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Ecosystems biology of microbial metabolism

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The metabolic capabilities of many environmentally and medically important microbes can be quantitatively explored using systems biology approaches to metabolic networks. Yet, as we learn more about the complex microbe–microbe and microbe–environment interactions in microbial communities, it is important to understand whether and how system-level approaches can be extended to the ecosystem level. Here we summarize recent work that addresses these challenges at multiple scales, starting from two-species natural and synthetic ecology models, up to biosphere-level approaches. Among the many fascinating open challenges in this field is whether the integration of high throughput sequencing methods and mathematical models will help us capture emerging principles of ecosystem-level metabolic organization and evolution.

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Introduction

Metabolism, in addition to being the engine of every individual microbe, lies at the heart of the myriad microbe–microbe and microbe–environment interactions that shape the dynamics and evolution of microbial ecosystems. Many of these interactions are directly mediated by the uptake and secretion of nutrients and byproducts, in a global process of 'molecular recycling'. Others, such as antibiotic warfare, and quorum sensing, are often ultimately attributable to nutrient-gathering cooperation or competition strategies. Metabolism fulfills this remarkable role at multiple scales in an ecosystem, from the physiology of biofilms [1,2], to the health-disease balancing role of the human microbiome [3,4], and up to global biogeochemical cycles [5–7]. While metabolic networks and their evolution are being unraveled in individual model organisms [8–10], an increasing number of groups have started to address the question of how to bridge the gap between individual metabolic networks and the larger networks of microbial communities. This question is highly relevant to fields as diverse as systems biology, biomedicine, metabolic engineering and earth-system sciences.

Integration of experimental and computational approaches [11••,12] is going to be an essential component of this endeavor. Metagenomic sequencing data can be leveraged to determine not only community-level phylotypes and functions [13,14], but also microbe co-occurrences [15], and evolutionary patterns [16]. In general, however, interpreting metagenomic information (as well as meta-transcriptomics [17] and meta-proteomics [18]) is hindered by our lack of knowledge on gene function [19], and by our preconceptions of what we should be looking for [20]. Furthermore, while metagenomic sequencing datasets provide a snapshot of the microbial communities genetic content [21•,22], they lack the resolution of how those genes are distributed among individual cells.

In this review, we will discuss some recent literature and ideas that address the emerging conundrum of whether cell-level approaches can be scaled to ecosystem-level studies. The evolution of microbial metabolism is greatly influenced by the many possible symbiotic interactions, and by the capacity for reshuffling and invention of metabolic pathways through horizontal gene transfer [23,24]. In parallel to microbial evolution, the chemical composition of the environment itself can be permanently changed due to microbial metabolic activity [25–27,28•]. Altogether the existence of such dynamism and fluidity in microbe–microbe and microbe–environment exchanges suggests that ecosystem-level metabolism may display yet to be discovered properties and organization principles indiscernible at the level of individual species.

Stoichiometric models enable ecological thinking

Computational biology has a strong tradition of building mathematical models for individual metabolic reactions and pathways. One of the recent and most influential developments of systems biology has been the construction and application of stoichiometric genome-scale models of metabolism [29•], which use simplifying assumptions to formally capture the metabolic capabilities of the entire metabolic network of an organism. Genome-scale metabolic reconstructions constitute mathematically encoded catalogs of biochemical transformations, which describe the molecular balance of each

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biochemical reaction, and, collectively, of the overall network. They allow one to estimate how a cell might allocate available resources to efficiently produce biomass, and what uptake/secretion routes might be compatible with cellular growth at steady state. Different methods have been developed to actually take a stoichiometric matrix and produce testable predictions about metabolic activity, ranging from approaches that decompose the network into basic pathways [30,31] to optimization-based algorithms that provide predictions of individual steady state rates (or fluxes), possibly integrating gene expression [32], proteomics [33], regulatory [34,35], or thermodynamic [36] data. Ten years after the first genome-scale model of *Escherichia coli* [37], more than 50 manually curated models have been built. Furthermore, recently developed automated model building pipelines [38•,39••,40] have extended the range of available models to more than 100 organisms, and have the potential to produce a draft model for any finished genome.

Thus stoichiometric flux balance models contain all the ingredients for the transition from single species to ecosystem analysis: they are potentially available for any sequenced organism, they provide a set of possible metabolic inputs and outputs, and they are inherently modular in their construction, so that it is feasible to combine multiple organisms into a single meta-organism model. This transition could give rise to the ecosystem analog of synthetic biology. One of the goals of synthetic biology is to build genetic modules with clear input–output properties (e.g. switches, counters, amplifiers) so as to facilitate subsequent engineering of more complex devices. In the same way, now that we have whole metabolic networks of individual species as input–output systems, we can think of how to combine such individual networks to build complex ecosystems. This new endeavor will help develop new applications for synthetic ecology [41••,42–44], and enhance our fundamental understanding of evolution and dynamics in natural microbial ecosystems.

From systems to ecosystems biology of biological networks

While several genome-scale network reconstructions have been built and validated against experimental data, the research frontier described here lies in the development of the rules and algorithms for putting these networks together. Some groups have recently studied naturally occurring and artificially induced cross-feeding interactions by developing novel approaches that include stoichiometric modeling. The examples reported below have extended stoichiometric models from individual species to multiple interacting species, using rules for cell–cell metabolite exchange analogous to the ones previously used for modeling subcellular compartments in stoichiometric models of eukaryotic cells [45,46].

A first two-species stoichiometric model [47], was aimed at capturing the known mutualistic interaction between the sulfate reducer *Desulfovibrio vulgaris* and the methanogen *Methanococcus maripaludis*, based on the hydrogen flow from the former to the latter. Hydrogen benefits the methanogen as an electron donor, while its depletion benefits the sulfate reducer by maintaining a downhill free energy gradient [48]. To model this interaction, Stolyar *et al.* [47], generated a joint flux stoichiometric model where metabolites present in both organisms are distinctly accounted for in each species. Metabolic exchange between the two microbes occurs through transport reactions that link individual cellular compartments to a common extracellular 'environment' compartment. This work demonstrated for the first time that stoichiometric models can be used to predict intercellular, in addition to intracellular metabolic fluxes, providing new testable predictions about the metabolic capabilities of small microbial ecosystems. Multiple joint pairs of stoichiometric models were recently analyzed also by Wintermute and Silver [41••], who addressed the prevalence of cross-feeding by performing high throughput experimental testing of symbiotic interactions in all the possible pairs of 46 *E. coli* gene deletion mutants. They found that 17% of the analyzed pairs displayed some level of metabolic synergy, and used a variant of the minimization of metabolic adjustment algorithm [49] to qualitatively predict mutualistic interactions.

The above efforts focused on joint stoichiometric analyses of organisms expected to cross-feed because of their naturally evolved lifestyle [47] or engineered metabolic limitations [41••]. In a recent computational work, we sought to develop a broadly applicable set of algorithms to ask whether and under what environmental condition any two given species may be expected to display metabolic symbiosis. Using a framework similar to the one of Stolyar *et al.* [47], we generated ecosystem-level stoichiometric models for all possible pairs among seven different species, and probed their symbiotic growth properties under a combinatorial number of different media compositions [50••]. Surprisingly, we found that for most organism pairs, it is possible to find a large number of putative environments that induce cross-feeding, i.e. that support growth of the joint model, but not of individual species. Hence, metabolism-based symbiotic interactions may be highly abundant in communities, and highly dependent on environmental composition and dynamics. This was further emphasized by a robustness analysis, which indicated that perturbations in environmental makeup may be more likely to make/destroy symbiotic relationships than enzyme repertoire perturbations do.

A different flavor of ecosystem-level stoichiometric modeling of microbial metabolism has been implemented by Taffs *et al.* [51•]. In this analysis the authors focus on a

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consortium composed of three functional classes (guilds) of organisms (oxygenic phototrophs, filamentous anoxygenic phototrophs, and sulfate-reducing bacteria) found in thermophilic phototrophic mat communities at Yellowstone National Park. Ecosystem-level metabolic networks constructed from individual simplified guild models are decomposed into elementary flux modes, which correspond to the minimal steady state pathways that can be active in a metabolic network [30]. The authors demonstrate that different specific assumptions about how the three guild networks should be joined produce different types of inferences about the metabolic functions of the microbial community. For example if the different guilds are conjoined as separate communicating compartments, the model provides predictions of possible exchange between guilds. If, on the contrary, the guilds are collapsed into an individual ecosystem-level network (as explored also in [46]), the model helps identify global rates in the community.

In single species models, flux balance analysis has been used, among others, for asking questions about the optimality of flux distributions in metabolic networks, and for predicting the possible outcome of evolutionary adaptation experiments [52]. Similarly one may wonder what (if any) optimality principles might best describe community-level objectives. For example the traditional objectives (e.g. biomass production maximization) may not be appropriate for many microbial ecosystems with little or no net production of biomass, but could be replaced by objective function associated with putative energetic and entropic extremum principles [53–55]. Another aspect to address is whether FBA models can account for issues related to the costs of cooperation, which are typically addressed using game theory or related approaches [56•,57]. Finally, these questions will likely cross-fertilize with parallel efforts aimed at building flux balance models of multicellular eukaryotic tissues [58,59•,60].

Evolution of metabolism in the biosphere

Genome-scale networks and algorithms are promising approaches toward studying small natural or engineered microbial ecosystems. An outstanding question is whether these approaches can be applied to the much larger number of interacting species present in most ecosystems, and whether large modular stoichiometric models are going to be useful and necessary. One potential answer to this question comes from metagenomic sequencing data, suggesting that while organism lineages fluctuate extensively through time and conditions, the functional (and more specifically metabolic) content of microbial communities displays dynamic stability [61] and correlations with environmental parameters [26,62]. On the one hand, it does not seem too surprising that the chemical make up of an environment (e.g. the available redox couples [63]) should, at evolutionary time scales, determine what metabolic functions will be present in the microbial community. However, if this 'metabolic determinism' has truly been shaping the microbial world, this would have profound consequences on our understanding of life and its evolutionary history on our planet. Given this prospective of potential ecosystem-level principles of metabolic organization, it may be useful to explore stoichiometric models that consider a whole (non-structured) microbial community as a single 'soup of enzymes', disregarding the boundaries of individual species [46].

In a different context, compartment-free ecosystem-level or even biosphere-level models of metabolism have already been explored with a focus on evolutionary questions. One notable example is the network expansion algorithm [64]. This approach takes advantage of the availability of complete collections of all known metabolic reactions in any living system to ask questions about the possible order and interdependencies in the gradual expansion of metabolic networks at a global scale. The network expansion algorithms recursively determines the set of all metabolites (the 'scope') that could be in principle produced by enzymatic reactions from an initial given seed of metabolites [65]. Variants of the network expansion approach have been used to study the role of oxygen-dependent pathways in the emergence of complex multicellular life [66], the co-evolution of metabolic networks and protein sequences [67], and the interaction potential of multiple microbial pairs [68,69•]. Further developments of similar ecosystem approaches to biochemical networks, and comparisons with bottom-up compartmentalized stoichiometric models may in the future shed light on these apparently dichotomous perspectives.

Future challenges and open questions

An emerging notion in systems biology is the value of phenomenological models that identify the right level of description to effectively generate quantitative testable predictions at a given scale. Genome-scale stoichiometric models have become a prototypical example of this tradeoff between completeness and accessibility. As more ideas are being put forward on how to extend these models to ecosystems, it will be interesting to see what types of approximation or tradeoffs will end up emerging, and enduring experimental testing. In addition, it will be important to remember that the limitations of the stoichiometric approaches, such as the difficulty of incorporating regulatory information, are propagated to the next levels, giving rise to novel challenges. Will the often large fluctuations in population dynamics dwarf the importance of regulatory dynamics within individual species? How can one model and understand the interplay between these two types of dynamic phenomena and their role in shaping microbial ecosystems? And how do these two scales influence evolutionary adaptation?

Finally, while metagenomic sequencing will continue to provide insight on microbial communities, parallel

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efforts in individual genome sequencing will boost our capacity to reconstruct increasingly accurate trees of life, taking into account metabolic innovations and horizontal gene transfer. For example, a recent work by David and Alm [70••] has provided a surprisingly detailed timeline of the relevance of different biochemical processes along the ancient history of life, with especially interesting insight on the early origin of respiration. Obviously all our current understanding is based on the metabolic universe we see today. Future efforts may address this issue either by inferring putative lost metabolic reactions based on current enzymatic functions or by exploring increasingly realistic artificial chemistry scenarios that could simulate early metabolic processes [71]. If ecosystem-level principles of biochemical organization really exist, we may find out that both present-day metabolism and its prebiotic ancestor are more readily understood from an ecosystem rather than a cellular perspective.

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