Emergent ecological advantage of sequential metabolic strategies in complex microbial communities

Zihan Wang[1,2], Yu Fu[1], Akshit Goyal[3,*], Sergei Maslov[1,2,4*],

[1] *Department of Physics, University of Illinois Urbana-Champaign, Urbana, Illinois, USA.*
[2] *Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, Urbana, Illinois, USA.*
[3] *International Centre for Theoretical Sciences, Tata Institute of Fundamental Research, Bengaluru 560089, India.*
[4] *Department of Bioengineering, University of Illinois Urbana-Champaign, Urbana, Illinois, USA.*

∗ Correspondence: `maslov@illinois.edu`, `akshitg@icts.res.in`

Abstract

Microbes adopt a variety of metabolic strategies to consume resources in fluctuating environments. Most work has focused on understanding these strategies physiologically for the growth of single species, rather than ecologically for the assembly of complex natural communities. Here, using a new consumer-resource framework incorporating dynamic proteome re-allocation, we compare the two dominant metabolic strategies among microbes: sequential (diauxie) and co-utilization of resources. We find that even when sequential strategies are worse in pairwise competitions, they can clearly dominate in complex multi-species communities. This emergent ecological advantage of sequential strategies lies in their growth rate distributions, specifically their wider upper tails despite lower average growth rates. While average growth rates are important when the level of competition is low, the tails matter in mature communities where the level of competition is high. We also find that both strategies stratify by species richness of communities, with sequential strategies dominating species-rich communities and co-utilizers dominating low-diversity communities with only 1–2 species. Sequential species can more efficiently pack resource niches due to their increased resilience to environmental fluctuations in resource ratios, i.e., their structural stability. Overall, our work provides an explanation for the long-standing puzzle of how sequential and co-utilizing strategies coexist in nature, and provides several testable predictions about their co-occurrence patterns.

Introduction

Microorganisms use a variety of metabolic strategies to utilize resources in their environments. They typically either consume the available resources all at once (co-utilization) or one after another (sequential utilization) [1, 2, 3]. Both strategies have different effects on physiology, i.e., on the growth rate of a single species in isolation. Even in this context, the answer to which strategy is superior remains disputed. On the one hand, sequential strategies have been theoretically shown to be growth-optimal for certain pairs of resources [4] and co-utilization optimal for others. On the other hand, experimental studies [5, 6, 7, 8, 9] show that microbes often deviate from growth-optimality, by allocating part of their proteome to resources that are not currently utilized [10].

While understanding the advantages of using different metabolic strategies when species are grown in isolation is important, microbes in natural environments rarely grow alone. Instead, they live in complex multi-species ecosystems, where emergent factors that are distinct from growth rate alone may favor some strategies over others. These ecological effects have never been systematically studied, either experimentally or theoretically. Thus, studies of both strategies in an ecological context may shed light on how and why they may coexist in natural environments.

An intuitive understanding of how ecological contexts might change the competitive advantage of a metabolic strategy is as follows. When growing in isolation, species can always count on resources being present. In contrast, in communities, some resources may be depleted by others before a species gets a chance to consume them [11]. This becomes only more likely as the number of species (community complexity) increases. Such an effect may lead to differences in the performance of a strategy between single species and community contexts.

Fluctuating environments such as in serial dilution experiments — where resource availability strongly changes with time — present a natural scenario in which to compare sequential and co-utilizing strategies. Indeed, in steady-state environments such as chemostats, resources reach low concentrations, where even sequential utilizers become co-utilizers [1, 3].

Here, using simulations and theory, we quantify the advantages of metabolic strategies in complex communities and decompose them into growth and ecological components. We find that sequential strategies have a large systematic ecological advantage over co-utilizing strategies. Furthermore, this ecological advantage increases with community complexity (number of species): in our simulations, low diversity communities are dominated by co-utilizers, while high diversity communities are dominated by sequential species. We then investigate the source of this behavior in terms of commonly used ecological properties, and find two key contributors. They are (1) the wider distribution of growth rates of sequential utilizers over co-utilizers, and (2) increased resilience to resource fluctuations (structural stability [12, 13, 14]) of communities enriched in sequential utilizers.

Results

Incorporating proteome allocation to study metabolic strategies

There are two major classes of metabolic strategies: (1) co-utilizing strategies where species consume multiple available resources simultaneously, and (2) sequential or diauxic strategies where species consume resources one at a time according to a species-specific hierarchy. The impact of utilizing either of these strategies on a species' growth rate has been extensively studied in the context of microbial physiology [4, 10]. One extreme point of view suggests that microbes optimize their

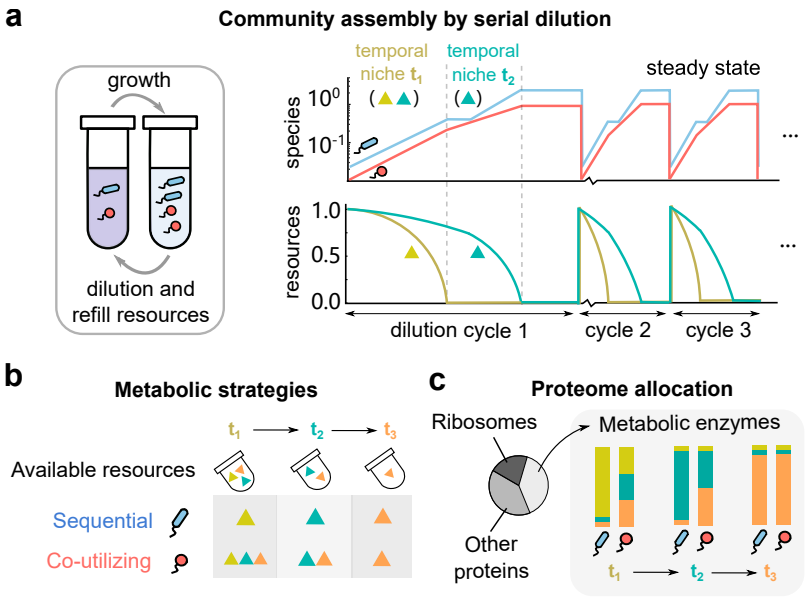

Figure 1: **Investigating sequential and co-utilizing metabolic strategies in boom and bust environments requires incorporating proteome allocation in consumer-resource models.** (a) Schematic illustration of species and resource dynamics in boom-and-bust (serial dilution) environments. In each growth-dilution cycle, resources are depleted in a specific temporal order, giving rise to "temporal niches" defined by which resources are currently available. Here, in the first niche lasting time t_1, both resources (gold and teal) are present, while in the second niche lasting time t_2, only one resource (teal) is present. After several growth-dilution cycles, both species (red and blue) reach a steady state where they grow by exactly the dilution factor D in each cycle. (b) Illustration of two major metabolic strategies: sequential (blue) and co-utilizing (red) used by the two species. Species using sequential strategies always consume one resource at a time, according to their idiosyncratically hardwired preference order (here, gold, then teal, then orange). Co-utilizing species use all available resources that they can consume at the same time. (c) Illustration of proteome (re)-allocation in our model. We assume that each species has a proteome broadly divided into three sectors: ribosomes, metabolic enzymes and other housekeeping proteins. Within the metabolic sector, all species allocate a fixed small fraction ϕ_{pre} to resources they are not currently utilizing. The rest of the metabolic sector is allocated according to the metabolic strategy: for sequential species all of the remaining metabolic sector is allocated to a single resource, while for co-utilizing species it is equally divided between all currently consumable resources.

growth rates on the currently available resources without allocating any proteome towards enzymes necessary to consume future resources [4]. This point of view leaves species vulnerable to long lag periods of no growth when resource availability changes, as may frequently occur during boom and bust cycles in nature and serial dilution experiments [5, 15]. The other point of view [10] cautions against pure optimality arguments using evidence showing that "a large fraction of proteomes is allocated towards proteins that are unnecessary for growth" including enzymes and transporters necessary to utilize resources that are not currently consumed. Indeed, experimental evidence in *E. coli* co-utilizing certain pairs of resources suggests similar allocation among enzymes used for each of these resources regardless of their current availability [16]. To capture the spectrum of possibilities with these two being extremes [4, 16], here we introduce a pre-allocation parameter ϕ_{pre} quantifying the fraction of the proteome always allocated towards the utilization of any resource regardless of their presence in the growth medium (Fig. 1c). Furthermore, in agreement with previous work, we

account for dynamic allocation between the ribosomal and metabolic sectors of the proteome [16] (Methods).

Growth and ecological advantages of metabolic strategies

To understand the relative advantage of different metabolic strategies in an ecological context, we simulated the outcomes of community assembly experiments starting from an equal number of species utilizing each of the different strategies. Any skew in the fraction of species utilizing a specific strategy among the final survivors in the community would quantify the relative advantage of that strategy. We will decompose the relative advantage of a strategy into two components: one arising from its growth rate (quantified using head-to-head competition) and the other arising from more complex ecological interactions within a multi-species community.

We focused on comparing two specific strategies: co-utilization and the best sequential strategy identified in previous work [11] — the "top smart" one, where a species first utilizes the resource with the fastest growth rate, then uses all other resources in an idiosyncratic order uncorrelated with growth rate. In what follows, we will compare the relative advantage of the sequential strategy over the co-utilizing one. The growth advantage was computed as the frequency with which sequential species outcompeted their co-utilizer opponents in random head-to-head competitions (Fig. 2a, top). To evaluate the ecological component, we assembled several multi-species communities, each starting from a large species pool with 500 species utilizing each strategy (Methods) and serially diluted until they reached steady state. The ecological advantage was defined as the fraction of sequential species among all survivors in the resulting communities. We computed the growth and ecological components for several values of the pre-allocation parameter ϕ_{pre} (Methods).

Growth advantage: In the limit of no proteome pre-allocation $\phi_{\text{pre}} \to 0$, sequential species had a systematic growth advantage (Fig. 2b, black), consistent with previous results [4]. As ϕ_{pre} increased, the growth advantage of sequential species decreased, reaching parity (fraction of 50%) at $\phi^*_{\text{pre}} \approx 0.044$. For $\phi_{\text{pre}} > \phi^*_{\text{pre}}$, co-utilizers gained a growth advantage over sequential species (Fig. 2b, black).

Ecological advantage: In the limit of no pre-allocation $\phi_{\text{pre}} \to 0$, sequential species comprised virtually 100% of surviving species in communities assembled from large species pool (Fig. 2b, green). This overwhelming ecological advantage of sequential species continued for larger values of ϕ_{pre}. Remarkably, at $\phi_{\text{pre}} = \phi^*_{\text{pre}}$, even though sequential species had no growth advantage in head-to-head competitions, they were virtually the only survivors in multi-species communities. Only at much larger values of $\phi_{\text{pre}} \approx 0.074$ did the ecological advantage of sequential species disappear, i.e., sequential species comprised $\approx 50\%$ of survivors across all communities.

Growth rate distributions explain the ecological advantage of sequential species

The ecological advantage of sequential species result can be explained by comparing the growth rate distributions for both strategies. The odds of a sequential species to win in a head-to-head competition with a co-utilizing species depend on which strategy's growth rate distribution has the larger median. At $\phi_{\text{pre}} = 0.07$, co-utilizers have a systematic advantage in head-to-head competition because they have a larger median growth rate: the typical co-utilizer grows faster than a typical sequential species (Fig. 2c). However, during community assembly from a large species pool (Fig. 2b, green), the level of competition is much greater. In this scenario, it is not the medians, but

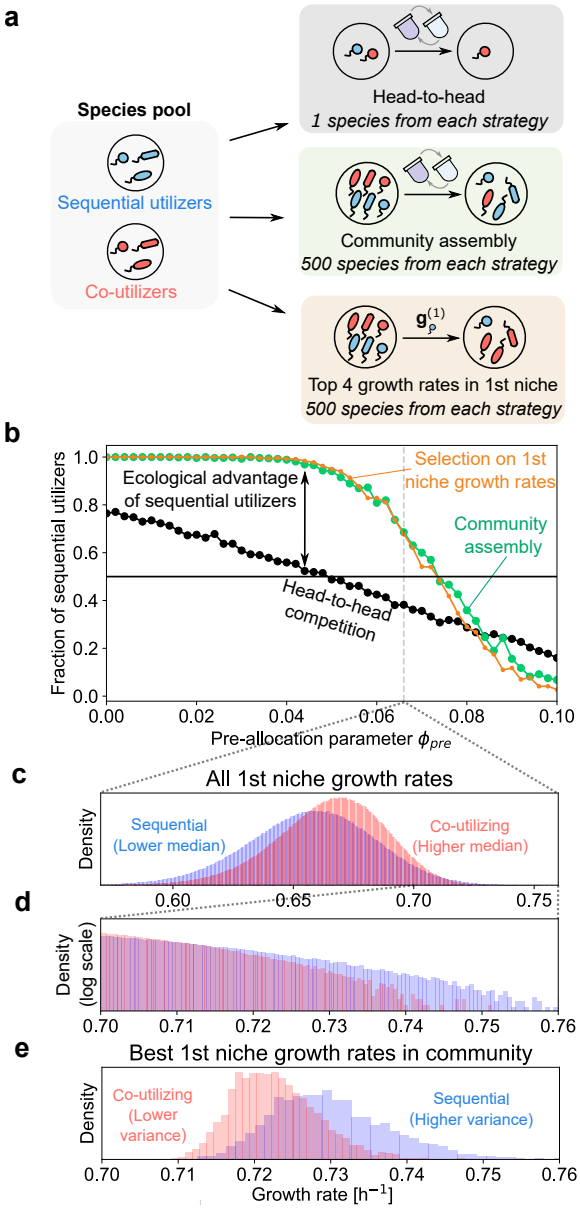

Figure 2: **Emergent ecological advantage of sequential metabolic strategies in complex communities.** (a) Schematic showing our simulations in two different scenarios: one where we compete 1 sequential and 1 co-utilizing species head-to-head (gray), and the other where we assemble a complex community seeded with 500 species from each strategy (green). For comparison we also pick the top 4 growth rates on the first temporal niche from the same pool (orange). (b) Fraction of surviving sequential utilizers in each of the two scenarios: head-to-head (black) and in complex communities (green), plotted as a function of the pre-allocation fraction $\phi_{\rm pre}$ common to all species in the pool. Sequential utilizers have a distinct ecological advantage in complex communities: the green curve is typically above the black curve. (c) Distribution of growth rates of sequential (blue) and co-utilizing (pink) species in the first temporal niche for $\phi_{\rm pre} = 0.07$. Sequential species have a lower median. (d) Zoomed in view of the upper tails of (c), showing that sequential species have a wider upper tail. (e) Distribution of top growth rates selected from each of the distributions in (c); sequential species have greater extreme values due to wider upper tails and thus win in complex communities where competition is strong. When accounted for, we can quantitatively predict the fraction of sequential species in (orange in (b)).

the extreme value of the growth rates that matter. The extremes are determined by the width of the upper tail of the growth rate distribution, which is affected not just by the median, but also the variance. At the same ϕ_{pre}, the upper tail of the growth rate distribution of sequential species is significantly wider than that of co-utilizers (Fig. 2d). There are two reasons for this difference. First, the growth rate of co-utilizers in the first temporal niche is an average of $n_R = 4$ growth rates, one for each resource; sequential species only grow on one resource at a time and thus don't have this averaging effect. This makes their growth rate distribution wider than that of co-utilizers by a factor $\sqrt{n_R}$. The other factor is that top-smart sequential utilizers pick the best of n_R growth rates to grow on the first temporal niche, where the competition is strongest. This skews their growth rate distribution further to the right, resulting in a wider upper tail (Fig. 2d). As a result, if we select the largest growth rates using each strategy from a pool of 500 randomly generated species, we observe that sequential species have a distinct edge (Fig. 2e). Thus, changing the level of competition from head-to-head to a large competing pool systematically shifts what is the important aspect of the growth rate distributions, from the median preferring co-utilizers (Fig. 2c) to the extreme tails preferring sequential species (Fig. 2e).

The fraction of sequential species can be quantitatively predicted by selecting the $n_R = 4$ largest growth rates among all 1,000 species (500 species each) in the pool on the first temporal niche. The fraction of the sequential utilizers to which these growth rates belong (Fig. 2b, orange) matches almost perfectly with the community assembly outcomes (Fig. 2b, green). Taken together, the wider tail of the growth rate distribution of sequential species explains their dominance in complex communities assembled from a large pool of competing species, even when they lose in head-to-head competition.

Sequential strategies promote diversity through increased structural stability

To better understand the forces guiding the assembly of complex communities of species using different metabolic strategies, we investigated the composition of communities with varying levels of species diversity or complexity. Because of the competitive exclusion, the final species diversity ranged between 1 and the number of resources $n_R = 4$, depending on the randomly generated species pool. Interestingly, the fraction of sequential species was strongly correlated with this final species diversity (Fig. 3a). That is, across all values of ϕ_{pre} tested, sequential species dominated in high-diversity assembled communities, while co-utilizers were prevalent in low-diversity communities (Fig. 3a). Even when there was no systematic ecological advantage of sequential species ($\phi_{\text{pre}} \approx 0.08$), both strategies continued to be segregated by final species diversity (Fig. 3b). Single-species communities almost entirely contained co-utilizers ($\approx 100\%$) while maximally diverse communities with 4 species were dominated by sequential species ($\approx 75\%$).

A possible explanation for the stratification of strategies by community richness/diversity (number of species) lies in the systematic difference in their structural stability, i.e., the likelihood that a given community can successfully in a given resource environment, e.g., when all resources are supplied in equal concentrations, as in our simulations. A given set of species may only stably coexist in a certain range of environmental conditions quantified by the supply of different resources (Fig. 3c, green shows range where coexistence is possible). This range quantifies the structural stability [12], and we developed a linear algebra approach to calculate it for any niche-packed community (Methods). By quantifying the structural stability of niche-packed communities composed of different number of sequential species (from 0 to $n_R = 4$), we found that structural stability progressively

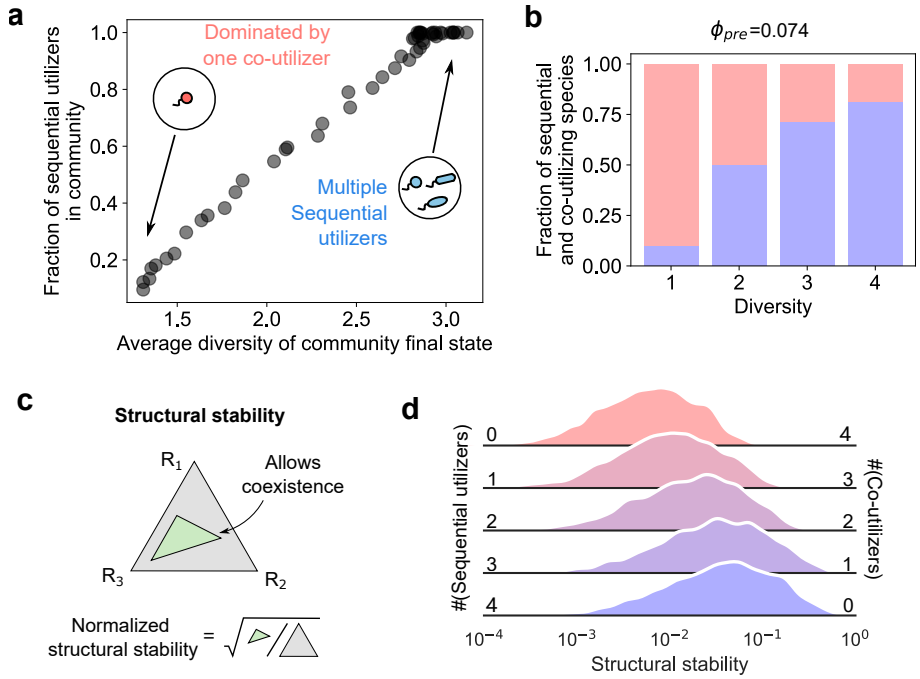

Figure 3: **Sequential strategies promote diversity through increased structural stability.** (a) Fraction of surviving sequential species as a function of the average diversity of the assembled communities (# of surviving species) for all simulated communities across all ϕ_{pre} values tested. As community complexity increases, sequential species become more dominant in communities. (b) Stacked bar plot showing how strategies are stratified across communities with different diversities at a fixed value of $\phi_{\text{pre}} \approx 0.074$. Here too, more complex communities are increasingly dominated by sequential species, while less complex communities are dominated by co-utilizing ones. (c) Illustration of structural stability of a community, representing the range of supplied resource ratios allowing the given set of species to stably coexist. (d) Distributions of normalized structural stability (Methods) for niche-packed communities ($n_S = n_R$ species). Increasing the number of sequential utilizers, from 0 to 4 systematically increases structural stability.

increased with increasing number of sequential species, becoming the largest in communities composed entirely of sequential species (Fig. 3d, bottom). This suggests that for fixed environmental conditions, sequential species would tend to be over-represented diverse niche-packed communities.

To understand what makes communities composed of sequential species more structurally stable, consider the following argument. Sequential species use only one resource at a time. In a community at steady state, different species would be using different subsets of resources. Importantly, some species may never get to utilize an available resource before it gets depleted by others in the community. For example, if the most preferred resource by a species gets depleted last by the community, this species will never get to use another resource, even though it might be capable of using all resources. In contrast, co-utilizing species always consume all available resources regardless of their depletion order. As a consequence, changing the concentration of any resource will affect all co-utilizers but only a fraction of sequential species. In more mathematical terms, the matrix M encoding which species consume which resources in the steady state cycle (Supple-

mentary Text) will be sparser for sequential communities than co-utilizing ones. The structural stability is proportional to the determinant of M. Because the matrix M must obey a mass conversation constraint — its columns must sum to $D-1$ (i.e., all species must grow by a factor D at steady state (Supplementary Text)) — sparser M's tend to have a larger determinant, yielding more structurally stable communities. As communities get more complex, the M matrix would get larger and tend to increase the systematic difference between sequential and co-utilizing species.

Discussion

Here, we showed that microbial species using sequential resource utilization (diauxie) have an emergent ecological advantage over co-utilizers (Fig. 2b, green). We found that the main source of this ecological advantage lies in the wider tails of the growth rate distributions of sequential species (Fig. 2d). Further, sequential species tend to dominate in species-rich communities, while co-utilizers dominate in species-poor communities (Fig. 3a), which may arise from systematic differences in the structural stability of both strategies (Fig. 3d).

A key innovation of our model is the pre-allocation parameter ϕ_{pre}, tuning which allowed us to systematically change the growth advantage of sequential species over co-utilizers in pairwise competitions (black curve, Fig. 2b). The inspiration for this parameter comes from experimental observations showing that microbes tend to pre-allocate enzymes for resources that they are not currently utilizing [9, 7, 6, 8]. This pre-allocation is important in fluctuating environments because it reduces the lag time it takes for a species to shift from one resource to another [17, 15, 5, 18]. Throughout this manuscript, we neglected these lag times. This approximation is valid for large dilution ratios D, when lag times are much shorter than durations of individual temporal niches. However, including lags makes the emergent ecological advantage of sequential species even stronger (Fig. S2a). Including a lag time only for sequential species significantly disadvantages them in pairwise competitions. In Fig. S2a, the gray line, representing the growth advantage of sequential species without lags relative to co-utilizers, is significantly above the black line, representing the same comparison between species with lags and co-utilizers without lags (see Methods). At the same time, lags barely affect the drastic ecological advantage of sequential strategies in complex multi-species communities, which can be seen by noting that the green line (with lags) almost overlaps with the light green line (without lags), and that both are significantly above the black and gray lines (pairwise competition). The reason for this advantage continues to be the wider upper tails of the growth rates of sequential species on their most preferred resource. Lags tend to reduce the importance of less preferred resources on which sequential species grow in later temporal niches, and thus the ecological advantage of sequential species only gets stronger. Further, lags also increase the structural stability of niche-packed communities with sequential species (Fig. S2b). This can be explained using the fact that for some species, lag times might be longer than the time it takes for the community to deplete a species' next preferred resources. This would make these species unable to use less preferred resources, thus increasing structural stability. To conclude, both our results are not only robust to, but are enhanced by the inclusion of lags.

In the results presented here, we kept the key parameters of our model fixed. To verify the robustness of our results to changing these parameters, we repeated our simulations with different dilution factors D, species pool size P, and the standard deviation of the distribution of growth rates (Fig. S1, S3, S4). We found that our results were qualitatively robust to changing these parameters across a broad, experimentally relevant range. In particular, communities assembled from larger pools showed a larger ecological advantage for sequential species (Fig. S1).

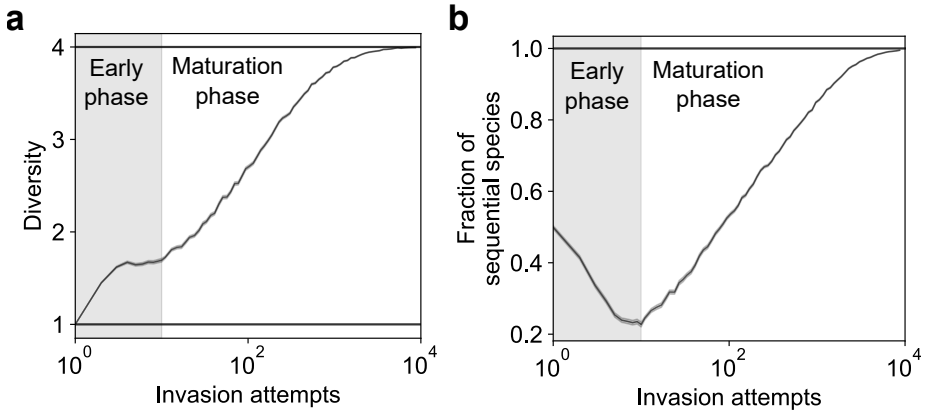

Figure 4: **Gradually assembled communities get progressively enriched in sequential species.** We assembled communities by gradually adding species using a mixture of metabolic strategies and a variety of pre-allocation fractions ϕ_{pre}. (a) Diversity (# of surviving species) gradually increases with the number of invasion attempts (# of species added). (b) Fraction of sequential species also increases with the number of invasion attempts, once the community is in the "maturation phase", i.e., after early colonization ends (gray region) and species start to compete strongly.

Testable predictions

Our results might potentially explain the diversity of metabolic strategies observed in nature, specifically why sequential (diauxic) and co-utilizing strategies coexist without one systematically outcompeting the other. In our model, both strategies stratify in communities with different diversity, or species richness, relative to the number of resources (Fig. 3a–b). This leads to the following testable prediction: niche-packed communities ($n_S/n_R \approx 1$) would tend to be richer in sequential species, while poorly packed ($n_S/n_R \ll 1$) communities would be dominated by co-utilizers. Future experimental or observational work could potentially test this prediction and help explain the diversity of observed metabolic strategies.

Another observation of our work is that the fraction of sequential species in communities increases with the species pool size (Fig. 2b, S1). The species pool size represents the "maturity" of a community, i.e., the number of species that have attempted to invade this community over time. To systematically investigate how communities comprising a mixture of metabolic strategies change over time, we gradually assembled communities by colonize them with species one at a time (Methods). In the early phase of colonization, species don't interfere with each other much since the niches are far from packed (Fig. 4, gray regions). In later phases, added species compete with residents. In this "maturation phase" of the community, we see that the number of species gradually increases with time (Fig. 4a). But both strategies are not equally represented in the species being retained in the community. Specifically, the fraction of sequential species gradually increases as the community becomes more mature (Fig. 4b). Hence we predict that more mature communities will tend to be enriched in sequential species.

Acknowledgements

We thank Y. Fridman, G. Chure and J. Cremer for valuable discussions. A.G. acknowledges support from the Ashok and Gita Vaish Junior Researcher Award, the Department of Atomic Energy, Government of India, under project number RTI4001, as well as the Government of India's DBT Ramalingaswami Fellowship.

Data and code availability

There are no data associated with this paper. All code is available as a GitHub repository at the following link: https://github.com/maslov-group/Ecol_adv_diaux

Methods

Model of microbial proteome allocation

To incorporate proteome allocation into our consumer-resource model in boom-and-bust environments, we extended previous proteome allocation schemes to include pre-allocation of metabolic enzymes. That is, just as in previous models, we assumed that the proteome of each individual in a species was composed of metabolic enzymes, ribosomes and other proteins [19]. We perform allocation in two steps. First, we assign the proteome into the ribosomal and metabolic sectors (assuming other sector(s) are fixed. Second, we subdivide the metabolic sector into distinct enzymes for different resources.

To describe the allocation between metabolic enzymes and ribosomes, we choose to adapt the model of Hermsen et al. [16]. Consider a species α growing in a particular resource environment. To grow on these resources, the species allocates a certain fraction of metabolic enzymes $E_1, \cdots, E_{n_R}$, normalized to 1. Note that in our model, species may allocate enzymes even towards resources that are not currently consumed by them. The growth rate g_α of the species is determined by the following equation:

$$\frac{g_\alpha}{1 - g_\alpha/g_C} = \sum_{i \in S_{\text{curr}}} \xi_{\alpha i} E_{\alpha i}. \tag{1}$$

where S_{curr} is the set of resources the species is currently consuming. This equation can be rearranged to obtain:

$$g_\alpha = \left(\frac{1}{\sum_{i \in S_{\text{curr}}} \xi_{\alpha i} E_{\alpha i}} + \frac{1}{g_C} \right)^{-1}. \tag{2}$$

Here, g_C is the theoretical upper bound on the growth rate which only depends on the species. We set it to the same value equal to 1 for all species for simplicity. Further $\xi_{\alpha i}$ is a species-specific coefficient representing the contribution of given resource i towards growth per unit enzyme fraction. In our simulations we select it from a truncated Gaussian distribution with mean 0.5 and standard deviation 0.1.

We then consider how species allocate their proteome towards different metabolic enzymes $E_{\alpha i}$. This proteome allocation is different for each strategy. Here, we assume that for each resource there is always a small portion of the proteome ϕ_{pre} that has been "pre-allocated" to the metabolic enzyme necessary to utilize this resource. This "pre-allocated" fraction of the proteome can never be reallocated to other resources.

For a co-utilizing species with n resources present in the environment ($n \leq n_R$), each such resource would initially have ϕ_{pre} fraction of the metabolic sector of the proteome pre-allocated to it. Hence, the total fraction of the metabolic enzyme pool that could in principle be reallocated is given by $(1 - n_R \phi_{\text{pre}})$. For co-utilizers, we assume that this amount is distributed among all n resources equally (for simplicity). In this case, each of the n resources currently utilized by the species has a total fraction of enzymes given by

$$E_i(\text{co-utilization}) = \phi_{\text{pre}} + \frac{(1 - n_R \phi_{\text{pre}})}{n}, \tag{3}$$

while each resource not currently utilized has a fraction ϕ_{pre} allocated to it. For a sequential species $n = 1$ because they always consume one resource at a time. Thus,

$$E_i(\text{sequential utilization}) = 1 - (n_R - 1)\phi_{pre}. \tag{4}$$

To understand how $\phi_{\rm pre}$ affects the balance between sequential and co-utilizing strategies in head-to-head competition, suppose there are two strains c and s sharing the same set of physiological parameters ($\xi_{ci} = \xi_{si} = \xi_i$), but c is a co-utilizing strain, while s is a top-smart sequential strain. These strains are grown in a boom-and-bust environment with n_R resources at the beginning of each cycle. When they compete against each other, our previous work shows that the competition occurs mainly during the first temporal niche where all resources are present [4]. To compare the growth rates of the sequential and co-utilizing species before correcting for the ribosomal proteome sector, we only need to compare the RHS in Eq. (1). The ratio r_{sc} quantifies the advantage of the top-smart sequential strategy over co-utilization one. When all substrates are present it is given by

$$r_{sc} \equiv \frac{\max_i(\xi_i E_{si})}{\sum_i^{n_R} \xi_i E_{ci}} = \frac{\max_i(\xi_i) \cdot n_R(1 - \phi_{\rm pre}(n_R - 1))}{\sum_i^{n_R} \xi_i}. \tag{5}$$

Note that for $\phi_{\rm pre} = 0$ the sequential strategy always has an advantage in the first temporal niche: $r_{sc} > 1$. Indeed, in this case r_{sc} is given by the ratio of the maximum over the mean growth rate, which is always larger than 1. As $\phi_{\rm pre}$ increases, a larger reallocation portion of metabolic enzymes starts to favor the co-utilization strategy over the sequential utilization one, so that at some value of $\phi_{\rm pre}$ the ratio would satisfy $r_{cs} < 1$. This argument qualitatively explains the behavior of the fraction of surviving sequential utilizers in head-to-head competition shown in Fig. 2 (black curve).

Model of microbial community dynamics in boom-and-bust environments

We model microbial community dynamics in a boom-and-bust environment where n_R substitutable resources are cyclically supplied at concentrations R_k ($k = 1, 2, \ldots n_R$). We attempt to assemble a community by adding species to this environment. Each species is assumed to be a generalist able to grow on each of these resources. We denote species by Greek symbols, so that the abundance of species α is represented by $N_\alpha(t)$; the concentration of resource k is represented by $R_k(t)$. Depending on the metabolic strategy of a species, it consumes certain resources quantified by a time-dependent consumption matrix $c_{\alpha k}(t)$. If species α consumes resource k at time t, we set $c_{\alpha k}(t)$ to 1; otherwise, we set it to 0.

We assume that resource concentrations are much higher than the species' half-saturating substrate concentrations throughout during the entirety of the temporal nice in which a resource is present. This is a reasonable assumption as long as initial resource concentrations are much larger than their corresponding half-saturating concentrations. Thus, in our model, in each temporal niche, species grow exponentially at constant growth rates given in the previous section as Eq. (2). Note that $E_{\alpha k}(t)$ is a function of $c_{\alpha k}(t)$'s, according to $E_{\alpha k}(t) = \phi_{\rm pre} + (1 - \phi_{\rm pre} n_R)/\sum_i^{n_R} c_{\alpha k}(t)$, which comes from Eq. (3). Thus, the dynamics of species abundance in our model can be written as follows

$$\frac{dN_\alpha}{dt} = g_\alpha(t) N_\alpha(t). \tag{6}$$

Similarly, the resource depletion dynamics can be written as follows

$$\frac{dR_k}{dt} = -\sum_\alpha \left(g_\alpha \cdot \frac{c_{\alpha k}(t)\xi_{\alpha k} E_{\alpha k}}{\sum_k^{n_R} c_{\alpha k}(t)\xi_{\alpha k} E_{\alpha k}} \cdot \frac{N_\alpha(t)}{Y} \right). \tag{7}$$

For the sake of simplicity, we define resource concentrations so that the yields Y all equal 1.

The "boom" phase of each cycle ends when all resources are depleted. It is then followed by a "bust" phase during which all microbial abundances are reduced (diluted) by the same factor $D > 1$

until the arrival of the next nutrient bolus, when the boom phase resumes. Our dynamical equations are inspired by the protocol of serial dilution experiments performed in many laboratories to study microbial communities in vitro. A version of this dynamics is also realized in natural microbial ecosystems living in boom-and-bust environments characterized by long intervals between bust phases and large boluses delivered at the beginning of each boom phase.

We are interested in computing the steady state of the boom-and-bust dynamics in which each species grows by exactly the same factor D by which it is subsequently diluted. To compute this steady state dynamics, it is convenient to divide the boom phase of the cycle into a sequence of n_R *temporal niches* where only a particular subset of resources is present [11, 20, 21]. The total number of possible temporal niches for n_R resources is given by $n_T = 2^{n_R} - 1$. Here we have excluded a temporal niche where all resources are absent and therefore no species can grow. The resources present in the environment disappear one by one, resulting in a particular subset of n_R temporal niches (out of n_T possible ones) realized for each of the $n_R!$ orders of resource depletion. For example, if three resources disappear in the order $2 \to 3 \to 1$, the corresponding temporal niches are $111 \to 101 \to 100$ (again, we do not show or count the last niche 000, which separates the end of the boom phase and the beginning of the bust phase of the cycle).

Note that we could have also assumed that species can die during the temporal niche where all resources are absent, e.g., due to starvation. Indeed, it is easy to generalize our model to incorporate the possibility of species dying (having a negative growth rate) during any temporal niche. In this situation, competition occurs even in the temporal niche where all resources are absent, adding one more temporal niche to n_T. Also, there will be $n_R + 1$ temporal niches between adjacent boom phases.

Model of lag times in sequential utilization

In the version of our model with lags, we assume that after a resource is depleted, species that were consuming it will enter a lag phase, during which they re-allocate their enzyme pools and do not grow at all. During this phase, enzymes necessary to utilize the species' next preferred resource will be synthesized through an exponential process, starting from its pre-allocated level ϕ_{pre}, and finishing as it reaches the maximum amount $(1-(n_R-1))\phi_{pre}$. This process corresponds to assuming that currently-existing enzymes synthesize precursors (charged tRNA) necessary for synthesis of additional enzymes of their own kind. We model the process as $dE/dt = \tau_0^{-1}E$, where E is the amount of the enzyme that is being synthesized, and τ_0 is a time-scale. For sequential utilizers, integrating from $t = 0$ to $t = \tau$, we get a lag time τ as

$$\tau(\text{sequential utilizers}) = \tau_0 \log\left(\frac{1-(n_R-1)\phi_{pre}}{\phi_{pre}}\right). \tag{8}$$

For co-utilizers, the log term will include a ratio of allocation fractions before and after the shift, resulting in considerably shorter lags compared with sequential utilizers.

In Ref. [5], it has been proposed and experimentally confirmed that when species switch from glycolytic to gluconeogenic carbon sources, the dynamics of enzymes necessary to utilize the latter resources are instead given by $dE/dt = \tau_0^{-1}E^2$; this is due to the peculiar biochemistry of these two complementary pathways. In this case we get a different expression for the lag τ of sequential utilizers as

$$\tau_{\text{glyco}\to\text{gluconeo}} = \tau_0\left(\frac{1}{\phi_{pre}} - \frac{1}{1-(n_R-1)\phi_{pre}}\right), \tag{9}$$

along with a corresponding variant for co-utilizers.

Details of numerical simulations

In all our numerical simulations in the main text, we use $D = 1000$, $g_C = 1\ \mathrm{hr}^{-1}$. We sampled $\xi_{\alpha i} = \xi_0 + \sigma_\xi Z_{\alpha i}$, where $Z_{\alpha i}$ is a random number selected from the standard normal distribution. For figures used in the main text of the study, we used $\xi_0 = 0.5\ \mathrm{hr}^{-1}$ and $\sigma_\xi = 0.1\ \mathrm{hr}^{-1}$. To prevent negative growth rates, and to keep our $\xi_{\alpha i}$ distribution symmetric around its mean, we truncated the normal distribution so that $0 < \xi_{\alpha i} < 1$.

In community assembly simulations, for each value of ϕ_{pre} we sampled N species pools, each with $P/2$ sequential and $P/2$ co-utilizing species ($N = 200, P = 1000$ in Fig. 2; $N = 1000, P = 10000$ in Fig. 4). For each species pool, 4 resources added at the beginning of each dilution cycle were sampled uniformly from the simplex $\sum_i R_i = 4$ at the beginning, and were fixed throughout the simulation. The span of each dilution cycle is set at 24 h. We attempted to invade the system with all species from the pool in a random order multiple times, until no more successful invasions were possible, i.e. an uninvadable state had been reached. All invaders from the pool were introduced at a small abundance of 10^{-8}, which is much lower than the abundance of any resident species in a steady state community.

For head-to-head competition, we selected 10 pairs of species from each species pool, one being top-smart sequential, and the other being co-utilizing species. We introduced each pair of species into the system in a random order and simulated their dynamics until steady state, with 1 or 2 surviving species. The elimination bound for a species was set at 10^{-8}.

In structural stability simulations, for each given metabolic strategy, we randomly sampled 10000 communities of $n_S = n_R = 4$ species. The ϕ_{pre} for these species were calculated from the condition of $r_{sc} = 1$ (see Methods equation 5). We then calculated the structural stability for these communities, as long as they were feasible and dynamically stable (see SI).

All simulations were carried out in Python (see Code Availability Statement).

References

1. Kovárová-Kovar, K. & Egli, T. Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. *Microbiology and molecular biology reviews* **62**, 646–666 (1998).

2. Bajic, D. & Sanchez, A. The ecology and evolution of microbial metabolic strategies. *Current opinion in biotechnology* **62**, 123–128 (2020).

3. Harder, W. & Dijkhuizen, L. Strategies of mixed substrate utilization in microorganisms. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences* **297**, 459–480 (1982).

4. Wang, X., Xia, K., Yang, X. & Tang, C. Growth strategy of microbes on mixed carbon sources. *Nature communications* **10**, 1279 (2019).

5. Basan, M. *et al.* A universal trade-off between growth and lag in fluctuating environments. *Nature* **584**, 470–474 (2020).

6. Mori, M. *et al.* From coarse to fine: the absolute escherichia coli proteome under diverse growth conditions. *Molecular systems biology* **17**, e9536 (2021).

7. Schmidt, A. *et al.* The quantitative and condition-dependent escherichia coli proteome. *Nature biotechnology* **34**, 104–110 (2016).

8. Balakrishnan, R., de Silva, R. T., Hwa, T. & Cremer, J. Suboptimal resource allocation in changing environments constrains response and growth in bacteria. *Molecular systems biology* **17**, e10597 (2021).

9. Price, M. N. *et al.* Indirect and suboptimal control of gene expression is widespread in bacteria. *Molecular systems biology* **9**, 660 (2013).

10. Okano, H., Hermsen, R. & Hwa, T. Hierarchical and simultaneous utilization of carbon substrates: mechanistic insights, physiological roles, and ecological consequences. *Current opinion in microbiology* **63**, 172–178 (2021).

11. Wang, Z. *et al.* Complementary resource preferences spontaneously emerge in diauxic microbial communities. *Nature communications* **12**, 1–12 (2021).

12. Rohr, R. P., Saavedra, S. & Bascompte, J. On the structural stability of mutualistic systems. *science* **345**, 1253497 (2014).

13. Butler, S. & O'Dwyer, J. P. Stability criteria for complex microbial communities. *Nature communications* **9**, 2970 (2018).

14. Dubinkina, V., Fridman, Y., Pandey, P. P. & Maslov, S. Multistability and regime shifts in microbial communities explained by competition for essential nutrients. *Elife* **8**, e49720 (2019).

15. Erickson, D. W. *et al.* A global resource allocation strategy governs growth transition kinetics of escherichia coli. *Nature* **551**, 119–123 (2017).

16. Hermsen, R., Okano, H., You, C., Werner, N. & Hwa, T. A growth-rate composition formula for the growth of e. coli on co-utilized carbon substrates. *Molecular systems biology* **11**, 801 (2015).

17. Wang, J. *et al.* Natural variation in preparation for nutrient depletion reveals a cost–benefit tradeoff. *PLoS biology* **13**, e1002041 (2015).

18. Julou, T., Zweifel, L., Blank, D., Fiori, A. & van Nimwegen, E. Subpopulations of sensorless bacteria drive fitness in fluctuating environments. *PLoS biology* **18**, e3000952 (2020).

19. Scott, M., Gunderson, C. W., Mateescu, E. M., Zhang, Z. & Hwa, T. Interdependence of cell growth and gene expression: origins and consequences. *Science* **330**, 1099–1102 (2010).

20. Fridman, Y., Wang, Z., Maslov, S. & Goyal, A. Fine-scale diversity of microbial communities due to satellite niches in boom and bust environments. *PLoS computational biology* **18**, e1010244 (2022).

21. Bloxham, B., Lee, H. & Gore, J. Biodiversity is enhanced by sequential resource utilization and environmental fluctuations via emergent temporal niches. *bioRxiv* 2023–02 (2023).