

The coming of age of microbial ecology

by

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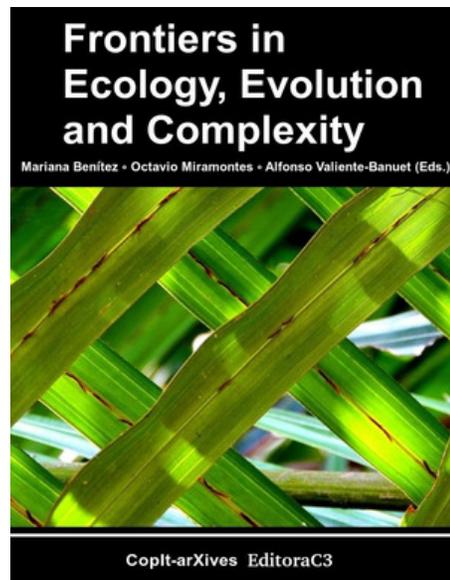
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1 Abstract

Research in microbial ecology is challenging due to its inherent complexity and, at the same time, is extremely important, because the relationships between microorganisms and their environments have a crucial role in the health of the planet and all of its inhabitants. Despite major challenges, new technological advances in genomic sciences have prompted microbial ecology into a revolution in data generation that has allowed us to move beyond studies of single isolates to the study of entire microbial communities without reliance on culture-dependent methods. Besides the data generation using these molecular tools, the adequate use of a theoretical framework is needed to test specific hypothesis and develop mechanistic models of microbial diversity. The development of a theory-based and hypothesis-driven research, along with a complex systems analytical approach, will result in appropriate models required to predict and possibly control the effect of environmental impacts on microbial diversity.

2 Resumen

La investigación en ecología microbiana es un gran reto debido a la complejidad inherente de su estudio y es, al mismo tiempo, extremadamente importante porque la relación entre los microorganismos y sus ambientes tiene un papel crucial en la salud del planeta y de todos sus habitantes. A pesar de los grandes retos, nuevos avances tecnológicos en las ciencias genómicas han impulsado a la ecología microbiana hacia una revolución en cuanto a la generación de datos, que han permitido movernos de estudios de cepas únicas, aisladas en cultivo, hacia el estudio de comunidades microbianas completas y sin necesidad de su aislamiento en cultivo. Además de la generación de datos con herramientas moleculares, es necesario el uso adecuado de un marco teórico que permita probar hipótesis específicas y desarrollar modelos mecanísticos sobre la diversidad mi-

robiana. El desarrollo de investigaciones enmarcadas en la teoría y que pongan a prueba hipótesis, junto con aproximaciones analíticas de sistemas complejos, resultará en modelos apropiados necesarios para predecir y posiblemente controlar el efecto de los impactos ambientales en la diversidad microbiana.

3 Introduction

Microorganisms play a primary role in a vast array of ecosystems, from soils to hydrothermal vents to the human body, in which microbes drive key processes, as the heart of ecosystem function and the keystone of global health. Despite the clear ecological importance of microbes, understanding of underlying mechanisms of microbial behavior, community assembly and functional consequences of diversity shifts is still limited.

The emergence of microbial ecology as an independent field of investigation in the 1960s was promoted by both the increasing public interest in environmental issues and the recognition of the essential role of microbes in the biosphere. Microbial ecology links those areas in which microbiologists are well trained (biochemistry, microbiology, molecular biology) with ecology or the study of the interactions between living organisms and their biotic and abiotic environment. Partly due to this dichotomy of disciplinary training (microbiologists vs ecologists), the field of microbial ecology has suffered a delay in its development as a scientific discipline, but other factors have contributed as well. In this essay we will review three main avenues that have delayed the advance of microbial ecology as a mechanistic and predictive discipline: (i) practical impediments related to observation and isolation of microorganisms from natural environments, (ii) problematic definition of species, and (iii) limited use of a theoretical framework. Nonetheless, in the recent years these challenges have been overcome or at least addressed, allowing microbial ecology to enter a new phase in which, beyond conducting important natural history descriptions, clear and well-defined hypothesis are now being posed and investigated.

4 Overcoming difficulties in the study of microbial communities

It has been estimated that the Earth hosts $> 10^{30}$ microbial cells [1], a figure that exceeds the number of stars in the universe by nine orders of magnitude [2]. Besides their great abundance, microorganisms are also immensely diverse and constitute about 60% of the Earth's biomass [1]. But this diversity is literally unseen under the naked eye, and even if seen, the morphological diversity of microorganisms is unrepresentative of their phylogenetic, metabolic and functional diversity.

As a consequence of these limitations, and until not so long ago, the only way to study and characterize microorganisms and their functions was through culture and isolation in selective or enrichment media in the laboratory. However, this approach introduces

important biases, as well as recovers a very small fraction of the naturally occurring microorganisms in any given environment, and loses the ecological context of the organisms under study [3]. Thus, the pure culture paradigm has not only limited what microbiologists have studied, it has also limited how they have thought about microbes.

In recent years, breakthrough advances in molecular biology and bioinformatics have permitted access to the uncultured microbial diversity of any given environment through the development of culture-independent methods (Figure

The analysis of microbial communities through genomic approaches, either with targeted genes (e.g. 16S rRNA as taxonomic standard) or by sequencing all the genomic content of a sample, has allowed a gene-based exploration of complex evolutionary processes and ecological interactions in microbial communities [11–13]. Furthermore, these technologies have revolutionized our understanding of the microbial diversity in our bodies [14, 15] and on our planet [16–18]. Specifically, culture independent approaches have permitted to unveil the existence of biogeographical patterns in microbes [19–21], which was a matter of great discussion during a good part of the last decade. The success of these first discoveries of the spatial distribution of microbial diversity prompted, in part, the evolution of culture independent approaches from giving data of only gene sequences to providing with both information on the taxonomic composition (metagenomes) as well as description of the metabolic routes (metabolomes) and functional capabilities (transcriptomes and proteomes) of a given community without losing the ecological context. Moreover, these new technical advances are opening the door to sophisticated studies for testing the functional responses of microbial communities to environmental stresses [22].

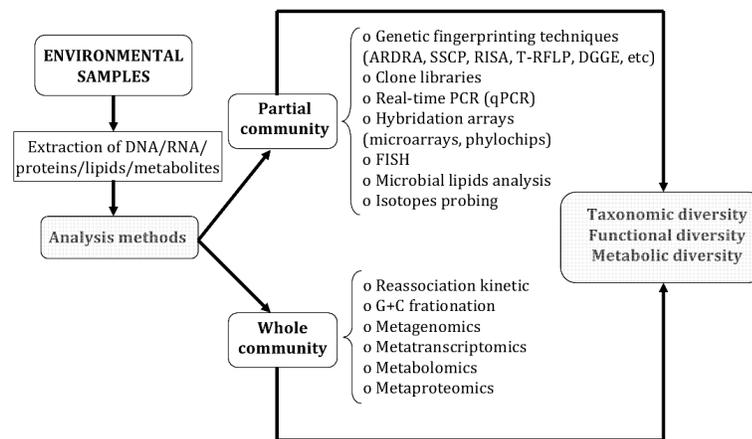


Figure 1: Culture-independent molecular methods to characterize microbial community diversity in the environment.

Nonetheless, the promise of genomic studies as a way to build a mechanistic knowledge that links microbial diversity with ecosystem function is challenging, given the ex-

tent of microbial diversity and the uncontrolled character of natural environments that complicate the ecological interpretation of genomic data [23]. A way to manage this challenge is to follow the example of classical ecological studies, where systematic experiments have been pursued under natural or laboratory controlled conditions, aimed to test specific hypotheses and simplify interpretations [24]. Despite the fact that examples of experimental approaches with microbes are still few, they are promising [25, 26], as microbial ecologists are aware of the need for a robust experimental design (e.g. replication, ecosystem characterization, controlled manipulations) across temporal and spatial scales [2]. Moreover, given the great metabolic plasticity, functional redundancy and adaptive potential of microorganisms [12, 22, 26], integrative multidisciplinary approaches including genomics, ecological and evolutionary theory will help develop stronger conclusions and better predictions in microbial ecology.

5 Problematic definition of species

Species is the fundamental unit of biological classification and is critical for describing, understanding and comparing biological diversities at different levels among ecological niches. However, what constitute a species is still controversial, especially in microbiology. This is because most ecological theory heavily relies on Mayr's biological concept of species [27] or groups with barriers to recombination. It is thus clear that microorganisms, particularly prokaryotes, with asexual reproduction do not fit to the biological species definition and make conceptually difficult the analysis of "species" diversity within the ecological theory.

Discussion around this conundrum has provided some insightful ideas and concepts that try to reconcile microorganisms with the species concept. One of these ideas is the alternative ecological species concept, which defines species as populations that are genetically cohesive and ecologically distinct. Frederick Cohan [28] has argued that this concept is appropriate for bacteria and has named bacterial ecological species as "ecotypes". The postulates of this proposal include that bacteria occupy discrete niches and that periodic selection purges genetic variation within each niche without preventing divergence between members of the different niches. Thus, genetically and ecologically distinct species can arise (given little recombination or clonality), permitting the application of the ecological theory of populations, communities or macroecology to prokaryotes.

Nonetheless, lack of recombination is an important assumption of prokaryotes that might not be absolute, given the overwhelming evidence of horizontal gene transfer in all prokaryotic phyla studied to date. A consequence of gene transfer is that prokaryotic genomes are thought to be the sum of the core genome and the accessory genome, called pan-genome [29]¹. The core genome includes all genes that encode essential metabolic housekeeping functions and can be regarded as the biological species, which maintains

¹see also Alcaraz's Chapter in this book.

coherence of species in the face of homologous recombination. The accessory genome encodes especial ecological adaptations in genes that can be readily interchanged. Microbes that belong to the same species, as defined by the core genome, can differ substantially in the accessory genome, and thus have different ecological capabilities or be different ecotypes, which makes it incongruent with the ecological species concept.

Thus, when reconciling species concepts, we face a pragmatic problem: how do we define the unit of study in microbial ecology? The current solution has opted for looking at genes of the core genome (e.g. 16S rRNA gene) that are indicators of what we understand as a coherent evolutionary unit that is robust in the face of homologous recombination. These evolutionary units, called Operational Taxonomic Units (OTUs), have been a practical solution that, however, is far from being a solution to the conceptual problem. Consequently, much research is required to form a coherent body of theory that relates the fluid nature of prokaryotic genomes to the ecology of their populations and communities [30].

6 Use of a theoretical framework

Without a theoretical framework, knowledge can be merely seen as a collection of observations and anecdotes with very little potential for generalizations and little predictive power. Theory helps understanding through the construction of a mechanistic knowledge of the world. If we do not understand how something works, it is impossible to predict its behavior or intervene to recover from perturbations.

The comprehension of the principles that govern the communities' dynamics is a central goal of the general science of ecology [30]. Ecology has its origins in natural history, and early publications tended to be very descriptive and site-specific [31]. Although many questions remain unanswered, modern ecology has progressed enormously from an almost anecdotal discipline to a very sophisticated model-based and hypothesis-driven science [31]. However, this theory has been developed mainly from plant and animal studies. On the other hand, traditionally, microorganisms have been considered "different" from macroscopic organisms, which implies that different rules, even different theories apply to them, thus limiting microbiology to separate and disconnected laboratory and ecology studies. Some of the arguments in favor of considering microbes different are their huge abundance, total biomass and diversity, their high rate of dispersal and global activities [30]. Moreover, the particular biology of microorganisms regarding sex and genetic isolation has limited the incorporation of a theoretical framework in microbial ecology research. These scenarios pose the challenge of investigating the applicability of ecological theory developed from macroorganisms to microbes.

In the course of the development of ecological theory, microbial model systems have played an important role in the evolution of more rigorous theory, which is also relevant for all types of organisms [25]. Despite the intrinsic value of bringing theory into the study of microbial ecology and the great potential of theoretical enrichment through controlled

experiments using microbial systems, there has been a delay in the development of theory in microbial ecology. One of the most important reasons for this is strongly related with the challenges described above (technical difficulties in studying microbes from natural environments and conflicting species concepts).

In recent years, however, microbial ecologists have begun applying macro-ecological theory into experimental design of hypothesis-driven studies, providing a reference point for the development of new theories. Specifically, works conducted at the community scale by Martiny and collaborators [22, 32] and by Fierer and collaborators [26, 33] are paradigmatic in the use and construction of ecological theory with microbial systems. Particularly, questions regarding the functional role of such vast microbial diversity and the drivers of dispersion and spatial distribution of microorganisms have guided their most recent investigations [7, 16, 34]. In line with these works, Bohannan and collaborators have also applied ecological theory in the study of spatial patterns in microbial diversity [35] and ecological dynamics of human microbiome [36]. These studies have provided important glimpses into the underlying mechanisms that structure ecological communities.

7 The coming of age, towards a predictive science

The development of bioinformatics and molecular genomics tools has replaced methodological barriers with conceptual barriers in the progress of microbial ecology. To date, microbial ecologists recognize a lack of unifying theories and concepts that can serve analysing and interpreting the vast amount of information and scattered results derived from metagenomic approaches [34, 37, 38]. Thus, despite many fundamental questions that remain to be answered (see Box 1), current avenues of research in microbial ecology are aimed in the direction to fill this conceptual gap.

The revolution in sequencing and advanced computational tools offers affordable access to functional and taxonomical inventories of microbial communities in any given space and time. On the other hand, despite unresolved debates on microbial species definition, practicality has prevailed by using 16S rDNA sequences as a proxy for microbial taxonomic diversity [17, 19]. However, recent studies in microbial ecology have been conducted to go beyond taxonomic or functional descriptions, using massive inventory information (i.e. metagenomics, metatranscriptomics, metabolomics) within a complex ecological and evolutionary framework to unveil mechanistic aspects of the microbial world through predictive models [11, 39].

Box 1. Challenges for microbial ecologists.

- The large fraction of microbial life that remains uncultivated.
- Accurate modification of natural microbial communities for study in the laboratory.

- Predicting the effects of disturbance in natural microbial communities.
- Unravelling the functions of unknown microbial genes.
- The importance of microbial community assembly in ecosystems.
- Spatial scale of microbial community functions and interactions.
- Co-evolution in interacting microbial species.
- Study of unexplored genetic and physiological diversity of the rare biosphere.
- Testing ecological theory in microbial model systems.
- Applying the principles of population biology and evolution to the study of microbial communities.

The transition towards a predictive science is not of minor importance, as it must consider the impact that microbial community modelling can have in almost every biological realm, from disease to ecosystem management. Examples of current avenues of research that are filling the technological-conceptual gap in microbial ecology come from studies of human and soil microbiomes [14, 40]. In both, we find notable efforts to develop general theories and models that aim to understand the relationship between community structure and ecosystem function in order to predict microbiome behaviour and its consequences [13, 34, 41].

Microbial ecologists are also conducting research around a key debate regarding the relevance of microbial taxonomic composition in the functioning of ecosystems. In other words, is it important to know who is there to predict what they will do? [22, 38]. To address this question, different approaches are being followed, from field samplings to laboratory experiments, most using new technologies available for sequencing, but more importantly with a theoretical ground for the study of microbial diversity patterns (see “use of a theoretical framework”). However, the full potential of massive sequencing technologies in the study of ecosystem functions may come also from other study approaches. Ecosystem functions (e.g. mineralization rates in soils) or system properties, such as resilience and robustness, may not be information readily accessible from inventory or multivariate analyses. Thus other approaches, such as network analyses, promise to advance our understanding of microbial systems properties and dynamics.

Recently, microbial ecology studies have started to incorporate analytical tools derived from complexity theory, among which network analysis coupled with dynamic modelling offer great promise in the search for a more predictive science [13, 34, 39, 42]. The reason, we believe, is because the relationship between microbial diversity and ecosystem functioning may lie greatly in the complexity of interactions that exists within any microbial consortia or community [43], as well as with abiotic parameters. The consequences of these interactions are nearly impossible to account for with standard multivariate analyses. Network theory approaches coupled with dynamic modeling of the interactions may aid in both experimental design, to test specific hypothesis, and visualizing consequences in scenarios where the structure of the network is altered.

Given current scenarios of environmental change and the major influence that microorganisms play in biogeochemical cycles, it is not only scientifically important to transcend a descriptive discipline towards a theory-based predictive science. Understanding and predicting microbial diversity responses facing environmental challenges is of major relevance because of the ecosystem consequences that microbial communities perturbations may have. Today, challenges are in the theoretical and analytical approaches to be taken and in the collective efforts of the scientific community to gather information and make it publicly available for its analysis. The massive data collection and analysis within a solid and systems-based theoretical framework in the study of microbial diversity will allow us to develop predictive models for improved strategies of study and concrete actions in ecosystem restoration and management, as well as in public health policies.

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