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Review

Contribution of single-cell omics to microbial ecology

S. Mauger,[1,2,*] C. Monard,[1] C. Thion,[2] and P. Vandenkoornhuyse[1,*]

Micro-organisms play key roles in various ecosystems, but many of their functions and interactions remain undefined. To investigate the ecological relevance of microbial communities, new molecular tools are being developed. Among them, single-cell omics assessing genetic diversity at the population and community levels and linking each individual cell to its functions is gaining interest in microbial ecology. By giving access to a wider range of ecological scales (from individual to community) than culture-based approaches and meta-omics, single-cell omics can contribute not only to micro-organisms' genomic and functional identification but also to the testing of concepts in ecology. Here, we discuss the contribution of single-cell omics to possible breakthroughs in concepts and knowledge on microbial ecosystems and ecoevolutionary processes.

Highlights

Microbes are involved in many ecosystems but remain understudied, mostly due to technical limitations.

Application of omics technologies to microbes involves changes in the scales of ecological studies.

Single-cell omics offer the opportunity to study microbes at a finer scale than meta-omics tools.

Single microbial cell omics enable the assessment and exploration of the dynamics of genetic changes from individuals to higher levels of ecological complexity.

Ecological scales

Interactions between organisms take place at all organizational levels, from molecules to communities and within or between species, and shape ecosystem dynamics. Ecological interactions are difficult to understand due to the number of biotic and abiotic parameters involved. Assembling knowledge at various ecological scales and from different standpoints is therefore crucial in the study of ecological and evolutionary processes. This is particularly true in the case of microbes, in which individuals can be seen as metabolic units involved in complex metabolic networks at much higher ecological scales (e.g., [1,2]). Therefore, accessing genetic and metabolic information of microbes is a necessary step to understand ecosystem functioning. In microbial metabolic units, 'small' changes in genomes and metabolic pathways may have significant impact on the microbial community organization and hence on ecosystems. Deciphering processes at large ecological scale therefore requires observation of fine ecological scale (i.e., at the individual cell level), which is the biggest challenge of current environmental microbiology [3] (Figure 1A).

A fundamental level of organization in ecology is the species. However, due to gene flow between cells that increases with ecological overlap and genetic similarity [4], the microbial species concept and thus also **populations** (see Glossary) are not clearly defined entities. Interactions and diversity at the population level (i.e., between individual cells of the same population) (Box 1) are still obscure because they are not often analyzed in environmental microbiology. Given the natural mutation rate in bacteria (~10^{-7} substitutions per nucleotide; e.g., [5]), even a single colony contains genetic variations (i.e., variants within a cell population). The population has been suggested to be more relevant than the species level for microbes [6,7], and species usually contain genetically divergent microorganisms. Considering the hierarchical levels of ecology, populations are keys to assessing genetic structure within species and, over time, changes therein. They thereby provide insights into ecoevolutionary processes and advance our understanding of microbiota composition dynamics (Box 1).

[1]Université de Rennes 1, CNRS, UMR 6553 ECOBIO, 35042 Rennes, France
[2]Cellenion, 60 Avenue Rockefeller, 69008 Lyon, France

*Correspondence:
solene.mauger@univ-rennes1.fr (S. Mauger) and
philippe.vandenkoornhuyse@univ-rennes1.fr (P. Vandenkoornhuyse).

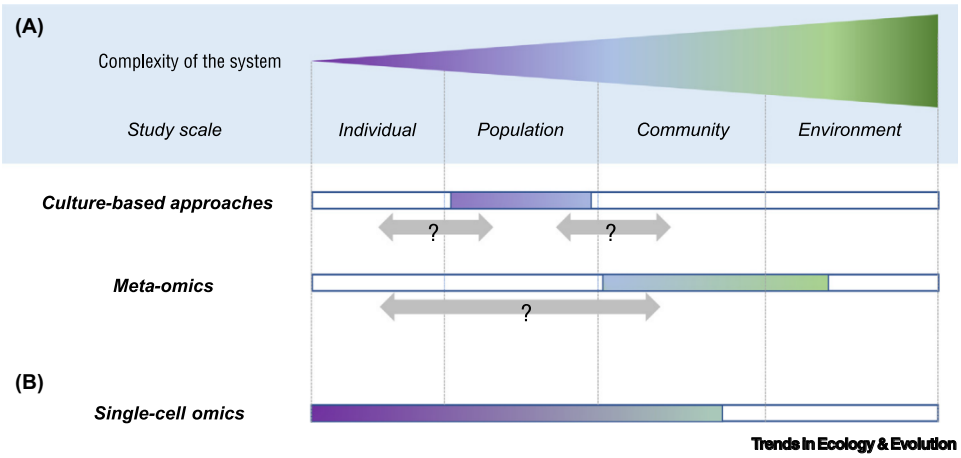

Figure 1. Gradient of ecological complexity, study scales, and associated approaches. Although other higher levels of ecology can be used, individual–population–community–environment scales describe much of the subject of ecology. () The culture-based approaches aim at studying microbial populations or a very limited number of strains; therefore, the outcoming data cannot be fully informative about higher levels of ecology. On the contrary, meta-omics approaches cover a range from the community to environmental scales of microbial ecology and do not provide finer information on the ecological gradient. In both cases, the individual scale is unattainable while being at the basis of ecological processes. B) Single-cell omics cover the scale from the individual microbe to the community from the same environmental sample, which allows one to connect the outcoming information of each ecological scale.

Our understanding of the microbial world and its ecological roles is still very limited [8]. Understanding the functions played by microbial cells in a complex community remains a frontier in microbial ecology. Beyond the technical limits that microbiology is facing, the information gathered from culture-based studies or from natural ecosystems can be difficult to interpret (Figure 1A). Laboratory experiments attempt to reproduce optimal ecological conditions for microbes by selecting from among the many biotic and environmental parameters [6] in order to understand specific processes such as trait trade-offs [9], interactions between strains [10], the production of metabolites [11], or genome evolution [12]. Extrapolating observations obtained *in vitro*, at restricted scales, to higher ecological scales such as natural communities and ecosystems requires particular attention. Conversely, observations made from environmental samples, including microbial community composition, diversity, or global functions, are less specific and represent an average of the microbial community. Ideally, we want to get the most information out of each level of approach (i.e., precise interactions and genetic dynamics from culture-based studies coupled with global function and diversity of a community with **meta-omics**). However, our

Box 1. Microbial population

In ecology, populations are individuals belonging to the same species living in the same environment, although the definition varies with different viewpoints [81]. Microbial populations represent a unit of diversity and selection. Within these populations, diversity can be either genetic or phenotypic. The diversity within a population to some extent buffers an environmental stress because existing variants are able to survive the stress and/or allow rapid phenotype switching (e.g., Bet-Hedging [31,82]), but positive selection of new variants can also be induced by the stress. This organizational level is therefore a key to understanding genetic structure; haplotype fitness; and the dynamics of ecological interactions, including associations of microbial species, symbioses, host–pathogen interactions, and ecosystem functioning, resilience, and stability. For instance, resistance to antibiotics can vary within populations [83], and the virulence pathogens can vary across subpopulations [84]. Genetic diversity and ecological features such as niches can vary between lineages [85], so that subpopulations are able to coexist through niche diversity. To capture the total genetic and phenotypic diversity and get a holistic view of populations, the scale must thus be tuned down to the individuals that compose the population in a given sample [84]. Otherwise, applying the current bacterial species concept to make population-level inferences may lead to false or partial interpretation of ecological phenomena.

Glossary

Metagenome-assembled genomes (MAGs): *in silico* reconstruction of an artificial microbial genome obtained from one or multiple binned metagenomes that represent the core genome of the population.

Meta-omics: group of molecular biology technologies, extensively used to access unculturable organisms, by studying the bulk pool of biomolecules from environmental samples to reveal genomes (metagenomics), transcriptomes (metatranscriptomics), proteomes (metaproteomics), and metabolites (metabolomics).

Niche complementarity/ partitioning: ecological concept describing how species differential specialization in different combinations of resource uses and functions allows them to coexist in the same environment.

Populations: applied to bacteria in natural communities for individuals with identical or different genomes from the same species gathered in a specific environment or sample. An isolated micro-organism culture also comprises a population.

ignorance of intricacies and interactions of ecological scales with one another still jeopardizes the assembly of the resulting information to answer specific ecological questions in environmental microbiology. Moreover, the uncertainty of community composition and the complexity of microbial interactions [2] make it even more difficult to target specific scientific hypotheses on natural communities and to choose the appropriate tools.

Specific tools for specific ecological questions

Like in any new field of exploration, ecological patterns within microbial communities are first observed and described but poorly understood [13], testifying to the enormous lack of knowledge concerning microbes [14]. The use of DNA- and RNA-based methods to study natural microbial communities has demonstrated the existence of a prodigious wealth of micro-organisms that remained unsuspected some years ago (e.g., [15]). Among meta-omics techniques, metagenomics and metatranscriptomics are the most widely used methods to explore microbiota. These techniques enabled a breakthrough in our understanding of microbial phylogenetic relationships [16], species diversity and abundance [17], metabolic abilities [18], and functional diversification [19]. The development and use of **metagenome-assembled genomes (MAGs)** led to discoveries that advanced our understanding of bacterial life and modified our perception of the tree of life [15,19]. Some studies attempted to reconstitute population-level genomes from metagenomes. For example, Crits-Christoph *et al*. [20] investigated genetic variation within populations of highly abundant soil bacteria by studying MAGs and observed spatial differentiation of alleles. However, inferring population features from meta-omics data remains limited, especially when genomes are inaccurately or incompletely reconstructed from short sequence fragments (Figure 2, Key figure). The use of MAGs becomes challenging when microbial richness and diversity within a community are high and taxa are phylogenetically close [21]. During genome assembly, stitching of fragments from different individual genomes and/or contaminant DNA can occur, creating chimeras that are irrelevant for the study of populations. In this case, the approach would necessarily conceal a considerable proportion of molecular diversity [22]. In addition, the molecular biology and bioinformatics methods used in meta-omics approaches are varied and based on different criteria and assumptions in the absence of a consensus, leading to contrasting results and interpretations [23,24]. Overall, it might be difficult to directly link the detected functions to their original microbial cell from meta-omics data, thereby limiting the identification of signaling pathways and trade-offs in gene regulation. Meta-omics approaches proved useful in describing communities using large-scale sampling and have made it possible to answer questions related to community composition and its associated global functions but not to fully understand the mechanisms underlying these patterns. Nevertheless, bioinformatics research has developed algorithms aiming to identify genetic variations in microbial populations: Vertically and horizontally inherited genes can be differentiated, and, from population-specific sweeps, SNPs can be detected (e.g., DiscoSNP, PopCOGenT) [4,25].

To complement meta-omics data, modeling approaches are used to explore microbial interactions and fluxes of metabolites and to reconstruct ecological networks in complex microbiomes [26–29]. These approaches provide a possible explanation and scenarios of interactions in natural communities, but they sometimes end in contradictions with culture-based experiments [1]. Indeed, models can predict a certain kind of interaction (e.g., cross-feeding) that is not verified or proven wrong in an experimental setup due to the oversight of key parameters such as growing conditions, space, and (very often) time [1]. They also rely on the co-occurrence of phenomena, which is more associated with correlations than cause–consequences relationships. Culture-dependent approaches may help reach the population level in simple community compositions through controlled and simplified laboratory-scale experiments [30] and can be effective for testing patterns observed in meta-omics studies and deconstructing

Key figure

Microbial communities observed through meta-omics versus single-cell omics

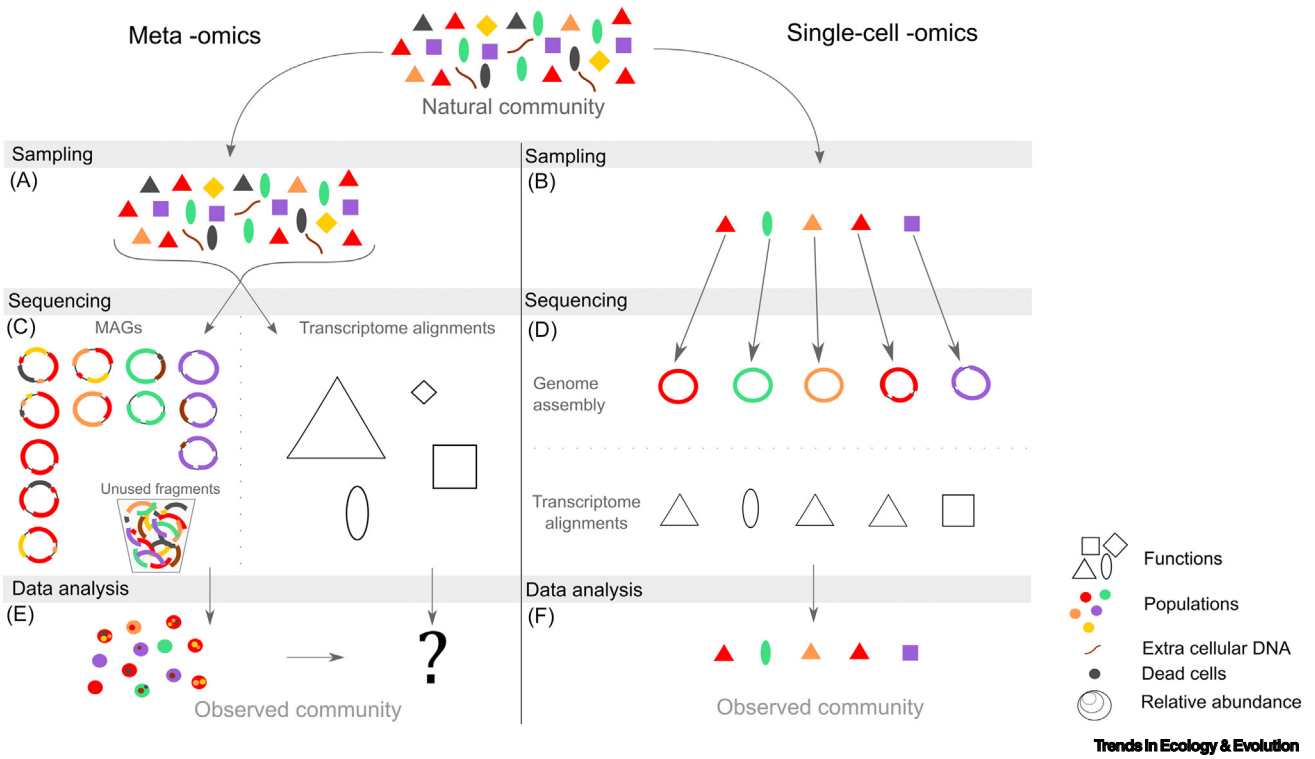

Figure 2. The key steps of meta-omics (left) and single-cell omics (right) approaches are shown, resulting in contrasting representations of a natural microbial community. Different genomes are represented by different colors: Red, orange, and yellow show genomes of close relatives (i.e., intrapopulation genomic variants). Dead cells are shown in gray and extracellular or host DNA in brown. Different functions are represented by different symbols (triangles, squares, diamonds, or ovals). (A) After meta-omics sampling, the cell proportions are maintained, but transient DNA and dead cells are not filtered. (B) In single-cell omics, a smaller proportion of the community is sampled, and dead cells can be excluded. (C) In meta-omics, the unit sequenced is the complete extracted sample. Metagenome-assembled genomes (MAGs) are partial and include chimeras (i.e., unreal collages of closely related genomes, dead cells, and extracellular material). Meta-transcriptomic analysis yields averaged relative abundances (represented by the size of the symbol) of functions within the sample. (D) By contrast, in single-cell omics, each cell is a sequenced unit and can be associated with its genome and/or transcriptome. (E) The community observed through meta-omics is representative of the composition of the whole community but not of the associated genes and functions. (F) With genome and transcriptome information from single cells, the observed community is undersampled but is closer to the natural community: If the sampling scale is appropriate, rare populations and functions are more likely to be detected.

mechanistic hypotheses (e.g., interactions, metabolism) (e.g., [31]). The lack of information on individual cells and mostly on populations (i.e., both functions and phylogeny) using existing methods limits our understanding of observed processes. Many studies aim at unraveling microbiotic diversity, primarily in plants, soil, water, and animal bodies, but few explain associated community assembly and evolutionary mechanisms [32–34]. In this context, microbiologists and ecologists are searching for other technical possibilities or approaches, such as single-cell omics, to complete the knowledge provided by current methods.

The alternative scope of single-cell omics

Single-cell whole-genome sequencing (scWGS) and single-cell transcriptomics [single-cell RNA sequencing (scRNAseq)] were first developed for eukaryotic cells and used in cancer research,

revealing both intrapopulation genetic diversity and heterogeneous genome expression. As in cancer research, where differentiation in space of the genome expression among cells has been observed [35], a pioneer paper on *Pseudomonas aeruginosa* biofilm using a fluorescence-based approach (i.e., parallel sequential fluorescence *in situ* hybridization) revealed a differentiation in space and time of cell expression [36]. Because spatial single-cell microbial approaches could allow the understanding of the drivers and mechanisms leading to the self-organization of these microbial structures, new developments are expected to expand in health science and many other fields of microbial ecology research. Single-cell approaches are promising candidates for microbial studies because they provide a complementary view to metagenomics and metatranscriptomics that have different strengths but also weaknesses (Figure 2).

Single-cell omics technologies require additional steps to prepare a sample for sequencing as compared with meta-omics techniques, especially with regard to cell isolation, for which different technical options are available [37,38]. Once the cells are lysed, DNA and RNA content from a single cell is in the femtogram scale for bacteria (i.e., 1000-fold less than in animal cells). Preparing the sequencing library, which typically requires nanogram ranges of material, will need an ultraefficient prior amplification step [e.g., multiple displacement amplification (MDA), the most widely used approach for bacteria] [39].

Single-cell approaches enable accurate access to genomic and transcriptomic information for each cell, so that the assembled cell information is highly representative of the original population (Figure 2). This enables the identification of heterogeneity in gene assemblage, gene expression, and metabolic pathways between cells. Single-cell transcriptomic and genomic information provides a link between phylogeny and functional traits and reveals the physiological status of an individual cell at the time of sampling. This is particularly important, considering that the individual gene expression profiles of genetically close cells may differ. What is more, some cell isolation tools, such as automated image-based isolation devices (cellenONE, Cellenion; and ICELL8, Takara Bio), make it possible to select cells on the basis of their integrity, their physiology, and/or their functional markers and to minimize contamination by the host or extracellular DNA. This is very promising for microbiology to, for instance, select active cells in the studied sample at the time of sampling and reveal which of them are taking part in the community productivity.

A seminal paper on single-cell microbial genome analysis was published in 2005 [40] and paved the way for further improvement of single-cell omics, notably on the amplification method (here MDA) and lysis buffer. Recent studies using single-cell omics have improved our understanding of intraspecific diversification and metabolic capacities at a limited scale [41,42]. Assessing the true individual cell gene assemblage and expression using single-cell omics will make it possible to study the hitherto unexplored microbial population level and the functioning of a given microbiome by linking the different ecological scales (Figure 1B). Indeed, single-cell omics enable access to information additional to that in culture-based and meta-omics studies (the single individual ecological scale) while also, from the environmental sample, giving information on the population and community interactions. To a broader extent, this will enable better access to ecoevolutionary pressures and evolutionary processes within microbial communities.

Applications of single-cell omics in microbial ecology

Single-cell omics provide information at the cell level by changing the camera angle when studying environmental communities and can contribute to microbiology and ecology at many levels by exploring microbial diversity or microbial interactions.

The tremendous diversity of single-microbe genomes

Observations of microbiota can complement/validate the diversity observed by meta-omics on fungi [43], human samples [8], and marine viruses [44] and can resolve cryptic bacterial species, which currently mainly rely on cultivable strains [45–47]. Single-microbe omics therefore contribute to the microbial inventory, which is still in its infancy in many ecosystems [48]. The use of single-cell–based approaches could demonstrate the existence of the discovered sequence-based lineages and discover possible new branches. Isolating cells from environmental samples can also cast light on rare organisms that might be obscured in millions of genome fragments using meta-omics. These rare organisms are considered to play key roles in community dynamics because they overproportionally contribute to the functions of the microbial community in fluctuating environments [49,50]. Although most studies that apply this approach use a limited number of cells, a recent survey of the marine microbiome recovered no less than 12 000 genomes from single cells [51], revealing a high degree of uniqueness and limited clonality in the analyzed samples of seawater and providing evidence for the ecological roles of uncultured microbial groups. Single-cell genomics, by looking at individual genomes instead of core genomes from meta-omics, from natural microbial communities represent an unprecedented opportunity to complete the identification and classification of microbes. This is a key step sometimes missing in environmental microbiology [52]: knowing what to look at and why to formulate hypotheses in ecology and better understanding processes involving microbes.

Ecological and evolutionary hypothesis testing using single-cell omics

Single-cell genomics and transcriptomics approaches therefore help to answer the questions 'What are these microbes?' and 'What are they doing (or capable of doing)?'. They also help to understand why and how observed patterns happen. Linking environmental and community parameters to individual gene expression and bacterial interactions enables a mechanistic understanding of underlying biotic and abiotic conditions to patterns. This represents an opportunity to explore multiple ecological theories and hypotheses, notably on interactions of microbes at many levels: within the community, with external microbes (i.e., viruses), and with their host. One of the hottest topics in microbial ecology is the link between diversity and function, including the productivity of the ecosystem that relies on the **niche partitioning** theory. This hypothesis states that species coexistence is enabled by species specialization in different available resources (or combinations of resources), thereby reducing interspecific competition [53] but likely modifying the microbial population structure [54]. Specialization in specific resources raises many questions concerning microbial interactions through the exchange of metabolites [55], loss of traits [56], and genome reduction [57]. The Black Queen Hypothesis (BQH), one of the ecological theories that conceptualized this phenomenon, states that microbial community assembly and complexity are at least partially determined by functional dependencies resulting from gene loss(es). Testing this hypothesis requires using environmental samples to evaluate functional redundancy within communities, the expression and distribution of the functions between interacting (micro-)organisms, and the impact of genotypic interactions on these functions. At the community level, it is impossible to access this information through meta-omics because BQH evolution is supposed to also occur at the population level [53,54], which is undetectable by most of the meta-omics tools used so far. Ecoevolutionary processes can so far be explicitly assessed only through ecological models [58] or from dedicated *in vitro* experiments [42] that require deciding which gene and which organisms to look at. Single-cell approaches can help to investigate such hypotheses and other theories in ecology and highlight patterns of interactions that shape microbial communities.

A breach to viral host ecology

The diversity of microbial communities is also influenced by the viral infections to which they are exposed, which is particularly difficult to evaluate in nature [59]. A recent study assessed viral infections

in the ocean thanks to single-cell genomics by identifying virus sequences in uncultured protist cells [44]. Such interactions are widespread but generally missing in current microbial ecology analyses. Among other roles, viruses are known to (i) control microbial community dynamics and drive microbial host evolution [60,61] and (ii) impact ecosystem changes and biogeochemical cycles [59,62]. Single-cell omics can help us understand the roles played by viruses in microbial populations by making it possible to assess both the prevalence of prophage sequences and possible lateral gene transfers [63,64], which would reveal preferential association of specific viruses with specific bacteria.

Single-cell insights into bigger-scale interactions

Generally speaking, single-cell omics can highlight diverse levels of interaction in natural habitats either between the microbes that compose a microbiota or between the microbes/microbiota and their host. Despite the great number of studies on the composition of human microbiota, very little is known about interactions between the microbes and with their host [65]. The single-cell study of host–pathogen interactions paves the way for understanding infectious processes through microbiota dynamics, metabolic capacities, and host resistance [66]. Dysbiosis is often shown to display a higher β-diversity interpreted as a higher stochasticity in the microbiota assembly [67]. Among other explanations, this apparent higher stochasticity may be the result of drastic pathogen-induced changes in the habitat (i.e., transitory state in the microbiota dynamics), modification of a component of the microbiota caused by genetic change(s), and/or a functional modification expressing a modified phenotype that leads to disequilibrium in the microbiota community. With the aim of understanding how a disorder of the microbiota leads to disease or the reverse (i.e., how a disease can modify the composition of a microbiota), single-cell microbiota analyses of genomes and transcriptomes would help better define the characteristics of dysbiosis (i.e., dysbiosis mechanisms). Using single-cell genomics makes it possible to address hypotheses related to changes in bacterial populations, whereas microbial single-cell transcriptomics may be more appropriate to decrypt the functions of microbes, metabolic abilities, and cellular states [68]. These two strategies are necessary to understand how microbial interactions occur within communities as well as their possible impact on the ecosystem [51,69].

Microbial single-cell omics are expected to improve understanding of not solely functional interactions but also the underlying evolutionary processes. Microbial single-cell omics should also promote a shift in standpoint from observation to interpretation and also offer new opportunities to test macroecological theories on microbes. The development of microbial single-cell omics will have high impacts on our understanding of microbial communities in many environments, such as (i) in freshwater and marine ecosystems, to define the interaction of bacteria and phytoplankton through the exchange of metabolites and to test links to blooms [70]; (ii) in soils, to better assess the provision of services by plant microbiota, including nutrient and water uptake and protection against pathogens [71]; and (iii) in plant, human, and animal health, to better decipher how dysbiosis could be a cause or consequence of a disease. However, it cannot be ignored that the wide development of single-cell omics applied to micro-organisms is subject to technical limits.

Limits of single-cell omics on microbes

The current limited number of cells studied in the published papers questions the representativeness of the analyses. Considering the number of microbial cells contained in a given environmental sample, one can wonder how many cells need to be isolated to cover the diversity of a sample, from hundreds [41] to thousands [51]. The limited number of analyzed cells is mostly due to technical problems and the cost of such experiments. It has to be emphasized that the current use of single-cell omics for microbes must solve many technical obstacles (Box 2), reviewed in [72,73], such as cell isolation, lysis, and a biased amplification step. The structure of the microbial cell wall is complex, and, unlike animal cells, they do not break easily. The diversity of cell wall

composition across phylogeny and physiological status (e.g., peptidoglycan layers, spores, capsules) makes it challenging to find a universal lysis method able to breach each cell without damaging its content or inhibiting enzymatic reactions downstream. Different protocols have been

Box 2. Single microbial cell omics approaches are technically challenging

Crucial but solvable issues (Table I) should be addressed at each step of single-microbe approaches (Figure I). First, microbial cells need to be properly isolated from complex environmental samples (1). The quality of this step is critical because it will directly impact the following applications, whether it concerns culturomics (2) or molecular analyses. The latter demands a prior lysis of microbial cells once isolated (3), which might appear simple but was and is still a major padlock in microbiology. Once the molecular material is available, whole-genome (4) and whole-transcriptome (5) sequencing can be performed, which both require particular attention to aspects listed in Table I. For each of these five steps, solutions are proposed in Table I to solve the associated challenges. However, the combination of the solutions represents an additional complication because the combined solutions might not be employable within the same protocol. The reduction of reaction volumes down to 'nanovolumes' is likely the most sensible solution, limiting contamination probability and allowing high throughput and cost reduction. Overall, single-cell omics applied to microbes need to focus on three guidelines: representativeness from molecules to samples, compatibility between the steps of sample preparation, and care throughout the process.

Table I. Padlocks and possible keys offered by single microbial cell omics approaches

Challenge	Possible solution
1. Isolation of single microbial cells	
Community representative sampling	High throughput
Cell isolation from complex matrices, such as soil, sediments, host tissue, feces, mucus, among others	Sonication, filtration, density gradient centrifugation
Exhaustive/targeted labeling/detection	Fluorescence, antibodies
Maintenance of axenic conditions	(As) clean (as possible) room + Ⓥ[a]
2. Culturomics experiments	
Maximum viability/cultivability	Gentle cell handling, liquid dispensing
Choice of culture conditions	High-throughput media screening + Ⓥ
Assessment of monoclonality (culture purity)	Microscopy, targeted sequencing
3. Microbial cell lysis/permeabilization	
Efficiency across phylogeny	(Ultra-)sonication, thermal shock, heat, enzymolysis, detergents, among others
Preservation of DNA/RNA quality and quantity	Gentle procedure, avoid purification
Minimum contamination from reagents and prevention of subsequent steps	Physical rather than biological/chemical + Ⓥ
4. Single-microbe WGS	
Superefficient (100 000– to 1 million–fold) amplification (1–10 f. DNA per prokaryotic cell)	Ⓥ, Molecular crowding, linear amplification (e.g., *in vitro* transcription)
Even/broad coverage, high fidelity, and no chimera creation	Minimum number of PCR cycles and/or primary template amplification (e.g., *in vitro* transcription)
Minimum contamination from reagents	Minimum reagent amount + Ⓥ
Cost reduction	Cell barcoding for multiplexing + Ⓥ
Bioinformatics	Dedicated tools for cell demultiplexing, monoclonality test, and so forth
5. Single-microbe RNA sequencing	
Same as WGS (up to 100 f. RNA per prokaryotic cell)	Ⓥ, Molecular crowding
Amplification bias	Unique molecular identifier
No polyadenylation tail on prokaryotic mRNA	RNA polyadenylation tailing, random priming, ribosomal RNA targeted depletion

[a]Ⓥ, nanovolume

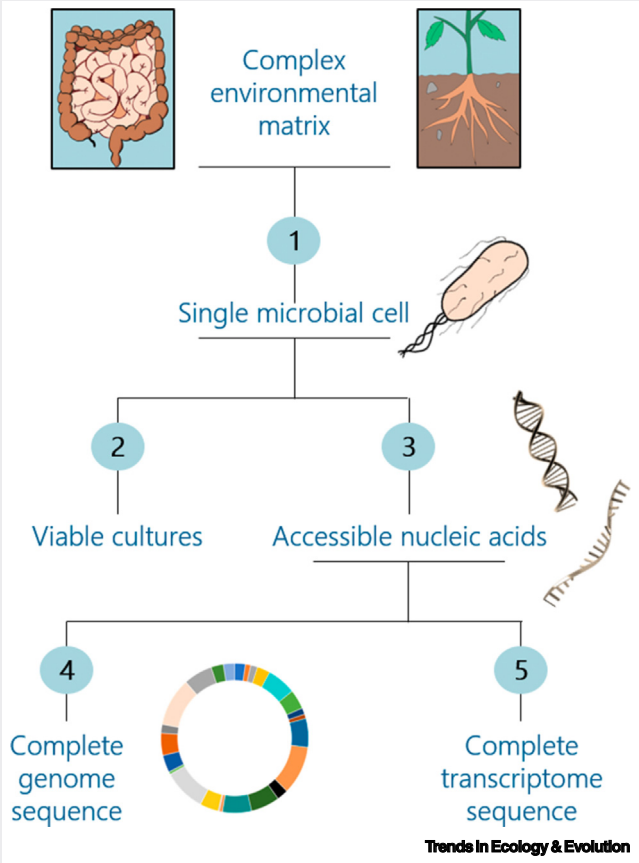

Figure I. Typical steps of single microbial cell omics approaches.

used in recent microbial single-cell–based studies, using either heat, temperature shocks, sonication, enzymes, detergents, or combinations of these [74,75]. In addition to technical issues, one can wonder how to be certain that cells are isolated and lysed equally and not preferentially, depending on their physical/physiological status. The amplification step, usually made via MDA, has been reported to be imprecise concerning the genome amplification uniformity, even though it presents a better genome coverage than other approaches [i.e., multiple annealing and looping–based amplification cycles (MALBAC) [39]]. This amplification step is highly relevant as it was suggested to be the cause of incomplete reconstruction of single amplified genomes [76], although solutions are being developed [77]. As the price of library preparation represents most of the cost of these new single-cell omics for micro-organisms, reduction of reaction volumes in 'nanolibraries' should be very cost-effective (Box 2). The probability of contamination decreases with the miniaturization of the reagent volumes [78] and associated robotics. Working in nanovolumes seems to be a convenient solution to solve multiple problems; however, this introduces new volume-related challenges such as pipetting or sample purification.

For these reasons, single-cell omics have sometimes been used in combination with meta-omics to combine the possibility of fine-scale analysis with high throughput [76,79]. It also represents a good opportunity to validate multiple aspects of single-cell omics: (i) the isolation and lysis universality, (ii) the sample preparation (genome amplification and library preparation for sequencing),

(iii) the lack of contamination, and (iv) the representativeness of the sample covered by single-cell omics (see Outstanding questions).

Concluding remarks

Soon, single-cell omics applied to micro-organisms could become a gold standard in microbial ecology thanks to the knowledge produced by focusing on individual genomes and transcriptomes and, possibly, individual proteomes and metabolomes. Today, technical problems prevent the testing of broad ecological hypotheses. Generalizing ecological single-cell studies on microbes requires the development of robust high-throughput techniques with a high cost-effectiveness ratio (see Outstanding questions). A knowledge upshot is expected in microbial interactions and ecoevolutionary boundaries through the enabling of mechanistic characterization of deterministic populations and community assembly processes. Currently, the use of metagenomics and single-cell genomics in the same study appears to be the best solution, combining the strengths of the two approaches: (i) high throughput and α/β diversity and (ii) fine-scale analysis by scWGS and/or scRNAseq [76,80]. Ideally, one would not overinterpret meta-omics data and rather would use those data to build hypotheses based on mechanisms, which can be tested using single-cell approaches. Approaches that will allow more accurate assessment of microbial genome diversity and genome functioning within complex microbiota are impatiently awaited. Still, the future of microbial single-cell omics will likely fuel a new perception of the world of micro-organisms.

Outstanding questions

To what extent can a single technical approach realistically reflect complex natural processes?

How many cells need to be isolated from a natural environment to accurately represent the population and/or community from which they originate? And how can this be assessed?

What criteria should be used to determine the scale of sampling in natural environments?

How can we use information obtained by single-cell omics to gain a better understanding of ecosystem functioning?

How can we make the application of proteomics, metabolomics, and multi-omics approaches to single microbes more realistic?

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Declaration of interests

The authors have no interests to declare.

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