Supplementary materials for "Accelerating returns from public goods favor quorum sensing in bacteria"

October 15, 2013

1 Relaxing the assumptions from the main text

In the main text we assume a well mixed population where timescales of cell growth and death is slower than that of degradation/depletion of signal and common good molecules. Below we discuss the implications of having a spatially structured system and the consequences of not having a clear separation of timescales between growth of cells and common good production and degradation/depletion.

1.1 Spatially structured systems

A similar calculation for an isotropically growing colony of cells in 1 dimension shows overall the same result as the calculation in the main text (eq. 1-4). The only difference is that as the population size grows very large, the optimal production rates do not fall to zero but stabilize at some finite non-zero value (see fig. 1). Intuitively, this is easy to understand. In a spatial setting, if the diffusion constant of the public good is D_E and its degradation rate is γ_E then public good produced at a specified position spreads only up to a distance of roughly $\sqrt{D_E/\gamma_E}$. Thus, any cell at the edge of a growing colony will only be influenced by other cells that are within this radius, and not all the cells in the colony like in a perfectly well mixed system. Initially, when the colony has a radius smaller than the length $\sqrt{D_E/\gamma_E}$ the system is like the well-mixed one where all cells influence the others. But later, even though the number of cells N continues to grow larger as the colony grows, the effective number of cells any given cells "sees" saturates at a finite value. The optimal production rate thus stops decreasing when the number of cells within a radius of $\sqrt{D_E/\gamma_E}$ stops increasing.

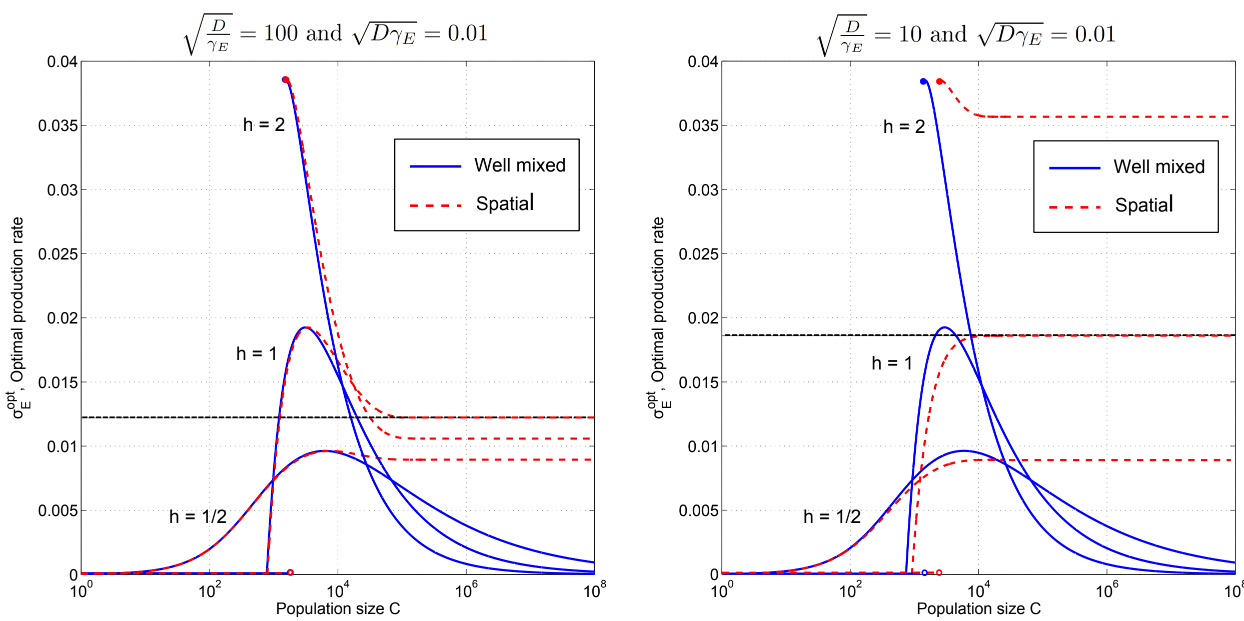

Figure 1: **Optimal production curves, as a function of population size C, for benefit functions with $h < 1$, $h = 1$ and $h > 1$,** For a well mixed system and a 1D system. Blue solid curves: the solution $\sigma_E^{opt}(C)$ plotted for $h = 1/2$, $h = 1$ and $h = 2$. Red dashed curves: the solution solution $\sigma_E^{opt}(C)$ for a 1D colony with equal spacing between cells. Units are arbitrary.

1.2 Not reaching steady-state

In the calculation in the main text, we used the assumption that cell growth and division occurs on a much slower timescale than public good production and degradation to replace E by $N\sigma_E/\gamma_E$ in equation (1) of the main text. If this assumption is not true, then there would not be enough time for E to get to this steady-state level. For example, in the well-mixed system, suppose at time 0 the system had N cells and a public good concentration of $E(0)$, then if the next cell division happened at a time t:

$$E(t) = E(0)e^{-\gamma_E t} + \left(1 - e^{-\gamma_E t}\right)\frac{N\sigma_E}{\gamma_E}. \tag{1}$$

So, even though E has not reached steady-state, it is nevertheless some increasing function of σ_E and N. Clearly, even a modest separation of timescales, such that the cell growth rate is a few times smaller than γ_E, will result in $E(t)$ being almost proportional to $N\sigma_E$ and the same conclusions as before. Only if the cell growth rate is comparable to γ_E will there be a significant deviation from our calculation above. But even in this case, $E(t)$ will nevertheless be some increasing function of σ_E and N, just not a linear function. The net effect would be to modify the nonlinearity of the benefit function to decrease(CHECK!!! sandeep is this right?) its initial convexity and so a critical population size at which to turn on public goods may appear only for an $h \geq h_c$, where $h_c > 1$, rather than for $h \geq 1$. This would also be true if we were looking at a spatially structured system, except that E may be a more complicated increasing function of N and σ_E. In general, we expect that cell growth will always be a few times slower than the rate of degradation/depletion of the public good and, thus, there will be at most a minor modification of the shape of the optimal production curve.

1.3 General requirements for a nonlinear cost and benefit function pair to produce a discontinuous optimal production function

In the main text we dealt primarily with the special case of a linear cost function and a sigmoidal benefit function. More general cases of nonlinear cost and benefit function pairs can be mapped onto the results of this simple case. When cost is linear, we saw that convex ($h > 1$) and concave ($h < 1$) sigmoidal benefit functions fall into two major categories: Either they have continuous (class 1) or discontinuous (class 2) optimal production curves. It turns out that general nonlinear pairs of (monotonically increasing) cost and benefit functions all fall into either of these two classes. In figure 2 we show examples of pairs of nonlinear cost and benefit functions from these two classes.

Assume a specific common good in a certain set of conditions have the benefit function $b([E])$ and cost the function $c(\sigma_E)$. (Both $b(E])$ and $c(\sigma_E)$ are given in units of $[time^{-1}]$). The benefit function gives the the positive contribution to the basic growth rate of a cell experiencing a common good concentration

$[E]$ in its surrounding and the cost function $c(\sigma_E)$ gives the negative impact on growth rate of a cell producing common good, E at the rate σ_E. If we assume separation of time scale (growth of cells slower than production and degradation/removal of common good) we have $\sigma_E \propto E/N$, where N is the number of cells. This allows us to rewrite the benefit function in terms of σ_E instead of $[E]$ as $b(\sigma_E)$. Its now understood that the benefit is due to production of common good by the population of N cells alone (i.e. no external source of common good and all cells are producing at the same rate σ_E). For each value of N the benefit function $b(\sigma_E)$ changes. Since $\sigma_E \propto E/N$ this change is basically just a rescaling of the x-axis and the result is that the function gets 'squeazed' and rises up as N is increased. The cost function does not change when N changes since it gives the cost per cell due to production of common good and this is not assumed to change due to production by other cells. Obviously it pays of to produce at a rate σ'_E at a certain population size N' if benefit is greater than cost: $b(\sigma'_E)|_{N=N'} > c(\sigma'_E)$.

If the functions $b(\sigma_E)$ and $c(\sigma_E)$ for a certain common good are known it is possible to determine whether a population of can get a positive fitness boost from producing the common good even at single cell level or whether they should wait until the population size has reached a critical level $N_c > 1$, e.i whether behavior is class 1 or class 2.

If production pays of even at single cell level (like the case of a concave benefit function and linear cost) is means that the optimal production curve will be continuos. The requirement for this to happen is simply that:

$$\left.\frac{dc}{d\sigma_E}\right|_{\sigma_E=0} \leq \left.\frac{db}{d\sigma_E}\right|_{\sigma_E=0,N=1}$$

The requirement for production only to pay off after a critical population size N_c has been reached is that:

$$\left.\frac{dc}{d\sigma_E}\right|_{\sigma_E=0} > \left.\frac{db}{d\sigma_E}\right|_{\sigma_E=0,N=1}$$

but this is not necessarily enough to produce a discontinuous optimal production curve. Functions which satisfy this can have a optimal production function which rises continuously from zero at population size $N_c > 1$, behaving like the limiting case where $h = 1$ with linear cost and sigmoidal benefit function. (Only the second derivate of the optimal production curve will be discontinuous in these limiting cases).

The additional requirement which ensures a discontinuous optimal production curve is that the second derivative of the benefit and cost functions have a relative shift in convexity at a non zero value of $\sigma^*_E > 0$, such that:

$$\left.\frac{d^2c}{d\sigma_E^2}\right|_{\sigma_E<\sigma*_E} < \left.\frac{d^2b}{d\sigma_E^2}\right|_{\sigma_E<\sigma*_E}$$

and

$$\left.\frac{d^2c}{d\sigma_E^2}\right|_{\sigma_E>\sigma*_E} > \left.\frac{d^2b}{d\sigma_E^2}\right|_{\sigma_E>\sigma*_E}$$

This can happen even if both functions are strictly concave. (See figure 2 lower panel).

2 Finding the critical α_c where the ODE model displays bistability

The ODE model presented in the main text of a well mixed population producing signal and public good (eq. 5 and 6) displays bistability in certain parts of the parameter space. We wish to determine the critical value of α_c above which the system will be bistable for a range of N values.

The rate of change of signal molecule is given by:

$$\frac{dS}{dt} = N\left(1 + \sigma_S^{max}\frac{S^\alpha}{S^\alpha + K_S^\alpha}\right) - S$$

at steady state $\frac{dS}{dt} = 0$, we have:

$$1 + \sigma_S^{max}\frac{S^\alpha}{S^\alpha + K_S^\alpha} = \frac{S}{N}. \tag{2}$$

We define

$$p(s) \equiv 1 + \sigma_S^{max}\frac{S^\alpha}{S^\alpha + K_S^\alpha}.$$

Fix points S^* are found when $p(S)$ intersect with S/N. The system will thus be bistable when it is possible for a straight line S/N (that passes through the origin and has a positive slope) to intersect with $p(S)$ for more than one value of S. Above a critical value of $\alpha = \alpha_c$ it becomes possible to get 3 intersections. (See fig. 3 where $p(S)$ is plotted for two different values of α (above and below the critical value), along with a range of straight lines, S/N).

We wish to determine the value of $\alpha = \alpha_c$ above which the system displays bistability.

We realize graphically that if a straight line l exists, which passes through a point $(S^*, p(S^*))$ and has a slope of $\left.\frac{dp}{dS}\right|_{S=S^*}$ can intersect the y-axis below zero, then we know that eq. (2) can have 3 solutions. The equation for the line l is:

$$l: \quad p(S') = S'\left.\frac{dp}{dS}\right|_{S=S'} + q$$

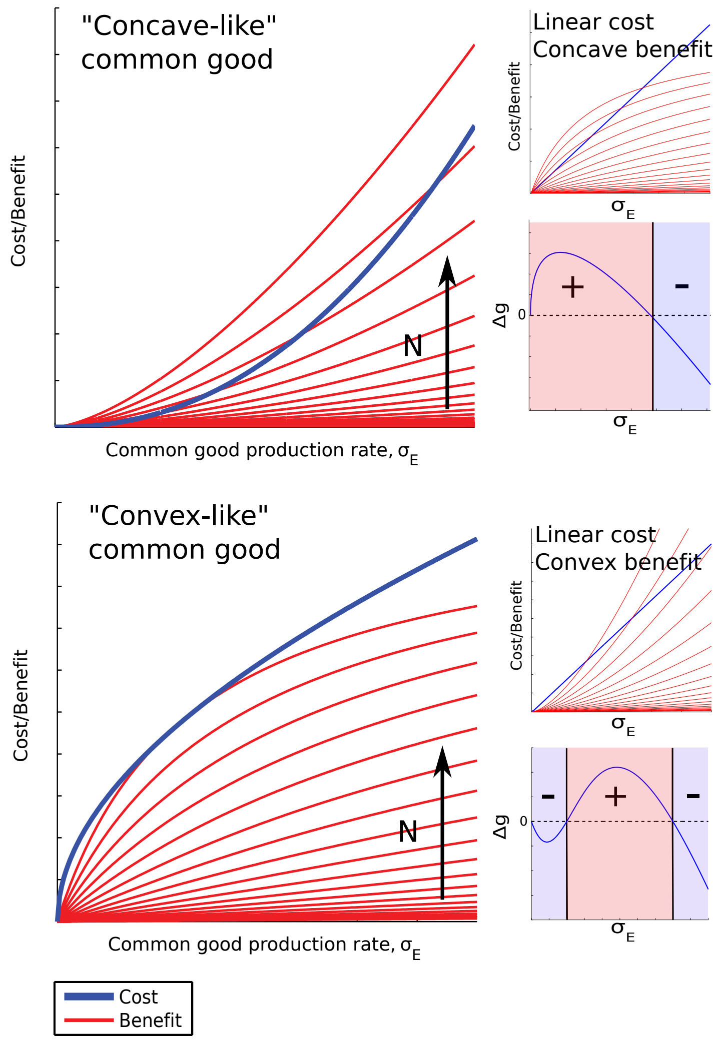

Figure 2: **General nonlinear cost and benefit curves:** Red curves: growth benefit per cell as a function of public good production rate for different values of the population size N. Blue curve: cost per cell as a function of public good production rate. When the population size, N, increases the x-axis is effectively squeezed for the benefit curve and the red benefit curve rises up to meet the blue cost curve. **Upper panel:** Example of a class 1 public good - a nonlinear cost/benefit function pair which has a continuous optimal production curve similar to the one depicted in fig. 1 in the main text, of a public good with a linear cost function and a sigmoidal benefit function with Hill-factor $h < 1$. **Lower panel:** Example of class 2 public good - a nonlinear cost/benefit function pair which have a discontinuous optimal production curve similar to the one depicted in fig. 3 in the main article, which has a linear cost function and a sigmoidal benefit function with Hill-factor $h > 1$.

where q is the intersection with the y-axis.

The first derivative of $p(S)$ is:

$$\frac{dp}{dS} = \alpha K_S^\alpha \sigma_S^{max} \frac{S^{\alpha-1}}{(S^\alpha + K_S^\alpha)^2}$$

For bistability (3 fix points) we must thus have:

$$\begin{aligned}
q &< 0 \Rightarrow \\
0 &> p(S') - S' \frac{dp}{dS}\Big|_{S=S'} \Rightarrow \\
0 &> 1 + \sigma_S^{max} \frac{S'^\alpha}{S'^\alpha + K_S^\alpha} - \alpha K_S^\alpha \sigma_S^{max} \frac{S'^{\alpha-1}}{(S'^\alpha + K_S^\alpha)^2} S' \Rightarrow \\
0 &> 1 + \sigma_S^{max} \left[\frac{S'^\alpha}{S'^\alpha + K_S^\alpha} - \alpha K_S^\alpha \frac{S'^\alpha}{(S'^\alpha + K_S^\alpha)^2} \right] \Rightarrow
\end{aligned}$$

We rename $S^\alpha \equiv x$ and rewrite to get a 2nd degree equation in x:

$$\begin{aligned}
0 &> x^2 (1 + \sigma_S^{max}) + x K_S^\alpha (2 + \sigma_S^{max} (1 - \alpha)) + K_S^{2\alpha} \\
&\equiv x^2 a + x b + c
\end{aligned}$$

there is exactly one solution $x_0 = S_0^\alpha$ to $x^2 a + x b + c = 0$ when the coefficients satisfy $d = b^2 - 4ac = 0$. This is when the line l will intersect the y-axis at zero at one value of $S^* \equiv S_0$ exactly (while for all other values of S^* the line l will intersect the y-axis above zero) and this thus defines the critical α_c above which the system starts to be bistable. This gives us the needed condition to find the critical α_c :

$$\begin{aligned}
d &= 0 \Rightarrow \\
0 &= b^2 - 4ac \Rightarrow \\
0 &= (K_S^\alpha (2 + \sigma_S^{max} (1 - \alpha)))^2 - 4 (1 + \sigma_S^{max}) K_S^{2\alpha} \Rightarrow \\
\alpha_\pm &= \frac{2 + \sigma_S^{max} \pm 2\sqrt{1 + \sigma_S^{max}}}{\sigma_S^{max}} \qquad (3)
\end{aligned}$$

it is of course only the positive solution $\alpha_+ \equiv \alpha_c$ which is relevant. For α_- the solution x_0 to $x^2 a + x b + c = 0$ is negative ($x_0 < 0$) which we cannot have since $S \geq 0$.

Note that the critical point α_c is independent of K_S. This is expected since we can always choose the unit of S such that $K_S \equiv 1$.

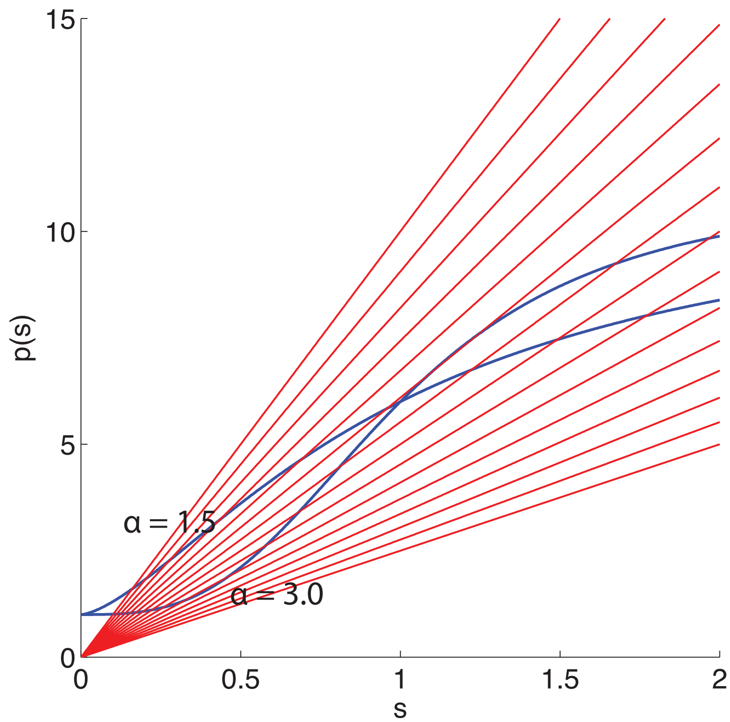

Figure 3: Blue lines shows $p(S)$ plotted for two different values of $\alpha = [1.5, 3.0]$, where the system can have only one or up to three fix points respectively. Red lines are S/N plotted fro a range of different N values (between 0.1 and 4.0). Fix points are values of S where $p(S)$ and S/N intersects. (Here $\sigma_S^{max} = 10$, and $K_s = 1$).

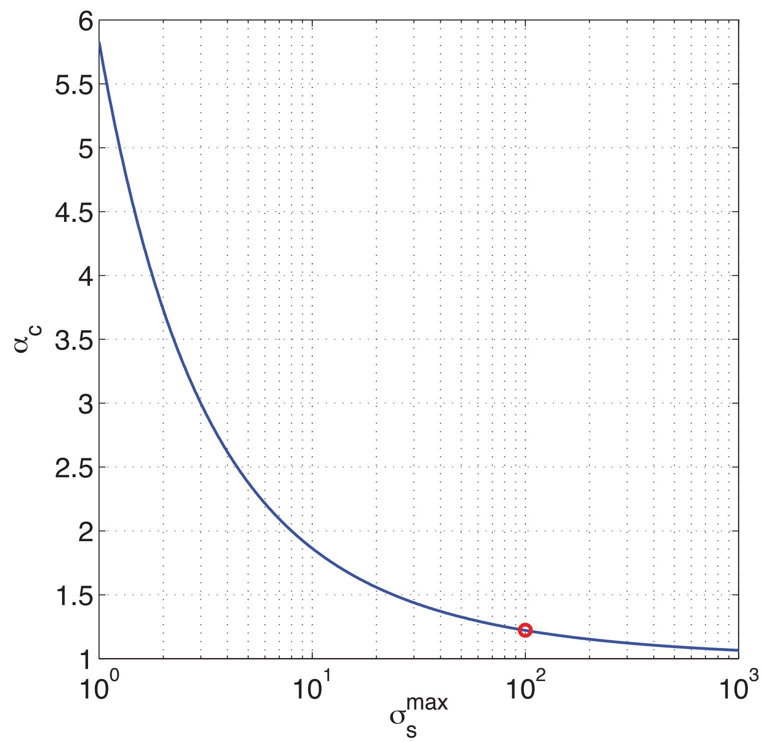

Figure 4: Blue line is $\alpha_c = \frac{2+\sigma_s^{max}+2\sqrt{1+\sigma_s^{max}}}{\sigma_s^{max}}$, (eq. 3) as a function of the ratio between basal and maximal signal production rates, σ_s^{max}. The red circles marks the value of σ_s^{max} used in the paper. The actual value of σ_s^{max} not well known for any bacterial species but it is reasonable to assume its lies in the range of $[5-1000]$ and possibly could be very different for different species.

3 Model for polymers degraded by an excreted endoprotease

An common example of an excreted public good are enzymes which act outside the cell to degrade long polymers into smaller pieces which subsequently can be transported over the cell membrane and metabolized. A well studied example is e.g. *Pseudomonas aeruginosa* which can excrete multiple endo-proteases [1] capable of degrading casein into casamino acids by breaking the polymers at sites of specific residues.

We wish to assess the shape of the benefit function for a well mixed environment with polymers that can be broken at random places by an excreted enzyme, (we assume that the specific sites where the enzymes can break the polymers are distributed randomly. [2] We will assume that the fitness increase is proportional to the number of pieces of polymer present which are small enough for transport over the cell membrane. Thus what we need to determine is the steady state distribution of polymers of different lengths, and specifically the concentration of polymers of the "edible length", as a function of the concentration of excreted enzyme. We set this "edible length" (the maximal length that still allows transport over the cell membrane), to one, and we assume a constant external source of polymers of length n, and a constant degradation rate equal for polymers of all lengths.

Concentration of a polymer of length i is denoted N_i. Longest polymers (the ones supplied by an external source) in the system has length n. Concentration of enzyme (common good) is denoted E. The production rate of polymers of maximal length n, is p. Degradation rate of all polymers is δ.

Equations describing the change in concentration of polymers of all lengths, for a given level of enzyme, E, are thus as follows:

$$
\begin{aligned}
\frac{dN_n}{dt} &= p - N_n(E+\delta) \\
&\vdots \\
\frac{dN_i}{dt} &= 2E\sum_{j=i}^{N-1}\frac{N_{j+1}}{j} - N_i(E+\delta) \\
&\vdots \\
\frac{dN_1}{dt} &= 2E\sum_{j=1}^{N-1}\frac{N_{j+1}}{j} - N_1\delta
\end{aligned}
$$

where $i = 1, 2, \ldots, n$

[1] LasB and AprA, the two major secreted proteases by *Pseudomonas aeruginosa*.

[2] The proteases LasB and AprA secreted by e.g. *Pseudomonas aeruginosa*, are endoproteases, which means they cut the protein next to specific residues. Endoenzymes are generally more common among QS-regulated secreted enzymes than enzymes which cleave molecules from the end (ref. Brook Peterson UW personal communication).

The steady state concentrations, $N_n^*, \ldots, N_i^*, \ldots, N_1^*$ can be found by setting $\dot{N}_n = \cdots = \dot{N}_i = \cdots = \dot{N}_1 = 0$.

For $n = 2$, the steady state concentration of N_1 will be:

$$N_{1,n=2}^* = 2\frac{Ep}{\delta(\delta + E)} \tag{4}$$

and for $n > 2$, the steady state concentration of N_1 is given by:

$$N_{1,n>2}^* = \frac{2}{n-1}\frac{Ep}{\delta(\delta+E)}\left[1 + \left(\frac{2}{n-2} + \sum_{k=2}^{n-2}\frac{1}{k-1}\prod_{j=k}^{n-2}\left(1+\frac{2}{j}\frac{E}{\delta+E}\right)\right)\frac{E}{\delta+E}\right] \tag{5}$$

Eq. 4 and 5 are plotted in figure 5. Note that it is only for a system with a maximal polymer length of $n = 2$ that the benefit function is *not* convex. (The second derivative of eq. (4) is negative while the second derivatives of eq. (5) are increasingly more positive for increasing n). This means that in general, benefits will accelerate with increasing concentration of enzyme if the polymers provided by the external source has a length of more than two 'edible' units (i.e. units small enough to be transported over the cell membrane).

This expression comes from combining the fact that:

$$N_1^* = 2\frac{E}{\delta}\left[\sum_{k=2}^{n}\frac{1}{k-1}N_k^*\right], \text{ for } n > 2$$

and

$$\begin{aligned}
N_i^* &= N_{i+1}^*\left(1+\frac{2}{i}\frac{E}{\delta+E}\right), \text{ for } 1 < i < n-1 \Rightarrow \\
N_k^* &= N_{n-1}^*\prod_{j=k}^{n-2}\left(1+\frac{2}{j}\frac{E}{\delta+E}\right), \text{ for } k > 1
\end{aligned}$$

and

$$\begin{aligned}
N_n^* &= \frac{p}{\delta+E} \\
N_{n-1}^* &= \frac{2}{n-1}\frac{E}{\delta+E}N_n^*, \text{ for } n > 2
\end{aligned}$$

which follows from setting $\dot{N}_n = \cdots = \dot{N}_i = \cdots = \dot{N}_1 = 0$, solving for $N_n^*, \ldots, N_i^*, \ldots, N_1^*$.

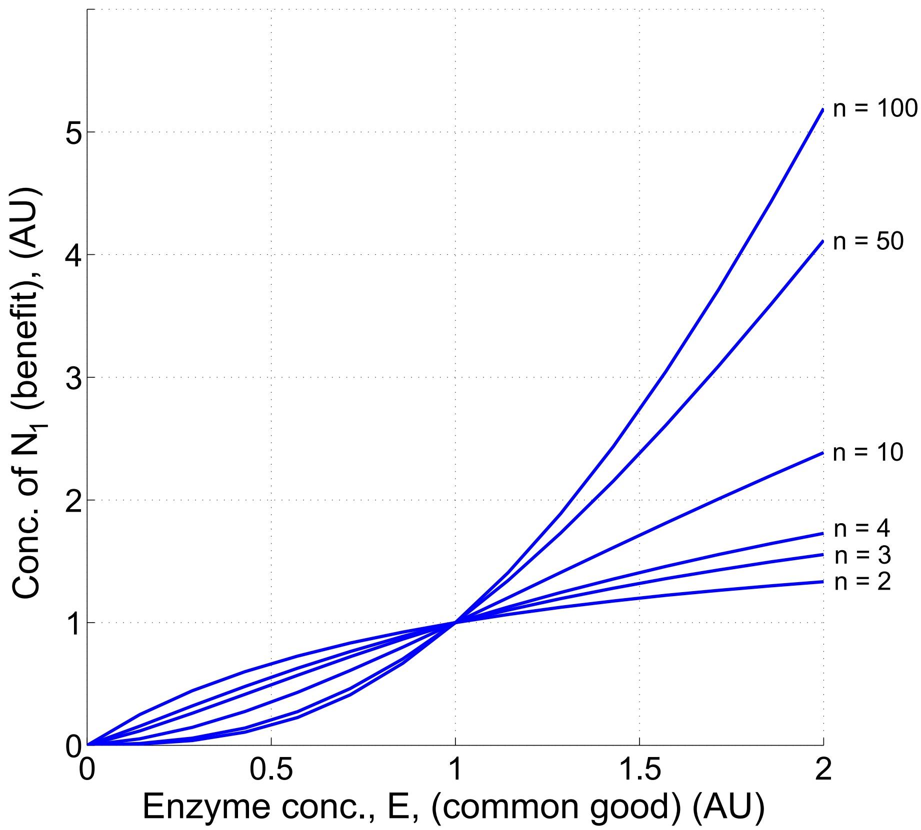

Figure 5: **Benefit function becomes increasingly convex as length of longest polymer in system increases, when degradation is done by an exoprotease.** Steady state concentration of N_1 as a function of enzyme concentration E, for systems with max polymer length of $n = [2, 3, 4, 10, 50, 100]$.

4 Maximum fitness as a function of α

In figure 6 in the main text, the maximum fitness value obtainable as a function of the feedback exponent α, is plotted for two different values of the benefit function hill factor h and for two different ecological scenarios ($x_{on} = 0.5$, $x_{off} = 0.5$ and $x_{on} = 1$, $x_{off} = 0$). In figure **??** we show examples of the fitness landscapes that the max fitness values in fig. 6 in the main text where picked from, (marked by white circles). For each value of α the value of the fitness peak was found in a 2D fitness landscape with the two other QS parameters σ_E^{max} and K_S on the $x-$axis and $y-$axis respectively.

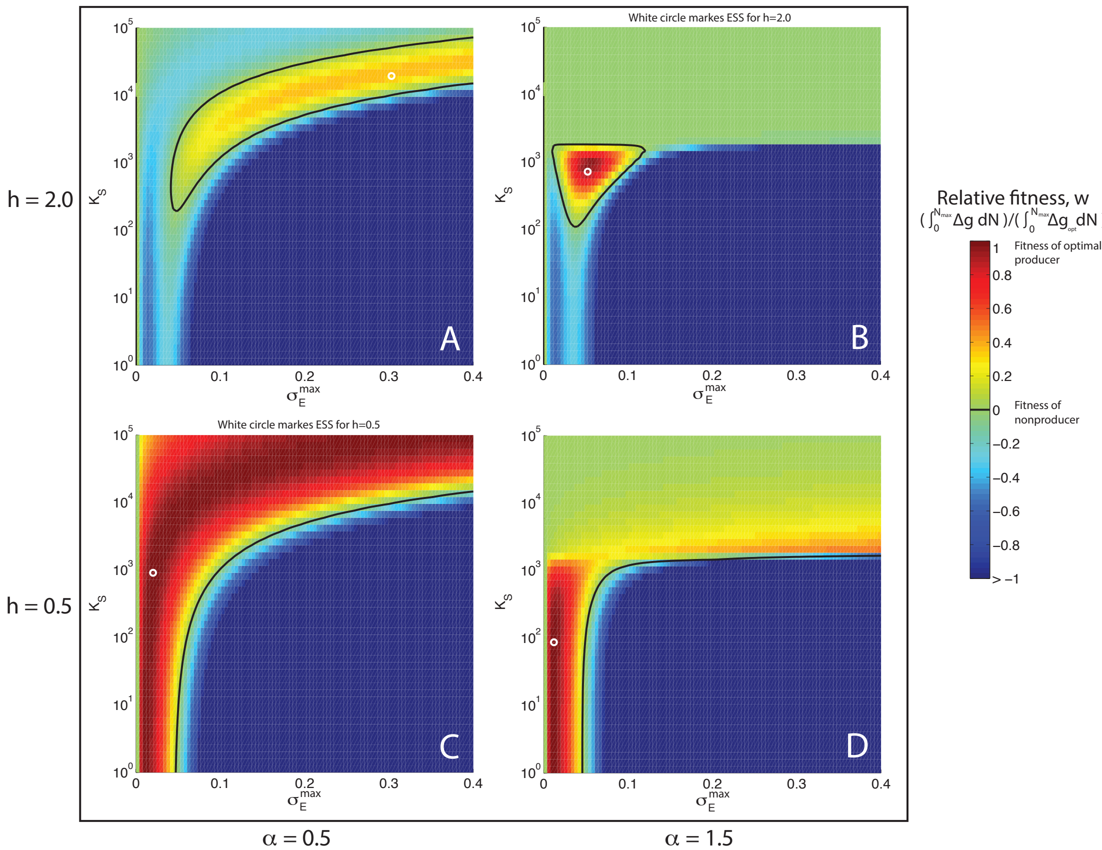

Figure 6: **Colormap in panel A, B C and D show relative fitness (assuming $x_{on} = 0.5$, $x_{off} = 0.5$) of a population as a function of the QS parameters (σ_E^{max}, K_S) for four different combinations of the benefit hill-factor h and the QS feedback exponent α.** **A**: when $h = 2.0$ and $\alpha = 0.5$, fitness peaks at ~ 0.4 meaning that here the best possible performance is well below the theoretical optimum of 1. **B**: When $h = 2.0$ and $\alpha = 1.5$, fitness peaks at ~ 0.90. This is the best possible fitness value obtainable for $h = 2.0$, (when both turn on and turn off scenarios are given equal weight) thus making $\alpha = 1.5$ the ESS for a scenario with a common good that has a convex benefit function. **C**: When $h = 0.5$ and $\alpha = 0.5$, fitness peaks at ~ 0.999. This is the best possible value obtainable for $h = 0.5$, making $\alpha = 0.5$ the ESS for for a scenario with a concave benefit function. D: When $h = 0.5$ and $\alpha = 1.5$, fitness peaks at ~ 0.98 meaning that the best possible performance is still pretty close to the theoretical optimum even though α is not at the ESS value. The relative fitness is defined here as $w \equiv 0.5w_{on} + 0.5w_{off}$ where $w_i \equiv \int \Delta g^i dN / \int_0^{N_{max}} \Delta g_{opt} dN$, $i = [on \vee off]$, (the indice 'on' refers to going from low to high N while the indice 'off' refers to going from high to low N). With this definition the fitness of a population which uses the theoretical optimal production strategy (for the relevant value of h) is one, while the fitness of a population which does not produce common good is zero. Black lines mark where fitness goes negative. All three panels has regions where the fitness goes much lower than $w = -1$, (dark blue lower right corner), but since we are not interested in the low fitness limit the color range was set to provide good contrast only for the range $w = [-1, 1]$. The fitness peak in each panel is marked by a white circle.