



# Metabolic interactions in microbial communities: untangling the Gordian knot

Olga Ponomarova and Kiran Raosaheb Patil

Metabolic exchanges are ubiquitous in microbial communities. However, detecting metabolite cross-feedings is difficult due to their intrinsically dynamic nature and the complexity of communities. Thus, while exhaustive description of metabolic networks operating in natural systems is a task for the future, the battle of today is divided between detailed characterizations of small, reduced complexity microbial consortia, and focusing on particular metabolic aspects of natural ecosystems. Detecting metabolic interactions requires methodological blend able to capture species identity, dependencies and the nature of exchanged metabolites. Multiple combinations of diverse techniques, from metagenomics to imaging mass spectrometry, offer solutions to this challenge, each combination being tailored to the community at hand.

## Address

Structural and Computational Biology Unit, European Molecular Biology Laboratory, Heidelberg, Germany

Corresponding author: Patil, Kiran Raosaheb ([patil@embl.de](mailto:patil@embl.de))

**Current Opinion in Microbiology** 2015, **27**:37–44

This review comes from a themed issue on **Microbial systems biology**

Edited by **Eric D Brown** and **Athanasios Typas**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 24th July 2015

<http://dx.doi.org/10.1016/j.mib.2015.06.014>

1369-5274/© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

## Introduction

Microbial communities are intertwined by metabolic links, whether viewed as narrowly as a pair of symbionts, or as broadly as the earth-wide ecosystem lined up with trophic chains. Understanding metabolic interactions at the global level is thus indispensable in microbial ecology and evolution. However, shifting attention from isolated metabolism of pure cultures to that of microbial communities is challenging and requires new tools and methods. And, as in the case of any complex network, when choosing a focus point in the large web of metabolic interactions, we have to compromise between resolution of detail and coverage.

Seeing microbial metabolism in the community context (as opposed to pure cultures) reveals new phenotypes [1<sup>\*</sup>], helps designing synthetic communities for biotechnology [2,3],

and enables cultivating the ‘uncultivables’ [4]. Accumulating examples of metabolic cross-feeding [5,6<sup>\*</sup>] and evidence from metabolic modeling [7<sup>\*</sup>] create an anticipation of many more to be discovered. Within the broad range of metabolic interactions, here we concentrate primarily on nutrient exchange. We aim to show how studying complex communities is shifting the paradigm of microbial metabolism and what methods and challenges await for those trying to disentangle inter-species connections.

## Metabolite exchanges provide group advantage

Multiple studies show that metabolite exchanges form a strategy for group success [6<sup>\*</sup>,8–11]. Metabolic interactions frequently contribute, through division of labor, to the emergent abilities at community level, such as biodegradation [12,13], faster growth [10] or increased virulence [9,14]. Outsourcing metabolic functions to fellow members embeds each pathway in a specialized micro-environment, hence avoiding biochemical conflict [15]. Moreover, under nutrient-poor conditions species can be readily prompted to share metabolites and thus complement each other’s biosynthetic capabilities [16,17<sup>\*\*</sup>,18]. Metabolic specialization can be found even within the same species, for example, filamentous cyanobacteria with specialized heterocyst cells for nitrogen fixation [19].

Despite benefits associated with cross-feeding, its evolution remains controversial, especially in case of metabolic cooperation [20,21]. Emergence and maintenance of metabolic exchanges depends on particular circumstances, such as spatial structure of microbial community, nutrient availability, diffusion constraints and cost effectiveness of concerned biosynthetic processes [22–24]. For example, aggregating or forming a biofilm maximizes efficiency of nutrient transfer and stimulates otherwise thermodynamically unfavorable metabolic processes [25]. In extreme cases, metabolic dependency results in endosymbiotic relationship, a popular solution for hydrogen-producing ciliates that harbor methanogenic archaea for H<sub>2</sub> outflow [26].

## Microbial metabolism is plastic and responsive to social cues

Microorganisms can often utilize and secrete a large number of metabolites [27,28]. This plastic network is readily adapted and regulated in response to nutrients, for example, to optimize resource allocation [29,30], but also in response to cues from other microorganisms [31<sup>\*</sup>]. Certain bacterial species can modulate yeast metabolism,

to reduce secretion of toxic ethanol, by deploying chemical signaling [1<sup>•</sup>]. Transcriptional response of *Streptococcus* species shows metabolic adaptations to other members of community [32].

## Discovering metabolic interactions

### Meta-omics analyses guide interaction discovery

Meta-omics technologies are culture independent and scalable in space/time. Metagenomics is a particularly powerful tool for discerning species identity and for detecting patterns of interspecies associations. These in turn can generate verifiable hypotheses about metabolic (and other) interactions between community members. Genotyping of associated microbes can reveal their functional palettes [33] and task distribution among community members [12]. For example, individual genomes of a co-aggregated pair of archaea showed that one of the symbionts is dependent on another for lipid, cofactor, amino acid, and nucleotide biosynthesis [34]. Following a specific community over time can also reveal metabolic dependencies as one species dynamically responds to change in abundance of the other, as shown in an activated sludge community [35]. Overlaying taxonomic data with other information, such as spatial distribution and geochemical profiles [36] or specific enzymatic function [37], can deepen insight into community co-metabolism. Beyond individual communities, metagenomics has allowed the identification of species co-occurrence structure across different habitats/samples [38,39] — associations that hint at interspecies interactions [7<sup>•</sup>,40].

Transcriptomics and proteomics are commonly used to complement metagenomics, to deduce what genome encoded metabolic potential is being used [41<sup>••</sup>,42]. For instance, analysis of transcriptional patterns in co-culture of a marine bacterium and a diatom, as well as ocean samples, pinpointed cross-feeding of 2,3-dihydroxypropane-1-sulfonate, a new link in marine microbial food web [41<sup>••</sup>]. Metabolic applications of meta-proteomics are more commonly used for relatively simple systems — it was used to demonstrate metabolic adjustments made by three species comprising a model oral biofilm [43] or to show how the presence/absence of *Aggregatibacter actinomycetemcomitans* modulates metabolism of other bacteria in a 10-species biofilm [44]. Although not distinguishing between species, these results give a sense of the complexity and scale of metabolic adjustments that happen in ‘real-world’ communities. On a larger scale, meta-proteomics, in combination with meta-genomics, allowed proposing differential flow of nitrogen, sulfur and hydrogen among the abundant taxa of marine microbial communities in response to oxygen availability [45].

### Isotope labeling for tracing community-scale pathways

Tracing of isotope labeled substrates, a standard approach in pathway discovery, can also be adapted to reveal flow of metabolites in microbial consortia. Although this is the

most conclusive method for showing metabolite exchange, the major challenge is to distinguish labeling fingerprints of different populations. To do so, one can use an artificially expressed reporter protein [46], species-specific peptides [47], or detect labeled DNA or RNA in conjunction with metagenomics analysis [48]. To give some examples, <sup>13</sup>C labeling served to experimentally prove bacterial feeding on fungal exudates [49], to suggest a chain of toluene degraders in methanogenic enrichment culture [13] and to identify key naphthalene-degrading bacteria *in situ* [50].

### Imaging community structure — clues from the neighbors

Efficient mass transfer between organisms is a prerequisite of successful metabolic interaction, therefore it is not uncommon for microbial partners to form tight aggregates and develop special structures that facilitate metabolite exchange. Microscopic detection of these structures can be a powerful tool in identifying interacting microorganisms. Illustrative is an example of nanotubes formed by cross-feeding *Escherichia coli* auxotrophs [51] or variety of formations in acid mine drainage community, such as cytoplasmic bridges, pili, and ‘synaps like connections’ [52].

Fluorescence *in situ* hybridization (FISH) based methods reveal spatial distribution of interacting partners, for instance showing stratification and co-aggregation patterns in biofilms [53] or bacterial groups attached to phytoplankton host [54]. In addition to resolving spatial structure, imaging, for example, based on fluorescent dyes, can be used to assess general metabolic state of community members [55,56<sup>•</sup>].

### Exploration using metabolomics

Mass spectrometry (MS) based methods can detect a broad spectrum of compounds and are being developed rapidly. This technique has a wide range of modifications, varying in application from a single cell to multiple colonies on a petri dish (reviewed by Watrous *et al.* [57]). Interestingly, MS can be used in an imaging set-up to study metabolic interactions [58]. The potential of imaging-MS unfolded, for example, in a study of chemical interactions on actinomycete bacteria, showing interactions through spectra of secondary metabolites [59<sup>•</sup>]. Application of MS to microbial interactions is, however, currently limited by various challenges in data analysis and compound identification [1<sup>•</sup>,59<sup>•</sup>,60,61]. Other methods that can facilitate interrogation of metabolic space of the community are reviewed by Maurice *et al.* [56<sup>•</sup>] and Wessel *et al.* [62<sup>•</sup>].

Metabolomics alone usually does not provide sufficient resolution to pinpoint exchanged molecules. Elucidating cross-feeding in a complex nutritional environment is possible only in combination with other techniques such

as stable isotope labeling and FISH. Such methodological blend allowed detecting nitrogen transfer from cyanobacteria to their symbiotic diatoms [63] or from methane-oxidizing archaea to sulfate-reducing bacteria in marine seeps [64,65]. Several examples of metabolic interactions detected through combination of different methods are described in Table 1.

### Synthetic communities as model systems

While natural consortia are still difficult to scrutinize, enrichment cultures offer a compromise between natural and synthetic communities. These are cultures obtained from natural samples by promoting growth of organisms of interest, typically by manipulating medium composition. Synthetic microbial communities provide further reduction in the complexity, creating a more tractable system for discovering metabolic exchanges [66,67]. Communities constructed with the isolates from the same environment maximize resemblance to the natural community and preserve indigenous interactions shaped by co-adaptation/evolution [68].

The pre-requisite for common history of member species can be relaxed when addressing fundamental questions like emergence and evolution of metabolic interactions [17\*,69]. To this end, one might also turn to engineered dependencies through genetic manipulation and/or laboratory evolution [16,18,70\*,71]. Despite being less ‘natural’, engineered interactions have the obvious advantage of knowing the identity of the transmitted metabolite (or at least of the involved pathways), as well as being easier to obtain, monitor, and control. Engineered communities are most common object to study synergistic growth effects of metabolic cross-feeding [10,70\*].

Another group of model systems for microbial interactions emerge from microbiota of fermented food [72]. These associations typically have reduced complexity compared with most environmental or host-associated systems, and can be grown in well controlled environment without loss of tractability, for example, cheese rinds [73]. Spatial organization, species succession, stability, resilience, and co-evolution history, such as those of water and milk kefir grains [74,75], create a rich ground in the search for metabolic interaction mechanisms.

### Divide and conquer through temporal/spatial compartmentalization

Using a defined assembly of microorganisms opens opportunities to employ methods inapplicable to complex systems. For example, species quantification can be done with selective plating, quantitative PCR or flow cytometry. However, for better control over metabolite production and consumption, as well as for discerning metabolic roles of different populations, modification to mixed cultures can be made. One of the simplest techniques is based on the cell-free culture filtrate — the so-called

conditioned or spent medium. This approach is frequently used to assay activity of secretome of the donor microorganism(s) by adding its conditioned medium to the recipient culture. This allows identifying non-induced dependencies such as an interaction network between seven gut symbionts knitted by polysaccharide degradation products [76].

Other approaches try to preserve real time molecule diffusion between species, but keep symbionts physically separated, for example, by means of a semi-permeable membrane [68], structuring their microenvironment in a microfluidics device [77], encapsulating cells in hydrogels [78], or co-culturing in a Petri dish [79]. Artificial barriers provide better control over conditions and more convenient quantification, separation and analysis of interacting populations, also in a high-throughput manner [80]. It is important to note that the co-culture conditions can have a profound impact on community metabolism [81] and hence caution is warranted when extrapolating the conclusions to other contexts.

### *In silico* hypothesis generation

Mathematical models of community metabolism are expanding the toolbox for discovering metabolic dependencies in microbial communities [82–84]. Although still in development, community models hold a distinct appeal due to broad applicability and scalability to the ecosystem level [85]. For identifying potential exchanged metabolites, steady-state models are of particular interest as these can be applied with as little information as the identity of community members and their genome sequences [7\*]. These can be further extended, albeit for small communities and with additional information on the metabolic physiology of the community and its members, to address more complex problems such as community dynamics [86].

Community models so far have been largely devoted to understanding general principles of community structure [24,83,87–89], but also have accurately captured experimentally observed metabolic dependencies [7\*,18,89]. The next frontier for the models will be to provide hypotheses verifiable with the experimental approaches discussed above. In particular, models hold a great potential to suggest cross-feeding scenarios and thus to narrow down the set of metabolites to be tested.

### Untangling the Gordian knot

Enumerating metabolic exchanges, being difficult even for small communities, becomes overwhelming for natural communities with hundreds of species living in fluctuating environment. One of the main underlying reasons for this difficulty is that metabolites cannot be directly attributed to a particular species or abiotic source. Furthermore, a large fraction of microbial diversity still remains largely undiscovered or uncharacterized for their

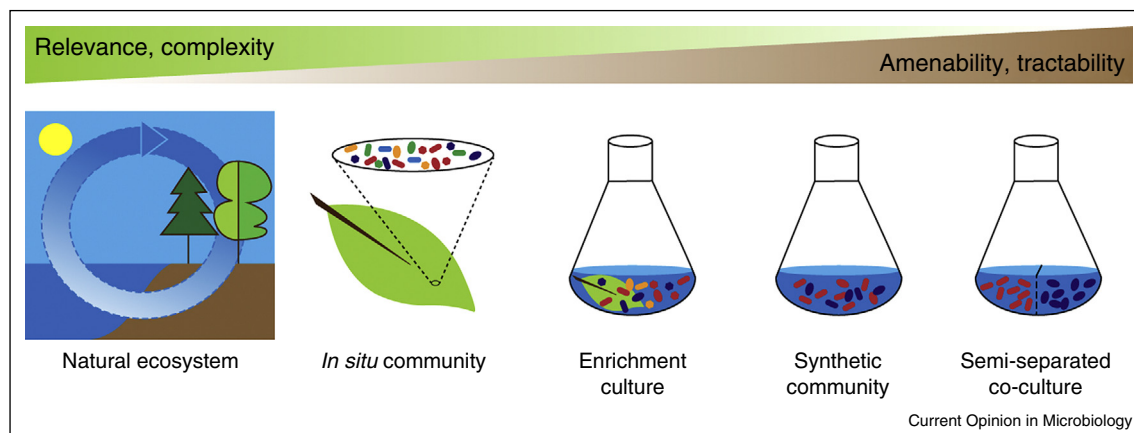
Table 1

## Examples of metabolic exchanges in microbial communities

Community type	(Eco-) system	Interacting taxa	(Potentially) exchanged metabolite(s)	Methods used to detect/infer			Reference
				Species identity	Inter-species dependency	Exchanged metabolite(s)	
Natural	Anoxic marine sediments	<i>Thioploca</i> (sulphur-oxidizing bacteria), anaerobic ammonium-oxidizing bacteria	NH <sub>4</sub> , NO <sub>2</sub>	FISH <sup>a</sup> , 16S rDNA sequencing	FISH analysis of spatial association	Inference from N isotope distribution	[90*]
Natural	Deep-sea sediments	ANME-2 archaea group (anaerobic methane-oxidizing archaea), <i>Desulfosarcina</i> / <i>Desulfococcus</i> (sulfate-reducing bacteria)	Reduced N species	FISH	Observed co-aggregation, previously described syntrophic relationship	FISH-coupled nanoSIMS <sup>c</sup> showing <sup>15</sup> N incorporation across aggregates	[64]
Natural	Ocean plankton	<i>Thalassiosira pseudonana</i> (diatom), <i>Roseobacter</i> clade bacteria	2,3-Dihydroxypropane-1-sulfonate (DHPS)	Metatranscriptome analysis, fractionation of marine biomass	Metabolic exchanges in model bacterial-phytoplankton system	Metatranscriptome analysis, targeted MS metabolomics of the eukaryotic plankton size fraction	[41**]
Enrichment culture <sup>b</sup>	Alkane-degrading methanogenic community	<i>Smithella</i> (bacteria); <i>Methanosaeta</i> and <i>Methanocalculus</i> (methanogenic archaea)	Acetate, electrons	Single-cell genome sequencing, community 16S rDNA analysis	Substrate dependent changes in community composition, <i>a priori</i> knowledge	Analysis of genome sequence and community metatranscriptome	[42]
Enrichment culture	Anaerobic terephthalate-degrading consortium	<i>Pelotomaculum</i> (anaerobic bacteria), <i>Methanosaeta</i> and <i>Methanolinea</i> (hyper-mesophilic methanogens)	CO <sub>2</sub> , H <sub>2</sub> , acetate	16S rDNA profiling, shotgun sequencing	FISH analysis of spatial association, <i>a priori</i> knowledge	Metagenome analysis, thermodynamic considerations	[12]
Synthetic	Isolates from a cellulose-degrading community	<i>Pseudoxanthomonas</i> , <i>Brevibacillus</i> , <i>Clostridium</i>	Acetate, ethanol, saccharides	Known; assessed by real-time PCR	Mixed culture dynamics, conditioned medium experiments	Targeted quantification of cellulose and cellulose degradation products	[91,92]
Synthetic	Isolates from water kefir	<i>Z. florentina</i> , <i>S. cerevisiae</i> , <i>L. hordei</i> , <i>L. nagelii</i>	Amino acids, vitamin B6, unknown factors	Known	Co-culture in transwell plates	Single component exclusion, growth in pairwise cultures	[68]
Synthetic	Human intestinal symbionts	<i>Bacteroides caccae</i> , <i>B. fragilis</i> , <i>B. ovatus</i> , <i>B. thetaiotaomicron</i> , <i>B. uniformis</i> , <i>B. vulgatus</i> , <i>Parabacteroides distasonis</i>	Polysaccharide degradation products (fructose, glucose, among others)	Known	Analysis of species growth in defined media, conditioned media and co-cultures	Assessment of carbohydrate breakdown products released by donors and consumed by recipients	[76]

<sup>a</sup> Fluorescence *in situ* hybridization.<sup>b</sup> Culture obtained from natural sample by promoting growth of organisms of interest, typically by manipulating medium composition.<sup>c</sup> Nanoscale secondary ion mass spectrometry.

Figure 1



Spectrum of microbial community study-systems directed by trade-off between complexity and tractability. Microbial interactions play a central role in biogeochemical cycles in numerous ecosystems, yet are difficult to investigate in molecular detail. In contrast, synthetic communities allow a controlled environment and ease of interpretation. Each study-system in this spectrum offers a choice of resolution to view microbial interactions.

metabolic needs and biosynthetic capabilities. These composite problems necessitate a trade-off between resolution and coverage (Figure 1).

An attractive means to achieve increased resolution of metabolic dependencies is through constructing a smaller manageable model system or by focusing on a particular interaction within a large network. On the other end of the spectrum, one can cover a large system by grouping individual players into higher-order units — guilds (e.g. methanotrophs, sulfur-reducers) and/or metabolite classes (e.g. electron equivalents, fixed nitrogen). Balancing between these two strategies can loosen the tangle and help tracing the main threads in the metabolic knot.

## Acknowledgements

We thank L. Rubinat Ripoll and S. Sheridan for fruitful discussions.

## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Jarosz DF, Brown JC, Walker GA, Datta MS, Ung WL, Lancaster AK, Rotem A, Chang A, Newby GA, Weitz DA *et al.*: **Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism.** *Cell* 2014, **158**:1083-1093.

Discovery of heritable modifications in yeast metabolism caused by chemical signal from bacteria. This interaction positively affects bacteria by reducing amount of ethanol produced by yeast and improves yeast growth on mixed carbon sources.

2. 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**:377-383.

3. Santala S, Karp M, Santala V: **Rationally engineered synthetic coculture for improved biomass and product formation.** *PLoS One* 2014, **9**:e113786.

4. Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, Mueller A, Schaberle TF, Hughes DE, Epstein S *et al.*: **A new antibiotic kills pathogens without detectable resistance.** *Nature* 2015, **517**:455-459.

5. Seth EC, Taga ME: **Nutrient cross-feeding in the microbial world.** *Front Microbiol* 2014, **5**:350.

6. Morris BE, Henneberger R, Huber H, Moissl-Eichinger C: **Microbial syntrophy: interaction for the common good.** *FEMS Microbiol Rev* 2013, **37**:384-406.

A comprehensive review on syntrophic interactions. Many examples of syntrophy are presented, including those between unculturable symbionts. Methods for analysing such interactions are also discussed.

7. Zelezniak A, Andrejev S, Ponomarova O, Mende DR, Bork P, Patil KR: **Metabolic dependencies drive species co-occurrence in diverse microbial communities.** *Proc Natl Acad Sci U S A* 2015, **112**:6449-6454.

Reports a novel modeling approach, SMETANA, which maps all possible interspecies metabolic exchanges in a community. It provides an unbiased estimate of the metabolic interaction potential as well as identifies likely exchanged metabolites.

8. Lawrence D, Fiegna F, Behrends V, Bundy JG, Phillimore AB, Bell T, Barraclough TG: **Species interactions alter evolutionary responses to a novel environment.** *PLoS Biol* 2012, **10**:e1001330.

9. McNally L, Viana M, Brown SP: **Cooperative secretions facilitate host range expansion in bacteria.** *Nat Commun* 2014, **5**:4594.

10. Pande S, Merker H, Bohl K, Reichelt M, Schuster S, de Figueiredo LF, Kaleta C, Kost C: **Fitness and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria.** *ISME J* 2014, **8**:953-962.

11. Ren D, Madsen JS, Sorensen SJ, Burmolle M: **High prevalence of biofilm synergy among bacterial soil isolates in cocultures indicates bacterial interspecific cooperation.** *ISME J* 2015, **9**:81-89.

12. Lykidis A, Chen CL, Tringe SG, McHardy AC, Copeland A, Kyrpides NC, Hugenholtz P, Macarie H, Olmos A, Monroy O *et al.*: **Multiple syntrophic interactions in a terephthalate-degrading methanogenic consortium.** *ISME J* 2011, **5**:122-130.

13. Fowler SJ, Gutierrez-Zamora ML, Manefield M, Gieg LM: **Identification of toluene degraders in a methanogenic enrichment culture.** *FEMS Microbiol Ecol* 2014, **89**:625-636.

14. Alteri CJ, Himpel SD, Mobley HL: **Preferential use of central metabolism in vivo reveals a nutritional basis for polymicrobial infection.** *PLoS Pathog* 2015, **11**:e1004601.
  15. Johnson DR, Goldschmidt F, Lilja EE, Ackermann M: **Metabolic specialization and the assembly of microbial communities.** *ISME J* 2012, **6**:1985-1991.
  16. Harcombe W: **Novel cooperation experimentally evolved between species.** *Evolution* 2010, **64**:2166-2172.
  17. Hom EF, Murray AW: **Plant-fungal ecology. Niche engineering demonstrates a latent capacity for fungal-algal mutualism.** *Science* 2014, **345**:94-98.
- Demonstration of readily established obligate mutualism without history of co-habitation. Illustrates how nutritional challenge stimulates the use of latent capacity for beneficial metabolic exchange.
18. Wintermute EH, Silver PA: **Emergent cooperation in microbial metabolism.** *Mol Syst Biol* 2010, **6**:407.
  19. Kumar K, Mella-Herrera RA, Golden JW: **Cyanobacterial heterocysts.** *Cold Spring Harb Perspect Biol* 2010, **2**:a000315.
  20. Oliveira NM, Niehus R, Foster KR: **Evolutionary limits to cooperation in microbial communities.** *Proc Natl Acad Sci U S A* 2014, **111**:17941-17946.
  21. Foster KR, Bell T: **Competition, not cooperation, dominates interactions among culturable microbial species.** *Curr Biol* 2012, **22**:1845-1850.
  22. Hol FJ, Galajda P, Nagy K, Woolthuis RG, Dekker C, Keymer JE: **Spatial structure facilitates cooperation in a social dilemma: empirical evidence from a bacterial community.** *PLoS One* 2013, **8**:e77042.
  23. Morris JJ, Lenski RE, Zinser ER: **The Black Queen Hypothesis: evolution of dependencies through adaptive gene loss.** *MBio* 2012:3.
  24. Allen B, Gore J, Nowak MA: **Spatial dilemmas of diffusible public goods.** *Elife* 2013, **2**:e01169.
  25. Agapakis CM, Boyle PM, Silver PA: **Natural strategies for the spatial optimization of metabolism in synthetic biology.** *Nat Chem Biol* 2012, **8**:527-535.
  26. Fenchel T, Finlay B: **Free-Living Protozoa with Endosymbiotic Methanogens.** In *(Endo)symbiotic Methanogenic Archaea*. Edited by Hackstein JHP: *Microbiology Monographs*, vol. 19. Springer Berlin Heidelberg; 2010:1-11.
  27. Barve A, Wagner A: **A latent capacity for evolutionary innovation through exaptation in metabolic systems.** *Nature* 2013, **500**:203-206.
  28. Paczia N, Nilgen A, Lehmann T, Gatzens J, Wiechert W, Noack S: **Extensive exometabolome analysis reveals extended overflow metabolism in various microorganisms.** *Microb Cell Fact* 2012, **11**:122.
  29. Gallie J, Libby E, Bertels F, Remigi P, Jendresen CB, Ferguson GC, Desprat N, Buffing MF, Sauer U, Beaumont HJ *et al.*: **Bistability in a metabolic network underpins the de novo evolution of colony switching in *Pseudomonas fluorescens*.** *PLoS Biol* 2015, **13**:e1002109.
  30. Xavier JB, Kim W, Foster KR: **A molecular mechanism that stabilizes cooperative secretions in *Pseudomonas aeruginosa*.** *Mol Microbiol* 2011, **79**:166-179.
  31. Estrela S, Whiteley M, Brown SP: **The demographic determinants of human microbiome health.** *Trends Microbiol* 2015, **23**:134-141.
- Discusses peculiarities of multi-species environments, including metabolic interactions, in the context of human gut microbiota. Highlights importance of metabolic feedback between community members.
32. Liu J, Wu C, Huang IH, Merritt J, Qi F: **Differential response of *Streptococcus mutans* towards friend and foe in mixed-species cultures.** *Microbiology* 2011, **157**:2433-2444.
  33. Shafquat A, Joice R, Simmons SL, Huttenhower C: **Functional and phylogenetic assembly of microbial communities in the human microbiome.** *Trends Microbiol* 2014, **22**:261-266.
  34. Waters E, Hohn MJ, Ahel I, Graham DE, Adams MD, Barnstead M, Beeson KY, Bibbs L, Bolanos R, Keller M *et al.*: **The genome of *Nanoarchaeum equitans*: insights into early archaeal evolution and derived parasitism.** *Proc Natl Acad Sci U S A* 2003, **100**:12984-12988.
  35. Ju F, Zhang T: **Bacterial assembly and temporal dynamics in activated sludge of a full-scale municipal wastewater treatment plant.** *ISME J* 2015, **9**:683-695.
  36. Fuchsman CA, Kirkpatrick JB, Brazelton WJ, Murray JW, Staley JT: **Metabolic strategies of free-living and aggregate-associated bacterial communities inferred from biologic and chemical profiles in the Black Sea suboxic zone.** *FEMS Microbiol Ecol* 2011, **78**:586-603.
  37. Bailey VL, Fansler SJ, Stegen JC, McCue LA: **Linking microbial community structure to beta-glucosidase function in soil aggregates.** *ISME J* 2013, **7**:2044-2053.
  38. Faust K, Sathirapongsasuti JF, Izard J, Segata N, Gevers D, Raes J, Huttenhower C: **Microbial co-occurrence relationships in the human microbiome.** *PLoS Comput Biol* 2012, **8**:e1002606.
  39. Friedman J, Alm EJ: **Inferring correlation networks from genomic survey data.** *PLoS Comput Biol* 2012, **8**:e1002687.
  40. Berry D, Widder S: **Deciphering microbial interactions and detecting keystone species with co-occurrence networks.** *Front Microbiol* 2014, **5**:219.
  41. Durham BP, Sharma S, Luo H, Smith CB, Amin SA, Bender SJ, Dearth SP, Van Mooy BA, Campagna SR, Kujawinski EB *et al.*: **Cryptic carbon and sulfur cycling between surface ocean plankton.** *Proc Natl Acad Sci U S A* 2015, **112**:453-457.
- Shows how metabolite exchange deciphered in laboratory co-culture guided discovery of trophic link in a marine ecosystem.
42. Embree M, Nagarajan H, Movahedi N, Chitsaz H, Zengler K: **Single-cell genome and metatranscriptome sequencing reveal metabolic interactions of an alkane-degrading methanogenic community.** *ISME J* 2014, **8**:757-767.
  43. Hendrickson EL, Wang T, Beck DA, Dickinson BC, Wright CJ, R JL, Hackett M: **Proteomics of *Fusobacterium nucleatum* within a model developing oral microbial community.** *Microbiologyopen* 2014, **3**:729-751.
  44. Bao K, Bostanci N, Selevsek N, Thurnheer T, Belibasakis GN: **Quantitative proteomics reveal distinct protein regulations caused by *Aggregatibacter actinomycetemcomitans* within subgingival biofilms.** *PLoS One* 2015, **10**:e0119222.
  45. Hawley AK, Brewer HM, Norbeck AD, Pasa-Tolic L, Hallam SJ: **Metaproteomics reveals differential modes of metabolic coupling among ubiquitous oxygen minimum zone microbes.** *Proc Natl Acad Sci U S A* 2014, **111**:11395-11400.
  46. Ruhl M, Hardt WD, Sauer U: **Subpopulation-specific metabolic pathway usage in mixed cultures as revealed by reporter protein-based <sup>13</sup>C analysis.** *Appl Environ Microbiol* 2011, **77**:1816-1821.
  47. Ghosh A, Nilmeier J, Weaver D, Adams PD, Keasling JD, Mukhopadhyay A, Petzold CJ, Martin HG: **A peptide-based method for <sup>13</sup>C metabolic flux analysis in microbial communities.** *PLoS Comput Biol* 2014, **10**:e1003827.
  48. Verastegui Y, Cheng J, Engel K, Kolczynski D, Mortimer S, Lavigne J, Montalibet J, Romantsov T, Hall M, McConkey BJ *et al.*: **Multisubstrate isotope labeling and metagenomic analysis of active soil bacterial communities.** *MBio* 2014, **5**:e01157-01114.
  49. Pion M, Spangenberg JE, Simon A, Bindschedler S, Flury C, Chatelain A, Bshary R, Job D, Junier P: **Bacterial farming by the fungus *Morchella crassipes*.** *Proc Biol Sci* 2013, **280**:20132242.
  50. Herbst FA, Bahr A, Duarte M, Pieper DH, Richnow HH, von Bergen M, Seifert J, Bombach P: **Elucidation of in situ polycyclic aromatic hydrocarbon degradation by functional metaproteomics (protein-SIP).** *Proteomics* 2013, **13**:2910-2920.
  51. Pande S, Shitut S, Freund L, Westermann M, Bertels F, Colesie C, Bischofs IB, Kost C: **Metabolic cross-feeding via intercellular nanotubes among bacteria.** *Nat Commun* 2015, **6**:6238.

52. Comolli LR, Banfield JF: **Inter-species interconnections in acid mine drainage microbial communities.** *Front Microbiol* 2014, **5**:367.
  53. Almstrand R, Daims H, Persson F, Sorensson F, Hermansson M: **New methods for analysis of spatial distribution and coaggregation of microbial populations in complex biofilms.** *Appl Environ Microbiol* 2013, **79**:5978-5987.
  54. Cruz-López R, Maske H: **A non-amplified FISH protocol to identify simultaneously different bacterial groups attached to eukaryotic phytoplankton.** *J Appl Phycol* 2014, **27**:797-804.
  55. Vila-Costa M, Gasol JM, Sharma S, Moran MA: **Community analysis of high- and low-nucleic acid-containing bacteria in NW Mediterranean coastal waters using 16S rDNA pyrosequencing.** *Environ Microbiol* 2012, **14**:1390-1402.
  56. Maurice CF, Turnbaugh PJ: **Quantifying the metabolic activities of human-associated microbial communities across multiple ecological scales.** *FEMS Microbiol Rev* 2013, **37**:830-848.
- Reviews different approaches used to assess species metabolic phenotypes in the context of host associated microbial community. Highlights techniques applied at different spatial scales.
57. Watrous JD, Alexandrov T, Dorrestein PC: **The evolving field of imaging mass spectrometry and its impact on future biological research.** *J Mass Spectrom* 2011, **46**:209-222.
  58. Shih CJ, Chen PY, Liaw CC, Lai YM, Yang YL: **Bringing microbial interactions to light using imaging mass spectrometry.** *Nat Prod Rep* 2014, **31**:739-755.
  59. Traxler MF, Watrous JD, Alexandrov T, Dorrestein PC, Kolter R: **Interspecies interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome.** *MBio* 2013, **4**.
- Illustrates application of mass-spectrometry for exploring metabolic changes stimulated by presence of other microorganisms.
60. Garg N, Kapon C, Lim YW, Koyama N, Vermeij MJ, Conrad D, Rohwer F, Dorrestein PC: **Mass spectral similarity for untargeted metabolomics data analysis of complex mixtures.** *Int J Mass Spectrom* 2015, **377**:717-719.
  61. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczar I, Bassler BL, Hughson FM: **Structural identification of a bacterial quorum-sensing signal containing boron.** *Nature* 2002, **415**:545-549.
  62. Wessel AK, Hmelo L, Parsek MR, Whiteley M: **Going local: technologies for exploring bacterial microenvironments.** *Nat Rev Microbiol* 2013, **11**:337-348.
- A thorough review of technologies for low-volume confinement of microbial populations. Also discusses methods for chemical characterization of microenvironments.
63. Foster RA, Kuypers MM, Vagner T, Paerl RW, Musat N, Zehr JP: **Nitrogen fixation and transfer in open ocean diatom-cyanobacterial symbioses.** *ISME J* 2011, **5**:1484-1493.
  64. Dekas AE, Poretsky RS, Orphan VJ: **Deep-sea archaea fix and share nitrogen in methane-consuming microbial consortia.** *Science* 2009, **326**:422-426.
  65. Green-Saxena A, Dekas AE, Dalleska NF, Orphan VJ: **Nitrate-based niche differentiation by distinct sulfate-reducing bacteria involved in the anaerobic oxidation of methane.** *ISME J* 2014, **8**:150-163.
  66. Grosskopf T, Soyer OS: **Synthetic microbial communities.** *Curr Opin Microbiol* 2014, **18**:72-77.
  67. Song H, Ding MZ, Jia XQ, Ma Q, Yuan YJ: **Synthetic microbial consortia: from systematic analysis to construction and applications.** *Chem Soc Rev* 2014, **43**:6954-6981.
  68. Stadie J, Gultiz A, Ehrmann MA, Vogel RF: **Metabolic activity and symbiotic interactions of lactic acid bacteria and yeasts isolated from water kefir.** *Food Microbiol* 2013, **35**:92-98.
  69. Andrade-Dominguez A, Salazar E, Vargas-Lagunas Mdel C, Kolter R, Encarnacion S: **Eco-evolutionary feedbacks drive species interactions.** *ISME J* 2014, **8**:1041-1054.
  70. Mee MT, Collins JJ, Church GM, Wang HH: **Syntrophic exchange in synthetic microbial communities.** *Proc Natl Acad Sci U S A* 2014, **111**:E2149-E2156.
- Example of engineered community of *Escherichia coli* amino acid auxotrophs, which readily establish synergistic cross-feeding. Supports the idea of division of labour in bacterial communities.
71. Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR: **Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria.** *Science* 2010, **330**:1413-1415.
  72. Wolfe BE, Dutton RJ: **Fermented foods as experimentally tractable microbial ecosystems.** *Cell* 2015, **161**:49-55.
  73. Wolfe BE, Button JE, Santarelli M, Dutton RJ: **Cheese rind communities provide tractable systems for in situ and in vitro studies of microbial diversity.** *Cell* 2014, **158**:422-433.
  74. Laureys D, De Vuyst L: **Microbial species diversity, community dynamics, and metabolite kinetics of water kefir fermentation.** *Appl Environ Microbiol* 2014, **80**:2564-2572.
  75. Marsh AJ, O'Sullivan O, Hill C, Ross RP, Cotter PD: **Sequencing-based analysis of the bacterial and fungal composition of kefir grains and milks from multiple sources.** *PLoS One* 2013, **8**:e69371.
  76. Rakoff-Nahoum S, Coyne MJ, Comstock LE: **An ecological network of polysaccharide utilization among human intestinal symbionts.** *Curr Biol* 2014, **24**:40-49.
  77. Kim HJ, Boedicker JQ, Choi JW, Ismagilov RF: **Defined spatial structure stabilizes a synthetic multispecies bacterial community.** *Proc Natl Acad Sci U S A* 2008, **105**:18188-18193.
  78. Connell JL, Ritschdorff ET, Whiteley M, Shear JB: **3D printing of microscopic bacterial communities.** *Proc Natl Acad Sci U S A* 2013, **110**:18380-18385.
  79. Kerr B, Riley MA, Feldman MW, Bohannan BJ: **Local dispersal promotes biodiversity in a real-life game of rock-paper-scissors.** *Nature* 2002, **418**:171-174.
  80. Leung K, Zahn H, Leaver T, Konwar KM, Hanson NW, Page AP, Lo CC, Chain PS, Hallam SJ, Hansen CL: **A programmable droplet-based microfluidic device applied to multiparameter analysis of single microbes and microbial communities.** *Proc Natl Acad Sci U S A* 2012, **109**:7665-7670.
  81. Goers L, Freemont P, Polizzi KM: **Co-culture systems and technologies: taking synthetic biology to the next level.** *J R Soc Interface* 2014:11.
  82. Mahadevan R, Henson MA: **Genome-based modeling and design of metabolic interactions in microbial communities.** *Comput Struct Biotechnol J* 2012, **3**:e201210008.
  83. Klitgord N, Segre D: **Environments that induce synthetic microbial ecosystems.** *PLoS Comput Biol* 2010, **6**:e1001002.
  84. Zengler K, Palsson BO: **A road map for the development of community systems (CoSy) biology.** *Nat Rev Microbiol* 2012, **10**:366-372.
  85. Hanemaaijer M, Roling WF, Olivier BG, Khandelwal RA, Teusink B, Bruggeman FJ: **Systems modeling approaches for microbial community studies: from metagenomics to inference of the community structure.** *Front Microbiol* 2015, **6**:213.
  86. Zomorodi AR, Islam MM, Maranas CD: **d-OptCom: dynamic multi-level and multi-objective metabolic modeling of microbial communities.** *ACS Synth Biol* 2014, **3**:247-257.
  87. Heinken A, Thiele I: **Anoxic conditions promote species-specific mutualism between gut microbes in silico.** *Appl Environ Microbiol* 2015, **81**:4049-4061.
  88. Levy R, Borenstein E: **Metagenomic systems biology and metabolic modeling of the human microbiome: from species composition to community assembly rules.** *Gut Microbes* 2014, **5**:265-270.
  89. Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E: **Competitive and cooperative metabolic interactions in bacterial communities.** *Nat Commun* 2011, **2**:589.
  90. Prokopenko MG, Hirst MB, De Brabandere L, Lawrence DJ, Berelson WM, Granger J, Chang BX, Dawson S, Crane EJ 3rd,

Chong L *et al.*: **Nitrogen losses in anoxic marine sediments driven by *Thioploca-anammox* bacterial consortia.** *Nature* 2013, **500**:194-198.

*In situ* cross-feeding of nitrogenous compounds between sulphur-oxidizing and ammonium-oxidizing bacteria in marine sediments. This metabolic interaction majorly contributes to benthic  $N_2$  production and loss of fixed nitrogen.

91. Kato S, Haruta S, Cui ZJ, Ishii M, Igarashi Y: **Stable coexistence of five bacterial strains as a cellulose-degrading community.** *Appl Environ Microbiol* 2005, **71**:7099-7106.
92. Kato S, Haruta S, Cui ZJ, Ishii M, Igarashi Y: **Network relationships of bacteria in a stable mixed culture.** *Microb Ecol* 2008, **56**:403-411.