

# Ecological firewalls for synthetic biology

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While rapidly becoming a main thread in the development of new therapies, the rise of synthetic biology is also tied to concerns about the potential impact on ecosystems. That is particularly relevant in the of deployment in natural habitats, including the human microbiome. These concerns have boosted the analysis of diverse strategies of containment, from engineered cell death to xenobiology to the creation of Xeno nucleic acids. However, little attention has been paid to the potential containment implicit in nonlinear ecological interactions and the lessons provided by the population dynamics models used in community ecology. If we consider synthetic strains as some class of "species" embedded within an ecological context, it is possible to show that some network interaction patterns and their associated nonlinear responses can offer a reliable source of containment. Here we present and discuss some simple examples of these *ecological firewalls* that could help provide a self-regulating biocontainment. Our firewall designs can help to ensure that engineered organisms have a limited spread while, when required, preventing their extinction. The basic synthetic designs and their dynamical behaviour 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 [11, 15]. This emerging discipline has been instrumental in providing new tools to interrogate nature [13, 53] and develop new biomedical applications [70, 95, 98] while expanding the reach of possible biology [30]. The success of the field is highlighted by the continuous expansion of its application domain towards higher-level problems, including the design of synthetic ecosystems [43, 58, 87, 88, 94, 96], biocomputation beyond simple circuits [39, 54, 67, 86], non-linear dynamics in cells [52, 92], multicellular designs [17, 28, 85]. 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 [50, 96]. Not surprisingly, community ecology -a traditional systems science- has inspired many relevant ideas within microbiome research, where concepts from ecology are easily translated into the microbiota context [16]. 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 [20, 26, 79]. 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 [18, 19, 24, 38, 51, 64, 71, 79, 90, 99]. However, too often the emphasis is placed in 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 the larger scales associated to the potential impact of synthetic biology on community dynamics? Is this an uncertain domain where little can be predicted or controlled?

A major issue of deployed engineered microorganisms concerns the potential implications for local ecosystems and unknown evolutionary outcomes [74, 76]. As a consequence of an almost complete lack of experimental evidence (due to 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 [12] or the use of urban organic waste [69] 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 has become a big issue within synthetic biology [49] as it was originally in the past century in relation with recombinant DNA technology.

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In this context, most proposed strategies deal with engineering tools that would minimise the interactions between synthetic strains and the potential life forms around it. Practical scenarios include microbes designed to degrade a given toxic substrate, modify the gut [33] or plant microbiomes [48] or cyanobacteria capable of improving soil [91], 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 [93]. 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 later would allow a process of directed codon emancipation leading to an artificial cell equipped with an unnatural genetic code [14].

In this paper we want to address the previous questions in relation with 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 [20, 79, 80, 82]. 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 and achieve 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 stabilisation of endangered communities (such as soils in drylands). This scenario raises immediate questions regarding unintended consequences, often to be compared with invasive species. In general, the assumption is that invaders will be harmful and thus designed "invaders" should also be detrimental to community organisation 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 [55]. 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 feedbacks (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 [57, 63, 97]. Can we design proper ecological interactions 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 [72, 73]. More generally, some universal properties of ecosystem organisation guarantee the presence of a well-defined set of robust attractor states [41]. In a nutshell, as a nonlinear dynamical system, every ecosystem has a finite number of possi-

ble 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.

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. In order 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.

## I. 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 to 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 sub-network 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 and sign of the interactions associated to each sub-network, we use the term "motif" to refer to them (as presented in [80]). 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.

Since we aim 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 a the

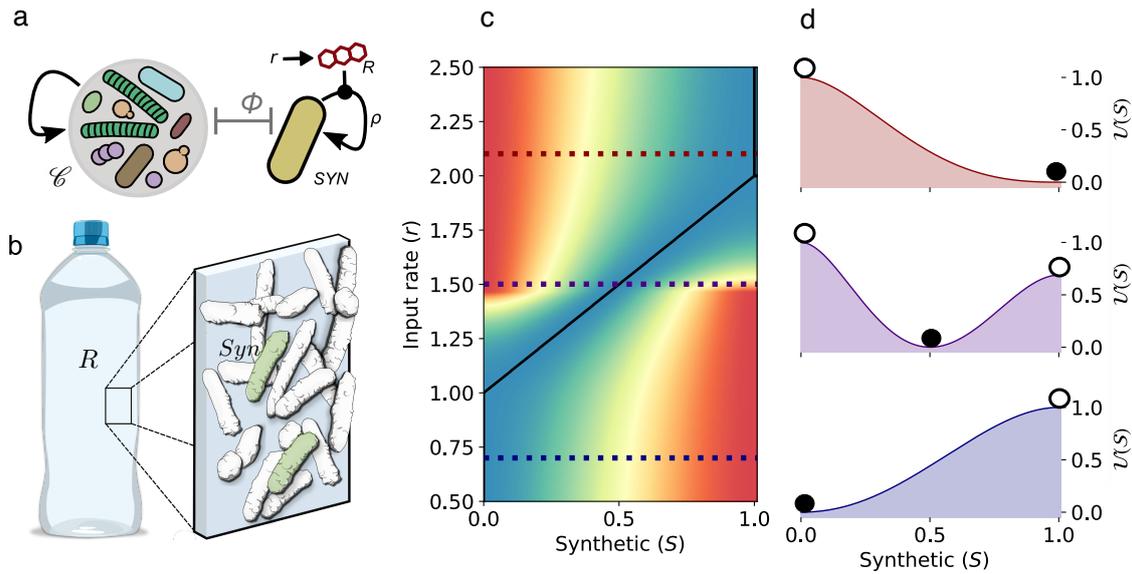


FIG. 1: **Resource-consumer firewall.** This motif is sketched in (a) and would be associated for example to plastic waste (b) where a synthetic strain  $S$  would compete for space on plastic surfaces with the resident community. Depending on the input rate of the resource ( $r$ ) the synthetic organism 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 overcome 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.

minimalist picture that helps understanding the outcome of each EFW in intuitive terms.

### A. Synthetic resource-consumer firewall

The first example of our systematic approach to ecological firewalls that affects many of the priorities of current bioremediation needs. It deals with the vast amount of Anthropogenic xenobiotics (such as oil spills, plastic or recalcitrant chemicals) and the difficulties associated to their safe removal. It was early suggested that synthetic biology could be the key for advanced avenues beyond classical bioremediation strategies [8, 100]. 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 stabilise a community of synthetic microorganisms able to degrade a given Anthropogenic substrate that is being injected inside the system at a given rate. We want the synthetic to become part of the community at intermediate levels of the xenobiotic while it becomes dominant at high levels. 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 [80].

The motif properties are summarised in figure 1. The model is inspired in classical models of resource-consumer dynamics but with an extra component rep-

resenting 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  $\delta$  and is removed from the system under the action of the synthetic strain (i. e. The term  $\eta SR$ ). The model starts from a whole community of microorganisms defined by means of a set of populations indicated as  $C = \{C_\mu\}$  with  $\mu = 1, 2, \dots, n$  indicating species index (figure 1a). Each one grows with a given rate  $\rho_i$  while competing with the synthetic strain  $S$  for some available (non-xenobiotic) resources and space (figure 1b), growing at a rate  $\rho$ . The previous rules can be summarised by the following set of differential equations:

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

In these equations the death rates of each species are indicated by  $\delta_j$ . The flux term  $\Phi$  is introduced to include the competition among  $S$  and  $C_i$  populations and is usually interpreted as an average fitness when total population size is conserved [61]. More precisely, if we assume that  $S + \sum_k C_k$  is constant (constant population constraint), we have  $d(S + \sum_k C_k)/dt = 0$  or, in other words,  $dS/dt + d(\sum_j C_j)/dt = 0$ . This allows (as we will see in our different examples) to explicitly find  $\Phi$  and solve our models.

### 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 summarise here using the resource-consumer firewall as a case study. In general, the stability of the fixed points associated to 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 to the linearised system  $dx_i/dt = \sum_j^d J_{ij}x_j$  namely:

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

and evaluated in each fixed point  $X_k^*$ . The set of eigenvalues  $\lambda_k$  is then determined from  $Det J = |(J_{ij}) - \lambda \delta_{ij}|$ , where  $\delta_{ij}$  is the Kronecker's delta [83]. 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  $2 \times 2$  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  $Det(J(X^*)) < 0$  and  $\Delta = Tr(J(X^*)) = (\partial \dot{X}_1/\partial X_1 + \partial \dot{X}_2/\partial X_2)(X^*) > 0$ . A detailed analysis shows that the first point where no synthetic is established will occur provided that this fixed point is always an attractor ( $\tau < 0$ ) and it will be a node if  $\rho_c < r\rho/\delta$ . 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.

This multidimensional system is difficult to analyse in all its complexity but we can take a shortcut 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:

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

By using a normalised sum  $S + C = 1$ , we find:

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

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 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:  $R(S) = r/(\eta S + \delta)$ .

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

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

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

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

## 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 [41, 73, 78]. 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 to 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  $f(S) \in \mathcal{C}^1(U)$  (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  $\mathcal{V}(S)$  [7, 78, 83] if we can write the dynamical system as:

$$\frac{dS}{dt} = -\frac{d\mathcal{V}}{dS} \quad (5)$$

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  $S^*$  towards  $S^* + s$  (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 (6) the right hand side of (5) is negative: growth is inhibited. The opposite occurs if we are in 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 (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.

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

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

[83] 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

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

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

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

In other words, the extinction of the synthetic strain depends on being unable to replicate if  $\delta/r < 1$ . 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

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

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

$$r_c = \frac{\rho_c}{\rho} (\eta + \delta) < 0. \quad (10)$$

In this case  $R$  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):

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

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 in order 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 figure 1c-d we use this potential function (and the information on stability gathered from  $\lambda(S)$ ) to capture the different equilibrium states associated to the synthetic population. In order to normalize the potentials (their exact values are irrelevant) we plot the normalised version (to be used in all the plots hereafter)

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

In the fig.1b diagram, the behaviour of the potential is displayed for different rates of pollutant input  $r$  (vertical axis). Bright and dark colours indicate higher and lower values of the potential, respectively. Darkest areas are thus associated to 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, while intermediate values of  $r$  create the conditions for a stabilised 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.

## B. 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 behaviour and help foster diversity, while they involve desirable dynamical properties and help construct systems with sharp responses. In some systems, the synthetic circuits are made in order to help the system to avoid extinction when the degradation of the community is increasing. On the other hand, the design of cooperative loops based on the creation of auxotrophic interactions has shown the reliable response of the designed consortia [2, 3, 45]. This is illustrated by drylands[20, 56, 79, 82].

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 the community diversity. One way of obtaining such result would be to use a 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 to our mutualistic design is depicted in figure 2a (same notation than in the previous EFW). As pointed out above, arid and semi-arid lands are one of the possible ecological contexts here this EFW would be helpful (fig. 2b). Depending on the parameters of synthetic replication ( $\rho$ ) and the increase of the positive symbiosis with the community ( $\eta$ ). As shown below, the mutualistic design allows 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:

$$\begin{aligned} \frac{dS}{dt} &= \left( \rho + \sum_k^n \epsilon_k \eta_k C_k \right) S - \delta S - S\Phi(S, \mathbf{C}) \\ \frac{dC_i}{dt} &= (\rho_c^i + \eta_i S) C_i - \delta_i C_i - C_i \Phi(S, \mathbf{C}) \end{aligned}$$

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, namely:

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

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 to obtain a third-order dynamical equation:

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

Where we define

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

The fixed points are: (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) for

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

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

$$\eta > \eta_c = \rho_c - \rho \quad (14)$$

Both conditions are the boundaries of existence the coexistence fixed point, which is the most common one.

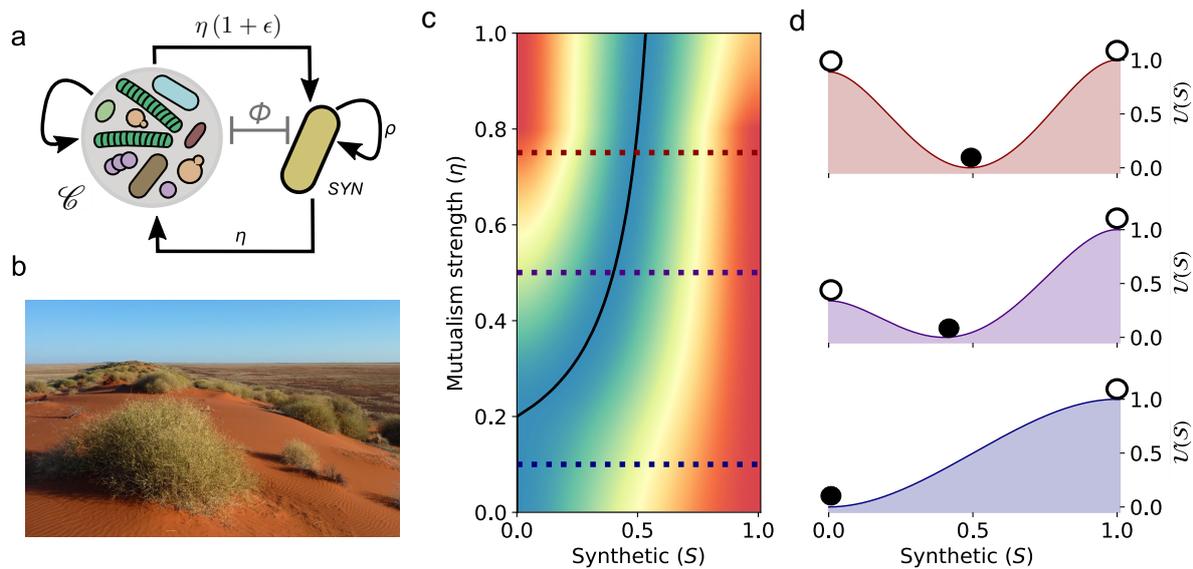


FIG. 2: **Synthetic mutualist firewall.** (a) Schematic representation of the interactions between the original community ( $\mathcal{C}$ ) and the synthetic mutualist organism ( $S$ ). One of the possible applications of this motif is the Terraformation of semi-arid ecosystems (b). Depending on the parameters of synthetic replication ( $\rho$ ) 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  $\delta=0.1$ .

The stability of these fixed points, and the dominance of coexistence for a broad range of conditions, is easily visualised by using the associated potential  $\mathcal{V}(S)$ :

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

The normalised potential  $\mathcal{V}(S)$  is displayed in figure 2c-d. The relevant parameter is now the efficiency of the cooperative interaction,  $\eta$ . Two major phases are now observed. If the strength of the cooperative link is weak (below a threshold value), the synthetic strain 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 behaviour. 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 [79, 80].

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 win over the second.

### C. Synthetic parasitic firewall

While coexistence is one of our design goals here, we might also want to guarantee that the synthetic population remains low. An EFW that favours this goal would help to preserve the resident community on high population levels. In the previous example, the mutualistic feedback typically pushes the synthetic towards 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 characterised by taking advantage of their hosts while not bringing back any benefit to the later. They can in fact be disruptive to the stability of a given species. However, we need to consider this possibility too and, as shown below, 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 2a: 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  $\text{CO}_2$  using as scaffold concrete from buildings (whose cracks can hosts dozens of bacterial species). This is one particularly important outcome of the An-

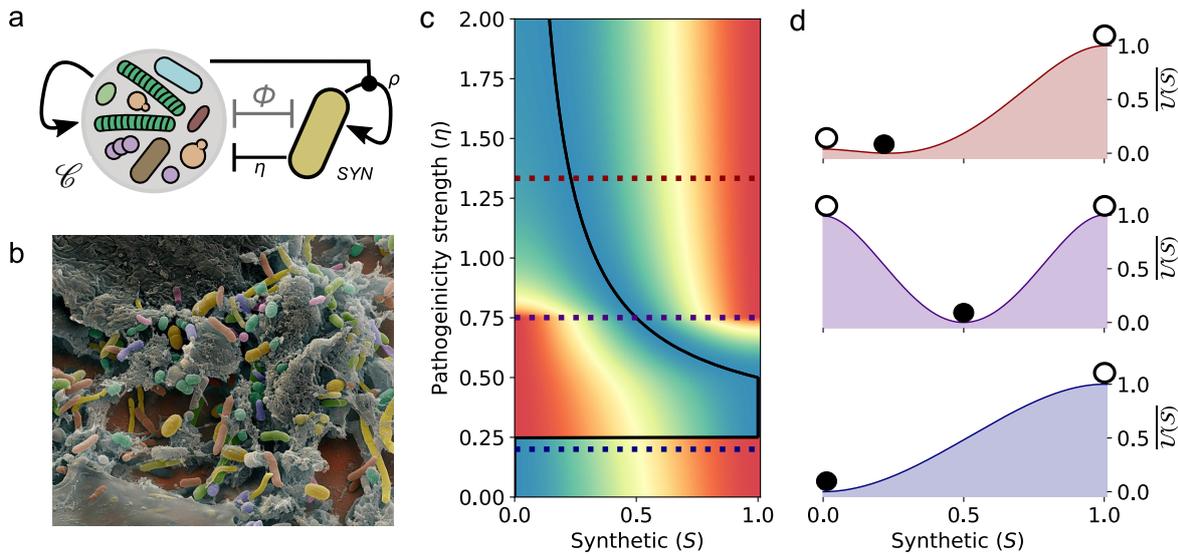


FIG. 3: **Synthetic parasite firewall.** (a) The schematic representation of the interactions between the original community ( $C$ ) and the synthetic parasite organism ( $S$ ). The target community here could be a diverse microbial ecosystem such as the gut microbiome (b, photograph by Ana Berges). Two important parameters for the synthetic parasite are how much profit takes from the ecosystem ( $\rho$ ) and how much inhibits the community ( $\eta$ ). Depending on this two parameters. different stable configurations can be obtained (see panel c). If  $\eta$  is small, the original community gets rid of the synthetic. For a small range of parameters, the synthetic can become dominant. However, when the inhibition  $\eta$  is greater enough coexistence is the only stable state. The parameters used for this study are:  $\rho_C=0.5$  and  $\rho=0.25$ .

thropocene: after water, it is the the second-most-used substance in the world. Our engineered organism may be able to take profit of 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 production.

These interactions can be introduced within another kind of EFW, where we again represent our community  $C$  as a set of species  $C_k$  that sustain the engineered organism by providing it with a given repertoire of nutrients or key chemical elements. 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, using the full community:

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

where in this case, following the same process as above, i. e. 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 now reads:

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

And as a consequence we obtain our one-dimensional system given by

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

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

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

The potential function associated to our parasitic 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 the corresponding normalised potential is shown as a function of the inhibition strength measured by the  $\eta$  parameter along with three representative snapshots (fig. 3d). By contrast with the mutualistic case, the valleys of the potential landscape

behave in a rather different way. For small  $\eta$ , 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  $\eta_c$  is achieved, a coexistence point is also generated and is robust over all the parameter space. As we can see, the  $S$  population decays in size with larger  $\eta$  but nevertheless coexists with the resident community.

#### D. Synthetic stigmergy firewall

While 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. It 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 behaviour. That includes the ways they keep building the scaffold. 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 [84]. Only recently the potential relevance of this concept has been acknowledged within the context of microbial colonies [35, 36] where stigmergy would be responsible for collective coordination of microbial assemblies.

This is a more sophisticated motif that takes advantage from the somewhat multicellular behaviour exhibited by bacterial communities. In particular, we can use their well-known niche construction strategy based on biofilm formation [32]. This is not a minor topic, since it has been estimated that a large majority of bacteria on the planet live in biofilms. Such 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), where we can take profit of the stones from the river bed or coating objects, respectively. The biofilm can also reduce the probability ecosystem decay. By contrast, the bacteria surrounded by the biofilm should be more stressed due to nutrient deprivation. Because of their prevalence in natural environments and their robust structural properties (that favour for example division of labour and enhanced persistence) they are becoming a major target for synthetic biology [46, 89].

In our model, the biofilm is represented by the state variable  $B$  produced at a rate  $\eta$  by the synthetic organism  $S$ . 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 normalised to one. In figure 4a the network motif that defines our firewall is sketched.

We will show below that, despite the difference design principles introduced in this EFW, it behaves very similarly as the parasitic motif 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. As we can see from figure 4a, the biofilm acts as an extra component (ECM, for extra-cellular matrix) that both limits the growth of the synthetic strain (due to metabolic trade-offs) while it reduces its effective death rate.

The equations used in our model (which, as before, is non-spatial) 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  $\eta$  rate by the synthetic strain  $S$ . At the same time,  $S$  have to compete for the resources with the original community  $\mathcal{C}$ . 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

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

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 long expression (see SM). From the previous equations, if we assume the rapid dynamics of the biofilm variable ( $dB/dt = 0$ ) then, we obtain the following expression for  $B$ , namely

$$B = \frac{\eta}{\delta} S. \quad (20)$$

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

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

with  $y = \eta S/\delta$ . For this single equation model we obtain four fixed points (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 as defined from the sign of  $\lambda(S^*)$  tell us that the synthetic will fail to establish if

$$\lambda(0) = \rho - \rho_C < 0 \quad (22)$$

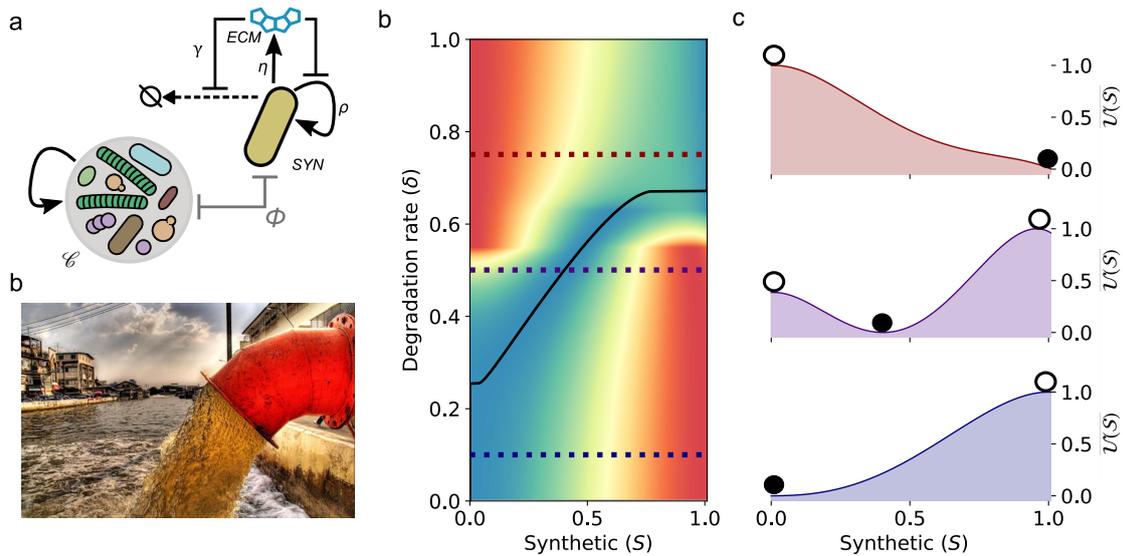


FIG. 4: **Synthetic stigmergy firewall.** (a) The schematic representation of the interactions between the original community ( $\mathcal{C}$ ) and the synthetic mutualist organism ( $S$ ). In some environments, the synthetic organisms could face physical aversions, i.e. the flow in a river or sewage (b). In this case, the effect is mediated by the synthetic organism replication ( $\rho$ ), the biofilm production ( $\eta$ ) thus reducing individual death while also limiting the growth ( $\gamma$ ) and noxious effect of being encapsulated. Depending on this characteristics, different ecosystem's configurations can be obtained (c) as different levels of biofilm production are used ( $\eta$ ). Low-level synthetic coexistence largely dominates the parameter space. The other parameters are:  $\rho_C=0.5$ ,  $\rho=0.25$ ,  $\delta=0.2$  and  $\gamma=0.5$ .

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

$$\lambda(1) = \rho_C - \delta \left( 1 - \frac{1}{1 + \frac{\eta\gamma}{\delta}} \right). \quad (23)$$

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

$$\eta_c = \frac{\delta}{\gamma} \left( 1 - \frac{1}{1 - \frac{\rho_C}{\delta}} \right). \quad (24)$$

For this system, the potential function  $\mathcal{V}$  involves also a lengthy expression (see SM) but its behaviour can be appreciated in figures 4b-c where we display how it changes with the biofilm production parameter  $\eta$  is increased. As we can see, a low- $S$  coexistence attractor dominates the parameter space, where higher synthesis of extra cellular matrix ECM), along with the nonlinear interactions associated with biofilm formation, provides the desired firewall for the synthetic population. As mentioned above, 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 [89] and references cited).

### E. Indirect cooperation firewall

Let us consider now an important scenario connected to many instances of environmental degrada-

tion associated to 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 local economies as key resources (such as fish) become contaminated. The costs of dealing with the cleaning of large spills can be staggering. Moreover, another large class of related problems, namely eliminating pharmaceuticals and endocrine disruptors from trophic chains [25]. 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  $\mathcal{C}$  (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 effects on the synthetic strain. This could be the case, for example, of a synthetic bacterium that uses the toxin as a source of carbon and has been evolved to do so. However, if we also introduce a toxic impact on  $S$ , the results derived below are recovered (with a shift in the parameter space for each threshold).

The previous system can be simplified if we assume that there is constant population constrain. Meaning that all the amount of species that decrease flow to

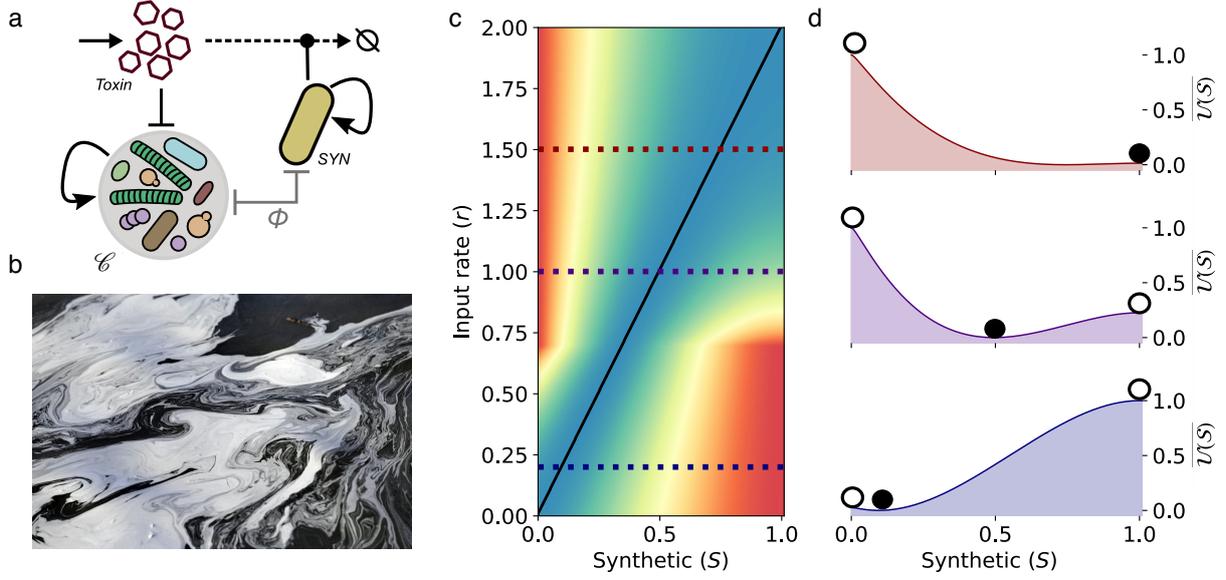


FIG. 5: **Indirect cooperation firewall.** In (a) we summarize the EFW motif. Interactions between the original community ( $C$ ) and the synthetic organism ( $S$ ) are coupled by the degradation of an incoming toxin ( $T$ ) resulting, for example, from a toxic spill (b, photograph by Gerry Broom). Here 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 behaviour all over the  $isr$  axis, as shown in the normalised potential (c-d). The parameters used here are  $\eta=1.0$ ,  $\beta=20.0$ ,  $\rho_c=1.0$ ,  $\rho=0.9$ , and  $\delta=0.1$ .

the other species (from  $S$  to  $C$  or from  $C$  to  $S$ ).

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

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  $\beta$ . 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  $\mathcal{C}$ , the overall effect of  $S$  on  $\mathcal{C}$  is positive. This is known within ecology as an indirect mutualism [59]. In other words, the net exchange between the engineered strain and our resident species is a cooperative one. And 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 for the resident community,  $\Phi(S, C)$  is simply

$$\Phi(S, C) = \rho_c + (\rho - \rho_c)S - \eta T(1 - S) - \delta. \quad (25)$$

Which allows 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:

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

Fixed points  $S^* = 0$  and  $S^* = 1$  and a coexistence point given by:

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

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

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

which will be always fulfilled if  $\rho_c > \rho$ . In this case, as illustrated by figures 5c-d, the potential function  $\mathcal{V}$  (see SM for its specific form) displays a single valley associated to 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

$$S \longrightarrow T \longrightarrow C \quad (29)$$

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

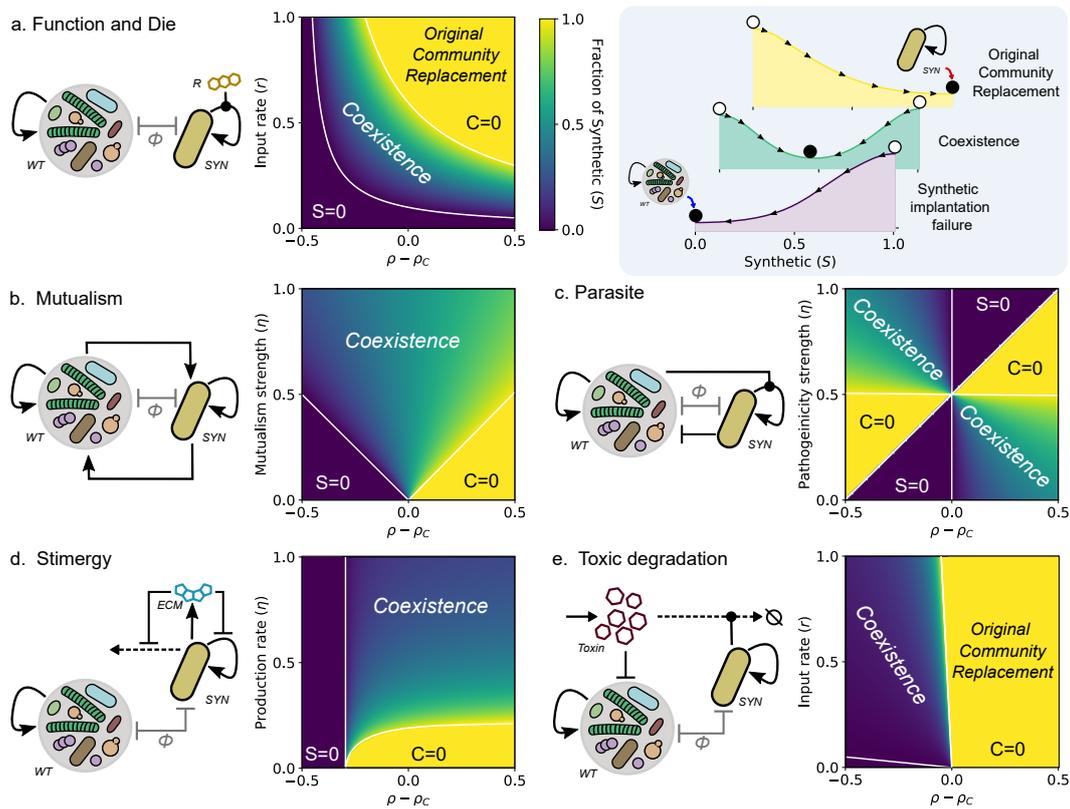


FIG. 6: **Parameter spaces and phases for the EFW.** In this five phase diagrams we display the domains associated to the three phases allowed by the minimal one-dimensional models. The parameters associated to each EFW are the following: **a** resource-consumer ( $\delta=0.1$ ,  $\eta=0.5$ , and  $r=0.1$ ), **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 to each phase.

improved removal of the toxin with increasing levels of toxin production is the obvious, direct consequence of the designed EFW. Additionally, the ecological, systems-level features of population interactions is 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 can also sustain the engineered strain in place when toxin levels might be very small.

This EFW (as well as other 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.

## II. DISCUSSION

In this paper we suggest an alternative path to address containment strategies of synthetic engineered. Instead of the usual approach based on gene circuit design. In the later, to address this biosafety issue, multiple mechanisms for constraining spread as well as horizontal gene transfer have been suggested. These include the use of host-construct dependencies, con-

ditional plasmid replication or the requirement for a specific metabolite to be present. While there is no doubt that refactoring of the existing genetic code offers many advantages, our suggestion (which can be complementary one) provides a different source of control based in this case in the top-level population dynamics.

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 [22, 42, 55]. Most invaders that end up as members of the resident community remain included in 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 such 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 to 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 sec-

tions are summarised for the five EFW, in figure 7. 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 parameter used in 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 stigmergic firewalls. The qualitative form of the potential function  $\mathcal{V}$  for each phase is also depicted. 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 artefact 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 dynamics of early expansion followed by extinction or major decay.

Although we have not pointed towards specific genetic circuits underlying the EFW designs, such connection can be made and tentative classes of circuit designs could be proposed. Such a connection between genetic circuits and population responses has done for example within the context of synthetic collective intelligence [81]. In this context, we can also envision more sophisticated EFW that use a multicellular definition of the synthetic population as a set of different, communicating strains. Finally, in relation to the mathematical modelling 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. 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.

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