EBRC Engineering Biology Research Consortium

Engineering Biology for Climate & Sustainability

A Research Roadmap for a Cleaner Future

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EBRC Engineering Biology Research Consortium

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Overview and Introduction

This EBRC technical roadmap, *Engineering Biology for Climate & Sustainability: A Research Roadmap for a Cleaner Future*, is a critical assessment of opportunities for engineering biology to contribute to tackling the climate crisis and long-term sustainability of products and solutions for health and well-being of Earth and its inhabitants. More extreme, frequent, and interconnected climate events are causing widespread vulnerabilities, damage, and loss to humans and nature, and these adverse impacts are compounding and more and more often becoming irreversible. As noted by the United Nations' Intergovernmental Panel on Climate Change (IPCC), the "magnitude and rate of climate change and associated risks depend strongly on near-term mitigation and adaptation actions, and projected adverse impacts and related losses and damages escalate with every increment of global warming" (IPCC, 2022). This roadmap identifies novel approaches, objectives, and aims for engineering biology research in climate change mitigation and adaptation that can help to lower greenhouse gases, reduce and remove pollution, and promote biodiversity and ecosystem conservation. This roadmap also identifies opportunities for engineering biology-enabled, sustainable replacements and alternatives in the food and agriculture sector, transportation and energy sectors, and for materials and industrial processes. These potential solutions include biobased alternatives to synthetic fertilizers, better energy storage with biobased batteries, and sustainable, climate-friendly biomaterials to replace non-degradable plastics and toxic textile dyes, among many others. The roadmap's opportunities and objectives are laid out as short-, medium-, and long-term milestones, to address the challenges of climate change and sustainability with both urgency and persistent ambition and vision for the development and translation of engineering biology tools to technologies and products for the current and next-generation bioeconomy. In addition to the roadmap, social and nontechnical dimensions case studies provide context and framing for the questions and considerations that can be asked and addressed during research and development, and are intended to be used as a discussion and learning tool by engineering biology researchers and their collaborators. Finally, a glossary provides a quick reference for the terms and concepts included in the technical roadmap.

The engineering biology tools and technologies described in this roadmap can only be a small part of the myriad solutions urgently and collectively needed to tackle climate change and challenges for sustainability. Like the global nature of the crisis, the solutions too must be global. We must support, leverage, and work in concert with advances in many other disciplines of science and technology – from ecology to climate science, environmental sciences to the renewable energy sector, geosciences, physics, chemistry, and materials science, and the social sciences, among many, many others. Each field will have their own answers and approaches, all of which are interconnected and must be combined with inclusive local engagement, informed regulations and policy, equitable education, and global connection and collaboration. Similarly, the engineering biology approaches herein are only a subset of opportunities, and should be considered for their ethical and economical risks and benefits, in addition to technical feasibility. Engineering biology has truly transformative potential and the biotechnologies envisioned by this roadmap, if established with longevity in mind and thoughtfully incorporated into existing and novel technologies, products, and processes, will greatly contribute to a robust global bioeconomy. This roadmap should serve as inspiration, driving passion and imagination towards solving the grand challenge of climate change and enabling a sustainable future for all.

While we will not solve all of the threats from climate change and challenges to sustainability with engineering biology, the capabilities and technologies envisioned by this roadmap could make significant contributions and advancements towards those goals. Sustainable solutions will require commercial and industrial sectors to partner with biology and transition to a biology-driven, circular economy that is respectful and inclusive of the diversity of ecosystems, environments, and all of their inhabitants. Moreover, while engineering biology can contribute to overcoming world-wide challenges, it can also be implemented at local, community-level scales, designed and tailored to fit regional ecosystems and economies, distributed to utilize local resources and solve smaller-scale problems, and provide materials, products, and solutions that fit the needs of diverse individuals. Biotechnologies imagined by this roadmap will help to capture, eliminate, store and sequester carbon, greenhouse gases and contaminants from the atmosphere, land, and water, and directly at point emission sources, to reduce global warming and the effects of climate change, and promote and ensure a cleaner Earth. Engineering biology can contribute to alternatives and modifications to those pollution and hazard sources, preventing harm in the first place. With engineering biology we can enable alternatives to carbon-intensive concrete and non-degradable plastics, reduce methane from agriculture and food production and processing, and find ways to create, store, and more efficiently use renewable energy. In concert with other solutions, targeted and creative investment, infrastructure, education, and engagement in engineering biology can ensure that we have a healthy, greener future.

Roadmapping Process and Project Development

This work represents the fifth of EBRC's technical roadmaps (which can be found at https://roadmap.ebrc.org) and the first dedicated to a specific application and global challenge. The topic of climate change and sustainability was identified by the EBRC membership and stakeholders as especially urgent and important and an area in which engineering biology is poised to significantly contribute. Other EBRC roadmaps have included objectives and opportunities related to environmental biotechnology, including climate change and sustainability, but never dedicated to the topic in such a way.

Addressing climate change and sustainability with engineering biology posed particular challenges as to the scope and framing of this roadmap. A roadmap for climate change must address a myriad of impacts on humans, animals, plants, infrastructure, and the physics and chemistry of the Earth's air, water, and land. Those impacts are felt immediately and at a distance, and some, if not many, are yet unknown and ever-changing. Climate change is a global challenge, meaning that biotechnologies must address local and regional challenges that impact everyday lives *and* impacts that span nations, oceans, and cross borders. Biotechnologies inspired by the roadmap must be accessible in a variety of resource settings, be contained to prevent adverse effects, be feasible (technically, economically, ethically, and politically), and be impactful on the necessary timescales. And the roadmap must speak to our expertise as the engineering biology community, with full acknowledgement of the research and understanding, technology and developments needed for systems-level solutions to the complex and interconnected challenges in climate and sustainability.

In the end, this roadmap only skims the surface of the potential for engineering biology to address climate change and sustainability. We chose to focus this roadmap on common themes found in other climate change-related publications, foremost being the work by the United Nations (UN) Intergovernmental Panel on Climate Change (IPCC) (https://www.ipcc.ch/), and informed by the UN Sustainable Development Goals (SDGs), and global climate change policy, particularly that of the United States. (EBRC roadmapping receives funding support from U.S. federal agencies and therefore typically focuses our efforts on U.S.-based opportunities and strategies; however, we hope that the engineering biology solutions envisioned by this roadmap are globally applicable and actionable.) One area not specifically called out in this roadmap is direct effects on human health; rather, opportunities to protect and improve human health are implicit in addressing other challenges. This roadmap also represents only a snapshot in time, with new challenges arising, and with new technologies and advancements continuing to be made daily. Thus, the milestones in this roadmap will be influenced by many factors affecting their attainment and should be taken as a point of reference for what the future can hold.

Roadmap stakeholders include the research community within and beyond engineering biology, in academia, industry, and government. Stakeholders also include policy- and decision-makers in government, industry, and nonprofit/non-governmental organizations and institutions. Educators, instructors, and the next generation of thought leaders are an important and integral part of the roadmap audience, necessary to realizing the advancements of engineering biology for climate and sustainability.

EBRC's roadmapping is an iterative process of brainstorming, discussion, drafting, review, and revision. *Engineering Biology for Climate & Sustainability* was created by over 90 individuals with expertise across engineering biology and other science and engineering disciplines (see **Contributors**). Scoping for this roadmap took place starting in early 2021, with adaptations made throughout the drafting process to account for new areas of interest and to ensure clear and concise communication of the challenges and opportunities [Figure 1]. Roadmap contributors participated in a number of virtual workshops and collaborative writing sessions between July 2021 and April 2022, building on the work of their colleagues and bringing new ideas and approaches to each strategy laid out in the roadmap's milestones and technical achievements. An Interim Report describing the anticipated scope and content of the roadmap was released in November 2021. The roadmap was reviewed by stakeholders and revised April through August 2022, edited for clarity and consistency, and prepared for publication in September 2022. EBRC roadmapping efforts are led by our Roadmapping Working Group, chaired by Dr. Michael Köpke (VP Synthetic Biology, LanzaTech), with staff direction from Dr. Emily Aurand.

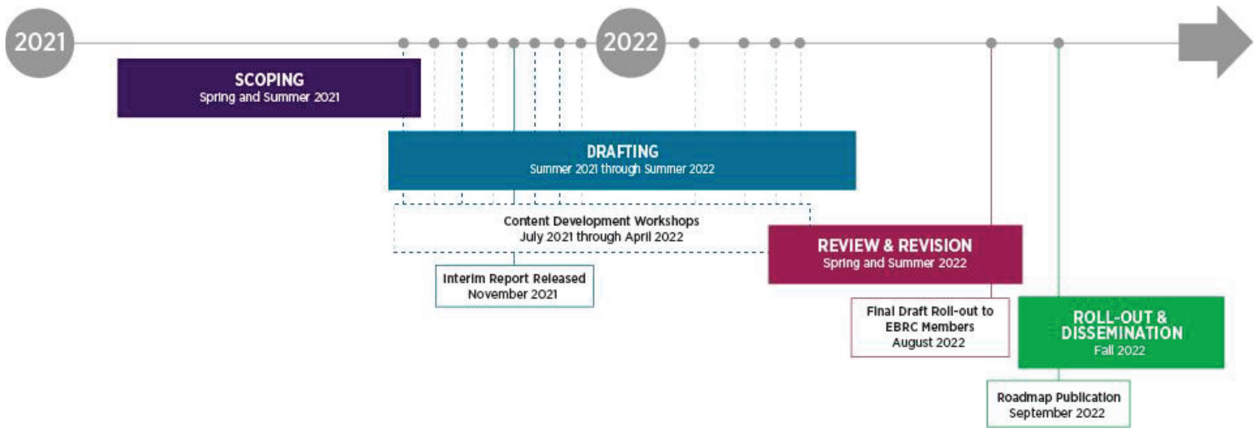

Figure 1. Timeline of Engineering Biology for Climate & Sustainability development. EBRC roadmapping consists of a scoping, drafting, revision, and review process to develop the final product, which is then published on our interactive website, https://roadmaps.ebrc.org/, and available for download as a PDF.

About the Roadmap

The **Technical Roadmap** is comprised of six themes that detail breakthroughs and milestones for engineering biology for climate and sustainability. Part 1 includes the first three themes, which focus on novel capabilities to mitigate and adapt to the effects of climate change and build and ensure resilient ecosystems. The **Biosequestration of Greenhouse Gases** theme addresses opportunities to capture and remove carbon dioxide, methane, and other harmful gases from the atmosphere and enable and strengthen carbon storage and conversion. The **Mitigation of Environmental Pollution** theme highlights opportunities to prevent and tackle pollution through bioremediation, biosequestration, and biodegradation of contaminants in the environment and from point-sources. And the **Conservation of Ecosystems and Biodiversity** theme addresses opportunities for engineering biology to contribute to the monitoring of ecosystem members and their health, distribution, and diversity, and pinpoints the need for strong biocontainment strategies that are necessary for all engineering biology applications. Part 2 includes the final three themes and focuses on climate-friendly, sustainable products and solutions for chief engineering biology application sectors. The **Food & Agriculture** theme addresses specific opportunities to reduce greenhouse gas emissions from food production and waste and towards making agriculture and food systems more robust to climate change. The **Transportation & Energy** theme addresses opportunities in biofuels, electricity production and storage, and reducing emissions from transportation, shipping, and aviation. Finally, the **Materials Production & Industrial Processes** theme identifies opportunities in the built environment, textiles, and other consumer products for reducing the anthropogenic carbon footprint, reducing toxins and wastes, and recovering economically-valuable resources sustainably.

Each theme is broken down into a series of roadmap elements (further described individually below). Considered from the top-down, the roadmap elements become progressively more technical. The higher-level elements, the Goals and Breakthrough Capabilities, are societal-level concerns and are written to be more approachable for non-technical audiences and those with expertise outside of engineering biology and related fields, identifying challenges they are likely to be familiar with regardless of their background or current role in addressing the climate crisis and sustainability challenges. The Milestones speak directly to the engineering biology tools and technologies that will need to be developed or enabled to achieve the Goals and Breakthrough Capabilities and are laid out over short-, medium-, and long-term timeframes, indicative of the resources, infrastructure, and other advancements necessary to their achievement. Finally, the Bottlenecks and Potential Solutions illustrate specific technical challenges that the engineering biology research community can attend to towards realizing each milestone. From the bottom-up, the roadmap elements provide a pathway for engineering biology questions and research topics to be applied towards mitigation, adaptation, and sustainability for the climate and global ecosystems. The roadmap elements build collectively, with the Milestones representing some of the engineering biology achievements necessary towards accomplishing the Breakthrough Capability, and the collection of Breakthroughs necessary, in part, towards achieving the overarching Goal.

Goals - The roadmap Goals are the “big-picture” objectives, what we hope to accomplish through science and technology to mitigate climate change and enable sustainability. Written in a way that is accessible to non-technical audiences, the Goals are intended to convey some of the biggest issues and opportunity areas in tackling the climate crisis and enabling long-term sustainability solutions.

Current State-of-the-Art **-** Each Goal is followed by a short summary of recent advances, what we can accomplish with engineering biology today, and where the biggest challenges are. The Current State-of-the-Art is intended to set the stage for further advancements in engineering biology and what opportunities are addressed in the roadmap.

Breakthrough Capabilities - The Breakthrough Capabilities identify how we can contribute to the Goal with engineering biology and are representative of major aims across the field. Typically written as what you might see in a *Science* or *Nature* publication headline, the Breakthrough Capabilities are the engineering biology achievements towards their higher Goal.

Milestones (Short-, Medium-, and Long-term) - The Milestones are the engineering biology tools and technologies that make a stepwise advancement towards achieving the Breakthrough Capability. *Short-term Milestones* are expected to be about 2-5 years away from achievement, representing research that is currently funded (or where funding opportunities exist) or could be accomplished with existing resources. *Medium-term Milestones* are tools and technologies anticipated to be achieved in approximately 5-10 years; these research areas likely need funding (including new grant/award programs) or infrastructure development, and other support at the institutional or federal level. *Long-term Milestones* are anticipated to be 10-20+ years from realization and, in most if not all cases, would require new funding, infrastructure, or other resources (including significant tool and technology development). All of the milestones are intended to be ambitious and visionary, representative of what engineering biology could accomplish with unconstrained resources and congruent advancements in other fields, so as to spur investment and action across the science, engineering, social, and political enterprise.

Bottlenecks and Potential Solutions - The Bottlenecks represent a specific technical challenge to achieving the milestone. Likewise, the Potential Solutions represent one or more ways in which we might overcome the bottleneck. These elements are not comprehensive, capturing only a few of the issues and approaches researchers may encounter and undertake.

In addition to the technical roadmap, this work also includes **Social and Nontechnical Dimensions Case Studies**. These case studies are intended to serve as a resource for technical researchers to encourage and guide these scientists and engineers in consideration of nontechnical issues, challenges, and approaches that should inform research and technology development. The case studies highlight a range of nontechnical dimensions through the lens of hypothetical engineering biology advancements drawn from the roadmap. Each case study presents questions of ethical, political, economic, and security dimensions that could impact technical design choices and approaches as researchers consider impact and feasibility of future tools and technology. Also included is a **Glossary** of important terms and concepts included, and in the context of, the technical roadmap. We hope the glossary enables greater understanding and a more common language among roadmap stakeholders and users.

Like all EBRC roadmaps, *Engineering Biology for Climate & Sustainability* is intended and anticipated to be a resource for scientists, engineers, educators, and policymakers considering how and where engineering biology and biotechnology can play a role in mitigating and adapting to climate change and enabling sustainable solutions, building a robust, global bioeconomy. The opportunities identified in the roadmap should be considered along with other solutions and developed in coordination and collaboration with other research fields, appropriate policy and regulation, and with input from local, national, and international communities.

About EBRC

EBRC is a non-profit, public-private partnership dedicated to bringing together an inclusive community committed to advancing engineering biology to address national and global needs. We showcase cutting-edge research in engineering biology, identify pressing challenges and opportunities in research and application, and articulate compelling research roadmaps and programs to address these challenges and opportunities. Our four focus areas, driven by member-led working groups, are Research Roadmapping, Education, Security, and Policy & International Engagement.

TECHNICAL ROADMAP

Part 1: Developing Novel Capabilities for Climate Change Mitigation and Ecosystem Resilience

Biosequestration of Greenhouse Gases

Introduction and Impact: Analysis by the Intergovernmental Panel on Climate Change (IPCC) shows that carbon dioxide removal (CDR), the process of removing and sequestering climate-damaging greenhouse gas (GHG) carbon dioxide (CO_2) from the atmosphere, is a crucial component to keeping global warming under 1.5ºC and achieving U.S. and global emissions reduction targets by 2050 (de Coninck et al., 2018). While Earth's land and ocean absorb roughly 50% of annual global CO_2 emissions (Folke et al., 2021; National Oceanic and Atmospheric Administration, 2015), the capacity for the biosphere to capture carbon is shrinking. Engineering biology could restore, or even increase, the biosphere's carbon uptake and the sequestration or removal of GHGs or other emissions that lead to increased GHG accumulation in the atmosphere, including methane (CH_4), nitrous oxide (NO_X), carbon oxides (CO, CO_2), and fluorinated gases. Engineering biology opportunities considered in this technical theme aim to capture, convert, and remove GHGs, including through improved photosynthetic efficiency, advancements and novel approaches for carbon fixation, and the recycling of captured carbon into value-added products [Figure 2].

Engineering biology could be used to engineer plants to store more carbon in their root systems or to engineer soil microbiomes and natural biocrusts to sequester larger amounts of carbon. This roadmap also addresses opportunities to deploy ice-nucleating microbes to maintain ice and snowpack, increasing albedo (reflection of light away from the earth) and helping prevent the thawing of permafrost, which stores massive amounts of carbon. Finally, this roadmap considers approaches to enhance ocean and coastal carbon capacity, such as through engineered macroalgae.

As with other carbon removal technologies, biobased carbon capture is neither a replacement for drastic emissions reduction nor a justification for delaying climate actions, and must be developed in conjunction with other approaches to deep decarbonization. This roadmap presents only a selection of potential engineering biology technologies that can be part of the solution and should be accompanied by research and development in ecology, geophysics, oceanography, agronomy, and many other fields. In addition, engineering biology-enabled carbon capture faces unique environmental implications that must be addressed, including the biocontainment of engineered organisms and the potential for competition between engineered organisms and non-engineered, native organisms.

Carbon sequestration in *Engineering Biology*

EBRC first addressed carbon sequestration in *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy*, published in 2019 {see: https://roadmap.ebrc.org/carbon-sequestration/}. Objectives included engineering soils to better sequester carbon, engineering plants for increased CO_2 removal, removing and recycling methane with engineered organisms and engineering marine microbes for long-term carbon storage.

Figure 2. Enabling the large-scale biosequestration of greenhouse gases. Engineering biology can contribute to improved capture and uptake of climate-damaging greenhouse gases (GHGs), including carbon dioxide, nitrous oxide, and methane, from the atmosphere and point-sources, such as industrial emissions. Advancements in the engineering of microbes, plants, and algae can help to sequester and store carbon in soils and other long-term carbon sinks, and to convert captured carbon into value-added chemicals and materials. Existing natural carbon storage could also be enhanced with engineering biology, increasing carbon sequestration capacity of soils, biocrusts, and marine environments. To do so, advancements need to be made in photosynthesis efficiency, design and engineering of carbon conversion enzymes, and organic GHG utilization capacity.

BIOSEQUESTRATION OF GREENHOUSE GASES

Goal	Breakthrough Capability	Milestone

At-scale capture, storage, and utilization of greenhouse gases (GHGs) by engineered organisms.

Improve CO_2 uptake by engineering more efficient photosynthetic organisms (plants, algae, cyanobacteria).

Short-term	Medium-term	Long-term
Engineer plants for optimized light collection and more efficient use of captured light for photosynthesis.	Engineer pathways and enzymes in photosynthetic organisms to increase the rate and efficiency of carbon fixation. Develop scalable carbon capturing platforms enabled by engineered green algae and cyanobacteria.	Combine and rewire native CO_2 fixation pathways (e.g., C3 and C4 pathways) and engineer organisms capable of utilizing multiple carbon fixation pathways.

Enable efficient carbon capture by engineered chemoautotrophs.

Short-term	Medium-term	Long-term
Map and identify parts in CO_2 fixation pathways to increase the efficiency of carbon fixation in chemoautotrophic organisms.	Engineer complexes and metabolic pathways in chemoautotrophs to improve carbon fixation.	Demonstrate use of engineered chemoautotrophs to capture more CO_2 in the context of environmental or industrial processes.

Enable organisms to utilize captured carbon to produce value-added chemicals and materials.

Short-term	Medium-term	Long-term
Engineer organisms to convert CO2, methane, or other C1 sources and intermediates (incuding methanol, formate, acetate) into value-added compounds.	Optimize the bio-utilization of CO_2 and methane emitted from point sources. Improve gas fermentation technologies.	Combine and rewire native carbon utilization pathways and engineer organisms capable of using multiple carbon metabolism pathways.

Enable carbon capture and utilization by enzymes or cell-free systems.

Short-term	Medium-term	Long-term
Develop efficient enzymes for concentrating carbon from the atmosphere. Develop scalable cell-free systems as platforms for carbon capture and bioconversion.	Develop efficient and scalable cell-free systems capable of utilizing methane, formate, or CO_2 to produce commodity fuels and chemicals.	Develop self-contained and/or standalone cell-free CO_2 fixation systems for bio-enabled artificial photosynthesis. Develop new platform tools for multienzyme immobilization in cell-free systems.

Increase carbon uptake and mitigate climate change by enhancing natural systems through engineered biology.

Enhance soil carbon storage capacity via engineered biology.

Short-term	Medium-term	Long-term
Understand the role of soil microbiome in modifying (specifically, increasing) soil carbon capacity.	Enable stable, long-term carbon storage in soil microbiomes, such as by introducing fungi to enhance weathering.	Develop methods for in situ modification of soil microbial communities to increase carbon storage in evolving at-risk soils.
Engineer model plants to increase root biomass contributing to below-ground carbon storage.	Identify and characterize exometabolites beneficial to increasing carbon storage capacity in plants or soil.	
Engineer the root systems of crop and non-model plants to store more carbon.	Enable microbial communities in permafrost to retain and/or capture greenhouse gases.	
Engineer plant roots to secrete metabolites that recruit microbes capable of converting labile plant exudates into stable soil carbon.		

Restore disturbed natural biocrusts and increase carbon sequestration in arid lands.

Short-term	Medium-term	Long-term
Assess the carbon removal potential of deploying artificial biocrust in a variety of arid environments.	Demonstrate engineered biocrust communities to sequester carbon in arid environments.	Engineer and deploy artificial biocrust to restore and increase climate-resilience of native biocrust and desert ecosystems.

Enhance albedo via engineered microbes.

Short-term	Medium-term	Long-term
Identify and engineer microbes with increased ice nucleation capabilities.	Demonstrate biological ice formation in simulated environments.	Deploy engineered microbes to nucleate ice and help preserve snowpack in the environment.

Enhance ocean and coastal carbon capacity via engineered biology.

Short-term	Medium-term	Long-term
Engineer anaerobic and halophilic microbes and planctomycetota to supplement coastal wetland soils for increased carbon storage.	Engineer macroalgae (including seaweed and kelp) for carbon capture and reduction of ocean acidification.	Systematically engineer marine biological carbon pumps to increase the amount of recalcitrant dissolved organic carbon in the ocean.
Engineer phytoplankton to be more robust to declining marine conditions, including increased water temperatures, acidification, eutrophication, and hypoxia.		

Goal: At-scale capture, storage, and utilization of greenhouse gases (GHGs) by engineered organisms.

Current State-of-the-Art: Removal of greenhouse gases – including carbon oxides, methane, nitrous oxide, and fluorinated gases – from the environment is one of the primary components to mitigating climate change. Using autotrophic organisms to capture GHGs, we can leverage the self-replication of biological organisms as a mechanism for continual capture, resulting in negative carbon emissions and a cleaner environment world-wide. Biology is uniquely suited to address GHG capture, storage and utilization. It is likely that the first complex molecules to emerge on Earth were all synthesized from CO_2 (Russell & Martin, 2004) and today several CO_2 fixation routes are known (Köpke, 2022; Berg, 2011; Bar-Even et al., 2012).

Photoautotrophs (plants, algae, cyanobacteria) absorb sunlight and CO_2 to make biomass. Engineering biology could increase the efficiency of this process and create more capacity for CO_2 drawdown by using genetic editing tools to optimize key complexes, enzymes, and pathways involved in photosynthesis and carbon fixation. Advances in engineering biology, especially the emergence and widespread use of CRISPR, have led to a series of recent successes in engineering plants, though major research questions and challenges still remain (Zhang et al., 2020). Extensive research efforts have been directed towards engineering RuBisCo - the enzyme responsible for catalyzing the first step of CO_2 uptake in carbon fixation and the most abundant protein on Earth turning over an approximate 400 gigatons of CO_2 per year - as a key target for improving plant photosynthesis efficiency to improve its catalytic efficiency (Erb and Zarzycki, 2018). In addition to improving enzymatic pathways for CO_2 conversion, engineering photosynthetic organisms (especially plants) to more efficiently capture light and tolerate dynamic lighting conditions will help to achieve higher rates of CO_2 conversion (Kirst et al., 2017). In addition to plants, photosynthetic organisms like cyanobacteria and algae are also valuable research targets for carbon capture. Importantly, cyanobacteria and algae contain carbon concentrating mechanisms (CCM) that make them more efficient at photosynthesis and carbon fixation than plants, and research is underway to embed CCMs into plants and other model organisms for carbon capture (Cai et al., 2021).

In addition to these photoautotrophs that require light as a source of electrons, there is a wide range of chemoautotrophs capable of utilizing carbon oxides or methane (Pavan et al., 2022; Dürre & Eikmanns, 2015). Efforts are underway to develop tools to efficiently engineer chemoautotrophic organisms including acetogens, hydrogenogens, or methanotrophs or even transfer into model organisms like *E.coli* or yeast (Bennett et al., 2021; Gleizer et al., 2019). This includes enhancing the seven known CO_2 fixation pathways with new-to-nature reactions or designing synthetic or *de novo* CO_2 fixation pathways. Researchers have aimed to circumvent the challenges posed by endogenous carbon fixation by focusing on designing synthetic metabolic pathways (Bar-Even et al., 2010; Scheffen et al., 2021) and identifying key enzymes other than RuBisCo that are critical for carbon fixation, such as carboxylation via 6-phosphogluconate dehydrogenase (Flamholz et al., 2019, Bar-Even, 2018). There is also work underway to rewire CO_2 fixation pathways (Wu et al., 2022; Köpke, 2022) or transplant engineered fixation pathways into (new) microbial chassis and engineering *in vitro* CO_2 fixation in cell-free systems (Scheffen et al., 2021). Key challenges include that there are still gaps in our understanding of CO_2 fixation pathways (Öppinger et al., 2022; Kremp et al., 2022; Köpke, 2022) and many pathways such as the Wood-Ljundahl pathway which is considered to be the most energy efficient CO_2 fixation pathway (Bar-Even et al., 2012; Claassens et al., 2019; Fast & Papoutsakis, 2012) are complex and require a network of hundreds of genes involved for chemoautotrophic growth and associated energy conservation (Kaster et al., 2011).

Most chemoautotrophs convert carbon oxides or methane into cellular biomass or simple molecules such as acetate (which are intermediates for other organisms in the global carbon cycle) (Drake et al., 2006; Zhuang et al., 2019). Engineered organisms and biobased systems could upgrade intermediates like acetate (Hu et al., 2016), or capture and convert carbon oxides directly, into more complex, value-added commodities (Köpke & Simpson, 2020; Fackler et al., 2021; Liew et al., 2022). Because many photosynthetic and chemoautotrophic organisms convert CO_2 into biomass through carbon fixation, essentially turning gaseous CO_2 into solid carbon, they conveniently achieve carbon capture and storage at the same time, enabling carbon negative manufacturing (Scown & Keasling, 2022). For instance, bacteria could be engineered to convert carbon oxides into precursors for acrylic glass (Liew et al., 2022), bioplastics (Ding et al., 2019), or solid compounds like calcium carbonate (Antunes, 2021), which could keep captured CO_2 sequestered for tens to hundreds of years (Chang et al., 2017). Such approaches could further help mitigate the risk of uncontrolled release from carbon capture and storage. Already, ethanol production from carbon-monoxide rich industrial off-gases with native chemoautotrophs is carried out at commercial scale by companies like LanzaTech. Charm Industrial, a carbon tech startup, aims to “permanently put CO_2 back underground” by making bio-oil from the pyrolysis of waste biomass and injecting the oil into deep geological formations. Recent research has demonstrated the biosynthesis of starch from CO_2 in cell-free systems (Cai et al., 2021), the production of cotton-alternative cellulose from CO_2 (RUBI Laboratories, n.d.), and the production of value-added chemicals in co-cultured microbial consortium (Cha et al., 2021). Similarly, a range of chemical production from methane has been described in engineered methanotrophs (Nazem-Bokaee et al., 2016; McAnulty et al., 2017; Nguyen et al., 2020; Strong et al., 2016). In addition to CO_2 and methane conversion, capturing and conversion carbon oxide containing off-gases from heavy industry (e.g. steel, ferroalloy) or syngas from gasification of various solid wastes via microbial gas fermentation into a range of chemicals has been demonstrated (Köpke & Simpson, 2020) and a recent study demonstrated production of platform chemicals acetone and isopropanol at high rates in an industrial pilot (Liew et al., 2022; and summarized by Scown & Keasling, 2022). Further, macroalgae could sequester nitrates and phosphates, followed by harvesting and use as low/negative-carbon fertilizers. Where no concentrated CO_2 or methane stream is available as required for many conversion or storage technologies, biology may also provide an opportunity to increase the concentration of gases, as an alternative to current direct air capture (DAC) methods (Talekar et al., 2022).

The processes described above could be used to store and utilize GHGs captured at emission sources. Concentrated streams, such as emissions from power plants, are easier to mitigate than diluted sources, such as diffuse GHGs in the atmosphere. While engineered organisms are currently tested in lab settings using controlled amounts of CO_2 or methane as input, we still need to develop engineering capabilities to enable the biosequestration of environmental and diffuse carbon at an industrial scale. Improving gas fermentation technology will be key to accomplishing this (Köpke & Simpson, 2020; Fackler et al., 2021). These capabilities will be important stepping stones towards enabling organisms to capture different types of GHGs from concentrated streams and ambient air and convert captured GHG molecules into value-added products.

Breakthrough Capability: Improve CO_2 uptake by engineering more efficient photosynthetic organisms (plants, algae, cyanobacteria).

Short-term Milestone: Engineer plants for optimized light collection and more efficient use of captured light for photosynthesis.

- Bottleneck: Chlorophylls have evolved to only absorb light in the wavelength range of 400nm to 700nm.
    - Potential Solution: Engineer and introduce into plants alternative chlorophylls with expanded absorption spectrum, such as by enabling the expression of bacteriochlorophylls, which have absorption maxima in the far-red region.
- Bottleneck: Light harvesting complexes (antennae proteins) trap more light than can be used for photochemistry and block leaves in lower layers from accessing more light.
    - Potential Solution: Engineer photosystems to have a reduced number of antennae or smaller antennae.
- Bottleneck: Photoprotective mechanisms, such as non-photochemical quenching (NPQ), protect the plant from excess light, but decrease the overall photosynthetic efficiency under high-light conditions.
    - Potential Solution: Introduce genes into plants to accelerate the relaxation rate of photoprotection and NPQ.
    - Potential Solution: Incorporate genes (and engineer new circuits and regulatory networks, as necessary) that enable plants to quickly adapt to fluctuating light conditions and turn off photoprotection, so excess light is used towards photosynthesis instead of being dissipated as heat.

Medium-term Milestone: Engineer pathways and enzymes in photosynthetic organisms to increase the rate and efficiency of carbon fixation.

- Bottleneck: Genetic engineering tools developed in model organisms are often ineffective or inefficient in photosynthetic organisms.
    - Potential Solution: Develop metabolic models and genetic engineering tools for photosynthetic organisms.
    - Potential Solution: Bioprospect for new organisms to expand basic understanding of the molecular biology of photosynthetic microbes.
- Bottleneck: RuBisCO is a large complex made of multiple subunits that require an array of chaperones for folding and assembling and that are sensitive to inhibition by sugar-phosphate ligands (Hayer-Hartl, 2017); engineering its catalytic biochemistry currently requires non-ideal tradeoffs.

    - Potential Solution: Develop better understanding of RuBisCo components, such as via high-throughput microfluidic enzyme kinetics, to enable editing multiple aspects simultaneously (Mokhtari et al., 2021; Scales et al., 2014).
    - Potential Solution: High-throughput characterization of the biodiversity of RuBisCos across photosynthetic organisms to identify those that have fewer subunits, simpler folding kinetics, and high efficiency; engineer existing elements of those systems into photosynthetic organisms that are or can be grown at scale.
- Bottleneck: RuBisCo has an error rate of more than 20% resulting in toxic 2-phosphoglycolate, with engineering efforts to improve has been challenging; 2-Phosphoglycolate salvage is an energetically expensive and wasteful process, losing a carbon in the form of CO_2 (Panich et al., 2021; Erb & Zarzycki, 2018).
    - Potential Solution: Modifying 2-Phosphoglycolate salvage to improve carbon efficiency.
    - Potential Solution: Engineer and transform plants with more efficient RuBisCO (Lin et al., 2014).
- Bottleneck: Challenges remain in expressing prokaryotic carbon concentrating mechanisms (CCM) in eukaryotic cells.
    - Potential Solution: Engineer fully reconstructed heterologous CCMs to enable successful expression of CCM in plants and model organisms.
    - Potential Solution: Engineer microbes to grow using captured carbon as substrates.

Medium-term Milestone: Develop scalable carbon capturing platforms enabled by engineered green algae and cyanobacteria.

- Bottleneck: For algae farms coupled to carbon emitters (e.g., power plants), there is more CO_2 emitted than the algae farm could fully capture and utilize.
    - Potential Solution: Select and engineer algal strains with high CO_2 uptake rates and/or carbon concentrating mechanisms.
    - Potential Solution: Engineer hydrogenases to increase carbon utilization in green algae (e.g., hydrogenases not inhibited by carbon monoxide).
- Bottleneck: Current photo-bioreactor design is insufficient to optimize carbon capture and bioproduction.
    - Potential Solution: Construct low-cost open bioreactors (e.g., ponds, photobioreactors, or gas fermentors) with organism-tailored geometries, flow rates, and media compositions.

Long-term Milestone: Combine and rewire native CO_2 fixation pathways (e.g., C3 and C4 pathways) and engineer organisms capable of utilizing multiple carbon fixation pathways (see for example Moreno-Villena et al., 2022).

- Bottleneck: Engineering C3 plants for C4 carbon fixation requires control of the precise spatial expression of many genes between mesophyll and bundle sheath cells, which would be challenging and very time intensive to engineer in plants (Ermakova et al., 2021).

  - Potential Solution: Improved techniques for transforming plants with multiple genes and/or pathways under precise spatial control.
- Bottleneck: Engineering many elements of a pathway is challenging; the expression and function of each element may need to be optimized, e.g., to avoid the production of undesirable intermediates or finetune pathway regulatory mechanisms (Schwander et al., 2016).
  - Potential Solution: Optimize rapid *in vitro* and *in vivo* pathway characterization.

Breakthrough Capability: Enable efficient carbon capture by engineered chemoautotrophs.

Short-term Milestone: Map and identify parts in CO_2 fixation pathways to increase the efficiency of carbon fixation in chemoautotrophic organisms.

- Bottleneck: Identity and understanding of the most rate-limiting step to CO_2 sequestration in chemoautotrophic model organisms and the missing energy-coupling sites and interaction in native carbon fixation pathways (e.g., Wood-Ljungdahl pathway).
  - Potential Solution: Understand the role of all genes involved in carbon fixation in chemoautotrophic organisms through omics approaches, enzyme studies, mutagenesis or knockout experiments to identify the rate-limiting step and missing links.
  - Potential Solution: Map and understand the flux and bioenergetic links between carbon, nitrogen, phosphorus, sulfur metabolism in chemoautotrophs.
- Bottleneck: Knowledge of how changes in enzyme expression levels affect function in C1 pathways.
  - Potential Solution: Map protein-protein interactions, characterize transcription factors and multienzyme complexes and their dynamics, and identify metabolic substrate channeling between relevant enzymes.

Medium-term Milestone: Engineer complexes and metabolic pathways in chemoautotrophs to improve carbon fixation.

- Bottleneck: Enzymes and cofactors optimized for recycling and energetics.
  - Potential Solution: Improve the efficiency of major CO_2 fixation or methane oxidizing enzymes.
  - Potential Solution: Discover or design new enzymes that are more efficient at capturing CO_2 or converting methane.
  - Potential Solution: Develop orthologous co-factors.
- Bottleneck: Limited molecular and genetic toolkits for domesticated chemoautotrophs.
  - Potential Solution: Develop broader toolsets (e.g., genome engineering, enzyme engineering, and cell-free systems) and high-throughput workflows for engineering chemoautotrophs, such as *Thermotoga neapolitana*, *Cupriavidus necator*, *Clostridia* species, and methanoarchaea.

        - Potential Solution: Develop high-throughput screening capabilities to reduce strain development cycle times.
- Bottleneck: High-throughput cultivation and product screening in context flammable and/or toxic gaseous substrates such as carbon oxides and methane.
    - Potential Solution: Develop new plate based or microfluidics based screening workflows that facilitate growth on gaseous substrates, while retaining or direct measuring of product concentrations.
    - Potential Solution: Develop analytics and sensor tools for dissolved concentrations of carbon oxide and methane gasses in screening assays.

Long-term Milestone: Demonstrate use of engineered chemoautotrophs to capture more CO_2 in the context of environmental or industrial processes.

- Bottleneck: Air and many other potential industrial streams (e.g., cement plants, landfills) have low CO_2 or methane concentrations requiring expensive steps for gas concentration or compression.
    - Potential solution: Engineer organisms for effective conversion at low or atmospheric CO_2 or methane concentrations.
- Bottleneck: Effective biocontainment strategies for deployed organisms.
    - Potential Solution: Develop low-cost methods to employ bio-orthogonal biochemistry.
    - Potential Solution: Develop risk analysis frameworks to define risk benchmarks.

Breakthrough Capability: Enable organisms to utilize captured carbon to produce value-added chemicals and materials.

Short-term Milestone: Engineer organisms to convert CO_2, methane, or other C1 sources and intermediates (incuding methanol, formate, acetate) into value-added compounds.

- Bottleneck: Optimal electro-biochemical routes for carbon conversion into value added compounds are not known.
    - Potential Solution: Design electro-biochemical routes for minimizing the loss of carbon through metabolism or to directly sequestering carbon for bioconversion into value-added compounds (Abel et al., 2022b).
    - Potential Solution: Develop approaches to evolve promising chemolithoautotrophic organisms to increase yield of desired products.
- Bottleneck: Lack of platforms for genome-wide engineering of non-model chemoautotrophs with metabolic and physiological capabilities needed for optimized carbon conversion.
    - Potential Solution: Develop new genome scale modeling and engineering tools for rapidly generating and implementing carbon-optimized designs.
    - Potential Solution: Develop machine learning algorithms, artificial intelligence tools, cell-free systems, and multi-omics workflows to enable faster data-driven DBTL cycles in non-model microbes.

- Bottleneck: While acetate is a universal carbon source for many microbes (including model organisms such as yeast or *E. coli*) that have been engineered to produce value-added chemicals, the current process releases CO_2 (Nielsen & Keasling, 2016).
    - Potential Solution: Chemoautotrophs are capable of producing acetate from CO_2 at high rates (Kantzow & Weuster-Botz, 2016); adapt efficient production strains for using acetate instead of sugars as substrate for value-added products and develop co-culture or coupled processes.

Medium-term Milestone: Optimize the bio-utilization of CO_2 and methane emitted from point sources.

- Bottleneck: High gas mass transfer is required; gases like methane, carbon monoxide or hydrogen are poorly soluble.
    - Potential Solution: Develop energy-efficient systems for harvesting products made by microbes grown in large-scale bioreactors.
- Bottleneck: Waste gas streams contain compounds that inhibit the activities of microbes and enzymes.
    - Potential Solution: Engineer and select microbes to tolerate different sources of greenhouse gas and metabolic byproducts.
    - Potential Solution: Improve enzymatic activity, stability, and reusability for converting CO_2 into chemicals.

Medium-term Milestone: Improve gas fermentation technologies.

- Bottleneck: Heterogeneity due to continuous gas feeding and gradients in bioreactor environments.
    - Potential Solution: Develop real-time, biobased monitoring tools (e.g., biosensors to detect and report dissolved gases such as carbon monoxide).
    - Potential Solution: Engineer microbes with focus on efficient utilization of variable, fluctuating gas ratios.

Long-term Milestone: Combine and rewire native carbon utilization pathways and engineer organisms capable of using multiple carbon metabolism pathways.

- Bottleneck: Flexible chassis organisms suitable for industrial scale cultivation.
    - Potential Solution: Engineer reversible flux-based CO_2 fixation, H_2 production and methanogenesis/methanotrophy in, for example, *Methanosarcinales* (Abel et al., 2022a).
    - Potential Solution: Engineer consortia that can capture and utilize the full carbon life-cycle in a circular manner.

Breakthrough Capability: Enable carbon capture and utilization by enzymes or cell-free systems.

Short-term Milestone: Develop efficient enzymes for concentrating carbon from the atmosphere.

- Bottleneck: Current methods for direct air capture (DAC) technologies to concentrate CO_2 from air are expensive (McQueen et al., 2021).

- ○ Potential Solution: Enzymes like carbonic anhydrase (CA) can facilitate the dissolution of atmospheric CO_2 but require improvement in efficiency, stability, and inexpensive ways to release CO_2 for downstream processes.

Short-term Milestone: Develop scalable cell-free systems as platforms for carbon capture and bioconversion.

- Bottleneck: Cell-free technologies are currently expensive at-scale.
  - Potential Solution: Identify organisms and components that can use carbon capture materials (carbon black, carbonate, etc.) as substrates.

Medium-term Milestone: Develop efficient and scalable cell-free systems capable of utilizing methane, formate, or CO_2 to produce commodity fuels and chemicals.

- Bottleneck: Modular capabilities within cell-free systems to produce high-value products.
  - Potential Solution: Engineer efficient multienzyme (plug-and-play, step-wise) cascade systems to convert CO_2.
- Bottleneck: Many methane-capturing enzymes, such as methane monooxygenase (MMO), are membrane-associated and thus more challenging to develop for cell-free technologies.
  - Potential Solution: Advance methods for creating vesicles or lipid discs enriched with functionally active MMOs that can be used to supplement cell-free systems with membrane-associated activities.

Long-term Milestone: Develop self-contained and/or standalone cell-free CO_2 fixation systems for bio-enabled artificial photosynthesis.

- Bottleneck: The high cost of cofactors and energy regeneration systems to support high-level activity.
  - Potential Solution: Develop the ability of cell-free systems to make all components necessary to support high metabolic rates.

Long-term Milestone: Develop new platform tools for multienzyme immobilization in cell-free systems.

- Bottleneck: Costs for enzyme production and maintaining catalyst/enzyme stability when immobilized.
  - Potential Solution: Establish new approaches for enzyme capture/immobilization that are cost-effective and facilitate high activity and stability.

Goal: Increase carbon uptake and mitigate climate change by enhancing natural systems through engineered biology.

Current State-of-the-Art: In addition to the active removal of greenhouse gases from the atmosphere, engineering biology can be used to bolster the uptake and storage of carbon in natural ecosystems. Agricultural ecosystems, wetlands and deserts all represent promising terrestrial ecosystems for carbon storage. Plant engineering, such as increasing carbon capture phenotypes through overexpression or engineering rhizosphere communities, could increase soil carbon capacity by modifying crops to store more carbon in their roots. Wetlands already represent major global carbon sinks (Nahlik & Fennessy, 2016) and a source of increasing greenhouse gas (GHG) emissions (Zhang et al., 2017c). Pollutant-degrading microbes could be deployed to help wetland plants fight pollution-related wetland degradation and support carbon sequestration. Engineering approaches that can rapidly restore wetlands, increase carbon storage, and reduce methane production (or increased methane utilization) could have a large beneficial climate effect.

Climate change is also contributing significantly to changes in terrestrial ecosystems conditions, particularly in the amount of heat they experience and the amount of water available. Arid ecosystems represent a promising target for soil carbon accumulation given that they account for ~40% of land area, are typically already very low carbon soils, and the limited water already stabilizes soil carbon pools (Rodríguez-Caballero et al., 2018). Engineering microbial communities that colonized these arid soils (biocrusts) provides a very promising approach to store soil carbon. Some large-scale projects in China have already demonstrated the feasibility of artificial inoculation of sands with biocrust cyanobacteria (hundreds of hectares, Zhou, 2020) for stabilizing soils, building soil carbon, and initiating ecosystem restoration. Given that large, and unfortunately growing, scale of arid ecosystems these approaches could have a massive impact and could potentially turn wastelands back into arable lands to help support Earth's growing population. Finally, microbial ice nucleation could be leveraged to help maintain snowpack, create more reflective surfaces in alpine and polar environments, and preserve permafrost and prevent carbon release (Brouillette, 2021).

Engineering biology could also enhance coastal and ocean carbon sequestration. Ocean and coastal environments account for significant amounts of CO_2 removal and storage, but are highly susceptible to damage caused by climate change. The processes of carbon cycling and storage in marine environments are less researched, but extremely productive (National Academies of Sciences, Engineering, and Medicine, 2019; Zhang et al., 2017b). Phytoplanktons and macroalgae, such as kelp, could be engineered to improve carbon capture in the ocean and mitigate ocean acidification. Similarly, planctomycetota (bacteria that carry out anammox, anaerobic ammonium oxidation, reactions), halophiles, and viruses could also play very important roles in marine carbon sequestration, and potentially be incorporated into microbiomes or otherwise be stably deployed into oceans to increase carbon capture.

Breakthrough Capability: Enhance soil carbon storage capacity via engineered biology.

Short-term Milestone: Understand the role of soil microbiome in modifying (specifically, increasing) soil carbon capacity.

- Bottleneck: The high complexity and multitude of soil microbes make it difficult to identify soil microbial community function using currently available -omics techniques.
    - Potential Solution: Develop high-throughput proteomics techniques to better understand biological functions in soil matrix.

  - Potential Solution: Improve soil metabolomics reference databases and metabolomics techniques to better understand biogeochemical cycling in soil.
  - Potential Solution: Integrate multiple -omics datasets into a single database.
- Bottleneck: Paucity of models for how soil microbiota regulate soil carbon capacity across different spatial (microscopic, mesoscopic, and macroscopic) and temporal scales.
  - Potential Solution: Develop tools to enable the measurement of biochemical reactions (microscale) in the field (macroscale).
  - Potential Solution: Consolidate datasets from lab- and field-based studies to create an integrated soil microbiome database.
  - Potential Solution: Develop computational models (informed by lab and field studies) to bridge the knowledge gaps between different spatial and temporal scales.
- Bottleneck: Limited understanding of how microbes deposit carbon as minerals and sediments in soil.
  - Potential Solution: Develop metabolite labeling and tracing tools that can be used to track carbon movement in soil microbiome first in laboratory conditions and later *in situ* (Watts-Williams, 2022).
- Bottleneck: Current poor understanding of the chemical and ecological factors that govern the residence time of specific molecules in soils (e.g., betaine).
  - Potential Solution: Highly controlled ecosystem studies coupled to high resolution mass spectrometry to determine the factors that affect the turnover of specific molecules in soils.

Short-term Milestones: Engineer model plants to increase root biomass contributing to below-ground carbon storage.

- Bottleneck: Avoiding undesired phenotypes associated with engineering metabolic flux (Mahmood et al., 2019; Baxter et al., 2009).
  - Potential Solution: Genetic determinants for some carbon-storing compounds are well characterized (see Harman-Ware et al., 2021); apply this understanding to systems/organismal engineering to enable control of compound synthesis, transport, and storage.

Short-term Milestone: Engineer the root systems of crop and non-model plants to store more carbon.

- Bottleneck: The synthesis of carbon-storing compounds can vary by cultivar in response to environmental conditions.
  - Potential Solution: Undertake large field trials using genomic, transcriptomic, proteomic, and metabolomic analyses to understand the contributions of genetics and the environment to the synthesis of key carbon-storing compounds, such as suberin, in non-model and crop plants (Harman-Ware et al., 2021).

- Potential Solution: Understand and engineer synthesis and transport mechanisms for carbon-storing compounds, such as suberin, that are synthesized above and below ground for consistent root accumulation across environmental conditions and relevant plant cultivars.
- Potential Solution: Engineer suberin to increase carrying capacity and retain carbon for longer time periods.

Short-term Milestone: Engineer plant roots to secrete metabolites that recruit microbes capable of converting labile plant exudates into stable soil carbon.

- Bottleneck: Relationships between plants and microbes vary under differing environmental conditions, potentially to the plant's benefit (see von Rein et al., 2016; Wipf et al., 2021); preferencing the recruitment of target microbes under stressful environmental conditions may disrupt interactions that support plant health.
  - Potential Solution: Engineer microbiomes that are responsive to changing conditions.
- Bottleneck: Plant metabolites may need to be converted to alternative forms or compounds by microbiome community members in order to recruit microbes that increase carbon capacity, which would be challenging to track and elucidate.
  - Potential Solution: Enhance capabilities for tracing metabolite transfer from plants through the microbiome, working toward greater resolution (e.g., Family or Genus) of involved community members.
  - Potential Solution: Harness microbial communities and knowledge of specific molecules with longer residence times under specific environmental and ecological conditions.
  - Possible Solution: Maximize microbial conversion of exudate to biomass (and subsequently, microbial necromass) by designing microbial communities that use all exudate components.

Medium-term Milestone: Enable stable, long-term carbon storage in soil microbiomes, such as by introducing fungi to enhance weathering.

- Bottleneck: Unstable soil aggregates (i.e., due to tilling) release captured carbon back into the atmosphere.
  - Potential Solution: Engineer soil microbes to produce biofilms to promote the formation of soil aggregates.
  - Potential Solution: Engineer biomaterials to stabilize soil micro-aggregates.
- Bottleneck: Fungal hyphal networks (e.g., arbuscular mycorrhizal fungi) have been shown to enhance mineral weathering in soil, but the most well-studied of such fungi – mycorrhizal fungi –need host plants to survive.
  - Potential Solution: Engineer arbuscular mycorrhizal fungi to survive independent of a host plant.
  - Potential Solution: Develop means to seed soil with fungi capable of mineral weathering (e.g., saprotrophic fungi) and surviving without host plants.

Medium-term Milestone: Identify and characterize exometabolites beneficial to increasing carbon storage capacity in plants or soil.

- Bottleneck: The exometabolome is challenging to characterize because of extensive cross-feeding/uptake by other community members.
  - Potential Solution: Develop improved tools to quantify the exchange of metabolites within complex microbiomes (Douglas, 2020).

Medium-term Milestone: Enable microbial communities in permafrost to retain and/or capture greenhouse gases.

- Bottleneck: Uncharacterized permafrost microbiome.
  - Potential Solution: Use multi-omics tools and machine learning to build comprehensive datasets of permafrost microbial communities.

Long-term Milestone: Develop methods for *in situ* modification of soil microbial communities to increase carbon storage in evolving at-risk soils.

- Bottleneck: Relevant soil microbiome constituents are under-characterized and we lack understanding of which species might be best for engineering approaches.
  - Potential Solution: Expand capabilities for culturing recalcitrant microbes.
  - Potential Solution: Develop techniques to characterize unculturable microbes *in situ*.

Breakthrough Capability: Restore disturbed natural biocrusts and increase carbon sequestration in arid lands.

Short-term Milestone: Assess the carbon removal potential of deploying artificial biocrust in a variety of arid environments.

- Bottleneck: More understanding is needed on the durability of sequestered carbon and how biocrust interacts with other parts of the carbon cycle.
  - Potential Solution: Identify biochemical factors that increase carbon sequestration in biocrusts.
  - Potential Solution: Measure carbon sequestration capacity in artificial biocrust over temporal and spatial scales, and under different environmental conditions (e.g., temperature, precipitation, and nutrient levels).

Medium-term Milestone: Demonstrate engineered biocrust communities to sequester carbon in arid environments.

- Bottleneck: It is difficult to isolate fast-growing and suitable cyanobacteria for inoculating biocrust.
  - Potential Solution: Identify, cultivate, and engineer filamentous cyanobacteria from a variety of dryland regions for desired growth rate and robustness under requisite environmental conditions (e.g., high summer heat).
  - Potential Solution: Develop and engineer consortia of cyanobacteria (different species) to more successfully inoculate artificial or native biocrusts.

- Bottleneck: Processes for inoculating engineered cyanobacteria into natural biocrusts are underdeveloped.

Long-term Milestone: Engineer and deploy artificial biocrust to restore and increase climate-resilience of native biocrust and desert ecosystems.

- Bottleneck: Biocrusts can be incredibly complex depending on the organisms/species and abiotic components involved; the interaction with native ecosystems would need to be structured and resolved.
    - Potential Solution: Characterize and engineer symbiosis between plants and artificially enhanced biocrusts to increase soil stability.

Breakthrough Capability: Enhance albedo via engineered microbes.

Short-term Milestone: Identify and engineer microbes with increased ice nucleation capabilities.

- Bottleneck: Mechanisms of microbial ice nucleation are not well understood.
    - Potential Solution: Use advanced -omics techniques to identify links between microbes/microbial communities and their ability to nucleate ice.
- Bottleneck: A lack of genetic editing tools to engineer ice nucleating biological systems.
    - Potential Solution: Develop genetic tools (e.g., transformation methods, genetic parts) for engineering cryophilic microbial chassis.
    - Potential Solution: Identify or design ice nucleating proteins with high efficiency and robustness of ice nucleation.

Medium-term Milestone: Demonstrate biological ice formation in simulated environments.

- Bottleneck: Poor understanding of environmental factors that inhibit or enhance microbial ice nucleation.
    - Potential Solution: Utilize machine learning and artificial intelligence to design models for microbial ice nucleation based on data from persistent, native microbial communities.

Long-term Milestone: Deploy engineered microbes to nucleate ice and help preserve snowpack in the environment.

- Bottleneck: Poor understanding how ice nucleating microbes interact with the broader ecosystem (for example, the role of ice nucleating bacteria in arctic marine environments).
    - Potential Solution: Develop and test strategies for biocontainment of engineered microbes in the polar/alpine environment.

Breakthrough Capability: Enhance ocean and coastal carbon capacity via engineered biology.

Short-term Milestone: Engineer anaerobic and halophilic microbes and planctomycetota to supplement coastal wetland soils for increased carbon storage.

- Bottleneck: Limited understanding of microbial carbon-cycling processes in anaerobic and/or high-salinity soils.

  - Potential Solution: Extend metagenomics, proteomics, and metabolomics tools for studying soils to wetland soil environments.

Short-term Milestone: Engineer phytoplankton to be more robust to declining marine conditions, including increased water temperatures, acidification, eutrophication, and hypoxia.

- Bottleneck: Limited understanding of how ecological stressors impact marine microbes in coastal ecosystems on a genetic and metabolic level.
  - Potential solution: Engineer field-deployable biosensors for local chemistry (e.g., salinity) and pollutants (e.g., agricultural fertilizer runoff) that specifically impacts wetlands, salt marshes, and other coastal ecosystems.

Medium-term Milestone: Engineer macroalgae (including seaweed and kelp) for carbon capture and reduction of ocean acidification.

- Bottleneck: Limited gene editing tools for engineering macroalgae (e.g., *Saccharina* and *Gracilaria*).
  - Potential Solution: Develop and improve direct bacterial transformation (such as in ways similar to agrobacterium transformation of plants) (Oertel et al., 2015).
  - Potential Solution: Gain better control of gametophyte hybridization and development of CRISPR-Cas systems (Wang et al., 2020a).
- Bottleneck: Macroalgae grown in offshore environments compete for nutrients with carbon-fixing phytoplankton.
  - Potential Solution: Deploy carbon-capturing microbes that produce nutrients necessary for macroalgae growth in coastal algal farms.

Long-term milestones: Systematically engineer marine biological carbon pumps to increase the amount of recalcitrant dissolved organic carbon in the ocean.

- Bottleneck: Paucity of information about (but ever-increasing volume of) refractory organic compounds and chemical structures.
  - Potential Solution: Develop further understanding of and engineer microbes that enhance processes involved in the biological carbon pump that leads to long-term sequestration of carbon from the surface ocean to the deep ocean interiors.
  - Potential Solution: Identify how conversion of short-lived organic pools to recalcitrant carbon impacts microbial-driven nutrient cycles.
  - Potential Solution: Develop further understanding of how bacteria, viruses, plankton, and other microbes interact to facilitate long-term carbon sequestration.

Mitigating Environmental Pollution

Introduction and Impact: Environmental contamination and pollution are unfortunate hallmarks of the anthropocene. Human population growth and inadequate environmental stewardship are causing significant impacts that ripple through human interactions with each other and the planet's land and water. Urbanization and advanced technological capabilities and capacity have resulted in human uses of natural resources in unsustainable ways that damage, pollute, deplete, or destroy natural environments. Further, climate change is worsening natural disasters (floods, storms, fires) that contribute to new and more frequent environmental disturbance and contamination. Fortunately, the rich diversity of biological systems can inspire biobased solutions to this grand challenge. This roadmap technical theme considers opportunities to leverage biology to help *protect* biology, as well as other elements of the broader biosphere, by engineering biological solutions to mitigate environmental pollution and contamination [Figure 3].

To adequately prevent and mitigate pollution, robust monitoring capabilities must be employed to identify pollutant sources and the locations of greatest accumulation and impact. Engineering biology can help to enable strategies to monitor these contaminants, ultimately informing and contributing to the development of tools for removal, mitigation, and remediation. For example, massively scalable point-of-use biosensing technologies could empower individuals to monitor their own environmental quality; such data would support efforts to advocate for environmental justice in marginalized communities. Affordable water quality biosensors could prevent acute and chronic illnesses. And continuous environmental quality monitoring capabilities could inform 'smart cities' approaches to tackle pollution challenges in urban environments.

Anthropogenic activities like mining, agriculture, and manufacturing introduce an assortment of contaminants into the environment, including plastic, heavy metals, excess nutrients, and harsh chemicals. These contaminants can cause significant disruptions to land (Alengebawy et al., 2021) and aquatic ecosystems (Bashir et al., 2020), damage wildlife in these environments (McCabe et al., 2016; Trainer et al., 2020), and adversely affect human health (Briffa et al., 2020; Jaishankar et al., 2014). Engineering biology can enable bioremediation through bio-enabled sequestration and degradation of harmful pollutants. Some of the most impactful pollutants we currently face, and have chosen to address in this roadmap, include plastic waste, per- and polyfluoroalkyl substances, and heavy metals, including electronics waste. This roadmap considers microbial engineering and other biotechnologies that can contribute to the capture and degradation of these pollutants.

Climate change is expected to exacerbate the risks these contaminants pose to the environment (Vadeboncoeur et al., 2021; Schiedek et al., 2007). In order to maintain healthy populations and ecosystems, we need to consider how we can mitigate pollution from select environments where the impacts of contaminants can have particularly wide ranging and harmful downstream effects. This technical theme considers three of those circumstances: contamination in municipal wastewater, contaminants stemming from agriculture and aquaculture, and industrial effluent, which often contains harsh chemical waste. Bioremediation

Figure 3. Mitigating environmental pollution with engineering biology. Climate change and human activity are leading to increased pollution and contamination of the environment. With engineering biology, we have the opportunity to collect and degrade many types of waste from many different sources. Through the use of embedded engineered biosensors, such as in biomaterials or engineered plants, or biosensing and reporting cell-free systems, we could continuously monitor and detect contaminants entering the environment. Engineering microbes and other biosystems could be used to sequester and degrade particularly problematic pollutants, including plastic waste, PFAS, and heavy metals. Pollution and contaminants could also be targeted at specific known sources ripe for integration of engineered biological technologies, including municipal wastewater and sites of industrial effluent. Advancements will need to be made in the biological detection and reporting of specific molecules and compounds, engineering of enzymes capable of targeted contaminant degradation, and the robustness of biosystems to complex and toxic environments.

can help to ensure safe drinking water, beaches and waterways free of harmful bacteria and algae, and safer industrial and urban environments.

Environmental remediation in *Microbiome Engineering*

EBRC's 2020 roadmap, *Microbiome Engineering: A Research Roadmap for the Next-Generation Bioeconomy*, identified a number of ways that engineered microbiomes, in particular, could be used to mitigate and remove pollutants from the environment {see: https://roadmap.ebrc.org/micro-enviro-biotech-remediation-recycling/}. These objectives and achievements include employing microbiomes to sense and sequester contaminants, degrade plastic waste, and improve water treatment processes.

MITIGATING ENVIRONMENTAL POLLUTION

Goal	Breakthrough Capability	Milestone

Rapid detection and continuous monitoring of environmental contaminants.

Enable the detection and continuous monitoring of pollutants and priority contaminants in the environment using biosensors.

Short-term	Medium-term	Long-term
Engineer highly specific, low-cost, and field-deployable biosensors for priority contaminants (e.g., paper-based cell-free systems).	Demonstrate next-generation biosensors with novel detection modalities.	Engineer autonomous, self-regulating biosensors that can detect and remediate pollutants.
Engineer biosensors to be compatible with digital infrastructure.	Enable the deployment of biosensors for field applications and long-term environmental monitoring.	

Mitigate targeted environmental pollutants through biosequestion and biodegradation.

Degrade plastic waste through engineered bioprocesses.

Short-term	Medium-term	Long-term
Engineer microbes, microbial consortia, or enzymes to efficiently degrade common plastic polymers.	Enable the integration of degradation enzymes for local, on-demand plastic waste processing.	Engineer organisms or enzymes to 'carbon-negatively' degrade plastics *in situ*, in different ecological niches (e.g., in soil, on the ocean floor).
	Develop microbial consortia and enzymatic cocktails for the efficient breakdown of mixed-waste plastics (e.g., multi-layer plastics such as those found in carpets or shoes) and 'dirty'/ used plastics (e.g., takeout containers).	Design bioplastics with innate degradation mechanisms (e.g., embedded enzymes).
	Incorporate engineered invertebrates (e.g., waxworm) into plastic degradation processes.	Engineer microbes and/or develop bioprocesses that recycle plastic hydrolysates into value-added products.

Enable the biodegradation of per- and polyfluoroalkyl substances (PFAS) in water and soil.

Short-term	Medium-term	Long-term
Demonstrate the efficient biodegradation of PFAS, perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS) in managed settings (e.g., water treatment plants, bioreactors).	Operationalize enzymes and microbes to degrade PFAS contaminants in dilute waste streams (concentration less than 200 ng/L), such as municipal water sources.	Demonstrate biodegradation of PFAS, PFOA, and PFOS in semi-managed and unmanaged settings (e.g., farmland, coastal areas and marine environments).

Short-term → Medium-term → Long-term

Enable the biosequestration of heavy metals in the environment and at scale.	
Identify and engineer microbes tolerant to biosequestration of heavy metals from the environment.	Enable cost-effective, industrial production of metal binding proteins or biomolecules at scale.
Engineer microbial pathways to efficiently express selective metal binding proteins and biomolecules.	Engineer plants to improve their capacity for heavy metal uptake and accumulation.
	Develop microbes to selectively mineralize or mobilize metals in the environment.

Mitigate pollutants from human-generated waste streams.

Enable the bioremediation of contaminants from municipal wastewater.		
Enable scale-up of highly-efficient, engineered microalgae bioremediation of municipal wastewater.	Engineer microbes/microbial consortia and enzymes that degrade and/or capture excess pharmaceuticals and endocrine disruptors (e.g., hormones from birth control, pesticide metabolites) in municipal wastewater.	Engineer living biofilms to continuously capture broad classes of pollutants in potable water streams.
Develop biological systems to capture waterborne pathogens (e.g., fecal coliforms).	Engineer microbes that can remove lubricants and fuels from municipal wastewater.	
	Engineer biobased filters for inorganic pollutant removal, for example to desalinate water and remove microplastics.	
	Engineer microbial conversion of municipal wastewater contaminants into value-added products.	
Short-term	**Medium-term**	**Long-term**

Enable the bioremediation of pollutants from agriculture and aquaculture.

Short-term	Medium-term	Long-term
Engineer soil microbes to sequester and concentrate inorganic contaminants in the soil column (for example, microbially induced calcite precipitation).	Engineer microbes, microbiomes, cell-free systems, or biomaterials designed to target and capture nitrogen, phosphate, and calcium runoff from synthetic fertilizers.	Engineer bioremediating microbes, microbiomes, cell-free systems, or biomaterials with programmable lifespans for agricultural and aquacultural environments.
	Engineer microbes, cell-free systems, or biomaterials that target, capture, and degrade agricultural/aquacultural antibiotics and insecticide contaminants.	
Engineer plants to detect soil contamination through interactions with the rhizosphere and produce a visible output of contamination status.	Enable biological recycling of captured runoff nutrients for new fertilizers.	
	Engineer macroalgae to bind and degrade toxins in marine environments.	

Enable the bioremediation of chemical waste from industrial effluent.

Short-term	Medium-term	Long-term
Enable *in silico* prediction of biodegradation pathways for toxic chemical compounds.	Incorporate engineered microbial or cell-free systems into chemical production processes to degrade toxic waste chemicals *in situ*.	Enable valorization of breakdown products and waste, such as via directly-coupled bioenergy generation or high-value product synthesis from engineered remediation organisms.
Identify and engineer microbes to biodegrade hazardous chemicals (for example, phenols, endocrine-disrupting chemicals, hydrocarbons, heavy metals).		
Design cell-free systems to degrade toxic waste chemicals generated from chemical production.		

Goal: Rapid detection and continuous monitoring of environmental contaminants.

Current State-of-the-Art: As we work to mitigate the impacts of climate change and ensure sustainability, the ability to detect and monitor pollution and contaminants in the environment will play a big role in our ability to mitigate and remove them. Currently, environmental contaminants are typically analyzed in lab settings through processes that are costly, labor-intensive, and slow. Compared to lab-based methods for contaminant detection and monitoring, biosensors are more affordable (Khanmohammadi, et al., 2020) and portable (Bilal & Iqbal, 2019), and could detect a wide range of contaminants rapidly at the point-of-need (Zhang et al., 2021). For example, microbes have evolved the ability to sense and respond to environmental cues, including nutrients and pollutants (Gupta et al., 2016). This sense-and-response ability can be leveraged for detection of pollutants in the environment (Inda & Lu, 2020) and ultimately coupled to remediation. However, using live organisms like *E. coli* or *S. cerevisiae* for sensing has practical challenges, such as their stability and "shelf-life," and raises additional concerns about biocontainment. Cell-free detection approaches, such as *in vitro* gene expression systems (Karig, 2017) and nucleic acid-based sensors (Wang et al., 2019) that decouple sensing from a host microbe could circumvent some of these issues (Jung et al., 2020; Silverman et al., 2020). [For a recent review of biosensor technologies for environmental monitoring, see Gavrilaș et al., 2022.]

Despite many of the latest advancements, environmental biosensing technologies still need to be more accurate, sensitive, and reliable to enable widespread adoption; moreover, we need to expand the range of analytes detectable. Specific short-term technical challenges include detecting contaminants at or below regulatory limits, providing fast readouts (e.g., < 15 minutes), performing multiplex detection of contaminants in a single device, and enabling automated *in situ* sample preparation. Further, biosensors are vulnerable to degradation and fouling in the environment, and many biosensors are only intended for single use. To overcome these challenges, more research is needed to improve sensor shelf-life to enable long term monitoring, such as designing more robust biosensing systems and compartmentalizing biosensor components (Li et al., 2022a). Existing biosensors also primarily rely on fluorescent or colorimetric outputs, which require additional instrumentation for analysis and quantitation. Developing new biosensing and reporting modalities, such as electrochemical readouts, will enable continuous, real-time monitoring, by allowing biosensors to be more easily integrated into existing digital sensor networks.

Breakthrough Capability: Enable the detection and continuous monitoring of pollutants and priority contaminants in the environment using biosensors.

Short-term Milestone: Engineer highly specific, low-cost, and field-deployable biosensors for priority contaminants (e.g., paper-based cell-free systems).

- Bottleneck: Reliable reporting from point-of-use, cell-free biosensors as to the presence/absence of heavy metal contaminants (e.g., Cd, Hg, Pb) below regulatory (i.e., EPA, WHO) recommended levels.
    - Potential Solution: Model and redesign the sequences of existing aptamer sensors for higher binding affinity to target heavy metal contaminants.

- Bottleneck: Biosensor devices and formats need to be designed to report results more quickly (i.e., in less than 15 minutes).
    - Potential Solution: For detection of biological analytes, enable the biosensor to perform *in situ* amplification of target genes for faster detection.
- Bottleneck: Sample preparation and biosensors can be difficult without specialized knowledge and/or prior training.
    - Potential Solution: Design and build platforms that simplify sample preparations, such as by combining filtration, pre-concentration, and/or solubilization steps, and that involve no chemical hazards and minimal/no power requirements.

Short-term Milestone: Engineer biosensors to be compatible with digital infrastructure.

- Bottleneck: Electrical responses from electrochemical reporters (e.g., horseradish peroxidase) currently used in biosensing applications are too weak to be detected by commonly available electronic components.
    - Potential Solution: Engineer or discover electrochemical reporters capable of generating electrical signals that are strong enough to be sensed by commonly available electronic components.

Medium-term Milestone: Demonstrate next-generation biosensors with novel detection modalities.

- Bottleneck: Current biosensors are limited in their range of detection of contaminants listed in EPA National Primary Drinking Water Regulations and WHO guidelines for drinking water.
    - Potential Solution: Develop high-throughput selection methods to find new contaminant-binding aptamers and proteins.
    - Potential Solution: Develop computational models using machine learning and artificial intelligence to design new contaminant-binding aptamers or proteins.
- Bottleneck: Biosensors that are capable of quantitative, multiplex analyses.
    - Potential Solution: Design biosensing molecules (e.g., proteins, aptamers) that have orthogonal sequences to minimize cross-interference.
    - Potential Solution: Engineer low-cost, microfluidic sensors with multiple compartments to house biosensors for detecting different analytes.
- Bottleneck: Point-of-use biosensors to detect highly toxic pollutants, such as mining byproducts and nuclear waste.
    - Potential Solution: Engineer robust biosensor molecules that can withstand high toxicity and pH extremes without denaturing.
- Bottleneck: Biosensors that function and detect priority contaminants in marine environments (nitrates, phosphates, microplastics).
    - Potential Solution: Design and engineer point-of-use biosensors for deployment in saline (marine) and wastewater applications.

Medium-term Milestone: Enable the deployment of biosensors for field applications and long-term environmental monitoring.

- Bottleneck: Biofouling and environmental degradation severely limit the capacity for biosensors to be used in environmental monitoring applications.
  - Potential Solution: Develop anti-fouling biomaterials to encapsulate and protect biosensors.
  - Potential Solution: Evolve biosensors for long-term functional robustness under complex conditions (pH, temperature, fouling agent fluctuations).
- Bottleneck: Strong biocontainment strategies for biosensors.
  - Potential Solution: Develop encapsulation materials that selectively allows the entering and exiting of certain molecules (e.g., analytes and other molecules are allowed to enter the flow cell, but biosensing components are prohibited from leaving).
- Bottleneck: Limited number of biosensors that work at application-relevant sensitivity and dynamic range.
  - Potential Solution: Develop transcription factors that can be induced by virtually any small molecule.
  - Potential Solution: Develop novel workflows that accelerate the design of key sensor response characteristics (e.g., half-maximal induction concentration ($K_{1/2}$), at appropriate dynamic range, among others).

Long-term Milestone: Engineer autonomous, self-regulating biosensors that can detect and remediate pollutants.

- Bottleneck: Enabling long-term passive monitoring in open systems requires consideration of biosafety and biocontainment.
  - Potential Solution: Develop stable engineered microbial consortia that can respond to and remove any detected pollutants within a limited timeline (e.g., using memory circuits to detect a threshold number of response events that triggers consortia death and release of enzymatic remediators).
  - Potential Solution: Enrich naturally-occuring communities from polluted environments, using -omics tools to characterize them and engineering/enhance their activity.

Goal: Mitigate targeted environmental pollutants through biosequestration and biodegradation.

Current State-of-the-Art: Biosequestration leverages biological organisms and biobased systems to recognize, bind, and absorb target contaminants. Examples of biosequestration are primarily seen with carbon, including CO_2, and bacterial and macroalgal binding of heavy metals (Giachino et al., 2021; Ankit et al., 2020; Mazur et al., 2018), and there has been demonstrated success of oil spill bioremediation by hydrocarbonoclastic bacteria (Adeleye et al., 2018; Ron & Rosenberg, 2014). While heavy metals are a significant environmental remediation challenge, so too are other recalcitrant materials, including plastics and per- and polyfluoroalkyl substances (PFAS), which are heavily abundant in waters and soils world-wide. The ability to sequester, and then degrade, these pollutants through biological processes will significantly impact all biospheres.

In general, biosequestration of inorganics tends to be a slow process because the biochemistries involved are inherently toxic to organisms. For example, toxic inorganics can compete with normal metal cofactors for binding in enzymes and damage important biomolecules (Dudev & Lim, 2014). Approaches to circumvent these issues include finding organisms and proteins that are resistant to metal toxicity, engineering cofactor binding competition, enabling organisms to compartmentalize toxins, engineering strong uptake systems, and accelerating enzymatic processes to mineralize inorganics, which can effectively detoxify the pollutant. Alternatively, once inorganic contaminants have been taken up through biosequestration, they could be reduced using conventional chemical processing into solid metal.

In contrast to biosequestration, biodegradation is the breakdown of organic materials by organisms and cellular/cell-free complexes. Importantly, organic contaminants are not necessarily toxic to organisms or disruptive to cells at the molecular level. Detoxification of organic contaminants often occurs via metabolic processes, such as when bacteria or enzymes break down an organic compound into chemicals the cell could use (e.g., acetate). Thus, it is important to engineer metabolic pathways for more efficient breakdown of organic contaminants, in addition to developing organisms and cell-free systems for better binding and recognition of target compounds.

Breakthrough Capability: Degrade plastic waste through engineered bioprocesses.[1]

Short-term Milestone: Engineer microbes, microbial consortia, or enzymes to efficiently degrade common plastic polymers.

- Bottleneck: Activity, stability, and reusability of PETase, and other (novel) enzymes to effectively depolymerize polyethylene, polypropylene, and polystyrene.
  - Potential Solution: Leverage protein engineering to design enzymes for desired traits, such as improved binding and hydrolysis of target polymers.
  - Potential Solution: Use metagenomic analysis to profile and select for microbial strains with high expression levels of PETase.
- Bottleneck: Efficiency of microbial production of hydrolytic enzymes, to make biodegradation viable at the industrial scale.
  - Potential Solution: Engineer microbes and other model organisms to increase the expression of desired hydrolytic enzymes.
- Bottleneck: Environmental safety of metabolites from degraded plastics.
  - Potential Solution: Carry-out extensive field trials to identify concerning side products and evolve hydrolytic enzymes to remove such side metabolites.

[1] For more on engineering biology to recycle/up-cycle plastic waste, please see **Goal: Enable sustainable production of plastics and chemicals. | Breakthrough Capability: Produce commodity chemicals by upcycling waste streams via bioprocessing.**

Medium-term Milestone: Enable the integration of degradation enzymes for local, on-demand plastic waste processing.

- Bottleneck: A wider-range of efficient and thermostable enzymes for plastic polymer degradation are needed (for example, see Lu et al., 2022).
    - Potential Solution: Further machine learning and deep design for protein engineering.
    - Potential Solution: Platforms for laboratory evolution of enzymes for plastic degradation.

Medium-term Milestone: Develop microbial consortia and enzymatic cocktails for the efficient breakdown of mixed-waste plastics (e.g., multi-layer plastics such as those found in carpets or shoes) and 'dirty'/used plastics (e.g., takeout containers).

- Bottleneck: Complex plastic mixtures contain polymers that require different environmental conditions and pretreatments to be properly degraded.
    - Potential Solution: Develop high-throughput screening methods to select microbes and enzymes that can function under the requisite environmental conditions for degrading a target polymer.

Medium-term Milestone: Incorporate engineered invertebrates (e.g., waxworm) into plastic degradation processes.

- Bottleneck: Incomplete digestion and degradation of plastics in the invertebrate gut could lead to the production of microplastics.
    - Potential Solution: Use multi-omics techniques and mass spectrometry to investigate and better understand polymer degradation pathways and byproducts in invertebrates that digest plastics.
    - Potential Solution: Develop secondary degradation processes that use engineered bacteria or fungi to further digest and degrade microplastic byproducts.

Long-term Milestone: Engineer organisms or enzymes to 'carbon-negatively' degrade plastics *in situ*, in different ecological niches (e.g., in soil, on the ocean floor).

- Bottleneck: Most plastic materials (synthetic polymers) are considered non-biodegradable.
    - Potential Solution: Engineer organisms or cell-free systems that specifically target labile or bioavailable polymer additives (including colorants, antioxidants, and plasticizers (Sheridan et al., 2022).

Long-term Milestone: Design bioplastics with innate degradation mechanisms (e.g., embedded enzymes).

- Bottleneck: Incorporation of hydrolytic enzymes and or cell-free systems could alter the properties of the bioplastic.
    - Potential Solution: Embed lyophilised cell-free systems that can be specifically activated to produce required hydrolytic enzymes.

Long-term Milestone: Engineer microbes and/or develop bioprocesses that recycle plastic hydrolysates into value-added products.

- Bottleneck: Most bioprocessing product yields and purities from plastic feedstocks are not economically-advantageous.
  - Potential Solution: New pathways and enzymatic cascades are needed to produce valuable products from plastic hydrolysates (Kim et al., 2019).

Breakthrough Capability: Enable the biodegradation of per- and polyfluoroalkyl substances (PFAS) in water and soil (Shahsavari et al., 2021).

Short-term Milestone: Demonstrate the efficient biodegradation of PFAS, perfluorooctanoic acid (PFOA), and perfluorooctanesulfonic acid (PFOS) in managed settings (e.g., water treatment plants, bioreactors).

- Bottleneck: Compared to alternative degradation methods, biodegradation of PFAS is more cost-effective, but still time consuming (takes days to fully degrade).
  - Potential Solution: Fully map the degradation pathways and enzymes of PFAS and PFOS degrading microbes (e.g., *Pseudomonas*) to enable biodegradation of PFAS in less than 24 hours.
- Bottleneck: The presence of other chemicals may decrease the rate at which microbes degrade PFAS.
  - Potential Solution: Develop high-throughput methods to test degradation efficiency of perfluorinated compounds in the presence of other common waste stream pollutants, to identify characteristics that allow microbes or complexes to function at high degradation efficiencies.

Medium-term Milestone: Operationalize enzymes and microbes to degrade PFAS contaminants in dilute waste streams (concentration less than 200 ng/L; Liu et al., 2013), such as municipal water sources.

- Bottleneck: Most PFOS and PFOA compounds are considered terminally degraded due to the high strength of their carbon—fluorine bonds.
  - Potential Solution: Engineer dehalogenation and defluorination pathways into the metabolic pathways of model organisms (Seong et al., 2019).

Long-term Milestone: Demonstrate biodegradation of PFAS, PFOA, and PFOS in semi-managed and unmanaged settings (e.g., farmland, coastal areas and marine environments).

- Bottleneck: Biodegradation of PFAS at low concentrations (<200 ng/L).
  - Potential Solution: Engineer metabolic pathways in native (anaerobic) microbes that exclusively require the relatively high oxidation states of perfluorinated chemicals (Kim et al., 2014).
- Bottleneck: Biodegradation of complex mixtures of PFAS contaminants that co-exist in soil or water matrices.
  - Potential Solution: Engineer bacterial consortia or plant-microbe-fungi symbiotic systems to enable the extraction of multiple PFAS contaminants.

Breakthrough Capability: Enable the biosequestration of heavy metals in the environment and at scale.[2]

Short-term Milestone: Identify and engineer microbes tolerant to biosequestration of heavy metals from the environment.

- Bottleneck: Toxicity tolerance and heavy metal uptake varies between bacterial strain and the type of metal.
    - Potential Solution: Leverage metagenomics to characterize organisms and enzymes that have adapted to heavily polluted environments to identify useful biological parts for toxin removal and remediation.
    - Potential Solution: Engineer microbes to take up and sequester multiple types of heavy metal simultaneously, such as through broad-spectrum metal chelating proteins or biomolecules with high affinity and capacity.
    - Potential Solution: As an alternative to whole organisms, develop protein-based materials for binding and recovering heavy metals, particularly from waste streams.

Short-term Milestone: Engineer microbial pathways to efficiently express selective metal binding proteins and biomolecules.

- Bottleneck: Binding and sequestration of some metals (e.g., iron) are well known; a wider range of metal binding capabilities is needed.
    - Potential Solution: Develop high-throughput screening methods to identify natural and/or engineered (or evolved) proteins with selective metal binding properties.

Medium-term Milestone: Enable cost-effective, industrial production of metal binding proteins or biomolecules at scale.

- Bottleneck: Challenges are similar to those experienced with all industrial protein production, including expression levels, separation and purification.
    - Potential Solution: Continued improvements to biomanufacturing and processing, including incorporation of automation and machine learning.

Medium-term Milestone: Engineer plants to improve their capacity for heavy metal uptake and accumulation.

- Bottleneck: Phytoremediation can be a particularly slow and/or laborious process.
    - Potential Solution: Engineer plants for faster growth and increased biomass.
    - Potential Solution: Inoculate plant root and/or rhizobia with filamentous fungi and microbes engineered to increase heavy metal uptake.
    - Potential Solution: Investigate the effects of overexpressing certain metal chelating ligands on the overall growth and metal toxicity tolerance of the plant.
- Bottleneck: Heavy metal accumulation causes oxidative stress in plants.

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[2] For more about enabling engineering biology for uptake and processing of metals, please see **Goal: Enable resource recovery through biomining.**

  - Potential Solution: Characterize oxidative stress response in hyperaccumulators, to identify effective antioxidant enzymes, pathways, and DNA repair mechanisms.
  - Potential Solution: Based on a fuller understanding of oxidative stress in plants, engineer specific genes, enzymes, and pathways to increase antioxidant activities in phytoremediation plants.

Medium-term Milestone: Develop microbes to selectively mineralize or mobilize metals in the environment.

- Bottleneck: Metal selectivity and tolerance is significantly variable across microbial species, thus microbes or consortia would need to be tailored for the environment (González Henao & Ghneim-Herrera, 2021).
  - Potential Solution: Engineer consortia with multiple mechanisms of soil detoxification to be broadly applied.
- Bottleneck: Not all contaminated soils are suitable for bioremediation, due to poor natural conditions, including lack of oxygen and extreme temperature (Kapahi & Sachdeva, 2019).
  - Potential Solution: Employ indigenous microbial strains, wherever possible, that have shown hardiness to local conditions.
  - Potential Solution: Consider engineering of extremophiles and anaerobic species for metal uptake.

Goal: Mitigate pollutants from human-generated waste streams.[3]

Current State-of-the-Art: Efficient biodegradation could prove especially useful for breaking down hydrocarbons (e.g., plastics and oils) and removing harmful chemicals from human-generated waste streams, before they reach the larger environment. For water treatment, in particular, biobased systems have been developed to capture fecal coliforms (Li et al., 2020), degrade nutrient runoff from agriculture and aquaculture (e.g., fish waste and feed) (Coppola et al., 2021), reduce antibiotics and insecticide contamination (Ferrando & Matamoros, 2020), and clean up fluorinated compounds (Moreira et al, 2018). Future advances in biodegradation could enable higher efficiency water purification, reclamation, and even desalination (such as occurs with mangrove trees, see Wang et al., 2020b), where halophilic bacteria could be incorporated into the desalination process to prevent biofouling and reduce chemical use. While some non-model organisms can grow on and process pollutants, more research is needed to identify novel organisms that can handle harsh and polluted environments (Sysoev et al., 2021; Yun et al., 2016). In addition to removing pollutants from waste streams, engineering biology can take the process further and upcycle pollutants by converting them into useful products (Cornwall, 2021; Lad et al., 2022).

[3] More detail about upcycling chemicals and materials can be found in **Materials Production & Industrial Processes.**

Breakthrough Capability: Enable the bioremediation of contaminants from municipal wastewater.

Short-term Milestone: Enable scale-up of highly-efficient, engineered microalgae bioremediation of municipal wastewater (Do et al., 2022).

- Bottleneck: Environmental conditions (e.g., pH, temperature, flow rate) of wastewater treatment sites.
    - Potential Solution: Engineer heterotrophic and mixotrophic microalgae robust to a wider range of climatic conditions (Aggarwal et al., 2021; Gao et al., 2021).

Short-term Milestone: Develop biological systems to capture waterborne pathogens (e.g., fecal coliforms).

- Bottleneck: Detecting and destroying specific pathogens and their toxic by-products at scale in wastewater and other municipal water sources.
    - Potential Solution: Engineer sensing and reporting predatory bacteria against known pathogen classes.
    - Potential Solution: Engineer organisms to produce coliphage or use other predatory mechanisms to control coliform populations.

Medium-term Milestone: Engineer microbes/microbial consortia and enzymes that degrade and/or capture excess pharmaceuticals and endocrine disruptors (e.g., hormones from birth control, pesticide metabolites) in municipal wastewater (Gavrilescu et al., 2015).

- Bottleneck: Metabolic pathways for pharmaceutical degradation are unknown.
    - Potential Solution: Screen municipal wastewater and other appropriate sources for microbes that degrade drugs of interest, for characterization with -omics and enrichment evolution.
    - Potential solution: Identify enzymes upregulated in the presence of pharmaceuticals (transcriptomics) and validate function via heterologous expression and/or knockouts.
- Bottleneck: Enzymes not expressed natively at sufficiently high levels to clear pharmaceuticals at appreciable rate (Chen et al., 2017).
    - Potential Solution: Upregulate protein expression in native hosts (see for example Ariste & Cabana, 2020).

Medium-term Milestone: Engineer microbes that can remove lubricants and fuels from municipal wastewater.

- Bottleneck: Microbial growth tolerance and efficient absorption of specific hydrophobic waste compounds are unknown.
    - Potential Solution: Enhance the chemical tolerance of microbes that have native abilities to tolerate and utilize toxic chemicals in wastewater.
    - Potential Solution: Enhance the production of surfactants that allow for solubilization and access of hydrophobic compounds by microbes.

- Potential Solution: Engineer alkane-activation mechanisms (e.g., fumarate) into facultative anaerobic bacteria to maximize processing under poor oxygen conditions (Rojo, 2009).

Medium-term Milestone: Engineer biobased filters for inorganic pollutant removal, for example to desalinate water and remove microplastics.

- Bottleneck: Filters would need to be able to quickly and efficiently trap pollutants/salts while allowing pure water to pass through.
  - Potential Solution: Design biomolecule or biopolymer based biofilms, perhaps continually replenished with associated (marine) microbes, with sufficient structural integrity and appropriate pore size to trap contaminants.
  - Potential Solution: Characterize biodegradation of organic contaminants in halophilic microbes.
  - Potential Solution: Engineer marine microbes that produce surfactants that flocculate or aggregate microplastics.

Medium-term Milestone: Engineer microbial conversion of municipal wastewater contaminants into value-added products.

- Bottleneck: Efficiency of purification and extraction of desired product.
  - Potential Solution: Engineer consortia capable of distributed metabolism for membrane bioreactors or moving bed biofilm reactors (Kumar Singh et al., 2020).

Long-term Milestone: Engineer living biofilms to continuously capture broad classes of pollutants in potable water streams.

- Bottleneck: Stability and maintenance of biofilms exposed to captured pollutants is unknown.
  - Potential Solution: Develop stable microbial consortia whose members are specialized in the capture of specific pollutants but will persist even when absent.

Breakthrough Capability: Enable the bioremediation of pollutants from agriculture and aquaculture.[4]

Short-term Milestone: Engineer soil microbes to sequester and concentrate inorganic contaminants in the soil column (for example, microbially induced calcite precipitation).

- Bottleneck: The mutual influences of the environment and soil microbes that affect precipitation speed, spatial distribution, and crystal properties have not been sufficiently elucidated.
  - Potential Solution: Engineer standard microbial hosts (e.g., *B. subtilis*) to express genetic drivers for inorganic contaminant concentration (e.g., urease enzymes), varying components of the media and study effects on

[4] For biotechnologies for reducing agricultural runoff (through more effective biofertilizers and more efficient crop uptake of nutrients), please see the **Food & Agriculture** theme.

precipitation speed, spatial distribution and crystal properties (Hoffmann et al., 2021).

Short-term Milestone: Engineer plants to detect soil contamination through interactions with the rhizosphere and produce a visible output of contamination status.

- Bottleneck: Requires optimization of plant-rhizosphere communication and reliable plant signal transduction and reporting.
    - Potential Solution: Engineer microbes to metabolize contaminants into compounds already known to be taken up by plant roots.

Medium-term Milestone: Engineer microbes, microbiomes, cell-free systems, or biomaterials designed to target and capture nitrogen, phosphate, and calcium runoff from synthetic fertilizers.

- Bottleneck: Biosystems need to be optimized to selectively and effectively sequester and concentrate inorganic contaminants.
    - Potential Solution: Optimize microbes that capture and store excess phosphate.
    - Potential Solution: Engineer nitrifying bacteria to control nitrogen losses in the soil (reduce oxidation of ammonia, thereby minimizing input fertilizer needs and runoff).
    - Potential solution: Engineer microbially induced calcite precipitation for agricultural soils.

Medium-term Milestone: Engineer microbes, cell-free systems, or biomaterials that target, capture, and degrade agricultural/aquacultural antibiotics and insecticide contaminants.

- Bottleneck: Enzymes not expressed natively in soil-associated organisms at sufficiently high levels to clear antibiotics or insecticides at appreciable rate.
    - Potential Solution: Upregulate protein expression in native hosts.

Medium-term Milestone: Enable biological recycling of captured runoff nutrients for new fertilizers.

- Bottleneck: Highly-mobile phosphorus and nitrogen fertilizer compounds leach quickly through soil.
    - Potential Solution: Engineer microbes to cycle phosphorus/nitrogen into less-mobile and bioavailable compounds.
- Bottleneck: Efficiency of employing anaerobic processes in aqueous, aquaculture-related environments.
    - Potential Solution: Identify and characterize tractable anaerobic organisms that thrive in aquaculture environments.

Medium-term Milestone: Engineer macroalgae to bind and degrade toxins in marine environments.

- Bottleneck: Macroalgae need to be able to selectively recognize toxins for uptake and sequestration or metabolism, without triggering pathways that would result in macroalgal death.

- Potential Solution: Surface display of toxin binding proteins that can be adapted and expressed in macroalgae.

Long-term Milestone: Engineer bioremediating microbes, microbiomes, cell-free systems, or biomaterials with programmable lifespans for agricultural and aquacultural environments.

- Bottleneck: Factors influencing microbial persistence are poorly understood in dynamic environments.
    - Potential Solution: Employ (meta)genomics, metabolomics, and other tools (especially emerging complementary activity measurements like qSIP, BONCAT, and PMA) to connect microbiome structure to environmental conditions and processes (Hungate et al., 2015; Couradeau et al., 2019; Wang et al., 2021).
- Bottleneck: Engineering individual microbes and consortia with the ability to persist and have defined activity (e.g., nitrogen fixation, phosphate mobilization or sequestration (depending on conditions), pathogen protection) within a defined field of action.
    - Potential Solution: Minimal *in situ* or *ex vivo* engineering of naturally persistent and ubiquitous subcommunities for defined activities (see Rubin et al., 2022) to take advantage of their natural adaptation to the particular environmental constraints.
    - Potential Solution: Encapsulated, stabilized cell- and nucleic acid-free engineered enzymes/cofactor systems that can be dispersed into water and soil while maintaining activity for defined periods in variable environments (Alves et al., 2018).

Breakthrough Capability: Enable the bioremediation of chemical waste from industrial effluent.

Short-term Milestone: Enable *in silico* prediction of biodegradation pathways for toxic chemical compounds.

- Bottleneck: Limited availability of databases on chemical toxicity and related biodegradation pathways.
    - Potential Solution: Use multi-omics technologies to identify and characterize biomolecules and metabolic pathways for toxic chemical biodegradation.

Short-term Milestone: Identify and engineer microbes to biodegrade hazardous chemicals (for example, phenols, endocrine-disrupting chemicals, hydrocarbons, heavy metals).

- Bottleneck: Bioremediation applications are still primarily limited to a few well-characterized model organisms.
    - Potential Solution: Identify non-model organisms that can survive and detoxify harmful chemical wastes and develop the tools to engineer them.

- Potential Solution: Sequencing and reuse of organisms and genetic systems indigenous (evolving and stable) to environments where these contaminants are released.
- Bottleneck: Fast-growing microbes favored for bioremediation are often not the most efficient at degrading toxic waste and could create more unwanted microbial biomass.
  - Potential Solution: Engineer microbes where growth is decoupled from catabolic activity.

Short-term Milestone: Design cell-free systems to degrade toxic waste chemicals generated from chemical production.

- Bottleneck: Environmental conditions in industrial effluents (e.g., extremely high or low pH) can cause enzymes to denature.
  - Potential Solution: Improve protein stability to enable enzymatic degradation of chemical waste under extreme conditions.
- Bottleneck: Lack of biocatalysts for specific toxic waste chemicals.
  - Potential Solution. Enzyme design and directed evolution to generate a range of required biocatalysts that can operate under specific environmental conditions.

Medium-term Milestone: Incorporate engineered microbial or cell-free systems into chemical production processes to degrade toxic waste chemicals *in situ*.

- Bottleneck: Microbial chassis are growth-sensitive to chemical waste components.
  - Potential Solution: Engineer microbes and enzymes with high tolerance to chemical waste.
- Bottleneck: Lack of suitable biocatalysts and microbial chasses for degradation of toxic byproducts.
  - Potential Solution: Identify microbes and enzymes that can degrade secondary toxic byproducts produced from biodegradation.
- Bottleneck: Engineered microbes highly-robust to toxic environments raise concerns for biocontainment breach.
  - Potential Solution: Develop tools to engineer microbes to be contained in the designated environment.

Long-term Milestone: Enable valorization of breakdown products and waste, such as via directly-coupled bioenergy generation or high-value product synthesis from engineered remediation organisms.

- Bottleneck: High level of impurities and concentration of toxins in waste streams.
  - Potential Solution: Selection and engineering of host strains tolerant to complex and toxic environments, such as halophiles, or strains engineered with high-lipase expression.

Conservation of Ecosystems and Biodiversity

Introduction and Impact: Climate change is a threat to ecosystems and biodiversity world-wide. Biodiversity is a measure of variation at the genetic, species, and ecosystem level. Biodiversity is an important element of the vibrance and health of our planet and supports human existence by providing food, medicine, shelter, and protection from diseases. Biodiversity is not distributed uniformly on Earth, but rather concentrated in particular ecosystems and hotspots that are vulnerable to mass extinctions due to rapid environmental changes produced by climate change. For example, the Great Barrier Reef — a biodiversity hotspot with thousands of known species — is threatened by mass bleaching events due to rising ocean temperatures. The Conservation of Ecosystems and Biodiversity technical theme focuses on engineering biology approaches that could complement nature-based solutions to restore and protect biodiversity and ecosystems, including addressing the issue of biocontainment [Figure 4]. The technical theme approaches supporting ecosystem health through the lens of engineering biology opportunities, however, like the rest of this roadmap, solutions must be considered in coordination with other fields of science and engineering, including the social sciences, as well as with local and indigenous communities who will experience the greatest impacts on their activities and livelihoods, and with the global community through appropriate policy and regulation to ensure risk reduction and limitation of harmful effects.

Approaches to preserving and restoring biodiversity should encompass tools and methods that support the resilience of both individual organisms or species and entire ecosystems that have been or could be adversely affected by climate change. In this roadmap we consider engineering biology-based approaches with a focus on three aspects of ecosystem resilience and health affected by climate change: supporting forest health, particularly where it has been impacted by drought and forest fire; resilience of marine ecosystems as they are being damaged by increased temperatures, acidification, and pollution; and the protection of ecosystems vulnerable to pathogens and invasive species that may be made worse by climate change.

Engineering biology also has the potential to help support the tracking and monitoring of existing and evolving biodiversity within ecosystems and reducing biodiversity loss by supporting keystone and foundational species. This roadmap describes technical opportunities in developing biosensors and reporting systems to detect changes in biodiversity and understand interactions within ecosystems to maintain their resilience. Through increased characterization of ecosystem components and biodiversity, engineering biology can enable genetic approaches toward protecting and enabling beneficial adaptation of species that are necessary for ecosystem health.

Finally, one of the most important factors in applying engineering biology, particularly on environmental scales, is preventing negative impacts of engineered organisms on biodiversity. Responsible and responsive biocontainment efforts are necessary to ensure that engineered organisms and systems are to the benefit of their environment and will not cause harm, whether through escape or biocontainment breach, or in persisting or acting beyond their intended target application. This roadmap identifies potential strategies for not only ensuring robust biocontainment, but also understanding the impact of engineering biology in the environment.

Figure 4. Engineering biology tools for ecosystem resilience and biodiversity conservation. Ecosystem damage and destruction is accelerating due to climate change and anthropogenic activity; however, there are many opportunities for engineering biology to support resilience and restoration of impacted environments and species. For example, engineering biology could help to restore forest soil microbiomes to aid recovery from forest fires, provide nutrient support and physical scaffolding to protect and support ocean corals damaged by heat and acidification, and with genetic and metabolic engineering, help to revive pollinator species. Engineering biology might also help to preserve genetic biodiversity in foundational, keystone, and threatened species, and could be used to monitor ecosystem health with sustainable, nature-friendly biosensors and reporters. Regardless of the potential application, robust biocontainment will be necessary for any engineering biology tool and technology intended for environmental use; biocontainment strategies should thoroughly be considered and implemented to prevent negative impacts. Importantly, advancements in genetic tools, consortia engineering, and data modeling need to be achieved in collaboration with ecologists and scientists and engineers from other fields, and local communities to understand system level impacts and priorities.

While biocontainment is considered in this technical theme, good practices in biocontainment should inform and apply to all biotechnologies envisioned by this roadmap.

Environmental biotechnology in *Engineering Biology*

EBRC's 2019 roadmap, *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy*, includes technical approaches to achieving ecosystem resilience {see: https://roadmap.ebrc.org/2019-roadmap/sectors/environmental-biotechnology/}. These objectives and achievements include enabling ecosystems to adapt to climate change, including monitoring impacts through biosensors, increasing drought-tolerance, improved nutrient uptake, and soil health and preservation. *Engineering Biology* also addressed controlled deployment of engineered organisms into the environment to support biodiversity and ecosystem robustness.

CONSERVATION OF ECOSYSTEMS AND BIODIVERSITY

Goal	Breakthrough Capability	Milestone

Increase ecosystem resilience to climate change.

Enhance forest restoration and recovery from fires and other environmental stressors.

Short-term	Medium-term	Long-term
Discover genes for conferring heat-stress and drought tolerance in microbes, fungi, plants, and insects.	Engineer symbiotic fungi (e.g., arbuscular mycorrhizal fungi) to enhance beneficial symbiotic traits (i.e., nutrient exchange, water retention, stress resistance) for seedling establishment in reforesting operations.	Deploy engineered organisms with robust regenerative capabilities to restore soils.
		Engineer plants and microbiomes to help plants adapt to new climate aspects as species migrate or are planted outside of their native range.
	Engineer and field-test heat and burn-resistant microbes in high-threat areas.	Engineer native plants and trees robust to invasive pathogens and pests.

Increase marine ecosystem resilience to adverse climatic factors through engineered biology and biomaterials.

Short-term	Medium-term	Long-term
Bioprospect and analyze marine species to determine potential marine model organisms, particularly those robust to changing climates, such as planctomycetota.	Engineer micro- and macroalgae to capture, degrade and remove pollutants, including pesticides, herbicides, and petroleum.	Engineer living biomaterials and biocoatings to protect aquatic organisms against pollutants and ocean acidification.
		Improve salt tolerance in coast-adjacent soils and plant species.
Engineer phytoplankton to be more robust to declining marine conditions, including increased water temperatures, acidification, eutrophication, and	Engineer kelp and plankton to have higher tolerance to heat, acid, and salinity stress.	Engineer adaptive living biomaterials that respond to fluctuations in marine salinity, acidity, and trace particles/ solutes.

Mitigate climate change-induced emergence of pathogens and invasive species.

Short-term	Medium-term	Long-term
Develop vaccines to protect endangered wildlife against newly-emergent pathogens.	Develop biosensors to enable detection of genetic and molecular hallmarks of known and emerging zoonoses.	Engineer the microbiome of pollinator insects (e.g., honey bees) to protect them against specific pathogens and pesticides.
Expand capabilities for monitoring/ detecting environmental DNA (eDNA) and protein, and metabolite profiling to monitor the spread of pathogens and invasive species.	Develop gene drives to control invasive species (e.g., mosquito, bark beetle, cane toad).	

Short-term → Medium-term → Long-term

Reduce biodiversity loss.

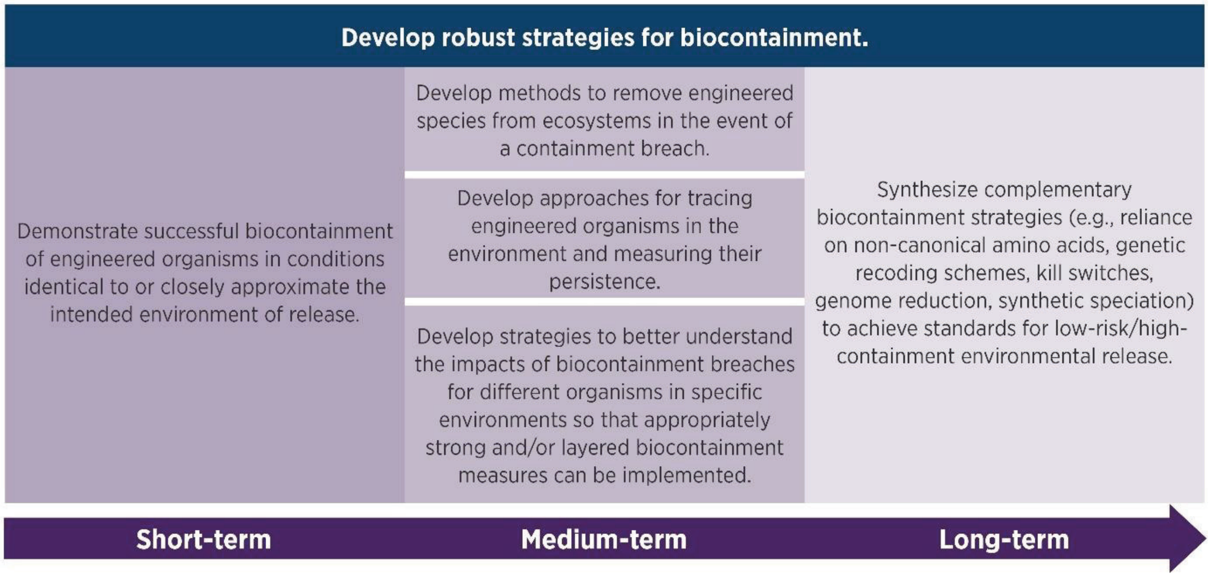
Enable monitoring of ecosystem health with bio-sensors and -reporters.		
Improve data collection and libraries of mitochondrial DNA for sensitive ecosystem members (i.e., threatened and keystone species).	Use genetic fingerprinting and marker surveillance to develop species demographic and evolutionary data libraries for conservation management strategies.	Track sourcing through supply chains by scaling-up biomolecular forensics (DNA, protein, and metabolic profiling) and genetic barcoding.
Increase application of genetic marker surveillance.	Expand activity-based monitoring (metafunctional genomics, metabolomics, stable tracer analyses) to track critical system function across diverse environments.	
	Develop integrated, continuous biosensors to track the effects of ecological forces (e.g., dispersal, drift, and selection) on community members in target biomes.	

Enable strengthening and protection of keystone and threatened species.		
Build reference genome libraries for currently threatened species.	Utilize genetic monitoring to determine best approaches for introducing and optimizing intrapopulation genetic variation.	Design and build targeted gene-drives for introducing beneficial or adaptive traits into threatened species.
		Engineer keystone and threatened species to be more adaptive or robust to climate change and anthropogenic impacts, such as through engineered microbiome robustness.

Ensure the availability of biocontainment approaches for engineered organisms.

Understand and model the potential and realized impacts of engineered organisms on the environment.		
Develop integrated field biosensors for detection and monitoring of engineered organisms.	Design and carry out environmental case-control studies comparing engineered biomes with control biomes in simulated environments to assess the impacts of engineered organisms on biodiversity.	Model and predict the dispersal of hyper-cultivated organisms and engineered biology (e.g., gene drives) and their impact on ecosystem diversity.

Short-term	Medium-term	Long-term


Develop robust strategies for biocontainment.
Demonstrate successful biocontainment of engineered organisms in conditions identical to or closely approximate the intended environment of release.
Develop methods to remove engineered species from ecosystems in the event of a containment breach.
Develop approaches for tracing engineered organisms in the environment and measuring their persistence.
Develop strategies to better understand the impacts of biocontainment breaches for different organisms in specific environments so that appropriately strong and/or layered biocontainment measures can be implemented.
Synthesize complementary biocontainment strategies (e.g., reliance on non-canonical amino acids, genetic recoding schemes, kill switches, genome reduction, synthetic speciation) to achieve standards for low-risk/high-containment environmental release.
Short-term
Medium-term
Long-term

Goal: Increase ecosystem resilience to climate change.

Current State-of-the-Art: Extreme climate events and disasters, such as wildfires, droughts, heat waves, hurricanes, and floods, have devastating effects on ecosystems and are poised to become even more frequent and intense as the planet continues to warm. Combined with ecology, geosciences, and other research disciplines, engineering biology provides options to help study and mitigate impacts from extreme weather events and to assist in ecosystem restoration.

One of the most ecologically devastating impacts from global warming has been an increase in intensity and frequencies of wildfires (U.N. Environment Programme, 2022). While wildfires are a natural part of forest ecosystem cycles, extreme wildfires diminish the ability for forests to recover post-fire. Forest fires affect the full landscape of an ecosystem, including everything from trees, grasses, and shrubs, to lichen and mosses, soils, and waterways. To aid the recovery of forests after catastrophic fires, trees and forest soils could be seeded with engineered fungi, microbes, and microbial communities to improve water-retention, combat soil erosion, and improve bio-recycling of detritus and undergrowth.

Ocean warming and ocean acidification have been detrimental to marine and coastal ecosystems. Heat waves damage coral reefs and kelp forests and heighten the likelihood of toxic algal blooms (Smale et al, 2019; EPA, 2013). Engineering biology approaches, such as genetically engineering keystone marine and freshwater species to increase resistance to environmental stressors, could help protect parts of the marine ecosystem from the effects of climate change. For example, to mitigate or reverse coral bleaching, researchers are helping corals tolerate higher heat and lower pH levels by engineering symbiotic microbes that can colonize coral to facilitate reactive oxygen species scavenging (Quigley et al., 2021). For coastal ecosystems, halotolerant plants, such as mangroves, could be developed to better adapt to rising sea levels (Menéndez et al., 2020).

Climate change is also altering the length and median temperature of seasons, with changing temperatures impacting population dynamics across ecosystems. These changes in population dynamics can lead to ecosystem collapses and catastrophes. In particular, research suggests warming summers and winters drive an increase in disease transmission of pathogens that target humans, including Zika, malaria, yellow fever, and dengue (Anwar, 2019; McDermott, 2022), animals (such as avian malaria; see Liao et al., 2017; U.S. National Park Service, 2017), and plants (such as bark and pine beetles; see Bentz et al., 2010; Sambaraju et al, 2012; Ungerer et al., 1999). Engineering biology could play a key role in mitigating risks from increased pathogen transmission and the spread of invasive species. For example, using gene drives, invasive insect species could be managed through engineered population control, such as altered mating success rate and fecundity based on desired traits (*e.g.,* host genetic markers). Additionally, endangered species could be engineered for increased resistance against biotic and abiotic stresses, such as engineering the microbiome of honeybees and other pollinators to help protect them against pathogens and pesticides.

Breakthrough Capability: Enhance forest restoration and recovery from fires and other environmental stressors.

Short-term Milestone: Discover genes for conferring heat-stress and drought tolerance in microbes, fungi, plants, and insects.

- Bottleneck: Undefined organism- and ecosystem-level metrics for heat and drought stress tolerance.
  - Potential Solution: Identify organisms that can thrive in burned soil and use multi-omics technology to identify genetic traits that enable their survival.

Medium-term Milestone: Engineer symbiotic fungi (e.g., arbuscular mycorrhizal fungi) to enhance beneficial symbiotic traits (i.e., nutrient exchange, water retention, stress resistance) for seedling establishment in reforesting operations.

- Bottleneck: Fungi have relatively small genomes, but more can be discovered about specific traits and interactions with other forest organisms.
  - Potential Solution: Develop the necessary tools to thoroughly characterize genetic and metabolic pathways in native forest fungi, such as to design synthetic genome approaches for engineering symbiotic organisms.

Medium-term Milestone: Engineer and field-test heat and burn-resistant microbes in high-threat areas.

- Bottleneck: Drought conditions make it difficult for new microbes to repopulate an area that has been depleted of soil microbial diversity by wildfires.
  - Potential Solution: Inoculate soil with engineered microbes that have improved water-holding capacities.

Long-term Milestone: Deploy engineered organisms with robust regenerative capabilities to restore soils.

- Bottleneck: Poor soil health after severe fire and drought makes it difficult for soil microbiomes to recover.
  - Potential Solution: Introduce organisms engineered (i.e., with improved nutrient exchange, stress resistance) to help stabilize soil consistency, prevent erosion, and contribute to soil regeneration.

Long-term Milestone: Engineer plants and microbiomes to help plants adapt to new climate aspects as species migrate or are planted outside of their native range.

Long-term Milestone: Engineer native plants and trees robust to invasive pathogens and pests.

Breakthrough Capability: Increase marine ecosystem resilience to adverse climatic factors through engineered biology and biomaterials.

Short-term Milestone: Bioprospect and analyze marine species to determine potential marine model organisms, particularly those robust to changing climates, such as planctomycetota.

- Bottleneck: Less is known about, and there is a paucity of engineering tools, for marine organisms.

    - Potential Solution: Leverage existing marine biological expertise and expertise in model organism selection and optimization in determining potential engineering targets.

Short-term Milestone: Engineer phytoplankton to be more robust to declining marine conditions, including increased water temperatures, acidification, eutrophication, and hypoxia.

- Bottleneck: Limited understanding of how ecological stressors impact marine microbes in coastal ecosystems on a genetic and metabolic level.
    - Potential solution: Engineer field-deployable biosensors for local chemistry (e.g., salinity) and pollutants (e.g., agricultural fertilizer runoff) that specifically impacts wetlands, salt marshes, and other coastal ecosystems.

Medium-term Milestone: Engineer micro- and macroalgae to capture, degrade and remove pollutants, including pesticides, herbicides, and petroleum.

- Bottleneck: Diversity and distribution of micro- and macroalgae means that they are less genetically and metabolically characterized compared with traditional model organisms.
    - Potential Solution: Extend metagenomics, proteomics, and metabolomics tools for engineering marine organisms.

Medium-term Milestone: Engineer kelp and plankton to have higher tolerance to heat, acid, and salinity stress.

- Bottleneck: Many marine species are not yet considered model organisms or have the toolkits necessary for efficient engineering.
    - Potential Solution: Develop genetic tools for engineering foundational marine species (e.g., kelp, plankton).
- Bottleneck: Effects of acute climate events (such as marine heat waves) on foundational marine species (e.g., kelp, plankton) are not well understood.
    - Potential Solution: Identify genetic markers for heat resistance, acidification, and salinity fluctuations in kelp, plankton, and other key species.

Long-term Milestone: Engineer living biomaterials and biocoatings to protect aquatic organisms against pollutants and ocean acidification.

- Bottleneck: Natural coral reef structures are losing integrity and coral ecosystems are collapsing at a rate much faster than they can be naturally replaced under changing climate conditions.
    - Potential Solution: Enable 3D printed coral structures made with biobased materials to function as a scaffold and to build robustness for natural corals.

Long-term Milestone: Improve salt tolerance in coast-adjacent soils and plant species.

- Bottleneck: Paucity of information about chemistry characteristics and dynamics of these environments and impacts on native organisms.

- Potential Solution: Characterize the relationship between the chemical diversity and the (micro)organisms that persist in these environments.

Long-term Milestone: Engineer adaptive living biomaterials that respond to fluctuations in marine salinity, acidity, and trace particles/solutes ([Liu et al., 2022](#)).

Breakthrough Capability: Mitigate climate change-induced emergence of pathogens and invasive species.

Short-term Milestone: Develop vaccines to protect endangered wildlife against newly-emergent pathogens.

- Bottleneck: Delivery to native populations of wildlife.
    - Potential Solution: Engineer natural vectors (e.g., mosquitos, effectively biocontained such as with gene drives) that may be safely released in the wild to inoculate wildlife.

Short-term Milestone: Expand capabilities for monitoring/detecting environmental DNA (eDNA) and protein, and metabolite profiling to monitor the spread of pathogens and invasive species.

- Bottleneck: Identity of the most significant, system-disrupting pathogens (e.g., microbial parasites) and invasive species.
    - Potential Solution: Use strain-agnostic sample and detection platforms (e.g., nucleic acid or protein nanopore sequencers, activity-based chemical probes) to monitor sensitive environments.
- Bottleneck: Performing eDNA tests requires extensive sample processing.
    - Potential Solution: Develop simplified, field-deployable sample-preparation kits and protocols for testing local water streams and middens.

Medium-term Milestone: Develop biosensors to enable detection of genetic and molecular hallmarks of known and emerging zoonoses.

- Bottleneck: Genetic stability of zoonoses hallmarks and ability to consistently identify and/or track emergence and location over time.
    - Potential Solution: More widespread use of genome surveillance tools to identify persistent and emergent threats.

Medium-term Milestone: Develop gene drives to control invasive species (e.g., mosquito, bark beetle, cane toad).

- Bottleneck: Paucity of tools for non-model, unique organisms, particularly those of higher order (i.e., animals).
    - Potential Solution: Develop/adapt genetic tools (e.g., CRISPR) to enable creation of genetically-modified species variants to control populations.

Long-term Milestone: Engineer the microbiome of pollinator insects (e.g., honey bees) to protect them against specific pathogens and pesticides.

- Bottleneck: We have a limited understanding of microbiome dysbiosis or pathology related to these conditions, constraining the solution space.

Goal: Reduce biodiversity loss.

Current State-of-the-Art: Biodiversity is necessary to maintain ecosystems, supply chains, and the health and persistence of all species, including humans. Engineering biology can help to reduce biodiversity loss through less-invasive and more-sustainable monitoring and by ensuring that we can protect and support the resilience of keystone species and those that are threatened, particularly due to climate change and human activities.

Measurement technologies (i.e., multi-omics techniques) could be leveraged to rapidly catalog existing biodiversity in field environments with regard to genetic expression, metabolomics, and species composition. By documenting microbial species and their strain level diversity and identifying keystone functional guilds within a given ecosystem, we can support foundational microbiomes that are essential, designing and engineering networks, pathways, and heterogeneity to support at-risk ecosystems and keystone guilds. Genome monitoring and editing can also help to conserve biodiversity by tracking at-risk organisms, informing adaptation approaches or genetic rescue, and helping to limit or prevent poaching (Phelps et al., 2020).

Breakthrough Capability: Enable monitoring of ecosystem health with bio-sensors and -reporters.

Short-term Milestone: Improve data collection and libraries of mitochondrial DNA for sensitive ecosystem members (i.e., threatened and keystone species).

- Bottleneck: Bioinformatics limitations for studying phylogenetics (Khan et al., 2008).

Short-term Milestone: Increase application of genetic marker surveillance.

- Bottleneck: Genetic data required for designing primers for microsatellite surveillance (Arif et al., 2011).
  - Potential Solution: Increase genetic sequencing and library generation for organisms in at-risk ecosystems.
  - Potential Solution: Increase use of single-nucleotide polymorphism (SNP) surveillance (Zimmerman, 2020).

Medium-term Milestone: Use genetic fingerprinting and marker surveillance to develop species demographic and evolutionary data libraries for conservation management strategies (Zimmerman et al., 2020).

- Bottleneck: Currently-available sequence libraries and marker datasets.

Medium-term Milestone: Expand activity-based monitoring (metafunctional genomics, metabolomics, stable tracer analyses) to track critical system function across diverse environments.

Medium-term Milestone: Develop integrated, continuous biosensors to track the effects of ecological forces (e.g., dispersal, drift, and selection) on community members in target biomes.

- Bottleneck: Short and long-term impacts of key abiotic and biotic stresses on the health of organisms are often unknown.

- Potential Solution: Create relevant laboratory model systems to study the effect of key environmental stressors on vulnerable cells/ organisms, and to test resistant engineered cells/ organisms (e.g., air pollution).

Long-term Milestone: Track sourcing through supply chains by scaling-up biomolecular forensics (DNA, protein, and metabolic profiling) and genetic barcoding.

Breakthrough Capability: Enable strengthening and protection of keystone and threatened species.

Short-term Milestone: Build reference genome libraries for currently threatened species (Paez et al., 2022).

- Bottleneck: Current sequences, when they exist, are error-prone and difficult to fully reassemble.
    - Potential Solution: Use the latest sequencing and genome writing technologies to improve genome assemblies for a wider range of threatened species.

Medium-term Milestone: Utilize genetic monitoring to determine best approaches for introducing and optimizing intrapopulation genetic variation (Kaczmarczyk, 2019).

Long-term Milestone: Design and build targeted gene-drives for introducing beneficial or adaptive traits into threatened species.

- Bottleneck: Current limitations in genetic data for targeting edit sites.
    - Potential Solution: Increase genetic sequencing and library generation for higher-order organisms, especially with regard to reproductive biology (Bier, 2022).
    - Potential Solution: Improve understanding of homologous recombination and non-homologous end-joining in mammals (Conklin, 2019).

Long-term Milestone: Engineer keystone and threatened species to be more adaptive or robust to climate change and anthropogenic impacts, such as through engineered microbiome robustness.

- Bottleneck: Requires thorough characterization and understanding of genomes and impact of genome editing.
    - Potential Solution: Extensive genome reference libraries, including for those of symbionts.

Long-term Milestone: Enable introduction of genetic diversity into threatened species populations.

Goal: Ensure the availability of biocontainment approaches for engineered organisms.

Current State-of-the-Art: Biocontainment of engineered organisms is a critical concern for engineering biology applications in the environment; the introduction of a new living organism to an ecosystem has the potential to cause adverse environmental impacts that are perpetuated by organism reproduction, out-crossing, persistence, and/or movement. Even though this Goal exists as a subsection of this theme, it is intended to inform all parts of the roadmap where engineered organisms might be released into the environment. Furthermore, though this Goal approaches biocontainment from an engineering biology perspective, there are significant overlaps with active research in ecology and other environmental sciences.

Before an engineered organism is released into the environment, researchers should be able to articulate mechanisms by which an organism could escape containment and what the consequences of that escape might be (Ellstrand, 2018; Mackelprang & Lemaux, 2020). To accomplish this, tools are needed to model and understand any potential impacts engineered organisms might have on the environment (Arnolds et al., 2021). There is ongoing research to examine how different engineered organisms interact with native populations and ecosystems, and how they will react to different climate change factors, over different time periods (see for example Allainguillaume et al., 2009, Rinkevich, 2015, and Lu et al., 2016), but more work is needed to model and study these interactions in simulated environments. Environmental release should be accompanied by means to monitor the organism(s) post-release, processes to collect evidence and inform risk assessment, strategies to contain the organisms in intended environments, and fail safes for eliminating the organisms in the event of a containment breach. Approaches to promoting resilience within existing populations will likely require the use of gene drive technologies, thus fundamentals of gene drive consequences, impacts, and control must be well understood. Additionally, means for detecting biocontainment breaches are needed, for example through the ubiquitous sensing and monitoring of unique biomarkers associated with an engineered organism. Furthermore, approaches are needed that confer lethality upon engineered organisms that escape biocontainment, such as auxotrophic bacterial strains with reliance on nutrients specific to an intended environment (Torres et al., 2016, Rottinghaus et al., 2022).

Social and regulatory uncertainties exist regarding affected communities' interests in the deployment of engineering biology tools. Coordination is also necessary to work directly with communities to find solutions that suit their needs and improve local economic conditions. Potential social, economic, and ethical implications—and how researchers can engage with such non-technical considerations—are discussed in more detail in the **Social and Nontechnical Dimensions Case Studies.**

Breakthrough Capability: Understand and model the potential and realized impacts of engineered organisms on the environment.

Short-term milestones: Develop integrated field biosensors for detection and monitoring of engineered organisms.

- Bottleneck: Engineering sensors that are robust to horizontal gene transfer of engineered genetic material.
    - Potential Solution: Engineer multi-component biosensors that can sense and report the presence of engineered sequences and the abundance of microbial genera.
- Bottleneck: Incorporation of biosensors at a density that is useful for detection and monitoring without negatively impacting field ecology.
    - Potential Solution: Engineer quantitative cell-free biosensors that provide more information at lower density.

Medium-term Milestone: Design and carry out environmental case-control studies comparing engineered biomes with control biomes in simulated environments to assess the impacts of engineered organisms on biodiversity.

- Bottleneck: Determining genetic systems responsible for environmental persistence of engineered organisms released to the environment (e.g., soil, waterways, animal gut).
    - Potential Solution: Specific tests of diversity maintenance, productivity maintenance/improvement and resilience for plant/microbe systems in environmental 'twin' formats such as EcoPODS.
    - Potential Solution: Identify relative genetic components of native organisms that are adaptive and resilient to climate stresses to incorporate into engineered systems.
- Bottleneck: Tracking and reporting (remotely, such as by release of detectable volatile compounds) of location and spread of engineered vs. native organisms within the study environment.
    - Potential Solution: Develop applicable biosensors.
- Bottleneck: Integrated risk-return models for releasing bioengineered organisms and systems into the field, including risk of dispersal and adverse ecological impacts, are needed.
    - Potential Solution: Environmental case studies deployed and standardized across a network of laboratories and centers to allow for comparative studies of ecosystem function and effects of intervention.

Long-term Milestone: Model and predict the dispersal of hyper-cultivated organisms and engineered biology (e.g., gene drives) and their impact on ecosystem diversity.

- Bottleneck: Dispersal indicators for key species, and the evidence backing the biotic and abiotic mechanisms of their constraint, are needed (currently under-developed).
    - Potential Solution: Develop open and integrated data and analysis systems for search and inferred relationships among biological data focused on prediction of key biological mechanisms most affecting key ecosystem health and productivity and pointing to engineering interventions.
    - Potential Solution: Identify the ecological impacts of gene drives, such as any adverse effects the gene drive might have on non-target populations.
- Bottleneck: Demonstrated effectiveness of deployment approaches.
    - Potential Solution: Scaled solutions (pilot and 1-2 years of field data) and assessments of risk criteria/considerations for deployment.
    - Potential Solution: Biodiversity tracking programs for the migration of hyper-cultivated and engineered biology to support studies of their impact on ecosystem function.

Breakthrough Capability: Develop robust strategies for biocontainment.

Short-term Milestone: Demonstrate successful biocontainment of engineered organisms in conditions identical to or closely approximate the intended environment of release.

- Bottleneck: Escaped cells could find an ecological niche in the natural environment where conditions allow the organisms to proliferate.
    - Potential Solution: Test biocontainment in conditions that simulate realistic environmental release.
    - Potential Solution: Develop growth conditions that simulate a worst-case scenario (for example, containing alternative nutrients the engineered organism could utilize that would enable its escape).
    - Potential Solution: Minimize the number of genes required for survival in broad conditions, so that the engineered host can only survive in the intended environment.
- Bottleneck: Small reactor sizes and short time-course measurements impose limits on approximating real-world conditions.
    - Potential Solution: Develop computer models that simulate industrial scale growth and/or environmental release of engineered organisms.

Medium-term Milestone: Develop methods to remove engineered species from ecosystems in the event of a containment breach.

- Bottleneck: Prevention of *in situ* evolution and gene transfer to and from engineered organisms to native organisms.
    - Potential Solution: Develop gene drives that can horizontally-transfer genes capable of limiting reproduction of escaped species.
    - Potential Solution: Develop mechanisms for planned senescence in engineered organisms.

Medium-term Milestone: Develop approaches for tracing engineered organisms in the environment and measuring their persistence.

- Bottleneck: Mutations may inactivate genetic tracing approaches.
    - Potential Solution: Use redundant tracing approaches.

Medium-term Milestone: Develop strategies to better understand the impacts of biocontainment breaches for different organisms in specific environments so that appropriately strong and/or layered biocontainment measures can be implemented (see Ellstrand, 2018).

- Bottleneck: The impacts of introducing an organism into an environment are determined by many factors and may take long time horizons to become apparent.
    - Potential Solution: Integration of controlled and contained field trials with existing environmental modeling approaches to design AI/ML algorithms that predict environmental impacts.

Long-term Milestone: Synthesize complementary biocontainment strategies (e.g., reliance on non-canonical amino acids, genetic recoding schemes, kill switches, genome

reduction, synthetic speciation) to achieve standards for low-risk/high-containment[5] environmental release.

- Bottleneck: Containment strategies might need to be used in parallel to sufficiently decrease risk, which might decrease organism fitness and create pressure for escape (see Gallagher et al., 2015; Moe-Behrens et al., 2013).
    - Potential Solution: Improve containment using individual strategies and identify determinants of any fitness costs associated with using multiple biocontainment strategies.

[5] Guidelines issued by the National Institutes of Health in 2019 stipulate that systems wherein recombinant or synthetic nucleotides escape at a rate of less than $1/10^8$ may be deemed to have a high level of biological containment (National Institutes of Health, 2019).

Part 2: Enabling Sustainable, Climate-friendly Production in Application Sectors

Food & Agriculture

Introduction and Impact: Agriculture and food systems are especially vulnerable to climate change. More intense and frequent droughts, floods, and heat waves have decimated agricultural output in all parts of the world, but have been especially detrimental to the Global South (Mbow et al., 2019; OECD, 2015; EPA, 2016). The impacts of these extreme climate events are compounded by a growing global population, leading to an acute need to improve food security. On top of this, many current agricultural practices even contribute to climate change and instability, through the production of greenhouse gases (particularly methane), production and over-application of synthetic nitrogen fertilizer, inefficient water use, and production of waste. The food and agriculture sector must leverage engineering biology to both minimize its impact on climate change and to sustain production in the face of abiotic and biotic stressors that result from climate change. The Food & Agriculture theme focuses on engineering biology research opportunities to enable the production of food and crops with lower greenhouse gas (GHG) emissions through climate-friendly biofertilizers, sustainable production of meat and meat-alternatives, crop and soil resilience, opportunities in 'smart agriculture', and opportunities to limit food waste or convert it to useful products [Figure 5]. Importantly, further advancement of engineering biology for agriculture requires ongoing stakeholder engagement between biotech researchers, legislators, consumers, and agriculture producers in order to identify tolerable risk thresholds, lower barriers to adoption, and incentivize scale-up.

Reducing GHG emissions from agriculture is now a top priority as part of the pathway to combat climate change (IPCC, 2021). Methane accounts for 11% of global GHG emissions, with the agricultural sector being the largest source of methane (EPA, 2020). Current agricultural practices also generate other GHGs such as nitrous oxide from synthetic fertilizer usage. Over-application of synthetic fertilizers also leads to nitrogen run-off and eutrophication of downstream ecosystems, as has been seen in the Gulf of Mexico (US Department of Commerce, 2021). This roadmap sets out breakthroughs and milestones toward developing more sustainable crops and fertilizers that prevent ecological disruption caused by run-off of water-soluble nitrates, engineering biology opportunities to reduce methane production from cattle and other ruminant sources of meat, and biobased alternative meats that reduce the need for water and other resources. Potential technical advances include engineering the plant rhizobiome to better capture nutrients from soils, engineering methanotrophs for ruminant feed or gut colonization, and generating lower-cost growth factors for cultured meat production.

This roadmap also identifies opportunities for engineering biology to be applied to the development of crops with enhanced resistance to biotic and abiotic stresses. As examples, plants could be engineered for greater drought and flood tolerance, soil microbiomes could be designed to improve plant health and survival under stressful environmental conditions (e.g., heat, high-salt content), and plants and soil microbiomes could be engineered for increased resistance to pathogens in the absence of environmentally harmful pesticides. Climate change also threatens global food supply chains by disrupting food transportation and increasing the likelihood of food spoilage; this roadmap addresses some opportunities to detect and prevent food spoilage through biosensors and advanced biomaterials.

Figure 5. Engineering biology for sustainable food & agriculture. The sustainability of agriculture practices and maintaining food security is rapidly decreasing due to climate change. Engineering biology has long been employed in the food and agriculture sector, and as tools and technology advance, there are more and more opportunities for novel engineering biology solutions. These include design and implementation of engineered crops, such as those that can better withstand climate extremes, alternative sources for meat and protein from sustainable sources, reduced food- and agriculture-related greenhouse gas release by engineering crop and soil efficiency and microbiome engineering, and conversion of food and agriculture waste, like manure. Advances in microbiome engineering, in particular, could enhance plant-rhizobiome interactions for more efficient water and nutrient uptake, help to curb harmful runoff from synthetic fertilizer, and could help to alter feed sources for animals and metabolism in ruminants to reduce methane emissions. Advances in biomaterials could help solve problems related to food spoilage or to induce or prevent the timing of ripening. Further research in engineering biology could accelerate and advance cellular agriculture and alternative meat production, conserving resources and providing the public with more climate-friendly food options.

Food & Agriculture in EBRC Roadmaps

Food & Agriculture has been a consistent theme in many EBRC Roadmaps. *Engineering Biology* (2019) addressed related tools, technologies, and processes including the production of "clean meat" and improving soils for more efficient crop production {see: https://roadmap.ebrc.org/2019-roadmap/sectors/food-agriculture/}. *Microbiome Engineering* (2020) also addressed reducing the environmental impacts of food production through alternative food sources and reducing reliance on chemical fertilizers {see: https://roadmap.ebrc.org/2020-roadmap-microbiomes/application-sectors-microbiomes/food-agriculture-microbiomes/}.

FOOD & AGRICULTURE

Goal	Breakthrough Capability	Milestone

Lower greenhouse gas emissions from food production.

Enable sustainable, climate-friendly biobased fertilizers.

Short-term	Medium-term	Long-term
Expand the toolbox for engineering rhizosphere microbes and communities (including isolation of tractable microbes, genetic parts).	Test biocontainment strategies for engineered microbes in rhizospheres.	Engineer nitrogen-intensive crops to form symbiotic relationships with nitrogen-fixing microbes.
Develop bacteriophage-based tools for detecting and engineering rhizosphere microbes in situ to avoid the need to culture these in a lab.	Engineer soil enzymes for nitrogen fixation, phosphorus assimilation, and other nutrients for improved activity.	
Improve understanding of gene expression dynamics in different bacterial growth phases under relevant soil conditions.		
Engineer genes and enzymes that facilitate symbiotic relationships between plants and soil microbes to promote nutrient fixation and reduce runoff.	Discover and engineer catabolic pathways for plant-specific root exudate compounds to control the persistence of microbiome members and to prevent their spread into off-target plant rhizospheres.	Establish obligate plant-microbiome ecosystems that provide containment of engineered microbes.
Assess the fidelity and longevity of biobased fertilizers and fertilizer components and formulations under field conditions.		

Engineer agricultural crops that are less emission-intensive.

Short-term	Medium-term	Long-term
Engineer additional crop varieties with plant-incorporated protectants to decrease pesticide inputs.	Engineer rice field microbiomes to minimize methane production by at least 50%.	Engineer weed-suppressing allelopathic crops that decrease the need for tillage and herbicide application by at least 50%.
Engineer additional common rice varieties with drought tolerance to reduce submergence time in irrigated rice paddies.	Engineer tunable and directed symbiosis between nutrient fixing bacteria (e.g., rhizobia) and non-legume plants (including engineering desired persistence time in the environment).	Develop crops with phosphorus and nitrogen biosensing and reporting capabilities to enable targeted and precise application of supplements/ fertilizers.

Short-term → Medium-term → Long-term

Reduce methane production through livestock and manure management.

Short-term	Medium-term	Long-term
Engineer ruminant gut methanotrophs.	Enable engineered methanotrophs to colonize the ruminant gut via feed or inoculation.	Develop forage crops for ruminant grazing that would result in lower methane production.
Engineer ruminant gut microbiome to produce less hydrogen, to reduce methane production by methanogens.	Develop enzymatic or microbial bioprocesses for manure management to produce biogas, bio-oil, biochar and recapture essential elements for fertilizer.	
Implement manure management strategies using existing anaerobic digestion technology to capture methane.		

Enable sustainable production of alternative meats and proteins.

Short-term	Medium-term	Long-term
Enable precision fermentation for commercial-scale production of microbe-derived milk proteins.	Engineer microbes or plants with lipid-biosynthesis pathways that produce fats identical to animal-derived.	Engineer complete nutrition crops, optimized for downstream processing into specialized meat and protein foods.
Predict and model functional and sensory performance of plant-derived proteins.	Engineer lower-cost and scalable growth media, specifically growth factors, for alternative meat production.	
Improve chemoautotroph protein expression tools.	Develop sustainable biological (vs. chemical or mechanical) processing methods for protein enrichment and extraction from crops.	
	Engineer crops for higher protein yields and functionality to decrease reliance on downstream processing steps.	

Enable engineered biology to convert food and agricultural waste to value-added products.	
Engineer efficient microbes, consortia, or cell-free systems for biogas and biofuel production from food and agriculture lignocellulose waste.	Enable protein and enzyme production from solid-phase fermentation of food processing byproducts or side-stream, such as spent grains.
Engineer inexpensive, multi-enzyme immobilized pathways for at-scale production of biodiesel from spent cooking oil and animal fats.	Engineer microbes capable of stabilizing and detoxifying biomass, enabling a longer conservation without spoilage.
Engineer microbes or microbial consortia to produce commodity and high-value chemicals from biogas released from anaerobic digestion.	Engineer highly-efficient hydrolytic pathways into consortia for recovery of fatty-acids, sugars, amino acids, and phosphates from mixed-waste streams.
Diversify feedstock for food additive production (e.g., amino acids, vitamins, etc.) to lignocellulosic biomass, one-carbon molecules, and industrial agricultural wastes via metabolic engineering.	Engineer consortia of microalgae that can grow on pure commercial food wastes for algal biomass/feedstock production.
	Enable bioprocessed recovery of nutrients from waste streams to reuse as fertilizer on industrial scales.

Enable a food and agriculture sector resilient to a changing climate.

Sense and report soil and crop health and response to climate stress.		
Develop biosensors detecting key metabolites found in soil/plant root exudates (like arabinose, salicylic acid, vanillic acid, naringenin) in soil microbes.	Implement engineered soil microbial biosensors in field-conditions to monitor soil health.	Engineer biosensors that sense environmental stressors or pathogens and activate soil microbiome remediation/self-regulation pathways.
Identify reporters of gene expression as biosensor outputs for use in soils and agricultural settings.		
Test deployment strategies for effectiveness and persistence of engineered biosensors and reporters in soil.		Link sensing networks across scales to facilitate exascale modeling linking soil microbiome perturbations to plant yields, nutrient content, and other indicators of soil and plant health.
Develop cell-based or cell-free biosensor systems to indicate the presence of crop pathogens.		
Short-term	**Medium-term**	**Long-term**

Engineer soils and crops resilient to a changing climate.

Short-term

- Identify and engineer genetic or metabolic pathways or processes (e.g., non-photochemical quenching, more efficient carbon fixation in C3 plants to decrease photorespiration) to improve plant health under stressful environmental conditions in model plants.
- Engineer crops and/or associated microbiomes to support more efficient nutrient and water capture from less-adequate growth environments.
- Further develop soil and plant microbial amendments (see for example, Bacillus thuringiensis) that suppress biotic stressors by expressing antagonistic compounds or by niche exclusion.
- Engineer crops that synthesize proteins or compounds that increase resistance to pests, particularly those increasing or encroaching due to climate change.

Medium-term

- Introduce genetic or metabolic pathways that improve plant health under stressful environmental conditions into species and varieties that are grown in regions most likely to experience given climate challenges.
- Introduce genetic diversity that cannot be achieved with breeding—or that cannot be achieved on a relevant time-horizon with breeding—into agricultural crops to improve resistance to pests and disease; for example, Resistance genes that recognize effectors and initiate effector-triggered immunity.
- Demonstrate synthetic microbial community promotion of plant resilience to environmental stresses in controlled (e.g., greenhouse) environments.

Long-term

- Design microbiomes for leaves and stems that protect against biotic and abiotic stressors, such as biofilms that minimize transpiration but are completely permeable to carbon dioxide and oxygen.
- Engineer genetic pathways into more diverse agricultural crops (beyond staple crops) to imbue resilience to environmental stressors.
- Engineer genetic pathways into more diverse agricultural crops (beyond staple crops) to increase crop resistance to pathogens, particularly those emerging due to climate change.
- Engineer entire phytobiomes (including crop, leaf microbiome, soil microbiome) for optimal resilience and yield.

Engineer foods and biomaterials to detect, reduce, and prevent spoilage.

Short-term

- Enable monitoring of early signs of food spoilage using cell-based or cell-free biosensor systems.
- Develop biomaterials or biobased coatings (e.g., cyclodextrin-containing) that inhibit molecules responsible for spoilage.

Medium-term

- Engineer fruits and vegetables which are less susceptible to spoilage pathogens.
- Engineer fruits and vegetables that can ripen on demand (e.g., controlled ethylene production).

Short-term → Medium-term → Long-term

Goal: Lower greenhouse gas emissions from food production.

Current State-of-the-Art: Current food production and agricultural practices contribute significantly to global greenhouse gas (GHG) emissions. From 2000 to 2014, CO_2 emissions from food production increased from 1 to 1.28 gigatonnes amongst 14 of the world's top 20 agricultural producers (Mrówczyńska-Kamińska et al., 2021). This production of GHGs includes nitrous oxide from synthetic fertilizer usage and agricultural production (Davidson, 2009; Timilsina et al., 2020), and methane produced by livestock as part of their natural digestion process (Tapio et al., 2017; Lassey, 2008).

The Haber–Bosch process for manufacturing fertilizers is a cornerstone of industrial agriculture. However, the Haber process is energy intensive and generates large amounts of N_2O, a potent and long-lived GHG with 300 times the warming potential of CO_2 (Ghavam et al., 2021). Engineering biology is developing alternatives to decrease or eliminate the need for industrial fertilizers and chemical supplements. The rhizosphere, the soil zone where plant roots influence biological and chemical features of the soil, supports natural symbiosis with bacteria that help the plant with nitrogen fixation. This ability could be extended to more crops (i.e., non-legume plants) by engineering enhanced nitrogen fixation capabilities to crop plants and engineering bacteria associated with crops as fertilizers (DeLisi, 2020; Bloch et al., 2020). Research is also underway to engineer microbial-plant interactions that enable lower use of phosphorus fertilization (Cheng, 2019; Barea et al., 2005; Ahmad et al., 2013). Some of this is already practiced commercially by companies such as Pivot Bio, though most technologies are still at an early stage of development. These new biobased fertilizers can help reduce the need for water, enable the use of currently non-arable land for plant growth, and prevent ecological disruption caused by run-off of water-soluble nitrates and the eutrophication of marine environments.

Advances in genetic engineering (e.g., CRISPR) and precision agriculture will lead to more efficient crop production that generates fewer greenhouse gases. Genetically engineered crops have been grown successfully since 1996 with important impacts on carbon emissions (Brookes & Barfoot, 2020). As an example of where engineering biology tools and technologies can impact the emissions from crop production, we can look at a world-wide staple crop: rice. Rice cultivation usually includes a period of time where fields are intentionally flooded, creating an environment where methanogens thrive. Methane from rice production accounts for approximately 11% of annual anthropogenic methane emission (Jiang et al., 2019). Rice engineered to maintain high yields with minimal flood time, or an engineered microbiome that suppresses methanogen activity during flooding, could lower total methane emissions (see Kumar et al., 2014; Scholz et al., 2020). As a specific example, a recent *Science* publication details the overexpression of a transcriptional regulator in rice, induced by light and low-nitrogen, resulting in increased photosynthesis and nitrogen utilization and higher yield (Wei et al., 2022). In addition to this example, the engineering of plant-incorporated protectants (PIPs) into more crop varieties could reduce emissions by decreasing the total production, transportation, and application of pesticides. Emissions caused by Conventional Tillage techniques can be reduced by engineering crops that enable transitions to Reduced Tillage or No Tillage (Brookes & Barfoot, 2020), for example by engineering enhanced crop allelochemical production (see Mahé et al., 2022). Crops that do not typically benefit from symbioses with nitrogen-fixing microbes could be engineered to do so. Plant biosensors could communicate phosphorus or nitrogen needs to enable their precise use, reducing overuse and runoff. Expanding the approaches, engineering goals, targeted crop species and varieties, accessibility, and general education about crop engineering will increase the ability to reduce emissions.

Food production from livestock is the largest anthropogenic source in the global methane budget, mostly from enteric fermentation of domestic ruminants (Chang, 2019). Engineering biology could reduce methane in agriculture by producing more climate friendly diets for livestock. To prevent methane emissions, methanotrophs could be introduced through feed or inoculation to stably colonize the ruminant gut microbiome. Colonized microbes could be engineered to secrete small molecule inhibitors (see Duin et al., 2016 and Patra et al., 2017, for examples) in ruminant guts to reduce methane formation or metabolize methane into non-gaseous compounds to enhance animal health (e.g., acetate, succinate, butyrate, amino acids, methanol) (Ungerfeld, 2020). Engineering biology could also be used to pretreat animal feed and increase animal feed efficiency. For example, engineered microbes could synthesize animal nutrients (e.g., essential amino acids, micronutrients, vitamins) not naturally found in unprocessed feed or maintain specific moisture content in hay, haylage, or silage to increase feed production efficiency.

Further development and expansion of the “cellular agriculture” sector, including current cultured/cultivated meat and algae food products, to produce a broader variety of meat alternatives would reduce climate change by providing substitutes for animal and crop use (agricultural footprint), and support resilience in the wake of the effects of climate change. Meat and protein alternatives enabled by engineering microbial, fungal, or plant production of proteins, fats, and flavorings offer another means to continue feeding a growing global population (Linder, 2019). These technologies can also help to reduce the consumption of resources, including land, water, fertilizers, and pesticides. For instance, the milk protein market relies on animals and plants as a source for dairy and dairy alternatives (e.g., oat and nut milks); a recent modeling study concluded that microbes could be cultured to scalably produce Bovine Alpha Lactalbumin, one of the most prevalent whey proteins in the market and used for human infant formula among other products (Vestergaard, 2016). Engineering biology to improve the taste, texture, and nutritional value of protein alternatives and lowering the cost of ingredients (e.g., flavorings, fatty acids, enzymes) will make these products more attractive and accessible to the global population. These proteins may ultimately be produced from CO_2 or methane using photo- and chemoautotrophic organisms, similar to previously demonstrated production of single cell protein (SCP) from methane (Marcellin et al., 2022; García Martínez et al., 2022).

Finally, the conversion or recycling of food and agricultural wastes into value-added products will help to circularize the sector, reducing greenhouse gas emissions, not just from the wastes themselves (e.g., emissions from rotting foods), but also waste management practices (Nayak & Bhushan, 2019). Engineered enzymatic pathways can be used to transform waste biomass into feedstocks, such as nutrients for biofertilizers or biomass for biofuels (Liew et al., 2022; Davis & Moon, 2020). To do so sustainably means ensuring that these systems can be implemented where wastes are generated and where the value-added products can be used or consumed locally.

Breakthrough Capability: Enable sustainable, climate-friendly biobased fertilizers.

Short-term Milestone: Expand the toolbox for engineering rhizosphere microbes and communities (including isolation of tractable microbes, genetic parts).

- Bottleneck: Lack of effective, scaleable screening strategies for genetic parts from diverse organisms.
  - Potential Solution: Apply cell-free lysate system for high-throughput automated prototyping of regulatory and other genetic elements.
- Bottleneck: Competition between existing soil rhizobia communities and inoculants decreases the viability of the inoculant rhizobium in the soil and access to host tissue.
  - Potential Solution: Identify native soil and rhizobia microbes (like *Azorhizobium caulinodans* and *Rhizobium* sp.) that can be genetically engineered.

Short-term Milestone: Develop bacteriophage-based tools for detecting and engineering rhizosphere microbes *in situ* to avoid the need to culture these in a lab (Zurier et al., 2020).

- Bottleneck: Phage engineering, currently mostly focused on killing microbes, needs to be technically adapted to engineer microbes to have new functions (Citorik et al., 2014).
  - Potential Solution: Development of systems using temperate phages and other integrative and conjugative elements (e.g., Brophy et al., 2018).
- Bottleneck: There is the potential for many different microbes and pathways to be used, but it is not clear where the best solutions are.
  - Potential Solution: Leverage current -omics data to identify effective targets for engineering (e.g., Mendes et al., 2011).

Short-term Milestone: Improve understanding of gene expression dynamics in different bacterial growth phases under relevant soil conditions.

- Bottleneck: Current gaps in understanding stationary phase gene expressions; genes and pathways are present, but not expressed in engineered organisms, or not expressed at relevant levels.
  - Potential Solution: Implement gene/enzyme expression pathways and engineer nitrogen, phosphorus, or sulfur fixing bacteria in the field.

Short-term Milestone: Engineer genes and enzymes that facilitate symbiotic relationships between plants and soil microbes to promote nutrient fixation and reduce runoff.

- Bottleneck: Lack of tools to non-disruptively study microbial behavior in complex, opaque environments like soils.
  - Potential Solution: Utilize interconnected fungal highways to study bacterial behavior via fluorescence expression (Young et al,. 2022).

Short-term Milestone: Assess the fidelity and longevity of biobased fertilizers and fertilizer components and formulations under field conditions.

- Bottleneck: Current laboratory and greenhouse practices are not representative of realistic field conditions.
  - Potential Solution: Design and implement semi-contained field trials, developing associated standards and controls for semi-contained engineered microbial release.

Medium-term Milestone: Test biocontainment strategies for engineered microbes in rhizospheres.

- Bottleneck: A reliable surveillance mechanism is needed that can capture biocontainment escape.
  - Potential Solution: Improved engineered reporter systems for continuous monitoring.

Medium-term Milestone: Engineer soil enzymes for nitrogen fixation, phosphorus assimilation, and other nutrients for improved activity.

- Bottleneck: Difficulty of testing the function of these enzymes in the heterogeneous and complex soil network.
  - Potential Solution: Enhanced enzyme modeling and engineering to increase enzyme specificity and function across conditions (e.g., decrease sensitivity to inhibition by common soil compounds).

Medium-term Milestone: Discover and engineer catabolic pathways for plant-specific root exudate compounds to control the persistence of microbiome members and to prevent their spread into off-target plant rhizospheres.

Long-term Milestone: Engineer nitrogen-intensive crops to form symbiotic relationships with nitrogen-fixing microbes.

- Bottleneck: Challenges in engineering these relationships without affecting other elements of plant growth, yield, and/or molecular or nutritive quality.
  - Potential Solution: Improved understanding of symbiotic relationships and spatial control of gene expression.

Long-term Milestone: Establish obligate plant-microbiome ecosystems that provide containment of engineered microbes.

- Bottleneck: Challenges in engineering plant-microbiome interactions that enable both systems to detect and respond to changes within the relationship and environment.
  - Potential Solution: Improved understanding of symbiotic relationships and spatial-temporal control of gene expression, metabolism, and microbiome guilds.

Breakthrough Capability: Engineer agricultural crops that are less emission-intensive.

Short-term Milestone: Engineer additional crop varieties with plant-incorporated protectants to decrease pesticide inputs.

- Bottleneck: Maintaining efficacy of these varieties amid constant selective pressure on pests to evolve resistance.
    - Potential Solution: Identify and/or develop additional plant-protecting sequences that are safe for human consumption and highly pest specific.
    - Potential Solution: Stack multiple protectants into plant varieties for durable pest resistance.

Short-term Milestone: Engineer additional common rice varieties with drought tolerance to reduce submergence time in irrigated rice paddies.

- Bottleneck: Yield decreases under lower water conditions.
    - Potential Solution: Engineer varieties that are able to maintain yield in unflooded or droughted conditions.
- Bottleneck: Higher weed pressure in unflooded or less flooded rice fields.
    - Potential Solution: Continue research on weed management techniques.

Medium-term Milestone: Engineer rice field microbiomes to minimize methane production by at least 50%.

- Bottleneck: Maintenance of engineered microbiome composition and balance over growing season or seasons.
    - Potential Solution: Engineer spatio-temporal control mechanisms for stimulating growth of desired microbiome community members.
- Bottleneck: Ensuring that engineered microbiome does not negatively impact crop yield or soil health over time.
    - Potential Solution: Longitudinal study of rice paddy mesocosm to investigate crop yield over seasons.

Medium-term Milestone: Engineer tunable and directed symbiosis between nutrient fixing bacteria (e.g., rhizobia) and non-legume plants (including engineering desired persistence time in the environment).

- Bottleneck: Symbioses are complex relationships with many genetic determinants in each organism.
    - Potential Solution: The diversity of existing nitrogen fixation symbioses provides many "blueprints" for engineering (Huisman & Geurts, 2020).
    - Potential Solution: Comparative genomics and iterations of the DBTL cycle may enable identification of minimal genes needed for symbiosis (Huisman & Geurts, 2020).
- Bottleneck: Symbioses can involve trade-offs that may decrease crop yield.
    - Potential Solution: Engineer symbiosis to be active only when nitrogen availability is low and may already impact plant yield.

Long-term Milestone: Engineer weed-suppressing allelopathic crops that decrease the need for tillage and herbicide application by at least 50%.

- Bottleneck: Engineering allelopathy with broad enough specificity to impact the variety of weeds found in fields across different regions.
    - Potential Solution: Engineer crops with the capability to inducibly synthesize weed-specific allelochemicals in response to weed presence.

- Bottleneck: Maintenance of suppression despite selective pressure for weeds to become insensitive to allelochemicals.
    - Potential Solution: Engineer crops with multiple allelochemicals and/or use in conjunction with other weed suppression approaches (e.g., engineered microbiomes).

Long-term Milestone: Develop crops with phosphorus and nitrogen biosensing and reporting capabilities to enable targeted and precise application of supplements/fertilizers.

- Bottleneck: Reporting of low phosphorus and nitrogen would need to be easily observed and acted upon by farmers in large fields.
    - Potential Solution: Development of clear visual reporter systems that do not affect plant growth or health.

Long-term Milestone: Engineer commodity crops that require less emissions-intensive field preparation and inputs each year, such as perennial varieties of annual crops.

- Bottleneck: The genetic determinants of annual vs. perennial growth are complex and not well-understood across crop species.
    - Potential Solution: Develop more robust genetic tools across commodity crops for understanding and engineering growth determinants.
- Bottleneck: Crops deplete soil nutrient composition.
    - Potential Solution: Engineer crops for seasonal rotations that maintain profits for farmers and preserve soil nutrient stability (e.g., improve our plant strain toolboxes so that crops can be mixed and matched based on demand but maintain or improve healthy soils).

Breakthrough Capability: Reduce methane production through livestock and manure management.

Short-term Milestone: Engineer ruminant gut methanotrophs.

- Bottleneck: Colonization of methane-consuming methanotrophs in the ruminant gut microbiome.
    - Potential solutions: Develop fast-growing thermophilic anaerobic methanotroph organisms that can stably colonize the rumen.
    - Potential Solution: Engineer methanotrophs to synthesize beneficial metabolites and compounds not naturally found in unprocessed feed to support the ruminant gut microbiome.
- Bottleneck: Databases and other knowledge necessary to develop next-gen animal health probiotics.
    - Potential Solution: Collaborations between animal scientists and synthetic biologists to better characterize and understand the ruminant gut microbiome and impacts of feed.

Short-term Milestone: Engineer ruminant gut microbiome to produce less hydrogen, to reduce methane production by methanogens.

- Bottleneck: Limited knowledge of physiology and metabolism of anaerobic and uncultured microbes in the rumen.
  - Potential Solution: Use new single-cell genomics, metabolomics, and physiology techniques alongside microbiome engineering and computational modeling to gain insight as to rumen microbiome dynamics.
- Bottleneck: Lack of genetic tools in keystone rumen microbes.
  - Potential Solution: Develop new culturing techniques and new methods of introducing DNA or mutations into non-model anaerobic microbes.
- Bottleneck: Expense of rumen studies, such as fistulated cattle and anaerobic culturing infrastructure, can be prohibitively expensive.
  - Potential Solution: Develop microscale rumen microcosm models to mimic rumen environment and enable experimental testing of hypotheses relating to microbiome interactions and rumen metabolism.

Short-term Milestone: Implement manure management strategies using existing anaerobic digestion technology to capture methane.

- Bottleneck: Current range cattle manure management practices are not compatible with harvesting manure for anaerobic digestion.
  - Potential Solution: Study manure management strategies and their lifecycle impacts to understand the contributions of manure to rangeland soil quality and productivity versus methane emissions.
  - Potential Solution: Improve and incentivise anaerobic digestion and biogas recapture using engineered microbial consortia during cattle finishing.

Medium-term Milestone: Enable engineered methanotrophs to colonize the ruminant gut via feed or inoculation.

- Bottleneck: Introduction of novel microbes is likely to disrupt ruminant metabolism.
  - Potential Solution: Develop bacteriophage-based genetic engineering of ruminant gut microbes *in situ* (see Voorhees et al., 2020).

Medium-term Milestone: Develop enzymatic or microbial bioprocesses for manure management to produce biogas, bio-oil, biochar and recapture essential elements for fertilizer.

Long-term Milestone: Develop forage crops for ruminant grazing that would result in lower methane production.

- Bottleneck: Alternative feed regimes often result in decreased feed efficiency of cattle.
  - Potential Solution: Engineer forage crops and probiotics to enhance cattle nutrition, not just lower methane production.

Breakthrough Capability: Enable sustainable production of alternative meats and proteins.

Short-term Milestone: Enable precision fermentation for commercial-scale production of microbe-derived milk proteins.

Short-term Milestone: Predict and model functional and sensory performance of plant-derived proteins.

- Bottleneck: Plant proteins currently exhibit high batch-to-batch variability and suppliers offer limited characterization data.
    - Potential Solution: Create industry-wide standards for analytical assays for characterizing plant protein ingredients to improve reproducibility.
    - Potential Solution: Refine and expand access to plant ingredient characterization techniques with established significance for predicting functional and sensory performance.
    - Potential Solution: Develop an open-access protein sequence, structure, and functionality database.
    - Potential Solution: Develop machine learning approaches to accelerate the designing process (protein sequence to texture and taste).

Short-term Milestone: Improve chemoautotroph protein expression tools.

- Bottleneck: Chemoautotrophs are used for single cell protein production, but expression of specific target proteins often requires advanced expression and secretion tools only developed in traditional host organisms.
    - Potential Solution: Improve and adapt systems for extreme protein overexpression and secretion systems for a range of chemoautotrophic organisms to allow target protein production from CO_2 or methane.
    - Potential Solution: Demonstrate production of alternative products (e.g., milk proteins) in chemoautotrophs and tailor protein content of chemotrophs.

Medium-term Milestone: Engineer microbes or plants with lipid-biosynthesis pathways that produce fats identical to animal-derived.

- Bottleneck: Current microbe-synthesized fats retain plant-oil characteristics.
    - Potential Solution: Novel enzymatic or microbial conversion processes to transform plant oils into more animal-like fats or to endow them with animal fat-like properties (e.g., saturations, longer chain length, etc.)

Medium-term Milestone: Engineer lower-cost and scalable growth media, specifically growth factors, for alternative meat production (see for example https://multus.media/).

- Bottleneck: Growth factors are currently too expensive to allow extensive alternative meat production at scale.
    - Potential Solution: Engineer yeast or other cost-effective hosts to produce growth factors and other small molecules useful in cell culture media.
- Bottleneck: Lack of clarity on best potential sources of low-cost feedstock, leading to uncertainty for rural producers about associated opportunities and challenges (Newton & Blaustein-Rejto, 2021; Post et al., 2020).
    - Potential Solution: Partnerships with rural producers and other partners to test traditional crops and alternative forms of biomass; surveys to study how new production systems used to grow on these inputs would affect rural landscapes and feedstock costs.

Medium-term Milestone: Develop sustainable biological (vs. chemical or mechanical) processing methods for protein enrichment and extraction from crops.

- Bottleneck: Limited digestibility of protein from crops without extensive pre-treatment.
  - Potential Solution: Design novel proteins that incorporate important amino acids that can be expressed in seed or improve protein solubility.
  - Potential Solution: Edit plant protein sequences to increase digestibility through changes to structure and amino acid content or reduce expression of anti-nutritive factors.

Medium-term Milestone: Engineer crops for higher protein yields and functionality to decrease reliance on downstream processing steps.

- Bottleneck: Plant structure, lignin content, and anti-nutritive factors impede protein yield and bioavailability.
  - Potential Solution: Modulate seed-specific pathway genes to enhance protein accumulation in seed without impacting germination.
  - Potential Solution: Edit plant protein sequences to increase digestibility through changes to structure and amino acid content or reduce expression of anti-nutritive factors.

Long-term Milestone: Engineer complete nutrition crops, optimized for downstream processing into specialized meat and protein foods.

- Bottleneck: Lignin content in crops limits the expression and accumulation of other nutrients (such as fatty acids and digestible fiber).
  - Potential Solution: Develop more effective enzymes that can reduce lignin content in crops and/or overexpress lignin-degradation enzyme(s) under an inducible promoter.

Breakthrough Capability: Enable engineered biology to convert food and agricultural waste to value-added products.

Short-term Milestone: Engineer efficient microbes, consortia, or cell-free systems for biogas and biofuel production from food and agriculture lignocellulose waste.

- Bottleneck: Food and agriculture wastes typically require pre-treatment to increase solubility and conversion.
  - Potential Solution: Engineer biosystems with increased expression of cellulase and hemicellulase that can work in concert with downstream anaerobic digestion (Zheng et al., 2014).

Short-term Milestone: Engineer inexpensive, multi-enzyme immobilized pathways for at-scale production of biodiesel from spent cooking oil and animal fats.

- Bottleneck: The enzymes necessary to accomplish this are currently too expensive for this process to be economically-viable.
  - Potential Solution: Further optimization of the enzyme pathways with machine-learning and protein design could reduce enzyme production costs.

Short-term Milestone: Engineer microbes or microbial consortia to produce commodity and high-value chemicals from biogas released from anaerobic digestion.

- Bottleneck: Biogas utilization organisms are usually slow growing, difficult to culture, or require mutualistic partners to work together as a consortia (Cha et al., 2021); engineering these systems usually takes more effort compared to model bacteria.
    - Potential Solution: Engineer unique metabolic traits of biogas-utilizing organisms to well-known microbes to enable faster development and optimization (for example, see Yu et al., 2022).
    - Potential Solution: Engineer consortia designed for extensive synergistic co-digestion (Zamanzadeh et al., 2017; Mata-Alvarez et al., 2000).

Short-term Milestone: Diversify feedstock for food additive production (e.g., amino acids, vitamins, etc.) to lignocellulosic biomass, one-carbon molecules, and industrial agricultural wastes via metabolic engineering.

- Bottleneck: Lignocellulose degradation is complex, requires multiple enzyme types, and is inhibited by numerous compounds.
    - Potential Solution: Engineer synthetic multifunctional cellulosomes (extracellular protein colocalization) in hosts that present all the enzymes needed for synergistic degradation of these feedstocks.

Medium-term Milestone: Enable protein and enzyme production from solid-phase fermentation of food processing byproducts or side-stream, such as spent grains.

- Bottleneck: Recovery of bio-active proteins and enzymes from food waste typically requires extraction via toxic organic solvents, and complex immobilization and purification steps.
    - Potential Solution: Scale-up of enzyme-mediated treatment and extraction under industrial bioreactor conditions.

Medium-term Milestone: Engineer microbes capable of stabilizing and detoxifying biomass, enabling a longer conservation without spoilage.

Medium-term Milestone: Engineer highly-efficient hydrolytic pathways into consortia for recovery of fatty-acids, sugars, amino acids, and phosphates from mixed-waste streams.

- Bottleneck: Microbial consortia population depends highly on feedstock and could be affected by the diverse compounds in mixed-waste streams.
    - Potential Solution: Develop regulatory systems in the (synthetic) consortia that help regulate the microbial population to tolerate environmental change and remain functional despite variations in mixed-waste streams (Li et al., 2022b).

Medium-term Milestone: Engineer consortia of microalgae that can grow on pure commercial food wastes for algal biomass/feedstock production (Pleissner & Lin, 2013).

Medium-term Milestone: Enable bioprocessed recovery of nutrients from waste streams to reuse as fertilizer on industrial scales (Wang et al., 2004).

Goal: Enable a food and agriculture sector resilient to a changing climate.

Current State-of-the-Art: One of the most significant effects of climate change on human health and well-being is the impact on food production and agricultural practices, and engineering biology has many opportunities to impact and improve resilience and sustainability throughout the entire food and agriculture sector. Climate change is impacting where we grow our food, how crops and livestock adapt to environmental conditions, and the quality of the food when it gets to our plates. Current practices in food and agriculture also contribute to climate stressors, including the use of fertilizers and pesticides, production of methane, and energy consumption and pollution for food processing, transportation, and storage.

Engineering biology can contribute to advanced "smart agriculture" tools to complement agricultural production by enabling sustainable biosensors and reporters to measure critical changes in soil and crop health in real time. This can help to ensure that farmers and growers can identify and resolve stresses or combat disease before it affects an entire crop. Biosensors and reporters could also be applied to monitoring livestock health.

Soil health is particularly important, not only for mitigating the effects of climate change but also to support sustainable growing practices. Soil microbiome engineering could enhance depleted soils by reconstituting nutrients (*e.g.,* nitrogen) needed for plant growth, increase bioavailability of nitrogen and phosphorus in the root rhizosphere, replenish nutrients that were removed during the previous growing season, and concentrate minerals and other micronutrients to improve crop nutrient content. Engineered soil microbiomes have been shown to improve plant health by mitigating soil pathogens (Schlatter, 2017). Further research could enable engineered soil microbiomes that help plants survive during stress-inducing environmental conditions (including heat, high-salt content, drought, flood, pollution, and disease).

Engineering biology could build crop resistance to biotic and abiotic stresses (*e.g.,* disease, drought, temperature, nitrogen limitation). For example, microbiomes could be engineered to increase drought tolerance in plants by creating biofilms on leaves to decrease transpiration without affecting carbon dioxide uptake and to increase water capture from atmospheric moisture. Alternatively, crops could be engineered for more robust photosynthesis with less perturbation when conditions rapidly change, such as by modifying non-photochemical quenching to increase yield (Souza et al., 2022). There is also a growing need to develop flood-resistant crops, especially for communities impacted by sea level rise and increased flooding due to climate change (Sasidharan et al., 2021). Plant microbiomes could also help to reduce pathogen disease pressure, such as by being engineered to secrete pathogen-specific cell-wall degrading enzymes.

In addition to threatening global or regional food yields, more intense and frequent extreme weather events caused by climate change disrupt food transportation and supplies, increasing the likelihood of food spoiling. There are several engineering biology approaches to mitigate food spoilage at different stages of the supply chain. Biobased systems could be developed to sense and report early biomarkers of food spoilage or the presence of pathogens or spoilage metabolites. Food-safe, novel bioprotectants applied to produce could lengthen shelf life; for example, ingestible biopolymer coatings could be developed to counteract spoilage-causing microbes or inhibit early stage biofilm formation (Marelli, 2022). Biopolymer coatings may also reduce energy consumption by reducing reliance on refrigeration to keep produce fresh. Novel biomaterials could be designed to express preservatives (such as benzoate) on-demand to respond to specific environmental signals (*e.g.,* time, temperature, pH, microbial activity). Engineered biomaterials could detect and control the ripening of produce. For example, cell-free or cell-based biosensors could be developed to detect molecules associated with over-ripening/spoilage in food storage facilities (*e.g.,* ethylene detecting sensors in apple warehouses). To control ripening, engineered biological systems could selectively release molecules that modulate ripening (*e.g.,* ethylene, methyl salicylate). For example, microbes could be engineered to dynamically produce and break down ethylene in response to local concentrations to accelerate ripening post-storage, but slow spoilage. And advances in food packaging could prolong shelf life of produce and reduce urban pollution and the associated GHG emissions.

Breakthrough Capability: Sense and report soil and crop health and response to climate stress.

Short-term Milestone: Develop biosensors detecting key metabolites found in soil/plant root exudates (like arabinose, salicylic acid, vanillic acid, naringenin) in soil microbes.

- Bottleneck: Detection technologies for soil-deployed biosensors are limiting.
  - Potential Solution: Research on reporter systems visible at a macro-scale.
- Bottleneck: Development of chassis with sensing and reporting capabilities that are suitable for environmental use (Del Valle et al., 2021).
  - Potential Solution: Expanded engineering biology chassis, tools, and parts.

Short-term Milestone: Identify reporters of gene expression as biosensor outputs for use in soils and agricultural settings.

- Bottleneck: Most output reporters (fluorescent proteins, pigments, etc.) require imaging, limiting their use in opaque environments like soils.
  - Potential Solution: Develop and refine macro-level reporters that can be inexpensively visualized or detected across space and time, and that do not negatively impact ecosystem members.

Short-term Milestone: Test deployment strategies for effectiveness and persistence of engineered biosensors and reporters in soil.

- Bottleneck: Unrefined capabilities to measure effectiveness and persistence in soil.
  - Potential Solution: High-throughput, field-deployable characterization capabilities for measuring effectiveness and persistence across space and time.

Short-term Milestone: Develop cell-based or cell-free biosensor systems to indicate the presence of crop pathogens.

- Bottleneck: Application and continued function of sensors across agricultural fields throughout a growing season.

- Potential Solution: Identify receptors/genes from plants known to respond to specific pathogens/pests that can be used as biosensors and engineer them into companion crops grown around a field or integrated at regular intervals into a field to provide lasting sensing capabilities.

Medium-term Milestone: Implement engineered soil microbial biosensors in field-conditions to monitor soil health.

- Bottleneck: Biosensor sensitivity needs to be improved to respond to physiologically relevant concentrations.
    - Potential Solution: Use automation and protein engineering capabilities to design sensors that function at low concentrations.

Long-term Milestone: Engineer biosensors that sense environmental stressors or pathogens and activate soil microbiome remediation/self-regulation pathways.

- Bottleneck: Controlling when, how, and at what level stress response pathways are activated (e.g., distinguishing between typical irrigation and flooding conditions).
    - Potential Solution: Characterize the extent of pathway activation and fine tune (using circuit controls like amplifier, band-pass filter, etc.) pathway activation to desired levels.

Long-term Milestone: Link sensing networks across scales to facilitate exascale modeling linking soil microbiome perturbations to plant yields, nutrient content, and other indicators of soil and plant health.

- Bottleneck: Data measuring many variables *in situ* are often very noisy, making it challenging to draw robust conclusions.
    - Potential Solution: Increase the number of variables for which information is collected (multiplex data collection and analysis) so the role that different variables (including gene expression level, temperature, soil composition) play can be better understood.

Breakthrough Capability: Engineer soils and crops resilient to a changing climate.[6]

Short-term Milestone: Identify and engineer genetic or metabolic pathways or processes (e.g., non-photochemical quenching, more efficient carbon fixation in C3 plants to decrease photorespiration) to improve plant health under stressful environmental conditions in commercially-relevant/model plants.

- Bottleneck: Because many crop plants are polyploid with significant genetic redundancy, pathways can be difficult to robustly and stably engineer.
    - Potential Solution: Plant whole-genome engineering approaches that enable specific editing of paralogs for precise control of expression and function (see Wang et al., 2014; Lv, 2020).

[6] Includes resiliency to biotic (e.g., pathogens, invasive species) and abiotic (e.g., excessive heat, flood, drought, high salinity) stressors that contribute to stressful environmental conditions and nutrient scarcity.

- Bottleneck: Delivery of gene editing machinery through plant cell walls can be challenging and limit uptake; plant transformation efficiencies are still often low despite decades of research (Ramkumar et al., 2020; Altpeter et al., 2016).
    - Potential Solution: Advances in delivery into protoplasts and plant regeneration.
    - Potential Solution: Development of nanoparticle-mediated delivery mechanisms that reliably make heritable genetic changes (see Sirirungruang et al., 2022).
- Bottleneck: Plant life cycles and regeneration times after genome editing or engineering are significant, which slows the pace of research and development compared to microbes.
    - Potential Solution: Engineer broadly useful approaches to speeding post-transformation regeneration (Aregawi et al., 2022).

Short-term Milestone: Engineer crops and/or associated microbiomes to support more efficient nutrient and water capture from less-adequate growth environments.

- Bottleneck: Nutrient capture from already-deficient soils can further deplete those soils.
    - Potential Solution: Engineer microbiomes that capture needed atmospheric compounds and/or convert compounds to bioavailable forms.
    - Potential Solution: Enhance cover crop symbioses that replenish nutrients.
- Bottleneck: Nutrients must be in bioavailable forms for plant uptake.
    - Potential Solution: Microbiome engineering to convert nutrients to forms that enable plant uptake (Deynze et al., 2018).
- Bottleneck: Plant roots may be unable to penetrate soils sufficiently to reach necessary nutrients and water.
    - Potential Solution: Design soil microbial communities to concentrate minerals and other plant nutritive compounds for uptake.
    - Potential Solution: Incorporation of hygroscopic microbes into soil surface for atmospheric water capture.
    - Potential Solution: Design genetic circuits that predictably control root architecture in non-model plants (Brophy et al., 2022).
- Bottleneck: Water and nutrient levels need to fall within preferred range to avoid over-abundance.
    - Potential Solution: Engineer rhizosphere microbes to modulate moisture levels in response to drought and/or flood conditions.

Short-term Milestone: Further develop soil and plant microbial amendments (see for example, *Bacillus thuringiensis*) that suppress biotic stressors by expressing antagonistic compounds or by niche exclusion.

- Bottleneck: Ensuring microbial amendments are sufficiently present and persistent to have the desired impact without out-competing other valued community members.

- ○ Potential Solution: Develop feedback mechanisms that influence microbial reproduction (e.g., growth when concentration of antagonistic compounds are low).

Short-term Milestone: Engineer crops that synthesize proteins or compounds that increase resistance to pests, particularly those increasing or encroaching due to climate change.

- Bottleneck: Identification of pest-specific resistance traits that provide durable resistance.
  - Potential Solution: Use of artificial intelligence-/machine learning-based algorithms to design proteins with insect-specific toxicity.

Medium-term Milestone: Introduce genetic or metabolic pathways that improve plant health under stressful environmental conditions into species and varieties that are grown in regions most likely to experience given climate challenges.

- Bottleneck: Understanding of the genetic determinants of phenotypic diversity observed in plant varieties internationally and how/if research in a model variety can be useful for other varieties.
  - Potential Solution: Incorporate an understanding of relevant crop varieties earlier in research development, matching tolerance or resilience pathways to the plants most likely to be impacted.

Medium-term Milestone: Introduce genetic diversity that cannot be achieved with breeding—or that cannot be achieved on a relevant time-horizon with breeding—into agricultural crops to improve resistance to pests and disease; for example, *Resistance* genes that recognize effectors and initiate effector-triggered immunity (Ngou et al., 2022).

- Bottleneck: Insufficient knowledge of the types of genetic diversity that might improve pest and disease resistance.
  - Potential Solution: Automated screening systems to rapidly advance knowledge of the genetic determinants of resistance to given pests.
- Bottleneck: Efficient expression and production of antimicrobial genes and compounds in response to pathogens that cannot easily be overcome by pathogen evolution.
  - Potential Solution: Better characterization of plant-pathogen interactions to identify and/or develop strategies for the durable production of pathogen-targeted antiviral molecules.
- Bottleneck: Some agriculturally valuable crops are perennials that do not produce a crop until several years of growth; thus, engineered solutions cannot immediately be implemented to alleviate crop loss from intensifying pest situations.
  - Potential Solution: Engineer perennial crop varieties that reach maturity more quickly.

Medium-term Milestone: Demonstrate synthetic microbial community promotion of plant resilience to environmental stresses in controlled (e.g., greenhouse) environments.

- Bottleneck: Engineering microbial communities that promote plant resilience in response to environmental stressors without compromising plant yield during ideal conditions.
    - Potential Solution: Design microbial communities whose growth and reproduction is induced by the environmental stressor for which they are engineered to promote resilience against.

Long-term Milestone: Design microbiomes for leaves and stems that protect against biotic and abiotic stressors, such as biofilms that minimize transpiration but are completely permeable to carbon dioxide and oxygen.

- Bottleneck: Understanding and preventing displacement of necessary functions of wild-type leaf microbiomes.
    - Potential Solution: Ensure communities retain members that perform necessary functions, for example through hygroscopic bacteria (Hernandez & Lindow, 2019).

Long-term Milestone: Engineer genetic pathways into more diverse agricultural crops (beyond staple crops) to imbue resilience to environmental stressors.

- Bottleneck: Plant transformation and regeneration are slow processes with low efficiency (particularly in less-researched crops), so engineering entire pathways is slow and technically challenging.
    - Potential Solution: Additional research that supports more efficient transformation processes across crop species and varieties.
- Bottleneck: Environmental stressors can affect many plant pathways and processes; imbuing resilience may require not just the engineering of a desired pathway but precisely controlling other pathways.
    - Potential Solution: As minimal bacterial genomes research advances, advance a "minimal plant genome" project to better understand and engineer complex organismal systems and processes (Hutchison et al., 2016).

Long-term Milestone: Engineer genetic pathways into more diverse agricultural crops (beyond staple crops) to increase crop resistance to pathogens, particularly those emerging due to climate change.

- Bottleneck: Every pathosystem is unique, and thus needs to be characterized to engineer resistance, especially as climate change impacts which pathogen species are present and lifecycle timing of both hosts and pathogens.
    - Potential Solution: Develop strategies for rapid characterization of host-pathogen relationships.
- Bottleneck: Plant transformation and regeneration are slow processes with low efficiency (particularly in less-researched crops), so engineering entire pathways is slow and technically challenging.
    - Potential Solution: Additional research that supports more efficient transformation processes across crop species and varieties.

- Bottleneck: Environmental stressors can affect many plant pathways and processes; imbuing resilience may require not just the engineering of a desired pathway but precisely controlling other pathways.
    - Potential Solution: As minimal bacterial genomes research advances, advance a "minimal plant genome" project to better understand and engineer complex organismal systems and processes (Hutchison et al., 2016).
- Bottleneck: Plant transformation and regeneration are slow processes with low efficiency (particularly in less-researched crops), so engineering entire pathways is slow and technically challenging.
    - Potential Solution: Additional research that supports more efficient transformation processes across crop species and varieties.

Long-term Milestone: Engineer entire phytobiomes (including crop, leaf microbiome, soil microbiome) for optimal resilience and yield.

- Bottleneck: Difficult to provide agronomically-feasible, durable spatiotemporal control of engineered microbiomes.
    - Potential Solution: Identify new strategies for rapid, non-invasive induction of genetic pathways across organisms.

Breakthrough Capability: Engineer foods and biomaterials to detect, reduce, and prevent spoilage.

Short-term Milestone: Enable monitoring of early signs of food spoilage using cell-based or cell-free biosensor systems.

- Bottleneck: Identity of key biochemical molecules responsible for causing food spoilage along different points of the supply chain.
    - Potential Solution: Develop high-throughput methods to identify spoiling agents (e.g., metabolites) in commonly consumed food products.
    - Potential Solution: Develop biobased trackers (e.g., DNA barcoding) to enable tracking of food along the supply chain and identify timepoints where food spoilage is more likely to occur.
- Bottleneck: Biosensors for detecting food spoilage need to be more specific, sensitive, reproducible, and easy-to-read.
    - Potential Solution: Engineer and test food-safe biorecognition molecules (e.g., synthetic enzymes, aptamers) targeting key molecular indicators of food spoilage (e.g., mycotoxins).
    - Potential Solution: Couple biosensors to food-safe colorimetric or electrochemical reporters.
- Bottleneck: Need to develop food-safe biosensors that can be applied on food or food packaging.
    - Potential Solution: Develop consumable and/or washable biomaterials (e.g., hydrogels, paper, silk) embedded with food-safe biosensors (e.g., synthetic nucleic acid-based sensors).

Short-term Milestone: Develop biomaterials or biobased coatings (e.g., cyclodextrin-containing) that inhibit molecules responsible for spoilage.

- Bottleneck: Current biomaterial technologies do not contain the necessary dynamics or complexity.
    - Potential Solution: Incorporate microbes that naturally inhibit the growth of spoilage bacteria and fungi.
    - Potential Solution: Discover and expand the collection of enzymes and small molecules that protect food against spoilage.

Medium-term Milestone: Engineer fruits and vegetables which are less susceptible to spoilage pathogens.

- Bottleneck: Foods can lose natural defenses against pathogens once harvested; these defenses need to be replicated for post-harvest foods.
    - Potential Solution: Engineer food microbiomes to produce preservatives in response to specific environmental signals (e.g., time, temperature, pH).

Medium-term Milestone: Engineer fruits and vegetables that can ripen on demand (e.g., controlled ethylene production).

- Bottleneck: It remains unclear how certain plant hormones, such as salicylic acid and jasmonic acid, are able to regulate plant ripening and drive ethylene production.
    - Potential Solution: Improve strategies for quantifying multiple plant hormones, measuring gene expression, and tying both to phenotypic outcomes.
    - Potential Solution: Develop and improve strategies to non-destructively measure plant hormone concentrations.

Transportation & Energy

Introduction and Impact: Together, transportation and energy production account for the vast majority of harmful greenhouse gas (GHG) production, including well more than half of the world's CO_2 production (data from https://www.climatetrace.org/explore). This roadmap's Transportation & Energy theme addresses engineering biology opportunities to shift these sectors towards sustainable, renewable fuels and energy sources, while also highlighting opportunities that will improve efficiencies during the transition to carbon-negative sources of energy [Figure 6].

Figure 6. Improving efficiency and sustainability in transportation and energy with engineering biology. While biofuels are one of the most recognizable uses of engineering biology, many other opportunities exist for biotechnology to reduce the impact of the energy and transportation sectors on climate change and to contribute to long-term sustainability. Engineering biology could revolutionize energy storage technologies, turning us away from environmentally-hazardous, and economically unsustainable, rare-earth batteries and enabling a "green" solution to storage of excess electricity from wind and solar. Advancements in microbial fuel cell technology could also enable wide-scale generation of electricity from biological systems. Progress is needed to increase the energy-density of fuel from biological feedstocks and to better capture and utilize organismal electron-transfer. For a different approach at reducing energy use, transportation efficiency could be improved with biomaterials and bio-derived coatings, such as biocoatings for ship hulls to reduce fouling and barnacle build-up, reducing friction and fuel utilization.

While there are existing solutions for carbon-free transportation (i.e., electric vehicles), engineering biology could play a key part in decarbonizing aviation, marine shipping, and heavy duty transport by enabling the production of low-emissions, energy-dense biofuels including sustainable aviation fuels (SAF) (Reinders, 2022; Tan et al., 2021). These fuels are made from biomass or other feedstocks (including carbon oxides, see Lanzajet, 2022) for use as "drop-in" fuels for incumbent engines (Balcombe et al., 2019). Assessments of the sustainability of such biofuels should incorporate: i) the potential for bioenergy feedstocks to compete with food crops for land and water use and ii) the capacity to make fuels sufficiently energy-dense, such that they are economical. This roadmap includes improving carbon utilization of feedstocks and enabling fuel production in closed-loop systems where all available carbon is captured. Another opportunity is to leverage advancements in biomaterials for surfaces and coatings that reduce friction and improve efficiency, reducing subsequent fuel expenditure.

Electricity generation is the second leading contributor to GHG production in the United States (EPA, 2015). As we turn to more renewable energy sources — such as wind, solar, and even the production of electricity from biology — sustainable energy storage technologies are becoming increasingly important. Advancements in engineering biology can enable bio-batteries and biobased fuel cells that are far more sustainable and climate-friendly than nearly all current battery technologies. This roadmap also addresses biotechnology opportunities to convert excess electricity produced by renewable resources at times of low demand into other value-added chemicals, materials, and products.

Energy in *Engineering Biology*

EBRC's 2019 roadmap, *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy*, includes approaches to advance biotechnology for transportation fuels, and other energy sources, including electricity {see https://roadmap.ebrc.org/2019-roadmap/sectors/energy/}. These primarily focus on efficiency of feedstocks and biomass for energy production and efforts to reduce global energy consumption, such as by developing bioprocesses to obtain energy from currently untapped sources.

TRANSPORTATION & ENERGY

Goal	Breakthrough Capability	Milestone

Reduce emissions from aviation, shipping, and heavy-duty transportation.

Enable the production of energy-dense biofuels from renewable feedstocks.

Short-term	Medium-term	Long-term
Improve the yield of bioenergy feedstocks (e.g., switchgrass, sorghum, miscanthus) on marginal lands.	Develop microbes and enzymes to degrade lignocellulose more efficiently to create economically-viable biofuels at scale for aviation and maritime industries.	Engineer microbiomes capable of distributed metabolism to capture all available carbon in a system (closed-loop production).

Enable the production of bio-coatings and biomaterials to improve transportation efficiency.

Short-term	Medium-term	Long-term
Develop non-toxic biobased coating or materials to prevent biofouling of ship hulls and reduce friction.	Engineer bio-components (strains, enzymes, or nucleic acids) that form biocoatings with increased lifetimes to reduce application frequency.	Develop novel, self-repairing microbial or cell-free biocoatings that minimize shear stress on land transportation vehicles, to reduce friction.
	Develop novel, self-repairing microbial or cell-free biocoatings for ship hulls.	

Enhance storage and generation of electricity from renewable resources.

Enable electricity production by engineered biological systems.

Short-term	Medium-term	Long-term
Improve microbial fuel cells to enhance electricity storage and generation.	Engineer efficient metabolic and microbial electron transfer pathways to funnel biological reducing cofactors to biocathode, biofilms, or nanowires.	Produce and maintain stable, large scale "microbial batteries" with high capacity.
Engineer biological electron transfer pathways (either in microbial or cell-free systems) that can efficiently interconvert electrons with biological reducing cofactors (e.g., NAD(P)H).	Develop a versatile microbial (or cell-free) electricity generation module that can efficiently couple any electrochemical oxidation reaction into electricity generation.	

Enable biological systems to store and utilize excess electricity generated by (intermittent) renewable energy sources.

Short-term	Medium-term	Long-term
Identify, understand, and mitigate rate limiting steps in microbial extracellular electron transfer (EET).	Demonstrate cost-effective microbial electrosynthesis (MES) systems that convert renewable electricity and CO_2 into C1 (e.g., methane) or multi-carbon molecules (e.g., alcohols).	Develop biological electrosynthesis systems that can be directly connected to the power grid.
	Enable biological systems to produce hydrogen from renewable resources.	

Short-term → Medium-term → Long-term

Goal: Reduce emissions from aviation, shipping, and heavy-duty transportation.

Current State-of-the-Art: Much of our transportation of goods and people currently relies on fossil fuels and, in 2020, transportation contributed to nearly 30% of greenhouse gas emissions in the United States (EPA, 2015). Recent actions by the Biden Administration in the U.S. federal government have highlighted the issue and put forth recommendations and commitments towards advancing sustainable aviation fuels (The White House, 2021).

There are several examples of energy dense molecules produced by engineered microbes that could be used as sustainable biofuels for aviation, marine, or heavy duty transportation, including isoprenoids farnesene or bisabolene (Liu et al., 2018), isobutene (Van Leeuwen et al., 2012), methyl ketones (Goh et al., 2012), or polycyclopropanated fuels (Cruz-Morales et al., 2022). There have been a few reported successful tests of marine biofuels, but the design of marine engines has limited this expansion (Tanzer et al., 2019). Tests that have been conducted on lignocellulosic biofuels include a soy biodiesel used by the Great Lakes Environmental Research Laboratory (Great Lakes Environmental Research Laboratory, 2017), a blended fuel that includes a sugar-based biodiesel provided by Amyris and tested by the US Maritime Administration (Risley and Saccani, 2013), and a wood residue-derived biofuel (UPM Biofuels, 2016).

More research is needed to improve carbon utilization from feedstocks (e.g., sugars, carbon dioxide, organic acids). For example, research should be undertaken to develop engineering biology approaches to more efficiently degrade lignocellulose, to improve carbon utilization and decrease processing time. Engineered ecological succession – creating a microbiome that progresses through different feedstocks – could help capture all available carbon in a system; such a system might include microbes that fix or degrade carbon dioxide or lignocellulose to sugars, combined with microbes that can ferment acids produced into other valuable compounds. Mixotrophic systems that co-consume sugars and gas have been shown to increase the carbon yield (Jones et al., 2016), and integrated bioprocesses have been shown to couple CO_2 conversion to acetate with acetate conversion to higher density molecules such as lipids or isoprenoids (Hu et al., 2016).

Making transportation vehicles travel more efficiently is another way to lower emissions from the shipping and transportation sector. This could be achieved in part by reducing dynamic friction on vehicle surfaces. Biomaterials, such as biofilms or biomolecular/cell-free biocoatings, could be used to cover surfaces and reduce friction or shear stress, and even provide a level of protection (efforts are already underway to achieve such technologies, see for example the DARPA Arcadia program). These biomaterials could be developed to contain antimicrobial compounds (e.g., antimicrobial peptides, antibiotics, anti-quorum sensing) to prevent fouling (e.g., barnacles on ships), physically modify surfaces to decrease bacterial attachment sites and prevent bacterial adhesion (Dang & Lovell, 2015), or to degrade bacterial holdfast structures to prevent “primary surface colonizers” from attaching and starting the biofilm formation process.

Breakthrough Capability: Enable the production of energy-dense biofuels from renewable feedstocks.

Short-term Milestone: Improve the yield of bioenergy feedstocks (e.g., switchgrass, sorghum, miscanthus) on marginal lands.

- Bottleneck: Most feedstock crops are not currently tolerant to environmental stressors that can exist in marginal lands, such as high-salinity or aridity.
  - Potential Solution: Engineer rhizobacteria to better support growth of bioenergy crops in marginal lands (e.g., nitrogen fixation, water retention).
  - Potential Solution: Develop feedstocks with genetic characteristics that enable tolerance to drought, high salinity, or high/low pH levels.
  - Potential Solution: Domesticate natural microbial communities that degrade lignocellulose (e.g., herbivore and insect gut microbiomes from wetlands/swamps).

Medium-term Milestone: Develop microbes and enzymes to degrade lignocellulose more efficiently to create economically-viable biofuels at scale for aviation and maritime industries.

- Bottleneck: Pretreating lignocellulosic biomass for downstream processing is costly, water-intensive, and energy-intensive.
  - Potential Solution: Select and engineer fast-growing, robust microbial strains for highly efficient lignin digestion, without consuming cellulose or hemicellulose.
  - Potential Solution: Enable enzyme production *in situ* by engineering microbial communities to process inhibitors found in crude plant feedstocks.
- Bottleneck: Enzymatic cocktails for digesting lignocellulose are too expensive to be commercially viable.
  - Potential Solution: Design multifunctional enzymes that can hydrolyze both cellulose and hemicellulose.
  - Potential Solution: Develop bioenergy crops with tailored lignin composition to optimize subsequent enzymatic deconstruction.
  - Potential Solution: Enable the production of desired enzymatic mixture from a single microbial strain.

Long-term Milestone: Engineer microbiomes capable of distributed metabolism to capture all available carbon in a system (closed-loop production).

- Bottleneck: The substrates in any given system are too diverse to ensure that needed microbiome community members are spatially and temporally present at sufficient levels to capture all source carbon.
  - Potential Solution: Improve computational microbiome design and subsequent high-throughput characterization of metabolic activity.

Breakthrough Capability: Enable the production of bio-coatings and biomaterials to improve transportation efficiency.

Short-term Milestone: Develop non-toxic biobased coating or materials to prevent biofouling of ship hulls and reduce friction.

- Bottleneck: Coating biomaterials need to be anti-corrosive, molluscicidal, anti-quorum-sensing, and/or have hydrophobic properties.
  - Potential Solution: Collect data about the interactions between potential biomaterials and biofouling species to tune material properties.

Medium-term Milestone: Engineer bio-components (strains, enzymes, ornucleic acids) that form biocoatings with increased lifetimes to reduce application frequency.

- Bottleneck: Challenges in creating dynamic materials with optimal physical properties that are robust to stress.
  - Potential Solution: Develop test-beds with adjustable and variable conditions for engineering biocoatings.

Medium-term Milestone: Develop novel, self-repairing microbial or cell-free biocoatings for ship hulls.

- Bottleneck: Challenges in creating materials that can sense *and* repair damage, while continuing to function as needed.

Long-term Milestone: Develop novel, self-repairing microbial or cell-free biocoatings that minimize shear stress on land transportation vehicles, to reduce friction.

- Bottleneck: Non-aqueous environments pose additional challenges for biobased solutions.

Goal: Enhance storage and generation of electricity from renewable resources.

Current State-of-the-Art: The accelerating pace at which renewable power generation capacity using wind and solar photovoltaics is being introduced presents a growing challenge: wind and solar-based power generation is inherently intermittent, necessitating that energy must be stored and delivered independently from generation. This represents a significant inefficiency in the power system, reduces revenue from renewable power generation facilities and ultimately slows the roll out of this sustainable infrastructure. The capacity of today's batteries is not suitable for the demand of the grid and batteries rely on the mining of rare earth metals, a process that is vastly detrimental to the environment (EARTH.ORG, 2020). Bio-powered energy storage represents an attractive alternative to this and can help to adapt and decentralize energy availability (Salimijazi et al., 2019). Biological systems are able to store excess energy in the form of polyhydroxyalkanoates (PHAs), glycogen, and triglycerides, and then use it on demand. Some biological systems are even able to generate electricity or hydrogen gas. The inherent process of microbes to oxidize organic materials and generate electricity has led to the development of microbial fuel cells; scale up of this technology could enable remote, persistent power sources (Kim et al., 2007). This goal aims to capture potential opportunities to enhance biological energy storage and generation in order to protect from the environmental and human health impacts of current, unsustainable energy sources.

Breakthrough Capability: Enable electricity production by engineered biological systems.

Short-term Milestone: Improve microbial fuel cells to enhance electricity storage and generation.

- Bottleneck: Current power-limiting factors of microbial fuel cells, such as biofouling and catalyst inactivation.
  - Potential Solution: Identify energy-efficient oxidation enzymes that do not involve oxygen as electron acceptor (e.g., alternative to methane monooxygenase (MMO) and/or RuBisCO).

Short-term Milestone: Engineer biological electron transfer pathways (either in microbial or cell-free systems) that can efficiently interconvert electrons with biological reducing cofactors (e.g., NAD(P)H).

- Bottleneck: The systems biology of electron transport systems is poorly resolved for engineering purposes (see Anand et al., 2022); participating proteins are known but their individual functions are not well-understood.
  - Potential Solution: Optimize electron transport pathways for engineering across relevant strains and systems, such that they can then be modified in concert with cofactors.

Medium-term Milestone: Engineer efficient metabolic and microbial electron transfer pathways to funnel biological reducing cofactors to biocathode, biofilms, or nanowires.

- Bottleneck: Instability/dynamic activity of biological fuel cell components.
  - Potential Solution: Adapt strains, enzymes, and/or nucleic acids to maintain or enhance function and maintain stability in fuel cell environments.

Medium-term Milestone: Develop a versatile microbial (or cell-free) electricity generation module that can efficiently couple any electrochemical oxidation reaction into electricity generation.

- Bottleneck: Extracellular electron transfer regulation mechanisms are poorly understood and highly dependent on organisms of interest.
  - Potential Solution: Metabolic flow analysis of different proteins on relevant microbes, using knockout systems and other genetic engineering tools (Sydow et al., 2014).

Long-term Milestone: Produce and maintain stable, large scale "microbial batteries" with high capacity.

Breakthrough Capability: Enable biological systems to store and utilize excess electricity generated by (intermittent) renewable energy sources.

Short-term Milestone: Identify, understand, and mitigate rate limiting steps in microbial extracellular electron transfer (EET).

- Bottleneck: A lack of screening methods hinders the discovery of novel electroactive microbes.
  - Potential Solution: Develop new high-throughput tools to screen for microbial EET activities.

    - Potential Solution: Engineer model chassis (e.g., *E. coli*) for implementation of extracellular electron transfer (EET) pathway.
- Bottleneck: Attempts to isolate electroactive microbes on non-selective media have led to the loss of electroactivity in selected microbes.
    - Potential Solution: Select microbes under anoxic environments, since most electroactive microbes are anaerobes (Bar-Even et al., 2010).

Medium-term Milestone: Demonstrate cost-effective microbial electrosynthesis (MES) systems that convert renewable electricity and CO_2 into C1 (e.g., methane; Jayathilake, 2022) or multi-carbon molecules (e.g., alcohols).

- Bottleneck: Currently available biocatalysts have small current densities (<100 mA/cm^2) that do not meet the requirements for industrial applications.
    - Potential Solution: Identify and engineer genetic circuits that control electron flux in electroactive microbes (e.g., *Shewanella oneidensis*) to increase current output.
- Bottleneck: Need to better understand the effects of operating parameters (e.g., pH, temperature, electrode potential) on the efficiency of MES systems.
    - Potential Solution: Measure MES system performance in a variety of operating environments, including under industrially relevant conditions.
- Bottleneck: Most MES studies focus only on acetate production (Scheffen et al., 2021), so there is a need to diversify products from MES systems.
    - Potential Solution: Map metabolic pathways in electroactive microbes to identify new pathways to synthesize multi-carbon molecules.
    - Potential Solution: Develop coupled systems that allow for efficient upgrading of acetate to a range of multi-carbon products via a secondary engineered system (Hu et al., 2016).

Medium-term Milestone: Enable biological systems to produce hydrogen from renewable resources.

- Bottleneck: High manufacturing costs of microbial electrolysis cells, their high internal resistance and methanogenesis, and membrane/cathode biofouling.
- Bottleneck: Efficient hydrogen evolution enzymes (e.g., hydrogenase, hydrogen lyase, etc.) need to be identified and engineered.

Long-term Milestones: Develop biological electrosynthesis systems that can be directly connected to the power grid.

Materials Production & Industrial Processes

Introduction and Impact: The manufacturing of materials and products, including plastics, textiles, cement and other building materials consumes large amounts of energy and is a substantial source of global greenhouse gas (GHG) emissions (International Energy Agency, 2020; EPA, 2015). This roadmap's Materials Production & Industrial Processes theme focuses on how to replace some of today's most energy-, resource-, and emissions-intensive — and environmentally-damaging — materials with sustainable, biobased alternatives [Figure 7]. One important approach is to embrace a circular bioeconomy: the current global economy is largely linear, meaning that consumables are mass produced, used, and then disposed of; in a circular economy, products at the end of their life cycles become the inputs for a new generation of materials or products. Utilizing engineering biology to degrade materials and waste and recycle their components and generate new, value-added products will help build opportunities for decreasing the emissions associated with industrial processes and taking an important step toward a sustainable future.

Some industrial processes, such as the production of building materials, are especially challenging to decarbonize. This roadmap identifies ways in which engineering biology can facilitate the production of sustainable building materials, reducing the amount of embodied carbon in the built environment. Cement production, iron and steel production, and chemical manufacturing are the three highest CO_2-emitting industrial sectors (Gross, 2021). These sectors are notoriously difficult to decarbonize because they rely on high-heat processes that cannot currently be electrified, and they also emit CO_2 as part of the fundamental stoichiometry of the process. Opportunities for engineering biology include enabling low-carbon, self-repairing bioconcrete, engineering trees for the production of high-density wood for a larger variety of construction applications, and scaling up the production of sustainable biobased wall materials, such as mycelium-based thermal insulation.

This roadmap also aims to advance solutions in biobased alternatives to replace fossil fuel-derived plastics. Globally, around 400 million tons of plastic are produced every year, emitting GHGs at every stage of their production (United Nations Environment Programme, 2022b), and less than 10% of all plastics are subsequently recycled (United Nations Environment Programme, 2021). Climate emissions reductions from bioplastics can be achieved primarily via two means: first, the carbon in bioplastics can be captured and fixed from the atmosphere, as opposed to being manufactured from fossil fuels; second, bioplastics can be produced with lower impacts (emissions), ensuring that the benefits of the biogenic carbon are realized. Additionally, bioplastics are much more likely to be fully degradable, helping to eliminate persistent waste, and accelerating recycling. Most bioplastics are used in packaging, but they are finding their way into everything from textiles to pharmaceuticals to electronics. Research continues to push the bounds of feedstocks, formulations, and applications. To address this, this roadmap highlights opportunities for replacing fossil fuel-derived plastics with bioplastics, improving the performance and cost of biobased packaging materials so that they may fully replace conventional plastic packaging, advancing biodegradation and bio-recycling processes, and advancing carbon-negative polymer and chemical manufacturing processes.

Figure 7. Engineering biology for materials production and industrial processes. Engineering biology can not only be used to create new material products such as coatings or packaging materials, but can also help to replace or increase recycling of existing materials, such as through the incorporation of biodegradable polymers. One of the most alluring attractions to biomaterials is the capacity for self-renewal or self-repair. Take, for example, bioconcrete: not only could this replace the carbon-intensive production of concrete we currently use, but dynamic biological activity could be imbued to enable self-healing repair of cracks or stress-damage. Engineered enzymes and organisms can be used to recycle materials and accelerate the processing of – or even eliminate – waste streams. Biomanufacturing and bioprocessing can help to produce fewer environmentally-damaging or -toxic materials like plastic polymers and textile dyes. Further, engineering biology has the potential to enable greater resource recovery, such as the extraction of valuable minerals and metals from waste streams or production of numerous commodity chemicals from a single biofermentation process. Advancements will be needed in engineering enzymes, predicting and designing biomaterial dynamics, and engineering persistence and containment of engineered organisms or biosystems in open and semi-open environments.

This roadmap also remarks on reducing the environmental footprint of the textile industry through engineering biology. It has been estimated that the textile industry is responsible for 8-10% of global GHG emissions, in addition to consuming massive amounts of water and generating chemical waste (Niinimäki et al., 2020). There is the potential for engineering biology to enable textiles and dyes that are more affordable, sustainable, and better-performing than existing products. Opportunities include engineering organisms to produce long-lasting bio-pigments and scaling up the production of biomaterials like engineered spider silk and mycelium-based leather alternatives.

Finally, with the increasing production and use of electronics and the associated economic prioritization of products containing rare-earth elements, the capacity to recover these materials is becoming more and more important. This roadmap considers opportunities to use engineering biology to extract and purify metals and ores from natural environments, such as current mining sites, and for recovering minerals and metals from waste-streams. Adoption of such technologies has the potential to reduce the environmental damage associated with traditional mining, such as ecosystem disruption and the production of toxic waste streams, and provide more secure and sustainable supply chains.

Biomaterials in *Engineering Biology & Materials Science*

EBRC's *Materials Science & Engineering Biology: A Research Roadmap for Interdisciplinary Innovation*, published in 2021, highlights tools, technologies, and processes for creating and enabling advanced materials using engineering biology {see: https://roadmap.ebrc.org/2021-roadmap-materials/}. *Engineering Biology & Materials Science* contains technical milestones and achievements directly related to many of the goals found in this roadmap, including production of environmentally-friendly materials, such as bioconcrete, and sustainable manufacturing practices, including utilization of waste streams.

MATERIALS PRODUCTION & INDUSTRIAL PROCESSES

Goal	Breakthrough Capability	Milestone

Reduce the embodied carbon in the built environment.

Enable the production of biocement and bioconcrete.

Short-term	Medium-term	Long-term
Enable scale-up of microbially-induced calcium carbonate precipitation (MICP) for biocement production and self-repair.	Engineer a greater number of environmentally-robust microbial species or consortia to biomineralize and deposit calcium carbonate.	Enable the production of robust bioconcrete from biocement that can withstand environmental stress.
Improve curing time of biodeposited calcium carbonate for biocement production and/or crack healing.		Engineer self-repairing biocement that can be used at scale.

Enable the production of sustainable, non-concrete building materials.

Short-term	Medium-term	Long-term
Develop biopolymers and bio-derived polymers to function as construction binders (such as glues, sealants).	Develop sustainable, biobased phase-change materials (PCM) for temperature regulation to reduce energy consumption in buildings.	Engineer trees to produce denser and fire resistant lumber for constructing high-rise buildings.
Design and engineer mycelium materials for construction, such as insulation materials.		Engineer paintable biocoatings with tunable albedo to optimize solar gain of buildings, or with radiation-resistant organisms, such as *Deinococcus radiodurans*.
Engineer biofilms, biomaterials, or biocoatings for built surfaces to capture CO_2, such as by functionalizing these materials with metal-organic framework compounds.	Engineer microbial consortia or other organisms to produce cellulose and similar polymers that could be used to repair woody materials.	Enable the wide-spread construction and adoption of living-architecture, for urban air and water filtration and purification.

Enable sustainable biobased production of plastics and chemicals.

Enable the at-scale production of biobased and biodegradable polymers for industrial purposes.		
Develop novel biosynthesis processes that use biomass feedstock to make commodity monomers and polymer precursors, such as 1,6-hexane-diamine (a precursor to nylon), lactic acid (a precursor to polylactic acid), and adipic acid.	Develop processes for commodity bioplastic synthesis from a wide range of feedstocks, such as industrial or agricultural waste.	Establish industry processes that maximize the circularity of bioplastics.
	Develop biopolymers that will fully degrade under designated environmental conditions, such as at specific temperatures or pH, or when in contact with salt water.	Imbue biofilms with tunable and responsive plastic-like material properties.
	Enable design and production of novel biopolymer materials for replacement of existing, non-degradable materials.	

Produce commodity chemicals by upcycling waste streams via bioprocessing.		
Engineer organisms or cell-free systems to make degradable bioplastics, such as polyhydroxyalkanoate (PHA), from waste plastics.	Engineer organisms or cell-free systems to make value-added (non-plastic) chemicals from waste plastics.	Enable engineered organisms or cell-free systems that consistently and predictably convert plastic waste to bioplastics and value-added chemicals at industrial scale.
	Develop biobased processes to make value-added chemicals from industrial chemical waste streams.	

Enable sustainable production of biomaterials for the textiles industry.

Industrial-scale production of sustainable textile dyes and pigments.		
Discover and develop microbial metabolites and plant biosynthetic gene clusters (BGCs) to widen the range of biopigments.	Engineer microbes to produce dyes with comparable or better color stability and brightness than synthetic dyes.	Introduce color by engineering physical attributes into biomaterials (i.e., fabrics that can change color in response or on demand).
	Enable commercial-scale production of sustainable biobased textile dyes.	
Short-term	**Medium-term**	**Long-term**

Commercial-scale production of sustainable biofabricated textiles.		
Enable the sustainable biosynthesis of biopolymer alternatives to synthetic fibers (e.g., polyester, nylon, and acrylic). Enable industrial-scale, sustainable fermentation or growth and processing of currently available biomaterials (e.g., mycelium, hemp, lyocell) at scale.	Enable industrial-scale production of biosynthetic spider silk fibers to make textile fabrics.	Design of new protein-, carbohydrate-, or lipid-based and hybrid materials that outperform synthetic fibers.

Enable resource recovery through biomining.

Mine and extract resources from the natural environment using engineering biology.		
Engineer organisms with key traits and tolerances for high-efficiency biomining.	Engineer organisms for biomining low-grade and complex ores.	Investigate and characterize bio-respiration as a form of metal extraction, as opposed to bioleaching.
Recover mineral and metal resources from waste using engineering biology.		
Design and construct biosorption-based, flow-through processes for continuous separation and recovery of valuable minerals from waste streams.	Develop engineered microbial or cell-free systems to recover valuable metals from electronic waste. Integrate biosorption into existing mineral recovery systems to enable sustainable and cost-effective co-extraction.	Diversify the global rare earth element (REE) supply through engineering biological systems (e.g., bioleaching, biosorption).
Short-term	**Medium-term**	**Long-term**

Goal: Reduce the embodied carbon in the built environment.

Current State-of-the-Art: The manufacturing of building materials is energy and carbon intensive - steel and cement productions already account for roughly 13% of global GHG emissions and are projected to grow in the coming decades (Fennell et al., 2022). Engineering biology could enable the production of sustainable building materials to significantly reduce the amount of energy and emissions required of the built environment. By engineering biocementing bacteria to grow faster and at-scale, it may be possible to eventually replace building materials like bricks and concrete with biobased alternatives, which would consume much less energy and lower emissions. To achieve this, more research is needed to increase the growth rate and 'curing' time of the primary biomass-generating microbe in microbial concretes and to improve the structural, load-bearing properties of biocement by enabling the engineering of crystalline structure of biocement. Bacteria capable of biocementation have already been used to repair cracks in concrete structures (Zhang et al., 2017a). To increase the efficiency and scale of biocementation, we need to develop a more advanced understanding on biodeposition of calcium carbonate, advance genetic toolbox of naturally occurring calcium carbonate depositors, and engineer and optimize biosynthetic carbonate deposition pathways. Similarly, microbes that secrete other compounds like iron could be used to make self-repairing surface coatings.

Biomaterials can also be engineered to attain physical and structural properties that enable them to replace existing carbon-intensive materials. For example, engineering biology could be used to make wood stronger, enabling its use in more structures as an alternative to materials that embody more carbon (Strain & FAIA, 2022). For example, trees could be engineered to produce denser wood for construction. Additionally, microbes could be engineered to produce cellulose and be used to repair and strengthen rotten wood. Mycelium-based products also have been successfully produced with desirable morphology and mechanical properties (Haneef, 2017; Ecovative, 2022). Mycelium biomaterials could be programmed to grow to sizes specified by boundaries in the built environment (*e.g.,* building walls or ceilings).

Biobased coatings that capture CO_2 can be applied to built surfaces to enhance carbon capture from the atmosphere (e.g., biofilms functionalized with CO_2 capturing particles such as metal-organic frameworks). Smart materials that can regulate moisture and temperature will help buildings and building residents adapt better to the changing climate. Potential technologies include phase change materials from organic fatty acid esters or protein-based materials that can store and release heat reversibly helping to keep indoor temperatures more consistent (Nazari et al., 2020). Buildings could even be outfitted with radiation-resistant biomaterials, such as materials that have incorporated *Deinococcus radiodurans* (Daly et al., 2007). Regardless of the biobased or bio-enabled material, biodegradability and recyclability should be considered; materials need to be designed for persistence during their functional period, but also with sustainable and climate-friendly reuse/recycling or degradation for end-of-life circumstances.

Breakthrough Capability: Enable the production of biocement and bioconcrete.

Short-term Milestone: Enable scale-up of microbially-induced calcium carbonate precipitation (MICP) (Castro-Alonso et al., 2019) for biocement production and self-repair.

- Bottleneck: If incorporated into the built environment, the harsh conditions (e.g., high pH, pressure, temperature, and nutrient deficiency) that microbes would be exposed to limits the scalability of MICP.
  - Potential Solution: Isolate or engineer new microbes capable of performing MICP and growing under harsh environmental conditions.
- Bottleneck: Production of toxic byproducts from MICP (e.g., ammonium, nitrous oxide) and the potential of uncontrolled microbial growth pose threats to the environment and human health.
  - Potential Solution: Engineer metabolic pathways to ensure the completion of biochemical reactions to limit the production of toxic byproducts.
  - Potential Solution: Engineer metabolic pathways to conditionally limit or stop microbial growth.
- Bottleneck: Urea, a primary feedstock for ureolytic MICP, is synthesized through energy- and carbon-intensive processes.
  - Potential Solution: Engineer microbes or consortia that can make urea from atmospheric nitrogen for use in ureolytic MICP.

Short-term Milestone: Improve curing time of biodeposited calcium carbonate for biocement production and/or crack healing.

- Bottleneck: Nucleation and crystallization can be influenced by deposition surface morphology, meaning that curing time may be a function of (limited by) the characteristics of the (damaged) deposition surface.
  - Potential Solution: Further investigation of conditions that elicit efficient and robust calcium carbonate deposition.

Medium-term Milestone: Engineer a greater number of environmentally-robust microbial species or consortia to biomineralize and deposit calcium carbonate.

- Bottleneck: More needs to be understood about which species are best suited for biocement and bioconcrete applications (particularly in harsher environments) and how to engineer consortia that can most efficiently be implemented.
  - Potential Solution: Engineer and optimize biosynthetic carbonate deposition pathways.

Long-term Milestone: Enable the production of robust bioconcrete from biocement that can withstand environmental stress.

- Bottleneck: Bioconcrete needs to meet construction standards (e.g., bioconcrete with compressive strength greater than 2500 psi).
  - Potential Solution: Characterize the materials properties of biocement and bioconcrete, including their durability, compressive strength, and fire resistance.

- Bottleneck: Biocement must be easily packaged, transported, and mixed (i.e., just add water).
  - Potential Solution: Develop biocement containing sporulating microbes and nutrients.

Long-term Milestone: Engineer self-repairing biocement that can be used at scale.

- Bottleneck: An engineered microbe, spore, or cell-free system has to be resilient to long periods of dormancy and become active in response to biocement damage.
  - Potential Solution: Optimization of microbial growth media and cement composition to support timely activation of repair in applicable environments.

Breakthrough Capability: Enable the production of sustainable, non-concrete building materials.

Short-term Milestone: Develop biopolymers and bio-derived polymers to function as construction binders (such as glues, sealants).

- Bottleneck: The stability of biopolymers in hybrid materials is a limiting factor.
  - Potential Solution: Design, develop, and test the relationship between genetic sequence and metabolic pathways and subsequent material mechanical properties of construction-relevant biopolymers.

Short-term Milestone: Design and engineer mycelium materials for construction, such as insulation materials.

- Bottleneck: Further study is needed to improve the physicochemical properties of mycelium composites, such as their compressive strength, density, and hydrophobicity.
  - Potential Solution: Engineer high-density and high-performance composites by incorporating biomineralizing microbes into mycelium-based materials.
  - Potential Solution: Experiment with genetic modification and changing growth conditions to improve hyphal density.

Short-term Milestone: Engineer biofilms, biomaterials, or biocoatings for built surfaces to capture CO_2, such as by functionalizing these materials with metal-organic framework compounds.

Medium-term Milestone: Develop sustainable, biobased phase-change materials (PCM) for temperature regulation to reduce energy consumption in buildings (Nazari et al., 2020; Naresh et al., 2020).

- Bottleneck: Currently available biobased PCM could undermine food security, as they are mainly sourced from food-grade fats and oils.
  - Potential Solution: Enable the manufacturing of biobased PCM from biowaste (e.g., discarded cooking oil).
  - Potential Solution: Design and engineer bioreactions to produce fats and oils with suitable chemical and thermal properties to make biobased PCM.

Medium-term Milestone: Engineer microbial consortia or other organisms to produce cellulose and similar polymers that could be used to repair woody materials.

Long-term Milestone: Engineer trees to produce denser and fire resistant lumber for constructing high-rise buildings.

- Bottleneck: Wood density is limited by photosynthetic efficiency of trees.
    - Potential Solution: Improve photosynthesis in lumber-source trees by increasing light capture and carbon fixation efficiency.

Long-term Milestone: Engineer paintable biocoatings with tunable albedo to optimize solar gain of buildings, or with radiation-resistant organisms, such as *Deinococcus radiodurans*.

- Bottleneck: Enabling controlled dynamic activity and persistence in biomaterials for environmental applications, particularly because of inconsistent feedstocks and metabolic resources.

Long-term Milestone: Enable the wide-spread construction and adoption of living-architecture, for urban air and water filtration and purification (see for example Living Architecture, 2020).

Goal: Enable sustainable biobased production of plastics and chemicals.[7]

Current State-of-the-Art: While greenhouse gas (GHG) emissions from the production of chemicals, including plastics, is not as significant as some other sectors and industries, it is still hugely impactful on the climate. Chemical manufacturing, excluding ammonia production, directly emits about half a gigatonne of CO_2 globally each year, which is less than 2% of the global GHG emissions, but is the single largest industrial consumer of oil and gas (International Energy Agency, 2021). One area of research and development that has progressed rapidly, especially within the last 5-10 years, is bioprocesses for chemical production. Microbial engineering has the capacity to enable the production of a wide array of diverse chemicals and compounds, reducing the amount of resources and toxins that might otherwise be consumed or produced, respectively. Today, most molecules produced commercially with microbes are high-value specialty chemicals such as flavors and fragrances, cosmetic additives and pharmaceuticals with only a handful of examples of commodity chemicals including ethanol, 1,3-propanediol, 1,4-butanediol, isobutanol, farnesene, lactic acid and succinic (Jullesson et al., 2015). If the thousands of chemicals derived from petroleum and natural gas could be produced instead with microbes, the annual savings in GHG emissions would be substantial. Most products manufactured through engineering biology approaches today generally have lower emissions than conventional petrochemical counterparts (Adom et al, 2014) but are not been fully carbon neutral, in part because they rely on yeast or *E. coli* and yeast that emit substantial amounts of CO_2 waste in order to generate highly reduced products from oxidized starting materials, namely, sugars. They must be engineered to be circular or, for example, combined into distributed metabolism systems with autotrophic bacteria (Scown & Keasling, 2022).

[7] For similar, related concepts, see the **Mitigating Environmental Pollution** theme in this roadmap.

Once the feedstock, whether it is sugars or a gaseous input, is converted to a product, the ultimate use and disposal (or recycling) of that product is the key to whether it will sequester carbon or simply release CO_2 back to the atmosphere. Certain bioproducts do have greater potential to lock carbon away in a stable form in particular polymer materials. Additionally, the persistence of materials such as plastics in the environment, they result in significant contribution to pollution, environmental damage, and worsening climate change. Millions of metric tons of plastics are created each year and that production is expected to continue, if not grow. However, some sources suggest that up to 90% of plastics could come from plant-derived alternatives (Hottle et al., 2020). Biobased alternatives, and chemicals and "plastic" materials produced through bioprocesses (biobased methods), can significantly reduce this impact.

Not only would this reduce the amount of petrochemicals that go into plastic production, but would enable greater flexibility for biodegradation, including into other value-added compounds (see Washington, 2021 in example). One of the biggest current technical challenges to bioplastics is attaining the strength and other physical and mechanical properties of plastics already on the market. While processes exist to create polylactic acid (PLA) from corn and sugarcane, and polyhydroxyalkanoate (PHA) from algae, these polymers and subsequent compounds aren't sufficiently tunable, or capable of being produced at economically-advantageous scales and prices (Robbins, 2020). These bioplastic materials also need to have controlled biodegradation pathways engineered and adapted to the appropriate conditions and environments.

One area of research and development that has progressed rapidly, especially within the last 5-10 years, is bioprocesses for commodity chemical production. Microbial engineering has the capacity to enable the production of a wide array of diverse, high-value chemicals and compounds (Jullesson et al., 2015), reducing the amount of resources and toxins that might otherwise be consumed or produced, respectively. However, these processes still produce significant amounts of CO_2, so to be efficient and sustainable, they must be engineered to be circular or engineered for distributed metabolism.

In addition to removing pollutants from the environment, engineering biology could go one step further and upcycle pollutants by converting them into useful products (Cornwall, 2021). For example, by using bacteria to recycle electronic waste, convert plastic waste into other, value-added compounds, sustainably remediate and extract heavy metals from waste waters, and more (Kwok et al., 2019; Washington, 2021; Sun et al., 2020; Yang, 2020). Key challenges to this are complex and compound metabolic engineering, such as engineering microbiomes capable of distributed metabolism, and attaining circular, closed-loop bioprocessing.

Breakthrough Capability: Enable the at-scale production of biobased and biodegradable polymers for industrial purposes.

Short-term Milestone: Develop novel biosynthesis processes that use biomass feedstock to make commodity monomers and polymer precursors, such as 1,6-hexane-diamine (a precursor to nylon), lactic acid (a precursor to polylactic acid), and adipic acid.

- Bottleneck: Monomer synthesis pathways need to be expanded to a larger array of model and non-model organisms, particularly those in high abundance in sustainable biomass feedstocks.
    - Potential Solution: Use bioinformatic and protein engineering strategies to discover new biosynthetic routes for making monomer analogs.

Medium-term Milestone: Develop processes for commodity bioplastic synthesis from a wide range of feedstocks, such as industrial or agricultural waste.

- Bottleneck: Industrial and agricultural waste need additional pretreatment steps to release polysaccharides from lignin.
    - Potential Solution: Engineer plants with activatable, integrated lignocellulose degrading enzymes.
- Bottleneck: Optimizing bioprocesses and production of post-translationally modified biomaterials.
    - Potential Solution: Identify enzymes and strategies for making post-translational modifications to protein and carbohydrate biomaterials.

Medium-term Milestone: Develop biopolymers that will fully degrade under designated environmental conditions, such as at specific temperatures or pH, or when in contact with salt water.

- Bottleneck: Tradeoffs between durability and degradability of biopolymers.
    - Potential Solution: Embed structurally-durable biopolymers with enzymes that initiate degradation under desired conditions.

Medium-term Milestone: Enable design and production of novel biopolymer materials for replacement of existing, non-degradable materials.

- Bottleneck: Constraints of native protein sequence and design space when using natural amino acids.
    - Potential Solution: Increased use of non-natural amino acids and novel designed proteins for biopolymer materials.
- Bottleneck: Design space for carbohydrate and lipid based materials is more difficult because the functionalization and accessible design space for lipids/carbohydrates is dependent on proteins.
    - Potential Solution: Increase collection and accessibility to protein-lipid and protein-carbohydrate interaction and function data, to enable design of biomaterials that incorporate lipids and carbohydrates.

Long-term Milestone: Establish industry processes that maximize the circularity of bioplastics.

- Bottleneck: Composting and recycling facilities are currently not equipped to sort or process bioplastics.
    - Potential Solution: Develop biodegradation processes that minimize the need to retrofit existing composting or recycling infrastructure.

Long-term Milestone: Imbue biofilms with tunable and responsive plastic-like material properties.

- Bottleneck: Understanding interplay between sequence, structure, and function of proteins, specifically de-novo designed proteins, with respect to material properties.
    - Potential Solution: Improve computational infrastructure to increase use and integration of functional/biophysical data that feeds models.

Breakthrough Capability: Produce commodity chemicals by upcycling waste streams via bioprocessing.

Short-term Milestone: Engineer organisms or cell-free systems to make degradable bioplastics, such as polyhydroxyalkanoate (PHA), from waste plastics.

- Bottleneck: Fully polymerized plastics, most common in waste streams, are difficult for model microbes to metabolize.
    - Potential Solution: Identify and engineer depolymerization enzymes (e.g., PETase, cutinase) and implement these enzymes as part of a biobased up-cycling process.
    - Potential Solution: Identify and engineer fungi or microbes that can directly metabolize plastic polymers to make bioplastics.
- Bottleneck: Plastics with high molecular weight (e.g., polyethylene, including high- and low-density polyethylene) are harder to metabolize.
    - Potential Solution: Identify and characterize gene clusters and metabolic pathways that enable certain microbes (e.g., *Brevibacillus borstelensis*) to use polyethylene as a carbon source, and transfer these traits to chassis organisms.

Medium-term Milestone: Engineer organisms or cell-free systems to make value-added (non-plastic) chemicals from waste plastics.

- Bottleneck: Few metabolic pathways are known for the bioconversion of plastic waste into value-added chemicals, such as high-value small molecules.
    - Potential Solution: Characterize the metabolic products from microbes that feed on plastic waste to identify microbes and metabolic pathways capable of producing target chemicals (e.g., using biosensors).

Medium-term Milestone: Develop biobased processes to make value-added chemicals from industrial chemical waste streams.

- Bottleneck: Chemical waste streams have different environmental conditions and contain different waste compounds, thus one-size fits all approaches are not suitable.
    - Potential Solution: Enable chassis organisms to metabolize compounds from different chemical waste streams.
    - Potential Solution: Develop microbiome systems with distributed metabolism and multiple pathways to waste processing.
    - Potential Solution: Scale up cell-free technologies for biobased chemical production, such as enzyme optimization, from dedicated waste streams.

- Bottleneck: Need to improve biological separation processes (e.g., enhanced secretion systems) to simplify downstream waste separation processes.
    - Potential Solution: Re-engineer common cellular machinery (e.g., efflux pumps, organelles) to capture and segregate relevant waste substrates.

Long-term Milestone: Enable engineered organisms or cell-free systems that consistently and predictably convert plastic waste to bioplastics and value-added chemicals at industrial scale.

- Bottleneck: Bioplastics production and biobased plastic up-cycling suffer from low-yield and high-cost of feedstock.
    - Potential Solution: Identify fermentation practices and genetic traits that result in high yield from certain types of microbes, and investigate how these practices or traits can be adapted to other bioconversion processes.
- Bottleneck: Need to enable and ensure cell-free enzyme robustness and recycling for industrial processes.
    - Potential Solution: Design, directed evolution, and synthetic metagenomics screening of suitable enzymes.

Goal: Enable sustainable production of biomaterials for the textiles industry.

Current State-of-the-Art: Sustainability is a growing trend in the fashion industry, with the understanding that the use of biobased materials can help brands and companies reduce their carbon footprint and diversify their supply chains. Engineering biology brings the potential to use different feedstocks (including end-of-life material) to biofabricate valuable materials for the textile industry as dyes, polyesters, among other materials. [Please see (Biofabricate, 2021) as a valuable resource.]

A number of biotechnology companies are producing biomaterials and using bioprocessing for the textile industry, including Bolt Threads, Huue, and Spiber; these include mycelium-based leather like fabrics, biosynthetic indigo dye for denim, and engineered microbial fermentation of silk proteins, respectively. This bioproduction is primarily limited by the ability to scale, diversifying the feedstocks and organisms that contribute to production, and ensuring that the products and byproducts of the process are not harmful to the biological components inside and outside the system. Like with other biomaterials, physical properties of the precursors and products also need to be carefully tuned.

Breakthrough Capability: Industrial-scale production of sustainable textile dyes and pigments.

Short-term Milestone: Discover and develop microbial metabolites and plant biosynthetic gene clusters (BGCs) to widen the range of biopigments.

- Bottleneck: Plant genomes are inherently complex.
    - Potential Solution: Leverage metabolomics and other -omics technologies to discover and characterize the functions of plant BGCs.

- Bottleneck: Some pigment-producing microbes also produce toxins (e.g., mycotoxin) as a byproduct.
    - Potential Solution: Bioprospect for non-toxic strains.
    - Potential Solution: Characterize and engineer metabolic pathways of pigment-producing microbes to reduce toxin production.

Medium-term Milestone: Engineer microbes to produce dyes with comparable or better color stability and brightness than synthetic dyes.

- Bottleneck: The same pigment produced from different bacterial strains does not show the same color stability when applied to certain textile fibers.
    - Potential Solution: Better characterize the interactions between biobased dyes and different textile fibers.
- Bottleneck: Certain biopigments (e.g., red) have lower color stability.
    - Potential Solution: Engineer biochemical protection strategies for less stable biopigments.

Medium-term Milestone: Enable commercial-scale production of sustainable biobased textile dyes.

- Bottleneck: The inherent toxicity of many textile dyes limit the maximum titer for microbially-produced dyes.
    - Potential Solution: Engineer microbes that use compartmentalization, efflux, and other stress-resistance strategies.
    - Potential Solution: Engineer extremophiles for use in dye production.
- Bottleneck: Repeatability of biobased dyes.
    - Potential Solution: Develop standardized protocols for dyeing textiles with a given biobased dye.

Long-term Milestone: Introduce color by engineering physical attributes into biomaterials (i.e., fabrics that can change color in response or on demand).

- Bottleneck: Constraints of polymers that possess both the desired textile properties and color properties.
    - Potential Solution: Better understand properties of textile materials that are inherently pigmented to recreate/engineer those properties into other materials.

Breakthrough Capability: Commercial-scale production of sustainable biofabricated textiles.

Short-term Milestone: Enable the sustainable biosynthesis of biopolymer alternatives to synthetic fibers (e.g., polyester, nylon, and acrylic).

- Bottleneck: Feedstocks for many biosynthetic fibers (e.g., polylactic acid) compete with food crops.
    - Potential Solution: Develop processes to make biosynthetic fibers that use waste or non-food biomass as feedstocks.
- Bottleneck: Due to its relative novelty, environmental and economic assessments on biosynthetic fibers are limited.

  - Potential Solution: Develop comprehensive TEA and LCA for biobased textile alternatives to identify points in biopolymer production that are carbon and energy intensive.

Short-term Milestone: Enable industrial-scale, sustainable fermentation or growth and processing of currently available biomaterials (e.g., mycelium, hemp, lyocell) at scale.

- Bottleneck: Inefficient, or undeveloped, bioprocessing for fiber removal, softening, etc.

Medium-term Milestone: Enable industrial-scale production of biosynthetic spider silk fibers to make textile fabrics.

- Bottleneck: Unlike natural spider silk, biosynthetic spider silk proteins (spidroin) need to be first spun into fibers to make fabric.
  - Potential Solution: Engineer host systems to produce spidroin in an environment that mimics the silk gland of spiders.
- Bottleneck: Spidroins produced by chassis organisms like *E.coli* and yeast are smaller and weaker than native spidroins.
  - Potential Solution: Extensively characterize and engineer the metabolic pathways responsible for spidroin production in chassis organisms to maximize spidroin size.

Long-term Milestone: Design of new protein-, carbohydrate-, or lipid-based and hybrid materials that outperform synthetic fibers.

- Bottleneck: Currently accessible protein and carbohydrate design space is limited.
  - Potential Solution: Could be alleviated by incorporation of unnatural amino acids; need new control for design of new/complex carbohydrates.

Goal: Enable resource recovery through biomining.[8]

Current State-of-the-Art: Current mining processes for heavy metals and the growing abundance of electronic waste opens a path for biology to enable more sustainable and environmentally-friendly capture of resources such as rare earth elements. The recent discovery that numerous environmental strains use rare earth elements as cofactors to alcohol dehydrogenases and naturally bioaccumulate rare earth elements, have opened an emergent area to design bacterial platforms for recovery of these critical metals (Skovran et al., 2019). Engineered microbes and cell-free systems can, for example, be used to rapidly detect heavy metals in the environment, sequester heavy metal wastes from many different sources, and process or recycle metals through accumulation and mineralization (see Bereza-Malcolm et al., 2015, Kachieng'a & Unuofin, 2021, and Giachino et al., 2021, respectively). Biomining occurs through the processes of bioleaching, in which the microbes solubilize the metal of interest, and biooxidation, in which a mineral sulfide matrix is oxidized to extract the metal of interest (Gumulya et al., 2018).

[8] For more about enabling engineering biology for the biosequestration of heavy metals, please see **Goal: Mitigate targeted environmental pollutants through biosequestion and biodegradation.**

The biggest challenges for this technology, particularly for achieving these processes at scale, is toxicity and how little we know about the (metabolic) regulatory processes of bioaccumulation and bioleaching. Many species are able to naturally utilize rare earth elements, but our understanding of where and how these organisms function is quite limited; bioinformatics approaches will be especially helpful in identifying strains able to chelate and bioaccumulate rare earth elements naturally (or are particularly amenable to engineering for such capacity). Approaches have been taken to overcome toxicity by engineering microbes with amplification circuitry to detect hazardous metals in low concentrations from diffuse sources (Cai et al., 2022), use cell-free systems more tolerant to harsh environments (Beabout et al., 2021), and by engineering environmental strains for biomining (Gumulya et al., 2018). Often these materials are found in complex mixtures and sources, and systems will need to be engineered to most efficiently process materials from other contaminants (Han et al., 2022).

Breakthrough Capability: Mine and extract resources from the natural environment using engineering biology.

Short-term Milestone: Engineer organisms with key traits and tolerances for high-efficiency biomining.

- Bottleneck: Conventional engineering biology hosts (e.g., *E. coli*) lack key traits and mechanisms for use in industrial mining applications, such as the ability to tolerate low pH, high temperatures, and high ionic concentrations.
    - Potential Solution: Identify organisms from relevant environments to serve as new chassis, taking advantage of microbes that naturally chelate rare earth elements in soils.
    - Potential Solution: Engineer model hosts with lanthanophores (Cotruvo, 2019).
- Bottleneck: Low transformation and selection efficiency of engineered biomining microbes with desired traits.
    - Potential Solution: Develop antibiotics that work efficiently in the growth media of the targeted strain.
    - Potential Solution: Develop nutritional-based selection mechanisms (e.g., new metabolic capability or complemetation of an auxotrophy).

Medium-term Milestone: Engineer organisms for biomining low-grade and complex ores.

- Bottleneck: Low-grade and complex ores contain high concentrations of metal and high levels of metal impurities.
    - Potential Solution: Increase metal resistance in organisms by engineering and evolving transport proteins and/or metal binding sites.
    - Potential Solution: Engineer hybrid microbial consortia that contain engineered strains capable of efficient metal bioaccumulation, to reduce metal concentrations for other biomining microbes in the consortium.
- Bottleneck: Valuable minerals (e.g., rare-earth metals) exist in low concentrations in low-grade and complex ores.

- Potential Solution: Identify new lanthanophores in different environmental strains and express heterologously in current bacterial platforms (Zytnick et al., 2022).
- Potential Solution: Identify regulatory mechanisms limiting rare earth transport in methylotrophs to enhance rare earth bioaccumulation and biomineralization to promote these strains as biorecovery platforms (Roszczenko-Jasińska et al., 2020).
- Potential Solution: Develop engineered strains that express proteins with high affinity for target metals.
- Potential Solution: Use protein design and engineering to synthesize enzymes with binding sites that selectively target desired minerals (e.g., rare-earth metals).
- Potential Solution: Develop new biosorption technologies, e.g., bacterial phages, to concentrate and separate rare-earth elements (REEs) from non-REEs.

Long-term Milestone: Investigate and characterize bio-respiration as a form of metal extraction, as opposed to bioleaching.

- Bottleneck: Some metals in their oxidized state form insoluble oxide minerals, which can have value themselves, or help sequester other metals of value.
  - Potential Solution: Extracellular electron transfer (EET) is a respiratory process that enables bacteria to reduce insoluble substrates; simple pathways (e.g., from *Shewanella oneidensis*) can be expressed heterologously, but more sophisticated EET pathways (e.g., from *Geobacter sulfurreducens*) have not yet been reconstructed.
  - Potential Solution: Identify new, genetically tractable microbes capable of EET that tolerate and/or thrive in extremophile conditions.

Breakthrough Capability: Recover mineral and metal resources from waste using engineering biology.

Short-term Milestone: Design and construct biosorption-based, flow-through processes for continuous separation and recovery of valuable minerals from waste streams.

- Bottleneck: Single-use adsorbents increase cost; treating depleted adsorbents could also lead to further environmental pollution.
  - Potential Solution: Develop reusable biosorbents (cell-, peptide-, protein-based, lipid, or phage) to selectively concentrate and recover mineral/metal resources from waste streams, such as by immobilizing biosorbents to fixed-bed columns.

Medium-term Milestone: Develop engineered microbial or cell-free systems to recover valuable metals from electronic waste.

- Bottleneck: Electronic waste contains high levels of toxic metals that reduces or inhibits growth of potential bacterial platforms for recovery.

    - Potential Solution: Increase heavy metal tolerance by genetic engineering or experimental evolution of microbes under appropriate selective pressure conditions.
- Bottleneck: Elemental composition of electronic waste is highly variable.
    - Potential Solution: Develop recovery processes at neutral pH that rely on specific metal ligands (siderophore-like molecules) instead of non-specific leaching processes that promote the solubility of toxic metals.
    - Potential Solution: Develop co-cultures or consortia to synergize recovery.

Medium-term Milestone: Integrate biosorption into existing mineral recovery systems to enable sustainable and cost-effective co-extraction.

- Bottleneck: Differentiation among rare earth elements is chemically challenging.
    - Potential Solution: Investigate the mechanism that environmental bacterial strains use to differentiate light from heavy lanthanides for integration into current microbial platforms used for recovery (Good et al., 2022).
    - Potential Solution: Engineer and construct biosorbents for separating different kinds of rare earth elements.
- Bottleneck: Biosorption and bioleaching may not be significantly economical or sustainable with just the incorporation of a biological processing component.
    - Potential Solution: Engineer microbes to reuse chemicals (e.g., residual sulfuric or nitric acids) for leaching minerals/metals from waste materials.

Long-term Milestone: Diversify the global rare earth element (REE) supply through engineering biological systems (e.g., bioleaching, biosorption).

- Bottleneck: Current understanding of natural lanthanide presence and environmental function is extremely limited; microbe-mediated rare earth recovery could be more widespread if we had a stronger understanding of existing natural recovery processes.

Social and Nontechnical Dimensions Case Studies

Overview

Climate change presents multifaceted challenges that require humanity's collective attention and commitment. Mitigating and adapting to the impacts of climate change will affect how individuals live their lives and interact with their planet. To have their desired impact, the technical solutions, approaches, and strategies presented in this roadmap must be considered with respect to the societal values and contexts in which they might someday be deployed. Across complex local, national, and international landscapes, personal and societal values and experiences can lead to disparate ideas about which challenges are most urgent to address and the appropriateness of any given approach. Uncertainty about outcomes and different tolerance for risk will lead individuals with the same factual information to come to different conclusions about appropriate uses of technology. Recognizing the complexity of this societal context and engaging with ethical, social, economic, political, and legal ideas and frameworks is necessary for the development of biotechnologies that can ultimately be accepted, implemented, and achieve their goals.

The following questions and case studies were developed for technical researchers who may be less- or unaccustomed to considering such nontechnical elements of their research. The case studies are intended to be used by such researchers as they consider how nontechnical dimensions can inform technical approaches to climate and sustainability challenges. Some nontechnical concerns can be alleviated with technical design choices or can help researchers identify target technical efficiencies/parameters needed to make an approach feasible. Case studies were selected to highlight a range of nontechnical issues, challenges, and considerations that permeate this *Engineering Biology for Climate & Sustainability* roadmap. Each case study consists of a hypothetical engineering biology-based technology drawn from this roadmap, an application area related to climate change and sustainability, and a geographical location where the technology could be deployed for context. Overarching **nontechnical considerations** are explored below, then applied to each case study. Within the case studies, questions are raised that highlight **ethical, political, economic, and security dimensions** of those considerations. We do not seek to answer these questions within the case studies—or even to identify all the necessary questions that should be considered—but rather use the case studies and associated questions as examples of how and why the consideration of social dimensions is important.

Addressing and adequately contending with these nontechnical considerations will, in many instances, necessitate consultation and collaboration with colleagues in the social sciences. Such partners have specialized knowledge, social research expertise, understanding, and context that can inform technical research approaches, techniques, and strategies. In partnership, technical and nontechnical researchers may successfully identify and engage appropriate stakeholders, such as local community members, understand and communicate regulatory needs and uncertainties, and seize opportunities to refine research approaches such that they are able to maximize positive impacts.

Unfortunately, well-trod pathways and funding are lacking for the development of partnerships, collaborations, and strengthening of professional networks between technical and social science researchers (see Viseu, 2015 and Carter & Mankad, 2021 for recommendations for integrating technical and social science). We encourage the development, funding, and use of such pathways, but presently envision these case studies as: i) a starting point for technical researchers to recognize and reflect upon how such nontechnical considerations might influence the trajectory of their own research and its application to climate and sustainability challenges; and ii) a tool for highlighting the value and necessity of interdisciplinary teams that do have the expertise to identify, develop, and implement solutions that work.

Engineering biology research is often motivated by a deep sense of curiosity and optimism for the opportunities that engineering biological systems present for making the world a better place. The research ecosystem incentivizes creative and optimistic perspectives on research applications; funders are interested in addressing challenges as well. Although incentives or even opportunities are lacking to think critically about the holistic impacts of the development and use of a technology and balance that with how quickly biotechnology can provide innovative solutions, we encourage technical researchers to commit to doing so.

Nontechnical considerations and social dimensions

Solutions landscape: Biotechnology in the landscape of other developing approaches and solutions

Researchers and innovators across many disciplines are working hard to identify and develop technologies to mitigate and adapt to climate change. Engineering biology-based solutions should be considered and weighed within that broader solutions ecosystem. Some challenges might best be addressed with a single, widely implemented approach, and other challenges must be met by the concerted efforts of many approaches in combination.

Feasibility: Practicality and feasibility of use and impact

Some biotechnologies may seem to offer innovative solutions to climate and sustainability challenges, but are impractical or not feasible at scale. Technical researchers might consider from the outset what impact a technology might have and how changes to different variables affect those impacts. For example, if carbon-capturing algae would need to be grown in high concentrations that negatively impacted other marine organisms and/or at a scale that required participation from all coastal nations, it may not be a practical solution. Additionally, the economic and technical feasibility of producing a biotechnology at scale should be considered at the outset to ensure a solution can be implemented or used at necessary scales, and that there could be a customer willing and able to pay for technology deployment.

Benefits and consequences: Uncertain or undetermined benefits and consequences of research and outcomes

The positive, negative, and neutral impacts of a technology can be difficult to fully predict in advance of its use. For example, the deployment of microbes engineered to capture and sequester carbon into soils could impact the soil microbiome and other ecosystem members including plants and insects. The microbes could enter waterways and affect downstream ecosystems. The extent of any ecosystem impacts and/or organism spread beyond a zone of application cannot be determined with complete certainty in advance of release. Uncertainty can be especially high early in technical research and development while the parameters and contexts for a technology's potential use are still unclear. Even closed systems, for example where an engineered microbe is used in a bioindustrial process, can have uncertain eventual benefits due to variables around yield, scaling efficiencies, resource inputs (e.g., water and energy), and economic realities. Identifying variables that may impact the balance of positive, negative, and neutral, consequences early in the research process can help illuminate opportunities to approach technological development in ways that shift that balance toward greater positive impacts. Researchers can also become familiar with the rich ecosystem of innovation and discovery within and outside of engineering biology. Doing so may inform their own approaches and/or lead to cross-disciplinary approaches that increase the certainty that a biotechnology's benefits will outweigh any negative impacts. Overall, the potential risks of using a technology should not be measured against 'no risk,' but against the likely outcomes and consequences of doing nothing or using alternative approaches that also have uncertain benefits and consequences.

Implementation: Regulatory or governance frameworks, access, and benefits-sharing

Engineering biology roadmaps look to shine a broad light on the possible technologies that could be developed in the coming years to decades. Cutting-edge technologies often move faster than regulatory frameworks can be developed or updated. Thus, when considering the development of innovative engineering biology technologies, researchers would be well-served by recognizing the local and international policy frameworks they must work within. Ultimately, policymakers and regulators have the authority to decide which technologies are deployed under what circumstances. Working with regulators to understand current and evolving frameworks (without seeking to unduly influence them) can help researchers develop technologies that fit within the current or likely bounds of policy.

Working with national regulators early in technical development is especially important when a biotechnology might have widespread or international impacts or implications. Diplomatic talks and negotiations may be necessary for the international community to align on accepted practices between countries. Researchers should also carefully consider the commercial viability and accessibility of their products. If partnerships with existing companies will be necessary for commercialization, researchers might explore which types of companies could be potential partners. For example, researchers might consider the benefits and challenges of licensing a biotechnology to an existing company and how that would influence who has or is given access to a product. Particularly in the context of this roadmap, researchers could consider the access and distribution of a biotechnology for its impacts and benefits to the climate globally.

Micro-level impacts: Effects on local populations, industries, environments, and economies

Some products of engineering biology might be used in specific regions or locales. Minimizing, mitigating, and/or eliminating negative impacts on local human communities and native flora and fauna should be a central priority for climate and sustainability efforts. The voices of local communities should be heard as policies are made about a technology's use. Additionally, if/when genetic resources from a region are utilized, appropriate benefits-sharing measures should be implemented in accordance with the *Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization (ABS) to the Convention on Biological Diversity* (Nagoya Protocol, 2014). The Nagoya Protocol recognizes that all members and parts of an ecosystem can be impacted by conservation and sustainability efforts, that local and indigenous populations have knowledge that can contribute to building and maintaining biodiversity and ecosystem health, and that the information, practices, and innovations that arise from this knowledge should be made accessible to all parties in a fair and equitable manner.

Macro-level impacts: Implications for macro (e.g., national, global) populations and geopolitical relationships

Some technologies identified within this roadmap would utilize engineered organisms in open environments and/or consumer products. While a single regulatory body might conclude that the use of technology is safe and appropriate, others might give more or less weight to contextual factors or philosophically prefer a precautionary approach. It is also possible for engineered microbes to maliciously or accidentally spread beyond their intended environments in ways that exacerbate international tensions or inequities. Awareness of global contexts and potential macro-level impacts can inform research approaches toward biotechnologies that are safe, effective, and also likely to be implemented.

Competing values and priorities: Recognizing trade-offs and divergent values between individuals, institutions, communities, and nations

The values, priorities, identities, and life experiences of individuals, communities, and nation-states will change how, or if, they think biotechnologies should be used in given circumstances. For example, the relative value placed on the preservation of unaltered lands and ecosystems, community decision-making, equitable access and outcomes, economic opportunity, etc., will vary. Reasonable, informed people who care deeply about a healthy planet can arrive at different conclusions. Recognizing some of these factors that underpin conclusions can be useful to determining how engineering biology can most productively be applied to climate and sustainability challenges.

Case Study 1: Release of engineered algae with increased carbon capture capability in U.S. coastal waters off California.

Capturing atmospheric carbon dioxide is a strategic priority for slowing the warming of the planet. Photosynthetic organisms naturally capture atmospheric carbon dioxide (CO_2) and, with engineering biology, could potentially become efficient enough at removing CO_2 to slow global warming. Annually, atmospheric carbon dioxide falls during the summer months of the northern hemisphere when most of the landmass on the planet experiences the warmer days and longer sunlight that contribute to photosynthetic biomass growth. Unfortunately, this natural carbon removal process is insufficient to stop the overall rise of atmospheric carbon dioxide.

Engineering plants or other photosynthetic organisms to capture more atmospheric carbon has been suggested as an approach to lower or prevent the rise of atmospheric carbon. One element of such an approach could be to leverage the vastness of Earth's oceans to capture more atmospheric carbon with marine photosynthetic organisms, for example by distributing engineered algae with increased CO_2 capture capabilities in coastal waters. However, a study by the National Academies of Science, Engineering, and Medicine found that if 63% of global coastlines were used to grow 100-meter-wide belts of seaweed for this purpose, it would still only capture 0.1 gigatons of carbon dioxide each year (National Academies of Sciences, Engineering, and Medicine, 2022). Engineered algae could potentially improve this trade-off, with less coastline required for more carbon dioxide capture. Assuming that engineering could make this strategy more feasible, high-efficiency carbon capturing algae would still very likely require growth around vast stretches of coastline where a large suite of biotic and abiotic factors could impact or be impacted by its presence. For example, the biomass and excess carbon of the engineered algae could impact marine food webs, including local seafood industries. Alternatively, some scientists are pursuing kelp growth on biodegradable rafts further out in the ocean. This would preserve valued coastlines and increase the likelihood of kelp sinking to leave its carbon on the ocean floor as opposed to washing up on beaches. Furthermore, because oceans are turbulent environments through which biomass can readily be moved, engineered algae is likely to spread beyond the areas where it is seeded or initially anchored, which could have impacts on geopolitical relationships.

Solutions landscape:

- **Ethical / societal -** If there is high uncertainty as to the ecosystem impacts of the release of engineered algae, should technologies with similar potential impact but more certain outcomes be preferenced (in terms of funding, research time)?
- **Ethical / societal -** How does this solution compare to other marine-based solutions like ocean fertilization or ocean alkalinity enhancement (National Academies of Sciences, Engineering, and Medicine, 2022)?

Feasibility:

- **Ethical / societal -** What scale (algae biomass, area and depth of the ocean) would be required for impact? Can open-air carbon capture systems (as opposed to point-source carbon capture) be sufficiently efficient?

- Can contained test environments be created and/or used that accurately predict impact on an environmental scale?

- **Economic -** Would the algae need to be biomanufactured? Or would small quantities successfully grow to relevant scale *in situ*? In either case, how would it be distributed?
- **Economic -** Who would pay for the production, distribution, maintenance, monitoring, etc., of engineered algae, especially considering the global movement of ocean water and its inhabitants?
    - What is the economic value of environmental carbon sequestration?

Benefits and consequences:

- **Ethical / societal -** How would coastal ecosystems be affected?
    - How do those effects compare to the impacts of higher atmospheric carbon levels if the technology is not used?
- **Ethical / societal -** Can engineered algae be used at a density that significantly lowers atmospheric carbon without compromising local ecosystems?
- **Security, Policy / regulatory -** How might the larger ocean ecosystem be impacted?

Implementation:

- **Policy / regulatory -** What existing policy and regulatory frameworks might impact the use of this technology?
    - Which treaties/international agreements would govern this release? What precedents exist for considering how the actions of one country impact the air, water, and/or organisms in another (e.g., nuclear power plants near international borders, dam construction on rivers that flow into other countries, genetically engineered mosquitos).
- **Policy / regulatory** - Are regulators aware of and considering their regulatory approach to using engineered organisms in the ocean?
- **Policy / regulatory, Ethical / societal -** Do relevant regulatory bodies require proof of safety? Or require that certain tests and experiments do not show evidence of harm?

Micro-level impacts:

- **Economic -** How would the use of engineered coastal algae for carbon capture impact coastal economic activities such as fishing and tourism?
- **Ethical / societal -** How would the algae affect the relationship of coast communities with the ocean?

Macro-level impacts:

- **Ethical / societal -** How could engineered algae alter the balance of marine food webs? What positive or negative effects could that have?
    - Could concerns about human health – regardless of the legitimacy of those concerns – ultimately lower global seafood consumption?
- **Security, Policy / regulatory -** Could the spread of engineered algae into the ocean food web and ecosystems foment international conflict based on real or perceived damages?

Competing values and priorities:

- **Ethical / societal -** If coastal engineered algae could capture significant atmospheric carbon, but would also cause ecological changes and/or economic damage to tourism communities, would it be worth it? How can all interests be represented and incorporated into decision-making and benefits-sharing?

Case Study 2: Application of biofertilizers based on engineered rhizobia to corn fields in the American Midwest.

The application of fertilizers to agricultural fields enables more crops for food or fiber to be produced on a given area of land. Globally, the use of nitrogen fertilizers has increased about 800% since 1961, which has contributed to a 30% increase in food supply per capita (Mbow et al., 2019). The production of synthetic nitrogen fertilizers uses the energy intensive Haber-Bosch process, accounting for around 2-3% of the world's energy supply (DeLisi et al., 2020). Nitrogen fertilizers can leach out of the soil into waterways, causing ecological problems such as eutrophication (Howarth, 2008; Bijay-Singh & Craswell, 2021), and they release nitrous oxide, a problematic greenhouse gas (Schwenke et al., 2015). Non-synthetic ("organic") fertilizers work well in many agro-systems but generally contain lower concentrations of nitrogen which often is not in a bioavailable form.

This case study considers how the beneficial relationships that plants such as legumes share with rhizobia can be extended to widely grown, nitrogen-intensive crops such as corn. It is likely that both the plant and bacteria would need to be engineered to support symbiosis, but because the nontechnical dimensions of plant engineering are well-explored elsewhere (e.g., Helliwell et al., 2019), we focus here on engineered rhizobia. Engineered rhizobia could reduce (or perhaps eliminate) the need for Haber-Bosch-derived fertilizers for growing corn, and potentially many other crops, as the key determinants of plant-rhizobia symbioses become fully elucidated. Then, such symbioses could be engineered to support the growth of other crops as well. Biofertilizers could further reduce agricultural emissions if they could be applied in the field at the same time as planting, for example as a seed coating for a symbiotic crop. Use in corn fields in the American Midwest could minimize nitrogen run-off and its downstream effects, such as toxic bacteria and algae growth impacting waters in the Gulf of Mexico. However, this might mean that more energy-intensive synthetic fertilizers no longer used in the Midwest might be sold and used elsewhere in the world. As a result, greenhouse gas emissions would not actually decrease, and those new markets might see higher food security coupled to negative environmental impacts. For maximal impact, engineered biofertilizers would need to be internationally available and economically competitive with synthetic fertilizers. If less expensive than synthetic fertilizers, it could additionally support global food security as an option for growers who cannot afford synthetic fertilizers.

Solutions landscape:

- **Ethical / societal -** This approach assumes that fertilizers for corn are necessary; are there farming practices or other advances in soil ecology that could minimize or eliminate the need for fertilizers?
    - What, if any, impacts would greater adoption of such practices (e.g., crop rotations, rotational livestock grazing to restore soil nutrients) have on the food supply chain and cost?
- **Ethical / societal -** Can other approaches, such as new chemistries for ammonia production or real-time sensing for precision fertilizer application, mitigate the energy-intensity and run-off challenges of synthetic nitrogen fertilizers?

Feasibility:

- **Economic -** Will biofertilizers be available and financially accessible to different types of farmers (large vs. small scale) in disparate international locations? Would incentives (and what kinds of incentives) increase uptake?
- **Economic -** Where can the biofertilizer be produced, and how expensive will it be? If it were only financially available in wealthier countries, would it still make an impact? What long term prospects might there be for lowering costs and distributing globally?

Benefits and consequences:

- **Ethical / societal -** Soil systems and microbial communities can vary greatly; is it possible to understand the impacts of engineered microbes in advance of applications in all these different environments?
  - Is a single engineered strain sufficient or would the solution require a community of engineered organisms for effective colonization and nitrogen fixation? If multiple strains are required, are all considerations compounded?
- **Ethical / societal -** What impacts (positive, negative, or neutral) might biofertilizers have on ecosystems? What might the impacts be on the micro- and macro-biomes where it is applied and where it may eventually move/be transported to?
- **Ethical / societal -** How do potential environmental risks of deployment compare to the better understood challenges of continued reliance on fertilizers made through the Haber-Bosch process?

Implementation:

- **Policy / regulatory -** Would the use of fertilizer composed of engineered microbes exclude a crop from organic certification? If not, how many years after application could the land be certified for organic production? Would there need to be testing to show no genetically engineered microbes remained?
  - At present, synthetic fertilizers cannot be used in organic farming; would such a fertilizer be considered "synthetic" because of the specific microbes used?
- **Security, Policy / regulatory -** What level of containment (if any) at the sequence and/or the organism level would be necessary? How would containment be demonstrated to regulators?
- **Policy / regulatory, Ethical / societal -** Will microbes used in biofertilizers be protected Intellectual Property (IP)? If so, will farmers be subject to legal action if protected microbes are found on their property?
  - Could the microbes be developed within an Open Source framework that allows for more equitable distribution?
  - What reasons might there be to have IP be open here?
- **Security -** If engineered organisms are sold as protected IP and are distributed in live culture, what measures protect from IP “theft” (i.e., reculturing and propagating engineered microbes)?
- **Ethical / societal, Economic -** Can technical decisions be made that ultimately increase biofertilizer accessibility globally?

- How can biofertilizers be made accessible to small-holder farmers to avoid contributing to the growing gap between small and large farming operations?

Micro-level impacts:

- **Economic -** Given potential persistence (or lack of persistence), would biofertilizers affect other environmentally-beneficial farming practices such as crop rotation?
- **Ethical / societal -** Where were the wild type microbes originally identified? Is there a historical context for the cultivation of these microbes in symbiosis with agricultural plants? Who benefits from their use? What measures need to be established to ensure benefits sharing and prevent biopiracy?

Macro-level impacts:

- **Ethical / societal -** Would engineered microbes be present in run-off and downstream waterways?
- **Security -** Could dependence on well-defined strains open up vulnerability to the evolution (or targeted attack) of neutralizing microbes (or plasmids, antibiotics)?

Competing values and priorities:

- **Ethical / societal -** If biofertilizers both meaningfully reduce the need for synthetic fertilizers and significantly alter soil ecology in the regions where they are applied, how should those benefits and costs be weighted?
    - Could metrics be developed by regulators and made available for public comment before implementation? What would be measured and where would that information be available?
- **Economic -** How might early research choices influence the industrial and economic models used later for commercialization?
    - Might linkage of engineered microbes to complementary engineered plants lock farmers into buying one (or few) plant varieties?
    - Would farmers need to reapply biofertilizers each season, or would microbes persist to recolonize roots in subsequent growing seasons?
- **Policy / regulatory -** The scale of biofertilizer production necessary to make a difference in the use of synthetic fertilizers would be significant; production and distribution on such scales is likely to be more feasible for large corporate actors. Should the economic beneficiaries of such technologies be a consideration in their development?

Case Study 3: High efficiency lithium biomining in Nevada with engineered microbes.

The inconsistency of some renewable energy sources, such as solar and wind, presents a significant challenge to their universal adoption. Strategies to efficiently store the electricity generated when sources are abundant are thus necessary. Currently, lithium-ion batteries are a common storage technology. They power electric vehicles and can be used in conjunction with solar panels to ensure electricity availability, day or night. Most lithium is mined outside the United States. To ensure a consistent supply chain and support U.S. clean energy goals, additional domestic mining is being pursued. This is controversial; despite the economic and supply chain benefits, mining can generate significant pollution (mining operations are generally powered by fossil fuels and generate waste), is water-intensive, and can disrupt existing ecosystems and valued lands.

Biomining has been used in the extraction of copper, gold, and other metals (Schippers et al., 2014), but there has been limited research toward biomining lithium. Bioleaching lithium from lithium ion secondary batteries by *Acidithiobacillus ferrooxidans* has been demonstrated, though recovery was low (Mishra et al., 2008). Microbes could potentially be engineered to more efficiently extract lithium from open pits, brines, and/or from recycled materials, thereby reducing the use of land, water, and energy. For example, a common mechanism of lithium mining is to inject water underground, where lithium and other salts are dissolved, then pump it back to the surface into lithium brine ponds and wait months to years for the water to evaporate, leaving the lithium behind. Using engineered microbes to recover the lithium from the brine could decrease land use and water lost from these typically arid environments.

Solutions landscape:

- **Ethical / societal -** Could efforts be better spent focused on alternatives to lithium mining, such as alternative battery systems (e.g., microbial fuel cell technologies)?

Feasibility:

- **Economic -** Can lithium mining microbes be produced cost effectively at large enough scale for use?

Benefits and consequences:

- **Ethical / societal -** Is there an environmental benefit to biomining as compared to traditional lithium mining? Is there an environmental harm? How can different perspectives on this be heard and taken into account?
- **Ethical / societal, Economic -** What are the benefits and consequences of biomining lithium in different environments/from different sources (i.e., brines, spent lithium batteries, ore)?
- **Economic -** Would this enable platform development for biomining other metals, e.g., rare-earth metals?

Implementation:

- **Policy / regulatory -** How would this interact with the existing federal land leasing or other forms of mineral acquisition rights?

Micro-level impacts:

- **Ethical / societal -** How does biomining lithium impact miners and local communities in terms of, for example, health, economic opportunity, environmental integrity, and changes to tourism and outdoor recreation?

Macro-level impacts:

- **Security -** Could engineered microbes for lithium mining be used intentionally or accidentally to destroy lithium batteries?
- **Policy / regulatory -** Could this technology make illegal lithium mining in protected environments easier?

Competing values and priorities:

- **Ethical / societal -** Lithium batteries enable the storage of renewable electricity, but lithium mining disrupts land and ecosystems (although there are efforts to minimize impacts and rehabilitate land). How can the disruption to an area of land and the micro- and macro-organisms that inhabit it be weighed against enabling a more consistent renewable energy supply?
    - Whose voices are heard and most valued? How might those voices weigh the value of the land compared to the value of mining lithium?
    - Can any benefits be disproportionately directed toward the people and lands that are disrupted?

Case Study 4: Engineering cattle gut microbiomes to reduce methane emissions in American agriculture.

Public awareness of the environmental impacts of meat consumption is growing, motivating some consumers to consider plant-based alternatives or lab-grown meat. However, many consumers are likely to continue to eat meat and consume dairy out of choice or necessity and it is thus useful to pursue opportunities to decrease the climate impacts of animal husbandry. Livestock produce 14.5% of the world's greenhouse gas emissions, and 61% of those emissions come from beef (41%) and cattle milk (20%). Reducing the emissions from cattle would thus be a significant contribution to global climate goals.[9]

Cows are ruminants, meaning they have four-chambered stomachs. One chamber, the rumen, ferments grass and other vegetation that is otherwise indigestible. Gases such as methane and carbon dioxide are produced as bioproducts of this fermentation. This case study considers the engineering of cattle microbiomes to reduce the release of such by-products. The public reception to meat or dairy from such cows is uncertain on both domestic and international fronts. For some, engineering ruminant microbiomes is a logical step in a long history of domesticating animals to serve human priorities; others may hold a more precautionary approach and purport that the potential impacts on cattle, environmental, and human well-being are unknown and potentially unknowable without assuming unacceptable risk.

Solutions landscape:

- **Ethical / societal -** Could methane be better managed with alternative farming practices?
- **Ethical / societal -** How will the economic and consumer viability of this approach compare to cultured meat production?
- **Policy / regulatory -** Could non-engineered microbes that could decrease methane emissions be used as a cattle "probiotic"?

Feasibility:

- **Economic -** How would the engineering of cattle microbiomes affect meat and dairy market value?

Benefits and consequences:

- **Ethical / societal -** What impacts might this have on the health and well-being of the cattle involved?
- **Ethical / societal -** How would engineered cattle microbiomes affect digestive nutrient uptake and therefore meat and dairy content? Would any differences impact taste, quality, or safety of food products?
- **Ethical / societal -** What are the anticipated impacts of cattle with engineered microbiomes on other fauna, compared to the impacts seen today?

Implementation:

- **Policy / regulatory -** How will meat and/or dairy from such cattle be regulated/labeled? Will meat or dairy from such cows be regulated as genetically engineered foods?

Micro-level impacts:
- **Economic -** What local industries or practices, such as commercial manure and compost manufacturing operations, might be impacted by this?
- **Ethical / societal -** How might excretions from cattle with engineered gut microbiomes affect micro- or macro-organisms in a given ecosystem?

Macro-level impacts:
- **Security -** Will international markets lead to global spreading of engineered microbes? If so, what consequences might that have?
- **Economic -** How could it impact market segmentation between organic and conventional meat and dairy?

Competing values and priorities:
- **Economic -** Do ranchers have any incentive to reduce methane emissions? Would a methane tax be an appropriate incentive?
  - What would the effects of a positive duty, such as a tax reduction for methane mitigation, be in comparison to that of a negative duty such as a methane tax? Which would be more effective and which would be easier to implement?
- **Ethical / societal -** How do consumer concerns about climate change intersect with the concerns of some consumers about the application of engineering approaches to livestock?

[9] Breeding and management approaches have already made cattle production significantly more efficient over recent decades, with fewer cattle being reared to feed more people.

Glossary

This glossary presents definitions and description of some of the key terms and concepts found in the roadmap. The glossary is specific to the context of this roadmap.

Abiotic stress is the negative impact (damage) non-living factors can have on living organisms in a specific environment. Abiotic stressors can include drought, salinity, low or high temperatures, and other environmental extremes.

Albedo is the ability or measure of a surface to reflect solar radiation. In environmental contexts, areas covered by ice and snow have high albedo, reflecting sunlight and helping to keep the earth cool; as climate change causes increased global warming, snow- and ice-covered regions, especially in the Arctic are melting, decreasing albedo and contributing to further warming.

Biobased (and *bio-derived*) processes and materials are those that function or occur through biological activity or are made of or derived from biological components, often through fermentation. Note: the United States Department of Agriculture's BioPreferred Program has a further definition of "biobased" that we find helpful, available at https://www.biopreferred.gov/BioPreferred/faces/pages/BiobasedProducts.xhtml

Biocement and *bioconcrete* are formed through the biological accumulation or precipitation of calcium carbonate/calcite, silica, or other minerals, to create limestone and other hard material products; biocement is a component of bioconcrete. While this process can occur naturally, engineering biology has been used to accelerate material formation and provide dynamic (i.e., self-repairing) activity.

Biocrusts, or biological soil crusts, are communities of living microbes that form a layer at the soil surface, most often in water-limited environments; biocrusts are typically comprised of mosses, lichens, and cyanobacteria (and sometimes also algae and fungi) that flourish in arid and semi-arid environments (Bowker et al., 2018).

Biofabricated materials are materials, such as textiles, produced by living cells and microbes, such as bacteria, yeast, and mycelium. For further information, please see Understanding 'Bio' Material Innovation: a primer for the fashion industry (Biofabricate, 2021).

Biofuel is any fuel derived from biomass, including plants (typically switchgrass or miscanthus, corn, soybean, or sugarcane) or algae.

Biomass is the amount of biological material that can be used for a process; when used directly for energy production, the term "biofuel" is often used interchangeably.

Biomaterial is any biological substance that has been engineered to interact with biological systems or derived from biological systems for non-biological use.

Biomining is the process of using microbes to extract economically-valuable materials from rock ores, mining waste, or other solid materials (including electronic waste).

Biomolecules are one of several major classes of biological molecules or complexes, such as proteins (including enzymes), nucleic acids, lipids, and glycans. For more about engineering biomolecules, please see EBRC's *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biosensor is a device or technology which uses living organism(s) or biological molecules or systems (including cell-free systems) to detect the presence of molecules, including chemicals or other cells.

Biosequestration is the process of storing or preventing escape of a specific substance (typically a pollutant) within a biological organism or biomaterial.

Biosorption is the process of binding or accumulating ions or other target molecules onto a surface, typically another biological surface such as cell membranes or biofilms.

Biosphere (or ecosphere) is the portion of Earth's surface, oceans and other bodies of water (hydrosphere), and atmosphere that contains life.

Biotic stress is the negative impact (damage) to an organism by other living organisms. Biotic stressors can include viruses, bacteria, fungi, and parasites, as well as insects, plants, and animals, particularly invasive species.

Carbon capture is the process of capturing carbon dioxide (CO_2) at its emission source, preventing it from entering the atmosphere.

Carbon carrying capacity is the amount of carbon that a system (organism or ecosystem) can absorb and store. "Carbon" in this sense is typically considered to be CO_2.

Carbon-Concentrating Mechanism (CCM) is a biological adaptation that enables a number of photosynthetic organisms to maximize their photosynthetic efficiency under low-CO_2 conditions (aqueous environments).

Carbon fixation is the process by which biological organisms convert inorganic carbon into organic compounds, which are then used for energy storage or biomolecule production.

Carbon flux is the rate of exchange of carbon between systems (a.k.a. carbon pools), such as carbon exchange between the oceans and the atmosphere. Carbon flux is typically measured in gigatons per year (GtC/yr).

Carbon negative is a process that achieves net carbon dioxide removal, effectively removing CO_2 from the atmosphere and locking it up in products.

Carbon oxides are molecules consisting only of carbon and oxygen including carbon monoxide (CO) and carbon dioxide (CO_2).

Carbon removal is the process of capturing and eliminating carbon, primarily CO_2 from the atmosphere, keeping the gas sequestered for long periods of time. Carbon removal, also referred to as carbon dioxide removal or CDR, can be a naturally-occurring process, or can be accelerated through technology.

Carbon storage is the long-term containment of captured or removed (sequestered) carbon (including CO and CO_2) in oceans, soils, vegetation, and geologic formations. Carbon storage typically occurs on the timeframe of centuries to millennia.

Carbon utilization is used to describe the many different ways that captured CO and CO_2 can be used or recycled to produce economically-valuable products (e.g., materials, chemicals, fuels).

Catabolism is the sequence of enzyme-catalyzed reactions by which relatively large molecules in living cells are broken down or degraded to release energy.

Cell-free systems are synthetic biological systems that consist of components to activate biological reactions without the environment of a living cell. Typically produced by isolating subcellular fractions, a cell-free system is an engineering biology tool for more controlled study of cellular reactions; simplified production of desired chemicals, biomolecules, or materials; or production or measurement in extreme or non-natural environments or with non-natural precursors or components.

Chassis, in engineering biology, is a cell/organism that serves as a foundation to physically house and support the genetic material and other biomolecules and materials necessary for biological function.

Distributed metabolism enables a biological system, such as a microbiome, to utilize many or all components of the system to cooperatively produce or degrade chemicals, materials, or compounds. For more, please see EBRC's *Microbiome Engineering: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2020).

Effector-triggered immunity, first identified in plants, refers to a second stage of plant defense against microbial pathogens, triggered when pathogen-associated effector proteins are recognized by cognate plant *Resistance* proteins. This is similar in microbes, where an internalized toxin triggers a direct transcriptional immune response (Rajamuthiah & Mylonakis, 2014).

Electroactive microbes are species that naturally, or through engineered mechanisms, transfer electrons across cell membranes; they are commonly used for microbial fuel cells and electrosynthesis (Sydow et al., 2014).

Emission intensity (or *carbon intensity*) refers to the amount of pollution emitted relative to the product (such as crop production, energy, or gross domestic product).

Engineering Biology is the design and construction of new biological entities such as enzymes, genetic circuits, and cells, or the redesign of existing biological systems. Engineering biology builds on the advances in molecular, cell, and systems biology and seeks to transform biology in the same way that synthesis transformed chemistry and integrated circuit design transformed computing. The element that distinguishes engineering biology from traditional molecular and cellular biology is the focus on the design and construction of core components (e.g., parts of enzymes, genetic circuits, metabolic pathways) that can be modeled, understood, and tuned to meet specific performance criteria, and the assembly of these smaller parts and devices into larger integrated systems to solve specific problems. Unlike many other areas of engineering, biology is incredibly dynamic, non-linear, and less predictable, and there is less knowledge of specific parts and how they interact. Hence, the overwhelming physical details of natural biology (e.g., gene sequences, protein properties, interactive biological components) must be organized and recast via a set of design rules that hide information and manage complexity, thereby enabling the engineering of many-component integrated biological systems. It is only when this is accomplished that designs of significant scale will be possible. The term “engineering biology” is often used synonymously with “synthetic biology;” EBRC considers engineering biology to encompass the field of synthetic biology.

Exometabolites are metabolic products, typically small molecules, that are lysed or diffused from the microbe or produced by processes that occur outside of the cell. Exometabolomics can be a powerful tool to measure activity of microbiomes and environmental impacts.

Feedstocks are the raw or unprocessed (biological) materials that are used or consumed. Feedstocks can be abiotic, including gases and metals, or biotic.

Foundational species are the organisms that play a major role in creating or maintaining a habitat in order to support other species in an ecosystem. Foundational species are often the most dominant or abundant organisms, and primary producers, within an ecosystem.

Genetic rescue is a strategy/tool to introduce or restore genetic diversity within a population, typically for species at (high) risk of extinction. Genetic rescue can include "genetically informed translocations of a species from one geographical region to another, other breeding strategies, and more extreme interventions such as gene editing” (Paez et al., 2022).

Greenhouse gases (GHG) are gases that absorb and emit radiant energy within the thermal infrared range, causing the greenhouse effect. The primary greenhouse gases in Earth's atmosphere are carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), and ozone (O_3).

Heat stress is defined as an increased temperature level sufficient to cause (sometimes irreversible) damage to an organism's growth and development or performance. For more, see Buckley & Huey, 2016.

Host is an organism that serves as a chassis or contained system for biological activity; typically a microbe, such as bacteria, plant or animal cell. For more about host engineering, please see EBRC's *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Hygroscopic means to attract and hold water molecules from the surrounding environment, whether by absorption or adsorption.

Indoor farming (see *Vertical farming*).

Keystone species are species that have an extremely high impact on a particular ecosystem relative to its population. Keystone species fill a critical niche in an ecosystem and have low functional redundancy – if they are lost, the ecosystem is likely to collapse.

Microalgae are photosynthetic algae/phytoplankton that are found in both marine and freshwater environments.

Microbial electrosynthesis is the process of providing electrons/electricity to microbes (from a cathode), which are taken up and used by the microbes to convert CO_2 into compounds and products through reduction; this is opposite of the activity of a *microbial fuel cell*. See also *Electroactive microbes* and *Microbial fuel cell*.

Microbial fuel cell is a system in which oxidation reactions within a microbe produce electrons for transfer (outside the cell/to an anode), generating electricity. See also *Electroactive microbes*.

Microbiomes are communities of diverse microbes that are found in a given environment. For more, please see EBRC's *Microbiome Engineering: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2020).

Non-photochemical quenching (NPQ) refers to a process by which photosynthetic organisms dissipate excess light that cannot be used for photosynthesis as heat.

Nutrient cycling (or ecological recycling) is the flux/pathway (movement and exchange) of nutrients and matter (biotic and abiotic) between an organism or system and the environment.

Photosynthetic capacity is a measure of the amount or maximum rate at which an organism is able to fix carbon (CO_2) during photosynthesis.

Rhizosphere is the area of soil around a plant root that is influenced by biochemicals associated with the plant and the surrounding microbes, the root microbiome.

Precision agriculture is a farming approach that leverages technology innovations, such as sensing technologies, to enable growers to increase crop yield through data. Precision agriculture aims to increase yield and quality of crops and reduce variability, while improving management of fertilizer and other resource use.

Protoplasts are plant cells where the cell wall has been removed, thus removing the challenge of penetrating the cell wall during transformation.

Regeneration (plants) is the process by which an individual engineered plant cell or protoplast can be grown into an entire plant.

Synthetic biology (See *Engineering biology*).

Transformation (plants) is the process by which DNA is delivered into a cell and causes a genetic change in the plant cell DNA.

Vertical farming/vertical agriculture or indoor farming is the practice of growing crops, most often indoors and in or close to urban centers, in vertical layers in a controlled environment (controlling for temperature, light, CO_2, and water levels) to optimize crop yield while reducing resource use. Vertical farming aims to reduce the negative environmental impacts of agriculture, particularly by growing food closer to where consumers live.

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