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REVIEW ARTICLE

Model Microbial Consortia as Tools for Understanding Complex Microbial Communities

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Abstract: A major biological challenge in the postgenomic era has been untangling the composition and functions of microbes that inhabit complex communities or microbiomes. Multi-omics and modern bioinformatics have provided the tools to assay molecules across different cellular and community scales; however, mechanistic knowledge over microbial interactions often remains elusive. This is due to the immense diversity and the essentially undiminished volume of not-yet-cultured microbes. Simplified model communities hold some promise in enabling researchers to manage complexity so that they can mechanistically understand the emergent properties of microbial community interactions. In this review, we surveyed several approaches that have effectively used tractable model consortia to elucidate the complex behavior of microbial communities. We go further to provide some perspectives on the limitations and new opportunities with these approaches and highlight where these efforts are likely to lead as advances are made in molecular ecology and systems biology.

Keywords: Microbial consortia, Ecosystems, Multi-omics, Interspecies interactions, Complex systems, Microbiome, Microbial ecology.

1. INTRODUCTION

The sheer complexity of most ecosystems has long attracted and baffled scientists. For instance, Charles Darwin represented the complexity of ecosystems as a "tangled bank" [1], and Thomas Brock once described the clarification of microbial diversity in nature as "mumbo jumbo" [2]. In the present post-genomic era of molecular ecology, we have been confronted with the realization that the immense taxonomic and functional diversity of most natural microbial communities render them intractable for comprehensive mechanistic studies. For example, natural soil microbiomes are critical for the functioning of terrestrial ecosystems [3] and are extremely diverse from both the taxonomic and functional aspects; for instance, 1 g of soil may harbor 10^9 microbial cells with representatives from 10^3-10^6 species [4, 5]. Furthermore, we know that microbes respond to each other to achieve emergent, higher order metabolic functioning and genetic adaptation [6-8] making it essentially impossible to translate the knowledge gained from isolates to complex natural systems.

At the turn of the century, synthetic ecology emerged as a nascent offshoot of synthetic biology [9]. Many consortial systems are being designed and/or adapted for biotechnological applications such as biofuel/bioproduct synthesis and management of greenhouse gases [10-12]. In addition, this movement has revealed a new paradigm for studying the natural microbial ecosystems. Rather than attempting to decompose the so-called "tangled bank", the complexity is managed in model microbial consortia which are designed and built [13-15] (Fig. **1**). This practice is now common, and the number of experimental systems continues to grow for testing and learning ecological theories and validating hypothesis. We should choose the appropriate model consortia that are useful to answer questions that we want to solve. Fig. (**2**) summarizes the microbial ecological methods applicable to microbial consortia, depending on complexity.

In this review, we introduce several approaches that have effectively used model communities (two-species co-cultures, three-species and higher member complex co-cultures, and enriched model systems) to elucidate the microbial community functions and behaviors from wide viewpoints, *i.e.,* gene regulatory networks, metabolic interactions, and ecological theory (Fig. **2**). In addition, we provide some perspectives on the limitations and emerging opportunities with these approaches as well as highlight the possible consequences of applying these approaches.

2. TWO-SPECIES CO-CULTURE - THE SIMPLEST COMMUNITIES

The reduction of the complexity in natural microbial communities is severe. However, the knowledge about interactions in one-to-one relationships (*e.g.,* styles and sign of interactions) is fundamental as "edges" (interaction) between "nodes" (species) in the network structure of the

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"nodes" (species) in the network structure of the microbial community. Thus, clarifying the actual, or at least potential, interaction between two species is a key step for untangling the complex network structure, and a two-species co-culture, *i.e.,* binary communities, offers numerous advantages as a model system (Fig. **2**).

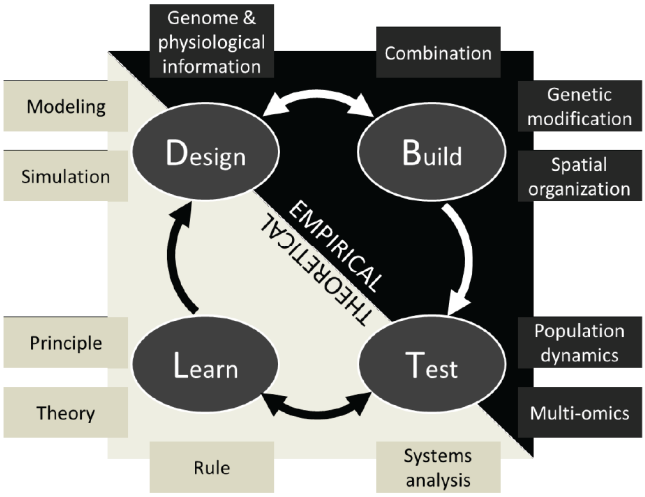

Fig. (1). An overview of the microbial ecological studies using the model microbial consortia.

Binary systems have been utilized for understanding ecological principles governing species coexistence and community dynamics (see below). The characteristics of microorganisms (*e.g.,* small individual size, large population size, and short generation time) are advantageous for performing experimental approaches for the establishment and verification of ecological theories, which are difficult to realize by using higher organisms. Beginning with the historical work by Gause [16], which describes the population dynamics of competitive and predator-prey relationships in co-culture experiments using protozoa and yeast, the empirical model microcosm approaches helped establish and improve the theoretical framework of general population ecology, which can be applied to all types of biological populations (*e.g.,* competitive exclusion principle [17] and Lotka-Volterra equation [18]). In addition to the trophic interactions, interspecies interactions mediated by secreted diffusible compounds such as antibiotics, organic acids, hydrogen peroxide, and growth factors have also been well characterized [19-23]. Studies using binary co-culture system have discovered contact-dependent interactions [24-27], which are difficult to be addressed using pure-culture systems or genome mining approaches. Nowadays, the Type VI Secretion System (T6SS), a contact-dependent interference system, is known to distribute among a wide variety of Gram-negative bacteria (more than one-fourth of species in which genomic information has been reported) [28].

Physiological, biochemical, and genetic approaches, and, recently, transcriptomic analysis have been implemented in several studies to reveal global expression profile upon syntrophic interactions (Fig. **2**). Such approaches contribute to obtaining more detailed molecular profiles as well as to achieve greater insights into interspecies interactions. Data obtained from syntrophic co-culture systems showed drastic changes in the gene expression profiles in both syntrophic bacterial [29, 30] and methanogenic archaeal populations [31], and the key genes for a syntrophic cooperative lifestyle were clarified. Transcriptome analysis was utilized in other model dual-culture systems [6, 32]. Bernstein *et al.* investigated the genes differentially expressed in a cyanobacterium in partnership with a heterotrophic bacterium and found that

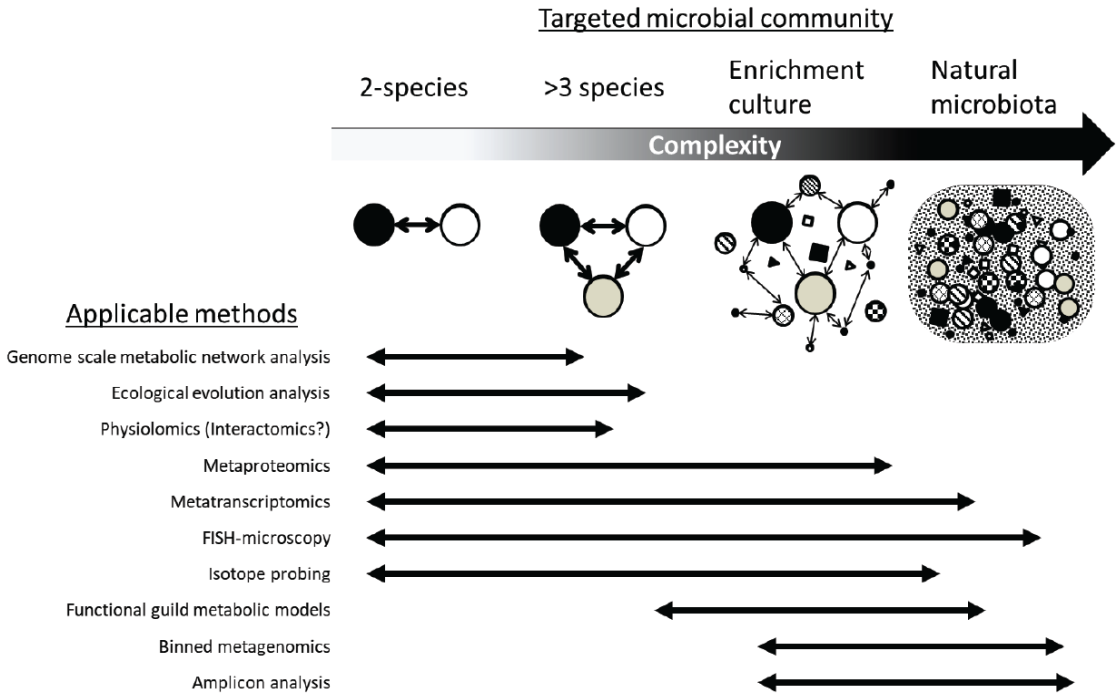

Fig. (2). Complexity-limited microbial ecological methods.

not only metabolism-related genes but also other genes such as stress-response genes are key factors for this partnership [6]. Thus, the analysis for the global gene expression profiles will provide a more comprehensive picture of interspecies relationships, including overlooked or unexpected information. Now the RNA-sequencing technique can extract information only about focal population from a mixed RNA sample, thereby contributing to the wide use of transcriptomics for mixed culture systems, where the separation of each population from the entire mixed population is often problematic. For detailed characterization of interaction, omics technologies other than transcriptome have been applied to binary co-culture systems (Fig. **2**): proteomics [33, 34] and metabolomics [35, 36]. These studies will elucidate the metabolic pathways in microbial consortia and their metabolic interaction network with the help of *in silico* approaches [13, 37, 38].

Binary systems have been also used to experimentally study the evolutionary aspects of multispecies communities. It is well-recognized that the ecological and evolutionary processes reciprocally influence each other (the eco-evolutionary feedbacks) [39]. The relatively small size of the bacterial genome and the recent advances in massive sequencing techniques have facilitated researchers to track the evolutionary history by detecting genetic variations in the course of laboratory evolution experiments (Fig. **2**). Harcomb demonstrated how cooperative behavior evolves [40]. Another example of the evolution of cooperation was provided by Hillesland and Stahl; they used the syntrophic bacterium *Desulfovibrio* sp. and its methanogenic archaea partner and found an increase in fitness during the course of the evolutionary experiment [41]. Hence, binary co-culture systems can be utilized to investigate the evolutionary origin and the developmental process of cooperative interactions. Similarly, antagonistic co-evolution, which is widely observed in natural ecosystem, also known as the evolutionary arms race, has also been investigated by the co-culture evolution experiments. Protozoa and bacteriophages are known to alter the evolutionary dynamics of bacteria [42-44]. Tognon *et al.* showed that evolution of *Pseudomonas aeruginosa* was affected by cohabiting *Staphylococcus aureus*, and *P. aeruginosa* clearly followed distinct evolutionary trajectories in the co-culture [45]. The evolutionary ecological studies on the antagonistic interactions are described below.

Most microbial cells are attached to interfaces to form a multicellular structure, namely biofilm, in natural settings. Biofilm offers a place for active interactions. Culture experiment of multispecies biofilms has suggested a unique way of spatial organization and coexistence of competing populations. It has been demonstrated that the structure and volume of the biofilm formed at the solid surface by pathogenic bacteria are altered by interspecies interactions. The opportunistic pathogen *P. aeruginosa,* shows a distinct biofilm structure from that formed by *P. aeruginosa* only when it coexists with another pathogen, *Stenotrophomonas maltophilia* [46]. In the case of *Escherichia coli*, the biofilm formation is promoted by signal molecules provided by other cohabiting enterobacterial species [47]. Wong *et al.* examined the population dynamics of *Vibrio cholerae* and *Aeromonas hydrophila* in a 2D plane and demonstrated that reciprocal antagonistic interactions (killing by T6SS) allowed them to coexist; each population exhibited a mosaic or patchy distribution as a result of contact-dependent killing of each other [48]. Thus, the model binary culture system combined with microscopic techniques (Fig. **2**) can be used to visualize spatial organization of interacting species to gain an insight into the distribution of each member in the complex biofilm community *in situ*.

3. THREE-SPECIES AND MORE COMPLEX CO-CULTURES

The utility of model microbial consortia is that they provide a simplified system that encapsulates some known properties of more complex microbiomes which are not easily studied. Hence, higher-member co-cultures, *i.e.,* more than two-species, are typically more relevant as model systems. However, systems composed of three or more elements are completely different from the two-element simplest system because the complexity of three-element systems considerably increases due to forming a complex network and mathematical simulation for three-element systems is extremely hard. It has been widely recognized that interspecies interactions in binary communities are context-dependent and variable depending on the environmental conditions and the third-party, *i.e.,* indirect effects [49, 50]. The challenge lies in the fact that the complexity of a microbial community - as defined by the number of potential physical and metabolic interactions - scales non-linearly with the number of participating species [51].

Advanced microbial ecological techniques such as NGS, digital PCR, DNA biochip, and multi-colored Fluorescence *In Situ* Hybridization (FISH) can enable the efficient tracking of the population dynamics of each member, even in >10 species mixed cultures (Fig. **2**). Furthermore, sophisticated microfluidic cultivation devices are available for real-time imaging of the spatiotemporal organization of microbial communities at the single-cell level [52-54]. A variety of metabolic modeling tools have been developed for complex consortia [15, 55-60]. As well as forward engineering approaches, *i.e.,* building-up of synthetic communities (*e.g.,* [61]), we are able to employ reverse engineering approaches to investigate the roles of the members of microbial communities, as Kato and colleagues generated "knock-out" communities in which one of the members was eliminated from the original five-member community [62].

Synergetic and complemental effects of the members allow them to stably coexist. Some studies using 3-7 species mixed cultures provided straightforward scenarios:

[Example 1] Rock-Paper-Scissors relationship among three genetically modified *E. coli* strains (a colisin producing strain with the slowest growth rate, a colisin resistant strain with the medium growth rate, and a colisin sensitive strain with the fastest growth rate) [63];

[Example 2] a decoy rescues a prey from a predatory bacterium through nonproductive attachments of the predator on the decoy [64];

[Example 3] Species A helps Species B, and Species B is required by Species C in a fermented drink [65];

[Example 4] Species A inhibits Species B but not Species C, and Species B is surrounded by Species C to keep away from Species A in a biofilm [66];

[Example 5] Species A inhibits Species B, Species B promotes Species C, and Species C suppresses the inhibitory effect of Species A in a cellulose-degrading microbial consortium [62, 67].

These studies indicate that the structural stability of microbial ecosystems requires not only cooperative interactions but also suppressive interactions to avoid over-growth of one of the members [68]. Narisawa *et al.* [66] found an attractive implication that suppressive interactions between some members through antibiotic production tighten the cell-cell association in biofilms. A theoretical and experimental study by Kelsic *et al.* [69] suggested that antibiotic production and degradation allows large numbers of species to coexist.

These synthetic approaches generalize the observations made in the microbial ecosystems and help developing and examining microbial ecological theory (Fig. **1**) [70]. In the following sections, we introduce sets of synthetic microbial ecological researches that investigated *diversity-productivity relationships*, *diversity-robustness relationships*, and *evolution-cooperativity relationships*.

Biomass production is frequently measured as ecosystem functions since it is a fundamentally important character for ecosystems including microbial ecosystems. It has long been suggested that biodiversity increases the productivity of the ecosystems [71]. Hodgson *et al.* constructed synthetic communities with genetically different *Pseudomonas fluorescens* strains to show that diversification increased productivity [72]. Bell *et al.* observed a similar trend by culturing mixtures of 72 bacterial species [73]. However, this diversity-productivity relationship may not be always applicable. Schmidtke *et al.* reported that an increase in the diversity of a synthetic phytoplankton community (cyanobacteria, green algae, diatom, and phytoflagellate) decreases biomass production [74]. The composition of synthetic communities should also be considered, as Venail and Vives showed that synthetic communities composed of phylogenetically distant species were less productive [75]. These synthetic ecological reports indicate the importance of additional factors as well, for example, the availability of a variety of nutrients, the growth yields of each member, competitive interactions among members, and homogeneity of environments. The effects of the availability of nutrients on the community members have been examined by comparison of synthetic bacterial communities dominated by generalists and specialists [76]. They detected more clear relationships between biodiversity and productivity in specialist-dominated communities.

Biodiversity probably enhances the robustness of the microbial ecosystems. Synthetic communities with higher diversity showed higher functional resistance against perturbation in a bacterial community investigated by Hodgson *et al.* [72] and in a green algal community by Li *et al.* [77]. Wittebolle *et al.* examined how initial species evenness affects the functional stability of a bacterial mixed culture containing 18 strains [78]. Their results show that communities with higher unevenness have lower functional stability, that is, lower robustness against environmental stress.

Since the 19th century, it has been recognized that interspecies interactions are an important factor of adaptation, and evolutionary ecology continuously highlights how interspecies interactions drive the evolution of organisms. Antagonistic interactions are believed to accelerate evolutionary changes. This has been known as the Red Queen hypothesis. Experimental coevolution studies using two species cocultures support this hypothesis and further indicate that coevolution results in diversification [79]. Recently, Baumgartner *et al.* [80] showed that genome reduction made bacteria adaptive to nutrient-limited environments and predation.

Environmental microbiological studies identified streamlined genomes in a variety of bacteria in nature [81]. Fiegna *et al.* synthesized a 12-species community and examined the effects of species richness on evolution. In their experimental setting, interactions among a limited number of species stimulated evolution, but not in the communities composed of larger number of species [82]. In 2012, the Black Queen hypothesis, which states that bacteria evolve to streamline their genomes by relying on other organisms, was proposed [83] and is currently being extensively discussed [84, 85]. To address this hypothesis, synthetic microbial communities are designed and studied to obtain useful implications. The keywords are “cooperativity” and “interdependency”. The extracellular secretion of public goods in the community is a sort of cooperative association, whereas the synthesis of goods incurs the cost of energy and nutrients [86, 87]. In microbial ecosystems, siderophore and polymer-degrading enzymes are good examples. Siderophore - an iron-chelating compound - is produced by a member in the community could help others to incorporate iron [88]. Polymer-degrading enzymes such as glycosidases and proteases make polymeric compounds smaller and easily utilizable as nutrients. Rakoff-Nahoum *et al.* found that glycosidase production enhanced the coexistence of 7 bacterial species in a synthetic community [89]. This was observed in trophic associations. Mee *et al.* used 14 types of auxotrophic *E. coli* and systematically combined them to synthesize a series of microbial communities [90]. Their findings indicate that the exchange of costly resources tends to strengthen the cooperation. Adequate knowledge about the types of interactions and their combinations in the ecosystems are necessary to develop ecological theory describing ecosystems dynamics and functions, as indicated in the theoretical study by Mougi and Kondou [91].

Very recently, Friedman *et al.* proposed a new assembly rule of microbial species and tested the rule using 2-3 species mixed cultures with comprehensive combinations of 8 bacterial species [92]. Their simple rule does not require a large number of parameter values but only the qualitative information about the outcome of competitions (*i.e.,* coexistence or extinction) among a limited number of species to predict the overall community structure composed of diverse species. As shown in their pioneering study, theoretical approaches will be helpful to explain how individual members or pairwise interactions build up microbial consortia and would open doors to research the complexity of microbial ecosystems.

4. ENRICHED MODEL SYSTEMS

In general, simplified model microbial community is recognized as a defined mixed culture composed of isolated

microorganisms. However, synthetic biological approach knocks out a part of the biological system or builds it up in order to simplify the systems in an organism. Considering this aspect, enrichment culturing from natural microbiota and engineering of enriched microbial consortia are also attractive approaches in the field of microbial ecology for understanding microbial ecosystems. Nowadays, several molecular ecological methods such as meta-transcriptomics and meta-proteomics are applicable to complex microbial ecosystems and the data obtained by these omics can be utilized for metabolic modelling of the ecosystems (Fig. **2**).

Enrichment cultivation is a traditional bioengineering method. Prior to realizing microbial life and developing isolation techniques, we obtain the desired microbial communities from nature by repetitive cultivation under appropriate selective pressures, for example, food fermentations, wastewater treatments, and composting. "Enrichment cultivation" refers to artificial selection performed to simulate a function or behavior of microbial communities in natural environments [93]. In this process, undesired members are subtracted (−), desired new members are combined (+), and desired and undesired functions are promoted or repressed by increasing or decreasing the population of members (×) respectively. In some cases, different members or functions are spatially divided (÷) (Fig. **3**). These "synthesized" microbial communities have been applied to several industries [94, 95], and these synthetic approaches also imply the microbial ecological rules. Examples for each of the synthesized processes (−, +, ×, and ÷) are described below:

− *(subtract)*: essentially, the "enrichment" processes remove undesired or unessential members from the natural microbiota by stimulating the growth of the required members and limiting dilution through repetitive cultivation. Knowledge of the appropriate selective conditions that promote the growth of the desired microbes helps (*e.g.,* [96-102]). The application of antibiotics, phages, predating protists, and physicochemical treatments such as heat, desiccation, and deoxygenation/oxygenation allow to subtract some microbial species from a consortium. Some chemicals are also available to inhibit certain reactions of microbes, for example, 2-bromoethanesulfonate is used to inhibit methanogenesis and molybdate to inhibit sulfate reduction. Analyses of the succession of microbial communities during enrichment processes are a good indicator for understanding relationships between the microbial community structure and functions. This information would help distinguish microbes or microbial associations that suppress the desired community function. However, only limited studies have reported the succession of microbial communities during the enrichment process. Swenson *et al.* systematically evaluated the community function of 3-chloroaniline degradation among the enrichment cultures and their observation proposed a relationship between the functional stability and the magnitude of the selective pressure [103]. Based on the experimental observations made by Swenson *et al.,* Williams and Lenton applied a simulation approach to the enrichment processes and reported that the responses of the microbial communities to environmental changes can be achieved not through independent responses of individual members, but

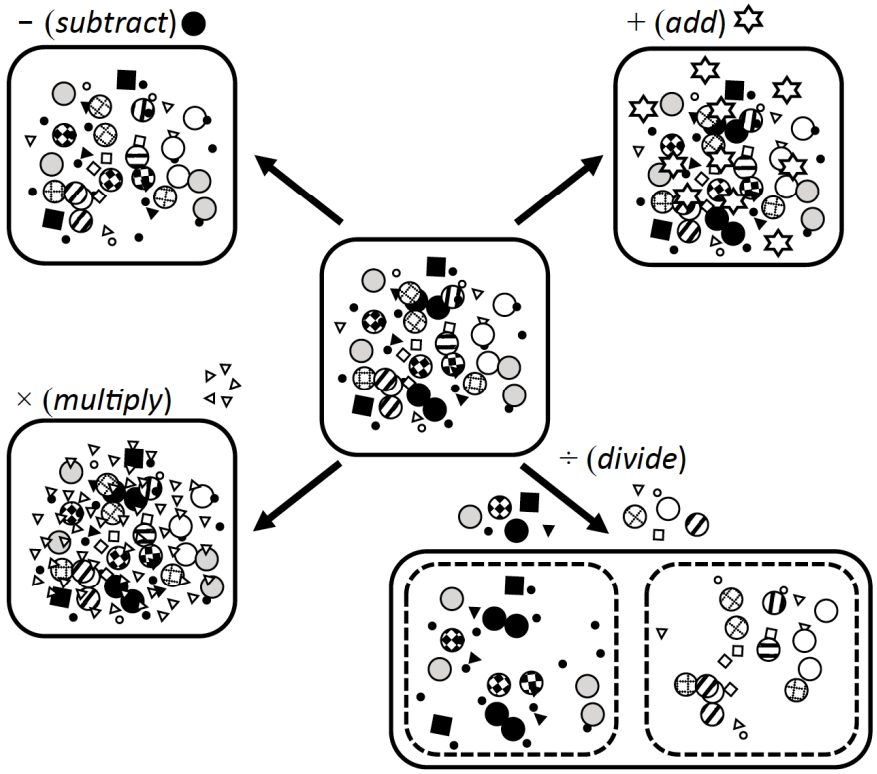

Fig. (3). Enrichment cultivation processes to obtain synthesized microbial communities **-**, subtract undesired members; +, add desired external microbes; ×, multiply the number of members, *i.e.,* promote (× >1) or suppress (× <1); ÷, divide a portion of members from others, *e.g.,* form biofilm on fixed beds separated from fluidized beds, and compartmentalize cultivation vessels into aerobic and anaerobic modules.

through interspecies interaction [104]. Garcia *et al.* successfully obtained microbial consortia that shows the productivity of freshwater microbiota through the limiting dilution approach [105]. Comparative metagenomic analyses of their microbial consortia provided useful information about interspecies interactions with evolutional aspects. Recently, comparative network analyses were widely applied to microbial consortia in the intestine to characterize topological changes of the microbial association network by application of antibiotics [106, 107].

***+ (add)*:** in this approach, several sources of microbial communities or several types of microbial consortia are empirically combined to efficiently synthesize the desired functional microbial consortia (*e.g.,* [96, 98, 100]). The integration of different microbial consortia would increase biodiversity and result in increased availability of nutrients and functional stability.

Another prominent example of the "add" approach is bioaugmentation, one of the methods of bioremediation, in which cultured microorganisms are inoculated into the natural microbiota to help remove contaminants [108]. Similarly, the administration of probiotics to animals and plants is also an example of the "add" approach [109, 110]. Most of these past studies showed that the effects of the addition of microbes did not last for long and the exogenous microbes were kicked out over time (*e.g.,* 3-chloroaniline degrading bacterium into activated sludge [111]). These observations imply that well-developed microbial ecosystems are highly resilient against the invasion by additional members, and the interspecies associations established in microbial ecosystems are hardly altered. However, Laurinavichene *et al.* successfully integrated a purple photosynthetic bacterium into an enriched microbial consortium [112]. Illumination possibly opened an additional niche for the purple bacterium in the established microbial consortium. Narisawa *et al.* introduced additional cellulose-degrading strain into a cellulose-degrading bacterial consortium which grew well on cellulose at 50°C, but not at 60°C [113]. The additional strain could not survive in the consortium cultivated at 50°C. However, cultivation at 60°C induced rearrangement of the community structure and allowed the consortium to accept the invasion by this exogenous bacterium. These observations indicate that the creation of a free niche to disturb the established community structure is required for invader species as suggested by Adam *et al.* [114] and Kinnunen *et al.* [115].

***× (multiply)*:** in the research field of ecology, the effects of changes in the nutrient supply and abiotic factors on the natural microbiota have been extensively studied [116]. These environmental disturbances affect ecosystems by allowing the population of some organisms to multiply. As a biotechnological application, biostimulation is a good example of "multiply by >1" approach. Suppressive disturbance described as "multiply by <1" (*e.g.,* starvation of a nutrient and RNAi) reduces the population or activity of particular members in a community. These "multiply" approaches may not completely break down the microbial association network in microbial communities, but may also strengthen or weaken some of the interspecies interactions. Analyses of the effects of "multiply" on enriched microbial consortia provide valuable information to understand the behaviors of microbial community as a system [117]. For example, a recent study introduced organic nutrients into an anaerobic sludge in a wastewater treatment process, followed by functional and structural analyses of the resultant microbial consortia [118]. In their study, they identified that amendment of molasses improved the community function without any significant impact on the community structure, but glucose amendment destabilized the function and induced a substantial change in the community structure. These approaches move microbial ecological studies forward to the further questions such as how competitive relationships contribute to the properties of a microbial consortia, for example, to resilience and robustness.

***÷ (divide)*:** in natural ecosystems, niche separation is a potent factor to conserve biodiversity [119]. In industrial engineering, compartmentalization of microbial communities can be achieved spatially and temporally, for example, in an aerobic chamber and anaerobic chamber installed into wastewater treatment plants. On a micro-scale, compartmentalization (a "divide") is achieved by forming granule and film [120-122]. During the enrichment cultivations, systematic analyses of granule/film formation will be desirable to clarify the self-organizing ability of a microbial consortia. In addition to the trophic interspecies interactions, tactic behaviors contribute to the spatial organization. Recent studies have reported that interspecies interactions drive cellular motilities [123, 124].

Compared with the natural microbiota composed of over thousands of prokaryotic species, viruses, micro-eukaryotes, and protists, the enriched microbial consortia described here are highly simplified to be understandable, but still highly complex. It is not easy to identify all the members of enriched microbial consortia, but distinctive behaviors of most members and their functional guilds are tractable. Enriched microbial consortia are a sort of complex systems. Further advancements in these studies using high throughput analytical techniques as shown in Fig. (**2**) may hopefully provide clues to build a complex systems theory.

5. GENERAL PERSPECTIVES ON MODEL MICROBIAL CONSORTIA

Advanced analytical techniques including DNA sequencing and mass spectrometry provide deep and wide insights into the profiles of mRNA, protein, and metabolite of microbial cells and microbial communities. We can analyze mixed cultures by considering them as a set of multi-organisms [125, 126]. Nowadays, researchers are attempting to elucidate *in vivo* microbial behaviors in host plants and animals by applying 7-38 species of bacteria to the system [127-129]. The number of molecules and cells that are tractable is increasing every year. In addition, the development of devices for co-cultivation is also proving helpful, for example, glass slide chambers and compartmented microfluidic devises realizing high-throughput cultivation and imaging with high resolution [53, 130]; 3D printing is also now available to make new micro-cultivation devices mimicking complex spatial structures of natural microbial habitats [131]. Of course, isolation techniques of not-yet-cultured microorganisms from natural microbiota are also quite important to reveal their functions [132]. Recently, high-throughput cultiva-

tion approaches, so-called "culturomics" were applied to characterize microbial communities [133].

The emerging reductionistic approaches always update microbial ecology. In this field, bioinformatics act as an indispensable tool to process huge datasets [134]. The integration of biological information and metabolic and thermodynamics flux balance analyses helps promote these researches [40, 135-140]. The biological information should include genome sequence of individuals and cell-cell interactions such as trophic interactions (*e.g.,* cross-feeding and competition), extracellular signaling (*e.g.,* antibiotics, quorum sensing chemicals, and membrane vesicles), and public goods. Mathematical modeling and simulation are crucial to design synthetic ecological experiments [141], as well as to predict the microbial behaviors in microbial ecosystems and to explore ecological theory (Fig. **1**) [117, 142]. In the meanwhile, it has been observed that unpredictable chaotic behavior of population dynamics emerges even in simple microbial ecosystems, such as in three-species mixed culture [143]. Chaotic behaviors may be attributed to subtle changes in conditions that are hard to detect and regulate.

We are currently observing microbial interactions and their assembly on a micro-scale level. We are also aware of microbial mats and granules as semi-microscale microbial world [144]. However, it is actually impossible to define the unit of microbial ecosystems. Recent studies are working on expanding the scale of target ecosystems beyond prokaryotic and micro-eukaryotic worlds, that is, to microecosystems involving protists and viruses [142] and macroecosystems as a part of fauna [145] and flora [146, 147]. Microbial diversity, even in a limited environment seems to be beyond practical calculation, although the genetic diversity can be extrapolated by deep sequencing. Beyond reductionism describing all elements in a system individually, we have to describe the complexity of a system and the dynamics of complexity and its ambiguity. Novel mathematical approaches are required for the next step to overcome the analytical limitations imposed by the integration of systems biology and complex systems science.

CONCLUSION

A variety of ecological theories have long been proposed for macro- and micro-ecosystems. Recent mathematical modeling studies have provided ecological rules and principles (*e.g.,* [84, 148, 149]). If we could obtain the appropriate microbes, synthetic microbial communities are the best tools to test the ecological theory, with the advantages of easy handling and tracking and an appropriate run time. Electricity-driven and photosynthesis-driven artificial microbial communities could provide desirable systems for testing the theory and rules, since the energy supply to the systems is easily regulated by electricity or illumination [150, 151]. Designed microbial communities could be a potential model to experimentally characterize not only ecosystems but also various complex systems such as economy and human society.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Darwin, C. *The Origin of Species*. John Murray: London, UK, **1890**.

[2] Brock, T.D. The study of microorganisms *in situ*: Progress and problems. In: *Symposium of the Society for General Microbiology: Ecology of Microbial Communities;* Fletcher M., Gray T.R.G., Jones J.G., Eds; Cambridge University Press: Cambridge, UK, **1987**, pp. 1-17.

[3] van der Heijden, M.G.A.; Bardgett, R.D.; van Straalen, N.M. The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecol. Lett.,* **2008**, *11*(3), 296-310.

[4] Roesch, L.F.W.; Fulthorpe, R.R.; Riva, A.; Casella, G.; Hadwin, A.K.M.; Kent, A.D.; Daroub, S.H.; Camargo, F.A.O.; Farmerie, W.G.; Triplett, E.W. Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME J.,* **2007**, *1*(4), 283-290.

[5] Torsvik, V.; Øvreås, L. Microbial diversity and function in soil: From genes to ecosystems. *Curr. Opin. Microbiol.,* **2002**, *5*(3), 240-245.

[6] Bernstein, H.C.; McClure, R.S.; Thiel, V.; Sadler, N.C.; Kim, Y.-M.; Chrisler, W.B.; Hill, E.A.; Bryant, D.A.; Romine, M.F.; Jansson, J.K.; Fredrickson, J.K.; Beliaev, A.S. Indirect Interspecies regulation: Transcriptional and physiological responses of a cyanobacterium to heterotrophic partnership. *mSystems,* **2017**, *2*(2), e00181.

[7] Foster, K.R.; Bell, T. Competition, not cooperation, dominates interactions among culturable microbial species. *Curr. Biol.,* **2012**, *22*(19), 1845-1850.

[8] Konopka, A.; Lindemann, S.; Fredrickson, J. Dynamics in microbial communities: Unraveling mechanisms to identify principles. *ISME J.,* **2015**, *9*(7), 1488-1495.

[9] Fredrickson, J.K. Ecological communities by design. *Science,* **2015**, *348*(6242), 1425-1427.

[10] Volmer, J.; Schmid, A.; Bü Hler, B. Guiding bioprocess design by microbial ecology. *Curr. Opin. Microbiol.,* **2015**, *25,* 25-32.

[11] Hill, E.A.; Chrisler, W.B.; Beliaev, A.S.; Bernstein, H.C. A flexible microbial co-culture platform for simultaneous utilization of methane and carbon dioxide from gas feedstocks. *Bioresour. Technol.,* **2017**, *228,* 250-256.

[12] Lindemann, S.R.; Bernstein, H.C.; Song, H.-S.; Fredrickson, J.K.; Fields, M.W.; Shou, W.; Johnson, D.R.; Beliaev, A.S. Engineering microbial consortia for controllable outputs. *ISME J.,* **2016**, *10*(9), 2077-2084.

[13] Bernstein, H.C.; Carlson, R.P. Microbial consortia engineering for cellular factories: *In vitro* to *in silico* systems. *Comput. Struct. Biotechnol. J.,* **2012**, *3,* e201210017.

[14] Dunham, M.J. Synthetic ecology: A model system for cooperation. *Proc. Natl. Acad. Sci. U.S.A.,* **2007**, *104*(6), 1741-1742.

[15] Taffs, R.; Aston, J.E.; Brileya, K.; Jay, Z.; Klatt, C.G.; McGlynn, S.; Mallette, N.; Montross, S.; Gerlach, R.; Inskeep, W.P.; Ward, D.M.; Carlson, R.P. *In silico* approaches to study mass and energy flows in microbial consortia: A syntrophic case study. *BMC Syst. Biol.,* **2009**, *3*(1), 114.

[16] Gauze, G.F. *The Struggle for Existence*. The Williams & Wilkins Company: Baltimore, USA, **1934.**

[17] Hardin, G. The competitive exclusion principle. *Science,* **1960**, *131*(3409), 1292-1297.

[18] Wangersky, P.J. Lotka-Volterra population models. *Annu. Rev. Ecol. Syst.,* **1978**, *9*(1), 189-218.

[19] Chanos, P.; Mygind, T. Co-culture-inducible bacteriocin produc-

tion in lactic acid bacteria. *Appl. Microbiol. Biotechnol.,* **2016**, *100*(10), 4297-4308.

[20] Tong, H.; Chen, W.; Merritt, J.; Qi, F.; Shi, W.; Dong, X. *Streptococcus oligofermentans* inhibits *Streptococcus mutans* through conversion of lactic acid into inhibitory H_2O_2: A possible counter-offensive strategy for interspecies competition. *Mol. Microbiol.,* **2007**, *63*(3), 872-880.

[21] Kell, D.B.; Young, M. Bacterial dormancy and culturability: The role of autocrine growth factors. *Curr. Opin. Microbiol.,* **2000**, *3*(3), 238-243.

[22] Tanaka, Y.; Hanada, S.; Manome, A.; Tsuchida, T.; Kurane, R.; Nakamura, K.; Kamagata, Y. *Catellibacterium nectariphilum* gen. nov., sp. nov., which requires a diffusible compound from a strain related to the genus *Sphingomonas* for vigorous growth. *Int. J. Syst. Evol. Microbiol.,* **2004**, *54*(Pt 3), 955-959.

[23] Ohno, M.; Shiratori, H.; Park, M.J.; Saitoh, Y.; Kumon, Y.; Yamashita, N.; Hirata, A.; Nishida, H.; Ueda, K.; Beppu, T. *Symbiobacterium thermophilum* gen. nov., sp. nov., a symbiotic thermophile that depends on co-culture with a *Bacillus* strain for growth. *Int. J. Syst. Evol. Microbiol.,* **2000**, *50*(Pt 5), 1829-1832.

[24] Aoki, S.K.; Pamma, R.; Hernday, A.D.; Bickham, J.E.; Braaten, B.A.; Low, D.A. Contact-dependent inhibition of growth in *Escherichia coli*. *Science,* **2005**, *309*(5738), 1245-1248.

[25] Pukatzki, S.; Ma, A.T.; Sturtevant, D.; Krastins, B.; Sarracino, D.; Nelson, W.C.; Heidelberg, J.F.; Mekalanos, J.J. Identification of a conserved bacterial protein secretion system in *Vibrio cholerae* using the Dictyostelium host model system. *Proc. Natl. Acad. Sci. U.S.A.,* **2006**, *103*(5), 1528-1533.

[26] Boyer, F.; Fichant, G.; Berthod, J.; Vandenbrouck, Y.; Attree, I. Dissecting the bacterial type VI secretion system by a genome wide *in silico* analysis: what can be learned from available microbial genomic resources? *BMC Genomics,* **2009**, *10,* 104.

[27] Basler, M.; Ho, B.T.; Mekalanos, J.J. Tit-for-tat: Type VI secretion system counterattack during bacterial cell-cell interactions. *Cell,* **2013**, *152*(4), 884-894.

[28] Jani, A.J.; Cotter, P.A. Type VI secretion: Not just for pathogenesis anymore. *Cell Host Microbe,* **2010**, *8*(1), 2-6.

[29] Kato, S.; Kosaka, T.; Watanabe, K. Substrate-dependent transcriptomic shifts in *Pelotomaculum thermopropionicum* grown in syntrophic co-culture with *Methanothermobacter thermautotrophicus*. *Microb. Biotechnol.,* **2009**, *2*(5), 575-584.

[30] Plugge, C.M.; Scholten, J.C.M.; Culley, D.E.; Nie, L.; Brockman, F.J.; Zhang, W. Global transcriptomics analysis of the *Desulfovibrio vulgaris* change from syntrophic growth with *Methanosarcina barkeri* to sulfidogenic metabolism. *Microbiology,* **2010**, *156*(Pt 9), 2746-2756.

[31] Browne, P.D.; Cadillo-Quiroz, H. Contribution of transcriptomics to systems-level understanding of methanogenic Archaea. *Archaea,* **2013**, *2013,* 586369.

[32] Beliaev, A.S.; Romine, M.F.; Serres, M.; Bernstein, H.C.; Linggi, B.E.; Markillie, L.M.; Isern, N.G.; Chrisler, W.B.; Kucek, L.A.; Hill, E.A.; Pinchuk, G.E.; Bryant, D.A.; Steven Wiley, H.; Fredrickson, J.K.; Konopka, A. Inference of interactions in cyanobacterial-heterotrophic co-cultures *via* transcriptome sequencing. *ISME J.,* **2014**, *8*(11), 2243-2255.

[33] Helliwell, K.E.; Pandhal, J.; Cooper, M.B.; Longworth, J.; Kudahl, U.J.; Russo, D.A.; Tomsett, E.V; Bunbury, F.; Salmon, D.L.; Smirnoff, N.; Wright, P.C.; Smith, A.G. Quantitative proteomics of a B_{12}-dependent alga grown in coculture with bacteria reveals metabolic tradeoffs required for mutualism. *New Phytol.,* **2018**, *217*(2), 599-612.

[34] Sedlacek, C.J.; Nielsen, S.; Greis, K.D.; Haffey, W.D.; Revsbech, N.P.; Ticak, T.; Laanbroek, H.J.; Bollmann, A. Effects of bacterial community members on the proteome of the ammonia-oxidizing bacterium *Nitrosomonas* sp. strain Is79. *Appl. Environ. Microbiol.,* **2016**, *82*(15), 4776-4788.

[35] Chen, T.; Zhao, Q.; Wang, L.; Xu, Y.; Wei, W. Comparative metabolomic analysis of the green microalga *Chlorella sorokiniana* cultivated in the single culture and a consortium with bacteria for wastewater remediation. *Appl. Biochem. Biotechnol.,* **2017**, *183*(3), 1062-1075.

[36] Zhou, J.; Ma, Q.; Yi, H.; Wang, L.; Song, H.; Yuan, Y.-J. Metabolome profiling reveals metabolic cooperation between *Bacillus megaterium* and *Ketogulonicigenium vulgare* during induced swarm motility. *Appl. Environ. Microbiol.,* **2011**, *77*(19), 7023-7030.

[37] Zhou, K.; Qiao, K.; Edgar, S.; Stephanopoulos, G. Distributing a metabolic pathway among a microbial consortium enhances production of natural products. *Nat. Biotechnol.,* **2015**, *33*(4), 377-383.

[38] Minty, J.J.; Singer, M.E.; Scholz, S.A.; Bae, C.-H.; Ahn, J.-H.; Foster, C.E.; Liao, J.C.; Lin, X.N. 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), 14592-14597.

[39] Post, D.M.; Palkovacs, E.P. Eco-evolutionary feedbacks in community and ecosystem ecology: Interactions between the ecological theatre and the evolutionary play. *Philos. Trans. R. Soc. B Biol. Sci.,* **2009**, *364*(1523), 1629-1640.

[40] Harcombe, W. Novel cooperation experimentally evolved between species. *Evolution*, **2010**, *64*(7), 2166-2172.

[41] Hillesland, K.L.; Stahl, D.A. Rapid evolution of stability and productivity at the origin of a microbial mutualism. *Proc. Natl. Acad. Sci. U.S.A.,* **2010**, *107*(5), 2124-2129.

[42] Buckling, A.; Brockhurst, M. Bacteria-virus coevolution. *Adv. Exp. Med. Biol.,* **2012**, *751,* 347-370.

[43] Koskella, B.; Brockhurst, M.A. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiol. Rev.,* **2014**, *38*(5), 916-931.

[44] Yoshida, T.; Jones, L.E.; Ellner, S.P.; Fussmann, G.F.; Hairston, N.G. Rapid evolution drives ecological dynamics in a predator-prey system. *Nature,* **2003**, *424*(6946), 303-306.

[45] Tognon, M.; Köhler, T.; Gdaniec, B.G.; Hao, Y.; Lam, J.S.; Beaume, M.; Luscher, A.; Buckling, A.; van Delden, C. Co-evolution with *Staphylococcus aureus* leads to lipopolysaccharide alterations in *Pseudomonas aeruginosa*. *ISME J.,* **2017**, *11*(10), 2233-2243.

[46] Ryan, R.P.; Fouhy, Y.; Garcia, B.F.; Watt, S.A.; Niehaus, K.; Yang, L.; Tolker-Nielsen, T.; Dow, J.M. Interspecies signalling *via* the *Stenotrophomonas maltophilia* diffusible signal factor influences biofilm formation and polymyxin tolerance in *Pseudomonas aeruginosa*. *Mol. Microbiol.,* **2008**, *68*(1), 75-86.

[47] Laganenka, L.; Sourjik, V. Autoinducer 2-dependent *Escherichia coli* biofilm formation is enhanced in a dual-species co-culture. *Appl. Environ. Microbiol.,* **2017**, *84*(5), e02638.

[48] Wong, M.; Liang, X.; Smart, M.; Tang, L.; Moore, R.; Ingalls, B.; Dong, T.G. Microbial herd protection mediated by antagonistic interaction in polymicrobial communities. *Appl. Environ. Microbiol.,* **2016**, *82*(23), 6881-6888.

[49] Wootton, J.T. The nature and consequences of indirect effects in ecological communities. *Annu. Rev. Ecol. Syst.,* **1994**, *25*(1), 443-466.

[50] Margolis, E. Hydrogen peroxide-mediated interference competition by *Streptococcus pneumoniae* has no significant effect on *Staphylococcus aureus* nasal colonization of neonatal rats. *J. Bacteriol.,* **2009**, *191*(2), 571-575.

[51] Wintermute, E.H.; Silver, P.A. Dynamics in the mixed microbial concourse. *Genes Dev.,* **2010**, *24*(23), 2603-2614.

[52] Kim, H.J.; Boedicker, J.Q.; Choi, J.W.; Ismagilov, R.F. Defined spatial structure stabilizes a synthetic multispecies bacterial community. *Proc. Natl. Acad. Sci. U.S.A.,* **2008**, *105*(47), 18188-18193.

[53] Goers, L.; Freemont, P.; Polizzi, K.M. Co-culture systems and technologies: Taking synthetic biology to the next level. *J. R. Soc. Interface,* **2014**, *11*(96), 20140065-20140065.

[54] Hays, S.G.; Patrick, W.G.; Ziesack, M.; Oxman, N.; Silver, P.A.; Betenbaugh, M.J.; Bentley, W.E. Better together: engineering and application of microbial symbioses introduction: The benefits of living together. *Curr. Opin. Biotechnol.,* **2015**, *36,* 40-49.

[55] Henry, C.S.; Bernstein, H.C.; Weisenhorn, P.; Taylor, R.C.; Lee, J.-Y.; Zucker, J.; Song, H.-S. Microbial community metabolic modeling: A community data-driven network reconstruction. *J. Cell. Physiol.,* **2016**, *231*(11), 2339-2345.

[56] Biggs, M.B.; Medlock, G.L.; Kolling, G.L.; Papin, J.A. Metabolic network modeling of microbial communities. *Wiley Interdisc. Rev. Syst. Biol. Med.*, **2015**, *7*(5), 317-334.

[57] Khandelwal, R.A.; Olivier, B.G.; Röling, W.F.M.; Teusink, B.; Bruggeman, F.J. Community flux balance analysis for microbial consortia at balanced growth. *PLoS One,* **2013**, *8*(5), e64567.

[58] Mendes-Soares, H.; Mundy, M.; Soares, L.M.; Chia, N. MMinte: An application for predicting metabolic interactions among the microbial species in a community. *BMC Bioinformatics,* **2016**, *17*(1), 343.

[59] Mee, M.T.; Wang, H.H. Engineering ecosystems and synthetic

ecologies. *Mol. Biosyst.,* **2012**, *8*(10), 2470.
[60] Zomorrodi, A.R.; Maranas, C.D. OptCom: A multi-level optimization framework for the metabolic modeling and analysis of microbial communities. *PLoS Comput. Biol.,* **2012**, *8*(2), e1002363.
[61] Kato, S.; Haruta, S.; Cui, Z.J.; Ishii, M.; Igarashi, Y. Effective cellulose degradation by a mixed-culture system composed of a cellulolytic *Clostridium* and aerobic non-cellulolytic bacteria. *FEMS Microbiol. Ecol.,* **2004**, *51*(1), 133-142.
[62] Kato, S.; Haruta, S.; Cui, Z.J.; Ishii, M.; Igarashi, Y. Stable coexistence of five bacterial strains as a cellulose-degrading community. *Appl. Environ. Microbiol.,* **2005**, *71*(11), 7099-7106.
[63] Kerr, B.; Riley, M.A.; Feldman, M.W.; Bohannan, B.J.M. Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors. *Nature,* **2002**, *418*(6894), 171-174.
[64] Hobley, L.; King, J.R.; Sockett, R.E. *Bdellovibrio* predation in the presence of decoys: Three-way bacterial interactions revealed by mathematical and experimental analyses. *Appl. Environ. Microbiol.,* **2006**, *72*(10), 6757-6765.
[65] Stadie, J.; Gulitz, A.; Ehrmann, M.A.; Vogel, R.F. Metabolic activity and symbiotic interactions of lactic acid bacteria and yeasts isolated from water kefir. *Food Microbiol.,* **2013**, *35*(2), 92-98.
[66] Narisawa, N.; Haruta, S.; Arai, H.; Ishii, M.; Igarashi, Y. Coexistence of Antibiotic-producing and antibiotic-sensitive bacteria in biofilms is mediated by resistant bacteria. *Appl. Environ. Microbiol.,* **2008**, *74*(12), 3887-3894.
[67] Kato, S.; Haruta, S.; Cui, Z.J.; Ishii, M.; Igarashi, Y. Network relationships of bacteria in a stable mixed culture. *Microb. Ecol.,* **2008**, *56*(3), 403-411.
[68] Haruta, S.; Kato, S.; Yamamoto, K.; Igarashi, Y. Intertwined interspecies relationships: Approaches to untangle the microbial network. *Environ. Microbiol.,* **2009**, *11*(12), 2963-2969.
[69] Kelsic, E.D.; Zhao, J.; Vetsigian, K.; Kishony, R. Counteraction of antibiotic production and degradation stabilizes microbial communities. *Nature,* **2015**, *521*(7553), 516-519.
[70] Loreau, M. Linking biodiversity and ecosystems: towards a unifying ecological theory. *Philos. Trans. R Soc. Lond. B Biol. Sci.,* **2010**, *365*(1537), 49-60.
[71] Duffy, J.E.; Godwin, C.M.; Cardinale, B.J. Biodiversity effects in the wild are common and as strong as key drivers of productivity. *Nature,* **2017**, *549*(7671), 261-264.
[72] Hodgson, D.J.; Rainey, P.B.; Buckling, A. Mechanisms linking diversity, productivity and invasibility in experimental bacterial communities. *Proc. R Soc. B Biol. Sci.,* **2002**, *269*(1506), 2277-2283.
[73] Bell, T.; Newman, J.A.; Silverman, B.W.; Turner, S.L.; Lilley, A.K. The contribution of species richness and composition to bacterial services. *Nature,* **2005**, *436*(7054), 1157-1160.
[74] Schmidtke, A.; Gaedke, U.; Weithoff, G. A mechanistic basis for underyielding in phytoplankton communities. *Ecology,* **2010**, *91*(1), 212-221.
[75] Venail, P.A.; Vives, M.J. Phylogenetic distance and species richness interactively affect the productivity of bacterial communities. *Ecology,* **2013**, *94*(11), 2529-2536.
[76] Gravel, D.; Bell, T.; Barbera, C.; Bouvier, T.; Pommier, T.; Venail, P.; Mouquet, N. Experimental niche evolution alters the strength of the diversity-productivity relationship. *Nature,* **2011**, *469*(7328), 89-92.
[77] Li, J.-T.; Duan, H.-N.; Li, S.-P.; Kuang, J.-L.; Zeng, Y.; Shu, W.-S. Cadmium pollution triggers a positive biodiversity-productivity relationship: Evidence from a laboratory microcosm experiment. *J. Appl. Ecol.,* **2010**, *47*(4), 890-898.
[78] Wittebolle, L.; Marzorati, M.; Clement, L.; Balloi, A.; Daffonchio, D.; Heylen, K.; De Vos, P.; Verstraete, W.; Boon, N. Initial community evenness favours functionality under selective stress. *Nature,* **2009**, *458*(7238), 623-626.
[79] Brockhurst, M.A.; Koskella, B. Experimental coevolution of species interactions. *Trends Ecol. Evol.,* **2013**, *28*(6), 367-375.
[80] Baumgartner, M.; Roffler, S.; Wicker, T.; Pernthaler, J. Letting go: Bacterial genome reduction solves the dilemma of adapting to predation mortality in a substrate-restricted environment. *ISME J.,* **2017**, *11*(10), 2258-2266.
[81] Giovannoni, S.J.; Cameron Thrash, J.; Temperton, B. Implications of streamlining theory for microbial ecology. *ISME J.,* **2014**, *8*(8), 1553-1565.
[82] Fiegna, F.; Scheuerl, T.; Moreno-Letelier, A.; Bell, T.; Barraclough, T.G. Saturating effects of species diversity on life-history evolution in bacteria. *Proc. R Soc. B Biol. Sci.,* **2015**, *282*(1815), 20151794.
[83] Morris, J.J.; Lenski, R.E.; Zinser, E.R. The black queen hypothesis: Evolution of dependencies through adaptive gene loss. *mBio,* **2012**, *3*(2), e00036.
[84] Oliveira, N.M.; Niehus, R.; Foster, K.R. Evolutionary limits to cooperation in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.,* **2014**, *111*(50), 17941-17946.
[85] Mas, A.; Jamshidi, S.; Lagadeuc, Y.; Eveillard, D.; Vandenkoornhuyse, P. Beyond the black queen hypothesis. *ISME J.,* **2016**, *10*(9), 2085-2091.
[86] Bachmann, H.; Fischlechner, M.; Rabbers, I.; Barfa, N.; dos Santos, F.B.; Molenaar, D.; Teusink, B. Availability of public goods shapes the evolution of competing metabolic strategies. *Proc. Natl. Acad. Sci. U.S.A.,* **2013**, *110*(35), 14302-14307.
[87] Bachmann, H.; Bruggeman, F.J.; Molenaar, D.; dos Santos, F.B.; Teusink, B. Public goods and metabolic strategies. *Curr. Opin. Microbiol.,* **2016**, *31*, 109-115.
[88] Butaitė, E.; Baumgartner, M.; Wyder, S.; Kümmerli, R. Siderophore cheating and cheating resistance shape competition for iron in soil and freshwater *Pseudomonas* communities. *Nat. Commun.,* **2017**, *8*(1), 414.
[89] Rakoff-Nahoum, S.; Coyne, M.J.; Comstock, L.E. An ecological network of polysaccharide utilization among human intestinal symbionts. *Curr. Biol.,* **2014**, *24*(1), 40-49.
[90] Mee, M.T.; Collins, J.J.; Church, G.M.; Wang, H.H. Syntrophic exchange in synthetic microbial communities. *Proc. Natl. Acad. Sci. U.S.A.,* **2014**, *111*(20), E2149-E2156.
[91] Mougi, A.; Kondoh, M. Diversity of interaction types and ecological community stability. *Science,* **2012**, *337*(6092), 349-351.
[92] Friedman, J.; Higgins, L.M.; Gore, J. Community structure follows simple assembly rules in microbial microcosms. *Nat. Ecol. Evol.,* **2017**, *1*(5), e0109.
[93] Swenson, W.; Wilson, D.S.; Elias, R. Artificial ecosystem selection. *Proc. Natl. Acad. Sci. U.S.A.,* **2000**, *97*(16), 9110-9114.
[94] Pandhal, J.; Noirel, J. Synthetic microbial ecosystems for biotechnology. *Biotechnol. Lett.,* **2014**, *36*(6), 1141-1151.
[95] Sivasubramaniam, D.; Franks, A.E. Bioengineering microbial communities: Their potential to help, hinder and disgust. *Bioengineered,* **2016**, *7*(3), 137-144.
[96] Carvalho, M.F.; Alves, C.C.T.; Ferreira, M.I.M.; De Marco, P.; Castro, P.M.L. Isolation and initial characterization of a bacterial consortium able to mineralize fluorobenzene. *Appl. Environ. Microbiol.,* **2002**, *68*(1), 102-105.
[97] Tashiro, Y.; Matsumoto, H.; Miyamoto, H.; Okugawa, Y.; Pramod, P.; Miyamoto, H.; Sakai, K. A novel production process for optically pure L-lactic acid from kitchen refuse using a bacterial consortium at high temperatures. *Bioresour. Technol.,* **2013**, *146,* 672-681.
[98] Haruta, S.; Cui, Z.; Huang, Z.; Li, M.; Ishii, M.; Igarashi, Y. Construction of a stable microbial community with high cellulose-degrading ability. *Appl. Microbiol. Biotechnol.,* **2002**, *59*(4-5), 529-534.
[99] Smith, D.; Alvey, S.; Crowley, D.E. Cooperative catabolic pathways within an atrazine-degrading enrichment culture isolated from soil. *FEMS Microbiol. Ecol.,* **2005**, *53*(2), 265-275.
[100] Wang, W.; Yan, L.; Cui, Z.; Gao, Y.; Wang, Y.; Jing, R. Characterization of a microbial consortium capable of degrading lignocellulose. *Bioresour. Technol.,* **2011**, *102*(19), 9321-9324.
[101] Wang, X.; Haruta, S.; Wang, P.; Ishii, M.; Igarashi, Y.; Cui, Z. Diversity of a stable enrichment culture which is useful for silage inoculant and its succession in alfalfa silage. *FEMS Microbiol. Ecol.,* **2006**, *57*(1), 106-115.
[102] Ozaki, S.; Kishimoto, N.; Fujita, T. Isolation and phylogenetic characterization of microbial consortia able to degrade aromatic hydrocarbons at high rates. *Microbes Environ.,* **2006**, *21*(1), 44-52.
[103] Swenson, W.; Arendt, J.; Wilson, D.S. Artificial selection of microbial ecosystems for 3-chloroaniline biodegradation. *Environ. Microbiol.,* **2000**, *2*(5), 564-571.
[104] Williams, H.T.P.; Lenton, T.M. Artificial selection of simulated microbial ecosystems. *Proc. Natl. Acad. Sci. U.S.A.,* **2007**, *104*(21), 8918-8923.
[105] Garcia, S.L.; Mcmahon, K.D.; Grossart, H.P.; Warnecke, F. Successful enrichment of the ubiquitous freshwater acI *Actinobacteria*. *Environ. Microbiol. Rep.,* **2014**, *6*(1), 21-27.
[106] Mahana, D.; Trent, C.M.; Kurtz, Z.D.; Bokulich, N.A.; Battaglia,

T.; Chung, J.; Müller, C.L.; Li, H.; Bonneau, R.A.; Blaser, M.J. Antibiotic perturbation of the murine gut microbiome enhances the adiposity, insulin resistance, and liver disease associated with high-fat diet. *Genome Med.,* **2016**, *8*(1), 48.

[107] Yang, G.; Peng, M.; Tian, X.; Dong, S. Molecular ecological network analysis reveals the effects of probiotics and florfenicol on intestinal microbiota homeostasis: An example of sea cucumber. *Sci. Rep.,* **2017**, *7*(1), 4778.

[108] Vogel, T.M. Bioaugmentation as a soil bioremediation approach. *Curr. Opin. Biotechnol.,* **1996**, *7*(3), 311-316.

[109] Pham, H.L.; Ho, C.L.; Wong, A.; Lee, Y.S.; Chang, M.W. Applying the design-build-test paradigm in microbiome engineering. *Curr. Opin. Biotechnol.,* **2017**, *48,* 85-93.

[110] Hardoim, P.R.; Overbeek, L.S. van; Berg, G.; Pirttilä, A.M.; Compant, S.; Campisano, A.; Döring, M.; Sessitsch, A. The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.,* **2015**, *79*(3), 293-320.

[111] Boon, N.; Goris, J.; Vos, P. De; Verstraete, W.; Top, E.M. Bioaugmentation of activated sludge by an indigenous 3-chloroaniline-degrading *Comamonas testosteroni* strain, I2gfp. *Appl. Environ. Microbiol.,* **2000**, *66*(7), 2906-2913.

[112] Laurinavichene, T.V.; Laurinavichius, K.S.; Tsygankov, A.A. Integration of purple non-sulfur bacteria into the starch-hydrolyzing consortium. *Int. J. Hydrogen Energy,* **2014**, *39*(15), 7713-7720.

[113] Narisawa, N.; Haruta, S.; Cui, Z.J.; Ishii, M.; Igarashi, Y. Effect of adding cellulolytic bacterium on stable cellulose-degrading microbial community. *J. Biosci. Bioeng.,* **2007**, *104*(5), 432-434.

[114] Adam, E.; Groenenboom, A.E.; Kurm, V.; Rajewska, M.; Schmidt, R.; Tyc, O.; Weidner, S.; Berg, G.; de Boer, W.; Falcão Salles, J. Controlling the microbiome: Microhabitat Adjustments for successful biocontrol strategies in soil and human gut. *Front. Microbiol.,* **2016**, *7*, 1079.

[115] Kinnunen, M.; Dechesne, A.; Proctor, C.; Hammes, F.; Johnson, D.; Quintela-Baluja, M.; Graham, D.; Daffonchio, D.; Fodelianakis, S.; Hahn, N.; Boon, N.; Smets, B.F. A conceptual framework for invasion in microbial communities. *ISME J.,* **2016**, *10*(12), 2773-2775.

[116] Hunt, D.E.; Ward, C.S. A network-based approach to disturbance transmission through microbial interactions. *Front. Microbiol.,* **2015**, *6*, 1182.

[117] Haruta, S.; Saito, Y.; Futamata. Editorial: Development of microbial ecological theory: stability, plasticity, and evolution of microbial ecosystems. *Front. Microbiol.,* **2016**, *7*, 2069.

[118] Liu, M.; Wang, S.; Nobu, M.K.; Bocher, B.T.W.; Kaley, S.A.; Liu, W.-T. Impacts of biostimulation and bioaugmentation on the performance and microbial ecology in methanogenic reactors treating purified terephthalic acid wastewater. *Water Res.,* **2017**, *122*, 308-316.

[119] Ward, D.M.; Ferris, M.J.; Nold, S.C.; Bateson, M.M. A natural view of microbial biodiversity within hot spring cyanobacterial mat communities. *Microbiol. Mol. Biol. Rev.,* **1998**, *62*(4), 1353-1370.

[120] Sekiguchi, Y.; Kamagata, Y.; Nakamura, K.; Ohashi, A.; Harada, H. Fluorescence *in situ* hybridization using 16S rRNA-targeted oligonucleotides reveals localization of methanogens and selected uncultured bacteria in mesophilic and thermophilic sludge granules. *Appl. Environ. Microbiol.,* **1999**, *65*(3), 1280-1288.

[121] Bagchi, S.; Lamendella, R.; Strutt, S.; Loosdrecht, M.C.M. Van; Saikaly, P.E. Metatranscriptomics reveals the molecular mechanism of large granule formation in granular anammox reactor. *Sci. Rep.,* **2016**, *6*(1), 28327.

[122] Tan, C.H.; Lee, K.W.K.; Burmlle, M.; Kjelleberg, S.; Rice, S.A. All together now: Experimental multispecies biofilm model systems. *Environ. Microbiol.,* **2017**, *19*(1), 42-53.

[123] Morohoshi, S.; Matsuura, K.; Haruta, S. Secreted protease mediates interspecies interaction and promotes cell aggregation of the photosynthetic bacterium *Chloroflexus aggregans*. *FEMS Microbiol. Lett.,* **2015**, *362*(3), 1-5.

[124] Müller, S.; Strack, S.N.; Ryan, S.E.; Kearns, D.B.; Kirby, J.R. Predation by *Myxococcus xanthus* induces *Bacillus subtilis* to form spore-filled megastructures. *Appl. Environ. Microbiol.,* **2015**, *81*(1), 203-210.

[125] Boon, E.; Meehan, C.J.; Whidden, C.; Wong, D.H.-J.; Langille, M.G.I.; Beiko, R.G. Interactions in the microbiome: Communities of organisms and communities of genes. *FEMS Microbiol. Rev.,* **2014**, *38*(1), 90-118.

[126] Klitgord, N.; Segrè, D. Ecosystems biology of microbial metabolism. *Curr. Opin. Biotechnol.,* **2011**, *22*(4), 541-546.

[127] Faith, J.J.; McNulty, N.P.; Rey, F.E.; Gordon, J.I. Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science,* **2011**, *333*(6038), 101-104.

[128] Lebeis, S.L.; Paredes, S.H.; Lundberg, D.S.; Breakfield, N.; Gehring, J.; McDonald, M.; Malfatti, S.; Glavina del Rio, T.; Jones, C.D.; Tringe, S.G.; Dangl, J.L. Plant Microbiome. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science,* **2015**, *349*(6250), 860-864.

[129] Niu, B.; Paulson, J.N.; Zheng, X.; Kolter, R. Simplified and representative bacterial community of maize roots. *Proc. Natl. Acad. Sci. U.S.A.,* **2017**, *114*(12), E2450-E2459.

[130] Hol, F.J.H.; Dekker, C. Zooming in to see the bigger picture: Microfluidic and nanofabrication tools to study bacteria. *Science,* **2014**, *346*(6208), 1251821-1251821.

[131] Connell, J.L.; Ritschdorff, E.T.; Whiteley, M.; Shear, J.B. 3D printing of microscopic bacterial communities. *Proc. Natl. Acad. Sci. U.S.A.,* **2013**, *110*(46), 18380-18385.

[132] Dewi Puspita, I.; Kamagata, Y.; Tanaka, M.; Asano, K.; Nakatsu, C.H. Are uncultivated bacteria really uncultivable? *Microbes Environ.,* **2012**, *27*(4), 356-366.

[133] Kaspar, U.; Kriegeskorte, A.; Schubert, T.; Peters, G.; Rudack, C.; Pieper, D.H.; Wos-Oxley, M.; Becker, K. The culturome of the human nose habitats reveals individual bacterial fingerprint patterns. *Environ. Microbiol.,* **2016**, *18*(7), 2130-2142.

[134] Hahn, A.S.; Konwar, K.M.; Louca, S.; Hanson, N.W.; Hallam, S.J. The information science of microbial ecology. *Curr. Opin. Microbiol.,* **2016**, *31,* 209-216.

[135] Embree, M.; Liu, J.K.; Al-Bassam, M.M.; Zengler, K. Networks of energetic and metabolic interactions define dynamics in microbial communities. *Proc. Natl. Acad. Sci. U.S.A.,* **2015**, *112*(50), 15450-15455.

[136] Henson, M.A.; Hanly, T.J. Dynamic flux balance analysis for synthetic microbial communities. *IET Syst. Biol.,* **2014**, *8*(5), 214-229.

[137] Mahadevan, R.; Henson, M.A. Genome-based modeling and design of metabolic interactions in microbial communities. *Comput. Struct. Biotechnol. J.,* **2012**, *3*(4), e201210008.

[138] Großkopf, T.; Soyer, O.S. Microbial diversity arising from thermodynamic constraints. *ISME J.,* **2016**, *10*(11), 2725-2733.

[139] Magnúsdóttir, S.; Heinken, A.; Kutt, L.; Ravcheev, D.A.; Bauer, E.; Noronha, A.; Greenhalgh, K.; Jäger, C.; Baginska, J.; Wilmes, P.; Fleming, R.M.T.; Thiele, I. Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota. *Nat. Biotechnol.,* **2017**, *35*(1), 81-89.

[140] Perez-Garcia, O.; Lear, G.; Singhal, N. Metabolic network modeling of microbial interactions in natural and engineered environmental systems. *Front. Microbiol.,* **2016**, *7,* 673.

[141] Zampieri, M.; Sauer, U. Model-based media selection to minimize the cost of metabolic cooperation in microbial ecosystems. *Bioinformatics,* **2016**, *32*(11), 1733-1739.

[142] Haruta, S. Rediscovery of the microbial world in microbial ecology. *Microbes Environ.,* **2013**, *28*(3), 281-284.

[143] Becks, L.; Hilker, F.M.; Malchow, H.; Jürgens, K.; Arndt, H. Experimental demonstration of chaos in a microbial food web. *Nature,* **2005**, *435*(7046), 1226-1229.

[144] Cordero, O.X.; Datta, M.S. Microbial interactions and community assembly at microscales. *Curr. Opin. Microbiol.,* **2016**, *31,* 227-234.

[145] McLean, A.H.C.; Parker, B.J.; Hrček, J.; Henry, L.M.; Godfray, H.C.J. Insect symbionts in food webs. *Philos. Trans. R Soc. B Biol. Sci.,* **2016**, *371*(1702), 20150325.

[146] Thijs, S.; Sillen, W.; Rineau, F.; Weyens, N.; Vangronsveld, J. Towards an enhanced understanding of plant-microbiome interactions to improve phytoremediation: Engineering the metaorganism. *Front. Microbiol.,* **2016**, *7,* 341.

[147] Toju, H.; Guimarães, P.R.; Olesen, J.M.; Thompson, J.N. Assembly of complex plant-fungus networks. *Nat. Commun.,* **2014**, *5*, 5273.

[148] Estrela, S.; Morris, J.J.; Kerr, B. Private benefits and metabolic conflicts shape the emergence of microbial interdependencies. *Environ. Microbiol.,* **2016**, *18*(5), 1415-1427.

[149] Zelezniak, A.; Andrejev, S.; Ponomarova, O.; Mende, D.R.; Bork, P.; Patil, K.R. Metabolic dependencies drive species co-occurrence in diverse microbial communities. *Proc. Natl. Acad. Sci. U.S.A.,*

2015, *112*(20), 6449-6454.

[150] Rabaey, K.; Rodríguez, J.; Blackall, L.L.; Keller, J.; Gross, P.; Batstone, D.; Verstraete, W.; Nealson, K.H. Microbial ecology meets electrochemistry: Electricity-driven and driving communities. *ISME J.,* **2007**, *1*(1), 9-18.

[151] Hays, S.G.; Yan, L.L.W.; Silver, P.A.; Ducat, D.C. Synthetic photosynthetic consortia define interactions leading to robustness and photoproduction. *J. Biol. Eng.,* **2017**, *11*(1), 4.