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Review article

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Synthetic microbiology in sustainability applications

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Abstract

Microorganisms are a promising means to address many societal sustainability challenges owing to their ability to thrive in diverse environments and interface with the microscale chemical world via diverse metabolic capacities. Synthetic biology can engineer microorganisms by rewiring their regulatory networks or introducing new functionalities, enhancing their utility for target applications. In this Review, we provide a broad, high-level overview of various research efforts addressing sustainability challenges through synthetic biology, emphasizing foundational microbiological research questions that can accelerate the development of these efforts. We introduce an organizational framework that categorizes these efforts along three domains — factory, farm and field — that are defined by the extent to which the engineered microorganisms interface with the natural external environment. Different application areas within the same domain share many fundamental challenges, highlighting productive opportunities for cross-disciplinary collaborations between researchers working in historically disparate fields.

Sections

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Introduction

Human activities are depleting natural resources and negatively impacting the environment, leading to a global environmental and climate crisis[1,2]. Tackling these challenges requires innovative solutions in areas as diverse as infrastructure, transportation, food management, and ecosystem conservation and restoration. Microorganisms offer a promising means to implement these solutions because of their diverse metabolic capabilities and their adaptability to a wide range of environments. However, there is often a disconnection between the priorities of microorganisms, which have evolved for their own survival, and the goals of human applications, such as minimizing environmental impact or maximizing efficiency in resource utilization. This is where synthetic biology, the field in which biological systems are engineered to perform tasks requiring programmed computation and actuation, can bridge the gap by enhancing the performance of natural microorganisms and tailoring them to address specific sustainability challenges (Fig. 1).

The diversity of potential roles that synthetic microbiology can play in sustainability applications is as diverse as the multifaceted nature of sustainability itself, ranging from new production techniques (for example, microbially derived foods or enhancing crop resilience) to environmental management (for example, bioremediation or carbon sequestration). To draw meaningful insights that hold across such a wide range of applications, it is useful to invoke a conceptual framework for organizing these applications.

We will base our framework on the property of environmental interfacing, the extent to which an engineered microbial system can affect and is affected by the external natural environment. The level of environmental interfacing of a given microbial system is closely tied and inversely related to the amount of control that operators can exert over the system and its local environment. To illustrate, a system wherein operators can control and monitor the local environmental variables very closely, such as a closed fermentation tank with manually supplied feedstocks, would have a low level of environmental interfacing. Conversely, a microorganism designed for a large-scale ecosystem engineering campaign would have a high level of environmental interfacing because operators can exert little control over relevant environmental variables such as temperature, moisture or the activities of the preexisting organisms.

The level of environmental interfacing, therefore, determines the design priorities and constraints for engineered microbial systems. In this Review, we will classify various sustainability applications into three major domains defined along a gradient of increasing environmental interfacing: the factory, farm and field domains (Fig. 2a). These three domains represent general distinctions in the nature of the design priorities and constraints associated with engineered microbial systems at these levels of environmental interfacing.

For example, consider biocontainment, the challenge of preventing unintended microbial escape into the wider environment. Although effective biocontainment is critical for all applications of engineered microorganisms, applications in each domain implement this requirement in different ways. For factory domain applications wherein the microorganism is physically sealed off from the outside environment, it is often sufficient to ensure that the microorganism does not persist within discarded waste materials. However, in typical farm domain applications, microorganisms are exposed to the external environment but must remain spatially contained within a particular area. As such, biocontainment becomes more complex and must be addressed by myriad strategies. Conversely, for field domain applications wherein microorganisms intentionally propagate widely throughout the environment, the design priorities of the system will focus less on enforcing spatial containment and more on ensuring that the microorganism does not persist beyond the intended timescale of the application. Similar differences can be found in other design properties, such as constraints on the readout modalities of the system or its resilience to specific environmental stressors.

We emphasize that the boundaries between these domains are not rigid and that specific applications can exhibit characteristics of multiple domains (Fig. 2b). For example, we classify agricultural applications in the farm domain because operators can exert some control over some environmental variables such as soil moisture and nutrient content but can exert little control over other variables such as temperature. Nonetheless, greenhouse hydroponic agriculture would share more properties with factory domain applications than open-air cropland agriculture because of its lower level of environmental interfacing and higher level of controllability.

In the following sections, we describe the various challenges and opportunities associated with the three domains and review the current state of research on selected application areas within these domains. We highlight shared properties among research areas typically considered to be separate and emphasize opportunities for cross-disciplinary collaboration. Because sustainability is a goal that is intimately associated with the natural ecosystems of earth, we place a particular focus on opportunities for fruitful interactions between environmental microbiologists and synthetic biologists to further accelerate the green transition of our society.

Factory domain

The factory domain comprises applications set in contained environments. Factory applications tend to be deployed in environments that are controlled and monitored at high spatiotemporal resolution. In the factory domain, sustainability-focused applications include the sequestration of greenhouse gases[3]; the extraction of valuable metals, such as copper, silver and gold from low-grade ores[4,5]; and the production of 'value-added' chemicals, such as biofuels[6–13], bioplastics[14–17] and fine chemicals[18], and food products such as vitamins[19] or single-cell protein[20–22].

Bioproduction using C1 gasses

Bioproduction for commodity and bulk chemicals is crucial for the green transition[23]. Whereas many sustainability-focused bioproduction processes resemble traditional methods and are reviewed extensively elsewhere[22–29], this section emphasizes C1 gas-based bioproduction in non-phototrophic organisms and the unique opportunities and challenges of C1 gasses. We refer readers interested in bioproduction in phototrophic organisms to excellent reviews elsewhere[10,30].

C1 gas emissions, including carbon dioxide, methane and carbon monoxide, are major contributors to global climate change. C1 gasses are produced in concentrated quantities by facilities such as power plants, wastewater treatment plants, steel mills and petroleum refineries[2]. Gas fermentation in factory settings can use these otherwise problematic emissions as a carbon-neutral substrate for microbial growth.

Many different organisms and metabolic pathways are used for carbon assimilation, but acetogens are used most frequently to ferment C1 gases. Acetogens such as *Clostridium ljungdahlii* are anaerobic organisms capable of fixing carbon through the Wood–Ljungdahl pathway using various energy sources, including carbon monoxide, which provides both carbon and energy[31]. This metabolic diversity allows acetogens to produce valuable commodity chemicals such as

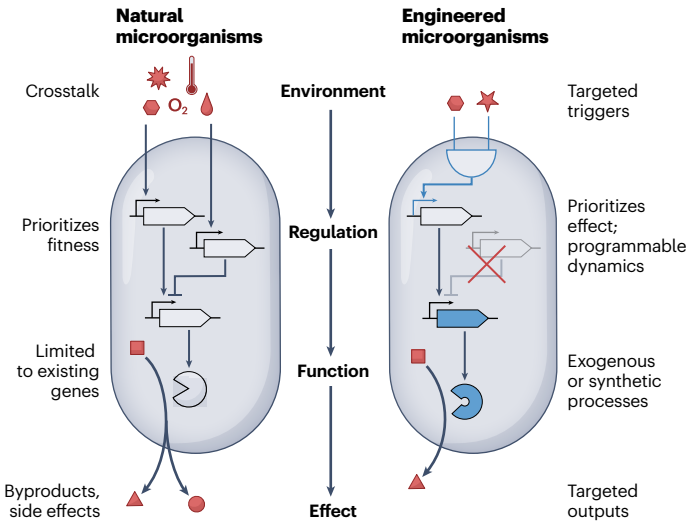


Fig. 1 | Synthetic biology enhances existing microbial applications. Although natural microorganisms (left panel) can perform functions that are useful for targeted applications, the fact that they evolved through natural selection means that their priority will be the fitness of the microorganism rather than the needs of the application. For instance, the functions of natural microorganisms are often concurrently regulated by multiple environmental factors ('crosstalk'), which makes them difficult to use in applications in which specific, targeted activation of the function is required. In engineered microorganisms (right panel), the regulatory pathways governing these functions can be rewired to enable their activation only by a desired set of targeted triggers. Moreover, the functions of natural microorganisms often have side effects that are undesirable for a particular application. Such side effects can be removed by engineering the microorganisms using synthetic biology techniques such as the rewiring of gene regulatory networks or the incorporation of exogenous or engineered enzymes and metabolic pathways. In concert, these changes enable the explicit prioritization of the desired application over the fitness of the host strain, thereby improving the target performance of the microorganism.

ethanol, acetone and isopropanol from substrates such as municipal waste and industrial emissions[6,9,32–34].

In a recent study, the acetogen *Clostridium autoethanogenum* was engineered to produce high titres (more than 2.5 g l^{-1} h^{-1}) of isopropanol and acetone in a 120-l reactor. This production was associated with an up to fivefold reduction in greenhouse gas emissions compared with production from fossil fuels, not accounting for the carbon stored in the product. To achieve high production rates and selectivity, the researchers applied innovative pathway engineering, fermentation process development and strain optimization using gene knockouts informed by kinetic modelling. These approaches were enabled by the existence of high-quality genetic engineering tools for acetogens and their ability to sequence 272 pre-collected strains and perform high-throughput prototyping of knockouts using cell-free systems[9].

Methanotrophs are another group of microorganisms that are used for gas fermentation. Methanotrophs are unique in that they can use methane as a sole carbon and energy source. Unlike acetogens, methanotrophs typically require the presence of at least some oxygen. Oxygen is required because methane is assimilated using the enzyme methane monooxygenase, which uses oxygen to convert methane to methanol. This methanol is converted to formaldehyde via methanol dehydrogenases and then assimilated through the serine or ribulose monophosphate cycles[35]. The commercial use of methanotrophs is less widespread than that of acetogens owing to several factors. These include the challenge of culturing them at high densities, the scarcity of genetic engineering tools, and the complexity of designing systems that can manage the explosive risk caused by oxygen–methane mixtures and highly exothermic metabolic reactions[7,36,37]. Although a few laboratory-scale experiments have attempted to use methanotrophs to generate commodity products such as single-cell protein[20,21], thus far, most production has concentrated on generating higher-value materials, such as biopolymers (polyhydroxylalkanoate and polyhydroxybutyrate), vitamins and antibiotics[14,18,36,38,39].

Acetogens and methanotrophs offer microbial solutions to the problem of fixing environmentally important C1 gases, such as carbon monoxide and methane. However, fixing carbon dioxide is less straightforward because it requires an additional energy source. For instance, energy can be provided by using electricity to produce hydrogen gas from water, providing a route for electricity-to-biomass production[8,11,40].

In particular, *Cupriviadus necator*, a gram-negative Betaproteobacteria that can grow on hydrogen and fix carbon dioxide through the Calvin–Benson–Bassham cycle, has been engineered to produce a variety of carbon-capturing products[8,11–13,15–17,40–42]. A prominent example is the development of an efficient solar-to-fuels production system by growing a genetically engineered strain of *C. necator* using the hydrogen generated from a water-splitting electrocatalyst. This integrated set-up initially achieved solar-to-biomass yields of up to 3.2% of the thermodynamic maximum, which exceeds the 1 to 2% annual solar-to-biomass yields of most terrestrial plants. It also exceeded previous bioelectrochemical fuel yields by more than 300% by producing 216 mg l^{-1} of isopropanol[8]. This work was then further improved in another study to reach solar-to-biomass yields of 9.8% and solar-to-fusel alcohol yields of 7.1% (ref. 11). This approach emphasized the potential of interfacing biotic and abiotic catalysts for achieving challenging chemical energy-to-fuels transformations.

An emerging alternative approach for generating biomass from electricity is to electrochemically convert gaseous C1 sources into soluble C1 sources that are more easily utilized by bacteria. For instance, carbon dioxide can be converted into formate or methanol, which can then provide energy for carbon fixation or be converted directly into biomass[40]. One advantage of soluble C1 sources is that new organisms require only minimal engineering to metabolize them. For example, the addition of three enzymes along with several months of continuous laboratory adaption enabled the autotrophic growth of *Escherichia coli* on formate alone[43,44]; similar work has led to *E. coli* strains capable of growth on methanol alone[44–46]. However, these engineered strains still grow much more slowly than microorganisms that naturally grow on formate or methanol. The ability to use these engineered strains in green industries, therefore, depends on whether their growth rates can be improved to near-native levels.

Biomining

Biomining is a broad term encompassing various biotechnological, hydrometallurgical and chemical techniques. In this Review, we define biomining as any process that uses microorganisms or microorganism-derived products to assist in producing refined metal, including processes such as mining and crushing ore, extracting metal from ore, purifying extracted metal and removing contaminants. As such, our definition of biomining includes techniques and

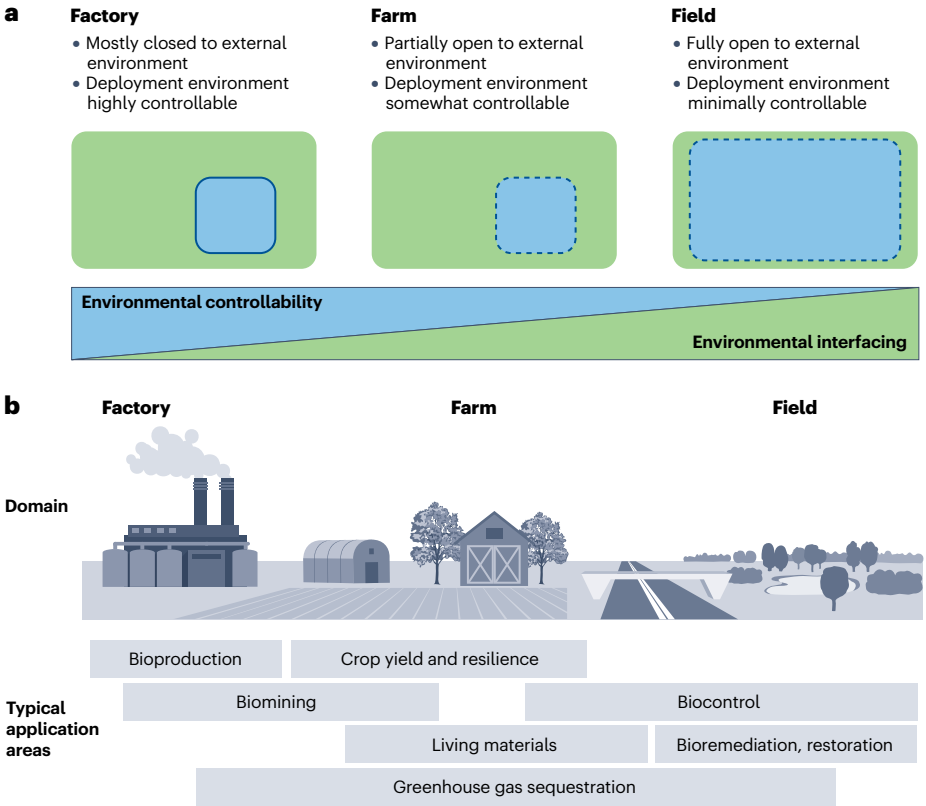


Fig. 2 | Domains of environmental interfacing for the organization of sustainability applications in synthetic microbiology. a, Synthetic microbiology applications can be broadly grouped into three different domains: the factory, the farm and the field. This classification is based on varying and opposite levels of environmental interfacing and controllability within each domain. Applications that are fully closed to the external environment (solid blue border) fall within the factory domain because they have minimal interfacing with the external environment (green region) and correspondingly have a high degree of control over the deployment environment (blue region). Applications that are open to the external environment (dashed borders) but still retain some level of controllability owing to a lower level of environmental interfacing are classified in the farm domain. By contrast, applications that exhibit a low level of controllability because they fully interface with the external environment fall within the field domain. The positioning of each domain along this gradient has distinct implications for design priorities and considerations of engineered microorganisms in that domain. **b**, Illustrative examples of typical application areas associated with different domains. Whereas some application areas such as bioproduction are closely associated with a single domain, other areas can have different implementations that lie in different domains. For example, greenhouse gas sequestration that occurs by feeding waste gas generated from an industrial process directly into a closed fermentation tank would be a factory domain application. By contrast, deploying microorganisms to drawdown atmospheric carbon into existing ecosystems would fall under the farm or field domains, depending on whether the microorganisms were deployed into more controllable environments (for example, cropland or other actively managed land) or into less controllable environments (for example, marine settings or across extremely large spatial scales).

approaches such as bioleaching, bioaccumulation, bio-sorption, biooxidation or bioreduction, and bioprecipitation[4,47,48].

Traditionally, metal production begins by extracting a metal-bearing rock or 'ore' from the earth. The ore is then crushed or ground and the desired metal is separated from impurities and concentrated using a combination of physical (for example, froth flotation or magnetic separation), chemical (for example, treatment with acid) and pyrometallurgical (for example, roasting) techniques. The concentrated metal is then refined, sometimes several times, to obtain a high-purity end product. Although the processes and techniques used vary based on the specific metal being produced and the characteristics of its ore, we can loosely group metal production into extraction, concentration and purification stages. There are biological approaches for each of the three stages (Fig. 3a).

To facilitate extraction, metal-bearing ore is excavated from the ground and comminuted (that is, broken down into smaller rock fragments). These steps are some of the most energy-intensive parts of the mining process. Comminution alone is estimated to make up between 3% and 5% of the world's total energy expenditure and between one-third to one-half of all energy used in mining[49,50]. The remainder of the energy used in the extraction process is mainly from the diesel fuel used in the heavy machinery that breaks up and transports the rock[49,50].

Although microorganisms are incapable of excavating, crushing and grinding rock, they could be used to bypass the energetically expensive and environmentally toxic extraction and comminution of rock via different techniques. One such proposed method is deep in situ bioleaching (Fig. 3b). In this process, the ore-bearing rock would first be disrupted, and then a solution generated by or containing

bioleaching microorganisms would be pumped in[51–53]. These microorganisms, typically bacteria such as those in the *Thiobacillus* genus, can break down sulfidic minerals through sulfide oxidation. This oxidation process solubilizes the minerals, releasing trapped metals such as gold and copper[5,54].

Bioleaching is currently used to extract metals after rocks are comminuted. This process is low-cost compared with traditional metal extraction methods, enabling its use with low-grade ores and mine tailings that would otherwise be unprofitable. Here, bioleaching typically occurs in either a stirred tank reactor or heap, wherein microorganisms break down the rock to free the metals, which are then extracted and purified. Commercial bioleaching has mainly utilized sulfidic ores such as low-grade copper and refractory gold ores[5,53]. In the case of refractory gold ores, the process is performed in a stirred tank and is often referred to as biooxidation[4]. Other metals, such as nickel, zinc and cobalt, are also leached through bioleaching[5,55,56].

There is great interest in establishing similar processes for non-sulfidic minerals, particularly those containing rare earth elements. Rare earth elements are of particular interest because they are crucial constituents of many electronic and green transition technologies and are environmentally expensive to produce[57–59]. However, rare earth elements are incompatible with existing bioleaching methods because they typically occur in non-sulfidic minerals such as those that contain phosphate, carbonate or fluoride[59,60]. Although some studies have investigated rare earth-leaching bacteria, mainly using phosphate-based minerals, our understanding of the mechanisms by which bacteria consume phosphate and solubilize rare earth elements is still limited[61–66]. For instance, a recent study has generated a whole-genome knockout collection of *Gluconobacter oxydans* and has demonstrated that disrupting genes involved in phosphate transport and pyrroloquinoline quinone synthesis enhanced rare earth bioleaching efficiency by up to 18% (ref. 67). Although such work provides an invaluable starting point for organism engineering efforts[68], more extensive studies of the mechanisms by which organisms solubilize lanthanide phosphates are needed. Such studies, when combined with microbial discovery efforts for solubilizers of other lanthanide-containing minerals, will provide a path to realize the full potential of microbial extraction of rare earth elements.

The biomining of rare earth elements also presents unique opportunities in the concentration and purification stages, as rare earth elements are often found in low concentrations, co-occur with one another and are difficult to separate owing to their similar chemical properties. Although biological-based methods are not currently in use, there is potential for the development of systems that can assist in the concentration or separation of rare earth elements. For instance, the discovery of lanthanide-dependent methanol dehydrogenases and corresponding proteins for lanthanide binding, uptake and storage has raised the possibility that microorganisms could aid in concentrating or purifying lanthanides[69–73] (Fig. 3c). This purification could potentially occur via binding, uptake, chemical reduction or precipitation in the form of lanthanide phosphates, similar to systems used for selenium or molybdenum[47,74,75]. In fact, a recent work has demonstrated that a

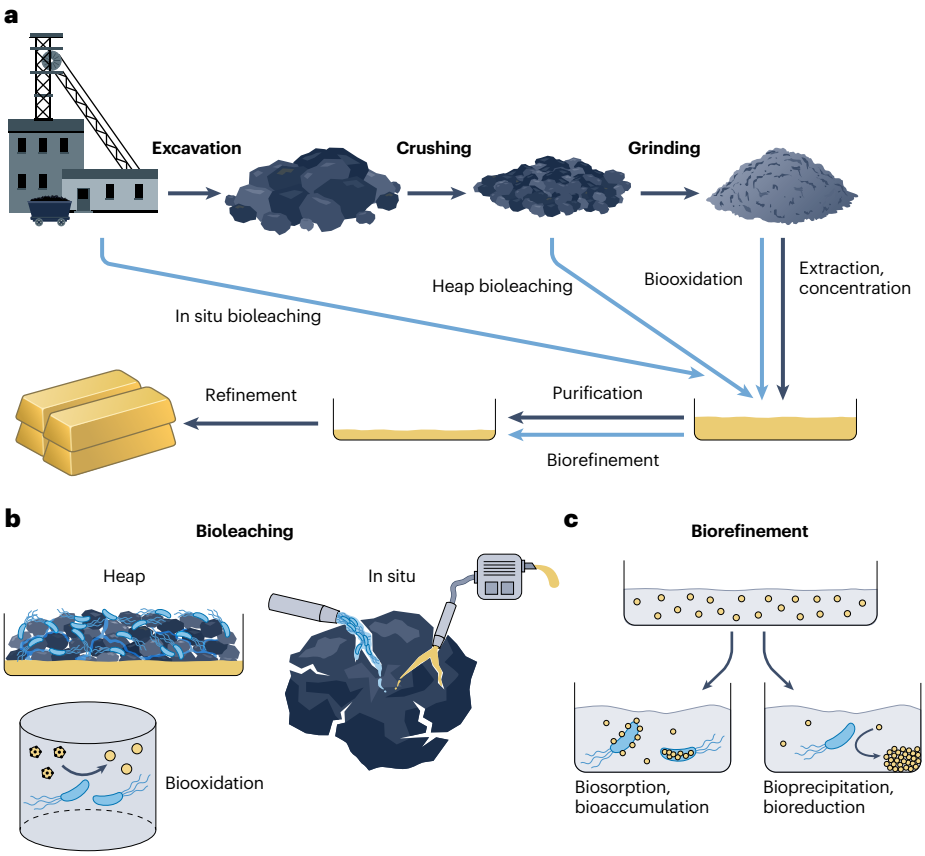


Fig. 3 | Microbially compatible steps in the mining process. a, Mining process (black arrows) and how microorganisms can be used (blue arrows) to either eliminate (for example, in situ bioleaching) or serve in place of or in addition to traditional processes (for example, bioaccumulation). **b**, Various forms of bioleaching. Bioleaching most commonly occurs in stacked heaps of crushed or ground ore in which liquid is circulated and occasionally removed (heap bioleaching), with microbial activity catalysing the dissolution of ores. When this process is performed in stirred tank reactors with refractory gold ores, it is typically termed 'biooxidation'. Recent developments have focused on the possibility of performing this extraction in situ, directly in the mine, using hydraulic fracturing (in situ bioleaching). **c**, Various methods that use microorganisms to purify or enrich metal ores. Microorganisms can bind to (biosorption), take up (bioaccumulation) or precipitate (bioprecipitation), or otherwise chemically react (bioreduction) with metals in solution.

lanthanide-binding protein from the methylotrophic bacterium *Hans-schlegelia quercus* could be used to purify a mixture of neodymium and dysprosium to greater than 98% individual element purities in a single-stage column separation[69,76].

Cybergenetics

One of the unique advantages of the factory domain is that designers and operators possess a high level of control and monitoring over the biological system. For example, by measuring the amount of key enzymes, metabolic intermediates and side products produced by cells as they produce a product, an operator can tune the environment of a biological system in a real-time, measurement-dependent way. When this control is applied, a feedback loop between environments, cells and operator is created. Here, a 'biological system' can be cultures or single cells, and an 'operator' can be a human or a computer. The latter approach, in which information processing is effectively offloaded from the microorganisms and managed by automated external systems such as computers, is termed cybergenetics[77] (Fig. 4a).

In principle, because our ability to perform information processing using computers far outstrips our ability to do so in cells, cybergenetics can enable new forms of precise and predictable bioengineering. Although integrated cybergenetic systems have not yet been implemented, numerous tools and technologies have been developed that can serve as the interface between machines and microorganisms. These tools include fluorescent reporters for monitoring various cellular parameters such as redox state, gene expression and cellular concentration of key molecules[78–84]. Optogenetic[85–93], temperature-dependent[94–96], electrical[97–100] and acoustic[101] tools are being developed alongside traditional small molecule-dependent tools[42,102–104], paving the way for the advancement of cybergenetics in synthetic microbiology.

One paradigm example of these tools is the Opto-T7 system, which combines a split T7 RNA polymerase with engineered Vivid homo-dimerization domains to create a blue light-inducible transcription system with high levels of temporal control[86]. It can easily be imagined how such a system, or a combination of similar systems, could be combined with a real-time readout of transcriptional activity and serve as the interface connecting the computer and the microorganism. However, one major limitation of these optogenetic systems is that there are only a few different families of light-inducible dimerization domains, which can have significant crosstalk, limiting multiplexability[105]. Efforts focusing on discovering or designing new chromatophores and domains with new or more precise absorption spectra are a key area for innovation and collaboration between basic biologists and synthetic biologists.

Adopting these tools and technologies is particularly important in large-scale bioreactors, in which traditional small molecule-based induction systems may be less efficient and require a significant replacement of media. However, many current tools for cybergenetics have difficulty with or an unproven ability to scale to larger bioreactors. For instance, optogenetic systems struggle with the limited ability of light to penetrate large-scale bioreactors. Although cybergenetics is still in its infancy and may require advances in basic biology, synthetic biology and chemical engineering, there is great potential in enabling designers to effectively and orthogonally control multiple aspects of an organism (Fig. 4b).

Farm domain

Unlike those in the factory domain, applications in the farm and field domains explicitly deploy engineered microorganisms into external environments. This leads to the two domains sharing many fundamental challenges, such as ensuring that the engineered microorganisms can reliably colonize a niche within the existing ecosystem[106,107]. Nonetheless, applications in the farm domain differ from their field domain counterparts because their deployment environments (such as cropland or structural infrastructure) retain an element of human controllability, as opposed to the fully natural environments found in bioremediation or ecosystem engineering applications. In this section, we will focus on application areas and challenges that are more specifically associated with the farm domain itself.

Synthetic microbiology in agriculture

Deploying engineered microorganisms in agricultural settings to promote the yield or resilience of crop plants is the dominant application area within the farm domain and has been reviewed thoroughly in the literature[106,108–112]. Here, we highlight key recent developments that illustrate the need for advances in our fundamental understanding of microbial physiology to enable further progress.

Developing nitrogen-fixing microorganisms that can cooperate with crop plants and alleviate their dependency on nitrogenous fertilizers is a flagship goal for synthetic microbiologists working in agriculture[110,112,113]. Nitrogenous fertilizers are energetically expensive to produce and directly lead to the emission of nitrous oxide. Together, these factors account for 2% of total anthropogenic greenhouse gas emissions[114]. Furthermore, fertilizers also cause damaging eutrophication in aquatic ecosystems[115].

Naturally occurring nitrogen-fixing bacteria do not colonize most major crop plants and tend to repress the expression of their nitrogen-fixing pathway in the presence of nitrate to minimize the metabolic burden of the pathway. Therefore, much work has been dedicated to increasing the expression of the pathway and transferring it into microbial hosts that stably colonize desired crop plants. For instance, a study has transferred 12 different nitrogenase clusters into 15 different bacterial strains, successfully introducing nitrogen fixation into three of them by performing additional engineering on the pathway: *Pseudomonas protegens* Pf-5 and the cereal endophytes *Azorhizobium caulinodans* ORS571 and *Rhizobium* sp. IRBG74 (ref. 116). Extensively throughout their study, the authors performed RNA sequencing and ribosome profiling to assess whether the expression levels of a rewired nitrogenase pathway matched the expression levels of the original pathway within its native host. Such an approach has been proposed as a general method for accelerating the transfer of metabolic pathways into non-native hosts[117]. A critical takeaway from this study is that although these approaches can be informative, they are insufficient on their own to predict nitrogenase activity in a new host[116]. For example, the authors found that the native *Klebsiella oxytoca* pathway was functional in *P. protegens* Pf-5 but not in *Rhizobium* sp. IRBG74, despite the fact that the expression profile of the pathway was better correlated to the original host in the *Rhizobium* background. Nonetheless, the authors were able to leverage the RNA sequencing and ribosome profiling data to achieve functional nitrogenase activity in *Rhizobium* sp. IRBG74. They accomplished this by rewiring the gene cluster to increase its transcription while preserving the translational coupling from the original operon structure. This work highlights the need for a deeper understanding of how all steps of the central dogma jointly regulate different metabolic pathways across microorganisms. This need becomes even more critical when cell-to-cell variability is considered. For example, another study has further engineered one of the cereal endophytes, *A. caulinodans*,

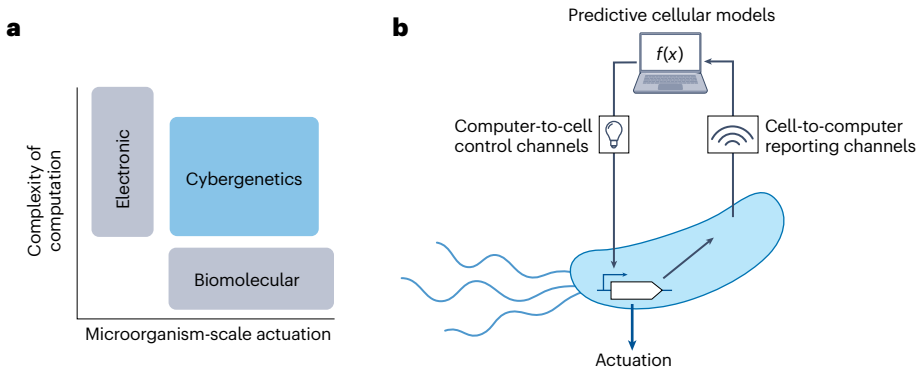


Fig. 4 | The benefits of, and requirements for, cybergenetic control in factory domain systems. a, Various computing modalities. Biomolecular systems such as engineered genetic circuits can perform functions such as metabolic reactions that enable the execution of target applications, but their ability to perform computation and information processing is rudimentary compared with conventional electronic computers. Computers, however, lack the means to interface with the microscale chemical world that is required for microbial applications. Cybergenetics provides a way to break this trade-off by offloading the computational requirements from cells to computers. **b**, Requirements for implementing cybergenetic control. Molecular reporters that accurately depict the cellular state at high temporal resolution are required to serve as inputs for mathematical models that integrate predictions about cellular physiology and the genetic circuit state to determine the appropriate control action at any given time. These instructions must be then communicated to the cell with high fidelity (for example, via optogenetic systems) to translate them into biomolecular actions (blue colour).

to express its exogenous nitrogenase pathway only in the presence of rhizopine, which was secreted by an engineered strain of barley[118]. The authors found that only a small subset (3% to 25%) of *A. caulinodans* cells grown on these rhizopine-secreting barley roots expressed the exogenous nitrogenase pathway. The fact that so few of the cells expressed the pathway, despite maintaining the engineered genetic cassette, suggests that the physiological state of individual microorganisms discouraged the activity of the pathway under these conditions. Microorganisms in the soil are thought to exist primarily under conditions of growth arrest, and the consequences of this physiological state on gene expression properties at multiple ecosystem levels — single cells, colonies and complex communities — are still poorly understood[119,120]. Understanding the relationship between the various environment-dependent survival strategies of microorganisms[121] and the activity of specific metabolic pathways, such as nitrogen fixation or phosphorus mineralization, will be essential for achieving the reliable long-term behaviour of engineered microorganisms in agricultural settings.

Engineered living materials

Developing engineered living materials (ELMs) is a rapidly emerging field of synthetic biology[122–127]. ELMs are macroscopic structures generated from living biological components and can be implemented through a combination of living and non-living substrates. These include autonomously self-assembling materials from interacting proteins expressed on cell surfaces[128,129], engineering properties of biofilms or cellulose matrices[130,131], embedding microorganisms into existing material scaffolds[132,133] and shaping fungal mycelia to grow into defined forms[132,134,135]. We classify ELMs into the farm domain for two reasons. First, because the resulting ELMs contain living cells when they are deployed in environmental settings, the cells are exposed to the open environment and are, therefore, not in the factory domain. Second, the cells are nevertheless designed not to propagate into the outer environment but rather to influence the properties of the material itself. ELMs, therefore, do not fit within the field domain.

ELMs can impact sustainability by displacing the use of energy-intensive or non-renewable materials, such as concrete or plastic. Although most studies focus on creating self-assembling ELMs, such materials are currently restricted to the centimetre length scale. For ELMs to serve as the basis for structural infrastructure, they must reach the metre length scale or greater. Currently, these length scales are only achievable with ELMs built by embedding microorganisms into existing structural matrices. In our review of this topic area, we focus on microbial concrete as it is one of the most developed examples of ELMs in this category.

Concrete production is estimated to generate 8% of total anthropogenic carbon dioxide emissions, which means that even modest improvements in the lifetime of newly produced concrete could yield significant reductions in long-term emissions[136]. The concept of self-healing biological concrete, in which spores of microorganisms that can precipitate calcium carbonate (typically by decomposing urea) are directly embedded into concrete, is one example of a biological solution to this challenge[137]. In this example, when cracks emerge in the concrete and expose the embedded spores to the outside environment, the spores germinate and induce calcium carbonate precipitation to re-fill the crack. This allows the microorganisms to extend the lifetime of the structure without requiring the use of additional concrete to repair the crack.

Microbial concretes must ensure that their material strengths exceed those of standard concrete over a long-time horizon to make their wider adoption economically viable. Although current implementations of microbial concretes almost exclusively use non-engineered organisms[138,139], microorganisms engineered to, for example, simultaneously persist over long timescales in the alkaline conditions of the concrete matrix while also efficiently precipitating calcite could lead to further improvements in self-healing concretes. As in the case of nitrogen fixation for agriculture, the ideal colonization and catalysis properties may not coexist in the same naturally occurring microorganism, but engineered microorganisms could perform effectively at both tasks. To illustrate this point, a study has discovered a biosilificating enzyme

(termed 'bioremediase') from a newly discovered hot spring bacterium, *Thermoanaerobactor*-like BKH1. When BKH1 was added to concrete, it increased the compressive and tensile strength of the concrete by up to 25% and 20%, respectively[140]. However, BKH1 was unable to stably persist within the concrete matrix. A later study has then cloned the bioremediase into the genome of the alkaliphile *Bacillus subtilis* to create a microorganism-embedded concrete with long-term persistence potential[141]. Microbial self-healing concrete was commercialized in 2017 (ref. 142), but the expense of its up-front costs still limits its wider adoption[143]. The ability to transfer and rewire the pathways governing cellular persistence and calcite precipitation and potentially incorporate evolved enzymes could enhance the effectiveness of microorganism-embedded concrete and pave the way towards its widespread adoption.

Biocontainment

Biocontainment efforts aim to ensure that organisms introduced into an area for a targeted application do not escape into the wider environment, potentially disrupting natural ecosystems. Although biocontainment is a vital concern for any engineered microbial system, it is of particular importance for applications in the farm domain because they cannot rely on physical barriers between the deployment range and the external environment (as in the factory domain) and the microorganisms are not designed to propagate widely across the environment itself (as in the field domain). The effectiveness of an engineered biocontainment system is typically determined by calculating the escape frequency, that is, the fraction of cells that survive and regrow outside of the intended deployment environment (for example, after the activation of a kill switch or the removal of an auxotrophic metabolite). Existing guidelines by the National Institutes of Health (NIH) define a safe escape frequency as one cell in 10^8 (ref. 144), and this is used as the standard for many biocontainment studies[145].

The process of escaping from biocontainment occurs in two steps. First, the engineered system must move outside its intended deployment region. This can occur spatially, via physical movement, or temporally, via persistence within the region beyond the intended deployment timescale (Fig. 5a and Box 1). In the second step, the engineered system must persist in a functional state within that external environment. Although biocontainment strategies can address either step in this process, historically, synthetic biologists have focused almost exclusively on the second step by developing systems that we will refer to as 'suicide containment' strategies. Instead of lowering the base rate at which the microorganisms leave their target deployment region, these strategies impose a penalty on escaping cells. This is typically implemented via programmed cell death (for example, kill switches or suicide vectors) or by preventing cell growth (for example, auxotrophies)[145,146] (Fig. 5b).

Genetic recoding has recently emerged as a new mechanism for the implementation of suicide containment that can simultaneously contain both the host microorganism and its potentially horizontally mobile DNA[147] (Fig. 5c). In genetic recoding, the entire genome of an organism is edited to remove all instances of specific codons. Then, these codons can be reassigned to noncanonical amino acids and incorporated into essential genes to create a synthetic auxotrophy to compounds not found in nature[148]. These altered genetic codes can also impede the influence of external genetic factors such as viruses. In 2021, a study developed an *E. coli* strain called Syn61Δ3 from which two serine codons and one stop codon, as well as their cognate transfer RNAs (tRNAs) and release factor, were deleted[149]. The authors observed that Syn61Δ3 was resistant to infection from a broad range of known bacteriophages because of the inability of the host strain to translate the phage genomes correctly. However, a later work has found that environmentally isolated phages that encoded their own serine tRNAs were still able to infect Syn61Δ3. This prompted the authors to introduce additional synthetic tRNAs that translated the originally serine codons as non-serine amino acids[150,151]. This modification not only conferred resistance to the tRNA-coding phages but also enabled a containment system for conjugative plasmids that recoded their essential genes to require synthetic tRNAs only expressed in the host strain.

These recent developments in the recoding field highlight a central challenge of biocontainment: in light of the vast diversity of biological adaptations and the ever-present destabilizing force of mutations, no single system can guarantee perfect containment across any environmental context[145]. Engineered systems that combine multiple different types of biocontainment strategies, therefore, should have a higher tendency to prevent the escape of engineered microorganisms or their DNA into natural environments.

One promising but underexplored category of biocontainment approaches is one we term 'nondispersive containment'. In contrast to suicide containment, nondispersive containment strategies lower the basal rate at which the microorganisms leave their deployment region. These strategies can be implemented by limiting the movement of the engineered cells (for example, by physical encapsulation) or by reducing the probability of mutational escape in the system (for example, by using nonreplicating entities such as synthetic cells)[152,153] (Fig. 5d). A recent work has highlighted the power of this approach by developing a semipermeable two-layer hydrogel capsule that permitted encapsulated microorganisms to sense metals and inducers such as acyl-homoserine lactones (AHLs) in river water while simultaneously preventing their ability to leave or conjugate plasmids out of the hydrogel[154]. The authors furthermore added a second orthogonal biocontainment mechanism to their system by engineering their cells to be auxotrophic to a noncanonical amino acid that was added to the inner hydrogel layer. This two-layer containment approach led to an undetectable (less than 0.5 cells ml^{-1}) escape frequency over 3 days. Future works in this field should continue in this direction, developing and characterizing nondispersive containment systems for various applications and assessing the composability of different biocontainment systems. Multidimensional measures of containment success that capture the spatiotemporal dispersal and persistence of the engineered microorganisms and their DNA should also be evaluated (Box 1).

Field domain

Applications in the field domain are intended to impact a large region of open environment, which means that the ability to control and monitor the microorganisms after deployment is limited. Applications in the field domain typically involve ecosystem engineering, either through the removal of target compounds from an environment (bioremediation)[155], the management or removal of a target population (biocontrol)[156], or the alteration of the entire ecosystem itself (for example, restoration of degraded environments)[157]. Currently, engineered microorganisms are not typically used in field domain applications because gaps in our knowledge preclude effective engineering.

Here, we highlight open questions relevant to the field domain using soils as an illustrative example. In particular, we will focus on the challenges that are particularly critical for field domain applications, as opposed to concerns such as biocontainment and niche colonization that are shared across both farm and field domain applications.

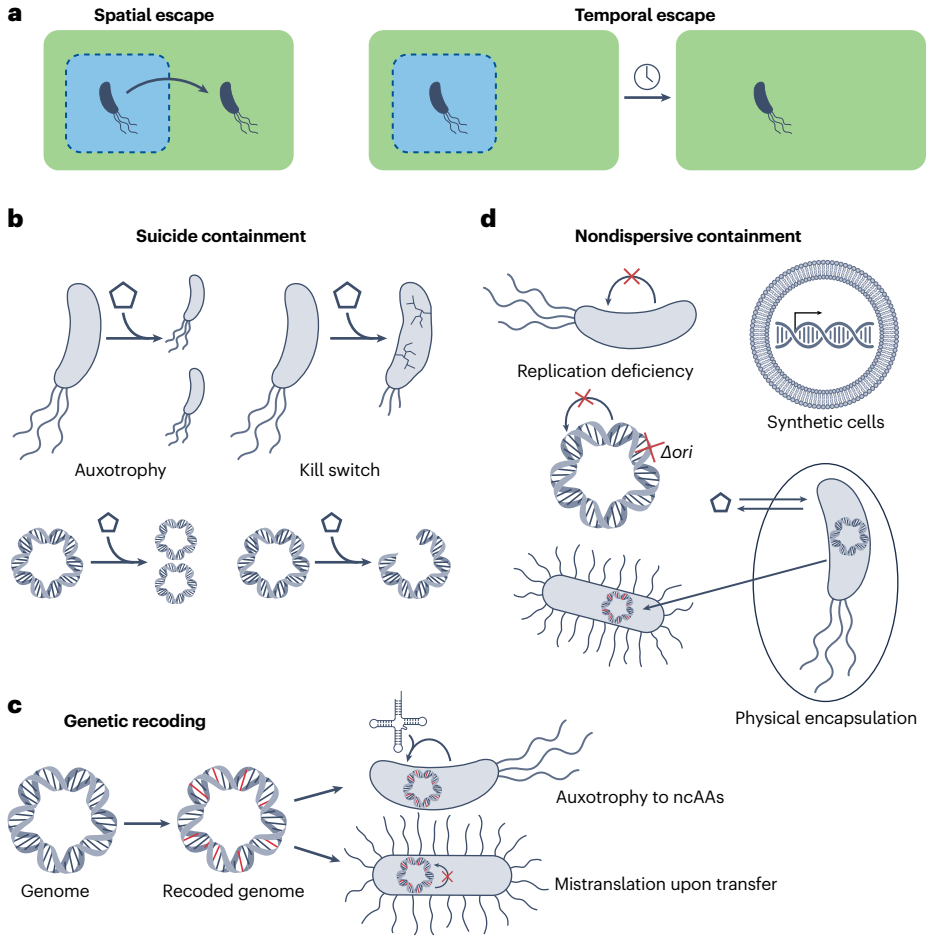


Fig. 5 | Synthetic microbial biocontainment systems. a, Ways through which microorganisms can escape biocontainment by moving outside of their target deployment region. Spatial escape involves the physical movement of the microorganism to a new location, whereas temporal escape involves the persistence of the microorganism in the same location after the intended deployment time window has ended. **b**, Examples of suicide containment strategies that prevent an engineered microorganism or its DNA from functionally persisting outside of the target deployment region. Auxotrophies engineer the system to require the presence of specific compounds (visualized as a pentagon) to propagate, allowing the user to present the compounds only within the deployment region. Kill switches induce cell death or autocatalysis of the genetic vector in the presence of a target signal, which can be used to define the spatiotemporal boundaries of the deployment region. **c**, Genetic recoding. Recoded organisms and DNA vectors can implement suicide containment systems with even greater effectiveness. For example, cells can be recoded to be auxotrophic to noncanonical amino acids (ncAAs) and DNA vectors can be recoded to be mistranslated outside of their target host. **d**, Examples of nondispersive containment systems that prevent the engineered microorganism or its DNA from exiting the target deployment region. Replication-deficient cells or DNA place a hard limit on the post-deployment persistence time of the system and also heavily reduce the probability of mutational escape. Synthetic cells, which consist of cell-free extract encapsulated in a membrane, exhibit neither replication nor active motility. Physical encapsulation systems that enable the interior microorganisms to interface with the outside environment while preventing their escape or gene transfer also prevent their spatial dispersal.

We consider the hypothetical problem of how to use an effective control and monitoring strategy for soil-associated microorganisms in the field domain. These general questions can be extended to other environments and ecosystems such as seawater, mycelia and animal microbiomes.

Model environmental substrates

Because microorganisms in a field domain application cannot easily be removed from their environment, it is essential to rigorously prototype their behaviour in field-like conditions prior to deployment. Doing so requires the development of model growth substrates that can accurately simulate environmental conditions within a laboratory context (Fig. 6a).

Attempts over the past decade to develop transparent soils for studying microbial processes illustrate the difficulty of managing this trade-off. Transparent soils are artificial substrates that consist of particles with a similar refractive index to their surrounding solution, enabling light to pass unhindered through both the solid and liquid components. A useful transparent soil should enable imaging deep within the substrate while reproducing the abiotic properties of natural soils, so that it can properly capture the influence of these properties on biological processes. Early attempts to use transparent soils to image microorganism–plant interactions[158,159] involved the use of the synthetic fluoropolymer Nafion. However, Nafion was

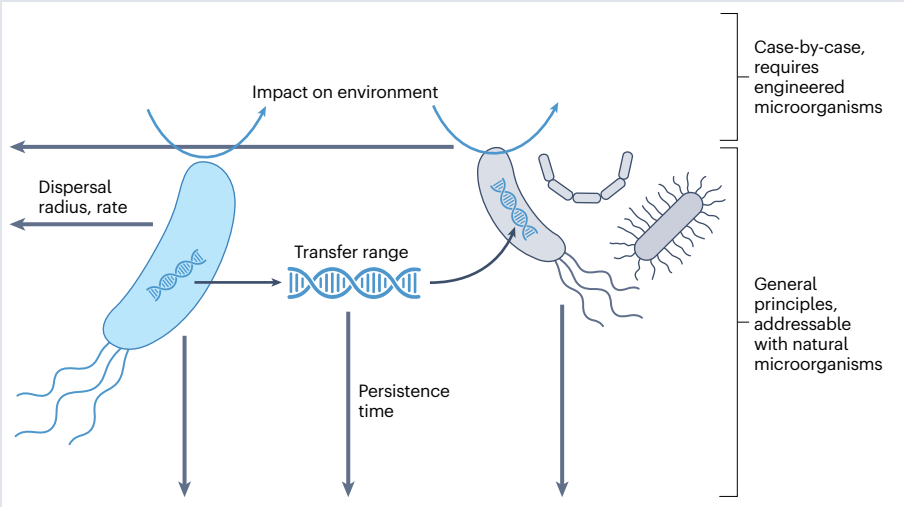
Box 1

Dispersal and persistence of microorganisms and DNA

Rather than abiding by the Baas Becking hypothesis that "everything is everywhere and the environment selects" (ref. 187), evidence increasingly suggests that the composition of natural microbial communities are limited by dispersal. This has prompted researchers to urge for renewed efforts to quantitatively investigate environmental microbial dispersal in natural environments across various scales[187–189].

In addition to understanding the natural dispersal and persistence properties of microorganisms, it is also critical that we understand the dispersal and persistence properties of the DNA carried within them. Horizontal gene transfer, either through active mechanisms such as plasmid conjugation and viral transduction or through passive mechanisms such as the uptake of extracellular DNA, could potentially enable foreign DNA molecules to exhibit even greater dispersal ranges and persistence times than their original host microorganisms. Extracellular DNA, in particular, is known to persist stably in natural environments such as soils[190,191], but more direct characterization of the spatiotemporal dispersal of various types of DNA within environmental contexts is needed[192].

Coupling ecological frameworks for microbial dispersal[188] and new tools for barcoding and tracking microorganisms and DNA in natural environments[193,194] should enable a wealth of knowledge about the principles governing the environmental dispersal and persistence of both microorganisms and their DNA. Furthermore, as illustrated in the figure, many of these principles can be determined through natural, unmodified microorganisms. This area of research, therefore, provides an excellent opportunity for synthetic biologists and environmental microbiologists to collaborate in the context of field-scale experiments. The insights from such studies will be essential in enabling well-informed risk assessments and multidimensional regulatory guidelines for the environmental deployment of engineered microorganisms, thereby shaping the extent and nature of the impact of synthetic microbiology on our society.



later found to be a plant stressor, making it incompatible with natural plant growth[160]. This prompted the development of a hydrogel-based system from alginate and gellan gum that permitted imaging several millimetres into the solution without introducing this plant stressor[160]. Unfortunately, the size distribution of the hydrogel beads (0.5 to 5 mm) was too large to replicate the microscale porosity of natural soils. This then led to the use of cryolite crystals for the development of a soil substrate with particles as small as 1 µm (ref. 161). However, this cryolite system was only validated using a microfluidic platform that was unable to image deeper than 100 µm. Currently, no single transparent soil substrate exhibits both a good imaging depth and a small particle size. Further research is needed to determine whether such trade-offs are intrinsic limitations of these substrates or whether they can be overcome.

Because a model substrate can never reproduce every environmental variable, it is essential to determine the subset of variables that most affects the biological processes relevant to an application. In an elegant example, a study has investigated the impact of soil moisture on plasmid conjugation rates by creating a sand microcosm whose water saturation level could be precisely controlled[162]. The authors found that conjugation rates increased 10-fold from the most saturated to the least saturated condition and determined that the increase in conjugation rates was driven by differences in local cell density in the soil. In dry soils, small fragmented pockets of moisture create regions of higher local cell density than are found in the diffuse, fully connected water networks in wet soils. Although a myriad of factors can impact conjugation rates across many environmental contexts[163–167], this work developed a tractable experimental system to isolate the mechanism of the impact of a single environmental variable on a specific biological process. This study has demonstrated that model substrates for applications relying on cell-to-cell contact must accurately capture soil moisture levels to predict system performance in the deployment environment.

In a complementary approach, another study has created a sequence of artificial soils that gradually approached the complexity of a true soil by successively matching its environmental properties such as mineralogy, pH and organic matter content[168]. By measuring the response curve of an AHL-inducible promoter along this sequence, the authors determined that specific properties of the response curve were primarily affected by different environmental variables. Specifically, the maximum expression level was most affected by the initial transition of the artificial soil from a liquid to particulate substrate and was relatively unaffected by other changes. Conversely, the half-maximal

AHL concentration (an indicator of its bioavailability) was unaffected by the initial transition and was instead most impacted by matching the mineralogy of the artificial soil to the mineralogy of the true soil.

These studies demonstrate that microorganisms engineered for different applications will be best served by different model environmental substrates to predict their behaviour in their deployment environment. Thus, a two-step process is required to reliably engineer microbial systems that can be safely deployed in the environment. First, the salient environmental variables that affect the microbial processes underlying the target application must be determined. This should be done through studies that isolate a process of interest and systematically investigate the influence of various environmental properties on that process. Second, laboratory-scale model substrates can then be developed to capture these salient environmental variables while still maintaining the experimental manipulability of the microbial system. Because both steps are intricately tied to the principles of microbial physiology and behaviour within environmental contexts, this research area provides a natural opportunity for synthetic biologists to collaborate meaningfully with environmental microbiologists.

Field-deployable control and monitoring strategies

Deploying microorganisms with autonomous functionality into the open environment is an enormously powerful but fundamentally risky proposition. The same autonomy that allows microorganisms to persist, grow and accomplish human-desired functions can also allow them to perform unwanted or unanticipated functions and escape containment. For synthetic biologists to safely deploy microorganisms into the open environment, we must be able to control and monitor them (Fig. 6b). Moreover, we must have a fundamental understanding of the mechanisms of our control and monitoring, as well as where they may break down. We define control as the process by which a human-derived signal is given to and interpreted by the microorganisms (signal induction and propagation) and monitoring as the ability for the microorganisms to produce a human-readable signal (signal detection).

Signal induction. Many chemical inducers used in synthetic biology are cost-prohibitive at the large scales necessary for applications in the field domain. Although economically feasible alternatives such as common sugars exist, they also tend to be abundant in environmental settings and, therefore, induce nonspecific or off-target cellular responses. Moreover, these inducers could be degraded or absorbed by the environment itself[169], further limiting their ability to communicate information to the engineered microorganisms. Systematic investigations of the specificity and effectiveness of low-cost inducers in environmental matrices, such as soils, would be beneficial. Such studies could also assess alternative means of gene induction such as sensors for temperature[170], osmolarity[171] or pH[172] that lie outside the natural range of variation for the target environment[173].

Signal propagation. Intercellular signals are typically propagated via either contact-based mechanisms (for example, plasmid conjugation or nanowires) or by diffusible molecules such as homoserine lactones. For field domain applications spanning large spatial scales, coordinating the activity of deployed microorganisms via intercellular communication must rely on strategies that can surpass the natural length scales of these mechanisms.

One approach that natural biological systems have implemented is the use of relay systems, wherein cells that sense a signal transmit that information to their neighbours, who themselves transmit the signal again to their neighbours, creating a travelling wave that can propagate faster than diffusion[174]. Such schemes enable locally induced signals to be transmitted across long ranges within a spatially dispersed population. For instance, a study has demonstrated that signal transmission in *B. subtilis* biofilms becomes possible when the fraction of signalling cells reaches a critical percolation threshold, forming a connected path that spans the system. They found that the signalling cells were spatially clustered, consistent with percolation theory predictions, enabling efficient long-range signal propagation[175].

In agricultural soil samples, the average distance between a bacterium and its nearest neighbour was found to be between 5 and

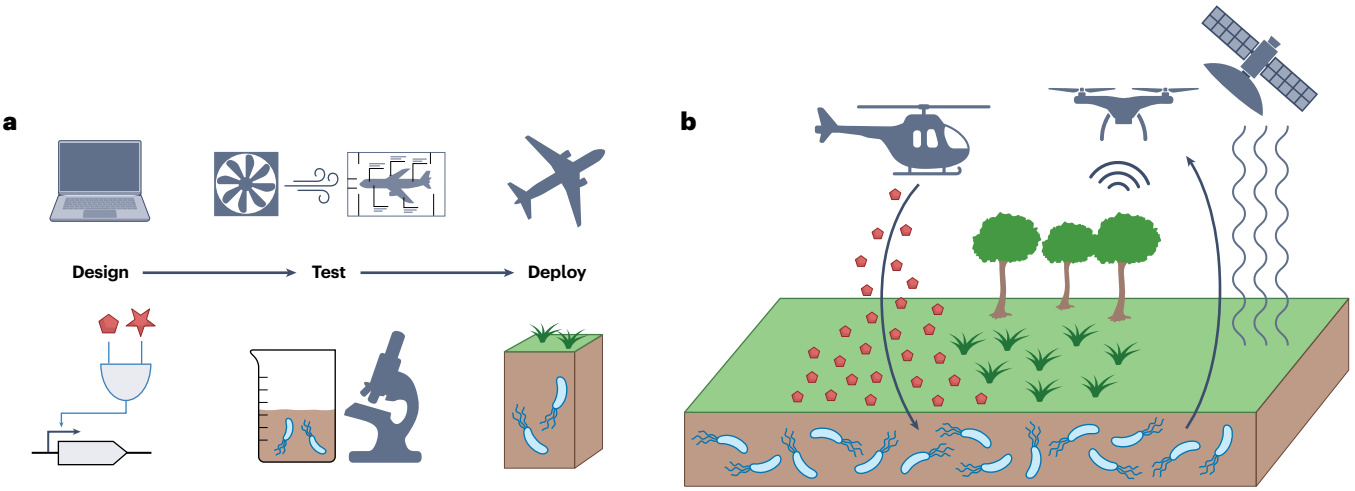


Fig. 6 | Grand challenges for applications of synthetic microbiology in the field domain. a, Model environmental substrates can serve as reliable and predictive testbeds within the design–test–deployment pipeline, analogously to wind tunnels in aerospace engineering. Such model substrates will be essential for accelerating the development of engineered microbial systems for applications in the field domain as they will provide insights into the relationship between environmental conditions and the behaviour of engineered microorganisms. **b**, In order to safely deploy microorganisms into a complex open environment, it is necessary to ensure that we can reliably control and monitor them within that environment. Such field-deployable strategies, such as determining chemical compounds (red shapes) that can reliably send instructions to microorganisms in the environment and determining effective channels for receiving information from engineered microorganisms across extensive spatial scales, are a major research priority for synthetic microbiology in the field domain.

30 µm (ref. 176). Meanwhile, AHL-based communication between engineered *Pseudomonas putida* cells can function at distances of up to 75 µm along plant roots[177]. This observation suggests that engineered signal relays may be feasible in soils, although the patchy distribution of soil microorganisms in microaggregates[178] and the requirement of continuous moisture channels to permit the diffusion of chemical signals through soil matrices[179] may present significant hurdles to their reliable implementation. Alternative approaches for crossing these inter-aggregate distances, such as relying on existing macroscopic soil entities such as plant roots or fungal hyphae[180], might be necessary to enable long-distance communication within soil environments.

Signal detection. Biosensors, biological components or genetically engineered microorganisms that report cellular or environmental states are ubiquitous in biological research. In the factory and field domains, these sensors are critical to our ability to monitor engineered microorganisms. However, in the field domain, microorganisms are distributed across a large amount of space and may persist or expand to unexpected locations, making traditional sensing difficult. Because effective, well-validated monitoring is a requirement for deployment, it is crucial that we continue to develop validated biosensors that are capable of being monitored at long distances over wide areas.

One promising recent approach to long-distance monitoring is the use of volatile gas reporters. By coupling a process of interest to the expression of a gas synthase rather than a fluorescent protein, the process can be continuously and non-disruptively monitored by measuring the gas concentration[181]. Among the microbially synthesizable volatile gasses, methyl halides have emerged as a particularly effective signal owing to the engineerability of their transferases[182] and their ability to achieve high signal-to-noise ratios in natural, metabolically active soil ecosystems[183]. Recent progress in this area enabled the detection of 1 mM exogenous inducer compound added to a non-sterilized soil using a concentration of only 1,000 biosensor cells per gram of soil[183], which is far lower than the approximately 10^9 cells g^{-1} densities typically used to detect fluorescent readouts from soil samples[184].

However, current gas reporters are still impractical to deploy in large-scale field conditions owing in part to their reliance on gas chromatography–mass spectrometry systems for detection. An important next step in developing this technology will be to create methods to translate the gas signal into a more easily monitored output. For example, a secondary biosensor with an optical output could be applied to the soil surface. Furthermore, the ecosystem impact of using methyl halides as reporters in soil environments must be more thoroughly assessed, as these compounds are often used as soil fumigants and are known to deplete atmospheric ozone[185]. Alternative monitoring strategies could also involve interfaces between microorganisms and plants, which already span the underground–aboveground interface. Machine learning approaches have been applied to satellite data of tree foliage to infer the presence and nature of the mycorrhizal fungi near their roots[186], suggesting that the activity of engineered microorganisms could potentially be encoded into optically detectable properties of aboveground plant tissue.

Conclusions

In this Review, we highlight the importance of developing a fundamental understanding of microbiological processes to enable continued progress in synthetic biology across multiple domains of sustainability applications. Natural microorganisms have evolved mechanisms that allow optimal functioning under environmental constraints; emulating them will require a systems-level understanding of microbial physiology, signalling and adaptation in diverse contexts. Sustainable applications will depend on deciphering how microorganisms sense and respond to biotic and abiotic factors and how their behaviours emerge from complex interactions between networks that govern metabolism, signalling and genetic regulation. Strengthening interdisciplinary collaboration among microbiologists, ecologists and engineers will be vital in unravelling these complexities, translating findings into responsible and effective designs, and developing tools to evaluate them rigorously. A rigorous understanding of the principles of microbial physiology and activity across various types of environments will be critical in ensuring that synthetic biology lives up to its promise of addressing pressing sustainability challenges in the decades to come.

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Competing interests
Pamela Silver is a founder of KulaBio and Circe Bioscience. The other authors declare no competing interests.

Additional information
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