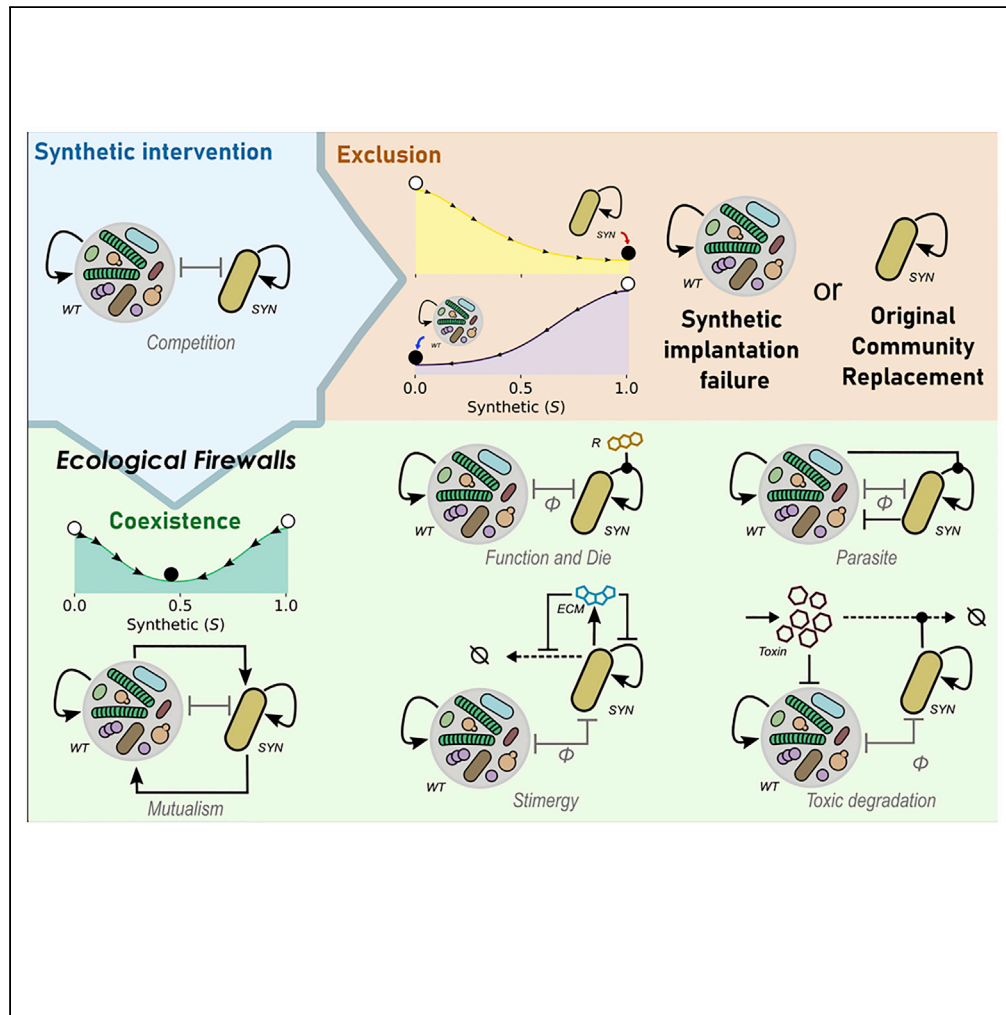


Article

Ecological firewalls for synthetic biology



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Highlights

Population control of synthetic strains can be achieved by engineering ecological links

We introduce ecological firewalls, inspired in four types of ecological interactions

Our firewalls are shown to maintain diversity while performing designed functions

Ecological firewalls will help to tackle future bioremediation strategies

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Article

Ecological firewalls for synthetic biology

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SUMMARY

It has been recently suggested that engineered microbial strains could be used to protect ecosystems from undesirable tipping points and biodiversity loss. A major concern in this context is the potential unintended consequences, which are usually addressed in terms of designed genetic constructs aimed at controlling overproliferation. Here we present and discuss an alternative view grounded in the nonlinear attractor dynamics of some ecological network motifs. These ecological firewalls are designed to perform novel functionalities (such as plastic removal) while containment is achieved within the resident community. That could help provide a self-regulating biocontainment. In this way, engineered organisms have a limited spread while—when required—preventing their extinction. The basic synthetic designs and their dynamical behavior are presented, each one inspired in a given ecological class of interaction. Their possible applications are discussed and the broader connection with invasion ecology outlined.

INTRODUCTION

Since the dawn of synthetic biology, scientists have been designing and modifying living systems in diverse ways, sometimes breaking the constraints imposed by evolution. In this context, synthetic biology is the last revolution within technological evolution: it deals with the creation of novel functional designs grounded in the use of living matter [Benner and Sismour, 2005; Cameron et al., 2014]. This emerging discipline has been instrumental in providing new tools to interrogate nature [Lim, 2010; Brophy and Voigt, 2014] and develop new biomedical applications [Ruder et al., 2011; Weber and Fussenegger, 2011; Wu et al., 2019] while expanding the reach of possible biology [Elowitz and Lim, 2010]. The success of the field is highlighted by the continuous expansion of its application domain toward higher-level problems, including the design of synthetic ecosystems [Weber et al., 2007; Mee and Wang, 2012; Großkopf and Soyer, 2014; De Roy et al., 2013; Widder et al., 2016; Jaramillo, 2017], biocomputation beyond simple circuits [Purnick and Weiss, 2009; Regot et al., 2011; Macía et al., 2012; Grozinger et al., 2019], nonlinear dynamics in cells [Levine et al., 2013; Vidiella et al., 2021] or multicellular designs [Davies, 2008; Toda et al., 2020; Duran-Nebreda et al., 2021]. Despite the major challenges ahead, everything seems to suggest that there is plenty of room for exploration and development. To a large extent, the success of this field has been tied to its deep connection with systems biology. Right from its initial steps, synthetic designs and their theoretical description (from Boolean networks to differential equations) came together. Over the last decade, major advances have been made within the context of engineered microbiomes thanks to the merging between the two disciplines [Widder et al., 2016; Leggieri et al., 2021]. Not surprisingly, community ecology—a traditional systems science—has inspired many relevant ideas within microbiome research, where concepts from population dynamics are easily translated into the microbiota context [Costello et al., 2012]. Can such ideas be also used to address the problem of climate change, where releasing modified microbes could be a potential future scenario?

It has been recently suggested that synthetic biology might actually help to protect, restore or “terraform” extant ecosystems that can be in danger of experiencing catastrophic transitions [Solé, 2015; de Lorenzo et al., 2016; Conde-Pueyo et al., 2020]. The rapid acceleration of Anthropogenic impacts on our biosphere, in particular in relation of marine and freshwater pollution, carbon dioxide removal, and biodiversity decay, has ignited a debate concerning the need of using synthetic biology as an emerging approach to these problems [Solé, 2015; Piaggio et al., 2017; Goold et al., 2018; Xiong et al., 2018; DeLisi et al., 2020; Rylott and Bruce, 2020; Cleves et al., 2018; Levin et al., 2017; van Oppen and Blackall, 2019; Coleman and Goold, 2019]. However, too often the emphasis is placed on the bottom, microscopic level, i.e., how genetic designs are made. In this context, our understanding of how synthetic designs can work within cell populations has been rapidly improving, particularly when dealing with microbial systems. However, what can be said about their impact on community dynamics? Is this an uncertain domain where little can be predicted or controlled?

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A major issue of deployed engineered microorganisms concerns the potential implications for local ecosystems and unknown evolutionary outcomes [Schmidt and de Lorenzo, 2012, 2016]. As a consequence of an almost complete lack of experimental evidence (because of moratoria and the absence of a proper theoretical framework) this issue has been very often discussed in non-scientific terms. The ethical debate has been prominent (as it should be) but not substantiated by evidence. Field interventions aimed at ecosystem restoration involving inoculation-based techniques [Bowker, 2007] or the use of urban organic waste [Pascual et al., 1999] are seldom questioned despite the fact that they actually bring multiple exotic microorganisms (even a whole microbiome) to the given habitat. Instead, the delivery of a single engineered microorganism strain is typically received with extreme caution.

Biocontainment designs are a big issue within synthetic biology [Lee et al., 2018] as it was originally in the past century in relation to recombinant DNA technology. In this context, most proposed strategies deal with engineering tools that would minimize the interactions between synthetic strains and the potential life forms around it. Practical scenarios include microbes designed to degrade a given toxic substrate [de Lorenzo, 2008; Nikel and Lorenzo, 2021], modify the gut [Foo et al., 2017] or plant microbiomes [Ke et al., 2021] or cyanobacteria capable of improving soil [Vidiella et al., 2020], to mention just a few. The key argument is that a properly defined genetic firewall could in principle provide a source of containment that is predictable [Wang and Zhang, 2019]. These genetic firewalls require some amount of orthogonality in relation to living designs as we know. Two major, conceivable routes to synthetic life are *de novo* cellular design or successive alienation of designed bacterial strains using *in vitro* evolution. The latter would allow a process of directed codon emancipation leading artificial cell equipped with an unnatural genetic code [Budisa, 2014].

In this paper, we want to address the previous questions in relation to the problem of population containment of synthetic organisms deployed in extant ecosystems. We start with the recent proposal of *terraformation* as applied to our biosphere [Solé, 2015; Conde-Pueyo et al., 2020; Solé et al., 2015, 2018]. The key concept here is that, ideally starting from a member of the resident community, a synthetic strain can be obtained to be reintroduced to perform a function while being constrained to a limited spread within the given habitat. This would include a broad range of goals, from removal of undesirable molecules (as in classic bioremediation) to the stabilization of endangered communities (such as soils in drylands). This scenario raises immediate questions regarding unintended consequences, often to be compared with those involving invasive species. In general, the assumption is that invaders will be harmful and thus designed “invaders” should also be detrimental to community organization or diversity. Is that the case? To properly address this question, we need to move to a systems-level picture.

The lessons from invasion ecology tell us a different story. First of all, invasion is usually constrained by community structure [Lockwood et al., 2013]. Typically, most invaders fail to get established. On the other hand, many historical examples of invaders have to do with species capable of fast growth facilitated by the lack of negative feedback (caused, for example by the disappearance of top predators) and the presence of a degraded resident community (caused, for example, by grazing). Importantly, we also know of other situations where the invader actually helps to sustain diversity and create opportunities for other species to survive [Marris, 2013; Wright et al., 2014; Pearce, 2016]. Can we design ecological webs where the introduced strains become effectively controlled by ecosystem-level interactions? Once again, lessons from the study and manipulation of ecological communities provide a positive answer. In particular, the work on tipping points and alternative states in ecosystems revealed that a limited set of potential “attractors” are possible and that the right manipulations can shift the system from a degraded state to a healthy one [Scheffer et al., 1993; Scheffer, 2009]. More generally, some universal properties of ecosystem organization guarantee the presence of a well-defined set of robust attractor states [Holling, 1973]. In a nutshell, as a nonlinear dynamical system, every ecosystem has a finite number of possible equilibrium states (or *attractors*) for a given set of parameters. The stability of each possible attractor is essentially determined by the nature of the interactions among the different species and sometimes only one attractor is possible. If that is the case, a very interesting possibility emerges: can an adequate design of synthetic interactions create a robust attractor state from which we cannot escape? If yes, can such engineering approach preserve species diversity? If that were the case, confinement would be the outcome of an *ecological firewall* resulting from the nature of ecological dynamics.

RESULTS

In the following sections, the concept of ecological firewalls is made explicit by considering a set of specific, potentially testable examples and how models predict the expansion or control of each firewall. None of

them involves a synthetic strain capable of triggering positive, uncontrolled feedback loops and can become extinct if functional traits (tied to their desired bioremediation goal) are not fit enough. In that case, it can transform into the original wild type or simply become extinct by competition. As shown in the next examples, this does not require special genetic orthogonal designs. Instead, the firewalls are obtained from a properly designed set of ecological interactions that include resource-consumer dynamics, mutualism, parasitism, niche construction and indirect cooperation. To provide a unified picture of our results in theoretical terms, we consider a population dynamics set of models where the multidimensional problem is collapsed to a single-species, one-dimensional equation.

Ecological firewalls

Five classes of ecological firewalls (EFWS) will be discussed that can be used to build novel synthetic communities under controlled conditions. The main goal here is to show that the synthetic population will be maintained in a self-controlled level associated with a suitable attractor state but can also scale up with the underlying problem (such toxin levels or plastic waste).

A given EFW will be described as a specific subnetwork of species interactions constructed to work within a larger web structure (a natural community) where a given functionality needs to be performed. Because of the specific topology of the interactions associated with each subnetwork, we use the term “motif” to refer to them (as presented in [Solé et al., 2015]). We do not suggest specific genetic constructs (and thus no explicit candidates are proposed) and instead assume their basic functional traits that are introduced in a population-level description. In this context, our goal is to show that the attractor dynamics resulting from the EFW is consistent with the containment goals that we aim to implement.

To provide a unifying picture of a diverse range of case studies, we chose to define population models that capture the dynamics of the synthetic strain as a single variable. That means a dimensional reduction from a multispecies ecosystem model to the minimalist picture that helps understanding the outcome of each EFW in intuitive terms.

Synthetic resource-consumer firewall

The first example of our systematic approach to ecological firewalls deals with the vast amount of anthropogenic xenobiotics (such as oil spills, plastic, or recalcitrant chemicals) and the difficulties associated with their safe removal. It was early suggested that synthetic biology could be the key for advanced avenues beyond classical bioremediation strategies [Zobell, 1946; Atlas, 1978]. Here we use the systems ecology approach to define the conditions of effectiveness and safety that should be at work.

The goal of this EFW is to stabilize a community of synthetic microorganisms able to degrade a given anthropogenic substrate that is being injected inside the system at a given rate (Figure 1A). We want the synthetic to become part of the community at intermediate levels of the xenobiotic while it becomes dominant at high levels. In additionally, when the levels of the substrate decay below some limits, the synthetic strain should disappear. In other words, if removal is efficient enough, once the function has been performed, the introduced strain gets extinct. In this context, our EFW acts as a “function-and-die” (FAD) motif [Solé et al., 2015].

The motif properties are summarized in Figure 1. The model is inspired by classical models of resource-consumer dynamics but with an extra component representing the synthetic strain that will perform the bioremediation task. Here R describes the amount of xenobiotic (such as polyethylene) which is generated at a rate r , decays at a rate δ_r and is removed from the system under the action of the synthetic strain (i.e., The term ηSR). The model starts from a whole community \mathcal{C} of microorganisms defined by means of a set of n populations, i.e., $\mathcal{C} = \{C_\mu\}$ with $\mu = 1, 2, \dots, n$ indicating species index (Figure 1A). Each one grows with a given rate ρ_i while competing with the synthetic strain S for some available (non-xenobiotic) resources and space (Figure 1B), growing at a rate p . The previous rules can be summarized by the following set of differential equations:

$$\begin{aligned}\frac{dR}{dt} &= r - \eta SR - \delta_r R \\ \frac{dS}{dt} &= \rho RS - \delta_s S - S\Phi(R, S, \{C_\mu\}) \\ \frac{dC_i}{dt} &= \rho_i C_i - \delta_i C_i - C_i \Phi(R, S, \{C_\mu\})\end{aligned}$$

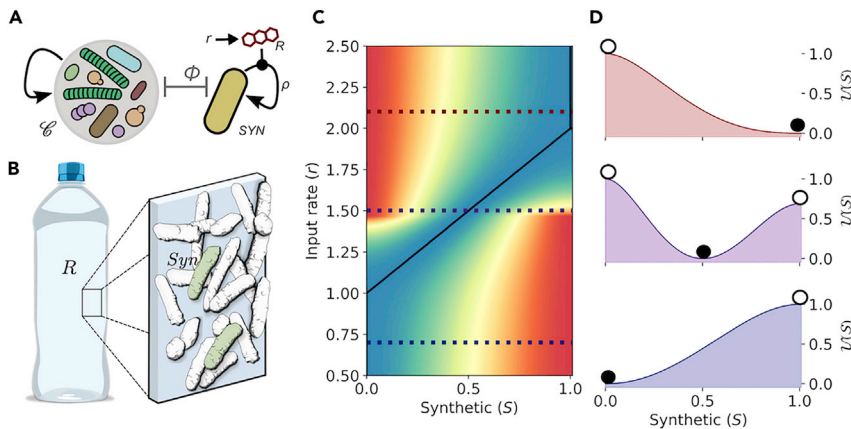


Figure 1. Resource-consumer (“function and die”) firewall

This motif is sketched in (a) and would be associated for example to plastic waste (b) where a synthetic strain S would compete for space on plastic surfaces with the resident community. Depending on the input rate of the resource (r) the synthetic organism will be more or less prevalent (c). The continuous line indicates the three regimes of stable states of the synthetic strain, from failure to establish itself (when r is too small), to overcoming the ecosystem when the input rate is really high, through a region of coexistence between the synthetic organism (S) and the original community (C). Notice that this happens even when the replication rate of the synthetic strain is very small compared to the original community growth (in this particular case $\rho_C = 10\rho$). The set of parameters used here are $\delta = 0.1$, $\rho_c = 0.5$, $\rho = 0.05$, and $\eta = 0.1$, respectively.

In these equations the decay and death rates are indicated by δ_j . The flux term Φ is introduced to include the competition among S and C and is usually interpreted as an average fitness when total population size is conserved [Nowak, 2006]. More precisely, if we assume that $S + \sum_k C_k$ is constant (constant population

constraint, CPC), we have $d\left(S + \sum_k C_k\right)/dt = 0$ or, in other words, $dS/dt + d\left(\sum_j C_j\right)/dt = 0$. This allows (as we will see in our different examples) to explicitly find Φ and solve our models.

This multidimensional system is difficult to analyze in all its complexity (see SM) but we can take a shortcut by considering that the resident community can be safely represented using average rates, i. e. assuming that $\rho_i \equiv \rho_c$ and that $\delta_i \approx \delta$ for all $i = 1, \dots, n$. Under this homogeneity assumption and using $C = \sum_k C_k$, we have:

$$\begin{aligned} \frac{dR}{dt} &= r - \eta SR - \delta R \\ \frac{dS}{dt} &= \rho RS - \delta S - S\Phi(S, C) \\ \frac{dC}{dt} &= \rho_c C - \delta C - C\Phi(S, C) \end{aligned}$$

and using a normalized sum $S + C = 1$, we find:

$$\Phi(S, C) = (\rho R - \rho_c)S + (\rho_c - \delta). \quad (\text{Equation 2})$$

The system is reduced to a two-dimensional set of equations including only the synthetic and the resource components, namely

$$\begin{aligned} \frac{dS}{dt} &= (\rho R - \rho_c)S(1 - S) \\ \frac{dR}{dt} &= r - \eta SR - \delta R \end{aligned}$$

This two-dimensional system can be studied using standard methods of phase-plane analysis (see Box 1). A further simplification of our model allows us to reduce it to a one-dimensional dynamical system. In this case, we assume that resources rapidly equilibrate (compared with cell populations), and thus we can use $dR/dt \approx 0$. This is a rather common approximation in dynamical systems that in this case gives a functional coupling between the resource and the synthetic population: $R(S) \approx r/(\eta S + \delta)$.

Box 1. Mathematical systems biology of ecological firewalls

The reduction from a high-dimensional model to a simplified, even one-dimensional equation requires different types of assumptions that we summarize here using the resource-consumer firewall as a case study. In general, the stability of the fixed points associated with a d -dimensional dynamical system $dX_i/dt = f_i(X_1, \dots, X_d)$ is determined by evaluating the eigenvalues of the ($d \times d$) Jacobian matrix (J), associated with the linearized system $dx_i/dt = \sum_j^d J_{ij}x_j$ namely:

$$J_{ij} = \left(\frac{\partial \dot{X}_i}{\partial X_j} \right)_{X^*} \quad \text{(Equation 1)}$$

and evaluated in each fixed point X_k^* . The set of eigenvalues λ_k is then determined from $\text{Det}J = |(J_{ij} - \lambda \delta_{ij})|$, where δ_{ij} is the Kronecker's delta [Strogatz, 1994]. For the FAD motif, we have $X_i \in \{R, S, C\}$. Once the CPC constraint has been applied to the original set of equations, and before we assume rapid dynamics for the resource R , the resulting $d = 2$ dynamical system involves three fixed points: $X^* = (S^*, R^*) \in \{(0, r/\delta), (1, r/(\delta + \eta)), ((r\delta_c - \delta\rho)/\rho\eta, \rho_c/\rho)\}$. Their stability can be studied using its associated 2×2 Jacobian matrix:

$$J_{S,R} = \begin{bmatrix} \frac{\partial}{\partial S} \left(\frac{dS}{dt} \right) & \frac{\partial}{\partial R} \left(\frac{dS}{dt} \right) \\ \frac{\partial}{\partial S} \left(\frac{dR}{dt} \right) & \frac{\partial}{\partial R} \left(\frac{dR}{dt} \right) \end{bmatrix}$$

For our specific set of equations, this reads

$$J_{S,R} = \begin{bmatrix} (1 - S)S\rho & (R\rho - \rho_c)(1 - 2S) \\ -S\eta - \delta & -R\eta \end{bmatrix}$$

The fixed points will be stable if $\text{Det}(J(X^*)) < 0$ and $\Delta = \text{Tr}(J(X^*)) = (\partial \dot{X}_1 / \partial X_1 + \partial \dot{X}_2 / \partial X_2)(X^*) > 0$. A detailed analysis shows that the first point where no synthetic is established will occur provided that $\rho_c > r\rho/\delta$, whereas the exclusion point where only the synthetic persists is obtained under the condition $\rho_c < r\rho/(\delta + \eta)$. See SM for a detailed derivation.

Using this expression, we obtain a single differential equation for the synthetic population, namely:

$$\frac{dS}{dt} \approx f(S) = S(1 - S) \left(\frac{r\rho}{\eta S + \delta} - \rho_c \right) \quad \text{(Equation 3)}$$

For this we have three fixed points, namely (i) no synthetic, i. e. $S^* = 0$, (ii) only-synthetic community, i. e. $S^* = 1$ and (iii) the coexistence point where the synthetic strain achieves an intermediate level:

$$S^* = \frac{r\rho - \delta\rho_c}{\eta\rho_c} \quad \text{(Equation 4)}$$

The stability of these fixed points is determined by the sign of

$$\lambda(S) = \frac{df(S)}{dS} \quad \text{(Equation 6)}$$

[Strogatz, 1994] evaluated at each S^* . A given fixed point S^* will be stable provided that $\lambda(S^*) < 0$ (and unstable if $\lambda(S^*) > 0$). For our system, we have

$$\lambda(S) = (1 - 2S) \left(\frac{r\rho}{\eta S + \delta} - \rho_c \right) - \frac{r\rho S(1 - S)}{(\eta S + \delta)^2} \quad \text{(Equation 7)}$$

The stability of the fixed point where the synthetic fails to establish itself ($S^* = 0$) is obtained under the inequality

$$\lambda(S^* = 0) = \frac{r\rho}{\delta} - \rho_c < 0 \quad \text{(Equation 8)}$$

In this case, the pollutant R will achieve a steady level $R = r/\delta$. On the other hand, the dominant synthetic state ($S^* = 1$) will be stable when

Box 2. Potential functions

A very common metaphor of a stable ecological state considers a physical analogy, where a marble (representing population size) is located at the bottom of a valley [Holling, 1973; Scheffer, 2009; Solé, 2011]. If perturbed, the marble will move around but eventually will settle back to the bottom state again. In contrast, a marble located on a peak in this landscape might stay there but the slightest perturbation makes it run away: the peak corresponds to an unstable state. Is there a way to define this landscape in some rigorous mathematical way? For a one-dimensional dynamical system defined as $dS/dt = f(S)$, with $f(S) \in C^1(U)$ (i. e. f and its derivative are continuous on the relevant set $U \subset \mathbb{R}$) the system is said to derive from a potential function $\mathcal{V}(S)$ [Arnold, 2013; Strogatz, 1994; Solé, 2011] if we can write the dynamical system as:

$$\frac{dS}{dt} = - \frac{d\mathcal{V}}{dS} \tag{Equation 5}$$

i. e. when the changes in the state of the system obey a *gradient response*: the steeper the derivative in the right hand side, the larger the damping in the opposite direction. If we move away from S^* toward $S^* + s$ (where s is small) the change in the potential with s will be $\Delta\mathcal{V}/s = (\mathcal{V}(S^* + s) - \mathcal{V}(S^*))/s$. If $\Delta\mathcal{V} > 0$ means that the potential grows (we are in a valley) and thus, from (Equation 6) the right hand side of (Equation 5) is negative: growth is inhibited. The opposite occurs if we are at an unstable point. From the previous equation, it is easy to see that

$$\mathcal{V}(S) = - \int \left(\frac{dS}{dt}\right) dS = - \int f(S) dS$$

At a given S^* , using (Equation 7) the first and second derivatives of \mathcal{V} are:

$$\left(\frac{d\mathcal{V}(S)}{dS}\right)_{S^*} = - f(S^*) \quad \left(\frac{d^2\mathcal{V}(S)}{dS^2}\right)_{S^*} = - \lambda(S^*),$$

respectively. The first derivative is zero (as it should be for an extremum of the function) and the second will be positive (negative) if the point is stable (unstable), consistently with a minimum (maximum) of the potential.

$$\lambda(S^* = 1) = \rho_c - \frac{r\rho}{\eta + \delta} < 0 \tag{Equation 9}$$

This can be achieved if the rate of pollutant input is higher than a critical value r_c . Where

$$r_c = \frac{\rho_c}{\rho} (\eta + \delta) \tag{Equation 10}$$

and now, R will reach a constant value $R = r/(\eta + \delta)$. The location of the stable states for our synthetic strain is indicated in Figure 1C by means of a continuous line. We can appreciate that three well-defined phases exist.

An alternative, complementary and often more intuitive way of studying the stability of these dynamical systems is grounded on the use of the so called potential function $\mathcal{V}(S)$. For a general system $dS/dt = f(S)$, it is defined as (see Box 2):

$$\mathcal{V}(S) = - \int f(S) dS \tag{Equation 11}$$

and it can be shown that the minima (maxima) of $\mathcal{V}(S)$ correspond to the stable (unstable) fixed points. In this paper we will transform all the examples into one-dimensional differential equations to provide a unified picture based on the properties of $\mathcal{V}(S)$.

For our FAD firewall, the potential function reads

$$\mathcal{V}(S) = \frac{r\rho}{\eta^2} (\delta + \eta) \left(\frac{\delta}{\eta} \log(\eta S + \delta) - S\right) + \rho_c S^2 \left(\frac{1}{2} + \frac{r\rho}{2\eta} - \frac{S}{3}\right)$$

In [Figures 1C](#) and [1D](#) we use this potential function (and the information on stability gathered from $\lambda(S)$) to capture the different equilibrium states associated with the synthetic population. In order to normalize the potentials (their exact values are irrelevant) we plot the normalized version (to be used in all the plots hereafter)

$$U(S) = \frac{\mathcal{V}(S) - \min_r\{\mathcal{V}(S)\}}{\max_r\{\mathcal{V}(S)\} - \min_r\{\mathcal{V}(S)\}} \quad (\text{Equation 12})$$

In the [Figure 1B](#) diagram, the behavior of the potential is displayed for different rates of pollutant input r (vertical axis). Bright and dark colors indicate higher and lower values of the potential, respectively. Darkest areas are thus associated with stable states of the synthetic population. Three snapshots of the $\mathcal{V}(S)$ plots are also displayed in [Figure 1C](#). As we can see, low levels of input (and thus low concentrations of R) are unable to sustain the synthetic, whereas intermediate values of r create the conditions for a stabilized coexistence attractor where both the synthetic and the resident community coexist. The dominance of the synthetic population can only occur at very high levels of waste concentrations.

The power of this simple model approach is illustrated by its application to a specific (but very relevant) case scenario associated with the dynamics of ocean plastic debris under a growing trend of plastic deployment, presented in [\[Solé et al., 2017\]](#). It was shown that a resource-consumer model similar to the one discussed here (but with a time-dependent input rate, i. e. $r = r(t)$) could explain a counterintuitive pattern displayed by sampled marine plastic concentrations, namely the lack of a growing trend despite the almost exponential rate of debris dumping. Assuming that a microbial compartment exploits the plastic substrate, two predictions were made. The first is that plastic concentrations would be much smaller than the expected from the uncontrolled scenario (i.e., a microbial-free context). Secondly, the model also predicted that the population dynamics of the synthetic strain $S(t)$ would positively correlate with plastic debris input rate. Both predictions have been recently confirmed by two different global metagenomic analyses. They include the detection of widespread presence of plastic-degrading microbes [\[Alam et al., 2020\]](#) and their correlation with pollution trends [\[Zrimec et al., 2021\]](#). The fact that plastic-degrading enzymes have been evolving and allowed for plastic containment (at least at some level) further supports the viability and scalability of the terraformation scenarios discussed here.

Synthetic mutualistic firewall

Cooperative interactions are particularly useful when designing ecological firewalls. On one hand cooperative loops are known to be relevant to maintain reliable behavior and help foster diversity, although they involve desirable dynamical properties and enhance stability. In some systems, synthetic circuits have been proposed in order to help the system to avoid undesired shifts. The design of cooperative loops based on the creation of auxotrophic interactions has shown the reliable response of the designed consortia [\[Johns et al., 2016; Amor et al., 2017; Rodríguez Amor and Dal Bello, 2019\]](#). This is illustrated by drylands [\[Solé, 2015; Maestre et al., 2017; Solé et al., 2018; Conde-Pueyo et al., 2020\]](#). In these arid and semiarid ecosystems, the goal of terraformation would be a synthetic design able to improve the conditions for the community driving it to a healthier situation while preserving community diversity. One way of obtaining such a result would be to use cyanobacteria from the resident community and engineer it to produce a molecule capable of improving soil moisture. This could be achieved by engineering some cyanobacteria already present within the community. By doing so, an improvement of soil quality would help the vegetation cover to be more stable and in return higher vegetation cover could also help improve the quality of the soil where the synthetic microorganism lives. A mutualistic dependency has been engineered.

The firewall motif associated with our mutualistic design is depicted in [Figure 2A](#) (same notation than in the previous EFW). As pointed out above, arid and semiarid lands are one of the possible ecological contexts where this EFW would be helpful ([Figure 2B](#)). Depending on the parameters of synthetic replication (ρ) and the increase of the positive symbiosis with the community (η) measures the strength of mutualistic exchanges. As shown in the following sections, the mutualistic design allows us to maintain or even increase diversity. Hereafter, as in previous sections, we consider the constraint $\sum_k C_k + S = 1$.

The multispecies model, as sketched in [Figure 2A](#), can be described by the following system of $n+1$ differential equations:

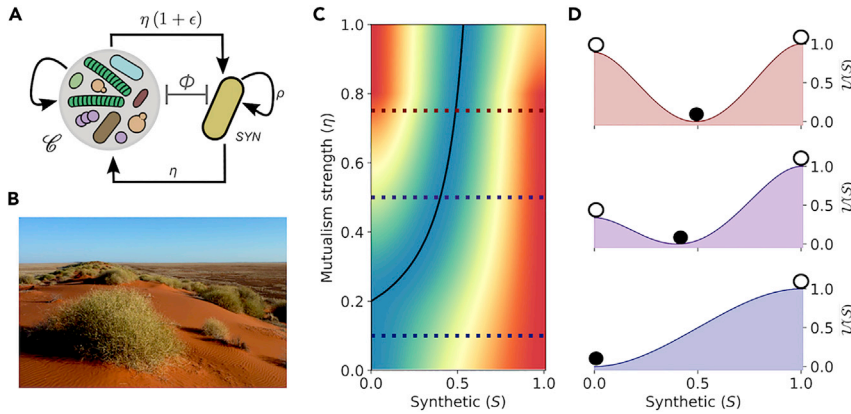


Figure 2. Synthetic mutualist firewall

(A) Schematic representation of the interactions between the original community (C) and the synthetic mutualist organism (S). One of the possible applications of this motif is the Terraformation of semiarid ecosystems (b). Depending on the parameters of synthetic replication (ρ) and the increase of the positive symbiosis with the community ($1 + \epsilon$). Different stable ecosystem configurations can be obtained (see panels c and d). As mutualism increases, the competition between the synthetic strain and the original community diminishes. Reaching a coexistence regime, an ecosystem where both are present. The parameters used for these simulations are $\rho = 0.1$, $\rho = 0.5$, $\epsilon = 2.0$ and $\delta = 0.1$.

$$\frac{dS}{dt} = \left(\rho + \sum_k^n \epsilon_k \eta_k C_k \right) S - \delta S - S\Phi(S, C)$$

$$\frac{dC_i}{dt} = (\rho_c^i + \eta_i S) C_i - \delta_i C_i - C_i \Phi(S, C)$$

Here the parameters ϵ_k stand for the gain associated with S as a result of mutualistic exchanges with each species from C. If we assume that $\eta_k \sim \eta$, $\delta_c^k \sim \delta$ and $\epsilon_k \sim \epsilon$, a new coarse-grained set of two equations for the synthetic-resident pair can be written, namely:

$$\frac{dS}{dt} = \rho C S - \delta S - S\Phi(S, C)$$

$$\frac{dC}{dt} = (\rho_c - \eta S) C - \delta C - C\Phi(S, C)$$

We end up again with a one-dimensional dynamical model:

$$\Phi(S, C) = \eta(1 + \epsilon)(1 - S)S + (\rho - \rho_c)S + \rho_c - \delta$$

where we used $C = 1 - S$. This allows us to obtain a third-order dynamical equation:

$$\frac{dS}{dt} = S(1 - S)(\Lambda - S)$$

Where we define

$$\Lambda = \frac{\rho - \rho_c + \eta\epsilon}{\eta(1 + \epsilon)} \tag{Equation 13}$$

The fixed points are thus: (i) $S = 0$ (no synthetic), (ii) $S = 1$ (synthetic dominates), (iii) $S = \Lambda$ (species coexistence). The stability of each S^* is now determined by the sign of

$$\lambda(S) = (1 - 2S) \left(\frac{\rho - \rho_c + \eta\epsilon}{\eta(1 + \epsilon)} \right) + S(2 - 3S)$$

For the first fixed point, it will be stable (and no synthetic will be present) if

$$\rho + \epsilon\eta < \rho_c$$

The dominant synthetic community will instead occur if $\rho - \eta > \rho_c$, which we can also write as a threshold condition:

$$\eta > \eta_c = \rho_c - \rho \tag{Equation 14}$$

Both conditions are the boundaries of existence, the coexistence fixed point, which is the most common one. The associated potential $\mathcal{V}(S)$:

$$\mathcal{V}(S) = \Delta S^2 \left(\frac{S}{3} - \frac{1}{2} \right) + S^3 \left(\frac{1}{3} - \frac{S}{4} \right) \quad (\text{Equation 15})$$

The normalized potential $\mathcal{U}(S)$ is displayed in [Figures 2C](#) and [2D](#). The relevant parameter is now the efficiency of the cooperative interaction, η . Two major phases are now observed. If the strength of the cooperative link is weak (below a threshold value, i. e. $\eta < \eta_c$), the synthetic train is unable to persist. Instead, once the threshold is overcome, a robust coexistence attractor is found.

The mutualistic firewall illustrates very well our main point concerning EFW behavior. It is defined not in terms of a given construct carried by a modified cell but as a designed network motif. The positive input provided by the synthetic mutualist to the whole community can be exemplified by the drylands case study: improving moisture levels can immediately improve the state of the components of \mathcal{C} . As a consequence, community level properties (such as soil carbon) can also improve to the benefit of the synthetic cooperator [[Solé, 2015](#); [Solé et al., 2015](#)].

The robustness of such design makes the coexistence attractor an inevitable outcome. More importantly, because cooperation requires a balance of two positive, dependent exchanges, none of the components of the system will displace the second.

Synthetic parasitic firewall

Although coexistence is one of our design goals here, we might also want to guarantee that the synthetic population remains low. An EFW that favors this goal would help to preserve the resident community on high population levels. In the previous example, the mutualistic feedback typically pushes the synthetic toward higher population values.

Along with competition (which we have implicitly introduced by means of the constant population constraint) and cooperation, parasitism is another main component of the space of ecological interactions. What if a parasitic-like interaction is considered as part of an EFW? Parasites are a dominant class of life forms that are characterized by taking advantage of a given species while not bringing back any benefit. They can in fact be disruptive to the stability of a given species. However, we need to consider this possibility too and, as shown in the following sections, a parasitic interaction is actually a good design principle for another kind of EFW.

Specifically, consider now the scenario where the synthetic strain performs a function while it takes advantage of the resident community from which it exploits a given set of resources while it acts as an inhibitor (for example, by means of an excreted molecule). The basic network motif for this firewall is depicted in [Figure 3A](#): the synthetic strain exploits some good from \mathcal{C} which it requires to replicate. One case study would involve to engineer an organism to fix CO_2 using concrete as scaffold from civil infrastructures (whose cracks can host dozens of bacterial species). This is one particularly important outcome of the Anthropocene: after water, concrete is the second-most-used substance in the world. Our engineered organism may be able to profit from the metabolic reactions done by the resident community using concrete as a substrate but needs to make sure not to become extinct (otherwise, its engineered function would be lost). This can be ensured by actively repelling the other organisms, by means for example of bacteriostatic antibiotics.

These interactions can be introduced within another kind of EFW, where we again represent our community \mathcal{C} as a set of species C_k that sustain the engineered organism by providing it with a given repertoire of nutrients or key metabolites. However, we do not ask the synthetic strain to provide anything in return. The question here is under what conditions the parasitic strain will be established in a stable manner.

The new set of equations reads:

$$\begin{aligned} \frac{dS}{dt} &= \rho \left(\sum_{\mu=1}^n \rho_{\mu} C_{\mu} \right) S - \delta S - S\Phi(C_k, S) \\ \frac{dC_k}{dt} &= (\rho_c^k - \eta_k S) C_k - C_k \Phi(C_k, S) \end{aligned}$$

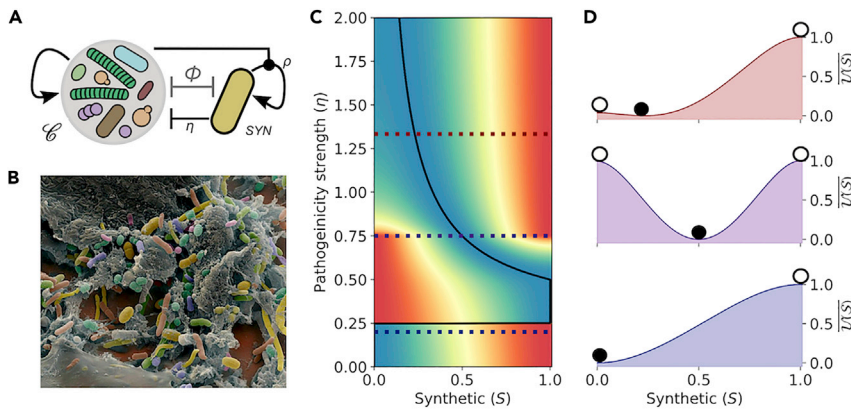


Figure 3. Synthetic parasite firewall

(A) The schematic representation of the interactions between the original community (C) and the synthetic parasite organism (S). The target community here could be a diverse microbial ecosystem such as the gut microbiome (b, photograph by Ana Berges). Two important parameters for the synthetic parasite are how much profit it takes from the ecosystem (ρ) and how much it inhibits the community (η). Depending on these two parameters. Different stable configurations can be obtained (see panel c). If η is small, the original community gets rid of the synthetic. For a small range of parameters, the synthetic can become dominant. However, when $\eta > \rho$ coexistence is the only stable state. The parameters used for this study are: $\rho_c = 0.5$ and $\rho = 0.25$.

The terms $\rho_k C_k S$ and $\eta_k C_k S$ stand for the advantages provided to S from the community and the inhibitory effect of the synthetic population on the resident community, respectively. Assuming that $\sum_k C_k = C$ and homogeneity in the parameters, we have now:

$$\begin{aligned} \frac{dS}{dt} &= \rho C S - \delta S - S\Phi(S, C) \\ \frac{dC}{dt} &= (\rho_c - \eta S) C - \delta C - C\Phi(S, C) \end{aligned}$$

Using $C = 1 - S$, the flow term reads:

$$\Phi(S, C) = (1 - S)(\rho_c - \eta S + \rho S) - \delta \quad \text{(Equation 16)}$$

and our one-dimensional system is:

$$\frac{dS}{dt} = S(1 - S) \left(\frac{\rho - \rho_c}{\rho - \eta} - S \right) \quad \text{(Equation 17)}$$

We have obtained again a simple model that has a factored form, with the fixed points: (i) $S = 0$, (ii) $S = 1$, (iii) $S = (\rho - \rho_c)/(\rho - \eta)$. The stability is determined from

$$\lambda(S) = (1 - S)(\rho - \rho_c) + S(2 - 3S)(\eta - \rho)$$

For the first fixed point, the existence condition is simply given by $\rho < \rho_c$, whereas the condition for complete invasion is $\rho < \eta$. The coexistence fixed point, its stability is achieved if two inequalities are met altogether, namely

$$\rho > \rho_c \quad \rho < \frac{3\eta + 4\rho_c}{7} \quad \text{(Equation 18)}$$

The potential function associated with this EFW now reads:

$$\mathcal{V}(S) = - \left(\frac{S^2}{2} - \frac{S^3}{3} \right) (\rho - \rho_c) - \left(\frac{S^3}{3} - \frac{S^4}{4} \right) (\eta - \rho)$$

In Figure 3C \mathcal{U} is shown as a function of the inhibition strength measured by the η parameter along with three representative snapshots (Figure 3D). By contrast with the mutualistic case, the valleys of the potential landscape behave in a rather different way. For small η , the parasitic strain is unable to survive and the $S^* = 0$ state is the only stable one. When the parasitic link grows, a short range of parameters is consistent with a full dominance of S, but again as soon as a threshold value η_c is achieved, a coexistence point is also

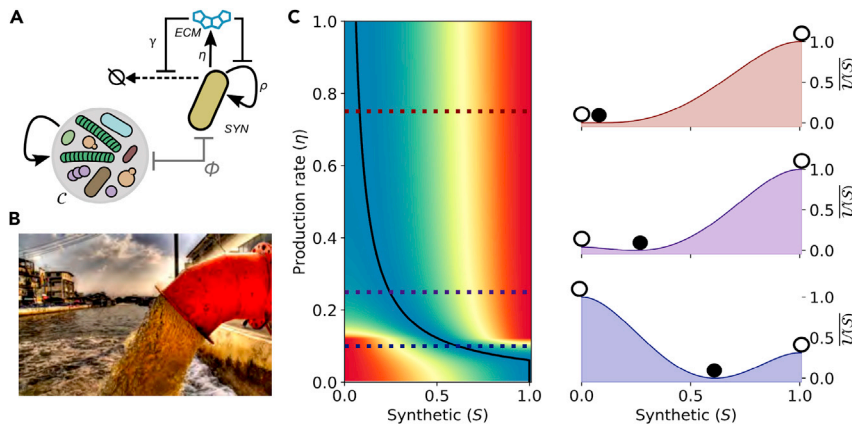


Figure 4. Synthetic stigmergy firewall

(A) The schematic representation of the interactions between the original community (C) and the synthetic mutualist organism (S). In some environments, the synthetic organisms could face physical aversions, i.e. the flow in a river or sewage (b). In this case, the effect is mediated by the synthetic organism replication (ρ), the biofilm production (η) thus reducing individual death while also limiting the growth (γ) and noxious effect of being encapsulated. Depending on these characteristics, different ecosystem's configurations can be obtained (c) as different levels of biofilm production are used (η). Low-level synthetic coexistence largely dominates the parameter space. The other parameters are: $\rho_C = 0.5$, $\rho = 0.25$, $\delta = 0.2$ and $\gamma = 0.5$.

generated. As we can see, the S population decays in size with larger η but nevertheless coexists with the resident community.

Synthetic stigmergy firewall

Although competition and cooperation are two well-known kinds of ecological interactions, less attention has been paid within synthetic and systems biology to niche construction processes as the one explored here. The next EFW deals with the phenomenon of *stigmergy*, i.e., the feedback between a given population that generates a material scaffold (an insect nest, for example) that then acts on the individuals, modifying their behavior. It is because of stigmergy that collective intelligence in social insects' works: coordinated, global activity emerges among individual agents thanks to the formation (and interactions with) an external substrate [Theraulaz and Bonabeau, 1999]. Only recently the potential relevance of this concept has been acknowledged within the context of microbial colonies [Gloag et al., 2013, 2016] where stigmergy would be responsible for collective coordination of microbial assemblies.

This is a more sophisticated motif that takes advantage of the multicellular behavior exhibited by bacterial communities. In particular, we can use their well-known niche construction strategy based on biofilm formation [Flemming et al., 2016]. This is not a minor topic, because it has been estimated that a large majority of bacteria on the planet live in biofilms. Such a strategy can be one of the most optimal ways of cooperation in environments such as rivers or the surface of the ocean (on plastic debris). Because of their prevalence in both natural and anthropogenic environments and their robust structural properties (that favor for example division of labor and enhanced persistence), they are becoming a major target for synthetic biology [Tran and Prindle, 2021; Kassinger and van Hoek, 2020].

In our model, the biofilm is represented by the state variable B produced by S at a rate η . At the same time, S competes for the resources with the resident community C . A maximum biofilm population (the carrying capacity) is assumed, to be associated with finite surface cover. As usual, we consider this maximum size normalized to one. In Figure 4A the network motif that defines our firewall is sketched.

We will show in the following sections that, despite the different design principles introduced in this EFW, its population dynamics are very close to the EFW presented in the previous section. One relevant bioremediation scheme where synthetic populations could be specially relevant concerns the potential for removing undesirable molecules from freshwater environments (including both natural and urban contexts, Figure 4B) or helping degrade plastic waste.

In general, creating a biofilm is a costly process that runs against the engineered strain, except when it provides a shelter against environmental fluctuations (Figure 4A). The biofilm acts as an extra component (ECM, for extracellular matrix) that both limits the growth of the synthetic strain (because of metabolic trade-offs) while it reduces its effective death rate.

The equations used in our model (which, as before, is nonspatial) are defined as follows:

$$\begin{aligned}\frac{dB}{dt} &= \eta S - \delta B \\ \frac{dS}{dt} &= \rho(1 - B)S - \frac{\delta}{1 + \gamma B} S - S\Phi(S, \{C_k\}) \\ \frac{dC_k}{dt} &= \rho_c^k C_k - \delta_k C_k - C_k \Phi(S, \{C_k\})\end{aligned}$$

where the biofilm is represented by the state variable B produced at η rate by the synthetic strain S . The positive effects of B are introduced by the parameter γ . Specifically, the γB in the equation for dS/dt introduces an inability to reduce mortality. At the same time, S have to compete for the resources with the original community C . Following the assumption of constant population constraint used above and assuming homogeneous constant rates for the resident species, it can be easily shown that the resulting two-dimensional model reads (using $C + S = 1$):

$$\begin{aligned}\frac{dB}{dt} &= \eta S - \delta B \\ \frac{dS}{dt} &= S(1 - S)(\rho(1 - B) - \rho_c + \delta(1 - G(B)))\end{aligned}$$

where $G(x)$ stands for the function

$$G(x) = \frac{1}{1 + \gamma x} \quad \text{(Equation 19)}$$

The fixed points of this system, are: (i) $X_0^* = (0, 0)$, (ii) $X_1^* = (1, \eta/\delta)$, and a coexistence attractor with a rather cumbersome expression (see SM). From the previous equations, if we assume a fast dynamics of the biofilm variable ($dB/dt \approx 0$) then, we obtain the following expression for B , namely

$$B \approx \frac{\eta}{\delta} S \quad \text{(Equation 20)}$$

which allows us to obtain our final, one-dimensional form for the synthetic population dynamics:

$$\frac{dS}{dt} = [\rho(1 - y) - \rho_c + \delta(1 - G(y))](1 - S)S \quad \text{(Equation 21)}$$

with $y = \eta S/\delta$. The three fixed points are: (i) $S^* = 0$, (ii) $S^* = 1$ and a pair of lengthy coexistence points (iii) S_{\pm}^* (see SM for their exact form).

The stability analysis indicates that the synthetic will fail to establish if

$$\lambda(0) = \rho - \rho_c < 0 \quad \text{(Equation 22)}$$

i.e., the extinction of the synthetic strain depends on being unable to replicate as fast as the resident community (C). Moreover, the stability of the synthetic exclusion fixed point depends on the sign of

$$\lambda(1) = \rho_c - \delta \left(1 - \frac{1}{1 + \frac{\eta \gamma}{\delta}} \right) \quad \text{(Equation 23)}$$

In this case, dominance of the synthetic is obtained if the rate of biofilm production is greater than a critical value η_c , where

$$\eta_c = \frac{\delta}{\gamma} \left(1 - \frac{1}{1 - \frac{\rho_c}{\delta}} \right) \quad \text{(Equation 24)}$$

For this system, the potential function \mathcal{V} involves also a lengthy expression (see SM) but its behavior can be appreciated in Figures 4B and 4C where we display how it changes with the biofilm production parameter η

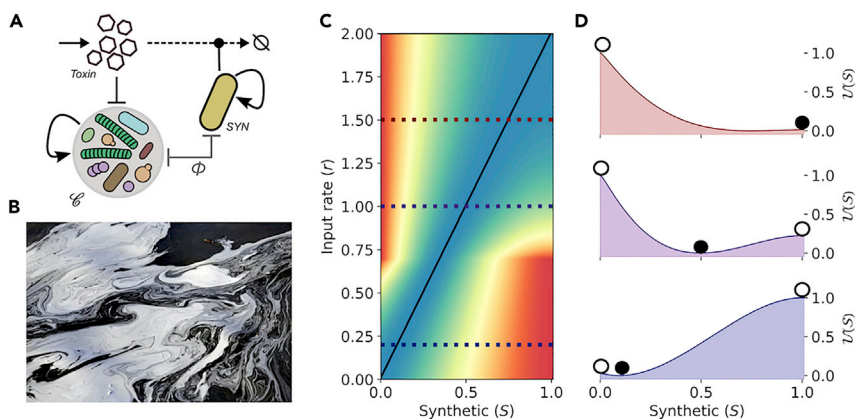


Figure 5. Indirect cooperation firewall

In (a) we summarize the EFW motif. Interactions between the original community (C) and the synthetic organism (S) are coupled by the degradation of an incoming toxin (T) resulting, for example, from a toxic spill (b, photograph by Gerry Broom). Here the toxin input rate is our relevant parameter (r). The amount of toxin is minimal when the complete ecosystem is synthetic and maximum when the whole ecosystem is the original community. The system displays full coexistence behavior all over the isr axis, as shown in the normalized potential (c-d). The parameters used here are $\eta = 1.0$, $\beta = 20.0$, $\rho_C = 1.0$, $\rho = 0.9$, and $\delta = 0.1$.

is increased. As we can see, a low- S coexistence attractor dominates the parameter space, where higher ECM production, along with the nonlinear interactions associated with biofilm formation, provides the desired firewall for the synthetic population. As mentioned earlier, one relevant application of this EFW is environmental bioremediation, where our synthetic strain would help with the removal hazardous components (from drugs in waste waters to heavy metals) that would end up safely stored in the extracellular matrix of the biofilm (see [Tran and Prindle, 2021] and references cited).

Indirect cooperation firewall

Let us consider now an important scenario connected to many instances of environmental degradation associated with toxic spills. In many cases, such spills can extend over long periods of time, damaging life in persistent ways. Each year, thousands of chemical spills occur in many places all around the planet.

Toxic spills are particularly damaging to coastal waters and rivers. Their effects are many and affect diverse scales. They kill wildlife, damage habitat and can wreak havoc on local economies as key resources (such as fish) become contaminated. Very often, the costs of dealing with the cleaning of large spills can be staggering. Moreover, another large class of related problems is eliminating pharmaceuticals and endocrine disruptors from trophic chains [de Lorenzo, 2017]. The ecological firewall described here would be addressed to these class or environmental problems.

The EFW includes three main compartments, sketched in Figure 5A. These are, as before, the resident community C (here the extant set of species representing the contaminated ecosystem), the synthetic strain S and the toxin concentration T . Here we consider that the toxin has no negative effects on the synthetic strain (a parameterized rate only shifts the location of threshold values). This could be the case, for example, of a synthetic bacterium that uses the toxin as a carbon source.

The EFW is in this case described by the following set of equations:

$$\begin{aligned} \frac{dT}{dt} &= r - \beta TS - \delta T \\ \frac{dS}{dt} &= \rho S - \delta S - S\Phi(S, \{C_j\}) \\ \frac{dC_k}{dt} &= (\rho_C^k - \eta_k T) C_k - \delta_k C_k - C_k \Phi(S, \{C_j\}) \end{aligned}$$

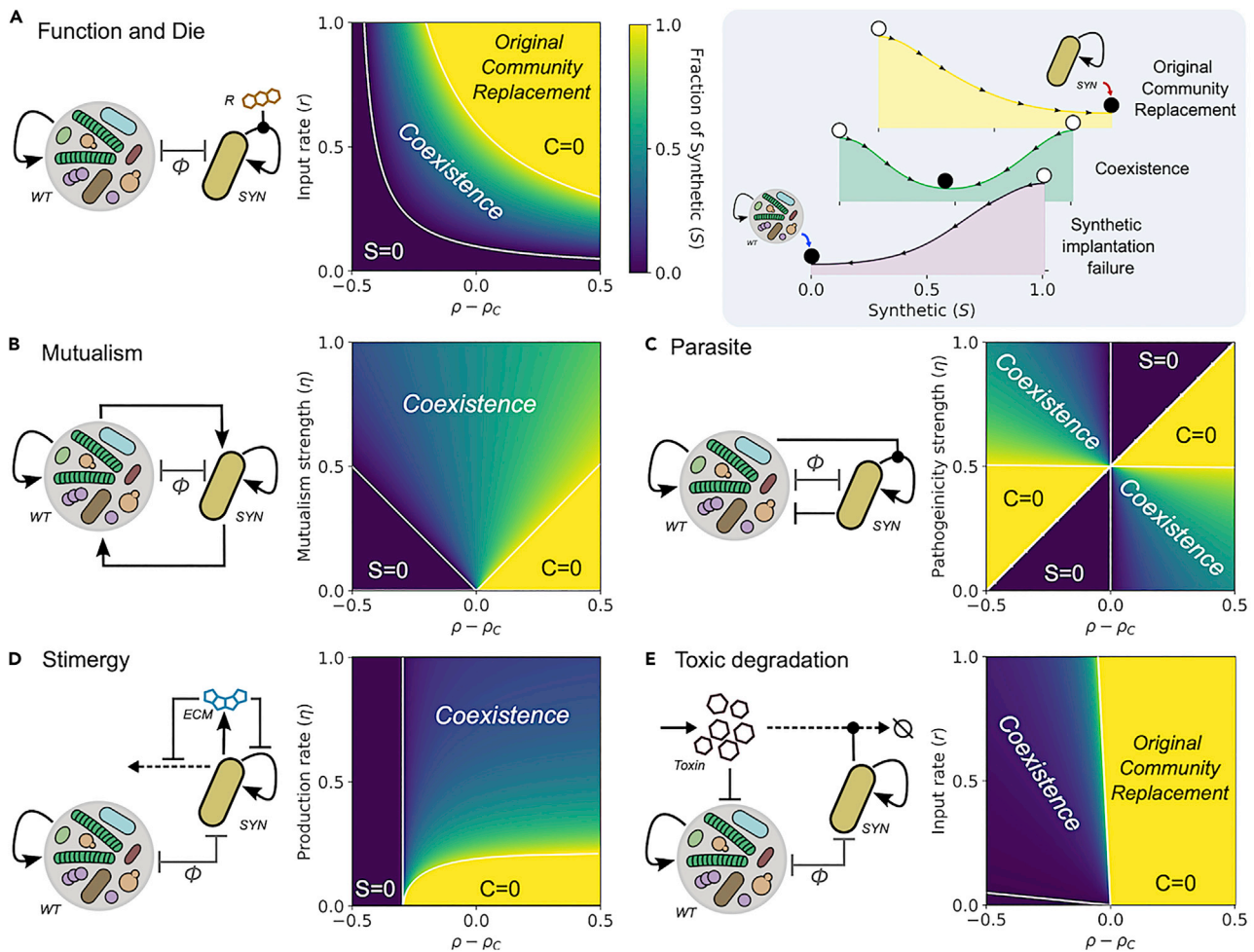


Figure 6. Parameter spaces and phases for the EFW

In these five phase diagrams we display the domains associated with the three phases allowed by the minimal one-dimensional models. The parameters associated with each EFW are the following: a resource-consumer ($\delta = 0.1$, $\eta = 0.5$, and $r = 0.1$), b synthetic mutualistic ($\epsilon = 1.0$), c synthetic parasitism ($\eta = 0.01$), d self-containment ($\delta = 0.25$, and $\gamma = 0.5$), and e synthetic indirect cooperator ($\beta = 20.0$, $\eta = 1.0$, $\delta = 0.1$). The original community growth rate is fixed at $\rho_c = 0.5$. The diagrams on the upper right sketch the qualitative classes of potential functions associated with each phase.

As defined by the model, the toxin enters the ecosystem at a rate r and is actively removed by the synthetic strain at a rate β . The parameter set η_k weights the negative impact of T on each species within the community. We will look at r as our key parameter to test its impact on the kinds of attractors displayed by the model.

What kind of ecological interaction class is involved here? Let us notice an important feature of this EFW: because the synthetic strain has an inhibitory effect on the toxin (driving its removal) T itself inhibits the resident community C , the overall effect of S on C is positive. This is known within ecology as an indirect mutualism [Morin, 1999]. In other words, the net exchange between the engineered strain and our resident species is a cooperative one. Besides, we have seen from previous examples that mutualism provides the basis for stable coexistence. Is that the case here?

Using the constant population constrain ($S + \sum_k C_k = 1$) and the assumption of homogeneous coefficients, $\Phi(S, C)$ is simply

$$\Phi(S, C) = \rho_c + (\rho - \rho_c)S - \eta T(1 - S) - \delta \quad (\text{Equation 25})$$

which allows us to write down the reduced system

$$\begin{aligned}\frac{dT}{dt} &= r - \beta TS - \delta T \\ \frac{dS}{dt} &= S(1 - S)(\rho - \rho_c + \eta T)\end{aligned}$$

As a final step, we consider the scenario where the toxin concentration has a rapid equilibration dynamics. Using $dT/dt \approx 0$, our one-dimensional model reads:

$$\frac{dS}{dt} = S(1 - S)\left(\rho - \rho_c + \frac{r\eta}{\beta S + \delta}\right) \quad (\text{Equation 26})$$

Once again the two fixed points $S^* = 0$ and $S^* = 1$ are present, along with a coexistence point

$$S^* = \frac{1}{\beta} \left(\frac{r\eta}{\rho_c - \rho} - \delta \right) \quad (\text{Equation 27})$$

which will be positive provided that the rate of toxin production is:

$$r > r_c = \frac{(\rho_c - \rho)\delta}{\eta} \quad (\text{Equation 28})$$

which will be always fulfilled if $\rho_c > \rho$. In this case, as illustrated by [Figures 5C and 5D](#), the potential function \mathcal{V} (see SM for its specific form) displays a single valley associated with a stable coexistence point for all the range of toxin input values.

The mathematical nature of this EFW is responsible for the dominant presence of a stable coexistence state. Because of the mutualistic input created through the chain of inhibitions



the synthetic strain helps to sustain the community while performing the toxic degradation task. These are two faces of the same motif, and illustrate the importance of considering the effects of nonlinear dynamics on the performance of designed strains. The improved removal of the toxin with increasing production rates is the obvious, direct consequence of the designed EFW. In addition, the ecological, systems-level features of population interactions are responsible for another, highly desirable outcome: maintaining biodiversity in place. Notice that, if the toxin levels decay, the synthetic strain also decays but remains present at low population values. In this way, we define an EFW that promotes diversity while also sustaining the engineered strain in place when toxin levels are very small.

This EFW (as well as others described in this paper) can be connected with other designs, such as the biofilm formation system. In this context, removal of toxins by biofilms (as we pointed out) would be part of a more complex consortium of designed bacteria. Future work should consider both the spatial extensions of these basic EFW as well as potential combinations.

DISCUSSION

In this paper we suggest an alternative path to containment strategies for synthetic microorganisms. The usual approach is based on a top-down gene circuit design, where biosafety is designed at the cellular level and different mechanisms for constraining spread as well as horizontal gene transfer have been suggested. These include the use of host-construct dependencies, conditional plasmid replication or the requirement for a specific metabolite to be present. Although there is no doubt that refactoring of the existing genetic code offers many advantages, our suggestion provides a different (bottom-up) source of control.

Although there is a general view of engineered microorganisms as similar to invasive species that can cause major harm, invasion ecology shows us that in fact the success of invaders—when they succeed—is very often limited [[Davis, 2012](#); [Lockwood et al., 2013](#); [Hui and Richardson, 2017](#)]. Most invaders that end up as members of the resident community remain reduced to small populations. The community thus is shifted to a new ecological state where diversity is maintained. In the previous examples, we have shown—using some assumptions—that the global state achieved by the engineered community can be made compatible with coexistence between the synthetic and the resident species pool. It is even possible to design the EFW in such a way that coexistence occurs at low levels of the engineered microorganisms. In most cases, the

presence of a toxic component largely influences the attractor state, and once removed, the synthetic can go away with it or remain at very low concentrations.

Each EFW shows three basic “phases” associated with the failure of the synthetic ($S^* = 0$), its dominance ($S^* = 1$) and, more generally, its coexistence ($S \in (0, 1)$). The results described in previous sections are summarized for the five EFW, in [Figure 6](#). The qualitative form of the potential function \mathcal{V} for each phase is also depicted (top right picture). In each case (a-e) the parameter space includes the difference between replication rates of the synthetic versus the resident (i.e., $\rho - \rho_c$ along with the control parameter chosen for each example). The success of each design is captured by the sum of two phases: the coexistence phase and the community replacement phase (yellow). Coexistence is feasible in a large area for all EFW and particularly dominant for the mutualistic and stigmatic firewalls. It is important to remind that, since we consider that each synthetic interacts with \mathcal{C} as a whole (i.e., with all members of the resident community) the full community replacement would be in reality an artifact of this assumption. In a realistic description, where interactions are necessarily limited to a subset of the ecosystem, the impact of the synthetic would also be limited.

Although our ecological, systems-level EFW approach is presented here by contrast with genetic firewalls, they are certainly not exclusive strategies. One can benefit from the other to make interventions as controllable as possible. Moreover, we are assuming that the synthetic is highly robust and able to thrive under the given conditions offered by the resident network and the surrounding environment. This might well not be the case, as illustrated by the failure of exotic strains when they invade microbial communities. Transient phenomena can be at work and our previous EFW might need to consider the possibility of a limited time span before the synthetic strain decays. This can be an advantage to add further control to the system and also reminds us that many invaders in the wild have a dynamic of early expansion followed by extinction or major decay.

We have not pointed toward specific genetic circuits underlying the EFW designs, such connections can be made and tentative classes of circuit designs could be proposed. Such a connection between genetic circuits and population responses has been done for example within the context of synthetic collective intelligence [[Solé et al., 2016](#)]. In this context, we can also envision more sophisticated EFW that use multi-cellular consortia using different, communicating strains. Finally, in relation to the mathematical modeling used here, future work will require the use of stochastic dynamics, a better and finer definition of models including other relevant variables (perhaps considering a more realistic introduction of community structure), the explicit use of spatial degrees of freedom and additional efforts to calibrate relevant parameters. Moreover, the nature of the physical environment and the integration between species and their material worlds deserves special attention. An important case study that illustrates this point is provided by soils and the emergent patterns that arise from the interaction between plants, the soil microbiome and abiotic soil characteristics [[Crawford et al., 2011](#)]. To a large extent, soils can be understood in fact as extended composite phenotypes, i. e. as expressions of the effects of genes through the effects of organisms [[Phillips, 2009](#); [Neal et al., 2020](#)].

Above all, these EFW should be used to test future terraformation strategies under a microcosm/mesocosm framework, where the presence and reliability of the previous attractor states could be studied under realistic conditions.

Limitations of the study

The models developed here are limited to a specific mathematical framework, namely deterministic, continuous sets of differential equations. Because we adopt a low-dimensional description of each ecological firewall, the heterogeneous nature of realistic ecosystems is not included, nor their intrinsic stochasticity. Prospective studies should also consider the use of these ingredients along with a connection between the cell/circuit level of design and its impact on large-scale population dynamics.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- [KEY RESOURCES TABLE](#)
- [RESOURCE AVAILABILITY](#)
 - Lead contact

- Materials availability
- Data and code availability
- **METHOD DETAILS**

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104658>.

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AUTHOR CONTRIBUTIONS

R.S. conceived the project and wrote the manuscript. B.V. performed the numerical simulations and data analysis. Both authors contributed to the development of the models, theory, and conceptualization.

DECLARATIONS OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS**KEY RESOURCES TABLE**

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
Phyton 3.8	NA	https://www.python.org/

RESOURCE AVAILABILITY**Lead contact**

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Ricard Solé (ricard.sole@upf.edu).

Materials availability

No materials were newly generated for this paper.

Data and code availability

No data and codes were newly generated for this paper.

METHOD DETAILS

A more detailed compilation of different alternatives to case studies is provided in the [Data S1](#), related to [STAR Methods](#).