Spatial self-organization of metabolism in microbial systems: a matter of enzymes and chemicals

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Abstract

Most bacteria live in dense spatially structured communities such as biofilms. The high density allows cells to alter the local microenvironment, while the limited mobility can cause species to become spatially organized. Together these factors can spatially organize metabolic processes within microbial communities, so that cells in different locations perform different metabolic reactions. The overall metabolic activity of a community depends both on how metabolic reactions are arranged in space, and on how they are coupled, i.e. how cells in different regions exchange metabolites. Here, we review mechanisms that lead to spatial organization of metabolic processes in microbial systems. We discuss factors that determine the length scales over which metabolic activities are arranged in space and highlight how the spatial organization of metabolic processes affects the ecology and evolution of microbial communities. Finally, we define key open questions that we believe should be the main focus of future research.

Introduction

Microbial communities perform important metabolic processes that shape the health of humans and the planet. These metabolic processes are composed of a large number of

individual metabolic reactions which are often distributed among cells in the community[1]. These metabolic reactions are then coupled to each other through the exchange of intermediate metabolites between cells. The metabolic activity of the community emerges from the activities of the individual cells and their interactions.

Most microbial communities are spatially structured biofilms, which are aggregates of cells with high density and limited mobility[2,3]. The high density allows cells to alter the local chemical microenvironment through the uptake and secretion of metabolites. The limited mobility can lead to clonal clusters of cells and reduces the mixing of species in multispecies communities[4,5]. Together these factors can spatially organize metabolic processes causing different metabolic reactions to take place in different locations within the community.

Spatial organization of metabolic processes can have important consequences for the ecology and evolution of microbial communities. For example, spatial variation in the chemical microenvironment can promote phenotypic variation within clonal populations, increasing their resilience to environmental changes[5–7]. In multispecies communities, spatial variation in the chemical microenvironment can promote the coexistence of species with incompatible niche requirements, for example allowing obligate aerobes and anaerobes to co-exist in close proximity[2]. In this *Perspective* we will review mechanisms that drive the organization of metabolic processes in space and discuss how this organization affects the ecology and evolution of microbial communities.

Components of spatial organization of metabolic processes

For a metabolic reaction to occur in a certain location, two requirements have to be met: First, the enzymes catalyzing the reaction have to be present; Second, the chemical microenvironment has to be permissive for the reaction, i.e., reaction substrates should be present and inhibiting compounds absent. To understand the spatial organization of metabolic processes, we thus have to understand how enzymes and chemicals are arranged in space (Fig 1A).

Spatial organization of enzymes

In clonal populations, all cells carry the same genes. The spatial organization of enzymes is thus determined by where cells express them, i.e. it is determined by gene regulatory networks. Cells regulate gene expression based on their local microenvironment[8,9]. In turn, cells change their local microenvironment through the uptake and release of chemicals[5]. This creates a feedback loop: a change in the arrangement of chemicals leads to a change in the arrangement of enzymes, which in turn leads to a change in the arrangement of chemicals,

and so on. In clonal populations the spatial arrangement of enzymes is primarily determined by this feedback loop (Fig 1B). One example for this process is the spatial separation of different catabolic reactions that is observed in spatially structured clonal populations of bacteria[6,10,11] and yeast[12].

Gene regulation also plays an important role in spatially organizing enzymes within multispecies communities. However, in these communities the arrangement of enzymes is subjected to an additional constraint: Each species carries genes for different sets of enzymes. Thus, the arrangement of species within the community, i.e. the community's biogeography, constrains where enzymes can be expressed (Fig 1C). Recent studies have identified many of the factors that shape the biogeography of multispecies communities[13–20]. A first important factor is the physics of cell growth: as cells grow and divide they push their offspring and neighbors around[4]. The spatial distribution of growth rates and the physics of cell movement thus shapes the arrangement of species in the community. Stochastic factors also play an important role: many biofilms grow by clonal expansion from a small number of seed cells. Thus, the spatial arrangement of the first colonizing cells can affect the biogeography of the community at later times[4,21,22].

The biogeography of a community is also shaped by physical properties of cells: Cells that differ in size or shape tend to self-organize into distinct regions. For example, rod and coccoid shape cells can sort into different regions[23,24]. Adhesive properties can also play a role in organizing cells[25]. Differential adhesion, where cells preferentially stick to cells of another species, can promote species mixing. Species specific adhesion instead leads to the sorting of different species[26]. Also the physical properties of the extracellular matrix can play an important role. For example, *Pseudomonas aeruginosa* and *Staphylococcus aureus* form biofilms that are either well-mixed or segregated depending on the make-up of the extracellular matrix[27]. Cells can also actively organize themselves in space through motility[28,29]. Undirected motility could increase species mixing. Directed motility, through chemotaxis, could allow cells to migrate to regions with preferred microenvironmental conditions. This could both promote species mixing or segregation, depending on how the different species move.

There is also a strong feedback between the spatial arrangement of species and chemicals (Fig 1C). The growth rate of a cell depends on its local microenvironment; this local microenvironment, in turn, is shaped by the metabolic reactions performed by the neighboring cells[2,5]. Species can thus be enriched in specific microenvironments where they grow well[4,19,30]. For example, when cells require metabolites produced by a different species, their growth becomes limited to regions where this other species is present. As the community grows, this feedback shapes the biogeography of the community (Fig 1C).

Spatial arrangement of chemicals

The spatial arrangement of chemicals depends both on the chemical and physical properties of the external environment and on the modification of this environment through the metabolic activity of microbial cells (Fig 1A). Biofilms typically have sizes that range from micrometers to millimeters. Over these lengths scales, diffusion can quickly homogenize the concentration of chemicals when there are no active sources or sinks. However, strong heterogeneities in concentration can form when there is active uptake and release of chemicals by cells[5,6,10,11,31]. Spatial variation in chemicals thus typically emerges as a consequence of the activity of cells.

The external environment can shape how chemicals organize in space. For example, nutrients typically enter a biofilm from the surrounding fluid or growth substrate. Cells in the outer biofilm layers, close to the nutrient source, can rapidly deplete the incoming nutrients and in the process secrete secondary metabolites. Cells can thus generate strong chemical gradients between the inner and outer layers of the biofilm[10,11,32]. Chemical gradients can also be formed by the amplification of external gradients, i.e. gradients that are also present in the absence of any microbial activity. For example, gradients can form around floating organic particles in water, or at the interface between a surface and a liquid flow[33]. These external gradients can also be caused by convection, i.e. by a flow of nutrients in the environment. Biofilms can alter these gradients, for example by altering the flow of nutrients[34,35], or by creating a nutrient depleted region downstream of the biofilm[36]. Finally, external gradients can be created by hosts, for example in the animal gut[37,38] or around plant roots[39]. The metabolic activity of cells strengthens these pre-existing, external, gradients, or in other words, reduces the length scales over which chemicals vary.

Stochastic variation in enzyme expression can also shape how chemicals organize in space. Protein levels can vary in the absence of genetic or environmental variation due to stochastic fluctuations in gene expression[40–42]. Stochastic fluctuations in enzyme levels in turn can cause fluctuations in the rate of metabolic reactions[43]. The feedback loop between a cell's metabolic activity and the state of its local environment can amplify these stochastic differences. The resulting spatial patterns depend on the type of feedback loop. With positive feedback, higher metabolic activity in one cell induces higher activity in neighboring cells. This can create clusters of cells with similar metabolic activities[44]. This is often seen in the regulation of exoenzymes where reaction products stimulate the production or export of the exoenzymes. Two examples are metal-bound siderophores, which increase siderophore production, and carbon monomers, which increase the production of enzymes that degrade polymers into monomers[45–47]. With negative feedback, higher metabolic activity in one cell reduces the activity in neighboring cells. For example, the metabolic products of one cell could inhibit the

reactions in neighboring cells through end-product inhibition. This can create patterns where neighboring cells specialize in different metabolic activities. For example, in filamentous cyanobacteria nitrogen fixation and photosynthesis are performed by distinct cell populations[48]. Stochastic gene expression combines with negative feedback to ensure that the nitrogen fixing cells are evenly spaced along the filament[49].

Difference in spatial arrangement of enzymes and chemicals

There is an important difference in how chemicals and enzymes are arranged in space: most enzymes are confined to cells, whereas most chemicals are freely exchanged through the environment. The confinement of enzymes to cells adds a strong constraint on which reactions can take place at different locations[7,10,50]. A metabolic reaction can only take place in a region if a species that can perform it is actually located there. This can generate correlations in the spatial organization of different enzymes, with enzymes produced by the same species co-occurring. Moreover, the production of multiple enzymes is often genetically co-regulated, and this also contributes to the spatial correlation of different enzymes.

Coupling metabolic reaction across space

When metabolic reactions are distributed in space, the overall metabolic activity of the community depends critically on the effectiveness with which these metabolic reactions are coupled in space. An important question thus arises: how are metabolites exchanged between cells[1,51]?

Metabolite exchange through direct cell-cell interactions

In some cases cells can directly exchange metabolites through physical connections. For example, in filamentous bacteria, neighboring cells can exchange molecules through pores in the membrane[48,52,53]. Direct cytosolic exchange, both within and between species, can also occur through membrane fusion or possibly nanotubes[54–56]. Finally, electrons can be exchanged through nanowires or extracellular DNA[57,58]. For example, in ocean sediments nutrients are located within the sediment layer, but oxygen (required as an electron acceptor) is located in the aqueous phase[59,60]. Centimeter long nanowires allow the microbial community to connect complementary reactions occurring in these two distinct regions.

Metabolite exchange through the environment

Most metabolites within microbial communities are exchanged by diffusion through the environment[5]. The effectiveness of metabolic coupling then depends on the length scales over which chemicals can be exchanged.

Length scales of spatial organization of enzymes and chemicals

We use the term *length scale* to refer to the characteristic distance over which the concentration of chemicals varies in the environment or the characteristic distance over which gene expression states change within the community. For example, in Fig 2 we defined the length scale as the distance at which the concentration of chemicals has decreased to 37% (=1/e) of that at the source. Here we will discuss the biological and physical factors that determine these length scales

Lengths scale of spatial organization of enzymes

An important driver of the spatial organization of enzymes is the feedback loop between the chemical microenvironment and gene regulatory dynamics. Due to the complexities of this feedback it is generally not feasible to predict the length scale over which enzymes vary. However, this length scale can be measured experimentally and for sufficiently simple and well studied systems it can even be predicted[61]. In multispecies communities an additional important length scale is that of spatial organization of species. In particular the size of clonal patches is an important quantity as it determines the distance between spatial domains where different metabolic reactions take place. The size of these patches depends on the physics of cell growth, as well as the other drives of biogeography[4,17,62–64].

Lengths scale of spatial organization of chemicals

Chemicals become arranged in space through the uptake and release of molecules by cells. Recent studies have identified a number of biophysical parameters that determine the length scale over which this happens[18,19,31,65–71]. Specifically, cell density, nutrient uptake rate, and diffusion rate are the most important parameters setting this length scale[19,72–74]. High cell densities and/or uptake rates lead to short length scales, while high diffusion rates lead to long length scales. The length scale can also depend on the external environment, for example on the external nutrient concentration. Finally, it also depends on the spatial organization of enzymes, as this determines the rate at which molecules are consumed and released at each location. Because of this complexity, we generally can only calculate this length scale for very simple communities.

For simple communities, we can analytically calculate the length scales over which chemicals vary in space. Specifically, we can do this when cells are densely and uniformly spaced, and when all cells take up the chemical of interest at the same rate. Under these conditions, we can calculate how far chemicals can—on average—travel before they are consumed (Fig 2A). This length scale depends on the ratio of the uptake to the diffusion rate, and on the cell

density (Fig 2B). These conditions are often met in cross-feeding communities, where mutually dependent strains exchange essential cellular building blocks. For example, mathematical models predict that yeast strains can exchange cellular building blocks across a range of about 100 µm, and experiments revealed that *Escherichia coli* bacteria exchange molecules on length scales of only a few micrometers[19,65,66,75]. Importantly, the length scale can vary dramatically between chemicals. While cell density alters the length scale of all chemicals in the same way, the other parameters modulate them differentially. Especially uptake rates of chemicals can vary by orders of magnitude, which in turn can lead to different length scales[19,76].

To derive the analytical length scale above we assumed that all cells take up nutrients at the same rate. However, often this assumption does not hold. For example, uptake rates often saturate at high nutrient concentrations (e.g., following Monod kinetics[77]). As a result, the length scale depends on the concentration of the supplied nutrient, and thus varies between environments (Fig 2C). Moreover, uptake rates depend on the enzymes and transporter proteins expressed by a cell and these could be regulated in response to the local nutrient concentration[9]. As long as the uptake rate only depends on the local concentration of the nutrient that is taken-up, we could potentially still calculate the length scale over which chemicals vary, provided that we have detailed physiological knowledge of the system. However, in many communities the uptake rate also depends on other factors. For example, uptake rates can vary between different species. In this case, we cannot even define a concept of a length scale over which chemicals vary in space because it will be different in each direction (Fig 2C). In this case the spatial arrangement of chemicals is determined by the location of the sources and sinks of these chemicals. In regions where cells take up chemicals, their concentration decreases exponentially, with a length scale that is set by the ratio of the uptake over diffusion rate (Fig 2D). In regions where chemicals are not taken up, they can rapidly spread by diffusion, and their concentration only slowly decreases with distance (Fig 2D)[31,78].

So far, we have only discussed metabolic interactions within a single biofilm, however chemicals can also be exchanged between biofilms. A striking example comes from *Bacillus subtilis* biofilms, where chemical and electrical oscillations can couple metabolic reactions in cells that are part of different biofilms. As a result, the metabolic activities of biofilms separated by several mm can fluctuate either in phase or anti-phase depending on nutrient concentrations[32,79].

Examples of spatial organization of enzymes and chemicals

In the previous sections we described the physical and biological factors that shape the spatial organization of enzymes and chemicals in microbial communities. As we showed, there are a number of important feedbacks that can lead to complex spatial patterns. In this section we discuss a number of examples to illustrate these points.

Anabolic cross-feeding in multispecies communities

Multispecies communities often contain auxotrophic species that cannot produce one or more essential metabolites. These auxotrophs can only grow by consuming metabolites that are leaked into the environment by nearby producers[80–82]. Producing these metabolites requires more energy than importing them from the environment, so typically all cells, producers and auxotrophs, take up these metabolites when they are present in the environment. The concentration of these metabolites thus rapidly (i.e. exponentially) decreases with distance from producing cells[78]. In the simplest scenario, when all species have comparable uptake pathways, it is possible to predict analytically the range over which metabolites are exchanged (Fig 2AB)[19]. Moreover, for two species communities it is possible to derive analytical predictions for the overall steady state properties of the community when the spatial arrangement of cell types is fully determined by the local growth dynamics[76].

Catabolic cross-feeding in multispecies communities

Catabolic pathways for both carbon and nitrogen are often divided between different species. Primary degraders partly catabolize the primary nutrient to intermediate metabolites that can be consumed by secondary degraders. The consumption of these metabolites is typically limited to distinct populations. As a result, consumers and producers do not have to be physically adjacent, as long as cells in between them do not take up the exchanged metabolite[10,83]. However, within each patch of consumer cells, the concentration of the metabolite again decreases exponentially. This sets a limit on how big these patches can grow. The overall arrangement of metabolic activity is thus primarily shaped by the growth dynamics of the different cell types[84–87].

Catabolic cross-feeding in clonal populations

Emergent gradients in chemicals can combine with gene regulatory networks to create metabolic interaction within clonal populations[83]. For example, *E. coli* switches to overflow metabolism when the carbon source (e.g., glucose) is available in growth saturating concentrations[88]. In this process cells ferment glucose to short chain fatty acids, such as acetate, which are released into the environment. Cells can respire this acetate, but only when

there is no glucose around: in the presence of glucose, acetate metabolism is inhibited by catabolite repression[8]. Catabolite repression thus drives the organization of metabolic activities in space, with glucose fermenting cells close to the nutrient source, followed at successively larger distances by glucose respiring cells, and acetate respiring cells[6,7,83]. More generally, gene regulation can cause cells to release metabolic intermediates when they grow in nutrient rich microenvironments[1,83,89,90]. These intermediates could then be consumed in cells in regions of the community where the primary nutrient has become depleted.

Coupling metabolic reactions across microenvironments

In spatially structured communities multiple gradients can form in different directions[5,83]. For example, at air-liquid interfaces, nutrients and oxygen are supplied from opposite sides. Similarly, in communities growing on nutrient rich substrates, nutrients and oxygen can enter the community from opposing directions. This creates distinct microenvironments each allowing for different reactions. This phenomenon has been well characterized in controlled laboratory conditions, for example by growing single-species colonies on agar. These colonies obtain nutrients from the bottom (i.e. from the agar), but oxygen from the top; in these conditions, a cross-feeding interaction based on carbon and/or nitrogen can form between fermenting cells at the bottom and respiring cells at the top[10,11]. Similarly, cross-feeding interactions can occur in more complex natural communities. For example, in sediments and wastewater biofilms, interactions are common between aerobic species growing near the oxygen rich liquid interface, and anaerobic species growing in oxygen depleted regions[91].

Consequences of spatial organization of metabolism

The spatial organization of metabolic activities can have important functional consequences for the ecology and evolution of microbial communities. Here we will give some examples.

Resolving metabolic incompatibilities

Incompatibilities between metabolic reactions can be resolved by segregating incompatible reactions to different spatial locations. These incompatibilities can have two main causes: First, the products of one reaction could inhibit a second reaction. In this case, splitting reactions between different cell types can resolve the incompatibility. For example, cyanobacteria require both photosynthesis and nitrogen fixation for growth. However, oxygen produced during photosynthesis deactivates the enzymes required for nitrogen fixation. Filamentous cyanobacteria thus separate these two processes into distinct cell types that are spatially organized along the filament[52,92]. Second, metabolic reactions could require fundamentally different microenvironments. In this case, metabolic coupling between cells growing in different microenvironments is required. For example, most sulfate reducing

bacteria are obligate anaerobes. In wastewater biofilms these bacteria grow within the anoxic core of the biofilm and interact metabolically with aerobic species growing in the oxic outer zone[93].

Incompatibilities can also be caused by gene regulation: two reactions might be biochemically compatible, but regulation might prevent both reactions from occurring simultaneously. As discussed above, *E. coli* performs overflow metabolism when grown on high glucose concentrations. Although cells could theoretically respire the produced acetate under these conditions, catabolite repression prevents them from doing so. In well-mixed systems this leads to a diauxic shift, where cells first ferment glucose and then respire acetate, provided that the acetate is not lost through flow. However, in spatially structured systems glucose fermentation and acetate respiration can occur simultaneously, as different regions specialize on different tasks[7,10]. As a result, the secreted acetate can be consumed even in the presence of flow.

Increasing resilience to environmental change

Spatial organization of metabolism often creates phenotypic variation which in turn might increase the resilience of a community or a clonal population to environmental change. The more phenotypic variation a community has, the higher the chances are that some cells have the ability to cope well with a rapid shift in the external conditions[94]. For example, biofilms often have a core of slow growing cells which has been linked to an increase in the tolerance to many environmental stressors[95,96]. Likewise, cells in different layers of the biofilm often specialize on different metabolic reactions, increasing the probability that some cells can adapt to a change in nutrient conditions[7,10,11].

Increasing species diversity

Chemical heterogeneities in the environment can promote biodiversity by allowing coexistence of species that specialize on different nutrients or that require different microenvironments for growth[62,90,97–99]. The resulting increase in diversity can promote ecosystem functioning across a variety of habitats, such as the soil[100,101] and the gut[102,103]. Moreover, spatial structure can facilitate interactions between species with incompatible niches. For example, obligate aerobes and anaerobes cannot grow together in a well-mixed system. However, the strong oxygen gradients in spatially structured communities create both oxic and anoxic niches allowing both to thrive within a short distance of each other[2].

Modulating interspecies interactions

Spatial organization can modulate how species within a community interact with each other. In a well-mixed system, all cells interact equally with all other cells. However, in spatially structured systems the strength of interaction depends on the distance between cells and on the distance over which chemicals can be exchanged. Typically, cells only interact with a small subset of other cells[19,104]. In multispecies communities this can moreover limit interactions to a subset of the other species in the community. This can have important consequences for the ecology of the community. For example, it can shield cells from negative interspecies interactions, but also prevent cells from interacting with the partner species they need to grow well[4,72,75,85,104]. Limiting interactions to a smaller subset of cells can also affect the evolution of microbial communities, for example by stabilizing cooperative interactions[4,72,73,75,85,104] by slowing the rate of adaptation[105], or by modulating the optimal production levels of inhibiting compounds[106].

Open questions

In recent years, we have gained much insight on how metabolic processes become spatially organized and on how this affects the functionality of the community. However, there are still many open questions that we believe are promising directions for future research.

How does the spatial organization of metabolic activities evolve?

The spatial organization of metabolism arises from the feedback between the organization of enzymes and chemicals. The arrangement of enzymes, in turn, is primarily the result of gene regulation and the spatial arrangement of cells. Both of these factors depend at least partly on cellular traits that are under genetic control. Did gene regulatory networks and other cell properties evolve to organize metabolic activities in space to improve the growth or survival of cells in these spatial communities?

Take, for example, overflow metabolism and catabolite repression. Previous studies have shown that overflow metabolism corresponds to a proteome allocation pattern that maximizes growth in well-mixed, high-nutrient, conditions[88]. Likewise, catabolite repression is often seen as a strategy for *E. coli* to adapt to temporally fluctuating environments[107]. However, these two regulatory pathways also give rise to a cross-feeding interaction within spatial populations[6,83]. Did selection on spatial patterns play any role in how these networks function? Or did they purely evolve for the previously stated reasons?

More generally, the evolution of gene-regulatory networks is often discussed in the context of how it allows cells to cope with temporal changes in the environment[108]. But in the natural

environment of bacteria, spatial variation is likely as important a factor as temporal variation. To what extent did gene regulatory networks evolve to organize metabolic activities in space? We believe this is an important question to address in future theoretical and experimental work.

Does spatial organization of metabolism follow some optimality principle?

The overall metabolic activity and growth of a community depends on how its metabolism is organized in space. This raises an important question: what arrangement of metabolic reactions does best achieve a certain objective function? This objective function can take many different forms: in a biotechnology setting maximizing the rate at which a certain chemical is produced or degraded might be the relevant objective function. While in an evolving community, the long term growth and survival rate might be the relevant objective function. Given an appropriate objective function for the system under consideration, the question is then how metabolic reactions should be arranged in order to maximize the objective. Theoretical approaches can help answer this question[61,109].

Once we know the optimal arrangement, we can also assess to what extent natural or engineered communities approach this optimal solution. If cells evolved to organize metabolism in space, we would expect a rather close match between the observed and expected arrangements. However, reactions are packed in individual cells, and the growth dynamics of these cells can cause the community to move away from an optimal arrangement. For example, interspecies cross-feeding interaction would work optimally in highly mixed systems, however cell growth decreases the degree of mixing of a community by creating clonal clusters[19,110]. To what extent did cells find strategies to maintain close-to-optimal arrangements? And for communities that are far from optimal, could we find strategies to make them function better? For this last question, experimental evolution and synthetic biology approaches can make important contributions[111–113]. These questions are not just of intellectual interest: a better understanding of how to optimally organize metabolism in space can also help improve engineered communities.

How are single- and multispecies biofilms different?

Metabolic processes can become spatially organized in both single- and multispecies biofilms, however there are some important differences. In single-species biofilms all cells share the same genome. The spatial organization of metabolic activities is thus fully under control of a shared gene regulatory-network. Moreover, each cell in the biofilm can found a new “offspring” biofilm with the same genetic makeup[114]. In this regard, single-species biofilms are thus similar to multicellular organisms that organize their activities in space through a developmental

process. Moreover, single-species biofilms are often formed by the clonal expansion of a single cell[114]. They thus undergo a single-cell bottleneck, which potentiates higher order selection (i.e. selection acting on group level properties such as the spatial organization of metabolic activity) by alleviating conflicts of interest between the individual and the community[115,116].

In contrast, in multispecies biofilms, the biogeography puts a strong constraint on how metabolic activities can be arranged. This can prevent a community from reaching a spatial organization that maximizes growth. Moreover, multispecies biofilms by definition require multiple cells to found a new biofilm, this makes it harder to produce "offspring" biofilms that have the same genetic make-up. In addition, in the absence of single-cell bottlenecks, mutations that give rise to conflicts of interests between individuals and the community are likely to spread[116]. As a result, higher order selection is typically much less effective in multispecies biofilms compared to single species biofilms. However, if multispecies biofilms "reproduce" by releasing small multicellular aggregates, higher order selection can still occur[117]. Moreover, even in the absence of higher order selection, spatial organization of metabolic activities and metabolic interactions can still (co)-evolve through selection at the individual level.

Higher order selection can allow for the evolution of spatial organization of metabolic activities that optimize the growth of the entire community, even when it (slightly) reduces the growth of individual community members. We would thus expect to find this kind of spatial organization primarily within single species biofilms. For example, many anabolic pathways show economies of scale: the cost of producing one extra molecule is lower the more molecules a cell produces[118]. In well-mixed systems, cross-feeding communities that divide anabolic labor grow faster than clonal populations that do not[51,80]. Could spatial structure allow clonal populations to benefit from a similar division of labor? For example, neighboring cells could potentially divide labor by producing complementary sets of metabolites. The ubiquity of post-translational regulation makes it hard to test this hypothesis experimentally, and so far only very circumstantial evidence has been found[44]. However, with new technologies it has become possible to revisit the question to what extent clonal populations have evolved to spatially organize metabolism[11].

How can we study spatial organization in natural systems?

Many insights into how metabolism becomes spatially organized have come from studies of synthetic lab systems of low complexity. We still have a poor understanding of how these findings translate to more complex natural communities. Bridging this knowledge gap should be a central research goal in the coming years. To reach this goal we need to measure the

metabolic activity of cells at high spatial resolution within natural communities. This remains highly challenging, but recent technological advances that allow for spatially resolved measurements of metabolic activity (e.g., Nanosims) and transcriptional activity (e.g., seqFISH), are starting to make this possible[11,69,119–123]. A promising way forward would be to combine bottom-up approaches, such as microfluidic systems, where we can measure the activity of cells at high spatiotemporal resolution in semi-realistic settings, with top-down approaches where we obtain more coarse-grained measurements of communities in more realistic settings. These approaches yield complementary insight and an effort should be made to connect them together.

What role does temporal variation play in spatially organizing metabolism?

So far we have only talked about space, but time is equally important[108]. Natural environments are hardly ever constant, and changes in the external environment can lead to substantial changes in the arrangement of chemicals and enzymes within the community. Moreover, microbial communities themselves can change drastically over time. Ephemeral particles, such as marine snow, show quick succession dynamics with a rapid turn-over of species as the particle is degraded and metabolites accumulate[33,124]. Many biofilms are seeded by a small number of cells that expand over time[114]. As cell number and species composition changes, so does the chemical environment. Moreover, metabolic activities can show complex spatiotemporal patterns, such as spiral waves of metabolic activity within biofilms[125]. Finally, vastly different time scales play a role: diffusion acts on the second scale, gene regulation on the minute scale, and cell growth on the hour scale. Thus, an important question is how all these temporal scales combine to determine the spatial organization of metabolic activities in microbial communities.

Conclusion

The metabolic processes performed by microbes are of critical importance for the health and wellbeing of humans and the planet. Many of these processes depend on the spatial organization of metabolic reactions within the microbial community. For example, the overall metabolic activity of wastewater biofilms depends critically on metabolic interactions between cells growing in the anoxic core with those in the oxic surface layer. We have learned a lot about how cells in lab conditions can spatially organize their metabolic activities and how this impacts the community function. However, we have only started to scratch the surface on how these processes work in natural communities and how these processes have evolved. We believe these are exciting and important questions that will drive the field forward in the years to come.

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Declaration of interests

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References

1. Fritts Ryan K., Fritts Ryan K., McCully Alexandra L., and McKinlay James B. (2021). Extracellular Metabolism Sets the Table for Microbial Cross-Feeding. Microbiol. Mol. Biol. Rev. *85*, e00135-20. 10.1128/MMBR.00135-20.

2. Flemming, H.-C., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., and Kjelleberg, S. (2016). Biofilms: an emergent form of bacterial life. Nat. Rev. Microbiol. *14*, 563–575. 10.1038/nrmicro.2016.94.

3. Flemming, H.-C., and Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms. Nat. Rev. Microbiol. *17*, 247–260. 10.1038/s41579-019-0158-9.

4. Nadell, C.D., Drescher, K., and Foster, K.R. (2016). Spatial structure, cooperation and competition in biofilms. Nat. Rev. Microbiol. *14*, 589.

5. Stewart, P.S., and Franklin, M.J. (2008). Physiological heterogeneity in biofilms. Nat. Rev. Microbiol. *6*, 199–210. 10.1038/nrmicro1838.

6. Dal Co, A., Van Vliet, S., and Ackermann, M. (2019). Emergent microscale gradients give rise to metabolic cross-feeding and antibiotic tolerance in clonal bacterial populations. Philos. Trans. R. Soc. B Biol. Sci. *374*. 10.1098/rstb.2019.0080.

7. Dal Co, A., Ackermann, M., and van Vliet, S. (2019). Metabolic activity affects the response of single cells to a nutrient switch in structured populations. J. R. Soc. Interface *16*, 20190182. 10.1098/rsif.2019.0182.

8. Chubukov, V., Gerosa, L., Kochanowski, K., and Sauer, U. (2014). Coordination of microbial metabolism. Nat. Rev. Microbiol. *12*, 327–340. 10.1038/nrmicro3238.

9. Shimizu, K. (2014). Regulation Systems of Bacteria such as Escherichia coli in Response to Nutrient Limitation and Environmental Stresses. Metabolites *4*, 1–35. 10.3390/metabo4010001.

10. Cole, J.A., Kohler, L., Hedhli, J., and Luthey-schulten, Z. (2015). Spatially-resolved metabolic cooperativity within dense bacterial colonies. BMC, 1–17. 10.1186/s12918-015-0155-1.

11. Díaz-Pascual, F., Lempp, M., Nosho, K., Jeckel, H., Jo, J.K., Neuhaus, K., Hartmann, R., Jelli, E., Hansen, M.F., Price-Whelan, A., et al. (2021). Spatial alanine metabolism determines local growth dynamics of Escherichia coli colonies. eLife *10*, e70794. 10.7554/eLife.70794.

12. Marinkovic, Z.S., Vulin, C., Acman, M., Song, X., Di Meglio, J.-M., Lindner, A.B., and Hersen, P. (2019). A microfluidic device for inferring metabolic landscapes in yeast monolayer colonies. eLife *8*, e47951. 10.7554/eLife.47951.

13. Welch, J.L.M., Hasegawa, Y., McNulty, N.P., Gordon, J.I., and Borisy, G.G. (2017). Spatial organization of a model 15-member human gut microbiota established in gnotobiotic mice. Proc. Natl. Acad. Sci. U. S. A. *114*, E9105–E9114. 10.1073/pnas.1711596114.

14. Shi, H., Shi, Q., Grodner, B., Lenz, J.S., Zipfel, W.R., Brito, I.L., and De Vlaminck, I. (2020). Highly multiplexed spatial mapping of microbial communities. Nature *588*, 676–681. 10.1038/s41586-020-2983-4.

15. Kolenbrander, P.E., Palmer, R.J., Periasamy, S., and Jakubovics, N.S. (2010). Oral multispecies biofilm development and the key role of cell–cell distance. Nat. Rev. Microbiol. *8*, 471–480. 10.1038/nrmicro2381.

16. Mark Welch, J.L., Rossetti, B.J., Rieken, C.W., Dewhirst, F.E., and Borisy, G.G. (2016). Biogeography of a human oral microbiome at the micron scale. Proc. Natl. Acad. Sci. U. S. A. *113*, E791-800. 10.1073/pnas.1522149113.

17. Azimi, S., Lewin, G.R., and Whiteley, M. (2022). The biogeography of infection revisited. Nat. Rev. Microbiol. *0123456789*. 10.1038/s41579-022-00683-3.

18. Darch, S.E., Simoska, O., Fitzpatrick, M., Barraza, J.P., Stevenson, K.J., Bonnecaze, R.T., Shear, J.B., and Whiteley, M. (2018). Spatial determinants of quorum signaling in a Pseudomonas aeruginosa infection model. Proc. Natl. Acad. Sci. *115*, 201719317. 10.1073/pnas.1719317115.

19. Dal Co, A., van Vliet, S., Kiviet, D.J., Schlegel, S., Ackermann, M., Dal Co, A., van Vliet, S., Kiviet, D.J., Schlegel, S., and Ackermann, M. (2020). Short-range interactions govern the dynamics and functions of microbial communities. Nat. Ecol. Evol. *4*, 366–375. 10.1038/s41559-019-1080-2.

20. Røder, H.L., Olsen, N.M.C., Whiteley, M., and Burmølle, M. (2020). Unravelling interspecies interactions across heterogeneities in complex biofilm communities. Environ. Microbiol. *22*, 5–16. 10.1111/1462-2920.14834.

21. Olsen, N.M.C., Røder, H.L., Russel, J., Madsen, J.S., Sørensen, S.J., and Burmølle, M. (2019). Priority of Early Colonizers but No Effect on Cohabitants in a Synergistic Biofilm Community. Front. Microbiol. *10*.

22. Debray, R., Herbert, R.A., Jaffe, A.L., Crits-Christoph, A., Power, M.E., and Koskella, B. (2022). Priority effects in microbiome assembly. Nat. Rev. Microbiol. *20*, 109–121. 10.1038/s41579-021-00604-w.

23. Maier, B. (2021). How Physical Interactions Shape Bacterial Biofilms. Annu. Rev. Biophys. *50*, 401–417. 10.1146/annurev-biophys-062920-063646.

24. Smith William P. J., Davit Yohan, Osborne James M., Kim Wook, Foster Kevin R., and Pitt-Francis Joe M. (2017). Cell morphology drives spatial patterning in microbial communities. Proc. Natl. Acad. Sci. *114*, E280–E286. 10.1073/pnas.1613007114.

25. Rickard, A.H., Gilbert, P., High, N.J., Kolenbrander, P.E., and Handley, P.S. (2003). Bacterial coaggregation: an integral process in the development of multi-species biofilms. Trends Microbiol. *11*, 94–100. 10.1016/S0966-842X(02)00034-3.

26. Kan, A., Del Valle, I., Rudge, T., Federici, F., and Haseloff, J. (2018). Intercellular adhesion promotes clonal mixing in growing bacterial populations. J. R. Soc. Interface *15*, 20180406. 10.1098/rsif.2018.0406.

27. Chew, S.C., Kundukad, B., Seviour, T., van der Maarel, J.R.C., Yang, L., Rice, S.A., Doyle, P., and Kjelleberg, S. (2014). Dynamic remodeling of microbial biofilms by functionally distinct exopolysaccharides. mBio *5*, e01536. 10.1128/mBio.01536-14.

28. Sweeney Emily G., Nishida Andrew, Weston Alexandra, Bañuelos Maria S., Potter Kristin, Conery John, Guillemin Karen, and Butler Geraldine (2019). Agent-Based Modeling Demonstrates How Local Chemotactic Behavior Can Shape Biofilm Architecture. mSphere *4*, e00285-19. 10.1128/mSphere.00285-19.

29. Laganenka Leanid, Sourjik Victor, and Drake Harold L. (2018). Autoinducer 2-Dependent Escherichia coli Biofilm Formation Is Enhanced in a Dual-Species Coculture. Appl. Environ. Microbiol. *84*, e02638-17. 10.1128/AEM.02638-17.

30. Momeni, B., Brileya, K.A., Fields, M.W., and Shou, W. (2013). Strong inter-population cooperation leads to partner intermixing in microbial communities. eLife *2*. 10.7554/eLife.00230.

31. van Gestel, J., Bareia, T., Tenennbaum, B., Dal Co, A., Guler, P., Aframian, N., Puyesky, S., Grinberg, I., D'Souza, G.G., Erez, Z., et al. (2021). Short-range quorum sensing controls horizontal gene transfer at micron scale in bacterial communities. Nat. Commun. *12*, 1–11. 10.1038/s41467-021-22649-4.

32. Liu, J., Prindle, A., Humphries, J., Gabalda-Sagarra, M., Asally, M., Lee, D.D., Ly, S., Garcia-Ojalvo, J., and Süel, G.M. (2015). Metabolic co-dependence gives rise to collective oscillations within biofilms. Nature *523*, 550–554. 10.1038/nature14660.

33. Cordero, O.X., and Datta, M.S. (2016). Microbial interactions and community assembly at microscales. Curr. Biol., 227–234. 10.1016/j.mib.2016.03.015.Microbial.

34. Drescher, K., Shen, Y., Bassler, B.L., and Stone, H.A. (2013). Biofilm streamers cause catastrophic disruption of flow with consequences for environmental and medical systems. Proc. Natl. Acad. Sci. *110*, 4345–4350. 10.1073/pnas.1300321110.

35. Persat, A., Nadell, C.D., Kim, M.K., Ingremeau, F., Siryaporn, A., Drescher, K., Wingreen, N.S., Bassler, B.L., Gitai, Z., and Stone, H.A. (2015). The Mechanical World of Bacteria. Cell *161*, 988–997. 10.1016/j.cell.2015.05.005.

36. Stewart, P.S. (2012). Mini-review: Convection around biofilms. Biofouling *28*, 187–198. 10.1080/08927014.2012.662641.

37. Chikina, A., and Matic Vignjevic, D. (2021). At the right time in the right place: How do luminal gradients position the microbiota along the gut? Quant. Cell Dev. Biol. *168*, 203712. 10.1016/j.cdev.2021.203712.

38. Nguyen, J., Pepin, D.M., and Tropini, C. (2021). Cause or effect? The spatial organization of pathogens and the gut microbiota in disease. Spec. Issue Intest. Microbiota Health Dis. *23*, 104815. 10.1016/j.micinf.2021.104815.

39. Sasse, J., Martinoia, E., and Northen, T. (2018). Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? Trends Plant Sci. *23*, 25–41. 10.1016/j.tplants.2017.09.003.

40. Elowitz, M.B., Levine, A.J., Siggia, E.D., and Swain, P.S. (2002). Stochastic Gene Expression in a Single Cell. Science *297*, 1183–1186.

41. Ozbudak, E.M., Thattai, M., Kurtser, I., Grossman, A.D., and van Oudenaarden, A. (2002). Regulation of noise in the expression of a single gene. Nat. Genet. *31*, 69–73. 10.1038/ng869.

42. Raj, A., and van Oudenaarden, A. (2008). Nature, nurture, or chance: stochastic gene expression and its consequences. Cell *135*, 216–226. 10.1016/j.cell.2008.09.050.

43. Kiviet, D.J., Nghe, P., Walker, N., Boulineau, S., Sunderlikova, V., and Tans, S.J. (2014). Stochasticity of metabolism and growth at the single-cell level. Nature *514*, 376–379. 10.1038/nature13582.

44. van Vliet, S., Dal Co, A., Winkler, A.R., Spriewald, S., Stecher, B., and Ackermann, M. (2018). Spatially Correlated Gene Expression in Bacterial Groups: The Role of Lineage

History, Spatial Gradients, and Cell-Cell Interactions. Cell Syst. *6*, 496-507.e6. 10.1016/j.cels.2018.03.009.

45. Leventhal, G.E., Ackermann, M., and Schiessl, K.T. (2019). Why microbes secrete molecules to modify their environment: The case of iron-chelating siderophores. J. R. Soc. Interface *16*. 10.1098/rsif.2018.0674.

46. Saha, M., Sarkar, S., Sarkar, B., Sharma, B.K., Bhattacharjee, S., and Tribedi, P. (2016). Microbial siderophores and their potential applications: a review. Environ. Sci. Pollut. Res. *23*, 3984–3999. 10.1007/s11356-015-4294-0.

47. Beier, S., and Bertilsson, S. (2013). Bacterial chitin degradation—mechanisms and ecophysiological strategies. Front. Microbiol. *4*.

48. Herrero, A., Stavans, J., and Flores, E. (2016). The multicellular nature of filamentous heterocyst-forming cyanobacteria. FEMS Microbiol. Rev. *40*, 831–854. 10.1093/femsre/fuw029.

49. Corrales-Guerrero, L., Tal, A., Arbel-Goren, R., Mariscal, V., Flores, E., Herrero, A., and Stavans, J. (2015). Spatial Fluctuations in Expression of the Heterocyst Differentiation Regulatory Gene hetR in Anabaena Filaments. PLOS Genet. *11*, e1005031. 10.1371/journal.pgen.1005031.

50. Gupta, S., Ross, T.D., Gomez, M.M., Grant, J.L., Romero, P.A., and Venturelli, O.S. (2020). Investigating the dynamics of microbial consortia in spatially structured environments. Nat. Commun. *11*, 1–15. 10.1038/s41467-020-16200-0.

51. D'Souza, G., Shitut, S., Preussger, D., Ghada, Y., Waschina, S., and Kost, C. (2018). Ecology and evolution of metabolic cross-feeding interactions in bacteria. Nat. Prod. Rep. *35*, 455–488. 10.1039/c8np00009c.

52. Fay, P. (1992). Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol. Rev. *56*, 340–373.

53. Kieninger, A.-K., and Maldener, I. (2021). Cell–cell communication through septal junctions in filamentous cyanobacteria. Curr. Opin. Microbiol. *61*, 35–41. 10.1016/j.mib.2021.02.002.

54. Ducret, A., Fleuchot, B., Bergam, P., and Mignot, T. (2013). Direct live imaging of cell-cell protein transfer by transient outer membrane fusion in Myxococcus xanthus. eLife *2*, e00868–e00868. 10.7554/eLife.00868.

55. Pande, S., Shitut, S., Freund, L., Westermann, M., Bertels, F., Colesie, C., Bischofs, I.B., and Kost, C. (2015). Metabolic cross-feeding via intercellular nanotubes among bacteria. Nat. Commun. *6*, 6238. 10.1038/ncomms7238.

56. Dubey, G.P., and Ben-Yehuda, S. (2011). Intercellular Nanotubes Mediate Bacterial Communication. Cell *144*, 590–600. 10.1016/j.cell.2011.01.015.

57. Nielsen, L.P., Risgaard-Petersen, N., Fossing, H., Christensen, P.B., and Sayama, M. (2010). Electric currents couple spatially separated biogeochemical processes in marine sediment. Nature *463*, 1071–1074. 10.1038/nature08790.

58. Slinker, J.D., Muren, N.B., Renfrew, S.E., and Barton, J.K. (2011). DNA charge transport over 34 nm. Nat. Chem. *3*, 228–233. 10.1038/nchem.982.

59. Bhattacharya, S., Roy, C., Mandal, S., Sarkar, J., Rameez, M.J., Mondal, N., Mapder, T., Chatterjee, S., Pyne, P., Alam, M., et al. (2020). Aerobic microbial communities in the sediments of a marine oxygen minimum zone. FEMS Microbiol. Lett. *367*, fnaa157. 10.1093/femsle/fnaa157.

60. Long, A.M., Jurgensen, S.K., Petchel, A.R., Savoie, E.R., and Brum, J.R. (2021). Microbial Ecology of Oxygen Minimum Zones Amidst Ocean Deoxygenation. Front. Microbiol. *12*.

61. Guo Yipei, Tikhonov Mikhail, and Brenner Michael P. (2018). Local growth rules can maintain metabolically efficient spatial structure throughout growth. Proc. Natl. Acad. Sci. *115*, 3593–3598. 10.1073/pnas.1801853115.

62. Li, S., Wang, P., Chen, Y., Wilson, M.C., Yang, X., Ma, C., Lu, J., Chen, X., Wu, J., Shu, W., et al. (2020). Island biogeography of soil bacteria and fungi: similar patterns, but different mechanisms. ISME J. *14*, 1886–1896. 10.1038/s41396-020-0657-8.

63. Labarthe, S., Polizzi, B., Phan, T., Goudon, T., Ribot, M., and Laroche, B. (2019). A mathematical model to investigate the key drivers of the biogeography of the colon microbiota. J. Theor. Biol. *462*, 552–581. 10.1016/j.jtbi.2018.12.009.

64. Stacy, A., McNally, L., Darch, S.E., Brown, S.P., and Whiteley, M. (2016). The biogeography of polymicrobial infection. Nat. Rev. Microbiol. *14*, 93–105. 10.1038/nrmicro.2015.8.

65. Christensen, B.B., Haagensen, J.A.J.J., Heydorn, A., and Molin, S. (2002). Metabolic commensalism and competition in a two-species microbial consortium. Appl. Environ. Microbiol. *68*, 2495–2502. 10.1128/AEM.68.5.2495-2502.2002.

66. Muller, M.J.I., Neugeboren, B.I., Nelson, D.R., and Murray, A.W. (2014). Genetic drift opposes mutualism during spatial population expansion. Proc. Natl. Acad. Sci. *111*, 1037–1042. 10.1073/pnas.1313285111.

67. He, X., Chadwick, G., Kempes, C., Shi, Y., McGlynn, S., Orphan, V., and Meile, C. (2019). Microbial interactions in the anaerobic oxidation of methane: model simulations constrained by process rates and activity patterns. Environ. Microbiol. *21*, 631–647. 10.1111/1462-2920.14507.

68. Drescher, K., Nadell, C.D., Stone, H.A., Wingreen, N.S., and Bassler, B.L. (2014). Report Solutions to the Public Goods Dilemma in Bacterial Biofilms. Curr. Biol. *24*, 50–55. 10.1016/j.cub.2013.10.030.

69. McGlynn, S.E., Chadwick, G.L., Kempes, C.P., and Orphan, V.J. (2015). Single cell activity reveals direct electron transfer in methanotrophic consortia. Nature *526*, 531.

70. Stump, S.M., Johnson, E.C., and Klausmeier, C.A. (2018). Local interactions and self-organized spatial patterns stabilize microbial cross-feeding against cheaters. J. R. Soc. Interface *15*, 20170822. 10.1098/rsif.2017.0822.

71. Stump, S.M., Johnson, E.C., Sun, Z., and Klausmeier, C.A. (2018). How spatial structure and neighbor uncertainty promote mutualists and weaken black queen effects. J. Theor. Biol. *446*, 33–60. 10.1016/J.JTBI.2018.02.031.

72. Dobay, A., Bagheri, H.C., Messina, A., Kümmerli, R., and Rankin, D.J. (2014). Interaction effects of cell diffusion, cell density and public goods properties on the evolution of cooperation in digital microbes. J. Evol. Biol. *27*, 1869–1877. 10.1111/jeb.12437.

73. Lindsay, R.J., Pawlowska, B.J., and Gudelj, I. (2018). When increasing population density can promote the evolution of metabolic cooperation. ISME J. *12*, 849–859. 10.1038/s41396-017-0016-6.

74. Ross-gillespie, A., and Kümmerli, R. (2014). Collective decision-making in microbes. Front. Microbiol. *5*, 1–12. 10.3389/fmicb.2014.00054.

75. Momeni, B., Waite, A.J., and Shou, W. (2013). Spatial self-organization favors heterotypic cooperation over cheating. eLife *2*, 1–18. 10.7554/eLife.00960.

76. van Vliet, S., Hauert, C., Fridberg, K., Ackermann, M., and Dal Co, A. (2022). Global dynamics of microbial communities emerge from local interaction rules. PLOS Comput. Biol. *18*, e1009877. 10.1371/journal.pcbi.1009877.

77. Monod, J. (1949). The Growth of Bacterial Cultures. Annu. Rev. Microbiol. *3*, 371–394. 10.1146/annurev.mi.03.100149.002103.

78. van Tatenhove-Pel, R.J., Rijavec, T., Lapanje, A., van Swam, I., Zwering, E., Hernandez-Valdes, J.A., Kuipers, O.P., Picioreanu, C., Teusink, B., and Bachmann, H. (2020). Microbial competition reduces metabolic interaction distances to the low µm-range. ISME J. 10.1038/s41396-020-00806-9.

79. Liu, J., Martinez-Corral, R., Prindle, A., Lee, D.-Y.D., Larkin, J., Gabalda-Sagarra, M., Garcia-Ojalvo, J., and Süel, G.M. (2017). Coupling between distant biofilms and emergence of nutrient time-sharing. Science *356*, 638–642. 10.1126/science.aah4204.

80. D'Souza, G., Waschina, S., Pande, S., Bohl, K., Kaleta, C., and Kost, C. (2014). Less is more: Selective advantages can explain the prevalent loss of biosynthetic genes in bacteria. Evolution *68*, 2559–2570. 10.1111/evo.12468.

81. Wintermute, E.H., and Silver, P.A. (2010). Emergent cooperation in microbial metabolism. Mol. Syst. Biol. *6*, 407. 10.1038/msb.2010.66.

82. Mee, M.T., Collins, J.J., Church, G.M., and Wang, H.H. (2014). Syntrophic exchange in synthetic microbial communities. 10.1073/pnas.1405641111.

83. Evans, C.R., Kempes, C.P., Price-Whelan, A., and Dietrich, L.E.P. (2020). Metabolic Heterogeneity and Cross-Feeding in Bacterial Multicellular Systems. Trends Microbiol. *28*, 732–743. 10.1016/j.tim.2020.03.008.

84. Mitri, S., Clarke, E., and Foster, K.R. (2015). Resource limitation drives spatial organization in microbial groups. ISME J., 1–12. 10.1038/ismej.2015.208.

85. Nadell, C.D., Foster, K.R., and Xavier, J.B. (2010). Emergence of spatial structure in cell groups and the evolution of cooperation. PLoS Comput. Biol. *6*. 10.1371/journal.pcbi.1000716.

86. Goldschmidt, F., Regoes, R.R., and Johnson, D.R. (2018). Metabolite toxicity slows local diversity loss during expansion of a microbial cross-feeding community. ISME J. *12*, 136–144. 10.1038/ismej.2017.147.

87. Hallatschek, O., and Nelson, D.R. (2010). LIFE AT THE FRONT OF AN EXPANDING POPULATION. Evolution *64*, 193–206. 10.1111/j.1558-5646.2009.00809.x.

88. Basan, M., Hui, S., Okano, H., Zhang, Z., Shen, Y., Williamson, J.R., and Hwa, T. (2015). Overflow metabolism in Escherichia coli results from efficient proteome allocation. Nature *528*, 99–104. 10.1038/nature15765.

89. Deutscher, J. (2008). The mechanisms of carbon catabolite repression in bacteria. Cell Regul. *11*, 87–93. 10.1016/j.mib.2008.02.007.

90. Roman, M.S., and Wagner, A. (2018). An enormous potential for niche construction through bacterial cross-feeding in a homogeneous environment. PLoS Comput. Biol. *14*, e1006340. 10.1371/journal.pcbi.1006340.

91. Jo, J., Price-Whelan, A., and Dietrich, L.E.P. (2022). Gradients and consequences of heterogeneity in biofilms. Nat. Rev. Microbiol. 10.1038/s41579-022-00692-2.

92. Ernst, A., and Maldener, I. (2001). Cyanobacterial Heterocysts. Life Sci., 1–7.

93. Okabe, S., Itoh, T., Satoh, H., and Watanabe, Y. (1999). Analyses of spatial distributions of sulfate-reducing bacteria and their activity in aerobic wastewater biofilms. Appl. Environ. Microbiol. *65*, 5107–5116. 10.1128/AEM.65.11.5107-5116.1999.

94. Veening, J.-W., Smits, W.K., and Kuipers, O.P. (2008). Bistability, Epigenetics, and Bet-Hedging in Bacteria. Annu. Rev. Microbiol. *62*, 193–210. 10.1146/annurev.micro.62.081307.163002.

95. Hall, C.W., and Mah, T.-F. (2017). Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. FEMS Microbiol. Rev. *41*, 276–301. 10.1093/femsre/fux010.

96. Ciofu, O., Moser, C., Jensen, P.Ø., and Høiby, N. (2022). Tolerance and resistance of microbial biofilms. Nat. Rev. Microbiol. 10.1038/s41579-022-00682-4.

97. Dubey, M., Hadadi, N., Pelet, S., Carraro, N., Johnson, D.R., and van der Meer, J.R. (2021). Environmental connectivity controls diversity in soil microbial communities. Commun. Biol. *4*, 1–15. 10.1038/s42003-021-02023-2.

98. D'Andrea, R., Riolo, M., and Ostling, A.M. (2019). Generalizing clusters of similar species as a signature of coexistence under competition. PLoS Comput. Biol. *15*, 1–19. 10.1371/journal.pcbi.1006688.

99. Bauer, E., Zimmermann, J., Baldini, F., Thiele, I., and Kaleta, C. (2017). BacArena: Individual-based metabolic modeling of heterogeneous microbes in complex communities. PLOS Comput. Biol. *13*, e1005544. 10.1371/journal.pcbi.1005544.

100. Pierre-Alain, M., Amadou, S., Aurore, K., Jean, L., Olivier, M., Julien, G., Battle, K., Laetitia, B., Samuel, D., Sébastien, T., et al. (2022). High Microbial Diversity Promotes Soil Ecosystem Functioning. Appl. Environ. Microbiol. *84*, e02738-17. 10.1128/AEM.02738-17.

101. Delgado-Baquerizo, M., Maestre, F.T., Reich, P.B., Jeffries, T.C., Gaitan, J.J., Encinar, D., Berdugo, M., Campbell, C.D., and Singh, B.K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. Nat. Commun. *7*, 10541. 10.1038/ncomms10541.

102. Giloteaux, L., Goodrich, J.K., Walters, W.A., Levine, S.M., Ley, R.E., and Hanson, M.R. (2016). Reduced diversity and altered composition of the gut microbiome in individuals with myalgic encephalomyelitis/chronic fatigue syndrome. Microbiome *4*, 30. 10.1186/s40168-016-0171-4.

103. Zouiouich, S., Loftfield, E., Huybrechts, I., Viallon, V., Louca, P., Vogtmann, E., Wells, P.M., Steves, C.J., Herzig, K.-H., Menni, C., et al. (2021). Markers of metabolic health and gut microbiome diversity: findings from two population-based cohort studies. Diabetologia *64*, 1749–1759. 10.1007/s00125-021-05464-w.

104. Mitri Sara, Xavier João B., and Foster Kevin R. (2011). Social evolution in multispecies biofilms. Proc. Natl. Acad. Sci. *108*, 10839–10846. 10.1073/pnas.1100292108.

105. Chacón, J.M., Shaw, A.K., and Harcombe, W.R. (2020). Increasing growth rate slows adaptation when genotypes compete for diffusing resources. PLOS Comput. Biol. *16*, e1007585. 10.1371/journal.pcbi.1007585.

106. Gerardin, Y., Springer, M., and Kishony, R. (2016). A competitive trade-off limits the selective advantage of increased antibiotic production. Nat. Microbiol. *1*, 16175. 10.1038/nmicrobiol.2016.175.

107. Khonsari, A.S., and Kollmann, M. (2015). Perception and regulatory principles of microbial growth control. PloS One *10*, e0126244–e0126244. 10.1371/journal.pone.0126244.

108. Nguyen, J., Lara-Gutiérrez, J., and Stocker, R. (2021). Environmental fluctuations and their effects on microbial communities, populations and individuals. FEMS Microbiol. Rev. *45*, fuaa068. 10.1093/femsre/fuaa068.

109. Gottstein, W., Olivier, B.G., Bruggeman, F.J., and Teusink, B. (2016). Constraint-based stoichiometric modelling from single organisms to microbial communities. J. R. Soc. Interface *13*, 20160627. 10.1098/rsif.2016.0627.

110. Mitri, S., Xavier, J.B., and Foster, K.R. (2011). Social evolution in multispecies bio fi lms. PNAS *108*, 10839–10846. 10.1073/pnas.1100292108.

111. Wang, T., Tague, N., Whelan, S.A., and Dunlop, M.J. (2021). Programmable gene regulation for metabolic engineering using decoy transcription factor binding sites. Nucleic Acids Res. *49*, 1163–1172. 10.1093/nar/gkaa1234.

112. Barbier, I., Kusumawardhani, H., and Schaerli, Y. (2022). Engineering synthetic spatial patterns in microbial populations and communities. OSF Prepr., 1–15.

113. Meijer, J., van Dijk, B., and Hogeweg, P. (2020). Contingent evolution of alternative metabolic network topologies determines whether cross-feeding evolves. Commun. Biol. *3*, 401. 10.1038/s42003-020-1107-x.

114. Rumbaugh, K.P., and Sauer, K. (2020). Biofilm dispersion. Nat. Rev. Microbiol. *18*, 571–586. 10.1038/s41579-020-0385-0.

115. Wein, T., and Dagan, T. (2019). The Effect of Population Bottleneck Size and Selective Regime on Genetic Diversity and Evolvability in Bacteria. Genome Biol. Evol. *11*, 3283–3290. 10.1093/gbe/evz243.

116. Roze, D., and Michod, R.E. (2001). Mutation, Multilevel Selection, and the Evolution of Propagule Size during the Origin of Multicellularity. Am. Nat. *158*, 638–654. 10.1086/323590.

117. Henriques, G.J.B., van Vliet, S., and Doebeli, M. (2021). Multilevel selection favors fragmentation modes that maintain cooperative interactions in multispecies communities. PLOS Comput. Biol. *17*, e1008896. 10.1371/journal.pcbi.1008896.

118. Schiessl, K.T., Ross-Gillespie, A., Cornforth, D.M., Weigert, M., Bigosch, C., Brown, S.P., Ackermann, M., and Kümmerli, R. (2019). Individual- versus group-optimality in the production of secreted bacterial compounds. Evol. Int. J. Org. Evol. *73*, 675–688. 10.1111/evo.13701.

119. Singhal, N., Kumar, M., Kanaujia, P.K., and Virdi, J.S. (2015). MALDI-TOF mass spectrometry: an emerging technology for microbial identification and diagnosis. Front. Microbiol. *6*.

120. Gao, D., Huang, X., and Tao, Y. (2016). A critical review of NanoSIMS in analysis of microbial metabolic activities at single-cell level. Crit. Rev. Biotechnol. *36*, 884–890. 10.3109/07388551.2015.1057550.

121. Batani, G., Bayer, K., Böge, J., Hentschel, U., and Thomas, T. (2019). Fluorescence in situ hybridization (FISH) and cell sorting of living bacteria. Sci. Rep. *9*, 18618. 10.1038/s41598-019-55049-2.

122. Dar, D., Dar, N., Cai, L., and Newman, D.K. (2021). Spatial transcriptomics of planktonic and sessile bacterial populations at single-cell resolution. Science *373*. 10.1126/science.abi4882.

123. Spencer, S.J., Tamminen, M.V., Preheim, S.P., Guo, M.T., Briggs, A.W., Brito, I.L., A Weitz, D., Pitkänen, L.K., Vigneault, F., Virta, M.P., et al. (2016). Massively parallel sequencing of single cells by epicPCR links functional genes with phylogenetic markers. ISME J. *10*, 427–436. 10.1038/ismej.2015.124.

124. Datta, M.S., Sliwerska, E., Gore, J., Polz, M.F., and Cordero, O.X. (2016). Microbial interactions lead to rapid micro-scale successions on model marine particles. Nat. Commun. *7*, 11965.

125. Zhai, X., Larkin, J.W., Süel, G.M., and Mugler, A. (2020). Spiral Wave Propagation in Communities with Spatially Correlated Heterogeneity. Biophys. J. *118*, 1721–1732. 10.1016/j.bpj.2020.02.007.

Figures

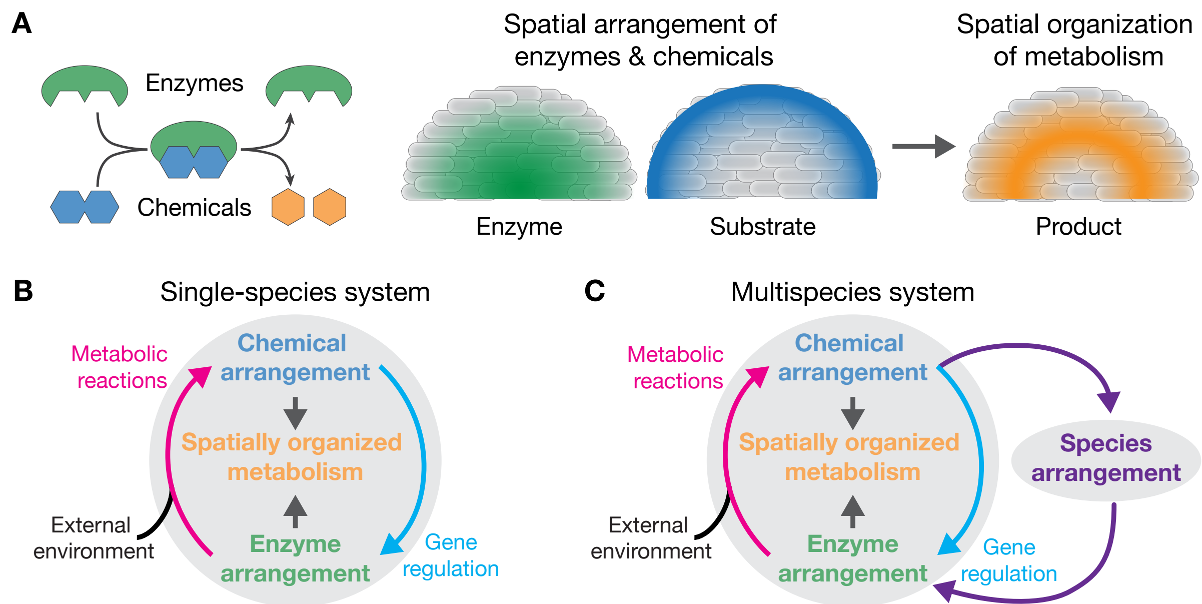

Figure 1

A) The spatial arrangement of enzymes and chemicals determines where metabolic reactions can take place. Together they thus determine the spatial organization of metabolic processes within spatially structured communities. B) In single species systems, the spatial arrangement of enzymes and chemicals is primarily the result of the feedback loop between the regulation of enzyme expression (governed by gene regulatory networks) and the local microenvironment that a cell experiences. The arrangement of chemicals is in addition shaped by the external environment. C) In multispecies systems, the spatial arrangement of species adds an important constraint on how enzymes can be arranged in space. In turn, the spatial arrangement of species depends on the arrangement of chemicals, adding a second feedback loop.

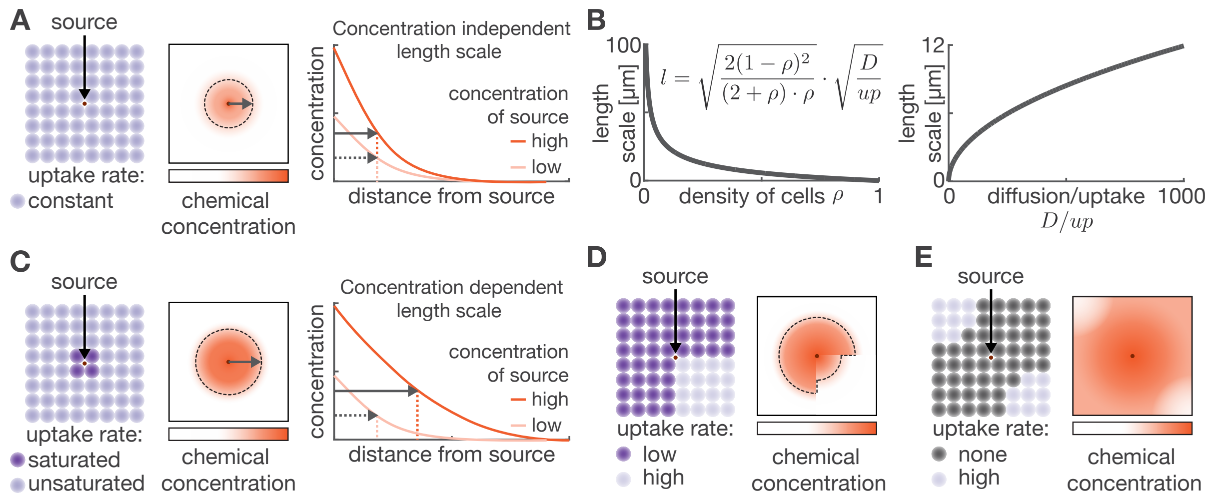

Figure 2

A) When chemicals are taken up by all cells at the same rate, their concentration decreases exponentially with the distance from the source. This decay has a length scale, which is independent of the source concentration (dark gray arrow, corresponding to the distance at which the chemical concentration has decreased by a factor of 1/e). B) This length scale (l) depends on cell density (ρ) and the ratio of the uptake rate (up) over the diffusion rate (D). Parameter values are based on those for small molecules such as amino acids[19]: up=10 1/s, D=800 $\mu m/s^2$, and ρ=0.65. C) When uptake rates are saturating (e.g., follow Monod kinetics), the length scale over which chemicals vary depends on the chemical concentration at the source. D) When uptake rates vary between cells, because they are of different species or use different uptake pathways, there is no longer a unique length scale over which chemicals vary in space. The length scale instead depends on the biogeography and is different in each direction. E) Chemical concentrations only decrease slowly with distance in regions where cells do not take up the chemicals. In regions where chemicals are taken up, their concentration decreases exponentially.