

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 community¹. 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 changes⁵⁻⁷. 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 proximity². 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 chemicals⁵. 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 yeast¹².

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 communities¹³⁻²⁰. A first important factor is the physics of cell growth: as cells grow and divide they push their offspring and neighbors around⁴. 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 cells²⁵. 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 species²⁶. 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 matrix²⁷. 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

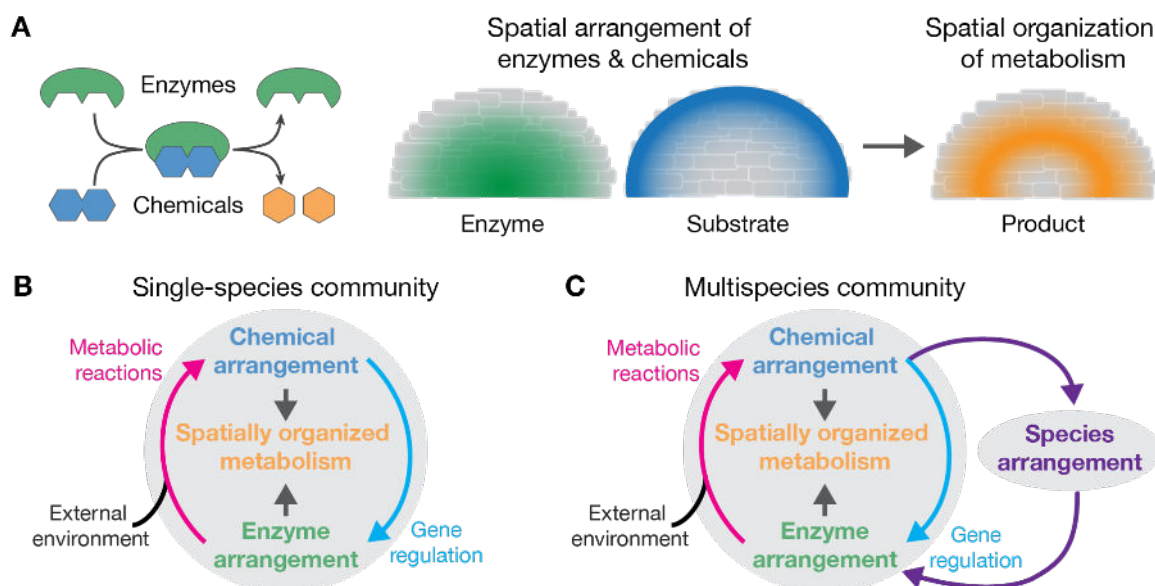


Figure 1. **A)** The spatial arrangement of enzymes and chemicals determines where metabolic reactions can take place. Together they thus determine the spatial organization of metabolic processes within spatially structured communities. **B)** In single species communities, the spatial arrangement of 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 communities, 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.

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 flow³³. 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 biofilm³⁶. Finally, external gradients can be created by hosts, for example in the animal gut^{37,38} or around plant roots³⁹. 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 expression⁴⁰⁻⁴². Stochastic fluctuations in enzyme levels in turn can cause fluctuations in the rate of metabolic reactions⁴³. 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 activities⁴⁴. 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 monomers⁴⁵⁻⁴⁷. 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 populations⁴⁸. Stochastic gene expression combines with negative feedback to ensure that the nitrogen fixing cells are evenly spaced along the filament⁴⁹.

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 nanotubes⁵⁴⁻⁵⁶. 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 environment⁵. 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 predicted⁶¹. 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 kinetics⁷⁷). 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 concentration⁹. 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

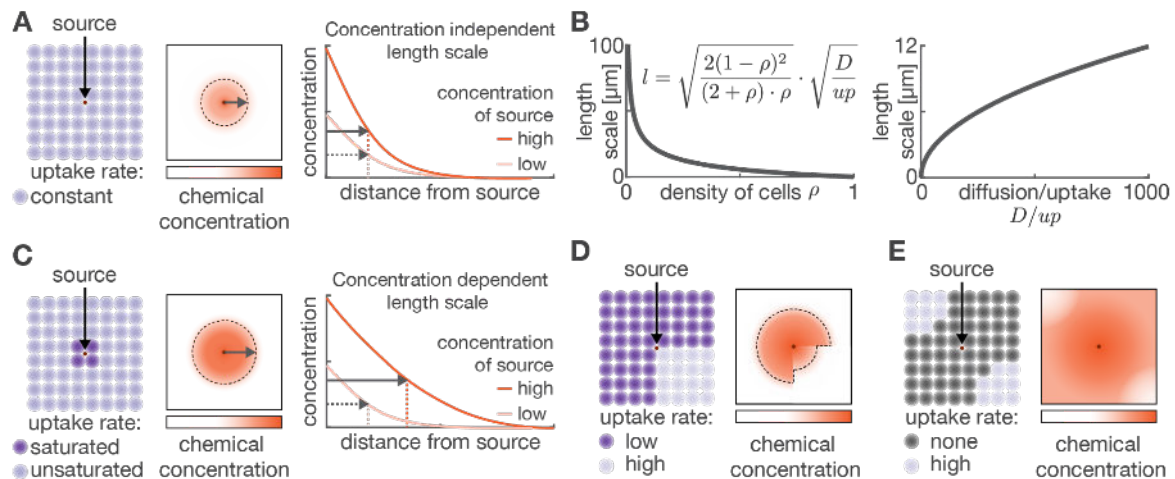


Figure 2. **A)** When chemicals are taken up by all cells at the same rate, their concentration decreases exponentially with the distance from the source. This decay has a length scale which is independent of the source concentration (dark gray arrow, corresponding to the distance at which the chemical 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: $up=10$ $1/s$, $D=800$ $\mu\text{m}^2/s^2$, and $\rho=0.65$ (Dal Co et al., 2020). **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.

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 producers⁸⁰⁻⁸². 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 cells⁷⁸. 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 dynamics⁷⁶.

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 types⁸⁴⁻⁸⁷.

Catabolic cross-feeding in clonal populations

Emergent gradients in chemicals can combine with gene regulatory networks to create metabolic interaction within clonal populations⁸³. For example, *E. coli* switches to overflow metabolism when the carbon source (e.g., glucose) is available in growth saturating concentrations⁸⁸. 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 repression⁸. 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 regions⁹¹.

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 zone⁹³.

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 conditions⁹⁴. 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 other².

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 adaptation¹⁰⁵, or by modulating the optimal production levels of inhibiting compounds¹⁰⁶.

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, conditions⁸⁸. Likewise, catabolite repression is often seen as a strategy for *E. coli* to adapt to temporally fluctuating environments¹⁰⁷. 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 environment¹⁰⁸. 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 contributions¹¹¹⁻¹¹³. 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 makeup¹¹⁴. 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 cell¹¹⁴. 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 spread¹¹⁶. 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 occur¹¹⁷. 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 produces¹¹⁸. 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 found⁴⁴. However, with new technologies it has become possible to revisit the question to what extent clonal populations have evolved to spatially organize metabolism¹¹.

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 important¹⁰⁸. 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 time¹¹⁴. 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 biofilms¹²⁵. 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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Alma Dal Co tragically and unexpectedly passed away on November 14th 2022, after the acceptance of this article. Alma was an extraordinary and visionary scientist and an exceptional and warm-hearted person and mentor. She brought a fearless passion to everything she did, inspiring all those around her. We are deeply saddened by the loss of our dearest friend and colleague, but are profoundly grateful for the many hours we got to work closely together with Alma, in which we got to experience the wonderful world of her exceptional mind. She has profoundly shaped our thinking and vision and will continue to do so for many years to come. We dedicate this article to Alma's memory, as we know of no better tribute to honor her past work and her vision for the future than these words that were so thoroughly shaped by her thinking.

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Author contributions

ADC, MA, SVV: wrote the paper

Declaration of interests

The authors declare no competing interests.

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