomes in the lab and natural environments.

While the two goals separate out spatial and temporal engineering, there will necessarily be significant interplay between the two. Spatially engineered microbiomes will be capable of organizing both in response to the environment and through predetermined spatial arrangements. Additionally, microbiomes will be engineered to interact with and influence the environment around them, through processes such as biofilm formation, to create biotic and cell-free structures. Microbiomes must also be engineered to function over time, particularly in cases when a microbiome's size is not changing or cannot change due to spatial constraints. Engineering microbiomes under such conditions requires understanding the physical, abiotic factors in the environment, as well as tightly regulated mechanisms to control cell growth and death within the microbiome. This, in turn, will help engineer microbiomes that continue to function over long periods of time.

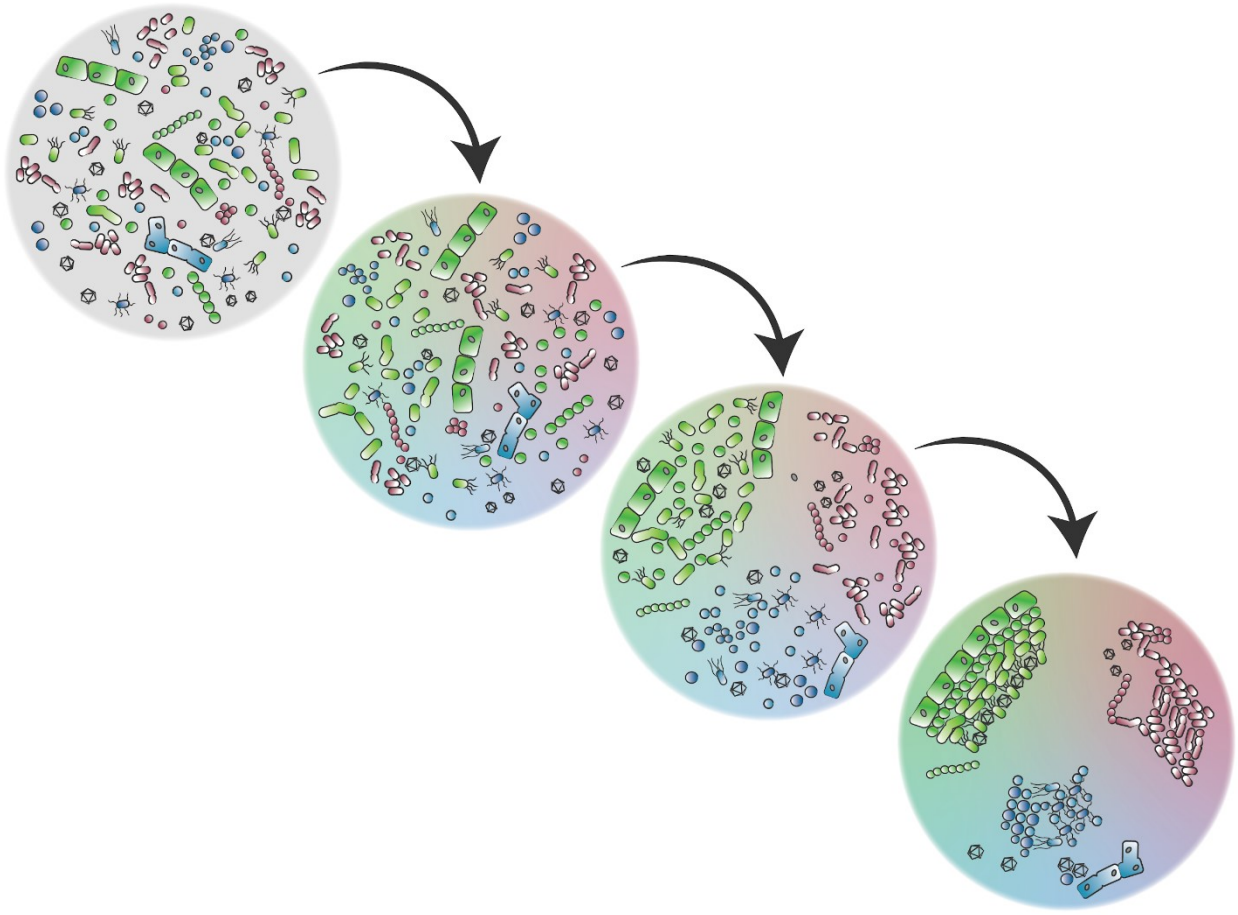


Figure 2. Engineering spatial and temporal dynamics of microbiomes. Microbiome engineering will require the ability to stably reorganize communities over time and to direct where and when individual cells or organisms exist within the microbiome. Initial measurements of a natural or randomly distributed microbiome (grey background) will help inform the rational design of a template that is applied to the microbiome (colored background). The template may alter the microbiome genetically, metabolically, or by other means, but will enable microbiome organization over time and space. Additional advances will allow for more detailed patterning that contributes additional functionality, such as enabling the microbiome to alter the environment in which it grows.

EBRC

MICROBIOME ENGINEERING: SPATIOTEMPORAL CONTROL

Goal | **Breakthrough Capability** | **Milestone**

Engineer the spatial characteristics of a microbiome.

Develop tools to engineer the spatial characteristics of microbiomes.

2022	2025	2030	2040
Generate standardized methods for determining basic physiological parameters (*e.g.,* growth rate, death rate, motility, metabolism) in pure cultures and correlate with measurements of in situ microbiomes (*e.g.,* genome ratio for replication rate).	Create large databases of three-dimensional imaged in situ microbiomes (*e.g.,* high-resolution sections of sediment columns characterized for geophysical, chemical and biological properties, tissue organoids with in vivo microbiomes) to help predict properties of novel microbiomes.	Engineer tools that reduce or prevent horizontal gene transfer in microbiomes to prevent reacquisition of genes that may allow for increased spatial spread.	Engineer technologies that can rapidly and robustly reduce microbiome genome sizes in natural environments to constrain spread.
Track allele-level genetic changes and transmission of mobile elements (*e.g.,* plasmids, phages) in hosts to measure population dynamics, genome evolution, and genetic exchange within a microbiome.			
Constrain environmental niche by removing genes critical for persistence in various environments (*e.g.,* eliminate biofilm formation or carbon utilization functions).	Design computational tools that identify gene loci or networks that can be removed without impacting organism growth or viability.	Design mechanisms to limit microbiome growth that are orthogonal to nutrient restrictions.	

Engineer a microbiome to change size in response to its environment.

2022	2025	2030	2040
Engineer structured microbial communities in an *x-y* plane across meter-scales in response to synthetic signals (*e.g.,* engineered metabolites, phages).	Engineer microbiomes that grow or conform to defined locations (*x-y-z* space) or can be specifically patterned or distributed in space.	Engineer microbiomes so growth and patterning is determined based on specific environmental cues (*e.g.,* surface texture changes, chemical or nutritional environment).	Engineer microbiomes so growth and patterning is determined based on specific environmental cues (*e.g.,* surface texture changes, chemical or nutritional environment).

Engineer spatially self-organizing communities (*i.e.*, organization is templated rather than shaped by the environment).			
Engineer microbiomes that can be used to produce a repeated pattern on a 2D surface.	Design 3D structured microbiomes in a controlled environment.	Engineer microbiomes that self-localize in complex environments (*e.g.*, soil, human gut) so they can be deployed to hard-to-access locations.	Engineer microbiomes with intrinsic Turing patterning so they form desired structures.
	Engineer microbiomes that stop growing once they grow to a fixed size.		Engineer microbiomes to create sophisticated three-dimensional structures with defined domains that interact and work together.

Design microbiomes that alter their extracellular environment.			
Program the formation of biofilms or other rigid structures that fix the spatial organization of a microbial community.	Generate different types of structured environments that extend beyond natural biofilms.	Create biofilms that function as autonomous organs.	
	Build in control of biofilm formation to create programmed, heterogeneous structures.		

Control the temporal dynamics of engineered microbiomes.

Determine the physical dynamics of a microbiome over time.			
Measure nutrient and water flow through diverse environments (*e.g.*, soil, soil to a plant, in vivo) and community members (*e.g.*, viruses, bacteria, fungi) in high-throughput.	Create high-throughput, multiplexed, and automated systems to quantify physiological parameters of a complex microbiome in a controlled environment.	Measure environmental dispersal and drift of microbes, phages, and chemicals across different environments to help generate predictive ecological models.	Generate methods that enable rapid, high-throughput quantification of physiological parameters in complex, natural microbiomes.

Engineer mechanisms to control the growth and spread of a microbiome over time.			
Engineer microbiomes that integrate programmed self-destruction with cell oscillators, so death is initiated based on time rather than cell density.	Use in situ measurements at a single point in time to predict future limiting factors (*e.g.*, space, nutrients, specific trace metals, essential amino acids) for a microbiome.	Engineer a microbiome that contains a 'universal clock' species that triggers self-destruction of the microbiome upon reaching a functional endpoint.	Design microbiomes that can operate at multiple timescales to enable more complex functions (*e.g.*, lay dormant for months to perform functions on second or minute timescales).
	Engineer cell-cell circuits that kill a counting strain, plus neighboring species in the microbiome, after a given number of cell divisions is reached.		

2022 **2025** **2030** **2040**

Engineer microbiomes that retain function on short to evolutionary (*i.e.*, months to years) time scales.

2022	2025	2030	2040
Model the fitness impacts of microbiome engineering and determine its contribution to escape frequency and competitive advantage.	Design and build genetic components that are more robust to mutation or loss than conventional genetic designs. Map evolutionary trade offs when multiple pressures are applied to a microbiome (including long-term continuous culturing) and use this information to design resilient organisms or combinatorial controls that prevent evolution away from a desired function.	Engineer microbiomes that remove or kill community members that lose their engineered function.	Engineer an organism that influences future successional dynamics in an environment (*e.g.*, niche construction, niche preemption, and priority effects), so the microbiome influences the surrounding environment past the microbiome's functional lifespan.

Spatiotemporal Control
Goal 1: Engineer the spatial characteristics of a microbiome.

[Current State-of-the-Art]: Uncontrolled spatial localization of engineered microbiomes, via growth, spread, or other mechanisms, could have detrimental health and environmental consequences, and limit the usefulness of engineered microbiomes. Additional tools are needed to help control the space occupied by engineered microbiomes, in response to external inputs and with autonomous feedback control. CRISPR-Cas circuits can be used to selectively kill bacteria (Bikard et al., 2014; Caliando & Voigt, 2015; Citorik et al., 2014). High-throughput functional genomics in controlled lab environments allows for identification of previously unstudied metabolic pathways (Thompson et al., 2019; Wetmore et al., 2015).

Measuring population and nutrient dynamics in a microbiome has been the subject of extensive research efforts (Cao et al., 2019). Recently, sophisticated culture systems have emerged using 3D printing and microfluidics to systematically environmental conditions (González et al., 2020; Kim et al., 2012; Zhalnina et al., 2018). These systems can be used to observe nutrient and population dynamics, but tools to accurately mimic natural environments are still emerging. Furthermore, most bacteria remain uncultured (Lloyd et al., 2018; Rappé & Giovannoni, 2003),particularly from non-human environmental samples. Some recent measurement successes include MaPS-seq (Metagenomic Plot Sampling by sequencing) which captures the spatial landscape of microbiota from “plots” of the microbiome host (Sheth et al., 2019), methods for ratiometric gas reporting to detect gene expression and cell counts in soil microbes in a quantitative manner without having to physically alter the soil matrix (Cheng et al., 2018), and mass spectrometry methods to measure the chemical makeup of human skin, a complex system that includes interaction between the host, a microbiome, and the surrounding environment (Bouslimani et al., 2015). Most methods, however, are destructive and there are limitations on what data can be captured from the same sample. Further, there is limited knowledge of how current model systems replicate variations that occur on short (sub-daily) timescales; and how technical errors/variations impact data produced from models.

Genomically recoded organisms can be engineered with effective growth control strategies (Lajoie et al., 2013; Ma & Isaacs, 2016; Rovner et al., 2015). Genome reduction approaches to date have largely focused on identifying minimal genomes for model organisms to improve bioproduction (*e.g., Escherichia coli* MDS42, *Bacillus subtilis*, *Pseudomonas putida*). Genome reduction techniques could be applied to shape the environmental niche of microbes and provide evolutionary robustness against complementation for populations deployed for human health, remediation, and plant growth.

Complete spatial and temporal control over individual species will enable more advanced pattern formation within a microbiome. Two-member bacterial populations that maintain the composition of the population (*e.g.,* proportions of two bacterial species) using feedback control via cell-to-cell signaling molecules have been designed (Dunlop et al., 2010; Hu & Murray, 2019; McCardell et al., 2019; Ren & Murray, 2018; You et al., 2004). Spatial patterning and Turing patterns have been created by single-species bacterial populations with synthetic circuits inside the individual cells (Baumgart et al., 2017; Bittihn et al., 2018; Dekas et al., 2009; Karig et al., 2018). *Escherichia coli* have recently been engineered to form templated two-dimensional patterns using optogenetics, but this functionality has not been demonstrated

in any other organism yet (Moser et al., 2019). More fine-tuned control must be developed for more ambitious applications, such as engineered living materials (Nguyen et al., 2018).

[Breakthrough Capability]: Develop tools to engineer the spatial characteristics of microbiomes.

- **2022 milestone: Generate standardized methods for determining basic physiological parameters (*e.g.,* growth rate, death rate, motility, metabolism) in pure cultures and correlate with measurements of *in situ* microbiomes (*e.g.,* genome ratio for replication rate (Brown et al., 2016)).**
    - [Bottleneck]: Cell-to-cell variation within a species could affect physiological parameters for particular cultures.
        - [Potential solution]: Establish benchmark culturing methods that can be used for both sample collections (*e.g.,* ATCC) and isolated strains.
    - [Bottleneck]: Some species cannot be grown in monoculture.
        - [Potential solution]: Design well-characterized helper strains that allow for growth in coculture, and enable measurements of physiological parameters in simplified systems (Morris et al., 2011).
- **2022 milestone: Track allele-level genetic changes and transmission of mobile elements (*e.g.,* plasmids, phages) in hosts to measure population dynamics, genome evolution, and genetic exchange within a microbiome.**
    - [Bottleneck]: Tracking will require affordable, high-throughput DNA sequencing and open source data analysis software.
        - [Potential solution]: Create sequencing consortiums to facilitate collaboration across academic and industrial groups, and can manage data repositories to ensure that sequences are easily accessible and interoperable.
- **2022 milestone: Constrain environmental niche by removing genes critical for persistence in various environments (*e.g.,* eliminate biofilm formation or carbon utilization functions).**
    - [Bottleneck]: Lack of efficient tools for effective transformation and genetic manipulation of arbitrary organisms.
        - [Potential solution]: Create pipelines to screen transformation conditions to deliver genome editing and knockout machinery.
- **2025 milestone: Create large databases of three-dimensional imaged *in situ* microbiomes (*e.g.,* high-resolution sections of sediment columns characterized for geophysical, chemical and biological properties, tissue organoids with *in vivo* microbiomes) to help predict properties of novel microbiomes.**
    - [Bottleneck]: Many existing microscopy techniques require substantial amounts of manual labor to section, stain, and analyze samples.
        - [Potential solution]: Use robotics to create automated workflows for existing spatial-imaging techniques (*e.g.,* three-dimensional mass spectrometry), and expand the use of image analysis programs to increase accuracy and throughput.
- **2025 milestone: Design computational tools that identify gene loci or networks that can be removed without impacting organism growth or viability.**

    - [Bottleneck]: Lack of functional annotation of genes that confer fitness in different environments.
        - [Potential solution]: Create databases for collecting high-throughput functional genomics in controlled and natural environments, and ensure that data is accessible for predictive model development.
- **2030 milestone: Engineer tools that reduce or prevent horizontal gene transfer in microbiomes to prevent reacquisition of genes that may allow for increased spatial spread.**
    - [Bottleneck]: Lack of genetic tools that enable a precise control of the degree and type of horizontal gene transfer.
        - [Potential solution]: Engineer exclusion systems (Avello et al., 2019), or recode genomes to eliminate tRNA codons (Lau et al., 2017).
- **2030 milestone: Design mechanisms to limit microbiome growth that are orthogonal to nutrient restrictions.**
    - [Bottleneck]: Microbes are very effective at continuing to grow in nutrient rich environments.
        - [Potential solution]: Use bacterial nanowires to design synthetic cell-cell circuits that halt microbiome growth, once the microbiome contacts two electrodes and closes the circuit.
- **2040 milestone: Engineer technologies that can rapidly and robustly reduce microbiome genome sizes in natural environments to constrain spread.**
    - [Bottleneck]: Gene editing technologies need to become significantly more specific, both in terms of spatial localization and to target specific organisms in a community.
        - [Potential solution]: Design genome engineering tools (*e.g.,* CRISPR, new technologies) that can target multiple organisms and multiple gene loci at the same time, with individual or pooled constructs.

[Breakthrough Capability]: Engineer a microbiome to change size in response to its environment.

- **2022 milestone: Engineer structured microbial communities in an x-y plane across meter-scales in response to synthetic signals (*e.g.,* engineered metabolites, phages).**
    - [Bottleneck]: Cells, viruses, and nutrients diffuse through space in a way that can be difficult to predict and control.
        - [Potential solution]: Design multi-signal inputs for growth, so microbiome has tighter control than a simple on-off switch (*i.e.,* if/and/or logic gates that direct growth).
        - [Potential solution]: Nutritional (or other) auxotrophies that prevent growth in absence of a small molecule (*e.g.,* microbiome fertilizer for fields).
- **2025 milestone: Engineer microbiomes that grow or conform to defined locations (*x-y-z* space) or can be specifically patterned or distributed in space.**

    - [Bottleneck]: Limited laboratory models for studying microbiome growth and spread in either homogeneous or heterogeneous three-dimensional environments.
        - [Potential solution]: Engineer optically clear, solid and semi-solid media to dynamically observe growth in various physical environments.
- **2030 milestone: Engineer microbiomes so growth and patterning is determined based on specific environmental cues (*e.g.,* surface texture changes, chemical or nutritional environment).**
    - [Bottleneck]: Strong evolutionary pressure for microbes in a microbiome to mutate or get rid of any growth-inhibiting genes and pathways.
        - [Potential solution]: Design “universal kill switches” that allow any microbe within a microbiome to kill the entire community, so there are multiple layers of redundancy.
- **2040 milestone: Engineer microbiomes that grow to fill, or shrink to fit, a functional niche (*e.g.,* nitrogen-fixing microbiomes that continue growing until they reach a patch of soil that has sufficient nitrogen).**
    - [Bottleneck]: Similar evolutionary pressures exist as for the 2030 milestone, but the stop/go input signals will be more complex than a single environmental cue.
        - [Potential solution]: Design “universal kill switches” that allow any microbe within a microbiome to kill the entire community in response to multiple external signals.

[Breakthrough Capability]: Engineer spatially self-organizing communities (i.e., organization is templated rather than shaped by the environment).

- **2022 milestone: Engineer microbiomes that can be used to produce a repeated pattern on a 2D surface.**
    - [Bottleneck]: *Escherichia coli* that were engineered to be templated onto materials had specific signaling pathways removed to prevent cross-talk. No other microbe’s signaling networks are characterized in a way that would allow for that problem to be identified and addressed *a priori*.
        - [Potential solution]: Create a catalog of signaling pathways that direct organism motility and adherence to enable more rapid design and engineering of templatable organisms.
    - [Bottleneck]: Most biological input signals to direct microbial cell localization (*e.g.,* chemicals, secondary metabolites, nutrients) diffuse readily and are not suitable for patterning.
        - [Potential solution]: Engineer plasmids or operons that can be inserted into diverse organisms to make them light-sensitive for optogenetics.
- **2025 milestone: Design 3D structured microbiomes in a controlled environment.**
    - [Bottleneck]: Self-organizing systems that use motility or chemotaxis to direct microbial movement will require more advanced mechanisms for restricting compound diffusion through space.

        - [Potential solution]: Create biodegradable materials that can be 3D printed into chemical-containing matrices that will facilitate growth of complex, patterned microbiomes.
    - [Bottleneck]: Optogenetic techniques are more precise than chemical-based techniques for controlling a cell's location, but most solid, microbial growth materials are not optically clear.
        - [Potential solution]: Engineer optically clear, biodegradable materials so optogenetics can be used to determine bacterial adhesion and microbiome structure.
- **2025 milestone: Engineer microbiomes that stop growing once they grow to a fixed size.**
    - [Bottleneck]: Individual species within a microbiome will have unique nutrient limitations, creating uneven bounds on spatial spread.
        - [Potential solution]: Introduce synthetic auxotrophy into all microbiome species, to limit growth to fixed spatial dimensions.
- **2030 milestone: Engineer microbiomes that self-localize in complex environments (*e.g.,* soil, human gut) so they can be deployed to hard-to-access locations.**
    - [Bottleneck]: In hard-to-access environments, it may be difficult to validate the correct self-localization and 3D construction has taken place.
        - [Potential solution]: Engineer compounds that are only synthesized when the microbiome achieves a desired spatial arrangement, and can be readily detected as a readout for microbiome organization.
- **2040 milestone: Engineer microbiomes with intrinsic Turing patterning so they form desired structures.**
    - [Bottleneck]: Designing Turing patterns requires robust control over extracellular diffusion and gradient formation.
        - [Potential solution]: Advance engineering of cell-to-cell signalling to enable tighter control over Turing pattern formation (Karig et al., 2018; Luo et al., 2019).
- **2040 milestone: Engineer microbiomes to create sophisticated three-dimensional structures with defined domains that interact and work together.**
    - [Bottleneck]: Creating a rigid and defined structure without an existing template, with underlying domains composed of different interlinking materials.
        - [Potential solution]: Design microbiomes that can transmit long-range signals, such as morphogen gradients, to form Turing patterns.

[Breakthrough Capability]: Design microbiomes that alter their extracellular environment.

- **2022 milestone: Program the formation of biofilms or other rigid structures that fix the spatial organization of a microbial community.**
    - [Bottleneck]: Microbiomes will naturally spread to fill an environmental niche, unless intentional mechanisms prevent its growth.
        - [Potential solution]: Design biofilm formation to be conditionally dependent on an exogenous signal or a signal from within the community.

- **2025 milestone: Generate different types of structured environments that extend beyond natural biofilms.**
    - [Bottleneck]: Biofilms are normally composed of specific extracellular components associated with natural biofilms.
        - [Potential solution]: Bring in capabilities in material synthesis and metabolic engineering to generate non-natural monomers that can form polymers once excreted outside of the cell.
- **2025 milestone: Build in control of biofilm formation to create programmed, heterogeneous structures.**
    - [Bottleneck]: The spatial composition of a biofilm is uncontrolled and relatively homogenous.
        - [Potential solution]: Apply spatial sensing, either through exogenous signals (*e.g.,* small molecules, light) or autonomous sensing, to generate different types of biofilms at different locations starting a single and otherwise spatially homogeneous microbial community.
- **2030 milestone: Create biofilms that function as autonomous organs.**
    - [Bottleneck]: Developing pre-programmed internal structures with a defined boundary between inside and outside.
        - [Potential solution]: Use synthetic genetic timers and other mechanisms to generate biofilms that grow to specific sizes, and then synthesize an external surface that serves as a semi-permeable barrier.

Spatiotemporal Control

Goal 2: Control the temporal dynamics of engineered microbiomes.

[Current State-of-the-Art]: Engineering temporal stability will be critical for the advancement of microbiome engineering. However, no framework for predicting or engineering such stability yet exists (Lawson et al., 2019). The development of a time resolved, high-throughput gut microbiome model system helped generate a predictive dynamic model to examine community evolution over time (Venturelli et al., 2018). The model was capable of predicting positive and negative interactions within the community, but was not sufficient to predict key species that impacted community assembly. Increasing engineered complexity also has shown to decrease stability over time (Venturelli et al., 2016; Haoqian Zhang et al., 2014) so the capacity to decrease a new technology's microbial cost (*e.g.,* metabolically, genetically) will be critical for its long-term maintenance in a microbiome. Greater temporal control over microbiomes will require a better understanding of the population dynamics and ecological interactions that shape the evolution of a microbiome, such as random mutations and horizontal gene transfer (Cao et al., 2019).

Temporal control will also be enabled by advances in molecular clocks that can regulate microbiome functions over time. A synchronized lysis circuit has been engineered in *Salmonella typhimurium* to induce population lysis at a set cell density, without regard to time (Din et al., 2016). Emerging tools have extended the time span that engineered genetic clocks can be used to days (Hussain et al., 2014; Riglar et al., 2019), but these must continue to be extended to obtain full temporal stability over engineered microbiomes. Some reactive transport models and other simulation tools to predict physical dynamics have been developed (Mills et al., 2007; Phillips et al., 2005). However, the state-of-the-art is a far reach from selectively manipulating or eliminating individual constituents of a microbial community, particularly in tailored-spectrum and spatiotemporal means.

[Breakthrough Capability]: Determine the physical dynamics of a microbiome over time.

- **2022 milestone: Measure nutrient and water flow through diverse environments (*e.g.,* soil, soil to a plant, *in vivo*) and community members (*e.g.,* viruses, bacteria, fungi) in high-throughput.**
    - [Bottleneck]: Limited tools to assay environments without specialized equipment/techniques (*e.g.,* isotope analysis) or at different scales.
        - [Potential solution]: Micro- and macro-scale controllable environments (*e.g.,* EcoFABs and EcoPods (Gao et al., 2018; Sasse et al., 2019) for microbiome engineering) to help test different environments at a smaller scale than field tests.
- **2025 milestone: Create high-throughput, multiplexed, and automated systems to quantify physiological parameters of a complex microbiome in a controlled environment.**
    - [Bottleneck]: It is challenging to determine how nutrients are utilized or exchanged between organisms, particularly in high throughput.
        - [Potential solution]: Expand the functionality of stable isotope probing methods (*e.g.,* increase sensitivity, improve sampling processes for

anaerobic environments) to enable more accurate metabolite tracing in a complex microbiome (Berry & Loy, 2018).

- **2030 milestone: Measure environmental dispersal and drift of microbes, phages, and chemicals across different environments to help generate predictive ecological models.**
    - [Bottleneck]: Current models do not adequately capture complex factors influencing natural microbiome environments to enable predictions.
        - [Potential solution]: Create coupled microbial/reactive transport type models suitable for prediction and design of consortia in situ in diverse environments (*e.g.,* sediment, plant roots, gut microbiome, oral microbiome).
- **2040 milestone: Generate methods that enable rapid, high-throughput quantification of physiological parameters in complex, natural microbiomes.**
    - [Bottleneck]: Methods for documenting large spatial volumes primarily rely on microscopy-based techniques, which are poorly suited to measurements in natural environments.
        - [Potential solution]: Design computational models that use measurements at the boundaries of microbiomes, which may be easier to sample, to predict responses (*e.g.,* transcriptional, translational, metabolic) at other locations in the microbiome.

[Breakthrough Capability]: Engineer mechanisms to control the growth and spread of a microbiome over time.

- **2022 milestone: Engineer microbiomes that integrate programmed self-destruction with cell oscillators, so death is initiated based on time rather than cell density.**
    - [Bottleneck]: Existing cell oscillators only function for a relatively small number of cell divisions *in vivo*.
        - [Potential solution]: Engineer synthetic oscillators that count for more generations, or have mechanisms by which the count can be reset by another organism or external signal.
- **2025 milestone: Use *in situ* measurements at a single point in time to predict future limiting factors (*e.g.,* space, nutrients, specific trace metals, essential amino acids) for a microbiome.**
    - [Bottleneck]: Many industrial processes are being converted to continuous fermentation (rather than batch fermentation) processes, where genetic drift is a more significant concern than growth-limitation.
        - [Potential solution]: Use genetic data from directed evolution experiments to anticipate mutations, and design genetic tools (*e.g.,* phages, CRISPR) that target and remove cells that acquire those mutations.
- **2025 milestone: Engineer cell-cell circuits that kill a counting strain, plus neighboring species in the microbiome, after a given number of cell divisions is reached.**

    - [Bottleneck]: In a large microbiome, organisms may be too far away from the counting strain to receive sufficient amounts of an antimicrobial compound to be killed.
        - [Potential solution]: Design counting strain to undergo multiple cycles of growth and killing to fully eliminate the other members of the microbiome.
- **2030 milestone: Engineer a microbiome that contains a 'universal clock' species that triggers self-destruction of the microbiome upon reaching a functional endpoint.**
    - [Bottleneck]: Complex microbiomes will contain diverse species that cannot normally be killed using the same mechanism, requiring a synthetic cell to encode multiple genetic pathways to destroy the microbiome.
        - [Potential solution]: Use orthogonal microbe-microbe signaling networks to trigger cell death, so a single signal from the synthetic cell clock can trigger self-destruction of the entire microbiome.
- **2040 milestone: Design microbiomes that can operate at multiple timescales to enable more complex functions (*e.g.,* lay dormant for months to perform functions on second or minute timescales).**
    - [Bottleneck]: No known methods for designing multiple interacting cellular clocks.

[Breakthrough Capability]: Engineer microbiomes that retain function on short to evolutionary (i.e., months to years) time scales.

- **2022 milestone: Model the fitness impacts of microbiome engineering and determine its contribution to escape frequency and competitive advantage.**
    - [Bottleneck]: Fitness impacts may be hard to predict in different strains or environments.
        - [Potential solution]: Iterate on models by pairing with high-throughput experimental data, to facilitate rapid design-build-test cycles.
- **2025 milestone: Design and build genetic components that are more robust to mutation or loss than conventional genetic designs.**
    - [Bottleneck]: Current natural mutational rates may be too high to overcome.
        - [Potential solution]: Engineer or select for strains that have reduced mutation rates, or select against high mutation rates.
- **2025 milestone: Map evolutionary trade offs when multiple pressures are applied to a microbiome (including long-term continuous culturing) and use this information to design resilient organisms or combinatorial controls that prevent evolution away from a desired function.**
    - [Bottleneck]: Designing around evolutionary pressures requires prior knowledge of the pressures and how they influence a microbiome (*e.g.,* coevolutionary design to prevent phage resistance (Kortright et al., 2019; Torres-Barceló, 2018), use multiple antibiotics to prevent antibiotic resistance (Baym et al., 2016)).
        - [Potential solution]: Experimental microbiome models that allow for high-throughput measurements over time, to examine how microbiomes evolve in response to different selective pressures.

- **2030 milestone: Engineer microbiomes that remove or kill community members that lose their engineered function.**
    - [Bottleneck]: Killing or removing groups of cheaters may disrupt spatial structure of microbiome.
        - [Potential solution]: Engineer strains that can recolonize or relocate to spaces where cheaters have been killed or removed.
    - [Bottleneck]: It may be difficult or impossible to engineer selective forces for some functions.
        - [Potential solution]: Create re-engineering schemes to overcome low levels of function loss without requiring selective pressure (*e.g.,* gene drives).
- **2040 milestone: Engineer an organism that influences future successional dynamics in an environment (*e.g.,* niche construction, niche preemption, and priority effects), so the microbiome influences the surrounding environment past the microbiome's functional lifespan.**
    - [Bottleneck]: Engineering succession requires in-depth knowledge of not only the engineered microbiome, but also the natural microbiome dynamics that would otherwise grow back.
        - [Potential solution]: Establish best-practices that require measuring and preserving natural microbiomes before engineered microbes are introduced, to always ensure that the original ecology can be reanalyzed.

Functional Biodiversity

Ultimately, a major goal of microbiome engineering is that these technologies will have functional purposes outside of the lab. Engineered microbiomes out in the world encounter new environmental conditions and different species from those used in the lab. This theme aims to address that technical challenge by examining how microbiomes can be engineered to harness the biodiversity present in these environments and focuses on a species' function, rather than the specific species present in the microbiome.

Underlying this is the ecological concept of "guilds." Loosely, guilds are basic structural units of communities and ecosystems. In the context of this roadmap, ecological guilds are a collection of microbes with similar roles/functions within a community and having similar resource requirements or exploiting resources in similar ways (Simberloff & Dayan, 1991; Stroud et al., 2015) (Figure 3). They can also exist in different niches (while still performing similar functions), resulting in potentially unique interactions with their neighbors compared to other members of the same functional guild. The end result is a holistic view of functional biodiversity – considering the functional diversity of an ecosystem at any level, with an agnostic view of what specific species are in these ecosystems.

This is particularly important as technologies are deployed outside controlled laboratory environments, where engineered microbiomes encounter new environmental conditions and microbial species. To maintain functionality, an engineered microbiome must persist in the face of these interactions. Engineering microbiomes based on guilds, rather than individual species, creates a framework in which guild functions can be maintained to ensure robust functionality, without requiring a complete redesign in every new environment.

The first goal of *Functional Biodiversity* addresses the conceptual shift that will need to occur, in order to design microbiomes based on functional guilds. Current methods for studying microbial communities rely heavily on metagenomics or other sequence-based techniques. This provides information on genetically related organisms, so called "taxonomic guilds," but developing a function-oriented understanding of microbiomes will require significant advancements in other -omics techniques and approaches for modeling microbial interactions within and between functional guilds (Dehal et al., 2010; Erbilgin et al., 2019; Gupta et al., 2020; Mutalik et al., 2019; Zengler et al., 2019). Subsequent goals focus on designing robust microbial communities based on their function (Lawson et al., 2019), and then harnessing the flexibility of functional guilds to engineer microbiomes that retain their function as they move from the lab into a natural environment.

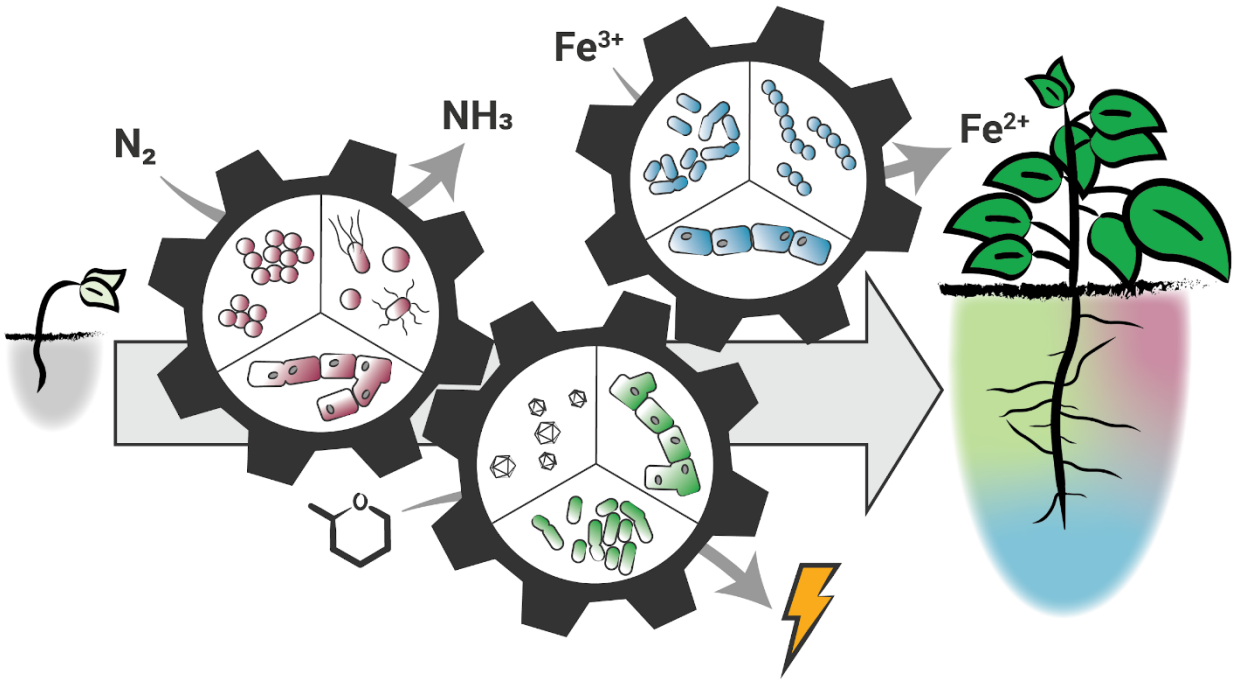


Figure 3. Functional biodiversity in natural and engineered microbiomes. Microbiomes consist of functional guilds - groups of potentially taxonomically distinct organisms that have similar roles in a community - which contribute to the microbiome's stability and function over time. Guilds (depicted as microbes similarly colored in red, green, and blue) may be genetically unrelated, but can all perform a function, such as nitrogen fixation, energy production, or iron reduction in a soil microbiome. Engineering functional guilds within a microbiome can harness biodiversity such that a task accomplished by any individual guild member (e.g., nitrogen fixation by red fungal cells) is redundant with another guild member (e.g., nitrogen fixation by red cocci) to make the whole community more resilient. Engineering guilds can be accomplished through either top-down (e.g., microbiome enrichment) or bottom-up (e.g., rationally designed consortia) approaches, both of which have unique benefits and drawbacks. Engineering microbiome guilds, rather than individual species, will create stable communities that resist evolutionary pressures.

EBRC

MICROBIOME ENGINEERING: FUNCTIONAL BIODIVERSITY

Goal | Breakthrough Capability | Milestone

Determine and manipulate the functional composition of microbiomes at the guild level (*i.e.,* top-down engineering).

2022	2025	2030	2040
Characterize the functional guild composition of any microbiome.			
Identify coherent functional subunits from metagenomic data and map activity (*e.g.,* metatranscriptomic data, stable isotope probing, metabolomics).	Rapid characterization of existing functional guilds before introducing or eliminating new members (*e.g.,* identify the microbiome members of the guild and their interactions, and characterize the interactions that an additional microbe would have with the existing members).	Map functional guild metabolic pathways and dependencies for growth, colonization, and anabolism. Non-destructive techniques to characterize functional guild composition and function (*i.e.,* avoid unnecessary -omics, find ways to reduce temporal, spatial, and cell-to-cell variability).	Rapidly characterize all species and their interactions within any natural microbiome, *in situ* and with high spatial resolution.
Remove or modify a functional guild to eliminate its function from a microbiome.			
Target and kill a single species in a microbiome in a controlled or natural environment.	Remove a function by altering or eliminating a gene or metabolic pathway from a controlled microbiome (*e.g.,* suppress amino acid synthesis to introduce synthetic auxotrophy).	Eliminate an entire functional guild from a natural microbiome, by either genetically removing the function or killing the function-consisting organisms.	Create rapid and robust computational methods to rationally design cocktails (*e.g.,* phages, phage tail-like bacteriocins, inhibitors, CRISPR) to remove desired functional guilds.
Add functional guilds to microbiomes to introduce a new function or modify the existing function.			
Design a guild that introduces a new function to a controlled microbiome.	Engineer a guild that changes an existing function in a controlled microbiome.	Readily edit a natural microbiome to create niches for introducing new guilds.	Manipulate any "native" species in situ in a natural microbiome and bypass culturing, model organisms, or model systems.

At-will manipulation of a specific microbial species or guild within a microbiome.			
Expand host range of existing DNA delivery vehicles and precisely define host breadth and efficiency under different environmental conditions.	Further functionalized delivery vehicles to allow for delivery of other biomolecules, such as RNA or protein. Achieve in vivo genetic manipulation of microbial communities. Manipulate DNA localization after DNA has been added to a microbiome.	Combine functionalities to make more sophisticated changes to a microbial community, or establish a pipeline where delivery can be achieved for any new bacterium and know the host range.	Rational modification of natural and structured communities in specific applications using multiple modalities (*e.g.,* nucleic acid manipulation, protein engineering, and introduction of new microbial species) guided by computational models.

Design microbiomes for any function or environment based on their component organisms or species (*i.e.,* bottom-up engineering).

Design and engineer functional microbiomes by adding or modifying individual species of microbes.			
Generate synthetic microbiomes with two to five characterized species (of different functional guilds) imitating functions of a natural community.	Control community functional dynamics (*i.e.,* abundance of species and/or expression profile) in a synthetic microbiome composed of two to five characterized species (of different functional guilds).	Design a community with a sufficient set of partially redundant guild members so that it is functionally robust against environmental or community variations.	
Predict and engineer interactions between different species within the microbiome.			
Establish methods for determining what metabolites are biologically available to a microbiome in any environment (*i.e.,* identify what a microbe can use to grow).	From metagenomic data, identify individual microbial species with related or coherent functions.	Engineer and optimize microbiomes where mutually-required interactions between species and functional guilds are implemented.	Generate predictive models and experimental frameworks to engineer microbiomes with defined species composition and interactions.
Predict and engineer interactions between a microbiome and the environment (*e.g.,* temperature, oxygen concentration, pH, small molecules or drugs, dietary compounds).			
Engineer non-model microbiome species to activate a genetic program in response to a single environmental perturbation.	Establish and validate experimental frameworks to engineer microbiomes responsive to the desired environmental perturbations.	Engineer a microbiome that responds to multiple environmental perturbations.	"Routine" prediction and manipulation of any species within a microbiome using environmental perturbations for desired functions.

2022 **2025** **2030** **2040**

Establish Design-Build-Test-Learn pipelines for bottom-up microbiome engineering – from culturing protocols, to genetic tools, to 'deployment' in controlled and natural microbiomes.			
Develop standard protocols for microbiome stock maintenance and propagation.	Expand use of and capacity of high-throughput platforms to allow for increased experimental reproducibility and accuracy.	Engineer methods that enable fully autonomous microbiome characterization (*e.g.,* grow in culture, measure metabolism) with limited prior knowledge of a sample.	Rapidly develop tools to control any organism (*e.g.,* identify culture conditions, genetic manipulation, spatial localization), including uncultivated organisms, after initial identification via sequencing.
Computationally predict behaviors of individual microbes within the biome and determine minimal nutrient requirements.			
Engineer markerless selection methods that allow for stable maintenance of an engineered strain in a microbiome.			

Engineer microbiomes robust to evolutionary and environmental pressures.

Engineer robust and stable microbiomes that are resilient to evolutionary and environmental stress (*e.g.,* environmental variations, dispersion forces, nutrient deprivation).			
Engineer high-throughput systems to measure coevolution of species within a guild over time.	Control community functional dynamics (*i.e.,* abundance of species and/or expression profile) in a synthetic microbiome composed of two to five characterized species (of different functional guilds).	Engineer microbiomes to kill cells that drift away from the guild's function (*e.g.,* producing a specific metabolite, degrading specific compounds).	Engineer microbiomes that contain diverse functional guilds (*i.e.,* multiple species per guild) and maintain their function in natural environments.
Use a model microbiome to examine responses to well-defined ecological stresses (*e.g.,* temperature change, moisture gradients, UV exposure) in a contained environment.	Engineer guilds that respond to an environmental stress to preserve function (*e.g.,* buffers environment in response to pH change, antibiotic/antiviral produced in response to predator, 'nutrient scavenging molecule' in response to decreases).	Engineer species that help a microbiome resist dispersion forces by producing biofilms or other materials.	Engineer guilds that evolve in a manner that is directed by environmental conditions or nutrient availability, so optimal function continues even as changes occur.

2022 **2025** **2030** **2040**

Functional Biodiversity

Goal 1: Determine and manipulate the functional composition of microbiomes at the guild level (*i.e.,* top-down engineering).

[Current State-of-the-Art]: Multiple approaches can be used to isolate and engineer functional microbiomes. Top-down engineering approaches have been applied to gut microbiomes (Zhao et al., 2018), microbiomes for environmental remediation (Löffler & Edwards, 2006), and other microbiomes. A significant benefit to this approach is that communities are enriched based on their function and ability to grow together. However, the current deficiency in broadly applicable genetic tools and culture techniques oftentimes prevents fine-tuned manipulation of these communities, because they usually contain non-model organisms. Antibiotic selection has been used to enrich communities that effectively degrade lignocellulose, at which point a bottom-up approach was used for more detailed study (Gilmore et al., 2019). Engineering functional guilds and tools to manipulate them will require improvements in data infrastructure, development of high-throughput microbiome models, and greater coordination between interdisciplinary scientific teams to effectively interpret data for model advancement (Lawson et al., 2019).

Techniques exist for delivering DNA to different organisms followed by varying means of genetic manipulation (*e.g.,* maintenance of exogenous DNA, genome editing, gene regulation). However, the ability to deliver DNA or perform genetic manipulations is normally performed in pure cultures in the lab and is limited to a small handful of "model" organisms. Some basic strategies to deliver DNA exist (*e.g.,* conjugation, phage delivery) and are being explored in the context of model microbial communities (Ando et al., 2015; Brophy et al., 2018; Ronda et al., 2019; G. Wang et al., 2019; Yosef et al., 2017). There are also ways available to selectively eliminate individual members of a community using lytic phages or the delivery of CRISPR-based antimicrobials (Fagen et al., 2017; Yehl et al., 2019).

[Breakthrough Capability]: Characterize the functional guild composition of any microbiome.

- **2022 milestone: Identify coherent functional subunits from metagenomic data and map activity (*e.g.,* metatranscriptomic data, stable isotope probing, metabolomics).**
    - [Bottleneck]: Lack of knowledge about the function and expression timing of many genes, as well as their impact on pathway/organism function, which may impact guild membership.
        - [Potential solution]: Incorporate activity-based probes coupled with proteomics to discover functions in complex communities.
- **2025 milestone: Rapid characterization of existing functional guilds before introducing or eliminating new members (*e.g.,* identify the microbiome members of the guild and their interactions, and characterize the interactions that an additional microbe would have with the existing members).**
    - [Bottleneck]: Predicting the downstream perturbations (*e.g.,* metabolic burden, community interactions) following the introduction of a new capability (*e.g.,* heterologous metabolic pathway) into an organism.

        - [Potential solution]: Machine learning approach using a metabolic flux model and trained using empirical data from perturbations.
- **2030 milestone: Map functional guild metabolic pathways and dependencies for growth, colonization, and anabolism.**
    - [Bottleneck]: Genome sequences are available, but we lack much of the necessary information (particularly for accessory genomes) needed to generate functional predictions (*e.g.,* cell morphology, metabolism, proteomics).
        - [Potential solution]: Design technologies that enable testing microbiomes at scale, so protein biochemistry, cellular phenotypes, and gene functions can be examined to increase the amount of data available for annotation prediction programs.
- **2030 milestone: Non-destructive techniques to characterize microbiome composition and function (*i.e.,* avoid unnecessary -omics, find ways to reduce temporal, spatial, and cell-to-cell variability).**
    - [Bottleneck]: Assays needed to take measurements are not well defined, or existing assays are poorly scaled to take appropriate measurements.
        - [Potential solution]: Identify minimal measurement requirements using inputs from knowledge base of measured trait/taxon associations.
- **2040 milestone: Rapidly characterize all species and their interactions within any natural microbiome, *in situ* and with high spatial resolution.**
    - [Bottleneck]: Significant advances (*e.g.,* microscopy, sample preparation, image analysis) will be needed to enable higher-resolution imaging in difficult-to-access environments (*e.g.,* gut microbiome, soil microbiomes), so it can be used to deconvolute complex biological structures such as biofilms.
        - [Potential solution]: Libraries of specifically labeled probes that allow tracking of many diverse metabolisms and activities simultaneously so functionally similar species can be defined at multiple levels of abstraction. The minimal set of these is partly defined by the 2030 milestone. Develop micron-scale magnetic and radioactive imaging *in situ*.

[Breakthrough Capability]: Remove or modify a functional guild to eliminate its function from a microbiome.

- **2022 milestone: Target and kill a single species in a microbiome in a controlled or natural environment.**
    - [Bottleneck]: Relatively few tools can specifically target individual species (*e.g.,* antibiotics target kingdoms of organisms).
        - [Potential solution]: Use a natural or specifically engineered mobile system (*e.g.,* phages, mobile plasmids) to deliver cytotoxic payloads (*e.g.,* phage tail-like bacteriocins, antimicrobial peptides).
- **2025 milestone: Remove a function by altering or eliminating a gene or metabolic pathway from a controlled microbiome (*e.g.,* suppress amino acid synthesis to introduce synthetic auxotrophy) (Lee et al., 2018).**

    - [Bottleneck]: Many existing technologies cannot fully penetrate a microbiome, so wild-type cells will grow to repopulate the community.
        - [Potential solution]: Expand the use of bacterial gene drive technology (Valderrama et al., 2019) or broad host range conjugative plasmids (Leonard et al., 2018; Sheth et al., 2016) to enable more consistent genetic manipulation in complex environments.
- **2030 milestone: Eliminate an entire functional guild from a natural microbiome, by either genetically removing the function or killing the function-consisting organisms.**
    - [Bottleneck]: Functional guilds may contain species from multiple kingdoms, and multiple techniques may be needed to manipulate a guild.
        - [Potential solution]: Engineer systems that allow for rapid manipulation and testing cycles, so different methods can be applied in succession to ensure that a function is eliminated from a microbiome.
- **2040 milestone: Create rapid and robust computational methods to rationally design cocktails (*e.g.,* phages, phage tail-like bacteriocins, inhibitors, CRISPR) to remove desired functional guilds.**
    - [Bottleneck]: Functional genomics are relatively new, so it is challenging to target functionally related species, especially across kingdoms, without off-target effects.
        - [Potential solution]: Use high-throughput microbiome model systems and genetic or small molecule inhibitor screens to improve databases of gene functions in complex environments.

[Breakthrough Capability]: Add functional guilds to microbiomes to introduce a new function or modify the existing function.

- **2022 milestone: Design a guild that introduces a new function to a controlled microbiome.**
    - [Bottleneck]: Most ecological communities are robust to intrusion, and new organisms will need to outcompete or displace other species in the microbiome.
        - [Potential solution]: Identify mild environmental (*e.g.,* pH change, heat shock), chemical (*e.g.,* low concentration antibiotics, nutrient stress), or biological (*e.g.,* phages, bacteria secreting antimicrobial peptides) perturbations that temporarily disrupt homeostasis and allow another organism to invade.
- **2025 milestone: Engineer a guild that changes an existing function in a controlled microbiome.**
    - [Bottleneck]: Existing organism functions will likely have more mechanisms to resist change, especially for mechanisms that contribute to growth or replication.
        - [Potential solution]: Engineer microbiomes to use multiple mechanisms to achieve its function (*e.g.,* stimulate increased glucose uptake while driving higher concentrations of biofuel production).
- **2030 milestone: Readily edit a natural microbiome to create niches for introducing new species.**

    - [Bottleneck]: Requires efficient *in situ* editing of a community, excellent metabolic knowledge of metabolic capabilities, timing of editing vs. augmenting with target microbes.
        - [Potential solution]: Engineer microbiomes that facilitate succession by using one engineered microbe to disrupt the natural microbiome, creating a niche for the new functional guild.
- **2040 milestone: Manipulate any "native" species *in situ* in a natural microbiome and bypass culturing, model organisms, or model systems.**
    - [Bottleneck]: Many factors limit our ability to identify what guilds exist in a natural community (*e.g.,* unculturable organisms, species may have overlapping functions, very limited models, limits methods to manipulate organisms physically/genetically).
        - [Potential solution]: Improve techniques for manipulating organisms *in situ* (see Spatiotemporal Control) and ensure that techniques function across kingdoms of life.
        - [Potential solution]: Introduce metabolic pathways for alternative carbon source utilization, to selectively enrich species organisms that can use specific nutrients (Shepherd et al., 2018).

[Breakthrough Capability]: At-will manipulation of a specific microbial species or guild within a microbiome.

- **2022 milestone: Expand host range of existing DNA delivery vehicles and precisely define host breadth and efficiency under different environmental conditions.**
    - [Bottleneck]: Current DNA delivery capabilities are limited to existing conjugative plasmids or specific "phagemid" systems with very defined and narrow host ranges. The efficacy of delivery is also dependent on many poorly defined factors (*e.g.,* environmental conditions, DNA methylation).
        - [Potential solution]: Isolate natural or engineered tail fiber proteins that interface with existing phagemid systems, harness new phagemid systems, and develop naturally-occurring or engineered conjugative systems to expand DNA delivery tools.
    - [Bottleneck]: Several environments have microbiomes of interest, but tools for delivering DNA are significantly underdeveloped (*e.g., Clostridiales spp.* in the human gut).
        - [Potential solution]: Create generalized genome editing tools (*e.g.,* vectors, genetic markers, gene promoters) and orthologous systems that are specifically designed to function under particular environmental conditions (*e.g.,* microaerophilic or anaerobic environments).
- **2025 milestone: Further functionalized delivery vehicles to allow for delivery of other biomolecules, such as RNA or protein.**
    - [Bottleneck]: Existing delivery vehicles only deliver DNA to microbes, while the ability to introduce other biomolecules could introduce other capabilities and circumvent some host defense systems.

        - [Potential solution]: Further develop existing phages known to deliver RNA or proteins. Combine with prior advances to expand or tailor the host range.
        - [Potential solution]: Use nanotechnology and extracellular vesicles to target cells specifically.
        - [Potential solution]: Combine cell-penetrating peptides with peptide nucleic acids or other smaller molecules to deliver DNA.
- **2025 milestone: Achieve *in vivo* genetic manipulation of microbial communities.**
    - [Bottleneck]: Standard approaches require culturing microbes under laboratory conditions, often to high densities.
        - [Potential solution]: Engineer a broad range of donor strains and conjugative proteins that can efficiently deliver genetic cargo to different hosts, even at low cell densities.
- **2025 milestone: Manipulate DNA localization after DNA has been added to a microbiome.**
    - [Bottleneck]: Existing DNA delivery technologies are uncontrollable once the delivery vehicle is added to an environment, but the ability to influence timing, location, and actuation of delivery would significantly improve manipulation.
        - [Potential solution]: Use optogenetics or spatially heterogeneous chemical components to direct DNA to specific locations.
        - [Potential solution]: Engineer specific delivery mechanisms, such as conditionally conjugative plasmids or DNA attached to tail fiber proteins, to control which organisms take up the DNA.
- **2030 milestone: Combine functionalities to make more sophisticated changes to a microbial community, or establish a pipeline where delivery can be achieved for any new bacterium and know the host range.**
    - [Bottleneck]: Combining components to evaluate how they potentially cross-talk.
        - [Potential solution]: Develop computational methods for considering interactions between delivery vehicles and delivered components.
- **2040 milestone: Rational modification of natural and structured communities in specific applications using multiple modalities (*e.g.,* nucleic acid manipulation, protein engineering, and introduction of new microbial species) guided by computational models.**
    - [Bottleneck]: Models that can capture changes from the level of nucleic acids up to whole cells will require significant computational and mathematical advances.
        - [Potential solution]: Develop conceptual and mathematical frameworks that specifically aim to determine how genetic changes impact cell physiology, with minimal computational processing.

Functional Biodiversity

Goal 2: Design microbiomes for any function or environment based on their component organisms or species (*i.e.,* bottom-up engineering).

[Current State-of-the-Art]: Basic bottom-up, *in vitro* models (three or four organisms) exist to manipulate rhizosphere microbiomes (Lozano et al., 2019), photosynthetic organisms (Hays et al., 2017), and co-dependent communities of gut bacteria (Ziesack et al., 2019). There has been recent success in engineering microbes found in important but experimentally challenging environments, such as *Bacteriodes thetaiotamicron* (Mimee et al., 2015) and *Eggerthella lenta* (Bisanz et al., 2020), both found in the gut microbiome. Nascent efforts have been made in oleaginous yeasts, *Neurospora*, *Aspergillus*, *Neocallimastigomycota*, and archaea (Wilken et al., 2020). A few studies have used engineered *Candida* spp. as a live vaccine or for host factor secretion (Nami et al., 2019; Saville et al., 2009). Standardized protocols, parts, and chassis for diverse organisms will allow for more predictable, fine-tuned control over metabolic outputs and functions in engineered communities (Scarborough et al., 2018).

There are still challenges in predictive computational models for bottom-up microbiome design (Feist & Palsson, 2016; Lawson et al., 2019). A predictive dynamic model of a small anaerobic microbial community was used to infer the design principles behind the microbiome's stable coexistence (Venturelli et al., 2018). Additional confounding factors have also been identified, such as microbial invaders that do not persist in the microbiome, but can still influence community evolution due to their impact on bioacid availability and pH (Amor et al., 2020).

[Breakthrough Capability]: Design and engineer functional microbiomes by adding or modifying individual species of microbes.

- **2022 milestone: Generate synthetic microbiomes with two to five characterized species (of different functional guilds) imitating functions of a natural community.**
    - [Bottleneck]: Few model systems exist for culturing and studying natural microbiomes.
        - [Potential solution]: Use dilution culturing to enrich co-occurring, minimal co-dependent functional assemblages (*e.g.,* syntrophic pairs) that form a stable overall function.
- **2025 milestone: Control community functional dynamics (*i.e.,* abundance of species and/or expression profile) in a synthetic microbiome composed of two to five characterized species (of different functional guilds).**
    - [Bottleneck]: Functionally insignificant members or non-specific interactions within the community can be difficult to identify, and may be a confounding factor when engineering synthetic interactions.
        - [Potential solution]: Improve deep sequencing and genome assembly methods to enable identification of microbial species occurring at low frequencies.
- **2030 milestone: Design a community with a sufficient set of partially redundant guild members so that it is functionally robust against environmental or community variations.**

    - [Bottleneck]: There is relatively little known about how environmental changes or other community members impact growth of even many model organisms.
        - [Potential solution]: Build a collection of well-annotated, diverse traits linked to the guild-definitive trait of a constituent taxa. Individual taxa that express a trait (*e.g.,* nitrate reduction), would be paired with a variety of linked "traits" (*e.g.,* differences in maximal flux, different carbon source requirements, different operational efficiency with temperature). These provide a starting point for designing more complex communities whose members can cover for one another as conditions change.

[Breakthrough Capability]: Predict and engineer interactions between different species within the microbiome.

- **2022 milestone: Establish methods for determining what metabolites are biologically available to a microbiome in any environment (*i.e.,* identify what a microbe can use to grow).**
    - [Bottleneck]: Tracing metabolites to specific microbes is dependent on culture conditions.
        - [Potential solution]: Use single-cell metagenomics or transcriptomics to reduce microbiome complexity without isolating individual species.
        - [Potential solution]: Systematically compare metabolite profiles of increasing microbial diversity to pure cultures to understand higher-order interactions and regulation of metabolites.
- **2025 milestone: From metagenomic data, identify individual microbial species with related or coherent functions.**
    - [Bottleneck]: Lack of data standards and integration across various microbe and microbiome databases (*e.g.*, GutFeeling KnowledgeBase, MGnify).
        - [Potential solution]: Open Access database with 'hidden Markov models' based on metagenome/activity data that define guilds.
    - [Bottleneck]: Many unsequenced or unidentified microbial species are in any given environment, ecosystem, or niche.
        - [Potential solution]: Use untargeted -omics surveys to classify and identify all the microbial species present in various environments.
    - [Bottleneck]: Genes with disparate sequence and hypothetical proteins may not be flagged as a particular guild leading to the functional mislabeling of microbial species.
        - [Potential solution]: Integrate traditional molecular biology and genetic techniques to the study of synthetic microbial communities to further classify and assign gene function for genes important for interactions and behavior of microbiomes.
- **2030 milestone: Engineer and optimize microbiomes where mutually-required interactions between species and functional guilds are implemented (Ziesack et al., 2019).**
    - [Bottleneck]: The presence of a given metabolite that is intended to trigger population growth of a mitigating community member (*e.g.,* production of a toxic

metabolic byproduct that can be neutralized by a complementary organism) may not be detectable as a direct trigger.
        - [Potential solution]: Engineer sensor capabilities into the responsive organism such that it is able to upregulate its activity in response to the metabolite of interest, or in response to another correlated metabolite that is more easily sensed.
- **2040 milestone: Generate predictive models and experimental frameworks to engineer microbiomes with defined species composition and interactions.**
    - [Bottleneck]: Significant technological advances will be needed to measure and predict the effect of new species members on a microbiome.
        - [Potential solution]: Combine high-throughput methods with sensor and response guilds developed in earlier milestones.

[Breakthrough Capability]: Predict and engineer interactions between a microbiome and the environment (e.g., temperature, oxygen concentration, pH, small molecules or drugs, dietary compounds).

- **2022 milestone: Engineer non-model microbiome species to activate a genetic program in response to a *single* environmental perturbation (Daeffler et al., 2017; Naydich et al., 2019; Piraner et al., 2017; Riglar et al., 2017).**
    - [Bottleneck]: Signal transduction can vary significantly between species, requiring fine-tuning for each new species.
        - [Potential solution]: Introduce synthetic signaling pathways so inputs/responses can be easily tuned between different guild/microbiome members.
    - [Bottleneck]: Disentangling environmental responses from effects caused by the loss of a microbiome's species, guilds, or symbionts.
        - [Potential solution]: High-throughput screening methods to determine the sensor-regulator responses employed across different environmental stimuli in varied community compositions.
- **2025 milestone: Establish and validate experimental frameworks to engineer microbiomes responsive to the desired environmental perturbations.**
    - [Bottleneck]: Designing sensor-regulator schemes that can be employed across species and functional guilds.
        - [Potential solution]: Identify universal systems or create orthogonal expression systems that modulate multiple species' responses simultaneously.
- **2030 milestone: Engineer a microbiome that responds to multiple environmental perturbations.**
    - [Bottleneck]: Integrating responses for all guilds across multiple environments, including conditions that have null or non-optimal responses.
        - [Potential solution]: Create modeling or machine learning pipelines that identify optimal design strategies depending on how individual guilds behave individually in response to complex environmental cues (*e.g.,* temperature/oxygen/moisture gradients, decreasing pH with growth).

- **2040 milestone: "Routine" prediction and manipulation of any species within a microbiome using environmental perturbations for desired functions.**
    - [Bottleneck]: Greater understanding of microbial ecology within the community and environment (*i.e.,* what are the underlying ecological rules and principles that drive communities that experience perturbations).
        - [Potential solution]: Design ways for microbes to gain selective advantages and then restore balance from the top down with previous guild implementation.

[Breakthrough Capability]: Establish Design-Build-Test-Learn pipelines for bottom-up microbiome engineering – from culturing protocols, to genetic tools, to 'deployment' in controlled and natural microbiomes.

- **2022 milestone: Develop standard protocols for microbiome stock maintenance and propagation.**
    - [Bottleneck]: Standard protocols are not well defined, even for many pure bacterial cultures.
        - [Potential solution]: Invest in more robust biometrology, to identify minimal culture parameters that are necessary to ensure consistent growth between labs.
- **2022 milestone: Computationally predict behaviors of individual microbes within the biome and determine minimal nutrient requirements.**
    - [Bottleneck]: Individual species of the microbiome may behave differently when cultured individually or in the presence of other species in the guild. Assembling microbiomes from scratch needs optimization.
        - [Potential solution]: Utilize high-throughput metabolomics with artificial intelligence to better predict how guild interactions impact growth.
- **2022 milestone: Engineer markerless selection methods that allow for stable maintenance of an engineered strain in a microbiome.**
    - [Bottleneck]: We lack the ability to perform rapid, high-throughput genetic manipulation on nonmodel organisms.
        - [Potential solution]: Engineer large collections of *Escherichia coli* cloning strains with unique DNA methylases, to increase the probability of success when transforming or conjugating DNA into a novel host.
    - [Bottleneck]: Plasmids can function slightly differently in different host organisms, requiring slight modifications or reengineering.
        - [Potential solution]: Design a broad range of well-defined, minimal, modularized vector systems with different conjugal proteins, markers, and GC-content.
- **2025 milestone: Expand use of and capacity of high-throughput platforms to allow for increased experimental reproducibility and accuracy.**
    - [Bottleneck]: Measurements often need to be performed under specialized conditions to prevent contamination and reduce safety concerns.

        - [Potential solution]: Create dedicated satellite facilities that allow for remote analysis of microbiomes and can train staff on specialized techniques or equipment.
    - [Bottleneck]: Equipment vendors often use various automation systems that need to be specially programmed to integrate properly.
        - [Potential solution]: Train programming experts to familiarize them with instruments and control softwares from different companies and develop algorithms for full integration from DNA assembly, transformation, outgrowth, and screening.
    - [Bottleneck]: Many high-throughput systems are engineered with model organisms (primarily *Escherichia coli*) in mind, and are not well-suited to the needs of non-model organisms (***e.g.,*** not built for long incubation time frames, anaerobic conditions, working with non-traditional substrates).
        - [Potential solution]: Engineer automated high-throughput systems that can handle non-model organisms better.
- **2030 milestone: Engineer methods that enable fully autonomous microbiome characterization (*e.g.,* grow in culture, measure metabolism) with limited prior knowledge of a sample.**
    - [Bottleneck]: Many untargeted -omics techniques require either large sample inputs or labor-intensive purification protocols due to limits of detection inherent in the technique.
        - [Potential solution]: Identify a limited set of compounds, genes, or metabolites that are sufficient for characterizing growth to allow for more targeted -omics that can be performed without hands-on interventions.
- **2040 milestone: Rapidly develop tools to control any organism (*e.g.,* identify culture conditions, genetic manipulation, spatial localization), including uncultivated organisms, after initial identification via sequencing.**
    - [Bottleneck]: Every new organism will have unique nutrient/growth requirements and physiology, that make it challenging for existing knowledge to be applied directly.
        - [Potential solution]: Improve tools used to generate metabolic models from metagenomics and metagenome-assembled genomes to enable more rapid cultivation of “uncultivated” organisms (The Genome Standards Consortium et al., 2017).

Functional Biodiversity

Goal 3: Engineer microbiomes robust to evolutionary and environmental pressures.

[Current State-of-the-Art]: Designing microbiomes that resist natural ecological and evolutionary forces will require a deeper understanding of both the dynamics within a microbiome and how environmental factors impact its growth and function. Lab grown microbiomes are still in their very early stages of development, limiting our understanding of how evolutionary and environmental pressures drive microbiome composition over time (Koskella et al., 2017). However, some advances in microbiome engineering have been made that will be useful for addressing known challenges.

Balancing the growth rates of microbial species within a microbiome has been challenging (McCardell et al., 2019; Shou et al., 2007), but differences have been mitigated by implementing feedback controllers on growth using synthetic genetic circuits (Ziesack et al., 2019). Predictive methods and computational models that can capture the landscape of fitness across various initial conditions and environmental perturbations are under development (Gutiérrez et al., 2017; Matyjaszkiewicz et al., 2017). A guild may also need to resist dispersion to maintain its function, which requires the ability to adhere cells into defined morphologies and patterns, as described by this cell-to-cell adhesion toolbox (Glass & Riedel-Kruse, 2018). Some progress has been made to engineer microbial social interactions (Kong et al., 2018), but additional progress will be needed to engineer interactions between kingdoms, which will be key for resisting evolutionary pressures in microbiomes.

[Breakthrough Capability]: Engineer robust and stable microbiomes that are resilient to evolutionary and environmental stress (e.g., environmental variations, dispersion forces, nutrient deprivation).

- **2022 milestone: Engineer high-throughput systems to measure coevolution of species within a guild over time.**
    - [Bottleneck]: Most efforts to observe and quantify coevolution have used simple, two-species communities (Hall et al., 2020).
        - [Potential solution]: In coordination with existing microbiome data repositories, establish quantifiable parameters to describe symbiotic relationships and mechanisms of succession between members of a functional guild (*i.e.,* define how guild members interact and compete with each other).
- **2022 milestone: Use a model microbiome to examine responses to well-defined ecological stresses (*e.g.,* temperature change, moisture gradients, UV exposure) in a contained environment.**
    - [Bottleneck]: Most microbiome models are low-throughput, making data collection slow and laborious.
        - [Potential solution]: Increase throughput of existing model systems, and use machine learning to determine minimum parameters for modeling microbiomes.

- **2025 milestone: Design guilds that maintain their function over time and when biologically destabilizing factors are introduced (*e.g.,* mobile genetic elements, plasmids, phages, nutrient deprivation).**
    - [Bottleneck]: Microbes have a tendency to lose genes that are redundant with others in the community.
        - [Potential solution]: Engineer guilds so their defining function can be exchanged between members (*e.g.,* via a plasmid, phage, mobile genetic element) to decrease the overall fitness cost.
    - [Bottleneck]: Competitive or antagonistic interactions between organisms will introduce strong selective pressures that may disrupt guild function.
        - [Potential solution]: Engineer mutualistic, positive interactions within a guild to help resist selective pressures.
        - [Potential solution]: Engineer succession within a guild, so as one guild member is outcompeted or otherwise impaired, another guild member grows up to fill the functional niche.
- **2025 milestone: Engineer guilds that respond to an environmental stress to preserve function (*e.g.,* buffers environment in response to pH change, antibiotic/antiviral produced in response to predator, 'nutrient scavenging molecule' in response to decreases).**
    - [Bottleneck]: A single environmental change can alter many different environmental parameters (*e.g.,* pH changes in response to temperature, nutrient availability may change in response to pH).
        - [Potential solution]: Many existing microbial signal pathways (*e.g.,* two-component systems) respond to multiple environmental signals and could be engineered to compensate for multiple different input signals.
- **2030 milestone: Engineer microbiomes to kill cells that drift away from the guild's function (*e.g.,* producing a specific metabolite, degrading specific compounds) (Stirling et al., 2017).**
    - [Bottleneck]: It may be difficult to properly tune the response and prevent accidental targeting due to natural fluctuations in activity or metabolism.
        - [Potential solution]: Microbiomes will need to be engineered so multiple input signals are received before targeting, similar to signal tuning in T cell receptor development.
- **2030 milestone: Engineer species that help a microbiome resist dispersion forces by producing biofilms or other materials.**
    - [Bottleneck]: Natural, multispecies biofilms are relatively poorly characterized, so significant advances will be needed to engineer biofilm formation (Røder et al., 2016).
        - [Potential solution]: Many of the tools developed to characterize microbiomes will contribute to studying biofilm communities. However, the unique physical properties of biofilms (*e.g.,* chemical resistance, adhesion to surfaces) mean that additional considerations will be needed for all experiments (*e.g.,* high-throughput liquid handling, -omics techniques).

- **2040 milestone: Engineer microbiomes that contain diverse functional guilds (*i.e.,* multiple species per guild) and maintain their function in natural environments.**
    - [Bottleneck]: Engineered microbiomes in natural environments may require stability over significantly longer time periods compared to controlled environments (*e.g.,* weeks in a bioreactor versus months or years in a field).
        - [Potential solution]: Design guilds that can go dormant (*e.g.,* halt growth, form spores) to reduce competition with other guilds, and are responsive to other guilds or the host environment for cues to start growing again.
- **2040 milestone: Engineer guilds that evolve in a manner that is directed by environmental conditions or nutrient availability, so optimal function continues even as changes occur.**
    - [Bottleneck]: Any organism designed to mutate and evolve will have a higher chance of losing engineered function in exchange for increased growth.
        - [Potential solution]: Use burden driven feedback control systems to broadly reduce mutation rates in engineered microbiomes (Ceroni et al., 2018), and further characterize mutation rates and evolutionary drivers to define necessary interventions in different environments (*e.g.,* antibiotic selection may not be needed) (Riglar et al., 2017, 2019).

Distributed metabolism

One of the distinguishing characteristics of a microbiome is that a community of microbes can work cooperatively to produce or degrade compounds, performing chemistry that could not be done by a single species on its own (Haoran Zhang et al., 2015) (Figure 4). Metabolic intermediates may be toxic to one species, but readily metabolized by another species. Unique enzyme functionalities may be easier to engineer in one species than another (Zhou et al., 2015). Engineering microbiomes, rather than individual microbes, makes it possible to optimize metabolic processes and expand synthesis and degradation outside the chemical compound space found in nature. This theme focuses on how to build and manipulate cooperative microbial communities that perform novel biochemistry, both for synthesis and degradation.

Distributed Metabolism breaks this challenge down into three goals. Goals 1 and 2 examine how to utilize fixed or reduced carbon and convert it into a new product. Goal 1 focuses specifically on synthesizing more complex molecules from simple inputs (*e.g.,* sugars, organic acids), while Goal 2 focuses on degrading more complex or recalcitrant molecules (*e.g.,* lignocellulose, pollutants) to simpler precursors for new syntheses (Minty et al., 2013). Goal 3 asks how microbiomes capable of harnessing alternative energy forms for growth (*e.g.,* photoautotrophs, lithoautotrophs) can use that energy to fix inorganic carbon and produce a desired compound.

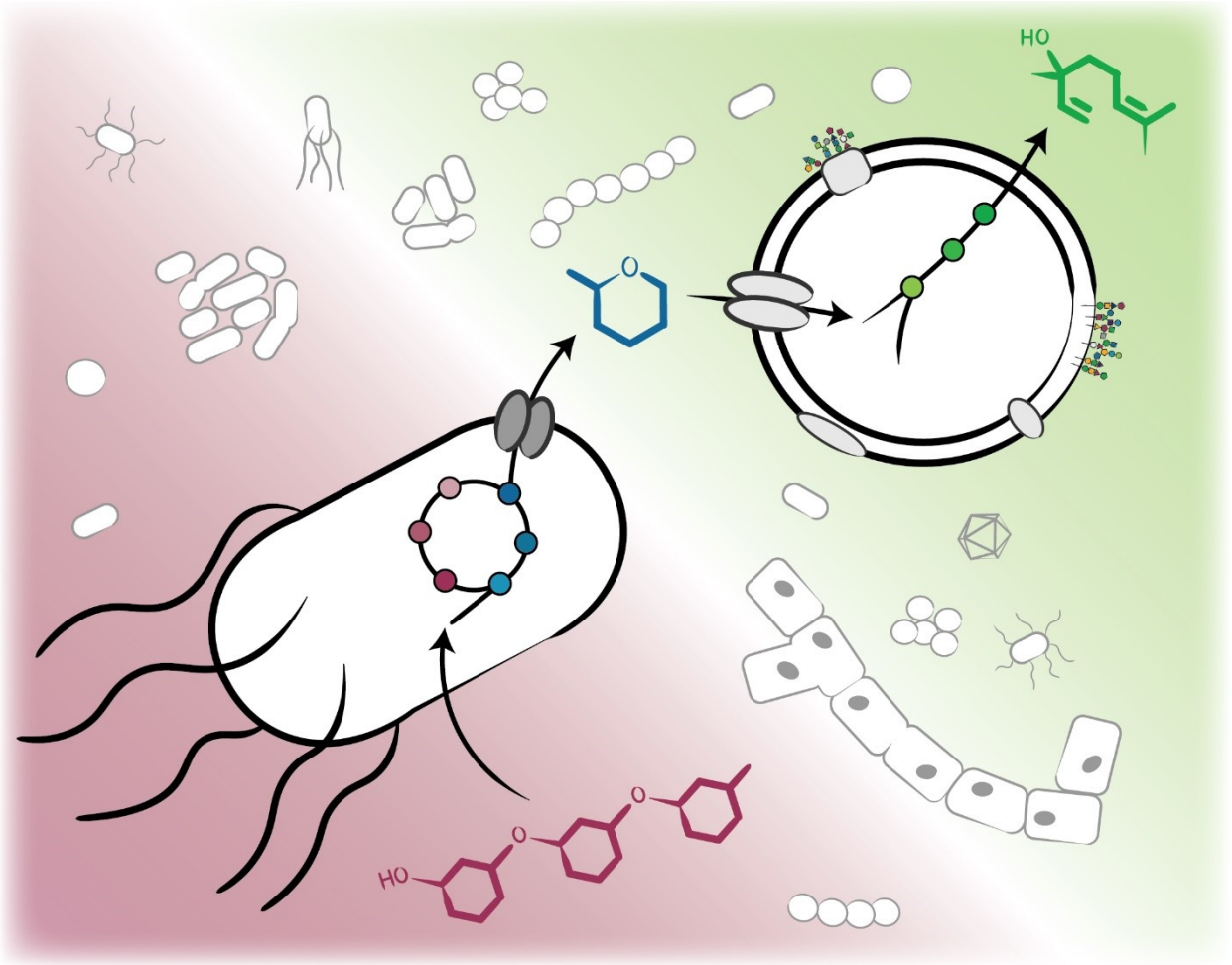


Figure 4. Distributed metabolic processes in an engineered microbiome can facilitate difficult or novel chemical transformations. Production of high-value compounds like biofuels, solvents, flavorings, and polymers can be facilitated by engineered microbiomes. For example as illustrated, lignocellulose degradation can be accomplished by one organism within the microbiome and directly converted into higher-value products, in this case linalool, immediately within the community, leading to more efficient and flexible metabolic processes. Further advances will also enable directed metabolic syntheses across disparate environments (e.g., aerobic and anaerobic) that are otherwise impossible using microbial monocultures.

EBRC

MICROBIOME ENGINEERING: DISTRIBUTED METABOLISM

Goal	Breakthrough Capability	Milestone

Produce natural and non-natural compounds using multi-species microbiomes.

Distributed compound biosynthesis using multiple microbial species.

Identify and engineer transporters, sensors, and/or extracellular enzymes that facilitate the movement of intermediate compounds within a microbiome.	Engineer species to convert common biological intermediates or electron carriers (*e.g.,* acetate, formate, butyrate, ethanol) into higher value precursor molecules for further transformations. Produce additional compounds (*e.g.,* addition of multiple functional groups) using a different microbial species to catalyze the reaction.	Engineer multi-step synthetic pathways spanning multiple organisms, to reduce accumulation of toxic intermediates.	Spatially organize communities with distinct catalysis environments (*e.g.,* reducing or oxidizing) to facilitate more efficient and specific biosynthesis.

Compound biosynthesis using community members that support a primary production strain.

Engineer microbiomes to remove inhibitory compounds (such as hydrogen or sulfur-containing compounds) to stimulate catalysis in a primary species.	Engineer microbiomes to maintain homeostasis in the local environment to ensure highest efficiency of the primary producer strain *in situ*.	Use existing microbial partnerships to synthesize value-added compounds (*e.g.*, anaerobic fungi convert plant biomass into ethanol, acids, and hydrogen gas. Methanogens and other hydrogenotrophs use the hydrogen gas as an electron donor to make something valuable, like methane).	Design communities of organisms consuming multiple different carbon sources, all of which transfer their reducing power to a universal carrier (*e.g.,* microbial species, extracellular protein, small molecule) that can be used by one or more producer organisms for biosynthesis.

Rapid design of engineered microbiomes that functionally complement existing natural microbiomes.

Develop sampling and analysis methods that can be adapted to diverse environmental niches.	Identify 'available' environmental niches based on the metagenomic, metatranscriptomic and metabolomic profiles of an existing natural community.	Design a microbiome capable of invading a natural community and producing a specific metabolite in the environment of interest.	Generate a modular toolbox of microbes with defined function to support the desired environmental niche (*e.g.,* provide resistance to compounds, generate specific products or byproducts).

2022 → 2025 → 2030 → 2040

Engineer microbiomes to transform recalcitrant materials into useful products.

2022	2025	2030	2040
Engineer communities metabolically tailored to capture and degrade recalcitrant materials.			
Develop tools to identify partner microbes for a given feedstock composition.	For a known compound, design a microbiome to degrade it and experimentally demonstrate partial degradation of the compound.	Develop retro biodegradation programs that can rationally design microbiomes to break down a non-natural compound with high efficiency.	Engineer dynamic community stability.
Engineer parallel degradation of inputs using normally incompatible chemistries (*e.g.,* combine anaerobic and aerobic processes).			
Identify organisms that independently break down different components of a compound (*e.g.,* one organism can perform one step aerobically, a second organism can perform a downstream step anaerobically).	Engineer microbiomes that can create and maintain distinct microenvironments (*e.g.,* anaerobic reaction vesicles, acidic microcompartments).	Design microenvironment-generating microbiomes, in combination with paired degradation organisms, to enable near simultaneous degradation by each organism (*i.e.,* it does not require diffusion between layers).	Create a physical linkage, or another close connection, between otherwise incompatible species that makes them dependent on each other for survival.
Engineer microbiomes that specialize in nutrient recapture and recycling to minimize inputs and create self sufficient environments.			
Design methods to identify 'usable' metabolites from bioreactor waste products.	Demonstrate process to "reuse" materials from completed biosynthesis (*i.e.,* whatever is left after the product is extracted from the bioreactor) as a feedstock for a new biosynthesis run.	Create methods to recycle dead biomass.	Engineer complex microbiomes that are self-sustaining ecosystems that recycle dead cell biomass, and are capable of transforming inputs to products.

Design synthetic microbiomes for photosynthetic and chemosynthetic electron and carbon capture for engineered bioprocesses.

Produce stable photoautotrophic or lithoautotrophic microbiomes that synthesize a value-added biochemical.

2022	2025	2030	2040
Generate a proof-of-concept photo/lithoautotrophic consortium that stably produces a value-added biochemical.	Increase energy capture efficiency in photo/lithoautotrophic communities.	Develop photoautotrophic communities that can assimilate multiple forms of carbon (*e.g.,* carbon dioxide, bicarbonate, methane) for biosynthesis.	Engineer industrial bioprocesses driven by microbiomes that harvest non-chemical energy to fix carbon and produce a value-added biochemical using a concentrated carbon dioxide or bicarbonate stream.
	Control biomass productivity-specific trade-offs using biphasic growth patterns in a proof-of-concept photo/lithoautotrophic community.		Create pilot-scale industrial processes with photo/lithoautotrophs using atmospheric carbon dioxide (~500 ppm).

Control electron flow into and within a microbiome to add exogenous reducing power to specific chemical reactions.

2022	2025	2030	2040
Design a microbiome powered by an external electron source (*e.g.,* electrofermentation, external supply of hydrogen gas or acetate) that couples electrical energy extraction to a biotransformation.	Determine experimental and modeling approaches to define critical functions of a model microbiome to improve efficiency and rate of biotransformations.	Engineer methods to tune energy or electron extraction from a microbiome based on demand (*i.e.,* limit growth rate to funnel energy into reaction processes), at lab to pilot scales.	Engineer microbiomes powered by light or electricity that are capable of performing industrial-scale biotransformations effectively.

Distributed Metabolism

Goal 1: Produce natural and non-natural compounds using multi-species microbiomes.

[Current State-of-the-Art]: Current efforts to use microbiomes for biosynthesis have focused on using consortia to improve production efficiency or to produce compounds that are difficult to synthesize using a single strain. Different strains or species have been co-cultured to optimize substrate utilization. Multiple species that are naturally well-suited to convert the molecule of interest can be co-cultured to identify optimal species compositions to achieve high titers. Mutualistic interactions can be engineered in synthetic multi-species consortia to increase fermentation efficiency (Mee et al., 2014; E.-X. Wang et al., 2016, 2019). Co-cultures of different species can also be used to convert multiple substrates into the desired products through different metabolic routes. This strategy was used to demonstrate complete conversion of glucose and xylose to ethanol (Fu et al., 2009). Similarly, when multiple sugars are available for fermentation, mixtures of "specialist" strains that ferment only one of each type of sugar can outperform individual strains that ferment multiple sugars (Eiteman et al., 2008). Microbial co-cultures have been demonstrated to use carbon dioxide and synthesis gas (syngas) to produce alcohols and other metabolites, while single cultures would only be capable of producing acetate (Haas et al., 2018; Molitor et al., 2019; Richter et al., 2016)

Another approach relies on consortia where biosynthetic reactions are distributed among naturally-occurring or engineered specialist organisms that are best-suited to perform select reactions within a metabolic pathway. Consortia have been engineered where engineered strains remove the metabolic byproducts generated by a primary producer strain (Bernstein et al., 2012). There are also several examples of production pathways split between different strains. Using this strategy, compounds previously too burdensome or toxic for using a single strain could be produced (Arai et al., 2007; J. A. Jones et al., 2017; Zhou et al., 2015). Quorum sensing regulation and division of labor have also been reported to engineer time-controlled expression of portions of the pathway and improve production (J. A. Jones et al., 2016).

Most current work has used a limited selection of model organisms (*e.g., Escherichia coli*, *Saccharomyces cerevisiae*, *Bacillus subtilis*) with well-established genetic tools. Significant advances in microbiome engineering will require expanding these models to include microbes from across the tree of life, including Archaea.

[Breakthrough Capability]: Distributed compound biosynthesis using multiple microbial species.

- **2022 milestone: Identify and engineer transporters, sensors, and/or extracellular enzymes that facilitate the movement of intermediate compounds within a microbiome.**
    - [Bottleneck]: Controlled movement of molecules within communities requires optimizing many processes with fundamentally different mechanisms (*e.g.,* diffusion, active transport, passive transport).
        - [Potential solution]: Design transporters with generic import/export functions, but have functional domains that can be altered for different compound specificities.

- **2025 milestone: Engineer species to convert common biological intermediates or electron carriers (*e.g.,* acetate, formate, butyrate, ethanol) into higher value precursor molecules for further transformations (Claassens et al., 2019).**
    - [Bottleneck]: Intermediates may be toxic to cells and inhibit microbial growth or production.
        - [Potential solution]: Engineer microbiome metabolism to decrease toxicity of specific compounds (*e.g.,* acetogenic consumption of methanol to bypass formaldehyde production).
        - [Potential solution]: Engineer microbiomes to simultaneously consume multiple intermediates for production, so they produce practical amounts of compounds without risk of cellular toxicity.
- **2025 milestone: Produce additional compounds (*e.g.,* addition of multiple functional groups) using a different microbial species to catalyze the reaction.**
    - [Bottleneck]: Importing and exporting a compound in a second cell may be difficult to achieve without it being metabolized or significant yield being lost.
        - [Potential solution]: Modifications could be performed outside the cell, by engineering a second microbe that secretes enzymes extracellularly.
- **2030 milestone: Engineer multi-step synthetic pathways spanning multiple organisms, to reduce accumulation of toxic intermediates.**
    - [Bottleneck]: Many bioactive compounds are the result of long metabolic pathways, so differentiating between toxicity and general cell stress (due to large gene clusters) may be challenging.
        - [Potential solution]: Improve existing metabolomics models to better distinguish between rate-limiting catalysis, metabolite toxicity, or additional factors.
        - [Potential solution]: Design biosensors to detect metabolic bottlenecks and indicate which parts of a pathway should be moved to a different organism.
    - [Bottleneck]: In natural environments, exported compounds may be metabolized by other non-engineered microbes.
        - [Potential solution]: Engineer microbes that self-associate into higher density clusters to outcompete non-engineered microbes.
- **2040 milestone: Spatially organize communities with distinct catalysis environments (*e.g.,* reducing or oxidizing) to facilitate more efficient and specific biosynthesis.**
    - [Bottleneck]: Microbiomes must be able to resist dispersive forces or reorganize a disrupted structure in natural environments.
        - [Potential solution]: Engineer exosomes/liposomes that can incorporate proteins from multiple organisms (*i.e.,* multiple vesicles fuse together and all cargo intermingles) to create spatially separated reaction compartments.
        - [Potential solution]: Engineer cell surface proteins that facilitate specific flocculation properties so sub-groups of microbes naturally reassociate when disrupted.

[Breakthrough Capability]: Compound biosynthesis using community members that support a primary production strain.

- **2022 milestone: Engineer microbiomes to remove inhibitory compounds (*e.g.,* hydrogen or sulfur-containing compounds) to stimulate catalysis in a primary species.**
    - [Bottleneck]: Anaerobic microbial communities frequently produce hydrogen as an electron sink for metabolism, but high levels of hydrogen inhibit enzyme function and reduce substrate to product conversion.
        - [Potential solution]: Design microbiomes containing hydrogenotrophic microbes alongside hydrogen-producing microbes, so that the hydrogenotrophs can detoxify excess concentrations of hydrogen.
- **2025 milestone: Engineer microbiomes to maintain homeostasis in the local environment to ensure highest efficiency of the primary producer strain *in situ*.**
    - [Bottleneck]: Environmental variables are unknown, including chemical composition and presence of pre-existing microbial communities in the area of deployment.
        - [Potential solution]: Develop flexible and modular consortia where individual components (taxa) are interchangeable and can be optimized to the particular environmental conditions in the area of deployment.
- **2030 milestone: Use existing microbial partnerships to synthesize value-added compounds (*e.g.,* anaerobic fungi convert plant biomass into ethanol, acids, and hydrogen gas. Methanogens and other hydrogenotrophs use the hydrogen gas as an electron donor to make something valuable, like methane) (Kracke et al., 2015).**
    - [Bottleneck]: Mechanisms of extracellular transfer of reducing power (*e.g.,* secretion of reduced compounds such as hydrogen, electrode-microbe interactions) may not be compatible between electron-donating and accepting organisms.
        - [Potential solution]: Heterologous expression of pathways for direct extracellular electron transfer or uptake to enhance efficiency of syntrophic partnerships.
- **2040 milestone: Design communities of organisms consuming multiple different carbon sources, all of which transfer their reducing power to a universal carrier (*e.g.,* microbial species, extracellular protein, small molecule) that can be used by one or more producer organisms for biosynthesis.**
    - [Bottleneck]: Identifying a universal carrier that is compatible with all organisms in the microbiome may be difficult.
        - [Potential solution]: Explore use of compounds that can pass through membranes such as neutral red and anthraquinone-2,6-disulfonate (AQDS).
    - [Bottleneck]: Electron transfer may be inhibited or quenched by compounds found in the local environment and/or may react to form toxic radicals.

        - [Potential solution]: Engineer constituents of the community to scavenge compounds created by rogue electron transfer events such as superoxide and hydroxyl radical.
        - [Potential solution]: Design community to include small numbers to respire or remove oxygen that may intrude into the system.
        - [Potential solution]: Identify reactive compounds in the local environment and engineer strains able to quench or transform these compounds.

[Breakthrough Capability]: Rapid design of engineered microbiomes that functionally complement existing natural microbiomes.

- **2022 milestone: Develop sampling and analysis methods that can be adapted to diverse environmental niches.**
    - [Bottleneck]: Many environments have unique challenges (*e.g.,* accessibility, sample size, sample density) that limit high-throughput sampling.
        - [Potential solution]: Design remote, core facilities that enable high-throughput sampling (*e.g.,* EcoFABs or EcoPods), and can invest in new high-throughput mammalian sampling as well.
- **2025 milestone: Identify 'available' environmental niches based on the metagenomic, metatranscriptomic and metabolomic profiles of an existing natural community.**
    - [Bottleneck]: Environmental niches are frequently colonized stochastically by the first microbes adapted to that environment. However, other microbes may exist that could thrive in those niches and improve community function.
        - [Potential solution]: Computational models that can predict community output (*e.g.,* metabolites consumed/produced, essential cofactors required) as a function of microbiota composition, metabolic function, and microbial competition.
- **2030 milestone: Design a microbiome capable of invading a natural community and producing a specific metabolite in the environment of interest.**
    - [Bottleneck]: Establishing new microbial members to introduce a new function that outcompetes the existing community is hard. Existing communities are frequently robust, and resist introgression of other species.
        - [Potential solution]: Engineer a microbe that temporarily induces dysbiosis (*e.g.,* secretes an antimicrobial compound) to destabilize the community and create a niche for the introduced species.
- **2040 milestone: Generate a modular toolbox of microbes with defined function to support the desired environmental niche (*e.g.,* provide resistance to compounds, generate specific products or byproducts).**
    - [Bottleneck]: Interactions between individual functions can be hard to predict and reduce performance.
        - [Potential solution]: Improved models - community databases of microbe functions that help create design rules for microbiome composition.

Distributed Metabolism

Goal 2: Engineer microbiomes to transform recalcitrant materials into useful products.

[Current State-of-the-Art]: Both natural (*e.g.,* lignin, cellulose, lignocellulose) and non-natural (*e.g.,* plastics, other polymers) recalcitrant materials are difficult to degrade into usable precursor molecules for other chemical processes (Ragauskas & Yoo, 2019). Microorganisms have been engineered to degrade several non-natural compounds in the laboratory but they tend to be non-model organisms with underdeveloped tool-sets to facilitate their engineering to use produced precursors for biosynthesis (Henske et al., 2018; Minty et al., 2013). We currently have poor control over the degradation products that are produced, which can make biosynthesis with the liberated precursors suboptimal. There are many natural organisms that have been shown to degrade non-natural compounds when exposed to them over time, including chlorinated alkenes and polychlorinated biphenyls (Bedard, 2008). Finally, much of the work on engineering the breakdown of non-natural products has been performed using single microorganisms, not in microbiomes.

[Breakthrough Capability]: Engineer communities metabolically tailored to capture and degrade recalcitrant materials.

- **2022 milestone: Develop tools to identify partner microbes for a given feedstock composition.**
    - [Bottleneck]: The needed info is distributed over dozens of enzyme- and species-specific databases and papers and not curated in useful functional databases.
        - [Potential solution]: Develop curated resources that link specific microbes with degradation and kinetics of specific metabolic inputs/outputs.
- **2025 milestone: For a known compound, design a microbiome to degrade it and experimentally demonstrate partial degradation of the compound.**
    - [Bottleneck]: Degradation pathways are less well-characterized than synthesis pathways in most organisms, so models will need significantly more input data.
        - [Potential solution]: High-throughput models that perform metabolomics on communities fed specific compounds, to track degradation products and composition ratios.
- **2030 milestone: Develop retro biodegradation programs that can rationally design microbiomes to break down a non-natural compound with high efficiency.**
    - [Bottleneck]: We do not have computational programs that can model the available metabolism in a community, or even in individual organisms, which will be necessary to predict the metabolism that is needed to completely mineralize a compound.
        - [Potential solution]: Utilize black box machine learning models to better understand metabolic inputs and outputs at the microbiome level, rather than tracking specific metabolites as they move within a community.
- **2040 milestone: Engineer dynamic community stability.**
    - [Bottleneck]: As inputs are consumed and exhausted, the need for intermediate transformations and their microbes may diminish. Current approaches to community stability rely on mutualistic exchange between all members which

destabilizes the community and its function as the importance of individual members diminishes.
    - [Potential solution]: Engineer universal degradation communities that autonomously enhance/suppress growth of individual species to enable efficient compound degradation.

[Breakthrough Capability]: Engineer parallel degradation of inputs using normally incompatible chemistries (e.g., combine anaerobic and aerobic processes).

- **2022 milestone: Identify organisms that independently break down different components of a compound (*e.g.,* one organism can perform one step aerobically, a second organism can perform a downstream step anaerobically).**
    - [Bottleneck]: A different environment is needed for each reaction, and degradation has to happen in a set order.
        - [Potential solution]: Design a reactor with different chemical zones (*e.g.,* aerobic top layer, anaerobic bottom layer) and have the first degradation step happen in the aerobic zone before metabolites sink to the anaerobic zone for the second step.
- **2025 milestone: Engineer microbiomes that can create and maintain distinct microenvironments (*e.g.,* anaerobic reaction vesicles, acidic microcompartments).**
    - [Bottleneck]: Environmental growth conditions must be compatible with the growth needs of both organisms.
        - [Potential solution]: Create a structured biofilm and use a third partner organism to mediate material transfer from one microenvironment to another.
- **2030 milestone: Design microenvironment-generating microbiomes, in combination with paired degradation organisms, to enable near simultaneous degradation by each organism (*i.e.,* it does not require diffusion between layers).**
    - [Bottleneck]: Resisting dispersive forces and environmental variations requires well-regulated microbe-microbe communication networks to maintain homeostasis.
        - [Potential solution]: Engineer biofilms that help physically maintain an intended microbiome composition, and are selectively permeable to compounds of interest.
- **2040 milestone: Create a physical linkage, or another close connection, between otherwise incompatible species that makes them dependent on each other for survival.**
    - [Bottleneck]: Organisms may have significantly different nutritional or environmental requirements that prevent them from growing well in close proximity.
        - [Potential solution]: Engineer synthetic toxin/anti-toxin systems, where the anti-toxin synthesizing protein is split between two species, placing a selective pressure on microbes to colocalize.

[Breakthrough Capability]: Engineer microbiomes that specialize in nutrient recapture and recycling to minimize inputs and create self sufficient environments.

- **2022 milestone: Design methods to identify 'usable' metabolites from bioreactor waste products.**
    - [Bottleneck]: Presence of potentially toxic byproducts and solvents that slow or inhibit microbial growth.
        - [Potential solution]: Develop extraction methods that limit the use of solvents and engineer microbiomes that can metabolize toxic intermediates.
- **2025 milestone: Demonstrate process to "reuse" materials from completed biosynthesis (*i.e.,* whatever is left after the product is extracted from the bioreactor) as a feedstock for a new biosynthesis run.**
    - [Bottleneck]: Presence of potentially toxic byproducts and solvents that slow or inhibit microbial growth.
        - [Potential solution]: Develop extraction methods that limit the use of solvents and engineer microbiomes that can metabolize toxic intermediates.
- **2030 milestone: Create methods to recycle dead biomass.**
    - [Bottleneck]: Dead biomass consists of complex biomolecules that must be broken down for reuse.
        - [Potential solution]: Develop microbiomes that persist in an ecosystem, but only break down dead cellular materials.
- **2040 milestone: Engineer complex microbiomes that are self-sustaining ecosystems that recycle dead cell biomass, and are capable of transforming inputs to products.**
    - [Bottleneck]: Ability to engineer needed inter-kingdom interactions is unprecedented. The dynamics of predator-prey interactions will lead to emergent bistability, which may be difficult to control.
        - [Potential solution]: Engineer microbial/bacterial apoptosis. Create a predator-prey system for recycling and maintaining stability by including microbes from all kingdoms (*e.g.,* Protists eat dead bacteria, dying protists release enzymes to break down their bodies to sustain the next generation of microbes in the community).

Distributed Metabolism

Goal 3: Design synthetic microbiomes for photosynthetic and chemosynthetic electron and carbon capture for engineered bioprocesses.

[Current State-of-the-Art]: Syntrophic anaerobic photosynthesis, via direct interspecies electron transfer, has been engineered between *Geobacter sulfurreducens* and green sulfur bacteria *Prosthecochloris aestaurii* (Ha et al., 2017). Butanol and hexanol can be produced using photovoltaic cells and *Clostridium* (Haas et al., 2018). However, practical application of this technology will require decreased solar energy production costs. Hybrid biological-inorganic (HBI) methods have been engineered to produce some chemicals and are rapidly increasing in efficiency (Nangle et al., 2017), but further advances are needed to scale up production and expand chemical diversity of outputs.

[Breakthrough Capability]: Produce stable photoautotrophic or lithoautotrophic microbiomes that synthesize a value-added biochemical.

- **2022 milestone: Generate a proof-of-concept photo/lithoautotrophic consortium that stably produces a value-added biochemical.**
    - [Bottleneck]: Maintaining microbiome stability and ensuring that energy is not redirected towards biomass production.
        - [Potential solution]: Engineer multiple functionally redundant pathways to prevent biomass generation and ensure continued biochemical production.
- **2025 milestone: Increase energy capture efficiency in photo/lithoautotrophic communities.**
    - [Bottleneck]: Only certain wavelengths of light are captured by any individual organism, so overall energy capture is low.
        - [Potential solution]: Engineer communities that capture multiple wavelengths (*i.e.,* a single wavelength band per organism) to maximize efficiency.
- **2025 milestone: Control biomass productivity-specific trade-offs using biphasic growth patterns in a proof-of-concept photo/lithoautotrophic community.**
    - [Bottleneck]: Lithoautotrophy does not use energy dense energy sources, and a lot of energy is used for biomass growth.
        - [Potential solution]: Engineer genetic circuits that allow for biomass accumulation initially, but then switch to compound production at the expense of growth.
- **2030 milestone: Develop photoautotrophic communities that can assimilate multiple forms of carbon (*e.g.,* carbon dioxide, bicarbonate, methane) for biosynthesis.**
    - [Bottleneck]: The 3-Hydroxypropionate bicycle accepts the most diverse selections of carbon substrates but is the most energy expensive, resulting in lower yields.

        - [Potential solution]: Use directed evolution to drive model microbiomes towards more energy-efficient enzymes within the 3-Hydroxypropionate bicycle.
- **2040 milestone: Engineer industrial bioprocesses driven by microbiomes that harvest non-chemical energy to fix carbon and produce a value-added biochemical using a concentrated carbon dioxide or bicarbonate stream.**
    - [Bottleneck]: Carbon dioxide or bicarbonate concentration/compression can be achieved today through engineering solutions, but are energy intensive processes.
        - [Potential solution]: Develop mechanisms to biologically concentrate carbon dioxide for efficient use.
- **2040 milestone: Create pilot-scale industrial processes with photo/lithoautotrophs using atmospheric carbon dioxide (~500 ppm).**
    - [Bottleneck]: Atmospheric carbon dioxide is present in low concentrations.
        - [Potential solution]: Develop mechanisms to biologically concentrate carbon dioxide for efficient use.

[Breakthrough Capability]: Control electron flow into and within a microbiome to add exogenous reducing power to specific chemical reactions.

- **2022 milestone: Design a microbiome powered by an external electron source (*e.g.,* electrofermentation, external supply of hydrogen gas or acetate) that couples electrical energy extraction to a biotransformation.**
    - [Bottleneck]: Most known extracellular electron transfer processes only function under anaerobic conditions.
        - [Potential solution]: Design microbiomes containing facultative anaerobes that can rapidly reduce/deoxygenate the growth environment to facilitate electrical energy production and extraction.
- **2025 milestone: Determine experimental and modeling approaches to define critical functions of a model microbiome to improve efficiency and rate of biotransformations.**
    - [Bottleneck]: Acquiring dynamic and species-specific data for electrochemical reactions in a microbiome is difficult.
        - [Potential solution]: Design methods to perform highly time-resolved mass spectrometry and capture reaction dynamics in a complex environment.
- **2030 milestone: Engineer methods to tune energy or electron extraction from a microbiome based on demand (*i.e.,* limit growth rate to funnel energy into reaction processes), at lab to pilot scales.**
    - [Bottleneck]: Energy can be lost, or slow to ramp up and down, when using naturally-occurring electron shuttles.
        - [Potential solution]: Engineer nanowires on microbial surfaces to directly shuttle electrons within a microbiome or to an electrode. This could allow for electron transfer between reactors and may facilitate biosynthesis of NADH or another energy-storing compound.

- **2040 milestone: Engineer microbiomes powered by light or electricity that are capable of performing industrial-scale biotransformations effectively.**
    - [Bottleneck]: Spatial organization of microbial species to maximize energy capture (*e.g.,* light-exposed volume for photosynthesis) and extraction (*e.g.,* contact surface area on electrodes).
        - [Potential solution]: Engineer interspecies nanowires to facilitate electron or energy transfer between organisms that are not exposed to an electrode or energy source.
    - [Bottleneck]: Evolutionary pressures will select for mutants/contaminating species that can use the 'excess' energy for biomass generation rather than biotransformation.
        - [Potential solution]: Engineer voltage-dependent kill switches, so cells that use excess energy for biomass are selectively removed from the microbiome.

Application Sectors

Industrial Biotechnology

Industrial Biotechnology focuses on technical challenges relevant to industrial use of synthetic biology and the establishment of the United States as a global leader in the bio-based economy. Applications of microbiome engineering in this sector focus on improving manufacturing of bio-based products by using the diversity of microbiomes to introduce greater flexibility to manufacturing processes. This flexibility comes in the form of both reduced infrastructure needs, to enable more distributed production, as well as more rapid process engineering by using custom-made microbiomes. The goal is to stimulate investment in infrastructure, streamline production, and advance our use of engineered microbiomes to improve lives.

Societal Challenge 1: Enable low-cost, distributed biomanufacturing.

- Scientific/Engineering Aim: Increase the diversity of outputs or products.
    - Microbiome Engineering Objective: Engineer 'one-pot' microbiomes that robustly produce multiple end-products from untreated or variable inputs.
        - Technical achievement: Engineer microbiomes made of bacteria with different carbon source preferences, so they can degrade complex mixtures of carbon sources and more efficiently utilize substrates without releasing carbon dioxide.
        - Technical achievement: Engineer microbiomes that utilize a single input precursor, but can produce multiple high-purity products depending on the species substituted into a community.
    - Microbiome Engineering Objective: Use microbiomes to expand the types of chemicals that can be synthesized.
        - Technical Achievement: Design microbiomes that have individual species capable of metabolizing or sequestering toxic intermediates to facilitate continued production.
        - Technical Achievement: Engineer microbiomes that can capture and scrub compounds (*e.g.,* sulfur species, carbon dioxide) that inhibit other industrial processes.
- Scientific/Engineering Aim: Increase manufacturing efficiency by reducing the need for energy- or chemical-intensive processes, and making processes more malleable and modular.
    - Microbiome Engineering Objective: Engineer microbiomes that can sense environmental or nutrient changes to regulate specific processes.
        - Technical Achievement: Engineer auto-inductive signal networks within a microbiome, so multiple gene or protein expression programs can be triggered without the need for exogenous inducers.
        - Technical Achievement: Use optogenetically engineered microbiomes to reduce the cost of chemical inducers, but maintain tighter control over cell processes than could be accomplished using environmental signals.

    - Microbiome Engineering Objective: Engineer microbiomes to secrete desired products from cells to enable rapid, low-cost separation of products from growth media.
        - Technical Achievement: Engineer microbiomes that "package" high-value chemicals in secretable liposomes or other extracellular structures.
        - Technical Achievement: Engineer synchronized microbiome lysis, so products are periodically released from the intracellular compartment without complex secretion systems or extraction methods.
    - Microbiome Engineering Objective: Engineer microbiome communities that can mitigate contamination between batches or during batch production, to allow for wider use of non-aseptic fermentation facilities.
        - Technical achievement: Engineer microbiomes that can be added to equipment post-processing to consume residual waste and nutritionally outcompete contaminants.
        - Technical achievement: Engineer microbiome biofilms that produce antimicrobial compounds (*e.g.,* antibiotics, antibacterial peptides) in the presence of contaminating microbes.
- Scientific/Engineering Aim: Optimize processes for handling more diverse bio-based inputs to reduce pretreatment and waste disposal costs.
    - Microbiome Engineering Objective: Engineer microbiomes that can accept waste as an alternative feedstock (*e.g.,* fruit/vegetable/plant waste biomass, animal byproducts) to utilize unused carbon and other compounds.
        - Technical achievement: Engineer ecological succession, so waste and microbial strains containing sensitive intellectual property are destroyed by a generic 'degradation' strain.
        - Technical Achievement: Design microbiomes that can utilize greenhouse gases (*e.g.,* carbon dioxide, carbon monoxide, methane) produced or generated by industrial processes.
        - Technical achievements: Create microbiomes that degrade waste into simple precursors (*e.g.,* sugar monomers, organic acids, amines, terpenes) that can be used to synthesize higher value products such as adhesives and polymers in remote environments (Roberts et al., 2019).
    - Microbiome Engineering Objective: Engineer microbiomes that can utilize process byproducts and low-value intermediate compounds as a feedstock.
        - Technical Achievement: Engineer strains to preferentially uptake process byproducts, instead of the inputs that will be converted into value-added products by main production strains in the microbiome.
        - Technical Achievement: Engineer microbiomes that simultaneously consume low-value metabolic intermediates (*e.g.,* acetate, formate) that may be toxic in high concentrations but can be converted to higher value intermediates or products.
        - Technical Achievement: Engineer microbiomes that can fix recaptured carbon dioxide or hydrogen gas generated by fermentative processes into usable compounds like acetone (S. W. Jones et al., 2016).

Industrial Biotechnology

Societal Challenge 2: Streamline industrial process development.

- Scientific/Engineering Aim: Enable more robust biomanufacturing processes.
    - Microbiome Engineering Objective: Engineer microbiomes to enable production in non-conventional industrial conditions.
        - Technical Achievement: Engineer microbiomes that enable production at ambient or near-ambient temperatures and pressures.
        - Technical Achievement: Engineer microbiomes that maintain their function at low or high pH.
        - Technical Achievement: Engineer microbiomes that continue functioning in high salt concentrations to reduce the need for dilution or desalination in industrial processes.
    - Microbiome Engineering Objective: Engineer microbiomes to reduce the need for aeration industrial processes.
        - Technical achievement: Design collections of gas-generating or gas-consuming microbes that grow/die in response to gas concentrations.
        - Technical achievement: Engineer 'universal buffer' strains that can prevent the buildup of toxic chemicals (*e.g.,* waste products, pH changes) in growth media.
- Scientific/Engineering Aim: Increase biomanufacturing process flexibility and adaptability, to shorten process development timeline for novel products and proof-of-concept translation into industrial workflows.
    - Microbiome Engineering Objective: Develop "modular" microbiomes that specialize in classes of metabolic functions for "plug and play" pathway engineering, so each species or strain is optimized for one step of the process.
        - Technical achievement: Create computational models that optimize microbiome composition based on the guilds needed for a given application.
        - Technical achievement: Produce libraries of strains with unique, but well-catalogued metabolic requirements and enzyme reaction capabilities, so communities can be developed based on available nutrients.
    - Microbiome Engineering Objective: Enable parallel processing (production of multiple outputs simultaneously) within a single microbiome (see also: Distributed Metabolism).
        - Technical Achievement: Design microbiomes to synthesize products that are easily separated to high purity using simple industrial processes (*e.g.,* phase separations, column separation).
        - Technical Achievement: Engineer microbiomes with distributed metabolic pathways to prevent accumulation of toxic intermediates and lost production capacity.

- Microbiome Engineering Objective: Design methods to analyze and improve performance of industrial microbiomes over time.
  - Technical Achievement: Couple product synthesis to an easily detectable readout (*e.g.,* fluorescence, luminescence, colored dye) so production can be monitored continuously or near-continuously.
  - Technical Achievement: Identify and add or remove microbes that are present in industrial fermentations at a background level to improve or modulate performance.

Health & Medicine

Health & Medicine focuses on technical challenges relevant to the well-being of humans, non-human animals, and populations. Applications of microbiome engineering in this sector focus on preventing non-communicable diseases, eradicating infectious diseases, and supporting longevity and quality of life. For related reading about tools and technologies in microbiome engineering that impact human and animal health, please see *Environmental Biotechnology* and *Food & Agriculture*.

Societal Challenge 1: Address non-communicable diseases and disorders.

- Scientific/Engineering Aim: Develop prophylactic or health-improving interventions for non-communicable diseases and disorders.
    - Microbiome Engineering Objective: Engineer microbiomes to prevent allergies and autoimmune diseases (*e.g.,* rheumatoid arthritis, inflammatory bowel disease, diabetes).
        - Technical Achievement: Engineer microbiomes to synthesize and secrete controllable levels of immune-stimulating antigens to tune down allergy-inducing immune responses.
        - Technical Achievement: Engineer microbiomes to sequester and degrade allergy-causing particles to prevent immune responses.
        - Technical Achievement: Identify changes in the gut microbiome that contribute to autoimmune diseases and engineer microbiomes to resist those shifts.
    - Microbiome Engineering Objective: Engineer gut microbiomes to reduce mental health problems and neurodegenerative diseases.
        - Technical Achievement: Engineer gut microbiomes to prevent dysbiosis associated with depression or other mental health disorders (Johnson, 2020; Valles-Colomer et al., 2019).
        - Technical Achievement: Engineer gut microbiomes to detect and break down aggregated proteins associated with Parkinson's Disease, to mitigate disease progression (Ghaisas et al., 2016).
    - Microbiome Engineering Objective: Engineer probiotic gut microbiomes.
        - Technical Achievement: Design gut microbiomes that produce beneficial molecules such as butyrate (Guo et al., 2019).
        - Technical Achievement: Engineer gut microbiomes that consume unwanted or unhealthy metabolites.
        - Technical Achievement: Engineer gut microbiomes that degrade or sequester toxins to prevent food poisoning.
    - Microbiome Engineering Objective: Design microbiomes that improve quality of life.
        - Technical Achievement: Engineer microbiomes that regulate caloric intake to prevent obesity.

        - Technical Achievement: Engineer microbiomes that allow humans to obtain energy from nontraditional carbon sources when food is not available (*e.g.,* consume grass and lignocellulosic material for energy).
        - Technical Achievement: Create microbiomes that enhance or alter mental health (*e.g.,* memory, mental acuity, emotional state) for short or long time periods.
        - Technical Achievement: Engineer microbiomes that prevent fatigue and increase alertness.
- Scientific/Engineering Aim: Dynamically treat diseases and disorders.
    - Microbiome Engineering Objective: Engineer microbiomes (*e.g.,* gut, skin, oral) to produce a missing metabolite or consume a toxic intermediate that builds up in a disease state.
        - Technical Achievement: Engineer microbiomes that compete with oral microbes to prevent plaque formation and degrade plaque-derived extracellular matrices.
        - Technical Achievement: Engineer microbiomes that monitor the levels of key metabolites and maintain homeostasis (*i.e.,* produce or degrade them if the levels are too low or high).
    - Microbiome Engineering Objective: Engineer microbiomes to enhance drug efficacy by preventing undesired drug metabolism or metabolizing leftover chemicals once treatment is complete.
        - Technical Achievement: Engineer gut microbiomes to couple their metabolism with metabolic or hormonal changes in a host (*e.g.,* human patient).
        - Technical Achievement: Engineer microbiomes to monitor, record, and report on drug metabolite and degradation product concentrations to improve drug dosing.
- Scientific/Engineering Aim: Rapid point-of-care diagnostic tools to assess patient health (*e.g.,* immune status, blood pressure, cholesterol level).
    - Microbiome Engineering Objective: Model individual health from native microbiome composition and function (*e.g.,* microbiome sequencing or transcriptomics for diagnosis).
        - Technical Achievement: Determine microbiome composition and individual species' functions under healthy conditions.
        - Technical Achievement: Generate computational models that can determine an individual's immune status from microbiome composition.
        - Technical Achievement: Model a host's disease or health status based on a microbiome's functional status (*e.g.,* transcriptional states).
    - Microbiome Engineering Objective: Engineer microbiome-based diagnostics.
        - Technical Achievement: Engineer ingestible microbiomes that capture environmental data from the human gut and provide a measurable read-out (*e.g.,* altered gene expression, protein modification) after excretion (Mimee et al., 2018; Naydich et al., 2019; Riglar et al., 2017).

- Technical Achievement: Engineer modular microbiome-based diagnostic systems to increase flexibility and adaptability of diagnostic tests (*e.g.,* one platorm that can test blood count, enzyme markers, and sexually transmitted diseases by switching out species in microbiome).
- Technical Achievement: Identify sensitive and specific microbiome biomarkers for any disease (*e.g.,* determine gut microbiome changes during co-located diseases like colon cancer or systemic diseases like lymphomas or leukemias).
- Technical Achievement: Engineer non-pathogenic microbiomes that can be administered to a person and used to assess immunosuppression or other alterations in immune status (*i.e.,* tuberculin skin test for a broader range of conditions).
- Technical Achievement: Engineer microbiomes to constitutively monitor immune-response biomarkers (*e.g.,* gut microbiome that changes color in response to low CD8+ T cell counts).
- Technical Achievement: Engineer skin microbiomes to detect biomarkers of early-stage skin diseases (*e.g.,* microbiome that produces dark pigment in response to UV damage).

- Microbiome Engineering Objective: Combine biomarkers into microbiome-based biosensor platforms.
  - Technical Achievement: Engineer gut microbiomes that can assess and report cardiovascular disease-associated microbiome shifts, such as increases in uric acid production (Tang et al., 2017).
  - Technical Achievement: Engineer oral microbiomes that can assess early stage periodontal disease by detecting pathogens (*e.g., Streptococcus mutans*) and salivary biomarkers (*e.g.,* MIP-1α) (Kc et al., 2020).

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Societal Challenge 2: Address environmental threats to health, including toxins, pollution, accidents, radiation, exposure, and injury.

- Scientific/Engineering Aim: Reduce toxicity of pollutants and other chemical and biological environmental stresses.
    - Microbiome Engineering Objective: Engineer skin and mucosal microbiomes that reduce or eliminate toxic chemical stresses.
        - Technical Achievement: Engineer lung microbiomes to quench reactive oxygen species, produced naturally and during inflammation, to reduce oxidative stress.
        - Technical Achievement: Engineer skin and mucosal microbiomes to degrade environmental carcinogens (*e.g.,* organophosphates, agrochemicals).
    - Microbiome Engineering Objective: Engineer skin and mucosal microbiomes to degrade allergens.
        - Technical Achievement: Engineer lung microbiomes that synthesize corticosteroid compounds in response to environmental inducers of asthma (*e.g.,* smoke particles, nitrogen dioxide, sulfur dioxide).
        - Technical Achievement: Engineer mucosal microbiomes that selectively capture allergens (*e.g.,* pollen, pet dander, mold, dust) in biofilms and secrete anti-IgE to reduce allergic immune responses.
- Scientific/Engineering Aim: Increase human resistance to physical environmental insults (*e.g.,* UV/Vis radiation).
    - Microbiome Engineering Objective: Engineer smart bandages containing microbiomes that promote wound healing and sense/prevent infections.
        - Technical Achievement: Engineer microbiomes that secrete antibacterial compounds in response to neutrophils or chemokines (*i.e.,* to prevent infection in a controlled manner, dependent on progression of tissue repair).
        - Technical Achievement: Engineer microbiomes that selectively secrete fibrinogen to promote blood clotting and wound healing.
    - Microbiome Engineering Objective: Engineer skin microbiomes that block or absorb UV/Vis radiation and persist for long periods after application.
        - Technical Achievement: Engineer microbiome biofilms that synthesize and secrete UV-absorbing metal iron complexes.
        - Technical Achievement: Engineer microbiomes that form biofilms to reduce moisture loss through skin and decrease the risk of dehydration in hot or dry environments.

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Societal Challenge 3: Detect and treat existing and emerging infectious diseases.

- Scientific/Engineering Aim: Modulate immune system to better recognize and respond to infectious agents.
    - Microbiome Engineering Objective: Engineer microbiomes to induce immunological tolerance to certain antigens or dampen down the severity of immune responses.
        - Technical achievement: Engineer microbiomes that express interleukins in response to inflammatory cytokines and chemokines, to reduce the risk of illness caused by cytokine storms.
        - Technical Achievement: Engineer microbiomes that facilitate phlegm and fluid transport out of the respiratory tract, to reduce the physiological impact of pneumonia.
        - Technical Achievement: Engineer immunologically silent microbiomes that localize to heart tissues and express surface proteins that bind coxsackievirus and adenovirus receptor (CAR) proteins, to decrease incidence of myocarditis-inducing viral infections.
    - Microbiome Engineering Objective: Engineer microbiomes to detect and degrade bacterial capsules in order to block immune evasion.
        - Technical Achievement: Engineer microbiomes to produce capsule degrading proteins in response to capsule-containing pathogens.
        - Technical Achievement: Engineer microbiomes that bind to and kill bacteria that produce specific capsule types.
    - Microbiome Engineering Objective: Engineer microbiomes to increase immune sensitivity to infection.
        - Technical Achievement: Engineer resident microbiomes that rapidly increase production of immunostimulatory molecules in response to pathogens (bacterial, viral, and fungal), so an immune response can be mounted prior to establishment of infection.
        - Technical Achievement: Engineer microbiomes so community members can bind to and be taken up with pathogens, to enable or inhibit cross-presentation of pathogen antigens.
- Scientific/Engineering Aim: Design prophylactic treatments that exclude pathogens and prevent or reduce virulence in opportunistic pathogens.
    - Microbiome Engineering Objective: Modulate the gut microbiome to reduce the risk of diseases caused by pathogen invasion or colonization.
        - Technical Achievement: Design microbiomes that exclude or kill cancer-causing bacteria (*e.g., Fusobacterium nucleatum*, *Helicobacter pylori*).
        - Technical Achievement: Engineer gut microbes that can eliminate bacterial pathogens (*e.g., Escherichia coli, Salmonella sp., Listeria monocytogenes*) in a strain-specific manner using a variety of mechanisms (*e.g.,* Type 6 secretion systems, antimicrobial peptides, microcins).

        - Technical Achievement: Design microbiomes that deprive pathogenic gut enterobacteria of lumenal niches through exploitative and interference competition.
        - Technical Achievement: Engineer gut microbiomes to constitutively produce antiviral compounds or to prevent viral adhesion to cells.
    - Microbiome Engineering Objective: Engineer gut microbiomes to prevent toxin-mediated diseases/pathogens.
        - Technical Achievement: Engineer microbiomes that degrade toxins before they can bind their target receptor in the gut.
        - Technical Achievement: Engineer microbiomes that produce anti-toxin proteins to neutralize toxins and prevent foodborne diseases.
    - Microbiome Engineering Objective: Engineer gut microbiomes to diminish virulence transitions in opportunistic pathogens.
        - Technical achievement: Engineer microbiomes that degrade quorum sensing molecules important for virulence transitions (*e.g., agr* cyclic peptides, acyl-homoserine lactones).
        - Technical achievement: Engineer microbiomes to prevent induction of inflammation or reduce inflammation after it is induced (*e.g.,* facultative tetrathionate respiration capabilities to mitigate pathogenic effects of *Salmonella* species).
        - Technical achievement: Engineer microbiomes to produce toxin-binding, neutralizing proteins (*e.g.,* mAb fragments against TcdAB).
    - Microbiome Engineering Objective: Engineer skin microbiomes to repel biting and stinging insects.
        - Technical Achievement: Engineer skin microbiomes to produce *Xenorabdus spp.*-derived mosquito repellant compounds (Kajla et al., 2019).
        - Technical Achievement: Engineer skin microbiomes to kill or exclude mosquito-attracting microbial species (Michalet et al., 2019).
        - Technical Achievement: Engineer skin microbiomes to produce compounds that repel stinging insects (*e.g.,* wasps, hornets).
        - Technical Achievement: Engineer skin or surface-adherent microbiomes (*e.g.,* to clothing, textiles, bed frames) that produce compounds that mask repel biting pests (*e.g.,* ticks, fleas, lice, bedbugs, spiders).
- Scientific/Engineering Aim: Design microbiomes that enhance or replace existing medicines (*e.g.,* drugs and vaccines) to protect against bacterial and viral pathogens.
    - Microbiome Engineering Objective: Engineer microbiomes to produce macromolecular biologics.
        - Technical Achievement: Engineer microbiomes that can colonize the gut, invade the gut epithelium, and produce antigens at will (*e.g.,* engineer a universal vaccine platform for foodborne pathogens).
        - Technical Achievement: Engineer microbiomes that secrete virus-trapping particles (*e.g.,* liposomes, extracellular compartments) to reduce virus infectivity.

- Microbiome Engineering Objective: Engineer microbiomes to produce small molecule drugs.
  - Technical Achievement: Engineer microbiomes that convert waste or inflammation-inducing molecules into pathogen-inhibiting compounds (*e.g.,* butyrate to suppress *Salmonella spp.*, antibiotics).
  - Technical Achievement: Engineer gut microbiomes that compete with pathogens for essential molecules (*e.g.,* secrete siderophores or chelators to sequester essential metal cofactors) without depleting the host of those same molecules.
- Microbiome Engineering Objective: Design microbiomes that use novel mechanisms to control infection.
  - Technical Achievement: Engineer microbiomes that chemotax towards antibiotic degradation products and sequester essential molecules, to nutritionally outcompete antibiotic-resistant bacteria.
  - Technical Achievement: Engineer microbiomes that express common cell-surface proteins used by pathogens to invade or enter cells, to reduce the likelihood of a successful infection.

Food & Agriculture

Food & Agriculture focuses on the tools and technologies impacting how we feed the Earth's people and animals. Microbiome engineering provides unique means and opportunities to support growing populations with more and different types of food, and address changes to food security, diet, and demand in a sustainable manner. In particular, engineering plant and soil microbiomes will be critical for managing agricultural productivity in the face of climate change and more frequent extreme weather events. Alternative proteins produced from engineered microbiomes also offer a means to continue feeding a growing population, while reducing the consumption of resources, including land, water, fertilizers, and pesticides.

Societal Challenge 1: Increase food production, security, and safety for a growing global population.

- Scientific/Engineering Aim: Engineer agricultural soils for improved plant growth (*e.g.,* improve nutrient content, improve physical qualities).
    - Microbiome Engineering Objective: Engineer agricultural soil microbiomes to increase crop nutrient capture, uptake, and assimilation.
        - Technical Achievement: Engineer microbiomes to produce plant hormones that encourage symbiotic growth between arbuscular mycorrhiza fungi and plant roots.
        - Technical Achievement: Engineer microbiomes with enhanced microbial sequestration (*e.g.,* of arsenic, heavy metals, soil toxins), that also facilitate metal deposition to create particles that can be easily removed from soils.
        - Technical Achievement: Engineer root-associated microbiomes to enhance depleted soils by reconstituting nutrients (*e.g.,* nitrogen) needed for plant growth (Van Deynze et al., 2018).
    - Microbiome Engineering Objective: Engineer soil microbiomes to locally concentrate minerals and other micronutrients to improve crop nutrient content.
        - Technical Achievement: Engineer soil microbiomes that increase bioavailability of nitrogen and phosphorus, regardless of plant host organism.
        - Technical Achievement: Design microbiomes that concentrate nitrogen, phosphorus, potassium, vitamins, and trace minerals in root rhizosphere.
        - Technical Achievement: Design microbiomes that autolyse under predetermined environmental conditions to release nutrients for plant growth.
        - Technical Achievement: Engineer microbiomes that can be grown at scale and applied to agricultural soils (*i.e.,* microbial fertilizers) to replenish nutrients that were removed during the previous growing season.

    - Microbiome Engineering Objective: Engineer microbiomes that help improve physical characteristics of soils (*e.g.,* texture, density, consistency, air).
        - Technical Achievement: Design microbiomes that produce biofilms or heat-resistant spores to help retain water in the soil.
        - Technical Achievement: Engineer synthetic soil microbiomes to produce low-cost, bio-based superabsorbents (*e.g.,* acrylic acid-secreting microbiomes to produce water-retaining polymers) on-demand.
        - Technical Achievement: Engineer microbiomes that secrete extracellular polymers to decrease erosion (*e.g.,* wind, water).
- Scientific/Engineering Aim: Improve crop resistance to biotic and abiotic stresses (*e.g.,* disease, drought, temperature, nitrogen limitation).
    - Microbiome Engineering Objective: Engineer phyllosphere and rhizosphere microbiomes to reduce plant pathogen infections.
        - Technical Achievement: Engineer microbiomes to secrete plant defense hormones (*e.g.,* salicylic acid) in response to pathogens, so the plant is protected against pathogens without altering plant growth or yield.
        - Technical Achievement: Engineer plant-associated microbiomes that produce antimicrobial compounds in response to pathogens.
    - Microbiome Engineering Objective: Engineer plant microbiomes to increase crop drought tolerance.
        - Technical Achievement: Design leaf microbiomes that produce biofilms to decrease transpiration without affecting carbon dioxide uptake.
        - Technical Achievement: Engineer microbiomes that facilitate increased water capture from atmospheric moisture, in drought or heat sensitive plants.
    - Microbiome Engineering Objective: Engineer disease suppressive soil microbiomes to improve plant health (Schlatter et al., 2017).
        - Technical Achievement: Design microbiomes that support plant resilience during stress-inducing environmental conditions (*e.g.,* drought, flood).
        - Technical Achievement: Engineer microbiomes that express decoy cell wall components that are targeted by pathogens.
        - Technical Achievement: Engineer microbiomes that secrete pathogen-specific cell-wall degrading enzymes to reduce pathogen disease pressure.
    - Microbiome Engineering Objective: Design microbiomes that facilitate above-ground nitrogen fixation through epiphytic interactions (Warshan et al., 2017).
        - Technical Achievement: Engineer microbiomes that support symbioses between plants and microbial epiphytes, such as cyanobacteria, that are capable of fixing nitrogen.
        - Technical Achievement: Engineer plant phyllosphere microbiomes to fix nitrogen via epiphytic interactions with the plant host.

- Scientific/Engineering Aim: Mitigate food spoilage.
    - Microbiome Engineering Objective: Engineer microbiomes to promote preservation and reduce reliance on refrigeration for food storage.
        - Technical Achievement: Engineer microbiomes to directly counteract spoilage-causing microbes.
        - Technical Achievement: Engineer microbiomes to dynamically express preservatives (such as benzoate) in response to specific environmental signals (*e.g.,* time, temperature, pH).
    - Microbiome Engineering Objective: Engineer microbiomes to sense and report early biomarkers of food spoilage, independent of foodborne pathogens.
        - Technical Achievement: Biosensors identified and integrated into a microbe which respond to spoilage-specific quorum sensing molecules.
        - Technical Achievement: Engineer inert microbiomes to produce propionate to inhibit the growth of other bacteria as a preservative and to prevent food spoilage.
    - Microbiome Engineering Objective: Engineer microbiomes to selectively release molecules that increase or decrease the speed of fruit ripening (*e.g.,* ethylene, methyl salicylate).
        - Technical Achievement: Design microbiomes that produce or breakdown ethylene in response to local concentrations (*i.e.,* fruits ripen quickly but are slow to spoil).
        - Technical Achievement: Engineer food-safe microbiomes that can be inoculated on the surface of fresh foods to prevent gas transfer via biofilm formation (*i.e.,* to slow oxidation or prevent reactivity to exogenous ripening hormones).
- Scientific/Engineering Aim: Reduce spread of foodborne pathogens.
    - Microbiome Engineering Objective: Design microbiomes to sense and report food-borne pathogens.
        - Technical Achievement: Design biosensors identified and integrated into a microbe which respond to toxins and/or quorum-sensing molecules (e.g. *Salmonella enterica*).
        - Technical Achievement: Engineer microbiomes to detect viral foodborne pathogens (*e.g.,* Hepatitis A) and produce colorometric indicators upon detection.
    - Microbiome Engineering Objective: Utilize engineered microbiomes to destroy environmental foodborne pathogens (*e.g., Listeria monocytogenes*, *Clostridium spp., Campylobacter spp., Toxoplasma gondii*), excluding pathogens that spread human-to-human.
        - Technical Achievement: Engineer microbiomes to break down known bacterial toxins involved in food-borne pathogens (*e.g.,* botulinum toxin).
        - Technical Achievement: Engineer microbiomes to break down known virus involved in food-borne pathogens (*e.g.,* Hepatitis A).

- Microbiome Engineering Objective: Develop engineered microbiomes that can be used for disease surveillance in animals.
  - Technical Achievement: Engineer microbiomes that produce an easily-detected (*e.g.,* colorimetric) and safely-excreted (*e.g.,* through urine or feces) metabolite when dysbiosis occurs, from pathogens or noninfectious diseases.
  - Technical Achievement: Engineer microbiomes that acquire and encode pathogen DNA to facilitate easier sequencing and tracking of pathogen evolution or recombination (*e.g.,* influenza A genome segments that get stored on a plasmid).
  - Technical Achievement: *In vivo* (*i.e.,* in animals) sensors of zoonotic diseases to improve animal health and reduce transmission to humans.

Food & Agriculture

Societal Challenge 2: Reduce the environmental impacts of food production.

- Scientific/Engineering Aim: Develop alternative food sources (*e.g.,* alternative meat, eggs, dairy, seafood) with a reduced environmental footprint that compete on taste, price, safety, and accessibility with their conventional counterparts.
    - Microbiome Engineering Objective: Leverage microbiome-based approaches to improve the production efficiency and reduce the cost of ingredients (*e.g.,* flavorings, fatty acids, enzymes) produced through industrial biotech for use in the alternative protein industry.
        - Technical Achievement: Identify plant-based or fermented food ingredients whose biosynthesis entails complex pathways and cannot reasonably be achieved with high efficiency within a single product host.
        - Technical Achievement: Engineer microbial communities that coexist in the appropriate ratios to synthesize ingredients requiring complex pathways with maximal efficiency, such that no community member represents a limiting step in biosynthesis.
        - Technical Achievement: Utilize microbiomes to create novel feedstocks, either for subsequent fermentation or for use in cell culture media for cultivated meat, from complex agricultural or industrial biotechnology residual biomass at low cost.
    - Microbiome Engineering Objective: Engineer modular, diverse microbiomes to improve the functionality of plant-based proteins and reduce the environmental impact of processing for protein-enriched ingredients used in plant-based meat products.
        - Technical Achievement: Identify food-safe microbial strains that express enzymes with functionalizing capabilities for improving the properties of plant-based proteins (*e.g.,* solubility, emulsification, gelling capacity, water- and fat-binding capacity).
        - Technical Achievement: Develop engineered microbial communities that can act simultaneously or sequentially to improve plant protein functionality (*e.g.,* a population with high hydrolysis activity may first be needed to improve solubility, followed by takeover by a population with high crosslinking activity to recapture gelling capacity).
        - Technical Advancement: Develop microbial communities that are optimized to efficiently utilize plant-based substrates for fermentation processes that have historically been used on dairy or meat to endow foods like plant-based cheeses and salamis with complex flavors.
    - Microbiome Engineering Objective: Develop new single-cell protein production platforms that offer optimized nutritional properties, decrease costs, and leverage nonconventional feedstocks (*e.g.,* methanotrophic bacteria, photosynthetic cyanobacteria, green algae).
        - Technical Achievement: Conduct high-throughput screening of a wide array of microbial species to identify a suite of candidates that are suitable as novel protein sources (*e.g.,* devoid of known toxins, low

nucleic acid content, high digestibility and protein content, neutral or savory taste).
        - Technical Achievement: Develop stable microbiomes that exhibit balanced amino acid ratios, desirable flavor palettes, and the ability to leverage a diverse array of feedstocks including agricultural or industrial biotechnology sidestreams.
        - Technical Achievement: Utilize metabolic engineering to identify toxic or undesirable metabolites produced by some candidate strains, and screen the candidate library for strains that are able to metabolize those products as carbon sources to convert into usable biomass.
        - Technical achievement: Engineer communities that are able to collectively combat contamination by undesirable strains (*e.g.,* develop synthetic-lethal systems whereby two strains must coexist in equal ratios to keep each other alive and together synthesize competitor-targeted toxic metabolites when competitive strains disrupt this balance).
    - Microbiome Engineering Objective: Engineer structured meat alternatives composed entirely or almost entirely of microbes.
        - Technical Achievement: Engineer microbiomes for partial conversion of lignocellulosic biomass into human-edible macromolecules (even as precursors for food production), such that the fibrosity of the starting material is maintained while improving palatability and digestibility.
        - Technical Achievement: Identify or engineer microbial communities that can collectively form sophisticated macro-scale structures (*i.e.,* biofilms) that recapitulate the anisotropy of meat, with discrete fibers, structural alignment, and non-heterogeneous regions of soft and firm tissue.
- Scientific/Engineering Aim: Reduce the environmental impact of animal husbandry.
    - Microbiome Engineering Objective: Reduce reliance on antibiotics for animal growth, weight gain, and health.
        - Technical Achievement: Engineer gut microbiome structure to be resilient to invasion by pathogenic bacteria.
        - Technical Achievement: Engineer microbiomes that grow in animal feed to detect and kill environmentally-occurring animal or human pathogens.
        - Technical Achievement: Control of rumen/hindgut (*e.g.,* cattle, sheep, goats) microbiome structure to optimize volatile fatty acids production that sustains animal growth (VFAs promote growth).
        - Technical Achievement: Engineer microbiomes for increased livestock growth and weight gain without antibiotics (*i.e.,* prebiotic introduction).
    - Microbiome Engineering Objective: Engineer rumen/hindgut-fermenting animal (*e.g.,* cattle, sheep, goat, cow) microbiomes so they release less carbon (*e.g.,* methane).
        - Technical Achievement: Engineer methanotroph-containing microbiomes that stably colonize cattle and livestock after a single inoculation, to reduce methane production.

        - Technical Achievement: Engineer methanogens in rumen microbiomes to continue oxidizing methane into more environmentally friendly compounds (*e.g.,* methanol).
        - Technical Achievement: Engineer microbiomes that secrete small molecules inhibitors to prevent methane production in ruminant guts.
    - Microbiome Engineering Objective: Utilize microbiomes to pretreat animal feed to increase animal feed efficiency
        - Technical achievement: Engineer microbiomes to synthesize animal nutrients (*e.g.,* essential amino acids, micronutrients, vitamins) not naturally found in unprocessed feed.
        - Technical achievement: Engineer microbiomes that maintain specific moisture content in hay, haylage, or silage to increase feed production efficiency.
        - Technical achievement: Engineer microbiomes that rapidly generate and maintain anaerobic environments to prevent rot.
- Scientific/Engineering Aim: Reduce reliance on chemical fertilizers and pesticides.
    - Microbiome Engineering Objective: Engineer phyllosphere microbiomes to produce pest repellents.
        - Technical Achievement: Design microbiomes that produce compounds that reduce insect herbivory.
        - Technical Achievement: Design microbiomes that inhibit the presence of salicylic acid-producing microbes, which increase insect herbivory (Costarelli et al., 2020).
    - Microbiome Engineering Objective: Engineer food-safe, nitrogen-fixing microbiomes that decrease or eliminate the need for fertilizers and chemical supplements.
        - Technical Achievement: Develop spatially structured soil microbiomes that have anaerobic nitrogen-fixation fed or driven by aerobic, energy-rich processes, thereby reducing energy inputs and costs of fertilizer production.
        - Technical Achievement: Engineer microbiomes that expand the plant-host range of symbiotic nitrogen-fixing bacteria so they grow in association with more diverse crops (*e.g., Rhizobia sp.* with non-legume plants).
        - Technical Achievement: Engineer metabolic pathways in root microbiomes to increase nitrogen-fixing activity, especially for crops that do not have naturally occurring nitrogen-fixing microbiomes.
        - Technical Achievement: Engineer microbiomes to form epiphytic interactions with agricultural crops and fix nitrogen above ground.

Environmental Biotechnology

Environmental Biotechnology focuses on the technologies and tools to enable deployment of engineered microbiomes in the land, air, water, and human landscapes for purposes related to remediation, natural resource management, environmental monitoring, and species management. In contrast to other sectors - including industry and agriculture - engineered microbiomes in the environment will inevitably be interacting with natural microbiomes. Scientific aims therefore reflect a desire not only to introduce new organisms, but also to engineer and preserve existing biodiversity.

Societal Challenge 1: Address and mitigate climate change.

- Scientific/Engineering Aim: Engineer tools for atmospheric and environmental manipulation to mitigate climate disturbances.
    - Microbiome Engineering Objective: Engineer aerosolizable microbiomes to facilitate cloud formation and alter sunlight irradiation or precipitation.
        - Technical achievement: Engineer nutritionally self-sustaining microbiomes (*i.e.,* with heterotrophs and autotrophs) so microbes can survive without reseeding.
        - Technical achievement: Produce microbiomes with ice-nucleating bacteria that can increase rainfall and snow in target areas.
        - Technical achievement: Engineer microbiomes to produce dimethyl sulfide and promote cloud condensation.
    - Microbiome Engineering Objective: Engineer microbiomes that help maintain or increase Earth's albedo.
        - Technical achievement: Deploy ice-nucleating microbiomes to help maintain snowpack and create more reflective surfaces in alpine and polar environments.
        - Technical achievement: Engineer microbiomes that concentrate salt on the surface of high-salinity soils, to increase albedo and reduce soil salinity.
- Scientific/Engineering Aim: Enable and advance carbon sequestration from the environment.
    - Microbiome Engineering Objective: Use microbiomes to enhance natural carbon sequestration processes.
        - Technical achievement: Develop computational models to characterize the role of viral shunts in oceanic carbon sequestration.
        - Technical achievement: Design microbiomes that can be stably deployed into oceans to increase carbon capture via viral shunts.
        - Technical achievement: Engineer methanogen-containing microbiomes to produce methane hydrates (*i.e.,* crystalline methane structures) as means of sequestering carbon in the deep ocean.
        - Technical achievement: Use ice-nucleating microbiomes to help preserve permafrost and prevent carbon release.

- Technical achievement: Create pollutant-degrading microbiomes that colonize wetland plants to prevent pollution-related wetland degradation and support carbon sequestration.

- Microbiome Engineering Objective: Engineer plant rhizosphere communities to promote carbon capture via plant root exudates.
  - Technical achievement: Increase carbon capture phenotypes of plants through overexpression of root exudate compounds.
  - Technical achievement: Engineer rhizosphere communities to convert root exudates into stable complexes.

Environmental Biotechnology

Societal Challenge 2: Expand tool sets for bioremediation and resource recycling.

- Scientific/Engineering Aim: Enable better, more advanced bioremediation of pollutants.
  - Microbiome Engineering Objective: Engineered environmental microbiomes to detect and respond to contaminants or spills.
    - Technical achievement: Engineer oil-consuming microbiomes that proliferate and chemotax towards chemicals used for contaminant cleanup (*e.g.,* surfactants, dispersants) to improve biocontainment in ocean environments.
    - Technical achievement: Engineer microbiomes for bioremediation that can clean up oil spills with a self-contained, single application (*e.g.,* secrete surfactants, produce sorbent extracellular matrices, and fully degrade oils) to decrease cost and increase efficacy of cleanup.
    - Technical achievement: Engineer microbiomes that can metabolize toxic chemical compounds to help neutralize or detoxify them *in situ*.
    - Technical achievement: Create modular microbiomes that contain environment-specific species to help the bioremediating organisms colonize any location where a spill occurs.
  - Microbiome Engineering Objective: Design microbiomes that facilitate degradation or removal of plastics.
    - Technical achievement: Engineer microbiomes that alter their composition for efficient plastic degradation in both aerobic and anaerobic environments.
    - Technical achievement: Engineer microbiomes that concentrate ocean microplastics in the ocean, so they can be physically removed and degraded or recycled using alternative methods.
    - Technical achievement: Engineer microbiomes to consume methane produced from plastic degradation in anaerobic environments.
    - Technical achievement: Engineer microbiomes that generate oxygen and prevent methane production from plastic degradation.
  - Microbiome Engineering Objective: Engineer microbiomes to breakdown pharmaceutical products (*e.g.,* antibiotics) to prevent active components from being distributed into the environment.
    - Technical achievement: Design microbiomes that have antibiotic resistance genes to breakdown compounds, but are synthetically dependent on the compound for survival to reduce the spread of resistance genes.
    - Technical achievement: Engineer microbiome filters that can be evolved to selectively remove/degrade compounds from the environment, based on the compound of interest and metabolites available in the environment.

- Scientific/Engineering Aim: Enable efficient treatment of chemical waste and contaminants (*e.g.,* heavy metals, rare-earth metals, radioactive materials, halogenated hydrocarbon mixtures).
    - Microbiome Engineering Objective: Engineer microbial communities such that waste and contaminants are efficiently biomined and concentrated into useful compounds.
        - Technical achievement: Engineer microbiomes to produce an easily detectable indicator (*e.g.*, color change) in the presence of pollutants.
        - Technical achievement: Design metabolic models to trace the dissemination of waste and contaminant breakdown products within ecological communities.
    - Microbiome Engineering Objective: Recycle radioactive products so that they can be reused for nuclear energy or facilitate radioactive waste degradation in the environment.
        - Technical achievement: Engineer microbiomes to produce radioisotope-specific enzymes that capture and concentrate radioactive material more quickly from contaminated environments (Martinez-Gomez et al., 2016).
        - Technical achievement: Engineer radiation-resistant microbiomes that fully detoxify solvents found in nuclear waste (*e.g.,* by fully degrading trichloroethylene into ethene under aerobic and anaerobic conditions).
        - Technical achievement: Design universal support microbiomes that evolve in inhospitable environments to produce nutrients for keystone bioremediation species (*e.g., Dehalococcoides* spp., *Deinococcus radiodurans*) to reduce the need for small-scale testing.
    - Microbiome Engineering Objective: Engineer magnetic microbiomes for improved extraction and bioremediation of rare-earth metals.
        - Technical achievement: Produce microbiomes that facilitate growth of lanthanide-binding methylotrophs capable of extracting rare-earth elements at extremely low concentration.
        - Technical achievement: Engineer aerobic microbiomes containing rare-earth element-binding enzymes from methylotrophs, so bioconcentration can occur at environmental sites.
    - Microbiome Engineering Objective: Engineer microbiomes to degrade halogenated hydrocarbon (*e.g.,* tetrahloroethylene, methylene chloride, Freon®) mixtures.
        - Technical achievement: Determine metabolic pathway maps halogenated hydrocarbon-degrading bacteria (*e.g., Desulfitobacterium hafniense*) to identify key enzymes involved in degradation.
        - Technical achievement: Design microbiomes that specifically support the metabolic needs (*e.g.,* nutrients, cofactors, reducing equivalents) of halocarbon-degrading bacteria to facilitate faster waste disposal.

- Scientific/Engineering Aim: Enable higher efficiency water purification, reclamation, and desalination.
    - Microbiome Engineering Objective: Engineering microbiomes to increase efficiency of water desalination (*e.g.,* decrease cost and maintenance required).
        - Technical achievement: Engineer halophilic microbiome coatings that prevent biofouling and reduce chemical use in desalination processes.
        - Technical achievement: Engineer microbiomes to purify or concentrate waste brine to facilitate more environmentally-friendly methods for disposing of waste.
        - Technical achievement: Engineer halophilic microbes capable of extracellular electron transfer (anode) and extracellular electron uptake (cathode) to decrease energy cost of desalination.
    - Microbiome Engineering Objective: Use microbiomes to improve water treatment processes.
        - Technical achievement: Engineer microbiomes that attract and capture fecal coliforms to facilitate coagulation, flocculation, and sedimentation.
        - Technical achievement: Engineer microbiomes to function as biosensors for water impurities (*e.g.,* viruses, metals, small molecule contaminants).
        - Technical achievement: Engineer microbiomes that can transform municipal waste into energy or higher-value products.
    - Microbiome Engineering Objective: Engineer microbiomes that reduce the chemical impact of ocean or river aquaculture on the surrounding environment.
        - Technical Achievement: Engineer ocean microbiomes that capture and degrade fish waste and chemical effluent in the pelagic zone, to increase concentrations of dissolved organic matter.
        - Technical Achievement: Engineer microbiomes that help fish resist pathogens and disease to decrease antibiotic use in aquaculture.
        - Technical Achievement: Engineer soil and water microbiomes to absorb and retain excess nitrogen, phosphorus, and potassium from agricultural runoff.

Environmental Biotechnology

Societal Challenge 3: Deploy engineered organisms to improve ecosystem biodiversity, robustness/resilience, and the well-being of inhabitants.

- Scientific/Engineering Aim: Genetically engineer insects to alter growth, protect against off-target pesticides, and improve the performance of insect genetic biocontrol against disease vectors.
    - Microbiome Engineering Objective: Engineer arthropod microbiomes to alter growth and reproduction.
        - Technical achievement: Develop persistent engineered microbiomes that can spread through vertical transmission.
        - Technical achievement: Engineer arthropod microbiomes to alter mate choice, mating success, or fecundity based on desired traits (*e.g.,* host genetic markers).
        - Technical achievement: Engineer arthropod microbiomes to increase or decrease host survival based on desired traits.
        - Technical achievement: Engineer dependent interactions between genetically engineered insects and genetically engineered microbiomes to prevent unintentional dissemination of engineer microbiomes (*i.e.,* design containment mechanisms for engineered organisms).
    - Microbiome Engineering Objective: Engineer arthropod microbiomes to reduce pathogen transmission (*e.g.,* viruses, bacteria, macroparasites).
        - Technical achievement: Engineer microbiomes that produce compounds that kill the vector host after sensing a pathogen.
        - Technical achievement: Engineer microbiomes to fill the ecological niche used by a pathogen, to prevent pathogen colonization (*e.g.,* nutrient competition, occupying the physical space).
        - Technical achievement: Protect honey bees and other pollinators by engineering their microbiomes to protect them against pathogens (Leonard et al., 2020) and pesticides.
- Scientific/Engineering Aim: Reduce or stop the spread of invasive species.
    - Microbiome Engineering Objective: Engineer soil microbiomes to resist introgression from invasive plant species.
        - Technical achievement: Engineered soil microbiomes that detect and degrade allelopathic chemicals produced by invasive species, to help preserve the native plant ecology.
        - Technical achievement: Engineer soil microbiomes that colonize native plant roots and produce allelopathic chemicals to kill native species.
    - Microbiome Engineering Objective: Engineer microbiomes to prevent the spread of invasive animal species.
        - Technical achievement: Design microbiome sprays that are specifically toxic to invasive insects or aquatic invasive species.
        - Technical achievement: Engineer microbiomes that use RNA interference (RNAi) to specifically target and kill invasive species of interest.

- Scientific/Engineering Aim: Design tools for environmental monitoring and conservation.
    - Microbiome Engineering Objective: Document and preserve microbial species and strain-level diversity for ecosystem health maintenance.
        - Technical achievement: Use computational microbiome models to identify keystone functional guilds within a given ecosystem/environment.
        - Technical achievement: Design microbiomes that can be supplemented into an ecosystem to support the growth of keystone guilds.
    - Microbiome Engineering Objective: Design microbial communities that produce biofilms or other structural materials to prevent erosion in sensitive environments.
        - Technical Achievement: Engineer microbiomes that absorb and retain water in diverse environments (*e.g.,* desert crusts, water-sensitive urban designs).
        - Technical Achievement: Engineer microbiomes that produce fibrous structures in and above soil/sand/dirt to prevent wind and water erosion.
    - Microbiome Engineering Objective: Engineered microbiomes to help restore damaged ecosystems (*e.g.,* bleached coral reefs).
        - Technical achievement: Engineer microbiomes that colonize coral reefs and facilitate reactive oxygen species (ROS) scavenging.
        - Technical achievement: Engineer microbial communities with improved thermotolerance to prevent bleaching.

Environmental Biotechnology

Societal Challenge 4: Enable sustainable, more environmentally-friendly materials and infrastructure development.

- Scientific/Engineering Aim: Enable the production of sustainable building materials.
  - Microbiome Engineering Objective: Engineer microbiomes to produce drop-in replacement chemicals for use in construction.
    - Technical achievement: Engineer microbial biofilms that adhere to diverse surface materials (*e.g.,* metal, latex paint, concrete) and resist environmental dispersion forces.
    - Technical achievement: Engineer microbiomes secrete compounds that replace surface coatings (*e.g.,* paint, anti-rust, optical).
  - Microbiome Engineering Objective: Engineer microbiomes to produce non-structural biomaterials (*i.e.,* microbiome-derived insulation to replace fiberglass).
    - Technical achievement: Design microbiomes that produce and secrete cellulosic fibers.
    - Technical achievement: Engineer microbiomes and microbiome-derived biomass to grow to sizes specified by boundaries in the built environment (*e.g.,* building walls, ceilings, concrete foundations).
  - Microbiome Engineering Objective: Engineer microbiomes to produce structural biomaterials (*i.e.,* replace concrete with microbial 'concrete').
    - Technical achievement: Engineer distributed metabolism within microbiomes to increase the growth rate and 'curing' time of the primary biomass-generating microbe in microbial concretes.
    - Technical achievement: Design microbiomes that form regular crystalline or lattice patterns, to improve their structural, load-bearing properties.
    - Technical achievement: Create living objects that grow and interact with the environment, such as an autonomous organism (Endy, 2008).
- Scientific/Engineering Aim: Enable sustainable production of environmentally-friendly consumable materials (*e.g.,* plastics, wood, fabrics).
  - Microbiome Engineering Objective: Engineer cellulose- or lignin-producing microbiomes to create wood-like materials.
    - Technical achievement: Design structured microbiomes that secrete cellulose or lignin in patterns that mimic and can replace cross-laminated wood materials.
    - Technical achievement: Engineer cellulose-producing microbiomes that can invade and grow on rotting wood, to repair and strengthen it.
  - Microbiome Engineering Objective: Engineer microbiomes that are integrated into or could be applied to fabrics/textiles, and repair them when damage occurs (*e.g.,* wear and tear, UV exposure).
    - Technical achievement: Enable rapid, directed production of complex polymers using microbes optimized for individual synthesis steps in polymer production.
    - Technical achievement: Engineer microbiomes that secrete pigments to prevent colors from fading or to restore faded colors.

        - Technical achievement: Engineer signal and response cascades in a synthetic microbiome so activity is specific to a damage signal.
    - Microbiome Engineering Objective: Engineer plastic-integrated microbiomes that trigger plastic degradation in response to environmental signals.
        - Technical achievement: Identify and heterologously express pathways for plastic degradation in chassis strains that can be incorporated into desired microbiome.
        - Technical achievement: Engineer plastic degrading microbiomes that form spores or remain otherwise dormant until a physical change in the plastic occurs (*e.g.,* UV damage triggers activity, disruption of a patterned microbiome indicates object breakage).

Energy

Energy focuses on the application of engineering biology tools and technologies to advance clean and affordable energy sources and to reduce overall energy consumption. To reduce the amount of carbon dioxide added to the atmosphere, energy will need to come from renewable sources, including waste gases (such as carbon dioxide and methane), microorganisms, and plants. Microbiomes can be used to produce highly energy-dense transportation fuels from biomass, increase the efficiency of conventional energy processes, and reduce energy consumption.

Societal Challenge 1: Produce affordable and clean energy.

- Scientific/Engineering Aim: Enable production of energy-dense and carbon-neutral transportation fuels from lignocellulosic feedstocks and oil crops.
    - Microbiome Engineering Objective: Design rhizosphere microbiomes that enable robust growth of feedstock crops (*e.g.,* switchgrass, sorghum) on marginal lands (*i.e.,* land with worse irrigation, fewer nutrients in soil).
        - Technical Achievement: Engineer soil microbiomes to capture atmospheric carbon and increase soil organic carbon in marginal lands.
        - Technical Achievement: Establish designer rhizospheres that selectively capture and concentrate chemicals to increase nutrient acquisition (*e.g.,* nitrogen, phosphorous, iron and other trace metals).
    - Microbiome Engineering Objective: Engineer microbiomes capable of producing fuel from lignocellulosic feedstocks and oil crops.
        - Technical Achievement: Design microbiomes that can hand off metabolites between organisms, so biocatalysis is performed by individual organisms optimized for each step (*i.e.,* bacteria are suboptimal for production of poly-ketide synthesis, but many biosynthetic gene clusters come from fungi so the natural host could be used).
        - Technical Achievement: Engineer and optimize extremophiles so that they can produce higher titers of butanol from biomass.
        - Technical Achievement: Engineer microbiomes that can withstand phase-separating concentrations of energy-dense fuels.
        - Technical Achievement: Build microbiomes that produce and secrete lignocellulolytic cocktails that can process all components of lignocellulose, rather than individual enzymes.
        - Technical Achievement: Develop communities that are resistant to inhibitors found in crude plant feedstocks to enable enzyme production *in situ.*
    - Microbiome Engineering Objective: Engineer microbiomes to increase carbon utilization from feedstocks (*e.g.*, sugars, carbon dioxide, organic acids).
        - Technical Achievement: Engineer mixotrophy/simultaneous utilization of all available carbon sources, rather than catabolite repression, to decrease processing time.

        - Technical Achievement: Engineer microbiomes to capture and recycle lost carbon of biomass generation for energy production, by catabolizing a given species once it is no longer needed.
        - Technical Achievement: Engineer ecological succession, creating a microbiome that progresses through different feedstocks (*e.g.,* carbon dioxide/lignocellulose to sugars, then fermentation acids from the first generation are used to produce compounds, then dead microbes from first generation are consumed) to capture all available carbon in a system (Brislawn et al., 2019).
        - Technical Achievement: Engineer co-fermentation processes to increase gas (*e.g.,* carbon dioxide, acetate) consumption rate and reduce microbiome toxicity arising from gas accumulation.
    - Microbiome Engineering Objective: Engineer microbiome pretreatments to break down feedstocks, rather than chemical treatments or heating.
        - Technical Achievement: Domesticate natural microbial communities that degrade lignocellulose (*e.g.,* herbivore and insect gut microbiomes, wetlands/swamps).
        - Technical Achievement: Using natural communities as a starting point, optimize microbiomes to degrade lignocellulose at increasing scales (*e.g.,* pilot-scale, mid-sized, industrial).
- Scientific/Engineering Aim: Reduce environmental impacts of conventional energy generation processes.
    - Microbiome Engineering Objective: Engineer microbiomes to reduce environmental impacts of mining (see also: Environmental Biotechnology).
        - Technical achievement: Engineer microbiomes to extract valuable metals (*e.g.,* rare earth elements) from electronic waste (Kwok, 2019).
        - Technical achievement: Engineer communities that can grow in mining waste and retain moisture, to reduce toxic dust from spreading in the environment.
        - Technical achievement: Engineer communities that can sequester valuable metals from tailings and/or waste streams. Program taxis cascades to assist in recovery of these cells and the sequestered metals.
        - Technical achievement: Program sentinel cells to detect pollutants in areas near mine to detect and mitigate problems at an early stage.
    - Microbiome Engineering Objective: Engineer microbiome-enhanced oil production at the source.
        - Technical achievement: Engineer microbial communities that can ‘clean’ dirty fossil fuels by removing contaminants or pollutants, or convert dirty fossil fuels to cleaner fuel sources (*e.g.,* coal converted to natural gas) (Sharma et al., 2018).
        - Technical Achievement: Develop communities that reduce souring (*e.g.,* as a result of hydrogen sulfide gas) production in oilfields to lower corrosion and capital costs.

- Technical Achievement: Develop communities that produce gases and/or surfactants *in situ* to mobilize trapped oil.
- Technical Achievement: Develop communities to produce organic acids *in situ* to dissolve rock formations and mobilize oil without using chemical surfactants or fracking.

Energy

Societal Challenge 2: Reduce global energy consumption.

- Scientific/Engineering Aim: Improve energy storage capabilities to allow for more efficient utilization of renewable energy sources.
    - Microbiome Engineering Objective: Engineer microbiome communities to temporarily store solar energy (*e.g.,* over hours, days), independent of their own biomass needs.
        - Technical Achievement: Engineer light-responsive microbiomes that track across surfaces to maximize energy capture over the course of the day.
        - Technical Achievement: Engineer microbiomes that interface with solar panels and utilize photovoltaic energy to produce biofuels, reducing the need for large battery storage, despite possibly lower efficiency.
    - Microbiome Engineering Objective: Engineer microbiome communities to store wind, hydroelectric, or other renewable energy (*e.g.,* over hours, days), independent of their own biomass needs.
        - Technical Achievement: Design biofuel production facilities so they can utilize excess mechanical or thermal energy (*e.g.,* for heating, cooling, mixing) in these renewable energy sources.
        - Technical Achievement: Engineer electroactive microbiomes so carbon fixation occurs across multiple species to increase energy capture (*e.g.,* an electroactive microbe makes a precursor that other community members metabolize for biofuel production).
- Scientific/Engineering Aim: Decrease energy consumption in infrastructure (*e.g.,* buildings, vehicles, pipelines, transportation).
    - Microbiome Engineering Objective: Design microbiome-derived surface coatings that increase efficiency (*e.g.,* prevent biofouling, reduce friction).
        - Technical Achievement: Engineer microbial biofilms that produce antimicrobial compounds (*e.g.,* antimicrobial peptides, antibiotics, anti-quorum sensing) to prevent fouling (*e.g.,* barnacles on ships).
        - Technical Achievement: Engineer microbial biofilms that physically modify surfaces to decrease bacterial attachment sites and prevent bacterial adhesion (Dang & Lovell, 2016).
        - Technical Achievement: Engineer microbial biofilms that degrade bacterial holdfast structures to prevent “primary surface colonizers” from attaching and starting the biofilm formation process.
        - Technical Achievement: Engineer microbiomes that produce biofilms to reduce shear (*e.g.,* wind, water) or friction.
    - Microbiome Engineering Objective: Create microbiomes to sense and respond to leaks and restrictions.
        - Technical Achievement: Engineer microbiomes that sense leaks in pipelines (*e.g.,* by detecting changes in laminar flow) and produce a dye to indicate damage.

        - Technical Achievement: Engineer microbiomes that produce fibrous, impermeable structures to patch leaks in pipelines/tubing (*e.g.* microbial platelets).
        - Technical Achievement: Engineer microbial consortia that target and degrade solid deposits or blockages in pipelines.
        - Technical Achievement: Engineer microbial consortia that deter growth of iron oxidizing and reducing microbes that promote corrosion in pipelines.
    - Microbiome Engineering Objective: Design microbiomes that transform waste into a non-transportation energy source (*e.g.,* heat generation, cooling).
        - Technical Achievement: Engineer microbiomes with metabolisms engineered to convert energy into heat rather than biomass growth.
        - Technical Achievement: Engineer microbiomes that absorb heat from the environment to reduce building cooling costs.

Addendum to *Engineering Biology* and *Microbiome Engineering*: Linking Societal Considerations and Integrating Public Values

The challenges and achievements presented in EBRC's roadmaps do not occur in a vacuum. Diversity within scientific disciplines, in all its forms (racial, gender, sexual identity, and more), strengthens research and helps advance science. Funding agencies have periodically made public efforts to support diversity in response to various current events, but they could better institutionalize practices to increase diversity, rather than enacting infrequent, reactionary policies. Advancing engineering biology will require significant parallel, non-technical efforts to address societal, legal, and regulatory challenges. In this section, we examine some of the broader considerations that need to be addressed to ensure that new technologies work in the best interests of society.

Diversity in Scientists

Funding agencies can play a key role in increasing diversity by providing a financial incentive for universities and labs to admit and support diverse trainees in the scientific workforce. Fully detailing the policy changes that could be implemented to increase diversity in science are beyond the scope of this roadmap, but a good starting point for any funding agency would be collecting more data from grant recipients and trainees supported by those grants. Grantee data on racial and gender diversity, academic outcomes of trainees, paper publication records, and community climate surveys are not adequate on their own, but can support future decision-making processes. It is essential that any data collected aims to capture the quality of training and mentorship, not simply the quantity of women or Black, Indigenous, and People of Color (BIPOC) scientists in a department or program. Additionally, collecting more information on scientists with disabilities will help universities understand how physical spaces may need to be redesigned (*e.g.,* if laboratory spaces are not wheelchair friendly). Like in engineering biology, good measurements can aid in designing interventions and quickly understanding whether they are effective or not. Over time, these data should help identify the programs and policies that best support diversity, allowing an agency to increase investments even further.

Diversity in Data

Additionally, scientists and policymakers should also strive to ensure that diversity is considered in scientific data itself. Facial recognition systems have already demonstrated the pitfalls of incomplete data sets, with some programs misidentifying non-white faces up to 100 times more often than white faces (Grother et al., 2019). As discussed throughout *Engineering Biology* and *Microbiome Engineering*, machine learning and artificial intelligence are essential for moving engineering biology forward. Regardless of whether a researcher is collecting human microbiome samples to study health, agricultural soil samples for enriching crop growth, or designing microbiomes to treat diseases, they should make a conscious effort to avoid a similar problem that currently exists in other artificial intelligence systems. Mitigating biases, especially for health-related research, oftentimes requires extra effort and outreach to populations that are underserved by or distrustful of healthcare services, a reasonable sentiment in light of the long history of bias and discrimination in medicine (Hoffman et al., 2016; Obermeyer et al., 2019). The example set by facial recognition software demonstrates the pitfalls of assuming that largely

computational science does not have the same concerns about bias that are more readily apparent in medical or public health research. Human genomics research offers a potential model for engineering biology (Guglielmi, 2019). Researchers are making efforts to get community members directly involved in study design and implementation, which helps ensure that cultural traditions are respected and helps broaden community engagement as a result.

Throughout the process of experimental design, development, and implementation, it will be important to carefully consider how accidental biases in sample selection may impact engineering biology, and consciously control for them or expand the data set to mitigate them. The exact interventions necessary will look different for every experimental setup, but as engineering biology moves toward more human-applied technologies, researchers and funding agencies should constantly push for as much diversity in data as possible.

Equitable Access to Science

Another important consideration is ensuring equitable access to benefits of engineering biology. This roadmap builds from state-of-the-art research and technology, looking forward to new discoveries that will benefit society. An unfortunate reality is that many existing technologies could have substantial benefits for society, but barriers such as cost or lack-of-infrastructure prevent people from reaping those benefits. As engineering biology advances, the inevitable trickle-down of technology will make some technologies more accessible, but this will not be true in all cases. For example, healthcare costs seem to resist the normal market forces that would drive down prices when new drugs or therapies are invented. Even if all of the engineering advances presented in our roadmaps are achieved, significant policy changes will be necessary to imagine that new therapies and drugs from engineered biology will be accessible for all people. The burden and responsibility for changing these systems falls mainly on policymakers, but scientists should also advocate for changes, if they want their work to have as broad an impact as possible.

In addition to costs, medical professionals and policymakers should ensure that patients have informed access to new technologies. Patients should be made aware of every potential therapy available and the efficacy of the treatment should be clearly explained. This is one component of a complex network of structures and individual choices that contribute to systemic racial and ethnic health disparities (Fiscella & Sanders, 2016), but medicines derived from engineered biology will require special attention for a healthcare provider to understand the treatment themselves, and then invest the time to explain it so their patient understands.

Public Education and Scientific Engagement

Community, stakeholder, and public engagement offers the chance to bridge the gap between technical experts and lay citizens in meaningful ways that go beyond one-way education (Committee on Gene Drive Research in Non-Human Organisms: Recommendations for Responsible Conduct et al., 2016). While science education and improving scientific literacy do play a role, scientists also need to engage with their communities to hear their concerns and address them accordingly. The long struggle for public acceptance of genetically modified organisms is a good example that boosting scientific literacy is not sufficient to quell public opposition. So scientists would benefit from drawing on the insights of social science to help navigate the challenge in gaining greater public buy-in for science like microbiome engineering

and engineered biology in general. Funding agencies should go out of their way to encourage and support scientists engaging with all scientific stakeholders, because public trust will be critical for the acceptance of new tools and technologies from engineering biology.

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