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Using metabolic networks to resolve ecological properties of microbiomes

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Abstract

The systematic collection, integration and modelling of high-throughput molecular data (multi-omics) allows the detailed characterisation of microbiomes *in situ*. Through metabolic trait inference, metabolic network reconstruction and modelling, we are now able to define ecological interactions based on metabolic exchanges, identify keystone genes, functions and species, and resolve ecological niches of constituent microbial populations. The resulting knowledge provides detailed information on ecosystem functioning. However, as microbial communities are dynamic in nature the field needs to move towards the integration of time- and space-resolved multi-omic data along with detailed environmental information to fully harness the power of community- and population-level metabolic network modelling. Such approaches will be fundamental for future targeted management strategies with wide-ranging applications in biotechnology and biomedicine.

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Microbial systems ecology

Microbial communities (microbiomes) are involved in all biogeochemical cycles by contributing functions which may be common to most ecosystems (underlined words are defined in Box 1), e.g. nitrogen fixation, or by being first-line to very specific ecosystem services, e.g. the degradation of particular xenobiotics. Although the global relevance of microbial activities for ecosystem functioning is now widely accepted, methods to study the ecology of the tremendous richness of the microbial realm are relatively recent. In order to model, predict and understand the behaviour of microbial constituents in their native environments, Microbial Systems Ecology heavily relies on high-throughput, high-fidelity and high-resolution measurements of microbial consortia (Figure 1A) as well as the integration of the resulting data [1]. Thereby, Microbial Systems Ecology relies on specialised wet- and dry-lab approaches to achieve coherent assessments of microbial community structure and function *in situ* [1–5]. In addition to the valuable insights on community structure and functional potential (metagenomics), expressed functions (metatranscriptomics and metaproteomics) and metabolic activity (metabolomics), the integration of the individual omic levels (Figure 1B) allows comprehensive resolution of the emergent properties of ecosystems [1,6]. Furthermore, integrative approaches can significantly reduce the current limitations associated with single omics by enhancing the interpretability of data [1], allowing for example to obtain improved genome reconstructions from constituent populations [7] and to link the expression of phenotype-associated microbial functions to distinct taxa [8].

Natural microbial communities are comprised of constituent, interacting populations. Therefore, to move from descriptive, comparative or statistical studies to ecological inferences [9], in Microbial Systems Ecology, microbial communities must be seen as networks of networks: community members (populations), consisting of collections of interwoven molecular networks, form the interacting units of higher-order ecological systems. Although different types of molecular networks exist (e.g. gene regulatory networks, co-occurrence networks, etc.), we particularly focus our review on metabolic network reconstruction and related modelling approaches as applied to microbial communities in view of resolving specific properties underpinning ecosystem functioning. We also present our opinion on how harnessing this ecological knowledge will facilitate

Box 1. Glossary

- Ecosystem: ecological self-supporting unit constituted of an environment (the biotope) and the living organisms inhabiting it (the biocoenosis). Despite flows of materials, organisms and energy occurring across the boundary of individual units, the two components of an ecosystem interact more strongly between each other than with the neighbouring units.
- Ecosystem functioning: all activities, processes and properties driving biogeochemical activities and leading to the relative ecological stability of an ecosystem.
- Ecological niche (Hutchinson): the hypervolume comprised of n dimensions representing the environmental conditions and resources gradients enabling a species to persist. This definition led to the subsequent description of the fundamental niche (the maximal usable space) and the realised niche (the actual used space).
- Ecological interactions (or biological interactions or symbiosis): long-term relationship between individuals of different species including mutualism (win–win), commensalism (win–neutral), parasitism/predation (win–lose) and amensalism (lose–neutral). Metabolic interactions represent a subset of these relationships when the interaction is mediated through one or multiple metabolite(s), as opposed to non-metabolic relationships.
- Metabolic models: in silico description of the metabolic potential of a biological unit (e.g. community, guild, species), often represented as a bipartite directed network consisting of metabolites and reactions/enzymes/genes [12]. While topological metabolic models represent a qualitative view of metabolism, stoichiometric metabolic models require the specification of each reaction's stoichiometry in a stoichiometric matrix, which forms the basis for quantitative metabolic modelling.
- Microbial Systems Ecology: the holistic study of microbial communities using systems biology approaches.
- Systematic measurement: "the standardised, reproducible, and simultaneous measurement of multiple features from a single sample. Resulting datasets are fully integrable and relate system-wide behaviours" [1].

targeted manipulations of microbial communities in the future. More specifically, space- and time-resolved integrated multi-omic datasets will allow us to define and subsequently alter the realised niches of constituent populations for the management of community–conferred traits.

Using metabolic networks to obtain meaningful ecological insights

Reconstruction, analysis and modelling of metabolic networks

Community-level metabolic modelling approaches are classified according to the unit being modelled (entire community, guilds, species or strains, see Figure 1B and C) [10] and the level of detail. Metabolic modelling approaches may be divided into i) stoichiometric approaches that model the metabolism quantitatively [11], and ii) topological (network-based) approaches, which are more suitable for qualitative metabolic modelling [12].

In any case, a prerequisite to metabolic modelling is metabolic network reconstruction, i.e. the assembly of a metabolic map for the unit of interest. A number of automatic pipelines generate metabolic reconstructions directly from the genome [13–15] or metagenome [16], which can subsequently serve as the starting point for manual curation [17]. Alternatively, a selected subset of pathways relevant in a particular environment can be targeted for metabolic reconstruction [18]. Two major challenges for metabolic reconstruction are i) the large number of genes without functional annotation, which can be partially overcome using gap filling methods [19], and ii) the association of genes to reactions. Semi-curated metabolic models are collected in repositories such as AGORA [20].

Once a metabolic network reconstruction has been obtained, the community's metabolism can be analysed qualitatively or quantitatively. For instance, a topological analysis can serve to identify specific metabolic pathways of interest or to extract the active part of a community's metabolism from metatranscriptomic [21], metaproteomic or (meta-)metabolomic data (Figure 1B). A widespread quantitative metabolic modelling approach is flux balance analysis (FBA), which calculates the metabolite flow through reactions such that a particular objective function, e.g. biomass production, is maximised [11]. While topological metabolic models can integrate omics data via node or edge weights, stoichiometric models can take them into account for instance by modifying flux distributions [22]. FBA, which was originally developed for single species, was recently extended to multiple species [23,24]. However, these approaches only provide a static picture of the community. Dynamic community-level metabolic modelling, which describes the change of species abundances and metabolite concentrations over time, currently is an active field of development [25,26].

In the following paragraphs, we will discuss some applications of metabolic modelling in more detail, namely the prediction of ecological interactions, identification of keystone species and functions as well as metabolic niche inference.

Metabolic interactions

Metabolic models can be exploited to predict ecological interactions between species via metabolic cross-feeding, for instance in the case of mutualistic growth on the toxic end-products of other species, or when two species compete for the same nutrients (Figure 1C and D). Importantly, the extracellular environment, which can be characterised by metabolomics and physicochemical measurements, needs to be taken into account when predicting interactions, since not all potential interactions will be actually realised particularly in nutrient-rich environments [27]. A number of stoichiometric interaction prediction approaches compare growth rates computed in the presence or the absence of

an interaction partner [28–30] or under different environmental condition [31] to determine the interaction type. Here, COMETS [26] also takes into account the impact of spatial structure on cross-feeding.

In contrast to analyses based on stoichiometric modelling, topology-based interaction prediction [32–34] first involves the inference of seed metabolites for a given microbial population, which include all metabolites that cannot be produced by the network itself [35]. It then assesses whether some of these seeds can be produced by the metabolic network of another species, which in turn allows quantification of the potential for commensalism or mutualism. The metabolic interaction potential measures the maximum number of essential nutrients that an organism can obtain by interacting with its community [34]. Furthermore, the competitive potential between two species can be determined by computing the overlap between their seed metabolites [36].

An alternative topological approach finds genome segments that maximise the number of consecutive enzyme-coding genes. The enzymes in turn catalyse metabolic transformations which are complementary across species [37]. Metabolic pathway complementarity or overlap can also be exploited to screen metagenomic data for interactions. This form of topological analysis has for instance been applied to explore metabolic strategies in human gut microbiota [38].

Recent work has involved the use of multi-omics to refine or validate model predictions in different environmental conditions [39–42]. Beyond interactions mediated through exchange or competition for metabolites, trophic interactions such as phage predation can also be inferred using omic data (see Box 2 for an example of non-metabolic interactions). Similarly, additional ecological insights such as keystone roles of some species can be inferred when metabolic networks are combined with other layers of knowledge such as co-occurrence of genes/transcripts/proteins/metabolites or to regression- and rule-based network analysis [43].

Keystone functions, genes and species

Ecological keystone species are commonly understood as species that have a pronounced impact on their environment independent of their abundance, i.e. they have a disproportionate deleterious effect on the community upon their removal [44,45]. This concept reflects the dependencies within a community governed by interactions among its members and is clearly context-dependent: the importance of any organism for stabilising the community is conferred by the particular group. Thus being a keystone species is not a Boolean trait, but it is rather a continuous property that emerges in the context of community function and different selection pressures. In order to predict which organism is a functional keystone species, the topological properties of networks derived from metabolic models that represent the community-wide organisation of microbial interactions may be used (Figure 1E) in synergy with co-occurrence networks [46,47]. Measures such as degree, clustering coefficient and closeness centrality reflect the scale of the embeddedness of the constituting organisms (nodes) in the microbial community ranging from direct ecological partners to local and global neighbourhoods, respectively [46].

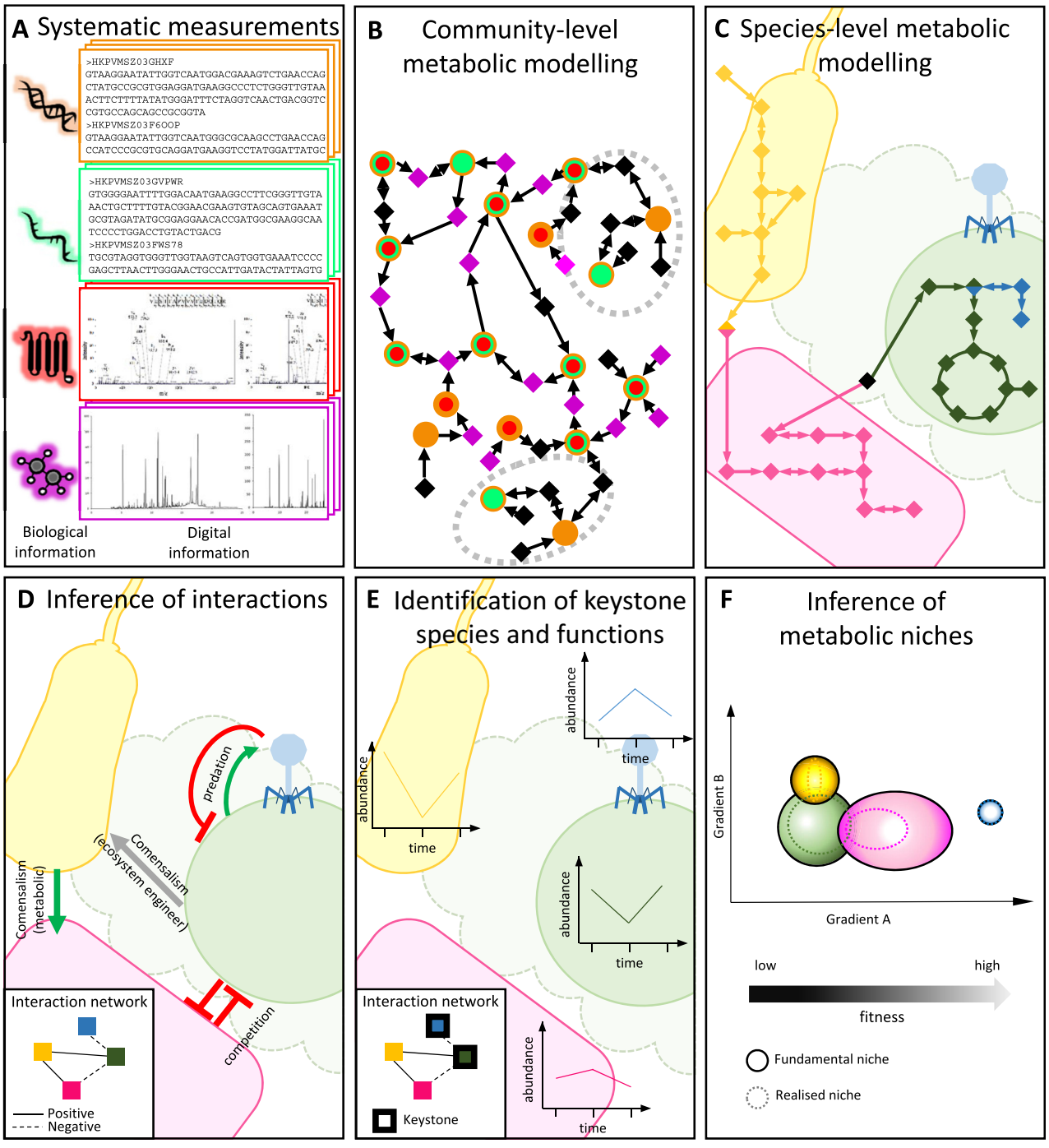
Different categories of keystone species have been proposed including ecosystem engineer (or modifier) keystone species (Figure 1E), trophic (prey or predator) keystone species or resource provider keystone species [48]. In any case, keystone species confer keystone functionalities to the ecosystem [49]. For example, the degradation of dietary fibres in the human gut is the result of a community-driven effort. However, the pivotal step is the breakdown of the complex resistant starches like amylopectin and amylose by primary degraders, which release simple sugar molecules to be fermented by the rest of the microbial consortium. *Ruminococcus bromii* is a keystone species in this context [50]. The organism possesses a highly specific cluster of keystone genes essential for efficient amylolysis [51].

Keystone metabolic genes are predicted to be highly expressed despite typically low gene copy numbers (reflecting the typical relatively low abundance of keystone species) and to catalyse key biochemical transformations (enzymes represent "load points" in the community-wide metabolic networks [52]). Therefore, a framework has been developed for the identification of such genes in reconstructed community-wide metabolic networks [49]. High relative gene expression (extracted from metatranscriptomic and/or metaproteomic data relative to gene abundance information derived from the corresponding metagenomic data) as well as specific network topological features (low relative degree and high betweenness centrality) are taken into account for the identification of such keystone genes which, through genomic linkage to reconstructed population-level genomes, can be linked to specific constituent populations which represent keystone species [49]. This approach has highlighted ammonia monooxygenase as a keystone gene in a biological wastewater treatment plant which is contributed to the community function by a specific keystone strain of *Nitrosomonas* spp [49]. Community-wide reconstructed metabolic networks are thereby particularly informative for the identification of keystone traits conferred by specific keystone species.

Microbial niche ecology

Even though it has been shown that clusters in a co-occurrence network based on 16S rRNA sequencing data reflect overlapping ecological niche preferences and common habitats of populations [53], the inference of niches of distinct bacterial populations in microbial

Figure 1



From metabolic models to ecological insights. (A) Following carefully adapted wet-lab procedures and systematic measurements of the purified biomolecules, (B) metabolic modelling (here resolved to the community level) by stepwise integration and modelling of the metagenomic (blue), metatranscriptomic (green), metaproteomic (red) and (meta-)metabolomic (pink) data, allows to detect, for example, parts of the metabolic network that are inactive (dotted line circle) at the sample collection. (C) Metabolic modelling (here resolved to the species level), often represented as a directed network consisting of metabolites (nodes) and reactions (edges), can be a starting point to determine (D) an ecological interaction network (nodes = species; edges = interactions). Although some non-metabolic interactions, such as commensalism by niche engineering (e.g. the green organism is a biofilm founder, allowing a secondary colonisation by the yellow microbe) or predation (see Box 2) cannot be predicted from inferred metabolic networks, other complimentary analyses, such as co-occurrence networks, will allow to predict such behaviour. (E) Topological analysis of metabolic, interaction and co-occurrence networks allow the detection of metabolic keystone species (highlighted in green; bacterial species) and trophic keystone species (highlighted in blue; phage). (F) The use of population-resolved metagenomic data to describe the fundamental niche is extended by the use of functional omic data to characterise the realised niche of different species. From this information, predictions can be made for example in relation to the fitness gradients of constituent populations.

Box 2. Causal inference of non-metabolic interactions (i.e. phage-host interactions from metagenomic data)

Phages are the most abundant and diverse entities in any environment, greatly influencing microbial community structure and dynamics through affecting the prokaryotic (host) metabolism [68,69], modulating nutrient cycles, and driving long-term host evolution [70]. The unculturability of the vast majority of host and phage strains can be circumvented by integrating meta-omic data [71,72]. Accordingly, computational methods have been developed to identify phages [73,74] and predict links to their putative hosts [75].
In addition, time-resolved datasets enable the inference of phage-host dynamics [76,77], which will result in improved knowledge and, thereby, the formulation of potential phage treatment strategies for biomedical and biotechnological applications [78].

communities remains a challenging task, due to the inherent complexity of trophic interactions and fluctuating environmental conditions. In that sense, integrated multi-omic approaches have been shown to be useful for studying microbial niche ecology. State-of-the-art binning approaches [54], or ensemble methods [55], allow near complete reconstruction of population-level genomes from assembled sequencing reads. By applying the traditional concepts of niche ecology by Hutchinson, the genomic functional potential of a microbial population reflects its fundamental niche [56,57]. Conversely, metatranscriptomic or metaproteomic data can be used to infer a population's realised niche at the time of sampling [57], while intra- and extracellular metabolomic data allows inferences regarding resource usage and the overall resource space available, respectively [57] (Figure 1F). Previous studies have relied on gene expression patterns to assess lifestyle strategies (generalists versus specialists) and the metabolic niche breadth of distinct populations [57,58]. Computational approaches that automatically predict phenotypic traits of reconstructed genomes [59] are an important resource for the in-depth characterisation of niche occupation. In this context, metabolic models can provide a detailed picture on growth conditions, such as available carbon or nitrogen sources and models have indeed been used to predict medium requirements reflecting niche breadths [60].

Apart from resource availability and usage, niche breadth also reflects tolerance ranges to physico-chemical variables, such as pH, temperature or dissolved oxygen, which are generally available only for cultured isolates. Currently, a popular approach involves the linking of inferred organismal abundances to environmental conditions, which can be challenging due to the compositional nature of rRNA amplicon sequencing data. Leveraging integrated multi-omic data and metabolic models may in turn provide a detailed mechanistic understanding of the adaptation to environmental factors for single organismal groups, as demonstrated for pH-dependent metabolic adaptations of *Enterococcus faecalis* [61].

Harnessing the power of data integration in Microbial Systems Ecology

The integration, contextualisation and analysis of multi-omic data using metabolic network approaches (in synergy with other network approaches) offer many exciting opportunities in the context of Microbial Systems Ecology, a few of which are highlighted above. While such tools are commonly used in systems biology [62], their utilisation in (microbial) ecology is still limited.

In order to move beyond associations and hypotheses derived from integrated multi-omic data, model predictions will have to be tested using combinations of detailed field and/or laboratory experiments [1,5,63], as described for example in Ref. [64]. A discovery-driven planning approach, wherein systematic measurements, data integration, model generation, hypothesis testing and new ecological hypotheses follow each other iteratively, should culminate in predictive models [1]. Thus, system-wide data has to be collected in a manner consistent with the subsequent integration and modelling to continuously improve the community models; ultimately we aim for models which allow the systematic and knowledge-guided control of different microbial community functions and/or structures. In this context, keystones functions, genes and species represent primary targets for community management, because of their disproportionate effect on ecosystem functioning. For example, lipid accumulating organisms present in wastewater treatment plants are an abundant source of lipids which may be directly converted into biodiesel [65], but as the community phenotype shows seasonal fluctuations, economical interest remains limited. Biostimulation of endogenous keystone specie(s) or targeted activation of keystone gene(s) would help tune the community towards the desired phenotype robustly around the year [63]. Conversely, a targeted removal of keystone functions may provoke a collapse of the community. In this context, the keystone concept was successfully used for the prediction of drug targets that control the pathological lung microbiome of persons with cystic fibrosis [66].

In the future, by determining the respective ecological niches of the constituent populations, we will be able to move beyond 'basic' ecological classifications of lifestyle strategy for microbes such as generalists and specialists towards more specific classifications such as the Universal Adaptive Strategy Theory (UAST) describing trade-offs between ruderal, stress tolerant and competitor behaviours [67]. This will further enable us to determine the metabolic basis of colonisation/immigration, successional stages and the community response to perturbations. In

order to establish such concepts, the field needs to move towards the integration of time- and space-resolved multi-omic data to unravel the functional dynamics of complex microbial communities. In our opinion, the elucidation of networks requires such longitudinal data and corresponding time-series analyses to model the populations' interplay as well as to highlight which parts of these networks are active under specific conditions. Hence, future augmented community-level metabolic models need to account for trophic interactions and changing environmental conditions, ideally by integrating dynamic community models with genome-scale metabolic models. Therefore, within the framework of Microbial Systems Ecology, we will in the future be able to systematically define and alter the realised niches of constituent populations *in situ* and manage community–conferred traits, leading to exciting prospects for biotechnology and biomedicine.

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References

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

- • of special interest
- •• of outstanding interest

1. Muller EEL, Glaab E, May P, Vlassis N, Wilmes P: **Condensing the omics fog of microbial communities**. *Trends Microbiol* 2013, **21**:325–333.

2. Pesant S, Not F, Picheral M, Kandels-Lewis S, Le Bescot N, Gorsky G, Iudicone D, Karsenti E, Speich S, Trouble R, *et al.*: **Open science resources for the discovery and analysis of Tara Oceans data**. *Sci Data* 2015, **2**:150023.

3. Roume H, Muller EEL, Cordes T, Renaut J, Hiller K, Wilmes P: **A biomolecular isolation framework for eco-systems biology**. *ISME J* 2013, **7**:110–121.

4. • Franzosa EA, Hsu T, Sirota-Madi A, Shafquat A, Abu-Ali G, Morgan XC, Huttenhower C: **Sequencing and beyond: integrating molecular 'omics' for microbial community profiling**. *Nat Rev Microbiol* 2015, **13**:360–372.

An overview of current multi-omic approaches in microbial ecology.

5. Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT, Cordero OX, Brown SP, Momeni B, Shou W, *et al.*: **Challenges in microbial ecology: building predictive understanding of community function and dynamics**. *ISME J* 2016, **10**:2557.

6. Abram F: **Systems-based approaches to unravel multi-species microbial community functioning**. *Comput Struct Biotechnol J* 2015, **13**:24–32.

7. Narayanasamy S, Jarosz Y, Muller EEL, Heintz-Buschart A, Herold M, Kaysen A, Laczny CC, Pinel N, May P, Wilmes P: **IMP: a pipeline for reproducible reference-independent integrated metagenomic and metatranscriptomic analyses**. *Genome Biol* 2016, **17**:260.

8. •• Heintz-Buschart A, May P, Laczny CC, Lebrun LA, Bellora C, Krishna A, Wampach L, Schneider JG, Hogan A, de Beaufort C, *et al.*: **Integrated multi-omics of the human gut microbiome in a case study of familial type 1 diabetes**. *Nat Microbiol* 2016, **2**:16180.

This article comprehensively describes for the first time disruption of ecosystem services in human disease as evidenced across the different omic levels.

9. Greenblum S, Chiu H-C, Levy R, Carr R, Borenstein E: **Towards a predictive systems-level model of the human microbiome: progress, challenges, and opportunities**. *Curr Opin Biotechnol* 2013, **24**:810–820.

10. Biggs MB, Medlock GL, Kolling GL, Papin JA: **Metabolic network modeling of microbial communities**. *WIREs Syst Biol Med* 2015, **7**:317–334.

11. Bordbar A, Monk JM, King ZA, Palsson BO: **Constraint-based models predict metabolic and associated cellular functions**. *Nat Rev Genet* 2014, **15**:107–120.

12. Faust K, Croes D, van Helden J: **Prediction of metabolic pathways from genome-scale metabolic networks**. *Biosystems* 2011, **105**:109–121.

13. Agren R, Liu L, Shoaie S, Vongsangnak W, Nookaew I, Nielsen J: **The RAVEN toolbox and its use for generating a genome-scale metabolic model for *Penicillium chrysogenum***. *PLoS Comput Biol* 2013, **9**:e1002980.

14. Devoid S, Overbeek R, DeJongh M, Vonstein V, Best A, Henry C: **Automated genome annotation and metabolic model reconstruction in the SEED and Model SEED**. *Methods Mol Biol* 2013, **985**:17–45.

15. Dias O, Rocha M, Ferreira EC, Rocha I: **Reconstructing genome-scale metabolic models with merlin**. *Nucleic Acids Res* 2015, **43**:3899–3910.

16. Konwar KM, Hanson NW, Bhatia MP, Kim D, Wu S-J, Hahn AS, Morgan-Lang C, Cheung HK, Hallam SJ: **MetaPathways v2.5: quantitative functional, taxonomic and usability improvements**. *Bioinformatics* 2015, **31**:3345–3347.

17. Thiele I, Palsson BØ: **A protocol for generating a high-quality genome-scale metabolic reconstruction**. *Nat Protoc* 2010, **5**:93–121.

18. Darzi Y, Falony G, Vieira-Silva S, Raes J: **Towards biome-specific analysis of meta-omics data**. *ISME J* 2016, **10**:1025–1028.

19. Thiele I, Vlassis N, Fleming RMT: **fastGapFill: efficient gap filling in metabolic networks**. *Bioinformatics* 2014, **30**:2529–2531.

20. Magnusdottir S, Heinken A, Kutt L, Ravcheev DA, Bauer E, Noronha A, Greenhalgh K, Jager C, Baginska J, Wilmes P, *et al.*: **Generation of genome-scale metabolic reconstructions for 773 members of the human gut microbiota**. *Nat Biotechnol* 2017, **35**:81–89.

21. Hamilton JJ, Garcia SL, Brown BS, Oyserman BO, Moya-Flores F, Bertilsson S, Malmstrom RR, Forest KT, McMahon KD: **Metabolic network analysis and metatranscriptomics reveal auxotrophies and nutrient sources of the cosmopolitan freshwater microbial lineage acI**. *mSystems* 2017, **2**.

22. Kim MK, Lun DS: **Methods for integration of transcriptomic data in genome-scale metabolic models**. *Comput Struct Biotechnol J* 2014, **11**:59–65.

23. Zomorrodi AR, Maranas CD: **OptCom: a multi-level optimization framework for the metabolic modeling and analysis of microbial communities**. *PLoS Comput Biol* 2012, **8**:e1002363.

24. Khandelwal RA, Olivier BG, Roeling WFM, Teusink B, Bruggeman FJ: **Community flux balance analysis for microbial consortia at balanced growth**. *PLos One* 2013, **8**:e64567.

25. Zomorrodi 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.

26. Harcombe RW, Riehl JW, Dukovski I, Granger RB, Betts A, Lang HA, Bonilla G, Kar A, Leiby N, Mehta P, *et al.*: **Metabolic**

resource allocation in individual microbes determines ecosystem interactions and spatial dynamics. *Cell Rep* 2014, **7**:1104–1115.
Harcombe and coauthors present a dynamic, multi-species metabolic modelling framework that takes spatial structure into account (COMETS – Computation Of Microbial Ecosystems in Time and Space).

27. Klitgord N, Segrè D: **Environments that induce synthetic microbial ecosystems**. *PLoS Comput Biol* 2010, **6**:e1001002.

28. Chiu H-C, Levy R, Borenstein E: **Emergent biosynthetic capacity in simple microbial communities**. *PLoS Comput Biol* 2014, **10**:e1003695.

29. 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.

30. Mendes-Soares H, Mundy M, Soares LM, Chia N: **MMinte: an application for predicting metabolic interactions among the microbial species in a community**. *BMC Bioinf* 2016, **17**:343.

31. Heinken A, Thiele I: **Anoxic conditions promote species-specific mutualism between gut microbes in silico**. *Appl Environ Microbiol* 2015, **81**(12):4049–4061.

32. Borenstein E, Feldman MW: **Topological signatures of species interactions in metabolic networks**. *J Comput Biol* 2009, **16**: 191–200.

33. Levy R, Borenstein E: **Metabolic modeling of species interaction in the human microbiome elucidates community-level assembly rules**. *Proc Natl Acad Sci U S A* 2013, **10**: 12804–12809.

34. • 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.
By analysing the composition of more than 800 communities, this article presents a mechanistic understanding for observed co-occurrence patterns among distinct populations through metabolic dependencies.

35. Carr R, Borenstein E: **NetSeed: a network-based reverse-ecology tool for calculating the metabolic interface of an organism with its environment**. *Bioinformatics* 2012, **28**: 734–735.

36. Kreimer A, Doron-Faigenboim A, Borenstein E, Freilich S: **NetCmpt: a network-based tool for calculating the metabolic competition between bacterial species**. *Bioinformatics* 2012, **28**:2195–2197.

37. Bordron P, Latorre M, Cortés M-P, González M, Thiele S, Siegel A, Maass A, Eveillard D: **Putative bacterial interactions from metagenomic knowledge with an integrative systems ecology approach**. *Mirobiol Open* 2016, **5**:106–117.

38. • Vieira-Silva S, Falony G, Darzi Y, Lima-Mendez G, Yunta RG, Okuda S, Vandeputte D, Valles-Colomer M, Hildebrand F, Chaffron S, *et al.*: **Species–function relationships shape ecological properties of the human gut microbiome**. *Nat Microbiol* 2016, **1**:16088.
Linkage of specific functional traits to constituent populations which are drivers of ecological networks.

39. Shoaie S, Karlsson F, Mardinoglu A, Nookaew I, Bordel S, Nielsen J: **Understanding the interactions between bacteria in the human gut through metabolic modeling**. *Sci Rep* 2013, **3**: 2532.

40. Lawson CE, Wu S, Bhattacharjee AS, Hamilton JJ, McMahon KD, Goel R, Noguera DR: **Metabolic network analysis reveals microbial community interactions in anammox granules**. *Nat Commun* 2017, **8**:15416.

41. Wrighton KC, Castelle CJ, Wilkins MJ, Hug LA, Sharon I, Thomas BC, Handley KM, Mullin SW, Nicora CD, Singh A, *et al.*: **Metabolic interdependencies between phylogenetically novel fermenters and respiratory organisms in an unconfined aquifer**. *ISME J* 2014, **8**:1452–1463.

42. Si Ishii, Suzuki S, Tenney A, Norden-Krichmar TM, Nealson KH, Bretschger O: **Microbial metabolic networks in a complex electrogenic biofilm recovered from a stimulus-induced metatranscriptomics approach**. *Sci Rep* 2015, **5**:14840.

43. Faust K, Raes J: **Microbial interactions: from networks to models**. *Nat Rev Microbiol* 2012, **10**:538–550.

44. Power ME, Tilman D, Estes JA, Menge BA, Bond WJ, Mills LS, Daily G, Castilla JC, Lubchenco J, Paine RT: **Challenges in the quest for keystones**. *Bioscience* 1996, **46**:609–620.

45. Paine RT: **A conversation on refining the concept of keystone species**. *Conserv Biol* 1995, **9**:962–964.

46. • Berry D, Widder S: **Deciphering microbial interactions and detecting keystone species with co-occurrence networks**. *Front Microbiol* 2014, **5**:219.
In this article, current approaches for identifying keystone species in microbial communities are comprehensively overview and tested on simulated communities with known interactions. It is demonstrated that the interpretability of co-occurrence networks can be lost when the effects of habitat filtering become significant.

47. Marcotte EM, Pellegrini M, Ng HL, Rice DW, Yeates TO, Eisenberg D: **Detecting protein function and protein-protein interactions from genome sequences**. *Science* 1999, **285**: 751–753.

48. Mills LS, Soulé ME, Doak DF: **The keystone-species concept in ecology and conservation management and policy must explicitly consider the complexity of interactions in natural systems**. *Bioscience* 1993, **43**:219–224.

49. •• Roume H, Heintz-Buschart A, Muller EEL, May P, Satagopam VP, Laczny CC, Narayanasamy S, Lebrun LA, Hoopmann MR, Schupp JM, *et al.*: **Comparative integrated omics: identification of key functionalities in microbial community-wide metabolic networks**. *NPJ Biofilms Microbiomes* 2015, **1**:15007.
This work takes a function-centric approach based on community-wide metabolic network reconstructions to identify "keystone genes" and links these to constituent keystone species.

50. •• Ze X, Duncan SH, Louis P, Flint HJ: ***Ruminococcus bromii* is a keystone species for the degradation of resistant starch in the human colon**. *ISME J* 2012, **6**:1535–1543.
By describing the first amylosome, a cell surface enzyme complex devoted to starch degradation, Ze and co-authors identified key functional attributes of keystone species within the human gut microbiome.

51. Ze X, Ben David Y, Laverde-Gomez JA, Dassa B, Sheridan PO, Duncan SH, Louis P, Henrissat B, Juge N, Koropatkin NM, *et al.*: **Unique organization of extracellular amylases into amylosomes in the resistant starch-utilizing human colonic Firmicutes bacterium *Ruminococcus bromii***. *mBio* 2015, **6**. e01058–01015.

52. Rahman SA, Schomburg D: **Observing local and global properties of metabolic pathways: 'load points' and 'choke points' in the metabolic networks**. *Bioinformatics* 2006, **22**:1767–1774.

53. Chaffron S, Rehrauer H, Pernthaler J, von Mering C: **A global network of coexisting microbes from environmental and whole-genome sequence data**. *Genome Res* 2010, **20**: 947–959.

54. Sedlar K, Kupkova K, Provaznik I: **Bioinformatics strategies for taxonomy independent binning and visualization of sequences in shotgun metagenomics**. *Comput Struct Biotechnol J* 2017, **15**:48–55.

55. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, Banfield JF: **Recovery of genomes from metagenomes via a dereplication, aggregation, and scoring strategy**. *bioRxiv* 2017, https://doi.org/10.1101/107789.

56. Mou X, Sun S, Edwards RA, Hodson RE, Moran MA: **Bacterial carbon processing by generalist species in the coastal ocean**. *Nature* 2008, **451**:708–711.

57. •• Muller EEL, Pinel N, Laczny CC, Hoopmann MR, Narayanasamy S, Lebrun LA, Roume H, Lin J, May P, Hicks ND, *et al.*: **Community-integrated omics links dominance of a microbial generalist to fine-tuned resource usage**. *Nat Commun* 2014, **5**:5603.
The first study to integrate metagenomic, metatranscriptomic, metaproteomic and (meta-)metabolomic data. The integrated data analysis provides unique insights into the lifestyle strategies of constituent populations and links the levels of genetic variation within populations to their ecological niche breadth.

58. Gifford SM, Sharma S, Booth M, Moran MA: **Expression patterns reveal niche diversification in a marine microbial assemblage**. *ISME J* 2013, **7**:281–298.

59. Weimann A, Mooren K, Frank J, Pope PB, Bremges A, McHardy AC: **From genomes to phenotypes: traitar, the microbial trait analyzer**. *mSystems* 2016, **1**.

60. Zarecki R, Oberhardt MA, Reshef L, Gophna U, Ruppin E: **A novel nutritional predictor links microbial fastidiousness with lowered ubiquity, growth rate, and cooperativeness**. *PLoS Comput Biol* 2014, **10**:e1003726.

61. Grosseholz R, Koh CC, Veith N, Fiedler T, Strauss M, Olivier B, Collins BC, Schubert OT, Bergmann F, Kreikemeyer B, *et al.*: **Integrating highly quantitative proteomics and genome-scale metabolic modeling to study pH adaptation in the human pathogen *Enterococcus faecalis***. *NPJ Syst Biol Appl* 2016, **2**: 16017.

62. Gomez-Cabrero D, Tegnér J: **Iterative systems biology for medicine – time for advancing from network signatures to mechanistic equations**. *Curr Opin Syst Biol* 2017, **3**: 111–118.

63. Narayanasamy S, Muller EEL, Sheik AR, Wilmes P: **Integrated omics for the identification of key functionalities in biological wastewater treatment microbial communities**. *Microb Biotechnol* 2015, **8**:363–368.

64. Sheik AR, Muller EE, Audinot JN, Lebrun LA, Grysan P, Guignard C, Wilmes P: ***In situ* phenotypic heterogeneity among single cells of the filamentous bacterium *Candidatus* Microthrix parvicella**. *ISME J* 2016, **10**:1274–1279.

65. Sheik AR, Muller EELL, Wilmes P: **A hundred years of activated sludge: time for a rethink**. *Front Microbiol* 2014, **5**:47.

66. Quinn RA, Whiteson K, Lim YW, Zhao J, Conrad D, LiPuma JJ, Rohwer F, Widder S: **Ecological networking of cystic fibrosis lung infections**. *NPJ Biofilms Microbiomes* 2016, **2**:4.

67. Grime JP, Pierce S: **Primary adaptive strategies in organisms other than plants**. In *The evolutionary strategies that shape ecosystems*. John Wiley & Sons, Ltd; 2012:40–104.

68. • De Smet J, Zimmermann M, Kogadeeva M, Ceyssens PJ, Vermaelen W, Blasdel B, Bin Jang H, Sauer U, Lavigne R: **High coverage metabolomics analysis reveals phage-specific alterations to *Pseudomonas aeruginosa* physiology during infection**. *ISME J* 2016, **10**:1823–1835.
In this work, the dynamics of phenotypic features of a bacterial host is tracked following viral infections using untargeted high coverage metabolomics. A clear phage-specific and infection stage-specific metabolic reshuffling was observed, highlighting the importance of the overlooked viral realm in Microbial System Ecology.

69. Zhao X, Shen M, Jiang X, Shen W, Zhong Q, Yang Y, Tan Y, Agnello M, He X, Hu F, *et al.*: **Transcriptomic and metabolomics profiling of phage-host interactions between phage PaP1 and *Pseudomonas aeruginosa***. *Front Microbiol* 2017, **8**:548.

70. Burmeister AR, Lenski RE: **Host coevolution alters the adaptive landscape of a virus**. *Proc Biol Sci* 2016, **283**.

71. Hayes S, Mahony J, Nauta A, van Sinderen D: **Metagenomic approaches to assess bacteriophages in various environmental niches**. *Viruses* 2017, **9**.

72. Motlagh AM, Bhattacharjee AS, Coutinho FH, Dutilh BE, Casjens SR, Goel RK: **Insights of phage-host interaction in hypersaline ecosystem through metagenomics analyses**. *Front Microbiol* 2017, **8**:352.

73. Roux S, Enault F, Hurwitz BL, Sullivan MB: **VirSorter: mining viral signal from microbial genomic data**. *PeerJ* 2015, **3**:e985.

74. Ren J, Ahlgren NA, Lu YY, Fuhrman JA, Sun F: **VirFinder: a novel k-mer based tool for identifying viral sequences from assembled metagenomic data**. *Microbiome* 2017, **5**:69.

75. Edwards RA, McNair K, Faust K, Raes J, Dutilh BE: **Computational approaches to predict bacteriophage-host relationships**. *FEMS Microbiol Rev* 2016, **40**:258–272.

76. Parsons RJ, Breitbart M, Lomas MW, Carlson CA: **Ocean time-series reveals recurring seasonal patterns of virioplankton dynamics in the northwestern Sargasso Sea**. *ISME J* 2012, **6**: 273–284.

77. Zhang J, Gao Q, Zhang Q, Wang T, Yue H, Wu L, Shi J, Qin Z, Zhou J, Zuo J, *et al.*: **Bacteriophage-prokaryote dynamics and interaction within anaerobic digestion processes across time and space**. *Microbiome* 2017, **5**:57.

78. Jassim SA, Limoges RG, El-Cheikh H: **Bacteriophage biocontrol in wastewater treatment**. *World J Microbiol Biotechnol* 2016, **32**:70.