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Review

Microbial Invasions: The Process, Patterns, and Mechanisms

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There has recently been a surge of literature examining microbial invasions into a variety of environments. These studies often include a component of biological diversity as a major factor determining an invader's fate, yet common results are rarely cross-compared. Since many studies only present a snapshot of the entire invasion process, a bird's eye view is required to piece together the entire continuum, which we find consists of introduction, establishment, spread, and impact phases. We further examine the patterns and mechanisms associated with invasion resistance and create a mechanistic synthesis governed by the species richness, species evenness, and resource availability of resident communities. We conclude by exploring the advantages of using a theoretical invasion framework across different fields.

Microbial Invasions

Invasive microbes run the gamut of microbial life, from viruses and bacteria to fungi and protozoa [1]. Although older work has addressed the survivability of bacteria released into soil [2,3], envisioning microorganisms as invaders is a poorly studied phenomenon in microbiology. It is preceded by the way the dispersal and biogeography of microbes have traditionally been viewed. In fact, the very idea of a **microbial invasion** (see Glossary) was until only recently inconceivable. A previous leading principle in microbial community ecology postulated that, in terms of incidence, microbes are homogeneously distributed on Earth and lack clear biogeographic patterns due to their high dispersability and large population sizes [4,5]. This consideration of ubiquity rendered any exploration of invasion behavior moot, because an invader could not invade a region where it already existed. Yet, the adage that "everything is everywhere but the environment selects" [6] has been challenged with the advent of direct high-throughput sequencing from environmental samples. These recent research efforts helped to reveal that microbes do, in many cases, exhibit biogeographic patterns and are also, albeit dependent on scale, dispersal-limited [5,7,8]. Although some studies report the existence of homogeneous distributions of microbes across certain ecosystems, such as the bacterial community in oceans [9], it is important to note that such conclusions are usually based on data originating from a small portion of the 16S rRNA taxonomic marker gene and not whole genomes. While the debate on microbial biogeography is not yet resolved, the high rates of horizontal gene transfer in microbial communities [10]; the great number of **ecotypes** with different physiological adaptations [11,12]; and the existing evidence of biogeographic patterns [13], especially when using multi-locus sequence typing [14], reveals that microbial communities and ecosystems alike are mosaics of genetically and phenotypically distinct organisms that are susceptible to invasion by fitter forms. Accordingly, the past 5 years has brought a surge of literature that documents the invasion of various ecological systems by microbes and the mechanisms that control this

Trends

Microbial invasions are widespread in nature and resemble a process of (i) introduction, (ii) establishment, (iii) growth and spread, and (iv) impact. This process is crucial in determining the diversity and distribution of microbes across the Earth.

Invasion resistance is a product of microbial community diversity. More diverse communities are better able to exploit available resources than less diverse communities. This limits any sustenance for an invading population and ultimately leads to its elimination.

The addition of resources to even a highly diverse community can temporarily uncouple the relationship between diversity and invasion.

The ecological principles that govern microbial invasions can be used to advance practical applications, such as biocontrol, biofertilization, and probiotic use.

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process [15–27]. A synthesis of these recent conclusions and their eventual merging into existing ecological and microbiological theory will enhance our understanding of the fundamental processes that govern microbial life.

The recent advent of microbial invasion research spans several fields of microbiology, including agricultural, medical, and environmental. Despite the fact that the entrance of a foreign micro-organism into a resident community of microbes (a microbial invasion) is a common phenomenon across these fields, there is a scarcity of general principles to foster cross-comparisons that can enhance the understanding, interpretation, and future research of this phenomenon. In fact, a major shortcoming is the failure of researchers to recognize a microbial invasion as a process rather than a mere snapshot of a longer frame. In the first part of this review, we examine different examples of invasion and fuse these into a unified framework. As dispersal is a key process in invasion, we highlight that the establishment of microbes in and throughout environments is a process mediated by passive and active dispersal, adaptability of the organism, and the biotic 'resistance to invasion' of the habitat. Once biotic resistance is breached, an invader will likely grow and spread, potentially leading to large impacts on the functioning of native communities. The second part of this review delves deeper into the patterns and mechanisms of invasion resistance, revealing a direct relationship with microbial diversity. We provide a mechanistic synthesis of how microbial diversity mediates invasion resistance and, in doing so, address another major shortcoming of microbial invasion research: the disconnect between short-term invasion experiments performed *in vitro* and long-term experiments usually performed *in vivo* or *in situ*. The former experiments most often utilize small diversity gradients with simulated invasions lasting anywhere from 24 to 48 hours, while the latter often employ larger diversity gradients and can last from weeks to months. This leaves us to wonder if the same patterns and mechanisms govern each scenario.

An Ecological Blueprint of Microbial Invasions: A Four-Step Process

To properly identify and understand the invasion of a system by a microorganism, it is important to comprehend the entire process leading to the success of an invader. Part of the difficulty in understanding the process lies in the fact that invasions are only rarely tracked from start to finish. Simply identifying studies pertaining to microbial invasions and collating them by phases reveals the emergence of a universal and stepwise invasion process that contains several barriers to a successful invasion. Although several variants of the invasion process have been concocted [28], our synthesis indicates that microbial invasions most closely follow a sequential process entailing (i) introduction, (ii) establishment, (iii) growth and spread, and (iv) impact (as hypothesized in [29]; Figure 1, Key Figure; see Box 1 for an *Escherichia coli* case study). Within each phase there are salient aspects that emerge, which provide insight into the ecological and evolutionary characteristics that define **microbial invaders** and their respective invasions.

Invasive microbes must first be transported from a source to foreign environment in order to commence the first invasion phase: introduction (Figure 1A). This is mediated by passive and active dispersal. Ocean currents passively transport and introduce endospores of bacilli over long distances throughout the world's seas and oceans [30]. This has resulted in the creation of communities of 'kin', whose networks stretch from Svalbard in the Arctic, the Baltic sea in Northern Europe, and even to the gulf of California in North America [30]. Several other types of marine bacteria are also circulated through regions of the Earth's oceans, forming distinct populations that are subject to invasion by neighboring taxa [31]. High above ocean waters, air samples collected from the Northwestern United States have been found to contain an average of 4.94×10^{-5} and 4.77×10^{-3} ng of microbial DNA m^{-3}, representing cells of bacteria and fungi, respectively [32]; these organisms were hypothesized to originate from the Gobi desert, being transported via transatlantic wind patterns that pushed the microbe-carrying, air-suspended matter [32,33]. Across large spatial scales, microbial dispersal rates are expected to be

Glossary

Biofilm: communities of microorganisms attached to a surface [77].

Community niche: a metric to estimate the resource use and functioning of an ecological community, which is based on the sum of the best performances of individuals on an array of different resources [78].

Competition-colonization tradeoff: refers to the ecological theory that species coexistence is mediated by the ability of some individuals to be better competitors while others are better colonizers [79].

Diversity–invasion effect: a common relationship witnessed between microbial community diversity (often measured in species richness or evenness) and the survival of an invading microbe, where more diverse communities better resist invasion than less diverse communities.

Ecotype: a genetically distinct form among a species, which may be adapted to a certain environmental condition.

Functional dissimilarity: the differential ability of species in a community to perform certain functions, such as the ability to use different resources.

Keystone species: a species that plays a disproportionately large role in maintaining the balance of an ecosystem or ecological community.

Microbial invader: any microorganism that is transported into a new environment or community where it has never before existed. Even if the invasion is not successful, it is still considered an invader.

Microbial invasion: a four-step process consisting of the (i) introduction, (ii) establishment, (iii) growth and spread, and (iv) impact of a microbial invader. While a successful invasion passes all four steps, reference to the term 'microbial invasion' could imply any one of the phases. Many variants of the invasion process exist; see [28] for an exhaustive list.
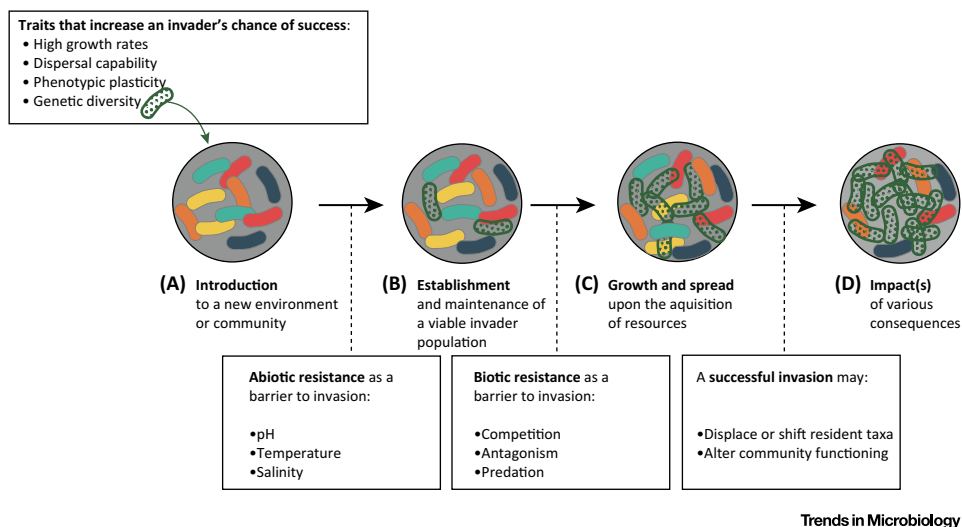
Microbiostasis: the inability of a microorganism to multiply in a particular environment.

Quorum sensing: the regulation of gene expression in response to cell density via chemical signaling [80].

Remaining niche available to the invader: a measure of the resources

Key Figure

The Process of a Microbial Invasion

Traits that increase an invader's chance of success:
• High growth rates
• Dispersal capability
• Phenotypic plasticity
• Genetic diversity

(A) Introduction to a new environment or community

(B) Establishment and maintenance of a viable invader population

(C) Growth and spread upon the aquisition of resources

(D) Impact(s) of various consequences

Abiotic resistance as a barrier to invasion:
• pH
• Temperature
• Salinity

Biotic resistance as a barrier to invasion:
• Competition
• Antagonism
• Predation

A **successful invasion** may:
• Displace or shift resident taxa
• Alter community functioning

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Figure 1. A successful invasion is depicted as a four-step process, envisioned through both the invader and resident community perspectives. The green, polka-dotted microbe represents an invader that is introduced into a community with various microbial taxa depicted in different colors. The same community is represented as it transitions through each phase of the invasion process. Each phase is a key step with a particular 'barrier value', meaning that there may be large (unsurmountable) or small (surmountable) barriers on the path to a successful invasion. From an invader's perspective, several of the listed factors may aid in bypassing the barriers of invasion resistance. (A) Should an invader be able to withstand the abiotic pressures of a new environment it is then considered to be introduced. (B) Biotic resistance will keep the invader in the establishment phase as it maintains a viable population. (C) If an invader gains access to resources that support its growth, it will pass to the growth and spread phase. (D) Growth and spread of the invader may cause impacts to the resident community. No list is a complete reference of all the factors and consequences related to the process of invasions but rather highlights a few important variables and outcomes.

unconsumed by the resident community and therefore available to an invader [26].
Secretome: the array of proteins secreted from a cell.
Species evenness: the distribution of abundance among species in a community [81].
Species richness: the number of phylogenetically distinct organisms in a community. When dealing with microbes, although we often use the terms 'species' or 'taxonomic' richness, these are operational definitions that are based upon the similarity of phylogenetic marker genes.

related to population densities in the source population; larger populations might disperse to a greater extent and their ability to withstand a variety of biotic and abiotic stresses may increase their chances of a successful invasion [13]. Overall, passive dispersal mechanisms transport bacteria over distances they themselves cannot travel and to locations where they otherwise would not be found. This results in the potential engagement of resource competition between members of the resident community and the invader.

Active dispersal mechanisms can also pick up where passive dispersal has left off. Flagellar motility, often in conjunction with **quorum sensing**, acts to propel microbial populations to available resources, which might result in the formation of **biofilms** that ensure resource capture and protect the adjoined cells from chemical or predatory pressures [34–38]. This ability to move provides invaders with a competitive advantage in the competition for resources when compared to nonmotile individuals across various environments, including plant [39], soil [40], marine [41], and vertebrate hosts [42]. And akin to **competition-colonization tradeoffs**, the ability to actively disperse allows for the migration to, and exploitation of, temporal resource hotspots rather than long-term investments into increased competitive abilities on stationary and patch-like resources, as has been shown between variants of *Vibrio cyclitrophicus* [43]. In effect, the colonization strategy implies an invasive lifestyle that is defined by dispersal to neighboring environments and the subsequent competition for resources.

Box 1. Case in Point: *Escherichia coli* **is an Invader of Soil Microbial Communities**

Primarily a creature of the vertebrate gut, *E. coli* is spread via the feces of its hosts into the food and water supply, where it must survive in such secondary (nonpreferred and nonhost) environments such as soil and water in the hope of getting transferred to a new host and continuing its lineage [82]. This introduction begins the invasion process, and, sensing its new environment, *E. coli* alters its cellular physiology to cope with increased abiotic stresses and nutrient depletion [45,82]. This represents a transition to the establishment phase. When in soils, Franz and colleagues [83] suggested that *E. coli* adaptations leading to an improvement in fatty acid metabolism may result in increased survival rates. The boundaries of *E. coli* survival in secondary environments are wide. It can survive in cow manure at levels of circa 5×10^7 cells/gram of soil for up to 15 days [84]; in strongly acidic soils (pH 4.57 to 5.14) it survives for up to 7 days; and in more neutral soils (pH 6.51 to 7.39) it can last up to 35 days [85]. In the laboratory, *E. coli* can also breach the biotic resistance of the establishment phase, and this has been shown when resources are ample and biotic counterpressure is low [26]. This represents a transition to the growth and spread phase where invader growth is detectable and exceeds the population's death rate. In nature, *E. coli* survival was monitored in the same watershed soil for a year-long period, and growth was positively related to seasonal temperatures, indicating that an invasion was successful and *E. coli* had become part of the autochthonous microbial community [86]. The invasion of *E. coli* can impact the structure of the resident community, likely by competition for resources, which stresses the resident community [87].

In nature, the acquisition of nutrients in a new environment is not guaranteed. Instead, the ability of invaders to resist the abiotic conditions of novel environments and then survive the biotic counterpressure of native organisms upon introduction represents a transition to the second phase of invasion: establishment (Figure 1B). In order to cope with the transitions to various environments, many microbes that have been reported or can be classified as invaders have evolved bi- and even tri-phasic lifestyles. For instance, *Salmonella enterica*, much like *E. coli*, harbors a suite of genes that allow for survival outside the mammalian host [44,45]. One such gene is *pcgL*, which allows growth on the bipeptide D-Ala-D-Ala, a substrate released from the cell walls of dead bacteria [44]. Once inside mammalian hosts, *S. enterica* can even occupy two distinct niches inside epithelial cells; that is, it can reside inside a vacuole as well as in the cytosol with the possibility of growth in both locations [46]. Moreover, the remarkable ability of endospores, such as those of different *Bacillus* species, to survive a long ocean journey and germinate once the spores sense nutrient availability is a clear token of a highly evolved characteristic of a successful invader [47]. Whatever the habitat in which a microbial invader is established, it is primarily in the establishment phase when invaders face the strongest biotic resistance by the resident community. The ability of microbes to maintain a viable population with a chance to spread and grow defines existence in the establishment phase and increases their chance of invasion success.

The growth and spread phase (Figure 1C) of an invasion is associated with the degree to which an invader can gain access to (previously unavailable) local resources. Observations on invaders that pass to the spread and growth phase reveal a dichotomy within their ranks – those that are pathogenic or nonpathogenic. They are both archetypical invaders, but their lifestyles are driven by different invasive strategies. Pathogenic invaders might directly manipulate their new environment in order to create ecological niches that perpetuate their transmission. The intestinal pathogen *S. enterica* induces inflammation of the gut that suppresses the growth of the commensal microbiota and triggers the release of *Salmonella*-specific substrates [48]. Thus, the organism is able to relieve the biotic pressure of the gut microbiota and promote its reintroduction into a new environment by living off custom-induced substrates [49,50]. Similar types of custom niche-inducing invasive strategies have been reported for other pathogenic invaders, such as *Agrobacterium tumefaciens* C58, the invader of plant tissues [51]. In contrast, nonpathogenic invaders profit from disturbances or alterations to their environment that make invasion favorable. In the laboratory, the ubiquitous soil bacterium *Pseudomonas fluorescens* is able to take advantage of heat shocks that temporarily impair the functioning of the resident community and lead to increases in resource availability, upon which *P. fluorescens* can grow [27]. In nature, the increase in water temperatures due to climate change has caused expansions of the cyanobacteria *Cylindrospermopsis raciborskii* and *Aphanizomenon ovalisporum* to

expand their ranges to now warmer subtropical and temperate waters from native tropical waters [52]. At higher temperatures, the superior growth rates of *C. raciborskii* and *A. ovalisporum* relative to residents allow them to invade [53]. Because a microbial invasion can take place on a range of scales – from micrometers to kilometers – we argue that the growth and spread phase of a microbial invasion should clearly be measured in dependency of scale. As a criterion, one should use the detection of net-positive growth whereby the growth of the population exceeds its death. This is because growth may occur during the establishment phase in an effort to maintain a viable population. However, such growth may be blurred by a general decaying trend of the entire invading population and may be difficult to detect. Net-positive growth would thus indicate a successful invasion where the invader has breached biotic resistance and spread among the native community, becoming an autochthonous resident.

Microbes that complete successful invasions may be selected for the occurrence of adaptations that increase their competitive ability and, ultimately, invasive potential. For example, several variants of the rare *E. coli* O104:H4 serogroup were responsible for the outbreak of enterohemorrhagic disease throughout Europe and Turkey in 2011 [54]. These variants did not evolve linearly but rather via horizontal gene transfer. Picking up different plasmids, prophages, and genomic islands that encoded different antibiotics and toxin-resistance genes, their survivability and fitness in low-resource and stressful environments was likely enhanced [54], as was their likelihood of transition back to the mammalian gut. Moreover, *Ralstonia solanacearum*, a potent plant pathogen associated with solanaceous plants and native to tropical habitats, also consists of many different variants, each corresponding with a different geographic origin [55,56]. One variant, named phylotype I, can survive for up to 6 months in potato tubers stored at 4 °C. Its recent evolution, deduced from molecular diversity data, suggests that it harbors some type of evolved fitness advantage that has aided its spread and invasion across the globe, including temperate climate regions [56–58]. Taken together, these results suggest that rapid adaptation and evolution of the invader may be a key factor that contributes to invasion success. While rapid evolution is frequent among invasive macroorganisms and contributes to successful invasions [59], microbial invasion research has so far emphasized the ecological interactions between species and is thus the focus of the second part of this review. However, given the rapid generation times of microbes and their propensity for horizontal gene transfer, such evolutionary aspects should not be ignored in the future.

Microbial invaders that manage to spread and grow may have neutral, beneficial or detrimental impacts in their new habitat (Figure 1D). Microbial communities form complex ecological networks of interacting species [60], and invaders have the potential to alter the structure of the interactions [61]. If the invader manages to displace a highly interacting, **keystone species** that facilitates interactions in the community, the network is prone to collapse [62]. Ecologically speaking, this means that the removal of a keystone species can result in strong shifts in community structure that severely alter ecosystem functioning [63]. Exactly how different microbial invaders might alter the structure and functioning of resident community networks has yet to be answered, but the invasion by *Limnohabitans planktonicus*, a freshwater bacterial invader, indicates that one possible consequence may actually be the promotion of species diversity via a competitive release mechanism [17]. In the invasion of a model microbial community, *L. planktonicus* appeared to compete with, and decrease the abundance of, the dominant *Arthrobacter hydrophila*, which, in turn, caused the subordinate species *Aeromonas agilis* and *Flavobacterium* sp. to increase their abundances by exploring the now vacant niches. Finally, an overlooked issue is the possibility that unsuccessful invasions might still lead to impacts since some, albeit little, growth may occur in the establishment phase. Although largely unexplored, this issue might have implications in areas where microbial invasions are sought, such as the efficacy of probiotic, biocontrol, and biofertilizing agents, because the legacy of past

invasion attempts may alter the present community structure, functioning, and, ultimately, mechanisms of resistance.

The Diversity–Invasion Relationship: Patterns and Mechanisms

Along the invasion process, within the biotic resistance of the establishment phase, recent research has revealed a deeper layer of resolution by which microbial invasions are characterized. This work was fostered by hypotheses that merged the well-rooted microbiological principle of **microbiostasis** [64] with macroecological observations, mostly from plant communities (e.g., [65,66]), which reported that biological diversity was inversely related to invasion success. Through testing the idea that diversity may also function as an invasion barrier in microbial communities, patterns and mechanisms of invasion resistance have since been revealed across many different microbial systems with a variety of experimental approaches. In the following, we synthesize these findings to show that biological diversity is a major factor determining invasion resistance.

Patterns

The origins and drivers of biotic invasion resistance present in a habitat have until only recently remained elusive. Early studies did indicate that removing portions of microbial communities from a soil system could increase plant productivity by relieving competition and increasing the abundance of organisms that provide nutrients (i.e., ammonia to plants [67]). This idea was later translated to the concept of microbiostasis, which could be further parsed into fungistasis (resistance to fungal invasion) and bacteriostasis (resistance to bacterial invasion) [64,68,69]. Recently, microbial, particularly bacterial, invasions have been studied in several different habitats, including *in vitro* [15–19,23], *in vivo* [20], and *in situ* [15,21,24,26,27,70]. All of these studies included a component of biological diversity, which was able to explain the population dynamics of the invader. Those experiments exploiting larger diversity gradients, often by removing diversity via a dilution-to-extinction approach, have found that **species richness** [15,26] and **species evenness** [24] are invariably negatively related to invader abundance. Interestingly, biotic resistance is also implicated in experiments using small diversity gradients. Experiments in model (constructed) communities have corroborated results from larger diversity gradients, indicating that richness [16,17,23] and evenness [18] are indeed factors that contribute to the invasion resistance of natural microbial communities. Overall, a pattern emerges indicating that the survival of an alien species is high when the diversity of the resident community is low and vice versa. This phenomenon has been coined the **diversity–invasion effect**.

Mechanisms

The mechanisms that drive invasion resistance and the observed diversity–invasion effect should be regarded in the context of the amazing diversity of most natural microbial communities, which can contain thousands of different species [71]. In any such community, the forces of natural selection are thought to have created a patchwork of individuals that contain motifs of resource use capabilities where each inhabitant occupies its own unique niche [72]. Concerning mechanisms, few experiments have addressed microbial systems, whereas most come from plant ecology [73–76]. These studies indicate that the availability of resources and the ability of the more diverse (plant) communities to best exploit those resources drives invasion resistance. The recent studies on microbes have supported such theories [18,19,21,23,26,27], and they have provided a window into how different components of biological diversity and the environment's resource availability create a resistance barrier.

Short-term invasion experiments in which communities are assembled from pure cultures have the advantage of separately quantifying each resident individual's contribution to the community's resource use profile. Experiments with diversity gradients consisting of one to eight *P. fluorescens* genotypes have shown that taxonomically richer communities could better resist

invasion by *Pseudomonas putida* and *Serratia liquefaciens* than poorer communities due to increased levels of resource exploitation [16,23]. Further analysis of resource use capabilities revealed that **functional dissimilarity** and, therefore, complementarity of resource utilization of the resident *Pseudomonas* genotypes could better explain the abundance of *P. putida* than could taxonomic richness [23]. This indicates that the community's resource use profile is not driven by species richness *per se* but instead by the ability of different species to exploit different types of resources. Furthermore, the invasion by a nondenitrifying *P. putida* strain into simple communities composed of 17 distinct denitrifying bacteria with a fixed level of richness but fluctuating levels of evenness revealed that more even communities resist invasion better than less even communities, most likely by blocking the invader's access to available resources [18]. Long-term invasion studies usually lack the capability to examine niche capabilities due to their *in situ* nature, but they are powerful in their ability to observe the effects of diversity over time. The effects of the resident community's richness on *E. coli* survival over a 60-day period have been found to become amplified with time [15], as have the effects of evenness on *Listeria monocytogenes* survival in soil over 30 days [24]. These long-term studies also evoked resource competition as a decisive mechanism governing invasion resistance.

Only one study so far has attempted to combine both short-term and long-term approaches, being instrumental in its ability to decipher the factors that contribute to invasion resistance. In this study, assemblages of pure bacterial strains were introduced into a sterile soil matrix, as were dilutions of natural soil [26]. The diversity gradient of assembled strains consisted of 5, 15, and 30 species; the gradient of diluted soil consisted of 10^{-1}, 10^{-3} and 10^{-6} dilution treatments, with estimates of richness pegged at 435, 438, and 153 bacterial species, respectively. The binding component was the quantification of niche capabilities from pure strains, including the invader, and diluted soil communities that were examined on the same array of 71 carbon sources. This allowed for a measure of the **community niche** metric. The breadth of the community niche increased with species richness, whereas invader survival decreased as the community niche expanded. Furthermore, an analysis of niche capabilities of the invader vis-à-vis all assembled and diluted communities showed that the **remaining niche available to the invader** decreased as species richness increased. This work helps to bridge the gap between short-term and long-term experiments because similar resource use mechanisms, measured by metrics such as **community niche** and **remaining niche available to the invader**, are also evidenced in long-term studies. Taking the results of short-term and long-term experiments together, it appears that, where species richness increases the multitude of resources used, evenness ensures their depletion. It is this choreography of diversity components working in concert that likely reduces the niche available to the invader and stems invasion.

Despite the power of diversity in establishing an invasion barrier, an important yet overlooked premise of ecological theory is that a high supply of resources should, at least temporarily, uncouple any relationship between diversity and invasion due to the relaxation of competition [73]. When resources are added to experimental communities, changing the resource availability from low to high, invaders may take full advantage and grow, even when confronted with highly diverse communities [26,27]. Strikingly, attenuated *E. coli* O157:H7 populations that are near extinction in soil communities can take advantage of resource pulses that suppress competition from residents, allowing the invasive population to regrow up to an astonishing four log units [26]. Resource pulses added in parallel with an invader also aid in their initial establishment [19]. And although the relaxed relationship between diversity and invasion is transient, resource pulses are expected to modulate local species richness by allowing an invader to successfully invade and integrate into a new locality [74]. Overall, there is an interplay between the components of biological diversity and resource availability that determine the community's invasion resistance barrier (Figure 2).
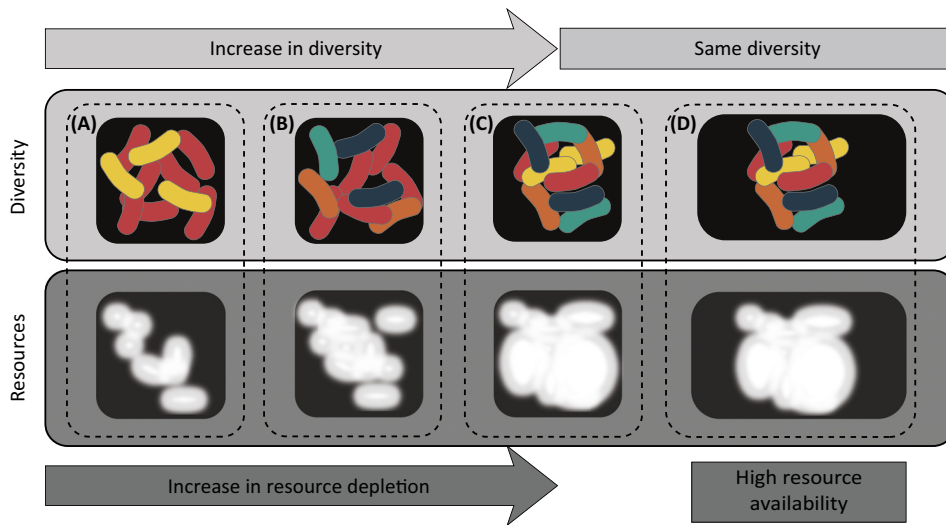
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Figure 2. The Interplay between Diversity and Resource Availability in Determining Invasion Resistance. The upper, light-gray area shows communities with increasing species richness and evenness (up to panel C) and similar cell abundances. The bottom, dark-gray area indicates the total amount of resources available to the community (black) and the amount that it has actually consumed (white). (A) Communities with lower diversity do not consume all resources available in a specific environment. Any foreign organism that reaches this area can then utilize the unconsumed resources, increasing the chances of a successful invasion. (B) As diversity increases, the amount of available resources decreases. The consequent decrease in resource availability thus increases competition between taxa and reduces the probability of a successful invasion. (C) As diversity increases even further, communities get close to reaching the carrying capacity of the environment, when resource depletion should approach a maximum level; here, a successful invasion is very unlikely to occur, due to the lack of available resources to sustain growth. (D) However, a resource pulse that increases the amount of available resources can temporarily open the window for a successful invasion to occur, even in highly diverse environments.

Concluding Remarks and Future Directions

The invasion resistance of a microbial community will ultimately be determined by the community's ability to best exploit and compete for available resources in their environment [74]. As we have shown, the chances of this occurring will increase with the species richness and evenness of the community. Despite the large body of evidence indicating that diversity is a major driver of invasion resistance, there are also other mechanisms besides resource use that can aid in the community's invasion resistance, such as predation, parasitism, and antagonism (Box 2). It remains to be seen how these mechanisms, as well as invader evolution and potential invader impacts upon introduction and establishment, influence the final outcome of invasion (see Outstanding Questions). So far, examining mechanisms such as antagonism has been difficult under *in situ* conditions, but metabolic and **secretome** analyses from natural environmental samples may be able to relate such activity to an invader's survival. By enhancing our knowledge of all the factors and mechanisms related to invasion resistance, we may be able to enhance the effectiveness of many practical microbiological applications related to probiotic, biofertilizer, and biocontrol use. The success of these agents is often limited by their low survival in novel environments, but it is possible to search for, or perhaps engineer, inoculants with effective ecological strategies (e.g., antibiotic production) and superior competitive abilities on limiting resources. In the same way that *Salmonella* infections are promoted by their custom-induced substrates, a superior competitor that is selected for or engineered to better capture those resources could drive an invading population to elimination and allow recolonization by the gut flora. In sum, the value of adopting an ecological perspective to the invasion phenomenon, which spans the spectrum of microbiology, has allowed for the discovery of general and unifying

Box 2. Other Mechanisms that Influence Invasion Resistance and Putative Links with Diversity.

Similar to resource availability, antagonism has been shown to limit invasion when low but promote invasion when high, most likely due to self-poisoning of the microbial community at high levels of diversity [16]. In the context of invasion and immunity, oral secretions from the spruce beetle *Dendroctonus rufipennis* contain bacteria that inhibit the colonization of several pathogenic fungi via the production of antifungal metabolites [88]. Furthermore, the microbiota of termite guts are able to produce actinomycin that likely helps to maintain a stable and uninvaded microbiome [89].

Parasitic attacks from organisms even more simple than bacteria may also influence invasion. Although the impact of bacteriophages was initially thought to be minimal in natural environments due to the high specificity required between phage and host [90], bacteriophages have been shown to locally adapt to natural bacterial communities on scales of centimeters [91]. However, most of the effects of bacteriophages have only been shown from an invader's perspective. For example, the prophages of *Enterococcus faecalis*, a mammalian gut resident, aid in the invasion of new communities and protect *E. faecalis*' niche through the production of a composite phage [92]. *Vibrio cholerae*, responsible for cholera, uses a polymeric protein nanotube encoded by a type VI secretion system (T6SS) to pierce the cell wall of competing intra- and inter-specific strains, killing the prey cell, using it as a resource, and growing within a new environment [93]. In this sense, bacteriophages could help invasive bacteria to invade a new environment or resident bacteria to protect their niche.

Predation from protozoa, which graze off bacteria, have also been shown to affect the fate of invading bacteria. Invading *Escherichia coli* populations are susceptible to grazing by protozoa [94], and their effects in soil ecosystems are thought to be dependent upon the localization of the invading bacteria within the soil pore. In the case of *Aerobacter aerogenes*, invading populations that were able to gain shelter and protection from protozoa within a soil pore were two times more abundant than populations lacking pore protection [95]. This is because bacteria can avoid predation from grazing protozoa if they can reach the most cavernous and deepest areas within the soil pore (<3 μm) that protozoa cannot enter [96]. In sum, the evidence presented above indicates that antagonistic, parasitic, and predatory mechanisms also play an important role in invasion resistance.

Outstanding Questions

Are microbial invasions a universal feature of microbes' lives, necessary for perpetuating their lineages and assembling into new communities and environments?

Does rapid evolution upon introduction make microbial invaders more invasive and contribute to invasion success?

Can growth of an invader occur despite an unsuccessful invasion? And can such growth impact the structure and functioning of the community?

How do other mechanisms, such as antagonism and predation, work either in concert or separately with diversity and resource use to influence invasion resistance?

principles upon which we can advance not only microbiological theory but also practical applications.

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