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Quantitative principles of microbial metabolism shared across scales

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Metabolism is the complex network of chemical reactions occurring within every cell and organism, maintaining life, mediating ecosystem processes and affecting Earth's climate. Experiments and models of microbial metabolism often focus on one specific scale, overlooking the connectivity between molecules, cells and ecosystems. Here we highlight quantitative metabolic principles that exhibit commonalities across scales, which we argue could help to achieve an integrated perspective on microbial life. Mass, electron and energy balance provide quantitative constraints on their flow within metabolic networks, organisms and ecosystems, shaping how each responds to its environment. The mechanisms underlying these flows, such as enzyme–substrate interactions, often involve encounter and handling stages that are represented by equations similar to those for cells and resources, or predators and prey. We propose that these formal similarities reflect shared principles and discuss how their investigation through experiments and models may contribute to a common language for studying microbial metabolism across scales.

Biological systems span and encompass many scales, from molecules to organisms, communities, ecosystems and the whole Earth biosphere[1–3]. The processes involved at each scale can seem very different, yet they are intimately linked[4–7]. Individual enzymes catalyse the chemical reactions that enable organisms to grow and reproduce. These organisms interact with their local environments and with each other, forming populations and ecosystems. In turn, biological communities can alter the environment beyond their immediate surroundings, shaping the global cycles of climatically important elements, such as carbon and nitrogen, as well as providing the resources that humans rely on[8–10]. This presents a bewildering challenge. For example, to understand complex phenomena such as climate change, we need to simultaneously comprehend and quantitatively model processes from the organization of a genome of a single bacterium to the global-scale flows in the carbon cycle.

A unique aspect of microbial and biogeochemical systems that transcend scales is their metabolism: the network of enzymatic chemical reactions that sustain life. It is responsible for the synthesis of complex molecules from simple precursors and the breakdown of these molecules to provide energy. The multi-scale nature of metabolic flow is apparent from the fact that it can be quantified for an individual reaction in a single cell (for example, the rate of oxidation of glucose by oxygen as part of respiration[11]), a whole multicellular organism (for example, the oxygen consumption rate of an elephant[6,12]) or a planetary-scale ecosystem (for example, the annual rate of carbon fixation in the global ocean[13]) (Fig. 1). Studying each of these metabolic fluxes (see the glossary in Box 1) would traditionally use different tools and models, which could suggest that a unified view of metabolism is a challenge too big for anyone to tackle and too remote from standard practices to be effectively addressed.

Yet, metabolism at different scales exhibits conceptual similarity and nested architecture, which can be organized around two simple principles. We argue that these shared principles can serve as unifying themes. The first is that each of the quantities transformed by a

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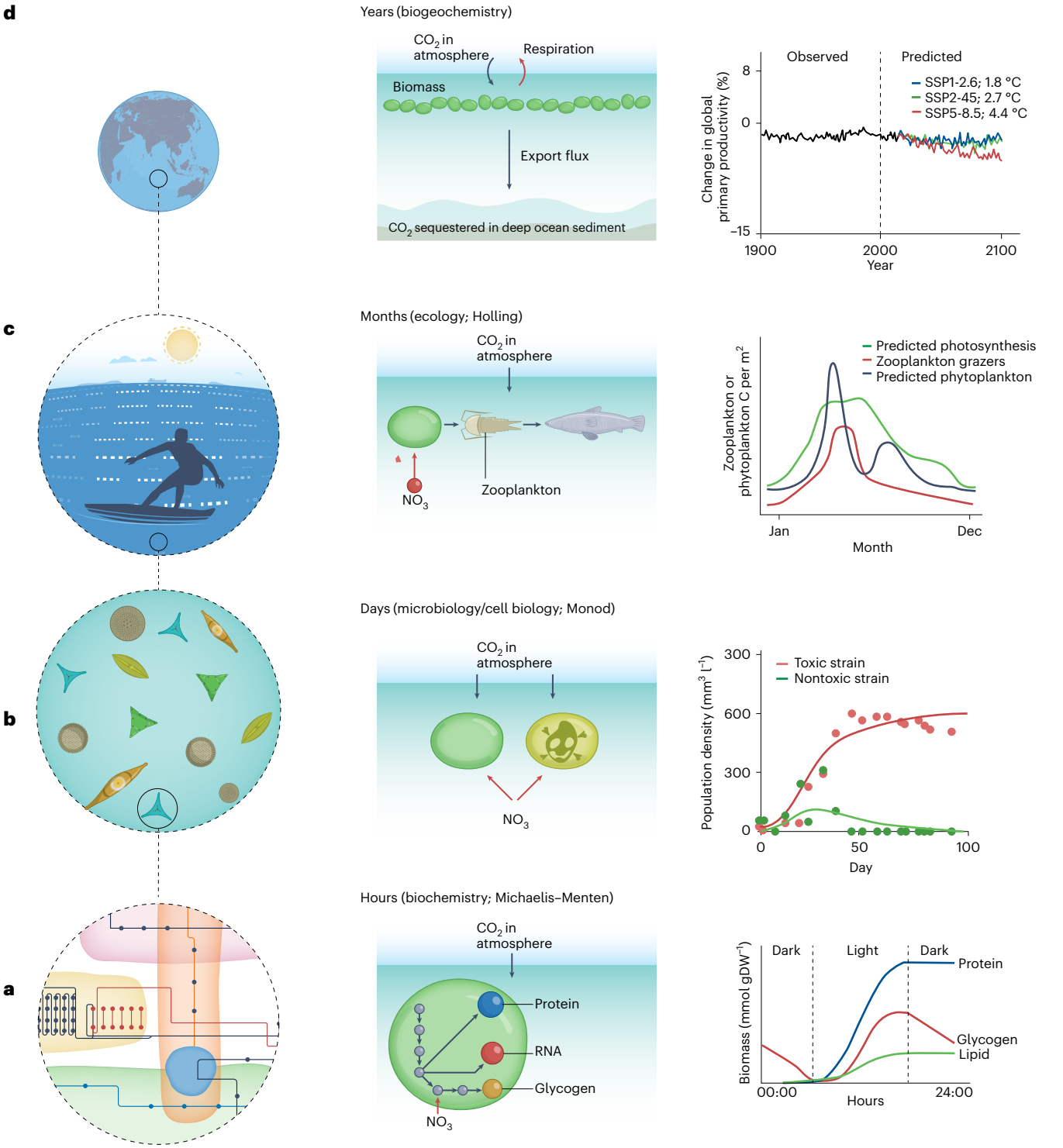

Fig. 1 | A cross-scale perspective of marine microbial systems. a, A genome-scale, FBA model can map changes in the cellular concentration of different biomass components in a cyanobacterium during the day–night cycle. Data from ref. 14. **b**, A cell-scale model describes competition between toxic and non-toxic cyanobacteria for bicarbonate during phytoplankton blooms in lakes over days. Data from ref. 15. **c**, A trophic-level (nutrient–phytoplankton–zooplankton) model describes the temporal progression of spring phytoplankton and zooplankton blooms over months. Highly simplified representations of photosynthesis and predation are used, as well as a physical description of how light changes with depth. Data from ref. 16. **d**, An ensemble of 13 climate models, each representing the physics, chemistry and phytoplankton physiology of the oceans, predicts changes in global marine primary production over the next century. The different colours show three of the five different climate change scenarios (that is, Shared Socioeconomic Pathways (SSPs)) used by the IPCC and the changes in mean temperatures they predict by the end of the century. DW, dry weight. Data from ref. 18.

metabolic flux (whether molecules, elements, electrons or energy) is subject to fundamental conservation principles and must therefore reconcile supply and demand. A second theme stems from the fact that fluxes at different scales often emerge due to the interaction between an agent of the transformation (an enzyme in a biochemical reaction or a predator in an ecosystem) and a substrate (a metabolite or prey item, respectively). This parallel in conceptual models leads to commonality between their mathematical descriptions.

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BOX 1

Glossary

Metabolic flux: The rate of conversion of substrates into products along a biochemical reaction or metabolic pathway. It is typically measured in units of the number of molecules per unit time, often also normalized to the amount of microbial mass.

Allelopathy: A biological phenomenon whereby one organism releases chemicals into the environment, affecting the growth or physiology of another. It is typically used to describe negative (inhibitory) interactions.

Monod equation: A mathematical model describing the growth rate of microorganisms as a function of the concentration of their limiting substrate in the local environment, commonly used in microbial ecology and biotechnology.

IPCC climate model: A comprehensive framework used by the IPCC to simulate and predict climate changes. It resolves multiple factors, including greenhouse gas emissions, socioeconomic scenarios, atmospheric and oceanic circulation, physics and biogeochemical cycles, to assess the potential impacts of human activities on Earth's climate.

FBA: An approach for predicting the metabolic fluxes of all reactions in an organism, based on the assumptions that the system is at steady state and has evolved towards an optimal metabolic goal.

Allosteric regulation: The modulation of an enzyme's activity by a molecule that binds to a site that is different from the active site, inducing a conformational change that modifies the enzyme's catalytic properties.

Resource Ratio Theory: An ecological concept proposing that the relative availability of multiple resources, such as nutrients, influences the composition and dynamics of ecosystems by determining the growth and competitive success of different species.

Michaelis–Menten equation: A mathematical model describing the rate of an enzymatic reaction as a function of substrate and enzyme concentrations, based on the notion that the substrate and enzyme form a complex before giving rise to the product.

Holling equations: A suite of three mathematical models that describe different scenarios of how the rate of a predator's consumption of prey changes with prey density. They help to characterize the dynamics of predator–prey interactions in ecological systems.

Nash equilibrium: In game theory, it is the solution of a game such that each participant's strategy is optimal given the strategies chosen by the other.

In this Perspective, we expand on and discuss these two unifying themes in the context of microbial metabolism, highlighting the commonalities between conceptual and mathematical descriptions at different scales. We suggest that recognition of these common themes has the potential to enhance multi-scale integration and decrease disciplinary barriers through the creation of new multi-scale models, the design of experiments linked more closely with theory, approachable cross-disciplinary education and the search for new cross-cutting principles in the study of Earth-level microbial metabolism.

Understanding metabolic flows from cells to ecosystems

We illustrate how microbial metabolism is interconnected across scales using the example of carbon and nitrogen cycles in the ocean, where tiny, photosynthetic marine microorganisms (phytoplankton) fix CO_2 into organic matter (Fig. 1). Although each individual microorganism typically contains 10^{-13} g of carbon, the aggregated activities of these organisms and others in marine food webs ultimately mediate very large reservoirs of oceanic and geologic carbon (10^{18} and 10^{22} g of carbon, respectively).

At the cellular scale (Fig. 1a), carbon fixation can lead to either the production of new functional cell biomass (for example, nitrogen-rich protein) or the storage of carbon-rich compounds, including glycogen[14]. This is determined by cellular allocation of metabolic fluxes in response to the local environment and community, as well as the genomic potential of each specific organism. Assuming that the internal metabolism of individual organisms equilibrates faster than environmental changes, steady-state genome-scale models of cellular metabolism can be used to understand and predict such fluxes. At the population level (Fig. 1b), competition for resources, metabolite exchange, allelopathy (for example, through antibiotic or toxin production; Box 1) and other processes determine the relative fitness of different species, leading to changes in population structure[15]. In the illustrated case, competition for common resources (for example, bicarbonate) between toxic and non-toxic cyanobacteria shapes the community composition. Dynamic changes can be recapitulated using kinetic models where population growth is related to the uptake rate of the limiting resource, described here using a modification of the Monod equation (Box 1). At the ecosystem scale (Fig. 1c), phytoplankton growth and primary production depend on resources, including light intensity and nutrient concentration, but phytoplankton are also preyed on by zooplankton[16]. Together, these forces shape the relative fitness of different phytoplankton phenotypes and determine the magnitude, timing and composition of large-scale phytoplankton blooms. Finally, globally integrated primary production (carbon fixation) in the ocean sustains marine food webs and fisheries and mediates a large store of carbon in the deep ocean, thus reducing atmospheric CO_2. Future changes in this store are of major societal interest and are thus represented in current Intergovernmental Panel on Climate Change (IPCC) climate models[17] (Box 1). Figure 1d illustrates an ensemble of 13 mathematical models that were used to predict future trends in global ocean primary production under several scenarios for atmospheric CO_2 (ref. 18). Each climate model in the ensemble includes different, highly simplified representations of the carbon cycle, including phytoplankton growth and death (biogeochemical components), which aim to model the collective response of the ocean ecosystem's metabolism.

Importantly, Fig. 1 also illustrates how—despite the cross-scale perspective needed to understand complex processes—research is still largely siloed within individual disciplines. The knowledge and language of scientists addressing different scales, from micrometres to kilometres, appear very different[19]. For example, understanding the function or regulation of a microbial enzyme requires very different experimental tools and training compared with those needed to understand currents that disperse and merge the cells carrying this enzyme in ocean microbial ecosystems (for example, refs. 20,21). Yet, important challenges, such as predicting the response of the carbon cycle to climate change, require a cross-scale perspective[22,23]. Similar quests for integration across scales are evident in other systems, including terrestrial/soil ecosystems and the microbiomes of animals, plants and humans[24–26].

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Balancing supply and demand to constrain complex metabolic fluxes

One of two core concepts that can serve as unifying themes across these scales and systems is the stoichiometric balance of resource supply and demand[27,28]. All living organisms have a set of metabolic demands. Specific elements and molecules serve as indispensable resources that are essential for the optimal functioning of cells, organisms and ecosystems. These resources are often needed in precise ratios, or stoichiometries, that vary by reaction. For example, nitrogen, carbon and sulfur are required in a specific stoichiometry to synthesize amino acids, whereas a different ratio of nitrogen, carbon and phosphorus is required for nucleic acids[28,29]. The environment around a cell typically does not supply all of the essential elemental resources at the optimal ratios. The mismatch of availability and requirements makes it necessary for cells, organisms or populations to carefully balance the uptake of external resources and their final fate. This balancing of resources imposes constraints on the metabolic flows within the system.

Elemental supply and demand in individual chemical reactions and total biomass

Balancing resources is familiar at the level of individual reactions. For example, here is a standard, balanced chemical equation for oxygenic photosynthesis, which leads to the production of carbohydrates:

$$CO_2 + H_2O + \text{photons} \rightarrow \frac{1}{6}C_6H_{12}O_6 + O_2 \quad (1)$$

Extending this notion to a whole cell, one can collectively write a single elementally balance equation to represent the reproduction of an individual organism or the production of biomass in a whole ecosystem:

$$106CO_2 + 16NO_3^- + H_2PO_4^{3-} + 122H_2O + \text{photons} \rightarrow C_{106}H_{246}O_{110}N_{16}P + 138O_2 \quad (2)$$

The left side of Eq. (2) represents the most common forms of inorganic nutrients in the ocean: NO_3^- as a source of nitrogen and PO_4^{3-} as a source of phosphorus, as well as the CO_2, water and photons used for the photosynthetic reaction shown in Eq. (1). On the right-hand side of the equation, $C_{106}H_{246}O_{110}N_{16}P$ represents the average composition of living biomass in marine plankton. This empirical average, known as the Redfield ratio[30], reflects the summed contributions of elements across the set of all molecules that form cellular biomass. These molecules include the carbohydrates from Eq. (1), as well as proteins, nucleic acids, lipids and thousands of other macromolecular structures and small metabolites. Importantly, the element-by-element conservation of mass (the same number of atoms on each side of Eqs. (1) and (2)) also imposes constraints on the fluxes of elements within individual chemical reactions, cells or ecosystems. Conservation of electrons and energy can also be accounted for (see, for example, refs. 31,32).

The Redfield ratio is typically applied in global-scale biogeochemical models to simulate the linked dynamics of carbon, nitrogen and other elements at the ecosystem scale. Yet, in any given environment, it is unlikely that the supply of resources exactly balances the organismal ratio, requiring modifications of Eq. (2). For example, in the case where there is insufficient NO_3^- in the ecosystem to balance the amount of CO_2, PO_4^{3-} and photons available, organisms will need to seek a different source of N, adding a new nitrogen source to the left-hand side of Eq. (2). Alternatively, they could change their biomass composition (for example, overproduce C-rich storage compounds, such as glycogen, resulting in a modified right-hand side of the equation) or reduce growth.

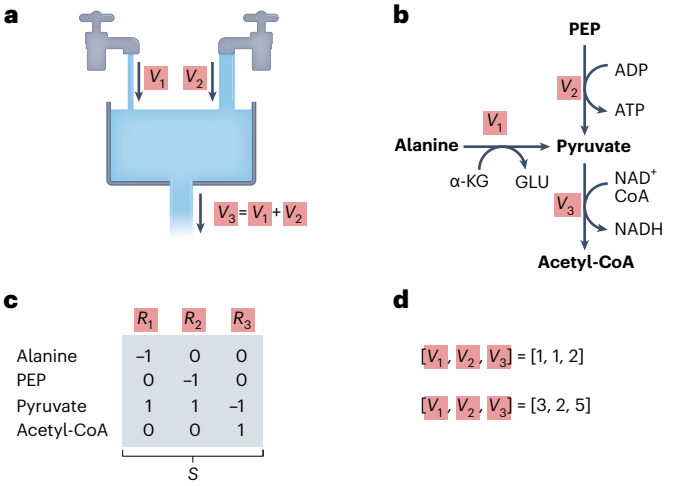

Fig. 2 | Mass balance imposes constraints on fluxes. a, A kitchen sink with two taps and one drain can be used as a simple analogy for balancing fluxes. This sink can be at a dynamical steady state, such that the amount of water in the sink does not change, despite water flowing in from the faucets and out of the drain. Conservation of (water) mass requires incoming and outgoing fluxes to be balanced, imposing a simple linear relationship between the fluxes. Note that the flux is not necessarily proportional to the amount of water in the sink (that is, there could be high flux with very little water or conversely a full sink with little flow through it) and that there are an infinite number of possible solutions. **b**, In a metabolic network, a given molecule can be produced and consumed by different reactions. If the network is at steady state (that is, the concentration of metabolites does not change in time), the flux producing a given metabolite ($V_1 + V_2$) must be equal to the sum of the fluxes consuming it (V_3). This relationship constitutes a constraint between the fluxes, meaning that once we choose two of the three fluxes the third is constrained to have a specific value. The metabolic network of a real bacterial cell comprises in the order of 1,000–3,000 reactions. **c**, The stoichiometric matrix (S) summarizes the constraints on each reaction (the balance of supply and demand) and is used (together with upper and lower bounds on the flux through each reaction, R) to solve the metabolic Sudoku. **d**, Examples of two possible solutions for the simplified network. α-KG, α-ketoglutarate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GLU, glutamate; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; PEP, phosphoenolpyruvate.

Balancing supply and demand in complex metabolic networks using flux balance analysis

Although Eqs. (1) and (2) balance atoms and electrons, what a cell is actually directly controlling is the uptake, production and loss of functional macromolecules (for example, amino acids, nucleotides, carbohydrates and so on) that lock together atoms of different elements, each with their own stoichiometry (for example, ref. 33). In fact, each cell constantly manages a complex metabolic network in which thousands of individual chemical reactions (such as Eq. (1)) are connected to each other through the usage of shared metabolites (substrates and products; Fig. 2). For example, although many cells use glycolysis and the tricarboxylic acid cycle to produce ATP and reducing power (for example, NADH/NADPH), the same pathways are also used to produce the macromolecular building blocks of biomass (for example, pyruvate, acetyl-CoA and tricarboxylic acid cycle intermediates used for the biosynthesis of amino acids). These equations all need to be balanced (Fig. 2a).

To understand how cells manage this complex balancing of resources, it is helpful to formulate the problem in terms of conservation laws and constraints. In metabolism, multiple reactions can concurrently contribute to the increase or decrease of a metabolite pool, resembling multiple sources and sinks of water in a reservoir (Fig. 2a). For the reservoir to be at steady state, all rates (or fluxes) of sources and sinks must balance each other. Similarly, for a cell to

maintain, on average, an internal metabolite at a fixed concentration, fluxes producing or consuming that metabolite must be balanced (Fig. 2b). Thus, each metabolite is associated with a constraint on fluxes. Extending this notion to all metabolites participating in a network of metabolic reactions requires simultaneously imposing multiple constraints. This gives rise to a large constraint satisfaction problem that is reminiscent of a giant, multidimensional Sudoku puzzle (Fig. 2b,c). As in a Sudoku, where the choice of a number in a column is constrained by the numbers we already have in that column, the choice of values for the fluxes that transform a given metabolite into downstream products is constrained by the net sum of the fluxes producing that metabolite.

This constraint satisfaction resource allocation approach is at the core of one of the most common approaches for modelling metabolism, known as stoichiometric modelling, constraint-based modelling or flux balance analysis (FBA; Box 1). The starting point for FBA is the construction of a stoichiometric matrix[34–36], which encapsulates the detailed stoichiometry of all molecules (rows) participating in each metabolic reaction (columns in Fig. 2b,c). Next, as illustrated above, FBA makes the simplifying assumption that all fluxes are in steady state (that is, there are no changes over time in the concentrations of the metabolites; Fig. 2a). This assumption implies that the flux variables, constrained by stoichiometry, are related to each other through a system of linear equations.

As this system of equations is typically under-determined, many possible solutions with balanced fluxes are possible, giving rise to a whole solution space called the feasible space (Fig. 2d). Additional constraints can be used to narrow down the solution space. In particular, specific fluxes in the metabolic network can be constrained to have values in a specified range. This type of constraint is generally used to limit the import of resources, based on availability in the extracellular environment or on transport capacity. If the system is still under-determined, an optimization step can be taken to identify, within the feasible space, the set of fluxes that maximize a biologically plausible objective function. Although the most commonly used function is the production of cell biomass (maximizing the growth rate), other objectives can also be used, such as increasing the production of a specific product (for example, in biotechnological applications[37]), minimizing overall flux[38] or maximizing ATP yield[39].

It is important to note, however, that behind the apparent simplicity of the FBA algorithm lie several complex and unresolved challenges. These include the process of constructing accurate microbial metabolic models based on genomic data. Most computationally generated metabolic networks have gaps that can either be real (for example, due to gene loss) or artefactual, caused by missing or erroneous annotation of gene function. Identifying and filling these gaps constitutes the subject of active research[40–42]. Moreover, for FBA to accurately predict growth, it is important to know the composition and abundance of the building blocks that compose biomass[43]. Biomass composition is taxon specific and requires different experimental techniques to characterize its different fractions (lipids, proteins, nucleotides and cofactors[44]). Additionally, although relationships between fluxes in FBA are linear, each individual flux depends on multiple factors adding hidden complexity. These include the expression level of relevant enzymes, their kinetic properties (for example, the half-saturation constant (K_m) and the maximum rate of the reaction when all of the enzyme's active sites are saturated with substrate (V_{max}); see below), post-translational modifications, allosteric regulation (Box 1) and the concentrations of reactants and products, none of which are explicitly considered in FBA. Thus, the elegance and value of steady-state stoichiometric models such as those used in FBA come at a price, namely the impossibility of predicting intracellular metabolite concentrations (but see refs. 45,46) and lack of representation of many of the mechanisms that are fundamental to the regulation of metabolism.

Environmental changes reorganize metabolic flux

Despite the internal constraints imposed by mass balance, cells and ecosystems respond to changing environments. In both cases, this is done by modulating the relative flow of metabolites through different parts of the network. For example, fertilizer overuse or other forms of pollution can lead to much more phosphorus entering lakes or coastal ecosystems relative to nitrogen, resulting in an imbalance of supply and demand for these two elemental resources. Under the resulting nitrogen-limited conditions, some organisms (including some toxic cyanobacteria) may assimilate or fix abundant atmospheric N_2 gas rather than utilize the limited amounts of fixed or reduced nitrogen (for example, in the form of NH_4^+, NO_3^-, urea or amino acids). Mole for mole, N_2 fixation is expensive relative to the assimilation of more reduced nitrogen forms, and most microorganisms do not have this metabolic capability[47]. For those that do, the extra costs may be worthwhile, enabling them to grow and consume other resources, such as phosphorus[48]. In this situation, at the cellular level, individuals with genomically encoded nitrogen fixing capability may reconfigure their internal metabolic network via changes in gene expression, leading to increased flux through the nitrogen-fixing pathway[49] (Fig. 3a). Nitrogen limitation also increases the relative fitness of nitrogen-fixing specialists (Fig. 3b), causing them to bloom, leading to a system-scale metabolic shift and enhanced nitrogen flow through a change in community composition[50]. Thus, both enzymes in an organism and organisms in a community can be viewed as dynamically adaptable catalysts of metabolic flux.

Stoichiometric models can capture some fundamental aspects of the physiological adaptation of organisms and the ecological changes in communities in response to different environmental conditions. In cellular FBA, constraints on the environmental availability (uptake fluxes) of specific metabolites translate into distinct sets of possible steady-state solutions that include or exclude, for example, nitrogen fixation[51,52]. Accurate estimation of environment-dependent redistribution of fluxes remains challenging because microbial cells alter not only their metabolic network but also their biomass composition as they acclimatize to different conditions[53–55]. Ecologists have developed similar modelling approaches, where equations describe the equilibrium fluxes of elements through organisms in an ecosystem. One key difference is that ecologists are often interested in how community composition shifts in response to external forcing and therefore focus on standing stocks (the concentrations or abundances of specific organisms or resources) and the process of competitive exclusion (Resource Ratio Theory[48]; Box 1). In contrast, cellular stoichiometry formulations typically focus on fluxes and do not delve into molecular abundances. Overall, although an aquatic microbial ecologist evaluating the water quality and plankton community in a lake and a bioengineer evaluating the production efficiency of a compound may consider different metrics and scales, they are probably using mathematical modelling frameworks that are remarkably similar.

In nature, environment-dependent regulation of genes within cells and dynamical changes of species abundances within ecosystems occur simultaneously and affect each other. In principle, these processes at different scales can be represented by a single underlying mathematical framework and used to implement more accurate predictive models. Extensions of mathematical frameworks that were developed for single organisms can be used to explore questions about ecosystem dynamics (for example, by simulating the emergent behaviour of multiple species, each of which is seeking to maximize its own growth rate)[56,57]. To bridge genome and ecosystem scales, however, several challenges need to be overcome. One of them is the differences in timescales, with chemical reactions taking fractions of seconds but cell reproduction taking minutes or hours. An approach called dynamic FBA[58] addresses this by calculating the steady-state, optimized fluxes within an organism at discrete time steps, assuming fast equilibration of intracellular

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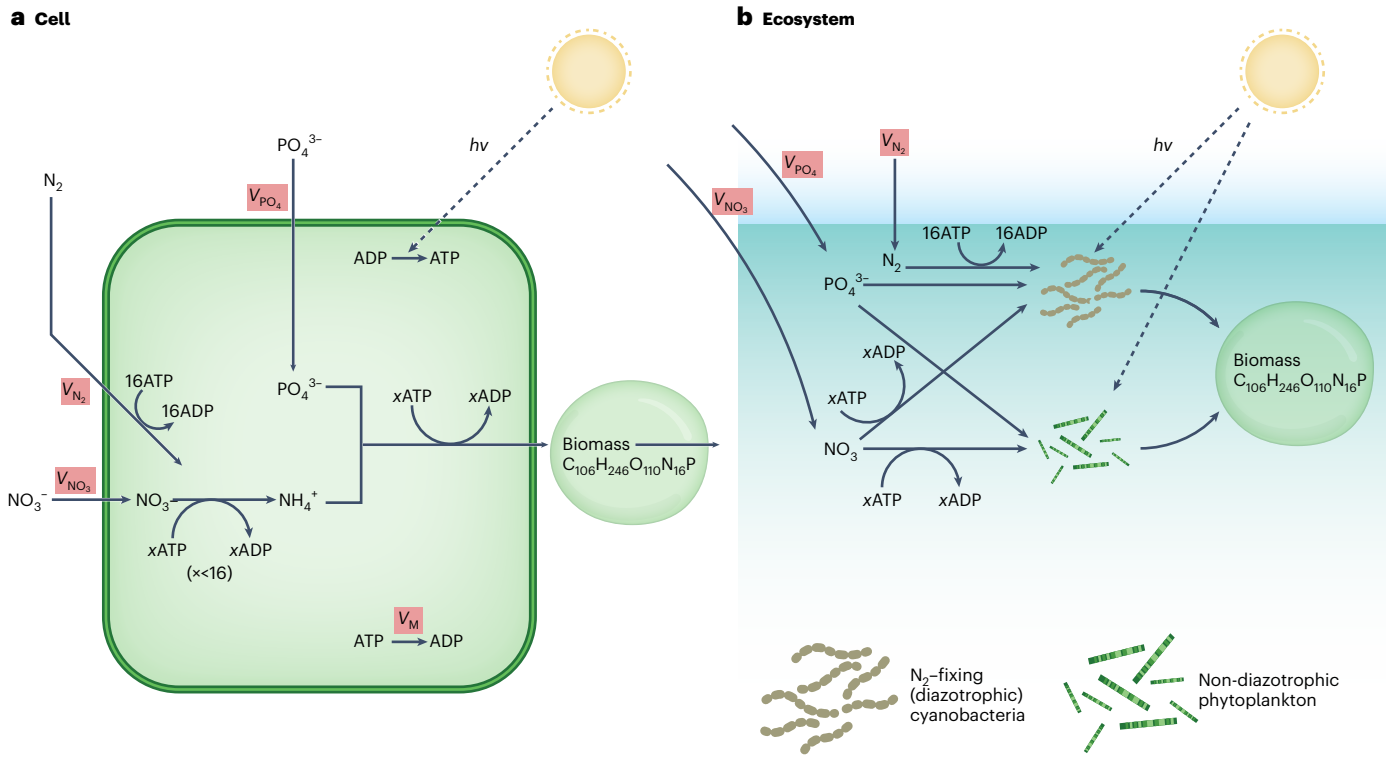

Fig. 3 | Parallel metabolic organization of a schematic cell and ecosystem. **a**, Many nitrogen-fixing cells can assimilate fixed nitrogen to build new biomass (shown here as NH_4^+ uptake) and fix N_2 only when the advantage outweighs the extra metabolic expense. These cells are essentially tackling an optimization problem and configuring the intracellular metabolic network to maximize the growth rate (see refs. 52,125 for examples of FBA simulations). In the illustrated cell, the expression of nitrogen fixation enzymes depends on the relative availability of phosphorus and ammonium; when the available NH_4^+:PO_4^{3-} ratio is low relative to demand (16:1), nitrogen fixation is cost effective provided that sunlight energy ($h\nu$) is plentiful. Usage of ATP for non-metabolic processes can be taken into account through a maintenance reaction flux (V_M). **b**, The cellular-scale metabolic reconfiguration is paralleled at the ecosystem scale (for example, in a lake). In this case, when the supply ratio of fixed N to bioavailable P (often measured as NO_3^-:PO_4^{3-}) is less than cellular demand (16:1), the extra cost of nitrogen fixation enables the assimilation of otherwise unused phosphate. This situation permits the co-existence of specialist nitrogen-fixing cells with relatively high growth costs alongside the fixed nitrogen users. When the supply ratio is less than demand, nitrogen-fixing cells are outcompeted.

metabolism[59,60]. This approach can track the abundance of different microbial populations and extracellular metabolites as a function of time, in simplified structured space[61,62]. Efforts have been made to implement FBA in complex natural ocean environments[63]. FBA can also be used to characterize and map metabolic niches, encoded in the ability of an organism to grow under different environmental conditions, and ongoing work aims to investigate how such niches can be mapped into an environmental space[64,65].

There are additional barriers to overcome before genome-scale models and Earth system models can be fully integrated, including differences in the molecular resolution at which organic matter is represented (for example, specific metabolites in genome-scale models and aggregated terms such as dissolved organic matter for Earth system models). Yet, the fact that researchers studying microbial metabolism at different scales share the use of flux as a fundamental quantity and mass balance as a universal constraint offers the opportunity to build increasingly efficient and insightful multi-scale models. Growth of this interdisciplinary area will require full partnership with experimental microbiologists and microbial ecologists, who could collaborate with theorists to incorporate estimates of metabolic supply and demand (budgeting) as standard components of experimental design (Box 2).

Understanding kinetics through encounter and handling

A second unifying concept is how the regulation of biological rates at different scales follows similar mechanistic constraints. Almost every process in biology requires an encounter between two entities, where one entity modifies (or handles) the other (Fig. 4)[66]. For example, an enzyme binds to and modifies its substrate, a cell takes up nutrients and incorporates them into macromolecules (growing) and a predator captures and eats its prey. Many biological questions revolve around how encounter and handling processes affect the concentrations of molecules, cells or organisms, and how these concentrations change over time, affecting the metabolic fluxes discussed above.

A common basis for kinetic modelling of enzymes, cells and predators

As shown in Fig. 4, enzymatic reactions, cellular nutrient acquisition and prey capture are often characterized by a saturating curve, where the rate of the process is determined by the concentration of a limiting factor. Here we emphasize that these functional response curves, which underlie several of the studies illustrated in Fig. 1, are qualitatively very similar across different processes: phenomena that were separately described for enzymes (the Michaelis–Menten equation[67]; Box 1), growing bacterial cells (the Monod equation[68]) and predator–prey interactions (Holling type II functional response[69]; Box 1) end up being represented by very similar—if not identical—equations[48,70,71] (Fig. 4). Despite taking place at distinct temporal and spatial scales, the relationships between fluxes and abundances in these phenomena can be viewed as special cases of the general concept of encounter and handling.

The generality of this concept, and of the ensuing saturation curve for enzymes, cells and predators, may be described in the form of a two-step process illustrated in Fig. 4. It is captured by the

BOX 2

Practical steps to integrate models and experiments for the study of multi-scale microbial processes

The concepts of mass balance and encounter and handling can be incorporated into the workflow of microbial ecosystem research in multiple ways, facilitating interactions between experimental and theoretical groups.

Write a mass balance (or budget) equation during experimental planning

The very act of writing an equation describing aspects of the supply and demand of a system, or formally describing its components as part of an overall budget (that is, resource allocation), helps to determine its key components and what is known about them[126]. Such an exercise may also help to identify which components can be measured easily, assess whether other components can be indirectly assessed (for example, based on mass balance) and identify gaps or inconsistencies that can hint at unconstrained or missing components.

Perform experiments in defined media where the limiting factors are known

Many microbiological experiments are performed under conditions designed to maximize experimental simplicity or biomass yield (for example, at the end of exponential growth in complex media). However, under these conditions, the environmental conditions sensed by the cells and their physiological adaptations are often unclear. Performing experiments in simple, defined media (for example, under conditions where the limiting nutrients are known) aids the calculation of fluxes or mass budget and, more generally, relates cell physiology to specific resources, including in the context of mathematical models.

Collect easily measurable data that can constrain mass balance even if they do not seem immediately useful

For example, protein, DNA and RNA concentrations can be relatively easily and sensitively measured using dyes and, being major components of biomass, can help to constrain resource allocation to other types of macromolecules (for example, ref. 127, but see ref. 128). The uptake, release or intracellular fluxes of metabolites or elements can be difficult to measure, but can sometimes be constrained by measurements of their concentrations in extracellular sources or sinks (for example, ref. 129) and can be extremely useful for testing and refining models[130]. Making such data findable, accessible, interoperable and reusable (that is, FAIR[131]) is also important.

Employ commonly used and/or experimentally measurable model parameters or variables

Many intellectually stimulating, informative and influential models explore fundamental aspects of biological systems using parameters that are either abstract (for example, generalized Lotka–Volterra interaction terms) or difficult to measure. A related issue is the use of radically different units in different fields, which can mask underlying similarities and natural connections (Fig. 5). For example, fluxes from genome-scale models are typically expressed in units of millimoles of metabolite transformed per gram of dry mass per hour. These units are very different from those used in biogeochemical models (for example, millimoles of carbon per m^3 per day). In some cases, the choice of units is dictated by technical limitations (for example, the availability of biomass or analytical limits of detection), whereas in other cases units are chosen to fit in a specific theoretical framework (for example, writing mass conservation equations for estimating carbon flow in an ecosystem). Overcoming these language barriers can be simple (for example, by clearly describing conversion factors in publications), but in some cases may require a concerted effort from scientists across disciplines to provide a community-approved set of standards and conversion utilities.

Use theory and computation to identify the most important variables to measure experimentally

A strength of computational models that can be leveraged in experimentation is the ability to perform thousands of in silico experiments, testing the sensitivity of a system to changes in individual parameters or conditions. This can help to prioritize specific experimental measurements, which account for the overall goal of the model. For example, although the importance of K_m for assessing competitive exclusion in ecosystems is clear (for example, refs. 132,133), sensitivity analyses show that this parameter (which is often difficult to measure experimentally) may be less important than loss processes, such as excretion, for describing growth in a batch bioreactor[134]. Sensitivity analyses can also be used to optimize experiments (for example, in identifying the minimum number of measurement points required to constrain a model parameter)[135].

Use fluxes and encounter and handling to teach about quantitative principles in biology

The relative simplicity and universality of the two notions discussed above—flux balancing and encounter and handling—offer a unique opportunity to motivate and foster better cross-talk between theory and experiment through education that fosters mathematical literacy in biology. For example, revisiting the Michaelis–Menten equation in basic microbiology courses and expanding the discussion to include a broader description of encounter and handling, as well as the Monod and Holling equations, can provide a concrete example for how mathematical principles apply across scales. Similarly, discussing the challenges of linking molecular genetic data and intracellular molecular fluxes, and relating these cell-scale views of metabolism to elemental fluxes, has the potential to motivate students to seek more opportunities for research at the junction between levels of organization.

following equation, which is related to that used in the derivation of the Michaelis–Menten equation:

$$\underset{\text{Encounter}}{C + X \xrightarrow{k_E}} \; C{\cdot}X \; \underset{\text{Handling}}{\xrightarrow{k_H}} \; C + Y \qquad (3)$$

Here, C is the catalyst (for example, predator), X is the reactant (or prey) and Y is the product (for example, new predator biomass). $C{\cdot}X$ is a complex in which the catalyst and reactant physically interact (for example, an enzyme–substrate complex or live prey in a predator's mouth; Fig. 4a) and has a short life span compared with the turnover times of the catalyst, reactant and product. k_E and k_H are the

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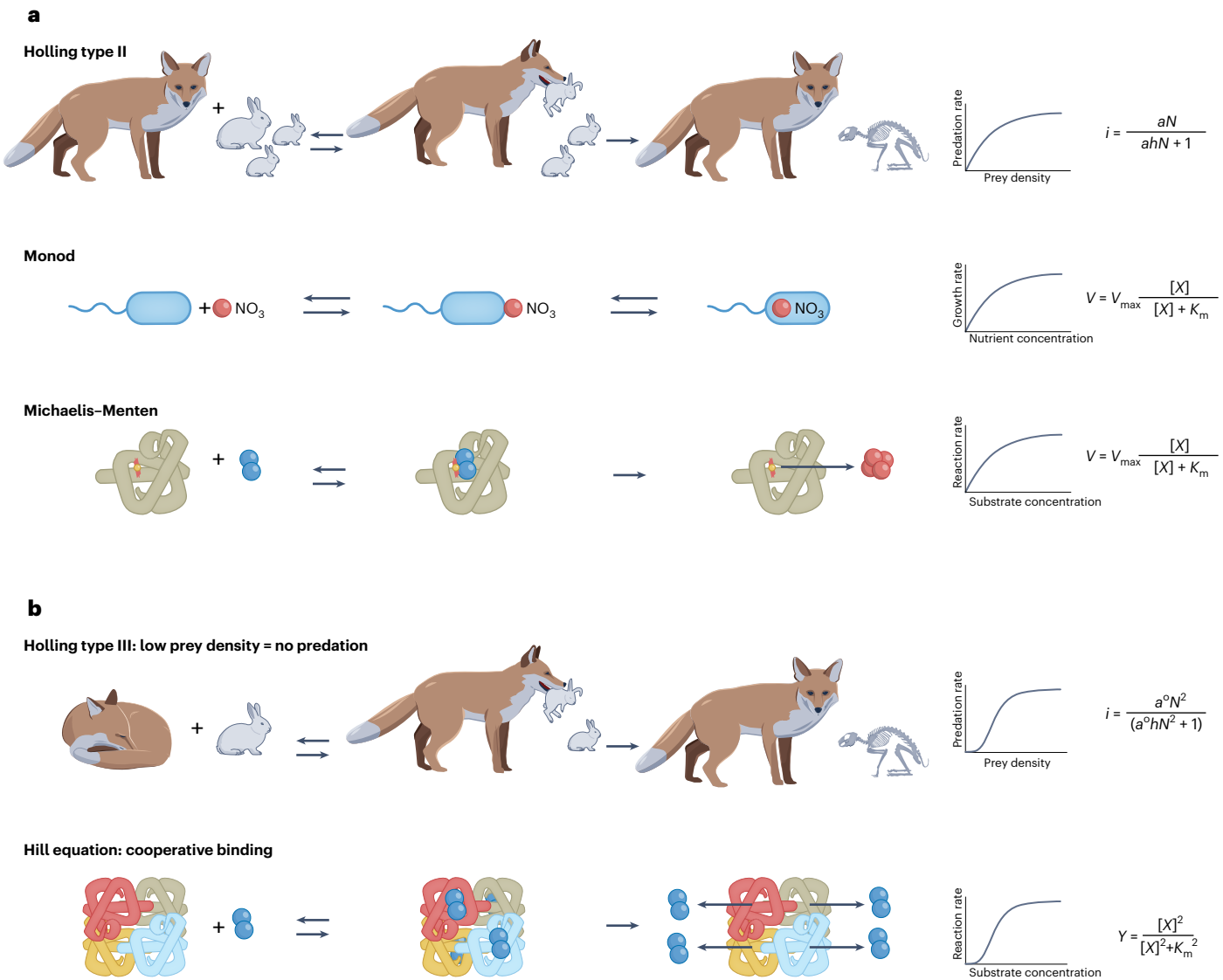

Fig. 4 | Encounter and handling processes and their representation in mathematical models. a, Schematic of biological encounter and handling processes at different scales, along with their characteristic graphs and equations. The catalysts are foxes (Holling type II equation), cells (Monod equation) and enzymes (Michaelis–Menten equation) and the reactants are prey, nutrients and substrates, respectively. In each case, the maximum rate of the process is the product of the total catalyst concentration and the rate at which it handles its reactant. The steepness with which the rate increases at low resource concentrations is characterized by the half-saturation coefficient, $K_m = \frac{k_H}{k_E}$, which is the ratio between handling and encounter rate coefficients (Eq. (3) in the main text). The encounter rate depends on, for example, the speed at which the fox searches its territory, the rate of diffusion of nutrient molecules in a medium and the rate at which substrate molecules diffuse within the cytoplasm of a cell. Increasing the encounter rate (k_E; see Eq. (3) in the main text) steepens the slope of rate versus resource. Hence, a fox increasing the speed at which it hunts might increase the rate of encounter with hares and its feeding rate at low hare densities. Note that in the Holling type II equation the parameters are explicitly described as encounter/attack (a) and handling (h) rates, while N is the prey density (equivalent to substrate concentration $[X]$ in the Michaelis–Menten and Monod equations) and i is the ingestion rate. **b**, The Holling type III equation and Hill function describe similar S-shaped relationships. The Holling type III form emerges when the encounter rate, k_E, is proportional to the resource density, $[X]$: for example, if the fox reduces its hunting effort at low prey densities. Other mechanisms lead to similar modifications of the rate–substrate relationship in enzyme kinetics. The Hill equation, for example, describes the fraction of a cooperative enzyme (Y) bound to its substrate, which depends on the cooperativity or Hill coefficient, n. The sigmoidal graphs shown here are for a Hill equation with $n = 4$ (when $n = 2$, the Hill and Holling Type III equations are identical). In the Holling Type III equation, a° is the density-dependent attack rate, defined by $a = a^{\circ}N$.

rate constants, which characterize the two stages—encounter and handling—respectively. In this simplified form, both reactions are assumed to be irreversible.

The first stage of this process is the encounter between the catalyst and reactant. In enzymatic reactions, the encounter rate depends on the diffusion of the enzyme and substrate; for microbial cells taking up nutrients, the encounter rate may additionally depend on the size of the cells, their motility and fluid flow[66,72]. In predator–prey interactions, the encounter rate depends on predator and prey motility, perception range and behavioural factors (for example, ref. 66). Following a successful encounter (production of the $C{\cdot}X$ complex), there is a handling stage during which the catalyst processes its reactant: the enzyme processes the substrate and releases it, or the predator consumes the prey.

Through a generic representation of a two-phase process (found in most biochemistry textbooks; for example, ref. 73), one can infer from

Eq. (3) a version of the commonly employed expression that relates rate, V, to the concentration of the limiting resource, $[X]$:

$$V = V_{\max}\frac{[X]}{[X]+K_m} \quad (4)$$

Here, $V_{\max}$ is the maximum flux, which depends on the handling rate of the catalyst. K_m is the concentration of the resource at which the flux is half of the $V_{\max}$ (the half-saturation). This expression has the form of a saturating function (Fig. 4) and variations of this equation have been used equally well to describe enzyme kinetics[67], predation rate as a function of prey density[69] and photosynthesis as a function of light intensity[74]. The Monod equation[68], which captures the empirical relationship between microbial growth rate and limiting resource concentration, is another well-known phenomenological law with the same functional shape, although its origin was entirely empirical rather than theoretical (see ref. 75 for extensive discussion on possible mechanistic interpretations). We note that for each of these equations the rate of generation of the product V is the flux through the reaction itself, which as discussed above is subject to mass conservation constraints when embedded in a steady-state network.

Extending the basic encounter and handling processes

Despite the commonalities highlighted above (and previously noted by refs. 48,70,71), each of these specific embodiments of the encounter and handling process (Michaelis–Menten, Monod and Holling type II) is associated with unique details and assumptions and its own rich literature. For example, for Michaelis–Menten kinetics to accurately capture real metabolic processes, it is often necessary to incorporate aspects such as reversibility, product inhibition, cooperativity and specific mechanisms for multi-substrate reactions, which modify the basic equation and behaviour of the saturation curve[73]. In some cases, similar modifications to the basic encounter and handling process were developed independently in different fields. For example, in biochemistry and pharmacology, the Hill equation captures the S-shaped functional responses when multiple substrates bind to the same enzyme or transporter (for example, haemoglobin; Fig. 4b)[59,76–78]. In ecology, a similar form, termed the Holling type III functional response, can describe predator–prey interactions affected by, for example, the predator's adaptive search effort, multiple prey types or spatial heterogeneity[66,70,79,80]. Recognizing that encounter and handling processes can provide a conceptual framework for biological processes across scales can provide the opportunity for researchers to apply knowledge gained in one field to a different one (for example, by expanding the possible variants of the relevant equations and their modulation by molecular and environmental factors).

A unified perspective for understanding microbial metabolism

We have revisited two fundamental quantitative principles of biology, highlighting their relevance across different scales and showing that the corresponding mathematical representations have much in common despite having developed independently in different fields. The concept of mass, electron and energy balance has led to constraint-based models of metabolic organization at scales from cells (at genomic resolution) to ecosystems, whereas the concept of encounter and handling lies at the core of mechanistic models for rate laws used in biochemistry, cell biology and ecology. Beyond their conceptual roots and intellectual appeal, can these similarities help to provide a common language for microbiologists across research areas and biological scales? Can they be utilized to focus experiments and provide a starting point for future efforts to construct multi-scale models of biology? We propose four practical steps that microbiologists can take to inform future research, as discussed below (see Box 2 for additional suggestions).

Using fluxes as universal connectors in metabolic modelling

Consider again the ocean carbon cycle example illustrated in Fig. 1d. Current Earth system models, which are used to simulate the coupled global carbon cycle and climate system (for example, ref. 81), typically use coarse parameterizations of the processes that are studied—and modelled—with more detail at the ecosystem, microorganism or enzyme scales (Fig. 1a–c). Although in principle it is possible to develop a highly resolved, genome-scale model of an organism and embed it within an ecological setting (for example, using dynamic FBA), this is currently computationally infeasible for global-scale models. More importantly, it is difficult to envisage a conceptually tractable model that captures every molecular process across hundreds or thousands of interacting organisms in a dynamic ocean setting. Moreover, many of the key currencies used in models of different scales are inherently different (for example, FBA resolves specific molecules, whereas biogeochemical models currently represent broader concepts, such as dissolved organic matter, which comprise thousands of (mostly unknown) molecules). As a result, models at the genome and ecosystem scale are not compatible; they require some form of translation or connection.

We propose that as Earth system models move towards incorporating more biological detail, carefully selected fluxes can serve as key connectors to mediate cross-scale integration (Fig. 5). For example, most current ocean simulations (for example, refs. 82,83) represent photosynthetic reactions using an idealized parameterization that takes into account temperature and nutrient inhibition, based on small laboratory populations[84]. To increase the biological realism of photosynthesis in ocean models, recent biophysical or molecular observations can be used (for example, ref. 85), as well as more detailed representations of photo-physiology. This could be achieved through a dedicated, high-resolution photosynthesis module, which connects to the main cell or ecosystem model via a compatible flux that can be used at both scales. For example, fluxes of glucose could provide a link between photosystem and cell scales, whereas a flux of fixed carbon could link photosystems and communities[86] (Fig. 5). An appropriate plug and play architecture, which builds on modular sub-models, each with its own relevant level of detail, could help to promote the incorporation of more detailed models that bridge scales, where appropriate, in an efficient and conceptually unified framework[87,88].

Importantly, this will require coordination between modellers and experimentalists to decide on the relevant, measurable fluxes and their units. Enforcing mass (and other) conservation laws brings powerful constraints to mathematical models of cells and ecosystems; hence, we root models in currencies for which we can enforce conservation (for example, carbon biomass or fluxes of carbon). In contrast, although molecular and genomic techniques are rapidly becoming the tools of choice with which to obtain a detailed molecular view of metabolism (for example, through changes in gene expression), these measurements cannot currently be used in the framework of mass conservation. Quantitative proteomics and metabolomics can, in principle, be translated into units of macromolecules or elements and are thus better suited for informing mass conservation[89]. Additionally, many measurements often collected as metadata for molecular or genomic experiments are in fact conservable currencies useful for modelling (see Box 2). Collaborative design of experiments, including theorists at the outset, could help to select appropriate measurements and maximize the longevity and overall value of both observations and the associated model development.

Exploring approaches to integrating stoichiometry and regulation

Beyond measuring conservable currencies, integrating gene and protein expression data with stoichiometric models of metabolism seems an obvious way of unifying metabolic and transcriptional networks towards a global predictive understanding of physiology. Unfortunately, this integration is very challenging for several reasons[90]. Gene

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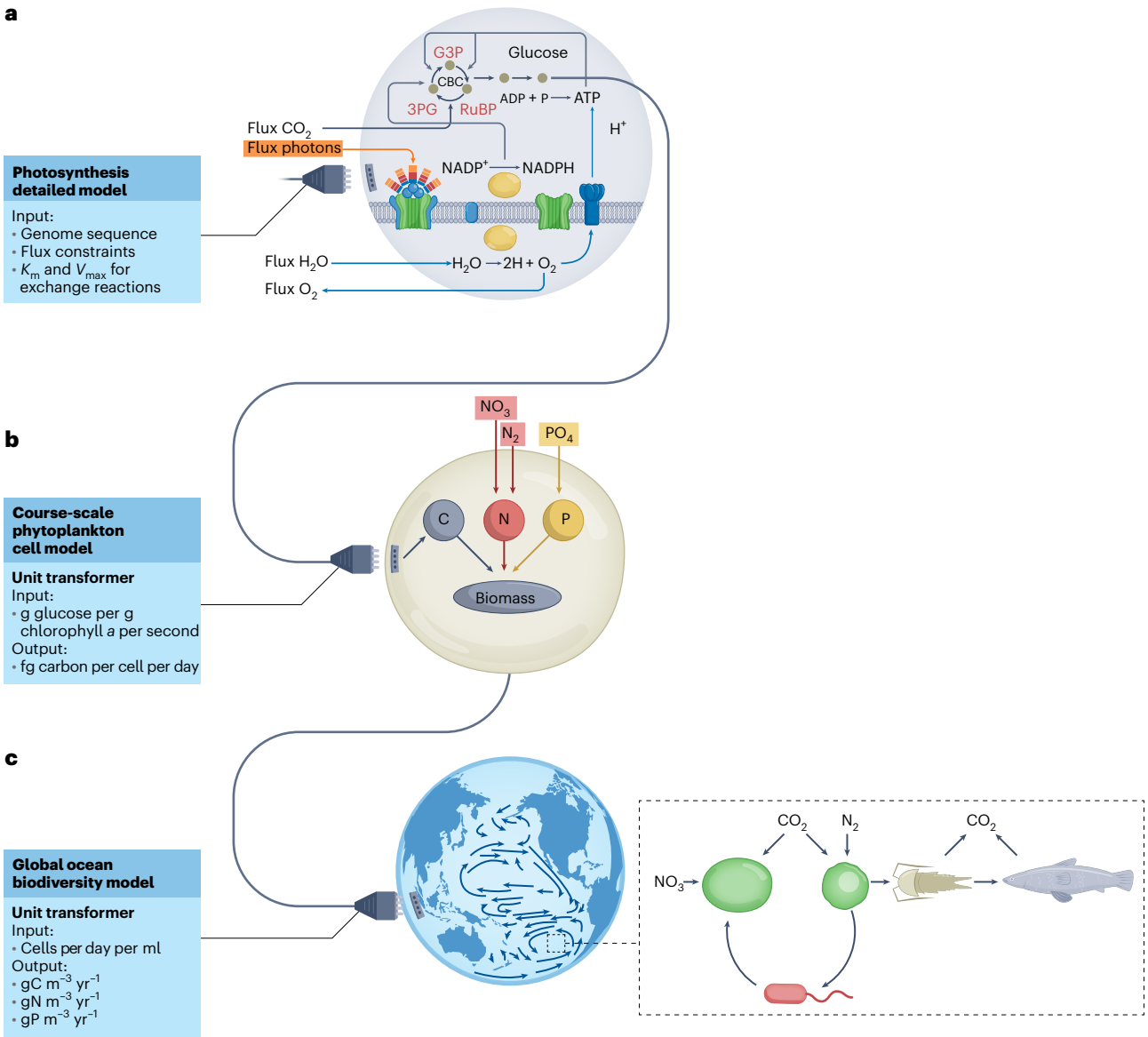

Fig. 5 | Examples of using fluxes and kinetics to connect models at different scales. a, A detailed biophysical model of photosynthesis coupled with a genome-scale model of metabolism could predict the flux of glucose produced per reaction centre. **b**, This flux could be used to link between this model (as a sub-module) and a coarse-scale model of a phytoplankton cell, but would require translation to carbon per cell. **c**, In turn, such a phytoplankton cell model could be linked with a global biogeochemical model, but again would require appropriate translation of fluxes. 3PG, 3-phosphoglyceric acid; CBC, Calvin–Benson cycle; G3P, glyceraldehyde 3-phosphate; RuBP, ribulose 1,5-bisphosphate.

expression and protein abundance do not always correlate and are rarely expressed as absolute concentrations (for example, the catalyst concentration in Eq. (3))[91]. Additionally, flux through an enzymatic reaction depends not only on enzyme concentration but also on its turnover rate and substrate affinity, which are often unknown, as well as on the substrate concentration, which is not modelled in FBA. Finally, enzyme activity is often allosterically modulated by its product or products of other reactions in the same metabolic pathway (for example, ref. 92).

The inherent limitations that hinder the integration of -omics data with flux-based models are very challenging but perhaps not unsurmountable. For example, systematic measurements of gene expression, protein abundance and metabolic fluxes from the same system may help to identify whether there are specific metabolic pathways in which gene expression or protein abundance measurements are consistently correlated with fluxes. Such pathways can serve as the focus of initial integration efforts. Moreover, theoretical and experimental approaches should explore new ways of integrating allosteric regulation in metabolic models (for example, by using thermodynamic constraints[93] and incorporating metabolite concentrations and their effects on enzymes for selected compounds). Systematic exploration of allosteric interaction networks and their representation in databases may enable an approximation of their effect on metabolic fluxes, possibly though modifying flux constraints in FBA (as is currently explored for kinetic parameters using algorithms such as GECKO[94]). Finally, it is possible that the rising amount of high-throughput data on gene expression, protein abundance and metabolic fluxes will help to build a new generation of hybrid machine learning–mechanistic models. Such approaches could, for example, use data-driven inference of regulation to impose constraints on metabolic fluxes in stoichiometric models of metabolism. The challenge of integrating -omics data is exacerbated in current ecosystem-scale models, where the link between the transcription rate of specific enzymes, for example, and coarse-grained

modelled flows of organic matter is even more tenuous and challenging to quantify. Yet, the potential for efficient, systematic sampling at the ecosystem scale using molecular metrics is clearly immense, and continued efforts to intercalibrate molecular and mass-based metrics of population are important[95–97].

Embracing evolutionary principles

In addition to aiding our understanding of the dynamics of molecules, cells and ecosystems, some of the mathematical approaches described above are strongly related to the role of evolutionary adaptation in shaping metabolism. For example, in its most frequent formulation, FBA describes the metabolic fluxes of an organism under the hypothesis that its regulatory mechanisms have evolved to support the objective of a maximally efficient production of biomass[43,98,99]. However, this hypothesis falls short of describing the incredibly diverse set of strategies employed by living organisms alone or in communities. In addition to environment-dependent variations in the composition of biomass, and alternative objectives that may best capture cellular goals during growth in noisy environments, fundamentally different optimization processes may occur during stress and starvation. Answering some of these questions will require broader definitions of condition-dependent objective functions that can be tested directly or inferred from experimentally measured fluxes[39,100]. This will require carefully designed experiments (for example, combining laboratory-controlled evolutionary experiments with detailed flux and biomass measurements)[99,101].

The role of evolutionary adaptation in shaping metabolism has also been explored at the ecosystem level. Extending the notion of optimality used in FBA, microbial community dynamics has been studied as an emergent property of multiple organisms each pursuing its own evolutionary objective[56,102]. However, the evolutionary trajectories of community members can be strongly coupled with each other. For example, key metabolic functions can be lost by some organisms, as long as others can still perform this function (a process termed the black queen hypothesis[103]). This kind of process can be studied using evolutionary game theory[104] and has been combined with flux balance modelling to predict Nash equilibria of multiple strains sharing resources[105]. At even larger scales, other approaches have asked whether optimality principles based on non-equilibrium thermodynamics can be identified for ecosystems and their metabolism[106,107]. This raises the question of how to reconcile the bottom-up view of ecosystems being shaped by Darwinian selection acting on individuals with the top-down notion of physical laws dominating the outcome at the ecosystem level[108].

Finally, it is not only the ecosystem-level flux that can be shaped by evolutionary adaptation, but also the structure of metabolism itself. In addition to modulating the regulation of gene expression or enzymatic kinetic parameters to affect fluxes[101], evolutionary processes can modify the shape of the metabolic network (for example, through gene gain or loss and through the acquisition of new functions). This can be investigated by performing simulations of horizontal gene transfer and adaptive gain or loss of metabolic functions[109]. Such analyses are beginning to explore both reductive evolution of organisms (for example, symbiosis within a host organism)[110] and expansion of metabolism on ecological and geologic timescales[4,111–117].

Seeking new principles

Beyond the recommendations discussed above, these formal similarities could suggest the existence of novel fundamental principles waiting to be discovered. Here, we can only speculate about the existence and nature of such principles. The scales described here are discrete levels within a much richer continuum of scales, for which a formal description is yet to be invented. It is possible that data-driven approaches will gradually help scientists to identify flux variables that are the most helpful descriptor of a system for each research question. These flux variables may encode aggregates of molecular fluxes constrained by environmental, metabolic or ecological constraints, somewhere in between detailed molecular fluxes and broad elemental fluxes (for example, fluxes of specific proportions of N and C). Natural candidates for such aggregates are metabolic pathways or modules, but one could envision other units of metabolism, perhaps evolving under similar driving forces (for example, ref. 118). One could then ask whether such fluxes satisfy specific flux balance constraints and if they can be expressed as the effective outcome of encounter and handling processes. Similarly, an ecological resource ratio model could be formed around consortia of organisms with different trophic strategies instead of single functional types (for example, mutualistic autotrophs and heterotrophs), yet similarly formulated in principles of flux balance and encounter and handling kinetics. Flux balance and kinetic equations may operate at any scale we may be interested in or are able to experimentally assess.

Conclusion

As the importance, challenges and ramifications of multi-scale models are becoming apparent in microbial ecology, we propose that two concepts—balancing supply and demand, and the ubiquity of encounter and handling processes—provide a common language for discussing metabolism across scales and disciplines. Clearly, we have not been able to cover every aspect of how metabolism operates and evolves (for example, group selection[119,120] and eco-evolutionary dynamics[121,122]) or other important concepts, such as the scaling of organism size and metabolic rates[123,124] and how they have evolved[6]. Yet, we hope that our effort to consider concepts and equations that are typically taught in different classes and discussed at different conferences will inspire new creative ways of understanding how metabolism links the microbial and planetary scales.

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