MINI REVIEW

Challenges in microbial ecology: building predictive understanding of community function and dynamics

Stefanie Widder1, Rosalind J Allen2, Thomas Pfeiffer3, Thomas P Curtis4, Carsten Wiuf4, William T Sloan6, Otto X Cordero7, Sam P Brown8, Babak Momeni9,10, Wenying Shou10, Helen Kettle11, Harry J Flint12, Andreas F Haas13, Béatrice Laroche14, Jan-Ulrich Kreft15, Paul B Rainey16, Shiri Freilich16, Stefan Schuster17, Kim Milferstedt18, Jan R van der Meer19, Tobias Großkopf20, Jef Huisman21, Andrew Free22, Cristian Picioroanu23, Christopher Quince24, Isaac Klapper25, Simon Labarthe14, Barth F Smets26, Harris Wang27, Isaac Newton Institute Fellows28 and Orkun S Soyer20

1CUBE, Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria; 2SUPA, School of Physics and Astronomy, University of Edinburgh, Edinburgh, UK; 3New Zealand Institute for Advanced Study, Massey University, Auckland, New Zealand; 4School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne, UK; 5Department of Mathematical Sciences, University of Copenhagen, Copenhagen, Denmark; 6Infrastructure and Environment Research Division, School of Engineering, University of Glasgow, Glasgow, UK; 7Department of Civil and Environmental Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA; 8Centre for Immunity, Infection and Evolution, School of Biological Sciences, University of Edinburgh, Edinburgh, UK; 9Department of Biology, Boston College, Chestnut Hill, MA, USA; 10Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; 11Biomathematics and Statistics Scotland, Edinburgh, UK; 12Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK; 13Biology Department, San Diego State University, San Diego, CA, USA; 14Département de Mathématiques Informatiques Appliquées, INRA, Jouy-en-Josas, France; 15School of Biosciences, University of Birmingham, Birmingham, UK; 16Newe Ya’ar Research Center, Agricultural Research Organization, Ramat Yishay, Israel; 17Department of Bioinformatics, Friedrich-Schiller-University Jena, Jena, Germany; 18INRA, UR0050, Laboratoire de Biotechnologie de l’Environnement, Narbonne, France; 19Department of Fundamental Microbiology, Université de Lausanne, Lausanne, Switzerland; 20School of Life Sciences, The University of Warwick, Coventry, UK; 21Department of Aquatic Microbiology, University of Amsterdam, Amsterdam, The Netherlands; 22Institute of Quantitative Biology, Biochemistry and Biotechnology, School of Biological Science, University of Edinburgh, Edinburgh, UK; 23Department of Biotechnology, Delft University of Technology, Delft, The Netherlands; 24Warwick Medical School, University of Warwick, Coventry, UK; 25Department of Mathematics, Temple University, Philadelphia, PA, USA; 26Department of Environmental Engineering, Technical University of Denmark, Kongens Lyngby, Denmark and 27Department of Systems Biology, Columbia University, New York, NY, USA

The importance of microbial communities (MCs) cannot be overstated. MCs underpin the biogeochemical cycles of the earth’s soil, oceans and the atmosphere, and perform ecosystem functions that impact plants, animals and humans. Yet our ability to predict and manage the function of these highly complex, dynamically changing communities is limited. Building predictive models that link MC composition to function is a key emerging challenge in microbial ecology. Here, we argue that addressing this challenge requires close coordination of experimental data collection and method development with mathematical model building. We discuss specific examples where model–experiment integration has already resulted in important insights into MC function and structure. We also highlight key research questions that still demand better integration of experiments and models. We argue that such integration is needed to achieve significant progress in our understanding of MC dynamics and function, and we make specific practical suggestions as to how this could be achieved.

Correspondence: RJ Allen, School of Physics and Astronomy, University of Edinburgh, Peter Guthrie Tait Road, Edinburgh EH9 3FD, UK.
E-mail: rallen2@staffmail.ed.ac.uk
or OS Soyer, School of Life Sciences, The University of Warwick, Coventry CV4 7AL, UK.
E-mail: O.Soyer@warwick.ac.uk

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Introduction

Microbes exist in complex, highly diverse and highly dynamic communities (Flint et al., 2007; Johnson et al., 2015). These microbial communities (MCs) have crucial roles in global climate regulation, human health and industrial biotechnology. Understanding, predicting and controlling MCs thus holds the key to a wealth of potential applications, from smart waste treatment plants, through probiotic treatments of gut-related diseases, to cheese and wine making (Shong et al., 2012; Johnson et al., 2015; Wolfe and Dutton, 2015). The need to predict MC dynamics has stimulated the development of ‘black box’ mathematical models, in which microbial population dynamics is represented by global empirical functions that do not attempt to address the inner workings of the MC. This approach has proved useful in fields ranging from food science to climate modelling and wastewater treatment (Orhon and Artan, 1994; Baranyi and Tamplin, 2004). However, such models do not aim to provide mechanistic insight, and are necessarily limited by the data sets to which they are fitted.

High-throughput sequencing, proteomics and metabolomics now allow us to catalogue the diversity of MCs to an unprecedented level of detail. These data represent a relatively unbiased compositional snapshot of the species, genes, metabolites and activities that are present in a given MC. The key challenge now is to convert this empirical knowledge into fundamental insights and testable predictions, which can be used to improve MC function for useful purposes. Here, we argue that addressing this challenge will require the development of mathematical models with a basis in mechanistic understanding, integrated with controlled experiments (Figure 1). In our view, this integration between theory and experiments is a crucial ‘missing link’ in current microbial ecology. Making this link is a key step on the way to discovering possible design principles of MC community assembly and function.

MCs as complex, interacting dynamical systems

The dynamics of MCs are driven by a multitude of interactions between their constituent microbial populations, as well as by environmental and host factors, such as immunological processes in gut microbiota or nutrient limitation in plant MCs (Klitgord and Segrè, 2010; Vorholt, 2012; Coyte et al., 2015). Species interactions within MCs can be metabolic, physical, regulatory and/or signalling based, and they can drive both temporal changes in MC composition and function, and spatial organisation. Phenomenologically, an interaction between two microbial populations can be defined as the dependence of one population’s growth or survival on the abundance of the other population. These interactions can be negative or positive, implying growth inhibition or facilitation. Negative interactions can arise from competition for resources such as electron donors and acceptors, nutrients, light or...
physical space (Hibbing et al., 2010), or via direct microbe–microbe interactions, or secretion of toxins or other inhibitory compounds (Riley and Wertz, 2002). Negative interactions have been observed in evolution and competition experiments with single or several species (Passarge et al., 2006; Brown et al., 2008), and are also widespread among different species in natural populations, such as soil and marine bacteria (Vetsigian et al., 2011; Cordero et al., 2012b). Positive interactions between microbial populations can occur via several mechanisms. From a metabolic standpoint, cross-feeding of metabolic by-products, in which one population benefits from the excreted metabolites of another, is a key mediator of positive interactions (Sieber et al., 2012); this also reduces the degree to which populations compete for resources (Kosaka et al., 2008). Other mechanisms include production of ‘public goods’ such as iron-scavenging molecules, which can be used not only by the producer population but also by others (West et al., 2006).

Identification and measurement of interactions within MCs, and accurate representation of these in theoretical models, is an important basis for building understanding, and thus presents a key challenge in microbial ecology. A second challenge is the converse: to use theoretical approaches to predict species interactions in MCs from proximal data such as taxon abundances. Both cases require direct integration of theory and data in specific ways. Although many advances have been made toward such integration (for examples, see Figures 2 and 3), a number of technical obstacles remain. In the following, we discuss key areas where integration theory and data can be highly productive. We then highlight a number of broader scientific questions, which we see as crucial for the longer-term development of models for MC structure, function and dynamics. Finally, we call for development of model systems where well-controlled experiments interrogating function–structure relation in communities can be more readily performed and information and methods among multiple laboratories can be shared.

Integrating theory and data to understand MC dynamics

Measuring population dynamics in MCs

A key objective of theoretical models for MC dynamics is to reproduce temporal trajectories of the populations within the community. However, state of the art high-throughput sequencing generates a snapshot of the relative abundances of taxa or genes within a MC; it does not provide absolute abundance information. Furthermore, there can be inherent biases even in this relative abundance data. These limitations mean that high-throughput sequencing on its own is not sufficient to track temporal and spatial population dynamics. Complementary experimental approaches such as quantitative PCR, flow cytometry, species-specific fluorescence in situ hybridisation or novel combinations of single-cell and functional-targeting methods with genomics (Berry et al., 2015) do yield information on absolute abundances. Yet, this information is limited, because these approaches are usually targeted, that is, need a priori knowledge of which populations or functions are to be investigated. Although some of these approaches can be used in an untargeted manner, for example, by using general primers for fluorescence in situ hybridisation or by combining single-cell separation with genome sequencing, this involves technical challenges. Up-scaling these absolute abundance measurements to

Figure 2  (a) The human large intestine and the rumen and caecum in herbivorous animals harbour dense MCs dominated by anaerobic microbes that cross-feed metabolites extensively. In recent models, these are approximated by a small number of functional groups (Muñoz-Tamayo et al., 2010; Kettle et al., 2015). (b) Comparison of this model to a fermenter experiment with a pH shift from 5.5 to 6.5 after 9 days for metabolic products (dashed lines are experiment data, solid lines are model results). (c) Comparison of temporal species dynamics between model predictions and data. The experimental data consists of phylogenetic groups (16S rRNA gene sequencing), simulations refer to functional groups; approximate correspondence between the two is indicated by colour coding (for example, Lachnospiraceae equivalent to B5 and B8, shown in green, Bacteroides belonging to B1 shown in blue and purple).
the community level is an important goal. We note that combining high-throughput sequencing with quantitative PCR analysis to measure total bacterial abundance should, in principle, allow absolute taxon abundances to be computed. Yet, we are not aware of studies that have used this approach to date.

We also note that the potential of sequencing approaches to predict aggregate community function is currently limited, because neither metagenomic data nor 16S RNA gene sequence data are a perfect predictor of metabolic function for an individual species. This is an area where new sequencing and bioinformatics approaches can result in significant improvement.

**Inferring species interactions from proximal data**

Direct measurement of interactions between species within a community (for example, for metabolic interactions, by tracking radioactively or isotopically labelled compounds) is an excellent way to collect the basic information needed for model building, but it is necessarily targeted to specific types of interaction. To obtain community-wide information on microbial interactions where direct evidence is lacking or restricted, one can use statistical inference based on correlations between taxon abundances from high-throughput sequence data (Fuhrman, 2009; Freilich *et al.*, 2010; Faust *et al.*, 2012). The resulting co-occurrence interaction networks can be used to make ecological predictions, such as the existence of metabolic dependencies, the fragility of communities to environmental change or the likely identity of keystone species that stabilise the community (Faust *et al.*, 2012; Berry and Widder, 2014; Widder *et al.*, 2014). Although correlational approaches can point to previously unknown interactions, they require confirmation by direct evidence.
given the concentrations of essential nutrients and/or inhibitory chemicals, and species-dependent parameters such as the maximal growth rate (Figure 3). Extending this approach to the community level is attractive because it is conceptually simple, computationally tractable and provides dynamical predictions. However, its success depends on identification of the most important microbial species to include in the model, and the interactions between them. Moreover, the reliability of the approach depends crucially on the underlying kinetic growth model and its parameters—thus large-scale measurement of kinetic growth parameters for a variety of microbial species would be extremely useful. More fundamentally, existing kinetic growth models are often ‘ad hoc’ and may miss aspects of the growth kinetics that are important in community function, such as product inhibition. To address this problem, new kinetic models are being developed that include features like condition-dependent changes in the maximal growth rate (Bonachela et al., 2011; Desmond-Le Quemener and Bouchez, 2014) and the thermodynamics of microbial metabolism (Jin et al., 2013). In particular, the inclusion of thermodynamics in kinetic growth models has contributed to improved modelling of MCs, for example in anaerobic waste treatment (Gonzalez-Cabaleiro et al., 2013).

Long-term challenges for developing an understanding of structure–function relation and dynamics in MCs

The need to include evolutionary processes

Traditionally, evolution has been ignored in ecological models, as it is assumed to occur only on long timescales. While this assumption might hold for animal and plant communities, ecological and evolutionary timescales can coincide in some MCs, because of their short generation times, large population sizes and high rates of gene transfer. Thus, models that address MC dynamics should take into account both ecology and evolution, for example, see (Post and Palkovacs, 2009). The intermixing of ecological and evolutionary dynamics is strikingly demonstrated by long-term growth experiments with *Escherichia coli* in glucose-limited chemostats. Here, genetic diversification produces two genotypes, which cross-feed; that is, it leads to a new ecological interaction. Functional diversification at the genome level is maintained by the novel ecological interaction and vice versa (Little et al., 2008). As ecological interactions evolve in MCs on the same timescales as the species themselves evolve (Cordero et al., 2012a; Hillesland et al., 2014), the development of modelling frameworks that include evolution of species’ traits and interactions should have an important role in microbial ecology. From a modelling perspective, simplified ‘toy-models’ have been developed, which include ecology and evolution, for example, in (Pfeiffer et al., 2001). The challenge is to make these
more realistic (for example, including more aspects of cellular metabolism). From an experimental perspective, attempts are being made to follow the evolution of selected species, for example, in natural soil MCs (Gomez and Buckling, 2013); the next step is to track more species simultaneously, and to extend to other environments, such as gut communities (for example, using axenic animal systems). An alternative approach is to use defined synthetic communities, constructed from known species, in which it is possible to track the ecological and evolutionary dynamics of individual species within the community (see for example, Mee and Wang, 2012; Bodenhausen et al., 2014; Faith et al., 2014).

Social evolution and bacterial strategies
An important challenge in model development is to correctly account for the complex social organisation that can occur amongst microbes and, for experimentalists, to test how frequently social interactions occur in natural settings. For example, secretion of ‘public goods’ such as toxins, enzymes, metabolic coreactors or signalling molecules can lead to intricate evolutionary dynamics (Leggett et al., 2014). Game theory provides a way to model complex social behaviours in mixed MCs. Here, different microbial behaviours are abstracted as simple ‘strategies’. As an example, some microbial populations produce extracellular enzymes such as cellulases that digest recalcitrant nutrients in the environment. ‘Cheater’ cells do not produce these enzymes, but nevertheless benefit from their release by ‘cooperative’ donor cells. Game theory maps this scenario on to a number of classic ‘games’, such as Prisoner’s Dilemma, the hawk-dove game or the mutual benefit game, depending on the values of the kinetic parameters (Gore et al., 2009). Extending such game theoretical models to include MC spatial structure remains a challenging area, although some important advances have been made using lattice-based simulations and continuum partial differential equation approaches (Reichenbach et al., 2007, 2008).

Community assembly and historical contingency
Community assembly, or the mechanism by which a community forms, is a widely studied topic in macro-ecology, but has been relatively little addressed for MCs (Woodcock et al., 2007). For some MCs it is known, however, that historical contingency—the order in which different species arrive in the community—can have a strong impact on community composition; examples include oral communities (Teles et al., 2012) and gut microbiota (David et al., 2014). Importantly, different patterns of species arrival can result in different long-term interaction networks within the community (Vannette and Fukami, 2014). These historical contingency effects are likely to have important consequences for the engineering and control of MCs in the environment, agriculture and medicine. Future work should systematically investigate these effects experimentally for complex MCs and, concurrently, integrate them into population dynamic models. Moreover revisiting suitably designed, older experiments with new methods may also contribute to understanding temporal processes of community assembly in MCs.

The importance of spatial structure
Another important feature of MCs is their complex spatial structure (Figure 4). Indeed, densely packed aggregates, which may be free-floating or in the form of surface-attached biofilms, are believed to be the predominant mode of life for many microbes in the natural environment, for example, MCs on marine snow particles (Kiorboe et al., 2003; Elias and Banin, 2012), and are crucial in processes such as wastewater treatment (Figure 3). Within these microbial aggregates, driving factors for spatial organisation include (i) metabolite gradients caused by consumption/production, diffusion and advection, (ii) gradients of abiotic factors such as light or temperature, (iii) physical adhesion and (iv) motility. A number of well-established methods exist for modelling spatial structure development within aggregated MCs (especially biofilms). Continuum spatial models predict how microbial biomass density and chemical concentrations change in space and time and typically include diffusion, advection and mechanical forces (Klapper and Dockery, 2010). At a more detailed level, individual-based models track the location and fate of individual microbial cells within the community, taking into account a plethora of features such as spatially resolved metabolite concentrations or electrochemical interactions with a surface (for example, an electrode). The use, and further development, of such spatially explicit models is important because spatial organisation of aggregated MCs is likely to have a drastic effect on their structure and function (Momeni et al., 2013). For example, biofilm infections are notoriously more resistant to antibiotic treatment than well-mixed planktonic cultures (Davies, 2003). Such improvements in modelling spatially structured MCs must go hand-in-hand with improvements in experimental interrogation of spatial structure within natural settings, and in particular development of non-invasive measurement methods.

A call for the development of model MCs and well-controlled experiments on them
In microbiology, fundamental understanding of microbial physiology and metabolism has been acquired by studying a set of standard microbial model organisms, for example, Saccharomyces cerevisiae, Escherichia coli, Bacillus subtilis and Pseudomonas aeruginosa, under well-defined conditions.
In microbial ecology, in contrast, data collection has mainly focused on characterisation of environmental samples from a multitude of different settings. We argue here that much can be gained by focusing efforts on a more limited set of well-defined ‘model’ MCs (Denef et al., 2010; Großkopf and Soyer, 2014; Estrela et al., 2015), including both synthetic communities constructed from known microbial species (Bodenhausen et al., 2014; Faith et al., 2014; Bai et al., 2015), and microcosm communities made from environmental samples (Foster and Bell, 2012; Pagaling et al., 2014; Wolfe and Dutton, 2015).

Such an approach offers many advantages. From a general point of view, focusing on a limited set of model systems would make results much more transferrable between studies, allowing multiple groups to work synergistically, and therefore speeding up progress toward mechanistic understanding. Lab experiments with well-defined MCs under controlled conditions, for example, in chemostats, should allow hypothesis testing via control of key external parameters such as substrate concentration, system size or temperature, and allow for multiple replicate experiments (Figure 5).

From a more specific point of view, synthetic ecological communities provide a way to limit the system to a manageable number of microbial constituents, making it simpler to analyse and model—it may even be possible to measure exhaustively kinetic and interaction parameters for an entire community, as input for theoretical models. Indeed, de-novo assembly of low diversity MCs (Mee and Wang, 2012) provides control of microbial interactions, non-linear effects because of adding traits or community members and evolutionary changes (Celiker and Gore, 2014; Fiegna et al., 2014).

Synthetic communities can also be used to test the role of particular ecological mechanisms, such as metabolic interactions, spatial heterogeneity (van Gestel et al., 2014) or induced cell death.
Asally et al., 2012). As a pathway toward more systematic development of synthetic model MCs, we advocate building on the success of early micro-biological studies in collecting data on specific model species under standardised culture conditions. In particular, we suggest that obtaining detailed physiological data (for example, kinetic growth parameters under different conditions) on a collection of ecologically relevant microbial model organisms, would greatly facilitate the community-wide construction of synthetic MCs. Although synthetic communities do not reproduce the full diversity and complexity of natural MCs, many of the principles of community organisation and dynamics, which we learn from them should be transferable to more complex MCs.

As a stepping stone from simplified synthetic systems to natural MCs, and to address questions concerning contingency, functional redundancy, species diversity and variability and the nature of interactions in highly diverse, complex communities (Foster and Bell, 2012), microcosms provide an excellent platform (Figure 5). A microcosm consists of an environmental sample that is cultured in the lab under well-defined conditions. The microcosm often retains much of the compositional and functional diversity of the seed community, and may also have spatial structure, allowing, for example, for nutrient cycles and redox gradients (Pagaling et al., 2014). As microcosm experiments can be replicated, sampled and perturbed under well-controlled conditions, they provide an excellent bridge between the simplicity of synthetic model ecosystems and the complexity of MCs in the natural environment (Pagaling et al., 2014; Wolfe and Dutton, 2015). We also advocate performing experiments on a limited set of standardized microcosm communities; for example, the Winogradsky column, in which an aquatic sediment-water sample develops under lab conditions into a self-sustaining, vertically stratified, nutrient-cycling community.

Figure 5 (a) (Left) Chemostat competition study between marine, nitrogen-fixing Cyanothec sp. and a non-nitrogen-fixing Synechococcus species (source: Department of Aquatic Microbiology, University of Amsterdam). (Middle) Schematic drawing of a chemostat. (Right) At high nitrate levels, the nitrogen-fixer (Cyanothec) is competitively excluded by the non-nitrogen-fixer (Synechococcus). Symbols are measurements; lines are model predictions (after Agawin et al., 2007). (b) (Left) Study in Winogradsky column microcosms. (Middle) Schematic of the vertically layered structure of a mature Winogradsky column. Principal microbial types are found in different layers, their ecological activities and the associated core chemical reactions are illustrated. As a result opposing gradients of sulphide and oxygen develop. (Right) Microbial activity leads to a transient drop in redox potential in the overlying water, and a long-term drop in the sediment, at high levels of added cellulose ('high C'). Low levels of added cellulose ('low C') induce only a short-term reduction in redox potential.
Conclusions

Despite impressive advances in our knowledge of the species composition of MCs, we are still far from achieving the level of fundamental understanding of the dynamics and function of MCs that is needed to predict and control MC behaviour. Here, we have argued that the key to achieving this level of predictive understanding is the integrative development of mathematical models with experimental data collection and method development. There are considerable challenges associated with both experimental method development and data collection, and mathematical model building in the study of MCs. These challenges are intertwined, such that tackling them effectively requires an integrated approach. To advance toward this goal, we advocate increased interaction between empirical and theoretical scientists, as well as the development of well-defined model MCs that can act as test-beds for the integrative development of experimental and modelling approaches.

How can this best be achieved in practice? Although many of the points that we make here are addressed to individual scientists, interactions between empiricists and theoreticians should be facilitated by the continuation of community-wide activities like the recent 4-month programme ‘Understanding Microbial Communities’ held at the Isaac Newton for Mathematical Sciences at Cambridge University. Moreover, some of the developments, which we call for here, such as the extensive characterisation of specific model systems, can best be done by groups of scientists working together rather than by individual groups. Specific funding of such community-directed research activities, in parallel with individual research efforts, would be a very welcome development.

Conflict of Interest

Dr Patrick B Warren holds equity (> $10k) in Unilever PLC. All other authors declare no conflict of interest.

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29Department of Ecology and Evolutionary Biology, University of California Irvine, USA; 30Department of Biology, University of York, UK; 31UMR7238 CNRS—Laboratoire de Biologie Computationnelle et Quantitative, Université Pierre et Marie Curie, France; 32Cavendish Laboratory, University of Cambridge, UK; 33INRA, UR0050, Laboratoire de Biotechnologie de l’Environnement, France; 34Mathematical Sciences, University of Southampton, UK; 35Division of Biological Sciences, University of California San Diego, USA; 36Department of Geological Sciences, University of Oregon, USA; 37Swiss Federal Institute of Aquatic Science and Technology (Eawag), Department of Environmental Microbiology, Switzerland; 38Systems Biology Program, Centro Nacional de Biotecnología, Spain; 39Department of Applied Mathematics, School of Mathematics, University of Leeds, UK; 40Unilever R&D Port Sunlight, UK, 41Department of Applied Mathematics and Theoretical Physics, University of Cambridge, UK; 42Institute of Biological and Environmental Sciences, University of Aberdeen, UK; 43Department of Biochemistry and Cambridge Systems Biology Centre, University of Cambridge, UK; 44The Francis Crick Institute, Mill Hill Laboratory, UK; 45Department of Plant Sciences, University of Cambridge, UK; 46School of Computer Science, University of Manchester, UK; 47Department of Ecology and Evolutionary Biology, Princeton University, USA; 48Luxembourg Centre for Systems Biomedicine, University of Luxembourg, Luxembourg.

Glossary: Modelling terms

Population dynamic models. These models deal with the long- and short-time changes in size and composition of biological populations, as well as the biological and environmental factors affecting these changes.

Stoichiometric models. These describe the set of metabolites taking part in cellular metabolism and their reactions based on chemical stoichiometry at steady state. The framework allows graph theoretical investigation of the cellular metabolism and linear optimisation of cellular fluxes. The latter approach includes FBA that optimises to predefined constraints such as biomass yield for given uptake rates and dynamic FBA, which adds a dynamical framework.

Individual-based models. These represent individual organisms (here microbes) as ‘agents’, whose behaviour depends on a predefined set of rules. The global dynamics of the system are a
consequence of the interplay between the local interactions of all agents in the system.

**Causal and correlational networks.** Networks (graphs) are general tools for representation and analysis of systems with a distinct interaction structure. Nodes represent the constituents of the system, edges between them their interactions that can be based on causal evidence or on statistical inference. Studies on network topology and dynamics can predict community properties and behaviour.

**Kinetic growth models.** These models seek to describe the growth rate of a microbial population as a function of the concentrations of one or more chemicals (nutrients/inhibitors). To represent MCs, several coupled equations are used, each describing a different population within the community. An example of a kinetic growth model is the classical Monod model.

**Well-mixed and spatially resolved approaches.** In ‘well-mixed models’, for example, ODEs, the spatial positions of the model constituents are not resolved; they are assumed to be homogenously distributed in space. If spatial structure is important for the dynamics of a system, for example, if its behaviour is controlled by motility or by chemical diffusion processes, then ‘spatially resolved’ approaches such as continuum spatial models or spatially resolved individual-based models should be considered. These models represent space explicitly.

**Evolutionary game theory.** Game theory considers strategic decision making in a group of competitors, resulting in conflict and cooperation. The application of game theory to evolving biological populations is called evolutionary game theory. Questions focus on evolving strategies (change of strategies and its frequency), such as the rock–paper–scissors dynamics in bacteriocin-producing MCs (Figure 4).

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