Biofilm community succession: a neutral perspective

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Abstract

Although biofilms represent one of the dominant forms of life in aqueous environments, our understanding of the assembly and development of their microbial communities remains relatively poor. In recent years, several studies have addressed this and have extended the concepts of succession theory in classical ecology into microbial systems. From these datasets, niche-based conceptual models have been developed explaining observed biodiversity patterns and their dynamics. These models have not, however, been formulated mathematically and so remain untested. Here, we further develop spatially resolved neutral community models and demonstrate that these can also explain these patterns and offer alternative explanations of microbial succession. The success of neutral models suggests that stochastic effects alone may have a much greater influence on microbial community succession than previously acknowledged. Furthermore, such models are much more readily parameterised and can be used as the foundation of more complex and realistic models of microbial community succession.

The theory of ecological succession is one of the earliest and longest-standing concepts in all of ecology [1–3]. Traditionally, the theory has been applied to plant communities to offer explanations of how the diversity and structure of an ecosystem changes once it starts to establish or re-establish. For example, after a forest fire has occurred, succession theory has been used to describe the patterns of the plants which are able to colonise the habitat in the immediate aftermath of the event and also in the following generations, as some initial colonisers become locally extinct, while others come to dominate or are joined by future immigrant species.

After this initial work, the idea of ecological succession was examined in other systems such as phytoplankton [4]. However, it is only really in the last decade or two that there has been significant research on how the theory might apply to microbial systems [5–8]. Largely, this has been due to technological and methodological limitations, which are only now being broken down. The advent of molecular methods to analyse and quantify microbial communities has allowed for a revolution in microbial ecology [9]. As such, in the last couple of decades, we have had for the first time the tools at our fingertips to examine the question of succession in microbial ecosystems.

In the past decade, there have been several studies looking at microbial ecological succession within biofilm communities [6, 10–13]. These previous works have examined community structure and its dynamics from the first establishing

colonisers through towards mature biofilms. Certain key signatures of these communities have been noted, which have been used as the basis for the development of conceptual models of microbial community succession. These features include:

(1) Community diversity increased rapidly during the first phase of biofilm establishment before dropping again in the intermediate stage as some of these initial colonising species become locally extinct.
(2) Diversity increases again during the third phase of biofilm development.
(3) The total biomass increased with time in all phases, but cannot grow unbounded.

Additionally, when multiple successional trajectories under the same conditions are examined, the community structures are seen to be initially similar, before diverging slightly and then finally converging. The period of diverging similarity is seen to coincide roughly with the intermediate period of biofilm development, after the initial colonisation but before establishing into a mature biofilm [6, 14, 15].

From these observed trends, conceptual models have described ecological succession in microbial systems [5, 16]. These have been largely niche-based theories, focussing on the roles that local competitive differences between species and the distribution of resources might have on biodiversity and its dynamics. For example, the model described by Jackson and co-authors [17] can be summarised thus.

Initially, the substrate is available to many colonisers who can briefly settle, irrespective of competitiveness. Once in an immature biofilm, then competition for near-universal resources (such as space) eliminates the poorest competitors. Finally, as the community matures, spatial heterogeneities arise and niches establish in which globally inferior competitors may find local advantages and establish again within niches. These mechanisms explain the first, second and third observed successional phases, respectively.

These explanations are certainly viable and offer plausible explanations of the mechanisms underlying microbial succession. They are, however, reliant upon many ecological concepts which, in practice, are extremely difficult to enumerate. For example, quantifying the number of available niches or the diversity of resources through time is far from a trivial task. The complexity involved in such concepts has proven prohibitive to the development of quantitative (and, therefore, testable or verifiable) models. As such, the proposed ecological models remain purely conceptual and have not been formulated mathematically with quantifiable parameters. Here, we demonstrate that a neutral community model [18–20] is able to offer an alternative explanation of observed patterns throughout all stages of biofilm community development. We propose this alternative to the previously suggested niche-based mechanisms to describe the drivers of microbial community succession.

In recent years, the development of neutral community models has been one of the most discussed and controversial theories in ecological modelling [21–26]. According to this theory, all individuals within a local community are assumed to be competitively equivalent and the population structure is shaped solely by chance migration and birth-death events. Some critics have attacked these models, finding the central assumption too strange and unrealistic [27]. However, a great number of studies have found that such models are able to reproduce abundance patterns of species across many scales, from trees to fish, and to micro-organisms [28–30].

The neutral community which we apply is the variant on Hubbell's original model [19], as described in Woodcock *et al.* [28]. In this model, the number of individuals in the ecosystem N_T, is assumed to be always constrained by finite resources and space, with all individuals equally able to compete for them. At regular time intervals, one individual is selected uniformly at random and removed from the community (death) and its space is instantaneously taken by a replacement, either an immigrant selected from the stream, which acts as the source community (immigration) or else a member of the local community is selected at random and replicates itself (birth). These alternative mechanisms occur with probabilities m and $1-m$, respectively (Fig. 1).

Initially, we assume that the underlying substrate hosts no bacterial community, as was the case with the experimental studies to which we compare our model results. The surface is then fully populated up to its initial maximum capacity via a pure immigration process from individuals within the source community. After the initial community is assembled, the system always remains saturated at size N_T individuals, with each death being replaced either by a new birth or immigration event. We make one additional assumption, namely that the community size per unit area initially increases but always remains bounded. This is consistent with observed data [6], as total population sizes increase as the biofilm community moves from being two-dimensional into three dimensions, but the biofilm cannot keep growing to infinite thickness. For the analyses presented, we assume that once the ecosystem reaches its first saturation, then additional growth as the biofilm matures and thickens follows a logistic growth curve. That is, we assume that N_T is described by

$$N_T(t) = N_{T_{min}} + \left[\frac{N_{T_{max}} - N_{T_{min}}}{1 + e^{-s\left(t - N_{T_{min}}\right)}} \right]$$

where $N_{T_{min}}$ is the maximum population size before the film begins to thicken, $N_{T_{max}}$ is the maximum population size in the mature biofilm and s is an appropriately chosen constant governing the rate of biofilm thickening. The exact nature of the growth function does not affect the analyses, provided it is monotonically increasing but bounded. For example, if Monod-like growth is assumed, the same phenomena are observed. The assumption of logistic growth is made here for simple proof of concept.

Using the neutral community model, we simulated (using MatLab) the early stage development of biofilms of approximately $1\,\text{mm}^2$ up to $100\,\text{mm}^2$, with bacterial cell densities increasing up to a maximal density of 10^8 cells cm^{-2}. These figures are consistent with the cell densities found in real stream biofilms [6]. Furthermore, we took the biodiversity parameter θ to be 15, which is consistent with previous findings for water-borne microbial systems [28]. The immigrant source (in this case, the stream water) community was simulated 100 times and the average of these repetitions used to minimise noise. Parameter values for $N_T(0)$ and s were more difficult to obtain from the literature, so many different values of these were utilised to test proof of concept. We ran simulations with many parameter pairs to see what effect varying these could have on our conclusions. We found that our results were not dependent upon selection from a narrow parameter range. For any sensible parameter values for $N_T(0)$ and s, the same mechanisms were in force and the resulting successional trajectories showed the same key signatures. The exact selection of these parameters did alter the points in time at which the diversity–time plots entered each of the three phases of development (initial growth – decline – second growth phase) and how pronounced the rates of diversity loss and gain were, but the same three phase diversity–time dynamics were observed (Fig. 2).

We found that our neutral model was able to reproduce the general trends which have been reported in the literature and only previously explained through more complex niche-based models. Not only were we able to reproduce all
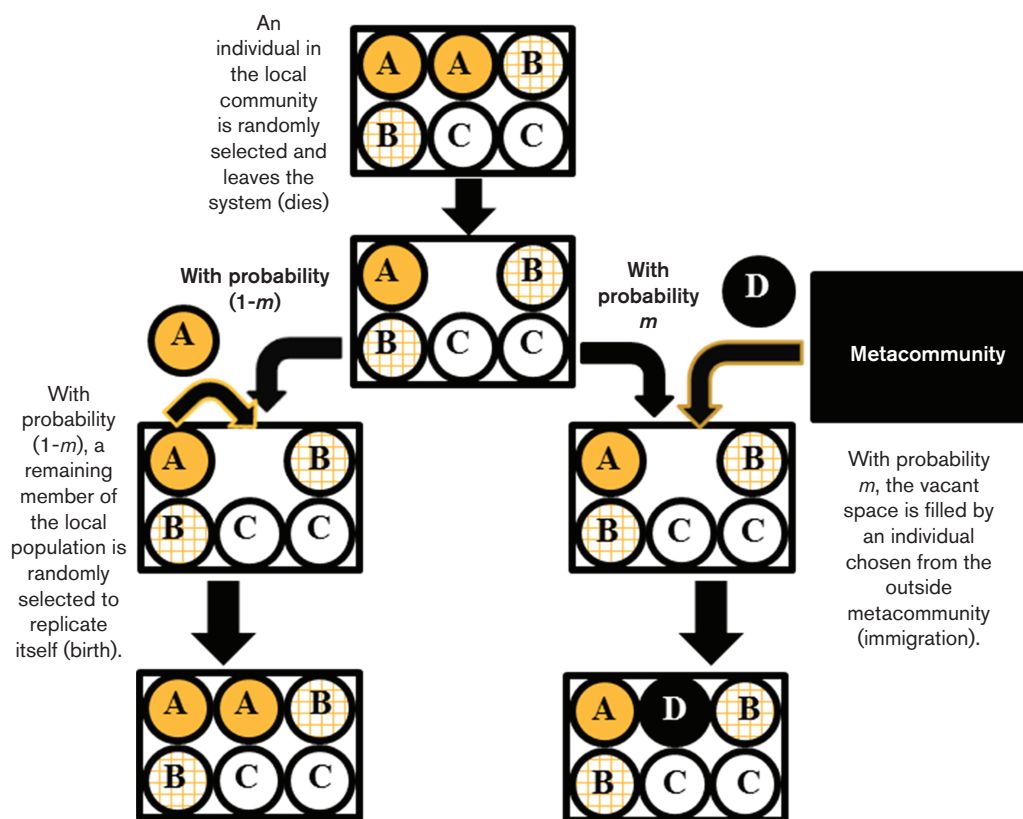
Fig. 1. Diagram illustrating the mechanisms governing community assembly for the neutral model. The left-hand channel shows new individuals being born into the system. The right-hand channel illustrates immigration from the source metacommunity.

three phases of the observed diversity–time dynamics, but the divergence and subsequent convergence of parallel trajectories through time were also generated by our model (Fig. 3). Using our neutral model, we replicated the observed trend that when biodiversity is seen to decline in the microbial community, trajectories became increasingly dissimilar before converging again in the later phase when the species richness increased for a second time.

The key features which forge the shapes of the diversity–time and similarity–time plots [31] relate to the accruement of rarer species and the space available in the biofilm to support them. In a purely neutral model, such as the one presented here, there are no competitive advantages for any given taxa in the system. However, purely on the basis of their inferior numbers in the community, it is the rarer taxa that are most likely to be excluded when there is increased competition for space or resources. Conversely, the chance of accumulating rare taxa increases as additional resources become available.

During the first phase of development, there is little competition for space or resources; any individuals arriving on the substrate can colonise and begin to grow. This drives the phase of initial biodiversity growth. Once the substrate is fully saturated, it is more difficult for new individuals to

invade and establish. There is now competition for space and new individuals are only able to immigrate into the space vacated by local deaths. During this phase, the space left by dying individuals of rarer taxa are most likely occupied by the offspring of individuals already in the community, and so total biodiversity is likely to decline. This forms the second phase of succession. Finally, as the biofilm matures and thickens, it increases in volume and can maintain a larger community size, leading to the sustenance of increased species richness.

Considering the similarity–time relationship, it is during the phase of biodiversity decline that parallel trajectories diverge. Again, this is driven by the change in the ability of rarer taxa to remain in each system. During this period, when rarer species are undergoing chance local extinctions in each trajectory, it is the case that a rare taxon which has suffered stochastic extinction in one system may still be established in another, hence their similarity declines. These rarer taxa can re-establish themselves in the majority of trajectories only when there is greater space available in the mature biofilm. It should be noted, however, that like many phenomena driven by the loss or accumulation of rare taxa, our observed picture of their behaviour may be distorted by limitations in current molecular methods [32].
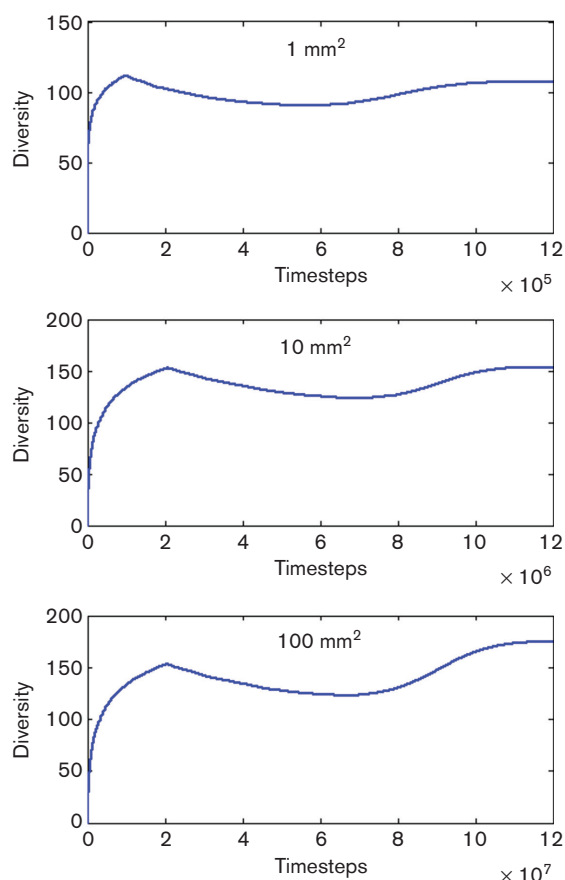
Fig. 2. Diversity–time plots for biofilms of area $1\,mm^2$, $10\,mm^2$ and $100\,mm^2$. The simulations were run until a stable equilibrium was reaches after time t_{max}. For all figures, the immigration rate was 10^{-6} and the diversity parameter θ was 15.

Fig. 3. Similarity–time plots for biofilms of area $1\,mm^2$, $10\,mm^2$ and $100\,mm^2$. Similarity is calculated using the Sorensen index. The parameter values used in these simulations were identical to those used for Fig. 2. Note that the time period during which the trajectories are seen to diverge in similarity coincides with that of overall decrease in biodiversity.

This demonstrates that a simple zero-dimension neutral model can replicate both the diversity–time relationship commonly seen in experimental studies of microbial community succession, and the similarity–time dynamics for parallel trajectories. Although some of the underlying assumptions may seem overly simplistic, it cannot be dismissed as a viable alternative explanation to niche-based models of community succession. What we have developed here is the first mathematical model of these phenomena in microbial systems, one based on quantifiable (and mainly directly measurable) parameters, rather than qualitative concepts which are difficult, or perhaps even impossible, to measure.

Just because we are able to explain many of the features observed in experimental systems does not mean that we regard our model as the most accurate description of biofilm community assembly. For example, there is considerable evidence for the effects of hydrodynamic regimes shaping biofilm architecture [6, 33, 34]. This feature is not incorporated into our zero-dimensional model and would be required if seeking a reasonable description of communities at a broader scale than presented here.
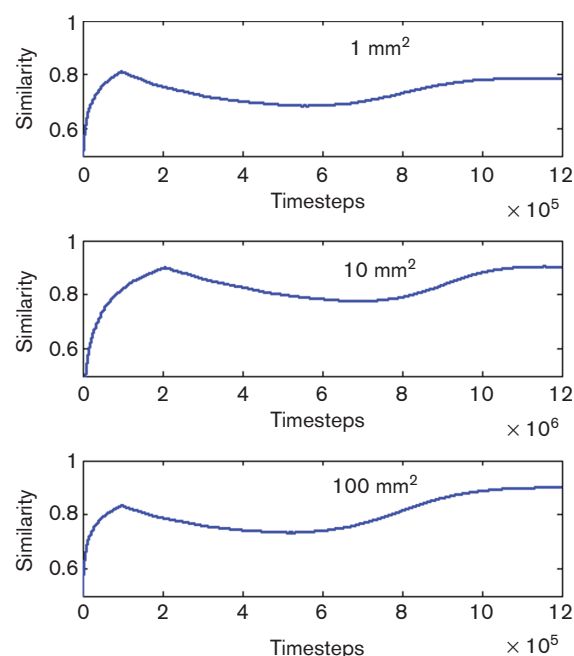
Nonetheless, we maintain that this simple neutral model is a good first step towards a more accurate description of microbial community succession. It may perhaps seem surprising to some just how much of the complexity of observed abundance patterns can be explained by purely stochastic birth-death-immigration events alone. Even when complex biodiversity patterns are observed, these do not preclude the possibility of the system being accurately described by a neutral model. In one of his works on neutral ecology [35], Houchmandzadeh describes the aim of the theory as being 'not to deny or diminish the role of environmental factors, but to stress that the observation of patchy spatial distributions of species in natural environments should be not be considered surprising or non-random by itself'. By looking at the stochastic birth-death-immigration events in isolation of the regional competitive advantages, we can first calculate how much of the observed community behaviour may be attributed to demographic stochasticity. Once this has been quantified, additional complexity can be incorporated into the model development, where it is shown to be required.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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