OPINION

Microbial landscapes: new paths to biofilm research

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Abstract | It is the best of times for biofilm research. Systems biology approaches are providing new insights into the genetic regulation of microbial functions, and sophisticated modelling techniques are enabling the prediction of microbial community structures. Yet it is also clear that there is a need for ecological theory to contribute to our understanding of biofilms. Here, we suggest a concept for biofilm research that is spatially explicit and solidly rooted in ecological theory, which might serve as a universal approach to the study of the numerous facets of biofilms.

The importance of microorganisms in human health and disease, and the massive impact of the pure-culture approach devised by Robert Koch and others, has understandably led to a philosophy in microbiological research that emphasizes the study of microorganisms in pure liquid culture. This approach has so prominently pervaded microbiology that biofilm research was long neglected until microbiologists 're-discovered' these fascinating communities almost 40 years ago[1–3]. Growing appreciation of the importance of biofilms has now led to the perception that these communities constitute the dominant mode of microbial life in most aquatic ecosystems[1–3]. Wherever free-swimming microbial cells encounter surfaces, they can switch from a planktonic lifestyle to form sessile communities that are enclosed by a slimy matrix. These communities often form striking architectures.

A growing body of excellent reviews[3–8] has plotted the highlights of biofilm research carried out over the last decade. Biofilms are now recognized as complex and dynamic communities in which substantial phenotypic diversification allows microorganisms to adapt to different environments. For instance, the hydrodynamic conditions and the availability of substrate and nutrients can shape biofilm architecture, whereas certain genes are essential for regulating the production of extracellular polymeric substances. Cell–cell communication can control biofilm development and architecture and, along with programmed cell death, seems to drive coordinated differentiation in mature biofilms and the release of dispersal cells. Microbiologists have discovered an unexpectedly high degree of coordinated multicellular behaviour that has led to the perception of biofilms as cities of microorganisms[5].

The intense research on single- or multi-species biofilms grown in flow cells[1–3] and the continuing attempts to understand the role of biofilms *in situ*[9–11] have both progressed our understanding of biofilm communities. Flow-cell work under standardized and optimized laboratory conditions echoes formal community theory that focuses on a single scale (in which scale is the temporal measure of a pattern or a process, or the level or degree of spatial resolution) and assumes that local communities are closed and isolated[12]. These laboratory experiments have unravelled many microbial interactions, including competition and cooperation, which are largely deterministic in nature and relate to theories of coexistence by niche differentiation.

In the wild, however, biofilms are open and highly dynamic communities and exist as part of a larger microbial network. In this network, local communities are linked by dispersal and multiple interacting species to form a metacommunity. This introduces an explicitly spatial dimension to biofilm research, and, at the same time, calls for biofilm research that is solidly rooted in ecological concepts and unifying principles. Yet a sober examination of the latest research reveals that microbiologists lack such a framework[13]. Undoubtedly this is largely due to the diversity of the biofilm inhabitants and the habitats they occupy. The absence of a theoretical framework severely restricts the extension of biofilm research across multiple scales and limits our understanding of biofilms and their community interactions at the systems level.

We have all walked through woods and seen seeds that are dispersed by the wind and carried away — and intuitively we accept the relationships between seed transport, vegetation patterns and the landscape. So why not import the tenets of landscape ecology and related fields into biofilm research? In this article, we propose that biofilms are microbial landscapes, to emphasize their spatially explicit dimension, and to lay the foundation for a unifying theoretical basis that supports and guides the relationships between biodiversity, ecosystem function and the effects of scales (composition, structure or function) in biofilm research.

Biofilms are landscapes

When we view a landscape, we look at the composition and expanse of elevations, valleys, rivers, vegetation and animals. Landscape ecology essentially studies the interactions between the spatial patterns of these landscape elements and the ecological processes operating within and between them[14]. Landscapes themselves can be defined as the configuration of patterns at any scale relative to the ecological processes or organisms under investigation, and the concept of landscape can therefore be applied to any scale or system[14]. The spatial patterns of organisms primarily result from abiotic factors, such as climate and landform, but organisms can also physically alter their environment and create spatial heterogeneity[14]. Resulting patches of resources and organisms are delineated by boundaries that control processes within the patches and regulate the flow of organisms and information through the landscape[14–16]. For instance, animals often move from one location to another because of the patchy distribution of resources and, at the same time, the heterogeneity of landscape elements (for example valleys and mountains) can enhance or inhibit their movement. This has logical implications for dispersal and the colonization of new niches or the invasion of existing communities. Patches and their boundaries also affect the transmission of communication signals, as canopy foliage, for instance, can absorb, attenuate and filter the song of birds. Yet boundaries in a patchy landscape also control material and energy fluxes and thereby ecosystem functioning. Landscape ecology therefore integrates various fields, such as neutral ecology, dispersal ecology, invasion ecology and ecosystem ecology, and is at the forefront of ecological science[16].

We advocate that biofilms should be viewed as microbial landscapes (FIG. 1a) and, at the same time, that they are interconnected parts of the larger landscape that they colonize. For instance, a biofilm in a stream is a system, but this system also interacts

with the surrounding landscape that is formed by the streambed and the catchment. Similarly, biofilms that form in the lungs of individuals with cystic fibrosis[3] are integrated into the lung landscape, and are exposed to the spatial heterogeneity of hydration and respiratory mucous viscosity.

What do forested and microbial landscapes have in common? In both landscapes, multiple physical and biological factors shape the spatial configuration of the biomass. For instance, flow-induced disturbance can erode biofilms and locally reduce biomass, which is comparable to the wind-fall of canopy trees and the resulting gaps in forests. Grazing by protozoa can further contribute to the patchy distribution of biomass[17] (FIG. 1a.). More generally, boundary conditions induced either by wind or water dynamics are analogous in diverse situations including: the canopy of forests[18], mosses[19], soil crusts[20] and biofilms[21]. Such boundaries not only determine gaseous and dissolved mass transfer, but also influence the transport of seeds and other propagules.

Whether trees grow protected on the valley bottom or are exposed to wind on a mountain, it is the basic landscape topography and the related aerodynamics that shape their canopies. Similarly, the texture of the colonized surface determines the primary structure of biofilm topography. Whether biofilms coat a flat or a rough surface determines their exposure to shear stress and mass transfer — and these sources of spatial variation are rarely encountered in flow cells. In addition to the resident biofilm
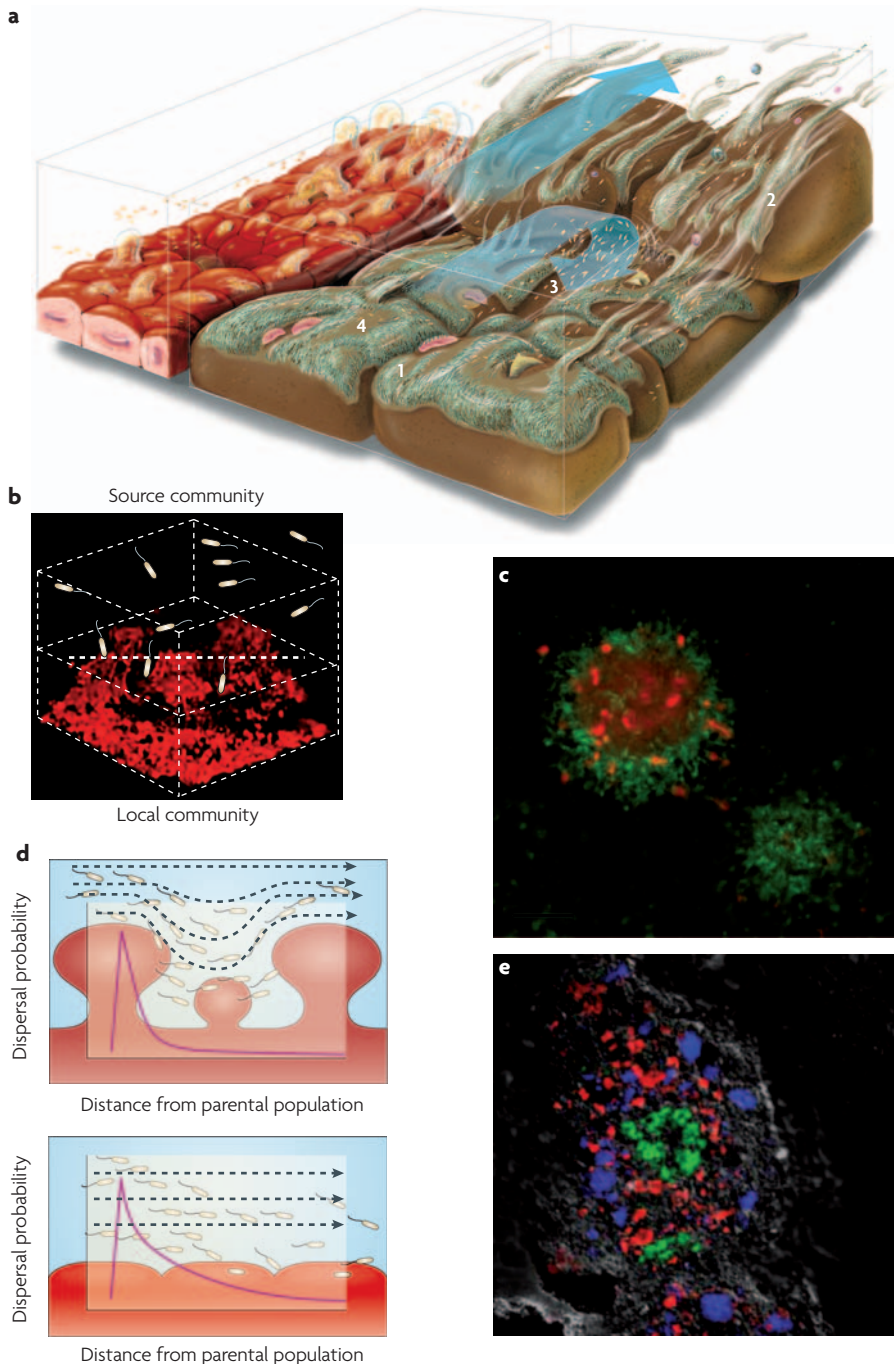
Figure 1 | **Biofilms as microbial landscapes. a** | Bacterial biofilms colonizing a lung epithelium (left) that could also represent a single- or multispecies biofilm grown in a flow cell, and a microbial biofilm colonizing a sedimentary environment in the 'wild' (right). Biofilms coalesce and accumulate biomass in slow flow (1), but predominantly develop filamentous streamers floating in the fast flow (2). Turbulent flow and eddies that are induced by the landscape topography might be preferential trajectories for dispersal cells to land (3). Grazing by protozoa increases the spatial heterogeneity of the microbial landscape (4). **b** | Neutral theory predicts that demographic stochasticity (death, reproduction) but also immigration from a source community (metacommunity) shapes local community assembly. Biotic interactions and cell–cell signalling might function as filters (dashed line) that differentially influence the invasion success of immigrating cells. **c** | Confocal micrograph of microcolonies of a *Pseudomonas aeruginosa* biofilm with localized regions where cell death occurs. Green fluorescent cells are viable, whereas red fluorescent cells have a compromised cell membrane and are dead. **d** | Dispersal behaviour of microorganisms as shaped by the interplay between biofilm topography and induced flow fields around landscape structures. The dispersal kernel shows the probability of encountering propagules as a function of the distance from their source community. The flow field around mushroom-like structures forces propagules to the surface, which narrows the dispersal kernel. Microbial landscapes with a less heterogeneous topography induce different flow fields and larger recruitment areas for propagules. **e** | Confocal image showing the localization of nitrifying bacteria in a wastewater biofilm visualized with fluorescence *in situ* hybridization (FISH) with probes targeting sublineage I *Nitrospira* (red), sublineage II *Nitrospira* (green) and ammonia oxidizing bacteria (blue). Part (**c**) is reproduced with permission from REF. 36 © (2003) American Society of Microbiology. Part (**e**) is reproduced with permission from REF. 45 © (2006) Blackwell Science.

Source community

Local community

Dispersal probability

Distance from parental population

Dispersal probability

Distance from parental population

organisms (bacteria and archaea) various architects, including algae, protozoa and even small metazoa, construct and reorganize the biofilm matrix and confer a further level of structural heterogeneity to biofilms. This level is characterized by the channel networks and protuberances, such as mushroom-like caps and filamentous streamers.

Our perception of biofilms as landscapes has several important implications for biofilm research. Biofilms that form part of a landscape are subject to the same basic rules that couple ecological patterns and processes at larger scales. This concept provides the framework to place biofilms in the context of microbial biogeography, the discipline that studies the large-scale distribution and movement of microorganisms[22,23]. This framework also paves the way for a spatial theory of the biodiversity of biofilms, and enables connections to neutral theory and the related fields of dispersal and invasion ecology.

Neutral theory and microbial landscapes

It has typically been assumed that environmental factors alone shape microbial biodiversity, an assumption that until recently also pervaded thinking in the ecology of large organisms, but has been challenged by the neutral theories of Hubbell[24] and Bell[25] (BOX 1). Neutral theory assumes that all individuals in a community are strictly equivalent regarding reproduction and death, and that community assembly is governed by the balance between the dynamics of these demographic processes and random dispersal from a metacommunity. Neutral theory is therefore a stochastic theory, and given that dispersal from a source to a local community (FIG. 1b) is the main mechanism controlling community assembly, it also is a spatial theory[26].

Although theories on the spatial patterns formed in microbial communities have been proposed[27,28], microbial ecologists have been slow to apply quantitative mathematical descriptions of biogeography to microbial communities. Yet the advent of neutral theory, itself rooted in biogeography, offers microbial ecologists the opportunity to test the importance of dispersal and chance in shaping microbial communities[29–31]. We are convinced that a landscape approach will further foster this development.

The hypothesis that the intrinsic stochastic demography of a biofilm affects its structure would seem to be a logical and attractive starting point in the exploration of biofilm landscapes. However, this approach is not trivial given the spatial heterogeneity that is inherent to biofilms. Nevertheless, Houchmandzadeh and Vallade[32] showed that stochastic processes alone can shape the spatial distribution of taxa with self-similarities at all scales and biomass clustering, as observed in real communities. Strikingly, their simulated landscapes are extremely similar to the spatial heterogeneity of stream biofilms (see Supplementary information S1 (figure)). Although new technologies are elucidating patterns in the topography of biofilms, we currently cannot explicitly observe biodiversity patterns at the scale of 'landscape elements' in biofilms. Unlike classical ecology, in which community patterns are easy to observe and in which models can give invaluable insights into the underlying mechanisms that drive community composition, biodiversity patterns in biofilms are more difficult to assess. Mathematical descriptions of ecological patterns need to be parameterized by observations at small scales, and only by integrating or 'up-scaling' them, will landscape descriptions emerge.

Neutral community models are surprisingly good at predicting the broad-scale patterns that are observed in microbial communities[29–31], but it is likely that neutral community assembly forms just one part of a more complex system in which the patterns from deterministic processes override those imposed by neutral processes. In fact, as pointed out by Alonso and colleagues[26], neutral theory is a first approximation to ecological reality, or a basic theory that provides the essential ingredients to further explore theories that involve more complex assumptions. Along the same lines, Chave[33] emphasizes that neutral and niche theories are complementary, not conflicting, and that this link should be contained in future improvements of community ecology. In the following sections, we consider the interplay between neutral and deterministic processes, and identify and describe dispersal, invasion and the possible role of cell–cell signalling in the invasion of biofilms as processes that

Glossary

Commensalism
Commensalism is an interaction between two organisms in which one organism benefits and the other is neither harmed nor helped.

Dispersal ecology
Dispersal ecology studies the processes by which a species maintains or expands the distribution of its populations. Dispersal implies movement away from an existing population (population expansion) or away from the parent organisms (population maintenance).

Dispersal kernel
The spatial distribution of offspring around a parent can be described as a function of the probability of the abundance of offspring and distance from the parental community. This function is represented by the kernel model.

Extracellular polymeric substance
Extracellular polymeric substances are the key components of the slime matrix of biofilms, and are composed of polysaccharides, proteins, nucleic acids, lipids and other macromolecules.

Landscape ecology
Landscape ecology addresses the causes and consequences of spatial heterogeneity; heterogeneity is a measure of how different parts of a landscape are from each another. This discipline also examines how spatial structure affects organisms.

Mass transfer
The transport of dissolved molecules and particles from the bulk liquid into the biofilm. Diffusion is the process by which molecules move from areas of higher concentration to areas of lower concentration. Advection is the process by which moving liquid actively carries molecules and particles.

Metacommunity
A metacommunity is a set of local communities that are linked by the dispersal of multiple, potentially interacting, species.

Mutualism
Mutualism is an interaction between two or more species in which both species derive benefit

Neutral ecology
Neutral ecology aims to explain the diversity and relative abundance of species in ecological communities. It assumes that the differences between members of an ecological community of trophically similar species are 'neutral,' or irrelevant to their success.

Niche differentiation
Niche differentiation refers to the process by which natural selection drives competing species into different uses of resources resulting in different niches.

Rhizosphere
The rhizosphere is the narrow region of soil that is directly influenced by roots and associated soil microorganisms. Abundant microorganisms feed on sloughed-off plant cells, and the proteins and sugars released by roots.

Self-similarity
A self-similar object is exactly or approximately similar to a part of itself.

Shear stress
Refers to the tangential force per unit area exerted by a fluid as it moves across a surface. Shear stress causes erosion and sloughing of biofilms, and initiates the formation of the filamentous streamers characteristic of many biofilms.

Stochastic theory
Stochastic theory deals with processes that are non-deterministic in that the next state of the environment is partially but not fully determined by the previous state of the environment; or, stochastic processes are largely governed by chance alone. By contrast, a deterministic process is causally determined by an unbroken chain of prior occurrences.

Tensoactive rhamnolipids
Rhamnolipids are tensoactive glycolipids that contain one or two rhamnose molecules.

potentially influence community assembly and that add further complexity to our neutral 'null hypothesis'. These processes essentially determine the immigration rate, which, in neutral models, can function as a 'barrier' or 'filter' for community assembly[31].

Dispersal in microbial landscapes

Recent advances in time-lapse microscopy have revealed the various modes of dispersal of biofilm organisms, such as rippling, rolling or detachment[3]. These dispersal modes mainly result from physical forces and are therefore passive. By contrast, evidence from monospecies biofilms shows that bacteria can also leave biofilms by several mechanisms, including cellular differentiation, which results in cells that have different functions and fates, matrix dissolution and the induction of motility functions[34–37]. For instance, prophage-mediated apoptosis-like death of biofilm cells was proposed to constitute an important mechanism of differentiation that facilitates the liberation of a subpopulation of cells by local lysis and the formation of motile dispersal cells[33,37] (FIG. 1c). Alterations in substrate availability can also induce similar dispersal behaviour, and both transcriptomics and proteomics have shown that the differentiation of specialized dispersal cells is highly regulated[34,35]. Proteomics also showed that dispersing cells of *Pseudomonas aeruginosa* are more similar to planktonic cells than to mature biofilm cells[34], which indicates that dispersing biofilm cells revert to the planktonic mode of growth. The confirmation of detachment as an intrinsic behaviour in biofilms has led to the appreciation of dispersal as an insurance policy to seed a new biofilm during resource limitation (or simply during ageing) of the parental biofilm[6,36,37].

Although detailed mechanisms that regulate dispersal have been identified, microbiologists lack conceptual models to predict the spread dynamics of propagules. Understanding spread dynamics is crucial for a dispersal-assembled theory such as the neutral theory[26], but also applies to understanding pathogen dispersal and disease gradients[38]. Microbiologists need experimentally testable models based on conditions and cellular traits for habitat selection, as derived from landscape ecology and dispersal ecology. One prediction from these models is that dispersal not only determines the potential area of propagule recruitment, but also functions as a template for post-dispersion processes, such as gene flow, competition, invasion and the assembly of new communities[39]. The spatial distribution of propagules can be represented by the dispersal kernel model, which is a

valuable tool for the prediction of invasive spread, and for understanding the mechanisms underlying spread dynamics[18]. Mechanisms underlying dispersal patterns are numerous and complex. For instance, structural features of the forest canopy can affect fluid dynamics above the canopy, which in turn affects seed dispersal patterns[18]. Furthermore, poor dispersers are predicted to spread better in landscapes in which fragmentation is low, whereas good dispersers

spread better in fragmented landscapes in which patches can create 'stepping stones' for dispersal[40].

Based on these concepts, we postulate that the interplay between biofilm topography, fluid dynamics and propagule properties shape spread dynamics in microbial landscapes. Two different scenarios with different dispersal kernels show this interplay (FIG. 1d). In heterogeneous microbial landscapes in which topography is

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Box 1 | Neutral community models
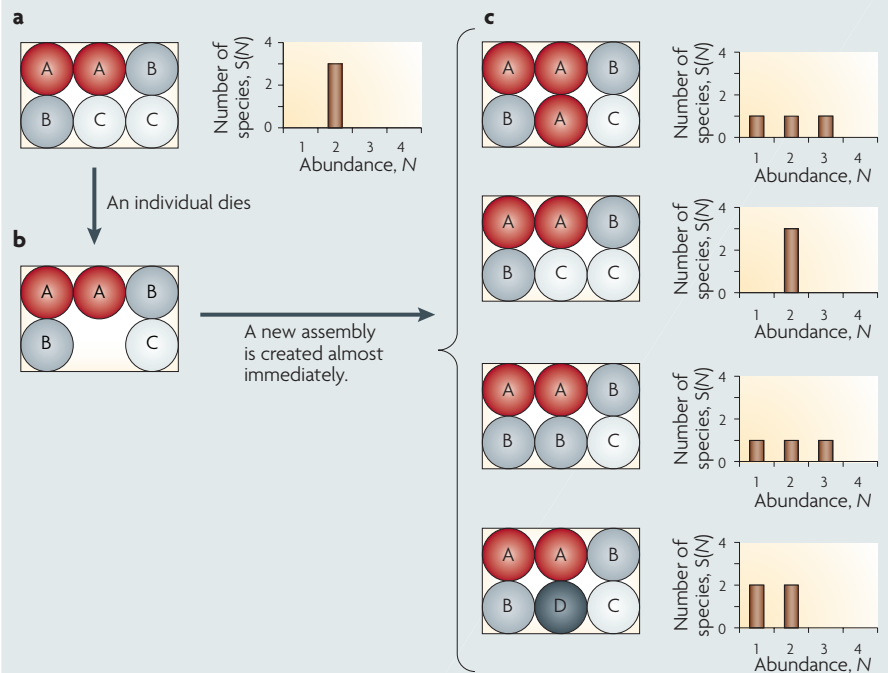
In common with the theory of island biogeography[60], neutral community models recognize a 'mainland' community that functions as a source pool for immigrants. Immigrants are taken randomly from this pool with their relative abundance being the sole arbiter of the probability that they become established in the local community. The degree of isolation of the local community dictates the probability that an immigrant, rather than a locally reproduced organism, replaces a dead individual in the local community. By attributing all the dynamics to chance and ignoring factors such as competition for resources, niche differentiation, disturbance, food webs and a multitude of other mechanisms that most ecologists consider should be represented in any comprehensive theory, Hubbell[24] and Bell[25] undoubtedly courted controversy when they proposed a neutral community model to explain biological community structure. However, despite its simplicity, the model can simulate community structures, in the form of taxa-abundance distributions, that are remarkably similar to those observed in plant communities in tropical forests, as well as communities of birds, insects, fish and bacteria.

The figure shows a schematic of neutral dynamics in a small community, with only 6 individuals, initially comprising 3 equally abundant taxa (part **a**). Hubbell assumes that communities are saturated with individuals. This means that for an assemblage to change, an individual must die or leave the system (part **b**). It is then immediately replaced by an individual, either by reproduction in the community or by an immigrant from a source community (part **c**). Therefore the community forms and develops through a continuous cycle of immigration, reproduction and death.

It is not only the relative abundance of taxa at a single site that is influenced by stochasticity and immigration, but also spatial patterns in relative abundance. Both Hubbell and Bell demonstrated the emergence of clusters in the spatial distribution of taxa by applying their neutral model to an array of local communities on a regular grid and allowing random dispersal between neighboring grid cells. The simulated decay in the similarity of community structure with the distance between microbial cells is similar to that observed in intensive surveys of trees, shrubs, herbs and birds.

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characterized by protuberances and voids, flow fields could affect the fate of propagules. By analogy with the fluid dynamics over vegetation canopies[18], propagules might leave the main current and, assisted by swimming, deposit through the bottom-ward trajectories of micro-eddies. Streamers that are particularly exposed to the flow might function as hotspots for propagule landing, as has been shown for suspended particles in stream biofilms[11]. This would result in 'short-distance' or 'intermediate' dispersal, and in a dispersal kernel with a typically reduced recruitment area. Flow fields over a less heterogeneous landscape would induce 'long-distance' dispersal and larger recruitment areas.

Collectively, different dispersal capabilities and landscape patterns might differentially affect dispersal-assembled communities. For instance, poor dispersal capabilities of rare species in the source community (BOX 1) mean that these species will be under-represented in local communities and might become extinct. Therefore, rare species might take longer to re-establish than more abundant species[41]. This differential barrier to dispersal is evidently important for the formation of diverse local communities[31], and for the spatial heterogeneity of diversity in microbial landscapes.

Invasibility in microbial landscapes

Once a propagule has successfully dispersed it can colonize barren land or invade a resident community. Factors that influence the invasibility (that is, the ease of invasion) of a resident community include the fitness of the resident organisms, their diversity, the available resources and the spatial configuration of these factors in the landscape[42,43]. Therefore, at this stage of dispersal-driven community assembly, deterministic processes related to interactions between newcomers and residents, and to coexistence through niche differentiation, might become important for the formation of biofilms and the landscapes that they form. Interactions between newcomers and residents can potentially range from commensalism and mutualism to parasitism, and these interactions contribute to the non-random spatial distribution that is observed in microbial landscapes[44]. For instance, Maixner and colleagues[45] showed that resource gradients in nitrifying biofilms can induce niche differentiation of coexisting *Nitrospira* populations (FIG. 1e). However, no one has addressed whether this coexistence might affect the invasion of biofilms. Furthermore, coaggregation is an important example of an interaction between resident cells and propagules. Coaggregation occurs when several bacterial species bind to each other through the interaction of cell-surface-expressed molecules, such as lectin–saccharide coupling, and is thought to produce the complex sequential structure of dental and aquatic bacterial biofilms[46].

The impact of the spatial organization that results from these interactions on the invasion of biofilms and community assembly has not been investigated. We propose that the response of propagules to a new environment in the microbial landscape depends on the mode of dispersal and phenotypic variation. Dispersed cell clusters that are enclosed in matrix fragments might not travel far, but after contact with 'barren land' or resident biofilms, they are better-suited to establish a new biofilm than single cells. Increased numbers of cells and the residual matrix might transiently buffer propagule cells against hostile environmental conditions. Single motile cells can actively seek suitable environments but are more susceptible to grazing by protozoa than clusters of cells.

Cell-to-cell signalling. The relationship of cell–cell signalling[47–49] to the colonization of microbial landscapes might also be relevant, as bacterial communication might enable self-recognition, intentional behaviour, 'decision-making' at the group-level and the recognition and identification of other colonies[50]. For instance, cell–cell signalling might allow landing propagules to differentiate between 'self' and 'non-self' (for example, resident cells), and bacteria might even have the ability to determine the ratio of self-to-non-self and modulate phenotypes accordingly[48]. Of equal importance in a new environment is the notion that signalling molecules provide information on the diffusion and mixing environment around cells and might function to control the expression or repression of extracellular enzymes[51].

How signalling molecules propagate through microbial landscapes remains poorly understood, yet it is of prime importance if the suppression of signalling is to be used to eradicate biofilms from indwelling devices or remove contaminating biofilms. Signalling molecules seem to function over short distances (on the order of 1 μm) in open systems such as dental plaque biofilms that are grown in flow cells[52], but long-range 'calling-distances' (mostly 4–5 μm, ranging up to 78 μm) have been identified in the rhizosphere environment[53]. Signalling molecules are typically small and, depending on their hydrophobicity, could diffuse freely through the biofilm matrix, which is highly hydrated. Diffusion is pathway-controlled[16], and chemical and physical heterogeneity can therefore affect signal transmission. For instance, the chemistry of extracellular polymeric substances is variable over small scales[54] and produces environmental gradients that might function as boundaries, filtering or deflecting signals. By contrast, the advective flow of water through channels might dilute the pool of signalling molecules but could also constitute a fast transmission lane.

Based on these considerations of signal propagation, we suggest that signalling by resident cells in response to invasion as some sort of defence mechanism would have to occur close to the preferential invasion site in order to be effective. This theory is supported by the quorum-sensing-induced production of tensoactive rhamnolipids in channels, which were at first thought to prevent clogging of channels in *P. aeruginosa* biofilms[55], but might also be a strategy to reduce the invasion of these biofilms[56]. Channels and pores in the biofilm matrix might be preferential invasion routes into biofilms, as propagules might be transported through them by advection, in a similar fashion to the function of rivers as conduits in terrestrial landscapes. Propagules might also preferentially land on canopy structures such as mushroom caps or streamers that are exposed to the bulk liquid flow. In this case, signalling by residents close to the surface of these structures would optimize signal transmission to invading cells. However, signal molecules would also be highly susceptible to flow-induced loss. This scenario complements the conceptual model that was proposed by Parsek and Greenberg[57], which predicts that the highest signal concentration occurs where biomass accumulation best protects signal molecules from flow-induced loss (for example, at the centre of mushroom caps). It is possible that cells expressing a 'guardian' phenotype that is specialized for cost-intensive signal production colonize the peripheral parts of biofilms. This would constitute an altruistic strategy that benefits the group by reducing the invasion of the biofilm.

More generally, incoming propagules might face chemical barriers synthesized by resident bacteria, such as antibiotics and other toxins. These molecules seem to have an essential role in determining microbial diversity[58] and in the structure of biofilm communities. Competition experiments with a marine epiphytic biofilm confirmed this, as strains that produce antibacterial extracellular compounds out-compete or invade biofilms of strains that do not produce such inhibitors[59].

Perspectives

Microbial ecologists need theories that can draw together simple models and concepts. We are optimistic that revisiting classical ecology can herald a new era in biofilm research that will enable microbial ecology to further develop as a field, and will bring microorganisms closer to the trees in the forest. Our framework can contribute to a better understanding of how biofilm communities are established. It is our hope that once the pathways to biofilm development are understood, it will be possible to manipulate the growth of biofilms. Biofilms might eventually function as model systems to address emerging questions surrounding invasion and landscape ecology that are inaccessible through field observations of communities of larger organisms.

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