Promoting diversity in ecological systems through toxin production

Ga Ching Lui[1] and Sidhartha Goyal[1,2,*]

[1]Department of Physics and [2]IBBME,
University of Toronto, Ontario, Canada, M5S 1A7
[*]Corresponding author: goyal@physics.utoronto.ca

Abstract

Toxin production is generally thought as a mechanism to increase competitiveness of the toxin producers over other strains, thus driving down the diversity in the system. However, the production of toxin would also incur costs to the producer, and thus the overall effect may not be one-sided. In this work, we investigate the regime in which the presence of toxins is required to maintain high diversity, either at steady state or at persistence where the strain abundances shows oscillations or chaotic behaviors. Complementary to cross-feeding, which is one of several mechanisms previously proposed to resolve the paradox of plankton, we suggest the regulation of toxin production could play a role in increasing diversity above the upper bound given by the competitive exclusion principle.

1 Introduction

Secondary metabolites are by-products produced and released to the environment that are not necessarily required to support growth. One class of secondary metabolites is toxins. Although the production of toxins has huge impact to the environment, such as in the case of algal bloom or succession [20], the role of toxins together with changes in nutrient levels in ecological structure has not been fully elucidated. For example, it has been conjectured that toxins can be used to suppress grazers and competitors, or in other cases facilitating the producer's growth [31]. In microbial ecology, it is a known mechanism that toxin-antitoxin pairs in bacteria can be used against phage infection, as well as mediating clonal suicide to avoid invasion of other strains [4]. To understand these relatively more complicated, counter-intuitive behaviors, it is important to first understand how toxins may affect diversity in simpler or more typical models. One of the typical roles of toxins is to increase the competitive advantage of strains that are resistant to the toxins through allelopathy, meaning the toxins released to the environment would negatively affect the competing strains [20]. This can be achieved by means such as suppressing protein biosynthesis, modifying and damaging cellular DNA ,or increasing permeability of cytoplasmic membrane which effectively kills the cells [32, 33, 9, 20, 41].

Apart from mediating allelopathic interactions, toxins are also conjectured to positively affect the producing strains. It has been suggested cylindrospermopsin, a common cyanotoxin associated with algae bloom in freshwater, induces an increase level in alkaline phosphatases (APase), which facilitates the uptake of inorganic phosphate[14]. For the purpose of this paper, we focus only on the role of allelopathy through causing inefficiency in protein synthesis, which leads to a drop in growth rate, in contrast to a number of models previously proposed [15, 24, 10] to describe the effects of toxins by modifying the death rate. A typical feature of these models is that during ecological timescale, the system always moves towards pure states, which we define as the states for which the entire population is out-competed by one single strain: either consistently dominated by the fittest strain, or bistable state for which the identity of the dominating strain depends on the initial conditions. In comparison with these models, the bacterial growth rate in our model is always non-negative and toxin does not lead to out-flux of the strains. This is to describe the effects of toxins on biosynthesis of proteins that leads to bacterial growth, such as decreasing the efficiency in translation. This framework allows us to apply interpretative constraints that can be mapped to a coarse-grained model

of metabolism and provides a rich behavior that ranges from multi-stability to coexistence, oscillations and chaos.

The production of toxins is costly and usually requires trade-offs with growth. This means toxin production is more effective against strains with very different phenotypes and metabolites [22]. Toxins should only be produced when necessary, and tight regulation is required to optimize the benefit-to-cost ratio. This can be achieved through the response to either quorum sensing [1] or abiotic stressors [28], which is known as the competition-sensing hypothesis [5]. The former case involves frequency dependent production of toxins, and therefore the outcome is sensitive to initial frequencies of the strains. For the latter case, there has been strong evidence that unfavorable growth condition due to low nutrient availability in the environment contributes to an increase level of toxin production [12], in contrast with non-nutrient related stressors. A rich body of literature has been dedicated to study the effect of toxin production under different nutrient conditions, at varying levels of Nitrogen and Phosphorous [12]. These effects would guide the ecological dynamics and dictates the community structure. Interestingly, in an experiment with Microcystis aeruginosa, the toxin producing strain out-compete the non-producer when nitrogen is depleted, and introducing pulses of nutrient supply further accelerate competitive exclusion; when nitrogen is depleted, non-toxin strain dominates vice versa when phosphorous is depleted and the supply pulses promotes coexistence [36]. The intuition is that the strains are competiting for these growth limiting nutrients, and toxins increase the competitive advantage through either allelopathic or non-allelopathic mechanisms. [28] shows strains with constitutive production is out-competed by strains with regulated production by either quorum sensing or competitive sensing such as nutrient sensing. The effect of regulation is also highlighted in [24], where the production rate is linearly dependent and monotonically decreasing on the growth rate of the producer.

One of the mechanisms to maintain diversity in the system is persistence at non-steady state [16, 17, 34, 8, 7]. The oscillatory behavior of cyanobacteria is important in investigating seasonality in harmful algal blooms. One way to generate cyclic behavior is through the rock-paper-scissor dynamics by introducing strains that (i) produce toxins; (ii) do not produce toxins but are resistent to toxins and ; (iii) do not produce toxins and are sensitive to toxins, with trade-offs in their growth rates [18, 19]. On top of temporal fluctuations, spatial structures have been considered to contribute to diversity [37, 30, 2, 27, 21]. The production of toxins requires trade-offs, and therefore at different environment, some of the regulations strategies is more advantages. In particular, [6] explores a lattice model in which evolution at different environment leads to different regulation strategies. Here we only explore the temporal heterogeneity and assume the system is spatially homogeneous.

For spatially homogeneous system, generally it is considered that at steady state there is a theoretical upper bound in diversity, which is known as competitive exclusion principle [13]. Different mechanisms has been proposed to circumvent around competitive exclusion principle. One of the mechanisms is to consider a fixed enzyme budget on alternative resources, which flattens the fitness landscape thus allowing more species to coexist [38]. Another way is to consider the effects of cross-feeding. Since cross-feeding mediates positive interactions between the strains and does not incur costs, it promotes diversity in the system. However, unlike cross-feeding, toxins mediates negative interactions. In this work, we hypothesize that similar to cross-feeding [26], toxins may counter-intuitively play a role in supporting diversity above the upper bound given by competitive exclusion principle, and lay down the groundwork to extend this framework to other modes of interaction. Firstly, we describe the relation of metabolic constraints with the choice of growth rate functional used in our model. Secondly, we explore the violation of competitive exclusion principle at steady state when there is no regulation. Thirdly, we illustrate the transition to persistence given regulation of toxin production. And finally, we discuss how toxin production sustain a richer diversity beyond what competitive exclusion principle suggests.

2 The model: growth rate functionals respecting proteome constraint

The model described in this work provides a linkage between two scales: metabolism and protein biosynthesis on the cellular scale, and species interactions through modification of the environment on the ecological scale. To understand how cellular activity is influenced by the environment, we adopt the model by [43, 35], which concerns with protein synthesis limited by two sub-processes, C and R, as shown in Fig. 1A, and takes

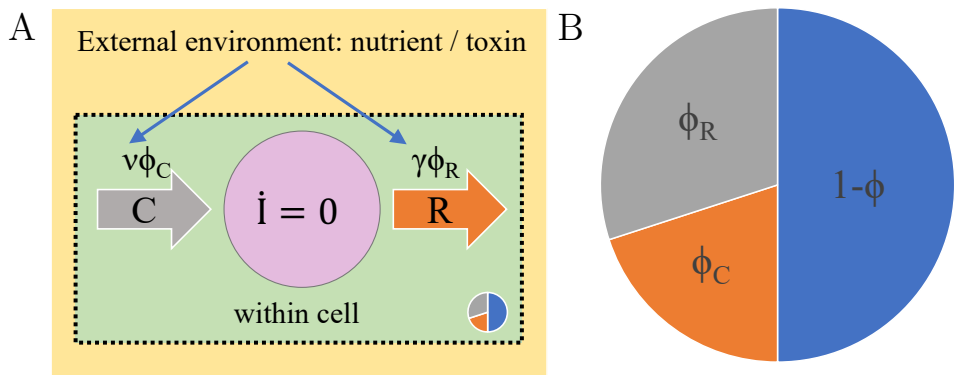

Figure 1: Rate of bacterial growth depends on both the abtiotic factors in the external environment and proteome partitioning within the cell. (A) Suppose the rate of bacterial growth is limited by two sub-processes C and R, contributing the the influx and ouflux of some intermediate, such as amino acids, with abundance I. The rate of the sub-processes is $\nu\phi_C$ and $\gamma\phi_R$. The efficiencies are affected by nutrient and toxin level in the external environment and the fraction of corresponding proteome partition. (B) The fraction of proteome $\phi = \phi_C + \phi_R$ associated with growth is fixed and is referred to as the proteome constraint. The sub-fractions ϕ_C and ϕ_R associated with sub-processes C and R varies to ensure steady state of the intermediate $\dot{I} = 0$.

the rate of protein production as a proxy of growth rate. Briefly, the model consists of an internal pool of amino acids with size I. The in-flux of the pool comes from sub-process C, which involves nutrient fixation and metabolism. Carbon is used as an example here, but other growth limiting factors such as nitrogen and phosphorous can also be included. Sub-process R contributes to the out-flux of the pool. It refers to translation which depletes amino acids to build polypeptides, and this requires ribosomal proteins to bind with mRNA to form ribosomes. The key determining factor of the rate of sub-process C is metabolic proteins such as enzymes and transporter proteins, whereas sub-process R is faster when there are more ribosomal proteins, which has a fixed stoichiometry with rRNA and is proportional to the amount of ribosomes. With these two bottlenecks, coordination is required to decide if more of ribosomal proteins or metabolic proteins should be produced. Fig. 1B illustrates the idea of proteome constraint, where ϕ is a fixed fraction of proteome which is growth associated and the remaining fraction $1 - \phi$ is growth rate independent. The fraction of metabolic proteins with respect to the entire proteome is ϕ_C and the fraction of ribosomal proteins is ϕ_R. By feedback regulation, ϕ_C and ϕ_R is determined to optimize growth rate and attain steady state, subjected to the constraint

$$\phi = \phi_C + \phi_R \ . \tag{1}$$

It turns out to there is only one single solution of the proteome fractions that satisfies the requirement of flux balance in Fig. 1B, and the rate of protein production or bacterial growth rate can be expressed in terms of ν and γ, which are the efficiencies of sub-processes C and R

$$g = \frac{\phi}{\nu^{-1} + \gamma^{-1}} \ . \tag{2}$$

The derivation is summarized in SM Sect. 1.A.1. Notice that in Hwa and Scott's formulation [35], the nutrient efficiency ν is held fixed irrespective of nutrient concentration in the external environment, which is to say the nutrients in the external environment is in excess compared to the reaction rates of sub-process C in the cell. In the limit where nutrient is scarce in the external environment, ν should depend on nutrient concentration n_N. In the presence of a single toxin, the translation efficiency γ may also depend on toxin concentration n_T. Consider ethanol as an example. It has been suggested ethanol toxicity can affect translation. For Escherichia-coli, applying stress to the system with ethanol leads to an increase in the level of ppGpp [39], which is an alarmone that leads to a decrease of transcription level of rRNA. When there are insufficient amount of rRNA to bind with ribosomal proteins, the excess ribosomal proteins self-regulate by repressing its own translation [35]. Experiments with directed evolution coupled with ribosome profiling and RNAseq further suggest sub-lethal level of ethanol can lead to translation error and ribosome stalling,

as well as repressing transcription inhibiting mRNA production [11]. Another example is colicin, a common type of bacteriocin that also affects some strains of E-coli, can disrupt cell growth by cleaving t-RNA and r-RNA [3]. These factors would lead to a decrease in translation efficiency. In our coarse-grained model, We take into account of the combined effect of these factors by expressing the translation efficiency with hill function, monotically decreasing with toxin concentration n_T. Since the sub-processes C (R) involve enzymes (or ribosomes) binding with a substrate (or mRNA), we use Michaelis-Menten kinetics (see SM Sect 1.A.2) to show that in the limits of low nutrient concentration and rich internal pool of amino acids, the bacterial growth rate for species i, with one growth-limiting nutrient and one translation inhibiting toxin can be written as

$$g_i = \frac{g_m}{K + \frac{k_N^{(i)}}{n_N} + \frac{n_T}{k_T^{(i)}}} \ , \tag{3}$$

where $k_N^{(i)}$ and $k_T^{(i)}$ are the Michaelis-Menten constants that determines the scale, and are interpretative in terms of the reaction rates. K is some constant due to other processes independent of the nutrient and toxin levels, and g_m is the maximal growth rate in an optimal environment when $n_N >> k_N^{(i)}$ and $n_T << k_T^{(i)}$. Here, the growth rate functional depends on two growth limiting factors. This is the minimal requirement to establish coexistence in a community since it allows the trade-off, in a sense a species that grows best in the community on a growth limiting factor has to be less adapted to grow on another growth-limiting factor, so that other species would not be out-competed.

Assuming in the ecological community of interest, such trade-off exists that allows all species in the community to attain similar fitness. We can decribe this by impose an ansatz to the growth rate functionals to describe this trade-off. For (3) involving toxins, we consider the following ansatz:

$$\frac{k_N^{(i)}}{\kappa_N} + \frac{\kappa_T}{k_T^{(i)}} = C \ , \qquad \forall i \tag{4}$$

where C is a constant, κ_N has a unit of nutrient concentration and κ_T has a unit of toxin concentration. This simply means if a species is well adapted at growing on the nutrient, with a small value of $k_N^{(i)}$, it is more susceptible to the toxin with a small value of $k_T^{(i)}$.

One of the approaches to investigate ecological diversity at steady state is to consider chemostat culture systems. Unlike serial dilution, chemostat is a continuous culturing device that pumps in fresh medium and removes stale medium at some constant rate F. This allows easy control over population size and environmental conditions such as resource availability in the culture with less fluctuation introduced externally, while keeping the volume of the system constant. More importantly, due to the flow, bacteria are suspended in the medium and do not grow and adhere at the wall of chemostat, and thus this provides a more realistic modelling of natural systems [42], yet simplified enough to recreate in-vitro and capture the diversity for biological systems such as gut microbiota [25]. In general, the system can be described by the following set of equations:

$$\begin{aligned} \dot{x}_i &= (g_i - F)x_i \ ; \\ \dot{n}_{\xi_j} &= (a_{\xi_j} - n_{\xi_j})F + \sum_i (\theta_{\xi_j}^{+\,(i)} - \theta_{\xi_j}^{-\,(i)})x_i \ , \end{aligned} \tag{5}$$

where x_i is the abundance of strain i, n_{ξ_j} is the concentration of some abiotic factor ξ_j, where $j = 1, 2, \cdots$ and $\xi = T$ for toxins and $\xi = N$ for nutrients available in the culture, and a_{ξ_j} is the concentration of the abiotic factor ξ_j in the fresh medium. The supply vector is defined as $\vec{a} = \Sigma_{\xi_j} a_{\xi_j} \hat{e}_{\xi_j}$, with $\hat{e}_{\xi_j}$ being the basis vectors. The production and consumption of abiotic factor j due to strain i per capita are denoted by $\theta_{\xi_j}^{+\,(i)}$ and $\theta_{\xi_j}^{-\,(i)}$. Equation (5) assumes the removal of the strains out of the system is dominantly dictated by the flow rate of chemostat. This is valid in the limit where the flow rate is much greater than the death rate of the strains. To survive in the chemostat, strains have to grow faster than the flow rate. By changing the flow rate F of the medium, and the supply concentrations a_{ξ_j} of the abiotic factors, we can adjust the selective pressure imposed on the strains and change the composition of the community. In turn, the strains can also modify the environment, which comes from the summation terms in (5). For a system with only

two abiotic factors, the impact of the strain i on the environment can be captured by the ratio

$$m_i = \left. \frac{\theta^{+}_{\xi'_{j'}}{}^{(i)} - \theta^{-}_{\xi'_{j'}}{}^{(i)}}{\theta^{+}_{\xi_j}{}^{(i)} - \theta^{-}_{\xi_j}{}^{(i)}} \right|_{g_i=F} , \tag{6}$$

which describes the change of levels of the abiotic factors per unit growth of cells when the system is in vicinity of the fixed point. For example, suppose both abiotic factors are growth-limiting nutrients with $\xi' = \xi = N$, $j' = 2$ and $j = 1$, species that consumes more of nutrient 2 would have a larger m- ratio. This indicates per unit growth of cell, it increases the competitive advantage of species that are adapted to growing on nutrient 2. The m- ratio is particularly useful when studying the partition in nutrient space, which we will discuss further in the next section.

3 Equalization and stabilization due to toxin production

We first consider the case with only one single growth limiting nutrient N and one toxin T with concentration n_N and n_T. For simplicity in the notation, we drop the sub-index j in this section since $j = 1$ is always fixed here. We assume the nutrient is available in the fresh medium with $a_N \neq 0$ and the toxins are produced by the strains and may or may not be supplied externally, or $a_T \geq 0$. When imposing flux balance to the coarse-grained model, there is a trade-off between production of the toxins θ^{+}_{2i} and the nutrient efficiency ν (see SM Sect. 1.C). More specifically, defining f_i to be the production fraction of toxins by strain i, such that $0 \leq f_i < 1$, and suppose that toxins are not consumed and nutrients are not produced, then the scaling parameters, in-flux and out-flux of the abiotic factors can be written as

$$\begin{gathered} k_N^{(i)} = \frac{\tilde{k}_N^{(i)}}{1-f_i} \; ; \quad \theta_T^{+(i)} = \frac{f_i}{1-f_i} g_i \; ; \quad \theta_T^{-(i)} = 0 \; ; \\ \theta_N^{+(i)} = 0 \; ; \quad \theta_N^{-(i)} = g_i \; , \end{gathered} \tag{7}$$

with the consumption ratio

$$m_i = \left. \frac{\theta_T^{+(i)} - \theta_T^{-(i)}}{\theta_N^{+(i)} - \theta_N^{-(i)}} \right|_{g_i=F} = \frac{1}{1-1/f_i} \; . \tag{8}$$

This means the species consume nutrient N for growth, and may or may not produce toxin T, depending on f_i. Notice with this set of parameters, the ratio m_i is a monotonic function of f_i when $f_i < 1$. When toxin production factor $f_i = 0$, no toxin is produced with $\theta_T^{+(i)} = 0$ and $k_N^{(i)} = \tilde{k}_N^{(i)}$. When the production factor f_i approaches unity, growth rate g_i approaches zero since most of the resources are used to produce toxin and not to sustain growth. Given that $f_i < 1$, both $k_N^{(i)}$ and $\theta_T^{+(i)}$ are monotonic in f_i.

Consider a system with five strains ($i = 1, \ldots, 5$), it is usually difficult to adopt a graphical approach in understanding the dynamics. However, because of the ansatz imposed on the growth rate functionals, we can now visualize the dynamics on a two-dimensional space spanned by the concentrations of the two abiotic factors. In Fig. 2A and 2B, the zero net growth isoclines (ZNGIs) are plotted in solid lines. These corresponds to the concentration at which biomass does not accumulate in the system, which can be expressed as $g_i(n_N, n_T) - F = 0$. By (4) and (7), there an environmental condition $(n_N, n_T) = (n_N^\star, n_T^\star)$ under which all strains have equal fitness set by the flow rate of the chemostat $g_i = F$ for all i. In other words, the underlying environmental condition of equalization is simply the intersection of every ZNGIs, which is only solvable because in addition to resource levels n_1, the growth rate also relies on toxin levels n_2.

The external supply of abiotic factors plays an important role in determining the fate of outcome of the community. In Fig. 2, region (iv) shaded in grey refers to the unsustainable region: at low supply concentration of growth-limiting nutrient n_N and high supply concentration of toxins n_T, net growth is negative and the biomass in the system is decreasing. If the supply vector $\vec{a}$ lies in region (iv), none of the strains are able to survive in the system. The remaining of the nutrient space can be divided into three regions: (i), (ii) and (iii). If supply vector $\vec{a}$ lies in region (i), since n_N is small, the strain ℓ that is most adapted at growing on the growth-limiting nutrient (with $k_N^{(\ell)} = \arg\min_i k_N^{(i)}$) would out-compete the entire population and dominate, with every other strain are driven to extinction. In region (iii) where nutrient is

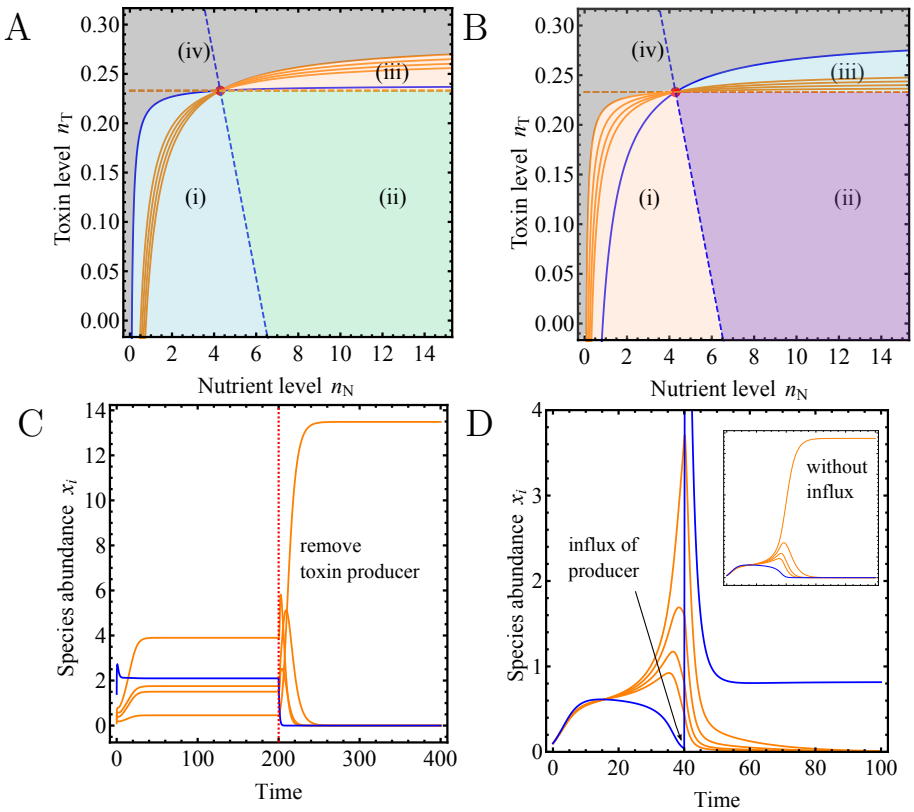

Figure 2: A system with 5 strains, with maximum growth rate $g_m = 1.20$ in a chemostat with flow rate $F = 1.03$. Orange color refers to strains that do not produce toxins with $f_i = 0, i = 2, \cdots, 5$.The Michaelis-Menten constants for the non-producers are $\tilde{k}_N^{(i)} = i/10$. All species are subjected to the subjected to constraint (4) with $C = 5$ and $\kappa_N = \kappa_T^{(i)} = 1$. Blue color notates the only toxin producer in the system with $f_1 = 0.1$. The phase diagrams in relation to nutrient supply for two different cases are plotted in (A) and (C), where grey area denotes the unsustainable region in region (iv) with high toxin level and low nutrient level, the blue (orange) region correspond the pure state region where only one species of either the producer (non-producer) survives. In region (ii) with low toxin level and high nutrient level, it can either be stable coexistence state (A) as denoted by green color, or bistable state (B) as denoted by purple color. The time series of species abundance in the green region are plotted in (C) and (D). The remaining of the partitions are region (i) and (ii). (A, C): The Michaelis-Menten constants of the producer is $\tilde{k}_N^{(1)} = 0.1$. With $a_T = 0$, there are two possible outcomes in Fig. 1a: (i) at small a_N, producer dominates; (ii) at large a_T, all strains coexist. This is due to two reasons: (1) the ansatz takes into consideration of the trade-offs between toxin production, toxin resistance and nutrient efficiency (2) The producer stabilizes the fixed point. The stabilization mechanism facilitated by toxin production is shown in (C). The supply vectors are $(a_N, a_T) = (14, 0)$ with initial conditions set to be $x_1(0) = 1.4$, $x_2(0) = 0.1$, $x_3(0) = 0.3$, $x_4(0) = 0.2$, $x_5(0) = 0.4$ with $n_N(0) = a_N$ and $n_T(0) = a_T$. Toxin producer (blue) is removed at $t = 200$ (red dotted line) by setting $\dot{x}_1(t > 200) = -x_1(t)$, which leads to the collapse of the community with only one surviving species. (B, D): The Michaelis-Menten constant of producer $\tilde{k}_N^{(1)} = 0.9$ The fixed point solution still exists due to equalization, but the role of the producer is to destabilize the system. (D) has a initial condition of $x_1(0) = x_2(0) = x_3(0) = x_4(0) = x_5(0) = 0.1$ with $n_N(0) = a_N$ and $n_T(0) = a_T$. It shows bistability in the system where the toxin producer is driven close to extinction at around $t = 40$. Momentarily an external flux of the producer strain is supplied to the system, which changes the fate of the community such that toxin producer dominates.

abundant with large n_N, resilience against toxins becomes the determining factor. Only the strain ℓ' that is least affected by the toxin (with $k_T^{(\ell)} = \arg\max_i k_T^{(i)}$) would survive. Region (ii) is case dependent. Suppose

there is only one single toxin producer with non-zero production fraction, or $f_{i\neq 1} = 0$, then in region (ii) the system is stabilized if the producer is the most efficient strain to utilize the resource, or $\ell = 1$, as shown in Fig. 2A. In contrary, if $\ell' = 1$ as shown in Fig. 2B, then in region (ii) the system is unstable and depending on the initial conditions, either the dominating strain in region (i) or (iii) would dominate. Fig. 2C shows the time series of strain abundances when the system is stabilized by the toxin producer. With abundant supply of resource a_N, the five strains coexist with equal fitness. In fact, the toxin producer is a keystone species in this system. As the toxin producer is removed at $t = 200$, the whole community collapse and all strains but one are driven to extinction since the system is no longer stable. More specifically, coexistence in this deterministic system without the toxin producer requires fine tuning of a_T since $m_i = 0$ for all i. Fig. 2D shows the time series of strain abundances when the system is bistable, leading to two different pure states in which the community collapses.

The above example shows two different strategies that a toxin producer can adopt to survive in the microbial consortium with very different outcomes. In the first case, the strategy of producing toxins sustain diversity and promotes the coexistence of all strains, such that the entire population would have a more diverse set of strategies to cope with fluctuations in the environment. As long as the nutrient supply is adequate, toxin producer can always survive. In other words, counter-intuitively a higher level of toxins in the environment points to a richer diversity. An interesting note is that compared with pure states where only the toxin producer survives, the coexistence states would have a higher level of both nutrients and toxins. In the second case, toxin production is used to remove competing strains. However, since toxin production is costly, this strategy does not always pay off. Depending on the initial condition, the toxin producer can dominate over the population only when its initial abundance is high. This is due to the fact that production of toxin is costly, and therefore when the initial abundance of toxin producers is low, each cell has to bear a higher cost, thus reducing its competitiveness. This dependency in initial condition due to costs in toxin production will be further discussed later in relation with toxin sensing.

4 Regulation of toxin production

Toxin production is costly, and the traditional view is that releasing toxins in an environment where the producer is the dominating species is wasteful. Competitive sensing is one of the feedback mechanism to reduce the wastage while maintaining the competitive advantage of the strain. Here, we consider two types of competitive sensing: nutrient sensing and toxin sensing. This refers to regulating toxin production either by sensing nutrient availability or toxin produced by its competitors in the environment. In both cases, we extend our previous framework to multiple toxins and sources of nutrients and consider the form of growth functional to be

$$g_i = \frac{g_m}{1 + \Sigma_j \left(\frac{n_{Nj}}{k_{N_j}^{(i)}}\right)^{-1} (1 - f_{ij})^{-1} + \Sigma_j \left(\frac{n_{Tj}}{k_{T_j}^{(i)}}\right)^{q}} , \tag{9}$$

where n_{Nj} are the concentration of the nutrients N_j and n_{Tj} are the concentration of toxins T_j in the environment. We introduce the sub-index j back to the expressions since there is now more than one type of toxins and nutrients. For strain i, the fraction f_{ij} denotes the trade-off that growth on nutrient Nj is less efficient during the production of toxin T_j. The influx and outflux of abiotic factors due to the species are

$$\begin{gathered} k_{N_j}^{(i)} = \frac{\tilde{k}_{N_j}^{(i)}}{1 - f_{ij}} \; ; \quad \theta_{T_j}^{+\,(i)} = \frac{f_{ij}}{1 - f_{ij}} g_i \; ; \quad \theta_{T_j}^{-\,(i)} = 0 \; ; \\ \theta_{N_j}^{+\,(i)} = 0 \; ; \quad \theta_{N_j}^{-\,(i)} = S_{ji} g_i \; . \end{gathered} \tag{10}$$

The simplification we have implicitly applied here is that species produces toxin T_j by sacrificing the efficiency in N_j, as noted by the fact that as f_{ij} increases, $k_{N_j}^{(i)}$ also increases. A new parameter S_{ji} is introduced here to descripe the stoichiometry of resource consumption per unit growth. Compared to the previous case, bacterial growth depends on more than one essential resources, and as such the ratio of consumption of these

resources matters. For a system with two nutrient sources, we define

$$m_i = \left. \frac{\theta^{+}_{N_2}{}^{(i)} - \theta^{-}_{N_2}{}^{(i)}}{\theta^{+}_{N_1}{}^{(i)} - \theta^{-}_{N_1}{}^{(i)}} \right|_{g_i=F} = \frac{S_{2i}}{S_{1i}} \ . \tag{11}$$

Different from the previous section, we will explore, within the limit imposed by competitive exclusion principle, how regulation affects diversity, from having extinction of certain species to maintaining survival of every species in the system. This would also establish some groundwork for the discussion in the subsequent section on further increasing diversity beyond the limit.

4.1 Nutrient sensing

We introduce the effect of nutrient sensing by considering dependence of f_{ij} on resource availability ofN_j, such that it takes the form of

$$f_{ij} = \frac{f_m^{(i,j)}}{1 + (\sigma_i / n_{N_{j'}})^p} \ ; \qquad j' = (i\%D) + 1, \tag{12}$$

where $f_m^{(i,j)}$ sets the maximum production fraction of toxin and σ_i is the Michaelis-Menten constant that sets the scale for sensing the resource level. As a set up, we assume species i is a producer of toxin T_i, while being the fittest at utilizing resource N_i and weakest at using resource $N_{j'}$. The Hill coefficient p can take either positive or negative sign, which is explored in SM Sect. 2. When p is negative, toxin is produced when the nutrient availability is low and the producer is under stress. When p is positive, toxin is produced when the environment is nutrient-rich, which is a relevant strategy when the competing strain has a fast growth rate. As an example, we consider a two-strain system and set $k^{(1)}_{N_1} < k^{(1)}_{N_2}$ and $k^{(2)}_{N_1} > k^{(2)}_{N_2}$, while $f_m^{(i,j)} = 0$ for $i \neq j$. This means each of the strain exclusively produces one toxin only. Furthermore, they determine the amount of toxins to produce by sensing the level of the resource that they use less efficiently. The producer should also be less susceptible to the toxin it produces: $k^{(i)}_{T_j} > k^{(i)}_{T_i}$.

For simplicity, we assume the external supply of resources into the system is symmetric to lower the degrees of freedom when exploring the phase space, meaning $a_{N_1} = a_{N_2} = \alpha$. This parameter is easily tunable in experiments. If α is too small, neither of the species can survive. As α increases, the fitness between the species becomes the same and both coexist. Further increasing α leads to oscillations. Fig 3 shows the transition from coexistence to persistence as the external resource supply α increases. Further increasing the external supply of resources breaks the oscillation as one of the species grow to dominance. Apart from changes in the external environment, the strength of nutrient sensing also controls the transition to persistence. The bifurcation graphs in Fig. S2 and the phase diagram in Fig. S5B in SM show the effects of changing p and σ on the dynamics of the system. Setting other parameters to be fixed, notice persistence is only possible when $m_1 > m_2$. When toxin is absent in the system, this correspond to the regime where the coexistence fixed point is stable. In other words, the effect of introducing toxin and regulation into our model is to destabilize the fixed point, leading to a transition from coexistence to persistence, with no increase in diversity of the system. When $m_2 > m_1$, the system is bistable when toxin is absent, such that only one of the two species dominates the population, depending on initial condition, and that coexistence cannot be reached. Even when toxin is introduced into the system, persistence cannot be sustained in this regime. Again, this is shown in SM Fig. S5B. To summarize, toxin production with nutrient sensing only changes how different species is sustained in the system, either through coexistence or persistence. It does not change the diversity, or number of species, in the system. In other words, there is no transition from pure states to persistence states by switching on regulation. As a side note, chaotic behavior is exhibited in this system, and is discussed in more details in SM Sect. 2.A.

In Fig. 4, similar trend can also be observed in a three-strain system. The system is set up in a similar fashion as the two-strain system, where the strains exhibit intransitive relations. In order words, we can map our model to a generic rock-paper-scissor game, which has applications outside of ecology. In general, at low resource supply, the strains coexist at steady state. As resource supply increases, strains persist either in limit cycles or in chaotic trajectories. At large external supply, the system collapse and is dominated by only one single strain. The effect of changing nutrient sensing is similar to that in a two-strain system.

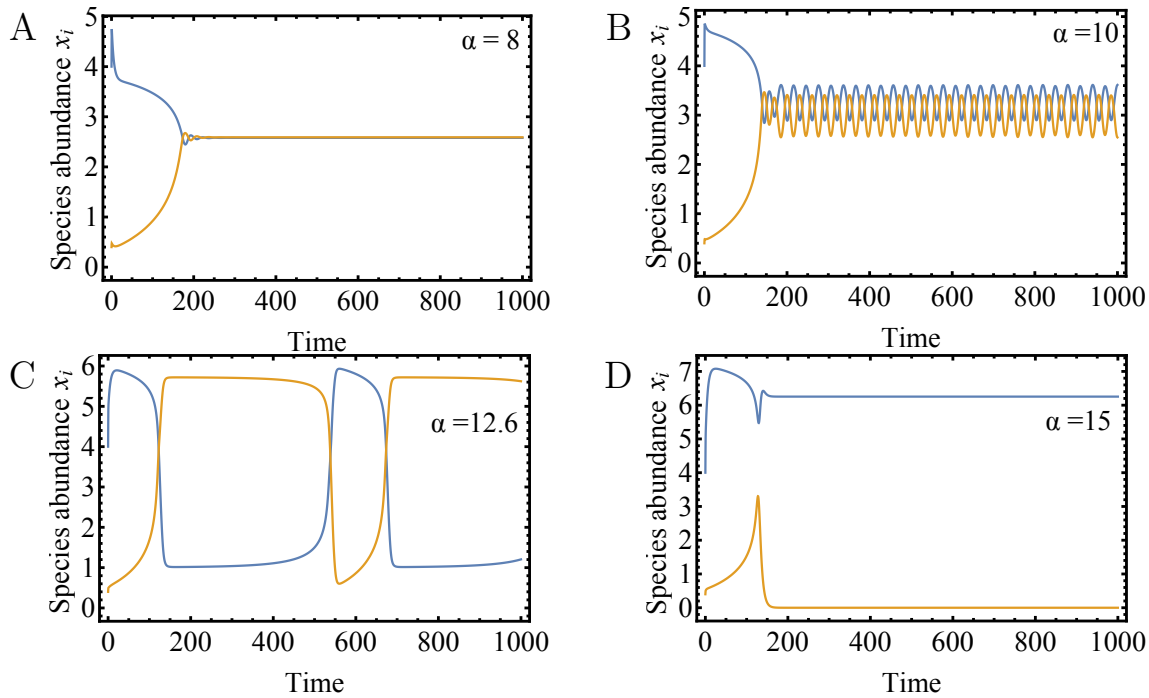

Figure 3: A system with 2 strains, 2 essential resources and 2 toxins. The supply for both resources are set to be the same $a_{N_1} = a_{N_2} = \alpha$. As α increases, the system changes from a stable coexistence state at $\alpha = 8$ in (A) to limit cycles at $\alpha = 10$ in (B). Further increase in α breaks the symmetry between the abundance of the two strains at $\alpha = 12.6$ in (C), where on average the orange strain is more abundant. At large supply $\alpha = 15$ in (D), persistence breaks and the abundance trajectory flows towards pure state, where the fitter strain takes over the population. Maximum growth rate is $g_m = 1.2$ and flow rate is $F = 0.2$. The Michaelis-Menten constants are set to be $k_{N_1}^{(1)} = 0.35$, $k_{N_2}^{(1)} = 0.65$, $k_{N_1}^{(2)} = 0.70$, $k_{N_2}^{(2)} = 0.30$, $k_{T_1}^{(1)} = 3.8$, $k_{N_2}^{(1)} = 2.2$, $k_{N_1}^{(2)} = 2$, $k_{N_2}^{(2)} = 4$. The toxin production factors are $f_m^{(1,1)} = f_m^{(2,2)} = 0.5$ and $f_m^{(1,2)} = f_m^{(2,1)} = 0$, with $\sigma_1 = 0.20278$, $\sigma_2 = 0.20316$. The Hill coefficients are $p = q = 3$. The stoichiometries for nutrient consumption are $S_{11} = S_{22} = 1$ and $S_{12} = S_{21} = 2$. Phase transition with other parameters are shown in supplementary

Furthermore, because it is a rock-paper-scissor game, there is a range of value of the parameters that falls into chaotic regime. When p is positive, increasing the strength of nutrient sensing by increasing p or decreasing σ changes the system from coexistence to persistence. This corresponds to rapid up-regulation of toxin production as nutrient level increases. When the environment is more advantageous to one of the strains due to high availability of a particular resource, the producer strain that senses that resource would produce more toxin to suppress that strain. This increase of toxin production would come with a cost in lowering the growth rate of the toxin producing strain, thus driving the oscillation or chaotic behaviors in both two- and three-strain systems. When p is negative, where toxin production is up-regulated at low resource level, strong nutrient sensing leads to tristability. This comes from the cost of toxin production introduced by the production fraction f_{ij}. If a producer strain is in abundance initially, the environment can be changed drastically even if each cell produces a small amount of toxin. If the abundance of the producer strain is low, each cell have to produce a greater amount of toxin, which severely lowers the growth rate. This is consistent to the observation in the next section when we consider the production fraction to be fixed.

4.2 Toxin sensing

Although the idea of generating persistence through competition over essential resources is not necessarily a novel idea [16, 17], for oscillations it requires at least three growth-limiting essential resources and three species to provide the delay in the system; for chaotic behaviors the minimum number of species is five. In

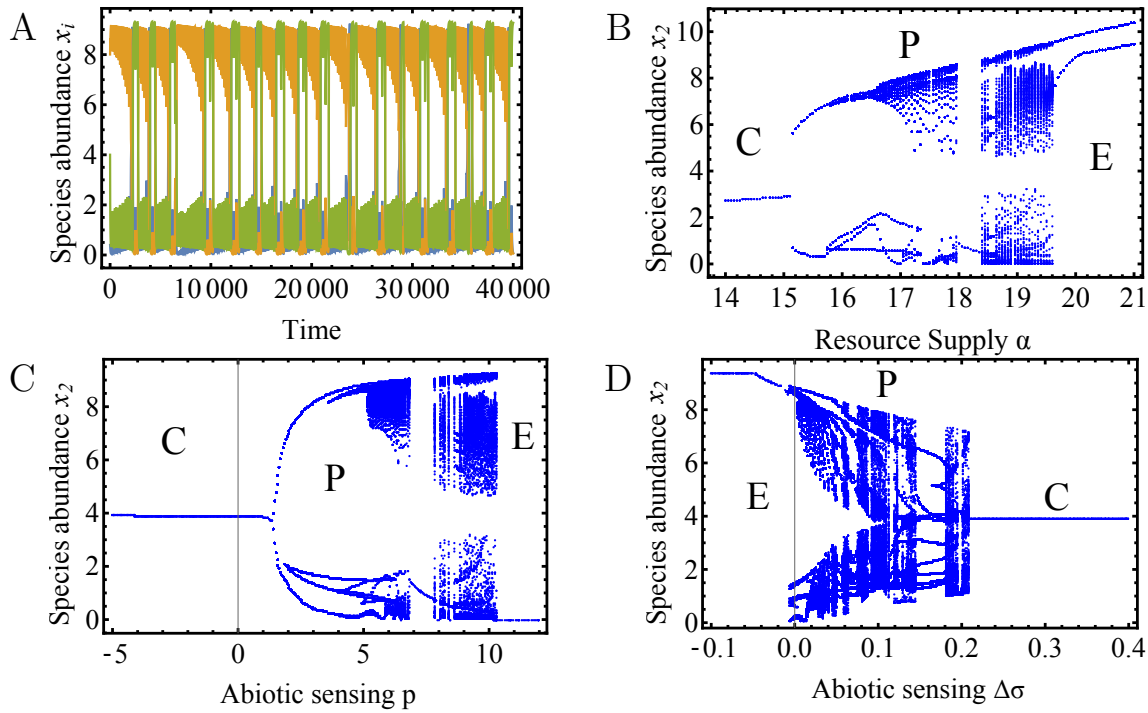

Figure 4: A system with 3 strains, 3 essential resources and 3 toxins. The parameters are set as follows unless specifically stated otherwise in each sub-graph captions: Maximum growth rate is $g_m = 1.2$ and flow rate is $F = 0.2$. External nutrient supply is $a_{N_j} = \alpha = 19$ and the toxin supplues are $a_{T_j} = 0.1$. The Michaelis-Menten constants for the nutrients are set to be $k_{N_1}^{(1)} = 0.10$, $k_{N_2}^{(1)} = 0.65$, $k_{N_3}^{(1)} = 0.25$, $k_{N_1}^{(2)} = 0.20$, $k_{N_2}^{(2)} = 0.15$, $k_{N_3}^{(2)} = 0.65$, $k_{N_1}^{(3)} = 0.70$, $k_{N_2}^{(3)} = 0.20$, $k_{N_3}^{(3)} = 0.10$. And the Michaelis-Menten constants for the toxins are $k_{T_i}^{(i)} = 4$ and $k_{T_j}^{(i)} = 2$ for $i \neq j$. The toxin production factors are $f_m^{(i,i)} = 0.5$ and $f_m^{(i,j)} = 0$ for $i \neq j$, with $\sigma_i = 0.2 + \Delta\sigma$ for all i. The Hill coefficients are $p = 9.36$ and $q = 1$. The stoichiometries for nutrient consumption are $S_{11} = S_{S22} = S_{33} = 1$ and $S_{12} = S_{23} = S_{31} = 1.8$ and $S_{13} = S_{21} = S_{32} = 2$.(A) Time series of all three strains showing persistence through chaos. (B-D) Bifurcation graph shows three different outcomes: C-coexistence, P-persistence and E-extinction of at least one species. (b) As α increases, the system changes from a stable coexistence state to limit cycles and chaos, until one of the strains extincts. (C) Increasing p causes transition from steady state coexistence to persistence before the strain is outcompete. (D) Setting the hill coefficient $p_n = 5$ to show transition by varying nutrient sensing.Smaller $\Delta\sigma$ corresponds to stronger nutrient sensing, which promotes persistence.

previous section, we show both types of persistence can be sustained with a smaller number of species, or to be more specific with only two species, competing for essential resources by introducing regulation of toxin production through nutrient sensing. Another common way of regulation is through toxin sensing. On top of sustaining persistence with few species, as we will demonstrate below, there is a major difference that sets toxin sensing apart from nutrient sensing: it can increase diversity. In this section, we first demonstrate the ability of toxin sensing to generate oscillations with only two species, in the limits where resource competition is not growth limiting. We then decouple the cost beared by each species in toxin production. Unlike nutrient sensing which involves only the transition from persistence to coexistence with no change in diversity of the system, increasing strength of toxin sensing can increase diversity and prevent the community from collapsing.

Here, we first look at a system with only two toxins T_1 and T_2 with concentrations n_{T_1} and n_{T_2}, and two strains X_1 (X_2) with abundances x_1 (x_2). Assuming resource availability in the environment is in abundance, the bottleneck of bacterial growth is described by ν, which is a constant. The growth rate functional can be

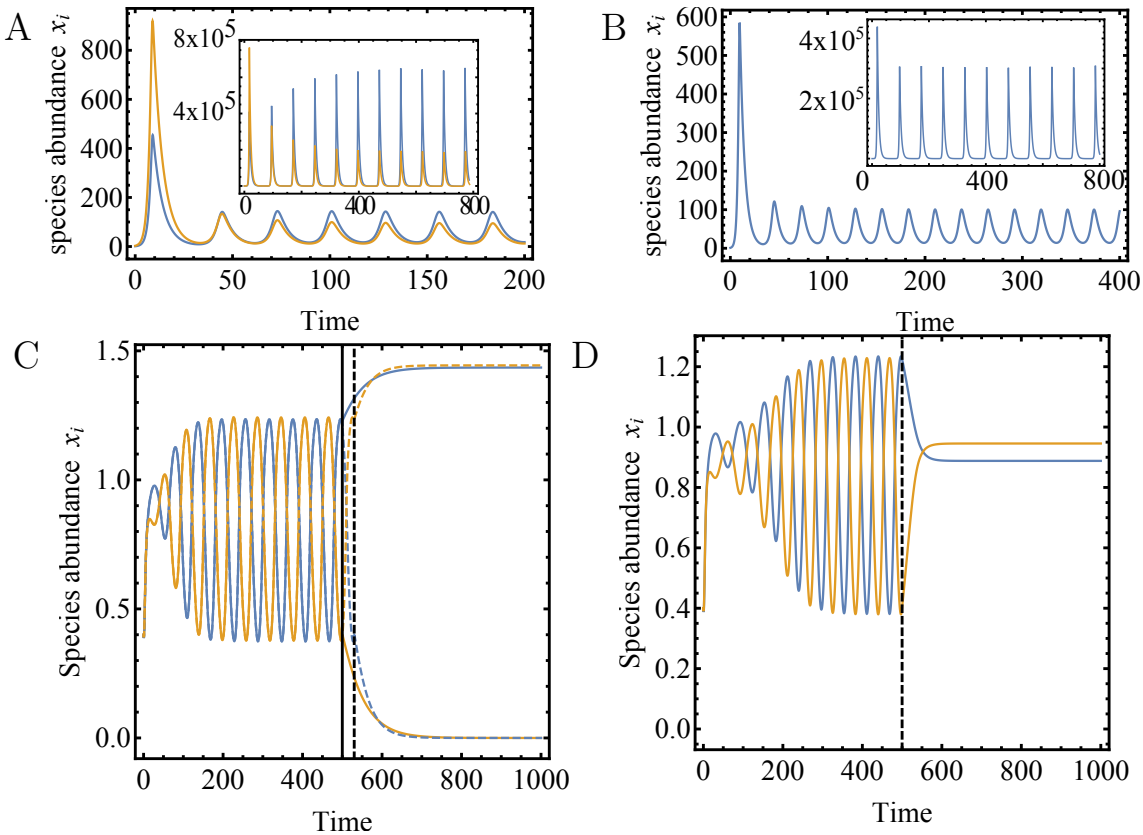

Figure 5: (A) A system with two toxins as abiotic factors in an environment with abundance nutrients and two species with maximum growth rate $g_m = 1.20$ in a chemostat with flow rate $F = 0.2$. Nutrient consumption is not a growth limiting factor here. The nutrient efficient is set to be $\nu = 5$, and the Michaelis-menten constants are $k_{T_1}^{(1)} = 4.0$, $k_{T_2}^{(1)} = 2.0$, $k_{T_1}^{(2)} = 2.2$, $k_{T_2}^{(2)} = 3.8$. The Hill coefficient for toxin sensitivity is $q = 3$. The maximum value of toxin production fraction is $f_m^{(i,i)} = 0.8$, and the scale is set to be $\sigma_1 = 6.74746$ and $\sigma_2 = 6.41009$. The Hill coefficient for toxin sensing are set to be $p = 4$ and $p = 8$ in inset to show dependence of fluctuation magnitude on sensing. (B) A system with one toxins as abiotic factors in an environment with abundance nutrients and one single species with maximum growth rate $g_m = 1.20$ in a chemostat with flow rate $F = 0.2$. Nutrient consumption is not a growth limiting factor here. The nutrient efficient is set to be $\nu = 5$, and the Michaelis-menten constants are $k_T = 2$. The Hill coefficient for toxin sensitivity is $q = 3$. The maximum value of toxin production fraction is $f_m = 0.8$, and the scale is set to be $\sigma = 6.4$. The Hill coefficient for toxin sensing are set to be $p = 4$ and $p = 8$ in inset to show dependence of fluctuation magnitude on sensing. (C-D) A system with two toxins, two essential nutrients and two species. Same parameters with Fig. 6A, except $p = 3$ and $p_s = -2$ with Michaelis-Menten constants for the nutrients to be $k_{N_1}^{(1)} = 0.35$,$k_{N_2}^{(1)} = 0.65$, $k_{N_1}^{(2)} = 0.70$,$k_{N_2}^{(2)} = 0.30$ and the stoichiometries are $S_{ii} = 2(1)$ and $S_{ij} = 1(2)$ for $i \neq j$ in Fig. 6C (D). The regulations are turned off either at $t = 500$ (black solid line) $t = 530$ (black dotted line) in (C), which leads to two different pure state outcomes showing bistability, or at $t = 500$ in (D) which leads to coexistence. In both cases, persistence can be maintained when there is strong regulation in toxin production.

written as

$$g_i = \frac{g_m}{1 + \nu^{-1}(1 - \Sigma_j f_{ij})^{-1} + \left(\frac{n_{T_1}}{k_{T_1}^{(i)}}\right)^q + \left(\frac{n_{T_2}}{k_{T_2}^{(i)}}\right)^q} . \tag{13}$$

Strain X_1 (X_2) is the sole toxin producer in the system that release T_1 (T_2). To generate persistence, the species are also sensitive to the toxins they produce, akin to the idea of ecological suicide [29]. Furthermore,

the amount of toxin a species produce depends on the toxin level produced by its competitor.

$$f_{ii} = \frac{f_m^{(i,i)}}{1 + (\sigma_i / n_{T_j})^p} \; ; \quad f_m^{(i,j)} = 0 \qquad j = (i\%d) + 1 \; . \tag{14}$$

The Hill coefficient for sensing is set to be positive, meaning that the production of toxins is regulated such that when the level of toxins T_j produced by its competitor exceeds certain level σ_i, the strain responds by increasing the toxin production fraction f_{ij}. The intuition is that as more toxins are produced by its competitor, the production fraction f increases as well, along with a drop in growth rate due to the cost incurred in toxin production. As abundance of the toxin producer drops, the amount of toxin also decreases, leading to a decrease in the production fraction f, which is the underlying mechanism to generate oscillations. Interestingly, as p becomes more positive, meaning the toxin sensing becomes more acute, the abundances can fluctuate between several orders of magnitude. The species abundance time-series is plotted in Fig. 5A, showing persistence behavior through oscillation. In particular, differ from previous section with nutrient sensing, the species abundances are in phase with each other. As toxin sensing is switched off, the fixed point is stable and the two species coexist at steady state. When $p < 0$, it is not as interesting as diversity is now maintained at coexistence. The effect of toxin regulation on generating oscillation is shown in supplementary (SM Sect. 3).

Using this scheme of toxin sensing, the abundance between two species are coupled together and locked in phase. It is suggestive that the underlying mechanism to oscillation does not require the presence of multiple species. To test this idea, we can look at a system with only one single species one and one toxin, meaning

$$g = \frac{g_m}{1 + v^{-1}(1 - f) + \left(\frac{n_T}{k}\right)^q} \; ; \qquad f = \frac{f_m}{1 + (\sigma / n_T)^p} \; . \tag{15}$$

The index i is dropped because there is only one single species. As expected, the single species has a qualitatively similar behavior compared to the two-species system, as shown in Fig 5B. Persistence of species abundance is caused by self-suppression due to toxin production, and can lead to huge fluctuations of orders of magnitude similar to observations in experiments involving ecological suicide [29]. It is difficult to increase the diversity through this type of oscillation without increasing the number of suppled nutrient sources, since small difference in average fitness will drive the system to pure state.

To decouple the costs of each species, we then treat v to be resource dependent, so that bacterial growth now relies on two different resources, and different toxins is produced using different resources. Instead of (15), we consider (9), (10) and (14), where the toxin producers are now using different growth limiting resources to produce toxins. In other words, we consider the following growth rate functional:

$$g_i = \frac{g_m}{1 + \Sigma_j \left(\frac{n_{N_j}}{k_{N_j}^{(i)}}\right)^{-1} \left(1 - \frac{f_m^{(i,j)}}{1 + (\sigma / n_{T_j})^p}\right)^{-1} + \Sigma_j \left(\frac{n_{Tj}}{k_{T_j}^{(i)}}\right)^q} \; . \tag{16}$$

Fig. 5C and D shows the case where diversity in the system is sustained by persistence. Notice in Fig. 5C there is a transition from persistence to pure state by switching off toxin production, and diversity is decreased. The phase diagram is plotted in SM Fig. S5A. Unlike nutrient sensing, persistence can be generated both with $m_2 > m_1$ and $m_1 > m_2$ in (11), which are controlled by the stoichiometries S_{ij} and in turn change the stability of the coexistence fixed point. As a result, when toxin production is switched off, the behavior is different under these two regimes, going to either pure state or coexistence. This has two implications: (i) in addition to the transition from coexistence to persistence as shown in nutrient sensing, there can now a change in number of coexistence species due to toxin production and; (ii) regulation allows species in the consortium to adopt strategies, defined in terms of the Michaelis constants, which would not be wiped out by competition, thus diversifying these strategies to cope against environmental fluctuations. There is, however, a fundamental difference between the resource-dependent and resource-independent toxin production scheme. In order to generate oscillations, the Hill coefficient for toxin sensing is negative, or $p < 0$, which results in an oscillation of the abundances of the two species that are out of phase. Intuitively, this means as the level of toxins produced by the competitor increases, the production fraction f decreases. However, the amount of toxin produced and the cost incurred in the producer, are not monotonic in f and

depend also on resources N, which bounds the amount of toxins that can be produced, and that in turn changes the environment due to the consumption of resources. Therefore, this results in the phase difference between the abundances of the two toxin producers, which is similar to nutrient sensing. However, unlike nutrient sensing, changing the strength of toxin sensing p can lead to a transition from a bistability to persistence and therefore a change of diversity; whereas in nutrient sensing we do not see transitions from bistability to persistence.

5 Discussion

In this paper, we explore how allelopathic interactions and costs incurred from toxin production can increase the diversity of a system. Using a coarse-grained model in metabolism and protein biosynthesis, we introduce metabolic constraints into the growth rate functional that depends on both nutrient and toxin levels, and apply it to chemostat systems. For steady-state coexistence, it requires all species to reach the same fitness, namely equalization. In a system with only a single growth-limiting factor, this is not possible since there is no trade-offs. By introducing toxins into the system, we expand the dimensionality of the abiotic factor space and apply trade-offs to the Michaelis constants such that equalization between a number of species is now possible even though the system is supplied externally with only one single nutrient. Depending on the ZNGIs of the producer in relation to other strains, the steady state behavior can either be bistability or stable coexistence state. This gives two very different strategies for the toxin producer to survive. For the former case, the initial species abundances plays an important role and can drive the system to dominance by either species that is most adapted to resource or to the toxin. For the latter case, as long as the nutrient supply is of abundance, coexistence can be reached at steady state, given that the toxin producer is present in the system. Without the toxin producer, the community would collapse and only one single species would survive: the species with the smallest Michaelis-Menten constant for nutrient uptake. Coexistence cannot be obtained even if toxin is externally supplied unless the the supply concentration of toxins is fine-tuned. In this sense, the toxin producer acts as a keystone species of the system. Further implication from equalization is discussed below.

Next, we incorporate the regulation of toxin production into our model. In particular, we consider nutrient sensing and toxin sensing. For nutrient sensing, it is shown that persistence can be maintained due to regulation, though this does not point to an increase of diversity. Indeed, the transition towards persistence is from a coexistence fixed point to either limit cycles or chaotic trajectories, with no change in the number of species surviving in the system. In particular, only two toxin producers are required to generate oscillations and chaos. We also show the case of a rock-paper-scissor game consists of three toxin producers to generate chaotic behaviors. With toxin sensing,by changing the parameters that affects these regulations, the system exhibits a transition from multi-stability to non-steady state persistence and/or steady state coexistence. We first demonstrate in a system where only toxin serves as the growth limiting factor that self-sustained oscillation is possible in the regime of bacterial suicide, meaning the presence of a single species can generate oscillation, with a variation of abundances of several orders of magnitude. Under this scheme, adding additional species would generate in-phase oscillations. To decouple the species, we added nutrients as growth limiting factors. Under this scheme, The abundances of the two species are out of phase, similar to nutrient sensing. In addition to that, since toxin regulation is decoupled from resource availability in the environment, transition from both either pure state and or to persistence is possible. As toxin production is increased, there is a transition from bistable state to oscillation in a two-species system, meaning there can be an increase in diversity due to toxin production. When toxin production is prohibited, the system simply collapsed such that only one species survives. Equivalently, this is to say that toxin sensing allows species to persist in regions in the phase space that would correspond to pure state in the case of nutrient sensing or the absence of toxin, and therefore can bring the system to these regions that are otherwise not attainable, and might have implications in evolutionary timescale which is beyond the scope of our work.

Both discussions above concerning systems with or without regulation has its implications in diversification beyond the upper limit set by competitive exclusion principle, which states that the maximum number of coexisting species is simply the number of abiotic factors. Based on our discussions above, there are two possible roles which toxin production can play to diversify the community composition against competitive

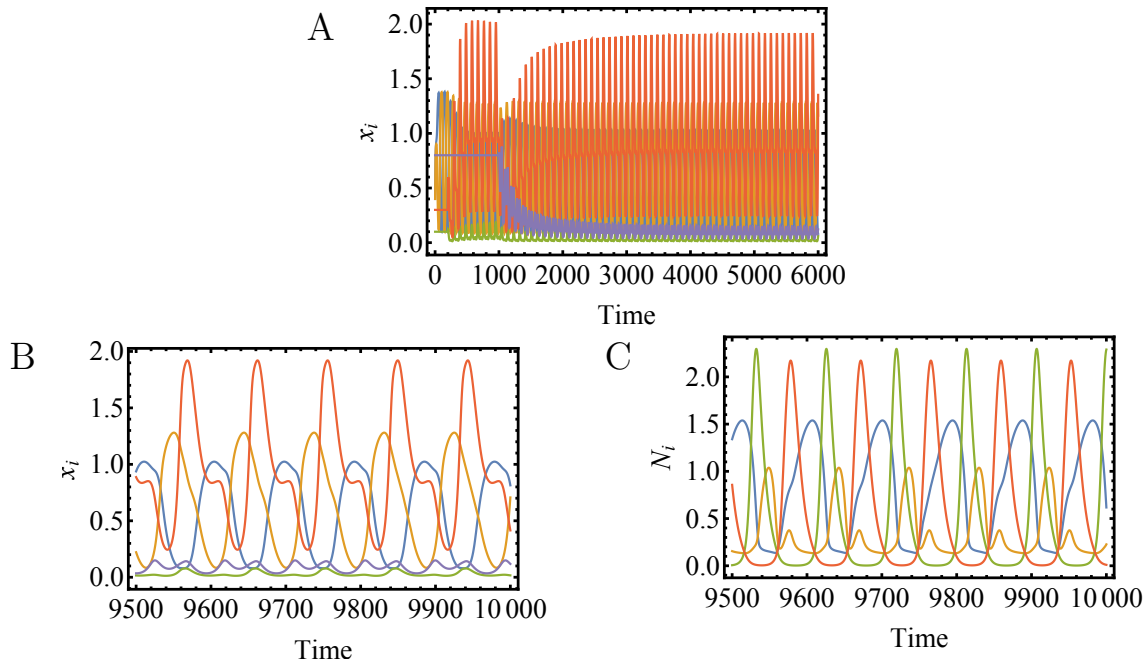

Figure 6: An example of violating competitive exclusion principle by persistence. The system consists of two toxin producers and 3 non-toxin producing strains, with four abiotic factors. (A) After transient dynamics, the five strains persist in limit cycle. Fluctuations of (B) strain abundances and (C) abiotic factors during $t = 9500$ and $t = 10000$. For parameters please refer to SM Sect. 5

exclusion: promoting coexistence at steady state and persistence. One of the ways to understand competitive exclusion principle is through equalization, which is addressed on our section regarding toxin production with no regulation. Assuming growth rate only depends on abiotic factors, every single strain that coexists at steady state has to attain equalized growth rate that is set by the flow rate of the chemostat:

$$g_i(\boldsymbol{n}) = F \ , \qquad i = 1, \cdots, M \ , \tag{17}$$

where M is the total number of species present in the system. This involves solving for $\dim(\boldsymbol{n})$ abiotic variables over M equations. Therefore, generally there is an upper bound to the total number of strains $M \leq \dim(\boldsymbol{n})$ that may survive in the system. As such, increasing the $\dim(\boldsymbol{n})$ is effectively increasing the upper bound. This idea of increasing the dimensionality of abiotic factors has been explored extensively with cross-feeding or syntrophy [26], but not so much in toxins. Increasing diversity through cross-feeding is natural since it mediates mutualistic interactions between the strains. On the other hand, the secretion of toxins harms both the toxin producing strain in the form of metabolic costs and its competitors, and its effects on diversity is not as intuitive as cross-feeding. Mathematically, both cross-feeding and toxins allow more strains to attain equalized growth rate without resorting to externally supplying more abiotic factors or nutrients into the system, since the species can produce additional abiotic factors, resulting in a larger M, or the dimensionality of the abiotic factor space. In addition to that, it allows trade-offs, in the form of Eq.(4). Such tradeoff is not possible if $\dim(\boldsymbol{n}) = 1$. For example, in systems where there is only one single growth-limiting resource supplied to the system, competitive exclusion should dictate the fate of the community when there is no dependency of bacterial growth on toxins. However, in our example, the inclusion of the toxin into our model now allows trade-off and equalization as it increases the dimensionality of $\boldsymbol{n}$. In addition to that, coexistence is only possible if the steady state solution is stable. In the deterministic system, this requires fine-tuning of the supplied vectors, which is defined as the ratio of the external supply of the growth limiting resource to that of the toxins; in the presence of noise long term coexistence is unlikely and the system are driven to pure states where only one species dominates. The production of toxins may stabilize or destabilize the steady state solution, depending on the toxin producer's fitness at different environment as defined by $\boldsymbol{n}$, which either leads to steady state coexistence or bistability.

Multi-stability has been explored in previous models under certain assumptions on the growth rates of the producers in relation to the environment [15, 24]. Here, we explore how a toxin producing strain can instead stabilize the system, and if the strain is the sole producer in the system, it can serve the role as a keystone species which sustains the diversity of the entire population. In combination with the trade-offs, the toxin producer can sustain a diversity with number of strains $N > \dim(\boldsymbol{n})$ at steady state.

In the previous section, we strictly impose the same global trade-off on the growth rate functional of every strains. This assumption may not be realistic, and other ideas of sustaining diversity without evoking a global trade-off is worth investigating. While competitive exclusion principle puts an upper limit on how many strains may survive in the system, it is only a statement on steady state behaviors and is not ascribed to non-steady state behaviors. In principle, we may have more persistent strains than the number of growth-limiting abiotic factors. In fact, this has been explored in [16] in resource competition models, although equalization and trade-offs or symmetries are often implicitly embedded in their models in the way to define these parameters. In particular, it has been shown that stable limit cycles can be sustained when there are three strains, since it introduces delay in the system, but not when there are only two strains which are "inherently unstable" [40]. As discussed in our results, by expanding the dimension of the system with toxin producers and introducing negative feedback, stable oscillations and chaotic trajectories can now be sustained in the presence of as few as two toxin producers, on the condition that toxin production is regulated. Our choice of considering only nutrient sensing and toxin sensing keeps the growth rates dependent only on growth limiting factors, which impose a more stringent condition to coexistence compared to quorum sensing as it keeps the degree of freedom in the growth rates small by not introducing dependencies on strain abundances. Notice however in the discussion above that the number of persistent strains are still less the the upper bound $N < \dim(\boldsymbol{n})$ in steady state coexistence. As a remark, fig. 6 showcases a system with four abiotic factors, which are the two essential resources and two toxins, each secreted by a single toxin producing strain. According the competitive exclusion principle, there can be at most four strains that coexist. However, since toxin production drivers internal oscillation of the environment, more than four strains can be packed into the system, thus accounting for a higher diversity than what is being traditionally allowed by competitive exclusion principle.

To conclude, in this paper we use a coarse-grained motivated model of metabolism, which exibits a diverse behavior from pure state to coexistence and persistence. The interplay between the effect of toxins and resource competition is explored in different regimes. Different from prior models, the production rate of toxins in our model satisfies proteome constraint while incoporating cost of production. Taking into account of regulation, oscillations can be generated, which mimics seasonality in algal bloom and the wide fluctuations of abundance in bacterial suicide. We also show chaotic attractor can exhibit in these system, implicating that abundance data that do not seem to be attracted to steady state may be explained by similar models. By introducing the toxins into our model as secondary metabolites, which are not necessarily supplied externally nor measured in experiments, we lower the number of required nutrient sources and species to generate persistence of both oscillation and chaos compared to [16, 17]. We have considered two different approaches which are used by toxin producing strains to enrich ecological diversity: either by equalizing and stabilizing the system at steady state, or by generating persistence. Both approaches can lead to a higher diversity than what is predicted by competitive exclusion principle. This opens up new directions to explore the role of toxins on diversity, as the bacterial arsenals is very diverse, and can hugely affect the dimensionality of the environmental space $\dim(\boldsymbol{n})$, not too dissimilar to cross-feeding in this regard. However, unlike cross-feeding which mediates positive interactions, toxin leads to negative interactions. In this work, effects of toxins on increasing diversity are illustrated when the number of abiotic factors is small and the system is highly ordered. As an extension of this work, it could be worthwhile to look at large systems by approaches similar to what have been done on cross-feeding[23, 26], and compares the structure of the communities driven by the two modes of interactions.

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