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Cross-Feeding Dynamics Described by a Series Expansion of the Replicator Equation

Torbjörn Lundh · Philip Gerlee

Abstract Understanding how ecosystems evolve and how they respond to external perturbations is critical if we are to predict the effects of human intervention. A particular class of ecosystems whose dynamics are poorly understood are those in which the species are related via cross-feeding. In these ecosystems the metabolic output of one species is being used as a nutrient or energy source by another species. In this paper we derive a mathematical description of cross-feeding dynamics based on the replicator equation. We show that under certain assumptions about the system (e.g., high flow of nutrients and time scale separation), the governing equations reduce to a second-order series expansion of the replicator equation. By analysing the case of two and three species we derive conditions for co-existence and show under which parameter conditions one can expect an increase in mean fitness. Finally, we discuss how the model can be parameterised from experimental data.

Keywords Syntrophy · Cross-feeding · Population dynamics

1 Introduction

Ecosystems contain a large number of species that interact either directly or indirectly, forming a complex web of interdependencies. The modes of interaction are many; ranging from predation, competition to parasitism and cooperation. In order to fully understand the properties of an ecosystem, and how it will react when perturbed,

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it is not sufficient to enumerate the constituent species, but one also needs to understand the dynamics that emerge from their mutual interactions. This is of particular importance if one wants to predict how ecosystems respond to human intervention.

Many functions carried out by microbes, such as degradation of man-made toxic compounds, require the joint metabolic effort of many bacterial species. In such bacterial communities the success of each species depends on the presence or absence of other species and chemical compounds, effectively joining the components of the community into a microbial ecosystem. A common mode of interaction in such ecosystems is cross-feeding or syntrophy, whereby the metabolic output of one species is being used as a nutrient or energy source by another species. This type of interaction is found in wide variety of systems, ranging from the human gut microbiota (Belenguer et al. 2006), where bacteria aid their host in degrading food stuff, to the oxidation of methane by ocean-living bacteria (Pernthaler et al. 2008), responsible for reducing the amount of methane, a potent greenhouse gas, in the atmosphere, and the cooperative degradation of pesticides by soil bacteria (Katsuyama et al. 2009).

Despite the global importance of the above-mentioned processes, still little is known, both experimentally and theoretically, about the dynamics of cross-feeding ecosystems. How do these systems evolve? Could they be made to function more efficiently? And how sensitive are they to external perturbations?

The phenomenon of cross-feeding was first observed by Helling et al. (1987) in a long-term evolutionary experiment with the bacteria *E. coli*. A bacterial colony consisting of only one strain and fed on a single source of nutrient, in this case glucose, repeatedly evolved to a state containing at least two co-existing strains. This observation contradicted the exclusion principle, stating that a single resource can only sustain a single species, and only after numerous replicates of the experiment were they convinced of the result.

Since then cross-feeding has been reported to occur in a variety of other systems such as methanogenic environments (Stams 1994), bacteria engaged in nitrification (Costa et al. 2006), the degradation of xenobiotic compounds (Katsuyama et al. 2009), and in the human gut microbiota (Belenguer et al. 2006). The reason why cross-feeding is so ubiquitous has not been satisfactorily answered yet and might seem puzzling. Why is the metabolic activity shared among several species and not performed by a single species? In the case of *E. coli* grown on glucose, one strain partially degrades the glucose to acetate, which after being excreted is consumed by the other strain. In this case the second strain is clearly subject to negative frequency-dependent selection (as it would starve in the absence of the primary degrader); however, it has been hypothesised that the first strain is dependent on the second one, as the secondary metabolite could be toxic at high concentrations (Pelz et al. 1999).

2 Previous Work

The emergence of cross-feeding was investigated by Pfeiffer and Bonhoeffer (2004) using a differential equation model that describes the dynamics of an evolving bacterial population within a chemostat. Their results showed that cross-feeding naturally emerges under the assumption that energy production is maximised while the total

concentrations of enzymes and intermediates are minimised. Further they showed that the evolution of cross-feeding depends on the dilution rate in the chemostat and that a stable co-existence between two strains is more likely to emerge at low dilution rates.

A different approach was taken by Doebeli ([2002]), who investigated the appearance of cross-feeding in the framework of adaptive dynamics. In this case the condition for evolutionary branching and the appearance of cross-feeding is that there is a trade-off between uptake efficiency of the primary and secondary metabolites and that this trade-off function has a positive curvature. The model also makes the correct prediction that cross-feeding is less likely to occur in serial batch culture, in which the primary resource is not replenished, a fact that has been experimentally observed (Rozen and Lenski [2000]). This highlights the necessity of the secondary metabolite being present for an extended period of time for cross-feeding to evolve.

Some instances of cross-feeding can be viewed as a type of cooperative behaviour, where the species involved reciprocate by producing secondary metabolites useful for the other species in the ecosystem. Such a system can always be invaded by mutants who only benefit from the cross-feeding and do not give anything in return. This scenario was investigated by Bull et al. ([2009]) in a two-species context, and it was shown that cross-feeding only emerges in a narrow parameter range and that it only occurs at intermediate population densities. These results have however been disputed, and the conditions under which cooperative cross-feeding relations can evolve and be stably maintained remain unclear (Estrela and Gudelj [2010]).

All the above theoretical studies have assumed a situation where the nutrients can only be degraded in a single way, thus creating a linear metabolic pathway spanning one or several species. A more interesting situation occurs when a metabolite can be degraded by several species in different ways, a common feature of real metabolic networks. This situation was tackled by Crombach et al. ([2009]) in an individual-based model, which makes use of an analogy between metabolism and binary computation. Instead of considering concentrations of metabolites and bacteria, they represent metabolites by binary strings and the bacteria feeding on them by simple computer algorithms that modify the strings. Using this model, they showed that cross-feeding evolves more easily when the ecosystem is spatially structured and when there is a strong selection for resource utilisation.

A similar approach was taken by Gerlee and Lundh ([2010]), who considered organisms represented by cellular automata rules, which transform binary strings, and where the information theoretic entropy of the strings was taken as a proxy for energy. Organism/rules that were able to increase the entropy of the binary strings present in the system were allowed to reproduce and spread in the population. Using this model, they could show that species diversity is higher when the flow of resources into the system is low and that this coincides with high ecosystem efficiency.

To summarise, the previous attempts at modelling cross-feeding have been constrained either to systems assuming linear metabolic chains or individual-based models with limited analytical insight. Our aim with the current paper is to present a general framework in which cross-feeding dynamics can be analysed, and we do this by deriving a population dynamic model from first principles. This general model can then be customised to fit a particular real-world situation, and we also carry out

such an analysis for the case where two and three species are present. This results in criteria on the interactions between the species which indicate when the species can co-exist and if the productivity of the ecosystem is maximised.

3 Derivation of Model

We will consider a consortium of n bacterial species engaged in cross-feeding relations through the metabolites they digest and excrete. For simplicity, we assume that the total number of individual bacteria is constant and therefore only need to consider the fraction of species x_i, $i = 1, \ldots, n$. The time evolution of the species composition is described by the replicator equation (Schuster and Sigmund 1983)

$$\frac{dx_i}{dt} = (\phi_i - \bar{\phi})x_i, \tag{1}$$

where ϕ_i is the fitness of species i, and

$$\bar{\phi} = \sum_{i=1}^{n} \phi_i x_i \tag{2}$$

is the mean fitness in the population. This means that if species i has a fitness above the average, its fraction in the population will increase, and if below average, decrease. In the following we will concern ourselves with a derivation of the fitness function when the consortium is engaged in cross-feeding.

The fitness is assumed to depend on the ability of the bacteria to harvest energy from the resources available. This means that we assume that the limiting factor is not the building blocks necessary for biomass growth, but the energy required to synthesise these molecules into new DNA, proteins or lipids. We further assume that the resources in the system consist of molecular species and that each bacterial species metabolises the molecules in a unique way, leaving, so to speak, a unique fingerprint on the transformed molecules. A single unmetabolised molecular type S_0 is added to system at a rate γ, and we will keep track of the metabolised molecules by labelling them according to which species they have been metabolised by. In this notation molecule S_{ijk} started its presence in the system as an S_0 molecule, was first metabolised by species i, then j, and finally by species k (see Fig. 1). This induces a natural hierarchy or (partial) ordering among the molecular types, where level l contains n^l distinct types of molecules. The inflow of S_0-molecules is counterbalanced by an outflow occurring on all levels, so that the number of molecules within in the system is kept constant. This means that we do not have to keep track of absolute numbers, but instead of the fraction, or relative concentration, s_i, each molecular type, S_i, constitutes.

The bacteria are assumed to be in a metabolic steady state, meaning that their uptake of a metabolite, metabolic conversion and excretion of the transformed metabolite are all equal, but not necessarily constant with respect to time. That is, we assume that the metabolic dynamics occur on a much shorter time scale than the replicator
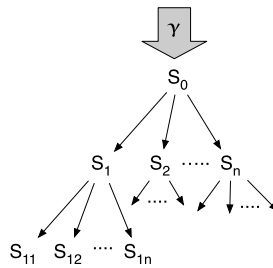
Fig. 1 A schematic of the dynamics of the metabolites. High-energy molecules S_0 flow into the system at rate γ and are transformed by the bacterial species $1, \ldots, n$. A molecule that has been metabolised first by species i and then by species j is denoted S_{ij}. The total outflow of metabolites is also γ, ensuring that the total number of metabolites is constant over time

dynamics. This means that the concentration of the primary metabolite S_0 can be described by the following ODE:

$$\frac{ds_0}{dt} = -\sum_i r_i(s_0)x_i + \gamma(1 - s_0), \tag{3}$$

where $r_i(\cdot)$ is a function that describes the uptake of S_0 by species i as a function of the media concentration, s_0. Thus the product $r_i(s_0)x_i$ corresponds to the total uptake of the metabolite of bacteria from species i, and lastly γ is the flow rate of metabolites into and out of the system. The concentration of a first-order metabolite S_i is given by

$$\frac{ds_i}{dt} = r_i(s_0)x_i - \sum_j r_{ij}(s_i)x_j - \gamma s_i, \tag{4}$$

where the first term corresponds to production of S_i by species i, the second to consumption by all bacterial species, and the last term stands for outflow.

To each metabolic step we associate an energy $\mathcal{E}$ that measures the amount of energy extracted in the metabolic reaction and use subscript to denote the particular step. Hence $\mathcal{E}_i$ denotes the energy extracted by species i when metabolising S_0 molecules.

The total fitness of a species is assumed to be proportional to the total harvested energy, but as an approximation, we will only consider the fitness contribution of zeroth- and first-order metabolites, valid when the flow rate γ is large. This means that the fitness of species i can be expressed as

$$\phi_i = r_i(s_0)\mathcal{E}_i + \sum_j r_{ji}(s_j)\mathcal{E}_{ji} + \Omega(2), \tag{5}$$

where the first term comes from the energy harvested from primary metabolites S_0, the second contribution comes from energy extracted from molecules produced by the other bacteria in the consortium, and $\Omega(2)$ stands, like an error term, for the fitness contribution from higher-order metabolic interactions.

Without any further simplifications we are left with a system of n equations for the bacteria and $1 + n$ equations for the metabolites giving in total $2n + 1$ coupled ODEs, whose analysis will be far from trivial, even when the effect from higher-order interactions, $\Omega(2)$, are disregarded. In order to arrive at a simpler system, we will make two final simplifications. Firstly, we assume that all uptake functions $r(s)$ are equal and of the form $r(s) = \kappa s$, which means that the uptake is linear with the medium concentration and implicitly assumes that metabolites are scarce and no saturation effects are present. Secondly, we assume that there is a separation in time scale between the dynamics of the metabolites and the bacterial population dynamics. This implies that the metabolite concentrations are in a steady state, which corresponds to setting the time derivatives of s_0 in Eq. (3) and s_i in Eq. (4) equal to zero. These two simplifications mean that we can solve for the steady-state concentrations to get

$$\hat{s}_0 = \frac{\gamma}{\gamma + \kappa} \tag{6}$$

and

$$\hat{s}_i = \frac{\kappa \gamma}{(\gamma + \kappa)^2} x_i, \tag{7}$$

where in the last expression the frequency of species i, x_i influences the steady-state molecular concentration linearly. Plugging these expressions for the metabolite concentrations into the fitness function (5), we get the following expression for the fitness of species i:

$$\widehat{\phi}_i = \frac{\kappa \gamma}{\gamma + \kappa} \mathcal{E}_i + \frac{\kappa^2 \gamma}{(\gamma + \kappa)^2} \sum_j \mathcal{E}_{ji} x_j + \Omega(2). \tag{8}$$

By introducing the parameter $\eta = \kappa/(\gamma + \kappa)$, we can rewrite the above expression as

$$\widehat{\phi}_i = \gamma \eta \mathcal{E}_i + \gamma \eta^2 \sum_j \mathcal{E}_{ji} x_j + \Omega(2). \tag{9}$$

This means that the fitness of a species depends both on how good it is at harvesting energy from the primary metabolite (the first term) and its interactions with other species (the second term). This interaction is quantified in the constant $\mathcal{E}_{ji}$ that describes how well species i can extract energy from metabolites previously metabolised by species j. This truncation of the fitness introduces an error, but from Theorem 1 in Appendix A we have that the sum of terms of degree two and higher, $\Omega(2)$, can be bounded

$$\Omega(2) \leq \frac{c_0 \gamma \eta^3 c^2}{1 - \eta c} = \frac{c_0 \gamma \kappa^3 c^2}{(\kappa + \gamma)(\kappa(1 - c) + \gamma)}$$

assuming that the sequence is so-called Level Limited of degree two (see Definition 1 in Appendix A).

4 Analysis of the Two Species Case

In order to gain insight into the above-derived system of equations, we will in this section analyse the special case of two and three species engaged in cross-feeding. Our analysis will focus on two important properties of ecosystems: species co-existence and the time-evolution of ecosystem productivity.

Let us denote the two species α and β, and let x be the fraction of species α, and consequently $1 - x$ the fraction of species β. The time dynamics of the system will be determined by the replicator equation (1):

$$\frac{dx}{dt} = \big(\phi_\alpha(x) - \bar{\phi}(x)\big)x = \big(\phi_\alpha(x) - \big(x\phi_\alpha(x) + (1 - x)\phi_\beta(x)\big)\big)x$$
$$= x(1 - x)\big(\phi_\alpha(x) - \phi_\beta(x)\big).$$

For convenience, we define the right-hand side of the above equation as the replicator function

$$\rho(x) = x(1 - x)\big(\phi_\alpha(x) - \phi_\beta(x)\big). \tag{10}$$

Based on the derivation in the previous section, Eq. (9) provides the following expressions for the fitness of the two species:

$$\phi_\alpha(x) = \eta\gamma\mathcal{E}_\alpha + \eta^2\gamma\big(\mathcal{E}_{\beta\alpha}(1 - x) + \mathcal{E}_{\alpha\alpha}x\big) \quad \text{and} \tag{11}$$

$$\phi_\beta(x) = \eta\gamma\mathcal{E}_\beta + \eta^2\gamma\big(\mathcal{E}_{\alpha\beta}x + \mathcal{E}_{\beta\beta}(1 - x)\big). \tag{12}$$

4.1 Co-existence

We will consider two different notions of co-existence, firstly the concept of permanence, which roughly corresponds to non-extinction, and secondly, the presence of an internal stable fixed point $x^\star \in (0, 1)$ for the replicator equation.

A replicator system is considered permanent if for all initial conditions, such that all species are present in non-zero frequencies, no species frequency will ever become zero. In ecological terms this simply means that no species will ever go extinct, or in the case of our system: $x(t) \in (0, 1)$ for all $t \geq 0$. We will approach the problem by analysing the stability of the two boundary fixed points. In our one-dimensional case we have permanence if the boundary point $x = 0, 1$ are repelling, i.e. if both these fixed points are unstable. This occurs when

$$\rho'(x) > 0 \quad \text{for } x = 1^- \text{ and } x = 0^+.$$

From (10) we have that

$$\rho'(x) = (1 - 2x)\big(\phi_\alpha(x) - \phi_\beta(x)\big) + x(1 - x)\big(\phi_\alpha'(x) - \phi_\beta'(x)\big). \tag{13}$$

So at the lower boundary point, $x = 0$, we have that

$$\rho'(0) = \phi_\alpha(0) - \phi_\beta(0) = \eta\gamma\big(\mathcal{E}_\alpha - \mathcal{E}_\beta - \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha})\big),$$

that is, the left-hand side limit point is unstable if

$$\mathcal{E}_\alpha - \mathcal{E}_\beta > \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}). \tag{14}$$

Similarly, for the upper boundary point, $x = 1$, we have that

$$\rho'(0) = -\big(\phi_\alpha(1) - \phi_\beta(1)\big) = -\eta\gamma\big(\mathcal{E}_\alpha - \mathcal{E}_\beta - \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha})\big).$$

Hence, we have that the boundary point $x = 1$ is unstable if

$$\mathcal{E}_\alpha - \mathcal{E}_\beta < \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}). \tag{15}$$

We conclude that we have permanence if

$$\eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}) < \mathcal{E}_\alpha - \mathcal{E}_\beta < \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}). \tag{16}$$

It turns out that we can say even more then that we have permanence. From (10) we see that $\rho(x)$ is a third-degree polynomial such that $\rho(0) = \rho(1) = 0$ and both $\rho'(0)$ and $\rho'(1)$ are strictly positive, which means that in a small right-hand neighbourhood of 0, $\rho(x)$ is strictly positive, and similarly $\rho(x)$ is strictly negative on a small left-hand neighbourhood of 1. Hence, there exists an $x^* \in (0, 1)$ such that $\rho(x^*) = 0$ and $\rho'(x^*) < 0$. Or, in other words, there exists a stable internal fixed point, and we have stable coexistence between the two species. Furthermore, the fixed point x^* can be given explicitly by solving Eq. (10). That is,

$$x^* = \frac{1/\eta(\mathcal{E}_\alpha - \mathcal{E}_\beta) + \mathcal{E}_{\beta\alpha} - \mathcal{E}_{\alpha\alpha}}{\mathcal{E}_{\alpha\beta} + \mathcal{E}_{\beta\alpha} - \mathcal{E}_{\alpha\alpha} - \mathcal{E}_{\beta\beta}}. \tag{17}$$
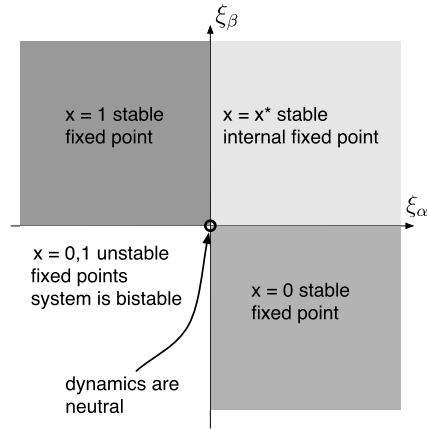
This means that in our simple system the two notions of coexistence coincide, i.e. if the system is permanent and the boundaries are repelling, then there exists a stable internal fixed point x^* given by the above expression (17).

4.2 Five Different Scenarios

By extending the above reasoning we can in fact classify the behaviour of the system into five distinct cases:

$$\begin{cases} x^* \text{ is a stable internal fixed point} \\ \quad \text{if } \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}) < \mathcal{E}_\alpha - \mathcal{E}_\beta < \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}); \\ 0 \text{ is a stable fixed point} \\ \quad \text{if } \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}) > \mathcal{E}_\alpha - \mathcal{E}_\beta < \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}); \\ 1 \text{ is a stable fixed point} \\ \quad \text{if } \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}) < \mathcal{E}_\alpha - \mathcal{E}_\beta > \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}); \\ 0 \text{ and } 1 \text{ are stable fixed points} \\ \quad \text{if } \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}) > \mathcal{E}_\alpha - \mathcal{E}_\beta > \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}); \\ \text{the dynamics are neutral, } \phi_\alpha = \phi_\beta \\ \quad \text{if } \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha}) = \mathcal{E}_\alpha - \mathcal{E}_\beta = \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}). \end{cases}$$

Fig. 2 A graphical illustration of the five possible scenarios for a two-species system. Each scenario corresponds to a quadrant in the (ξ_α, ξ_β)-plane, except the neutral case, which only occurs at the origin, and implies that the fitnesses of α and β are equal for all x

Note that in the second scenario where 0 is a stable fixed point, 1 is a unstable fixed point, and x^* is outside $(0, 1)$. In the third case, 1 is a stable fixed point, 0 unstable, and again x^* is outside $(0, 1)$. In the fourth case where both 1 and 0 are stable fixed points, x^* is an unstable fixed point in $(0, 1)$. Finally, the fifth case is valid if and only if $\mathcal{E}_{\alpha\beta} + \mathcal{E}_{\beta\alpha} = \mathcal{E}_{\alpha\alpha} + \mathcal{E}_{\beta\beta}$ and $\mathcal{E}_\alpha - \mathcal{E}_\beta = \eta(\mathcal{E}_{\beta\beta} - \mathcal{E}_{\beta\alpha})$ holds.

By defining the quantities

$$\xi_\alpha = \eta(\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\alpha\alpha}) - (\mathcal{E}_\alpha - \mathcal{E}_\beta) \quad \text{and} \tag{18}$$

$$\xi_\beta = \eta(\mathcal{E}_{\beta\alpha} - \mathcal{E}_{\beta\beta}) - (\mathcal{E}_\alpha - \mathcal{E}_\beta) \tag{19}$$

we can illustrate the five scenarios graphically shown in Fig. 2. Each case corresponds to a quadrant, except the neutral case, which only occurs at the origin.

4.3 A Generalized Permanence Analysis

Let us briefly mention that the problem of permanence can be approached from a different angle. It has been shown, see for example (Jansen 1987), that if one can find a function $P(x)$ such that $P(x) = 0$ for all x on the boundary of the system and $P(x) > 0$ for all x in the interior, then the system is permanent. The function $P(x)$ is required to satisfy

$$\frac{dP(x(t))}{dt} = P(x)\Psi(x),$$

where $\Psi(x)$ is an average Lyapunov function, which satisfies $\Psi(x^*) > 0$ for all rest points x^* on the boundary. In the case of $n = 2$ a good choice is $P(x) = x^a(1-x)^b$, where $a, b > 0$ are constants, and the average Lyapunov function becomes $\Psi(x) = aw_\alpha(x) + bw_\beta(x)$, where $w_i(x) = \phi_i(x) - \bar{\phi}(x)$. In order to determine the permanence of the system, we thus need to find out if there exists constants $a, b > 0$ such that $\Psi(0) > 0$ and $\Psi(1) > 0$ since the rest points on the boundary in this case reduce to $x^* = 0$ and 1. This yields two inequalities, which in fact are independent of a and b, but instead put constraints on the model parameters and result in precisely the same inequalities (16) as the above analysis produced. However, this technique to

show permanence has the advantage that it can easily be extended to higher dimensions, and one can in fact show that a sufficient condition for permanence for three species that form an intransitive triple (i.e. in isolation, species i outcompetes j, j outcompetes k and k outcompetes i) is given by

$$\Gamma_{12}\Gamma_{23}\Gamma_{31} < 1, \tag{20}$$

where

$$\Gamma_{ij} = \frac{\mathcal{E}_j - \mathcal{E}_i + \eta(\mathcal{E}_{jj} - \mathcal{E}_{ji})}{\mathcal{E}_j - \mathcal{E}_i + \eta(\mathcal{E}_{ji} - \mathcal{E}_{ii})}$$

(see Appendix B for details).

4.4 Productivity

We now return to the two-species case to investigate another important concept, namely that of ecosystem productivity. There are several ways in which this property can be defined, but a convenient measure is to consider the total amount of energy that the species within the ecosystem can assimilate per unit time. Since we assume that the reproductive rate of a species is proportional to its energy uptake, the productivity of the ecosystem is proportional the average fitness in the population $\bar{\phi}(x) = x\phi_\alpha + (1-x)\phi_\beta$. A natural question to ask about the productivity is if it increases over time or, in other words, if the dynamics of the system pushes the ecosystem towards an increase in average fitness. An answer to this question can be found by applying a theorem by Shashahani, which states that if the condition

$$\frac{\partial \phi_i}{\partial x_j} = \frac{\partial \phi_j}{\partial x_i}$$

holds for all species i and j, then the average fitness always increases along trajectories of the system (Shashahani 1979), i.e.

$$\frac{d\bar{\phi}(x(t))}{dt} > 0.$$

In the case of cross-feeding with only two species present, this reduces to the surprisingly simple condition $\mathcal{E}_{\alpha\beta} = \mathcal{E}_{\beta\alpha}$, which means that the two species need to be equally good at extracting energy from each others left-overs. This reasoning can be extended to the three species scenario, and in this case the condition for increasing productivity takes the form

$$\mathcal{E}_{\alpha\beta} + \mathcal{E}_{\beta\gamma} + \mathcal{E}_{\gamma\alpha} = \mathcal{E}_{\alpha\gamma} + \mathcal{E}_{\gamma\beta} + \mathcal{E}_{\beta\alpha},$$

which, as one might expect, is a considerably stricter condition, and involves all second-order energy terms.

   If we combine the condition for the two-species case with the previously derived conditions for co-existence (16), we get the following requirements for a two-species

system that evolves towards a stable state of coexistence that coincides with a maximum in the productivity of the ecosystem:

$$\mathcal{E}_{\alpha\beta} = \mathcal{E}_{\beta\alpha},$$
$$\mathcal{E}_{\alpha\beta} > \mathcal{E}_{\alpha\alpha}, \quad \text{and}$$
$$\mathcal{E}_{\alpha\beta} > \mathcal{E}_{\beta\beta}.$$

5 Discussion

In this paper we have considered the problem of cross-feeding, or syntrophy, and derived a model of this phenomenon based on the replicator equation. In the case of a high flow of energy into the system and under the assumption of a separation in time-scale between metabolic and population dynamics, we could show that the fitness of each species in the ecosystem is given by a constant term plus a linear combination of the species frequencies, whose coefficients correspond to the ability of a species to extract energy from previously metabolised resources. In our analysis of the system we have focused on the case of a two-species ecosystem and were able to derive conditions for coexistence and increase in productivity. The latter condition in fact tells us when Fisher's fundamental theorem of natural selection (Fisher 1930) applies in this system, where selection is frequency dependent.

Two-species systems are in fact the typical systems considered in laboratory experiments on cross-feeding, and it would be interesting to observe if the conditions derived in this paper hold for real bacterial ecosystems. In order to make such a comparison, one would need to characterise the bacterial species according to their ability to extract energy from the metabolites provided and manufactured by the other species in the ecosystem.

This can actually be achieved by measuring the growth rate of the bacteria in various assays. For example, the $\mathcal{E}_i$ can be directly related to the growth rate of species i in isolation in the limit of high flow γ. The second-order interactions terms $\mathcal{E}_{ji}$ could be estimated by letting media pass from species j to species i under appropriate flow conditions and by measuring the growth rate of species i. By measuring the lowest order interactions terms first, higher-order terms could be similarly determined. A potential system in which these ideas can be tested is an experimental microcosms of *E. coli* grown on minimal media with glucose (Saxer et al. 2009).

However, as a complement to in vivo experimentation, the possibility exists to test the model and its predictions on a computational system. An ideal candidate for this would be the agent-based system *Urdar*, in which agents represented by cellular automaton rules that engage in cross-feeding interactions by metabolising binary strings. This system exhibits complex dynamics, and some of these might be explicable by applying the framework presented in this paper. For example, one could examine to what extent truncation of higher-order interaction terms in the fitness function affect the accuracy of the model predictions. In particular, it would be interesting to investigate how closely the productivity (or average fitness) maxima is attained for species that only to an approximate degree satisfy the criterion $\mathcal{E}_{\alpha\beta} = \mathcal{E}_{\beta\alpha}$. In other

words, if we let $\varepsilon = |\mathcal{E}_{\alpha\beta} - \mathcal{E}_{\beta\alpha}|$ and $\delta = |x^{\star} - x_p|$, where $x^{\star}$ is the fixed point, and x_p is the point at which productivity is maximised, how does δ depend on ε?

This paper presents a first step towards a more thorough understanding of cross-feeding dynamics. With the methodology developed in this paper it will be possible to characterise microbial ecosystems involved in cross-feeding and to asses their potential in achieving optimal ecosystem efficiency. Furthermore, it will provide tools for manipulating existing microbial ecosystems, by for example introducing foreign species, so that the ecosystem as a whole can achieve a higher efficiency.

Appendix A: An Estimate of the Higher-Order Terms

In order for the truncated series in Eq. (8) to be valid, we need to control the tail, i.e. the higher-order terms.

First we need to define the notation of multi-index I. Let $I = i_1 i_2 \ldots i_k$ where all $i_j \in \{1, \ldots, n\}$, and let x_I be the product $x_{i_1} x_{i_2} \ldots x_{i_k}$ of fractions of species. We will also use the notation Ii for the concatenation $i_1 \ldots i_k i$. Finally, we denote the length of the multi-index I by $|I|$.

Definition 1 We say that the cross-feeding process is *Level Limited of degree d* if there are positive constants c_0 and c, where $c < 1$ such that for every multi-index I with $|I| \geq d - 1$ and every index i,

$$\mathcal{E}_{Ii} \leq c_0 c^{|I|}.$$

Note that if the cross-feeding process is monotone in the following way:

$$\frac{\mathcal{E}_{Ii}}{\mathcal{E}_I} \leq c < 1$$

for all multi-indices I with $|I| \geq d - 1$, then it is Level Limited of degree d.

Theorem 1 *The sum of the higher-order terms of degree d, $\Omega(d)$, is bounded above by $\kappa^{d+1}/(\kappa + \gamma)^d$. Furthermore, if the process is level limited of degree d, we have that the sum of the higher-order terms of degree d are bounded in the following way*:

$$\Omega(d) \leq \frac{c_0 \gamma \kappa^{d+1} c^d}{(\kappa + \gamma)^{d+1} (\kappa(1 - c) + \gamma)}. \tag{21}$$

Before we prove this, we need a lemma as a generalisation of Eqs. (6) and (7), which is easily proved using induction.

Lemma 1 *For any multi-index I*,

$$\widehat{s_I} = \frac{\kappa^{|I|} \gamma x_I}{(\kappa + \gamma)^{|I|+1}}.$$

Let us now prove the proposition starting with Eq. (21).

Proof of Theorem 1 To simplify the notation, let us consider the case where $d = 2$ and recall that $\eta = \kappa/(\kappa + \gamma)$. We start by noting that the sum over every multi-index I with fixed length p is

$$\sum_{|I|=p} x_I = \sum_{i_1}\sum_{i_2}\cdots\sum_{i_{|I|}} x_{i_1} x_{i_2}\ldots x_{i_{|I|}} = \sum_{i_1} x_{i_1}\sum_{i_2} x_{i_2}\cdots\sum_{i_{|I|}} x_{i_{|I|}}$$
$$= 1\cdot 1\cdots 1 = 1.$$

By then using the lemma and the assumption that the process is Level Limited of degree $d = 2$, we have that

$$\Omega(2) = \gamma\eta^3\sum_k\sum_j x_k x_j \mathcal{E}_{kji} + \gamma\eta^4\sum_l\sum_k\sum_j x_l x_k x_j \mathcal{E}_{lkji} + \cdots$$

$$= \gamma\sum_{p=2}^{\infty}\eta^{p+1}\sum_{|I|=p} x_I \mathcal{E}_{Ii} \le \gamma\sum_{p=2}^{\infty}\eta^{p+1} c_0 c^p\sum_{|I|=p} x_I \le \gamma\sum_{p=2}^{\infty}\eta^{p+1} c_0 c^p$$

$$= \gamma\eta^3 c^2\sum_{p=0}^{\infty}(\eta c)^p = \frac{c_0\gamma\eta^3 c^2}{1 - \eta c} = \frac{c_0\gamma\kappa^3 c^2}{(\kappa + \gamma)^2(\kappa(1 - c) + \gamma)}.$$

Now, if we cannot assume that the sequence is Level Limited, we always have the trivial estimate $\mathcal{E}_I \le 1$ for any multi-index I. Repeating the train of inequalities above, we end up with the following estimate:

$$\Omega(2) = \gamma\sum_{p=2}^{\infty}\eta^{p+1}\sum_{|I|=p} x_I \mathcal{E}_{Ii} \le \gamma\left(\eta^3 + \eta^4 + \cdots\right) = \frac{\gamma\eta^3}{1 - \eta} = \frac{\kappa^3}{(\kappa + \gamma)^2}.$$

It is now straightforward to extend the proof to any degree d other than two. $\qquad\square$

Appendix B: Intransitivity and Permanence for Three Species

In this section we present a conditions for intransitivity and permanence for three interacting species.

Definition 2 We say that a set containing N species is intransitive if the constituent species can be ordered in such a way that the pairwise dynamics between species i and $i + 1$ for $i = 1, \ldots, N - 1$ are such that the fixed point $(x_i, x_{i+1}) = (1, 0)$ is unstable and the fixed point at $(x_i, x_{i+1}) = (0, 1)$ is stable, and where the fixed point $(x_N, x_1) = (1, 0)$ is unstable and $(x_N, x_1) = (0, 1)$ is stable.

Theorem 2 *Consider three species involved in cross-feeding described by* (1) *and* (9) *and assume that* $\Omega(2) = 0$. *If the inequalities*

$$\mathcal{E}_i - \mathcal{E}_j > \eta(\mathcal{E}_{jj} - \mathcal{E}_{ji}) \tag{22}$$

and

$$\mathcal{E}_i - \mathcal{E}_j > \eta(\mathcal{E}_{ij} - \mathcal{E}_{ii}) \tag{23}$$

hold for all pairs of species i and j, then the three species form an intransitive triple. Further, if the inequality

$$\Gamma_{12}\Gamma_{23}\Gamma_{31} < 1, \tag{24}$$

where

$$\Gamma_{ij} = \frac{\mathcal{E}_j - \mathcal{E}_i + \eta(\mathcal{E}_{jj} - \mathcal{E}_{ji})}{\mathcal{E}_j - \mathcal{E}_i + \eta(\mathcal{E}_{ji} - \mathcal{E}_{ii})},$$

holds, then the system exhibits permanence.

Proof We begin by noting that inequality (22) is equivalent to condition (14), which describes the criteria for the fixed point $x = 0$ being unstable in the two species system, and that the second inequality (23) is the reverse of (15), hence a condition for the fixed point at $x = 1$ being stable. Since this is assumed to hold between all three species, we have, according to the definition, an intransitive set.

For the second part of the theorem, we will make use of the technique described in Sect. 4.3 (Jansen 1987), here extended to the case of three species. First we define the function $P(x, y) = x^a y^b (1 - x - y)^c$, where x is the fraction of species 1, y is the fraction of species 2, and the fraction of species 3 is given by $1 - x - y$. The constant a, b, c are assumed to be real and positive. Now P satisfies

$$\frac{dP(x(t), y(t))}{dt} = P(x, y)\Psi(x, y)$$

with $\Psi(x, y) = aw_1(x, y) + bw_2(x, y) + cw_3(x, y)$, where $w_i(x, y) = \phi_i(x, y) - \bar{\phi}(x, y)$. In order to determine the permanence of the system, we need to determine if there exists constants $a, b, c > 0$ such that $\Psi(x^\star, y^\star) > 0$ for all fixed points $(x^\star, y^\star)$ on the boundary of the system. Since we know that the system is intransitive, we also know that the fixed points on the boundary are located at the corners of the simplex, i.e. at $(x^\star, y^\star) = (0, 0), (0, 1)$ and $(1, 0)$. Evaluating Ψ at these points, we get, by using (9),

$$\Psi(0, 0) = aw_1(0, 0) + bw_2(0, 0) + cw_3(0, 0)$$
$$= a\left(\gamma\eta\mathcal{E}_1 + \gamma\eta^2\mathcal{E}_{31} - \gamma\eta\mathcal{E}_3 - \gamma\eta^2\mathcal{E}_{33}\right)$$
$$+ b\left(\gamma\eta\mathcal{E}_2 + \gamma\eta^2\mathcal{E}_{32} - \gamma\eta\mathcal{E}_3 - \gamma\eta^2\mathcal{E}_{33}\right) > 0.$$

For notational convenience, we now define

$$\lambda_{ji} = \gamma\eta\mathcal{E}_i + \gamma\eta^2\mathcal{E}_{ji}.$$

We can now rewrite the above inequality as

$$\Psi(0, 0) = a(\lambda_{31} - \lambda_{33}) + b(\lambda_{32} - \lambda_{33}) > 0, \tag{25}$$

and similarly for the two other fixed points, we get

$$\Psi(0, 1) = a(\lambda_{21} - \lambda_{22}) + c(\lambda_{23} - \lambda_{22}) > 0 \tag{26}$$

and

$$\Psi(1, 0) = b(\lambda_{12} - \lambda_{11}) + c(\lambda_{13} - \lambda_{11}) > 0. \tag{27}$$

We will now try to eliminate the constants a, b, c and determine for which values of the λs these inequalities hold. We proceed by rewriting (25) as

$$a > b\frac{\lambda_{33} - \lambda_{32}}{\lambda_{31} - \lambda_{33}}, \tag{28}$$

where we have assumed that $\lambda_{31} > \lambda_{33}$. However this assumption corresponds to (22). We now rearrange (27) into

$$a < c\frac{\lambda_{23} - \lambda_{22}}{\lambda_{22} - \lambda_{21}}, \tag{29}$$

under the warranted assumption that $\lambda_{22} > \lambda_{21}$ i.e. (23). Combining (28) and (29), we obtain

$$b\frac{\lambda_{33} - \lambda_{32}}{\lambda_{31} - \lambda_{33}} < a < c\frac{\lambda_{23} - \lambda_{22}}{\lambda_{22} - \lambda_{21}}.$$

This eliminates a from the system, and we can instead write

$$\frac{c}{b} > \frac{(\lambda_{33} - \lambda_{32})(\lambda_{22} - \lambda_{21})}{(\lambda_{31} - \lambda_{33})(\lambda_{23} - \lambda_{22})},$$

where we have assumed that $\lambda_{22} < \lambda_{23}$, i.e. (22). Lastly (26) gives us, under the assumption that $\lambda_{11} > \lambda_{13}$, i.e. (23),

$$\frac{c}{b} < \frac{\lambda_{12} - \lambda_{11}}{\lambda_{11} - \lambda_{13}}.$$

We now have lower and upper bounds for c/b, and we can conclude that constants a, b, c exist that satisfy the original inequalities if

$$\frac{(\lambda_{33} - \lambda_{32})(\lambda_{22} - \lambda_{21})}{(\lambda_{31} - \lambda_{33})(\lambda_{23} - \lambda_{22})} < \frac{\lambda_{12} - \lambda_{11}}{\lambda_{11} - \lambda_{13}},$$

where in the last step we made use of the fact that $\lambda_{12} > \lambda_{11}$, i.e. (22). This last inequality is equivalent to (24), and hence we know that the system is permanent in this case. □

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