



**HAL**  
open science

## Contribution of single-cell omics to microbial ecology

S Mauger, C Monard, C Thion, P Vandenkoornhuys

► **To cite this version:**

S Mauger, C Monard, C Thion, P Vandenkoornhuys. Contribution of single-cell omics to microbial ecology. *Trends in Ecology & Evolution*, 2022, 37 (1), pp.67-78. 10.1016/j.tree.2021.09.002 . hal-03414494

**HAL Id: hal-03414494**

**<https://hal.science/hal-03414494>**

Submitted on 15 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18

**Contribution of single-cell omics to microbial ecology**

**Authors:** Mauger, S.<sup>1,2</sup>, Monard, C.<sup>1</sup>, Thion, C.<sup>2</sup>, Vandenkoornhuyse, P.<sup>1</sup>

<sup>1</sup> Université de Rennes 1, CNRS, UMR 6553 ECOBIO, 35042 Rennes (France)

<sup>2</sup> Cellenion, 60 Avenue Rockefeller, 69008 Lyon (France)

Corresponding authors : Solène Mauger (solene.mauger@univ-rennes1.fr); Philippe Vandenkoornhuyse (philippe.vandenkoornhuyse@univ-rennes1.fr)

**Keywords:** Microbiota; single cell; omics; population; scales

19 **Highlights**

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

22 Applying -omics technologies to microbes involve changes in the scales of ecological  
23 studies.

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

26 Single microbial cell omics enable to assess and explore the dynamics of genetic  
27 changes from individuals to higher levels of ecological complexity.

28

29 **Abstract**

30 Microorganisms play key roles in various ecosystems but many of their functions and  
31 interactions remain undefined. To investigate the ecological relevance of microbial  
32 communities, new molecular tools are being developed. Among them, single-cell  
33 omics assessing genetic diversity at the population and community level and linking  
34 each individual cell to its functions is gaining interest in microbial ecology. By giving  
35 access to a bigger range of ecological scales (from individual to community) than  
36 culture-based approaches and meta-omics, single-cell omics can contribute to  
37 microorganisms genomes and functions identification but also to the testing of  
38 concepts in ecology. Here, we discuss the contribution of single-cell omics to  
39 possible breakthroughs in concepts and knowledge on microbial ecosystems and  
40 eco-evolutionary processes.

## 41 **Main Text**

### 42 **Ecological scales**

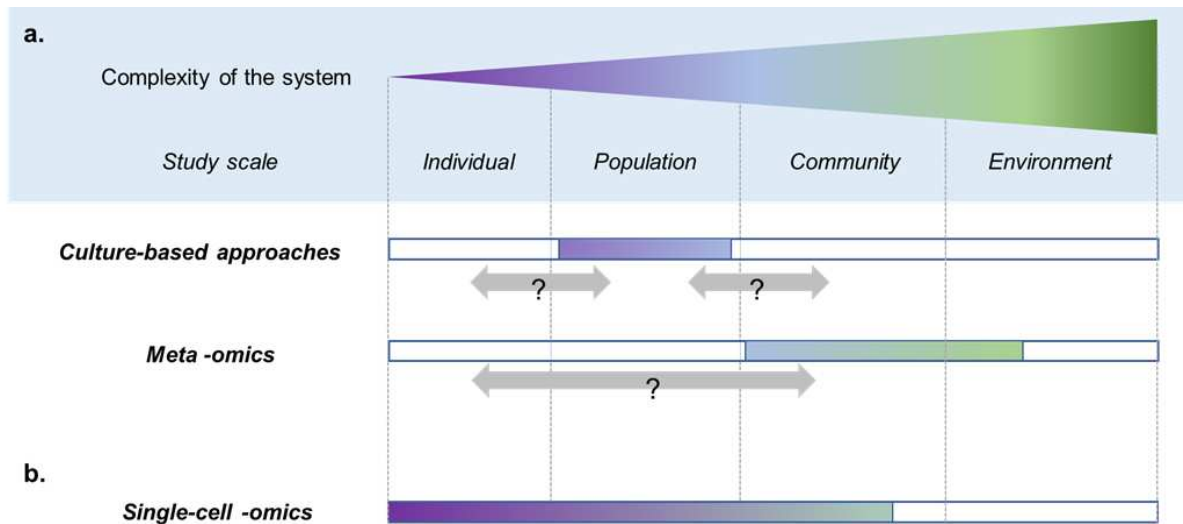
43 Interactions between organisms take place at all organizational levels, from  
44 molecules to communities, within or between species, and shape ecosystem  
45 dynamics. Ecological interactions are difficult to understand due to the number of  
46 biotic and abiotic parameters involved. Assembling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

57 A fundamental level of organization in ecology is the species. However, due to gene  
58 flow between cells that increases with ecological overlap and genetic similarity [4],  
59 microbial species concept and thus, **populations** (see Glossary), are not clearly  
60 defined entities. Interactions and diversity at the populational level, i.e. between  
61 individual cells of the same population (Box 1), is still obscure since it is not often  
62 analyzed in environmental microbiology. Given the natural mutation rate in bacteria  
63 ( $\sim 10^{-7}$  substitution per nucleotide, e.g. [5]), even a single colony contains genetic  
64 variations, i.e. variants within a cell population. The population has been suggested

65 to be more relevant than the species level for microbes [6,7] and species usually  
66 contain genetically divergent microorganisms. Considering the hierarchical levels of  
67 ecology, populations are keys to assessing genetic structure within species and over  
68 time, changes therein. They thereby provide insights into eco-evolutionary  
69 processes and advance our understanding of microbiota composition dynamics (Box  
70 1).

71 Our understanding of the microbial world and its ecological roles is still very limited  
72 [8]. Understanding the functions played by microbial cells in a complex community  
73 remains a frontier in microbial ecology. Beyond the technical limits that microbiology  
74 is facing, the information gathered from culture-based studies or from natural  
75 ecosystems can be difficult to interpret (Figure 1a). Laboratory experiments attempt  
76 to reproduce optimal ecological conditions for microbes by selecting among the  
77 many biotic and environmental parameters [6] in order to understand specific  
78 processes such as trait tradeoffs [9], interactions between strains [10], the production  
79 of metabolites [11] or genome evolution [12]. Extrapolating observations obtained in  
80 vitro, at restricted scales, to higher ecological scales such as natural communities  
81 and ecosystems requires particular attention. Conversely, observations made from  
82 environmental samples, including microbial community composition, diversity or  
83 global functions are less specific and represent an average of the microbial  
84 community. Ideally, we want to get the most information out of each level of  
85 approach (i.e precise interactions and genetic dynamics from culture-based studies  
86 coupled with global function and diversity of a community with meta-omics).  
87 However, our ignorance of intricacies and interactions of ecological scales with one  
88 another still jeopardize the assembly of the resulting information to answer specific  
89 ecological questions in environmental microbiology. Moreover, the uncertainty of

90 community composition and the complexity of microbial interactions [2] make it even  
 91 more difficult to target specific scientific hypotheses on natural communities and to  
 92 choose the appropriate tools.



93

94 **Figure 1:** Gradient of ecological complexity, study scales and associated  
 95 approaches.

96 Although other higher levels of ecology can be used, individual-population-  
 97 community-environment scales describe much of the subject of ecology. a) The  
 98 culture-based approaches aim at studying microbial populations or a very limited  
 99 number of strains, therefore the outcoming data cannot be fully informative about  
 100 higher levels of ecology. On the contrary, meta-omics approaches cover from the  
 101 community to environmental scales of microbial ecology and does not provide  
 102 finer information on the ecological gradient. In both cases, the individual scale is  
 103 unattainable while being at the basis of ecological processes. b) Single-cell omics  
 104 cover the scales from the individual microbe to the community from the same  
 105 environmental sample which allows to connect the outcoming information of each  
 106 ecological scale.

107

## 108 **Specific tools for specific ecological questions**

109 Like in any new field of exploration, ecological patterns within microbial communities  
110 are firstly observed and described but poorly understood [13], testifying to the  
111 enormous lack of knowledge concerning microbes [14]. The use of DNA- and RNA-  
112 based methods to study natural microbial communities have demonstrated the  
113 existence of a prodigious wealth of microorganisms which remained unsuspected  
114 some years ago (e.g. [15]). Among **meta-omics** techniques, metagenomics and  
115 metatranscriptomics are the most widely used methods to explore microbiota. These  
116 techniques enabled a breakthrough in our understanding of microbial phylogenetic  
117 relationships [16], species diversity and abundance [17], metabolic abilities [18] and  
118 functional diversification [19]. The development and use of **metagenome-**  
119 **assembled genomes (MAGs)** led to discoveries that advanced our understanding  
120 of bacterial life and modified our perception of the tree of life [15,19]. Some studies  
121 attempted to reconstitute population level genomes from metagenomes. For  
122 example, Crits-Christoph et al. [20] investigated genetic variation within populations  
123 of highly abundant soil bacteria by studying MAGs and observed spatial  
124 differentiation of alleles. However, inferring population features from meta-omics  
125 data remains limited, especially when genomes are inaccurately or incompletely  
126 reconstructed from short sequence fragments (Figure 2, Key Figure). The use of  
127 MAGs becomes challenging when microbial richness and diversity within a  
128 community are high and taxa are phylogenetically close [21]. During genome  
129 assembly, stitching of fragments from different individual genomes and/or  
130 contaminant DNA can occur, creating chimeras that are irrelevant for the study of

131 populations. In this case, the approach would necessarily conceal a considerable  
132 proportion of molecular diversity [22]. In addition, the molecular biology and  
133 bioinformatics methods used in meta-omics approaches are varied and based on  
134 different criteria and assumptions in the absence of a consensus, leading to  
135 contrasting results and interpretations [23,24]. Overall, it might be difficult to directly  
136 link the detected functions to their original microbial cell from meta-omics data,  
137 thereby limiting the identification of signaling pathways and trade-offs in gene  
138 regulation. Meta-omics approaches proved useful in describing communities using  
139 large-scale sampling and make it possible to answer questions related to community  
140 composition and its associated global functions but not to fully understand the  
141 mechanisms underlying these patterns. Nevertheless, bioinformatics research has  
142 developed algorithms aiming to identify genetic variations in microbial populations:  
143 vertically and horizontally inherited genes can be differentiated, and from population-  
144 specific sweeps, single nucleotide polymorphisms can be detected (e.g. DiscoSNP;  
145 PopCOGenT) [4, 25].

146 To complement meta-omics data, modelling approaches are used to explore  
147 microbial interactions, fluxes of metabolites, and to reconstruct ecological networks  
148 in complex microbiomes [26-29]. These approaches provide a possible explanation  
149 and scenarios of interactions in natural communities, but sometimes end in  
150 contradictions with culture-based experiments [1]. Indeed, models can predict a  
151 certain kind of interaction (e.g. cross-feeding) that is not verified or proven wrong in  
152 an experimental setup due to the oversight of key parameters such as growing  
153 conditions, space and (very often) time [1]. They also rely on the co-occurrence of  
154 phenomena, which is more associated with correlations than cause-consequences  
155 relationships. Culture-dependent approaches may help reach the population level in



156 simple community compositions through controlled and simplified laboratory-scale  
157 experiments [30] and can be effective for testing patterns observed in meta-omics  
158 studies and deconstructing mechanistic hypotheses (e.g. interactions, metabolism)  
159 (e.g. [31]). The lack of information on individual cells and mostly on populations (i.e.  
160 both functions and phylogeny) using existing methods limits our understanding of  
161 observed processes. Many studies aim at unravelling microbiota diversity, primarily  
162 in plants, soil, water, and animal bodies, but few explain associated community  
163 assembly and evolutionary mechanisms [32-34]. In this context, microbiologists and  
164 ecologists are searching for other technical possibilities or approaches such as  
165 single-cell omics to complete the knowledge provided by current methods.

#### 166 **The alternative scope of single -cell omics**

167 Single-cell whole genome sequencing (scWGS) and single-cell transcriptomics  
168 (scRNA-seq) were first developed for eukaryotic cells and used in cancer research,  
169 revealing both intra-population genetic diversity and heterogeneous genome  
170 expression. As in cancer research where differentiation in space of the genome  
171 expression among cell has been observed [35], a pioneer paper on *Pseudomonas*  
172 *aeruginosa* biofilm using a fluorescent based approach (i.e. parallel sequential  
173 fluorescence in situ hybridization (par-seqFISH)), revealed a differentiation in space  
174 and time of cell-expression [36]. Because spatial single-cell microbial approaches  
175 could allow the understanding of the drivers and mechanisms leading to the self-  
176 organization of these microbial structures, new developments are expected to  
177 expand in health science and many other fields of microbial ecology research.  
178 Single-cell approaches are promising candidates for microbial studies, as they

179 provide a complementary view to metagenomics and metatranscriptomics that have  
180 different strengths, but also weaknesses (Figure 2).

181 Single-cell omics technologies require additional steps to prepare a sample for  
182 sequencing as compared to meta-omics techniques, especially with regards to cell  
183 isolation, for which different technical options are available [37,38]. Once the cells  
184 lysed, DNA and RNA content from a single cell is in the femtogram scale for  
185 bacteria, i.e. 1,000-fold less than in animal cells. Preparing the sequencing library,  
186 which typically requires ng ranges of material, will need an ultra-efficient prior  
187 amplification step (e.g. Multiple Displacement Amplification (MDA), the most widely  
188 used approach for bacteria [39]).

189 Single-cell approaches enable accurate access to genomic and transcriptomic  
190 information for each cell, so that the assembled cell information is highly  
191 representative of the original population (Figure 2). This enables the identification of  
192 heterogeneity in gene assemblage, gene expression and metabolic pathways  
193 between cells. Single-cell transcriptomic and genomic information provide a link  
194 between phylogeny and functional traits and reveal the physiological status of an  
195 individual cell, at the time of sampling. This is particularly important considering that  
196 the individual gene expression profiles of genetically close cells may differ. What is  
197 more, some cell isolation tools such as automated image-based isolation devices  
198 (CellenONE Cellenion; icell8 Takara) make it possible to select cells based on their  
199 integrity, their physiology and/or functional markers, and to minimize contamination  
200 by the host or extracellular DNA. This is very promising for microbiology to, for  
201 instance, select active cells in the studied sample at the time of sampling and reveal  
202 which of them are taking part in the community productivity.

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

## 216 **Applications of single-cell omics in microbial ecology**

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

### 220 *The tremendous diversity of single-microbe genomes.*

221 Observations of microbiota can complement/validate the diversity observed by meta-  
222 omics on fungi [43], human samples [8], and marine viruses [44], and resolve cryptic  
223 bacterial species, which currently mainly rely on cultivable strains [45-47]. Single-  
224 microbe -omics therefore contribute to the microbial inventory which is still in its  
225 infancy in many ecosystems [48]. The use of single-cell based approaches could  
226 demonstrate the existence of the discovered sequence-based lineages and discover

227 possible new branches. Isolating cells from environmental samples can also cast  
228 light on rare organisms that might be obscured in millions of genome fragments  
229 using meta-omics. These rare organisms are considered to play key roles in  
230 community dynamics since they over-proportionally contribute to the functions of the  
231 microbial community in fluctuating environments [49,50]. While most studies that  
232 apply this approach use a limited number of cells, a recent survey of the marine  
233 microbiome recovered no less than 12,000 genomes from single cells [51], revealing  
234 a high degree of uniqueness and limited clonality in the analyzed samples of sea  
235 water and providing evidence for the ecological roles of uncultured microbial groups.  
236 Single-cell genomics, by looking at individual genomes instead of core genomes  
237 from meta-omics, from natural microbial communities represent an unprecedented  
238 opportunity to complete the identification and classification of microbes. This is a key  
239 step sometimes missing in environmental microbiology [52]: knowing what to look at  
240 and why to formulate hypotheses in ecology and better understand processes  
241 involving microbes.

242 *Ecological and evolutionary hypothesis testing using single-cell -omics.*

243 Single-cell genomics and transcriptomics approaches therefore help to answer the  
244 questions “What are these microbes?” and “What are they doing (or capable of  
245 doing)?” but also to understand why and how observed patterns happen. Linking  
246 environmental and community parameters to individual gene expression and  
247 bacterial interactions enables a mechanistic understanding of underlying biotic and  
248 abiotic conditions to patterns. This represents an opportunity to explore multiple  
249 ecological theories and hypotheses, notably on interactions of microbes at many  
250 levels: within the community, with external microbes (i.e. viruses) and with their host.

251 One of the hottest topics in microbial ecology is the link between diversity and  
252 function, including the productivity of the ecosystem that relies on the **niche**  
253 **partitioning** theory. This hypothesis states that species coexistence is enabled by  
254 species specialization in different available resources (or combinations of resources),  
255 thereby reducing interspecific competition [53] but likely modifying the microbial  
256 population structure [54]. Specialization in specific resources raises many questions  
257 concerning microbial interactions through the exchange of metabolites [55], loss of  
258 traits [56] and genome reduction [57]. The Black Queen Hypothesis (BQH), one of  
259 the ecological theories that conceptualized this phenomenon, states that microbial  
260 community assembly and complexity are at least partially determined by functional  
261 dependencies resulting from gene loss(es). Testing this hypothesis requires using  
262 environmental samples to evaluate functional redundancy within communities, the  
263 expression and distribution of the functions between interacting (micro-)organisms  
264 and the impact of genotypic interactions on these functions. At the community level,  
265 it is impossible to access this information through meta-omics since BQH evolution is  
266 supposed to also occur at the population level [53,54] which is undetectable from  
267 most of the meta-omics tools used so far. Eco-evolutionary processes can so far be  
268 explicitly assessed only through ecological models [58] or from dedicated *in-vitro*  
269 experiments [42] that require deciding which gene and which organisms to look at.  
270 Single cell approaches can help to investigate such hypotheses and other theories in  
271 ecology and highlight patterns of interactions that shape microbial communities.

272 *A breach to viral-host ecology.*

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

275 recent study assessed viral infections in the ocean thanks to single cell genomics by  
276 identifying virus sequences in uncultured protist cells [44]. Such interactions are  
277 widespread but generally missing in current microbial ecology analyses. Among  
278 other roles, viruses are known to (1) control microbial community dynamics and drive  
279 microbial-host evolution [60,61] and (2) impact ecosystem changes and  
280 biogeochemical cycles [59,62]. Single-cell omics can help us understand the roles  
281 played by viruses in microbial populations by making it possible to assess both the  
282 prevalence of prophage sequences and possible lateral gene transfers [63,64] which  
283 would reveal preferential association of specific viruses with specific bacteria.

284 *Single-cell insights into bigger scale interactions.*

285 Generally speaking, single-cell omics can highlight diverse levels of interaction in  
286 natural habitats either between the microbes that comprise a microbiota or between  
287 the microbes/microbiota and their host. Despite the great number of studies on the  
288 composition of human microbiota, very little is known about interactions between the  
289 microbes and with their host [65]. The single cell study of host-pathogen interactions  
290 paves the way for understanding infectious processes through microbiota dynamics,  
291 metabolic capacities, and host resistance [66]. Dysbiosis is often shown to display a  
292 higher beta-diversity interpreted as a higher stochasticity in the microbiota assembly  
293 [67]. Among other explanations, this apparent higher stochasticity may be the result  
294 of drastic pathogen-induced changes in the habitat (i.e. transitory state in the  
295 microbiota dynamics), modification of a component of the microbiota caused by  
296 genetic change(s) and/or a functional modification expressing a modified phenotype  
297 that leads to disequilibrium in the microbiota community. With the aim of  
298 understanding how a disorder of the microbiota leads to disease or the reverse, i.e.

299 how a disease can modify the composition of a microbiota, single-cell microbiota  
300 analyses of genomes and transcriptomes would help better define the characteristics  
301 of dysbiosis (i.e. dysbiosis mechanisms). Using single cell genomics makes it  
302 possible to address hypotheses related to changes in bacterial populations, while  
303 microbial single-cell transcriptomics may be more appropriate to decrypt the  
304 functions of microbes, metabolic abilities, and cellular states [68]. These two  
305 strategies are necessary to understand how microbial interactions occur within  
306 communities as well as their possible impact on the ecosystem [69,51].

307

308 Microbial single-cell omics are not solely expected to better understand functional  
309 interactions but also the underlying evolutionary processes. Microbial single-cell  
310 omics should also promote a shift in standpoint from observation to interpretation  
311 and also offer new opportunities to test macroecological theories on microbes. The  
312 development of microbial single-cell omics will have high impacts on our  
313 understanding of microbial communities in many environments. For instance (i) in  
314 freshwater and marine ecosystems, to define the interaction of bacteria and  
315 phytoplankton through the exchange of metabolites, and to test links to blooms [70],  
316 (ii) in soils, to better assess the provision of services by plant microbiota including  
317 nutrient and water uptake and protection against pathogens [71], (iii) in plant, human  
318 and animal health, to better decipher how dysbiosis could be cause or consequence  
319 of a disease. However, it can't be ignored that the wide development of single-cell  
320 omics applied to microorganisms is limited by technical limits.

321

322 **Limits of single-cell omics on microbes**

323 The current limited number of cells studied in the published papers questions the  
324 representativeness of the analyses. Considering the number of microbial cells  
325 contained in a given environmental sample, one can wonder how many cells need to  
326 be isolated to cover the diversity of a sample, from hundreds [41], to thousands [51].  
327 The limited number of analyzed cells is mostly due to technical problems and the  
328 cost of such experiments. It has to be emphasized that the current use of single-cell  
329 omics for microbes have to solve many technical obstacles (Box 2), reviewed in  
330 [72,73] such as cell isolation, lysis, and a biased amplification step. The structure of  
331 the microbial cell wall is complex, and unlike the animal cells, they do not break  
332 easily. The diversity of cell wall composition across phylogeny and physiological  
333 status (peptidoglycan layer(s), spores, capsules, etc.) makes it challenging to find a  
334 universal lysis method able to breach each cell without damaging its content or  
335 inhibiting enzymatic reactions downstream. Different protocols have been used in  
336 recent microbial single-cell based studies, using either heat, temperature shocks,  
337 sonication, enzymes, detergents, or combinations of these [74,75]. In addition to  
338 technical issues, one can wonder how to be certain that cells are isolated and lysed  
339 equally and not preferentially depending on their physical/physiological status. The  
340 amplification step, usually made via MDA, has been reported to be imprecise  
341 concerning the genome amplification uniformity, even though it presents a better  
342 genome coverage than other approaches (i.e MALBAC [39]). This amplification step  
343 is highly relevant, as it was suggested to be the cause of incomplete reconstruction  
344 of single amplified genomes (SAGs) [76], although solutions are being developed  
345 [77]. As the price of library preparation represents most of the cost of these new  
346 single- cell omics for microorganisms, reduction of reaction volumes in  
347 “nanolibraries” should be very cost-effective (Box 2). The probability of contamination

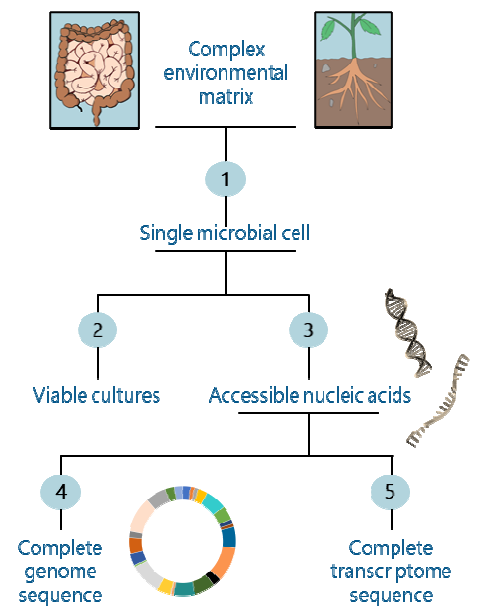


348 decreases with the miniaturization of the reagent volumes [78] and associated  
 349 robotics. Working in nanovolumes seems to be a convenient solution to solve  
 350 multiple problems, however this introduces new volume-related challenges such as  
 351 pipetting or sample purification.

352 For these reasons, single-cell omics have been sometimes used in combination with  
 353 meta-omics to combine the possibility of fine scale analysis with high throughput  
 354 [76,79]. It also represents a good opportunity to validate multiple aspects of single-  
 355 cell omics: 1) the isolation and lysis universality, 2) the sample preparation (genome  
 356 amplification and library preparation for sequencing), 3) the lack of contamination,  
 357 and 4) the representativeness of the sample covered by single -cell omics (See  
 358 outstanding questions).

359 **Box 2:** Single microbial cell -omics approaches are technically challenging.

Challenge	Possible solution
<b>1. Isolation of single microbial cells</b>	
Community representative sampling	High throughput
Cell isolation from complex matrices, such as soil, sediments, host tissue, feces, mucus...	Sonication, filtration, density gradient centrifugation
Exhaustive/targeted labelling/detection	Fluorescence, antibodies
Maintenance of axenic conditions	(As) clean (as possible) room + Ⓟ
<b>2. Culturomics experiments</b>	
Maximum viability/cultivability	Gentle cell handling, liquid dispensing
Choice of culture conditions	High throughput media screening + Ⓟ
Assessment of monoclonality (= culture purity)	Microscopy, targeted sequencing
<b>3. Microbial cell lysis/permeabilization</b>	
Efficiency across phylogeny	(Ultra-) sonication, thermal shock, heat, enzymolysis, detergents...
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 + Ⓟ
<b>4. Single-microbe WGS</b>	



5 steps of single microbial approaches.

Super-efficient (100K- to 1 Mo-fold) amplification (1-10 fg DNA prokaryotic cell <sup>-1</sup> )	Ⓟ, molecular crowding, linear amplification (e.g. <i>in vitro</i> transcription)
Even/broad coverage, high fidelity, and no chimera creation	Minimum number of PCR cycles and/or primary template amplification (e.g. <i>in vitro</i> transcription)
Minimum contamination from reagents	Minimum reagent amount + Ⓟ
Cost reduction	Cell barcoding for multiplexing + Ⓟ
Bioinformatics	Dedicated tools for cell demultiplexing, monoclonality test...
<b>5. Single-microbe RNA-seq</b>	
Same as WGS (up to 100 fg RNA prokaryotic cell <sup>-1</sup> )	Ⓟ, molecular crowding
Amplification bias	Unique molecular identifier (UMI)
No poly-A tail on prokaryotic mRNA	RNA poly-A tailing, random priming, ribosomal RNA targeted depletion

360 **Table i.** Padlocks and possible keys offered by single microbial cell –omics  
361 approaches.

362 Crucial, but solvable, issues (Table i) should be addressed at each step of single  
363 microbe approaches (Fig. i). The reduction of reaction volumes down to  
364 “nanovolumes” (see Ⓟ symbols), is often the most sensible solution, limiting  
365 contamination probability and allowing high throughput and cost reduction.

### 366 **Concluding Remarks**

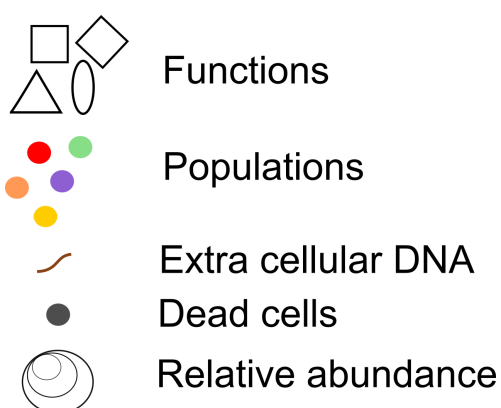
367 Soon, single-cell omics applied to microorganisms could become a gold standard in  
368 microbial ecology thanks to the knowledge produced by focusing on individual  
369 genomes and transcriptomes, and, possibly, individual proteomes and metabolomes.  
370 Today, technical problems prevent the testing of broad ecological hypotheses.  
371 Generalizing ecological single-cell studies on microbes requires the development of  
372 robust high throughput techniques with a high-cost effectiveness ratio (See  
373 Outstanding Questions). A knowledge upshot is expected in microbial interactions

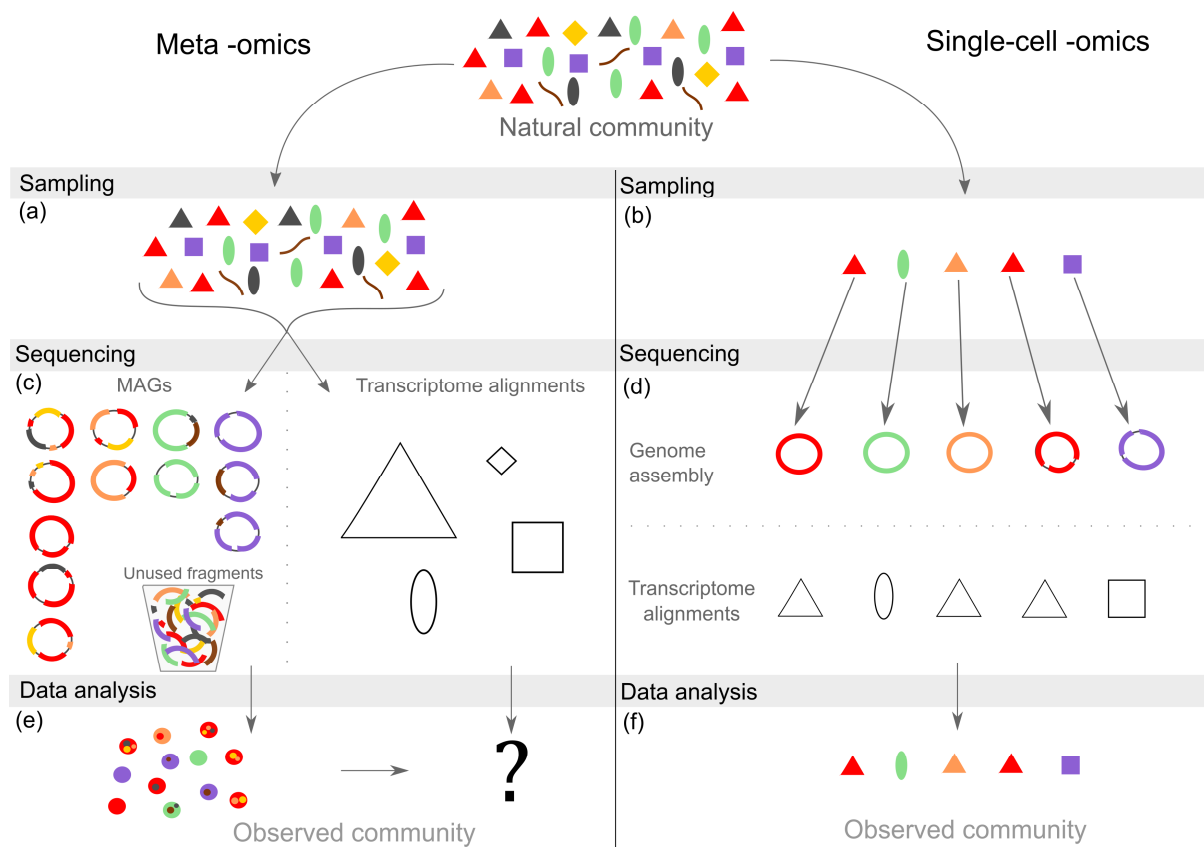
374 and eco-evolutionary boundaries through the enabling of mechanistic  
375 characterization of deterministic populations and community assembly processes.  
376 Currently, the use of metagenomics and single cell genomics in the same study  
377 appears as the best solution, combining the strengths of the two approaches, i.e. (i)  
378 high throughput and alpha/beta diversity, and (ii) fine scale analysis by scWGS  
379 and/or scRNAseq [76,80]. Ideally, one would not over-interpret meta-omics data and  
380 rather use those to build hypotheses on mechanisms, which can be tested using  
381 single-cell approaches. Approaches that will allow more accurate assessment of  
382 microbial genome diversity and genome functioning within complex microbiota, are  
383 impatiently awaited. Still, the future of microbial single-cell omics will likely fuel a new  
384 perception of the microorganisms world.

### 385 **Acknowledgements**

386 We would like to thank LABCOM 'Microscale' for funding.

### 387 **Key figure**





388

389 **Outstanding Questions**

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

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

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

397 How can we use information obtained by single-cell omics for a better understanding  
 398 of ecosystem functioning?

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

401

## 402 **Glossary**

403 **Metagenome-assembled genome (MAG):** in-silico reconstruction of an artificial  
404 microbial genome obtained from one or multiple binned metagenomes that represent  
405 the core genome of the population

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

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

414 **Population:** applied to bacteria in natural communities, individuals with identical or  
415 different genomes from the same species gathered in a specific environment or  
416 sample. An isolated microorganism culture also comprises a population.

417

## 418 **Box 1: Microbial population**

419 In ecology, populations are individuals belonging to the same species, living in  
420 the same environment, although the definition varies with different viewpoints  
421 [81]. Microbial populations represent a unit of diversity and selection. Within  
422 these populations, diversity can be either genetic or phenotypic. The diversity  
423 within a population to some extent buffers an environmental stress because  
424 existing variants are able to survive the stress and/or allow rapid phenotype  
425 switching (e.g. Bet-Hedging, [82,31]) but positive selection of new variants can  
426 also be induced by the stress. This organizational level is therefore a key to  
427 understanding genetic structure and haplotype fitness, the dynamics of ecological  
428 interactions, including associations of microbial species, symbioses, host-  
429 pathogen interactions, and ecosystem functioning, resilience, and stability. For  
430 instance, resistance to antibiotics can vary within populations [83] and the  
431 virulence pathogens can vary across subpopulations [84]. Genetic diversity and  
432 ecological features such as niches can vary between lineages [85], so that  
433 subpopulations are able to co-exists through niche diversity. To capture the total  
434 genetic and phenotypic diversity and get a holistic view of populations, the scale  
435 must thus be tuned down to the individuals that comprise the population in a  
436 given sample [84]. Otherwise, applying the current bacterial species concept to  
437 make population level inferences may lead to false or partial interpretation of  
438 ecological phenomena.

#### 439 **Reference list**

- 440 1- Coyte, K.Z., and Rakoff-Nahoum, S. (2019) Understanding competition  
441 and cooperation within the mammalian gut microbiome. *Curr. Biol.* 29(11),  
442 538–544. DOI: <https://doi.org/10.1016/j.cub.2019.04.017>

- 443 2- Pacheco, A.R., and Segrè, D. (2019) A multidimensional perspective on  
444 microbial interactions. *FEMS Microbiol. Lett.* 366(11), 1–11. DOI:  
445 <https://doi.org/10.1093/femsle/fnz125>
- 446 3- Dini-Andreote, F. *et al.* (2020) Towards meaningful scales in ecosystem  
447 microbiome research. *Environ. Microbiol.* 23(1), 1-4. DOI:  
448 <https://doi.org/10.1111/1462-2920.15276>
- 449 4- VanInsberghe, D. *et al.* (2020) How can microbial population genomics  
450 inform community ecology? *Phil. Trans. R. Soc. B.* 375(1798), 20190253.  
451 DOI: <https://dx.doi.org/10.1098%2Frstb.2019.0253>
- 452 5- Westra, E.R. *et al.* (2017) Mechanisms and consequences of diversity-  
453 generating immune strategies. *Nat Rev Immunol*, 17(11), 719-728. DOI:  
454 <https://doi.org/10.1038/nri.2017.78>
- 455 6- García-García, N. *et al.* (2019) Microdiversity ensures the maintenance of  
456 functional microbial communities under changing environmental  
457 conditions. *ISME J.* 13(12), 2969-2983. DOI :  
458 <https://doi.org/10.1038/s41396-019-0487-8>
- 459 7- Niccum, B.A. *et al.* (2020) Strain-level diversity impacts cheese rind  
460 microbiome assembly and function. *mSystems* 5(3) e00149-20. DOI:  
461 <https://doi.org/10.1128/msystems.00149-20>
- 462 8- Marcy, Y. *et al.* (2007) Dissecting biological “dark matter” with single-cell  
463 genetic analysis of rare and uncultivated TM7 microbes from the human  
464 mouth. *PNAS* 104(29), 11889–11894. DOI:  
465 <https://doi.org/10.1073/pnas.0704662104>

- 466 9- Moreno-Gómez, S. *et al.* (2020) Wide lag time distributions break a trade-  
467 off between reproduction and survival in bacteria. *PNAS* 117(31), 18729-  
468 18736. DOI: <https://doi.org/10.1073/pnas.2003331117>
- 469 10-Ratzke, C. *et al.* (2020) Strength of species interactions determines  
470 biodiversity and stability in microbial communities. *Nat. Ecol. Evol.* 4(3),  
471 376-383. DOI: <https://doi.org/10.1038/s41559-020-1099-4>
- 472 11-Rütting, T. *et al.* (2021) The contribution of ammonia-oxidizing archaea  
473 and bacteria to gross nitrification under different substrate availability. *Soil*  
474 *Biol. Biochem.* 160, 108353. DOI:  
475 <https://doi.org/10.1016/j.soilbio.2021.108353>
- 476 12-Hall, J. P. *et al.* (2018) Competitive species interactions constrain abiotic  
477 adaptation in a bacterial soil community. *Evol. Lett.* 2(6), 580-589. DOI:  
478 <https://doi.org/10.1002/evl3.83>
- 479 13-Tripathi, A. *et al.* (2018) Are microbiome studies ready for hypothesis-  
480 driven research? *Curr. Opin. Microbiol.* 44, 61–69. DOI:  
481 <https://doi.org/10.1016/j.mib.2018.07.002>
- 482 14-Lloyd, K.G. *et al.* (2018) Phylogenetically novel uncultured microbial cells  
483 dominate earth microbiomes. *mSystems* 3(5) e00055-18. DOI:  
484 <https://doi.org/10.1128/msystems.00055-18>
- 485 15-Hug, L.A. *et al.* (2016) A new view of the tree of life. *Nat. Microbiol.* 1(5),  
486 16048. DOI: <https://doi.org/10.1038/nmicrobiol.2016.48>
- 487 16-Alteio, L.V. *et al.* (2020) Complementary metagenomic approaches  
488 improve reconstruction of microbial diversity in a forest  
489 soil. *mSystems*, 5(2), e00768-19. DOI:  
490 <https://doi.org/10.1128/mSystems.00768-19>



- 491 17-Daims, H. *et al.* (2015) Complete nitrification by *Nitrospira* bacteria. *Nature*  
492 528(7583), 504. DOI: <https://doi.org/10.1038/nature16461>
- 493 18-Cernava, T. *et al.* (2017) Deciphering functional diversification within the  
494 lichen microbiota by meta-omics. *Microbiome* 5(1), 82. DOI:  
495 <https://doi.org/10.1186/s40168-017-0303-5>
- 496 19- Parks, D.H. *et al.* (2017) Recovery of nearly 8,000 metagenome-  
497 assembled genomes substantially expands the tree of life. *Nat. Microbiol.*  
498 2(11), 1533-1542. DOI: <https://doi.org/10.1038/s41564-017-0012-7>
- 499 20-Crits-Christoph, A. *et al.* (2020) Soil bacterial populations are shaped by  
500 recombination and gene-specific selection across a grassland meadow.  
501 *ISME J.* 14(7), 1834-1846. DOI: [https://doi.org/10.1038/s41396-020-0655-](https://doi.org/10.1038/s41396-020-0655-x)  
502 [x](https://doi.org/10.1038/s41396-020-0655-x)
- 503 21-Nayfach, S. *et al.* (2019) New insights from uncultivated genomes of the  
504 global human gut microbiome. *Nature* 568(7753), 505-510. DOI:  
505 <https://doi.org/10.1038/s41586-019-1058-x>
- 506 22-Nelson, W.C. *et al.* (2020) Biases in genome reconstruction from  
507 metagenomic data. *PeerJ* 8, e10119. DOI:  
508 <https://doi.org/10.7717/peerj.10119>
- 509 23-Li, L. *et al.* (2015) Comparing viral metagenomics methods using a highly  
510 multiplexed human viral pathogens reagent. *J. Virol. Methods*, 213, 139-  
511 146. DOI: <https://doi.org/10.1016/j.jviromet.2014.12.002>
- 512 24-Mande, S.S. *et al.* (2012) Classification of metagenomic sequences:  
513 methods and challenges. *Brief. Bioinform.* 13(6), 669-681. DOI:  
514 <https://doi.org/10.1093/bib/bbs054>

- 515 25-Uricaru, R. *et al.* (2015) Reference-free detection of isolated  
516 SNPs. *Nucleic Acids Res.* 43(2), e11-e11. DOI:  
517 <https://doi.org/10.1093/nar/gku1187>
- 518 26-Antoniewicz, M.R. (2020) A guide to deciphering microbial interactions and  
519 metabolic fluxes in microbiome communities. *Curr. Opin. Biotechnol.* 64,  
520 230–237. DOI: <https://doi.org/10.1016/j.copbio.2020.07.001>
- 521 27-Belcour, A. *et al.* (2020) Metage2Metabo, microbiota-scale metabolic  
522 complementarity for the identification of key species. *eLife* 9, e61968. DOI:  
523 <https://doi.org/10.7554/eLife.61968>
- 524 28-Gorter, F.A. *et al.* (2020) Understanding the evolution of interspecies  
525 interactions in microbial communities. *Philos. Trans. R. Soc. Lond., B, Biol.*  
526 *Sci.* 375(1798), 20190256. DOI: <https://doi.org/10.1098/rstb.2019.0256>
- 527 29-Perez-Garcia, O. *et al.* (2016) Metabolic network modeling of microbial  
528 interactions in natural and engineered environmental systems. *Front.*  
529 *Microbiol.* 7, 673. DOI: <https://doi.org/10.3389/fmicb.2016.00673>
- 530 30-Morin, M. *et al.* (2018) Changes in the genetic requirements for microbial  
531 interactions with increasing community complexity. *eLife* 7, e37072. DOI:  
532 <https://doi.org/10.7554/eLife.37072>
- 533 31-Beaumont, H. J. E. *et al.* (2009) Experimental evolution of bet hedging.  
534 *Nature* 462(7269), 90–93. DOI: <https://doi.org/10.1038/nature08504>
- 535 32-Kiers, E.T. *et al.* (2011) Reciprocal rewards stabilize cooperation in the  
536 mycorrhizal symbiosis. *Science* 333(6044), 880–882. DOI:  
537 <https://doi.org/10.1126/science.1208473>

- 538 33-Werner, G.D., and Kiers, E.T. (2015) Partner selection in the mycorrhizal  
539 mutualism. *New Phytol.* 205(4), 1437-1442. DOI:  
540 <https://doi.org/10.1111/nph.13113>
- 541 34-Amin, S.A. *et al.* (2015) Interaction and signaling between a cosmopolitan  
542 phytoplankton and associated bacteria. *Nature* 522(7554), 98–101. DOI:  
543 <https://doi.org/10.1038/nature14488>
- 544 35-Alon, S. *et al.* (2021) Expansion sequencing: Spatially precise in situ  
545 transcriptomics in intact biological systems. *Science* 371(6528). DOI:  
546 [10.1126/science.aax2656](https://doi.org/10.1126/science.aax2656)
- 547 36-Dar, D. *et al.* (2021) Spatial transcriptomics of planktonic and sessile  
548 bacterial populations at single-cell resolution. *Science* 373(6556). DOI:  
549 [10.1126/science.abi4882](https://doi.org/10.1126/science.abi4882)
- 550 37-Gawad, C. *et al.* (2016) Single-cell genome sequencing: current state of  
551 the science. *Nat. Rev. Genet.* 17(3), 175. DOI:  
552 <https://doi.org/10.1038/nrg.2015.16>
- 553 38-Woyke, T. *et al.* (2017) The trajectory of microbial single-cell sequencing.  
554 *Nat. Methods* 14(11), 1045. DOI: <https://doi.org/10.1038/nmeth.4469>
- 555 39-Chen, Z. *et al.* (2017) Tools for genomic and transcriptomic analysis of  
556 microbes at single-cell level. *Front Microbiol.* 8, 1831. DOI:  
557 <https://doi.org/10.3389/fmicb.2017.01831>
- 558 40-Raghunathan, A. *et al.* (2005) Genomic DNA amplification from a single  
559 bacterium. *Appl. Environ. Microbiol.* 71(6), 3342–3347. DOI:  
560 <https://doi.org/10.1128/AEM.71.6.3342-3347.2005>

- 561 41-Engel, P. *et al.* (2014) Hidden diversity in honey bee gut symbionts  
562 detected by single-cell genomics. *PLoS Genet.* 10(9), e1004596. DOI:  
563 <https://doi.org/10.1371/journal.pgen.1004596>
- 564 42-Hennon, G.M. *et al.* (2018) The impact of elevated CO<sub>2</sub> on  
565 *Prochlorococcus* and microbial interactions with ‘helper’ bacterium  
566 *Alteromonas*. *ISME J.* 12(2), 520–531. DOI:  
567 <https://doi.org/10.1038/ismej.2017.189>
- 568 43-Ahrendt, S.R. *et al.* (2018) Leveraging single-cell genomics to expand the  
569 fungal tree of life. *Nat. Microbiol.* 3(12), 1417-1428. DOI:  
570 <https://doi.org/10.1038/s41564-018-0261-0>
- 571 44-Castillo, Y.M. *et al.* (2019) Assessing the viral content of uncultured  
572 picoeukaryotes in the global ocean by single cell genomics. *Mol. Ecol.*  
573 28(18), 4272–4289. DOI: <https://doi.org/10.1111/mec.15210>
- 574 45-Hahn, M.W. *et al.* (2016) Complete ecological isolation and cryptic  
575 diversity in *Polynucleobacter* bacteria not resolved by 16S rRNA gene  
576 sequences. *ISME J.* 10(7), 1642–1655. DOI:  
577 <https://doi.org/10.1038/ismej.2015.237>
- 578 46-Hoetzinger, M. *et al.* (2019) *Polynucleobacter paneuropaeus* sp. nov.,  
579 characterized by six strains isolated from freshwater lakes located along a  
580 3000 km north-south cross-section across Europe. *Int. J. Syst. Evol.*  
581 *Microbiol.* 69(1), 203–213. DOI: <https://doi.org/10.1099/ijsem.0.003130>
- 582 47-Pitt, A. *et al.* (2019) *Aquirufa antheringensis* gen nov., sp nov. and  
583 *Aquirufa nivaísiívae* sp nov., representing a new genus of widespread  
584 freshwater bacteria. *Int. J. Syst. Evol. Microbiol.* 69(9), 2739–2749. DOI:  
585 <https://doi.org/10.1099/ijsem.0.003554>

- 586 48-Bernard, G. *et al.* (2018) Microbial dark matter investigations: how  
587 microbial studies transform biological knowledge and empirically sketch a  
588 logic of scientific discovery. *Genome Biol. Evol.* 10(3), 707-715. DOI:  
589 <https://doi.org/10.1093/gbe/evy031>
- 590 49-Shade, A. *et al.* (2014) Conditionally rare taxa disproportionately contribute  
591 to temporal changes in microbial diversity. *MBio* 5(4), 1371–1385. DOI:  
592 <https://doi.org/10.1128/mBio.01371-14>
- 593 50-Xu, Q. *et al.* (2021) Rare Bacterial Assembly in Soils Is Mainly Driven by  
594 Deterministic Processes. *Microb. Ecol.* 1–14. DOI:  
595 <https://doi.org/10.1007/s00248-021-01741-8>
- 596 51-Pachiadaki, M.G. *et al.* (2019) Charting the complexity of the marine  
597 microbiome through single-cell genomics. *Cell* 179(7), 1623-1635.e11.  
598 DOI: <https://doi.org/10.1016/j.cell.2019.11.017>
- 599 52-Prosser, J.I. (2020) Putting science back into microbial ecology: a question  
600 of approach. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 375(1798),  
601 20190240. DOI: <https://doi.org/10.1098/rstb.2019.024>
- 602 53-Mas, A. *et al.* (2020) Reflections on the predictability of evolution: towards  
603 a conceptual framework. *iScience*, 23(11), 101736. DOI:  
604 <https://doi.org/10.1016/j.isci.2020.101736>
- 605 54-Mas, A. *et al.* (2016) Beyond the Black Queen Hypothesis. *ISME J*, 10(9),  
606 2085–2091. DOI : <https://doi.org/10.1038/ismej.2016.22>
- 607 55-Zelezniak, A. *et al.* (2015) Metabolic dependencies drive species co-  
608 occurrence in diverse microbial communities. *PNAS* 112(20), 6449–6454.  
609 DOI: <https://doi.org/10.1073/pnas.1421834112>

610 56- Ellers, J. *et al.* (2012) Ecological interactions drive evolutionary loss of  
611 traits. *Ecol. Lett.* 15(10), 1071–1082. DOI: [https://doi.org/10.1111/j.1461-](https://doi.org/10.1111/j.1461-0248.2012.01830.x)  
612 [0248.2012.01830.x](https://doi.org/10.1111/j.1461-0248.2012.01830.x)

613 57-Morris, J.J. *et al.* (2012) The Black Queen Hypothesis: evolution of  
614 dependencies through adaptive gene loss. *MBio*, 3(2) e00036-12. DOI:  
615 [10.1128/mBio.00036-12](https://doi.org/10.1128/mBio.00036-12)

616 58-Wang, M. *et al.* (2020) Selfishness driving reductive evolution shapes  
617 interdependent patterns in spatially structured microbial communities.  
618 *ISME J.* 15(5), 1387-1401. DOI: [https://doi.org/10.1038/s41396-020-](https://doi.org/10.1038/s41396-020-00858-x)  
619 [00858-x](https://doi.org/10.1038/s41396-020-00858-x)

620 59-Weitz, J.S., and Wilhelm, S.W. (2012) Ocean viruses and their effects on  
621 microbial communities and biogeochemical cycles. *F1000 Biol. Rep.* 4(1),  
622 17. DOI: <https://doi.org/10.3410/B4-17>

623 60-Frada, M. *et al.* (2008) The “Cheshire Cat” escape strategy of the  
624 coccolithophore *Emiliana huxleyi* in response to viral infection. *PNAS*  
625 105(41), 15944–15949. DOI: <https://doi.org/10.1073/pnas.0807707105>

626 61-Scanlan, P.D., and Buckling, A. (2012) Co-evolution with lytic phage  
627 selects for the mucoid phenotype of *Pseudomonas fluorescens* SBW25.  
628 *ISME J.* 6(6), 1148–1158. DOI: <https://doi.org/10.1038/ismej.2011.174>

629 62-Rousk, J., and Bengtson, P. (2014) Microbial regulation of global  
630 biogeochemical cycles. *Front. microbiol.* 5, 103. DOI:  
631 <https://doi.org/10.3389/fmicb.2014.00103>

632 63-Roux, S. *et al.* (2014) Ecology and evolution of viruses infecting  
633 uncultivated SUP05 bacteria as revealed by single-cell-and meta-  
634 genomics. *eLife*, 3, e03125. DOI: <https://doi.org/10.7554/eLife.03125>

- 635 64-Labonté, J.M. *et al.* (2015) Single-cell genomics-based analysis of virus–  
636 host interactions in marine surface bacterioplankton. *ISME J.* 9(11), 2386–  
637 2399. DOI: <https://doi.org/10.1038/ismej.2015.48>
- 638 65-Hassani, M.A. *et al.* (2018) Microbial interactions within the plant holobiont.  
639 *Microbiome* 6(1)58. DOI: <https://doi.org/10.1186/s40168-018-0445-0>
- 640 66-Mills, E. and Avraham, R. (2017) Breaking the population barrier by single  
641 cell analysis: one host against one pathogen. *Curr. Opin. Microbiol.* 36, 69–  
642 75. DOI: <https://doi.org/10.1016/j.mib.2017.01.005>
- 643 67-Zaneveld, J.R. *et al.* (2017) Stress and stability: Applying the Anna  
644 Karenina principle to animal microbiomes. *Nat. Microbiol.* 2(9),1–8. DOI:  
645 <https://doi.org/10.1038/nmicrobiol.2017.121>
- 646 68-Hatzenpichler, R. *et al.* (2020) Next-generation physiology approaches to  
647 study microbiome function at single cell level. *Nat Rev Microbiol.* 18(4),  
648 241-256. DOI: <https://doi.org/10.1038/s41579-020-0323-1>
- 649 69-McNulty, R. *et al.* (2021) Droplet-based single cell RNA sequencing of  
650 bacteria identifies known and previously unseen cellular states. *BioRxiv*  
651 2021.03.10.434868. DOI: <https://doi.org/10.1101/2021.03.10.434868>
- 652 70-Landa, M. *et al.* (2016) Shifts in bacterial community composition  
653 associated with increased carbon cycling in a mosaic of phytoplankton  
654 blooms. *ISME J.* 10(1), 39–50. DOI:  
655 <https://doi.org/10.1038/ismej.2015.105>
- 656 71-Vandenkoornhuyse, P. *et al.* (2015) The importance of the microbiome of  
657 the plant holobiont. *New Phytol.* 206(4), 1196-1206. DOI:  
658 <https://doi.org/10.1111/nph.13312>

- 659 72-Kaster, A.K., and Sobol, M.S. (2020) Microbial single-cell omics: the crux  
660 of the matter. *Appl. Microbiol. Biotechnol.* 104(19),8209–8220. DOI:  
661 <https://doi.org/10.1007/s00253-020-10844-0>
- 662 73-Clingenpeel, S. *et al.* (2015) Reconstructing each cell's genome within  
663 complex microbial communities - dream or reality? *Front. Microbiol.* 5, 771.  
664 DOI: <https://doi.org/10.3389/fmicb.2014.00771>
- 665 74-He, J. *et al.* (2016) Improved lysis of single bacterial cells by a modified  
666 alkaline-thermal shock procedure. *Biotechniques* 60(3), 129-135. DOI:  
667 [10.2144/000114389](https://doi.org/10.2144/000114389)
- 668 75-Liu, Y. *et al.* (2018) The development of an effective bacterial single-cell  
669 lysis method suitable for whole genome amplification in microfluidic  
670 platforms. *Micromachines* 9(8), 367. DOI:  
671 <https://doi.org/10.3390/mi9080367>
- 672 76-Alneberg, J. *et al.* (2018) Genomes from uncultivated prokaryotes: a  
673 comparison of metagenome-assembled and single-amplified genomes.  
674 *Microbiome* 6(1), 1-14. DOI: <https://doi.org/10.1186/s40168-018-0550-0>
- 675 77-Kogawa, M. *et al.* (2018) Obtaining high-quality draft genomes from  
676 uncultured microbes by cleaning and co-assembly of single-cell amplified  
677 genomes. *Sci Rep.* 8(1), 1-11. DOI: [https://doi.org/10.1038/s41598-018-  
678 20384-3](https://doi.org/10.1038/s41598-018-20384-3)
- 679 78-Blainey, P.C. (2013) The future is now: single-cell genomics of bacteria  
680 and archaea. *FEMS Microbiol. Rev.* 37(3), 407-427. DOI:  
681 <https://doi.org/10.1111/1574-6976.12015>
- 682 79-Probst, A.J. *et al.* (2018) Differential depth distribution of microbial function  
683 and putative symbionts through sediment-hosted aquifers in the deep



684 terrestrial subsurface. *Nat. Microbiol.* (3), 328-336. DOI:  
685 <https://doi.org/10.1038/s41564-017-0098-y>

686 80-Santoro, A.E. *et al.* (2019) Contributions of single-cell genomics to our  
687 understanding of planktonic marine archaea. *Philos. Trans. R. Soc. Lond.,*  
688 *B, Biol. Sci.*374(1786), 20190096. DOI:  
689 <https://doi.org/10.1098/rstb.2019.0096>

690 81-Waples, R.S. and Gaggiotti, O. (2006) What is a population? An empirical  
691 evaluation of some genetic methods for identifying the number of gene  
692 pools and their degree of connectivity. *Mol. Ecol.* 15(6), 1419–1439. DOI:  
693 <https://doi.org/10.1111/j.1365-294X.2006.02890.x>

694 82-Veening, J.-W. *et al.* (2008) Bistability, Epigenetics, and Bet-Hedging in  
695 Bacteria. *Annu. Rev. Microbiol.* 62(1), 193–210. DOI:  
696 <https://doi.org/10.1146/annurev.micro.62.081307.163002>

697 83-Chua, S.L. *et al.* (2016) Selective labelling and eradication of antibiotic-  
698 Tolerant bacterial populations in *Pseudomonas aeruginosa* biofilms. *Nat.*  
699 *Commun.* 7(1), 1–11. DOI : <https://doi.org/10.1038/ncomms10750>

700 84-Davis, K.M., and Isberg, R.R. (2016) Defining heterogeneity within  
701 bacterial populations via single cell approaches. *BioEssays* 38(8), 782–  
702 790. DOI: <https://doi.org/10.1002/bies.201500121>

703 85-Garcia, S.L. *et al.* (2018) Contrasting patterns of genome-level diversity  
704 across distinct co-occurring bacterial populations. *ISME J.* 12(3), 742–755.  
705 DOI : <https://doi.org/10.1038/s41396-017-0001-0>

706

707 **Figure 2 – Key Figure: Microbial communities observed through meta-**  
708 **omics versus single-cell omics**

709 The key steps of meta-omics (left) and single-cell omics (right) approaches are  
710 shown, resulting in contrasting representations of a natural microbial community.  
711 Different genomes are represented by different colors, red, orange and yellow  
712 show genomes of close relatives, i.e. intra-population genomic variants. Dead  
713 cells are shown in grey, and extracellular or host DNA in brown. Different  
714 functions are represented by different symbols (triangles, squares, diamonds, or  
715 ovals). (a) After meta-omics sampling, the cell proportions are maintained but  
716 transient DNA and dead cells are not filtered. (b) In single-cell omics, a smaller  
717 proportion of the community is sampled, and dead cells can be excluded. (c) In  
718 meta-omics, the unit sequenced is the complete extracted sample. Metagenome  
719 assembled genomes (MAGs) are partial and include chimeras, i.e. unreal  
720 collages of closely related genomes, dead cells, and extracellular material. Meta-  
721 transcriptome analysis yields averaged relative abundances (represented by the  
722 size of the symbol) of functions within the sample. (d) By contrast, in single-cell  
723 omics, each cell is a sequenced unit and can be associated with its genome  
724 and/or transcriptome. (e) The community observed through meta-omics is  
725 representative of the composition of the whole community but not of the  
726 associated genes and functions. (f) With genome and transcriptome information  
727 from single cells, the observed community is under-sampled but is closer to the  
728 natural community: if the sampling scale is appropriate, rare populations and  
729 functions are more likely to be detected.