Microbial redox interplay enhances biodiversity and ecosystem productivity

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Microbial redox interplay enhances biodiversity and ecosystem productivity

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Summary paragraph

A significant portion of the Earth's biodiversity and biomass is from the subsurface biosphere, where chemotrophic microbial species harness diverse inorganic oxidation-reduction reactions (redox reactions) as a major source of metabolic energy while driving biogeochemical cycles. Given the limited availability of energy in the anaerobic environment, a fundamental question concerns what interplay between the chemical environment and chemotrophic community enables the persistence of whole biogeochemical systems. Here, using a thermodynamics-based mechanistic model that maps the interplay between diverse chemotrophic species and chemical compounds onto a redox network, we show that cycles of redox reactions mediate chemotrophic interactions in a way that increases the complexity of both redox reaction networks and microbial communities and enhances the community-level efficiency of energy metabolism. The high efficiency and complexity of biogeochemical systems arises from the self-organised ecological niche segmentation of microbes. More specifically, a

25 consortium of chemotrophic species that subdivide a long-reaction pathway into
26 shorter-reaction segments enhance each other's population growth, replaces the
27 species that monopolises the long-reaction pathway, and increases ecosystem
28 productivity. An ecologically driven 'division of metabolic labour' in the
29 chemotrophic community provides a novel mechanism through which an intimate
30 life-environment interplay concurrently enhances biodiversity and ecosystem
31 productivity.

Main

Microbial cells exist up to 2.5 km under the surface of marine sediments^{1,2}, and the subsurface biomass contributes to a significant portion of the Earth's biomass^{3,4}. According to the previous estimations, the microbial biomass hidden beneath the surface is several to tens of Pg C^{3,5,6}, which accounts for 70% of the total microbial cells on Earth⁷. The major energy sources for the subsurface biosphere, given the limited supply of light and photosynthetic products, are redox gradients (e.g., oxic-anoxic interfaces or chemical gradients within deep-sea hydrothermal vent systems), which provide chemical energy via redox reactions^{8–10}.

The maximum energy production per redox reaction, or the negative Gibbs energy change of reaction ($-\Delta_r G_r$), available in the subsurface anaerobic environment is, in general, significantly lower than aerobic respiration. Thus, there is a question of how chemotrophic microbial communities efficiently yield energy from the thermodynamically less favourable environment and maintain their biodiversity^{11,12}. Current theory suggests that redox reactions can mediate mutualistic interactions between chemotrophic organisms and allow for the efficient utilisation of redox reactions^{13,14}. However, earlier studies investigated relatively simple systems, thus leaving the role of eco-geochemical interactions in a more realistic multi-species, multi-compound system unanswered.

Thermodynamic understanding of chemotrophic community dynamics

To understand the eco-geochemical interplay mapped onto a complex redox network, we constructed a mechanistic model that linked the population dynamics of chemotrophic species to a chemical environment based on thermodynamic and

electrochemical properties. Assuming that there are elements X ($X = A, B, C, \dots$) with X -containing compounds, denoted by X_j ($1 \leq j \leq N_X$), the half-reaction $X_j + (z-j)e^- \rightleftharpoons X_z$ holds where $j < z$ and e^- denote an electron. A model redox network consists of redox reactions formulated from all possible combinations of two half-reactions that are available in the system (see Supplementary Information, section S.1 and Extended Data Tables 1 and 2). The topological structure of a redox network and the number of possible redox reactions, or links, forming the network (N_{reac}) are uniquely determined for a given composition of compounds of elements, or nodes (Fig. 1a, b and Extended Data Table 3). We assumed that there are as many chemotrophic microbial species (a group of species that utilise an identical redox reaction as an energy source, and hereafter referred to as a species (Sp.)) as N_{reac} . The population dynamics of Sp. i , which specifically harness reaction i for population growth is described as:

$$\frac{dM_i}{dt} = q_i(c_i(-\Delta_r G_i)f_i - m_i)M_i \quad (1a)$$

where M_i is the biomass of Sp. i ; $-\Delta_r G_i$ (kJ mol⁻¹) is the negative Gibbs energy change of reaction ($-\Delta_r G$) of the i th reaction; f_i is the microbial catalytic rate of the i th reaction (see Methods); q_i is the biomass yield per energy; c_i is the fraction of energy that can be used for adenosine triphosphate (ATP) synthesis ($0 \leq c_i \leq 1$); and m_i is the maintenance energy of Sp. i ¹⁵. According to thermodynamics, $-\Delta_r G_1$ for a reaction with compounds A_1 and B_2 as reactants and A_2 and B_1 as products in Fig. 1 is given by:

$$-\Delta_r G_1 = -\Delta_r G_1^\circ + RT \ln \frac{\{A_1\}\{B_2\}}{\{A_2\}\{B_1\}} \quad (1b)$$

where $-\Delta_r G_i^\circ$ denotes the negative standard Gibbs energy change of reaction i , the value of which is reaction-specific and determined by the compounds associated with reaction i at a given temperature and pressure (see Methods); R is the gas constant; T is the absolute temperature; and $\{X_j\}$ is the activity of the chemical compound X_j . We assumed that the dynamics of the molar concentration of X_j were determined by the input from and output to the surroundings and the abiotic and microbial reactions (see Methods and Supplementary Information, section S.2).

Equations 1a and b have two ecological implications. First, species that harness reactions with higher $-\Delta_r G^\circ$ can obtain larger ATP yields per reactant and, therefore, enjoy larger growth rates. Each redox reaction can be regarded as an ecological niche whose potential quality at the standard condition is given by $-\Delta_r G^\circ$, and a species using a high-quality reaction (high-quality niche) is more likely to grow faster. Second, the population growth rate is modified by the chemical environment, as indicated by the dependence of $-\Delta_r G$ on the activity of chemical compounds $\{X_j\}$, with the population growth rate increasing with increasing amounts of reactants or decreasing amounts of products. These formulas provide a basis for understanding chemotrophic community dynamics in terms of community ecological theory.

Interspecific interactions in chemotrophic communities and reaction segmentation

Interspecific interaction is a key concept in community ecology and provides a mechanistic basis for understanding multi-species population dynamics. The dependence of population growth on the chemical environment, as depicted in equation

1b, identifies three types of compound-mediated interspecific interactions operating in chemotrophic communities. The first is ‘reactant-mediated competition’, which is a negative interaction arising between species that consume the same resource (e.g., Sp. 1 and 3 in Fig. 1). The second is ‘product-mediated competition’, which is another kind of negative interaction (and unknown in animal and plant communities) arising between species that produce the same product (e.g., Sp. 2 and 3 in Fig. 1). The third is ‘mutualism’, which is a positive interaction that occurs when a species (the donor) supplies its product to another species (the recipient) as a reactant (e.g., Sp. 1 and 2 in Fig. 1)^{14,13}. Note that the positive effect is not only from the donor species to the recipient species; rather, the donor also receives a benefit from the recipient as the removal of product increases $-\Delta_r G$ of the donor’s energy-source reaction.

The model analysis revealed that the three types of interspecific interactions can jointly lead to reaction segmentation, which was identified as a key process for understanding the organisation of chemotrophic communities. A redox reaction can be divided into sequential reactions in the presence of appropriate electron carriers (B_1 and B_2 in Fig. 1a) or intermediates (A_2 in Fig. 1b) that mediate electron transfer between the two reactions. When a reaction is segmented, energy production yielded from either subdivided reaction is lower than the overall reaction at the standard condition (see $-\Delta_r G_i^\circ$ values in Fig. 1). Therefore, it is reasonable to assume that the overall reaction should generate more metabolic energy and, thus, be preferred to either subdivided reaction. However, this is not the case with consortium species (Sp. 1 and 2 in Fig. 1) that use the subdivided reactions and may competitively exclude solitary species (Sp. 3 in Fig. 1) that use the overall reaction. The replacement can be explained by the combined effects of the above-mentioned three kinds of interactions. Each consortium

species is in a competitive relationship (reactant- or product-mediated) with the solitary species, while the two consortium species are in mutualistic relationship, as mentioned above. If the right conditions are met, the consortium species may replace the solitary one, and the overall reaction would be segmented into several subdivided reactions. Reaction segmentation can be regarded as the result of an ecological division of metabolic labour, which enables more efficient resource—and energy—use (see Supplementary Information, section S.4.3 and Extended Data Fig. 3).

Chemical environments and chemotrophic community structure

Ecological theory predicts that resource availability and composition are essential determinants of community structure. The same is true for chemotrophic communities for which redox reactions serve as energy sources, and the structure of the redox network has a substantial impact on consortium formation and the division of metabolic labour. Key parameters that strongly affect the occurrence of the division of metabolic labour are identified through the analysis of model communities with varying chemical environmental conditions.

First, the amount of chemical compounds in a system should be large enough for the mutualistic division of metabolic labour to take place. For example, in a model system with ≤ 2 species at a steady-state, mutualistic consortium is more likely to emerge when compound availability (X_{tot}), as measured by the ratio of input rate to output rate, is larger (Fig. 2a; see Methods). This is because compound availability increases the likelihood that sufficient amounts of electron carriers or intermediates, which are necessary to subdivide an energy-providing redox reaction, are present in the system. If compound availability is not large enough, the community will be dominated

by one species or two species that are in a competitive relationship. The same is true for a multi-species system, where the benefit a species receives from the entire community is larger in a community with higher compound availability (Fig. 3a; see Methods for the definition of ‘the degree of mutualism’).

Second, due to the similar reason, the diversity of chemical compounds favours the division of metabolic labour. For model systems with ≥ 2 species, we set a 3-element chemical environment with a varying number of compounds per element (2 to 4 compounds each) and evaluated how the community structure responded to the changed compound diversity ($N_{tot} = N_A + N_B + N_C$) (the steady-state number of species at N_{tot} is summarised in Extended Data Fig. 4). The model analysis revealed that a mutualistic consortium was more likely to be formed in a redox network with higher compound diversity, as suggested by increased mutualism (Fig. 3a). This occurs as a larger N_{tot} achieves more complex pathways within a metabolic network and increases the likelihood that appropriate electron carriers or intermediates are present in the system.

Third, the catalytic effectiveness at which chemotrophs accelerate redox reactions also affects community structure. The maximum catalytic rate constant (r_i ; see Methods) is the maximum rate at which a chemotroph catalyses a redox reaction. The model analysis revealed that r_i had a considerable effect on the establishment of a mutualistic consortium in a system (Fig. 2b). More specifically, when the maximum catalytic rate constant was higher, the division of metabolic labour was more likely to take place (Fig. 3b). This can be explained by the coupling strength between chemotrophic community dynamics and chemical reactions. For a consortium species to outcompete a solitary species, the mutualism-driven positive feedback between

consortium species should be strong enough. The maximum catalytic rate constant determines how strongly a consortium species can enhance the population growth of the other consortium species and, therefore, can strongly affect the competitive outcome and the emergence of the division of metabolic labour.

Ecosystem productivity enhanced by mutualistic consortiums

Segmentation of the reaction pathway caused by the mutualistic division of metabolic labour can improve energy acquisition efficiency at the community level and enhance ecosystem productivity. In both systems with ≤ 2 species and multi-species, the $-\Delta_r G_i^\circ$ values of the reactions used by chemotrophic species were smaller when the mutualistic division of labour was favoured (larger X_{tot} or larger N_{tot}) (Fig. 3c and Extended Data Fig. 5). This is because the establishment of mutualistic relationships enables the chemotrophic community to persist with reactions that have a smaller energy production in the standard state ($-\Delta_r G_i^\circ$). That is, reactions with smaller $-\Delta_r G_i^\circ$, which are left unused when non-mutualistic, solitary species dominate ($X_{tot} = 10^{-4}$ in Fig. 3c), can be efficiently utilised when a mutualistic consortium is established. As a result, ecosystem productivity, as measured by the median of the total biomass, is also improved under conditions that favour the mutualistic division of metabolic labour (Fig. 3d).

Discussion

We have shown how the chemotrophic community is organised under the thermodynamic rules that govern chemical reactions and energy transformations. The eco-geochemical interplay gives rise to three kinds of interspecific interactions:

198 reactant-mediated and product-mediated competitions and mutualism. These lead to the
199 key process of the division of metabolic labour, which subdivides an energy-providing
200 redox reaction into several segmented reactions and, as a result, increases complexity in
201 a reaction network and is accompanied by increased ecosystem productivity and
202 microbial diversity. It is the explicit incorporation of free energy in formulating the
203 chemotrophic dynamics that reveals the ecological properties of the chemotrophic
204 community.

205 Giving consideration to eco-geochemical interplay is essential to
206 understanding why some redox reactions are favoured, while other are not. Redox
207 reactions are regarded as niches for chemotrophic species. Therefore, one might expect
208 that a species would favour a reaction with a high $-\Delta_r G^\circ$ as its energy source. However,
209 the redox reaction network is shaped through niche construction processes such that
210 utilization of one reaction changes the amount of energy available from other reactions,
211 i.e., not $-\Delta_r G^\circ$ but $-\Delta_r G$. Therefore, as shown by the model analysis, reactions with
212 relatively low $-\Delta_r G^\circ$ could be more favoured due to the biological niche construction
213 effect. The interplay between chemotrophs and the chemical environment might explain
214 why cultivation techniques have not been established for some chemotrophs, especially
215 chemolithoautotrophs living in oligotrophic environments. What may be necessary for
216 successful cultivation might not only be unknown physicochemical conditions, but also
217 the co-presence of key species that support the survival of the target species through the
218 modification of the redox network.

219 The division of metabolic labour can be regarded as a kind of niche
220 partitioning. Yet, it is fundamentally different from those in animal or plant
221 communities in that, due to the nature of the redox network, it is accompanied by the

establishment of mutualistic interactions. The mutualistic nature of the division of metabolic labour has implications for the classic issue concerning the relationship between community complexity and ecosystem functioning. The model community of chemotrophic microbes demonstrates that chemotrophs can acquire energy more efficiently, and the total biomass becomes higher, when the number of compounds or species is larger. This suggests that the interplay between chemotrophs and chemical energy can act as a mechanism for a positive complexity-productivity relationship to emerge. A complex metabolic network with more subdivided pathways may increase the stability of chemotrophic ecosystems because the fragmentation of a metabolic network is inseparable from increases in mutualistic interactions and the subsequent enhanced productivity.

The present study explored the basic nature underlying how a chemotrophic community in an anaerobic environment is organised via the explicit consideration of thermodynamic constraints. Further model verification and extension will help us to understand more specific inter-relationships between microbial and biogeochemical networks. Aerobic microbes that gain energy from aerobic respiration with a high $-\Delta_r G_i^\circ$ appear to be dominant under the high availability of organic matter and oxygen, which may be further addressed by investigating the effect of the availability of specific compounds on a microbial community. Moreover, the redox reactions available for ATP production are not only limited by thermodynamics but by kinetics (e.g., activation energy) and other physico-chemical properties of compounds (e.g., oxygen toxicity) related to the reaction¹⁶.

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Figure Legends

Fig. 1 | Structures of redox networks and examples of metabolic interactions. a, When $(N_A, N_B, N_C) = (2, 2, 2)$ and **b**, when $(N_A, N_B, N_C) = (3, 2, 0)$. The number i in the circle stands for the i th reaction and species. Blue and red arrows correspond to oxidation and reduction reactions, respectively. The right panel illustrates the half-

reactions that build overall redox reactions 1, 2, and 3 (see Supplementary Information, section S.1) and the metabolic relationships among species 1, 2, and 3.

Fig. 2 | The number and relationships of species when ($N_A, N_B, N_C = 2, 2, 2$). **a**, The steady-state responses to the number of compounds X_{tot} , and **b**, the maximum catalytic rate constant, r_i . During the simulation in (**b**), r_i for all species was fixed to the same value. 0 and 1 meant that either no species or 1 species existed in a steady-state, respectively. Two species existed in a steady state for cases 2C and 2M and could either compete for the same resource and produce the same compound (C) or mutually divide the metabolic labour (M).

Fig. 3 | Steady state responses to changes in the availability of compound X_{tot} , and the number of compounds, N_{tot} . **a**, The degree of mutualism (see Methods for the definition of ‘the degree of mutualism’), **b** and **c** are the median of $-\Delta_r G_i^\circ$ of the energy-source reactions and the maximum catalytic rate, r_i , of all species surviving at a steady-state with other species. **d**, the median of the total biomass, $\sum M_i^*$, for 1000 iterations.

Methods

An algorithm for formulating redox reactions and constructing a redox network model

We developed an algorithm in the Wolfram language in Mathematica 12, which automatically formulates redox reactions when all of the numbers of the compounds of X element, N_X , are provided (see Supplementary Information, section S.3). The programs are available in the *Programs* folder. By running the processes 1 to 3 in *Simulation.nb*: i)

the standard Gibbs energy change of the compounds is randomly selected, ii) half reactions with corresponding standard reduction potentials are formulated (Extended Data Table 1), and iii) redox reactions are generated for all of the possible combinations of two half reactions (Extended Data Table 2). The number of possible redox reactions, N_{reac} , were uniquely determined depending on the number of compounds of elements (Extended Data Table 3).

The microbial catalytic rate and the standard Gibbs energy change of formation

The microbial catalytic rate of a Sp. i , f_i (in units of $\text{mol } X_j \text{ L}^{-1} \text{ mg}^{-1} \text{ time}^{-1}$) was determined by the consumption rate of compound X_j in the first term of the left side of its energy-source redox reaction. When a species harnesses a disproportionation reaction, the microbial catalytic rate follows the Monod (or Michaelis-Menten) equation. Using reaction 9 in Extended Data Table 2 as an example,

$$f_9 = r_9 \frac{[A_2]}{K_{9A_2} + [A_2]} \quad (\text{M.1})$$

where $[X_j]$ denotes the molar concentration of X_j , r_9 is the maximum catalytic rate constant of Sp. 9 ($\text{mol mg}^{-1} \text{ h}^{-1}$), and K_{9X_j} is the half-saturation constant for X_j of Sp. 9. For species harnessing the rest of the redox reactions, their microbial catalytic rates follow the dual Monod equation¹⁷. Using reaction 1 in Extended Data Table 2 as an example,

$$f_1 = r_1 \frac{[A_1]}{K_{1A_1} + [A_1]} \frac{[A_3]}{K_{1A_3} + [A_3]} \quad (\text{M.2})$$

The standard Gibbs energy change of the i th reaction, $-\Delta_r G_i^\circ$, is equal to the sum of the standard Gibbs energy change of formation of reactants minus that of the products. Thus, for reaction 1 in Extended Data Table 2,

$$-\Delta_r G_i^\circ = \Delta_f G_{A_1}^\circ + \Delta_f G_{A_3}^\circ - 2\Delta_f G_{A_2}^\circ \quad (\text{M.3})$$

where $\Delta_f G_{X_j}^\circ$ denotes the standard Gibbs energy change of formation of compound X_j .

The dynamics of the molar concentration of a compound

The dynamics of the molar concentration of the j th compound of element X, X_j , follows

$$\frac{dX_j}{dt} = I_{X_j} + [\text{Abiotic supply rate}] - [\text{Abiotic consumption rate}] + [\text{Microbial supply rate}] - [\text{Microbial consumption rate}] - D_{X_j}X_j \quad (\text{M.5})$$

where I_{X_j} and D_{X_j} are the inflow rate ($\text{mol L}^{-1} \text{h}^{-1}$) and the outflow rate constant (h^{-1}) of X_j , respectively. X_j is abiotically and microbially supplied and consumed, and the abiotic reaction rate is proportional to the product of the molar concentration of the reactants. The microbial catalytic rate is given by the product of $f_i M_i$ and the stoichiometry constant.

Default values of the parameters and the initial conditions of the variables

All simulations in this study were conducted using Mathematica 12. Extended Data Table 4 summarises the symbols, definitions, units, values of the initial conditions of the variables, and the default values of the parameters. The values of the standard

Gibbs energy change of formation of compound X_j , $\Delta_f G_{X_j}^\circ$, the maximum reaction rate constant of the i th species, r_i , and the abiotic reaction rate constant of forward reactions k_i , were regarded as random variables (see Supplementary Information, sections S.1 and S2 for the abiotic and backward reactions). The values of $\Delta_f G_{X_j}^\circ$, r_i , and k_i in $\log_{10}$ were assumed to follow a uniform distribution within the range shown in Extended Data Table 4. They were generated by the *RandomReal* function and were eventually converted to antilog values. An example of the dynamics of the model when $(N_A, N_B, N_C) = (3, 3, 3)$ is shown in Extended Data Fig. 1a (see Supplementary Information, section S.4.1). Within the given range of values of the parameters, the inflow and outflow rates of compound X_j significantly exceeded the abiotic reaction rates in the system. Consequently, the molar concentration of compounds in the system without microbes ($M_i = 0$ ($1 \leq i \leq N_{\text{reac}}$)) was almost determined by the balance between inflow and outflow. The molar concentration of compounds at the abiotic steady state was $X_j = I_{X_j}/D_{X_j}$. We defined the availability of compounds as $X_{\text{tot}} = \sum I_{X_j}/D_{X_j}$.

The simulations at the given compound diversity $N_{\text{tot}} = N_A + N_B + N_C$ and X_{tot} condition were repeated 1000 times and only those results that reached a steady-state were extracted. $N_{\text{tot}} = 6, 7, 8, 9, 10, 11$, and 12 in Fig. 3 correspond to $(N_A, N_B, N_C) = (2, 2, 2), (3, 2, 2), (3, 3, 2), (3, 3, 3), (4, 3, 3), (4, 4, 3)$, and $(4, 4, 4)$, respectively. Although the system had multi-stability since the microbial community at the steady-state depends on the invasion history (see Supplementary Information, section S.4.2), the same initial conditions were used to obtain the steady states.

The degree of mutualism

To assess the degree of mutualism of a community at a given N_{tot} and X_{tot} condition, and

for all of the species that could exist at a steady state for iterations, we evaluated the steady-state biomass of Sp. *i* when it was solely present in a redox network, $\overline{M}_i$, and the steady-state biomass of Sp. *i* when it exists with other species in the redox network with the same structure and abiotic settings, M_i^* . If $\frac{\overline{M}_i}{M_i^*} < 1$, the steady-state biomass of Sp. *i* was collectively, positively affected and supported by the presence of other species. The degree of mutualism of a community was defined as the ratio of Sp. *i* that satisfied $\frac{\overline{M}_i}{M_i^*} < 1$ for all species surviving at a steady state with other species for iterations.

Data availability

The parameters and differential equations for the *NDsolve* function for numerical differentiation in Mathematica that generated Extended Data Figure 1a are provided in the *Programs/FigS1/data* folder. The default values of the parameters and initial conditions of the variables are summarised in Extended Data Table 4.

Code availability

All codes were written in the Wolfram Language platform using Mathematica 12. The codes are available in the *Programs* folder. See details in Supplemental Information S.3.

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Author contributions

M.S and M.K conceived of the presented idea. M.S designed the model and the computational framework and analysed the data. M.S and M.K contributed equally to the interpretation of the results and writing the manuscript.

Competing interests

The authors declare no competing interests.

Supplementary Information is available for this paper.

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Extended data figures and tables

Extended Data Figure 1 | An example of the dynamics of microbes and the Gibbs energy when $(N_A, N_B, N_C) = (3, 3, 3)$. **a**, The dynamics of the biomass of species i ($1 \leq i \leq 60$), M_i . **b**, The dynamics of the biomass of five species (top) and the negative Gibbs energy change of reaction (bottom) when species survive at a steady state when **(a)**

invaded, in order, every 300 hours (see Supplemental Information S.4.1). The values of the parameters were set as the default values listed in Extended Data Table 4. The values of random parameters are listed in *Program/FigS1/data*.

Extended Data Figure 2 | Simulation when a species is randomly and sequentially added to a system. a, The flowcharts represent Steps 2 and 3 in Supplementary Information, section S.4.2. **b**, The identifier number of species surviving at t ($x_i(t) > 10^{-6}$) when each species was randomly selected and added to the system in order every 1000 hours when $(N_A, N_B, N_C) = (4, 4, 4)$ and $X_{tot} = 1$. Filled circles denote species when no more species could replace them. Black, blue, and red show the results of the trials out of 10. **b (left)**, The same combination of species exists at a steady state, suggesting that there is a single, stable, steady state. **b (right)**, The combination of species existing in a steady state depends on the invasion history, suggesting the presence of multi-stable, steady-states.

Extended Data Figure 3 | The combinations of species surviving in a steady state (a, b, and c) and R_s^*/R_c^* (d) in response to various (I_{B1}, I_{B2}) conditions when $(N_A, N_B, N_C) = (2, 2, 2)$. The energy-source reactions of species (Sp.) 1, 2, and 3 are reactions 1, 2, and 3 in Fig. 1(a), respectively. **a**, Sp. 3 is solely present at $t = 0$. **b**, Sp. 1 and 2 are present at $t = 0$. **c**, Sp. 1, 2, and 3 are present at $t = 0$. In (a-c), black: Sp. 3 only exists; pink: Sp. 1 only exists; cyan: Sp. 2 only exists; blue: Sp. 1 and 2 exist; green: Sp. 1 and 3 exist; red: Sp. 2 and 3 exist. Sp. 1 and 2 were unable to exist in the white region in (b) and (d). We let $R^* = A_1^* C_2^*$ where * denotes the steady state. R^* when Sp. 3 only exists is referred to as R_s^* and when Sp. 1 and 2 exist it is referred to as

R_c^* , respectively. s stands for solitary (Sp. 3) and c stands for the two consortium species (species 1 and 2). The solid contour in (d) shows $R_s^*/R_c^* = 1$, above which species 2 and 3 can lower the steady state abundance of $A_1^*C_2^*$ more than species 3 can alone.

Extended Data Figure 4 | The steady-state number of species at different total number of compounds, N_{tot} , and compound availability, X_{tot} . $N_{tot} = 6, 7, 8, 9, 10, 11$, and 12 correspond to $(N_A, N_B, N_C) = (2, 2, 2), (3, 2, 2), (3, 3, 2), (3, 3, 3), (4, 3, 3), (4, 4, 3)$, and $(4, 4, 4)$, respectively. Filled circles show the median and the solid and dotted error bars show the 25 and 75 percentiles and the minimum and maximum values.

Extended Data Figure 5 | The distribution of $-\Delta_r G_i^\circ$ from the energy-source reactions of species i surviving in a steady state when $(N_A, N_B, N_C) = (2, 2, 2)$. Decreases in $-\Delta_r G_i^\circ$ as X_{tot} increases means species existing in a steady-state harness reactions with lower $-\Delta_r G_i^\circ$ due to the division of metabolic labour.

Extended Data Table 1 | Half reactions and overall cell reactions with a half reaction as a reduction reaction and the standard hydrogen electrode as an oxidation reaction. $N_A = 3, N_B = 2$, and $N_C = 2$

Extended Data Table 2 | Possible redox reactions when $(N_A, N_B, N_C) = (3, 2, 2)$. The standard Gibbs energy change of reactions were calculated by setting $\Delta_f G_{A1}^\circ = 9.71$, $\Delta_f G_{A2}^\circ = 135.47$, $\Delta_f G_{A3}^\circ = 409.13$, $\Delta_f G_{B1}^\circ = 53.1$, $\Delta_f G_{B2}^\circ = 155.25$, $\Delta_f G_{C1}^\circ = 70.33$, and $\Delta_f G_{C2}^\circ = 6.33$

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484 **Extended Data Table 3 | The total number of possible redox reactions at a given (N_A ,**

485 **N_B , N_C) condition**

486

487 **Extended Data Table 4 | Symbols, definition, units, values of the initial conditions,**

488 **and default values or ranges of the parameters**

Figure 1

Figure 1: **Structures of redox networks and examples of metabolic interactions.** **a**, When $(N_A, N_B, N_C) = (2, 2, 2)$ and **b**, when $(N_A, N_B, N_C) = (3, 2, 0)$. The number i in the circle stands for the i th reaction and species. Blue and red arrows correspond to oxidation and reduction reactions, respectively. The right panel illustrates the half-reactions that build overall redox reactions 1, 2, and 3 (see Supplementary Information, section S.1) and the metabolic relationships among species 1, 2, and 3.

Figure 2

Figure 2: **The number and relationships of species when $(N_A, N_B, N_C = 2, 2, 2)$.** **a**, The steady-state responses to the number of compounds X_{tot} , and **b**, the maximum catalytic rate constant, r_i . During the simulation in (b), r_i for all species was fixed to the same value. 0 and 1 meant that either no species or 1 species existed in a steady-state, respectively. Two species existed in a steady state for cases 2C and 2M and could either compete for the same resource and produce the same compound (C) or mutually divide the metabolic labour (M).

Figure 3

Figure 3: **Steady state responses to changes in the availability of compound X_{tot} , and the number of compounds, N_{tot} .** **a**, The degree of mutualism (see Methods for the definition of ‘the degree of mutualism’), **b** and **c** are the median of $-\Delta_r G_i^\circ$ of the energy-source reactions and the maximum catalytic rate, r_i , of all species surviving at a steady-state with other species. **d**, the median of the total biomass, $\sum M_i^*$, for 1000 iterations.

Supplementary Files

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