Synthetic Circuit Designs for Earth Terraformation

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Background Mounting evidence indicates that our planet might experience runaway effects associated to rising temperatures and ecosystem overexploitation, leading to catastrophic shifts on short time scales. Remediation scenarios capable of counterbalancing these effects involve geoengineering, sustainable practices and carbon sequestration, among others. None of these scenarios seems powerful enough to achieve the desired restoration of safe boundaries.

Hypothesis We hypothesise that synthetic organisms with the appropriate engineering design could be used to safely prevent declines in some stressed ecosystems and help improving carbon sequestration. Such schemes would include engineering mutualistic dependencies preventing undesired evolutionary processes. We hypothesise that some particular design principles introduce unescapable constraints to the engineered organisms that act as effective firewalls.

Implications Testing this designed organisms can be achieved by using controlled bioreactor models and accurate computational models including different scales (from genetic constructs and metabolic pathways to population dynamics). Our hypothesis heads towards a future anthropogenic action that should effectively act as Terraforming agents. It also implies a major challenge in the existing biosafety policies, since we suggest release of modified organisms as potentially necessary strategy for success.

Keywords: Synthetic Biology, Ecological Engineering, climate change, catastrophic shifts, mutualism

The future cannot be predicted,
but futures can be invented
Dennis Gabor

I. BACKGROUND

Climate change, along with a rapid depletion of natural resources and biodiversity declines is driving the biosphere towards unstable states. Widespread evidence indicates that increasing rise of average temperatures is leading to local, regional and global modifications of extant habitats, seriously endangering the future of our planet [1,2]. Given the large scale of the problem, suggested scenarios based on human intervention might fail to properly address the ongoing changes. Additionally, the time evolution of these changes can rapidly accelerate due to runaway effects associated to the nonlinear nature of these phenomena. In other words, current continuous changes might end up in so called *catastrophic shifts* [3-5]. Are we going to be capable to avoid them?

Along with a better understanding of these changes, scientists and engineers have also come up with potential remediation scenarios to ameliorate and even stop the current trends. Different strategies involving mitigation [6] geoengineering [7-9] or adaptation [10] have been proposed. Mitigation implies measures that slowdown ongoing emission rates or provide ways for limiting emissions while geoengineering explicitly requires directed change. Geoengineering has been questioned due to staggering costs, unknown outcomes and limited impact (particularly in relation with CO_2) which make unclear their potential for counterbalancing current trends [7,11,12]. Adaptation scenarios place us in a future world where we will need to cope with new environmental and economic constraints. None of these suggested solutions might be a definite solution, but clearly the price for inaction will be much larger than any of the previous possibilities.

It has been recently suggested that an alternative possibility would involve actively acting on the biosphere through the use of synthetic biology [13]. This approach could be used, among other things, as a way to curtain the accumulation of greenhouse gases, enhance nitrogen fixation or slow down degradation in arid and semiarid ecosystems. The key point of this proposal is that engineering living systems allows to reach large scales thanks to the intrinsic growth of the synthetic organisms. This makes a big difference in relation to standard engineering schemes, where artefacts need to be fully constructed from scratch. Instead, once a designed population is released, appropriate conditions will allow the living machines to make copies of themselves and expand to the desired spatial and temporal scales.

This approach, which is an effective way of "Terraforming" the biosphere, needs to consider potential scenarios that guarantee an efficient result as well as a limited evolutionary potential. Designed microbes capable of functioning only under specific conditions have been constructed and strategies to incorporate genetic safeguards explored [16]. One avenue, to be used in biomedical ap-

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plications, is to force the need for xenobiotic (unnatural) molecules that need to be supplied along with the genetically modified bacteria [17]. In this context, target habitats for designed organisms should be chosen as an additional, ecological-level containment strategy. Moreover, limits to the impact of synthetic organisms can be obtained using ecological interactions that are based on either cooperative loops or habitat constraints that are specially well met by different classes of anthropogenic-modified scenarios. In this paper we consider four possible engineering motifs that can cope with these two constraints. We do not consider explicit case studies (i. e. detailed genetic constructs or designed organisms) but instead the logic design schemes.

II. PRESENTATION OF THE HYPOTHESIS

The obvious criticism to the scenario presented in [15] has to do with the unknown consequences of ecological and evolutionary dynamics on the engineered ecosystems. Actually, it can be argued that well known cases of exotic species introduced in some ecosystems caused large-scale disasters [18,19]. The list includes the introduction of different kinds of species into a novel habitat where they have benefited from a higher efficiency to exploit available resources. This situation corresponds (at least transiently) to a population positive feedback loop that involves an accelerated expansion (typically exponential in its first phase). Is there a rational strategy that can minimise the impact of an engineered species?

One way of preventing undesired explosive growth is to use a modified version of an extant organism that exhibits a strict relationship with another species associated to the target habitat. This means engineering a strong ecological link that makes spread limited. That would result in population dynamical processes preventing undesired growth of the modified organism. Moreover, using the appropriate context, strong habitat constraints can act in synergy as ecological firewalls.

Here we suggest that two main avenues can be followed. One is engineering mutualistic relationships with resident organisms through the modification of already extant microorganisms or fungi. Recent experimental studies indicate that such designed mutualistic link can be created by artificially forcing a strong metabolic dependence and also with the help of genetic engineering. These studies have shown that the end product can be a physically interacting, stable pairwise relationship.

Another possibility is to use a modified organism that grows on a given waste-related substrate that can be preferentially (or exclusively) used, and may be degraded, by the synthetic organism. Such substrate can be plastic garbage, sewage and other sources of human-created waste. Additionally, some special habitats might be ideal to grow strains of engineered microbes capable of performing a given functional task and unable to survive outside their restricted environment.

In the next section, we consider a list of candidate engineering designs (and their variants) that could fit the previous description. We will define their basic logic and outline potential scenarios for their implementation, as well as potential drawbacks.

III. SYNTHETIC TERRAFORMATION MOTIFS

In this paper we introduce four potential bioengineering schemes. Hereafter, H and SYN indicate the target host and a synthetic microbe, respectively. Here SYN might have been obtained from some existing wild type strain (WT). Similarly, R is used to indicate some sort of resource, while W stands for water. The basic designs are intended to represent the logical organisation of our proposed constructs, and not the specific genetic designs. For this reason, since they are introduced as logic graphs, we choose to call them *Terraformation motifs* (TMS) to indicate this logic nature.

The first two motifs deal with the engineering of cooperative interactions, either directly or indirectly. The third incorporates a design principle grounded in a tight dependence of the engineered microbes with a specific class of available resource or physical support. The fourth involves the use of an existing, human-generated waste habitats as the substrate of engineered microbes, will be controlled through some class of lethality outside their niche.

A. Engineered mutualism

In this case, an engineered candidate organism is used to modify ecological systems through an engineered mutualistic relation in such a way that it will spread only if associated to its mutualistic partner. Mutualism requires a double positive feedback where the synthetic species benefits -and is benefited by- its host. Ideally, failure should end in the disappearance of the modified species. In figure 1a we display the TM associated to this approach. Here the host and the synthetic organism have been designed to enhance each other's growth. Moreover, the synthetic species has been derived from an existing wild type strain and it can thus mutate into WT. This will be the case if the engineered part is not enough advantageous and instead becomes a burden for the microorganism.

This scenario is tied to the symbiotic relationships that characterise several types of natural associations, such as nitrogen-fixing bacteria living in plant root nodules (fig 1b). Several experimental approaches have shown that such mutualistic relationship can be enforced by coevolving plants and bacteria under strong selection together with genetic engineering. Engineering mutualistic symbiosis is already a reality. Proper manipulation of free-living species allow to force them to become obligate mutualists. This includes synthetic cooperative strains

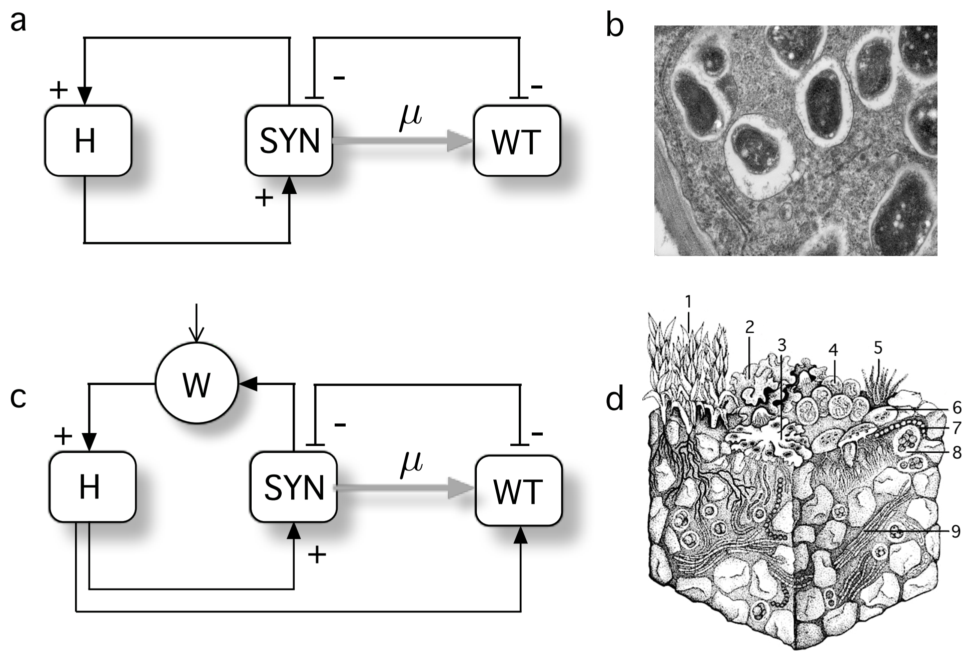

FIG. 1 Terraformation motifs involving closed cooperation among players. Two main classes of potential engineered synthetic microbes (SYN) interacting with their hosts (H) are indicated. Assuming that the engineered species has been obtained from an existing one in the same environment, the wild type (here indicated as WT) can be obtained from SYN if the engineered construct is lost by mutation (here indicated as a fray arrow, and as a rate μ) In (a) we display a logic diagram of positive interactions among both partners defining a mutual dependency. In (b) such cooperative interaction is mediated through some class of physical factor, such as water (W). These two classes correspond, for example, to exclusive mutualistic interactions displayed by plant cells within root nodules (c) where nitrogen-fixing bacteria are physically embedded (image from wikipedia commons). On the other hand, the need for survival under stressful conditions, as those common in arid ecosystems, makes water a major player and limiting resource. An engineered microbe capable of improving moisture retention can have a very strong effect on the underlying plant species, expanding their populations. In soil crusts (d) a whole range of species exist, adapted to water-poor conditions (drawing adapted from Belnap et al 2001). Here we indicate (1) mosses (2,3) lichens, (4,5,7,9) cyanobacteria, (6) fungi and (8) green algae.

[20] evolving a plant pathogen into a legume symbiont [21,22], fungal-plant mycorrhizal symbiosis [23] yeast-alga and fungi-alga associations created through a forced environmental change [24] or by means of long-term selection experiments enforcing metabolic dependencies [25] among others.

B. Indirect cooperation

Cooperation can also arise from an interfaced interaction where one of the species modifies the existing medium in such a way that the partner can thrive and create more growth opportunities for the first. The canonical example can be a species of microbe that has been engineered to excrete a molecule capable of enhancing water retention in arid conditions (Figure 1c). Here a microbe that exists in the chosen context can be engineered in order to release some kind of protein capable of enhancing water retention. Potential candidates would be engineered cyanobacteria that are known to produce extracellular polysaccharides [27,28]. Enhanced production of these molecules by synthetic strains could easily improve dry land soils and yield. The soil crust in particular (figure 1d) involves a rich ecosystem composed by lichens, mosses and cyanobacteria [29, 30] and constitute a crucial regulator of soil respiration in dryland ecosystems. Strategies oriented to soil rehabilitation and carbon sequestration could be implemented through the engineering of soil crust [31,32].

In an arid ecosystem, plants can improve their growth thus expanding their population and providing further opportunities for microbial populations also to grow. In arid and semiarid habitats, plants typically develop local interactions involving so called *facilitation*: the presence of neighbouring plants favours the establishment of others and the preservation of a healthy soil [33]. Given the constraints imposed by water shortage and overgrazing, patchy distributions of plants are the common pattern [34-37]. Mounting evidence suggests that the conditions allowing these ecosystems to survive and the nonlinear nature of facilitation implies the existence of breakpoints and catastrophes: once reduced water availability or grazing pressure cross a given threshold, a rapid transi-

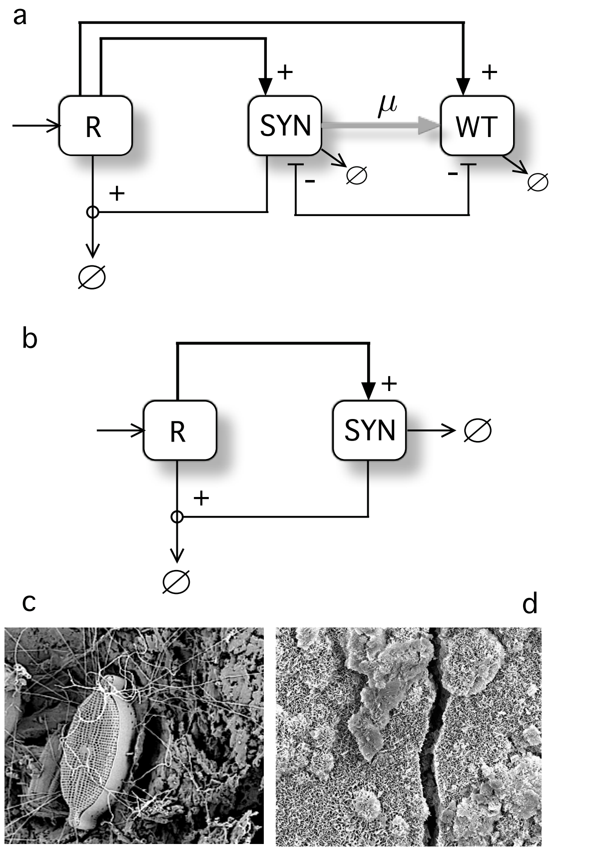

FIG. 2 Function-and-die Terraformation motif. Here a given substrate R is being generated at a given rate and provides physical substrate to the synthetic population. The TM motif in (a) is based on the modification of an extant species, whereas in (b) we just assume that the engineered species has been improved to attach efficiently to the substrate. in both cases, the engineered species could perform a function while degrading the waste material. Candidate examples are plastic ocean debris, where many species are known to live (c) or concrete cracks (d). Figures (c) and (d) have been adapted from [43] and [44], respectively.

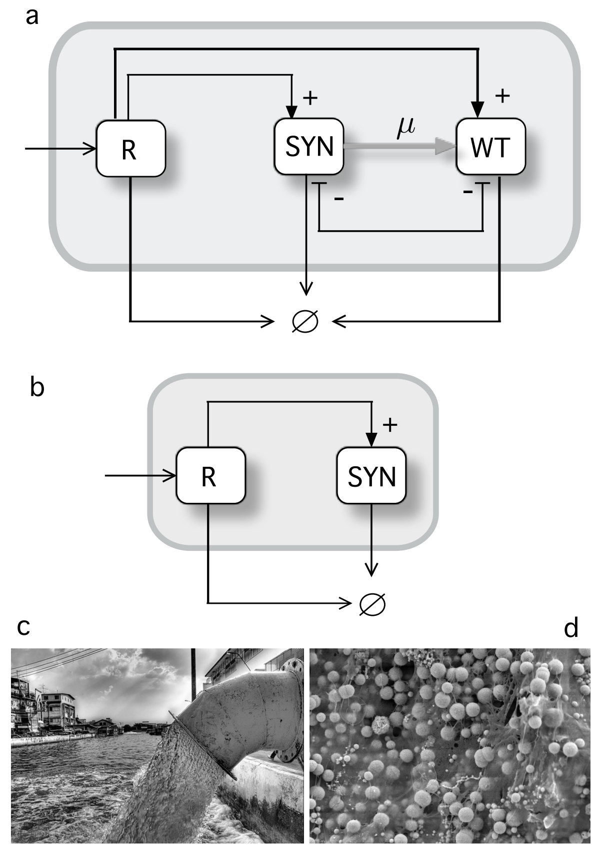

FIG. 3 Sewage-based terraformation motif. In (a) we consider a situation where an artificial environment is created as a byproduct of human activities producing waste. Our two strains are both sustained by available nutrients and physical conditions but now all of them are removed (burned or released) at a given rate. A simpler alternative (b) does not require engineering of extant species. A typical scenario would be sewage-related infrastructures (c) where a rich microbial community (d) is known to exist.

tion to the desert state should be expected. Modified organisms capable of building the indirect co-operative loop outlined above would easily increase facilitation. The increasing role of arid and semi-arid ecosystems as carbon sinks [38] makes them a specially relevant target for our terraformation proposal.

C. "Function and die" design

An engineered microbe performing a given functionality (such as carbon sequestration) can be coupled to the degradation of a given resource, such as plastic garbage or other long-living byproducts of human activities. This scenario is strongly tied to the problem of bioremediation [39-40] Here a non-living resource (R) is produced from anthropogenic actions and it provides the physical substrate where individuals can attach. In figure 2a we consider a TM that follows our previous scheme (again, a synthetic strain is derived form an existing one). In this case, however, no mutualistic loop is at work. Instead, both SYN and WT would attach to the substrate R and thus their populations deepen on such potential for adhesion, which could be improved in the designed strain.

A good candidate could be plastic garbage in the ocean [41,42] which is known to be colonised by many different species, including several microbial genus, such as Vibrio [43]. In this context, it is worth noting that, despite the rapid increase in plastic waste dumped in the ocean, the observed amount of plastic in open waters is much less than expected [44] suggesting (among other possibilities) that some microbial species capable to attach to plastic polymers are also degrading them. This observation indicates that evolutionary forces might have favoured plastic-eating strains which could be used as engineering targets. If the only goal of the SYN is degrade the waste material, we could use a modified organism that might not be normally attached to this substrate (figure 2b). Different species, both prokaryotes and eukaryotes, are known to persist in plastic (figure 2c). Since removal of plastic debris might actually part of the goal, it might be unnecessary to use existing species associated to this substrate. Instead, it could be more efficient to simply design or evolve a highly-efficient species capable of attaching to the plastic surface and to over-compete other species.

A different scenario that can be represented by our motif is provided by engineered bacteria that can be used to repair concrete cracks (figure 2d). The alkaline environment makes difficult for most species to thrive but some species can be used to this purpose [45]. Here the designed bacteria would enter, grow and replenish cracks with calcium carbonate until the task is finished. Several strategies have been used to this end and major improvements have been obtained [46,47]. A major advantage of this problem is that anaerobic bacteria are not going to survive outside the crack and thus selection immediately acts once the task is finished. Once again, the right combination of genetic design and ecological constraints create a powerful safeguard against undesired evolution.

D. Sewage synthetic microbiome

Urban centres are the largest human structures and as such they also incorporate massive infrastructures associated to treatment of waste as an end part of the city metabolism. Sewage systems and landfills offer a specially interesting opportunity to apply our approach. It is known that sewage systems involve their own micro biomes [48] and that some evolved microbes are currently causing damage to the concrete [49]. On the other hand, the sewage-based scenario is specially useful in our context, since microbes are eventually removed once they reach the open sea due to osmotic shock. If the same basic scheme is used, namely engineering an existing species, the TM can be summarised in figure 3a. Here a constant removal of both waste waters and microbes is represented by the arrows ending as $\rightarrow \emptyset$.

Here too it might be less relevant to preserve the existing species of microbes, thus making unnecessary to engineer from wild type (figure 3b). Being part of the human infrastructures of developed countries (figure 3c) the sewage TM is also relevant to asses the potential dynamical responses of bioengineered ecosystems. The existing sewage and urban microbiomes (figure 3d) provide a rich repertoire of candidate species, although we just start to grasp their richness [50]. An interesting connection between these potential engineered strains and the gut microbiome has been pointed in [13]. The later defines an enormously rich microbial ecosystem that has coevolved with our species through our long evolutionary history. Ongoing biomedical research starts to be oriented towards intervening in the microbiome by means of both drugs but also microbial strains that might act like exotic invaders aimed to restore lost functionalities [51-53].

IV. DISCUSSION

The three major classes of TMs presented above provide a framework to design synthetic biology alternatives to existing strategies aimed to fight against climate change and its consequences. A main departure from geo-engineering is the fact that designed living machines are by definition capable of self-replication. From an engineering perspective, that implies that the designed biomachines will be capable of making new replicas and thus scale up the problem. The synthetic organisms associated to the TMs act as *ecosystem engineers*, capable of modifying the flows of energy and matter through the ecosystem [54,55]. This is actually an approach to restoration ecology that is based in the existence of multiple alternative states in complex ecosystems [56,57].

A major objection to developing this framework in the real natural habitats is the potential for evolving undesirable (or unexpected) traits. This could be labelled as the "Jurassic Park Effect": even designed systems aimed to population control can eventually escape from genetic firewalls [58]. This is a claim that is supported by the unescapable potential of microbial systems for evolution. However, two important points need to be made. One is that microbes are being constantly dispersed on a global scale without special impact on extant ecosystems. As it occurs with most invaders, they either fail to survive or simply become part of the receptor habitats, where they are over competed by resident species. Secondly, the design principles proposed in this paper consider engineering extant organisms under a cooperation-based framework (thus enhancing mutualistic loops) or taking advantage of human-generated waste that can act as an artificial substrate to support the synthetic organisms. In all cases, a synergetic interaction between design and niche context is at work.

Redesigning our ecosystems requires a modification of nature, and deal with ecosystem complexity face to face [59]. But we should not forget that most biomes in our planet have already been deeply transformed by human activities [60]. Far from what we could expect, they can be diverse, robust and more efficient in terms of nutrient cycling and other components of ecosystem services [61]. Despite the long, sustained and profound anthropogenic impact on many of these novel ecosystems, they can display a richness and resilience that reminds us the potential of nature to reconstruct itself. It is time to decide what we want and why is our role in the future of nature. If we want humans to be part of the biosphere, we need to foresee the future impact of climate change on our planet. Here too slow response can trigger shifts. In this case, social collapse [62]. Synthetic biology can play a major role, along with all other strategies, to modify ongoing trends. That means redesign nature, but perhaps too to safely exit the Anthropocene with a renewed relationship with ecological systems.

Authors contributions

RS conceived the original idea. All authors contributed to develop it, made a literature research and designed the final figures. RS, in close collaboration with SDN and RM, wrote the manuscript, which was approved by all authors .

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