CHAPTER 15

Interpreting and Designing Microbial Communities for Bioprocess Applications, from Components to Interactions to Emergent Properties

A.E. Beck[*,†,1], **K.A. Hunt**[*,‡,1], **H.C. Bernstein**[§], **R.P. Carlson**[*,‡]

[*]Center for Biofilm Engineering, Montana State University, Bozeman, Montana, USA [†]Department of Microbiology and Immunology, Montana State University, Bozeman, Montana, USA [‡]Department of Chemical and Biological Engineering, Montana State University, Bozeman, Montana, USA [§]Chemical and Biological Signature Science, Pacific Northwest National Laboratory, Richland, Washington, USA

[1]Contributed equally

15.1 Introduction

Most natural microbial ecosystems are the result of millions of years of natural selection in spatially and temporally dynamic landscapes. These ecosystems possess effective, highly evolved functions and are almost exclusively organized as polymicrobial communities. The study of natural and engineered microbial communities has benefited from new technologies such as increased resolution and throughput of omics measurements, development of new genetic systems for establishing model organisms, advanced cell isolation methods such as flow cytometry and cell sorting,[1] comprehensive databases such as KBase (http://kbase.us), and ever-growing computational power for performing *in silico* experiments such as community-scale metabolic network modeling.[2,3] Interest in using polymicrobial systems for applied bioprocesses stems largely from an effort to mimic and ultimately control the beneficial emergent properties that are often observed in natural ecosystems. These attractive attributes of communities have a potential to enable superior catalytic function compared to traditional monocultures based on simultaneous optimization of multiple tasks, increased productivity, and greater stability.

While the study of applied microbial communities is growing in popularity, the appreciation and use of communities for societal purposes is not new. In fact, applications of microbial communities date back at least 5000 years to early food preservation via lactic acid-producing bacterial communities used for yogurt production,[4] and evidence suggests that directed biogas production was practiced in Assyria and China going back at least 3000 years.[5] The significance of polymicrobial systems was also observed and tested by the earliest pioneers

Biotechnology for Biofuel Production and Optimization. http://dx.doi.org/10.1016/B978-0-444-63475-7.00015-7

of modern microbiology. In 1683, Antoine van Leeuwenhoek recorded observations of morphologically distinct "animalcules" collected from oral scrapings and, in 1877, Louis Pasteur tested antagonistic interactions between medically relevant bacteria.[6–9]

Numerous reviews reiterate, with overlapping content, the relevance and use of polymicrobial systems for human health, biological circuit synthesis, microbial computing, synthetic ecology, biomass degradation, and a myriad of other biological applications (Table 15.1). This review provides a generalized discussion of ecological foundations useful in understanding polymicrobial systems and highlights classical theories applicable to future microbial community engineering efforts. No attempt is made here to exhaustively outline every relevant study in this rapidly growing field; rather, case studies are selected to epitomize ecological themes and design motifs within the context of the current and future state of polymicrobial bioprocessing.

15.2 Definitions

The study of microbial communities has expanded from traditional biological disciplines to include a wide cross section of applied sciences. This broad expansion has resulted in the merging of concepts and terms from classical disciplines including biochemistry, computational biology, ecology, engineering, genetics, and microbiology. These fields use vernacular with varying connotations; hence, a list of terms is provided with the definitions used here to facilitate a unified discussion.

Table 15.1: Recent community-relevant review articles categorized by focus

Focus	Reference
Artificial symbiosis	[100]
Biodegradation	[101]
Bioenergy, biomaterials	[102]
Bioengineering	[46]
Bioprocess	[103, 104]
Bioprocess, experimental and theoretical	[11]
Biotechnology, algal biofuels	[105]
Cellulose degradation, bioprocess	[106]
Cyanobacteria/microalgae and bacteria	[107]
Ecological interactions, symbiosis	[108]
Engineering, bioprocess	[109]
Food fermentations	[110]
Industrial bioprocessing	[111]
Microbiome	[112]
Mining	[113]
Polymicrobial infections	[114]
Synthetic biology	[115]
Synthetic biology, bioprocess	[116]
Synthetic communities	[117]
Synthetic ecosystems	[22]
Viral interactions	[12]

A **microbiological community** is a collection of populations that may be comprised of prokaryotic, eukaryotic, or viral components; definable interactions are not a requirement. A **microbial community** is a microbiological community limited to prokaryotic and eukaryotic populations with no explicit accounting for viruses and no constraints on interactions. However, the ubiquity of viruses suggests that the vast majority of microbial species hosts viruses, and the existence of virus-free microbial communities is therefore thought to be exceedingly rare. A microbial community exhibiting positive interactions is referred to as a **consortium** (plural consortia)[10]; some uses of the term are more generic, referring to any interacting community. The origin of community populations can be used to further categorize the system. A **natural community** is defined as a collection of wild-type populations that have interacted in nature on evolutionary time scales. An **artificial community** is a collection of unmodified populations that have been assembled through manual intervention and are not thought to interact in natural habitats. A **synthetic community** is defined as a collection of genetically modified populations, whereas a **semisynthetic community** is a collection of populations with at least one wild-type population and at least one genetically modified population. A review of recent case studies exemplifying these different organizations can be found in Bernstein and Carlson.[11]

15.2.1 Community Components

Communities are ensembles of populations that serve as the system components. Populations can be classified by phylogenetic and/or phenotypic distinction. **Phylogenetically distinct** community populations are different species that can range across the domains of microbial life and can include viruses. Table 15.2 provides examples of components comprising interacting communities ranging from virus-virus systems to archaeon-eukaryote systems. Virus-virus and host-virus interactions are often overlooked in reviews of applied microbial communities despite their common distribution across most natural systems and their potential to impact engineered systems.

Table 15.2: Representative microbial communities organized by system components

Community members	Description	Reference
Virus-virus	Enhanced pathogenicity	[118]
Virus-bacterium	Enhanced infectivity of human cells	[119]
Virus-bacterium	Lysis of competitors	[120]
Virus-eukaryote	Suppression of additional virus infection (HIV)	[121, 122]
Virus-eukaryote	Prevention of diabetes	[123]
Virus-eukaryote	Thermal stress tolerance	[124]
Bacterium-bacterium	Differentiation of cyanobacteria to fix nitrogen	[125]
Archaeon-bacterium	Enhanced function via hydrogen exchange and methanogenesis	[21]
Bacterium-eukaryote	Cellulose conversion to value-added biochemicals	[126]
Archaeon-eukaryote	Enhanced cellulose degradation via hydrogen exchange and methanogenesis	[97]

These community interactions are not limited to pathogenicity and can represent a spectrum of interaction outcomes, including mutually beneficial effects as reviewed in Roossinck.[12]

Phenotypically distinct populations are not differentiable on the species level but exhibit separate expression patterns that often lead to niche differentiation. An **ecological niche** is the function or location of an organism within an ecosystem. The cyanobacterial strategy of cellular differentiation into specialized nitrogen-fixing heterocysts and vegetative cells is a well-studied example of a single-species population functioning as a community.[13] Another example is synthetic or adapted communities of *Escherichia coli* strains with different substrate specificities which can lead to niche partitioning in biofilms (e.g., oxic or anoxic separation).[14–17] **Biofilms** are polymer-encapsulated microbial populations attached to biological or abiotic surfaces.[18] Mass transfer typically limits the availability of resources within a biofilm, resulting in spatial heterogeneity which, in turn, leads to phenotypic differentiation.[16,18,19]

15.2.2 Interaction Outcomes

Community interactions can be classified as one of six outcomes. These interactions may be unidirectional, bidirectional, or of higher order; they can also be obligatory or facultative. Each of the interaction outcome categories presented here is symbolically summarized for a two-population community using "+" to indicate a population benefit, "–" to indicate an adverse effect, and "0" to indicate no effect. In practice, these interaction outcomes are often observed in communities comprised of more than two populations.[20]

(1) **Mutualism** (+/+): both populations benefit from the interaction(s). Syntrophy is a specific instance of mutualism associated with the cross-feeding of essential resources between populations.[21] (2) **Commensalism** (+/0): one population benefits from the interaction(s) while the other is unaffected. (3) **Ammensalism** (–/0): one population is adversely affected by the interaction(s) while the other is unaffected. (4) **Competition/antagonism** (–/–): both populations are negatively affected by the interaction(s), which can be due to functional redundancy or antagonistic interactions. (5) **Parasitism** (+/–): one population benefits and one population is adversely affected by the interaction(s). (6) **Neutralism** (0/0): neither population is affected by the interaction(s); alternatively, there is no interaction between populations. Synthetic ecology, another scientific field witnessing rapid growth, often attempts to assemble tractable, albeit constrained, systems to test these interaction outcomes.[22]

15.2.3 Interaction Mechanisms

The mechanisms that mediate known interactions can be divided into three generalized categories: metabolite exchange, physical interaction, and environmental modification. These mechanisms are typically combinatorial and interrelated within natural and engineered microbial communities. **Metabolite exchange** is any transfer of material and/or chemical energy between community populations and requires some combination of active transport (e.g., ABC-type transporters)

Table 15.3: Representative microbial communities organized by system interaction mechanisms

Interaction mechanism	Community	Description	Reference
Anabolic metabolite exchange	Filamentous anoxygenic phototrophs/cyanobacteria	Vitamin	[127]
Anabolic metabolite exchange	Uncultured marine bacteria	Siderophore	[128]
Anabolic and catabolic metabolite exchange	Sulfate-reducing bacteria/ methanogens	Alanine and hydrogen/ formate	[95]
Catabolic metabolite exchange	Sulfate-reducing bacteria/ methanogens	Hydrogen/formate	[129]
Catabolic metabolite exchange	Colon microbiota	Acetate cross-feeding	[130]
Quorum sensing exchange	Sludge community	Granulation and EPS production	[131]
Antibiotic/antimicrobial peptide exchange	*K. pneumoniae*/*E. coli*	Microcin-mediated ammensalism	[132]
Environment sensing exchange	*Vibrio* pathogen/crustacean hosts	Differential virulence impacts	[133]
Direct physical interaction	Cellulose-fed soil biofilm community	Filamentous structures for electron transfer	[134]
Environment modulation	Marine phytoplankton community	Hydrogen peroxide scavenging	[135]

and/or passive transport (e.g., diffusion). Exchanges of **anabolic resources,** resources used in biosynthetic processes (e.g., amino acids, nucleotides, vitamins, cofactors, and siderophores), are widely distributed among natural systems and represent targets for metabolically engineered polymicrobial systems (Table 15.3).[23,24] Exchanged metabolites can also serve as **catabolic resources**, resources used to produce cellular energy. Examples include electron donors or acceptors (e.g., hydrogen or oxygen, respectively), which are used in biologically mediated redox reactions to facilitate production of energetic molecules like NAD(P)H or ATP.

Exchanged material can serve functions other than anabolic or catabolic roles, such as modulating the behavior of community populations. **Quorum sensing**, a process by which organisms secrete and receive specific soluble metabolites that act as regulatory signals, is associated with a wide variety of natural multicellular functions, including coordinated biofilm formation, microbial pathogenicity, and culture bioluminescence.[25] In addition, quorum sensing has been proposed as a means of interrogating local environments, permitting a feedback mechanism for regulating phenotype (e.g., resource-intensive strategies such as enzyme secretion).[26,27] Modulating community population activity can also be realized through competitive strategies like the exchange of inhibitory or toxic metabolites.[28,29] Secretion of antibiotics and antimicrobial peptides is widely distributed and provides both offensive and defensive mechanisms within some communities.

Direct **physical interactions** between community populations can dictate community structure and function. These interactions are a hallmark mechanism of biofilms[19,30]; this physical association can be controlled or constrained by metabolite exchange.[31] Evidence suggests that

direct physical contact between community populations can also be used for the transfer of electrons through materials such as cytochrome-rich extracellular structures, which have been characterized as outer membrane and periplasmic extensions.[32] Similarly, filamentous cable bacteria in microbial sediment communities permit electron transfer across centimeter-length scales.[33] Direct interpopulation electron transfer remains an active area of investigation.

Environmental modification is a mechanism of interaction in which a community population influences the local environment and thereby alters the niche(s) that other populations can inhabit. Modulation often occurs when one population consumes a chemical species (e.g., organic acids, oxygen, or hydrogen sulfide) that inhibits other populations.[14,34,35] Table 15.3 provides examples of natural and engineered systems organized by mechanism of interaction.

15.2.4 Emergent Properties

A major advantage of polymicrobial systems over traditional monoculture biotechnology is the potential for emergence of higher order properties. **Emergent properties** are attributes that are either not present or increased in magnitude from those properties characteristic of the individual system components. Contrary to many connotations, emergent properties are not always beneficial to a community; however, the discussions here are limited to positive attributes. **Stability** is defined by a community's response to perturbations,[36] although many other interpretations have been used for the concept of ecological stability and related properties such as robustness.[37] Generally, a stable community returns to its initial state after a small perturbation, while an unstable community does not. Stable community behavior is attributed to the two quantifiable metrics, resistance and resilience.[38] **Resistance** is defined as the degree to which community behavior is insensitive to a perturbation, and **resilience** is the rate at which community behavior returns to its original condition.[39] An increase in any combination of resistance and resilience is desirable for bioprocessing and often for **ecological fitness**, defined as the ability of an organism to survive and reproduce in an environment. Another set of properties that can emerge from community interactions is productivity and uptake. **Productivity** is defined by the rate at which material is produced; **uptake** is the material consumption rate. Many ecological studies have observed correlations between productivity and diversity (or species richness),[40] although a universally accepted mechanism has not been identified.

15.3 Ecological Theories for Interpreting and Designing Communities

Ecologists have historically studied multiscale material and energy flows between populations and their environments. Resource scarcity has influenced fundamental aspects of biological organization, including the elemental and macromolecular composition of microorganisms.[41–43] Hence, theories focused on themes of resource acquisition, resource investment, energetic efficiencies, and tradeoffs have been developed to explain observations of natural phenomena. Fundamental theories help organize observations, describe community

behaviors, and enable strategic engineering of community composition or manipulation of environmental factors to improve natural and biotechnological processes.[44–46] The current section details three distinct but complementary ecological theories that provide explanations for the competitive basis of component organization and interaction outcomes found in many natural and engineered communities. These classical theories will remain relevant to ecologists and bioengineers as their respective fields further mature. Table 15.4 outlines some existing studies of microbial communities employing these theoretical concepts.

15.3.1 Maximum Power Principle

The maximum power principle describes community interactions based on acquisition of available energy. The maximum power principle asserts that the fitness of biological systems will increase with the rate of available energy harvest, resulting in a maximization of metabolic power (units of J/s).[47–49] From an evolutionary viewpoint, the principle predicts selection of systems that capture previously unutilized energy sources.[50] The ability to acquire available energy from the environment at faster rates leads to enhanced fitness by enabling more energy to be apportioned to survival and reproduction, while reducing available energy for competitors. Interactions between populations that increase the overall metabolic power of a system can lead to coexistence via cooperation[49] or niche differentiation such as the use of different substrates.[50] Additional related postulates have been proposed over the years, including maximization or minimization of entropy.[51,52] Box 15.1 graphically demonstrates interaction outcomes for two populations with different metabolic powers.

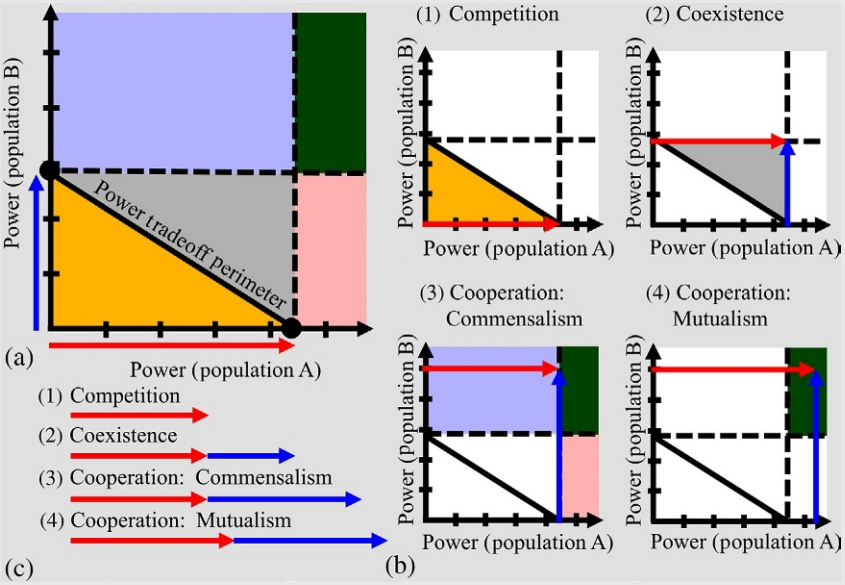
15.3.2 Resource Ratio Theory

Resource ratio theory is an ecological theory describing resource consumption, competition, and niche partitioning in which interpopulation interactions are defined with respect to shared resources.[53,54] These resources are often essential, but hemi-essential and substitutable resources

Table 15.4: Ecological theory studies

Theory	Community	Description	Reference
Maximum power	Aquatic planktonic microcosms	Effect of pH-controlled light on power acquisition	[50]
Maximum power	Two closely related picocyanobacteria	Stable coexistence due to light partitioning	[136]
Resource ratio	Competition studies with bacteria, phytoplankton, and zooplankton	Meta-analysis of prediction consistency	[137]
Resource ratio	Nine phytoplankton species	Competition for nitrogen, phosphorus, and light	[60]
Pareto/Tradeoff	Three-member hot springs mat community	Community productivity and inhibitory byproduct tradeoffs	[3]
Pareto/Tradeoff	Syntrophic *Geobacter* species	Interspecies electron transfer	[138]

BOX 15.1 Maximum Power Principle



(a) *Graphical representation of key concepts from the maximum power principle*. Populations A and B are each characterized by a steady-state metabolic power when growing in monoculture (black dots on axes; red and blue arrows represent magnitude of power for A and B, respectively). The line connecting these two points, the **power tradeoff perimeter**, represents the partitioning of metabolic power between the two populations. Any point within the plot represents a cumulative metabolic power of the community through summing the individual metabolic powers for populations A and B.

(b) *Interaction outcomes interpreted with maximum power principle*.

(b1) Populations A and B are competing for resources within the same ecological niche. According to the maximum power principle, the fittest population has the largest metabolic power. Therefore, population A will outcompete population B. The total metabolic power of any mixture of two competing populations in the same niche is a nonnegative linear combination of the individual metabolic powers and by definition cannot exceed the power tradeoff perimeter at steady state. For competition-based unsteady state scenarios, the community power will shift toward the population capable of the most metabolic power, which will eventually dominate (population A here).

(b2) Coexistence can occur when populations obtain energy from separate resources, allowing each population the potential to achieve its respective maximum power. Total community power may exceed the power tradeoff perimeter but is bounded by the magnitude of the individual population maximum powers. The gray region represents antagonism, as neither population is able to achieve its maximum power. A neutralistic community exists at the upper right corner (both populations achieve their maximum powers), while the edges bounding this region indicate an ammensal community in which one population realizes its maximum power and one does not.

BOX 15.1 Maximum Power Principle—cont'd

(b3) Cooperating populations can exceed the maximum power of neutralistic populations if one population facilitates the acquisition of energy by the other population (e.g., commensal interaction) (boundary of the green region). Parasitic interactions may reduce such gains in community power because the increased power acquisition of one population occurs at the expense of the other population (red and blue patterned regions indicate parasitism favoring populations A and B, respectively).
(b4) The total community power can exceed the maximum power of neutralistic populations through mutualistic interactions. The two populations facilitate acquisition of metabolic power by each other, placing the community power in the green region. The final magnitude of an unsteady state trajectory is unknown without further information about the interaction.
(c) *Comparing community metabolic powers*. The example metabolic powers of populations A and B shown in (b1)–(b4) are summed and aligned to visualize the increase in metabolic power that may occur through community interactions.
Figure adapted from DeLong.[51]

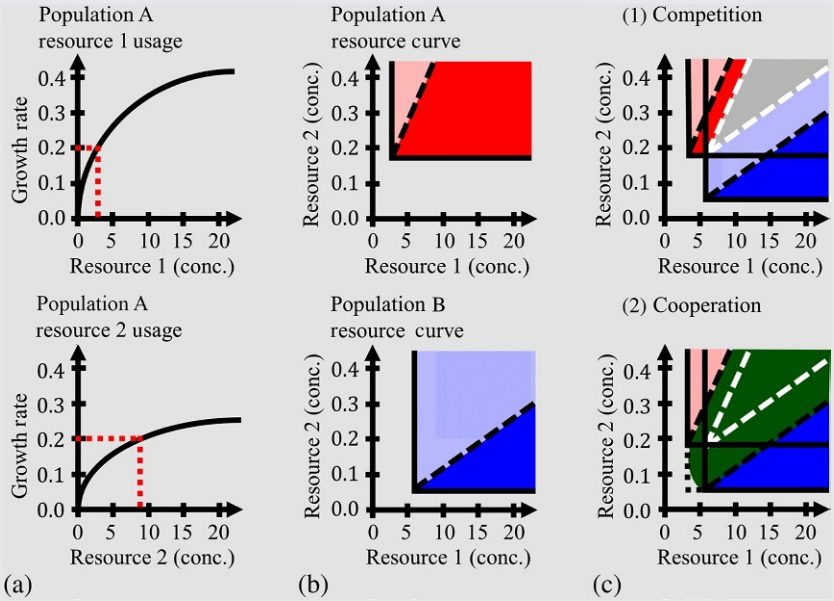
have also been studied.[55] Resource ratio theory postulates that the population best capable of depleting a limiting resource while maintaining a positive growth rate will be most competitive. The resource ratio theory has been used to assess outcomes (competitive exclusion or coexistence) between populations competing for shared limiting resources and can predict resource levels that will permit coexistence of multiple populations.[53,55–57] The theory can be consistent with classic chemostat theory, which states that it is possible to sustain multiple stable populations when there are multiple limiting substrates, provided that each population is limited by a different resource.[58–60]

Resource ratio theory can describe cooperative populations by accounting for mutualistic resource exchange. Cooperating populations that exchange limiting resources can exist in a wider range of resource environments than is possible for either population individually. This scenario describes what has been termed a super-competitor unit,[54] a community with the emergent property of enhanced resource utilization that can deplete limiting resources more effectively than the individual monocultures. This ability to survive at reduced resource availabilities highlights an evolutionary advantage of cooperation that has been observed in natural ecosystems.[54] Resource ratio theory extended to cooperation is analogous to the economic concept of comparative advantage, where specialization and resource exchange enable enhanced community function.[61] Box 15.2 describes a generalized example of resource ratio theory applied to two different populations, each of which is more effective at depleting a different essential resource.[54]

15.3.3 Resource Allocation Theories: Pareto Surfaces and Metabolic Tradeoff Analysis

Biological systems from enzymes to communities represent competitive resource allocation.[62] Phenotypic plasticity, the ability to change phenotype with changing environment, can enable different relationships between cellular function and resource allocation. However,

BOX 15.2 Resource Ratio Theory



Graphical representation of key concepts from resource ratio theory.
(a) *Determination of resource requirements*. The resource concentrations required to support steady state growth of population A at different growth rates are tabulated using Monod expressions; an analogous set of data for population B is not shown. Resources 1 and 2 are essential for both species (e.g., nitrogen or phosphorus sources). The red dotted lines denote a particular growth rate selected for study and the corresponding resource concentrations required to support steady state growth.
(b) *Population growth responses to limiting resources*. The minimum requirements for resources 1 and 2 to maintain a steady state growth rate are indicated by the solid black edges for populations A (top) and B (bottom). Population A has a higher affinity for resource 1 and a lower affinity for resource 2 as compared to population B. Thus, populations A and B are more effective at depleting resources 1 and 2, respectively. The shaded areas represent conditions sustaining positive growth for each population. The slope of the dashed line separating the lightly shaded region (resource 1-limited) from the darkly shaded region (resource 2-limited) represents the resource ratio requirement (resource 2 per resource 1 necessary for growth). If both resources are in excess, the population will maintain positive growth and consume resources as governed by its resource ratio requirement, until its consumption reaches the steady-state resource level indicated by the solid lines.
(c) *Interaction outcomes interpreted with resource ratio theory*.
(c1) Resource ratio theory describes community interactions by combining the growth responses of the two populations and examining the resulting regions. If populations A and B are competing for resources 1 and 2, the population that can drive a resource concentration below the acquisition capability of the other will dominate the ecological niche. Population A will dominate the community in the red region due to its superior ability to deplete resource 1,

BOX 15.2 Resource Ratio Theory—cont'd

and population B will dominate the community in the blue region due to its superior ability to deplete resource 2. These regions are bounded by intersecting the resource ratio requirements of the two populations (white dashed lines). The gray region (coexistence), bounded by the white dashed lines, represents an excess of resource 1 for population A and an excess of resource 2 for population B. The ratio of resources permits coexistence as both populations are limited by their noncompetitive resources.
(c2) Resource ratio theory can describe cooperating populations utilizing mutualistic resource exchange. The region of coexistence (green region) is expanded (c1 gray region) due to resource trading. In the light red region, population A is resource 1-limited, leaving no excess resource 1 to trade with population B. Therefore, population A will dominate the community since it is more competitive not to trade with population B. In contrast, to the right of the light red region, population A is resource 2-limited and has excess resource 1, making it more competitive to exchange resource 1 with population B for resource 2. Analogous reasoning explains the dark blue region. Additionally, through resource trading, populations A and B are able to exist in a wider range of resource environments than is possible for either population individually (dotted box, lower left). In this region, both resources 1 and 2 are limiting for both populations. Thus, resource exchange is more competitive since neither population can maintain growth under these resource conditions without the other. The rectangular boundary around this extended resource availability region represents an ideal one-to-one trading scenario; however, the shape of this region may take on an ellipsoidal form depending on the costs and relative fluxes of resource exchange.
Figure adapted from de Mazancourt and Schwartz.[54]

phenotypic plasticity requires additional resources for the genes and regulatory systems to express different phenotypes under different environments. This investment cost for phenotypic plasticity may be ecologically justified for populations that persist in spatially and/or temporally dynamic environments.[63]

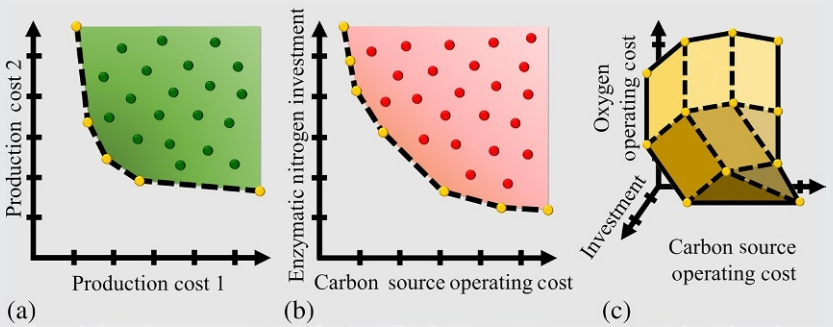
Tradeoff theories analogous to those used in economics have been applied to metabolic systems to assess the competitive use of phenotypic plasticity.[64] For many cellular behaviors, a benefit in one objective is realized only at the detriment of another objective. These tradeoffs are postulated to have tamed the "Darwinian demon," a superspecies that can optimize all objectives simultaneously and which has never been observed in nature or the laboratory.[65] The boundary that describes the tradeoff between resource uses is referred to as the Pareto front, efficiency frontier, or tradeoff surface.[64,66–68] Analysis of these resource allocation strategies is often paired with mathematical analysis such as stoichiometric modeling approaches (e.g., elementary flux mode and flux balance analyses) and/or kinetic models (Table 15.5).[66,69] Phenotypic phase plane analysis and multi-objective optimization have been used in stoichiometric modeling approaches to examine the effects of resource allocation on competitive network function.[70–75] The tradeoff concept can be represented graphically via rates or efficiencies of resource utilization, a measure of cost. Box 15.3 demonstrates a generalized

Table 15.5: Microbial community *in silico* analysis studies

Focus	Description	Reference
Review of modeling	Application of genome-scale models to microbial communities	[2]
Cyanobacterial mat	Three EFMA modeling approaches	[3]
Geobacter electron transfer	Multi-omic modeling	[138]
Synthetic microbial ecosystems	Prediction of symbiotic communities via environmental constraints	[139]
SRB-methanogen interactions	FBA	[140]
Spatial community dynamics	Reaction-diffusion FBA	[141]
Three case studies of OptCom algorithm	Community FBA	[142]
Consortial studies	Dynamic community FBA	[143–145]

EFMA, elementary flux mode analysis; FBA, flux balance analysis

BOX 15.3 Resource Allocation Theory: Pareto/Tradeoff Analysis



(A) *A generalized Pareto tradeoff curve* between two economic costs associated with the production of a good (for instance, the cost of fertilizer and pesticide required to produce a bushel of corn). The plot quantifies the tradeoff between minimizing the two different costs. In the example, the yellow points and connecting line segments represent optimal cost-minimizing relationships between the variables, while green interior points represent higher cost alternative strategies.
(B) *A metabolic tradeoff curve* analogous to a generalized Pareto tradeoff curve between two metabolic costs: (1) the carbon source operating cost, or the amount of carbon source required to synthesize a unit of bioproduct, and (2) enzymatic nitrogen investment, or the amount of nitrogen required to synthesize the enzymes in the utilized pathways. The yellow points represent the continuum of optimal pathways for producing the bioproduct, given the objectives of minimizing the cost of carbon source or nitrogen investment; interior red points represent less efficient alternative pathways for producing the bioproduct.
(C) *Three-dimensional metabolic tradeoff surface* considering the requirements for three resources (carbon source, nitrogen, and oxygen). The resulting Pareto surface represents the optimal relationship between these three costs for synthesizing a bioproduct. For ease of visualization, the interior volume of the tradeoff surface is omitted.

example of a Pareto optimization tradeoff curve for economic costs, with an analogous scenario translated into metabolic terms.

Single population examples of the predictions from resource allocation tradeoffs include the predicted use of the glyoxylate shunt and the Entner-Doudoroff glycolysis pathway during *E. coli* nutrient-limited growth. These alternative pathways produce less ATP than the citric acid cycle and Embden-Meyerhof-Parnas glycolysis pathway, respectively, but also require fewer anabolic resources, such as nitrogen and carbon, to synthesize the pathway enzymes as compared to the higher ATP-yielding pathways.[74,76,77]

In addition to modulating metabolic pathways, resource investment transitions are predicted to occur based on allocation of resources to either enzyme or substrate pools. Classic Michaelis-Menten kinetics describe the driving of flux via enzyme ($v_{max} = k_{cat} \cdot [E]$) and substrate pools. These two pools create a continuum of drivers for a single flux, ranging from high relative substrate concentration to high relative enzyme concentrations (Figure 15.1a); these different flux-driving mechanisms are referred to as push- or pull-based mechanisms, respectively.[78–80] The optimal distribution of resources between substrate and enzyme pools can vary depending on the required flux (Figure 15.1a, dotted line). Taking the FumC enzyme as an example, relatively small fluxes are best driven by a substrate-based push mechanism which minimizes overall resource requirements, while higher fluxes are best driven by a pull mechanism in which elevated enzyme concentrations represent a more efficient use of anabolic carbon.

Resource allocation between enzyme and substrate pools can also benefit microbial communities when populations exchange metabolites such as amino acids, vitamins, or nucleotides. Maximization of flux per investment of a valuable resource can be applied to a community exchanging metabolites such as cyanobacterial heterocyst-vegetative cell interactions.[13] As a numerical illustration, five interacting populations can each individually support a flux of 50 µM/s through enzyme X, which would require a total substrate and enzyme carbon investment of 1.51 CmM (millimolar concentration of carbon). Alternatively, one population can specialize in that function and drive a flux of 250 µM/s with a total carbon investment of 4.43 CmM and then exchange the product with the community. The division of labor strategy with product exchange reduces the total community carbon investment requirement by ~41%, neglecting the cost of metabolite exchange. Figure 15.1b demonstrates the general principle of flux as a function of total carbon investment into both enzyme and substrate pools. The net community investment savings continue to improve as the flux magnitude increases. Extrapolating to entire pathways will likely offset the involvement of transport proteins while maintaining resource investment savings. While this economy of scale holds from a purely reaction kinetics basis, additional factors such as diffusion rates and protein synthesis machinery could play substantial roles.

15.4 Case Studies of Communities with Interpretation

The maximum power principle, resource ratio theory, and resource allocation theory are useful for developing and interrogating the design principles of microbial communities. The current section provides an analysis of natural and engineered communities with dissection of relevant components, interactions, and theories.

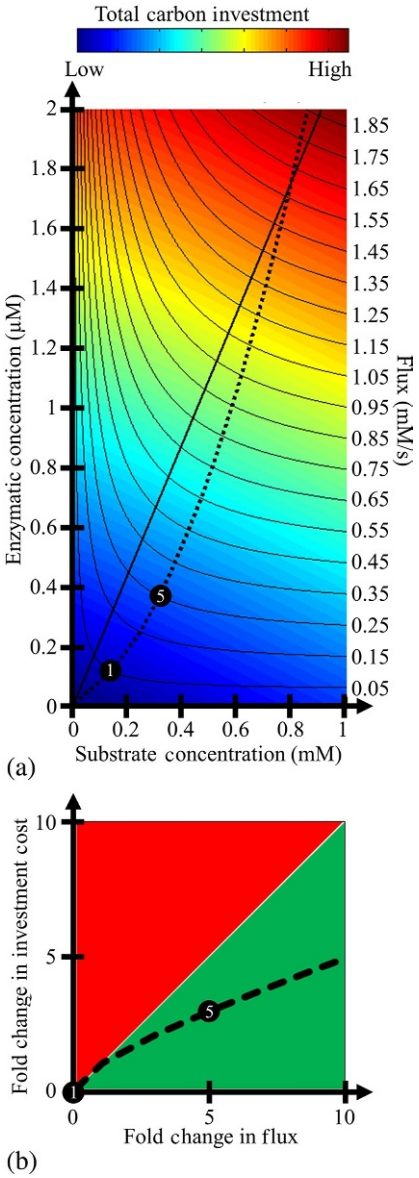


Figure 15.1
See figure legend on next page

15.4.1 Case Study: Multilevel Interactions in a Naturally Occurring Phototrophic Community

Community Description

The naturally occurring phototrophic community "*Chlorochromatium aggregatum*" is comprised of the phototrophic green sulfur bacterium *Chlorobium chlorochromatii* physically attached to the heterotrophic β-proteobacterium *Candidatus* Symbiobacter mobilis (Figure 15.2). This community is typically arranged as 13 green sulfur bacteria surrounding a single central β-proteobacterium and is found in sulfide-containing, oxic-anoxic interfaces of stratified lakes.[81,82] The green sulfur bacteria are nonmotile anoxygenic phototrophs which use light energy and electrons from sulfide to fix carbon and nitrogen. The heterotrophic β-proteobacterium is motile and possesses genetic evidence of phototactic and chemotactic capabilities but cannot harvest light energy and has limited respiratory capacities.[83] The green sulfur bacterium, although culturable in the laboratory, is not found free-living in nature, and the β-proteobacterium is unculturable independent of the green sulfur bacteria.[83,84]

Interaction Mechanisms

"*C. aggregatum*" interacts via a number of mechanisms. First, the green sulfur bacteria exchange anabolic and catabolic metabolites (reduced carbon and nitrogen) with the β-proteobacterium. Metabolite exchange is hypothesized to be bidirectional; the β-proteobacterium may synthesize compounds such as acetate that could be assimilated by the green sulfur bacteria due to limited respiratory capabilities.[83] Additionally, the β-proteobacterium and green sulfur bacteria are connected by periplasmic tubules. The

Figure 15.1

Resource investment and economies of scale for enzymatic flux. (a) A target enzymatic flux described by Michaelis-Menten kinetics can be achieved by a continuum of substrate or enzyme concentrations. Thin solid curves depict reaction isoflux lines that increase in 100 μM/s increments moving up and to the right. The substrate and enzyme concentrations each have associated resource costs related to the size and composition of the molecules. The total resource investment cost, depicted in color, sums the carbon investment from both the substrate and enzyme pools. The heavy solid line marks equal carbon investment contributions from substrate and enzyme pools. The dotted line represents the minimum total carbon investment for each isoflux line. Enzyme and substrate calculations model FumC from *E. coli* using $K_{cat} = 1150\ s^{-1}$, $K_m = 0.207$ mM, assuming fumarate, and 8800 carbon atoms per functional enzyme. This principle can be extrapolated to entire pathways, posing a mechanism for improved resource efficiency with increased specialization, as represented by the economies of scale. (b) The relationship between increasing flux and resource investment can be described based on the optimal minimization of investment. Total carbon investment per flux can be higher (red), lower (green), or the same (boundary) at larger fluxes as compared to smaller fluxes. In the example depicted, an increase in flux from 50 μM/s (point 1) to 250 μM/s (point 5) requires only threefold more resources.

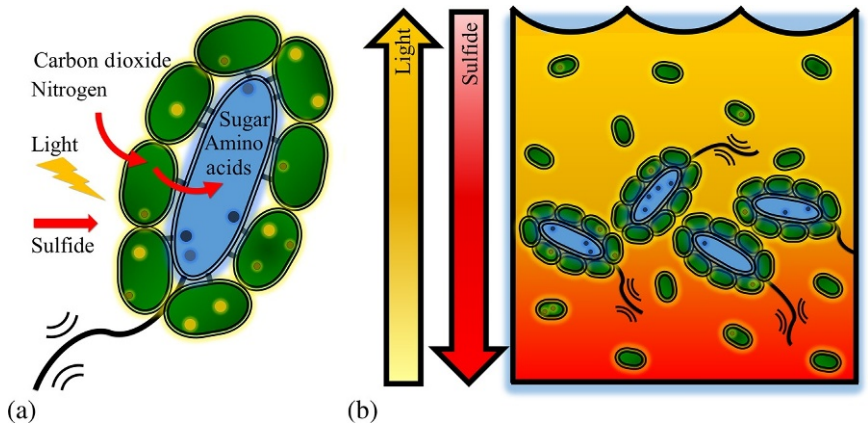


Figure 15.2
Phototrophic community interactions. (a) Green sulfur bacteria fix carbon dioxide and nitrogen from the environment and exchange reduced products with the heterotrophic β-proteobacterium. In return, the β-proteobacterium maintains a flagellum and provides taxis toward light and sulfide, which the green sulfur bacteria require for photosynthesis. (b) In a stratified lake environment, light is more intense at the top of the lake, and sulfide concentration increases toward the bottom. The motile community is able to take advantage of ideal concentrations of both resources. Free-living green sulfur bacteria cannot actively direct their movement toward resources and the β-proteobacterium is not able to survive independently.

direct physical interaction between the bacteria enables the β-proteobacterium to provide community motility toward light and sulfide.[85,86] These intercellular contacts could also permit exchange of electrons via soluble carriers,[87] enabling community-wide energy transfer and redox balancing.[83]

Theory Applications

Resource ratio and resource allocation theories provide an evolutionary rationale for the interactions of these two bacteria. In an environment with scarce resources (e.g., light, sulfide, reduced carbon, reduced nitrogen), this cooperative strategy confers a selective advantage.[54] The physical interactions and metabolite exchange are consistent with the resource ratio theory concept of a super-competitor unit. The motility contributed by the β-proteobacterium enables a competitive advantage for growth at low sulfide concentrations and lower light intensities via directed movement toward essential resources.[88] Additionally, the community configuration minimizes the associated resource investment costs. The direct physical connection between the bacteria permits 14 cells to benefit from the ability to move actively toward light and sulfide while only one cell needs to maintain the sensing and motility genes, synthesize flagellar proteins, and power flagellar operation.[83]

15.4.2 Case Study: Anaerobic Syntrophy in Methanogenic Communities

Community Description

Methane is a major focus of renewable energy efforts and plays an essential role in ecological food webs. Anaerobic communities produce methane by catabolizing organic feedstocks like biomass via a cascade of cross-feeding microorganisms. Sulfate-reducing bacteria (SRBs) and methanogenic archaea are key members of these anaerobic communities found in aquatic sediments and anaerobic digesters.[89] The SRBs oxidize organic acids and produce hydrogen or formate in the absence of alternative electron acceptors. The methanogens catabolize hydrogen and formate for cellular energy, ultimately producing methane as a by-product (Figure 15.3).[90,91]

Interaction Mechanisms

Methanogenic communities have a strong requirement for metabolite exchange due to the thermodynamic constraints of hydrogen synthesis.[92–94] Hydrogen partial pressures above a very small critical threshold (~10 Pa) shift the chemical reaction equilibrium from hydrogen synthesis to hydrogen consumption, stalling SRB metabolism in the absence of sulfate. The consumption of hydrogen by methanogens reduces local hydrogen partial pressures below the critical threshold, enabling further SRB catabolism.[94] The mutualistic interactions can extend further. In addition to providing the methanogens with substrate, the SRBs can also modify the local environment by reducing sulfate to sulfide rather than forming hydrogen. Sulfide is highly reactive with oxygen and reduces the environmental concentrations of oxygen,

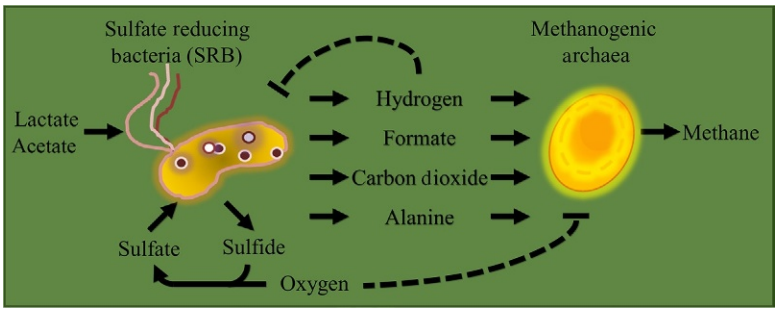


Figure 15.3

Sulfate-reducing bacteria (SRBs)—methanogen anaerobic syntrophy. SRBs create a reducing environment conducive to methanogenesis by reducing sulfate to sulfide, which can then abiotically react with oxygen. In the absence of sulfate, SRBs produce hydrogen; this metabolism becomes unfavorable without a hydrogen sink. Methanogens consume the hydrogen, facilitating SRB oxidation of carbon. SRBs cross-feed anabolic resources like amino acids to facilitate methanogen growth, resulting in larger hydrogen sink.

benefiting the oxygen-inhibited methanogens. In addition, research suggests that SRBs enhance methanogen consumption of hydrogen by exchanging anabolic resources like amino acids.[95]

Theory Applications

SRB-methanogen communities are a well-studied example of anaerobic syntrophy.[21] The cross-feeding of hydrogen, which can also be viewed as an environmental modification interaction, represents a classic example of the maximum power principle by collectively increasing the potential for energy acquisition from the environment. Metabolite exchange allows the SRBs to consume substrate at higher rates, thus increasing the collective community metabolic power. The exchange of anabolic resources such as amino acids is proposed to facilitate methanogen growth by providing an energetically expensive metabolite, thus increasing methanogen consumption of hydrogen.[95] The amino acid cross-feeding strategy represents an example of improved function per anabolic resource investment according to resource allocation theory (Figure 15.1b).

The syntrophic interactions of methanogens are not strictly limited to hydrogen-producing SRBs. Hydrogen-producing fermenters, which provide the substrate for SRBs, also benefit from methanogens.[96] Hydrogen transfer has also been observed in communities of hydrogen-producing anaerobic fungi and methanogens, which demonstrate the emergent property of more complete degradation of cellulosic biomass.[97]

15.4.3 Case Study: Cross-Feeding Chemostat Communities

Community Description

Culturing a clonal population of *E. coli* in a glucose-limited chemostat for hundreds of generations results in the reproducible formation of multiple, phenotypically distinct *E. coli* populations which cross-feed secondary metabolites (Figure 15.4).[17,98,99] The interacting community consists of three distinct functional populations: a glucose-catabolizing glycerol- and acetate-producing specialist, an acetate-catabolizing specialist, and a glycerol-catabolizing specialist. Both the acetate- and glycerol-catabolizing specialists completely oxidize their respective substrates. The population functions are based primarily on mutations in gene regulatory sequences, resulting in altered gene expression.[98] For instance, the glucose-catabolizing specialist expresses the high-affinity Mgl sugar transporter and has reduced expression of citric acid cycle enzymes, resulting in acetate and glycerol secretion. This strain, which represents 80% of the community, grown in isolation has a lower specific growth rate and a lower biomass per glucose yield than the parent monoculture; yet the community demonstrates a 15% improvement in biomass per glucose yield. The reproducibility of the interacting consortium suggests that the organization of components and interactions is more competitive and stable than an *E. coli* monoculture in a homogeneous environment like a chemostat.

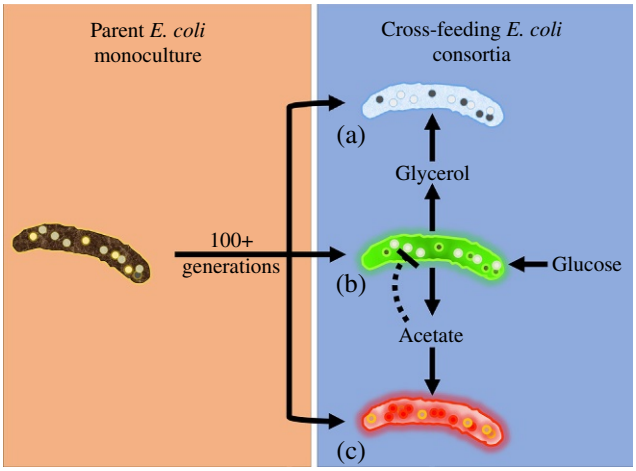


Figure 15.4
Naturally occurring *E. coli* crossing-feeding communities. *E. coli* monocultures grown long-term in glucose-limited chemostats evolve into cross-feeding communities. Community members include a glucose-catabolizing specialist that secretes acetate and glycerol (b), an acetate-catabolizing specialist (c), and a glycerol-catabolizing specialist (a). The cross-feeding community has an ~15% improvement on biomass per glucose yield as compared to the parent monoculture and depletes available substrates more completely than the parent monoculture.

Interaction Mechanisms

The community populations interact through the cross-feeding of catabolic resources. Additionally, consumption of acetate represents an environmental modification interaction that reduces the inhibition of the glucose-catabolizing strain.

Theory Applications

The cross-feeding *E. coli* community highlights key features of the maximum power principle, resource ratio theory, and resource allocation theory. The consortium has a higher metabolic power than the monoculture because inhibitory by-products (e.g., acetate) are removed during growth, allowing for a higher community metabolic rate. The community, as a whole, demonstrates the emergent property of a super-competitor unit defined in resource ratio theory; through metabolite exchange, the community drives the concentrations of carbon-based resources to levels unattainable by the parent monoculture. The populations also demonstrate an enhanced resource allocation configuration through division of metabolic labor. Each respective population increases expression of enzymes associated with a portion of central metabolism. The glucose-consuming specialist has higher glycolytic expression but lower citric acid cycle fluxes, resulting in the secretion of acetate and glycerol.[17,98,99] The strategy is representative of the tradeoff between substrate-driven and enzyme-driven fluxes; driving a reduced number of higher fluxes can be less resource intensive than driving a greater number of smaller fluxes.

A synthetic biology analog of the cross-feeding community has been created based on gene deletion rather than altered gene regulation.[14] The synthetic system demonstrated improved biomass productivity under batch, chemostat, and biofilm culturing conditions.

15.5 Conclusions

The ebbs and flows of scientific efforts build largely on achievements of the predecessors of the modern scientific community. While gaining popularity in recent years, the importance of microbial communities is not new. At this time of renewed interest in a systems-focused future, it is critical to keep a broad view. The early reductionist scientists are to be commended for laying a solid foundation that enables the current trajectory toward systems studies. In fact, reductionist approaches have a bright future because systems cannot be constructed solely on noble postulations but must be grounded in well-described components. It is not possible to define an emergent property until the basic components and interactions are appropriately catalogued.

A paramount goal and current challenge facing microbial community design is to mechanistically understand the design principles that govern higher order attributes of polymicrobial communities. The definitions presented here lay a foundation for a common language to bridge gaps across varying disciplines and enable greater collaboration between fields studying polymicrobial communities. The three ecological theories discussed—maximum power principle, resource ratio theory, and resource allocation theory—distill a broad range of literature regarding ecological theory into a concise synthesis and highlight the relevance of these theories to community interaction outcomes. The usefulness of these theories is illustrated through application to specific case studies in both natural and engineered environments. The tenets of resource acquisition, concentration, and allocation will remain useful for developing and examining the design principles of microbial communities and guide the future of polymicrobial bioprocess engineering.

Acknowledgments

The development of this work was supported by the National Institutes of Health awards (1U01EB019416-01, P20GM103474) and the Genomic Science Program (GSP), Office of Biological and Environmental Research (OBER), U.S. Department of Energy (DOE), and is a contribution of the PNNL Foundational Scientific Focus Area (Principles of Microbial Community Design) subcontracted to Montana State University. Ashley E. Beck was supported by the Office of the Provost at Montana State University through the Molecular Biosciences Program. Hans C. Bernstein was supported by the Linus Pauling Distinguished Postdoctoral Fellowship which is a Laboratory Directed Research and Development program at PNNL, operated for the DOE by Battelle Memorial Institute under Contract DE-AC05-76RLO 1830. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Muller S, Nebe-von-Caron G. Functional single-cell analyses: flow cytometry and cell sorting of microbial populations and communities. *FEMS Microbiol Rev* 2010;**34**(4):554–87.
2. Mahadevan R, Henson MA. Genome-based modeling and design of metabolic interactions in microbial communities. *Comput Struct Biotechnol J* 2012;**3**:e201210008.
3. Taffs R, et al. In silico approaches to study mass and energy flows in microbial consortia: a syntrophic case study. *BMC Syst Biol* 2009;**3**:114.
4. Tamime AY, Robinson RK. *Yoghurt: science and technology*. Cambridge: Woodhead; 1999.
5. Bond T, Templeton MR. History and future of domestic biogas plants in the developing world. *Energy Sustainable Dev* 2011;**15**(4):347–54.
6. Dobell C. *Antony van Leeuwenhoek and his "Little Animals." Being some account of the father of protozoology and bacteriology and his multifarious discoveries in these disciplines*. Collected, translated, and edited, from his printed works, unpublished manuscripts, and contemporary records. Published on the 300th anniversary of his birth, Great Titchfield Street, London W.C.1: John Bale, Sons & Danielsson, Ltd; 1932, p. 83–91.
7. Korgaonkar A, et al. Community surveillance enhances Pseudomonas aeruginosa virulence during polymicrobial infection. *Proc Natl Acad Sci U S A* 2013;**110**(3):1059–64.
8. Florey HW. The use of micro-organisms for therapeutic purposes. *Yale J Biol Med* 1946;**19**(1):101–18.
9. Pasteur L, Joubert J. Charbon et septicemie. *C R Acad Sci* 1877;**85**:101–5.
10. Madigan MT, Martinko JM, Bender K, Bender KS, Clark DP, Buckley DP, et al. *Brock biology of microorganisms*. 13 ed. San Francisco: Benjamin Cummings; 2010.
11. Bernstein HC, Carlson RP. Microbial consortia engineering for cellular factories: in vitro to in silico systems. *Comput Struct Biotechnol J* 2012;**3**(4):e201210017.
12. Roossinck MJ. The good viruses: viral mutualistic symbioses. *Nat Rev Microbiol* 2011;**9**(2):99–108.
13. Kumar K, Mella-Herrera RA, Golden JW. Cyanobacterial heterocysts. *Cold Spring Harbor Perspect Biol* 2010;**2**(4):a000315.
14. Bernstein HC, Paulson SD, Carlson RP. Synthetic Escherichia coli consortia engineered for syntrophy demonstrate enhanced biomass productivity. *J Biotechnol* 2012;**157**(1):159–66.
15. Eiteman MA, Lee SA, Altman E. A co-fermentation strategy to consume sugar mixtures effectively. *J Biol Eng* 2008;**2**:3.
16. Poltak SR, Cooper VS. Ecological succession in long-term experimentally evolved biofilms produces synergistic communities. *ISME J* 2011;**5**(3):369–78.
17. Rosenzweig RF, et al. Microbial evolution in a simple unstructured environment—genetic differentiation in Escherichia-Coli. *Genetics* 1994;**137**(4):903–17.
18. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat Rev Microbiol* 2004;**2**(2):95–108.
19. Stoodley P, et al. Biofilms as complex differentiated communities. *Annu Rev Microbiol* 2002;**56**:187–209.
20. Holland JN, DeAngelis DL. Consumer-resource theory predicts dynamic transitions between outcomes of interspecific interactions. *Ecol Lett* 2009;**12**(12):1357–66.
21. McInerney MJ, Sieber JR, Gunsalus RP. Syntrophy in anaerobic global carbon cycles. *Curr Opin Biotechnol* 2009;**20**(6):623–32.
22. De Roy K, et al. Synthetic microbial ecosystems: an exciting tool to understand and apply microbial communities. *Environ Microbiol* 2014;**16**(6):1472–81.
23. Wintermute EH, Silver PA. Emergent cooperation in microbial metabolism. *Mol Syst Biol* 2010;**6**:407.
24. Phelan VV, et al. Microbial metabolic exchange—the chemotype-to-phenotype link. *Nat Chem Biol* 2012;**8**(1):26–35.
25. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 2005;**21**:319–46.
26. Hense BA, et al. Does efficiency sensing unify diffusion and quorum sensing? *Nat Rev Microbiol* 2007;**5**(3):230–9.

27. Decho AW, Norman RS, Visscher PT. Quorum sensing in natural environments: emerging views from microbial mats. *Trends Microbiol* 2010;**18**(2):73–80.
28. Hibbing ME, et al. Bacterial competition: surviving and thriving in the microbial jungle. *Nat Rev Microbiol* 2010;**8**(1):15–25.
29. Fajardo A, Martinez JL. Antibiotics as signals that trigger specific bacterial responses. *Curr Opin Microbiol* 2008;**11**(2):161–7.
30. Paerl HW, Pinckney JL. A mini-review of microbial consortia: their roles in aquatic production and biogeochemical cycling. *Microb Ecol* 1996;**31**(3):225–47.
31. Brenner K, Arnold FH. Self-organization, layered structure, and aggregation enhance persistence of a synthetic biofilm consortium. *PLoS One* 2011;**6**(2):e16791.
32. Pirbadian S, et al. Shewanella oneidensis MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components. *Proc Natl Acad Sci U S A* 2014;**111**(35):12883–8.
33. Pfeffer C, et al. Filamentous bacteria transport electrons over centimetre distances. *Nature* 2012;**491**(7423):218–21.
34. Carlton RG, Richardson LL. Oxygen and sulfide dynamics in a horizontally migrating cyanobacterial mat: black band disease of corals. *FEMS Microbiol Ecol* 1995;**18**(2):155–62.
35. Kuhl M, Jorgensen BB. Microsensor measurements of sulfate reduction and sulfide oxidation in compact microbial communities of aerobic biofilms. *Appl Environ Microbiol* 1992;**58**(4):1164–74.
36. Rykiel EJ. Towards a definition of ecological disturbance. *Aust J Ecol* 1985;**10**(3):361–5.
37. Grimm V, Wissel C. Babel, or the ecological stability discussions: an inventory and analysis of terminology and a guide for avoiding confusion. *Oecologia* 1997;**109**(3):323–34.
38. Pimm SL. The complexity and stability of ecosystems. *Nature* 1984;**307**(5949):321–6.
39. Shade A, et al. Fundamentals of microbial community resistance and resilience. *Front Microbiol* 2012;**3**:417.
40. Waide RB, et al. The relationship between productivity and species richness. *Annu Rev Ecol Syst* 1999;**30**:257–300.
41. Dekel E, Alon U. Optimality and evolutionary tuning of the expression level of a protein. *Nature* 2005;**436**(7050):588–92.
42. Elser JJ, Acquisti C, Kumar S. Stoichiogenomics: the evolutionary ecology of macromolecular elemental composition. *Trends Ecol Evol* 2011;**26**(1):38–44.
43. Zinn M, Witholt B, Egli T. Dual nutrient limited growth: models, experimental observations, and applications. *J Biotechnol* 2004;**113**(1–3):263–79.
44. Konopka A. What is microbial community ecology? *ISME J* 2009;**3**(11):1223–30.
45. Prosser JI, et al. The role of ecological theory in microbial ecology. *Nat Rev Microbiol* 2007;**5**(5):384–92.
46. Brenner K, You L, Arnold FH. Engineering microbial consortia: a new frontier in synthetic biology. *Trends Biotechnol* 2008;**26**(9):483–9.
47. Lotka AJ. Contribution to the energetics of evolution. *Proc Natl Acad Sci U S A* 1922;**8**(6):147–51.
48. Lotka AJ. Natural selection as a physical principle. *Proc Natl Acad Sci U S A* 1922;**8**:151–4.
49. Sciubba E. What did Lotka really say? A critical reassessment of the "maximum power principle". *Ecol Model* 2011;**222**(8):1347–53.
50. Cai TT, Montague CL, Davis JS. The maximum power principle: an empirical investigation. *Ecol Model* 2006;**190**(3–4):317–35.
51. DeLong JP. The maximum power principle predicts the outcomes of two-species competition experiments. *Oikos* 2008;**117**(9):1329–36.
52. Martyushev L. Entropy and entropy production: old misconceptions and new breakthroughs. *Entropy* 2013;**15**(4):1152–70.
53. Tilman D. *Resource competition and community structure*. Princeton: Princeton University Press; 1982.
54. de Mazancourt C, Schwartz MW. A resource ratio theory of cooperation. *Ecol Lett* 2010;**13**(3):349–59.
55. Tilman D. Resources—a graphical-mechanistic approach to competition and predation. *Am Nat* 1980;**116**(3):362–93.
56. Tilman D. Tests of resource competition theory using four species of Lake Michigan algae. *Ecology* 1981;**62**(3):802.

57. Passarge J, et al. Competition for nutrients and light: Stable coexistence, alternative stable states, or competitive exclusion? *Ecol Monogr* 2006;**76**(1):57–72.
58. Bull AT. The renaissance of continuous culture in the post-genomics age. *J Ind Microbiol Biotechnol* 2010;**37**(10):993–1021.
59. Taylor PA, le BWPJ. Theoretical studies on the coexistence of competing species under continuous-flow conditions. *Can J Microbiol* 1975;**21**(1):90–8.
60. Brauer VS, Stomp M, Huisman J. The nutrient-load hypothesis: patterns of resource limitation and community structure driven by competition for nutrients and light. *Am Nat* 2012;**179**(6):721–40.
61. Enyeart PJ, Simpson ZB, Ellington AD. A microbial model of economic trading and comparative advantage. *J Theor Biol* 2014;**364**:326–43.
62. Sterner RW, Elser JJ. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton, NJ: Princeton University Press; 2002.
63. Agrawal AA. Phenotypic plasticity in the interactions and evolution of species. *Science* 2001;**294**(5541):321–6.
64. Kitano H. Violations of robustness trade-offs. *Mol Syst Biol* 2010;**6**:384.
65. Law R. Optimal life histories under age-specific predation. *Am Nat* 1979;**114**(3):399–417.
66. Molenaar D, et al. Shifts in growth strategies reflect tradeoffs in cellular economics. *Mol Syst Biol* 2009;**5**:323.
67. Carlson RP, Taffs RL. Molecular-level tradeoffs and metabolic adaptation to simultaneous stressors. *Curr Opin Biotechnol* 2010;**21**(5):670–6.
68. Shoval O, et al. Evolutionary trade-offs, Pareto optimality, and the geometry of phenotype space. *Science* 2012;**336**(6085):1157–60.
69. Song H-S, et al. Mathematical modeling of microbial community dynamics: a methodological review. *Processes* 2014;**2**(4):711–52.
70. Edwards JS, Ramakrishna R, Palsson BO. Characterizing the metabolic phenotype: a phenotype phase plane analysis. *Biotechnol Bioeng* 2002;**77**(1):27–36.
71. Oh YG, et al. Multiobjective flux balancing using the NISE method for metabolic network analysis. *Biotechnol Prog* 2009;**25**(4):999–1008.
72. Schuetz R, et al. Multidimensional optimality of microbial metabolism. *Science* 2012;**336**(6081):601–4.
73. Carlson R, Srienc F. Fundamental Escherichia coli biochemical pathways for biomass and energy production: identification of reactions. *Biotechnol Bioeng* 2004;**85**(1):1–19.
74. Carlson RP. Metabolic systems cost-benefit analysis for interpreting network structure and regulation. *Bioinformatics* 2007;**23**(10):1258–64.
75. Carlson RP. Decomposition of complex microbial behaviors into resource-based stress responses. *Bioinformatics* 2009;**25**(1):90–7.
76. Flamholz A, et al. Glycolytic strategy as a tradeoff between energy yield and protein cost. *Proc Natl Acad Sci U S A* 2013;**110**(24):10039–44.
77. Fischer E, Sauer U. A novel metabolic cycle catalyzes glucose oxidation and anaplerosis in hungry Escherichia coli. *J Biol Chem* 2003;**278**(47):46446–51.
78. Carlson RP, Oshota OJ, Taffs RL. Systems analysis of microbial adaptations to simultaneous stresses. *Subcell Biochem* 2012;**64**:139–57.
79. Tepper N, et al. Steady-state metabolite concentrations reflect a balance between maximizing enzyme efficiency and minimizing total metabolite load. *PLoS One* 2013;**8**(9):e75370.
80. Valenzuela J, et al. Potential role of multiple carbon fixation pathways during lipid accumulation in Phaeodactylum tricornutum. *Biotechnol Biofuels* 2012;**5**(1):40.
81. Overmann J. The phototrophic consortium "Chlorochromatium aggregatum"—a model for bacterial heterologous multicellularity. *Adv Exp Med Biol* 2010;**675**:15–29.
82. Overmann J, et al. The ecological niche of the consortium "Pelochromatium roseum". *Arch Microbiol* 1998;**169**(2):120–8.
83. Liu Z, et al. Genomic analysis reveals key aspects of prokaryotic symbiosis in the phototrophic consortium "Chlorochromatium aggregatum". *Genome Biol* 2013;**14**(11):R127.

84. Vogl K, et al. Chlorobium chlorochromatii sp. nov., a symbiotic green sulfur bacterium isolated from the phototrophic consortium "Chlorochromatium aggregatum". *Arch Microbiol* 2006;**185**(5):363–72.
85. Bryant DA, et al. Comparative and functional genomics of anoxygenic green bacteria from the taxa chlorobi, chloroflexi, and acidobacteria. In: Burnap RL, Vermaas WFJ, editors. *Functional Genomics and Evolution of Photosynthetic Systems*, 33. New York: Springer; 2012. p. 47–102.
86. Muller J, Overmann J. Close interspecies interactions between prokaryotes from sulfureous environments. *Front Microbiol* 2011;**2**:146.
87. Wanner G, Vogl K, Overmann J. Ultrastructural characterization of the prokaryotic symbiosis in "Chlorochromatium aggregatum". *J Bacteriol* 2008;**190**(10):3721–30.
88. Frostl JM, Overmann J. Physiology and tactic response of the phototrophic consortium "Chlorochromatium aggregatum". *Arch Microbiol* 1998;**169**(2):129–35.
89. Offre P, Spang A, Schleper C. Archaea in biogeochemical cycles. *Annu Rev Microbiol* 2013;**67**:437–57.
90. Li X, et al. Metabolism of H2 by Desulfovibrio alaskensis G20 during syntrophic growth on lactate. *Microbiology* 2011;**157**(Pt 10):2912–21.
91. Meyer B, et al. Variation among Desulfovibrio species in electron transfer systems used for syntrophic growth. *J Bacteriol* 2013;**195**(5):990–1004.
92. Thauer RK, et al. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nat Rev Microbiol* 2008;**6**(8):579–91.
93. Schink B. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* 1997;**61**(2):262–80.
94. Stams AJ, Plugge CM. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Microbiol* 2009;**7**(8):568–77.
95. Walker CB, et al. Functional responses of methanogenic archaea to syntrophic growth. *ISME J* 2012;**6**(11):2045–55.
96. Bizukojc M, et al. Metabolic modelling of syntrophic-like growth of a 1,3-propanediol producer, Clostridium butyricum, and a methanogenic archeon, Methanosarcina mazei, under anaerobic conditions. *Bioprocess Biosyst Eng* 2010;**33**(4):507–23.
97. Cheng YF, et al. Diversity and activity of enriched ruminal cultures of anaerobic fungi and methanogens grown together on lignocellulose in consecutive batch culture. *Bioresour Technol* 2009;**100**(20):4821–8.
98. Kinnersley M, et al. Ex uno plures: clonal reinforcement drives evolution of a simple microbial community. *PLoS Genet* 2014;**10**(6):e1004430.
99. Treves DS, Manning S, Adams J. Repeated evolution of an acetate-crossfeeding polymorphism in long-term populations of Escherichia coli. *Mol Biol Evol* 1998;**15**(7):789–97.
100. Momeni B, et al. Correction: strong inter-population cooperation leads to partner intermixing in microbial communities. *Elife* 2014;**3**:e02945.
101. Mikeskova H, Novotny C, Svobodova K. Interspecific interactions in mixed microbial cultures in a biodegradation perspective. *Appl Microbiol Biotechnol* 2012;**95**(4):861–70.
102. Ortiz-Marquez JC, et al. Genetic engineering of multispecies microbial cell factories as an alternative for bioenergy production. *Trends Biotechnol* 2013;**31**(9):521–9.
103. Jagmann N, Philipp B. Design of synthetic microbial communities for biotechnological production processes. *J Biotechnol* 2014;**184**:209–18.
104. Bader J, et al. Relevance of microbial coculture fermentations in biotechnology. *J Appl Microbiol* 2010;**109**(2):371–87.
105. Pandhal J, Noirel J. Synthetic microbial ecosystems for biotechnology. *Biotechnol Lett* 2014;**36**(6):1141–51.
106. Zuroff TR, Curtis WR. Developing symbiotic consortia for lignocellulosic biofuel production. *Appl Microbiol Biotechnol* 2012;**93**(4):1423–35.
107. Subashchandrabose SR, et al. Consortia of cyanobacteria/microalgae and bacteria: biotechnological potential. *Biotechnol Adv* 2011;**29**(6):896–907.

108. Hussa EA, Goodrich-Blair H. It takes a village: ecological and fitness impacts of multipartite mutualism. *Annu Rev Microbiol* 2013;**67**:161–78.
109. Shong J, Jimenez Diaz MR, Collins CH. Towards synthetic microbial consortia for bioprocessing. *Curr Opin Biotechnol* 2012;**23**(5):798–802.
110. Smid EJ, Lacroix C. Microbe-microbe interactions in mixed culture food fermentations. *Curr Opin Biotechnol* 2013;**24**(2):148–54.
111. Sabra W, et al. Biosystems analysis and engineering of microbial consortia for industrial biotechnology. *Eng Life Sci* 2010;**10**(5):407–21.
112. Thiele I, Heinken A, Fleming RM. A systems biology approach to studying the role of microbes in human health. *Curr Opin Biotechnol* 2013;**24**(1):4–12.
113. Brune KD, Bayer TS. Engineering microbial consortia to enhance biomining and bioremediation. *Front Microbiol* 2012;**3**:203.
114. Murray JL, et al. Mechanisms of synergy in polymicrobial infections. *J Microbiol* 2014;**52**(3):188–99.
115. Goers L, Freemont P, Polizzi KM. Co-culture systems and technologies: taking synthetic biology to the next level. *J R Soc Interface* 2014;**11**(96).
116. Song H, et al. Synthetic microbial consortia: from systematic analysis to construction and applications. *Chem Soc Rev* 2014.
117. Grosskopf T, Soyer OS. Synthetic microbial communities. *Curr Opin Microbiol* 2014;**18**:72–7.
118. Lopez-Ferber M, et al. Defective or effective? Mutualistic interactions between virus genotypes. *Proc Biol Sci* 2003;**270**(1530):2249–55.
119. Kuss SK, et al. Intestinal microbiota promote enteric virus replication and systemic pathogenesis. *Science* 2011;**334**(6053):249–52.
120. Sandaa RA, et al. Viral control of bacterial biodiversity–evidence from a nutrient-enriched marine mesocosm experiment. *Environ Microbiol* 2009;**11**(10):2585–97.
121. Tillmann HL, et al. Infection with GB virus C and reduced mortality among HIV-infected patients. *N Engl J Med* 2001;**345**(10):715–24.
122. Ernst D, et al. Impact of GB virus C viraemia on clinical outcome in HIV-1-infected patients: a 20-year follow-up study. *HIV Med* 2014;**15**(4):245–50.
123. Oldstone M. Prevention of type I diabetes in nonobese diabetic mice by virus infection. *Science* 1988;**239**(4839):500–2.
124. Marquez LM, et al. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. *Science* 2007;**315**(5811):513–5.
125. Zhang CC, et al. Heterocyst differentiation and pattern formation in cyanobacteria: a chorus of signals. *Mol Microbiol* 2006;**59**(2):367–75.
126. Minty JJ, et al. Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass. *Proc Natl Acad Sci U S A* 2013;**110**(36):14592–7.
127. Rodionova IA, et al. Genomic distribution of B-vitamin auxotrophy and uptake transporters in environmental bacteria from the Chloroflexi phylum. *Environ Microbiol Rep* 2014.
128. D'Onofrio A, et al. Siderophores from neighboring organisms promote the growth of uncultured bacteria. *Chem Biol* 2010;**17**(3):254–64.
129. Sieber JR, McInerney MJ, Gunsalus RP. Genomic insights into syntrophy: the paradigm for anaerobic metabolic cooperation. *Annu Rev Microbiol* 2012;**66**:429–52.
130. Falony G, De Vuyst L. *Ecological interactions of bacteria in the human gut*. 2009 p. 639–79.
131. Tan CH, et al. The role of quorum sensing signalling in EPS production and the assembly of a sludge community into aerobic granules. *ISME J* 2014;**8**(6):1186–97.
132. De Lorenzo V, Martinez JL, Asensio C. Microcin-mediated interactions between Klebsiella pneumoniae and Escherichia coli strains. *Microbiology* 1984;**130**(2):391–400.
133. Pande GS, et al. The Vibrio campbellii quorum sensing signals have a different impact on virulence of the bacterium towards different crustacean hosts. *Vet Microbiol* 2013;**167**(3–4):540–5.

134. Ishii S, et al. Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell. *BMC Microbiol* 2008;**8**:6.
135. Morris JJ, et al. Dependence of the cyanobacterium Prochlorococcus on hydrogen peroxide scavenging microbes for growth at the ocean's surface. *PLoS One* 2011;**6**(2):e16805.
136. Stomp M, et al. Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature* 2004;**432**(7013):104–7.
137. Wilson JB, Spijkerman E, Huisman J. Is there really insufficient support for Tilman's R* concept? A comment on Miller et al. *Am Nat* 2007;**169**(5):700–6.
138. Nagarajan H, et al. Characterization and modelling of interspecies electron transfer mechanisms and microbial community dynamics of a syntrophic association. *Nat Commun* 2013;**4**:2809.
139. Klitgord N, Segre D. Environments that induce synthetic microbial ecosystems. *PLoS Comput Biol* 2010;**6**(11):e1001002.
140. Stolyar S, et al. Metabolic modeling of a mutualistic microbial community. *Mol Syst Biol* 2007;**3**:92.
141. Harcombe WR, et al. Metabolic resource allocation in individual microbes determines ecosystem interactions and spatial dynamics. *Cell Rep* 2014;**7**(4):1104–15.
142. Zomorrodi AR, Maranas CD. OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities. *PLoS Comput Biol* 2012;**8**(2):e1002363.
143. Zomorrodi AR, Islam MM, Maranas CD. d-OptCom: dynamic multi-level and multi-objective metabolic modeling of microbial communities. *ACS Synth Biol* 2014;**3**(4):247–57.
144. Hanly TJ, Henson MA. Dynamic flux balance modeling of microbial co-cultures for efficient batch fermentation of glucose and xylose mixtures. *Biotechnol Bioeng* 2011;**108**(2):376–85.
145. Hanly TJ, Urello M, Henson MA. Dynamic flux balance modeling of S. cerevisiae and E. coli co-cultures for efficient consumption of glucose/xylose mixtures. *Appl Microbiol Biotechnol* 2012;**93**(6):2529–41.