Environmental microbiology to the rescue of planet Earth

by

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Abstract

Environmental Microbiology has undergone a dramatic transition from being a somewhat marginal branch of Life Sciences to becoming one of the most vibrant and visible areas of contemporary research. The homonymous journal has not only borne witness of the growing interest in environmental microbes that bloomed since the mid-1980s but it has helped also to give visibility to the field and nucleate an active and influential community of authors and readers. During the past 20 years the focus has shifted from individual isolates to communities and microbiomes, from single genomes to metagenomes and from small/medium-scale experimental systems to large/very large scenarios. New challenges that were somewhat marginal when the journal was founded have acquired an unanticipated relevance owing to their impact on the global Earth's homeostasis. They include the unacceptably high atmospheric levels of greenhouse gases, the worrying pollution of the oceans with very recalcitrant plastics and microplastics and the noxious effects of micropollutants on many ecosystems. Global problems ask for global solutions and the environmental microbiome—because of its dimension and its amazing activities—may end up being our best instrument to both counter the impact of industrial development and enable a new, sustainable partnership with Nature.

Originality-Significance Statement: Same as Abstract
Introduction: When Molecular Microbiology reached out beyond *E. coli*¹

There was a time when the only microbes worth to tackle for molecular biologists were *Escherichia coli* K12, its phages and its plasmids. *Bacillus* was still a curiosity, yeasts merely a source of enzymes, and the rest of the microbial world mostly names of the Bergey's manual, interesting only to clinicians and naturalists. *E. coli* K12 and phage lambda remained for many years as the top stars of the list of experimental systems for addressing key biological questions. During that time a large number of scientists were convinced that *E. coli* K12 held all the secrets of the biological universe (*What is true for *E. coli* is true for an elephant*, Monod dixit) and thus other microbes and other experimental organisms could basically be ignored. Colony morphology on Petri dishes was proposed as a genetically determined developmental program, and the lysis vs. lysogeny cycle of lambda as the one paradigm of complex biological circuitries. Why did we need more? But this *E. coli* protagonism came to an end by the mid-1980s. Novel experimental systems emerged rapidly as molecular tools became available for examining not only other microbes (*Bacillus, Pseudomonas, Streptomyces*, several yeasts) but also higher organisms (mouse, *C. elegans*, zebrafish, *Arabidopsis* ...), where a wealth of biological phenomena could be properly addressed. In a few years, numerous microbiologists felt that their work was losing ground and not a small number of them migrated to what appeared to be more complex systems. Those remaining in the field of microbiology often felt that other Molecular Biologists despised their work as too undemanding and not of sufficient general interest. Many succumbed to the fear and chagrin of not being sufficiently recognized because of working with prokaryotes.

Fortunately, also during the mid-1980s the whole realm of Microbiology started to bring Molecular Biology tools and tactics to species other than *E. coli*. Such approaches started to propagate well beyond the traditional realm of fundamental bacterial genetics and its cognate phage/plasmid biology to reach out new questions and real-world challenges. Many of these developments coincided with a series of local environmental calamities (the Exxon Valdez spill, the Bhopal disaster, the consequences of Vietnam's Agent Orange) that highlighted the aptitude of environmental bacteria to execute extraordinary chemical reactions and heralded its potential as instruments for *in situ* bioremediation—whether as such or enhanced through genetic

¹ An extended account of the transition from an *E. coli*-centered microbial genetics to the growing focus on environmental bacteria can be found in [https://goo.gl/vYmu9U](https://goo.gl/vYmu9U)
engineering. Also, this time framed the work of Stanley Falkow, the father of molecular microbial pathogenesis. His pioneering research on the genetic and molecular basis of bacterial infections through genetically tractable virulence factors (Finlay and Falkow, 1989) opened a new field that has since reached out a large number of microbial agents, including plant pathogens. Finally, the 1980s started to grasp the immense diversity of the microbial world, both the culturable and the non-culturable, which could be increasingly explored through the by then emerging techniques of PCR and DNA sequencing. During the period mid-1980s to end-1990s, molecular studies on biodegradation, pathogenesis and diversity enjoyed a considerable attention that brought Microbiology back to the core agenda of frontline scientific research. Microorganisms left behind their status as mere model systems, proxies of something else and became a genuine source of pivotal biological questions that belonged to the core of modern Life Science research. This scenario set the stage for the birth of a new journal and explains its success from its very foundation.

20 years of Environmental Microbiology

Besides better understanding of bacterial virulence, the same period of time (mid 1980s-end 1990s) witnessed amazing discoveries in the environmental front along with the identification of their molecular determinants. Besides identification of numerous pathways for catabolism of xenobiotics (and other recalcitrant compounds) and the intricacies of their regulation, this epoch framed findings as consequential as the process known anammox or anaerobic oxidation of ammonium (Kuenen, 2008). The cognate biochemical reactions are run by bacteria that combine ammonium and nitrite or nitrate to produce nitrogen gas, thereby contributing very significantly to the nitrogen cycle at a global scale. The process has been later domesticated for a large number of very successful wastewater treatment strategies (van Kessel et al., 2018). Another remarkable breakthrough of the time was the finding of rhodopsines in marine bacteria (Beja et al., 2000). These light-sensitive receptor proteins bind retinal and mediate phototransduction in eukaryotes. In the prokaryotic world, rhodopsines were formerly believed to be present only in some halophilic archaea and function as light-triggered proton pumps (Pinhassi et al., 2016). That they were frequent also in marine bacteria exposed a new and entirely unexpected mode of light-driven energy generation in surface waters. The decade also witnessed the recognition of biofilms as
the favourite lifestyle of most environmental microorganisms. The field, pioneered by Bill Costerton (Flemming et al., 2016) had a major boost with the publication in 1998 of the now classical paper by O'Toole and Kolter with their simple method to inspect the molecular basis of biofilm formation (O'Toole and Kolter, 1998). The subject is to this day one of the most transversal topics of research through the whole microbial field, as it has connections to subjects as diverse as bacterial pathogenesis, industrial fermentations, biocatalysis and waste treatment schemes.

As the 1990 decade advanced and DNA sequencing became easier and more affordable, a brand new opportunity to penetrate the invisible, non-culturable world opened to environmental microbiologists: the access to metagenomes (Rondon et al., 2000). For the first time we were able not only to enumerate the members of a community that could or could not be cultivated individually in the Laboratory but even reconstruct their complete genetic complement without having ever had the biological object proper in our hands. Later developments of meta-transcriptomics and meta-proteomics have largely converted microbial ecology into an information-processing endeavour—leaving the romanticism of sampling expeditions to exotic places as a marginal activity. The metagenomes of a Nespresso® coffe machine (Vilanova et al., 2015), a kitchen sponge or a chopping board can be as fascinating as those of a volcano or a deep sea vent. The biological data on strains and communities that was earlier extracted after painful experimentation, generation of mutants and chemical analyses of metabolites could now be generated through the mere inspection of the genomes (i.e. the DNA) of the samples at stake.

When the publication that is now celebrating its 20th anniversary was founded in 1998 Environmental Microbiology was leaving behind an earlier excitement on genetically engineered (GE) microorganisms as agents for bioremediation. Although the green hype of the mid 1980s helped enormously to bring attention to the possibility of tailoring GE bacteria for environmental cleanup and on microbial ecology as a whole, reality is that the level of knowledge was (still is) insufficient to make it work under real field conditions (Cases and de Lorenzo, 2005). Instead, the ground started to move towards new questions that were largely ignored (if not despised) by the high-profile publishers of the time. There was then a clear need to have a high-quality community journal to be the home of the best research on the interplay of microorganisms among
themselves and their environment. Such questions have been later shown to be not just the matter of scientific curiosity but also a source of transcendental body of information for the future of our planet.

It would be difficult to summarize in this short commentary the awesome quantity of knowledge that Environmental Microbiology has hosted and delivered over these years. New questions, new technologies and a large number of fresh biological systems at every scale (from specific genes and proteins to large, global scenarios) have found in the authors and the readership of Env Microbiol the right audience. Once in a while, the field even has gone beyond the pure microbiological realm and has become a source of model systems to study intricate sociological questions on collaboration vs. competition, cheating, division of labour and other matters difficult to experiment with human subjects (McDonald et al., 2017; Mavridou et al., 2018). That the journal has been at the top of the ranking of primary microbiology publications for a quite some time—way ahead of other well established counterparts—indicates not only the wisdom of its sustained editorial policy and the efficacy of its Editors and Editorial Board at large. Also, that the field keeps on gaining momentum in the contemporary landscape of Life Sciences research. This is not alien to the onset of a fresh upsurge of global environmental awareness in more recent years. Unlike the first wave of the 1980s, this time the driver is not the concern about local spills of toxic chemicals, bad as they can be. It is about the fate of our planet as a whole: the realization that only the environmental microbiome has the capacity to mitigate (and even revert) the damage that industrial development, land use and human overpopulation has done to Earth. We had better understand what could ultimately be our best, perhaps only, ally to hand over the planet in good shape to future generations!

What is going on and what may come next

As Niels Bohr\(^2\) put it ‘... *It's hard to make predictions, especially about the future* ...’: But it is tempting to speculate on what will be the research attractors around which Environmental Microbiology (both the field and the journal) will rotate in the next decades. Some of the ongoing topics are likely to still enjoy a long lifespan. Environmental metagenomics will tackle virtually

\(^2\) In fact, the origin of the quote often attributed to Bohr is disputed (Arthur K. Ellis, Piet Hein, Robert Storm Petersen and many others) and probably reflects an old Danish proverb on how risky it is to make predictions.
every niche that microorganisms may inhabit, from remote geographical locations to domestic hotspots. This will generate more and more ORFs with unknown functions and thousands—if not millions—of new OTUs. The avalanche of viral sequences added in recent times and the realization of the awesome functional scope of the environmental virome (Paez-Espino et al., 2016; Paez-Espino et al., 2017) will do nothing but also increase in the next few years. This will likely swamp and collapse databases much more than they are now. To counter this, new computational and experimental methods for predicting reliably the functions of the thereby discovered genes need to be badly developed. The challenge goes much beyond the capabilities of human managers and will ask for adopting big data handling strategies and artificial intelligence (AI), in particular for exploring the unknown unknowns of genomes and metagenomes. Hopefully the resulting wealth of information will help us to have a fair functional view of the global microbiome of Earth that accounts for a very large share of its total biomass (Kallmeyer et al., 2012) and how much insult it can take from us on the basis of its merged catalytic capabilities. As early as 2003, systems biology approaches suggested that the combination of catabolic routes of environmental bacteria could form a super-metabolic network much more powerful than the sum of the biochemical complements of individual bacteria (Pazos et al., 2003; Gomez et al., 2007). This is something really worth to inspect, quantify and whenever possible domesticate in our favour. One first feature of the Env Microbiol to come will thus be the leading role of computation and modelling to make sense of the flood of data that will keep on growing. The growing availability of personal, small-size DNA sequencers will do nothing but exacerbate this trend.

The interest on the metagenome, which originally started in the environmental field, has since expanded very quickly into the human realm. Research on the gut microbiome is one of the most active and fascinating endeavours of our time. It seems that every day new discoveries shake the former views on our interplay with our bacterial guests—or are they in reality our hosts? Mounting evidence indicates that the human microbiome controls a large number of key vital functions and in fact it seems to behave as the third brain of the body, in close interaction with the other two: the nervous system (Cryan and O’mahony, 2011; Cho and Blaser, 2012; Huttenhower et al., 2012) and the immune system (Round and Mazmanian, 2009). Despite the large number of publications on the matter, we are still scratching the surface and the field is bound to boom in
the next few years. Understanding, manipulating and even programming the association with our microorganisms may altogether transform medicine and healthcare (Ruder et al., 2011; Sheth et al., 2016).

But at the end, the microbiome is a bacterial community that is necessarily ruled by the same adaptive principles that operate in any other environmental niche. Furthermore, virtually all members of the microbiome have an environmental origin and life cycle outside the animal body. There is thus a continuum between the environmental and the human microbiomes, a sort of Ariadne's thread that connects the two types of microbial communities and somehow nail us up further to mother Earth. Under this view, studies on the human microbiome can gain momentum from adopting microbial ecology principles and methods. But also environmental studies can adopt and scale-up medical concepts such as dysbiosis (i.e. breakdown of homeostasis of the gut microbiome (Myers, 2004) to comprehend ecological crises and the collapse of natural cycles run by microorganisms. In sum, investigating the wealth of environmental microbiomes—including the viromes—their composition and functions in diverse habitats and scales could be a second pillar of the research agenda of the field for the next few years. Specific questions include the interplay between the environmental and the human microbiome as well as the emergence and flow of antibiotic resistance genes and other virulence determinants between the environmental and the human/animal niches. These connections are still very poorly understood and they are likely at the basis of unexpected infection outbreaks in humans, animals and plants. Systems and Synthetic Biology tools to reprogram the microbiome for medical and therapeutic purposes thrive at the time of writing this article. Could we not think on extending the same to the much larger Earth's microbiome for the sake of fixing pollution and other large-scale environmental dysbiosis problems as well?

As a matter of fact (see above), one of the founding motivations of Env Microbiol is the study of the impact, microbial catabolism and eventual remediation of chemical pollution. Unlike 20 years ago, better industrial procedures, growing environmental awareness, new prevention/cleanup technologies and implementation of legal measures make the pollution cases that made headlines before to be less and less frequent (Dvorak et al., 2017). However, much bigger challenges have emerged in the meantime. Paramount among them is the rise of the
atmospheric levels of greenhouse gases, specifically CO2. This has accompanied by the continuous discharge of plastics to aquatic ecosystems (Law, 2017) and the realization of noxious effects of micropollutants i.e. bioactive molecules that are typically found at low levels but still enough to have a devastating impact in the corresponding ecosystems (Luo et al., 2014). CO2, plastics and micropollutants, which were basically ignored not far back, now threaten not just specific sites but also the functioning of the planet’s homeostasis at a global scale. The environmental fate of new xenobiotic compounds, such as ionic liquids (Jordan and Gathergood, 2015) remain a question mark as well. Studies on biodegradation and development of new bioremediation technologies will thus have to go much beyond the earlier focus on specific catabolic pathways (which be required in any case), include in the agenda new strategies for efficient CO2 capture (whether photosynthetic or not) and take into account the multi-scale complexity involved in possible interventions. This mission intersects with studies on the environmental microbiome discussed above and the possibilities of engineering it with the tools of Systems and Synthetic Biology. The focus on new ways of removing CO2, degrading plastics and eliminating micropollutants (and new xenobiotics) could then shape the third pillar of the new Env Microbiol that we will see developing in the upcoming decades. But every table still needs a fourth leg and below comes a possible vision for it.

Environmental microbiology to the rescue of planet Earth

Before the industrial revolution, the surface of planet Earth evolved over billions of years as a balanced bio-geological system ultimately sustained by sunpower and a large-scale cycling of elements largely run by the global environmental microbiome. Yet, during the last century human action has impacted such balances to an unprecedented degree. The problem has nothing but exacerbated in the last 20 years, with chemical emissions like those mentioned above being largely the culprits of the state of affairs. Drastic changes in the use of territory, with growing deforestation and expansion of arid, desertic lands accompany and aggravate the pollution problem as well. The traditional way of looking at these troubles is to remediate the impact of industrial activities to the best we can and get away with it. Alas, this mere mitigation mentality may just achieve the relief or at least containment of the undesirable consequences of our lifestyle, but not really addressing their causes. A different approach is needed. The question is
whether we can keep and an even expand the well being of advanced societies (that is linked to industrial development) while making the corresponding processes cost-effective and environmentally welcoming.

In reality, industry can be considered an artificial metabolic system in which generally abiotic feedstocks are converted into products. But unlike the biological metabolism or the geochemical cycles of the planet, transformations occur mostly in just one direction with little or no recycling that makes natural metabolism a sustainable process (de Lorenzo et al., 2016). Furthermore, the industrial metabolism badly collides with the global biological metabolism at least in four friction points: [i] emissions of CO2 and other greenhouse gases, [ii] spreading of non-degradable polymers and xenobiotics, [iii] demand of nitrogen and phosphorus for intensive agriculture and [iv] surplus of lignocellulosic waste (de Lorenzo, 2017). In my view, there is no mission as important as reverting human impact on the Biosphere, with a focus on relapsing climate change. But not by just mitigating the environmental impact of development but by creating a new, beneficial connectivity between industrial metabolism and the biogeochemical cycles of the Biosphere (Fig. 1).

Many of such reactions or processes needed to re-establish a sustainable element cycling have been identified. Alas, some of them (e.g. CO2 capture) occur naturally with low efficiency, certainly much lower than required to have a real impact. Others (e.g. degradation of polyethene or biological mobilization of abiotic chemical elements: Si, B) do not occur at any significant degree. And others (e.g. fixation of N2 in the presence of O2, or recovery of diluted phosphate) may not happen biologically at all. We thus need to expand (micro) biological metabolism towards elements and compounds produced by industry with chemical bonds that natural, biochemistry cannot thus far handle—not only plastics. The work of David Baker (Huang et al., 2016), Frances Arnold (Arnold, 2017) and Tom Ward (Rebelein and Ward, 2018) to invent new biochemistries are steps in the right direction, but much more is needed. And then such transmetabolic reactions need to be scaled up and delivered to an unprecedented environmental level for amicably connecting global industry to global biology. In sum: the issue is not about only remediating or containing the wrongdoings of existing industry, but to work out a new partnership with Nature that only the environmental microbiome can bring about. One can just grasp the
amount of fundamental science, technology and innovation that such biologisation of industry could unleash.

Conclusion

It is difficult to track the birth of Environmental Microbiology as a distinct discipline in a specific date. For a long time it was seen as a sort of lesser brother of the real stars of the microbiological show: E. coli as a model system for in Molecular Genetics, the suite of pathogens that caused disease in humans, animals and plants, and the microbes that produced antibiotics (e.g. Streptomyces), fermented edibles (e.g. Lactobacteria) or bulk chemicals (e.g. Clostridium). But things have evolved along with the lifetime of the homonymous journal and now the field is one of the most thriving domains of research in our time. Unlike other areas of Microbiology where the emphasis lays on the individual isolate, the environmental counterparts focus mostly on community structure and functions, so that IDs of individuals becomes less important. This asks necessarily for technologies that enable the management of multi-scale complexity, as well as conceptual frames that depart from traditional Koch's postulates on causality by single, isolated bacteria. That systems biology has found in Environmental Microbiology one of its most fertile applications cannot come as a surprise. Interfacing Env Microbiol with Synthetic Biology and scale up interventions could then be the next breakthrough in the way of fulfilling the promise that motivates the entire field: to know more about the microbes that run the Earth in order to preserve the planet as an habitable place. And I have no doubt that this journal will keep on being a key instrument to this end.

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References


Fig. 1. Reconnecting the industrial cycle to the natural biological and geochemical cycles of the Biosphere.

(A) Natural geochemical and biological element cycling. Both overlap in a balanced fashion to maintain the homeostasis of carbon, nitrogen and phosphorus at a global scale. (B) Industrial metabolism perturbs natural cycling of elements. Chemical industry, manufacturing, urban activity and land use generates products and residues that cannot be naturally recycled, thus colliding at distinct sites with the natural rounds of element. (C) Large scale bioremediation interventions for restoring balance. Systems-guided strategies somewhat reminiscent Terraforming could be designed for uncoupling growth from non-sustainable use of natural resources and recoupling rationally the connections to have a new scenario with the 3 cycles duly connected.