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Review Article

REVIEW ARTICLE

Synthetic microbial ecology and the dynamic interplay between microbial genotypes

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One sentence summary: Interactions between different microbial genotypes can generate complex dynamics and promote community-level functions that might not be readily predicted from investigating each genotype in isolation.

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ABSTRACT

Assemblages of microbial genotypes growing together can display surprisingly complex and unexpected dynamics and result in community-level functions and behaviors that are not readily expected from analyzing each genotype in isolation. This complexity has, at least in part, inspired a discipline of synthetic microbial ecology. Synthetic microbial ecology focuses on designing, building and analyzing the dynamic behavior of 'ecological circuits' (i.e. a set of interacting microbial genotypes) and understanding how community-level properties emerge as a consequence of those interactions. In this review, we discuss typical objectives of synthetic microbial ecology and the main advantages and rationales of using synthetic microbial assemblages. We then summarize recent findings of current synthetic microbial ecology investigations. In particular, we focus on the causes and consequences of the interplay between different microbial genotypes and illustrate how simple interactions can create complex dynamics and promote unexpected community-level properties. We finally propose that distinguishing between active and passive interactions and accounting for the pervasiveness of competition can improve existing frameworks for designing and predicting the dynamics of microbial assemblages.

Keywords: synthetic ecology; microbial ecology; population dynamics; microbial interactions; community assembly

INTRODUCTION

Consider an ecosystem consisting of two different microbial genotypes that live within close spatial proximity to each other. Given the apparent simplicity of this ecosystem, one might presume that the dynamic behaviors of these two microbial genotypes are relatively easy to predict and explain from basic principles and measurable properties. Recent theoretical and experimental investigations, however, suggest that even a simple assemblage of two microbial genotypes can exhibit surprisingly complex and unexpected dynamics (Nowak and Sigmund 2004; Nadell and Foster 2012; Allen and Nowak 2013). Moreover, the dynamic interplay between the microbial genotypes can result in community-level functionalities and properties (e.g. robustness, resilience, complementarity, facilitation, competition, antagonism, etc.) that might not be readily expected from analyzing each genotype in isolation (Korb and Foster 2010; Celiker and Gore 2012; Großkopf and Soyer 2014; Escalante *et al.* 2015; Fredrickson 2015). Clearly, our understanding of the

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general rules and principles that govern the dynamics and emergent properties of microbial assemblages is at its infancy.

This knowledge gap has, at least in part, inspired a developing discipline of synthetic microbial ecology (Brenner, You and Arnold 2008; Wintermute and Silver 2010b; Momeni *et al.* 2011; Chuang 2012; Mee and Wang 2012; Bacchus and Fussenegger 2013; De Roy *et al.* 2013; Großkopf and Soyer 2014; Zomorrodi and Segrè 2015; Lindemann *et al.* 2016). Synthetic microbial ecology can be viewed as an extension or subdiscipline of synthetic biology. While synthetic biology typically focuses on designing, building, quantitatively analyzing, and predicting the dynamic behavior of metabolic and regulatory circuits (i.e. a set of interacting molecules), synthetic microbial ecology focuses on designing, building, quantitatively analyzing and predicting the dynamic behavior of 'ecological circuits' (i.e. a set of interacting microbial genotypes). In addition, whereas synthetic biology seeks to understand how cellular-level properties emerge as a consequence of molecular interactions, synthetic microbial ecology seeks to understand how community-level properties emerge as a consequence of microbial interactions.

From an experimental perspective, synthetic microbial ecology can be broadly delineated into bottom-up and top-down approaches (however, see alternative delineations by De Roy *et al.* 2013; Großkopf and Soyer 2014). Bottom-up approaches focus on the 'design and build' principle, which is elegantly summarized by Richard Feynman in his statement that 'What I cannot create, I do not understand'. The main approach is to assemble different microbial genotypes with pre-determined properties together in order to achieve a desired set of interactions. The researcher then measures the dynamics and properties of both individual genotypes and the synthetic assemblage itself and attempts to understand or control specific features or higher level properties that emerge as a consequence of those interactions. It therefore closely aligns with conventional synthetic biology, where the researcher often assembles genes, enzymes or other molecules together within a cell, measures the dynamics and properties of individual molecules and the cell itself, and attempts to understand or control specific features or higher level properties that emerge as a consequence of the molecular interactions (Smolke and Silver 2011; Lanza, Crook and Alper 2012). Top-down approaches, in contrast, are not based on designing and building a set of particular ecological interactions. Instead, genotypes are selected from a predefined set and assembled together randomly to obtain a set of synthetic assemblages with certain compositional aspects (e.g. assemblages with varying levels of functional, taxonomic or phylogenetic diversity) (Bell *et al.* 2005; Cardinale *et al.* 2006; Wittebolle *et al.* 2009; Cardinale 2011; Reich *et al.* 2012; De Roy *et al.* 2013). The researcher then measures the properties of the synthetic assemblages (e.g. resource consumption rates, biomass production, susceptibility to invasion, response to perturbations etc.) and tests whether the controlled aspects relate to those properties. In some cases, the measured properties can provide insight into the interactions within those synthetic assemblages and how those interactions affect specific features of the assemblages (e.g. Foster and Bell 2012; Zuppinger-Dingley *et al.* 2014). We note that synthetic microbial ecology is not an entirely new discipline, but instead builds upon many decades of research with microbial isolates and defined assemblages (Fredrickson 1977; Fredrickson and Stephanopoulos 1981; Schink 2002). Nevertheless, modern mathematical modeling and genetic tools together with insights from the last three decades of research on environmental microbiology and experimental ecology are transforming synthetic ecology into a distinct and exciting new discipline.

GOALS AND SCOPE

The goals of this review are the following. First, we introduce typical objectives of synthetic microbial ecology and review the main advantages and rationales of using synthetic microbial assemblages. We primarily focus on bottom-up approaches, as in our view these approaches align most closely with the 'design and build' principle that has been central to the broader field of synthetic biology (Church *et al.* 2014; Way *et al.* 2014). Second, we briefly summarize the main methodologies of synthetic microbial ecology, including experimental, theoretical and mathematical techniques. We emphasize that synthetic microbial ecology often benefits when experimentation and mathematical modeling are combined. Third, we summarize the main findings of current synthetic microbial ecology investigations. Namely, we focus on the causes and consequences of the dynamic interplay between different microbial genotypes. Finally, we propose alternative terminology for defining interactions that may stimulate new perspectives and discussions. Throughout the review, we illustrate how simple ecological interactions can create complex dynamics, and how those dynamic behaviors can lead to community-level functionalities that might not be readily predicted from analyzing each genotype in isolation. We do not attempt to define the scope of synthetic microbial ecology, but to instead provide a perspective for discussing the main concepts, approaches and questions relevant to the field.

WHAT ARE TYPICAL OBJECTIVES OF SYNTHETIC MICROBIAL ECOLOGY?

The typical objectives of a synthetic microbial ecology investigation can often be assigned to one of the following two types.

(i) Understand the general principles and rules that govern the dynamics, functioning and higher-order community-level properties of microbial assemblages. How do community-level properties emerge from the dynamic interplay between different microbial genotypes (e.g. ecological and evolutionary feedbacks)?
(ii) Rationally engineer synthetic microbial assemblages to enable, control or optimize a desired biotransformation, such as the production of a valuable product from a low-cost source material or the transformation of a pollutant into an innocuous end product.

The objectives are therefore broad and encompass both fundamental questions and tangible applications. While these objectives are not mutually exclusive, the main focus of this review is on objective i. We acknowledge that applications (objective ii) have been a major driving force for the field, and we therefore refer the reader to previous reviews and perspectives that provide more emphasis on applications (e.g. Markx, Andrews and Mason 2004; De Roy *et al.* 2013; Ortiz-Marquez *et al.* 2013; Jagmann and Philipp 2014; Song *et al.* 2014; Lindemann *et al.* 2016).

Why use synthetic microbial assemblages?

To achieve the objectives listed above, the following features of synthetic assemblages are of significant value.

(i) Microbial interactions can be controlled and monitored. Different microbial genotypes can be obtained or genetically engineered that interact with each other in a desired manner.

(ii) Experimental conditions can be controlled. Synthetic microbial assemblages can be propagated under carefully maintained experimental conditions where the physical and chemical properties of the environment are well defined. Experimental conditions can also be modified to perturb, promote or prohibit specific types of microbial interactions, thus, allowing for carefully controlled manipulation experiments.
(iii) Synthetic microbial assemblages are less complex than natural microbial communities, and are therefore generally more amenable to mathematical modeling. The growth and metabolic properties of each genotype can be measured in isolation and, in some cases, within the assemblages themselves. It is therefore easier to obtain a comprehensive description of their dynamic properties, which is important for accurate and reliable model predictions. Moreover, because the frequencies of each genotype can typically be monitored over time, it is possible to compare model predictions with experimental observations at a high level of detail.

All three of these features are less likely to be available when investigating microbial assemblages in their natural environment. Natural microbial assemblages often contain many hundreds to thousands of different genotypes (Curtis, Sloan and Scannell 2002; Pedrós-Alió 2012), most of which are not readily amenable to isolation or phenotypic characterization. The metabolic properties of individual genotypes and the interactions between them must therefore typically be deduced using predominantly indirect tools, such as (meta) genomic (Ponomarova and Patil 2015; Stepanauskas 2015) or stable-isotope probing (Neufeld, Wagner and Murrell 2007; Dolinšek *et al.* 2013) methods. The interactions are consequently often hypothetical in nature, incompletely described, of high dimensionality (i.e. the number of interactions within a natural assemblage may be exceedingly large), and difficult to manipulate in a desired manner, thus, creating confounding factors that make it difficult to understand how interactions affect community dynamics. In addition, environmental parameters available for manipulation are relatively limited when investigating natural microbial assemblages in their native environment. For example, while one can add a resource to an existing nutrient pool to promote or prevent a particular interaction, it can be difficult to completely remove a particular resource, which is less of an issue when working with synthetic microbial assemblages propagated in defined medium in the laboratory. Finally, it is typically more difficult to track the abundances or frequencies of different genotypes within natural microbial assemblages, and the measured dynamics are therefore of coarser resolution.

Imposing interactions and analyzing synthetic microbial assemblages

There are two main approaches for imposing interactions between different microbial genotypes. One approach is to use different species or strains that mimic a simplified natural microbial assemblage of interest. For example, if one is interested in fermentation processes, one might assemble together a species that ferments an organic substrate to hydrogen with another species that consumes hydrogen (Stolyar *et al.* 2007). This approach is advantageous in that the synthetic assemblage may more accurately mimic a natural community. The use of different species also allows for a wide range of interactions. However, the approach also suffers in that the two genotypes will likely have many genetic differences between them. This could create confounding factors and lead to non-intuitive or unwanted interactions, thus resulting in some loss of experimental control (Hansen *et al.* 2007). An alternative approach is to genetically engineer and assemble together a set of genotypes that were all derived from the same parental strain (i.e. isogenic mutants) (e.g. Wintermute and Silver 2010a; Lilja and Johnson 2016). Isogenic mutants have fewer genetic differences between them, thus reducing (but not eliminating) the probability of generating unexpected or unwanted interactions that could emerge from numerous physiological differences between more distantly related genotypes. Interactions are therefore more controlled and effectively limited to a few well-defined types. For example, one can create interactions by deleting or inactivating genes encoding essential biosynthetic machinery (e.g. for amino acids or nucleotides biosynthesis), thus forcing two isogenic mutants to cross-feed specific biosynthetic building blocks (Wintermute and Silver 2010a; Mee *et al.* 2014; Pande *et al.* 2014). A potential disadvantage of using isogenic mutants, however, is that they may not accurately mimic natural microbial assemblages, and thus provide less insight into natural processes. Moreover, even if isogenic mutants only contain a few genetic differences, these genetic differences may have large phenotypic effects and also lead to the generation of unexpected interactions and confounding factors.

After creating a synthetic microbial assemblage, the main behaviors of the assemblage are measured, such as resource consumption, growth, productivity, etc. Properties of the individual genotypes might also be measured, such as changes in their abundances or frequencies and their spatial positioning relative to each other. To quantify the abundances or frequencies of different genotypes, genetic or phenotypic traits (e.g. fluorescent protein-encoding genes, antibiotic resistance genes etc.) can be introduced into the genotypes, which are then assayed using microscopic or phenotypic assays (e.g. by selective plating). Alternatively, it may be possible to use native traits to distinguish and quantify different genotypes, such as the production of pigments, the ability to use certain resources, or the requirement for specific nutrients (e.g. Lenski *et al.* 1991; Kassen *et al.* 2000). Finally, for synthetic assemblages containing larger numbers of genotypes, sequencing-based techniques may be useful for quantifying the abundances of individual genotypes based on engineered or natural genetic differences. The decision about how to distinguish and quantify different genotypes depends on the main questions and objectives of interest. If spatial arrangement at the microscale or the single cell-level is central for an investigation, then the use of fluorescent protein-encoding genes is especially powerful because one can readily quantify spatial metrics such as intermixing and cooccurrence patterns (Daims, Lücker and Wagner 2006; Hansen *et al.* 2007; Momeni, Waite and Shou 2013; Müller *et al.* 2014). However, if one is interested in spatial arrangement at the macroscale or in behaviors in completely mixed systems, then the use of native traits may be sufficient and additionally avoids the possibility that the genetic markers themselves might impact the biology of the organisms (e.g. Lenski *et al.* 1991; Kassen *et al.* 2000).

Mathematical models are often used to generate predictions and hypotheses that can be tested with synthetic microbial assemblages (Zomorrodi and Segrè 2015; Widder *et al.* 2016). Mathematical models are powerful because interactions between different genotypes often result in non-linear behaviors and asynchronous growth of the different genotypes, which can lead to complex and non-intuitive predictions (e.g. Yoshida *et al.* 2003). Dynamical models based on differential equations have been widely applied, where the goal is to predict how system

features change over time. These system features may include the abundances of different genotypes, the concentrations of resources or the abundances of specific biological molecules (e.g. enzymes, transcripts, ATP, toxins etc.). While powerful, dynamical models typically require the estimation or measurement of a relatively large number of biological parameters, such as maximum reaction rates, half-saturation coefficients, inhibition coefficients and carrying capacities. They also model population-level behaviors rather than individual-level behaviors. An advantage of dynamical models is that they have a fixed set of equations that are typically more tractable to mathematical analyses. The outcomes are therefore typically easier to generalize and the main drivers of system behavior are often easier to identify.

Alternative types of models include game theoretical or agent-based models. In these models, individuals are treated as agents that obey by certain rules. These rules are the processes that govern how different individuals interact with each other and with their environment. They determine the types of strategies that individuals may take and the costs and benefits for implementing those strategies. For example, an individual may be a 'cooperator' that secretes a molecule that is beneficial for the entire population or a 'cheater' that consumes the secreted molecule but does not secrete it itself. If an individual engages in a strategy that has greater benefits than costs, then that individual is more likely to reproduce and, in turn, increase in frequency or abundance. Different strategies can therefore be competed against each other and the outcomes compared. Thus, they differ from dynamical models in that they focus on individual-level behaviors rather than on population-level behaviors. One disadvantage of agent-based models, however, is that they are often complex. The outcomes are therefore sometimes more difficult to generalize and the main drivers of system behaviors are more difficult to identify.

More recently, metabolic models have been used to comprehensively predict the metabolism of a genotype and how genotypes are likely to interact with others within synthetic assemblages (Stolyar *et al.* 2007; Klitgord and Segrè 2010; Wintermute and Silver 2010a; Harcombe *et al.* 2014; Chubiz *et al.* 2015). These models differ from the previous two in that they are inherently stoichiometric and/or thermodynamic in nature, and they therefore do not require measurement or adjustment of kinetic parameters. They are also capable of generating accurate descriptions of metabolism and emergent ecological interactions from genomic data using stoichiometric balancing and by considering thermodynamic properties of metabolic reactions. However, they suffer from relying on questionable optimality assumptions and are often incomplete in nature (for a detailed overview of the utility and limitations of metabolic models, see e.g. Ataman and Hatzimanikatis 2015; O'Brien, Monk and Palsson 2015). Continuing development of these models and ongoing translation of genomic data into mathematically described metabolic networks (O'Brien, Monk and Palsson 2015) hold promise for identifying novel interactions and improving the rational design of synthetic microbial assemblages (Zomorrodi and Segrè 2015).

TYPES OF SYNTHETIC MICROBIAL ASSEMBLAGES

An important objective of many synthetic microbial ecology experiments is to create a reduced ecosystem that retains key functionalities and properties of a more complex microbial assemblage. This reduces confounding factors and complexity that might otherwise prohibit detailed analyses and the confident testing of hypotheses and theoretical predictions. The first task of the synthetic microbial ecologist, therefore, is to define the biological properties of the genotypes that will be assembled together and how they are likely to interact in the context of the experimentally imposed environment. This may seem straightforward, but interactions are context dependent and can change in strength and nature over both time and space. The dynamics and emergent properties of a synthetic microbial assemblage are therefore sometimes difficult to predict. Nevertheless, defining initial interactions is a typical starting point. We therefore summarize the types of interactions that have been imposed between two genotypes. We propose two major criteria to distinguish different types of interactions: whether the interaction is passive or active in nature (see below for definitions) and whether the interaction has positive or negative effects on each of the involved genotypes.

Passive interactions refer to interactions where the growth of one genotype is affected by the inadvertent activities of a second genotype. For example, one genotype may produce a waste product (i.e. the passive promoter) that is then consumed by another genotype (i.e. the beneficiary), which is sometimes called a by-product interaction (Sachs *et al.* 2004; West, Griffin and Gardner 2007) or a commensalism (i.e. accidental effect, see Mitri and Foster 2013). Here, we use the term 'passive' to emphasize that the passive promoter does not actively invest metabolic resources into promoting the growth of the beneficiary, but instead promotes the growth of the beneficiary as an inadvertent consequence of its own metabolism. Another example of a passive interaction is the inadvertent leakage of metabolites from one cell and consumption of those metabolites by another cell.

Active interactions refer to interactions where one genotype actively invests resources into metabolic processes or behaviors that affect other genotypes. When positive in nature, such interactions are sometimes classified as cooperative interactions (West, Griffin and Gardner 2007; Mitri and Foster 2013) or directed reciprocation (Sachs *et al.* 2004). For example, one genotype may divert cellular resources away from its own growth to produce a metabolite or provide a service that promotes or supports the growth of a second genotype, while the second genotype may divert cellular resources away from its own growth to produce a different metabolite or provide a service that promotes or supports the growth of the first genotype. There is therefore a reciprocal exchange of resources or services. Because of their active nature, these interactions pose interesting evolutionary dilemmas. How do active investments of resources into partner genotypes emerge in the first place? What prevents genotypes that benefit from active investments but do not pay any of the costs (i.e. 'cheater' genotypes) from exploiting and disrupting an active interaction? The susceptibility of active interactions to cheating has therefore stimulated a large amount of research into how active interactions originate and persist over time (West *et al.* 2006; West, Griffin and Gardner 2007; Mitri and Foster 2013).

We emphasize that strictly positive interactions (active or passive) are unlikely to occur and that most positive interactions will have competitive elements at the same time, as the genotypes may simultaneously compete for other shared resources (e.g. oxygen, nitrogen and phosphorous; see Fredrickson 1977, Fredrickson and Stephanopoulos 1981 or Mitri and Foster 2013). In order to achieve a strictly positive interaction, each genotype must occupy a non-overlapping ecological niche that prevents competition for shared resources, which is experimentally difficult to implement (however, see Weber, Daoud-El

Baba and Fussenegger 2007, for a successful implementation). Importantly, it may not be immediately clear whether the positive or the competitive elements have the dominant effect on the dynamics and behaviors of the assemblage (Hansen *et al.* 2007). We therefore do not describe resource competition in a separate section but instead try to identify competitive elements in the examples we provide below.

Passive unidirectional positive interactions (commensalisms)

Passive unidirectional positive interactions refer to interactions where the growth of one genotype (designated as the beneficiary) is promoted by the growth of a second genotype (designated as the passive promoter) (Fig. 1). Among the most extensively investigated examples of a passive unidirectional positive interaction is acetate cross-feeding within populations of *Escherichia coli*. When clonal populations of *E. coli* are propagated with glucose as the growth-limiting resource, intermediate metabolites (such as acetate) transiently and inadvertently leak from the cell and accumulate in the medium (for recent insight of this phenomenon; see Basan *et al.* 2015, and references therein). However, over evolutionary time, metabolically specialized genotypes repeatedly emerge (Helling, Vargas and Adams 1987; Rosenzweig *et al.* 1994; Treves, Manning and Adams 1998; Rozen and Lenski 2000; Rozen *et al.* 2009). In one case, two coexisting genotypes emerged that compete for glucose and acetate, but one genotype consumes glucose more effectively while the other consumes the secreted acetate more effectively (Rosenzweig *et al.* 1994; Treves, Manning and Adams 1998). Further, experiments identified the genetic basis of these two genotypes, where genetic changes in a single gene are sufficient to increase glucose uptake while simultaneously decreasing acetate uptake (Treves, Manning and Adams 1998). Thus, the intrinsic tradeoff between glucose and acetate uptake creates a dynamic interplay between the two genotypes that enables their coexistence and results in accelerated glucose consumption via substrate cross-feeding without abolishing competition for shared resources.

Such interactions were recently engineered *de novo* in synthetic assemblages of *E. coli* strains, where the spatial structure of the environment was experimentally controlled. An acetate-consuming specialist genotype was genetically engineered in the laboratory, where glucose consumption was completely abolished by introducing loss-of-function mutations into *E. coli* (Bernstein, Paulson and Carlson 2012). When the acetate-consuming specialist genotype was then assembled together with its glucose-consuming parental genotype, the two genotypes coexisted via cross-feeding acetate and other metabolites (Bernstein, Paulson and Carlson 2012). Moreover, the cross-feeding assemblage achieved 15% greater biomass than the parental strain when grown in batch culture and 50% greater biomass than the parental strain when grown in biofilm. The two cross-feeding genotypes self-organized within the biofilm, where the upper well-oxygenated layer of the biofilm was populated predominately with the acetate-consuming genotype, while the glucose-exposed base of the biofilm was populated predominantly by the glucose-consuming genotype (Bernstein, Paulson and Carlson 2012). This structuring is therefore an emergent property of the interaction and likely improved the productivity of the biofilm, emphasizing the benefits of compartmentalizing different metabolic processes into different cell types. Thus, if conflicts are known to exist between different metabolic processes [e.g. in this case between glucose and acetate consumption; see Rosenzweig *et al.* (1994) and Treves, Manning and Adams (1998)], then one can engineer and assemble genotypes together that prevent the emergence of those conflicts and consequently accelerate community-level metabolic processes (Bernstein, Paulson and Carlson 2012).

Passive unidirectional positive interactions need not always be based on cross-feeding of metabolic waste products or inadvertent cell leakage. An example is the depletion of a growth-inhibiting molecule, such as an antibiotic (Yurtsev *et al.* 2013). Antibiotic-resistant genotypes can create 'antibiotic-free' local environments thorough their metabolic activities, where a resistant genotype secretes an enzyme such as β-lactamase that inactivates the antibiotic and enables the growth of a sensitive genotype (e.g. Perlin *et al.* 2009). In one study with ampicillin-resistant and -sensitive strains of *E. coli*, the frequencies of the two populations reached equilibrium over time (Yurtsev *et al.* 2013). However, the equilibrium frequencies of the resistant strain were dependent on the initial ampicillin concentration (proportional dependence) and on the initial population size (inverse-proportional dependence). When resistant cells were initially rare, their relative abundance 'overshot' the equilibrium point and then descended back towards it. The authors proposed a model that recapitulated the system dynamics and revealed non-intuitive and unexpected behaviors. For example, the addition of a β-lactamase inhibitor (which is used in clinical practice) increases rather than decreases the proportion of ampicillin-resistant cells (Yurtsev *et al.* 2013).

An important feature of the interaction described above is that it involves the production of a public good (i.e. β-lactamase). There is therefore an active 'cooperative' intrapopulation interaction between genetically identical cells that produce β-lactamase. The interpopulation interaction (i.e. the interaction between the β-lactamase-producing and the non-producing genotypes), however, is nevertheless a passive unidirectional positive interaction with competition. The genotype that produces β-lactamase did not evolve this trait because it provides a benefit to the genotype that does not produce β-lactamase. Instead, that trait was likely selected to benefit the β-lactamase producing strain itself. The interaction between the two genotypes is therefore passive, which in turn permits competition between the two genotypes for other metabolic resources. We therefore emphasize that the terminology discussed here refers to interactions between different genotypes and not to interactions between cells of a single genotype.

Passive unidirectional positive interactions based on the secretion or leakage of metabolites from one genotype and exploitation by other genotypes may be pervasive in the natural environment as predicted by the Black Queen hypothesis (Morris, Lenski and Zinser 2012). The Black Queen hypothesis states that if one genotype provides a reliable source of a metabolite (e.g. via inadvertent cell leakage) or a service (e.g. the consumption or inactivation of a growth-inhibiting molecule), then another genotype may exploit those metabolites and services, and cease to biosynthesize or perform those services itself (Morris, Lenski and Zinser 2012; Morris, Papoulis and Lenski 2014). One outcome, then, is the origin of a passive unidirectional positive interaction between the two genotypes (Morris, Lenski and Zinser 2012; Morris, Papoulis and Lenski 2014). Synthetic microbial ecology has successfully tested some of the main predictions of the Black Queen hypothesis (Morris, Papoulis and Lenski 2014). Namely, genes should be continuously lost from a community, and thus passive unidirectional positive interactions should originate (Fig. 2) as long as at least one genotype performs a leaky function that other genotypes can reliably exploit (Morris, Lenski and Zinser 2012).

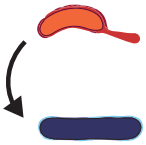
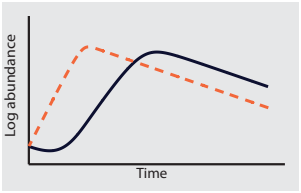
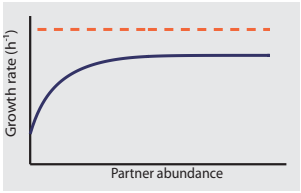
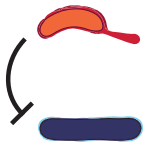
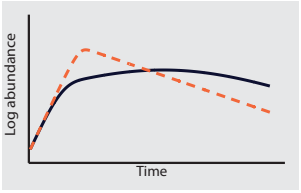
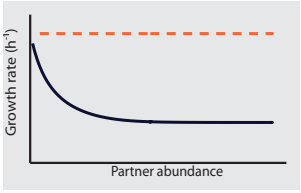
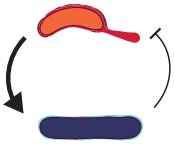
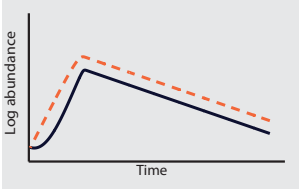
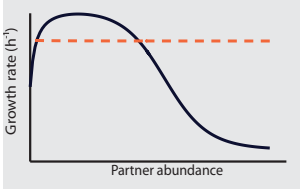
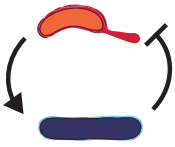
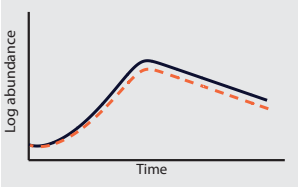
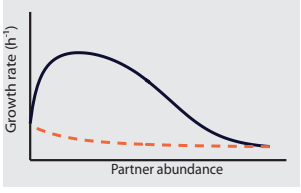
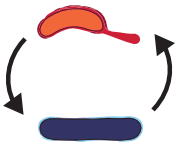
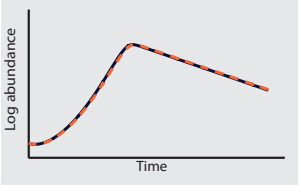
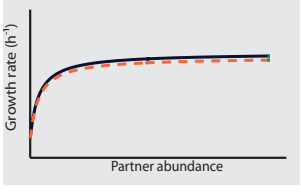
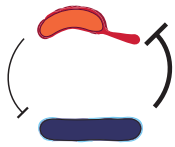
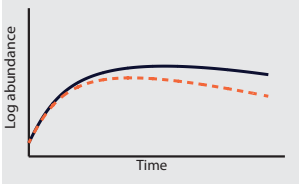
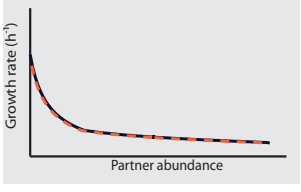
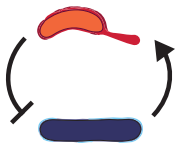
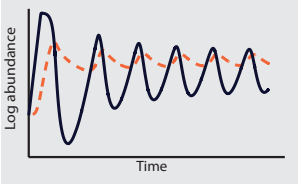
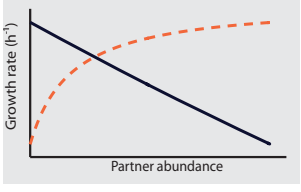
Interaction type	Abundance vs. Time	Growth rate (μ) vs. Partner abundance	Examples from literature
Positive unidirectional 			Weber, Daoud-El Baba and Fussenegger 2007; Lilja and Johnson 2016
Negative unidirectional 			Kerr *et al.* 2002; Cordero *et al.* 2012; Kelsic *et al.* 2015
Positive unidirectional with competition 			Rosenzweig *et al.* 1994; Hansen *et al.* 2007; Bernstein, Paulson and Carlson 2012
Positive unidirectional with competition for primary and produced substrates. 			Yurtsev *et al.* 2013; Morris, Papoulis and Lenski 2014
Positive bidirectional 			Stolyar *et al.* 2007; Harcombe 2010; Kazamia *et al.* 2012; Pande *et al.* 2014; Lilja and Johnson 2016; Zhou *et al.* 2015; Summers *et al.* 2010; Koch *et al.* 2015
Negative bidirectional 			Vetsigian, Jajoo and Kishony 2011
Predation /parasitism 			Yoshida *et al.* 2003; Weber, Daoud-El Baba and Fussenegger 2007; Balagaddé *et al.* 2008

Figure 1. Dynamics of binary assemblages. We simulated population dynamics for binary assemblages with different interactions between the two distinct genotypes. The initial ratio of both genotypes was 1:1 (cell number:cell number). The first column describes the interaction type, the second column shows how populations change over time, the third column shows how growth rate depends on the partner's abundance and the last column refers to studies where similar dynamics took place. Arrow thickness corresponds to the interaction strength. All models are based on the limited substrate model for microorganisms growing in batch culture, with the exception of the predator–prey interaction where microorganisms are growing in chemostat culture. The model includes a constant mortality term, and thus results in the decline of all abundances at later time points. Our models assume that evolution does not occur over the time scale of the simulations. A full description of the models, an explanation of the underlying assumptions for each model, and further discussions of the dynamics are provided in the Supporting Information.

Passive bidirectional positive interactions (non-cooperative mutualisms)

Passive bidirectional positive interactions refer to interactions where the growth of one genotype (designated as passive promoter A) is promoted by the growth of a second genotype (designated as passive promoter B), while the growth of passive promoter B is also promoted by the growth of passive promoter A (Fig. 1). Thus, there are reciprocal beneficial effects. We again use the term 'passive' to emphasize that the passive promoters do not actively invest metabolic resources into promoting the growth of others, but they instead promote the growth of others as inadvertent consequences of their own metabolism (e.g. generating waste products or inadvertent leakage of molecules that can be consumed by others). The interactions therefore do 'not' constitute as cooperative interactions in the sociological sense, where active investments in partners are necessary (West, Griffin and Gardner 2007). A typical example is unidirectional cross-feeding of a self-inhibiting waste product. One genotype produces a metabolic waste product that can inhibit its own growth if it accumulates to sufficient concentrations, while another genotype consumes the waste product and relieves inhibition of the first genotype. The main dynamic behavior of such a system is typically the approximate convergence of growth rates between the two interdependent genotypes (Fig. 1).

A canonical example of this type of interaction is interspecies hydrogen transfer. Some microorganisms ferment organic substrates to hydrogen. However, if hydrogen accumulates to sufficient concentrations, then the metabolic reaction can become thermodynamically unfavorable and stop. Thus, a partner microorganism is required that consumes the hydrogen to sufficiently low concentrations to maintain thermodynamically favorable conditions (McInerney *et al.* 2008; Morris *et al.* 2013). In one synthetic microbial assemblage, the sulfate-reducing bacterium *Desulfovibrio vulgaris* was grown together with the methanogenic archaeon *Methanococcus maripaludis* (Stolyar *et al.* 2007). In the absence of sulfate, *D. vulgaris* can ferment lactate to hydrogen, while the methanogen *M. maripaludis* maintains the metabolic activity of *D. vulgaris* by transforming the hydrogen into methane. Thus, the growth rates of the two genotypes spontaneously converge based on the production and consumption rates of hydrogen. Initially, the growth of synthetic assemblages was somewhat erratic, and a small subset of the assemblages went extinct (Hillesland and Stahl 2010; Hillesland *et al.* 2014). Over evolutionary time, however, the remaining synthetic assemblages began to improve in productivity. Further, experiments demonstrated that the improved performance was a consequence of genetic changes in both *D. vulgaris* and *M. maripaludis*, suggesting potential evolutionary responses to the passive bidirectional positive interaction.

Another example of this type of interaction is nitrite cross-feeding. Nitrifying communities are well known to cross-feed nitrite, where ammonia-oxidizing microorganisms convert ammonia to nitrite and nitrite-oxidizing bacteria consume the secreted nitrite (Costa, Pérez and Kreft 2006; Maixner *et al.* 2006). Under low pH conditions, both microorganisms may benefit from this interaction because the cross-fed intermediate nitrite can become growth-inhibiting while also serving as a growth substrate for the nitrite-oxidizing bacteria. A similar scenario sometimes occurs within denitrifying communities. Many microorganisms are capable of using nitrogen oxides as terminal electron acceptors to support their growth (Zumft 1997). While some can completely respire nitrate to nitrogen gas, others specialize at specific steps of the pathway (Heylen *et al.* 2006) and, in some cases, assemble together into nitrite or nitrous oxide cross-feeding consortia (Martienssen and Schöps 1999; Van de Pas-Schoonen *et al.* 2005). In one recent study, two isogenic mutant strains of *Pseudomonas stutzeri* were constructed, where one strain consumes nitrate to nitrite and another consumes nitrite to nitrogen gas (Lilja and Johnson 2016). The authors found that segregating the two parts of the pathway into different genotypes eliminated competition between the nitrate and nitrite reductases for intracellular resources and consequently reduced the accumulation of the intermediate nitrite. Moreover, under low pH conditions, when nitrite has growth-inhibiting effects (Almeida *et al.* 1995; Baumann *et al.* 1997), nitrite cross-feeding accelerated substrate consumption, presumably because nitrite accumulated to lower concentrations and had reduced deleterious effects on growth (Lilja and Johnson 2016). This study again emphasizes that if conflicts are known between different metabolic processes (e.g. in this case between the nitrate and nitrite reductases), then one can engineer and assemble genotypes together that avoid those conflicts and consequently accelerate community-level metabolic processes (Johnson *et al.* 2012; Lindemann *et al.* 2016).

Passive bidirectional positive interactions have also been designed for biosynthetic applications, where metabolic interdependencies were engineered to control system dynamics. In one study, synthetic cocultures were engineered to produce oxygenated taxanes, which are precursors for the anti-cancer drug taxol (Zhou *et al.* 2015). The cocultures consisted of an *E. coli* strain that produced and secreted a precursor and an *S. cerevisiae* strain that oxygenated the precursor to produce the desired oxygenated taxane (Zhou *et al.* 2015). An important aspect of the system is that the two genotypes were not initially dependent on each other but instead had competitive and negative interactions. Both organisms competed for glucose while *S. cerevisiae* produced ethanol that inhibited the growth of *E. coli*. These competitive and negative interactions resulted in reduced taxane production. To overcome this, the authors re-engineered the environment to minimize these competitive and negative interactions. First, the authors provided xylose rather than glucose as a carbon substrate. Only *E. coli* could consume the xylose, thus preventing the main competitive interaction. Moreover, *E. coli* produced acetate as a waste product that *S. cerevisiae* then consumed as a growth-substrate, thus creating a passive positive interaction between the two genotypes. Finally, acetate has growth-inhibiting effects on *E. coli* if it is not consumed by *S. cerevisiae*, thus creating a second passive positive interaction in the opposite direction. Together, the minimization of competition and the promotion of the passive bidirectional positive interaction resulted in improved taxane production, demonstrating that simple but well informed engineering of the environment can provide control over system behavior.

Active bidirectional positive interactions (cooperative mutualisms)

Active bidirectional positive interactions pose interesting evolutionary dilemmas concerning their origin and maintenance, but are nevertheless found in nature. One such example is the vitamin B_{12} (cobalamin)-based interaction between many algal species and bacteria. Approximately one-half of cultivated algal species require vitamin B_{12} for their growth but cannot biosynthesize vitamin B_{12} *de novo* (Croft *et al.* 2005). Instead, they depend on bacteria to provide vitamin B_{12}, which is metabolically costly to biosynthesize and secrete (Raux *et al.* 1996).

Kazamia *et al.* (2012) assembled the vitamin B_{12}-dependent green alga *Lobomonas rostrata* with the bacterium *Mezorhizobium loti* and found that the two species could be cocultivated with each other in the absence of exogenous vitamin B_{12}. The alga supported the growth of the bacterium by providing it with fixed carbon, while the bacterium supported the growth of the alga by providing it with vitamin B_{12}. The authors further reported evidence that the bacterium actively diverts cellular resources from its own growth into supplying vitamin B_{12} to the algae, thus meeting the criteria for an active 'cooperative' interaction. First, the bacterium produced more vitamin B_{12} when in the presence than in the absence of the algae (Grant *et al.* 2014). Second, mathematical modeling indicated that bacterial lysis alone could not explain the extent of algal growth, as more bacterial cells would have needed to lyse than were present in the culture to support the observed growth of the algae (Grant *et al.* 2014). Dissecting the vitamin B_{12}-based interaction between algae and bacteria further, Xie *et al.* demonstrated that the synthesis of the vitamin B_{12}-independent methionine synthase is repressed during heat stress in *Chlamydomonas reinhardtii* and that survival of heat stress depends on the functional vitamin B_{12}-dependent methionine synthase. By cocultivating *C. reinhardtii* with diverse bacteria, they showed that algal stress tolerance is restored in the presence of vitamin B_{12}-producing bacteria, thus, providing an example of an emergent community property (i.e. stress resistance) that might not be readily expected from analyzing each genotype in isolation (Xie *et al.* 2013).

While the example above attempts to mimic a natural system, likely the most widely engineered active bidirectional positive interaction (i.e. cooperative interaction) is the reciprocal exchange of amino acids or nucleotides between genotypes with different auxotrophic requirements (i.e. genotypes that require exogenous sources of different amino acids or nucleotides to support their growth). Genotypes that cannot biosynthesize certain amino acids or nucleotides are readily obtained from existing mutant libraries (Baba *et al.* 2006) or can be created by targeted gene deletions. Interactions are then established by assembling two genotypes together that require different amino acids or nucleotides, thus forcing each genotype to provide the essential resource required by the other (Shou, Ram and Vilar 2007; Wintermute and Silver 2010a; Hosoda *et al.* 2011; Park *et al.* 2011; Kerner *et al.* 2012; Waite and Shou 2012; Momeni, Waite and Shou 2013; Mee *et al.* 2014; Pande *et al.* 2014). The questions addressed with these synthetic assemblages are diverse. They range from exploring conditions under which such interactions are likely to arise (Shou, Ram and Vilar 2007; Wintermute and Silver 2010a; Mee *et al.* 2014; Pande *et al.* 2014) to examining the factors that prevent the emergence and proliferation of mutants that exploit but do not contribute towards the active interaction (i.e. cheaters) (Waite and Shou 2012; Momeni, Waite and Shou 2013).

The leitmotif that emerges from many of these studies is that costly metabolites are often not readily released, and genetically engineered auxotrophs do not typically release sufficient amounts of amino acids or nucleotides to support the growth of partner auxotrophs (Shou, Ram and Vilar 2007; Harcombe 2010; Pande *et al.* 2014, 2015). For example, pairs of *Saccharomyces cerevisiae* mutants that were deficient in either lysine or adenine biosynthesis repeatedly went extinct when they were grown together in the absence of lysine and adenine unless feedback inhibition mechanisms that prevent the overproduction of the shared metabolites were disrupted (Shou, Ram and Vilar 2007). In a study, discussed in more detail below (Harcombe 2010), extensive mutagenesis was required to obtain a *Salmonella typhimurium* mutant that secretes sufficient methionine to support the growth of an *E. coli* genotype that cannot biosynthesize methionine. The necessity for engineered overproduction (and thus active secretion) of exchanged amino acids was further elaborated in Pande *et al.* (2014), where four *E. coli* amino acid auxotrophs were assembled in pairwise combinations. They used metabolic modeling to identify genes that, upon deletion or inactivation, would contribute the most towards the overproduction of complementary amino acids. When the auxotrophic mutants were then assembled together in pairwise combinations, the majority of the synthetic assemblages had significantly higher growth rates when compared to the ancestral strain. Thus, while not easily achieved, segregating different amino acid biosynthetic pathways into different genotypes and imposing active positive interactions via targeted genetic engineering can sometimes improve the overall productivity of synthetic assemblages (Pande *et al.* 2014).

An important point regarding the above-mentioned studies is that they only investigated the exchange of a limited number of metabolites, and the outcomes of active bidirectional cross-feeding might therefore be quite different for alternative auxotroph pairs. This knowledge gap was partially addressed by Wintermute and Silver (2010a) and Mee *et al.* (2014). Wintermute and Silver constructed extensive libraries of *E. coli* auxotroph pairs and observed substantial growth for only 17% of the auxotrophic pairs (Wintermute and Silver 2010a). They found that the metabolites most likely to be exchanged were those where the ratio of costs of secretion to benefits of uptake was a minimum, a finding that could be predicted via stoichometric modeling. Mee *et al.* (2014) performed a similar analysis and found a two-parameter predictor: the metabolites more likely to be exchanged in cocultures of auxotrophic mutants were those that were costly to biosynthesize and needed in small amounts. Together, the relatively low prevalence of cross-feeding resulting in enhanced (or even possible) growth (Wintermute and Silver 2010a; Mee *et al.* 2014) seems to support the notion that, in general, costly metabolites are not readily shared, and active positive interactions are therefore difficult to impose or engineer.

A main question that arises, then, is the following. If segregating different amino acid biosynthetic pathways into different genotypes sometimes improves productivity relative to the prototrophic genotype (Pande *et al.* 2014), how prevalent are these types of active reciprocal (i.e. cooperative) cross-feeding interactions in the natural environment? Mee *et al.* (2014) addressed this question by relating reciprocal amino acid cross-feeding in synthetic *E. coli* assemblages to the importance of such interactions in natural systems. Genome analyses of more than 6000-sequenced bacterial genomes identified numerous instances of apparent auxotrophies (Mee and Wang 2012; Mee *et al.* 2014; D'Souza *et al.* 2014). Additionally, genome analyses of 32 *E. coli* strains suggested high phenotype variability among closely related genotypes—more than half of the *E. coli* strains could not biosynthesize at least one amino acid and approximately one-third of the strains could not biosynthesize two or more amino acids (Pande *et al.* 2014). Together, these studies suggest that, while somewhat difficult to impose in the laboratory, active reciprocal cross-feeding interactions might indeed be widespread in nature and contribute towards the assembly and functioning of microbial communities. It should be noted, however, that the isolation histories of laboratory strains were not considered in these studies and amino acid auxotrophs are known to have advantages during growth in rich medium (D'Souza *et al.* 2014).

Negative interactions (passive or active)

While positive interactions have received substantial attention, it is generally unclear whether positive or negative interactions dominate the dynamics and properties of natural microbial assemblages. Likely, the most common negative interaction between to coexisting genotypes is resource competition. Competition is an inadvertent consequence of one genotype, using the same resources as another genotype (i.e. each genotype occupies a niche space that, at least in part, overlaps with the niche space of another genotype). Pure competition is thus passive in nature. Synthetic assemblages were recently used to quantify resource competition among different genotypes (Foster and Bell 2012; Wei *et al.* 2015).

If both genotypes inhabit the same ecological niche and no trade-offs exist, then competition among them will likely result in one genotype being displaced by another genotype (Hardin 1960). Typically, organisms then evolve to reduce niche overlap, and thereby reduce interspecies competition. Thus, negative interactions can impose selection toward evolutionary outcomes that reduce competition and promote coexistence (Lawrence *et al.* 2012). Metabolites produced by one genotype may have negative effects on the growth ability of another genotype. When secreted metabolites are an inadvertent result of one genotype's metabolism (i.e. metabolic by-products), then the negative interactions are again passive. One prominent example is alcoholic fermentation under aerobic conditions (De Deken 1966; Dashko *et al.* 2014; Pfeiffer and Morley 2014). In one example described in more detail above, passive bidirectional negative interactions were observed between *E. coli* and *S. cerevisiae* strains growing in coculture. On one hand, they were competing for glucose as a growth-limiting substrate. However, while fermenting glucose, *S. cerevisiae* released ethanol that had an additional negative effect on the *E. coli* strain (Zhou *et al.* 2015).

Negative interactions may also be active in nature, in that one genotype actively diverts metabolic resources away from its own growth to antagonize another genotype (Ratcliff and Denison 2011). For example, a genotype may produce antibiotics or other inhibitory secondary metabolites that are costly to synthesize and require an active investment of metabolic resources. Several studies explored competitive and antagonistic interactions in binary assemblages among somewhat related strains (Vetsigian, Jajoo and Kishony 2011; Cordero *et al.* 2012; Pérez-Gutiérrez *et al.* 2012; Wright and Vetsigian 2016). For example, inhibition by small molecules was often observed in pairwise cocultures sampled from a set of 185 *Vibrio* isolates (Cordero *et al.* 2012). Some additional examples of active negative interactions are described below (Kerr *et al.* 2002; Kelsic *et al.* 2015).

Many interactions are more complex than those discussed above and may contain both positive and negative components. Predator–prey interactions provide a well-studied example of such an interaction. Predator–prey interactions are ubiquitous and occur among all kingdoms of life, including between different bacteria (Sockett 2009; Velicer and Vos 2009; Pasternak *et al.* 2014), between bacteria and protozoa (Tsuchiya *et al.* 1972; Jost *et al.* 1973; Fredrickson 1977; Fredrickson and Stephanopoulos 1981), between rotifers and algae (Yoshida *et al.* 2003) and between bacteria and phage (Hall, Scanlan and Buckling 2011; Friman and Buckling 2014). The typical dynamic that emerges is oscillations in the abundances of predator and prey. The prey population collapses as the predator population increases. However, when the prey reaches sufficiently low abundances, the predator population collapses and the prey population recovers (see Fig. 1). Such population oscillations are not unique to predator–prey-type interactions, but have also been engineered in other types of mixed interaction systems that include negative interactions (Weber, Daoud-El Baba and Fussenegger 2007; Balagaddé *et al.* 2008; Song *et al.* 2009; Li, Wang and Wang 2011).

Interactions may not be readily defined

A central assumption of the terminology described above is that interactions between different genotypes can be precisely defined. This is not always the case. Distinguishing between passive and active interactions is especially challenging; yet, this distinction has critical implications for predicting the long-term evolutionary dynamics of synthetic assemblages. Namely, active positive interactions are susceptible to cheating, while passive positive interactions based on unidirectional cross-feeding of an inhibitory waste product are not. The amino acid exchange studies described above, are a prominent example of this challenge (Mee *et al.* 2014). Amino acid synthesis is costly and is clearly neither a by-product nor a waste-product. Yet, while the tendency is to view reciprocal amino acid exchange as an active process, amino acids may be exchanged via purely passive processes, such as inadvertent cell leakage. Without understanding whether the interaction is active or passive, however, it is difficult to make predictions about the long-term evolutionary maintenance of the interaction, where passive interactions are more likely to be maintained than active interactions.

One example of the ambiguity of the nature of interactions occurs within a consortium of an ammonia-oxidizing bacterium and a nitrite-oxidizing bacterium (Koch *et al.* 2015; Palatinszky *et al.* 2015). The ammonia-oxidizing bacteria convert ammonia to nitrite, while the nitrite-oxidizing bacteria then consume the secreted nitrite. When either urea (Koch *et al.* 2015) or cyanate (Palatinszky *et al.* 2015) was supplied to a consortium of the two bacteria, urea or cyanate were first decomposed to ammonia via the activity of the nitrite-oxidizing bacteria rather than by the ammonia-oxidizing bacteria themselves. The nitrite-oxidizing bacteria therefore depended on the ammonia-oxidizing bacteria for nitrite, while the ammonia-oxidizing bacteria depended on the nitrite-oxidizing bacteria for ammonia. Whether the nitrite-oxidizing bacteria actively diverted cellular resources into the production of ammonia for the ammonia-oxidizing bacteria, however, remains unclear. For example, cyanases may be required by the nitrite-oxidizing bacteria for the detoxification of their own metabolism (Palatinszky *et al.* 2015). It is therefore unknown whether this interaction is active or passive in nature, or whether it is susceptible to the emergence and proliferation of 'cheating' genotypes over evolutionary time-scales.

BEYOND BINARY INTERACTIONS

A number of studies have investigated the dynamics of assemblages that consist of more than two genotypes, and thus have more than a single interaction (Kerr *et al.* 2002; Harcombe *et al.* 2014; Mee *et al.* 2014; Abrudan *et al.* 2015; Kelsic *et al.* 2015). An important aspect of these studies is that they often result in unexpected dynamics that might not be readily predicted from interactions between any two-member assemblage. One example is the synthetic construction of rock–paper–scissors dynamics (Kerr *et al.* 2002; Kelsic *et al.* 2015). In one case, three genotypes were investigated *in silico*, each of which produced a different antibiotic while being sensitive to the antibiotic produced by one of the other genotypes (Kelsic *et al.* 2015). Thus,

each genotype had a negative effect on only one other genotype. The authors discovered that all three genotypes could coexist in a spatially structured environment and undergo predictable frequency oscillations. However, they could not coexist in a homogeneous environment, as every genotype succumbed to the antibiotic produced by another genotype, but due to inherent fitness differences, one genotype eventually prevailed. This finding was in line with previous experimental and theoretical results (Kerr *et al.* 2002). Perhaps, unexpectedly, however, when the system was extended such that each genotype had a negative effect on 'one' genotype but a positive effect on 'another' genotype (i.e. when antibiotic degradation was 'shared'), then the strains could coexist in a homogeneous environment (in this context see also Coyte, Schluter and Foster 2015).

These experiments with more than two genotypes then raise a critical question: If all possible binary interactions are known for a set of genotypes, can the dynamic properties and behaviors of mixtures of more than two genotypes be predicted? Recent theoretical studies suggest that the answer might typically be no (Gokhale and Traulsen 2010). As the number of genotypes and strategies increases, then the set of binary interactions may not be useful for predicting the dynamics of more complex assemblages. More specifically, the likelihood of successfully predicting the dynamics from binary interactions rapidly decreases as the number of genotypes within an assemblage increases. This could have profound implications for designing and applying synthetic microbial communities in concrete applications, as many applications require more than two genotypes to achieve a desired design objective (e.g. see Kato *et al.* 2008).

INTERACTIONS ARE THEMSELVES DYNAMIC

Ecological context is an important determinant of interactions between different genotypes (e.g. Samuel and Gordon 2006). When the biological activities of the genotypes themselves alter the ecological setting, interactions may change in strength and nature over time (Fig. 2). For example, while interactions between two genotypes might be initially positive, they can become negative as nutrients are depleted over time (Bull and Harcombe 2009). Moreover, one genotype may modify the environment by producing certain molecules, which in turn may affect how another genotype interacts with the first. This dynamical nature of interactions can lead to novel selection pressures, the emergence of new genotypes with different behaviors, and the further evolution of interactions (eco-evolutionary feedbacks). As each new genotype that becomes fixed within a population can interact with each existing genotype, the behavior of an initially simple assembly can quickly become exceedingly complex and challenging to understand.

A canonical example is Red Queen dynamics, which may occur when one genotype preys on another genotype. Predation rapidly selects for mutants of the prey that are no longer susceptible to predation. This, in turn, rapidly selects for mutants of the predator that can again predate on newly resistant mutants of the prey (for recent review; see Liow, Van Valen and Stenseth 2011). In a series of studies with cocultures of the predatory rotifer *Brachionus calyciflorus* and its algal prey *Chlorella vulgaris*, the predator reached its maximum abundance when the prey was at its minimum abundance and vice versa (Turchin 2003; Yoshida *et al.* 2003). This is different from typical predator–prey oscillations, where the predator population reaches its maximum abundance shortly after the prey reaches its maximum abundance and the predator population reaches its minimum abundance shortly after the prey reaches its minimum abundance (Fig. 1, but see also Turchin 2003). Mathematical modeling suggested that these unexpected oscillations could have resulted from diversification of the prey into two strains with different susceptibilities to predation (Shertzer *et al.* 2002). Yoshida *et al.* (2003) then replayed these dynamics *in vitro*. In parallel synthetic assemblages, rotifers could either graze on clonal algal populations (resulting in a typical predator–prey oscillations) or on mixed algal populations (i.e. an assemblage of more than one algal clone). The analysis of mixed algal populations showed that rotifers preferentially graze on the larger and faster growing algae strain and less on the smaller and slower growing strain, resulting in the same unexpected oscillations. Thus, predation pressure resulted in rapid algal diversification and the evolution of new interactions and atypical dynamics (Yoshida *et al.* 2003).

Another example of the dynamic nature of interactions is the benzoate cross-feeding interaction between strains of *P. putida* and *Acinetobacter* (Christensen *et al.* 2002; Hansen *et al.* 2007). The *Acinetobacter* strain can completely consume benzyl alcohol, while the *P. putida* strain can only consume the intermediate benzoate. Above certain benzyl alcohol concentrations, *Acinetobacter* inadvertently leaks benzoate out of the cell, thus allowing *P. putida* to consume the leaked benzoate and persist within the assemblage. When grown in mixed biofilms, the *Acinetobacter* strain typically had a positive effect on the *P. putida* strain by providing benzoate as a growth substrate. However, a mutant strain of *P. putida* emerged that had a deleterious effect on the *Acinetobacter* strain but a positive effect on the productivity of the assemblage as a whole. Thus, by accumulating particular mutations, an initially unidirectional positive interaction evolved into an exploitive interaction that was nevertheless more productive at the community level (Hansen *et al.* 2007). A similar outcome was observed between *S. cerevisiae* and *Rhizobium etli*, in that an initially unidirectional and positive interaction rapidly evolved to become competitive and antagonistic (Andrade-Domínguez *et al.* 2014). In both cases, a common feature is that the nature of the interactions changed rapidly, emphasizing that ecological and evolutionary processes may occur at similar and experimentally observable time scales (Post and Palkovacs 2009; Schoener 2011).

A final example is the effect of cheating genotypes that exploit an active bidirectional positive interaction between two cooperating genotypes. Waite and Shou (2012) extended a synthetic reciprocal cross-feeding assemblage consisting of two yeast strains, each of which produced an essential metabolite (lysine or adenine) required by the other (Shou, Ram and Vilar 2007). The authors then added a third strain that consumed lysine but did not reciprocate by overproducing adenine, and could therefore be viewed as a 'cheater' (Waite and Shou 2012). While theoretical considerations predicted that the ecosystem should collapse in the absence of spatial structure, cross-feeding unexpectedly persisted. This was a consequence of rapid evolution of the cooperating genotype that improved its metabolite uptake, which in turn reduced the relative fitness of the cheating genotype and thus prevented the cheating genotype from reaching sufficient abundance levels to disrupt the ecosystem.

A common feature of the examples above is that the interactions evolved to become more competitive or antagonistic in nature, which is not always the case (Summers *et al.* 2010; Lawrence *et al.* 2012; Fiegna *et al.* 2015). An opposite dynamic was recently observed in studies using randomly assembled synthetic assemblages, containing species isolated from a single

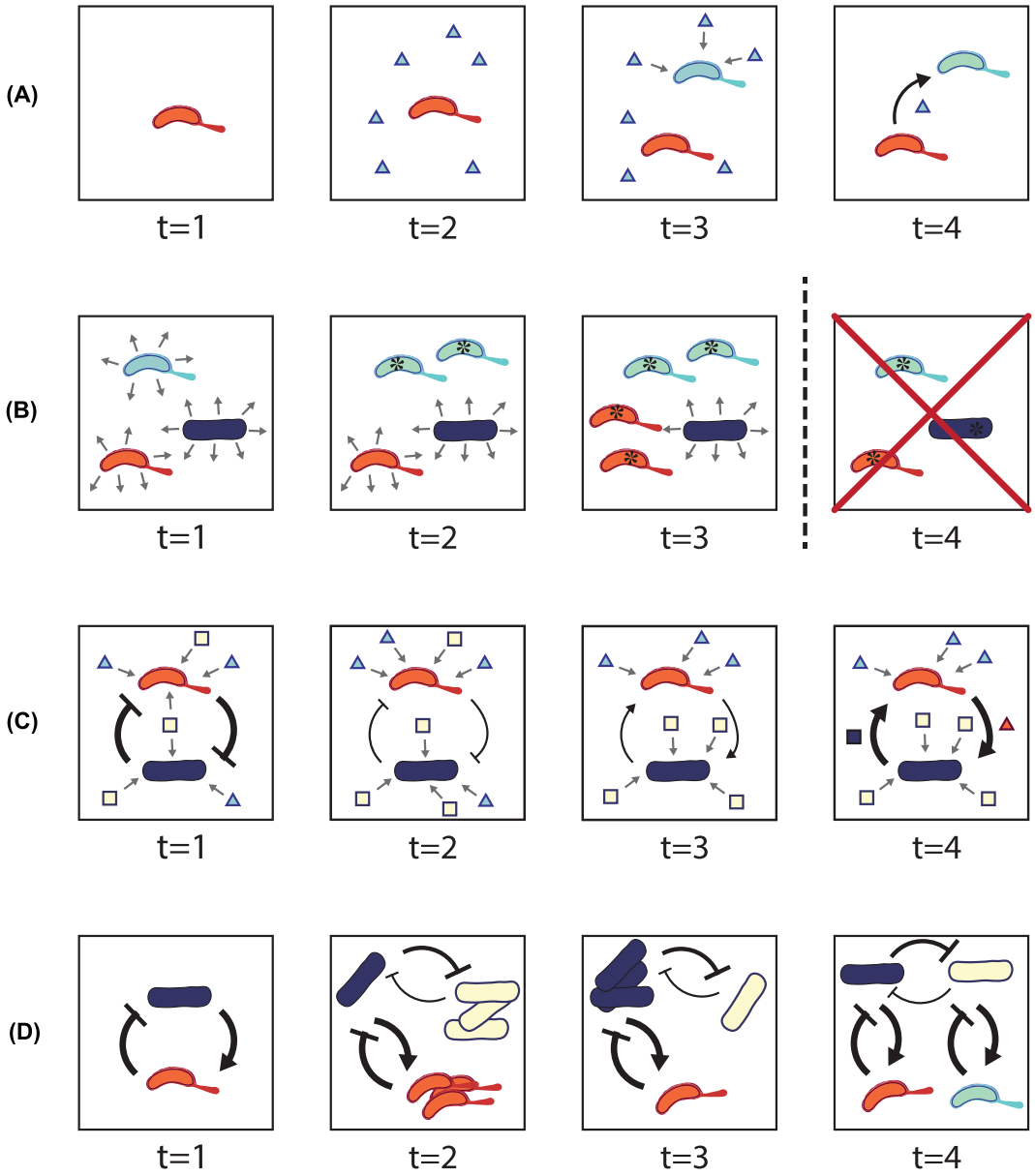


Figure 2. Effect of ecological and evolutionary feedbacks on dynamics. We consider some scenarios that illustrate how ecological and evolutionary feedbacks can affect ecosystem behaviors. (**A**) Consider a genotype that consumes a substrate into a waste product. If the waste product accumulates to substantial concentrations ($t = 2$, blue triangles), then this could promote the emergence of a mutant genotype that then consumes the metabolic waste product ($t = 3$, blue genotype). Thus, the metabolic activities of the red genotype created a new niche allowing the emergence of the blue genotype. (**B**) The Black Queen hypothesis predicts that genotypes will lose essential functions (gray arrows) whenever another genotype reliably provides those functions. However, as the number of service providers shrinks ($t = 2$ and $t = 3$, asterisks denotes those genotypes that have lost a previously shared function), the selective pressure on the remaining service providers increases, and ultimately any further loss of this function (e.g. via environmental perturbations) will result in collapse of the entire ecosystem ($t = 4$). (**C**) Organisms residing in a similar environmental niche may show considerable overlap in resource use. Thus, immediately after reassembly in a synthetic setting, resource competition is likely to be a predominant interaction between such organisms ($t = 1$). However, after some rounds of experimental evolution, competitive interactions may weaken as resource specialization evolves ($t = 2$). Subsequent evolution of positive interactions may then emerge (e.g. via Black Queen dynamics) and shift the net negative interaction ($t = 1$ and $t = 2$) into a net–positive interaction ($t = 3$ and $t = 4$). (**D**) Predator–prey interactions are particularly prone to evolutionary fluctuations. Predation can lead to the emergence of new prey genotypes that are resistant to the predator ($t = 2$, beige genotype). However, this could create a competitive interaction between the two prey genotypes ($t = 2$ and $t = 3$, beige and purple genotypes), which in turn could affect predator abundance. Moreover, if the new prey genotype proliferates ($t = 2$ and $t = 3$, beige genotype), then this could promote the evolution of a new predator genotype ($t = 4$, blue genotype), thus, accelerating diversification.

environment (Lawrence *et al.* 2012; Fiegna *et al.* 2015). The authors found that the interactions were initially competitive. However, after ~60 generations of experimental evolution, the assemblages tended to improve in productivity. The authors demonstrated that species interactions within the assemblages evolved to become less competitive. While the underlying mechanisms that reduced competition were unclear, possible explanations include the emergence of mutants that consumed the waste products of others, thus providing an example of how the biological activities of some species can create novel ecological niches for different genotypes to occupy and thus reduce competition (Fiegna *et al.* 2015).

SPATIAL STRUCTURE CAN STABILIZE INTERACTIONS

Spatial structure refers to the presence of solid–liquid, liquid–liquid or gas–liquid interfaces that prevent complete mixing within a particular environment. The prevalence of spatial structure is intuitive in systems that typically lack turbulent flows and contain abundant surface area such as soils. However, spatial structure also occurs in environments that are often assumed to be spatially homogenous such as seawater, where incomplete mixing and particulate matter can create small-scale spatial structuring (Stocker 2012). When one considers microbes residing in other spatially structured environments, such as plant and animal epithelia, sediments, stratified lakes and the subsurface (Whitman, Coleman and Wiebe 1998), it is clear that spatial structure is a general environmental factor that affects the majority of microbes in nature. There is therefore growing interest in understanding how spatial structure affects interactions and, in turn, community dynamics. In the section below, we review scenarios where spatial structure can impact interactions, community dynamics and ultimately the evolutionary trajectories of microbial assemblages (Fig. 3). Synthetic ecology can be used to investigate the role of spatial structure on interactions and community dynamics because spatial structure can be experimentally manipulated in defined manners. For example, spatial structure has been experimentally imposed by growing synthetic assemblages on agar plates (Harcombe 2010; Momeni, Waite and Shou 2013; Hol *et al.* 2015), by varying water channel connectivity on hydrated porous surfaces (Dechesne *et al.* 2010), by using flow chambers with heterogeneous flow velocities (Cardinale 2011), or by growing cells isolated in well plates while allowing them to interact via volatile substrate (Weber, Daoud-El Baba and Fussenegger 2007).

Spatial structure can maintain positive interactions

In stirred or shaken liquid systems with extensive mixing, mass transfer of secreted or leaked molecules is dominated by convection rather than by diffusion. Typically, each cell within the system therefore experiences somewhat similar concentrations of those molecules (however, see Gore, Youk and van Oudenaarden 2009, for exception). In contrast, in spatially structured systems, diffusion may become the main process governing mass transfer of secreted or leaked molecules. The concentrations of those molecules are therefore highest immediately adjacent to the producing cells, and concentration gradients are created between producing cells and the bulk liquid or consuming cells.

The higher local concentrations of secreted or leaked metabolites in spatially structured environments can have important effects on interactions between different microbial genotypes. This is because the concentrations of the secreted or leaked molecules may have to reach threshold concentrations before they can have significant effects on the dynamics of a microbial assemblage. This is particularly important when the secretion of the molecule occurs in limited amounts. In a completely mixed system and at low cell densities, the rapid dilution of a growth-promoting molecule into the bulk medium may prevent the molecule from accumulating to sufficiently high local concentrations to have biologically significant effects (Bull and Harcombe 2009). In a spatially structured system, however, diffusion localizes substrate competition; thus, dense localized populations will be able to produce sufficient growth-promoting molecules and still coexist with each other (Kim *et al.* 2008). However, distribution of such patches is important—when cell clusters are sufficiently close to each other, substrate competition might inhibit growth. But when they are sufficiently far apart, interactions based on secreted or leaked molecules might suffer from diffusion limitations (Kim *et al.* 2008).

Spatial structure and its localizing effects have been identified as one mechanism that could prevent 'cheating' genotypes from disrupting active secretion-based interactions (Fig. 3). Such genotypes consume the secreted molecule but do not contribute towards the secretion of the molecule itself. In other words, they exploit the active interaction without paying any of the costs (for a review of a broader topic of cooperation see e.g. Sachs *et al.* 2004 or West *et al.* 2006). In the absence of spatial structure, conceptual and experimental considerations suggest that such exploitation can cause the active interaction to completely collapse and disappear (Harcombe 2010; Mitri, Xavier and Foster 2011; Momeni, Waite and Shou 2013). The effects of spatial structure on preventing the emergence and proliferation of cheating genotypes were explored in detail using the yeast adenine-lysine cross-feeding system described above (Waite and Shou 2012; Momeni, Waite and Shou 2013). When the environment was sufficiently structured (such as when the assemblages were grown on agar plates), the active 'cooperative' interaction persisted and was resilient toward the addition of cheating genotypes. Further, experiments and mathematical simulations indicated that in environments where mixing is incomplete, patches of genotypes that actively secrete molecules were not displaced by cheating genotypes, and this was due to the preferential access of the actively secreting genotypes to the benefits of secretion (i.e. spatial segregation prevented the cheating genotype from having equal access to the benefits of active secretion, while local positive interactions between the cooperating genotypes ensured their faster growth). Therefore, spatial structure alone is sufficient to promote and maintain positive active-secretion-based interactions (Momeni *et al.* 2013; Momeni, Waite and Shou 2013), and this result has been observed repeatedly in other systems (Harcombe 2010; Datta *et al.* 2013; Harcombe *et al.* 2014).

Spatial structure itself can readily emerge

Spatial structure may also be created by interactions between the genotypes themselves via cell–cell contact, and thus maintain higher local concentrations of secreted or leaked metabolites (Pande *et al.* 2015). In one example, synthetic assemblages of amino acid auxotroph pairs rapidly improved in productivity when grown within completely mixed environments. Upon microscopic analysis, the authors discovered that the different genotypes were connected via nanotube-like structures. Further experiments suggested that the nanotubes promoted transfer of cytosolic components from one genotype to the other (Pande *et al.* 2015, but see also Benomar *et al.* 2015), and that nanotube formation is regulated (i.e. nanotubes did not form when amino acids were supplemented to the medium). Thus, if insufficient spatial structure is present in the abiotic environment, assemblages can sometimes create the necessary spatial structure to maintain positive bidirectional interactions based on the exchange of essential metabolites (in this case by producing nanotube connections).

Spatial structure can mitigate competitive interactions

The diffusional gradients of molecules in spatially structured environments can create a diversity of quantitatively or even

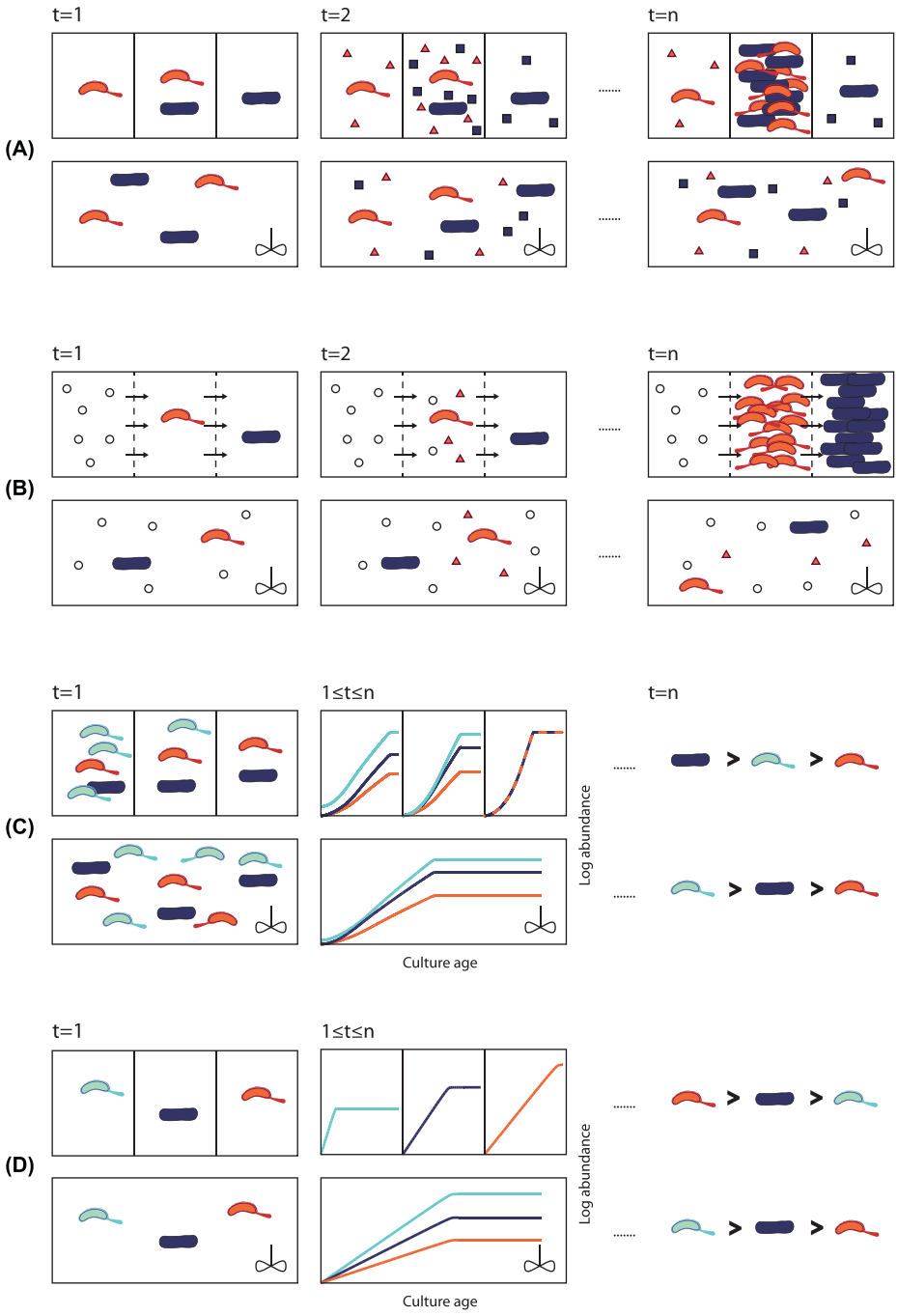


Figure 3. Effect of spatial structure on dynamics. We consider some scenarios, where spatial structure can affect community-level dynamics. Upper scenarios in each panel show spatially organized environments (dashed lines separate different spatial patches, and solid lines separate different compartments), whereas lower scenarios depict well-mixed systems. **(A)** For a bidirectional positive interaction between two genotypes, spatial structure can slow the loss of exchanged metabolites into the bulk medium (red triangles and purple squares), and thus, increase their local concentrations and promote growth (center compartment). However, spatial structure can also physically prevent different genotypes from interacting, thus, reducing or preventing growth (left and right compartments). **(B)** Spatial structure can physically separate incompatible processes. In this hypothetical case, the red genotype consumes oxygen (empty circles), while the purple genotype is sensitive to oxygen. However, the purple genotype depends on a molecule secreted by the red genotype. In a spatially structured environment, the red genotype consumes the oxygen before it encounters the purple genotype, thus, promoting growth. Analogous to sediments in nature, in this scenario oxygen penetrates only from one direction (i.e. from left to right). However, in a completely mixed environment, oxygen inhibits the purple genotype, and the red genotype suffers from product inhibition caused by the accumulation of the secreted metabolite. **(C)** Physical barriers can effectively protect cooperating genotypes (purple and red cells) from invasive 'cheating' genotypes (light blue cells). In a completely mixed system, the cheating genotype may achieve higher abundance than the cooperating genotypes. However, in a spatially fragmented system, cooperators may achieve higher abundances than the cheating genotype because of the localization of positive interactions. Note that the starting frequency of strains is equal for both scenarios, and that possible evolution is not considered in our models. **(D)** Spatial structure can modify the relative frequencies of different genotypes. Consider a mixture of three genotypes, competing for the same substrate, but whose rates and yields are negatively related to each other (i.e. a rate-yield trade-off). At the completion of substrate consumption, the spatially structured environment could have greater biomass than the completely mixed environment. This is because the spatially structured environment may prevent competition between faster and slower growing genotypes, thus, allowing the slower growing genotypes to proliferate and achieve higher cell densities. Again, our models assume that all genotypes remain the same over the course of simulation.

qualitatively different habitats for genotypes to proliferate within, and these niches may occur over very small distances. A canonical example occurs in sediments, where the chemical and physical environment may change rapidly over small distances as a consequence of the metabolic activities of the resident microorganisms and mass transfer phenomena. Such spatial structure allows for the apparent coexistence of species with different environmental adaptations. As an example, synthetic assemblages of different algal isolates were constructed, where the isolates competed in artificial flumes operated under homogenous or heterogeneous flow regimes (Cardinale 2011). System productivity was measured by the uptake rate of NO_3^- and primary production (algal biomass). When the assemblages were inoculated into a homogeneous environment, one genotype rapidly displaced the others. In contrast, when the assemblages were inoculated into a spatially structured environment, more genotypes persisted within the system, presumably, because they occupied different ecological niches created by mass transfer gradients. Interestingly, the mixed assemblages always performed worse than monocultures in the homogeneous environment, pointing toward competitive and antagonistic interactions becoming dominant between species. On the other hand, the mixed assemblages generally performed better than monocultures in the heterogeneous environment, suggesting the maintenance of specialists via niche complementarity.

Lack of environmental connectivity can also create different habitats that enable competing genotypes to apparently coexist. A set of theoretical studies recently investigated how water availability affects environmental connectivity and, in turn, influences the coexistence of different genotypes (Wang and Or 2012a,b). In a wet and highly connected environment, a rapidly growing phenotype displaced a slower growing phenotype within the assemblage. In dry conditions, however, where patches were less connected, the slower growing phenotype could coexist with the rapidly growing phenotype over the time-course of the mathematical simulations. One reason for coexistence in dry environments is reduced cell motility (for experimental observations, see also Dechesne *et al.* 2010) and reduced numbers of interconnecting channels for substrates to diffuse through. As a consequence, both cell types were spatially separated, effectively preventing competition. When a similar *in silico* community was designed to cross-feed a metabolic waste product on hydrated surfaces (Wang and Or 2014), the assemblages organized into persistent patterns. Importantly, the spatial organization depended on both the interaction network topology and on the connectedness of the environment.

Space itself can also be a resource for which microbes compete (Saxer, Doebeli and Travisano 2009; Estrela and Brown 2013; Müller *et al.* 2014; Lloyd and Allen 2015). As mixed colonies expand over surfaces, only a proportion of cells at the growing front may contribute to colonization of unoccupied space. Thus, genetic drift can have a major effect on the local community composition. In the absence of additional interactions, genotypes will typically demix, and one population will go locally extinct (Hallatschek *et al.* 2007). When two auxotroph yeast strains grew in a synthetic assemblage, reciprocal exchange of amino acids was necessary for them to proliferate on a solid minimal medium. However, efficient nutrient exchange dictated continuous mixing of both populations and opposed genetic drift as the colonies expanded (Müller *et al.* 2014). On the other hand, gradual addition of leucine or tryptophan (i.e. effectively shifting the active interaction from bidirectional toward unidirectional), resulted in an increasing segregation of populations. In other words, with decreasing strength of the positive interaction component, competition for space and resources may become the dominant factor, and can even lead to competitive exclusion of one genotype over time.

TEMPORAL HETEROGENEITY CAN MAINTAIN INTERACTIONS

The activities of microorganisms themselves can rapidly change the local environment over time, which can have consequences on microbial interactions. Such dynamic changes and their effects on ecosystem processes are readily observed within synthetic assemblages and over time-spans of only a few microbial generations where evolutionary adaptations are unlikely to have significant effects. These dynamic changes can create new habitats that allow otherwise competing genotypes to coexist. For example, while all cells experience the same environment at any given point in time in a completely mixed system, one genotype may have higher fitness at one time point while the other genotype may have fitness advantage at another time point. If conditions oscillate over time, then neither of the genotype may be able to completely displace the other. Examples of such dynamics include temporal oscillations in resource availability (Rosenzweig *et al.* 1994; Rozen and Lenski 2000; Zhou *et al.* 2015), chemical/physical parameters (Kato *et al.* 2005, 2008), and when growth alters density and/or frequency-dependent interactions (Weber, Daoud-El Baba and Fussenegger 2007; Chuang, Rivoire and Leibler 2009; Celiker and Gore 2012; Yurtsev *et al.* 2013). Below we highlight some examples of such dynamics.

A canonical example of the importance of temporal oscillations occurs when *E. coli* was evolved in completely mixed batch culture. During batch growth, resources were initially in excess but rapidly changed to become scarce. After serial transfers, at least two genotypes emerged. One genotype had higher fitness when resources were abundant while the other genotype had higher viability during starvation (i.e. during stationary phase) (Rozen *et al.* 2009). Synthetic assemblages of the two genotypes together combined with modeling demonstrated that both genotypes could be maintained by temporally segregated fitness benefits (Rozen and Lenski 2000; Rozen *et al.* 2009; Saxer, Doebeli and Travisano 2009; Ribeck and Lenski 2015). The temporal fitness segregation is context dependent. The genotype that had greater viability during starvation (i.e. during stationary phase) only benefits from this phenotype when the other genotype is present (Rozen *et al.* 2009).

Another example occurs between organisms that exhibit different rate-yield properties. In one study, assemblages were constructed of a yeast strain that could both ferment and respire glucose with another strain that could only respire glucose (MacLean and Gudelj 2006). The former strain grew faster but achieved lower yields, while the latter strain grew slower but achieved higher yields (Otterstedt *et al.* 2004). When inoculated into a chemostat, the faster growing strain displaced the slower growing strain. However, in batch culture, both strains could coexist. The explanation for coexistence was that the fermenting activity of the faster growing strain was more susceptible to product inhibition. They experimentally confirmed that when glucose was abundant, the intermediates accumulated and slowed the growth of the faster growing strain, thus providing a fitness benefit to the slower growing strain. Also, after the glucose was completely consumed, the slower growing but more

efficient strain had already extracted energy from the metabolites, thus depleting the secondary resource pool available to the faster growing strain (MacLean and Gudelj 2006).

Temporal oscillations combined with spatial structure were also critical for maintaining rock–scissor–paper-type dynamics with *E. coli* (Kerr *et al.* 2002). This interaction was mediated by colicin, which is a plasmid-encoded bacterial toxin produced by some *E. coli* strains. When colicin-sensitive *E. coli* was exposed to the toxin, resistant genotypes occasionally emerged. Note that both colicin production and colicin resistance carry significant fitness costs compared to colicin-sensitive genotype. The colicin-resistant genotype could invade the spatial patch initially occupied by colicin-sensitive *E. coli*, but only after this spatial patch had been invaded by colicin-producing variant. The same was true for the other two genotypes; the invasion into a spatial patch occupied by an otherwise more competitive strain could proceed only in a periodic manner, thus, requiring a particular succession history (i.e. a particular ordering of immigration events).

CONCLUSION

The reader might have noticed that we avoided using conventional terminology for interactions between different genotypes, such as commensalism and mutualism. While this terminology is likely useful for macroecology, we believe that it may be less useful for microbial ecology and might instead create confusion. The main problem is that conventional interaction terminology oversimplifies the true nature of interactions between different genotypes.

We argue this on four points. First, the terms commensalism and mutualism do not indicate whether the positive interaction results from passive or active processes. Yet, whether the interaction is passive or active can profoundly affect the selection pressures that act on that interaction, and thus on its properties and stability over evolutionary time scales. The lack of such a distinction between passive and active processes can consequently create confusion, such as equating the terms mutualism and cooperation with each other, which are not necessarily the same (i.e. a mutualism need not be based on active processes while cooperation must be based on active processes). Second, the terms commensalism and mutualism may suggest that the interaction is purely positive. However, an interaction is almost never purely positive. Instead, the interaction will almost always have competitive elements at the same time. Importantly, it is often unclear whether the positive or competitive elements have the dominant effect on ecosystem dynamics and community properties. Also, these terms describe interactions as a net effect and generally do not attempt to dissect interactions into individual components. We feel that identifying the individual components of an interaction lends us power to predict how synthetic assemblages may develop over evolutionary time. Finally, terms such as cooperation or commensalism leave the appearance that interactions are static. However, interactions can change abruptly over both ecological and evolutionary time-scales. The challenge with this dynamical nature of interactions is enormous. Consider that the number of interactions within an assemblage scales exponentially with the number of genotypes, where the addition of an additional genotype adds n new potential interactions to already existing $n*(n-1)/2$ interactions within that system. If even one of these interactions changes in strength or sign (e.g. positive to negative) over time, then it could have cascading effects on all other interactions.

Given these limitations, we argue that an alternative framework for describing interactions might be useful, and we suggest a new set of terminology here. We refrained from simple single-word terminology and instead stated explicitly whether an interaction is active or passive and whether the interaction also contains competitive elements. Unfortunately, our terminology presented here is admittedly cumbersome and does not address the issue with the dynamical nature of interactions; further advances and discussions are clearly needed. Perhaps bleakly, we note here that our arguments for developing a more precise terminology for interactions are not new, and similar suggestions regarding the nature of interactions and the pervasiveness and complexity of competition were made nearly 40 years ago (Fredrickson 1977; Fredrickson and Stephanopoulos 1981), yet widespread adoption of more precise terminology generally remains absent.

Another important outcome of our review is that what we name today as 'synthetic microbial ecology' is not an entirely new discipline; it is rather an ongoing effort in microbiology. Perhaps the biggest advance in recent years is the predictive power of modern mathematical and metabolic modeling and the availability of elegant genetic systems to control and analyze the dynamic behavior of synthetic assemblages. Taken together, these tools allow us to premeditate and then impose interactions of our choosing, and here we are not necessarily limited by native constraints of microbes at hand. It is also stimulating a new generation of microbiologists to integrate analytical, mathematical and experimental approaches to improve our basic understanding of microbial communities. These skills have largely been distributed among different research fields rather than brought together within a single research effort.

OUTLOOK

Synthetic microbial ecology will have continuing potential to understand how interactions between different microbial genotypes can lead to community-level processes and higher order-level properties. This information is not only important for basic scientific advances, but also has potential to be applied in concrete biotechnological applications. For example, we have emphasized the susceptibility of active interactions to cheating, which can lead to their eventual collapse. Thus, if one wishes to engineer a system to achieve a specific design objective, it is clear that one should avoid active interactions or engineer environments that protect active interactions from exploitation. We have also emphasized how interactions can be engineered or imposed to control system dynamics for the production of valuable bioproducts. Continuing advances at the basic level should help to establish general engineering design principles that predict how best to distribute different metabolic processes across different genotypes to optimize a desired biotransformation (Johnson *et al.* 2012). They will also help to identify how to impose new or additional interactions to control stability and resiliency, which should lead to important biotechnological advances.

Another great challenge for future research is to translate the advances in synthetic microbial ecology to natural microbial assemblages. This poses enormous challenges, as system complexity scales exponentially with the number of genotypes present in the system (i.e. each additional genotype present within a community can, directly or indirectly, interact with every other genotype). Still, the general principles and rules derived from synthetic microbial ecology may be useful for understanding which interactions have a critical role within

complex assemblages and which do not. This could help establish truly predictive and comprehensive models of system dynamics for real-world environmental communities, such as those residing in the human gut or in wastewater treatment plants. Thus, the bridging of synthetic ecology with natural systems will be a grand challenge that also could have profound effects on a variety of biological disciplines, including microbiology, ecology, evolution and the environmental sciences.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSRE online.

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