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Environmental microbiome engineering for the mitigation of climate change

Michael R. Silverstein[1,2] | Daniel Segrè[1,2,3,4,5] | Jennifer M. Bhatnagar[1,3]

[1]Bioinformatics Program, Boston University, Boston, Massachusetts, USA

[2]Biological Design Center, Boston University, Boston, Massachusetts, USA

[3]Department of Biology, Boston University, Boston, Massachusetts, USA

[4]Department of Biomedical Engineering, Boston University, Boston, Massachusetts, USA

[5]Department of Physics, Boston University, Boston, Massachusetts, USA

Correspondence
Michael R. Silverstein, Bioinformatics Program, Boston University, Boston, MA, USA.
Email: msilver4@bu.edu

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Abstract

Environmental microbiome engineering is emerging as a potential avenue for climate change mitigation. In this process, microbial inocula are introduced to natural microbial communities to tune activities that regulate the long-term stabilization of carbon in ecosystems. In this review, we outline the process of environmental engineering and synthesize key considerations about ecosystem functions to target, means of sourcing microorganisms, strategies for designing microbial inocula, methods to deliver inocula, and the factors that enable inocula to establish within a resident community and modify an ecosystem function target. Recent work, enabled by high-throughput technologies and modeling approaches, indicate that microbial inocula designed from the top-down, particularly through directed evolution, may generally have a higher chance of establishing within existing microbial communities than other historical approaches to microbiome engineering. We address outstanding questions about the determinants of inocula establishment and provide suggestions for further research about the possibilities and challenges of environmental microbiome engineering as a tool to combat climate change.

KEYWORDS
bioinoculant, climate change mitigation, microbial inoculum, microbiome engineering, microbiome transplant

1 | INTRODUCTION

Climate change continues to threaten the stability of the biosphere, increasing the demand for mitigation strategies. In the event that emissions were to immediately cease, current atmospheric carbon dioxide (CO_2) levels would threaten to continue atmospheric warming (Frölicher et al., 2014), but may be curbed by the sequestration of atmospheric carbon (C) into stable reservoirs such as soil (Farrelly et al., 2013; Ghommem et al., 2012; Paustian et al., 2019). One exciting opportunity for mitigation is environmental microbiome engineering, that is, the delivery of a microbial inoculum to a natural environment in order to induce enduring stable modifications to a natural microbial community and the ecosystem functions it regulates. In both terrestrial (Kallenbach et al., 2016; Sokol et al., 2022) and aquatic (Jiao & Zheng, 2011; Tara Ocean Foundation et al., 2022) environments, microbial activities are responsible for transforming C into microbial products that can stably remain out of the atmosphere for hundreds to thousands of years (Kögel-Knabner et al., 2008). As a result, enhancing the rate of these natural C stabilization processes has been proposed as a climate change mitigation strategy (Paustian et al., 2019). Because the structure of a microbial community (i.e., its taxonomic composition or "who is there") can be coupled with its function (i.e., "what they are doing") (Gowda et al., 2022; Greslehner, 2020), appropriate perturbations to microbial community structure can in principle improve C stabilization and contribute to climate change mitigation.

Environmental microbiome engineering is a rapidly developing area, whose applicability will require addressing several open questions. Are certain ecosystem functions more susceptible than others to successful modification through microbiome engineering? What inoculum design strategies can improve the chances that microbial inocula will establish themselves in resident communities and confer ecosystem-level changes? Is it safe to inoculate ecosystems with nonnative microbes? Confronting these questions with the current science on environmental microbiomes will bring us closer to understanding the opportunities and barriers of manipulating these complex systems for ecological gains.

The promise and challenge of microbiome engineering are well illustrated by projects in medicine and agriculture. Fecal microbiota transplants, where feces (and the gut microbiota contained within) from a healthy donor are delivered to a patient, was a medical practice in fourth century China and has now resurfaced as a modern medical success (Borody & Campbell, 2011; Zhang et al., 2012). With a 90% efficacy in treating recurrent *Clostridium difficile* infection, fecal transplants far outpace the 20%–30% success rates of prolonged antibiotic therapy, the previous leading standard of care (Wang et al., 2019). In agriculture, microbial inocula have been commercially available for at least a century and have been successful in boosting the yield of many crops from ~5% to 80% (Hijri, 2016; Santos et al., 2019). While these results are encouraging for future microbiome engineering applications, not all attempts have been as successful and the drivers of inoculation failure are typically unclear (Hijri, 2016; Kaminsky et al., 2019).

The efficacy of microbial inocula in any system is limited by biotic and abiotic factors related to the capacity for the inoculum to establish in its target environment (Albright et al., 2021; Kaminsky et al., 2019). What is currently lacking in the field of microbiome engineering is the development and adoption of a mechanistically informed framework for understanding the properties of successful inoculum establishment and efficacy when incorporated into a natural environment. Recent experimental and theoretical studies provide some guidance, suggesting that inoculum community diversity (Vila et al., 2019), metabolic interconnectedness (Lechón-Alonso et al., 2021), and niche preferences (Eisenhauer et al., 2013) can increase community stability and can determine an inoculum's success in establishing and engineering target microbiomes (Bano et al., 2021). Unsurprisingly, the properties that increase microbial community stability, especially community diversity, are also implicated in the resistance of microbial communities to microbial invasion (Eisenhauer et al., 2013; Mallon et al., 2015).

In this review, we explore the potential and challenges of environmental microbiome engineering to mitigate climate change. First, we provide an overview of the history and definition of environmental microbiome engineering, next we review literature pertaining to each of a series of steps that we view as key components of the environmental microbiome engineering process. We then discuss safety concerns, and finally we offer recommendations for future directions in environmental microbiome engineering. While this review focuses on the engineering of soil microbiomes, many of the same principles can be applied to microbiomes in aquatic and other ecosystems.

2 | ENVIRONMENTAL MICROBIOME ENGINEERING—FROM THEN TO NOW

Environmental microbiome engineering can be broadly defined as the modification of a natural microbial community toward a specific practical goal and is a well-established practice in agriculture, where the most extensive use has been in promoting plant growth (Ahmad et al., 2018; Bano et al., 2021; Ellis et al., 1984; Hamilton et al., 2016; Hart et al., 2018; Howeler & Sieverding, 1983; Kaul et al., 2021; Ke et al., 2021; Liu et al., 2022; Maitra et al., 2021; Mawarda et al., 2020; Nadeem et al., 2014; Policelli et al., 2020; Qiu et al., 2019; Rodriguez & Durán, 2020; Ryan & Graham, 2018; Santos et al., 2019; Trabelsi & Mhamdi, 2013; Vishwakarma et al., 2020). The first commercially produced agricultural microbial inoculant was patented in 1896 and today, the use of microbial inocula has exploded (Santos et al., 2019). For example, microbial inocula are delivered to about 78% of Brazil's soybean cropping area (36.5 million hectares; about the size of Germany) annually, resulting in tens of millions of inoculation doses over the past two decades (Santos et al., 2019). In addition to soybeans, different inocula are commercially available for various crops such as wheat, maize, rice, tomatoes, peas, bananas, alfalfa, clover, sunflower, sugarcane, millet, safflower, cassava, potatoes, and more (Hijri, 2016; Howeler & Sieverding, 1983; Maitra et al., 2021; Santos et al., 2019). The earliest inocula were delivered in gelatin, which failed to sustain inocula members and was replaced with peat, which became environmentally destructive and limited in supply, so since the 1990s, most inocula have been delivered in a liquid medium (Santos et al., 2019). Early agricultural microbial inoculants contained a single rhizobacterium (root-associated bacterium) strain, and while the majority of modern agricultural inocula still contain a single bacterial strain, more products now contain a consortium (Santos et al., 2019), with some including mycorrhizal fungi (Hoeksema et al., 2010).

Despite its relatively long history, the science of microbial community engineering has been transformed by the rise of high-throughput approaches such as "omics" (metagenomics, metatranscriptomics, metabolomics, etc.) technologies, which have allowed for the detailed characterization of the taxonomic and functional composition of microbial communities (Jansson et al., 2012). These new detailed maps of environmental microbial communities have provided insight into the mechanisms by which a microbial inoculum establishes in a natural environment and transfers its functional capabilities to that new space. These molecular technologies, together with advances in theory and computational modeling of microbial ecology (Grilli, 2020; Marsland et al., 2020; van den Berg et al., 2022) are opening new directions for more informed inoculum design and monitoring of establishment.

The modern process of environmental microbiome engineering can be viewed as the overall output of a series of distinct, but mutually interdependent steps. These steps include: (1) choosing an ecosystem function to target, (2) gathering microbes or a microbial community to use for inoculum design (i.e., sourcing), (3) constructing the microbial inoculum (i.e., inoculum design), (4) inoculating a resident community, and (5) monitoring the establishment, or long-term persistence, of the inoculated community and changes to the ecosystem function target (Figure 1). While environmental microbiome engineering has historically been practiced in agriculture, these steps are generalizable to other ecosystems (forests, oceans, etc.), industries, and research applications.

The steps of the modern microbiome engineering process are well illustrated through a classic experiment in which a microbial inoculum was designed to modify the flowering time of host plants (Panke-Buisse et al., 2015) (step 1, ecosystem function target). Microbial communities were sourced from farm, forest, and grassland soils (step 2, sourcing) and a single plant species (*Arabidopsis thaliana*) was grown for multiple generations in a greenhouse experiment where the unsterilized soils from early or late flowering plants were used as the growth medium for each subsequent plant generation. By repeating this iterative artificial selection process for 10 generations, the researchers designed microbial inocula with the capacity to influence plant flowering time (step 3, inoculum design). These microbial inocula were then delivered to four novel plant hosts potted in sterile soil: three genotypes of the original plant and one different plant species (step 4, delivery) which were grown until they flowered. Ultimately, the microbial inocula established in recipient communities (the taxonomic composition of recipient communities reflected that of the inocula they received), host plants' flowering time was impacted by their respective inocula, and plants that received late flowering inocula also had higher biomass and enzyme activity than controls (step 5, establishment and impact). This experiment displayed the precision with which microbiome engineering can be used to modify complex, seemingly indirect traits, such as host plant flowering time. Other controlled experiments have been performed to engineer microbiomes with more climate-related ecosystem function targets in mind such as C sequestration (Ahmed et al., 2019; Kheirfam, 2020; Nie et al., 2015; Paustian et al., 2019), nitrogen (N) cycling (Calderón et al., 2017), and bioremediation (Baghapour et al., 2013; Chen et al., 2014; Dvořák et al., 2017; Mrozik & Piotrowska-Seget, 2010; Patowary et al., 2016) using a variety of inoculum design strategies (Table 1).

A well-designed environmental engineering procedure is one that considers how decisions made for each step of the process have potential consequences for other steps. Recent opinions argue that microbial ecology studies performed in idealized laboratory settings have led researchers to neglect the importance of inoculum establishment, one of the main barriers to inoculum success in the field (Albright et al., 2021). Translating these discoveries to industry and field applications will require the need for inocula design (step 3) to include considerations for establishment (step 5), which has been commonly neglected in laboratory studies that have solely focused on designing inocula to maximize the performance of a particular ecosystem function (step 1) (Albright et al., 2021; Kaminsky et al., 2019). In the next sections, we review possible avenues for each one of the environmental microbiome engineering steps outlined above, noting how the choices in a given step may influence the effectiveness of other steps (Figure 1).

3 | STEPS OF THE ENVIRONMENTAL MICROBIOME ENGINEERING PROCESS

3.1 | Choosing an ecosystem function target (step 1)

Microorganisms perform various ecosystem functions that are critical to the long-term stabilization of C and could serve as microbiome engineering targets for climate change mitigation (Figure 2). Microbial activities stabilize C both directly, through the deposition and association of microbial products on soil particles (Cotrufo et al., 2013), and indirectly, through processes like modulating plant growth (Trivedi et al., 2020), contributing to nutrient cycling (Nelson et al., 2016), and bioremediation of pollutants (Dvořák et al., 2017).

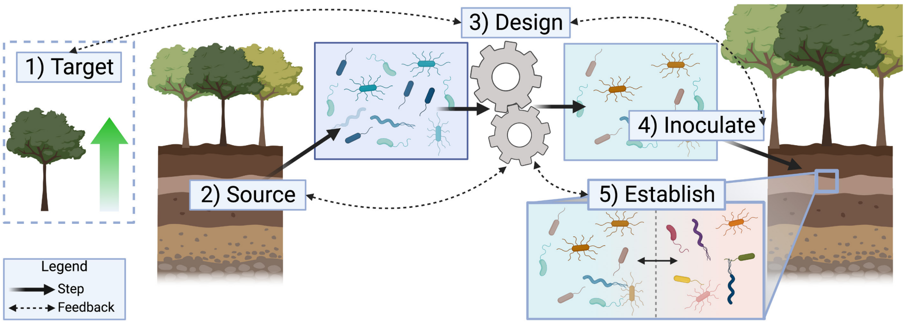

FIGURE 1 The modern process of environmental microbiome engineering. An ecosystem function target is chosen (1, such as plant growth promotion, as shown here), microbes are sourced (2, from an environmental site or from a strain collection), an inoculum is designed (3), the inoculum is delivered (4), and establishment is monitored (5, represented here by the mixing of inoculum (blue) and resident (brown) communities). While these steps are performed separately, considerations for some steps can feed back into the performance of others as indicated by the dashed lines.

TABLE 1 Examples of environmental microbiome engineering studies with details of each step of the environmental microbiome engineering process.

Study	Target	Source	Design	Inoculation	Establishment	Evaluation	Reference
Selection on soil microbiomes reveals reproducible impacts on plant function	Plant flowering time	Agricultural, forest, and grassland soils	Directed evolution	Soil mixed into sterile soil	Taxonomic and function (flowering time associated with inocula)	In vitro success	Panke-Buisse et al. (2015)
Increasing soil potential for carbon sequestration using microbes from biological soil crusts	C sequestration	Isolated from dryland soils	Assembled consortia	Liquid sprayed onto field soil	Taxonomic and function (increased soil C)	In vitro success	Kheirfam (2020)
Increased plant productivity and decreased microbial respiratory C loss by plant growth-promoting rhizobacteria under elevated CO_2	C sequestration	Isolated from soil	Single strain	Liquid into nutrient leached and dried soil	Taxonomic and function (increased soil C)	In vitro success	Nie et al. (2015)
Effectiveness of ecological rescue for altered soil microbial communities and functions	N cycling	Forest soils	Whole community	Liquid into irradiated soil	Taxonomic but not function (N did not increase)	In vitro failure	Calderón et al. (2017)
Atrazine removal from aqueous solutions using submerged biological aerated filter	Bioremediation of atrazine	Wastewater	Enriched consortia	Liquid into synthetic wastewater (containing atrazine)	Function (removal of atrazine, taxonomic not assessed)	In vitro success	Baghapour et al. (2013)
Development of an Efficient Bacterial Consortium for the Potential Remediation of Hydrocarbons from Contaminated Sites	Bioremediation of crude oil	Isolated from crude oil contaminated soil	Assembled consortia	Liquid into crude oil	Taxonomic (OD) and function (degradation of crude oil)	In vitro success	Patowary et al. (2016)
Artificial Selection on Microbiomes To Breed Microbiomes That Confer Salt Tolerance to Plants	Plant growth promotion under salt stress	Grass rhizosphere	Directed evolution	Liquid into sterilized soil	Function (increased plant biomass, taxonomic not assessed)	In vitro success	Mueller et al. (2021)
Potentials and limitations of mycorrhizal inoculation illustrated by experiments with field-grown cassava	Plant growth promotion with nonnative mycorrhizal	Cassava rhizosphere	Single strain	Spores into field soil	Function (increased root yield, taxonomic not assessed)	In situ success	Howeler and Sieverding (1983)
Potentials and limitations of mycorrhizal inoculation illustrated by experiments with field-grown cassava	Plant growth promotion with native mycorrhizal	Cassava rhizosphere	Single strain	Spores into field soil	No increase to root yield	In situ failure	Howeler and Sieverding (1983)
Enhanced Biodegradation of Alkane Hydrocarbons and Crude Oil by Mixed Strains and Bacterial Community Analysis	Bioremediation of crude oil	Isolated from crude oil contaminated soil	Assembled consortia	Liquid into crude oil	Taxonomic and function (degradation of crude oil)	In situ success	Chen et al. (2014)
Immobilized Synthetic Pathway for Biodegradation of Toxic Recalcitrant Pollutant 1,2,3-Trichloropropane	Bioremediation of 1,2,3-trichloropropane (TCP)	Type strains	Synthetic biology	Liquid into TCP	Function (degradation of TCP, no cells)	In vitro success	Dvorak et al. (2014)

TABLE 1 (Continued)

Study	Target	Source	Design	Inoculation	Establishment	Evaluation	Reference
Evaluation of microbial inoculants as biofertilizers for the improvement of growth and yield of soybean and maize crops in savanna soils	Plant growth promotion of soybean and maize	Isolated from soil and commercial products	Single strain	Liquid, powder, and peat into unsterilized and unsterilized soils	No increase to shoot yield. No increase in nitrogen or phosphorus content	In vitro failure	Laditi (2012)
How effective are "Effective microorganisms® (EM)"? Results from a field study in temperate climate	Plant growth promotion on potato, barley, alfalfa, wheat	Commercial product	Assembled consortia	Liquid sprayed onto field soil	No increase in microbial biomass nor plant yields	In situ failure	Mayer et al. (2010)

Note: Establishment refers to the long-term residence of inocula community members and impact to the ecosystem function target. Studies are evaluated as a success if the ecosystem function target has increased activity in inoculated conditions compared to controls.

Increasing the rate of C stabilization in ecosystems could effectively reduce the net fluxes of C from the biosphere to the atmosphere, minimizing the greenhouse effect. Understanding the mechanisms of these activities can aid in the choice of the specific ecosystem function to target for a desired microbiome engineering outcome, informing the choice of inocula design strategies. For both direct and indirect C stabilization activities, we review the natural role of microbial communities and the opportunities for microbiome engineering for climate change mitigation.

Soil microbes directly contribute to stable C pools through the deposition of necromass (e.g., high-molecular weight products, such as cell wall components) onto clay particles, which can protect C from subsequent degradation (Cotrufo et al., 2013; Yang et al., 2021) (Figure 2). Soil matrix-associated microbial products, which in isolation are relatively labile, can last in soils an order of magnitude longer than molecules that are more chemically recalcitrant but do not associate with soil minerals (Cotrufo et al., 2013). Microbes simultaneously expel C to the atmosphere (as CO_2) through respiration, the rate of which is determined by properties of the organism and its responses to the environment. Recently, the Y-A-S framework has been developed to conceptualize how microbes generally dedicate C toward yield (Y; growth), acquisition (A; e.g., extracellular enzyme production), and stress tolerance (S) (Malik et al., 2020). All three of these activities incur an energetic cost and result in respiration, but only yield-related activities directly set C down the path toward stabilization as necromass (Anthony et al., 2020; Malik et al., 2020). For example, when faced with moisture stress (drought), some organisms produce osmolytes to increase osmotic pressure resulting in a 90% reduction in growth yield as C is diverted from biomass production (Schimel et al., 2007). Given some input, the fraction of C that microorganisms assimilate into biosynthetic products versus the fraction that is respired is called carbon use efficiency (CUE) and can be used to compute C fluxes at the microorganism, community, and ecosystem scale (Manzoni et al., 2018). Designing inocula to increase community CUE could increase rates of C stabilization and remove atmospheric C over time.

Soil microbes also indirectly contribute to stable C pools through activities such as plant growth promotion, N cycling, and bioremediation that indirectly stabilize C and could serve as ecosystem function targets (Figure 2). Soil microbes play a key role in the development, growth, and protection of plants from pathogens (Trivedi et al., 2020). Plant microbiome assembly commences when seeds germinate and as plants grow, plants "exchange" C-based photosynthate for nutrients made available through microbial decomposition. This symbiosis enables plants and microbes to access otherwise inaccessible substrates while funneling C into stable reservoirs in the process. Soil microbes participate in the biogeochemical cycling of N, transforming N into bioavailable products (e.g., ammonium, nitrate, or amino acids and small peptides) that are available to microbes and plants, maintaining ecosystem processes that direct C into stable forms (Nelson et al., 2016). Soil microbes have been used for the bioremediation of sites contaminated with ecologically harmful pollutants, such as crude oil, phenol, and polycyclic aromatic hydrocarbons (Mrozik & Piotrowska-Seget, 2010), promoting

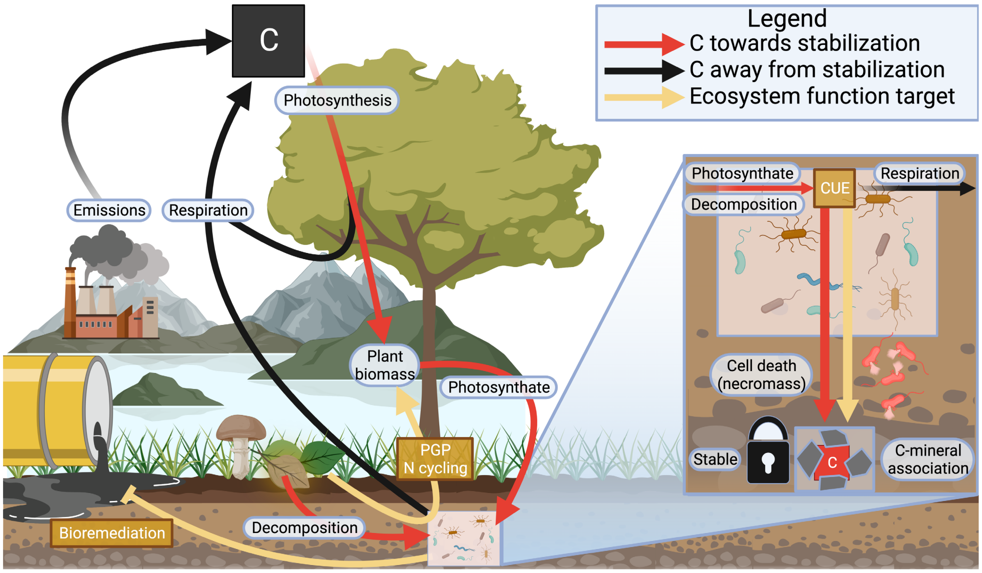

FIGURE 2 Soil microbiome ecosystem function targets for climate change mitigation. Different processes control the flow of C into the atmosphere away from stabilization (black arrows) and into the biosphere toward stabilization (red arrows). Atmospheric C flows to microbes where C can be deposited as necromass onto minerals and stabilized for hundreds to thousands of years (inset on right). Microbial community carbon use efficiency (CUE) is the fraction of uptaken C that becomes biomass. This C can then stabilize following cell death. Plant growth promotion (PGP), nitrogen (N) cycling, and bioremediation support microbial growth and indirectly promote C stabilization. These ecosystem functions (gold) can be targeted to increase C fluxes from the atmosphere to stable reservoirs in the biosphere.

otherwise inhibited ecosystem activities that stabilize C. Designing inocula to promote plant growth, N cycling, or bioremediation would ultimately benefit the C stabilizing activities of soil microbiomes.

Some ecosystem functions may be more susceptible to modification than others. In a study where different microbial communities were grown in the same metabolically complex environment, community activities converged for "core" functions, such as CO_2 production which showed little variation between communities, but diverged for "auxiliary" functions, such as the utilization of complex substrates like chitin which correlated with the abundance of chitinase-producing species in each community (Bittleston et al., 2020). This study concluded that "auxiliary" functions, which are performed by a narrow set of microbes (like those capable of chitinase production), are more sensitive to community membership than "core" functions (like respiration), which are redundant across all microbes. As a result, ecosystem function targets like bioremediation, which involve the degradation of a complex substrate (an auxiliary function), may be more susceptible to microbiome engineering, where the presence of the right microbe could be sufficient to eliminate a pollutant, while targets involving respiration like CUE (a core function) may be more challenging to modulate (Bittleston et al., 2020).

3.2 | Sourcing a microbial inoculum (step 2)

Designing a microbial inoculum requires sourcing individual microbial strains or whole microbial communities. Individual microbial strains can be purchased through vendors such as the nonprofit organization American Type Culture Collection (ATCC; atcc.org) (Research et al., 1996) or can be isolated from environmental samples. For design strategies that benefit from a diverse collection of microorganisms, whole community taxonomic diversity can be maximized by collecting samples hundreds to thousands of kilometers apart or during different seasons (Averill et al., 2019). Sourcing from the target environment allows for the inclusion of strains that are already adapted to site conditions (Policelli et al., 2020). Individual microbial strains are typically isolated by culturing environmental samples on different types of growth media at varying dilutions to enrich the abundance of different organisms. When grown on agar petri dishes, distinct colony forming units containing single strains can be isolated. Growth media can consist of single carbon sources, such as glucose or chitin, or can be more complex and less defined, such as soil extract media. The vast majority of microbes have yet to be isolated in culture (Lloyd et al., 2018), however, new protocols, such as ones to improve whole microbial community extraction from soil (Liu et al., 2010) and generate soil extract media (Nguyen et al., 2018), continue to enable researchers to cultivate previously uncultured organisms. Which strains (for bottom-up inocula design strategies where individual strains are assembled into a consortium) or whole communities (for top-down design strategies where microbial communities are enriched, Figure 3) to source is contingent upon the ecosystem function target and the microbial inoculum design strategy.

3.3 | Designing a microbial inoculum (step 3)

Microbial inocula are communities designed using one of a variety of strategies to be delivered into natural environments and confer

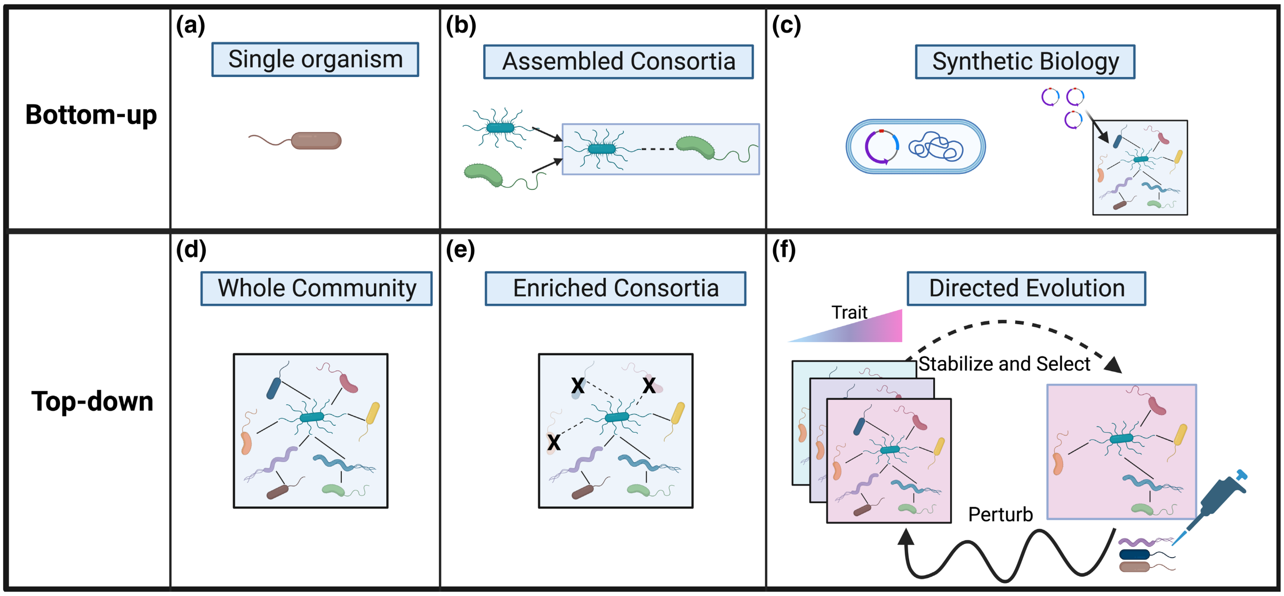

FIGURE 3 Inoculum design strategies. Bottom-up strategies (top row) include a single organism (a) an assembled consortium where individual organisms are pooled together (b), and synthetic biology where the genomes of individual organisms or whole communities can be engineered (c). Top-down strategies (bottom row) include a whole unaltered microbial community (d), an enriched consortium where growth conditions select for a subset of the community (faded organisms) (e), and directed evolution where communities are enriched under iterative rounds of artificial selection (f).

changes to ecosystem function targets. Inoculum design proceeds through the bottom-up assembly of small consortia or through the top-down enrichment of whole communities and either can include elements of synthetic biology (Lawson et al., 2019; Liang et al., 2022) (Figure 3). Top-down and bottom-up strategies to inocula design offer trade-offs in functional specificity, maintenance, and chance of establishment following inoculation (Table 2).

3.3.1 | Bottom-up versus top-down approaches

Bottom-up microbial inocula design consists of culturing a single strain or small consortia with members selected based on their individual roles as keystone taxa or their capacity to interact with one another. Keystone taxa are not necessarily the most abundant taxa within a community, but they do co-occur with many community members and are believed to be drivers of community structure and function (Banerjee et al., 2018). However, there is skepticism about the physiological role of keystone species and whether they alone are able to persist over longer, ecologically relevant timescales following inoculation (Albright et al., 2021) or whether the traits of keystone taxa are maintained in the context of a new community, such as when delivered as an inoculum. Recent opinions have advocated for assembling consortia more methodically, by characterizing species interactions through culturing assays and computational simulations in order to incorporate these community dynamics into consortium design (Lawson et al., 2019; Liang et al., 2022; Rodríguez Amor & Dal Bello, 2019).

Another approach for constructing consortia is to enrich a community from the top-down, as opposed to assembling isolated strains from the bottom-up. Top-down design proceeds by culturing an existing microbial community in selective conditions that result in a new community with a different taxonomic and functional composition. Unlike communities designed with bottom-up strategies, top-down communities emerge through the selective pressures of their growth conditions and do not require a deep understanding of the mechanisms governing the ecosystem function target of interest which can simplify design (Lawson et al., 2019). In addition, by starting with stable whole communities, top-down strategies implicitly account for species interactions, the assembly of which are often the goal and challenge of bottom-up approaches. Species interactions have been shown to be a critical consideration for establishment both from an ecological perspective, since cooperative and competitive dynamics are drivers of species survival in a community (Albright et al., 2021). Interactions are also important from an evolutionary perspective, because species have been shown to evolve to utilize each other's waste products (i.e., cross-feed) and display higher productivity when grown in communities compared to monoculture (Lawrence et al., 2012). More interconnected communities are expected to better establish, such that top-down design may be more promising for conferring long-term ecosystem change generally (Albright et al., 2021). In a simulation that compared how different communities responded to a coalescence event (mixing of entire microbial communities) (Rillig et al., 2015), communities designed from the top-down resisted compositional changes more than communities designed from the bottom-up (Chang et al., 2021) suggesting that inocula designed from the top-down may be more likely to establish and persist in a new environment than those designed from the bottom-up. However, due to the stochasticity of microbial

TABLE 2 Qualities of bottom-up and top-down inoculum design strategies.

Quality	Bottom-up	Top-down
Definition	Single strain or collection of isolated strains	Enriched community
Species interactions	Strains of assembled consortia do not necessarily interact	Stable interactions emerge through culturing
Mechanistic insight	Can use known mechanisms to design consortia and well-defined consortia allows for mechanistic analysis	Does not require understanding of mechanism a priori. Analysis on emerged communities can be performed to uncover mechanisms
Maintenance	Well-defined consortia are relatively trivial to assemble and maintain	Long-term cultivation of complex communities can be challenging/noisy
Establishment	Can establish where open niches are known a priori	Higher efficacy in modifying target ecosystem functions in situ

community dynamics, larger (typically top-down) communities are more challenging to maintain in culture than smaller (typically bottom-up) ones (Liang et al., 2022) presenting significant challenges to developing consortia at industry scales.

An overwhelming majority of microbiome engineering studies design consortia from the bottom-up, by choosing single strains or assembling small consortia based on functional traits, rather than from the top-down, by culturing whole microbial communities (Hoeksema et al., 2010). Below, we synthesize concepts from these engineering studies and showcase recent successful examples with climate change mitigation applications.

3.3.2 | Bottom-up approaches

3.3.2.1 | *Single organism*

The simplest inoculum is a single microorganism, typically one that is believed to be a keystone taxon within a given ecosystem and relevant to the ecosystem function target (Figure 3a). For example, one greenhouse study found that the addition of the plant growth-promoting rhizobacteria *Pseudomonas fluorescens* to a common grass species increased plant productivity without increasing microbial respiration under elevated CO_2 treatment (Nie et al., 2015). This study used sterilized (nutrient leached and then dried) soils, so whether this effect would persist in natural communities is unclear. Another study found that inoculating field-grown cassava with the arbuscular mycorrhizal fungi (AMF) *Glomus manihotis* had no significant effect to plant growth yield in soils well populated with AMF, while soils that were poorly populated with AMF showed significant responses, even outcompeting inoculations with native AMF (Howeler & Sieverding, 1983) (Table 1). Many plant species have obligate symbioses with ectomycorrhizal fungi and field inoculations with single (and multiple) strains have proven successful in restoring forests following disturbances such as mining, erosion, fire, or pollution (Policelli et al., 2020). These results suggest that single member inocula may be well suited for applications where an open niche is known a priori, whether due to natural ecological processes or disturbances.

Nevertheless, single member inocula may be less effective at impacting natural ecosystems than multi-species inocula. A meta-analysis investigating the effect of mycorrhizal fungi inocula on plant growth provides some perspective on the frequency of these inocula design strategies and their efficacy (Hoeksema et al., 2010). In one analysis of 306 studies, 264 were single strain inocula, 25 were small consortia, and 17 were whole soil. Small consortia and whole soil inocula had double the effect on plant response than single strain inocula, which only had an effect similar to that of uninoculated controls (Hoeksema et al., 2010).

3.3.2.2 | *Assembled consortia*

A collection of isolated microorganisms can be pooled to form an assembled consortium (Figure 3b). Like single organism innocula, assembled consortia commonly involve keystone species, with an aim to reflect the diverse composition and function of microbial communities. Assembled consortia may be capable of performing the same functions as whole communities with higher efficiency through metabolic division of labor (Liang et al., 2022). Techniques for assembling consortia range from mixed culturing of isolates to sophisticated bioreactors that attempt to recapitulate the natural spatial structuring of soil microbial communities (Ben Said & Or, 2017).

Typically, bottom-up consortia have been assembled with microbes that are independently high performing in a trait of interest. For example, one study isolated bacteria from dryland biocrusts, assembled consortia of the most abundant microbes and those most implicated in the literature to improve soil qualities, and found that after 60 days, inoculated field soils sequestered significantly more C than uninoculated controls in the laboratory (Kheirfam, 2020). Other laboratory studies assembled consortia of petroleum-degrading bacteria isolated from multiple contaminated soils; one study found a consortium that was able to degrade 84% of crude oil in culture after 5 weeks (Patowary et al., 2016) and another study assembled a two-strain consortia that outperformed its source community in crude oil degradation (Chen et al., 2014) (Table 1). Generally, consortia that are functionally effective, but may face challenges with establishment, could still provide climate change mitigating effects if implemented in controlled processing environments where contaminated materials could be treated ex situ with consortia and then returned.

Consortia can also be strategically assembled to include members with known interactions. Metabolizing nutrients through cross-feeding interactions can lower the overall metabolic cost of living for individual organisms when compared to a single organism performing the same metabolic process (Tsoi et al., 2018), and potentially lead to environment-dependent obligate interdependence (Klitgord & Segrè, 2010; Shou et al., 2007). The efficiency of this metabolic division of labor has been hypothesized to explain the emergence of cross-feeding interactions (Thommes et al., 2019) and suggests that assembled consortia may be more efficient and establish better in a resident community than single organism inocula performing the same function. However, assembling consortia require searching a large combinatorial space of community membership, a challenge that can be ameliorated by top-down strategies, which search this space implicitly.

3.3.3 | Top-down approaches

3.3.3.1 | *Enriched consortia*

Enrichment treatments can select for microbial communities that specialize in certain functions (Figure 3e). For example, an enriched consortium was developed on wheat grass to specialize in lignocellulose degradation, which the authors proposed could be used for transforming agricultural waste into commercial products (Díaz-García et al., 2021). The production of such "biocommodities" has been advocated as a market-based climate change mitigation strategy (Jatain et al., 2021). Another study enriched a community of bacteria on low concentrations of the ubiquitous herbicide atrazine and found that the resulting community was able to remove up to 96.1% of a higher concentration of atrazine in 24 h (Baghapour et al., 2013) (Table 1). In addition to selecting taxa adapted to particular conditions, enriched consortia have been shown to select for taxa that were lowly abundant or undetected in source communities offering a means of working with otherwise neglected microbes (Naylor et al., 2020). While easier to design, enriched consortia lack the flexibility of assembled ones, which can be arbitrarily modified through the addition of more organisms to attempt boosts in function or establishment.

3.3.3.2 | *Directed evolution*

Directed evolution reconciles issues with enriched and assembled consortia by incorporating elements of assembly and enrichment to construct a community with the optimal capacity to perform a particular trait (Figure 3f) (Arias-Sánchez et al., 2019; Sánchez et al., 2021; Xie & Shou, 2021). Directed evolution of a microbial community is a process akin to selective breeding, where iterative enrichment of naturally variable communities and artificial selection on a trait of interest gives rise to a community with increased expression of that trait (Arias-Sánchez et al., 2019; Sánchez et al., 2021; Xie & Shou, 2021). Examples of microbiome directed evolution include communities that reduce CO_2 emissions (Blouin et al., 2015), degrade 3-chloroalanine (Swenson, Arendt, & Wilson, 2000), increase plant biomass (Swenson, Wilson, & Elias, 2000), confer plant salt tolerance (Mueller et al., 2021), enhance chitinase activity (Wright et al., 2019), and regulate the flowering time of different plant species (Panke-Buisse et al., 2015) (Table 1).

In a recent review (Sánchez et al., 2021) and simulation study (Chang et al., 2021) Sánchez et al. (2021) analyzed previous directed evolution studies and proposed a framework for performing it successfully. There are four keys to successfully directly evolving microbial communities: (1) the trait of interest is heritable, (2) the trait of interest displays natural variation among communities, (3) the communities reach "generational stability," and (4) the communities are perturbed to enhance taxonomic and functional variation. Microbial directed evolution studies from the past 20 years (Blouin et al., 2015; Mueller et al., 2021; Panke-Buisse et al., 2015; Swenson, Arendt, & Wilson, 2000; Swenson, Wilson, & Elias, 2000) have performed iterative artificial selection, but have rarely directly considered generational stability and perturbations in their experimental design. Generational stability is achieved by incubating communities for long enough that after each passage, they converge to the same taxonomic and functional composition from one generation to the next, which tightens the relationship between community structure and function (Sánchez et al., 2021). Generational stability can take between 12 and 21 days, depending on the environment (Bittleston et al., 2020; Goldford et al., 2018; Zegeye et al., 2019), and longer incubation periods (2 and 6 months) generate more community diversity than short incubation periods (1 week) in soil (Čaušević et al., 2022). Perturbing these stable communities with the delivery of a diverse pool of species or nutrients regenerates trait variation and increases the chances of finding a community with even higher trait expression on the subsequent selection cycle.

In practice, the directed evolution of microbial communities proceeds with cyclic iterations of (i) seeding, (ii) stabilizing, (iii) ranking, and (iv) perturbing communities (Sánchez et al., 2021). Seeding (i) refers to the initial plating of various microbial communities, which ideally differ in composition and community-level expression of the trait of interest. Once plated, communities are propagated in serial batch culture until they reach generational stability (ii), when the successional dynamics of a child community matches those of its parent batch. Following generational stability, communities are ranked (iii), or artificially selected, based on their expression of the trait of interest. If the performance of these selected populations is satisfactory, then the inoculum design can conclude; if not, each of the top ranked communities can be perturbed (iv) and the entire process can be reiterated using these communities as new seeds (i) in hopes of directly evolving communities with higher performance. Perturbations to the top ranked communities, such as a shift in resources or a spike-in of new microbes, induce variability that can increase the chances of finding an even more desirable community in the next cycle. These communities emerge through shifts in community composition and through evolution of community members. Regardless of which of these forces dominates, directed evolution can generate communities with improved expression of a trait, as long as the trait of interest is heritable. However, since directly evolved communities emerge through complex dynamics, their assembly can be noisy (the activity of the trait of interest does not always increase monotonically) and understanding which ecosystem target functions are susceptible to directed evolution is a topic of ongoing research (King et al., 2022).

For appropriate ecosystem target functions, directed evolution manages to maximize functional and, as a top-down strategy, establishment traits. In simulations using a consumer-resource model (Marsland et al., 2020), microbial communities were designed top-down with directed evolution or bottom-up by assembling small consortia of individual top performing taxa (Chang et al., 2021). While assembled consortia started with higher activity than directly evolved communities, upon facing a simulated coalescence with a resident community, the assembled consortia collapsed, while directly evolved communities remained unperturbed. This result highlights an advantage of top-down design approaches more broadly: whole communities may be more robust (resistant) than small consortia to perturbations or inoculation into a resident community.

3.3.3.3 | *Whole community*

The easiest design strategy is the use of a whole microbial community sourced from a natural environment without any in vitro modifications (Figure 3d). Although an environmentally sourced whole community may display lower activity of an ecosystem function than a directly evolved one, the activity displayed by these natural communities may be sufficient for intended environmental impacts.

Previous studies have sourced communities and isolates from particularly harsh environments to serve as inocula for other communities experiencing similar stresses (Rodriguez & Durán, 2020). In one experiment, willow cuttings growing in petroleum-contaminated soils were inoculated with rhizosphere soil from a field willow that grew well in contaminated soil or with bulk soil from a willow that died (Yergeau et al., 2015). Unexpectedly, cuttings that received the bulk soil from dead willows grew larger than those that received rhizosphere soils from optimally growing willows, despite containing equal microbial biomass. Initially, bulk soil inocula had a greater bacterial and fungal diversity than the rhizosphere inocula, leading the authors to consider if inocula diversity offers more opportunities for fitness than a less diverse pool of niche-specialized microbes. Following 100 days, there was no difference in rhizosphere microbial diversity between treatments despite differences in growth, providing support for the link between initial inocula diversity and plant growth promotion.

Other studies have sourced communities from stable ecosystems to restore degraded ones. In two experiments, degraded grasslands received a soil inoculation from different donor sites following topsoil removal, resulting in donor site-specific plant community compositions (Han et al., 2022; Wubs et al., 2016) and significant increases to C, nitrogen, phosphorus, and root biomass compared to controls after 3 years (Han et al., 2022). These studies highlight how, in addition to targeting single ecosystem functions (as is typical in microbiome engineering projects), whole community inocula can readily transfer far more intricate phenotypes than other inocula design strategies, such as multiple aspects of plant health and diversity. Without the need to enrich communities in vitro, this strategy trades-off maximizing phenotypic expression for ease of design without sacrificing establishment traits.

3.3.4 | Synthetic biology

Recent advances in synthetic biology have enabled the precise modification of microbes and their traits toward climate change mitigation applications (Cambray et al., 2011; DeLisi, 2019; Miller et al., 2020; Schwander et al., 2016; Voigt, 2006). Since 1987, genetically modified microorganisms have been tested and used in agriculture to enhance plant growth-promoting traits (Ke et al., 2021). Strategies involve isolating, engineering, and reintroducing engineered microbes to a resident community or engineering a broad range of microbes in situ through horizontal gene transfer (Ke et al., 2021). Synthetic biology approaches are now expanding to other ecosystem function targets, including the design of traits that do not exist naturally. One study constructed a synthetic pathway using three enzymes (one engineered) from two different organisms to transform 1,2,3-tricholopropane, an anthropogenic groundwater contaminant, into the nontoxic compound glycerol over 2.5 months with 78% efficiency (Dvorak et al., 2014). In another experiment with profound implications, *Escherichia coli*, an obligate heterotrophic microbe, was genetically engineered to be autotrophic and generate all of its biomass C from carbon dioxide (Gleizer et al., 2019). Not only does this experiment represent a landmark for the field of synthetic biology, but it enables the development of new C sequestration technologies.

The safety of introducing genetically engineered elements into the field is a delicate topic and regulation of these new technologies is critical (DeLisi, 2019; Ke et al., 2021). In addition, genetically modified microbes face the same challenges of establishing in resident communities as other single strain inocula. For this reason, microbial synthetic biology approaches may be most impactful in industrial or highly controlled microbiome engineering applications, such as the production of biocommodities and agriculture.

3.4 | Inoculum development and delivery (step 4)

Following design, microbial inocula are developed and delivered into the environment. Developing inocula requires choosing a delivery substrate (or carrier) that balances many factors such as manufacturability, maintenance of inoculum community, scalability, and chances of establishment (Johns et al., 2016; Malusá et al., 2012). The trade-offs between these factors vary by the type of carrier that inocula are developed in and delivered by, which include various options, from solid to liquid substrates such as soil, soil-like matrices, soil slurries, powders, polymers, seed coatings, encapsulated cultures, as spores (for sporulating organisms), or liquid culture (Malusá et al., 2012; Santos et al., 2019). Solid inocula carriers, like soil, have been shown to increase chances of establishment compared to liquid media (Howard et al., 2017) due to increased contact time between inocula and recipient soils (Trexler & Bell, 2019). Solid carriers have also been used to overcome common challenges with maintaining inoculum community composition and long-term storage, because they can be lyophilized (in the case of cultures (Malusá et al., 2012)) or frozen (in the case of soil (King et al., 2022)) after inoculum design. However, solid carriers are generally more challenging to manufacture and deliver at scale (Santos et al., 2019). By contrast, culturing inocula in liquid carriers is fast, scalable, has a longer shelf life, and can be easier to deliver to the environment than solid carriers (Allouzi et al., 2022). However, liquid can restructure inocula (Čaušević et al., 2022; Howard et al., 2017; Zegeye et al., 2019) and reduce the capacity for establishment into a solid, spatially structured target environment like soil. In one study, soil communities were enriched with chitin and then cultured with its monomer N-acetylglucosamine in sterile soil and liquid minimal media at serial dilutions (Zegeye et al., 2019). Overall, liquid communities exhibited lower diversity; however, they displayed less volatile dynamics than soil communities over time, which may prove beneficial in maintaining inocula communities at industrial scales (Howard et al., 2017; Zegeye et al., 2019). Many of the same bacterial phyla were shared between treatments, especially at lower dilutions (Zegeye et al., 2019) or with longer incubation times (Čaušević et al., 2022). While scaling the production of inocula in liquid will impose selective pressures, these results showed that liquid cultures stabilize more quickly than those grown in a solid substrate and that nutrient supplementation can preserve the deeper lineage structure of communities (Zegeye et al., 2019). Nutrient supplementation before, during, and after inoculation has also been shown to increase the chances of establishment (Li & Stevens, 2012).

The dispersal capacity of the inoculum community members is a valuable consideration for determining inoculum delivery frequency and density. Microorganisms utilize a diversity of dispersal strategies that influence establishment by enabling inocula members to spread further, locate open niches, and mediate the activity of ecosystem function targets (King & Bell, 2022). Even nonmotile microbes have been observed to engage in "hitchhiking," where they utilize motile partners in order to disperse (Muok & Briegel, 2021). Leveraging these dispersal strategies in inocula design could benefit industrial scale microbiome engineering projects which seek to inoculate large geographic areas (King & Bell, 2022). Considering the options for inoculum development and delivery can feedback into inoculum design in order to improve the chances of delivering communities designed in the laboratory to the field.

3.5 | Establishment and impact (step 5)

Microbiome engineering requires new microorganisms to establish into an existing community in order to induce targeted changes to community activities. A successful inoculant is one that modulates an ecosystem function optimally and stably. While optimizing function has been a common goal of many microbiome engineering studies, inoculum establishment has been less well studied (Albright et al., 2021; Kaminsky et al., 2019). An inoculum establishes in an environment when it coalesces with the resident community, restructuring its taxonomic and functional composition (Rillig et al., 2015, 2016). If an inoculum establishes, its effects may persist, leading to longer-term ecosystem changes. Importantly, the properties of a microbial community that determine its activity (functional traits) are not necessarily related to its chance of establishment (establishment traits). Establishment requires that niches in the environment match those of the inoculum (environmental filtering) and that the inoculum community successfully coalesces with the resident community (biotic interactions) (Albright et al., 2021; Rillig et al., 2015, 2016) (Figure 4).

More complex environments are capable of sustaining a wider variety of microbial communities, enabling the establishment of inocula within natural (complex) ecosystems. A recent elegant study explored the strength of environmental filtering by culturing microbial communities from different sources (soils and leaves) in the same

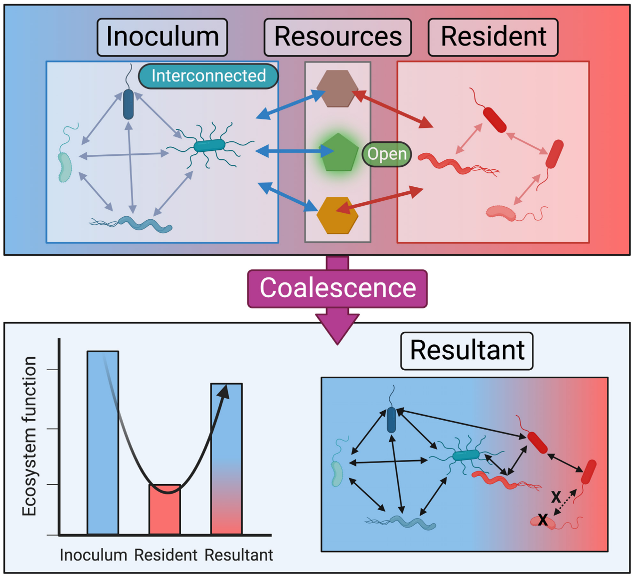

FIGURE 4 Properties of establishment. Biotic interactions and environmental filtering are key determinants of establishment. When two communities mix (inoculum in blue; resident in red), the community with more metabolic interconnections (more interactions among inoculum members) and preferences for open niches (inoculum consumption of green resource) is more likely to comprise a larger fraction of the resultant community (more blue in resultant; extinction of one resident species marked by X and dashed line). Here, the inoculum community establishes and imparts its expression of a trait of interest, resulting in a higher level of ecosystem function.

simple environment (single C source minimal media) and found that communities clustered taxonomically by growth environment, independent of where they were sourced from (Goldford et al., 2018). At first, this result may seem to threaten the ability of inocula to establish: how can a new community establish in an environment if the environment determines community composition? However, a similar experiment that cultured microbial communities from different pitcher plants in the same *complex* environment (acidified cricket media to mimic the native pitcher plant environment) found that taxonomic composition and substrate use remained distinct (unlike with simple environments) (Bittleston et al., 2020). This combination of results suggests that the strength of environmental filtering is contingent on the complexity of the environment (Bittleston et al., 2020); as more niches are available in an environment, more diversity can be accommodated, such as the presence of specialists who can occupy those niches (Bittleston et al., 2020). Because natural environments like soils are metabolically complex (compared to single C media, for example), well-designed inocula should in principle stand a chance of establishing and restructuring native communities without returning communities to their original state, as would happen in simpler laboratory environments.

There is growing support for the relationship between inocula community complexity and establishment. Simulations of microbial community coalescence have revealed that larger, diverse, and more metabolically cooperative communities comprise a larger fraction of the resultant community (Lechón-Alonso et al., 2021; Vila et al., 2019) suggesting that these properties are critical to establishment. Increasing or maintaining high levels of biodiversity, in particular, is a common goal for impactful ecosystem management, including recent calls for defending Earth's microbiomes which highlight the relationship between terrestrial microbial biodiversity and ecosystem function (Averill et al., 2022). Increased diversity has been shown to support certain ecosystem functions, such as functions that are narrowly distributed across microbial kingdoms, such as nitrification, denitrification, and methanogenesis (Trivedi et al., 2019). Increasing soil microbiome diversity can also correlate with multifunctionality, or communities with increased capacity to perform multiple ecosystem functions, such as C sequestration (Wagg et al., 2014). In addition, increased microbial community diversity is often associated with increased resistance to invasion and resilience in the face of environmental disturbances, both of which are critical for establishment and maintenance of ecosystem functions (Shade et al., 2012). Along with inoculum diversity and metabolic interconnectedness, invading species with preferences for open niches have been shown to have higher invasion success, particularly in metabolically complex environments, suggesting that preference for open niches also promotes establishment (Eisenhauer et al., 2013).

Collectively, these studies suggest that inocula that incorporate diversity (Vila et al., 2019), metabolic interconnectedness (Lechón-Alonso et al., 2021), and preferences for open niches (Eisenhauer et al., 2013) are more likely to establish in resident communities (Figure 4). Specifically, a community of noncompeting specialists may be ideal for establishment, as its members would theoretically be able to utilize resources efficiently and either integrate with or outcompete resident community members (Lechón-Alonso et al., 2021). We reemphasize recent opinions that claim that prioritizing biotic interactions in inoculum design is more likely to lead to establishment than increasing inoculation dose and frequency (propagule pressure), which has been shown to have minimal impact (Albright et al., 2021).

Establishment is not necessarily sufficient to impart changes to the ecosystem function target. A restructured microbial community can exhibit functional redundancy with its original community, where the taxonomic composition of the community changes, but the functional activity remains the same (Allison & Martiny, 2008; Louca et al., 2018). As a result, it is important to monitor changes in both community structure and the ecosystem function target to ensure that both change in the intended direction (Kaminsky et al., 2019; Liu et al., 2022). Changes to ecosystem functions may also not necessarily require establishment. In one laboratory study, "transient invaders," microbes which temporarily invade but do not establish, were shown to stably shift environmental pH and the community composition despite their short residency (Amor et al., 2020).

4 | ENVIRONMENTAL MICROBIOME ENGINEERING SAFETY

Microbiome engineering, like any environmental intervention, holds the potential to present debilitating unintended consequences. While abiotic environmental stresses are chiefly associated with destabilizing microbial communities (Hernandez et al., 2021; Schimel et al., 2007; Shade et al., 2012), it is possible that new taxa could destabilize a microbial community or ecosystem. As just mentioned, even transient invasions can modify environmental features, like pH (Amor et al., 2020), and restructure microbial communities with potential consequences to key ecosystem functions. Even when deliberate, nonnative fungal inoculations have been reported to both promote and impede plant growth, increase or decrease the concentration of toxic compounds, and enhance or deplete soil C storage, highlighting the importance of testing inocula in target environments before widespread distribution (Policelli et al., 2020; Schwartz et al., 2006).

Recent critiques of microbiome engineering have expressed concerns surrounding threats to biodiversity and the subsequent hampering of ecosystem processes. A recent meta-analysis reports that 80% of soil inoculations result in long-term taxonomic shifts to microbial communities which leads the authors to raise concerns about the potential impacts to biodiversity and ecosystem functioning, despite not directly reporting effects to these features (Mawarda et al., 2020). While threats to biodiversity and ecosystem functioning should not be taken lightly, it is important to note that taxonomic shifts occur routinely in ecosystems through natural coalescence events (Rillig et al., 2015), such as dispersal after rain, and these shifts do not necessarily alter community diversity nor ecosystem functioning detrimentally since new members, if they establish, can occupy existing niches (functional redundancy). The replacement of native taxa with selected mixtures of naturally occurring nonpathogenic strains does not necessarily disrupt ecosystems and can stably modify ecosystem functions. For example, in a greenhouse study, clover grown in natural soil under drought conditions was inoculated with native and nonnative fungi and bacteria and all microbial inocula were effective in increasing plant drought tolerance independent of whether the taxa were native or not (Ortiz et al., 2015). Designed inocula should be sourced locally when possible, trialed in vitro with target soils and screened for pathogens before any large-scale implementation is considered, but ultimately, more research is needed to design appropriate environmental microbiome engineering safety protocols (Schwartz et al., 2006).

5 | CONCLUSION

Environmental microbiome engineering offers an exciting opportunity to mitigate the effects of global climate change by modulating microbially regulated ecosystem functions. Carbon use efficiency, plant growth promotion, nitrogen cycling, and bioremediation serve as microbiome engineering targets to either directly or indirectly contribute to the stabilization of C and combat the greenhouse effect. Inocula design for microbiome engineering that includes considerations for the functional traits associated with these targets, as well as establishment traits (diversity, metabolic interconnectedness, and preferences for open niches) will have maximum efficacy. By inherently optimizing functional and establishment traits, directed evolution may be the ideal inoculum design strategy and deserves further exploration in this space. Where many inocula design strategies can struggle with balancing functional and establishment traits, directed evolution can generate inocula that express high activity of an ecosystem function target and, as a top-down strategy, inherently possess properties (diversity and interconnectedness) that maximize the chance of establishment. In this review, we focus on biotic approaches to microbiome engineering based on microbial inoculations. Further research is needed to understand how abiotic modifications during inoculum delivery, such as nutrient supplementation (Li & Stevens, 2012), impact the efficacy of the inoculum design strategies discussed here. Overall, experimental evidence and emerging principles in microbial ecology suggest that, despite the complexity of the Earth's microbiomes and the ecosystems they regulate, environmental microbiome engineering has the potential to contribute as another stable, long-term intervention for combating global climate change.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study

ORCID

Michael R. Silverstein https://orcid.org/0000-0003-2205-3624
Daniel Segrè https://orcid.org/0000-0003-4859-1914
Jennifer M. Bhatnagar https://orcid.org/0000-0001-6424-4133

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