Microbial invasions and inoculants: a call to action

Joshua Ladau[1,⋆], Ashkaan K. Fahimipour[2], Michelle E. Newcomer[3], James B. Brown[1,4,5], Gary J. Vora[6], Melissa K. Melby[7,8], and Julia A. Maresca[9]

[1]*Computational Biosciences Group, Lawrence Berkeley National Laboratory, Berkeley, California, USA*

[2]*Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida, USA*

[3]*Earth and Environmental Sciences Area, Lawrence Berkeley National Laboratory, Berkeley, California, USA*

[4]*Statistics Department, University of California, Berkeley, California, USA*

[5]*Arva Intelligence, Inc., Salt Lake City, Utah, USA*

[6]*Center for Bio/Molecular Science and Engineering, U.S. Naval Research Laboratory, Washington DC, USA*

[7]*Department of Anthropology, University of Delaware, Newark, Delaware, USA*

[8]*Canadian Institute for Advanced Research, Toronto, Canada*

[9]*Department of Civil and Environmental Engineering, University of Delaware, Newark, Delaware, USA*

[⋆]*Correspondence to: jladau@gmail.com*

Abstract

The use of non-genetically modified microbial inoculants for beneficial purposes in agriculture, bioremediation, medicine, and infrastructure is increasing. The intentional introduction of plants and animals for similar purposes has a long history, but despite successes, has resulted in thousands of plant and animal species becoming invasive, with catastrophic consequences for the environment, public health, and society. Hundreds of microbial invasions are known, and although microbial inoculants can provide benefits, they have similar potential to negatively impact ecosystems. Little action has been taken to guard against the threat of microbial invasions from non-genetically modified microbial inoculants. Now is the time to develop an effective research and management infrastructure for these microbial inoculants to avoid catastrophic outcomes similar to those caused by intentionally-introduced plants and animals. Here, we propose a unified research and management approach to spur action by regulators and practitioners. Three aims need to be addressed: developing (1) a coherent mechanistic understanding of how microbial inoculants effect invasions, (2) predictive models forecasting which microbes pose risks of invasion, and (3) effective management strategies. To guide mechanistic understanding, we develop seventeen key hypotheses. For predictive modeling, quantitative trait analysis and risk maps will be critical. Management strategies will depend on both understanding and predictions, but prevention rather than eradication or control of invasive microbes is likely to be most effective, and a precautionary regulatory approach should immediately be applied to inoculants. Multiple data types will be instrumental for understanding and predicting which microbial inoculants have invasive potential: experimental data from microcosm and mesocosm experiments, and large-scale observational data from microbial surveillance. Both phenomenological and mechanistic modeling approaches will be key in achieving these fundamental and applied research aims. Moreover, each stage of the invasion process — transport, establishment, spread, and impacts — often need to be investigated separately. The unified approach developed here provides a roadmap for developing a research and management infrastructure to guard against the threat of microbial invasions from non-genetically modified microbial inoculants.

KEYWORDS: Invasive species | Microorganisms | Microbial inoculation | Microbial amendment

Introduction

In the late 1800s, the chestnut blight fungus, *Cryphonectria parasitica*, accompanied Asian chestnut trees imported to the United States, where it infected American chestnut trees. Within twenty years, it decimated forests and the timber industry in the eastern United States, which have never recovered (*1*). Thousands of other **invasive species**† are documented, many microbial and many caused by the intentional introduction of organisms for food, crops, biocontrol, conservation, horticulture, and other purposes (*2*, *3*). Now we are inoculating **microbes** into many environments in enormous numbers – over 800 patents in China for agricultural microbial inoculants, over 200 million doses of agricultural **microbial inoculants** applied between 2014 and 2019 in

†Bold terms defined in the glossary.

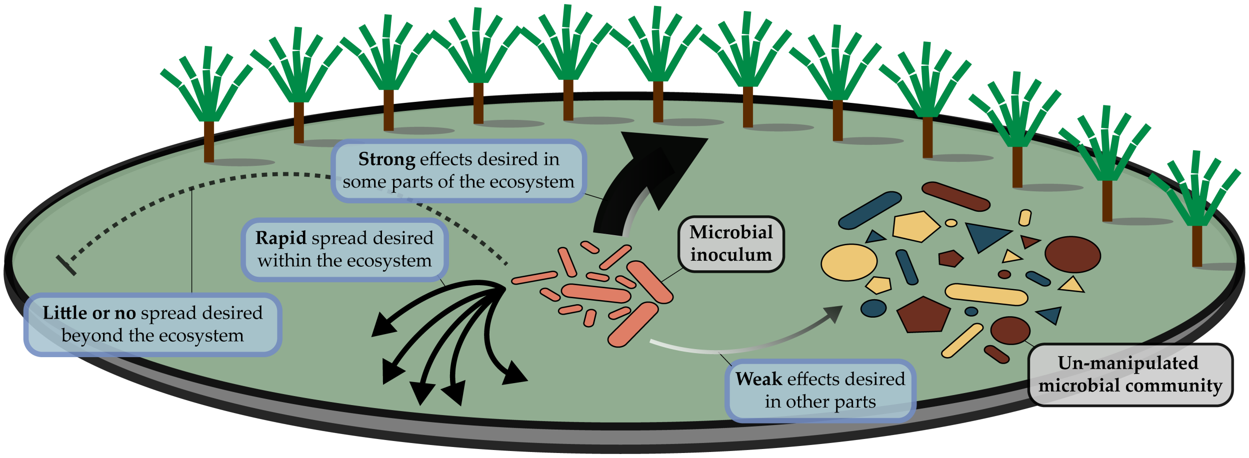

Figure 1: **The *Goldilocks Zone* of microbial inoculants.** Potentially conflicting outcomes characterize many microbial inoculants. A hypothetical agricultural example is illustrated, wherein a microbial inoculation is expected to simultaneously strongly benefit agricultural crops and spread widely through the system, while also have a minimal impacts on other components of the ecosystem (e.g., resident microorganisms, animal species) and remain confined within community borders.

Brazil (*4*), and in some cases up to billions of cells per cubic centimeter in soil – to fertilize agricultural soils, remediate contaminated groundwater, control eutrophication, precipitate minerals, restore ecosystem functions, and improve human health (*5*–*8*). Because microbes are autonomous, self-replicating systems, they are attractive tools for solving these problems. However, given the astronomical numbers and myriad environments that are intentionally inoculated with microorganisms, these manipulations risk creating microbial invasions with cascading effects throughout ecosystems, in much the same way that catastrophic invasions of plants and animals have long been precipitated by intentional introductions by humans (*9*–*13*).

Globally, invasive plants, animals, and microbes dominate among the threats to biodiversity and ecosystems, (*14*) inflicting over $1TUSD in damage annually (*15*). Despite over a century of extensive efforts to predict, prevent, eradicate, and control them, invasive plants and animals continue to multiply and impact the environment, infrastructure, public health, and agriculture (*16*, *17*). Moreover, despite numerous documented detrimental invasions of microbes, due to both intentional and unintentional introductions (Box 1), our understanding of the mechanisms, prediction, control, and complete extent of microbial invasions remains nascent (*18*–*22*).

Microbial inoculants pose a special challenge: microbes are sought that effectively exist in a "**Goldilocks Zone**," where they become established, but do not spread and have unintended impacts beyond their site of introduction (Figure 1). On one hand, microbial inoculants must modify existing communities, at least temporarily, to have the desired outcomes (*4*, *12*). Simultaneously, microbes in microbial inoculants should not become too established or spread beyond their location of introduction, where they may become invasive and have negative unintended consequences (*10*). A deeper understanding of the potential for microbial inoculants to cause invasions is needed to balance their benefits with their risks of causing harmful effects. However, little research or regulatory action has been taken (*10*). Here, we present a roadmap for developing (1) a mechanistic understanding of how microbial inoculants can cause microbial invasions, (2) predictions of which microbial taxa have the potential to become invasive and where, and (3) management strategies to mitigate these threats. We develop seventeen testable, key hypotheses for developing a mechanistic understanding, and identify eight management, data, and modeling priorities to guide the development of approaches to predict and manage **invasive microbes**. Our framework highlights interdisciplinary, immediate research needs essential for actionable approaches and policy to guard against microbial invasions.

Box 1: Microbial invasions

Anthropogenically introduced microbes have a long history of becoming invasive. These microbes fall into two categories: microbes without direct impacts to human health, and human pathogens. Non-pathogenic microbial invasions initiated by deliberate introductions include ectomycorrhizal symbionts of *Pinus* spp. trees that were intentionally introduced in the Southern Hemisphere and Hawaii, resulting in both the ectomycorrhizal symbionts and *Pinus* spp. becoming invasive (*23*–*25*), with broad negative environmental consequences, including depleted soil carbon stocks (*26*). Invasions of microbes stemming from accidental introductions abound as well, including *Cryphonectria parasitica*, which decimated American chestnut forests (*27*); *Phytophthora infestans*, which was responsible for 19th Century potato famines in Europe (*28*, *29*); *Batrachochytrium* spp., which are causing global amphibian declines (*30*); and microbes from human microbiomes potentially invading the microbiomes of captive primates (*31*). At least 375 species of fungi have been introduced and have become established in Austria alone (*32*), and hundreds of other microbial invasions are known; e.g., (*19*, *22*, *33*–*43*). Many invasive human pathogens were introduced as acts of biowarfare: for instance, in 1346 the Mongol army tossed cadavers infected with *Yersinia pestis* into the city of Caffa, deliberately starting a plague of the Black Death (*44*); *Francisella tularensis* was intentionally introduced in Anatolia in the 14th century, starting an epidemic (*45*); blankets infected with Variola major or V. minor were deliberately given to Native Americans, causing outbreaks of smallpox (*46*); and *Salmonella typhimurium* was deliberately introduced in 1984 in the Dalles, Oregon, sickening the population (*46*). Additional examples of intentionally introduced microbes causing outbreaks include *Bacillus anthracis* in Scotland (*47*) and the U.S.S.R (*48*), and *Mycobacterium leprae* in Italy (*46*). In the 1950s and 1960s, the United States military deliberately released *Serratia marcescens* and *Bacillus globigii* in tests in San Francisco and New York, creating microbial invasions that caused lasting negative public health and societal outcomes (*48*, *49*). Individuals have also deliberately spread microbial pathogens instigating microbial invasions, including Mary Mallon spreading *Salmonella enterica* (*50*), and "pox parties" spreading the Varicella-zoster virus and Measles morbillivirus (*51*). The threat of invasions caused by intentionally introduced pathogenic microbes has been deemed sufficiently severe that it motivated the creation of two international biowarfare treaties (*48*). In addition, the application of manure as fertilizer in agriculture is recognized as a major source of invasive human pathogens in water, on food, and in connected environments (*52*). Despite the plethora of known nonpathogenic and pathogenic invasive microbes, the scope of microbial invasions likely remains underestimated due to inadequate surveillance (*53*).

Four dimensions of mitigating microbial invasions

Four dimensions, or factors, circumscribe identifying knowledge gaps and mitigating emerging threats from microbial invasions. The dimensions are the (1) stage of the invasion process under consideration (transport, establishment, spread, or impacts of invasive microbes); (2) overall aim (2A, mechanistic understanding; 2B, prediction; and 2C, management); (3) data collection approach (3A, surveillance of microbial communities and 3B, experimental microcosms, mesocosms, landscape-scale manipulations); and (4) analysis strategy (statistical or mechanistic modeling). An investigation, for example, might develop approaches to *predict* the *establishment* of introduced microbes using *mesocosm experiments* analyzed via *statistical modeling*. Here, we examine these dimensions in detail and highlight key research needs for each.

Dimension 1: The stages of biological invasions

All biological invasions, including microbial invasions, pass through a series of common stages, separated by barriers: (i) transport and introduction of a species out of its native range, (ii) establishment at the point of introduction, (iii) spread beyond the point of introduction, and (iv) impacts on ecosystems, the environment, public health, agriculture, or infrastructure (*2*, *20*). The barriers between stages are: (i) geographic and (ii) captivity barriers for transport and introduction; (iii) survival and (iv) reproduction barriers for establishment; and (v) dispersal and (vi) environmental barriers for spread (*54*). Considering these stages explicitly is important because microbial inoculants may have inconsistent affects on each stage and barrier, making mechanistic understanding, predictions, and management strategies stage- and barrier-dependent.

Dimension 2A: Mechanistic understanding of how microbial inoculants cause invasions

Along with predictive models, described in Section 2B, a mechanistic understanding of how microbial inoculants instigate microbial invasions will underpin effective management strategies. Here, building on relevant knowledge from microbial ecology and **invasion ecology**, we develop a list of seventeen guiding hypotheses to catalyze research on how microbial inoculants affect microbial invasions.

2A.1 Microbial inoculants reduce geographic barriers

Geography constitutes the first barrier against invasion: not all species can reach every habitable location (*54*). Microbes were historically hypothesized to not face geographic barriers (*55*), and under such a scenario microbial invasions would be impossible because microbes already disperse everywhere. However, numerous studies have demonstrated that microbial dispersal is geographically limited and indeed influences community composition (e.g., (*56–60*)). This point is conclusively demonstrated by the many documented examples of microbial invasions (Box 1). Because microbial dispersal is naturally limited, introducing exogenous microbes into an environment can breach geographic barriers.

Specifically, microbial inoculants have the potential to breach geographic barriers via four routes. First, inoculations typically entail intentionally introducing microbes into new locations where they may become invasive, as has been the case with numerous macroorganisms that were intentionally introduced with positive intentions but detrimental results (Tables 1.1 and 2.1) (*10*, *13*). Second, microbial inoculants may breach geographic barriers by providing transport for hitchhiking microbes, as has been the case with many plants and animals: microbial inoculants are subject to little oversight to ensure that they are pure cultures, or that they contain only the microbes that they claim, suggesting that they may be particularly prone to transporting hitchhikers (Tables 1.2 and 2.2). Third, rather than introduce non-native microbes, some microbial inoculants increase the relative abundance of rare microbes or genes that are already present in a community, a manipulation that is liable to cause microbial invasions by (i) radically increasing the population size of native rare microbes or and/or (ii) creating widespread disturbances that may prime the landscape for invasions by other microbes (Tables 1.3 and 2.3; detailed below). Last, microbes have been introduced for harmful purposes throughout history (Box 1), and microbes that have deleterious effects could be deliberately introduced to disrupt agriculture, infrastructure, or public health (Table 1.4). Although this potential has previously existed, the development of increasingly sophisticated techniques for applying microbial inoculants may provide bad actors with an expanded arsenal of approaches for instigating malicious invasions (Table 2.4).

2A.2 Microbial escape during the formulation and transport of microbial inoculants

In some cases, after surmounting geographic barriers, macroorganism species must escape from captivity or cultivation to invade. Microbes are difficult to contain: biosafety containment laboratories are costly and challenging to construct (*61*), and microbial inoculants are rarely formulated in laboratories with strict containment controls nor transported securely (Table 1.5). In general, microbes are inoculated into uncontained agricultural or contaminated natural environments with the assumption that their spread will be limited either in time or in space. However, unintentional escapes may be likely in environments with high invasibility, even when the intended releases are localized in environments that have low invasibility (Table 2.5). Alternatively, microbes inoculated into soil or groundwater that feed into local surface waters can be transported long distances into more invasible environments (*52*).

2A.3 Microbial inoculants alter the likelihood of survival and reproduction

Once a species surmounts geographic barriers, and, if necessary, escapes cultivation, to become invasive it must next overcome barriers to survival and reproduction at its location of introduction (*54*). Microbial inoculants may modify the barriers to survival and reproduction of introduced microbes in five ways.

2A.3.1 Propagule pressure

Among the traits that can predispose certain species to become invasive while others remain uninvasive, elevated **propagule pressure** typically most strongly predicts whether a species will become established (*62–64*). Many microbial inoculants consist of tremendous numbers of microbial individuals introduced widely which can allow microbes in microbial inoculants to become established (Table 1.6) (*11*). Furthermore, because the propagule pressure exerted by microbial inoculants can greatly exceed propagule pressure from dispersal through wind currents, water, dust deposition, and other natural routes, even if microbes naturally disperse to locations where microbial inoculants are applied, the extreme propagule pressure exerted by microbial inoculants may allow non-native microbes to establish in regions where it would otherwise be impossible (Table 2.6).

2A.3.2 Disturbed environments

Although the link between disturbance and the invasibility of environments can be complex (*65*, *66*), heavily disturbed environments – where the level of disturbance is well beyond what native species would usually experience – are consistently highly invasible (*2*). Microbial inoculants are applied in heavily disturbed environments, where they may easily overcome survival and reproduction barriers (Tables 1.7 and 2.7). Moreover, even when microbial inoculants are not applied in environments that are a priori disturbed, they may be accompanied by disturbances that increase invasibility. These disturbances may be deliberate, meant to increase the chances that the inoculant "takes" (*13*), or they may be caused by the introduced microbes themselves (Tables 1.8 and 2.8). Indeed, introduced **foundation species** can act as a disturbance, which increases the invasibility of a community, leading to cascades of additional invasions due to positive feedback. These **invasional meltdowns** can fundamentally reorganize ecological communities (*67*, *68*), and may present a risk of microbial inoculants because the taxa in inoculants are specifically chosen to have substantial ecosystem impacts (Table 1.9). Microbial inoculants that do not introduce new taxa, but rather increase the abundance of taxa that are already present may be particularly prone to this problem because such disturbances have the potential to further restructure communities (*69*) (Table 2.9).

2A.3.3 Interspecific interactions

The establishment of non-native species is often blocked by missing interaction partners (e.g., mutualists) that benefit survival or reproduction (*2*). Many microbial inoculants include numerous microbial taxa or even entire microbial communities, and multiple microbial inoculants may be applied simultaneously (Table 1.10) (*70*). Hence, microbial inoculants may aid in survival and reproduction when they preserve facilitative ecological interactions (Table 2.10).

When microbial inoculants are applied for bioremediation or biocontrol, once microbes have consumed all of the contaminants or target organisms, they are often assumed to be incapable of further proliferation (*71*). However, this may be a strong assumption, as many microbes are generalists with some flexibility in substrate use, particularly those chosen as microbial inoculants. Introduced generalist animal consumers can be among the most damaging, causing mass extinctions and having a disproportionate impact on ecological networks (*72*). A tendency toward resource generalists in microbial inoculants may make them both likely to become established and particularly impactful (*73*) if they become invasive (Tables 1.11 and 2.11). The further impacts of native species diversity on invasibility – viz competition, which has incorrectly been assumed to protect microbial communities from invasion – are discussed in Box 2.

2A.3.4 Reduced founder effects

Population bottlenecks caused by founder effects can inhibit the establishment (and spread; see below) of introduced species. This inhibition is generally contingent on few individuals being introduced sparsely (Table 1.13). However, the aforementioned immense numbers of cells, widespread and repeated application, and lack of purity of microbial inoculants may overcome population bottlenecks and founder effects that would normally inhibit the survival and reproduction of naturally or anthropogenically dispersed microbes, leading to establishment (Tables 1.13 and 2.13).

2A.3.5 High potential for evolution and hybridization

Frequently, intentionally introduced plants and animals initially struggle to survive and reproduce in their new environments because they are maladapted there. However, they then evolve or hybridize to become invasive, for instance by capitalizing on hybrid vigor. Microbes can undergo high rates of evolution and horizontal gene transfer, predisposing them for evolutionary changes that enable survival and reproduction in new environments, even when the originally introduced microbes may be locally maladapted (Table 1.14 and 2.14) (*10*).

Box 2: Links between diversity, competition, and invasibility

Evidence for this prediction across microbial, plant, and animal communities has been equivocal.

An oft-made prediction is that the high diversity of microbial communities imparts high competitive resistance, and thus microbial communities are robust to invasions. Evidence for this prediction across microbial, plant, and animal communities is equivocal. For some plant and animal communities, high diversity is associated with reduced invasibility (e.g., (*74*)). In others, high diversity is associated with increased invasibility (e.g., (*75*); reviewed in (*2*)). At small spatial scales intraspecific interactions tend to govern establishment, potentially leading to a negative relationship between diversity and invasibility, while at large scales habitat filtering and other processes lead to positive associations (*76–78*). Hence, at regional to global scales (*79*), the high diversity of microbial communities may actually translate to high invasibility, and more microbial invasions.

The role of competition in determining microbial community assembly dynamics has been questioned relative to facilitation (e.g., cross-feeding), top-down pressures (e.g., phage), neutral processes, dispersal limitation, priority effects, and habitat filtering (*57*, *59*, *80*–*85*). These latter processes can act both to make communities more and less resistant to invasion (*2*, *12*), but there is little reason to suspect that their preponderance in microbial communities should decrease invasibility relative to plant and animal communities where similar processes also often predominate.

Finally, among plants and animals, biotic interactions alone (e.g., competition, facilitation) are rarely sufficient to halt invasions, although they can slow them (*2*, *86*). Hence, even if microbial communities were structured by high levels of competition, evidence suggests that would be insufficient to prevent microbial invasions. In fact, competitors can sometimes promote invasions via indirect effects (e.g., (*87*, *88*)). Existing evidence supports the hypothesis that non-mutualistic interspecific interactions should neutrally affect or increase the invasive potential of microbial inoculants rather than limit it (Tables 1.12 and 2.12).

2A.4 Microbial inoculants impact barriers to dispersal within new environments

Once a non-native species has established at its site of introduction, to become invasive, it must spread within its new environment. Two factors often determine whether this spread is successful. First, dispersal limitation can impede spread: how will the organism get to new locations (*54*)? Dispersal can occur via natural or anthropogenic mechanisms, and is often contingent on long-distance dispersal events (*2*). Microbial inoculants are applied in environments with numerous avenues for long-distance anthropogenic dispersal – for instance, on exported farm machinery and livestock (Table 1.15) (*13*). These avenues create an opportunity for locally established non-native microbes to rapidly disperse broadly (Table 2.15).

Second, growth rates can determine whether a species spreads beyond its location of establishment: high local growth rates facilitate the dispersal and spread of introduced plants and animals. Many microbial taxa exhibit high growth rates, and treatments accompanying inoculants often are designed to increase growth rates. Importantly, microbial inoculants that increase the abundance of rare taxa and genes without introducing non-native microbes also risk increasing dispersal of the rare taxa beyond their natural limits. These rare taxa, which are likely to have substantial impacts on ecosystem processes, may then become invasive in the regions where they can newly disperse. Both the naturally high growth rate of many microbes and biostimulation may enable the microbes in microbial inoculants to disperse if they become established (Tables 1.16 and 2.16).

2A.5 Microbial inoculants remove environmental barriers

Introduced species face a final barrier after dispersal in their new environments: the availability of suitable habitat patches beyond the sites of introduction (*54*). Many microbial inoculants – both those that introduce non-native microbes and those that increase the abundance of rare taxa – are deployed densely across large regions. The disturbances that they produce may inadvertently create numerous habitat patches susceptible to invasion by microbes both from the same inoculants, and other inoculants. As a result, widely applied microbial inoculants may prime the landscape for the large-scale spread of introduced microbes (Tables 1.17 and 2.17).

Dimension 2B: Predicting the threats of microbial invasions

Coupled with mechanistic understanding, predictive tools will underlie successfully managing inoculants and invasive microbes. Here, we discuss tools whose development should be prioritized.

2B.1 Predicting hazardous microbes: quantitative trait analysis

Quantitative trait analysis seeks to identify which traits make certain species more likely to become invasive than others. It relies on data indicating which species have and have not become invasive upon introduction; the latter are currently scant for microbes. It can be complicated by interspecific differences in time-since-introduction and propagule pressure, both of which are strong predictors of invasiveness, and a given trait can also act both for and against the same species at different stages of the invasion process. Nonetheless, quantitative trait analysis presents an important tool for predicting and preventing invasions, and it needs to be applied to candidate microbes for microbial inoculants. Innovative approaches are needed to overcome the aforementioned hurdles.

2B.2 Predicting susceptible geographic regions: risk maps

In addition to their identity, knowing the places where microbes are likely to invade underpins accurately targeting prevention efforts: a given microbe is likely to have invasive potential only in certain regions where environmental conditions are favorable. A key approach for identifying invasible regions are risk maps. Risk maps are created by assessing the environmental conditions that are suitable for a species (its niche) via either (1) regressing observations of the occurrence or abundance of the species on environmental conditions or (2) laboratory experiments designed to assess its range of suitable conditions. These inferred niches are then projected geographically using maps of environmental conditions to create maps of potential distributions; more sophisticated variations can also integrate the effects of interspecific interactions. Locations within the projected distribution that are currently uncolonized can be flagged as locations where invasions can occur: where inoculations should be avoided and biological surveillance increased. Similar environmental niche modeling has been applied to microbial systems (*89*, *90*), but studies applying it to identify where microbes in inoculants are likely to become invasive are needed for prediction and prevention. Quantitative Microbial Risk Assessments may also be adaptable for predicting the impacts of invasions (*91*, *92*).

2B.3 Prediction and invasion stages

To quantify risks, it is important to predict which stages of the invasion process provide the most effective checkpoints for invasive microbes. For plants and animals, the "rule of tens" posits that species have a 10% chance of passing each stage in the invasion process, so that overall 0.1% of species are likely to pass through all stages and become invasive. Although numerous exceptions to this rule exist, whether a similar rule applies to microbes remains an open question. Indeed, even if a rule of "hundreds" applied to microbes in inoculants, implying that 0.0001% of microbial taxa would become invasive, that could still translate into numerous invasive microbes because microbial diversity is high (e.g., 0.0001% of 10^8 taxa is 100 taxa). However, perhaps more pertinent to assessing risks is assessing which invasion stages pose the strongest checkpoints, and whether these stages are predicted by taxonomic grouping, functional genes, and/or environmental factors. The hypotheses discussed in Section 2A provide a roadmap for identifying how microbial inoculants may impact the filtering effect of each stage. Identifying and quantifying the bottlenecks in the microbial invasion process will be instrumental in predicting which microbial taxa are prone to become invasive, and where and how to prevent those invasions.

Dimension 2C: Management and strategies to prevent invasions

Ecology has a long history of prevention, eradication, and control of invasive plants, animals, and some microbial pathogens. Generally, prevention has been more successful and cost-effective than eradication and control: indeed, examples of successful eradication campaigns are sparse and primarily restricted to instances where invasive species occupy less than 10 km^2. As demonstrated by the immense efforts needed to control infectious diseases, the challenges of eradicating or controlling invasive microbes are likely to be extreme. Hence, developing strategies to prevent microbial invasions is of paramount importance.

Although understanding and predictions of how microbial inoculations effect microbial invasions are nascent, given the hazards, certain preventative management options should be implemented immediately. The recent United States Executive Order on Advancing Biotechnology and Biomanufacturing Innovation explicitly calls for regulations that "elevate biological risk management as a cornerstone of the life cycle of biotechnology and biomanufacturing"(*93*). Risks of generating invasive species from microbial inoculations should be included in these regulations. Regulatory action should include requiring inoculant manufacturers to report standardized hazard data – based on quantitative trait analysis and risk maps – in much the same way that pesticides, chemicals, and pharmaceuticals require hazard documentation. Furthermore, microbial inoculants should proactively be treated as "guilty until proven innocent:" prior to deployment, developers should be required to demonstrate that inoculants do not present a risk of causing invasions, a paradigm that has successfully been enacted in New Zealand to prevent plant and animal invasions (*94*).

Dimension 3: Data collection approaches

Multiple types of data will be central for pursuing the aforementioned research areas; this section highlights important data collection approaches.

Dimension 3A: Experiments

Data on which microbial taxa have been successful and unsuccessful at invading following introduction underlie quantitative risk assessments to predict which microbial taxa are likely to be safe and unsafe for future inoculations. Unfortunately, data on failed microbial invasions are largely non-existent due to a lack of records of introduction and surveillance. Microcosm and mesocosm experiments can help fill this gap by allowing experimental introductions to be performed and monitored in controlled settings (*95*). In addition, ongoing commercial field-scale inoculations can provide long-term data on microbial community dynamics (*70*, *71*, *96*, *97*). With monitoring, the invasions that these inoculations cause (or do not cause) can help guide future decision making to prevent additional invasions. Human-associated microbial communities in well-studied model systems may also provide promising insights about invasive microbes (e.g., *C. difficile* in the human gut).

Dimension 3B: Surveillance

Complementing experimentation, the need for large-scale and long-term surveillance of microbes in the environment cannot be overstated. The geographic ranges of most microbes – both native and invasive – remain largely unknown. However, new sequencing technologies have made it possible to easily survey microbial communities, allowing the current ranges of microbes to be assessed (*89*, *90*) and microbial invasions to be detected. Such assessments are important for quantitative risk assessments, allowing identification of the traits that make certain microbes invasive. Furthermore, microbial surveillance comprises a key early warning system for detecting microbial invasions, when mitigation is still possible, and provides the data to outline contemporary microbial distributions, a cornerstone of developing microbial risk maps. As the cost of sequencing continues to plummet, the practicality and cost-effectiveness of large-scale, long-term microbial surveillance continues to increase (*98*). In addition, it may soon be possible to use remotely-sensed indices of surface biophysical properties (e.g., plant productivity) to evaluate patterns of microbial diversity (*99*–*101*). Potential exists to leverage stakeholders and local governments to implement innovative sampling solutions (e.g. wastewater sampling, agricultural runoff, citizen scientists). A key component of invasive microbe surveillance will be a publicly-accessible data infrastucture, following a FAIR data and model framework (*2*, *102*). For a relatively modest public investment, microbial surveillance can provide enormous benefits to preventing and predicting microbial invasions, while simultaneously providing data to identify which microbes are safe to use in inoculants. Moreover, the same microbial surveillance data used to guard against microbial invaders can provide dual benefits of informing basic ecological science and detecting pathogens threatening public health (*103*–*109*).

Dimension 4: Modeling approaches

Mathematical modeling can both allow predictions to be derived phenomenologically from data and the plausibility of potential mechanisms to be investigated. Phenomenological statistical modeling approaches will underlie inferring the niches of microbes from observational data for risk maps, and developing predictive models of invasive potential from experimental and observational data. These approaches will include both classical statistical tools and advanced artificial intelligence tools. Methods that include dimensionality reduction (*110*), equation-free models (*111*), fuzzy models (*112*), time-series analysis and forecasting (*113*), deep-learning and machine learning approaches appropriate for complex interactions and feedbacks are clear near-term opportunities (*114*). Much insight can also be gained from novel interdisciplinary bioinformatics and numerical modeling frameworks that combine physics, chemistry, geology, and biology to predict microbial traits and microbial community shifts over time (*115*, *116*).

Mechanistic modeling approaches will be important for gaining understanding of the general principles governing microbial invasions stemming from microbial inoculants. Toward this end, modeling frameworks designed to describe the spatial spread, establishment and demographics, and ecological impacts of invasive plants and animals will be useful. Examples of theories for spreading processes and spatial dynamics include island biogeography (*117*), neutral (*118*), and metacommunity ecological theories (*119*, *120*); and models of contagia on networks from epidemiology and physics (*121*, *122*). Niche-based theories (*110*, *123*), habitat suitability models (*124*), ecological scaling (*125*), and community assembly theories (*126*) will be important for understanding large-scale abundance patterns of invasive microorganisms, and their potential to establish in unditurbed communities. Finally, understanding how the establishment of invasive microorganisms impacts intact ecosystems and their constituent species will benefit from dynamical systems models of ecological network dynamics (*127*), methods for coarse-graining biological interactions (*128*), and generalized models (*129*), which are especially useful for predicting invasions in systems for which few empirical details are available (*130*).

Conclusion

Microbes introduced through inoculations pose a threat of creating invasions. Existing evidence suggests that a perfect storm may be brewing: microbial inoculants are designed with features that may allow microbes in them to overcome most or all barriers to invasion. Even if only a small fraction of introduced microbes become invasive, the consequences may be catastrophic to the environment, agriculture, and public health. The time to act is now, via a threefold approach: the development of a better understanding of how inoculants can effect invasions, generation of quantitative predictions about the invasive potential of inoculants, and implementation of proactive and preventative management strategies. A unified approach is needed to tackle these challenges quickly. The hypotheses and methods highlighted here provide a roadmap toward this end.

Tables

Table 1: Hypotheses regarding the effects of microbial inoculants on microbial invasions

Barrier Affected	Hypothesis
Geographic	(1) **Transportation:** transporting and introducing microbes intentionally in microbial inoculants can overcome geographic barriers, allowing microbes to reach new locations. (2) **Hitchhiking:** adventitious, hitchhiking microbes in microbial inoculants can overcome geographic barriers to be introduced in new locations. (3) **Bioaugmentation:** microbial inoculants that increase the abundance of already present rare microbes can induce invasions by permitting rare microbes to disperse to new locations due to elevated growth rates. (4) **Bad actors**: methods developed for creating microbial inoculants can be used by bad actors to deliberately generate harmful microbial invasions.
Captivity	(5) **Escape:** microbes in inoculants have the potential to escape into the environment from laboratories and during transport to their sites of application.
Survival and Reproduction	(6) **Propagule pressure:** extreme propagule pressure exerted by microbial inoculants can permit microbes to become established where they could not otherwise. (7) **Disturbed environments:** microbes in inoculants are likely to become established at their sites of introduction because they are applied in highly disturbed environments. (8) **Disturbance:** disturbances – both deliberate and incidental – accompanying application of microbial inoculants can increase the likelihood that microbes in the inoculants will become established. (9) **Invasional meltdowns:** microbes in microbial inoculants are chosen to have high community-level impacts, and are therefore likely to precipitate invasional meltdowns and have indirect effects. (10) **Mutualistic consortia:** consortia of microbes introduced in microbial inoculants often include mutualists, facilitating establishment at the site of introduction. (11) **Generalists:** generalist microbes in microbial inoculants will continue to proliferate and become established once they have consumed target resources (bioremediation) or organisms (biocontrol) because generalists can subsist on various food sources. (12) **Competition:** relatively low competitive structuring of microbial communities fails to make microbial communities resistant to the establishment of microbes in microbial inoculants. (13) **Founder effects:** large numbers of individuals in applications of microbial inoculants will overcome population bottlenecks caused by founder effects that would otherwise limit survival and reproduction of introduced microbes. (14) **Gene transfer:** the ubiquity of hybridization and horizontal gene transfer among microbes can allow microbes in microbial inoculants to locally adapt, and thereby become established.
Dispersal	(15) **Infrastructure:** extensive transportation infrastructure which can carry abiotic substrates, plants, and animals between locations where inoculants are applied can facilitate long-distance dispersal of introduced microbes that have become established. (16) **Growth rates:** high growth rates of microbes in microbial inoculants can promote the dispersal of microbes beyond their sites of introduction.
Environmental	(17) **Widespread application:** widespread application of microbial inoculants can create numerous habitat patches for introduced microbes, priming the landscape for microbial invasions.

Table 2: Examples supporting the hypotheses in Table 1

Hypothesis	Select Examples Motivating Hypothesis	Inoculant Risk Factor
(1) **Transportation**	Cane toads, mongooses, rosy wolfsnails, and Nile perch became invasive after being intentionally transported to and released in Australia, Hawaii, Pacific Islands, and Lake Victora, respectively. Numerous other invasive species have similar histories (*2*).	Many microbial inoculations involve intentionally transporting and introducing microbes to new regions: for instance, the bacterial consortium KB-1, initially enriched from Ontario (*131*, *132*), has been introduced globally for remediation of chlorinated solvents (*71*); *Penicillium bilaii* was isolated from soil in Alberta (*133*) and has been marketed and introduced globally as JumpStart (*134*); and the entomopathogenic fungus *Metarhizium anisopliae* was originally found in Ukraine and has been applied globally for biocontrol (*135*).
(2) **Hitchhiking**	Examples of hitchhiking organisms include cheatgrass seed in grass seed shipments to North America (*136*), pathogenic fungi accompanying horticultural plants (*137*), and chytrid fungus on amphibians in the pet trade (*138*).	The microbes in microbial inoculants are often only partially characterized, with non-sterile additives, such as worm castings, providing uncharacterized microbial components (*139*). Among four commercial microbial inoculants selected for study, 20%-35% of bacterial cells were from adventitious species (*140*). When complex microbial mixtures are introduced, even after screening, some unwanted microbes may be included, for instance in the case of FMTs and drug-resistant *E. coli* (*141*, *142*).
(3) **Bioaugmentation**	Reference (*143*) reviews numerous instances of increased growth rates promoting dispersal of native species to new locations, where they become invasive.	Numerous microbial inoculants increase growth rates of microbes that are already present: *Acinetobacter* SZ-1 strain KF453955 coupled with N and P to enhance growth rates is used for petroleum hydrocarbon degradation (*144*), and inoculants with a mixture of bacterial strains and N, P, and K fertilization are used for bioremediation of PAH-contaminated soil (*145*).
(4) **Bad actors**	Box 1 lists historical examples of deliberate introductions of non-native species by bad actors, leading to invasions.	The technology and methods used to develop microbial inoculants are becoming increasingly sophisticated, widely available, and effective: advances which could be co-opted by bad actors.
(5) **Escape**	Bighead and silver carp, initially cultivated in captivity in the United States to purify water in aquaculture facilities, escaped and are now highly invasive (*146*).	Microbial inoculants are often formulated in laboratories with weak containment controls and transported insecurely (*140*); invasive disease-causing organisms have escaped from labs (*147*–*150*); and quality control is lax for the manufacture of some inoculants (*151*).
(6) **Propagule pressure**	Which European bird species became established in New Zealand was primarily determined by the number of introduced individuals and the frequency of introductions – (their propagule pressure) with large numbers of individuals increasing the likelihood of establishment (*152*).	Large microbial inoculants are often applied in biological remediation to ensure that added microbes persist and are sufficiently active to consume contaminants (*71*, *153*, *154*); mixes of microbes are added at recommended rates of over 100 cfu per mL (*155*–*157*), up to an order of magnitude in excess of what would be encountered naturally (*158*, *159*).
(7) **Disturbed environments**	Large-scale conversion of land to agriculture in Puerto Rico (high disturbance) permitted persistent invasions of non-native trees, despite native species being better adapted to natural environmental conditions (*160*).	Microbial inoculants are regularly applied in highly-disturbed environments, including agricultural environments with high levels of pesticide application (*161*), sites requiring bioremediation (*144*), and human gut ecosystems with persistent pathogenic infections (*162*).
(8) **Disturbance**	In California, the establishment of invasive figs was made possible by farming-related disturbances accompanying their introduction (*163*).	Disturbances often accompany microbial inoculant applications: Antibiotics are applied before fecal microbiome transplants to reduce existing gut microbiome diversity and abundance, promoting establishment of introduced microbes (*164*); granular microbial inoculants are applied in agriculture during planting when soil is tilled (*165*); wasterwater is often aerated to promote the effectiveness of microbial inoculants for bioremediation (*166*).
(9) **Invasional meltdowns**	Invasive crazy ants, *Anoplolepis gracilipes*, on Christmas Island extirpated native land crabs, precipitating the collapse of native plant communities, allowing the establishment of additional invasive species (*67*).	The microbes in microbial inoculants are selected to have extreme impacts: they change fundamental biogeochemical processes including nitrogen fixation and phosphate solubilization; are ecosystem engineers, for instance affecting water retention; and modify top-down control ecosystem control mechanisms, including disease dynamics (*134*).
(10) **Mutualistic consortia**	When fig trees were introduced to Florida, their mutualistic pollinator wasps were excluded, preventing reproduction, but subsequent introductions of the mutualsitic fig wasps allowed the figs to become established and invade (*167*).	The development and use of polymicrobial inoculants is increasing and viewed as a major area for innovation (*168*). These polymicrobial inoculants are designed to for increased the stability and resistance of introduced microbial communities, the same features that are known to endow mutualistic consortia with high invasive potential. Existing inoculants contain over ten microbial taxa, including both bacteria and fungi (*169*).

Table 2: (Continued)

Hypothesis	Select Examples Motivating Hypothesis	Inoculant Risk Factor
(11) **Generalists**	Invasive generalist predators are prone to causing mass extinctions: for instance, the introduction of generalist Nile perch in Lake Victoria resulted in the extinction of hundreds of native cichlid fish species (*170*).	The biopesticide *Bacillus thuringiensis* kills numerous native lepidopteran species in addition to target species (*171*); other biopesticides are likely to have extensive ecosystem-wide effects due to their lack of specificity (*172*); the fungal biocontrol agents *Trichoderma* spp. produce broad-spectrum anti-microbial compounds that are likely to affect numerous non-target native microbes (*173*).
(12) **Competition**	Non-mutualistic interspecific interactions neutrally or positively affect invasibility: for instance, grazing by livestock (predation) on the Carrizo Plain promotes the recruitment of non-native grasses (*174*).	Non-competitive community assembly processes – facilitation (*175*), predation, habitat filtering, priority effects (*176*), neutral processes (*177*) dominate the assembly of many microbial communities including communities where microbial inoculants are applied.
(13) **Founder effects**	Invasive fire ants (*Solenopsis invicta*) only spread in the United States when a genetic variant was introduced for multi-queen colonies (*178*); low genetic diversity initially prevented the spread of the invasive potato late-blight *Phytophthora infestans* (*2*).	Microbial inoculants usually include multiple strains due to limited purification; these mixtures include high genetic diversity and may include both beneficial and pathogenic strains (*134*).
(14) **Gene transfer**	*Spartina alterniflora* was deliberately introduced to the San Francisco Bay in the 1970s from the Eastern U.S., where it remained non-invasive. However, upon hybridizing with the native *S. foliosa*, it became highly invasive due to hybrid vigor (*179*). Invasive species are also documented to have indirect evolutionary impacts on communities (*180*).	Many microbes undergo high rates of evolution and may also provide new genes to resident community members through horizontal gene transfer (*19*, *181*); hybridization is leading to high rates of evolution of fungal plant pathogens (*182*).
(15) **Infrastructure**	Long-distance dispersal events can be key in driving the spread of introduced species beyond their sites of initial establishment (*2*): for example, people traveling on trains and airplanes were pivotal in spreading SARS-CoV-2 globally during the COVID-19 pandemic (*183*).	Environments where microbial inoculants are applied likely have high levels of long-distance transport mechanisms; e.g., movement of agricultural machinery and livestock between farms; shipping near bioremediated aquatic environments; and international travel by people taking probiotics. Microbial inoculants are often applied in environments with potential for microbial spillover to other environments (*184*); for instance, dispersal of microbes via effluents from from agricultural lands (*185*), including microbes from fertilizer (*52*). Microbial inoculants may also be explicitly designed to promote microbial dispersal (*186*).
(16) **Growth rates**	The dispersal of invasive Sparrowhawks in Europe was largely determined and promoted by population growth rates: 86% of the variation in the dispersal was accounted for by variation in growth rates (*187*).	Bioaugmentation accompanying microbial inoculants often greatly increase microbial growth rates (*70*, *97*, *188*). Microbial inoculants are also often applied at times of year and in micro-environments – e.g., adjacent to plant roots – when and where they are most likely to proliferate (*134*).
(17) **Widespread application**	Invasions can be enabled (or disabled) by the availability of numerous suitable habitat patches at landscape and regional scales: for instance, zebra mussels (*Dreissena polymorpha*) may be unable to spread into the U.S. Intermountain West due to unsuitable habitat and environmental conditions (*189*).	Microbial inoculants are often applied across large regions: in Brazil, soybean application of microbial inoculants is estimated over of 75% of the land area (*190*).

Glossary

- An **invasive species** is defined as a species that has been introduced to a new region by humans, become established, spread, and had harmful impacts (ecological, environmental, health, agricultural, or otherwise). A species can be invasive in some regions and at some times, but not others; e.g., chestnut blight, *Cryphonectria parasitica*, is invasive in North America and Europe, but not Asia, where it is native (*2*).

- **Microbes** are defined here as Bacteria, Archaea, Viruses, Fungi, and Protists. We omit prions from our definition of microbes (*191*), but the possibility of environmental prion invasions caused by humans is an interesting, potentially important, and apparently unexplored area.

- A **microbial inoculant** is a collection of microbes with specific metabolic properties introduced into an environment to perform some function (*134*).

- The **Goldilocks zone of microbial inoculants** is the 'sweet spot' where the microbes in an inoculant become established and have desirable impacts where they are introduced, without spreading and having unintended impacts elsewhere. While it is possible to create inoculants in the Goldilocks zone, there is no reason to believe that all – or even most – microbial inoculants will fall into it.

- **Propagule pressure** is the number of introduced individuals of a species; the product of the size of each introduction event and the number of introductions (*2*).

- **Microbial invasion ecology** is the study of microbes, introduced outside of their native ranges by humans, which have become established and spread, and have ecological, environmental, health, agricultural, or other impacts (*2*).

- A **foundation species** is a species that plays a dominant role in structuring ecological communities (*192*).

- **Invasional meltdowns** occur when invasive species create conditions favorable to additional invasive species in a positive feedback loop (*68*).

- An **invasive microbe**, like an invasive species, is a microbe that has been moved outside of its natural range by humans, become established, spread, and caused impacts (*19*). Because microbial species can be poorly defined, in many cases it may be more relevant to consider strains, higher taxonomic groupings, or genes as the unit of invasion in a microbial context.

References

1. D. Rigling, S. Prospero, *Molecular Plant Pathology* **19**, 7–20 (2018).
2. J. L. Lockwood, M. F. Hoopes, M. P. Marchetti, *Invasion Ecology, 2nd Edition* (Wiley-Blackwell, West Sussex, 2013).
3. D. M. Lodge *et al.*, *Ecological Applications* **16**, 2035–2054 (2006).
4. M. S. Santos, M. A. Nogueira, M. Hungria, *AMB Express* **9**, 1–22 (2019).
5. D. Drekonja *et al.*, *Annals of Internal Medicine* **162**, 630–638 (2015).
6. J. E. Green *et al.*, *Gut microbes* **12**, 1854640 (2020).
7. P. Markowiak, K. Śliżewska, *Nutrients* **9**, 1021 (2017).
8. M. R. Silverstein, D. Segré, J. M. Bhatnagar, *Global Change Biology* **29**, 2050–2066 (2023).
9. S. Basiru, M. Hijri, *Microorganisms* **10**, 404 (2022).
10. C. N. Jack, R. H. Petipas, T. E. Cheeke, J. L. Rowland, M. L. Friesen, *Trends in Microbiology* **29**, 299–308 (2021).
11. M. M. Martignoni, J. Garnier, M. M. Hart, R. C. Tyson, *Ecological Modelling* **438**, 109321 (2020).
12. P. C. Mawarda, X. Le Roux, J. Dirk van Elsas, J. F. Salles, *Soil Biology and Biochemistry* **148**, 107874 (2020).
13. C. N. Thomsen, M. M. Hart, *Biological Invasions* **20**, 2695–2706 (2018).
14. S. Pagad, P. Genovesi, L. Carnevali, D. Schigel, M. A. McGeoch, *Scientific Data* **5**, 170202 (2018).
15. R. N. Cuthbert *et al.*, *Science of The Total Environment* **819**, 153404 (2022).
16. H. Seebens *et al.*, *Global Change Biology* **27**, 970–982 (2021).
17. H. Seebens *et al.*, *Nature Communications* **8**, 14435 (2017).
18. C. Kruk *et al.*, *Oikos* **130**, 571–586 (2021).
19. E. Litchman, *Ecology Letters* **13**, 1560–1572 (2010).
20. C. A. Mallon, J. D. v. Elsas, J. F. Salles, *Trends in Microbiology* **23**, 719–729 (2015).
21. W. H. van der Putten, J. N. Klironomos, D. A. Wardle, *The ISME Journal* **1**, 28–37 (2007).
22. M. P. Thakur, W. H. van der Putten, M. M. P. Cobben, M. van Kleunen, S. Geisen, *Nature Reviews Microbiology* **17**, 621–631 (2019).
23. D. M. Richardson, N. Allsopp, C. M. D'Antonio, S. J. Milton, M. Rejmánek, *Biological Reviews* **75**, 65–93 (2000).
24. E. C. Vellinga, B. E. Wolfe, A. Pringle, *New Phytologist* **181**, 960–973 (2009).
25. A. R. Wood, *Bothalia-African Biodiversity & Conservation* **47**, 1–16 (2017).
26. I. H. Chapela, L. J. Osher, T. R. Horton, M. R. Henn, *Soil Biology and Biochemistry* **33**, 1733–1740 (2001).
27. A. C. Miller, K. E. Woeste, S. L. Anagnostakis, D. F. Jacobs, *AoB Plants* **6** (2014).
28. P. R. J. Birch, S. C. Whisson, *Molecular Plant Pathology* **2**, 257–263 (2001).
29. M.-L. Desprez-Loustau *et al.*, *Trends in Ecology & Evolution* **22**, 472–480 (2007).
30. S. J. O'Hanlon *et al.*, *Science* **360**, 621–627 (2018).
31. L. K. Fuerniss *et al.*, *Journal of Animal Science* **100**, skac252 (2022).
32. H. Voglmayr, A. Schertler, F. Essl, I. Krisai-Greilhuber, *Biological Invasions* **25**, 27–38 (2023).
33. A. J. Alverson *et al.*, *Biological Invasions* **23**, 2191–2204 (2021).
34. S. A. Spaulding, E. Elwell, "Increase in nuisance blooms and geographic expansion of the freshwater diatom Didymosphenia geminata", English, tech. rep. (Reston, VA, 2007).
35. C. Wiedner, J. Rücker, R. Brüggemann, B. Nixdorf, *Oecologia* **152**, 473–484 (2007).
36. C. Zachow *et al.*, *The ISME Journal* **3**, 79–92 (2009).
37. C.-S. Chin *et al.*, *New England Journal of Medicine* **364**, 33–42 (2011).
38. S. Rodríguez-Echeverría, *Journal of Biogeography* **37**, 1611–1622 (2010).

39. M. Edwards, A. John, D. Johns, P. Reid, *Journal of the Marine Biological Association of the United Kingdom* **81**, 207–211 (2001).
40. J. Jussila, L. Edsman, I. Maguire, J. Diéguez-Uribeondo, K. Theissinger, *Frontiers in Ecology and Evolution* **9** (2021).
41. S. Pertola, H. Kuosa, R. Olsonen, *Harmful Algae* **4**, 481–492 (2005).
42. R. Piarroux, S. Moore, S. Rebaudet, *La Presse Médicale* **51**, 104136 (2022).
43. D. M. Rizzo, M. Garbelotto, E. M. Hansen, *Annual Review of Phytopathology* **43**, 309–335 (2005).
44. M. Wheelis, *Emerging Infectious Disease journal* **8**, 971 (2002).
45. S. I. Trevisanato, *Medical Hypotheses* **69**, 1371–1374 (2007).
46. V. Barras, G. Greub, *Clinical Microbiology and Infection* **20**, 497–502 (2014).
47. J. Guillemin, *Biological weapons: From the invention of state-sponsored programs to contemporary bioterrorism* (Columbia University Press, 2004).
48. F. Frischknecht, *EMBO reports* **4**, S47–S52 (2003).
49. L. A. Cole, *Clouds of secrecy: The army's germ warfare tests over populated areas* (Rowman & Littlefield, 1988).
50. F. Marineli, G. Tsoucalas, M. Karamanou, G. Androutsos, *Annals of Gastroenterology: Quarterly Publication of the Hellenic Society of Gastroenterology* **26**, 132 (2013).
51. A. Kitta, *Vaccinations and public concern in history: legend, rumor, and risk perception* (Routledge, 2012).
52. Z. Black *et al.*, *Frontiers in Microbiology*, 3873 (2021).
53. C. A. Guerra *et al.*, *Nature Communications* **11**, 3870 (2020).
54. T. M. Blackburn *et al.*, *Trends in Ecology & Evolution* **26**, 333–339 (2011).
55. R. De Wit, T. Bouvier, *Environmental Microbiology* **8**, 755–758 (2006).
56. W. Chen, S. Jiao, Q. Li, N. Du, *Journal of Applied Ecology* **57**, 402–412 (2020).
57. K. G. Peay, M. Belisle, T. Fukami, *Proceedings of the Royal Society B: Biological Sciences* **279**, 749–758 (2012).
58. K. G. Peay, M. Garbelotto, T. D. Bruns, *Ecology* **91**, 3631–3640 (2010).
59. J. B. H. Martiny *et al.*, *Nature Reviews Microbiology* **4**, 102–112 (2006).
60. R. J. Telford, V. Vandvik, H. J. B. Birks, *Science* **312**, 1015–1015 (2006).
61. T. M. L. Souza, C. M. Morel, *Biosafety and Health* **03**, 1–3 (2021).
62. J. L. Lockwood, P. Cassey, T. Blackburn, *Trends in Ecology & Evolution* **20**, 223–228 (2005).
63. D. Simberloff, *Annual Review of Ecology, Evolution, and Systematics* **40**, 81–102 (2009).
64. G. Á. Mhuireach *et al.*, *Environmental Microbiome* **17**, 61 (2022).
65. J. W. Fox, *Trends in Ecology & Evolution* **28**, 86–92 (2013).
66. A. T. Moles *et al.*, *Journal of Ecology* **100**, 116–127 (2012).
67. D. J. O'Dowd, P. T. Green, P. S. Lake, *Ecology Letters* **6**, 812–817 (2003).
68. D. Simberloff, B. Von Holle, *Biological Invasions* **1**, 21–32 (1999).
69. X. Jia, F. Dini-Andreote, J. F. Salles, *ISME Communications* **2**, 96 (2022).
70. M. Tyagi, M. M. R. da Fonseca, C. C. de Carvalho, *Biodegradation* **22**, 231–241 (2011).
71. D. Y. Lyon, T. M. Vogel, *Bioaugmentation for groundwater remediation*, 1–37 (2013).
72. T. Gross, L. Rudolf, S. A. Levin, U. Dieckmann, *Science* **325**, 747–750 (2009).
73. C. Averill *et al.*, *Nature Microbiology* **7**, 1717–1725 (2022).
74. J. J. Stachowicz, R. B. Whitlatch, R. W. Osman, *Science* **286**, 1577–1579 (1999).
75. T. J. Stohlgren, D. T. Barnett, J. T. Kartesz, *Frontiers in Ecology and the Environment* **1**, 11–14 (2003).
76. K. F. Davies *et al.*, *Ecology* **86**, 1602–1610 (2005).
77. J. D. Fridley *et al.*, *Ecology* **88**, 3–17 (2007).
78. P. Pillai, A. Gonzalez, M. Loreau, *Proceedings of the National Academy of Sciences* **108**, 19293–19298 (2011).

79. J. Ladau, E. A. Eloe-Fadrosh, *Trends in Microbiology* **27**, 662–669 (2019).
80. M. S. Datta, E. Sliwerska, J. Gore, M. F. Polz, O. X. Cordero, *Nature Communications* **7**, 11965 (2016).
81. M. Gralka, R. Szabo, R. Stocker, O. X. Cordero, *Current Biology* **30**, R1176–R1188 (2020).
82. J. L. Green, B. J. Bohannan, R. J. Whitaker, *Science* **320**, 1039–1043 (2008).
83. C. A. Hanson, J. A. Fuhrman, M. C. Horner-Devine, J. B. H. Martiny, *Nature Reviews Microbiology* **10**, 497–506 (2012).
84. D. R. Nemergut *et al.*, *Microbiology and Molecular Biology Reviews* **77**, 342–356 (2013).
85. R. E. Szabo *et al.*, *Proceedings of the National Academy of Sciences* **119**, e2117748119 (2022).
86. M. B. N. Albright, S. Sevanto, L. V. Gallegos-Graves, J. Dunbar, *mBio* **11**, e02089–20 (2020).
87. J. M. Facelli, A. M. Temby, *Austral Ecology* **27**, 422–432 (2002).
88. A. Ricciardi, F. G. Whoriskey, J. B. Rasmussen, *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 2596–2608 (1997).
89. J. Ladau *et al.*, *The ISME journal* **7**, 1669–1677 (2013).
90. N. Fierer *et al.*, *Science* **342**, 621–624 (2013).
91. A. Joshi *et al.*, *Food Research International*, 111323 (2022).
92. W. H. Organization *et al.*, *Quantitative microbial risk assessment: application for water safety management*, 2016.
93. *Executive order on advancing biotechnology and biomanufacturing innovation for a sustainable, safe, and secure American bioeconomy*, 2022.
94. R. P. Keller, D. M. Lodge, *Bioinvasions and globalization: Ecology, economics, management, and policy*, 220 (2010).
95. T. G. Benton, M. Solan, J. M. Travis, S. M. Sait, *Trends in Ecology and Evolution* **22**, 516–521 (2007).
96. E. Barter, A. Brechtel, B. Drossel, T. Gross, *Proceedings of the Royal Society A* **477**, 20200742 (2021).
97. J. C. Underwood *et al.*, *FEMS Microbiology Ecology* **98**, fiac077 (2022).
98. C. Noecker, A. Eng, E. Muller, E. Borenstein, *Bioinformatics* **38**, 1615–1623 (2022).
99. E. Brodie *et al.*, presented at the NSG2021 2nd Conference on Geophysics for Infrastructure Planning, Monitoring and BIM, vol. 2021, pp. 1–5.
100. K. D. Chadwick *et al.*, *Methods in Ecology and Evolution* **11**, 1492–1508 (2020).
101. Y. Hamada, J. A. Gilbert, P. E. Larsen, M. J. Norgaard, *Photogrammetric Engineering & Remote Sensing* **80**, 311–321 (2014).
102. S. Lowe, M. Browne, S. Boudjelas, M. De Poorter, *100 of the world's worst invasive alien species: a selection from the global invasive species database* (Invasive Species Specialist Group Auckland, 2000), vol. 12.
103. N. L. Fahrenfeld, W. R. Morales Medina, S. D'Elia, A. S. Deshpande, G. Ehasz, *Water Environment Research* **94**, e10768 (2022).
104. K. G. Kuhn *et al.*, *Science of The Total Environment* **812**, 151431 (2022).
105. L. Li *et al.*, *Science of The Total Environment* **817**, 152958 (2022).
106. R. Link-Gelles, *MMWR. Morbidity and Mortality Weekly Report* **71** (2022).
107. G. Mohan, S. Shrestha, S. Chapagain, C. Payus, K. Fukushi, *Strengthening Wastewater Monitoring and Surveillance for COVID-19 and Other Infectious Diseases*, 2022.
108. M. J. Wade *et al.*, *Journal of Hazardous materials* **424**, 127456 (2022).
109. M. K. Wolfe *et al.*, *medRxiv* (2022).
110. A. K. Fahimipour, T. Gross, *Nature Communications* **11**, 1–8 (2020).
111. H. Ye *et al.*, *Proceedings of the National Academy of Sciences* **112**, E1569–E1576 (2015).
112. A. Salski, *Ecological Modelling* **63**, 103–112 (1992).
113. H. Kantz, T. Schreiber, *Nonlinear time series analysis* (Cambridge university press, 2004), vol. 7.
114. Y. Cheng *et al.*, *Scientific Reports* **11**, 1–11 (2021).
115. U. Karaoz, E. Brodie, *Frontiers in Bioinformatics* **2** (2022).

116. R. L. Rubinstein *et al.*, *Bioinformatics* **38**, 778–784 (2022).
117. R. H. MacArthur, E. O. Wilson, *The theory of island biogeography* (Princeton University Press, 1967).
118. S. P. Hubbell, in *The Unified Neutral Theory of Biodiversity and Biogeography (MPB-32)* (Princeton University Press, 2011).
119. T. Gross *et al.*, *Philosophical Transactions of the Royal Society B* **375**, 20190455 (2020).
120. M. A. Leibold *et al.*, *Ecology letters* **7**, 601–613 (2004).
121. H. Sayama *et al.*, *Computers & Mathematics with Applications* **65**, 1645–1664 (2013).
122. D. J. Watts, *Proceedings of the National Academy of Sciences* **99**, 5766–5771 (2002).
123. A. Ryabov, B. Blasius, H. Hillebrand, I. Olenina, T. Gross, *Proceedings of the National Academy of Sciences* **119**, e2118156119 (2022).
124. A. H. Hirzel, G. Le Lay, V. Helfer, C. Randin, A. Guisan, *Ecological Modelling* **199**, 142–152 (2006).
125. J. H. Brown, J. F. Gillooly, A. P. Allen, V. M. Savage, G. B. West, *Ecology* **85**, 1771–1789 (2004).
126. J. D. Yeakel *et al.*, *Nature Communications* **11**, 1–10 (2020).
127. M. Pascual, J. A. Dunne, J. A. Dunne, *et al.*, *Ecological networks: linking structure to dynamics in food webs* (Oxford University Press, 2006).
128. M. E. Muscarella, J. P. O'Dwyer, *Theoretical Ecology* **13**, 503–518 (2020).
129. T. Gross, U. Feudel, *Physical Review E* **73**, 016205 (2006).
130. H. Aufderheide, L. Rudolf, T. Gross, K. D. Lafferty, *Proceedings of the Royal Society B: Biological Sciences* **280**, 20132355 (2013).
131. M. Duhamel *et al.*, *Water Research* **36**, 4193–4202 (2002).
132. M. Duhamel, E. A. Edwards, *FEMS Microbiology Ecology* **58**, 538–549 (2006).
133. R. Kucey, *Canadian Journal of Soil Science* **63**, 671–678 (1983).
134. M. O'Callaghan, R. A. Ballard, D. Wright, *Soil Use and Management* **38**, 1340–1369 (2022).
135. G. Zimmermann, *Biocontrol Science and technology* **17**, 879–920 (2007).
136. S. J. Novak, R. N. Mack, *Bioscience* **51**, 114–122 (2001).
137. M. E. Palm, A. Y. Rossman, *et al.*, *Invasive species: vectors and management strategies*, 31–43 (2003).
138. S. J. O'hanlon *et al.*, *Science* **360**, 621–627 (2018).
139. S. Pal, H. B. Singh, A. Farooqui, A. Rakshit, *Agriculturally Important Microorganisms*, 97–105 (2016).
140. L. Dong *et al.*, *Frontiers in Medicine* **9**, 963988–963988 (2022).
141. Z. DeFilipp *et al.*, *New England Journal of Medicine* **381**, 2043–2050 (2019).
142. S.-Y. Park, G. S. Seo, *Clinical Endoscopy* **54**, 157–160 (2021).
143. D. T. Iles, R. Salguero-Gómez, P. B. Adler, D. N. Koons, *Journal of Ecology* **104**, 399–408 (2016).
144. M. Wu *et al.*, *International Biodeterioration & Biodegradation* **107**, 158–164 (2016).
145. P. Innemanová, A. Filipová, K. Michalıková, L. Wimmerová, T. Cajthaml, *Ecological Engineering* **118**, 93–96 (2018).
146. S. J. Sampson, J. H. Chick, M. A. Pegg, *Biological Invasions* **11**, 483–496 (2009).
147. M. Furmanski, *A Brief, Terrifying History of Viruses Escaping From Labs* (2023; https://slate.com/technology/2014/04/how-dangerous-viruses-could-escape-from-laboratories.html).
148. K. Piper, *How deadly pathogens have escaped the lab — over and over again* (2023; https://www.vox.com/future-perfect/2019/3/20/18260669/deadly-pathogens-escape-lab-smallpox-bird-flu).
149. R. Jacobsen, *The Non-Paranoid Person's Guide to Viruses Escaping From Labs* (2023; https://www.motherjones.com/politics/2020/05/the-non-paranoid-persons-guide-to-viruses-escaping-from-labs).
150. *Infections caught in laboratories are surprisingly common* (2023; https://www.economist.com/graphic-detail/2021/08/24/infections-caught-in-laboratories-are-surprisingly-common).
151. I. E. Garcıa de Salamone, R. Esquivel-Cote, D. J. Hernández-Melchor, A. Alarcón, in *Microbial interventions in agriculture and environment* (Springer, 2019), pp. 37–74.
152. R. P. Duncan, *The American Naturalist* **149**, 903–915 (1997).

153. M. A. Ramadan, O. El-Tayeb, M. Alexander, *Applied and Environmental Microbiology* **56**, 1392–1396 (1990).
154. E. French, I. Kaplan, A. Iyer-Pascuzzi, C. H. Nakatsu, L. Enders, *Nature Plants* **7**, 256–267 (2021).
155. *Bioremediate Canada: microbial product for eutrophied water* (2023; http://www.bioremediate.com/eutroclear.html).
156. *Bioremediate Canada: Petro-Clear for the bioremediation of hydrocarbons* (2023; http : / / www . bioremediate.com/petroclear.html).
157. *CL Solutions: Munox SR* (2023; https://cl-solutions.com/cl-munox-sr/).
158. J. D. Van Elsas *et al.*, *Proceedings of the National Academy of Sciences* **109**, 1159–1164 (2012).
159. P. E. Corneo *et al.*, *FEMS Microbiology Ecology* **84**, 588–602 (2013).
160. A. E. Lugo, *Frontiers in Ecology and the Environment* **2**, 265–273 (2004).
161. S. Yates, D. Wang, F. Ernst, J. Gan, *Environmental Science and Technology* **31**, 1136–1143 (1997).
162. S. M. D. Baunwall *et al.*, *EClinicalMedicine* **29**, 100642 (2020).
163. K. A. Holmes, S. E. Greco, A. M. Berry, *Invasive Plant Science and Management* **7**, 46–58 (2014).
164. T. L. Freitag *et al.*, *Frontiers in Microbiology* **10**, 2685 (2019).
165. Y. Bashan, L. E. de-Bashan, S. Prabhu, J.-P. Hernandez, *Plant and Soil* **378**, 1–33 (2014).
166. M. Vrints, S. Bertrand, J.-M. Collard, *Journal of Applied Microbiology* **103**, 2006–2015 (Nov. 2007).
167. H. Nadel, J. H. Frank, R. Knight Jr, *Florida Entomologist*, 29–38 (1992).
168. C. A. Reddy, R. S. Saravanan, in *Advances in applied microbiology* (Elsevier, 2013), vol. 82, pp. 53–113.
169. *Sigma Bio Liquid* (2023; https://sigmaagriscience.com/catalog/product/sigma-bio/).
170. F. Witte *et al.*, *Environmental Biology of Fishes* **34**, 1–28 (1992).
171. J. W. Peacock, D. F. Schweitzer, J. L. Carter, N. R. Dubois, *Environmental Entomology* **27**, 450–457 (1998).
172. F. Cappa, D. Baracchi, R. Cervo, *Science of the Total Environment*, 155714 (2022).
173. A. Schuster, M. Schmoll, *Applied Microbiology and Biotechnology* **87**, 787–799 (2010).
174. S. Kimball, P. M. Schiffman, *Conservation Biology* **17**, 1681–1693 (2003).
175. E. Borenstein, M. Kupiec, M. W. Feldman, E. Ruppin, *Proceedings of the National Academy of Sciences* **105**, 14482–14487 (2008).
176. D. Sprockett, T. Fukami, D. A. Relman, *Nature Reviews Gastroenterology & Hepatology* **15**, 197–205 (2018).
177. A. R. Burns *et al.*, *The ISME Journal* **10**, 655–664 (2016).
178. S. D. Porter, D. A. Savignano, *Ecology* **71**, 2095–2106 (1990).
179. D. R. Ayres, D. L. Smith, K. Zaremba, S. Klohr, D. R. Strong, *Biological Invasions* **6**, 221–231 (2004).
180. H. A. Mooney, E. E. Cleland, *Proceedings of the National Academy of Sciences* **98**, 5446–5451 (2001).
181. I. L. Brito, *Nature Reviews Microbiology* **19**, 442–453 (2021).
182. C. M. Brasier, *Bioscience* **51**, 123–133 (2001).
183. P. Zhu, Y. Guo, *Travel Medicine and Infectious Disease* **42**, 102097 (2021).
184. T. Bell, J. M. Tylianakis, *Proceedings of the Royal Society B: Biological Sciences* **283**, 20160896 (2016).
185. F. J. Dıaz, A. T. O'Geen, R. A. Dahlgren, *Agricultural water management* **97**, 1813–1821 (2010).
186. W. L. King, T. H. Bell, *Trends in Biotechnology* **40**, 12–21 (2022).
187. H. Caswell, R. Lensink, M. G. Neubert, *Ecology* **84**, 1968–1978 (2003).
188. L. He *et al.*, *Environmental Science and Pollution Research* **26**, 16906–16922 (2019).
189. J. M. Drake, J. M. Bossenbroek, *BioScience* **54**, 931–941 (2004).
190. A. M. d. Oliveira *et al.*, *Revista Brasileira de Ciência do Solo* **46** (2022).
191. S. B. Prusiner, *Science* **252**, 1515–1522 (1991).
192. P. K. Dayton, presented at the Proceedings of the colloquium on conservation problems in Antarctica, pp. 81–96.