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An Exclusion Principle and the Importance of Mobility for a Class of Biofilm Models

Isaac Klapper · Barbara Szomolay

Abstract Much of the earth's microbial biomass resides in sessile, spatially structured communities such as biofilms and microbial mats, systems consisting of large numbers of single-celled organisms living within self-secreted matrices made of polymers and other molecules. As a result of their spatial structure, these communities differ in important ways from well-mixed (and well-studied) microbial systems such as those present in chemostats. Here we consider a widely used class of 1D biofilm models in the context of a description of their basic ecology. It will be shown via an exclusion principle resulting from competition for space that these models lead to restrictions on ecological structure. Mathematically, this result follows from a classification of steady-state solutions based on a 0-stability condition: 0-stable solutions are in some sense determined by competitive balance at the biofilm base, whereas solutions that are not 0-stable, while less dependent on conditions at the biofilm base, are unstable at the base. As a result of the exclusion principle, it is argued that some form of downward mobility, against the favorable substrate gradient direction, is needed at least in models and possibly in actuality.

Keywords Microbial ecology · Biofilms · Exclusion principle

1 Introduction

Biofilms are surface-associated collections of microorganisms held together by self-secreted polymeric matrices in communities that are believed to play key

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Fig. 1 Cross-section of a bacterial mat (a large biofilm) from Octopus Spring, Yellowstone National Park. *The top*, *green* layer is dominated by cyanobacterial phototrophs. Heterotrophic bacteria dominate lower layers. Reproduced from Ward et al. (1998) with permission from American Society for Microbiology

roles in many environmental, industrial, and medical contexts (Costerton 2007; Stoodley et al. 2002). As such, they have been subjects of frequent modeling studies, see Klapper and Dockery (2010), Wanner et al. (2006). While biofilms are three-dimensional structures adhering typically to two-dimensional surfaces, for many questions of interest, it is useful and reasonable to simplify to one dimension by allowing only variation in the transverse (to the surface) direction. In one dimension, continuum mechanics platforms are particularly tractable. Partly in consequence, a number of biofilm models have been one-dimensional, continuum-mechanics based, in many cases following the influential study (Wanner and Gujer 1984, 1986) as well as earlier ones (Atkinson and Davies 1974; Kissel et al. 1984; Williamson and McCarty 1976). For example, the widely used software system BIOSIM (Reichert et al. 1989) for simulating multispecies biofilm communities was developed based on this setup, and we will designate a class of models with similar characteristics to be of *basic* type.

As consequence, we will also consider extending the basic model with the purpose of circumventing the exclusion principle to allow increased ecological hetero-

Here we present a rather general version of the one-dimensional basic models and propose an exclusion principle for it stating, roughly, that (within the general model we formulate) species that cannot cohabitate at the base of the biofilm cannot cohabitate elsewhere within the biofilm either. That is, we show that steady-state solutions of basic models tend toward a kind of spatial ecological homogeneity where environmental conditions at the biofilm base are determining. More specifically, in the context of 1D models, spatial heterogeneity means horizontal layering of some kind, see Fig. 1 for an (actual) example, layering that is absent and excluded in a homogeneous ecology. While exclusion principles are common and useful in ecological theory, we argue though that this one may present a modeling flaw when applied to the study of multispecies ecology in biofilms. Indeed, evidence widely suggests, e.g., Ramsing et al. (2000), Zhang et al. (1994), that spatial ecological heterogeneity is the norm in multispecies biofilms, and even that this heterogeneity can be driven by environmental variability inherent to the biofilm spatial structure (Boles et al. 2004; Rainey and Travisano 1998).

As a consequence, we will also consider extending the basic model with the purpose of circumventing the exclusion principle to allow increased ecological hetero-

geneity. For illustrative purposes, the focus will be on one addition in particular, namely allowance for diffusivity of biomaterial. Diffusivity was included in the software system AQUASIM (Reichert 1994; Wanner and Riechert 1996), a replacement for BIOSIM (in fact, exclusion was predicted by the authors of BIOSIM in some circumstance Wanner and Gujer 1986), but is often not included in biofilm models. We refer to the resulting class of models as *extended* type and present numerical evidence that sufficiently large (but not too large) biomaterial diffusivity can break ecological exclusion.

We note that ecological principles such as competitive exclusion have a long history of study, including in well-mixed (batch and chemostat) microbial systems (Smith and Waltman 1995; Waltman 1983). Indeed, one of the pioneering studies of exclusion was made in just such a system (Gause 1934). However, it seems that most microbes live in spatially structured communities that are not well mixed. Theory for these systems is less explored (Prosser et al. 2007). As mentioned, recent studies indicate that microbial communities generally have complex species structures. So, though it is likely that spatial structure introduces new and difficult challenges, it would nevertheless seem worthwhile to extend the theory of microbial ecology where possible to include spatially structured communities such as biofilms.

2 The Basic Model

We consider a one-dimensional biofilm model consisting of N active species (with volume fractions $X_j(x, t)$, $j = 1, \ldots, N$), one inert species (with volume fraction $I(x, t)$), and M substrates (with concentrations $C_i(x, t)$, $i = 1, \ldots, M$). Active species material can "deactivate" into the inert state, but not vice versa. Reactivation of inert material into active material could also be allowed without altering our main conclusions, as could inclusion of additional inert species (such as extracellular matrix or free water for example). The spatial coordinate is $x \in [0, L(t)]$ where $L(t)$ is the moving boundary at the top of the biofilm and $x = 0$ is the location of the base of the biofilm, assumed to be an impermeable wall. Consideration could also be made of substrate transport in a bulk fluid domain above $L(t)$, to for example allow for effects of a diffusive boundary layer, but again this addition would not alter our main results. The final model ingredient is a growth-induced advective velocity v determined by the volume conservation constraint $I + X_1 + X_2 + \cdots + X_N = 1$.

Altogether, the basic model system takes the form

$$0 = D_i \frac{\partial^2 C_i}{\partial x^2} - \sum_{j=1,\ldots,N} r_{ij}(\mathbf{C}, \mathbf{X}, I), \quad i = 1, \ldots, M, \tag{1}$$

$$\frac{\partial X_j}{\partial t} + \frac{\partial}{\partial x}(X_j v) = \big(g_j(\mathbf{C}, \mathbf{X}, I) - k_j(\mathbf{C}, \mathbf{X}, I)\big) X_j, \quad j = 1, \ldots, N, \tag{2}$$

$$\frac{\partial I}{\partial t} + \frac{\partial}{\partial x}(I v) = \sum_{j=1,\ldots,N} k_j(\mathbf{C}, \mathbf{X}, I) X_j, \tag{3}$$

$$\frac{dL}{dt} = v\big(L(t), t\big) - \sigma L^2, \tag{4}$$

$$v = \int_0^x \sum_{l=1,\dots,N} g_l(\mathbf{C}, \mathbf{X}, I) X_l \, dx', \tag{5}$$

where $\mathbf{C}$ is the M-vector of substrate concentrations, and $\mathbf{X}$ is the N-vector of species volume fractions. Substrates C_i satisfy reaction–diffusion equations (1), where the time derivatives $\partial C_i / \partial t$ are neglected due to fast substrate equilibration relative to other system processes. The functions r_{ij} quantify the consumption rate of substrate i by species j. Note that r_{ij} could be negative, indicating production of substrate i by species j. In advection–reaction equations (2) and (3), the functions g_j and k_j are rates of growth and deactivation, respectively, of species j. We assume that $g_j, k_j \geq 0$. Using the requirement that $I + X_1 + X_2 + \cdots + X_N = 1$, (5) for v is obtained by summing (2) and (3) and integrating over x (together with the condition $v(0) = 0$). The term $-\sigma L^2$ in (4) indicates erosion, necessarily included to allow non-trivial equilibrium states; its quadratic form follows standard usage (Stewart 1993; Xavier et al. 2005). The boundary conditions for (1) are

$$\frac{\partial C_i}{\partial x}(0, t) = 0, \qquad C_i\big(L(t), t\big) = C_i^0, \quad i = 1, \dots, M. \tag{6}$$

We will assume that each C_i^0 is constant in time. Equations (2), (3), and (4) take initial conditions for L as well as $\mathbf{C}$ and $\mathbf{X}$ on $[0, L(0)]$. With respect to boundary conditions for the species volume fractions, it has been shown in a similar, though somewhat less general model, that such boundary conditions are not required (Szomolay 2008). Further, in that same reference, the existence and uniqueness of solutions has been proved. The existence and uniqueness of solutions to the initial-value problem (1)–(5), together with initial and boundary conditions as described above, can be shown in the same way provided adequate smoothness requirements are met.

In many cases, interest lies with the long-time behavior of system (1)–(5). Hence we focus here on properties of steady states, i.e., solutions (independent of t) of the equations

$$D_i \frac{d^2 C_i}{dx^2} = \sum_{j=1,\dots,N} r_{ij}(\mathbf{C}, \mathbf{X}, I), \quad i = 1, \dots, M, \tag{7}$$

$$\frac{dX_j}{dx} v = \left(g_j(\mathbf{C}, \mathbf{X}, I) - k_j(\mathbf{C}, \mathbf{X}, I) - \sum_{l=1,\dots,N} g_l(\mathbf{C}, \mathbf{X}, I) X_l \right) X_j,$$

$$j = 1, \dots, N, \tag{8}$$

$$\frac{dI}{dx} v = \sum_{j=1,\dots,N} k_j(\mathbf{C}, \mathbf{X}, I) X_j - \sum_{l=1,\dots,N} g_l(\mathbf{C}, \mathbf{X}, I) X_l, \tag{9}$$

$$\frac{dv}{dx} = \sum_{l=1,\dots,N} g_l(\mathbf{C}, \mathbf{X}, I) X_l, \tag{10}$$

where $0 \leq x \leq L$, and L is the steady-state biofilm thickness. Note that L is also an unknown. The corresponding boundary conditions are

$$v(0) = 0, \qquad v(L) = \sigma L^2, \qquad \frac{dC_i}{dx}(0) = 0, \qquad C_i(L) = C_i^0, \qquad (11)$$

for $i = 1, \ldots, M$. We do not address the question of existence of solutions to (7)–(11). For discussion of steady-state solution existence, see Szomolay (2008). In particular, rather, we will show that those species volume fractions that are zero at the bottom of a steady-state biofilm solution are in fact zero throughout the entire biofilm domain $0 \leq x \leq L$ if the given steady state is 0-stable (defined below). The requirement of 0-stability is necessary to rule out a class of solutions that include what we designate as *spontaneously generated* species, described later.

Note the following intuition about steady solutions of the basic model (7)–(11). The advection velocity v is growth-driven and hence is always nonnegative. Thus, biomaterial always moves upward and never down, so that the ecology, i.e., the species make-up, of steady-state solutions might be expected to be determined by the ecology at $x = 0$. That is, we expect that if a species, or species combination, is unable to survive at the bottom of the biofilm, it will not be able to survive anywhere within the biofilm (although this is not quite true, see below). In preparation, we introduce the following:

Definition We say that a steady-state solution $\mathbf{C}(x), \mathbf{X}(x), I(x), L$ of (7)–(11) is 0-*stable* if for each j such that $X_j(0) = 0$, we have $\Gamma_j < 0$, where

$$\Gamma_j = g_j\big(\mathbf{C}(0), \mathbf{X}(0), I(0)\big) - k_j\big(\mathbf{C}(0), \mathbf{X}(0), I(0)\big)$$
$$- \sum_{l=1,\ldots,N} g_l\big(\mathbf{C}(0), \mathbf{X}(0), I(0)\big) X_l(0). \qquad (12)$$

Note that at $x = 0$, (2), together with the boundary conditions, reduce to

$$\frac{\partial X_j}{\partial t}(0, t) = \Gamma_j X_j(0, t), \qquad (13)$$

so that 0-stability is equivalent to the requirement that, restricting dynamics to $x = 0$ only, the steady-state solution in question be stable to perturbations of species that are extinct at $x = 0$, suggesting that a steady-state solution that is nontrivial and not 0-stable is itself linearly unstable with respect to dynamics on the full domain $[0, L]$. We extend the definition of 0-stability to steady solutions of (13). That is, a solution of $0 = \Gamma_j X_j(0)$ is called 0-stable if for each j with $X_j(0) = 0$, condition (12) holds.

Example 1 The 0-stability condition is necessary in order to disallow spontaneous generation solutions. To illustrate, consider a basic-type model with no limiting substrates (i.e., with substrate saturation) and two noninteracting species with volume fractions X_1, X_2. We also suppose linear growth and no species inactivation ($I = 0$). For a more detailed study of this type of system, see Overgaard (2010).

Then the steady-state equations become

$$\frac{d}{dx}(X_1 v) = c_1 X_1,$$

$$\frac{d}{dx}(X_2 v) = c_2 X_2, \tag{14}$$

$$v = \int_0^x \left(c_1 X_1(x') + c_2 X_2(x') \right) dx',$$

with condition $v(L) = \sigma L^2$, where c_1 and c_2 are constant growth rates. We suppose that $c_1 > c_2$. Along the characteristic $x(s)$ (defined as before by $dx/ds = v(x(s))$, $x(0) = L$, $-\infty < s \le 0$), (14) can be written, using $X_1 + X_2 = 1$, as

$$\frac{d}{ds} X_1(x(s)) = \left(c_1 - \frac{dv}{dx}(x(s)) \right) X_1(x(s))$$

$$= \alpha X_1(x(s)) \left(1 - X_1(x(s)) \right), \tag{15}$$

where $\alpha = c_1 - c_2 > 0$. Equation (15) has three admissible solution classes: I: $(X_1(s) = 1, X_2(s) = 0)$, II: $(X_1(s) = 0, X_2(s) = 1)$, and III:

$$X_1(s) = \frac{e^{\alpha s}}{e^{\alpha s} + C},$$

$$X_2(s) = \frac{C}{e^{\alpha s} + C},$$

where $C > 0$ is an integration constant determined by $v|_{x=L} = \sigma L^2$. (Note that by taking the limit $C \to 0$ we can observe that the largest possible value of L is bounded by c_1/σ.) For this third solution, $X_1 > 0$ for $x > 0$ but $X_1 \to 0$ as $x \to 0$ (i.e., as $s \to -\infty$). Thus the third solution represents what we designate as a spontaneous generation solution: species 1 rides through the biofilm along characteristic $x(s)$, but this characteristic originates from a location where species 1 does not exist. Hence species 1 in some sense emerges from nothing. An example is shown in Fig. 2.

Spontaneous generation solutions are not desirable; however we observe that solution class III (as well as solution class II, $X_1 = 0$, $X_2 = 1$) is unstable to perturbations that make $X_1|_{x=0} > 0$. This can be verified from (2), which reduce in this example to

$$\frac{\partial X_1}{\partial t} = -\frac{\partial}{\partial x}(X_1 v) + c_1 X_1 = -v \frac{\partial X_1}{\partial x} + \alpha X_1 (1 - X_1).$$

As $\alpha > 0$ and v is small near $x = 0$, small perturbations for which initial perturbation values $X_1(0) > 0$ and $\partial X_1/\partial x$ are small enough near $x = 0$ will grow in time.

We are now able to state the main result, from which an exclusion principle for the basic model follows. We assume the existence of a steady-state solution and study its properties.

Fig. 2 Steady states X_1 (*solid*), X_2 (*dash*) for Example 1 with $c_1 = 1$, $c_2 = 0.5$, $\sigma = 0.18$. *Horizontal axis is the spatial coordinate* x; *vertical axis is* volume fraction. Note that $X_1(x)$ is spontaneously generated
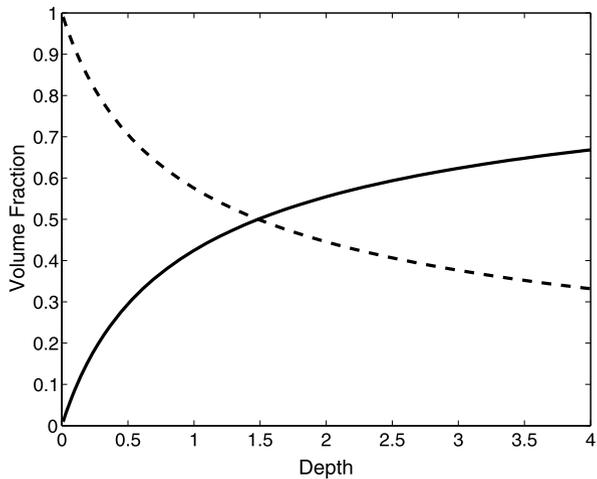
Theorem *Let* $r_{ij}, g_j, k_j, 1 \leq i \leq M, 1 \leq j \leq N$, *be bounded and Lipschitz continuous with all* g_{ij}, k_j *nonnegative. Suppose that* $C_i(x), X_j(x), I(x), L, 1 \leq i \leq M, 1 \leq j \leq N$, *is a 0-stable, steady-state solution of* (7)–(11). *Then, for each* $j, 1 \leq j \leq N$, *if* $X_j(0) = 0$, *then* $X_j(x) = 0$ *for* $x \in [0, L]$.

Proof Suppose that $C_i(x)$ for $1 \leq i \leq M$, $X_j(x)$ for $1 \leq j \leq N$, $I(x)$, L comprise a steady-state solution of (7)–(11). Suppose for a given value of j that $X_j(0) = 0$.

Let $x(s)$ be the characteristic defined by the equation

$$\frac{dx}{ds} = v(x(s)), \qquad x(0) = L, \quad -\infty < s \leq 0.$$

(Note: use of the term characteristic to label $x(s)$ is nonstandard, as the system is time-independent, but useful—technically, rather, we are changing domain from $[0, L]$ to $(-\infty, 0]$.) Writing the steady state in the new variable s, we see that X_j satisfies

$$\frac{d}{ds} X_j(x(s)) = \left(g_j(x(s)) - k_j(x(s)) - \frac{dv}{dx}(x(s)) \right) X_j(x(s)). \tag{16}$$

By nonnegativity of g_j, $j = 1, \ldots, N$, it follows that dv/dx is also nonnegative, see (5). We may assume in fact that $dv/dx > 0$ at $x = 0$ and hence that $v > 0$ for $x > 0$. Also, observe that v is an increasing function of x. (Note: it is possible that $v = 0$ on $[0, a]$ and $v > 0$ on $(a, L]$, but this only means that $[0, a]$ is an inactive layer which can be ignored.)

Since

$$\frac{dv}{dx} = \sum_{l=1,\ldots,N} g_l X_l,$$

(16) can be rewritten as

$$\frac{d}{ds}X_j = \left(g_j - k_j - \sum_{l=1,\dots,N} g_l X_l\right)X_j.$$ (17)

Now, choose $\varepsilon > 0$. There is an $A > 0$ such that for all $s \in (-\infty, -A)$, due to the continuity of functions g_j, k_j and of solutions C_i, X_j, it holds that

$$\left| \frac{d}{ds}X_j(x(s)) - g_j(\mathbf{C}(0), \mathbf{X}(0), I(0))X_j(x(s)) \right.$$

$$\left. + k_j(\mathbf{C}(0), \mathbf{X}(0), I(0))X_j(x(s)) + \frac{dv}{dx}(0)X_j(x(s)) \right| < \varepsilon/2.$$

Also, there is a $B > 0$ such that, for $s \in (-\infty, -B)$, we have

$$\left| \frac{d}{ds}X_j(x(s)) \right| < \varepsilon/2$$

due to the fact that X_j is necessarily almost constant in some interval $s \in (-\infty, -B)$ on which $x(s)$ only takes values near 0. Together we obtain

$$\left| -g_j(\mathbf{C}(0), \mathbf{X}(0), I(0))X_j(x(s)) + k_j(\mathbf{C}(0), \mathbf{X}(0), I(0))X_j(x(s)) \right.$$

$$\left. + \frac{dv}{dx}(0)X_j(x(s)) \right| = \left| \Gamma_j X_j(x(s)) \right| < \varepsilon.$$

It follows that $|X_j| < \frac{\varepsilon}{|\Gamma_j|}$ for s on some interval $(-\infty, -C)$ (note that $\Gamma_j \neq 0$ by 0-stability). Now, since $\Gamma_j < 0$ by 0-stability, the continuity of solutions and (17) implies that $\frac{d}{ds}X_j(x(s)) \leq 0$ for s on some interval $(-\infty, -D)$. Finally, by the uniqueness of solutions we must have $X_j = 0$ for $-\infty < s \leq 0$.   □

Remark An alternative proof goes as follows. By the assumptions of the theorem we have that

$$\frac{d}{ds}X_j(x(s)) \leq (1 - \epsilon)\Gamma_j X_j(x(s))$$

for s on some interval $-\infty < s < -A$, where Γ_j is the constant from the 0-stability definition, and $\epsilon > 0$ is small. Hence, for $s > s_0$ and $s_0 < -A$,

$$X_j(x(s)) \leq e^{(1-\epsilon)\Gamma_j(s-s_0)}X_j(x(s_0)).$$

Since X_j is bounded and $\Gamma_j < 0$, taking the limit as $s_0 \to -\infty$, we get that $X_j = 0$ for $s \in (-\infty, -A)$, and thus, by the uniqueness of solutions, $X_j = 0$ for $s \in (-\infty, 0]$.

Exclusion We interpret the theorem as implying an exclusion principle in the following sense: in order for a species to survive in a given steady state, the species must be able to survive in the $x = 0$ environment of that steady state, noting that basic-type models do not allow transport of material downward from $x > 0$ to $x = 0$.

Example 2 To illustrate the exclusion principle, we present a system of two species with volume fractions X_1, X_2, competing for the same limiting substrate with density C_1 and now allowing inactivation ($I \geq 0$). Including inactivation makes the example more similar to systems often found in the literature. Inactivation rate functions k_1 and k_2 are set to be constants $k_1 = k_2 = 0.1$.

We suppose growth functions g_j, $j = 1, 2$, to be of the Monod forms

$$g_j(C_1) = r_j \frac{C_1}{K_j + C_1},$$

where $r_1 = 1$, $K_1 = 1$, and $r_2 = 0.5$, $K_2 = 0.15$, see Fig. 3. Note that species 1 outcompetes species 2 when substrate is at large concentrations but species 2 outcompetes species 1 at low substrate concentrations. We thus designate species 1 as the high substrate specialist and species 2 as the low substrate specialist. The critical, crossover concentration occurs for these parameters at $C_1^* = 0.7$ where $g_1(C_1^*) = g_2(C_1^*)$. We approximate solutions of (7)–(11) by computing solutions of (1)–(5) for long times and with varying applied substrate C_1^0 (the upper boundary condition for C_1) with other parameters held fixed ($D_1 = 1$, $r_{11} = \gamma_1 g_1$ with $\gamma_1 = 1$, and $r_{12} = \gamma_2 g_2$ with $\gamma_2 = 1$ and $\sigma = 0.3$) using initial volume fractions $X_1 = X_2 = 0.5$ and $I = 0$.

In this example setup, we might expect the steady-state to be a layered biofilm in which species 1 dominates near the top, where substrate levels are relatively high, and species 2 dominates near the bottom, where substrate levels are relatively low. However, except for one special value $\hat{C}_1^0$ of applied substrate, the exclusion principle precludes survival of more than one species so that layering cannot occur. Figures 4 and 5 illustrate the two typical cases. For applied substrate less than the special value $\hat{C}_1^0$, lying somewhere between 0.95 and 1.0 for the chosen parameter values, only the low substrate specialist survives (e.g., Fig. 4), while for higher applied substrates, only the high substrate specialist survives (e.g., Fig. 5). Note in Fig. 4 that $C_1(x)$ crosses the critical value C^* so that the high substrate specialist might be expected to

Fig. 3 Growth rates $g_1(C_1)$ and $g_2(C_1)$ as functions of substrate for species 1 (*solid*) and species 2 (*dash*) for Example 2. Note that species 1 outcompetes species 2 for substrate at large substrate concentration but species 2 outcompetes species 1 at low substrate concentrations. The critical, crossover substrate concentration in this example occurs at $C_1^* = 0.7$ where $g_1(C_1^*) = g_2(C_1^*)$
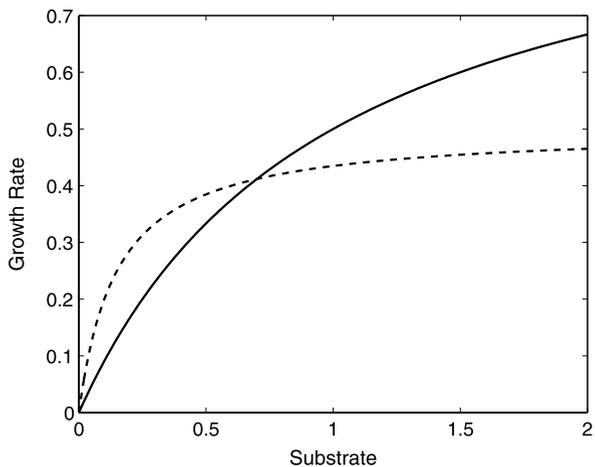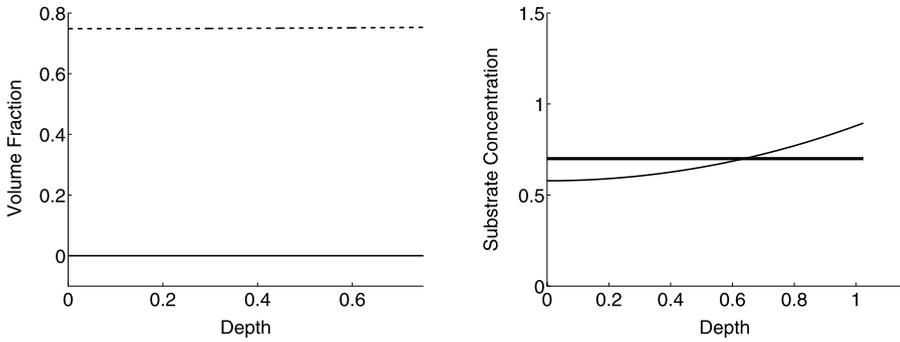
Fig. 4 Exclusion principle example. *Left panel*: species 1 (*solid*) and species 2 (*dash*) volume fractions at steady state in the biofilm as functions of height. *Right panel*: limiting substrate at steady state (*thin line*) as a function of height. *The thick, horizontal line in the right panel* is at the critical substrate value 0.7: for values of substrate above the critical value, species 1 outcompetes species 2, while for values of substrate below the critical value, species 2 outcompetes species 1. However, despite the fact that substrate is above the critical value in the upper regions of the biofilm, species 1 is excluded. In this computation, the applied substrate level is 0.95
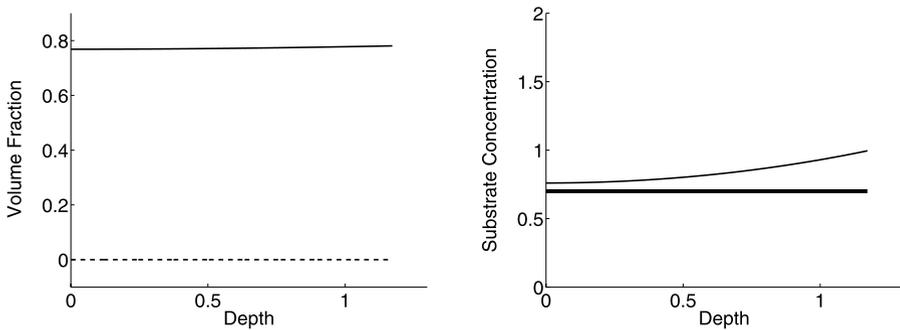

Fig. 5 Exclusion principle example. *Left panel*: species 1 (*solid*) and species 2 (*dash*) volume fractions at steady state in the biofilm as functions of height. *Right panel*: limiting substrate at steady state (*thin line*) as a function of height. *The thick, horizontal line in the right panel* is at the critical substrate value 0.7: for values of substrate above the critical value, species 1 outcompetes species 2, while for values of substrate below the critical value, species 2 outcompetes species 1. Substrate level is always above the critical value, and species 2 is excluded. In this computation, the applied substrate level is 1.0

be present at least in the upper biofilm layer where $C_1 > C^*$. In fact, the exclusion principle states that only the substrate value $C_1(0)$ at the bottom of the biofilm $x = 0$ matters; if $C_1(0) < C^*$, then $X_1(0) = 0$, and $X_2(0) > 0$ is the stable equilibrium so that the high substrate specialist (species 1) cannot survive in the biofilm, and vice versa for $C_1(0) > C^*$. Observe, by the way, that in both computations the solution is 0-stable: Γ_j for the extinct species (the quantity of relevance for 0-stability) reduces to $-|g_1(C_1(0)) - g_2(C_1(0))| < 0$. Note that at the special value of applied substrate $\hat{C}_1^0$, it happens that $C_1(0) = C^*$. In this single case, species 1 and 2 are equally competitive at $x = 0$, so both can survive there, and hence both can survive throughout the biofilm (Wik and Breitholtz 1996).

3 An Extended Model

The exclusion principle follows as a consequence of the fact that characteristics transport material upward (at the characteristic velocity $dx/ds = v(x(s)) > 0$) with the result that biomaterial from below displaces biomaterial up above. The velocity v is positive because new growth requires space, thereby pushing material upwards, away from the wall; exclusion is thus a result of spatial competition. Consequently we now consider downward microbial mobility. Downward material transport is known to occur in microbial communities, e.g., Doemel and Brock (1977), Drury et al. (1993). The mechanisms are unclear, though there are a number of possibilities. For example, phototaxis has been observed in response to changing environmental conditions such as daily light cycles (Doemel and Brock 1977). A dramatic example of the impact of twitching motility on biofilm structure was presented by Klausen et al. (2003). However, the simplest mode of mobility is that is be random; such motion can be modeled by introduction of diffusion (which has in fact been observed and measured in laboratory-grown biofilms Rogers et al. 2008) into the equations for motile species. This effect was included in the AQUASIM model, an extension of BIOSIM, where it was argued that mechanical stresses lead to "temporary detachment of single cells or particles from the matrix" followed by "subsequent reattachment at another location" (Wanner and Riechert 1996). Though not directly linked to the possibility of ecological exclusion in that paper (rather, in fact, the authors motivated inclusion of material diffusion as a way to improve penetration of surface-attaching cells by allowing them to move downward; such transport appears to have been observed in practice, e.g., Klayman et al. 2008), we wish to claim that material diffusion can break the basic model exclusion principle; with diffusion, ecology in the biofilm is not solely determined by ecology at the biofilm base. Hence we focus on mobility through diffusion, not intending to exclude the possible importance of other mobility mechanisms, but rather in part to connect to AQUASIM and also in part to exploit the relative simplicity and illustrative value of diffusive transport. Note that nonlinear diffusion-based biofilm models of a rather different sort than those presented here have been introduced by Eberl and coworkers (Eberl et al. 2001).

Adding diffusion terms to the equations for the material volume fractions, the extended model with material diffusion takes the form

$$0 = D_i^c \frac{\partial^2 C_i}{\partial x^2} - \sum_{j=1,\dots,N} r_{ij}(\mathbf{C}, \mathbf{X}, I), \quad i = 1, \dots, M, \tag{18}$$

$$\frac{\partial X_j}{\partial t} + \frac{\partial}{\partial x}(X_j v) = \big(g_j(\mathbf{C}, \mathbf{X}, I) - k_j(\mathbf{C}, \mathbf{X}, I)\big)X_j + D_j^x \frac{\partial^2 X_j}{\partial x^2},$$
$$j = 1, \dots, N, \tag{19}$$

$$\frac{\partial I}{\partial t} + \frac{\partial}{\partial x}(Iv) = \sum_{j=1,\dots,N} k_j(\mathbf{C}, \mathbf{X}, I)X_j + D^I \frac{\partial^2 I}{\partial x^2}, \tag{20}$$

$$\frac{dL}{dt} = v\big(L(t), t\big) - \sigma L^2, \tag{21}$$

$$v(x,t) = \int_0^x \sum_{l=1,\ldots,N} g_l(\mathbf{C}, \mathbf{X}, I) X_l \, d\zeta + \sum_{l=1,\ldots,N} D_l^x \frac{\partial X_l}{\partial x}(x,t) + D^I \frac{\partial I}{\partial x}(x,t). \quad (22)$$

Additional boundary conditions are no flux of I and X_j, $1 \le j \le N$, at $x = 0$ and $x = L$. The upper boundary flux condition is chosen so that material loss through the biofilm-bulk fluid interface occurs solely through erosion via (21). Observe that if the species diffusion coefficients are equal, i.e., $D_1^x = D_2^x = \cdots = D_N^x = D^I$, then the diffusive flux terms in (22) combine to vanish by conservation of volume. We will restrict ourselves to equal diffusivities in the following, but note the possibility that unequal diffusivities can lead to interesting effects in systems dominated by diffusive transport (Brown et al. 2005) and might also do so here.

Rewriting (19) as

$$\frac{\partial X_j}{\partial t} + \frac{\partial}{\partial x}\left(X_j v - D_j^x \frac{\partial X_j}{\partial x}\right) = \left(g_j(\mathbf{C}, \mathbf{X}, I) - k_j(\mathbf{C}, \mathbf{X}, I)\right) X_j$$

and considering the net flux term $X_j v - D_j^x \partial X_j / \partial x$ suggests that sufficiently large diffusion coefficients may allow downward flux of material, particularly near the top of the biofilm, where species volume fraction gradients tend to be larger (and positive). Hence material diffusion may be able to negate conditions leading to the exclusion principle.

Example 3 To illustrate, we return to the system of Example 2 where two species with volume fractions X_1, X_2 compete for the same limiting substrate C. As then, we suppose growth functions g_j, $j = 1, 2$, to be of the Monod forms

$$g_j(C_1) = r_j \frac{C_1}{K_j + C_1},$$

where $r_1 = 10$, $K_1 = 1$, and $r_2 = 5$, $K_2 = 0.45$. Again, species 1 outcompetes species 2 for substrate at large substrate concentration, but species 2 outcompetes species 1 at low substrate concentrations. The critical crossover concentration, where $g_1(C_1^*) = g_2(C_1^*)$, occurs at $C_1^* = 0.1$. We approximate, by computing to large times, steady-state solutions of (18)–(22) for varying biomaterial diffusion constants (although all are set equal to the same constant κ, i.e., $D_1^x = D_2^x = D^I = \kappa$) with other parameters held fixed ($k_1 = 0.5$, $k_2 = 0.4$, $D_1^c = 1$, $r_{11} = \gamma_1 g_1$ with $\gamma_1 = 0.1$, and $r_{12} = \gamma_2 g_2$ with $\gamma_2 = 0.2$). In all cases, initial volume fractions are set at $X_1 = X_2 = 0.5$, $I = 0$, and the boundary condition $C_1^0 = C(L) = 1$ is applied. Note that $C(L) > C_1^* = 0.1$; we also find that $C(0) < C_1^*$ in all computations presented. That is, in all example steady states, substrate level favors species 1 at the top of the biofilm but species 2 at the bottom. In the basic model (Example 2), without inclusion of material diffusion, only species 2 was able to persist in this circumstance.

Figure 6 shows an approximation to steady state with $\kappa = D_1^x = D_1^x = D^I = 0.1$. The left panel shows population volume fractions: note that both species 1, the high substrate concentration specialist, and species 2, the low substrate concentration specialist, are able to persist (see Fig. 6, left panel, and Fig. 7). This contrasts to Fig. 8, an example with identical parameters except that $D_1^x = D_1^x = D^I = 0.001$. Here the
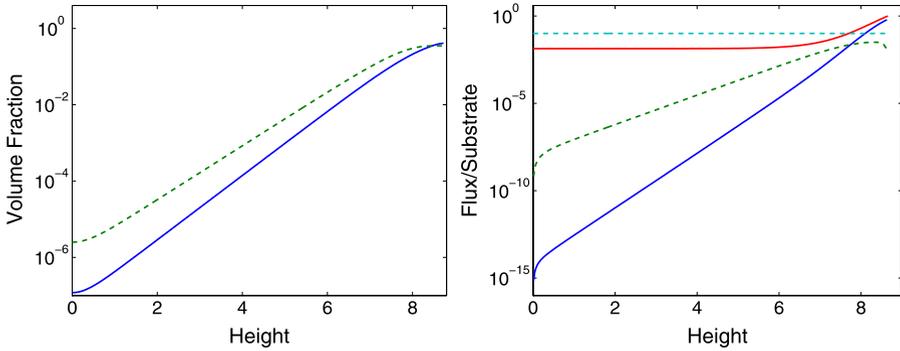
Fig. 6 Steady-state niche creation example, material diffusivity $\kappa = 10^{-1}$ (otherwise, all parameters are the same as in the case shown in Fig. 8). *Left panel*: volume fractions in the biofilm (in log coordinates) of species 1 (*solid*) and species 2 (*dash*) as functions of height. Both populations are able to persist. *Right panel*: comparison of flux rates for species 1 as functions of height. Working from the bottom curve upward in log coordinates, (*solid blue*) absolute value of the advective flux $X_1 v$ of species 1, (*dash green*) absolute value of the diffusive flux $-\kappa \partial X_1 / \partial x$ of species 1, (*solid red*) substrate concentration C, (*dash cyan*) critical substrate C^* above which value species 1 is favored over species 2. Note that the sign of the diffusive flux is negative (it transports organisms from larger populations at the top toward smaller populations at the bottom), while the advective flux is positive (growth pushes upwards). Observe that (see also the close-up in Fig. 7) downward diffusive flux dominates over upward advective flux in a neighborhood of the transition point between high and low substrate
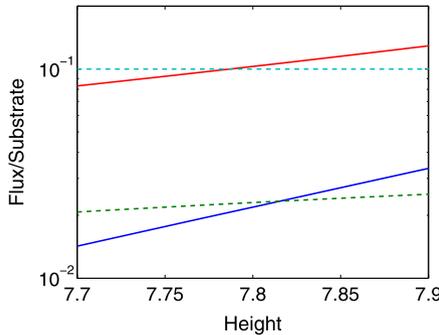

Fig. 7 Close-up of the critical region from Fig. 6, right panel. Working from the bottom upward in log coordinates of height in the biofilm, (*solid blue*) absolute value of the advective flux $X_1 v$ of species 1, (*dash green*) absolute value of the diffusive flux $-\kappa \partial X_1 / \partial x$ of species 1, (*solid red*) substrate concentration, (*dash cyan*) critical substrate C^* above which value species 1 is favored over species 2. Species 1 can outcompete species 2 in the region where the *red curve* is above the *cyan one*, but only with the help of the domination of downward, diffusive flux over upward, advective flux from the subregion (approximately $x \in [7.785, 7.82]$) just above the crossover from subcritical to supercritical substrate

left panel suggests that only species 2 persists. In fact, if we follow the solutions over longer and longer times, the blue curve, the logarithm of species 1 volume fraction, appears to decrease toward 0. Thus it seems that exclusion persists for small enough diffusivity.

The key to understanding the difference between the two cases lies in examining the diffusive and advective components of flux of species 1. In both cases, advective
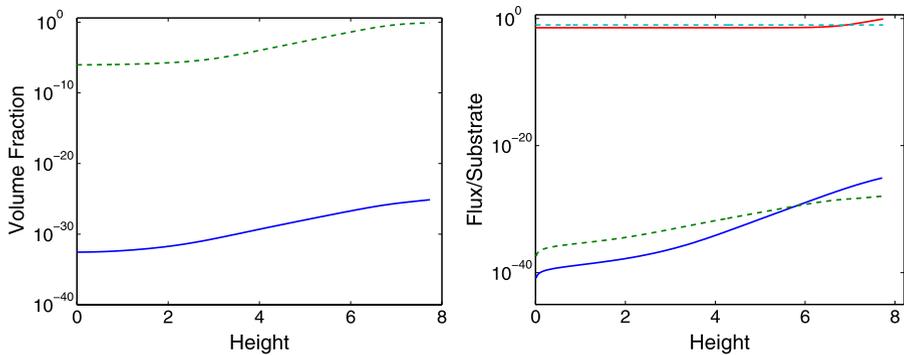
Fig. 8 Steady-state non-niche creation example, material diffusivity $\kappa = 10^{-3}$ (otherwise, all parameters are the same as in the case shown in Fig. 6). *Left panel*: volume fractions in the biofilm (in log coordinates) of species 1 (*solid*) and species 2 (*dash*) as functions of height. The population level of species 1 is very low; at later times, it in fact appears to approach extinction. *Right panel*: comparison of flux rates for species 1 as functions of depth. Working from the bottom upwards, in log coordinates, (*solid blue*) absolute value of the advective flux $X_1 v$ of species 1, (*dash green*) absolute value of the diffusive flux $-\kappa \partial X_1/\partial x$ of species 1, (*solid red*) substrate concentration C, (*dash cyan*) critical substrate C^* above which value species 1 is favored over species 2. Note that the diffusive flux is negative (it transports organisms from larger populations at the top toward smaller populations at the bottom), while the advective flux is positive (growth pushes upward). Observe that the downward, diffusive flux of species 1 is insufficient to overcome its upward, advective flux throughout the region at the top of the biofilm where substrate level is above critical (and hence favorable to species 1)

flux is *directed* upward, while diffusive flux is directed downward nearly everywhere, and, in fact, in both cases advective flux dominates near the top of the biofilm, and diffusive flux dominates below the top region (see Figs. 6 and 8, right panels). The difference is that in the larger diffusivity case, see Fig. 6, the region where diffusive flux dominates extends to include the critical substrate crossover location, see Fig. 7. That is, in the larger diffusivity case, species 1 is able to transport material downward across the critical substrate interface, thus avoiding extinction through growth transport exclusion. Note that it seems that downward transport is only required in that narrow transition region. On the other hand, this is exactly the location (where conditions go from unfavorable to favorable) where anti-taxic motion might be considered unlikely.

As a final observation, if we continue to increase the magnitude of the diffusivity, eventually diffusive transport dominates over growth-generated advection through most of the biofilm, see Fig. 9. In this case, species 1 is able to outcompete species 2 throughout the biofilm by transporting organisms from the top of the biofilm, where its growth is relatively fast, to the bottom of the biofilm, where its growth is relatively slow. Roughly speaking, the biofilm becomes well mixed when the diffusion time is sufficiently small.

4 Discussion

We have presented an exclusion principle for a class of biofilm models, designated basic, showing that these models predict overall ecological structure of steady states to
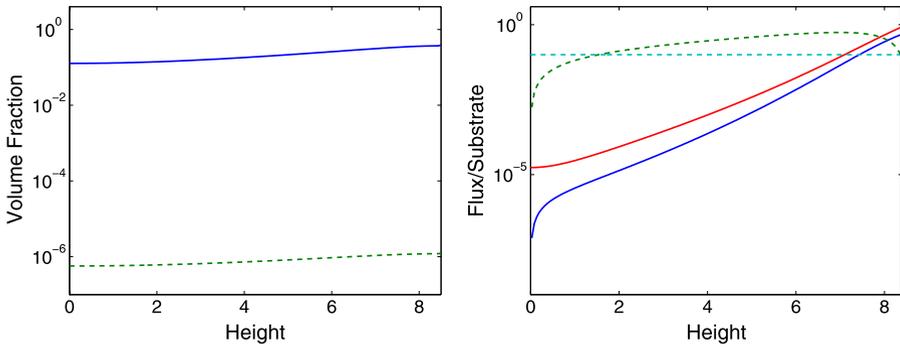
Fig. 9 Steady-state non-niche creation example, material diffusivity $\kappa = 10^1$ (otherwise, all parameters are the same as in the cases shown in Figs. 6 and 8). *Left panel*: volume fractions in the biofilm (in log coordinates) of species 1 (*solid*) and species 2 (*dash*) as functions of height. The population level of species 2 is very low; at later times, it appears to approach extinction. *Right panel*: comparison of flux rates for species 1 as functions of height. Working from the bottom upward, in log coordinates, (*solid blue*) absolute value of the advective flux $X_1 v$ of species 1, (*solid red*) concentration of substrate C, (*dash green*) absolute value of the diffusive flux $-\kappa \partial X_1 / \partial x$ of species 1, (*dash cyan*) critical substrate C^* above which value favors species 1 over species 2. Note that the diffusive flux is negative (it transports organisms from larger populations at the top toward smaller populations at the bottom), while the advective flux is positive (growth pushes upward). Observe that the upward, advective flux of species 1 is insufficient to overcome its downward, diffusive flux throughout the region where substrate level is below critical (and hence favorable to species 2)

be essentially controlled by competitive outcomes at the biofilm base. This exclusion principle is probably undesirably restrictive; observations suggest rather that biofilm niche structure is considerably more complicated. In particular, vertically structured ecological structure is generally observed as a presumed consequence of reaction–diffusion limitation of substrate transport through the biofilm. Thus we suggest that basic models should be utilized with caution when ecological considerations are of interest.

Mathematically, exclusion follows from the 0-stability classification of steady-state solutions. Ecological diversity in 0-stable steady states for the basic model is spatially homogeneous and, most importantly, is determined by competitive balance at the biofilm base $x = 0$. We note that solutions of the extended model trivially have spatially homogeneous ecology as well since material diffusion spreads all species everywhere, at least in the case where all diffusion coefficients are nonzero. However, this diversity is no longer determined by competition at $x = 0$; species that are noncompetitive there can still survive in the steady state.

Exclusion (in the basic model class) results from unbalanced growth-generated upward advection of biomaterial. Hence we have argued that some means of downward-directed mobility is important, certainly in 1D models and possibly in actuality, in order to maintain complex ecological structure. Although the mobility rate need not be large, it does need to be large enough to overcome growth-generated advection in the critical transitions between niche regions. While this advection rate is small—significant biofilm growth typically occurs on time scales of a day or longer—we remark, though, that it would seem that microbes need to somehow be transported

downward through a transition from more favorable to less favorable conditions, opposite to what one might expect from chemotaxis.

Material diffusion, as presented, is one means of introducing downward transport and breaking the exclusion principle. Another thought, one that was in fact already suggested in the presentation of the BIOSIM system (Wanner and Gujer 1986), is to allow biomaterial decay and consolidation. Loss and consolidation has the potential to cause biofilm contraction and hence downward-directed advective transport, and in models can even support steady states without erosion (Pritchett and Dockery 2001). Note that downward moving material presumably resulting from decay and consolidation has in fact been observed in reality, e.g., Doemel and Brock (1977). We can add decay to the basic model by, for example, introducing a loss term for the inert material volume fraction, thus changing (3) to

$$\frac{\partial I}{\partial t} + \frac{\partial}{\partial x}(Iv) = -d(\mathbf{C}, \mathbf{X}, I) + \sum_{j=1,\dots,N} k_j(\mathbf{C}, \mathbf{X}, I) X_j, \tag{23}$$

where the decay function $d(\mathbf{C}, \mathbf{X}, I)$ is nonnegative. With this change, the velocity (5) becomes

$$v = \int_0^x \left[-d(\mathbf{C}, \mathbf{X}, I) + \sum_{l=1,\dots,N} g_l(\mathbf{C}, \mathbf{X}, I) X_l \right] d\zeta, \tag{24}$$

which, with the addition of the decay contribution, can now be negative, due to consolidation, and indeed in many cases will quite likely be so in the lower regions of the biofilm, where limiting substrate levels and hence growth are generally at low levels.

The new model (1)–(5) with (3) replaced by (23) and (5) by (24) does indeed break the conditions of the exclusion principle and presumably may allow spatially nontrivial ecologies at least in some circumstances. However in many cases, while no longer uniformly nonnegative, $v(x)$ can be expected to be negative at the bottom of the biofilm and positive at the top with a single null between, say at x_0, since often growth increases with increasing x, while at the same time decay decreases. Then characteristics will originate from $x = x_0$ (rather than $x = 0$); for $x < x_0$, characteristics move downward to $x = 0$, while for $x > x_0$, characteristics move upward to $x = L$. Hence an exclusion principle might still apply, this time with ecology determined solely by what occurs at the location $x = x_0$. In order to avoid this, it seems necessary that $v(x)$ have at least two nulls, which in turn requires growth regions separated by decay ones (or vice versa). While this may occur in certain cases, it seems generally unlikely, and, further, ecology would still be determined by the environment at only a few locations. Hence, while decay may be a reasonable addition to the basic model system, it is not clear that decay alone really resolves the exclusion difficulty.

It should also be noted that the only removal mechanism we consider is erosion from the biofilm bulk fluid interface. In actuality, material can be lost through detachment of chunks of material; in fact, detachment might be the most significant cause of loss (Wilson et al. 2004). However, this removal mechanism still would seem to advantage material lower down within the biofilm, so it does not necessarily make downward mobility unimportant. Another mechanism that might have relevance is that of dispersion—the tendency sometimes observed for inner regions to

somehow and in some sense liquify—and escape (again, in some manner not well understood) (Purevdorj-Gage et al. 2005). It is currently not yet clear, though, how significant dispersion is generally.

We present only 1D results; however, numerics suggest that the same issues are evident for basic models in two and three dimensions (Alpkvist and Klapper 2007). One possibility that we have not mentioned though, namely that biofilm frictional properties may vary spatially (perhaps, for example, as functions of species makeup), may be significant in the present context in 2D and 3D. In 1D, material, generally, is pushed upward by growth-generated advective displacement; in higher dimensions there is the possibility that growth-related displacement is concentrated in less viscous pathways and that more viscous regions are less subject to displacement toward the surface and resulting erosion. Thus, and from observations of the previous paragraph as well, we note that multidimensional influences on material properties may be of importance ecologically and should also be considered.

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