# INTERFACE

## Research

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# Insights into resource consumption, cross-feeding, system collapse, stability and biodiversity from an artificial ecosystem

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Community ecosystems at very different levels of biological organization often have similar properties. Coexistence of multiple species, cross-feeding, biodiversity and fluctuating population dynamics are just a few of the properties that arise in a range of ecological settings. Here we develop a bottom-up model of consumer–resource interactions, in the form of an artificial ecosystem 'number soup', which reflects basic properties of many bacterial and other community ecologies. We demonstrate four key properties of the number soup model: (i) communities self-organize so that all available resources are fully consumed; (ii) reciprocal cross-feeding is a common evolutionary outcome, which evolves in a number of stages, and many transitional species are involved; (iii) the evolved ecosystems are often 'robust yet fragile', with keystone species required to prevent the whole system from collapsing; (iv) non-equilibrium dynamics and chaotic patterns are general properties, readily generating rich biodiversity. These properties have been observed in empirical ecosystems, ranging from bacteria to rainforests. Establishing similar properties in an evolutionary model as simple as the number soup suggests that these four properties are ubiquitous features of all community ecosystems, and raises questions about how we interpret ecosystem structure in the context of natural selection.

## 1. Introduction

Ecosystems are defined as the sum of a community of living organisms and the abiotic factors they interact with [1]. Distinct ecosystems are found at all levels of biological organization: prey, predators and parasites interact within a habitat [2,3]; microbes cross-feed and compete for resources within artificial cultures or natural communities [4–6] and different types of cells interact by exchanging metabolites within an organism body [7,8]. In understanding the evolution of ecosystems, we have to consider how all its components interact [9–12]. This study leads us to questions such as: what determines the demographic structure or species composition [13], what results in lost of resilience or triggers collapse of the whole ecosystem [14] and how does biodiversity come about and be maintained [15]?

A common way of investigating ecosystem evolution is using evolutionary game theory models (reviewed in [16]). A classic example of this approach is the derivation of the competitive exclusion principle that two species competing for exactly the same resource cannot coexist [17]. More recently, evolutionary game theory has been used to look at microbial cross-feeding [13,18–21]. For instance, Doebeli [18] uses an adaptive dynamics model to show that when there is a trade-off between uptake efficiency of the primary and secondary metabolites, single strains can evolve to become two separate cross-feeding polymorphisms. In the evolutionary game theory approach, the fitness function for individuals is typically predefined, providing a top-down view of ecosystem structure. This approach allows us to investigate why certain trade-offs arise in ecosystems, but does not address the bottom-up question of how evolution acts to shape communities in the first place.

One bottom-up method to investigate evolution are 'artificial life' simulations [22–26]. Avida is one of the most notable models of this type [27]. In the virtual world of Avida there are a fixed number of grid points occupied by different digital organisms. Each has its own self-replicating and evolvable code, and they compete for their living space on the grids. Although interactions between digital organisms are taken into account, they are typically weak [28]. As such, Avida simulations investigate the evolution of individual complexity, rather than community-level patterns.

Inspired by the artificial life approach, several authors have started to look at artificial ecosystem evolution. For example, Bell makes a model of an artificial ecosystem composed of resources, producers and predators, and investigates a wide range of system-level properties such as, evolutionarily stable food webs can exist only when there is a small number of species [29]. Williams & Lenton [30,31] design a 'Flask' model, which explicitly considers the change in the environment arising as a side effect of microbes' metabolism, showing that ecosystem responses, in most cases, are originated from ecological interactions among species, rather than the additive combination of responses from individual species. In the context of consumer–resource interactions, Gerlee & Lundh [28] investigate digital organisms that transform metabolites from which they extract energy for self-maintenance and reproduction. The metabolites are represented by binary strings, and the energy extracted is defined equal to the increment of Shannon entropy of the metabolite before and after transformation. They find that diversity of the digital organisms is negatively correlated with biomass production and energy uptake, while it correlates positively with energy-uptake efficiency. Another example of artificial ecosystems comes from the model created by Crombach & Hogeweg [32]. They represent a metabolite as a binary string, and a digital organism transforms a metabolite by rotating it in a certain way defined by its genome. The organism then gets a 'score' that is used to calculate fitness. Organisms compete for space on a grid in a virtual world and the fitness decides whether an organism can occupy a grid point. Unlike Gerlee and Lundh's model, where metabolites can only be transformed in an entropy-increasing way, metabolites can be transformed backwards too. This allows for resource cycling, widely observed in nature, where resources or elements are cycled through the entire ecosystem [33,34].

One drawback of the 'artificial life'-style models is that the model definition is very complicated. This is in stark contrast to the mathematical elegance of evolutionary game theory models, which are defined in terms of a small number of equations. The complexity of artificial life models is problematic for three reasons. Firstly, results are difficult to reproduce and verify. To reproduce a study involves correctly understanding and implementing the details of the model, which is often far from straightforward. Secondly, mathematical analysis of the results is all but impossible. Artificial life models consist of large numbers of interrelated equations that cannot be easily simplified. Finally, and most importantly, model complexity obscures the generality of the insights that can be drawn from the simulations. While artificial life models appear to produce complex ecosystems, it is difficult to disentangle how much of that complexity is a result of complicated individual genotypes and how much of it emerges from mutual interactions. In studies of ecosystem complexity, we are usually more interested in the latter form of emergence.

In this paper, we introduce a bottom-up model of consumer–resource interactions. The model is based on one of the simplest possible mathematical structures, namely modular addition. In our model, each resource corresponds to an integer and each organism corresponds to an addition operation. Our choice of modular addition is made primarily because it is a simple model of how resources are transformed, via metabolism, by organisms. Moreover, modular addition forms a cyclic (mathematical) group, which means that they give a general representation of the problem of resource cycling.

We use our model to identify common properties of living ecosystems. In particular, we look at resource consumption, reciprocal cross-feeding, system collapse, stability and biodiversity. We now describe each of these properties in turn in the context of biological systems.

## 1.1. Resource consumption

There are many examples where ecosystems with a continuous inflow of resources tend to self-organize so that the inflowing resources are efficiently consumed. An example is the biotransforming granules formed in upflow anaerobic sludge blanket (UASB) reactors, which are the most commonly used reactors for degrading organic substances in waste water to $CH_4$ and $CO_2$ [35,36]. Different bacteria in UASB granules carry out sequential metabolic processes and the degradation can usually be achieved irrespective of the waste water composition, which only affects the size and composition of the granules. Another example comes from the guts of mice, which can be colonized by the microbiota of zebrafish and vice versa [37]. After colonization, the non-native microbiota self-organize to resemble the native microbiota composition, and the host animal responds very similarly to the non-native microbiota [9]. Because the diet of the host animal does not change after non-native microbial colonization, the inflow of the ecosystem in the guts is almost the same as before. So the non-native microbiota have to self-organize to adapt.

## 1.2. Reciprocal cross-feeding

Cross-feeding occurs when a microbe uses metabolites excreted by other microbes [16,20,21]. Generally, there are two types of cross-feeding: sequential and reciprocal. A well-known example of the former type is the polymorphism of *Escherichia coli* [4]. In a glucose-limiting chemostat culture, different strains of *E. coli* coexist, where one strain dominantly degrades glucose to acetate, and another strain degrades the acetate to final metabolites. One example of reciprocal cross-feeding is between *Salmonella enterica* ser. Typhimurium and an *E. coli* mutant that is unable to synthesize methionine, in a lactose-limiting environment [38]. In this case, the *E. coli* mutant metabolizes lactose and excretes the by product acetate, on which *Salmonella* feeds, while *Salmonella* excretes the amino acid necessary for the growth of *E. coli*. These are just two examples out of many [6], including complex networks of metabolite exchange in humans [39], termite gut microbiota [40], the diverse syntrophic microorganisms in deep-sea methane vents [41] and corrinoid cross-feeding in bacteria [40,42]. The question raised by reciprocal cross-feeding is how, given that species need each other to survive, this type of interaction begins in the first place [38].

## 1.3. System collapse

Sudden collapses are frequently observed in various ecosystems. These range from large spatial and temporal scales, such as coastal ecosystem collapses triggered by prolonged overfishing [14] and rainforest collapse in late Moscovian-Kasimovian [43], to small scales, such as sudden disintegration of sludge granules in UASB reactors [36]. Ecosystems are commonly said to be 'robust yet fragile' [44,45]. One demonstration of this property is the keystone species hypothesis [46–48]. That is, removal of one single non-dominating species, with respect to its relative small abundance, would cause dramatic degeneration of the whole ecosystem, e.g. the starfish *Pisaster* in rocky intertidal zones [49], and sea otters in coastal communities [50].

## 1.4. Stability and biodiversity

The classic question about biodiversity is the 'paradox of the plankton': how can a limited range of resources support dozens of phytoplankton species [51]. This biodiversity seems to violate the competitive exclusion principle [17]. Besides various factors attributed to complex and complicated external environment [52–56], Huisman and co-workers [10,15] showed, both theoretically and empirically, that even in a constant and well-mixed environment, the plankton community has rich biodiversity. The basic mechanism for this biodiversity is thought to be the chaotic nature of population dynamics driven by resource competition. While this provides a possible explanation to the biodiversity puzzle the question remains in which situations do we expect natural selection to produce stability or biodiversity?

We now investigate all four of these properties in the context of our own model of cyclic resources, with the aim of explaining how ubiquitous we expect these properties to be.

## 2. Model

Our model consists of *metabolites* which are resources for *species*. Each metabolite is an integer labelled $\bar{1}, \bar{2}, \ldots, \bar{n}$. The species, $s_{ij}$, of each organism is defined in terms of the two metabolites ($\bar{i}$ and $\bar{j}$) which it requires to reproduce. So, for example, species $s_{12}$ requires both a $\bar{1}$ and a $\bar{2}$. And if we take, for example, $n = 3$, there are three metabolites and six species in total. When an organism feeds on two metabolites, it excretes one or two new metabolites. The excreted metabolite is based on modulus-$(n+1)$ addition. Taking $n=3$ as an example, we then define the following chemical equations for how metabolites are transformed by each species:

$$\left.\begin{array}{l} s_{11}: \bar{1} + \bar{1} \to \bar{2} \\ s_{12}: \bar{1} + \bar{2} \to \bar{3} \\ s_{13}: \bar{1} + \bar{3} \to \bar{1} \\ s_{22}: \bar{2} + \bar{2} \to \bar{1} \\ s_{23}: \bar{2} + \bar{3} \to \bar{1} + \bar{1} \\ s_{33}: \bar{3} + \bar{3} \to \bar{1} + \bar{2} \end{array}\right\} \tag{2.1}$$

and

Generally, in the $n$-metabolite system, the chemical equation is

$$s_{ij}: \bar{i} + \bar{j} \to \begin{cases} \overline{i+j}, & \text{if } i+j < n+1 \\ \bar{1}, & \text{if } i+j = n+1 \\ \bar{1} + \overline{(i+j)\bmod(n+1)}, & \text{if } i+j > n+1 \end{cases} \tag{2.2}$$

Note that there is no metabolite $\bar{0}$ in the system. Instead, the $\bar{0}$ corresponds to energy leaving the system. The metabolite $\bar{1}$ also plays a special role because it is produced whenever the

modular operation produces a 'quotient'. From a biological point of view, the modular addition gives us a way of describing how metabolites are transformed cyclically, e.g. in (2.1), $\bar{1} + \bar{1} \to \bar{2}$ and also $\bar{2} + \bar{3} \to \bar{1} + \bar{1}$. In a microbial cross-feeding experiment, for example, *Salmonella* feeds on acetate and excretes methionine, while an *E. coli* mutant lives on methionine and lactose, and excretes acetate [38]. So comparing with (2.1), metabolite $\bar{1}$, $\bar{2}$ and $\bar{3}$ could be acetate, methionine and lactose, respectively, while species $s_{11}$ is *Salmonella* and $s_{23}$ is *E. coli*.

In order to reproduce, an organism uses both of its required metabolites. For example, in the 3-metabolite system, one organism of species $s_{23}$ reproduces according to the following chemical equation:

$$s_{23} + \bar{2} + \bar{3} \to 2s_{23} + \bar{1} + \bar{1}, \tag{2.3}$$

making a copy of itself and changing the composition of metabolites. From a physical point of view, this model does not violate the energy conservation law. To see this assume that every metabolite $\bar{i}$ contains $(i + 1)$ units of energy. For example, in (2.3), on the left-hand side metabolite $\bar{2}$ and $\bar{3}$, we assume that, contain 7 units of energy, and on the right-hand side, two metabolites $\bar{1}$ contain 4 units of energy. The 'missing' 3 units of energy can be interpreted as being used to reproduce one organism $s_{23}$. See the electronic supplementary material for details and a proof that the system conserves energy.

In the $n$-metabolite system, the reproduction of one organism of $s_{ij}$ can be written as the following chemical equation:

$$s_{ij} + \bar{i} + \bar{j} \to \begin{cases} 2s_{ij} + \overline{i+j}, & \text{if } i+j < n+1 \\ 2s_{ij} + \bar{1}, & \text{if } i+j = n+1 \\ 2s_{ij} + \bar{1} + \overline{(i+j)\bmod(n+1)}, & \text{if } i+j > n+1. \end{cases} \tag{2.4}$$

This gives a full set of possible transformations that can occur in our system, although we will later describe how we add mutations to the species. In the systems we study here, only one type of metabolite is externally added to the system. We denote it as the metabolite $\bar{u}$, and $\bar{u}$ is added at a constant rate $\mu$.

We let $R_i(t)$ be a variable giving the amount of metabolite $\bar{i}$ in the system at time $t$. Similarly, $S_{ij}(t)$ is the (discrete) number of organisms of species $s_{ij}$. We denote the total population size $N(t) = \sum_{i,j} S_{ij}(t)$. To simulate the changes in metabolites and organisms, we use the Gillespie algorithm. The steps of the simulation are as follows:

(i) Set $R_i(0) = 0$ so initially there is no metabolite and $S_{ij}(0) = 1$ so there is one of each species. Set $t = 0$.

(ii) On each step of the model we generate a time interval $\Delta t$ until the next event from an exponential distribution with parameter $N(t)$, i.e.

$$P(\tau \le \Delta t \le \tau + d\tau) = N(t)e^{-N(t)\tau} d\tau.$$

(iii) We increase the amount of metabolites in the system due to inflow according to

$$R_u(t) = R_u(t) + \mu \cdot \Delta t.$$

Note that here we update the metabolites at the current time step, rather than at time $t + \Delta t$. This potentially allows added metabolites to be immediately consumed by the organisms.

(iv) We select one individual organism from the entire population, so that the probability of selecting species $s_{ij}$ is $S_{ij}(t)/N(t)$.

(v) The selected organism either reproduces with probability $q(R_i(t), R_j(t))$ or dies with probability $1 - q(R_i(t), R_j(t))$. This probability is determined by the availability of metabolites, as follows:

$$q(R_i(t), R_j(t)) = \begin{cases} \dfrac{R_i(t)}{a + R_i(t)} \cdot \dfrac{R_j(t)}{a + R_j(t)}, & \text{if } i \neq j \\ \dfrac{R_i(t)}{a + R_i(t)} \cdot \dfrac{R_i(t) - 1}{a + R_i(t) - 1}, & \text{if } i = j, \end{cases}$$
(2.5)

where $a$ is a constant measuring the difficulty of obtaining metabolites. Here we employed the Monod equation (similar with the type II functional response), commonly used for describing the growth of microorganisms, to be the probability of uptake. An exception to this rule occurs when either $R_i(t) < 1$ or $R_j(t) < 1$ (if $i \neq j$), or $R_i(t) < 2$ (if $i = j$). In this case, the organism dies, as the metabolites do not exist in sufficient amounts.

If the selected organism dies, we set

$$S_{ij}(t + \Delta t) = S_{ij}(t) - 1.$$

If the selected organism reproduces, we update the metabolites and the organisms according to (2.4). For example, in the three-metabolite system, if the selected organism is $s_{23}$ and it reproduces, then

$$\begin{aligned} R_1(t + \Delta t) &= R_1(t) + 2, \\ R_2(t + \Delta t) &= R_2(t) - 1, \\ R_3(t + \Delta t) &= R_3(t) - 1 \end{aligned}$$
and
$$S_{23}(t + \Delta t) = S_{23}(t) + 1.$$

Those species and metabolites not involved in the reproduction take the same value at time $t + \Delta t$ as they did at time $t$. To introduce mutations, we assume that the new copy of $s_{ij}$ can mutate to another species with a constant probability $p$. For example, in the reproduction of $s_{23}$, shown in (2.3), there is a probability $p$ that the new copy of $s_{23}$ becomes one organism of species $s_{11}, s_{12}, s_{13}, s_{22}$ or $s_{33}$.

(vi) Update the time step, $t = t + \Delta t$. As a result of this updating, after one unit time of the simulation, on average, every individual has been selected, and completed a life cycle. We thus call one unit time, one generation.

(vii) Iterate from step (ii) until the simulation is complete.

In this paper, we mostly work with these stochastic simulations, but we also approximate and study our system using deterministic differential equations. The equations for $n$-metabolite system can be written as follows:

$$\left. \begin{aligned} \frac{dR_u}{dt} &= \mu + \sum_{i=1}^{n} \sum_{j=1, j \geq i}^{n} A_{uij} S_{ij} q(R_i, R_j), \\ \frac{dR_k}{dt} &= \sum_{i=1}^{n} \sum_{j=1, j \geq i}^{n} A_{kij} S_{ij} q(R_i, R_j), \\ & \quad k = 1, 2, \ldots, n, \quad \text{and} \quad k \neq u \\ \frac{dS_{ij}}{dt} &= S_{ij}(2q(R_i, R_j) - 1), \\ & \quad i, j = 1, 2, \ldots, n, \quad \text{and} \quad j \geq i, \end{aligned} \right\}$$
(2.6)

where $A_{kij}$ is the net production of metabolite $\overline{k}$ for one organism of species $s_{ij}$, which could be worked out according to (2.2). Example equations for the 3-metabolite system can be found in the electronic supplementary material.

The number of metabolites, $n$, and the inflowing metabolite $u$ are key parameters in the model. We choose four specific examples on which we focus our study. For investigating resource consumption, we set $n = 2$ and $u = 1$. For investigating reciprocal cross-feeding, we set $n = 3$ and $u = 2$. For investigating system collapse, we set $n = 3$, $u = 2$ and $n = 6$, $u = 2$. For investigating stability and biodiversity, we set $n = 6$, $u = 2$ and $n = 9$, $u = 7$. We could use other settings, but the choices here provide good illustrations of the main outcomes that occur in the models. A simulator is available here (http://collective-behavior.com/apps/numbersoup/).

The other parameters for the model are $a$, $\mu$ and $p$. For all the simulations in this paper, we set $a = 5$. Provided $a > 1$, the results are not sensitive to the value of $a$. For the setting $n = 2$ and $u = 1$, we set $\mu = 500$ and $p = 0.001$. The choice of $\mu$ is quite arbitrary, but just to make the total population of all species not too small and the computing time not too long. The choice of $p$ is to tune the number of mutated newly-born organisms so that it is small enough but not zero, that is, $N(t) \cdot p \approx 1$. For the same reason, we set $\mu = 500$ and $p = 0.001$ for the setting $n = 3$ and $u = 2$. When $n$ is larger, there are more species potentially coexisting, so the inflow rate $\mu$ should be larger accordingly, in order that the populations of different species are not too small. Thus, we set $\mu = 2000$ and $p = 0.0002$ for the setting $n = 6$ and $u = 2$; $\mu = 5000$ and $p = 0.00005$ for the setting $n = 9$ and $u = 7$.

# 3. Results

## 3.1. Resource consumption

We first consider a system of two metabolites ($n = 2$) with inflowing metabolite $\overline{1}$ ($u = 1$). An example of the system's evolution is shown in figure 1. Initially, species $s_{11}$ consumes the readily available metabolites $\overline{1}$ and grows exponentially (figure 1$a$,$b$). At generation $t = 11$, metabolite $\overline{1}$ is depleted and the population of $s_{11}$ decays. However, when a mutation produces an individual of species $s_{22}$, its population grows exponentially, consuming metabolite $\overline{2}$ produced by $s_{11}$. As $s_{22}$ produces $\overline{1}$, the $s_{11}$ population recovers and starts to grow again. By generation $t = 16$, both metabolites are now fully exploited, and there is a decay in both species. Around generation $t = 20$, the system reaches an equilibrium where the metabolites are nearly all consumed, and the populations of each species are relatively stable: $S_{11} \approx 2N/3$, $S_{12} \approx 0$ and $S_{22} \approx N/3$. An example of this demographic structure is illustrated in figure 2$a$.

Although stable for several hundred generations, this first demographic structure is transient. Around generation $t = 800$, there is a sharp decrease in both $S_{11}$ and $S_{22}$ and increase in $S_{12}$ (figure 1$c$). The system is then nearly stable at the second demographic structure, shown in figure 2$b$, where $S_{11} \approx N/2$, $S_{12} \approx N/2$ and $S_{22} \approx 0$.

Although this demographic structure is quite stable, it is invaded now and again by species $s_{22}$ (see e.g., generation $t = 1130$ and 2900). However, $s_{22}$ does not persist for a long time and the proportion of $s_{22}$ varies a lot. These fluctuations are not negligible and correspond to a demographic structure with all three species coexisting. An example of such a demographic structure is illustrated in figure 2$c$.

In order to check that these demographic structures, seen in a single simulation, are representative of the typical evolution of the system, we repeated the simulation 1000 times. Figure 3 shows how the frequency of different demographic structures
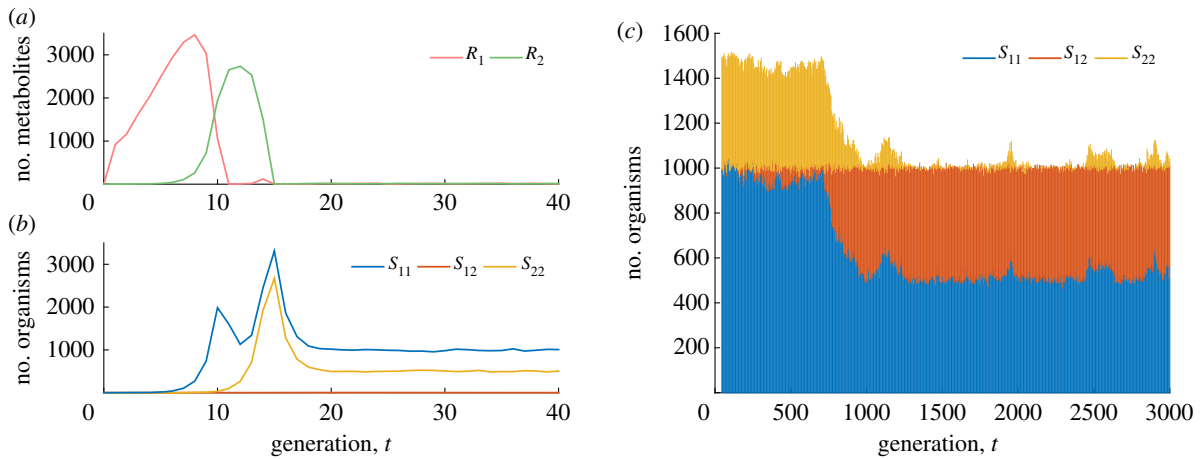
**Figure 1.** Example evolution for the system with $n = 2$ and $u = 1$. (a) The number of metabolites for the first 40 generations of the simulation. (b) The number of organisms of the three possible species for the first 40 generations. (c) The total population divided into species type over 3000 generations of the simulation. The populations here are cumulative so that, for example, at generation $t = 500$, $S_{11} = 949$, $S_{12} = 35$ and $S_{22} = 460$. (Online version in colour.)
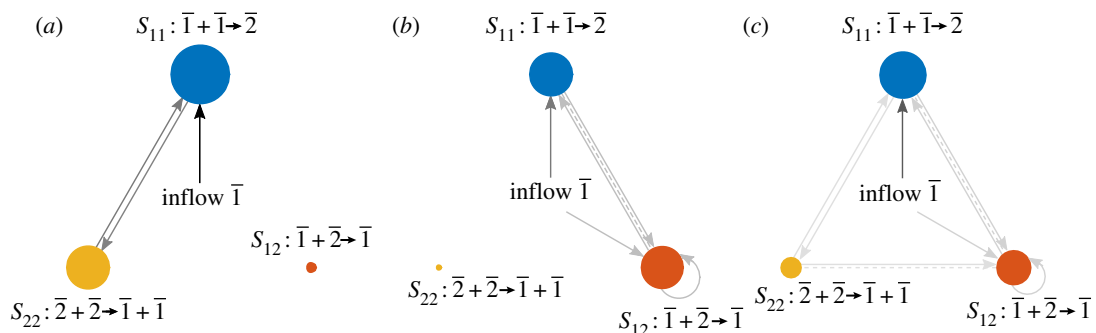


**Figure 2.** Demographic structures arising when $n = 2$ and $u = 1$. Species are represented by coloured circles, with the area of the circle proportional to the size of the population. A dash line between two species indicates that they both require the same metabolites. An arrow indicates that the species at the source of the arrow produces metabolites consumed by the species at the point of the arrow. The shading of the arrow is proportional to the total number of metabolites the species at the point of the arrow receives from the species at the source of the arrow (see the electronic supplementary material for details). (a) The first demographic structure occurring at generation $t = 200$. It has $S_{11} = 963$, $S_{12} = 26$ and $S_{22} = 517$. (b) The second demographic structure occurring at generation $t = 1600$. It has $S_{11} = 518$, $S_{12} = 495$ and $S_{22} = 7$. (c) The demographic structure occurring at generation $t = 1130$. It has $S_{11} = 642$, $S_{12} = 355$ and $S_{22} = 133$. All parameter values as in figure 1. (Online version in colour.)

changes over time. After 100 generations, 68.0% of simulations resulted in the first demographic structure, where $s_{11}$ and $s_{22}$ coexisted (figure 2a). This demographic structure decreased over time, however, and by 3000 generations the first demographic structure almost always disappeared. After 3000 generations, 84.9% of simulations resulted in the second demographic structure where $s_{11}$ and $s_{12}$ coexisted (figure 2b). The other 15.1% of simulations resulted in the demographic structure where all the three species coexist (figure 2c).

The differential equations corresponding to this system ($n = 2$ and $u = 1$) have two steady states (see the electronic supplementary material for details). The first steady state has $S_{11} = 2\mu$, $S_{12} = 0$ and $S_{22} = \mu$, corresponding to the first demographic structure (figure 2a). This steady state is unstable, explaining why the demographic structure does not persist in the simulations. The second steady state of the differential equations has $S_{11} = \mu$, $S_{12} = \mu$ and $S_{22} = 0$, corresponding to the second demographic structure (figure 2b). This second steady state is stable, explaining why it is the predominant outcome of our simulations. There is no stable steady state corresponding to the coexistence of all three species (figure 2c), and thus the 15.1% of simulations where $s_{22}$ coexists with $s_{11}$ and $s_{12}$ can be primarily attributed to short-lived mutations spreading, but then failing to compete with the existing demographic structure.

In all the simulations, the available metabolites are fully consumed. From the steady states of the differential equation, we find that $R_1 = R_2 = (2a + 1 + \sqrt{8a^2 + 1})/2$ for the first (unstable) steady state, while $R_1 = (2a + 1 + \sqrt{8a^2 + 1})/2$ and $R_2 = (2a - 1 + \sqrt{8a^2 + 1})/2$ for the second (stable) steady state. So the number of metabolites at both steady states is not related to the inflow rate $\mu$, but only relates to $a$, the parameter that determines the difficulty for organisms to obtain metabolites. In the electronic supplementary material, we show that every steady state of the underlying differential equations for a particular system corresponds to a state where all the inflowing metabolites are constantly consumed up by the whole population. As a result, for any number of metabolites, there is self-organization of species abundance to consume all available resources. This is a general feature of all $n$-metabolite systems with any inflow.

## 3.2. Species loops

For two metabolites, it is possible to use the differential equation model to analyse the system. However, the complexity of the underlying differential equation increases with the number of species, and mathematical analysis becomes complicated. We thus now develop a concept, which we call the species loop, for identifying properties of
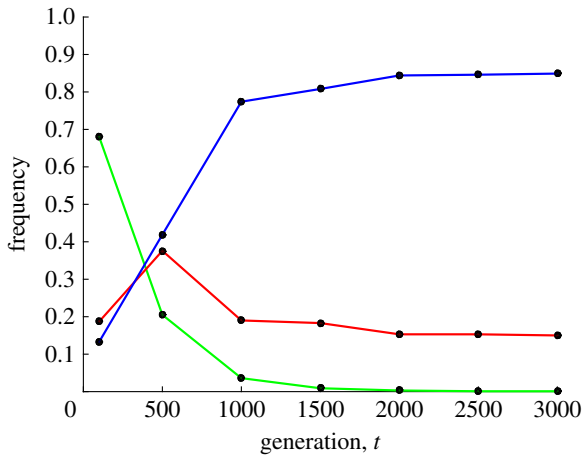
**Figure 3.** The frequency over generations of three different demographic structures ever occurred during simulations for $n = 2$ and $u = 1$. When classifying demographic structures from the stochastic data of simulations, we only consider the main species which together constitute more than 90% of the whole population (see the electronic supplementary material for details). The green line (the line starting with frequency 0.68) is the demographic structure where $S_{11} \approx 2N/3$, $S_{12} \approx 0$ and $S_{22} \approx N/3$, illustrated in figure 2a. The blue line (the line starting with 0.12) corresponds to the demographic structure where $S_{11} \approx N/2$, $S_{12} \approx N/2$ and $S_{22} \approx 0$, illustrated in figure 2b. The red line (the line starting with 0.19) corresponds to the demographic structure with all the three species coexisting. One example of this demographic structure is illustrated in figure 2c. (Online version in colour.)

the demographic structures in $n$-metabolite systems. The name species loop arises from the term 'microbial loop' that is used to describe a group of trophic interactions among marine microbes that primarily serve as a sink of the fixed carbon in the system [57,58].

We define a *species loop* as the minimal set of species that, when provided with its required metabolites, after every organism in the set has reproduced the only metabolite that has been consumed is the inflowing metabolite and no additional metabolites are produced. This is a rather complicated definition and is best illustrated with an example. Consider a set containing two organisms of $s_{11}$ and one of $s_{22}$. When all of the organisms have reproduced, the following chemical reaction has been performed

$$2s_{11} + s_{22} + \bar{1} + \bar{1} + \bar{1} + \bar{1} + \bar{2} + \bar{2}$$
$$\rightarrow 4s_{11} + 2s_{22} + \bar{2} + \bar{2} + \bar{1} + \bar{1}.$$
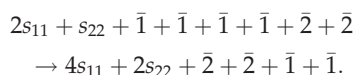
Thus the only metabolite that has been consumed is $\bar{1}$, and we write that $2s_{11} + s_{22}$ is a species loop for $u = 1$. We further define $\Omega$ to be the number of inflowing metabolites that a species loop consumes. The species loop $2s_{11} + s_{22}$ consumes two metabolites $\bar{1}$, so $\Omega = 2$.

Whether or not a set of organisms is a species loop depends on the inflowing metabolite. For example, $s_{11} + s_{12}$ has the chemical reaction

$$s_{11} + s_{22} + \bar{1} + \bar{1} + \bar{2} + \bar{2} \rightarrow 2s_{11} + 2s_{22} + \bar{2} + \bar{1} + \bar{1}$$
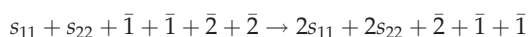
and is thus not a species loop for $u = 1$, but is a species loop for $u = 2$ with $\Omega = 1$. A similar calculation shows that $s_{11} + 2s_{22}$ is not a species loop for either $u = 1$ or $u = 2$, because it consumes metabolite $\bar{2}$ and also produces $\bar{1}$. We also require that a species loop is minimal. For example, $4s_{11} + 2s_{12} + s_{22}$ consumes six metabolites $\bar{1}$. However, because it can be broken down into a $2s_{11} + s_{22}$ and two instances of $s_{11} + s_{12}$, it is not minimal and thus not a species loop.
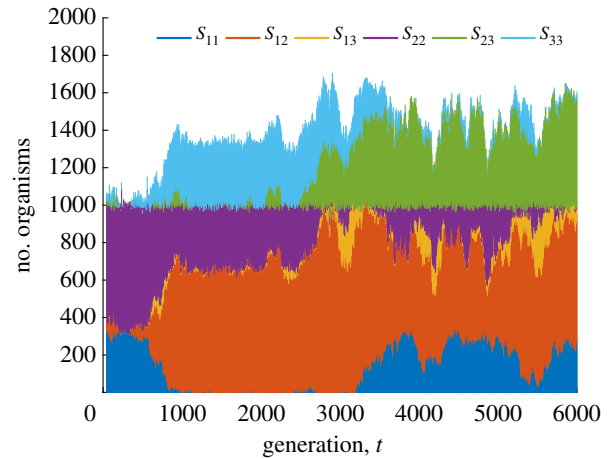


**Figure 4.** Example evolution over 6000 generations for the system with $n = 3$ and $u = 2$. (Online version in colour.)

The first demographic structure (figure 2a) is composed of the species loop $2s_{11} + s_{22}$. Similarly, the second demographic structure (figure 2b) is composed of the species loop $s_{11} + s_{12}$. Note that in both cases, the number of species loops fluctuates around $2\mu/\Omega$ (for detailed derivation, see the electronic supplementary material). Moreover, the demographic structure in figure 2c is made of 72.2% of the species loop $s_{11} + s_{12}$ and 27.8% of the species loop $2s_{11} + s_{22}$. When $n$ is small, it is relatively straightforward to work out the compositions of species loops given a certain demographic structure, while it is far from straightforward for large $n$ systems. We have developed an algorithm that calculates species loops for systems with arbitrary $n$ and $u$ (see the electronic supplementary material for details). In the electronic supplementary material, we show that the algorithm works well for systems with $n$ smaller than 7 (there are in total 28 possible species in the 7-metabolite system), but it takes a very long computational time if $n$ is larger.

## 3.3. Reciprocal cross-feeding
Now we investigate the system with three metabolites ($n = 3$) and inflow of metabolite $\bar{2}$ ($u = 2$). Figure 4 shows one example evolution over 6000 generations. Up until generation 700, the system stays at the demographic structure where $S_{11} \approx N/3$, $S_{22} \approx 2N/3$ and other species are found at only low levels. This demographic structure is illustrated in figure 5a. It is composed of the species loop $s_{11} + 2s_{22}$ with $\Omega = 3$, and corresponds to one set of steady states of the differential equations that $S_{11} = 2\mu/3$, $S_{22} = 4\mu/3$ and $S_{12} = S_{13} = S_{23} = S_{33} = 0$ (see the electronic supplementary material for details regarding analysing the differential equations, and why it corresponds to one set of steady states).

From the linear stability analysis of the differential equations for this system, we see that this set of steady states is unstable. So after generation 700, in this particular example, species $s_{12}$ invades. Then the population of $s_{22}$ shrinks and $s_{11}$ is driven towards extinction. This is because when metabolites are scarce, $s_{12}$ is more efficient in obtaining $\bar{2}$ than $s_{22}$, and it is also more efficient in obtaining $\bar{1}$ than $s_{11}$ (see (2.5)). At the same time, as $s_{12}$ produces $\bar{3}$, species $s_{33}$ starts to flourish, consequently producing the metabolites $s_{12}$ and $s_{22}$ need. Then another equilibrium, the demographic structure where $S_{12} \approx N/2$, $S_{22} \approx N/4$, $S_{33} \approx N/4$ and other
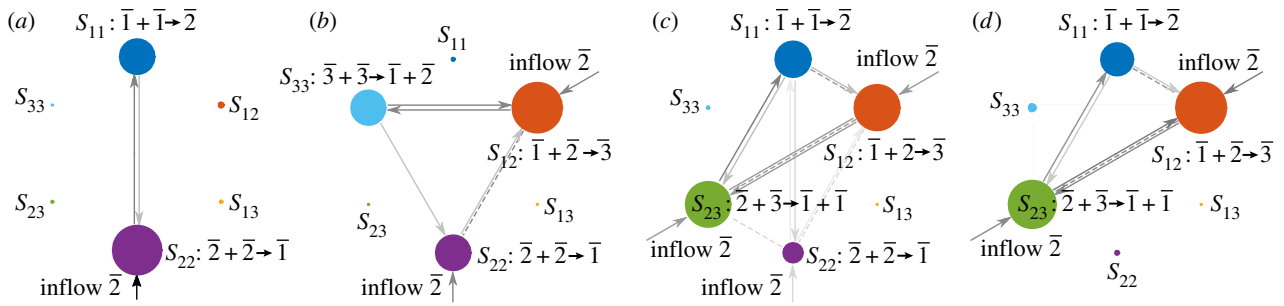
**Figure 5.** Demographic structures arising in the example evolution (as shown in figure 4). (a) The demographic structure occurred at generation $t = 400$. (b) The demographic structure occurred at generation $t = 1500$. (c) The demographic structure occurred at generation $t = 4000$. (d) The demographic structure occurred at generation $t = 6000$. (Online version in colour.)
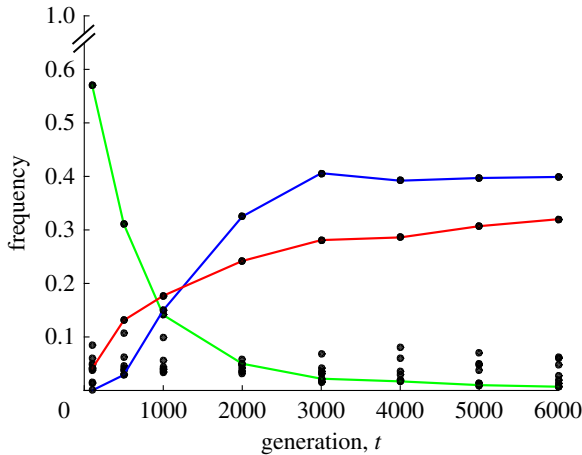


**Figure 6.** The frequency over generations of several most frequent demographic structures occurred during simulations for the system with $n = 3$ and $u = 2$. The green line (the line starting with frequency 0.58) corresponds to the demographic structure where $S_{11} \approx N/3$, $S_{22} \approx 2N/3$ and other species are at low levels, illustrated in figure 5a. The blue line (the line starting with 0) corresponds to the demographic structure where $S_{11} \approx N/5$, $S_{12} \approx 2N/5$, $S_{23} \approx 2N/5$ and other species are at low levels, illustrated in figure 5d. The red line (the line starting with 0.05) corresponds to the demographic structure where the four species $s_{11}$, $s_{12}$, $s_{22}$ and $s_{23}$ coexist. One example is illustrated in figure 5c, but note that the proportions of different species can vary a lot among simulations. The black dots off the three lines represent other infrequent demographic structures. (Online version in colour.)

species are at low levels (illustrated in figure 5b), is established. It is composed of the species loop $2s_{12} + s_{22} + s_{33}$ with $\Omega = 3$, and corresponds to a steady state where $S_{12} = 4\mu/3$, $S_{22} = 2\mu/3$, $S_{33} = 2\mu/3$ and $S_{11} = S_{13} = S_{23} = 0$. This state is also unstable and thus transient.

After generation 2500, the demographic structure is invaded by species $s_{23}$, as during the last equilibrium, metabolite $\bar{2}$ and $\bar{3}$ are always available. $s_{23}$ is more efficient in obtaining metabolites than $s_{22}$ and $s_{33}$. Then there is a decay in both species $s_{22}$ and $s_{33}$, followed by an increase in the population of species $s_{11}$. Around generation 4000, the system stays at the demographic structure where the four species $s_{11}$, $s_{12}$, $s_{22}$ and $s_{23}$ coexist, but the proportions of different species vary a lot. One example of this demographic structure is illustrated in figure 5c. There is, however, no steady state of the differential equations corresponding to this demographic structure.

As $s_{22}$ is less efficient in obtaining metabolite $\bar{2}$ than $s_{12}$ and $s_{23}$, its population remains only at a low level and it sometimes goes extinct. At the end of 6000 generations, the

demographic structure where $S_{11} \approx N/5$, $S_{12} \approx 2N/5$, $S_{23} \approx 2N/5$ and other species are at low levels, is established, illustrated in figure 5d. It corresponds to a steady state where $S_{11} = 2\mu/3$, $S_{12} = 4\mu/3$, $S_{23} = 4\mu/3$ and $S_{13} = S_{22} = S_{33} = 0$, thus composed of the species loop $s_{11} + 2s_{12} + 2s_{23}$ with $\Omega = 3$. This steady state is unstable too, so the system will still evolve to other demographic structures.

Now we see that the demographic structure where the four species coexist is a combination of the species loop $s_{11} + 2s_{22}$ and $s_{11} + 2s_{12} + 2s_{23}$. Particularly, for the demographic structure in figure 5c, it is composed of 17.4% of the species loop $s_{11} + 2s_{22}$, and 82.6% of the species loop $s_{11} + 2s_{12} + 2s_{23}$.

The evolution in figure 4 and the transitions between different demographic structures are just one specific example, and the evolution could have various trajectories. Figure 6 summarizes the outcomes over 1000 simulations. The demographic structure in figure 5a dominates in the beginning, but its frequency decreases quickly with generations. At 6000 generations, it occurs in as few as 0.7% of cases. The frequency of the demographic structure in figure 5d increases through the generations. By 6000 generations, it dominates with frequency of 39.9%. The demographic structure in figure 5c shows a similar pattern. Its frequency at generation 6000 is 32.0%.

It is interesting to look at the demographic structure in figure 5d. There, species $s_{12}$ needs metabolite $\bar{1}$ besides of the inflowing metabolite $\bar{2}$, but the metabolite $\bar{1}$ is only produced by species $s_{23}$. On the other hand, species $s_{23}$ needs $\bar{3}$ which is only produced by $s_{12}$. So species $s_{12}$ and $s_{23}$ cross-feed reciprocally. The evolution in this example shows that reciprocal cross-feeding evolves in a number of stages, with many transitional species involved in these stages, later driven to extinction [11,38].

## 3.4. System collapse

There are 48 simulations, out of the 1000 simulations of $n = 3$ and $u = 2$, where the whole system collapses over a very short time. An example is shown in figure 7, where the whole system undergoes a very sharp degeneration from generation $t = 5763$, and then all species die. Figure 7b shows that from generation $t = 5730$ to 5763, just before the collapse, the demographic structure is mainly composed of three coexisting species: $S_{11} \approx N/5$, $S_{12} \approx 2N/5$ and $S_{23} \approx 2N/5$ (this situation is similar to that shown in figure 5d).

To understand why the system collapses, we need to consider what happens when certain metabolites become unavailable in a system composed only of $s_{11}$, $s_{12}$ and $s_{23}$. If metabolite $\bar{1}$ is fully consumed, species $s_{11}$ and $s_{12}$ will start to die. However, species $s_{23}$ survives, with then $\bar{1}$ being
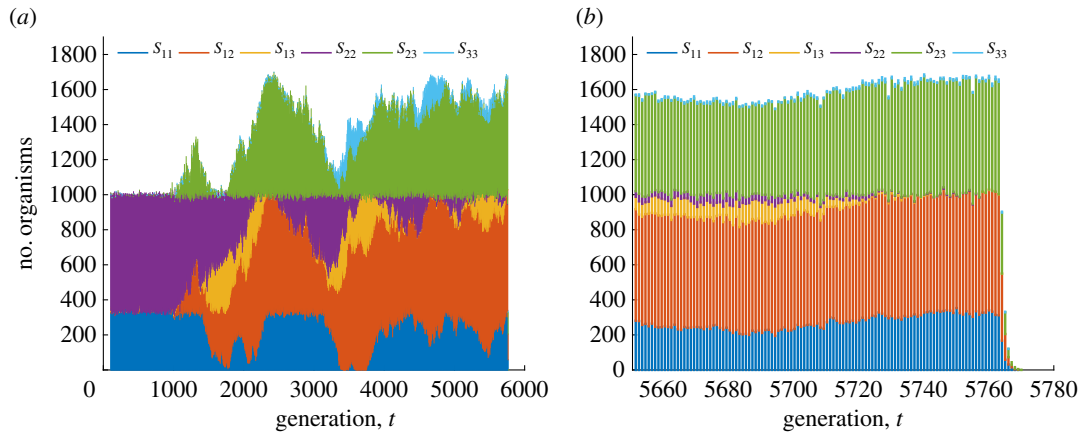
**Figure 7.** Example of the evolution with collapse for the system with $n = 3$ and $u = 2$. (a) Evolution over 6000 generations. (b) The enlarged figure of (a) for the time period just before collapse. (Online version in colour.)
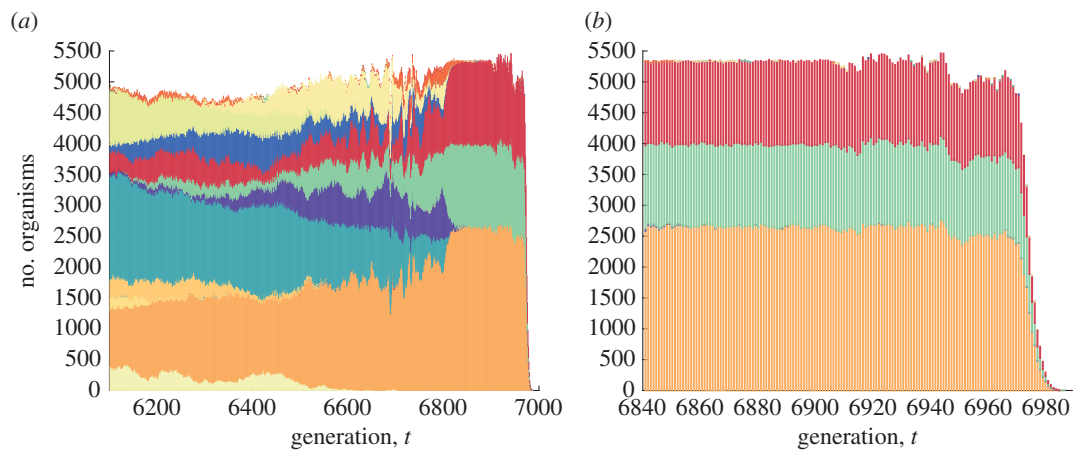


**Figure 8.** Example of the evolution with collapse for the system with $n = 6$ and $u = 2$. (a) The evolution from generation $t = 6000$ to 7000. (b) The enlarged figure of (a) for the duration just before collapse. (Online version in colour.)

replenished, and species $s_{11}$ and $s_{12}$ increase in number again. The system can also recover if metabolite $\overline{3}$ is completely consumed. However, if both $\overline{1}$ and $\overline{3}$ are fully consumed, all three species start to die and the system collapses. Because there are few metabolites in the system, the complete consumption of either $\overline{1}$ or $\overline{3}$ is relatively common, but the case where both are simultaneously unavailable is much rarer. In this sense, we can say that the three species system is 'robust yet fragile' [44,45]. It is robust to common perturbations (complete consumption of either $\overline{1}$ or $\overline{3}$) but potentially fragile to rare perturbations (complete consumption of both $\overline{1}$ and $\overline{3}$).

The system, however, does not collapse if species $s_{22}$ is present. In the critical situation where both $\overline{1}$ and $\overline{3}$ are consumed up, $s_{22}$ can always obtain the inflowing metabolite $\overline{2}$ and produce $\overline{1}$. Indeed, only a small population of $s_{22}$ is needed to produce enough $\overline{1}$ and prevent system collapse. This observation explains why the system did not collapse when $s_{22}$ was present (i.e. in figure 7b before generation $t = 5730$). It also explains why the demographic structure with the four species $s_{11}$, $s_{12}$, $s_{22}$ and $s_{23}$ coexisting (figure 5c) is common (figure 6). Demographic structures without $s_{22}$ (figure 5d) is more likely to collapse than those with $s_{22}$. The two properties of species $s_{22}$—its necessity for the continued existence of the whole system and the small relative abundance—make it the keystone species in this artificial ecosystem [47].

A similar phenomena of collapse is observed in larger $n$-metabolite systems. Figure 8 shows an example in the system
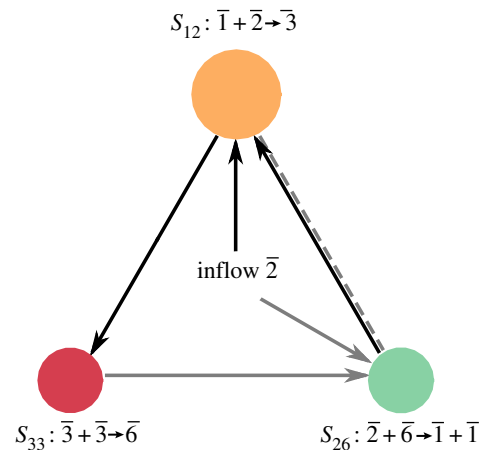


**Figure 9.** The demographic structure just before collapse at generation $t = 6880$ in the example evolution in figure 8, where $S_{12} \approx N/2$, $S_{26} \approx N/4$, $S_{33} \approx N/4$ and almost no other species. All the nearly-zero species are not shown. (Online version in colour.)

when $n = 6$ and $u = 2$. The system collapses when it stays at the demographic structure where $S_{12} \approx N/2$, $S_{26} \approx N/4$, $S_{33} \approx N/4$, and almost no other species (illustrated in figure 9). This demographic structure is composed of the species loop $2s_{12} + s_{26} + s_{33}$ with $\Omega = 3$. Figure 9 shows that the three species cross-feed: $s_{12}$ requires the $\overline{1}$ produced by $s_{26}$, which requires the $\overline{6}$ produced by $s_{33}$, which requires the $\overline{3}$ produced by $s_{12}$. By similar arguments of the $n = 3$ system, this system will not collapse if
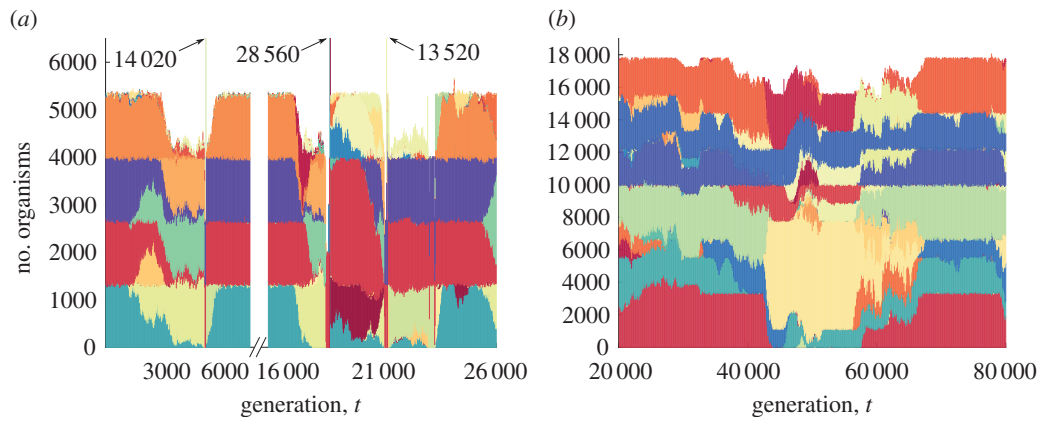
**Figure 10.** (a) Typical evolution for the system with $n = 6$ and $u = 2$, over 26 000 generations. For this system, in total there are six types of possible metabolites and 21 possible species. (b) A typical duration in a long-term evolution for the system with $n = 9$ and $u = 7$. For this system, in total there are nine types of possible metabolites and 45 possible species.
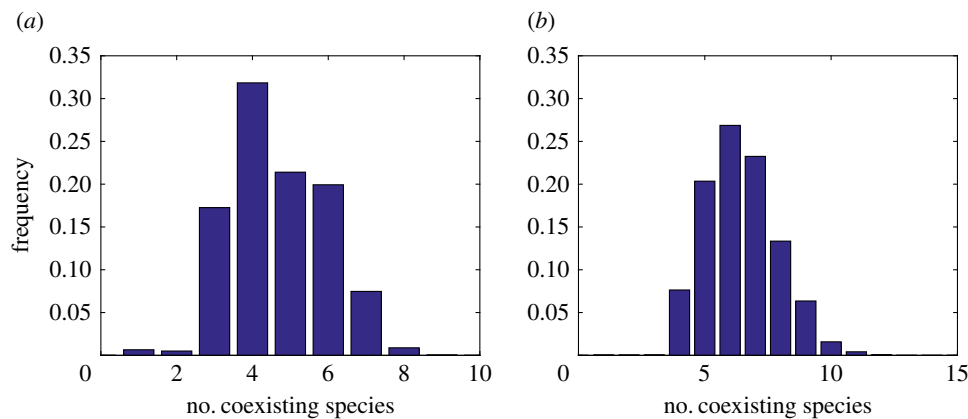


**Figure 11.** Histograms of the number of coexisting species. Note that we first classify the demographic structures by the same technique as in figure 3, and then count the number of coexisting species. That means, we only consider the main species which together constitute more than 90% of the whole population. In addition, when counting the number of coexisting species, the first 500 generations are excluded. (a) The histogram for the system with $n = 6$ and $u = 2$. (b) The histogram for the system with $n = 9$ and $u = 7$. (Online version in colour.)

only one or two of the three types of metabolites $\bar{1}$, $\bar{3}$ and $\bar{6}$ are completely consumed. However, in the rare event that all $\bar{1}$, $\bar{3}$ and $\bar{6}$ are totally consumed then extinction occurs (e.g. see figure 8b after generation $t = 6965$).

The keystone species would be the ones able to produce either the metabolite $\bar{1}$, $\bar{3}$ or $\bar{6}$, by taking in the inflowing metabolite $\bar{2}$. However, there is no such species in this system. Instead, by the cooperation of species $s_{22}$ ($\bar{2} + \bar{2} \rightarrow \bar{4}$) and $s_{44}$ ($\bar{4} + \bar{4} \rightarrow \bar{1} + \bar{1}$), the metabolite $\bar{1}$ can be produced; or by the cooperation of $s_{22}$ and $s_{24}$ ($\bar{2} + \bar{4} \rightarrow \bar{6}$), the metabolite $\bar{6}$ can be produced. Therefore, the role of the keystone species is played by either the pair of $s_{22}$ and $s_{44}$, or the pair of $s_{22}$ and $s_{24}$, rather than only one species.

## 3.5. Stability and biodiversity

All systems with different $n$ exhibit changes in their demographic structures. For example, we saw that the system with $n = 3$ and $u = 2$ switches between the demographic structure in figure 5c and that in figure 5d.

Figure 10a shows a typical evolution for the system with $n = 6$ and $u = 2$. The most frequent demographic structure is the one observed between generation 6000 and 16 000. Another demographic structure, for example, that seen at generation 22 000, is stable for around 2000 generations. On the other hand, the timescale of the switch between different demographic structures varies. It can be very rapid, e.g.

around generation 4600, 18 400, 21 000 (corresponding to the three population peaks) and 23 000, which occur over around 50 generations. Or it can be slow, e.g. around generation 2800, 17 000 and 25 000, which occurs over around 500 generations.

Figure 10b shows another example for a more complex system where $n = 9$ and $u = 7$. Over a long timescale, we see both slow and fast changes between different demographic structures, and no single equilibrium is always stable. These non-equilibrium dynamics and chaotic patterns are general properties of the evolution for all values of $n$ and $u$. In aquatic ecosystems, such as plankton communities, these properties are also observed, and proposed to be one of the mechanisms for the rich biodiversity of those communities [10,15].

To investigate the relationship between the number of coexisting species and all possible species, we repeated the simulation for the two systems above for 200 times, namely $n = 6$, $u = 2$ and $n = 9$, $u = 7$. The histograms of the number of coexisting species are shown in figure 11. There are 21 and 45 possible species, respectively, in the two systems. We see that although coexistence is common, not all the species are able to coexist. The most probable value of the number of coexisting species is near to or slightly below the number of metabolites, and the more possible species there are, the larger the number of coexisting species is.

# 4. Discussion

Our artificial ecosystem, based on a simple description of how resources are transformed by organisms, reproduces a whole range of complex ecological phenomena, such as cross-feeding and biodiversity. Our results help us understand how phenomena of community ecology emerge and why certain features are ubiquitous to all community ecosystems. The phenomena observed in our model emerge from minimal assumptions about how resources are consumed and recycled.

Our first result is that, for any number of resources and independent of the inflow rate, the artificial ecosystem self-organizes to consume all of the available resources. This property has been widely observed in real ecological systems, such as the microbial community in granules in the UASB reactors [35,36], and microbiota of zebrafish [9,37]. A similar theoretical result was obtained by Gedeon & Murphy [12], in relation to a generalized differential equation system for simple food webs. They found that the stable community always maximizes utilization of available resources. Self-organization to full consumption of resources are thus likely to be a general property of all ecosystems.

The emergence of reciprocal cross-feeding in our system gives some insights into the problem of 'missing' species, such as the corrinoid-producing bacteria. Around 75% of bacteria are predicted to encode corrinoid-dependent enzymes, but at least half of them cannot produce corrinoids *de novo* [40,42]. They either cross-feed with corrinoid-producing partners, get corrinoids from the surrounding environment or modify corrinoid precursors in their own cells. It has been speculated that cross-feeding requires spatial structure, preexistence of one-way benefit, intermediate population density, etc. [13,20]. In our artificial ecosystem, cross-feeding is a common evolutionary outcome. For example, in a three-metabolite system an early epoch of simple sequential cross-feeding (figure 5*a*) is outcompeted by a species loop of reciprocal cross-feeding (figure 5*d*). Similar reciprocal cross-feeding outcomes are seen in systems with more metabolites, with reciprocal dependencies readily evolving from an earlier stage of sequential dependence between species. While our results do not directly explain the lack of corrinoid-producing bacteria, they illustrate that it is common for 'intermediate' species to be driven extinct on the way towards a system of reciprocal cross-feeding.

Our model also reproduces an emergent system collapse, where even without external perturbations, populations suddenly die out. A biological example of this sudden disintegration, without any obvious external reason, are sludge granules in UASB reactors [36]. Our model demonstrates how the extinction of one specific non-dominating species makes the whole ecosystem much more fragile, although it does not trigger collapse directly. Because of its importance and small relative abundance, it is the keystone species [46]. The concept of keystone species is important for conservation in ecology [48]. However, the empirical evidence, such as the starfish *Pisaster* [49] and sea otters [50], of the keystone species remains debated due to its 'poor' definition [47]. While our model did not clarify the controversy on keystone species, it explicitly shows that this certain kind of species with small relative abundance but significant importance on the continued existence of the whole system are commonly observed in artificial ecosystems.

Lastly, our model shows that non-equilibrium dynamics and chaotic patterns are general properties of resource dependent evolution, providing one part of an explanation of the 'paradox of the plankton' and biodiversity [10,15]. Our results support the claim that natural selection does not necessarily maximize stability or biodiversity [29]. We also find limitations to biodiversity in our model, although the demographic structures change over time. As the ecosystem always self-organizes to consume all available resources, then only species loops tend to persist. A demographic structure is usually dominated by one species loop, with several other non-dominating species loops surviving at lower numbers. Thus biodiversity is bounded by the number of coexisting species loops, and the types of species loop determine the limits of biodiversity, rather than species themselves.

We defined a useful ecological concept for looking at interdependencies in ecosystems: the species loop. Our definition arises from the term 'microbial loop', that acts as a sink of carbon in a water column [58]. It is an influential concept in biological oceanography [57]. In our artificial ecosystem, at any one time various species loops persist, self-organizing to consume up all the available resources. In our model, natural selection directly acts on individual organisms rather than on species loops, but finally various species loops are spontaneously selected for. Species loops could thus be considered as a higher level unit of selection than individual species, as a quasi-unit of community ecology, which natural selection indirectly acts on.

Our model assumes a constant external environment and well-mixed system [15]. This allows us to show that temporal and spatial heterogeneity are not necessary for complex patterns of coexistence, cross-feeding and collapse in natural ecosystems. We have, however, only considered the commensal interactions, and not syntrophic interactions [12]. That is, metabolites excreted by the organism are waste products or byproducts, which require no cost or have no other effect on the producer. Nor do we consider the substances of which organisms are made, i.e. after an organism dies, it just disappears from the system. That means we do not consider predator–prey interactions [29]. As in natural ecosystems, dead organisms could be food of others, and living organisms could be food of predators and parasites. Lastly, in our current model, each species is defined as one chemical reaction, while in reality, organisms may have different potential metabolic pathways that are able to adapt to different environments [4].

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