

The impact of resource dependence of the mechanisms of life on the spatial population dynamics of an *in silico* microbial community

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Biodiversity has a critical impact on ecosystem functionality and stability, and thus the current biodiversity crisis has motivated many studies of the mechanisms that sustain biodiversity, a notable example being non-transitive or cyclic competition. We therefore extend existing microscopic models of communities with cyclic competition by incorporating resource dependence in demographic processes, characteristics of natural systems often oversimplified or overlooked by modellers. The spatially explicit nature of our individual-based model of three interacting species results in the formation of stable spatial structures, which have significant effects on community functioning, in agreement with experimental observations of pattern formation in microbial communities. *Published by AIP Publishing.*

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Biodiversity plays a crucial role in promoting and preserving the proper functioning of ecosystems.¹ As a result, significant attention has been devoted to understanding the mechanisms that maintain biodiversity,² in particular, the role of cyclic competition. Existing microscopic models of communities with cyclic competition often significantly oversimplify or even neglect the resource-dependent nature of demographic processes.^{3–6} We therefore extend existing models by considering this key factor in a spatially heterogeneous landscape, to align them more closely with real-world microbial ecosystems, and to investigate how this more realistic approach affects community productivity and biodiversity. We demonstrate the consequent dramatic effects on the population dynamics of this community of three species, consistent with other modelling and experimental studies. These results have implications for the maintenance and development of natural ecosystems, as well as for pattern formation in microbial communities.

I. INTRODUCTION AND BACKGROUND

A. Maintaining biodiversity

The issue of promoting and maintaining biodiversity has gained urgency in recent years with the recognition of an ongoing biodiversity crisis, with human actions resulting in significant numbers of species across a wide range of ecosystems being threatened with extinction.⁷

Theoretical models have demonstrated that biodiversity can be maintained in ecosystems if ecological processes such as competition and dispersal occur over small spatial scales.⁸ This is also true for communities where the competition between species is non-transitive, meaning that a strict competitive hierarchy does not exist.⁹ The rock-paper-scissors

game is the classic example of non-transitive competition, which is also known as cyclic competition. There are many examples of communities in nature that demonstrate this type of competition; two examples from microbiology are populations of colicinogenic *E. coli* strains¹⁰ and certain bacterial species engaging in antibiotic production.³

When modelling such microbial communities, recent work has focused on microscopic models describing interactions at the individual rather than the population level, see for example, Refs. 8, 11–13 and reviews in Refs. 14 and 15. The cyclic competition setting that has attracted the most attention is that of three species, being the simplest case. This competition structure of a simple loop can be generalized to higher numbers of species, where the species dominate each other cyclically, e.g., species 1 beats species 2, species 2 beats species 3, ..., and finally species N beats species 1.¹⁶ As the number of species increases, the behaviour becomes more complex¹⁷ and studies often turn to mean field approximations rather than individual-based models to draw conclusions about the system's behaviour.¹⁸

One study that has attracted particular attention in the field is due to Reichenbach *et al.*; these authors used a stochastic grid-based model to demonstrate that coexistence of three species in cyclic competition is mediated by their dispersal. Once species' mobility exceeded a critical rate, biodiversity was lost regardless of the spatial environment and details of the cyclic competition.³ This critical effect of mobility persists as the number of species in the cyclic competition scheme increases; studies including up to n species have observed that higher numbers of species produce more and more complex behaviour.^{17,19}

Empirical evidence confirming this result in the three species case can be found in a study that employed a non-transitive model community containing three populations of *E. coli*.¹⁰ The study found that diversity was rapidly lost in the experimental community when dispersal and interaction occurred over relatively large spatial scales, whereas all

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populations could coexist when these ecological processes were localized.¹⁰

In such experimental and modelling studies, a key proxy of biodiversity is species diversity, which is generally understood to be composed of species richness and species evenness.²⁰ Species richness refers to the absolute number of species present in the community, whereas species evenness refers to the relative abundances of the different species.²¹ Thus, if a population is completely even, all species are equally abundant. Richness and evenness play different roles in community functioning²² and have therefore often been treated separately. Of the two, richness has attracted far more attention in experimental and modelling studies, while evenness has been comparatively overlooked.²³

The relative neglect of the role of evenness in community functioning and dynamics is unfortunate since it has been shown to be a key factor in preserving the functional stability of ecosystems,^{23–25} while also having a positive impact on productivity by increasing the representation of each species' functional traits.²² Thus, while the importance of evenness for proper community functioning is well established, evenness is rarely accounted for in experimental and modelling studies where it is typically assumed to be maximal.¹⁶ This implies that all species are present in equal proportions, despite a growing body of experimental evidence suggesting that perfectly even communities are rarely found in natural systems.^{26–28}

B. Resource dependence

In addition to high evenness, community diversity can also be promoted (see Section IA) by non-transitive competition. In the particular case of competition in a microbial setting, nutritional resources are a particular focus.²⁹ The resource ratio theory of competition suggests that the prevalence of species in a community is mediated by the available nutrients, in particular, their availability and rate of consumption.³⁰ Thus, competing microbial species can coexist for certain ratios of nutrient concentrations. But if nutrient availability is limited, some species can die out as a result of being outcompeted, leading to a biodiversity loss. The resource ratio competition model has been shown to explain, for various ecosystems including microbial communities,^{31,32} some of the most typical dynamics between resource competitors, including, for example, that the species better able to survive at lower levels of a limiting resource will be the best competitor for that resource.³³

Competition for a limiting resource can be categorised as either scramble competition or contest competition.²⁹ Scramble competition occurs when one species deprives its competitor(s) of the communal resource by depleting said resource, whereas contest competition occurs when one species actively harms its competitor(s), for example, by producing harmful toxins or otherwise attacking other individuals.³⁴ An example of scramble competition is the non-interference competition between different microbial strains for a common but limited carbon source needed to drive their growth.³² An example of contest competition is the secretion, by bacteria such as *Streptococcus thermophilus*, of toxic antimicrobial

compounds which directly reduce the growth rates of its competitor species.²⁹

In microscopic models, the typical approach to resource limitation has been to represent it by imposing a constant limit on population size, rather than modelling the resource dynamics explicitly.^{35,36} More recently, this approach has been altered to consider resource fluxes and dynamic population sizes.^{37–40} This echoes recent developments in the more specific case of modelling biofilm formation, where both the growth of cells and the diffusion of nutrients through the bulk liquid are taken into account.^{41–43} This approach has in recent years been extended to individual-based models,⁴⁰ where a typical example admits a limiting resource that constrains individuals' reproduction.⁴⁴ However, some models of resource-limited reproduction assume that the population in question is well mixed, a typical yet significant simplifying assumption which by design does not permit any effects of spatial structure to emerge. This is despite the fact that spatially structured environments have been acknowledged to result in a significantly different population dynamics than well-mixed environments.⁴⁵

C. Motivation and scope

The mechanisms described in Sections IA and IB are acknowledged as playing significant roles in community functioning, but there is a lack of models integrating them.⁶ In addition, the role of space in promoting biodiversity, by permitting and maintaining species coexistence, has attracted much attention in ecology generally, and more particularly in the case of non-transitive competition.⁴⁶ We therefore extend the approach presented in a previous paper,⁴⁷ in order to present in this paper a comprehensive study of how resource dependence impacts biodiversity maintenance for *in silico* communities in heterogeneous space. We thus focus on studying the emergence of spatial patterns and the population dynamics of the community, as well as their underlying mechanisms and the interplay between them. For this purpose, we employ a spatially explicit individual-based model of a community of three species engaged in cyclic competition.

The formulation and implementation of the model is described in Section II, along with the *in silico* experimental set-up. Results of these experiments are presented and discussed in Section III, before conclusions are drawn in Section IV.

II. MODEL FORMULATION AND IMPLEMENTATION

The foundational framework of our model is a spatially explicit individual-based model due to Reichenbach *et al.*³ (see Section IA). We modified and extended this model by incorporating resource dynamics, individual resource uptake and biomass growth, variable initial community evenness, and by linking the demographic processes to internal resource (substrate) level.

A. Variable initial evenness

To determine the impact of variable evenness on the *in silico* community dynamics, our model must be initialized

with communities of different evenness, so that their evolution can be compared. Evenness can be measured using a wide range of indices, and there is no consensus choice in the literature.⁴⁸ We opted for the Gini evenness index due to its frequent use in both modelling and microbial studies.^{24,49} This index is based on Lorenz curves, where the cumulative proportion of species is plotted against the cumulative proportion of individuals.⁵⁰ The index ranges from zero (perfect inequality) to 1 (perfect equality) and is given by

$$G(\mathbf{p}) = \frac{2}{N-1} \left(N - \sum_{i=1}^N i p_i \right), \quad (1)$$

where N is the number of species in the community and p_i is the relative abundance of species i , sorted such that $p_i \leq p_{i+1}$ for $i = 1, \dots, N-1$. To obtain population abundances resulting in a given evenness, the Gini index can be sampled using a consistent estimator,⁵¹ for which we have previously designed and implemented a sampling algorithm.¹⁶ In this way, we may choose an initial evenness value and then obtain a vector of species abundances to construct a community with the desired degree of evenness.

B. Resource-dependent demographic processes

Individuals represented in our model are subject to the three key demographic processes typically incorporated in microscopic models: reproduction, competition, and mobility. We denote these processes as occurring at rates μ , σ , and ϵ , respectively, and we assume they are independent of the particular species in the modelled community. For simplicity, we consider equal rates for competition and reproduction, and (without loss of generality) determine the time unit by fixing $\sigma = \mu = 1$. The mobility parameter is set at $\epsilon = 4.25$, since this value is below the system's critical mobility rate above which coexistence of species is not possible.³

1. Substrate dynamics

The model also incorporates an environmental substrate that individuals can consume and convert into biomass. Each demographic process is linked to individuals' biomass, in this way mimicking the resource-dependent nature of such demographic processes in the real world.⁶ The amount of substrate in the *in silico* environment is maintained via a constant inflow from a source in the centre of the grid. A diffusion process displaces substrate around the grid.

The growth of an individual is modelled using Monod kinetics.⁵² The values of the two key growth parameters (the maximum growth rate μ_{max} and the half-saturation constant K_s) were based on literature relating to *E. coli* strains,⁴ along with the substrate conversion efficiency E_c , and are, respectively, $\mu_{max} = 8.3 \times 10^{-4} \text{ s}^{-1}$, $K_s = 4.5 \times 10^{-3} \text{ g L}^{-1}$, and $E_c = 0.44 \text{ g mass per g substrate}$. The Monod growth model implies that substrate uptake occurs at a rate proportional to an individual's biomass, until a maximum total biomass is reached ($2.5 \times 10^{-15} \text{ g}$) after which an individual is unable to consume any more substrate. Thus, individuals are

engaged in scramble competition for the environmental substrate.

2. Individual-based modelling approach

We used an individual-based approach to study this system of three interacting species and one environmental substrate. In this approach, interactions take place at the individual level, and the demographic processes occur on a local scale in order to permit long-term coexistence.³ Employing such a "bottom-up" model construction by using individuals rather than populations as our basic unit both admits variation between individuals and allows the spatial heterogeneity of this system to emerge naturally as a result of the localised interactions.⁵³ For these and other reasons, the use of individual-based modelling in microbiological studies has gained increasing favour in recent years.⁵⁴⁻⁵⁷

To localize the interactions in our model, two-dimensional space is represented by a square regular grid and individuals may only interact with their nearest neighbours, which are defined as the four grid cells located in their von Neumann neighbourhood. These interactions occur stochastically, as they are simulated using the Gillespie algorithm.⁵⁸ The algorithm proceeds using a Monte Carlo step, where the next demographic process to occur is determined by generating random numbers. The outcomes of interaction events are governed by game-theoretic rules,⁸ where the success of an individual depends on the behaviour of its neighbours⁵⁹ (see Figure 1).

To permit mobility, an individual may exchange grid cells with a neighbour if its internal substrate $I'(i, j)$ is above a certain threshold τ_m , where $I'(i, j)$ denotes the internal substrate of the individual located at grid cell (i, j) at time step t . Similarly, an individual may reproduce into a neighbouring empty grid cell once its internal substrate has passed a certain threshold τ_r . In this case, the parent's biomass splits equally between itself and its offspring (minus a certain amount since reproduction is assumed to be less than fully efficient). Finally, an individual may compete with one of its neighbours if its internal substrate is above a certain threshold τ_c . Whichever individual loses the competition is killed, and the grid cell it occupied becomes empty. The victor of the competition is determined by the cyclic competition scheme: species A beats species B, which beats species C, which beats species A. If one individual's internal substrate is above the competition threshold τ_c while the other individual's is below it, then the second individual is assumed to be too weak to compete and loses the competition. When an individual dies, its internal substrate is instantaneously released into the environment. Hence, individuals are engaged in contest competition in addition to the scramble competition for the common resource.

After linking the demographic processes to substrate uptake as described, we investigated the consequent effects on community biodiversity first by varying the amount of substrate required for a demographic process to occur (the substrate threshold τ) and second by assigning different substrate utilization profiles to the species in the community.

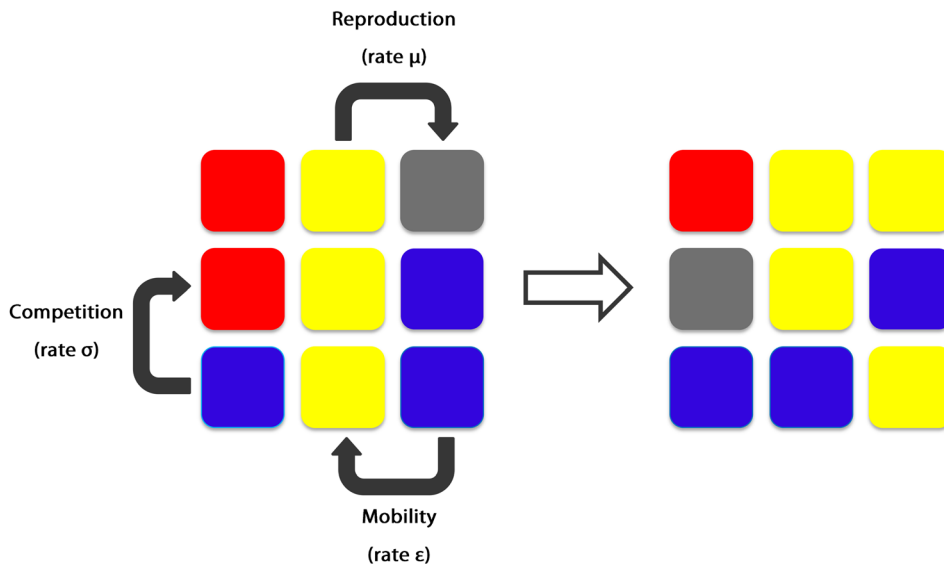


FIG. 1. Mechanisms of demographic processes, following Reichenbach *et al.*³ Whether an interaction occurs or not depends on the individuals' internal substrate levels.

3. Substrate utilization scenarios

Motivated by examples of microbial communities in nature,^{45,60,61} we selected three substrate utilization scenarios. To facilitate analysis and comparison of their impacts, we also investigated a benchmark case.

In the benchmark case, demographic processes occur regardless of individuals' internal substrate level, at the rates μ , σ , and ϵ introduced in Section II B. Individuals absorb environmental substrate and convert this into biomass, but their biomass has no impact on interaction events. This is analogous to the original Reichenbach model,³ where there is no influence of biomass on interaction events, since in this model there is no substrate and individuals have no mass. Thus in the benchmark case, a pair of neighbours is selected randomly, an interaction occurs and its outcome is calculated, the grid is updated and the process repeats.

In the following three substrate utilization scenarios, we impose a substrate threshold on a specific demographic process as described in Section II B 2.

In the first scenario, all three species are subject to the same substrate threshold and therefore have the same reproductive/competitive/mobile capacity: $\tau_A = \tau_B = \tau_C$. This symmetric limitation scenario represents the simplest case of an ecosystem with similar species that all depend on a common environmental resource in the same way.⁶²

In the second scenario, one species is subject to a substrate threshold τ , while the other two species are not (asymmetric limitation). Then, for example, species A has substrate threshold $\tau_A > 0$ while species B and C have substrate thresholds equal to zero, i.e., $\tau_B = \tau_C = 0$. Thus, only one species is constrained in its capacity for reproduction/competition/mobility. This scenario can represent, for example, a community with one species that must produce an extracellular enzyme to degrade the substrate into a usable form, whereas the other species do not and hence do not face the same cost in synthesizing the substrate to drive their growth.⁶⁰

In the third scenario, a hierarchy in terms of substrate limitation is imposed on the community, e.g., $\tau_A > \tau_B > \tau_C$.

This scenario can represent, for example, a community where the common strategy of "cheating" is present.⁶¹ Two species require an extracellular enzyme to degrade the substrate, but one of them intercepts the reaction products secreted by the other species, in this way avoiding the need to produce its own enzyme, and cheating to benefit more from the common resource.⁴⁵

C. *In silico* experimental set-up

In our simulation study, we employed a 100×100 grid evolved over 500 generations, where a generation is defined as the number of interaction events required so that on average each individual is the focus of one interaction. Fixed boundary conditions were used for both the bacteria and the substrate, to mimic a closed environment typically found in *in vitro* experiments. The grid was initialized with all cells containing a small amount of environmental substrate (10^{-17} g), and then populated with individuals (without internal substrate) in a random spatial configuration. A fixed proportion of cells in the initial grid (10%) was left empty so that the simulated environment was not initially at carrying capacity. The initial abundances of the different species were calculated using a specified initial evenness with the Gini index, as described in Section II A.

After the grid was initialized, substrate flowed into the grid at each time step at a fixed rate (8.3×10^{-20} g μm^{-2} s $^{-1}$) via a source located in the centre of the grid, and a fixed number of diffusion steps (with coefficient $D = 1.7 \times 10^{-3}$ μm^2 s $^{-1}$) was then carried out. The time scales of the diffusion and individual interaction processes were separated so that diffusion occurred at a faster time scale than individual interactions, since otherwise all substrate would be very quickly consumed.

The diffusion process was implemented using the following scheme:

$$S^{t+1}(i,j) = S^t(i,j) + \frac{\Delta t}{\Delta x^2} D [S^t(i-1,j) + S^t(i+1,j) + S^t(i,j-1) + S^t(i,j+1) - 4S^t(i,j)], \quad (2)$$

where Δt and Δx are, respectively, the time and space discretization step size. These parameters are chosen so that their ratio $\frac{\Delta t}{\Delta x}$ satisfies the Courant-Friedrichs-Lewy (CFL) condition which is necessary for stability of the time-explicit numerical scheme.⁶³

An excess of environmental substrate (an atypical situation in natural systems) was avoided through the choice of the inflow and diffusion parameters. After the diffusion steps took place, the environmental substrate concentration was updated for each grid cell. Then, each individual consumed substrate if it was not yet at maximum biomass. Substrate uptake was governed by the Monod equation (see Section II B 1), which was implemented as follows:

$$r^t(i, j) = \mu_{\max} \frac{I^{t-1}(i, j) S^{t-1}(i, j)}{K_s + S^{t-1}(i, j)}, \quad (3)$$

where $r^t(i, j)$ is the uptake rate of the individual at grid cell (i, j) at time step t , and the Monod parameters are as described in Section II B 1.

The individual's internal substrate concentration $I^t(i, j)$ and the environmental substrate concentration $S^t(i, j)$ were then updated for each grid cell where uptake occurred. After substrate diffusion and uptake were carried out, individuals were stochastically selected for an interaction event: either reproduction, competition, or mobility. The interaction event occurred if the focal individual's internal substrate was above the appropriate threshold. These interactions were governed by the game-theoretic rules described in Section II B 2. The outcome of the interaction event (if any) was determined, the grid updated accordingly, and the procedure repeated for the specified number of time steps until the simulation ended.

For each substrate utilization scenario (symmetric, asymmetric, and hierarchical), we limited separately each of the three demographic processes (reproduction, competition, and mobility) to avoid confounding effects. Thus, for example, reproduction was limited asymmetrically among the species, while competition and mobility were not substrate-limited. With the addition of the benchmark scenario, this resulted in ten scenarios. For each of these scenarios, we investigated three initial evenness settings: maximal ($E_0 = 1$), intermediate ($E_0 = 0.5$), and low ($E_0 = 0.2$).

We additionally investigated the effects of two demographic processes being simultaneously substrate-dependent, to gain insight into confounding effects. In this case, we assigned the same substrate threshold to both demographic processes. This was also done for the three scenarios: symmetric limitations, asymmetric limitations, and hierarchical limitations.

Once the simulation set-up was specified by fixing the initial evenness and type of substrate limitation, a set of simulations was carried out using a substrate threshold τ systematically varying from the lowest threshold (10^{-16} g) to the highest (2×10^{-15} g), so that 20 different substrate threshold values were tested. The identity, biomass, and location of each individual, the amount of environmental substrate per grid cell, a spatial aggregation measure (per individual), and total community evenness were tracked at every time step.

The model was implemented using Mathematica (version 10.0, Wolfram Research Inc., USA). Simulations were carried out using the High Performance Computing infrastructure at Ghent University.

III. RESULTS AND DISCUSSION

In a previous short paper,⁴⁷ we used this model to study the impact of substrate limitation on community productivity, as a proxy for ecosystem functioning. We briefly summarize the main results here. Most notable was the observation of a trade-off between maintaining community diversity and increasing community productivity: the resource limitation scenarios that gave rise to the highest biomass yields also resulted in the least diverse communities. The difference in biomass production between monocultures and more diverse communities was due to a negative dominance effect that originated from a trade-off between growth rate (due to a high substrate threshold) and final biomass level. In monocultures, species with a high substrate threshold were able to produce more biomass than fast-growing ones (with a lower substrate threshold), although this occurred more slowly. However, in more diverse communities a fast-growing but less productive species would monopolize most of the substrate and prevent its competitor species from achieving the high biomass levels seen in corresponding monocultures. This effect agrees with experimental studies which observed an underyielding of diverse communities compared to their component monocultures due to the competitive suppression of highly productive species.^{64–67}

In this paper, we present a more comprehensive study of the model's behaviour, and the insights this can lead to with regard to the mechanisms under investigation. We present and discuss results related to pattern formation, population dynamics, the effects of more than one demographic process being simultaneously substrate-limited, and the effect of resource dependence on the system's critical mobility rate.

A. Pattern formation

To study the role of space in maintaining biodiversity in our system, we examined the spatial evolution of our modelled communities under the different resource limitation scenarios.

In the benchmark case, the different species arrange themselves into stable rotating spirals, as seen in the Reichenbach model,³ and experimental results for cyclically competing *E. coli* strains.¹⁰ Introducing resource dependence restricts the development of these spiral structures to those areas of the grid where there is first sufficient substrate (which we recall is diffusing outwards from the central source) and second individuals with the ability to exploit such substrate (Figure 2). These emergent spatial patterns broadly agree with those observed in experimental studies of three bacterial strains in cyclic competition.⁶⁸

We notice a significant difference in behaviour between resource-dependent reproduction and competition when compared with resource-dependent mobility. In the first two cases, a notable zone of activity emerges where interactions occur more frequently due to the relatively higher

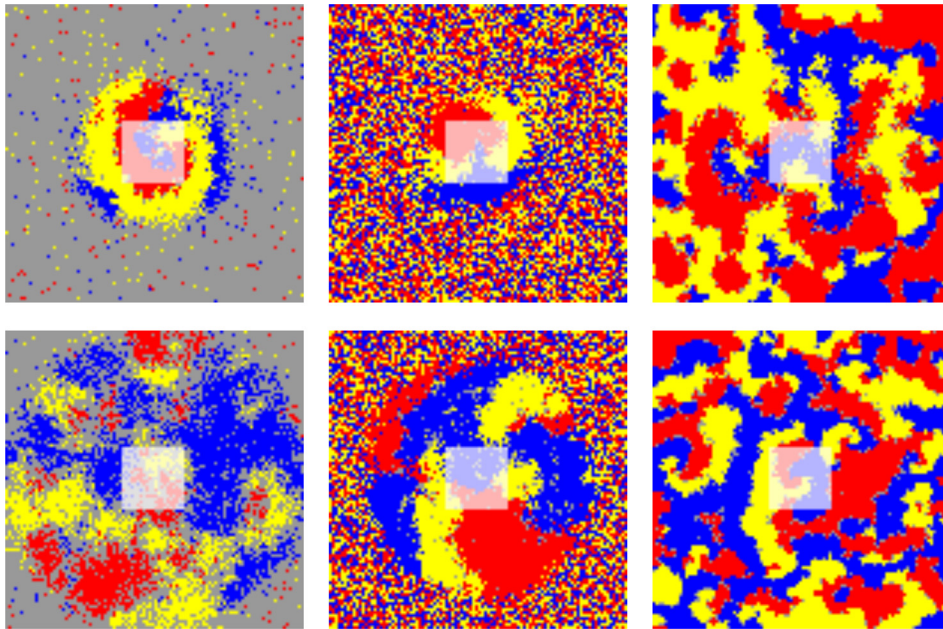


FIG. 2. Comparison of pattern formation for resource-dependent demographic processes, after 500 generations. The box in the centre indicates the size of the substrate source. Top: substrate threshold $\tau = 10^{-16}$ g. Bottom: substrate threshold $\tau = 2 \times 10^{-15}$ g. Left to right: substrate-limited process—reproduction, competition, and mobility.

concentration of environmental substrate compared to the outer areas of the grid.

We observe little impact of resource dependence on mobility. As noted previously, strong effects are only observed when mobility exceeds a critical rate, allowing individuals to move over distances too great to permit local interactions. In this case of high mobility, we approach a setting where the population can be considered well mixed, and therefore the mean field approximation becomes relevant. This approximation predicts that the coexistence equilibrium is not asymptotically stable and therefore extinctions are frequent.¹⁸ Hence, as long as mobility in our model does not decline to zero nor exceed the critical rate, we notice little difference between low mobility and even lower mobility (as induced by increasing the substrate threshold).

In the case of substrate-limited reproduction, the substrate gradient implies that most reproductive events occur closer to the centre of the grid, where the high-biomass individuals are predominantly located. Outside this zone, reproductive events occur rarely since there is less environmental substrate to fuel growth. Thus, individuals killed in competition events in this zone are not replaced, and the grid becomes depopulated. When the substrate threshold is increased (see bottom row of Figure 2), the zone of activity expands due to the increased substrate cost to reproduce.

The existence of the zone of activity is confirmed using the density plots in Figure 3, which illustrate the number of

interaction events that occurred per cell over the course of a simulation. We observe similar behaviour in the cases of substrate-limited reproduction and competition (hence we show only one example of each in Figure 3): most of the interaction activity occurs in a central zone, which expands when the substrate threshold is increased. In the case of substrate-limited mobility, the interaction activity is not confined to any particular area but occurs throughout the grid. It is possible in this case to distinguish the characteristic spiral formations, since there is much activity occurring at their edges, being the interfaces of different species aggregations.

The mechanism driving the formation of the zone of activity is the following: individuals require more time to grow sufficient biomass to reproduce, and in this extended time the substrate has diffused farther away from the centre, expanding the zone of activity. However, within this expanded zone, comparatively fewer reproductive events occur. Hence individuals become more disaggregated, since empty grid cells are not filled as easily as for lower substrate thresholds. Thus, increasing the substrate threshold does not greatly affect spatial aggregation per species, as measured by patchiness,⁶⁹ since the expansion in the zone of activity is counterbalanced by the decrease in aggregation.⁷⁰

An analogous phenomenon is seen for substrate-limited competition, where instead of depopulation in the outer areas of the grid, we observe a lack of spatial structure. The spiral structures characteristic of this type of model emerge as a

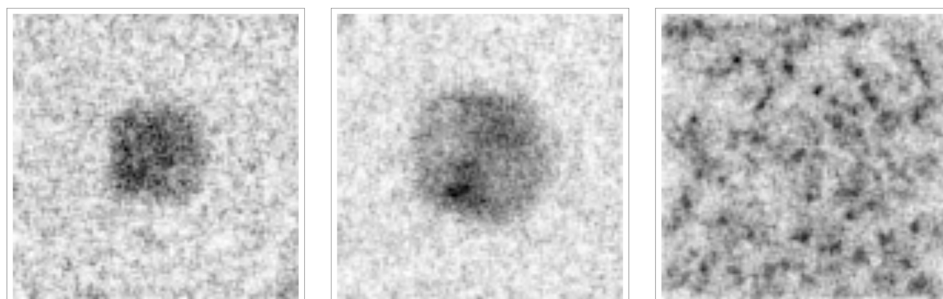


FIG. 3. Activity for resource-dependent demographic processes, after 500 generations. Darker colour indicates that a higher number of interaction events were executed during the course of the simulation. Left to right, substrate-limited process: competition (threshold $\tau = 10^{-16}$ g), reproduction (threshold $\tau = 1.2 \times 10^{-15}$ g), and mobility (threshold $\tau = 5 \times 10^{-16}$ g).

result of the local cyclic competition scheme and hence are confined to the zone of activity where individuals can find sufficient substrate to fuel competition. Outside this zone, few competition events occur and thus the community remains well mixed. Again the zone of activity expands when the substrate threshold is increased, while there is no disaggregation as seen for substrate-limited reproduction.

These results support the mechanistic explanation of spatial structures in microbial biofilms, which describes the formation of various structures in biofilms as a consequence of differences in local substrate availability,⁷¹ more specifically as occurring under substrate-limited conditions such as those modelled here.⁷²

B. Population dynamics

The spatial structures described in Section III A also have significant effects on the overall population dynamics. The mean field approximation of the rock-paper-scissors model predicts a single coexistence equilibrium (which is stable but not asymptotically so) and three stable homogeneous equilibria representing the three possible monocultures.⁷³ However, the mean field approximation relies on the assumption that the population is well mixed, which is not relevant in the spatially explicit case we consider. This explicit consideration of space therefore produces significantly different population dynamics, as does the resource dependence of the demographic processes.

An example of the evolution of the species proportions for hierarchically limited competition is shown in Figure 4. In this scenario, species A is subject to the highest substrate threshold for successful competition, with $\tau_A = 2 \times 10^{-16}$ g, while species B has a substrate threshold that is half that of species A, and species C has no substrate threshold and may therefore compete with no regard to its internal substrate level. The simulation is initialized with a completely even community. As the simulation evolves, species B and C benefit from lower substrate thresholds than species A, and prosper and persist in oscillating proportions. Meanwhile species A declines but does not collapse to extinction.

The mechanism underlying this behaviour has previously been noted in a voter-type model of cyclic competition

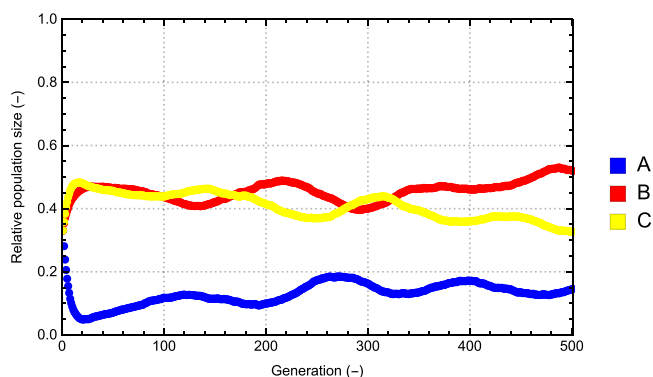


FIG. 4. Evolution of species proportions for hierarchically limited competition ($\tau_A > \tau_B > \tau_C$) in a community which is initially completely even.

between three populations, with the additional process of one population type being “externally supported,” in the sense of having stronger competitive and reproductive ability than the other two types.⁷⁴ In our model, this “externally supported” species is species C, since it is not resource-limited in contrast to the other two species. Thus, species C kills more of its prey (species A), whose population decreases. Thus, species A kills fewer of species B, which thereby achieves an advantage in the dynamical balance since it has more prey (species C) and less predators (species A). The advantage of species B is less dramatic in our model than that of Tainaka⁷⁴ and other models,¹⁸ since in our model the externally supported species enjoys an advantage in only one process (competition) as opposed to two, as in the voter model where reproduction and competition are coupled, which is characteristic of such models and in contrast to rock-paper-scissors models.

Thus, all three species may coexist in unequal proportions, behaviour not seen in the cases without a substrate limitation, or homogeneous substrate inflow. In these cases, the species can only coexist in roughly equal proportions, since any small differences in population proportions unbalance the rock-paper-scissors dynamics.⁴⁶ These differences in proportions (due to stochasticity or otherwise) are quickly magnified and inevitably lead to the extinction of two species, with the third species persisting alone. A clue to the mechanism underlying this behaviour can be found in the mean field approximation of the three species model in the substrate-less case, where the only stable equilibria are those representing equal coexistence, or survival of a single species.¹⁶

The existence of a stable unbalanced community also agrees qualitatively with the predicted outcome of siderophore-mediated bacterial competition between two species.²⁹ In this system, both species require a compound, called a siderophore, to chelate iron. When one species does not produce a siderophore itself, but rather uses that produced by the other species, the predicted outcome of the competition for iron is that the “cheating” species will dominate the “honest” species, since it benefits from a lower cost by not producing its own siderophore.²⁹ Our model produces analogous behaviour for a three-species equivalent, where the third species C also benefits from cheating.

The existence of this type of stable unbalanced community in our model is facilitated by the emergence of spatial structures discussed in Section III A, where most of the interaction events occur in a central “zone of activity” determined by the substrate gradient, where all three species are present. Outside this zone, dynamics are significantly different (see Figure 5). This behaviour agrees qualitatively with patterns observed in juvenile mussel banks, where it was noted that “self-organization allows mussels to persist at algal concentrations that would not permit survival of mussels in a homogeneous bed.”⁷⁰ In our model, the spatial structures permit the persistence of species at lower levels than would be possible under homogeneous spatial conditions, as the spatial heterogeneities provide “refuges” for these species, notably in the central zone of activity.

The fact that these unbalanced communities are stable and persisting agrees with microbiological studies showing

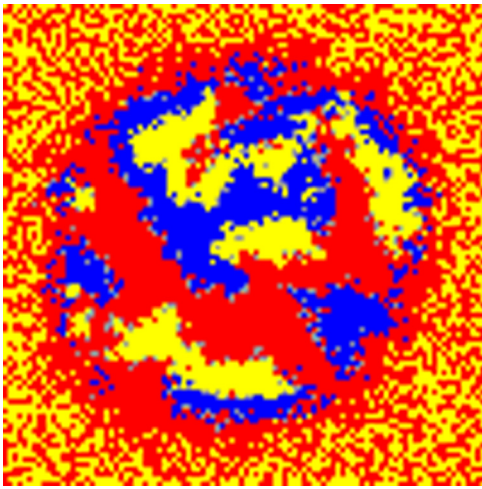


FIG. 5. Grid configuration after 500 generations for hierarchically limited competition ($\tau_A > \tau_B > \tau_C$) with high initial evenness.

that the presence of spatial refuges can enhance community resistance to stress and disturbance, providing a buffer against adverse effects on community composition and function.⁷⁵

We note that the formation of spatial refuges observed in our model is qualitatively different from a phenomenon described as “coexistence by small numbers,” which has been observed in other individual-based modelling studies of non-transitive competition.⁷⁶ This term refers to the situation where a species collapses to near extinction due to competition effects, but does not disappear completely—a very small number of individuals remains present, completely disaggregated from other individuals of the same species, and entirely surrounded by non-competitors (e.g., an individual of species A survives in a neighbourhood consisting entirely of individuals of species B). Such a scenario is theoretically consistent with experimental studies that suggest that communities in nature are often dominated by only a few species, with many other species present in low quantities,⁷⁷ but has also been suggested as an artefact of the neighbourhood structure that was used.⁷⁶

The stable unbalanced communities evolved using our model are much less unbalanced than those in the case of “coexistence by small numbers,” where the least abundant species may number only a handful of individuals. In our case, the least abundant species is still present in a significant proportion relative to the rest of the community, and individuals remain fairly aggregated with their conspecifics (Figure 5).

A different type of behaviour is illustrated in Figure 6, where the evolution of the species proportions is shown for a simulation of the asymmetrically limited reproduction scenario with substrate thresholds $\tau_A = 2 \times 10^{-16}$ g and $\tau_B = \tau_C = 0$. In this case, a cheater species (B) again dominates the honest species (A), but here the second cheater species (C) does not prosper. This is due to the imposed cyclic competition structure, which in this case is not substrate limited. Hence, species C is more vulnerable to its predator (species B) than in the case shown in Figure 4 and is depressed by the increased competitive pressure despite its stronger reproductive capacity relative to the honest species (A). The stable persistence of all

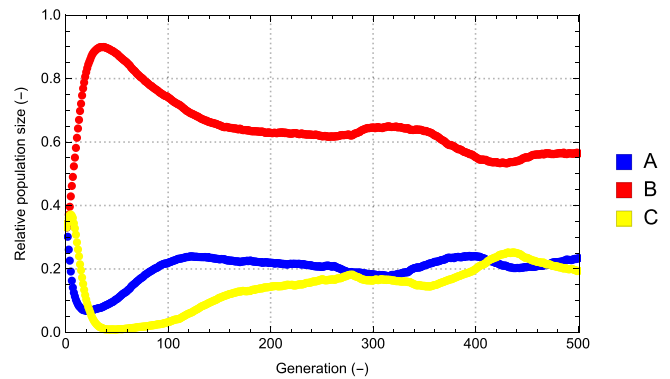


FIG. 6. Evolution of species proportions for asymmetrically limited reproduction ($\tau_A > \tau_B = \tau_C = 0$) in a community which was initially completely even.

three species in unbalanced proportions is again permitted by the emergent spatial structure discussed in Section III A, which resembles the one shown in Figure 5, but now species C is also absent from the outer regions of the grid.

In a third and final example, we show in Figure 7 the evolution of the species proportions for the scenario of asymmetrically limited reproduction with substrate threshold $\tau_A = 2 \times 10^{-16}$ g and $\tau_B = \tau_C = 0$, with intermediate initial evenness. The behaviour again agrees with results observed in siderophore-mediated competition,²⁹ where a species able to monopolize the available iron will force the other species into extinction. In our three species case, the species with the highest cost (A) quickly collapses to extinction as the others monopolize the available substrate. The subsequent resource competition between the remaining two species is then once more determined by the cyclic competition scheme, which explains why species B outcompetes species C until the latter species collapses to extinction. In this case, the unstable dynamics are magnified by the lower initial evenness, which we previously observed as increasing the probability of species extinctions by increasing the amplitudes of the population oscillations.⁴⁷

C. Effect on probability of extinction

In the benchmark case of no resource dependence (analogous to the model of Reichenbach *et al.*³), the biodiversity

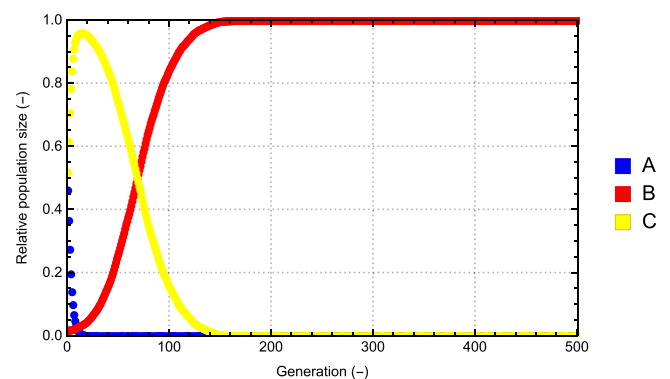


FIG. 7. Evolution of species proportions for asymmetrically limited reproduction ($\tau_A > \tau_B = \tau_C = 0$) in a community with initially intermediate evenness.

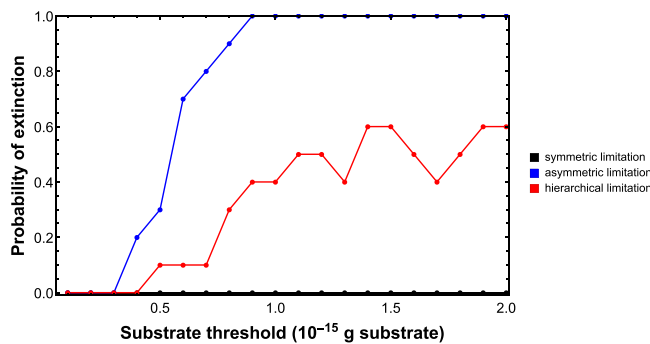


FIG. 8. Probability of extinction as a function of substrate threshold. The symmetric, asymmetric, and hierarchical limitation scenarios were tested, for the competition process. The mobility rate of the system was fixed at $\epsilon = 4.5$.

of the system is moderated by the mobility rate; once the mobility exceeds a certain critical rate ϵ_c (which scales with system size), the interactions are no longer sufficiently localized to permit long-term coexistence, and the system will suffer extinctions and tend to monoculture.

When the demographic processes become resource-dependent, we have seen in Section III B that extinction events become more common, and the tendency to monoculture increases, particularly for the asymmetric and hierarchical limitation scenarios. This raises the question of what effect the substrate threshold has on the extinction probability of the system, and whether strength of resource limitation or mobility rate takes precedence in moderating long-term coexistence.

Therefore, selecting a mobility rate significantly smaller than the critical value of the benchmark case ($\epsilon_c = 10.63$), which would in the benchmark case ensure long-term coexistence, we calculated the extinction probabilities obtained for simulations with varying substrate thresholds. In this way, we observed the impact of the different resource limitation scenarios and substrate thresholds on the extinction probability of the system. The results are shown in Figure 8. For the symmetric limitation scenario, imposing a substrate threshold does not increase the probability of extinction past the benchmark level (approximately zero, implying long-term coexistence). This agrees with previous results for this model,⁴⁷ which show that imposing a substrate threshold reduces biodiversity levels slightly below benchmark levels, but does not provoke extinction events to reduce biodiversity more dramatically. We observe a similar effect here.

For the asymmetric and hierarchical scenarios, the results of increasing the substrate threshold are more

dramatic, again in agreement with our previous results where it was observed that above very small substrate thresholds, for these two scenarios there was a significant decrease in biodiversity due to extinction events, in particular, for the asymmetric limitation scenario.⁴⁷ There remains a significant range of the tested values of τ where extinction probabilities remain low or close to zero, permitting the type of coexistence seen, for example, in Figures 4, 5, and 6.

We therefore conclude that the main determinant of long-term coexistence becomes first the resource limitation scenario: if the scenario is asymmetric or hierarchical, this will imply significant extinctions despite a low mobility rate. Individuals may benefit from spatial refuges and persist in unbalanced but coexisting communities (as seen in Section III B), but these scenarios are vulnerable to stochastic effects which may provoke extinctions, and thus extinction probabilities for these cases can be significant. If the limitation scenario is symmetric (mirroring the more balanced dynamics of the benchmark case), increasing the substrate threshold within the same range does not induce extinctions, and therefore the mobility rate remains the principal determinant of long-term coexistence.

D. Simultaneous substrate limitations

After studying the nine possible resource limitation scenarios in isolation to avoid confounding effects, we turned our attention to simultaneously substrate-limited processes—that is, scenarios where two demographics are both substrate-limited. Given that imposing a substrate limitation on mobility did not produce noticeable effects in isolation, we concentrated on the remaining processes: competition and reproduction.

To investigate the interaction between these two phenomena, we assigned the same substrate threshold in the same way (symmetrically, asymmetrically, or hierarchically between species) to both processes. For example, in the simultaneous symmetric scenario, we assign the thresholds for reproduction as $\tau_A^r = \tau_B^r = \tau_C^r = 10^{-16}$ g, and the thresholds for competition as $\tau_A^c = \tau_B^c = \tau_C^c = 10^{-16}$ g. In an asymmetric scenario, we assign, for example, $\tau_A^r = 10^{-16}$ g and $\tau_A^c = 10^{-16}$ g to species A while the other species are not subject to substrate thresholds.

Examples of the grid configurations produced by these simulations are shown in Figure 9, where a clear separation of regions can be observed. When reproduction and competition are both symmetrically limited, we observe two central zones: the characteristic spiral formations in the centre,

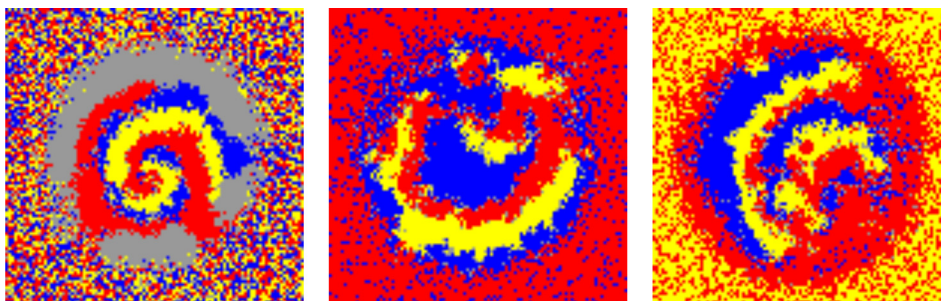


FIG. 9. Grid configuration after 500 generations for a community where both competition and reproduction are (left to right) symmetrically, asymmetrically, and hierarchically substrate-limited ($\tau = 10^{-16}$ g).

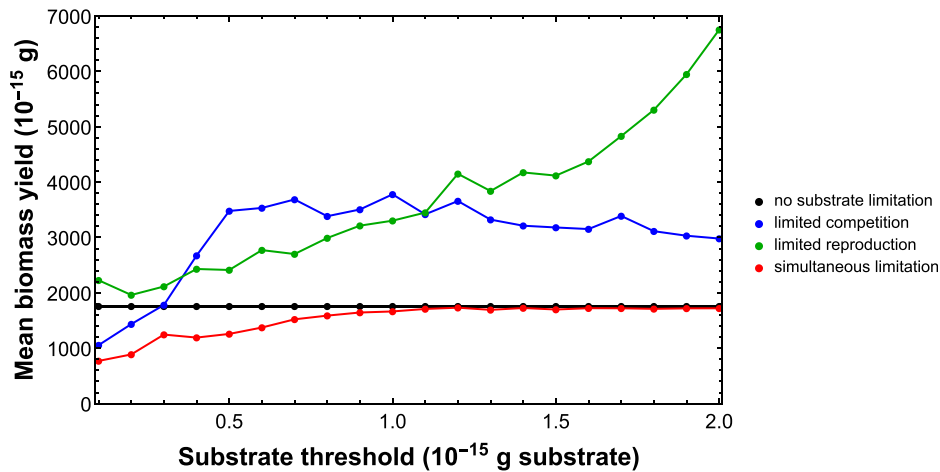


FIG. 10. Comparison of average biomass yield for the symmetrically limited scenarios. Each curve represents the mean of 200 simulations.

enclosed first by empty space (as was seen for substrate-limited reproduction) and second by a region where individuals are present but randomly mixed (as was seen for substrate-limited competition).

In the cases where reproduction and competition are both asymmetrically or hierarchically limited, we again observe unbalanced communities due to the spatial heterogeneities providing refuges for a vulnerable species. For asymmetric simultaneous limitations, the vulnerable species is

species C; compare with Figures 6 and 7 where in the case of a single substrate-limited process, for high initial evenness species C persisted in similar proportion to species B and for lower initial evenness species C collapsed to extinction. In this simultaneous limitation case, the effect is somewhere in between: species C persists but at a lower proportion than the other two species.

When both reproduction and competition are hierarchically limited, the vulnerable species is species A, which in

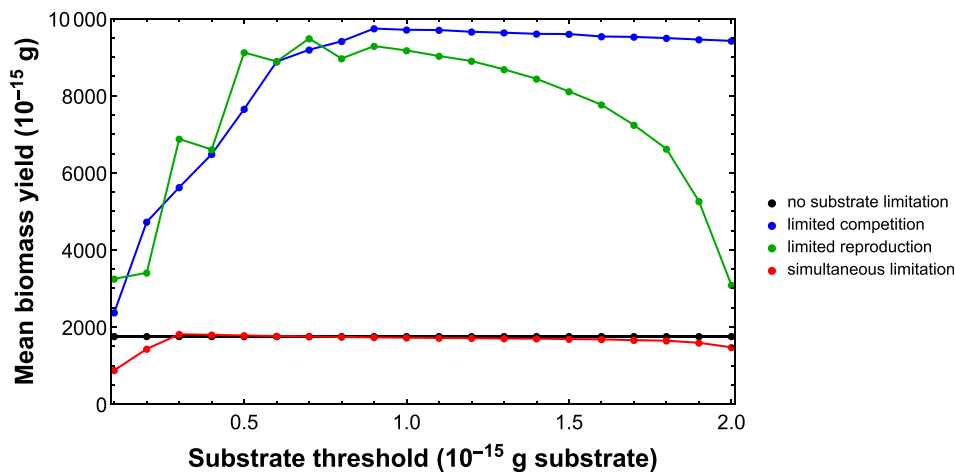


FIG. 11. Comparison of average biomass yield for the asymmetrically limited scenarios. Each curve represents the mean of 200 simulations.

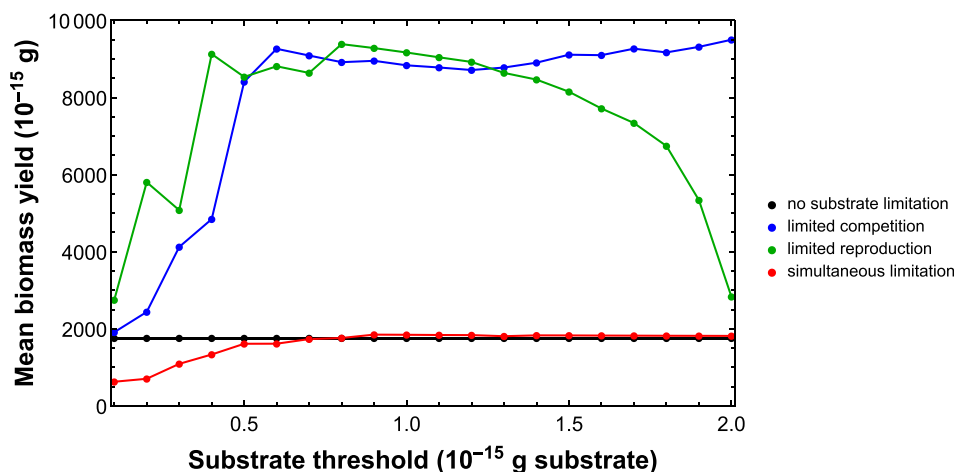


FIG. 12. Comparison of average biomass yield for the hierarchically limited scenarios. Each curve represents the mean of 200 simulations.

this scenario is subject to the highest substrate threshold. This behaviour is similar to the effects of a single hierarchically limited process (see Figure 5).

Making reproduction and competition simultaneously resource-dependent also has a significant effect on community productivity, which collapses compared to the cases of individual processes being resource-dependent. This is shown in Figures 10–12, where community productivity is compared for symmetric, asymmetric and hierarchical scenarios, respectively. In all cases, the productivity of communities subject to simultaneous process limitations never exceeds the productivity of the benchmark case.

Thus, while resource-limiting two processes simultaneously do not greatly alter the population dynamics by way of significantly increased extinctions compared to individual limitation scenarios, in the simultaneous case individuals must divide their resource allocation between reproducing and competing with their neighbours. This trade-off significantly depresses community productivity, although this effect is not greatly increased by increasing the substrate threshold, having reached a plateau at intermediate threshold values. This suggests that for high substrate thresholds, the resource allocation trade-off between reproduction and competition has constrained biomass growth to its minimum, and thus further increasing the substrate threshold has little effect on productivity.

IV. CONCLUSIONS

We have extended existing microscopic models of three cyclically competing species by considering resource-dependent demographic processes in a spatially heterogeneous landscape, thereby providing a more realistic *in silico* representation of natural systems. The explicit treatment of space, which permits resource gradients, can induce dramatic effects in the system population dynamics. These effects, consistent with other modelling and experimental studies, are not seen in well-mixed models due to the absence of spatial heterogeneities in such models, thereby neglecting this key facet of natural systems. Our findings have implications for the formation and maintenance of spatial patterns in microbial populations such as biofilms.

Further steps include calibration of the model, which awaits appropriate experimental data. We also envision the implementation of more realistic competition structures. This extension would remove the need to explicitly impose the cyclic competition structure. A more realistic representation of competition can be achieved by incorporating, for example, individual fitness measures. Then, competitive interactions would be mediated by individual fitness, a mechanism which can produce an emergent cyclic competitive balance typical of those observed in natural ecosystems.

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