American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Microbial Ecology in the Age of Omics: Exploring Microbe-Environment Interactions

Muhammad Khudair Obyes
University of Babylon - College of Science Department of Microbiology

Heba Ahmed Juma
University of Wasit / College of Science/ Department of biology

Tebai Abbas Hassan
University ofAl-Qasim Green College of science Department of biology

Munir Falah Hassan
University of Babylon - College of Science Department of Microbiology

Abdul Khaleq Muhammad Zuwaid
University of Babylon - College of Science Department of Microbiology

Received: 2024, 29, Jan
Accepted: 2025, 29, Feb
Published: 2025, 31, mar

Abstract: Microbial ecology has undergone a paradigm shift with the emergence of omics technologies, offering unprecedented insights into microbe-environment interactions. Despite major advancements, challenges remain in integrating multi-omics data to fully understand microbial community structure, function, and dynamics within complex ecosystems. This review highlights recent progress in genomics, metagenomics, transcriptomics, proteomics, and metabolomics, and their combined application to study microbial communities. Findings show that microorganisms are essential drivers of biogeochemical cycles, host-microbe interactions, and ecosystem resilience, with omics-based methods uncovering previously unknown microbial diversity and metabolic capabilities. However, the complexity of data analysis,

limited culturing of environmental microbes, and incomplete understanding of microbial functional roles persist as key barriers. The results emphasize the necessity for interdisciplinary approaches, improved bioinformatics tools, and systems biology integration to achieve a predictive understanding of microbial ecosystems and their role in global environmental and health-related processes.

Keywords: microbial ecology, omics technologies, microbe-environment interactions, systems biology, metagenomics, microbial diversity, ecosystem function

1. Introduction to Microbial Ecology

Microorganisms are the main drivers of biogeochemical cycles in Earth ecosystems. They are involved in the breakdown and transformation of carbon, phosphorus, nitrogen, and sulfur for the maintenance of life. Four-fifths of the world biomass consists of microbial cells, which in sum contain the largest fraction of organic carbon stored on Earth. Life on population level can be taken as the result of natural selection and as the adaptation of organisms to their environmental challenges through expression of sets of functionally relevant (adaptive) genes. Because the fitness of organisms from the same or different domains of life is often coupled, a deep understanding of their complementing adaptive strategies requires the context of the interacting partner community, known as eco-system or - in the case of microorganisms - as microbiome. Through selective and adaptive processes, microbial communities are composed of phylotypes that establish complex networks of taxon-taxon interactions. They negotiate the uptake of resources, protection against predators, entry competitors and viruses. Prediction of properties emerging out of the interactions as well as of community succession, assembly and evolution in consortia is a major challenge of TTSystems [1]. Prospectively, through the integration of high throughput observations with mathematical models it will be possible to simulate metaorganisms, engineered to optimize a multitude of biological processes. There has been a paradigm shift in microbiology: from investigating pure cultures under laboratory conditions to applying culture-independent systems for elucidating the ecology of environmental as well as host-associated microbial consortia. Combined data from molecular-biological, observational and experimental techniques will be integrated to become a predictive understanding of the overall process.

2. The Omics Revolution

Since the time Anton van Leeuwenhoek scraped his teeth and visualized the result with an early microscope, it has been understood that microorganisms are everywhere and are incredibly important. Modern studies of microbial ecology adopt a broader perspective, aiming to understand how these simple organisms fit into the larger environment and with each other. There have been a number of exciting breakthroughs in recent years regarding microbial interactions. For example, a

group discovered complementarity in microbial environments, where one set of organisms is responsible for degrading one resource and a different set is responsible for degrading the other. Similarly, there have been advances in understanding why seemingly simple spatial arrangements of microbes can promote surprisingly complex behavior.

While the new understanding of multifaceted microbe-environment interactions has been welcome, it has been harder to study multilevel intermicrobial interactions. The best examples of such interactions are in the context of a group, where they are described as high-dimensional dynamical systems with a classification of up to six different kinds of interactions on average. It is important to understand the full impact of this classification on the predictability of microbial systems. However, because of technological advances, the focus of microbial ecology has been on the "macroscopic" interaction of microbial systems with either simple environments or macroscopic descriptions of the environment. Consequently, when microbial interaction studies appeared they attempted to simplify interaction networks such that only a collection of population growth rates was used to describe the entire system, losing the detailed resolution at which actual microbial systems interacted. Most recently, understanding of the physical interplay between bacteria and their environment has been augmented, particularly in the case of many cellular systems. [2][3][4]

3. Microbial Diversity and Function

Microorganisms represent the vast majority of the Earth's biodiversity and form complex communities in every ecosystem. The current availability of omics technologies – genomics, metagenomics, metatranscriptomics, metaproteomics, and metabolomics – enables comprehensive analyses of microbial consortia. However, the application and integration of omics technologies to study microbial ecosystems, from basic research to biotechnology, are still restricted by various limitations, viz. network complexity, stochastic processes, data treatment and integration. Systems biology has been proposed as a means to understand the function of biological systems through network-based analyses, with its mathematical developments enabling the identification of the most prominent nodes of a network. It is hypothesized that omics technologies, when combined in network-based approaches, can significantly boost the understanding of microbial community functioning and of the functions of unculturable microorganisms in the environment [1].

Culture-independent, high-throughput molecular techniques have revolutionized the field of microbial ecology over the past two decades. Since the advent of 16S rRNA-based community analyses, researchers have gained fundamental insights into the taxa comprising microbial ecosystems. However, interrogations into the physiological or functional roles of distinct microbial populations lags considerably behind [5]. It is paramount to develop techniques that can link taxonomic fingerprints with in situ activities of environmental microorganisms. Achromobacteria have been recognized globally as key players in biogeochemical cycles located in anoxic niches. This has largely been attributed to their ability to lower the thermodynamically required oxygen concentration and thus to enable a favorable growth environment for other anaerobes. Several distinct methanogenic groups that are a part of the Euryarchaeota have been successfully isolated. They are represented in the Orders Methanobacteriales, Methanococcales, and Methanomicrobiales - the latter classified as Methanoculleus and Methanospirillum species. It has been proposed that Methanoperedens species are capable of ANME-kind of methane oxidation via the reduction of nitrate as terminal electron acceptor. This process has been much less investigated compared to AOM, in general and for the key organisms involved. The reduced metabolites that form CH4 and N2 are not known, but have been proposed in silico. [6][7][8]

3.1. Taxonomic Classification

With the advent of new sequencing technologies, the exploration of metagenomes and whole genome short reads has rapidly increased. This resulted in the launch of many new bioinformatics tools and methods to computationally analyze the large volume of generated data. Still, there is no all-purpose solution and analysis tasks demand the judicious selection of suitable programs and

databases, with the specific choice depending on the data at hand and research questions. The most frequently used strategies for metagenomic sequence analysis are taxonomic classification and functional assignment. On the first layer of metagenomic analysis, the taxonomic classification of metagenomic sequences aims at identifying all ribosomal and protein-coding markers in the metagenome projects' sequences. The significant problem with traditional BLAST- and HMM-based classifications of environmental sequences is that most of them have no reliable hits against protein databases. This is why a great number of strategies aim at functional classification based on the identification of other informative sequences [9]. In metaproteomics, metatranscriptomics, and single cell genomics, sequences from environmental organisms are not analysed, but peptides, mRNA or metagenomes of single cells or whole communities. The functional assignment of those sequences usually is not difficult, since most of the proteins in public databases contain at least some conserved domains for which the recognition is straightforward. In parallel to the analysis of environmental samples, interest to explore the karyotype, transcriptome or proteome of a single cell prevails in the scientific community. Further, for metagenomic and single cell research projects a specific problem arises: So far, less than 1% of all prokaryotes could be cultured. Consequently, the basic steps in bioinformatic analysis of whole genome short reads coming from single cells are the taxonomic classification and the comparative analysis of those reads. In a natural community distribution of an unknown amount of novel organisms is considered therefore the choice of a metagenomic classifier, databases and criteria for threshold setting is also challenging. The primary goal of the taxonomic classification of environmental sequences is to distinguish between the "new lineages" and the "known organisms." Several bioinformatic methods are used to classify the environmental sequences: BLAST, phylogeny-based neighbor joining and Bayesian methods of RDP Classifier tool, and comparison of tetranucleotide frequencies [10].

3.2. Functional Genomics

Genome-enabled metagenomics has well outpaced culture-based approaches in providing examples for microbial communities in which the speed of biologically relevant data acquisition. The insights into the function of the participants, the organisms that dominate activities in the community and drive community assembly, would be more realistically attained by an iterative application of other omics approaches like metatranscriptomics, (meta)proteomics and metabolomics [1].

Metatranscriptomics reveal subsets of expressed genes that may paint a more accurate depiction of ongoing processes than gene content alone. Despite the challenge of mRNA sampling compared to DNA, a series of success stories illustrate the power of this approach in: finding candidate nitrate transporters in freshwater Deltaproteobacteria-auxotrophic for nitrate based on checks of the expression of experimental Rs transcripts; closing the nitrogen cycle in a groundwater bioremediation system by identifying complete denitrification pathway expression in a Pseudomonas-dominated consortium; elucidating the denitrification story of syntrophic organisms in cocultures using DAMO reactors; cataloging active microbial guilds in the poplar rhizosphere and their functional potential to scavenge mannitol, l-arabinose and phenolic compounds.

Metaproteomics offers the promise of characterizing the active molecular machinery of a microbial community. Regardless of challenges from the complexity of the whole community extract, low abundance of interesting proteins, databases not encompassing relevant taxa, and discussions on protein-centric omics unfairly missing out on RNA-sequencing-like novel discovery opportunities, the past five years have yielded some pioneering studies showcasing the biological relevance of metaproteomics. Examples are: the elucidation of acetate oxidation in an anaerobic granular sludge bioreactor using deuterium-labeled substrates and identifying the expressed acetyl-CoA synthetase, a key enzyme that differentiated the dominant Clostridia from other acetate oxidizers. [11][12][13]

4. Microbe-Environment Interactions

In the environment, microorganisms can live in close contact with many different hosts and with each other in communities, usually including many species. Indeed, microbial communities can comprise more than cells/g and represent the highest division of life in terms of genetic and metabolic diversity. The interactions among microorganisms and also between microorganisms and hosts are highly complex, and a wide diversity of molecules plays a key role in this molecular cross-talk. Exploring the interaction at a molecular level has led to the understanding of many ecological processes. Microorganisms are exposed to spatial and temporal variation in environmental conditions and have evolved a wide range of molecular mechanisms to survive in such environments. Thus, environmental heterogeneity is a key factor in the understanding of microbe-environment interactions. A central question is to what extent these environments can be moulded by microorganisms. On the other hand, environmental variation can also affect the interaction among microorganisms and also between microorganisms and the host.

Microorganisms are capable of detecting environmental changes and activating a portfolio of adaptations at the molecular level. One of the insights gained from the application of techniques in microbial ecology was a further understanding of the molecular mechanisms involved in the physiological response of microorganisms exposed to complex environmental variables. The mechanisms underlying the interaction between pathogens and hosts are still far from understood, despite the continuing analysis of the genomes of numerous organisms. Indeed, this represents one of the most challenging issues in the field of host-pathogen interactions. Thus, molecular studies have focused on the identification of the components of microbial pathogens that are important for the infection process. This field has been greatly facilitated by the post-genomics era since access to sequences has allowed the development of high throughput methods and a systems biology approach to aid in the identification and functional analysis of genes. Furthermore, it has explored the new adaptations that have arisen in the course of rapid adaptive evolution of pathogenic microorganisms to escape the immune responses of the host. [14][15][16]

4.1. Biogeochemical Cycles

Since the advent of the large-scale, sequence-based metagenomics, progress in microbial ecology has followed two closely related directions: (1) broad-scale, pattern-oriented ecology and (2) community efforts in describing the ecological and metabolic roles of the individual species or groups of co-occurring species within the community context. Only consistent results complying with the general rules of conservation of matter and energy, as well as the distinction between essential and condition-dependent features, have chance to prove useful in predicting behavior in novel, uncharacterized systems. Present challenges regarding microbial communities are threefold: (1) to further develop the conceptual basis of community systems biology, (2) to develop and exploit adequate technology for community characterization, and (3) to develop and validate cogent approaches to mathematical modeling of microbial communities. Cross-fertilization between progress in these three directions will be needed to make real world microbial ecology clearly beyond the descriptive stage and advance towards a genuine predictive science [1].

4.2. Microbial Symbiosis

Microbial communities are found everywhere on Earth, ranging from top layers of Earth's crust to microbial communities found beneath layers of ice. These microbial communities are not limited to one subsample. Since microbial communities vary, the goal is to analyze two particular microbial communities and seek to understand how the community structure reflects the geochemistry [17].

While not much is known about the ‘palaeosomes’ that were generated during the Paleoproterozoic, they likely represent a primary origin of microbial sulfur communities. These communities may have been established under near-surface conditions if Ferruginous Seawater

was insufficient to quantitatively precipitate Precambrian dissolved sulfide, as has been proposed previously. Existing observations of Recent deposits of macroscopic pyrite and associated sulfides support this notion. During Paleoproterozoic glacial conditions, biotic S-Metabolism communities like Chlorobium sp. proliferated, thereby contributing significantly to the local S-Isotope composition of the deposits. Isotopic mapping of these 'palaeosomes' may allow to follow these original communities into the sediment. Similarly, if the Black Sea picnoclyne is of microbial origins, then mapping of the isotope compositions of these layers in regions with high Pore-Water Venting might also provide insights into the community structure and the S-Metabolism of the contributing organisms.

Binary microbe-microbe interactions have been found to be essential ecological mechanisms in understanding the assembly of complex communities. In bioenvironmental research, binary microbe-microbe interactions are inevitable when microorganisms are used to treat polluted sites through bioremediation processes. For instance, the biodegradation of toxic organic pollutants often involves the catabolic activity of two or more microbial species arranged in a particular sequential metabolic way because of the exudate activity and limited substrate-scope of participating organisms. In this work, a simple model to estimate the population dynamics of an imbalanced growth-mediated binary interaction taking into account cell-cell adhesion and organism-soluble substrate/side-product exchanges from one bacterial species to the other is presented. It was found that a minimal adhesion coefficient should be reached for the mutual benefit relationship to materialize.

5. Metagenomics and Its Applications

Metagenomics, also referred to as environmental genomics, ecogenomic or community genomics, has been defined as function-based or sequence-based cultivation-independent analysis of the collective microbial genomes (the metagenome) present in a given habitat [18]. This rapidly growing research area has soon provided unprecedented access to the hitherto hidden universe of (meta)genes and provided new insights into the unimagined metabolic and physiological capacities of microbial life. Furthermore, metagenomics gave access to novel biomolecules providing a natural source of a rapidly increasing number of unique gene products for biotechnological exploitation. To a certain extent, many of the established and emerging metagenomic technologies can be perceived as complementary with, or aiming at complementing, classical, culture-based techniques in environmental microbiology or as groundbreaking tools to achieve a more complete understanding of the often complex processes underlying microbe–microbe and microbe–environment interactions.

Most of the 15–20% of all estimated prokaryotic cells on Earth occurring in biofilms play a central role in the global cycling of inorganic elements, such as iron, sulfur, and nitrogen. Recent metagenomic databases reveal insights into the functional diversity of microbial consortia in natural and engineered systems currently not possible by any other available tool. Community genomic, metagenomic and biometric data can be integrated for a system biology understanding of naturally occurring biofilms. More than 100 Leptospirillum group II cells could be sorted from an acid mine, their community DNA sampled to provide a low-resolution insight of this part of the biofilm population [19]. Further, DNA-seq captured novel functions such as cobalamin biosynthesis and reconstructed a metabolic blueprint, augmented by proteomic analyses. Modularity analysis using this metabolic information revealed the treble clef as a crucial interface in the deep biofilm.

5.1. Data Acquisition and Processing

Understanding the functioning of complex microbial communities will be one of the main preoccupations of microbial ecologists in the coming years since this comprehension will be essential to predict the responses of metaorganisms to environmental changes. The exponential growth of experimental data obtained by high-throughput techniques should help in this regard. However, while data production is getting easier and faster, data processing and analysis lags

behind. This lag translates the difficulty in extracting relevant biological information from those data. Indeed, the different levels of biological information provided by high-throughput technologies are complex and present specific mathematical, computational and technical challenges. In addition to that, the biology of metaorganisms is complex and ecosystems present by essence emergent properties, which difficult the establishment of links between the different processes studied at various scales of observation (organisms, populations, communities, ecosystems). Another difficulty stems from the integration gap existing between observation scales and experimental protocols. This integration needs the development of sophisticated sampling and measurement techniques to obtain consistent and complementary measurements of the relevant processes. Broadly, the emerging complexity of experiment designs to infer on microbial communities from experimental observations is the main current limitation to ecologists to unravel the fine mechanisms linking structure and function of microbial communities.

5.2. Case Studies in Metagenomics

The large scale sequence analysis of DNA isolated directly from the environment, or shotgun metagenomics, has generated considerable excitement since it has become possible to access the vast majority of the genes present in a sample [19]. The genes captured in this way can be the basis for reconstructions of the metabolic potential of organisms within environments as diverse as soil, seawater and the human large intestine. Indeed the value of such sequence-based predictions has been confirmed by complementary analysis of community transcriptomes. Unfortunately, relatively large changes in environmental conditions or community structure may have subtle effects on such community-level measures of gene expression. If only a relatively small fraction of the metabolically relevant members of a community show altered gene expression, this may be obscured when analyzing total RNA extracted from a mixture of species. A promising future approach is the direct analysis of gene expression by individual cells [1].

6. Transcriptomics in Microbial Ecology

Knowledge about microbial communities and their functions are largely derived from culture-dependent and -independent analyses of the 16S rRNA gene, core genes, functional genes, the metagenome, or the metatranscriptome. Recently, also, focus has been on metabolites and the metaproteome [20]. Due to the overwhelming number of biologically relevant genes encoded in microbial communities, knowledge about the metabolic potential of prokaryotes is only fragmentary with a predicted function of 10% of detected proteins. Even less is known about gene expression in complex communities. Irrespective of these limitations, the importance of gene expression studies in microbial ecology has been recognized from the beginning of the “omics era”. Microbial ecologists have realized that the mere knowledge of the potential functions encoded in a community without considering gene expression is inadequate. Also, the idea of microorganisms as single entities with defined functions has become obsolete. Rather, microorganisms are now recognized as being part of “superorganisms” within which small scale population heterogeneities are present. The following discussion of objectives should be seen in that context. Besides the composition of the prokaryotic community, it is important to extract information about diversity and gene expression. Community diversity will be analyzed by sequencing the 16S rRNA gene and by a metatranscriptome approach. In addition, prokaryotic gene transcript sequences selected for their potential interest to the topic under study will be determined. Soil characteristics and the cultivation-independent analyses of a prokaryotic community are also approached in combination with mugC type expression plasmids. Prospective improvements by continuing this approach are at least combination with genes marker of metabolic traits, and, preferentially, a time dependent investigations on the same culture. Further ammonia oxidation experiments on cultures performing anammox are hampered by a yet insufficient knowledge on the biology of closely related species.

6.1. Gene Expression Analysis

Microorganisms are so ubiquitous that it would be surprising, and exceptional, to find one of them

in isolation in nature; the studies of Koch in the 19th century were conducted in the laboratory under highly artificial conditions [1]. One could argue that any effort to unravel the secrets of biology, or the workings of the natural world that gives rise to life, should adopt an approach that emphasizes the study of environmental microorganisms. The last decade has seen important technological advances, in particular in DNA analysis, leading to the study of entire microbial communities. This is known as environmental microbial ecology. When it comes to omic technologies, the proteomic approach has lagged behind the genomic and transcriptomic approaches, which in their time lagged behind the development of more traditional microbiological methods that enable the isolation and cultivation of bacterial strains. However, and as is almost a rule with timescale in biology, the analysis of environmental proteomes is booming. Two proteomic approaches are currently used for environmental samples: either a metaproteomic approach or the analysis of crude extracts. The first approach essentially consists in the extraction of total cellular proteins, followed by protein separation, mass spectrometry analysis and peptide sequencing, typically using high-throughput methods. The sequence data generated is exploited using bioinformatic tools on sequence databases, that are composed of nucleotide or protein sequence data predicted from genome sequences. However, and although metagenetics and metatranscriptomics benefited from the early development of high throughput DNA sequencing techniques, protein sequencing is considerably far more complex and prone to contaminants; obtaining clean and reliable cell extracts from environmental samples is a much more tedious and sophisticated task. As reported in the pioneering works of environmental proteomics, finding proteins that are at the same time abundant, soluble in commercial electrophoresis solutions and avoid colloidal crystalline nanospheres has proven a challenge. In consequence, a second approach has been developed that is capable of analyzing crude extracts. The goal of this analysis is the detection of several or even a single protein (or group of proteins) indicative of the physiological status or of the target metabolisms of a particular organism in a complex microbial community. Several methods have been explored for this task, namely: immunological-based techniques, targeted proteomics, 2D protein electrophoresis, and protein-based DNA superarrays. As compared to the previously mentioned shotgun proteomics, these techniques are straightforward and simple to perform. [21][22]

6.2. Environmental Stress Responses

The response of biological systems to environmental perturbations is characterized by a rapid and appropriate adjustment of the physiology on every level of the cellular and molecular network. On a first level, there are mechanisms detecting changing states in the environment and transmitting the related information into the cell. On a further level, this signal often results in systematic changes of gene regulation. The initial environmental stress response (ESR) results in a vast re-wiring of the wiring of the gene regulatory network (GRN) of the cell, including the rearrangement of sigma factor, two-component, and sRNA communication networks, as well as in changes in the regulation of cellular processes such as signal transduction, tRNA modification, cell, and DNA repair, antibiotic production, and various transport systems. Most of these re-arrangements are the result of alternative use of pre-existing connections, as only a few new regulatory interactions are induced. Adjustments in protein production occur at different levels of the underlying regulatory network. On a physiological level, rapid response strategies need to be established to ensure conservation of energy reserves upon stress, and to minimize the pathogenic liability of the bacterium [23].

7. Proteomics and Microbial Function

An ecosystem is generally defined as a community of living organisms together with the nonliving components of their environment, interacting as a system. It is important to identify the functions of an organism/ community in their given ecosystem. This is especially true for those living organisms we cannot grow in laboratory conditions and those which have not been characterized in any form. Here we focus on the consideration of high-resolution “omics” technologies and their application to microbiology. Omics technologies are high-throughput analytical techniques that

monitor at a global level the presence and relative abundance of cellular RNAs (transcriptomics), proteins (proteomics) and metabolites (metabolomics) and the DNA (genomics) of living organisms [24]. Efforts to make this concept a reality have primarily focused on the development of metagenomics, the study of the collective genomes of the microorganisms in an ecosystem and have facilitated great advancements in our understanding of the type and potential function of organisms in a given environment.

Whilst metagenomics has provided a comprehensive view of the genetic content of an ecosystem, it only offers predictions of function. An environment and its accompanying microorganisms have the capacity to rapidly change with variations in conditions, and consequently, the function of an organism in one environment may not be the same in another. An additional challenge is that an estimated 70% of the functional genes of any community are carried by the unculturable fraction. “Omics” technologies are an approach to apply fine-scale functional characterization to the broad biodiversity encompassed by the metagenome, and can also be used in the exploration of biochemical events in biology. Metaproteomics is the identification of all the proteins expressed at a given time within an ecosystem. Through mass spectrometry-based analysis, it allows the determination of microbial functionality. At present, there remains significant challenges in this field, including but not limited to: protein extraction, interpretation of data, and the assignment of metadata with proteome data. While the great majority of species in a community will remain unsequenced, it is likely the same will not be true for the proteins they express.

7.1. Protein Expression Profiling

Microorganisms occupy virtually every habitat on our planet and their activities largely determine the environmental conditions of today’s world. However, very little is known about most microbial species and, to make matters worse, only a small fraction of these can be isolated and grown. Moreover, and even when isolation is possible, a single species removed from its natural environment might not necessarily display the same characteristics under laboratory conditions as it does within its ecological niche. Therefore, the study of mixed microbial communities within their natural environment is key to the investigation of the diverse roles played by microorganisms [24]. An emerging field of research in microbial ecology encompasses system approaches. Recent technological advances, including the development of high-throughput ‘omics’ methods, make such system approaches possible, where mixed microbial communities are viewed as one meta-organism. Metagenomics, metatranscriptomics, metaproteomics and metametabolomics are employed to determine respectively the DNA sequences of the meta-organism under study, the collectively transcribed RNA, the translated proteins and the metabolites resulting from cellular processes. All of the generated data can then be used to identify the metabolic pathways and cellular processes at work within an ecosystem. Yet another level of information is required to access the molecular interactions occurring within the ecological niche under investigation, and this is achieved by the application of metainteractomics. Ultimately, system approaches aim to develop mathematical models that can be used to predict the behaviour of a biological system in response to environmental stimuli. An overview of recent advances in metaproteomics technology and software created. It is concluded that metaproteomics has proven its worth as an important and essential tool in microbial investigations and offers exciting research prospects. [25][26]

7.2. Functional Characterization of Microbial Communities

Much of the natural history of microorganisms is encoded in the functional traits of the majority of prokaryotic microorganisms that resist laboratory culturing. Although knowledge about broad-scale patterns of microbial diversity has grown, an appropriate theoretical framework for understanding microbial ecology is still lacking. A central concept in ecology is the niche: entities of similar form or function that compete for similar resources are expected to generally exclude one another. However, this concept is less easily applied in the microbial world where interactions are difficult to observe and are likely multifarious. Inference about the workings of microbial communities has typically been done through the lens of taxonomic relationships, either between

organisms or between organisms and their environment. As a resolution of discrimination, microbial communities are often studied as bins, defined by operational concepts such as OTUs or gene tags which are quantified to represent the number of gene tags in the bin, or sequence counts mapping to it. Analyses of such microbial bins based on taxonomic or phylogenetic relationships often produce uno-corroborated, high-variance results. The pursuit of an ecological role for each and every bonafide biological entity is a tall order that empirical studies have struggled to fulfill. Broad patterns of function in the environment emerge from the collective activity of diverse organisms, some of which differ substantially in their form, function and ecological requirements. In groups of organisms, organisms interact by the dynamics of genes as well as by the emergent consequences of their metabolic activity.

8. Metabolomics in Microbial Studies

Metabolomic studies of bacterial species are integrated with techniques like BONCAT, which exploit the genetic principles of life and death. The literature is reviewed about the abundance of putatively competitive loci and the genomic frequency of those loci in Pseudomonas aeruginosa. Broadly, it’s found that bacterial growth is associated with increased genetic competitiveness with the exception of some highly toxic loci like those encoding type VI secretion systems. The field of microbial ecology entered into the age of metagenomics more than a decade ago, in which DNA extraction and targeting sequencing can reveal taxonomic composition of complex microbial communities. The development of GFP-based biosensors for metabolomic studies revolutionize the culture-based metabolic profiling of bacterial populations in the laboratory. Bacterial cultures heterogeneously express metabolic pathways both temporally and among cells, and this metabolic division of labor can promote bacterial survival in the presence of competitors. These observations raise the question of how, and by what classes of metabolites, nutritional competition can lead to the outgrowth of a small subset of individuals in a previously clonal population [1]. To understand these mechanisms, metabolomic studies of competitions between bacterial species are integrated with established techniques for modulating bacterial fitness using antibiotics, with the genetic principles underlying life and death in a bacterial population predicting the metabolomic environment that would accrue to late-surviving individuals.

The findings therefore have large implications for clinical and industrial efforts to combat recalcitrant, competitive infections, and the broader evolutionary frameworks for understanding subpopulations within microbial communities in the context of nutrient competition. Despite a wealth of unprecedented detail in individual species, these studies have brought little understanding to the larger-scale networks in which genes, proteins, metabolites, and cells interact and influence community function. Nonetheless, it has been increasingly clear that bacteria are intensely social organisms, capable of a proliferative lifestyle that depends upon complex, coordinated interactions at the genotypic, phenotypic and metabolic level. The ecological success of both pathogens and environmental bacteria similarly arises from a capacity to remodel their growth environment via the secretion of diverse, growth-altering metabolites such as exotoxins, siderophores, and surfactants [27]. Infection and competition studies in the past decade have revealed surprising complexities in the metabolic intertwining of mammalian and bacterial physiology via the utility and production of diverse metabolites.

9. Integrative Approaches in Microbial Ecology

Scientific expertise typically is developed over time, creating an environment in which integration between concepts is a priority, with the end result likely being consideration of a concept in the context of a larger picture. The culture of microorganisms from the environment is discussed from the point of view of a microbial ecologist, and vice versa. The goal is to provide specialists from different fields with the capacity to further their ambition in the framework of both disciplines by promoting consistent application of terminology and by addressing harmful "urban legends".

Microbial communities are ubiquitous in natural environments, in humans, and also in industrial processing and control with implications for our daily life, starting with water quality or health

compromising in a variety of ways. The traditional microbiological approach to assess the in-situ functioning of a microbial population is to bring it back to the laboratory as pure cultivation to survey controlled conditions without interacting community members. Yet about 99% of environmental bacteria are non-culturable with standard techniques. Despite great success in cultivation, the development of axenic cultures from nature was seen as an approach restricted to the exceptional chance. The limitations of axenic culture are further pointed out by the incapacity of microorganisms to express certain specific phenotypic traits associated with their fitness cost.

This perception extended to microbial ecologists that then developed methods based on the assumption that, for cell- and community- level properties of microbial life, as for macro-organisms, an organism is characterized by the genotype. Microbial ecologists began to consider phenotypical properties of microbial populations to comply with ecological definitions of species and population. As a result, microbial ecologists conventionally referred to bacterial assemblages with a phylogenetic monitiousness indistinguishable by sequencing as "strain" and to phenotypically distinguish communities with the same genome representation as "species". Strains as ecologically delineated units of a bacterial population were thus defined as cooperator bow-ties frames long-term, high-frequency occupational relationship across intra- and inter species boundaries.

9.1. Multi-Omics Integration

Post-genomic technologies are significantly advancing the ability to measure the genome, transcriptome, proteome, and metabolome of species in a variety of ecosystems through approaches known as 'omics'. The dual development of 'omics methodologies, in terms of ease of use and reduced costs, and cost-effective stewardship of high performance computing resources has expanded the utility of these approaches to microbial ecosystems and multi-species systems.

The establishment of two bacterial isolates grown together, an amalgamation of Pseudomonas aeruginosa growths with an Escherichia coli mutant, exemplifies microbe-microbe interactions. Through a multi-omics approach, with DNA, RNA and metabolite measurements, the connection between the two bacterial lifestyles affected by killing has been uncovered. This investigation illustrates the potential to unravel cause-and-effect relationships and inter-organism connectivity via the multifaceted application of 'omics technologies [28].

Microorganisms are critical components of every ecosystems, contributing to ecosystem function in diverse ways ranging from global nutrient cycling to food spoilage and infection. The ability to model and predict the effects of biological changes to aquatic ecosystems would revolutionize their understanding and management. However, microorganisms are complex and their activities are influenced by that of other microorganisms around them, forming complex consortia composting multiple trophic levels. It is thus important to have a good understanding of the system's players: which microorganisms are important and what are they doing. Visualizing such complex systems is impossible through classical means, hence the advent of metagenomics and other high-throughput bio-omics approaches is well timed. Appreciating these technical approaches could revolutionize the understanding of aquatic ecosystem function.

9.2. Systems Biology Perspectives

As systems biologists experienced in model systems, microbes have been highly amenable to omics technologies and lab experiments. Both their phylogeny and physiology are accessible from culture collections or reconstructed from sequencing data. Microbial ecologists have therefore taken the lead in exploring the population genetics and biogeography of environmental communities across parallel and independent studies. Genomic and metagenomic methods are now widely exploited to progress our understanding of the microbial role in ecosystem functioning. In contrast, plant-related biological functions need to be unraveled from complex gene networks and in a context-dependent manner that is far from easy [1]. Fallacies associated with the linear reasoning derived from a wet-bench-oriented mindset are likely responsible for a

series of flawed conclusions over the past decade. Although omics approaches offer a promising window for microbial biodiversity and interactions with their environment, in-depth knowledge of complex plant-microbe interactions also requires a more focused probing of networks and experimental validation. Such a gap between modeling and experimental approaches is currently limiting systems biology perspectives in microbial ecology.

Like environmental microbial communities, plant microbiota is also characterized by the coexistence of many microbial species with possible asymmetric interactions. Unraveling community structure-function relationships first rely on the perspective developed at the eco-system level, usually by correlating biodiversity indices or experimental parameters with the throughput data generated by metagenomics, metatranscriptomics, meta-metabolomics, and meta-proteomics. In parallel to computational modeling, microbial ecologists devoted to plant nutrition have developed empirical models to summarize datasets of microbial density and diversity that enhance plant fitness. Most such attempts take advantage of the well-documented omics avalanche but are generally devoid of modeling hypotheses.

10. Microbial Ecology in Extreme Environments

More than a century of microbial ecology based on isolation and cultivation has provided a limited view of how microorganisms interact with each other and their environments. Recently, we have been enabled to explore our microbial world in a revolutionary new way with culture-independent and high-throughput environmental genomic approaches. In combination with other “omics” approaches, these culture-independent techniques have revealed additional complexity of microbial ecosystems and increased insights into the identity, genetic composition, function, and dynamics of microbial communities in ways that were not thought possible a human generation ago. With the rapidly decreasing costs of omic approaches and the development of analysis pipelines and data resources, these approaches are widely accessible to microbial ecologists. It is, therefore, an exciting time to be studying microbial life, as obtaining new coinages and a deeper understanding of “microbial dark matter” (i.e. uncultivated or poorly understood microbial lineages). In light of these technical advances, this special focus issue is dedicated to the broad theme “Microbial Ecology in the Age of Omics: exploring more microbes and environmental interactions”. The articles published in this special focus issue highlight the use of omic approaches for studies on microbial communities in various ecosystems and environments, including the human body, soil and sediment, extreme environments, and with model organisms. These studies describe and discuss current and innovative methods, challenges and limitations, and future directions for generating and analyzing omics data in microbial ecology. Truthfully addressing these aspects has the potential to realize the full potential of environmental genomic approaches to more completely understand the ecological complexity and evolutionary history of microbial ecosystems. These studies were contributions from diverse scientists working together or independently with “omic” analyses. Together with Perspectives and Reviews on the field, this issue aimed to offer broader community with an overview of the state of the art for the use of omic approaches in microbial ecology and insightfully associated and innovative research topics.

10.1. Extremophiles and Their Adaptations

10.1.1. Thermophiles and Hyperthermophiles Microorganisms that grow optimally above 50 degrees Celsius are generally classified as thermophiles, with those above 80 degrees being hyperthermophiles, and utilized as important sources of thermostable enzymes in industry [29]. Adaptations to high temperatures involve thermostable DNA, protein molecules, enzymes, metabolic pathways, and peptides. High GC content in Thermus thermophilus and Aquifex aeolicus genomes encodes proteins with positively charged surface to stabilize the structure of macromolecules that interact with negatively charged DNA. Furthermore, there is an enzyme mechanism from Thermus thermophilus where elongation factors EF-Tu and EF-G function at high temperatures in a simple way. These proteins are lost their structural elements under heat shock and are less effective in complex processes. EF-Ts in Thermus thermophilus is not present,

EF-Tu with GTP will form a complex with the release factor RF-R, so the discharge efficiency will be significantly reduced, and protein synthesis will continue to avoid cold shock [30].

10.1.2. Halophiles The salt concentration is another important environmental factor that affects living systems. There are ones that grow optimally in 0.5 to 2.5 M (moderate), which are halotolerant, then can grow under moderate salt stress conditions. Aerobic bacteria that require salt to grow are halophiles. They usually appear as moderate halophiles and grow in salt concentrations between 0.5 and 2.5 M. There are also extreme (or obligate) halophiles that grow only at salt concentrations above 2.5 M. An additional growth intractable for other microorganisms occurs in the hypersaline (above 5 M) environments of ancient marine saltern crystallizer ponds.

10.1.3. Acidophiles and Alkaliphiles Like the others, microorganisms can adapt to different pH values are called acidophiles, those that grow best in acidic conditions are called acidophiles. Acidophiles are generally fungi, yeasts, but there are also prokaryotes with optimum growth value of pH 2-5.5. They usually use iron and sulfur compounds as an energy source. Likewise, there are microorganisms that can grow in and around alkaline conditions, these are called alkaliphiles. Generally, they prefer pH between 8 and 11.5, but there are also alkaliphile genera that grow better above this value. Many of them use nitrite, nitrate, sulfur or organic amines as electron donors.

10.2. Ecological Roles in Harsh Conditions

Microbial ecosystems remain a mostly hidden world. However now, with the availability of next generation sequencing and other omics technologies (collectively referred to as omics), it is possible to describe and begin to understand the functional roles of members in complex assemblages. For some habitats omics approaches have been transformative, in particular for those such as the deep ocean and subsurface environments, where traditional tools have failed to provide insights. However, despite the current enthusiasm for these tools, which some have compared to the arrival of the first microscopes in the history of microbiology, they are only part of the methodological toolbox.

It is a powerful combination of biophysical techniques, including PCR-based methods, automated ribosomal intergenic spacer analysis (ARISA), metagenomic DNA sequencing, and fluorescence in situ hybridization (FISH), that has been used to catalogue a surprising diversity of bacteria in compact on anti-tangle devices. That system serves as a model for an ompanion environment that is relatively extreme in terms of hydorothermal stress, and the microbial assemblages are of essentially unknown composition. The majority of phylotypes recovered from on anti-tangle devices were either known to be or, in the case of the novel phylotypes detected, are likely to be sulfate-reducing bacteria (SRB). These organisms are ubiquitous at low-temperature vent systems, where they often dominate the bacterial population, forming mats and symbiotic mutualists often with vestimentiferan worms [31]. As a biogeochemical guild or using syntrophy they maintain a co-dependent and reductive relationship with sulfur compounds, including hydrogen sulfide and sulfate. Quantification of functional gene markers for SRB, dsrAB, in samples revealed an overall increase in SRB gene content on the tangle device. This appears to have been driven by a growth or entrainment increase of SRB taxa present in assemblages.

11. Human Microbiome Research

Understanding the impact of microbiota on health, eco-systems and biotechnological processes has become an important goal. The surge of high-performance approaches and the unravelling of poor unculturable microbes through synthetic ecology offers to understand complex microbial networks. The generation of representative communities tasked to accomplish specific functions through design principles could improve microbial ecosystem health. A virtuous loop of multi-omics and models is possible for the rational design of synthetic communities as workhorses for bioprocesses requiring a defined level of ecosystem stability, robustness and spatial architecture.

After the initial focus on unculturable microbes and simple communities of cultured species, current research is addressing complex microbiota interactions using a new generation of cultivation under diverse sample conditions and culture settings. Progresses in diversity and composition of human microbiota, functional microbiology, and associated multi-omics experiments are reviewed. This creates an opening to synthesize knowledge on microbe-microbe and microbe-environment interactions, as well as on culture-enabled omic methods, which are emerging for microbiota research. Such knowledge is discussed in the context of synthetic community design. These community settings seek an integration of high-throughput experimental data with metabolic modeling to develop design principles for synthetic communities.

There has been a remarkable transformation in the field of human microbiome research since the declaration of the human microbiome project. The human microbiome, defined as the unique microbial community that colonizes different human body habitats, primarily the gastrointestinal tract, skin, and oral cavity, has been firmly linked to human health and diseases, including inflammatory bowel diseases, obesity, diabetes, and bacterial infections. The gut microbiome in particular has been receiving extensive scientific study, and there is growing recognition that this hidden organ interacts with host metabolism, immunity, and other physiological processes. Because of the rapid development and decreased cost of omics technologies, including metataxonomics, metagenomics, metatranscriptomics, metaproteomics, and metabolomics, integration of multi-omics data has become feasible and present opportunities for studying microbial functionality within the complex gut ecosystem.

11.1. Health Implications of Microbial Diversity

The era of microorganism exploring has long been opened since Anton Van Leeuwenhoek invented the first light microscope. Leeuwenhoek’s discovering of microorganisms in 1675 opened the era of microorganism researches, the controversy of spontaneous generation and theory of bio-genesis, early demonstrations of microbes in the surrounding flora, and discovery and practices of penicillin by Fleming.

The researches of microorganisms are predominantly involved in the pure microbiology. In recent years, based on the growing technology, it is easy and efficient to acquire the comprehensive lists of microbial community compositions. Omics studies have become one hot point and help microbial ecology research to be entered a new era. Many intricate microbial communities have been investigated by a variety of omics approaches, including virome, metagenome, metatranscriptome, metaproteome, metabolome with or without culturing method .

Microbial diversity is highest in soil, sediment, ocean and plant, while lower in upper atmosphere, clouds, rain, snow, glacier and snow. Microbial diversity could affect nutrient cycling, pollutions removal and so on. The surface of everything such as humans, animal, plant and surrounding environment is covered by complex microbial ecosystem acting as a co-organism and exo-gene because microbiota diversity effect is health effect and vice versa health benefit effect behaves as safety-depth for ecosystem; also surrounding good health. This consideration, and the “one world, one health, one science” concept officially adopted by the Food and Agriculture Organization of the United Nations, the World Organization for Animal Health and the World Health Organization should examine hygiene-theory to consider complementary animal, human, plant and ecosystem parallelism.

11.2. Microbial Interactions with Host

Microorganisms mainly do not exist as pure cultures or solely but form complex communities of single or mixed species, denoted as microbial populations [32]. Within a microbial community, and between the microbes and an eukaryotic host, or the environment, a large variety of different microbial interactions occur. In nature, most of these interactions are currently unknown or have not yet been described. Microbial interactions range from simple bacterial interactions with other bacteria, fungi or viruses, to complex associations of bacteria or viruses with a eukaryotic host at

the molecular, cellular and organizational level. For many microbes, the existence within well-organized populations is the basis for colonizing different environments. Various organisms are already able to interact during the early steps of colonization in order to establish a successful community at high abundance and for persistent time periods. Microbes can express different compounds or structures like liposomes, extracellular polysaccharides or adhesins for cell colonization, often triggered by a signal of the same or another species. Bacteria can take advantage of their motility, such as flagellar movement to approach each other or the host cell surface and consequently induce biofilm formation and adhesion following a so called `quorum sensing` mechanism. Within the biofilm, or complex structure of aggregates at living surfaces like teeth or body fluids, cells are encased and mostly undisturbed by external factors like antibiotics or the host immune system [33]. Given that only the successful establishment of a microbial population within or upon a host guarantees a persistent colonization, the host permits colonization of coherent units by a kind of detents or biofilm formation. This complex structure enables intramicrobial nutrient sharing, enhanced signaling and effector concentration, and cell-cell interaction like T4P-T4P-mediated exchange of genetic material among cells. In the search of additional nutrient sources or other advantages, a variety of other microbial interactions can occur within the population. Extracellular digestion of complex macromolecules is excreted into the environment and the products used by other cells including those of different species. Mostly, the individual microbial settlement consists of structures with porous morphology, decreasing the distance between the reacting cells or strands. Microorganisms could promote different effects on the host fitness through temporal sequence of stages: first generation effect, alteration of the interaction niche; second generation effect, alteration of other microbes niche including pathogens and; third generation effect, interference on host genes related to the development of the first or second effects.

12. Biotechnological Applications of Microbial Ecology

Microorganisms are found in every environment on the planet, including human bodies, and are involved in many biogeochemical processes [20]. New microbiological techniques based on the diversity and genetics of environmental bacteria have led to the study of complex microbial ecosystems in nature. All these ways of understanding microbial ecosystems should result in an understanding of the behavior of the system as a whole. This approach, however, may not succeed since the basic properties of complex systems are not expected to scale from individual parts to the whole, or they are poorly understood even in much simpler multicellular biological systems. Thus, it would be necessary to explore other ways of understanding microbial community structure, like the analysis of the "emergent properties" from its interaction network. In the last decade, the birth and growth of network science brought a new perspective to the understanding and analysis of complex systems. Recent work has analyzed the topology of the jointly weighted biochemical reaction networks in the bacteria and the yeast. However, bipartite biological networks, whose nodes can be divided into two sets with links dedicated to connecting nodes from the opposite sets, have been overlooked in the literature compared to monopartite networks. Ortholog clusters depict a bipartite network where the nodes are divided into two different types: species and genes. The analysis of this kind of networks allows focusing on local properties for the species (genes) nodes which would not be easily identifiable with a monopartite projection. This kind of network represents the gene conservation among a group of species and can be used to analyze the evolutionary dynamics of microbial ecosystems. In this article the ortholog clusters for a wide variety of bacteria are derived, an ortholog groups network is constructed and analyzed, and a model to generate bipartite networks based on a stochastic forms of duplication and diversification is presented.

12.1. Bioremediation Strategies

Microbial Ecology in the Age of Omics: Exploring Microbe-Environment Interactions

Bioremediation is one of the principal strategies for pollution confrontation and ecological

restoration. The primary techniques, including contamination isolation, dredging, ventilation, and adsorption, leachate collection and treatment, and landfill recovery face many problems due to the complexity of contaminations. As such, they only transfer or move pollutant from one medium to another medium, or they cannot treat pollutant in situ but require a trans-location process. By contrast, bio-treatment mainly uses various microorganisms and their microbial metabolites or enzyme systems to remove pollutants, either degrading or converting them into harmless compounds. This technology has become a research hotspot in the remediation of polluted soil, sewage, and groundwater because of its high degradation efficiency, low cost, negligible secondary pollution, and non-destructive characteristics.

Over the past decades, progress in bioremediation has been amazing. Each novel gene, enzyme, and even some strains were identified/ran in the previous decade or two. The rapid development and application of DNA sequencing technologies and bioinformatics tools/services have facilitated timely identification of novel functional genes or microbial resources from genomes. To enhance the efficiency of exploring those pollution degradation genes or microbial resources, it is then proposed that future research should focus on the optimization of multi-omics technologies. While a comprehensive strategy is provided to derive the immediate need for researchers through this technical review, more attention should be directed to: optimize the sample processing methods of omics; develop and refine the sequences databases, and some biological analysis platform/services; develop a high throughput and automatic screen platform, which can be freely and easily customized; and more seriously, the abstract schedule should be strengthened to validate experimentally, which requires a highly integrated effort between computational simulation and biochemical analysis [34]. Only in this way can it escape the cycle of making promises of increasing efficiency but remaining out to sea, and improve the efficiency of microbial gene and strain mining and discovery for future environmental remediation.

12.2. Industrial Microbiology Innovations

Industrial Microbiology Innovations the Rise of Microfludics, High-Throughput Screening, Systems Engineering, Bioinformatics and Regulatory Innovations but Challenges Lie Ahead with Eco-Friendly Biotech and Ethics for the Built Environment

13. Ethical Considerations in Microbial Research

Microbiomes are microbial communities that can be found on and in all multicellular organisms, and also throughout natural and manmade environments, including host-associated habitats. As a result, microbiomes—and their importance for health, ecology, and biotechnology—are now considered as the next frontier in life sciences. After intense research on genetics, transcriptomics, and proteomics, the science of microbiomes is termed ‘‘omics’’ [35]. The reason for this reluctance can be manifold. One can fear the ever-increasing amount of sample and sequence data that needs to be processed, absence or lack of experimental control, spatial and temporal complexity of microbe-environment interactions, and, as with any emerging field, scarce knowledge on how to interpret these data are common arguments. Indeed, it has been much less studied, and developing simple and tractable model systems is rather difficult. Our purpose is threefold: First, a short overview of the pre-”omics” modeling approach to host-dependent microbiomes. It is shown that this conventional modeling, often involving ordinary, partial, or stochastic differential equations or structured population models, is highly incomplete, typically neglecting the microbial part of the system, for instance by imposing an exogenous microbial diversity. Rather, microbiome-aware modeling frameworks, and especially discrete-generation individual-based models of host-microbiome systems, are needed. Second, a set of seven steps establishing a general strategy to implement innovative modeling to microbiomes in the host context. Third, this strategy is applied to the simplest model example, the case of no microbial interactions, and we demonstrate how host-microbe interaction analysis can be performed, leading to valuable ecological information. A brief discussion on further, ongoing, model applications and on pertinent future modeling directions concludes the article.

14. Future Directions in Microbial Ecology

Microbial ecology is without doubt the Microbiology discipline that has applied the highest number of techniques hitherto reserved for ecology. In fact, culture independent molecular tools to identify microbial taxa within environmental samples have been used since the hijacking of the PCR by microbial ecologists as the method of choice for amplification and quantification of the 16S rRNA genes of Bacteria, Archaea and chloroplasts [10]. Such methods have allowed breakthrough discoveries and provided unprecedented insights into the microbial communities of the planet. What appeared to be a fertile scientific marriage, would turn into a scientific superorganism sparkling astonishing discoveries driven by novel technologies that eventually made traditional microbiology cumbersome. Nevertheless, due to novel genetic fingerprint techniques and next generation sequencing techniques, this line of investigation has survived [20]. Although analyses of microbial diversity within environmental samples will remain important forever, it no longer represents the main focus of research articles in mainstream journals devoted to microbial ecology. The time-consuming and mostly ineffective attempts to isolate and culture novel microorganisms from environmental samples has always represented an important and legitimate aspect of microbial ecology and it must be acknowledged that an incredible number of microorganisms that have enriched human knowledge and technological developments was isolated exactly by such approach. However, for the large part such classical approach has taken advantage of microbial populations growing in model environments. Classical microbiology contributions were promptly or belatedly transformed into ecological questions or pursued in more complex and less predictable environments.

It is undeniable that culture independent approaches are powerful tools which have revealed that a significant part (often the vast majority) of the environmental microbial community is intransient. Nevertheless, in spite of theoretical and technological opportunities, the great undiscovered microbial diversity, or the rare biosphere, in the environment appears not inclined to give in to the contemporary standards of molecular microbial ecology. Moreover, culture independent approaches are still limited in several aspects: bias of DNA extraction, primer selection and coverage, PCR bias, downstream data processing artefacts, chimeras, phylotypes clustering, sequencing errors, database limitations or inaccuracies. Most importantly, readers' comprehension and validity of reports is often hampered by the lack of descriptive adequacy of the experimental design and methods. empadec is introduced as an intended modest effort to take back the field to an appropriate methodological and theoretical standard so as to provide reliable and informative ecological insights.

15. Conclusion

Microbial life dominates Earth, driving nutrient biogeochemical cycles and maintaining ecosystems. Integrated biogeochemical cycles arise out of collective activities of microorganisms and other organisms in their environments. Systems biology offers a powerful experimental strategy to tackle the daunting task of deciphering microbial interactions between the components—microorganisms and molecules—of microbial communities underpinning emergent properties of ecosystems. In this framework, entire microbial communities are envisioned as metaorganisms and every level of biological information (DNA, RNA, proteins and metabolites) is comprehensively considered along with in situ environmental characteristics. Each type of biological information (DNA, RNA, proteins and metabolites) provides a different level of characterisation of the microbial communities.

In this era of molecular biology, understanding life on Earth entails the central challenge of understanding the collective activities of microbes in Earth's systems. Many intermingled microbial species and populations inhabit every environment on this planet, the overwhelming majority of which is not readily accessible to culture. Furthermore, microorganisms in their environments are not autonomous agents. Indeed, different organisms are constantly interacting with one another and their environment—whether through competition, cooperation,

communication, exchange of resources, exchange of genes, etc.. These transfer of molecules and genetic material mean that only considering the direct environment of a microorganismos ignores a potentially rich set of influences. Systematic interactions between the different parts of microbial ecosystems are ultimately responsible for their emergent properties—those that manifest at a scale much larger than that of the individual components. These scales can be spatial or temporal.

References:

1. F. Abram, "Systems-based approaches to unravel multi-species microbial community functioning," 2014. ncbi.nlm.nih.gov
2. M. S. Matchado, M. Lauber, S. Reitmeier, "Network analysis methods for studying microbial communities: A mini review," *Computational and Structural Biotechnology Journal*, vol. 19, pp. 1-10, 2021. sciencedirect.com
3. E. Parente, T. Zotta, and A. Ricciardi, "A review of methods for the inference and experimental confirmation of microbial association networks in cheese," International Journal of Food Microbiology, 2022. biorxiv.org
4. E. Mandolini, M. Probst, and U. Peintner, "Methods for studying bacterial–fungal interactions in the microenvironments of soil," Applied Sciences, 2021. mdpi.com
5. N. H. Youssef, M. B. Couger, A. L. McCully, A. Eduardo Guerrero Criado et al., "Assessing the global phylum level diversity within the bacterial domain: A review," 2015. ncbi.nlm.nih.gov
6. Y. Li, Z. Guo, and S. Q. Ni, "Dynamic stratification and biogeochemical cycling response of microbial communities in east indian ocean ridge," Chemical Engineering Journal, 2024. [HTML]
7. Z. Wang, S. Chen, L. Yang, Q. Wang, N. Hou, "Remediation Strategies of Biochar and Microbial Inoculum for PAHs-contaminated Soil: Quorum Sensing-Mediated PAHs Degradation and Element Cycling," Journal of Hazardous Materials, 2025. [HTML]
8. N. Kamal and B. S. Saharan, "Microbial dynamics in soil: Impacts on fertility, nutrient cycling, and soil properties for sustainable geosciences—people, planet, and prosperity," Arabian Journal of Geosciences, 2025. researchgate.net
9. W. Gerlach, "Taxonomic classification of metagenomic sequences," 2012. [PDF]
10. M. Mühling, "Assessment of complex microbial assemblages: description of their diversity and characterisation of individual members: Assessment of complex microbial assemblages: description of their diversity and characterisation of individual members," 2017. [PDF]
11. H. K. Shrestha, M. R. Appidi, M. I. Villalobos Solis, J. Wang, et al., "Metaproteomics reveals insights into microbial structure, interactions, and dynamic regulation in defined communities as they respond to environmental disturbance," BMC Microbiology, vol. 21, no. 1, 2021. springer.com
12. H. Pan, R. Wattiez, and D. Gillan, "Soil Metaproteomics for Microbial Community Profiling: Methodologies and Challenges," Current Microbiology, 2024. umons.ac.be
13. A. Mathuria, K. Jain, A. Saini, C. Verma, and I. Mani, "Metatranscriptomics, Metaproteomics, and Metabolomics Approaches for Microbiome Characterization," in Multi-Omics Analysis of the …, 2024, Springer. [HTML]
14. A. K. Wani, N. Akhtar, F. Sher, A. A. Navarrete, et al., "Microbial adaptation to different environmental conditions: molecular perspective of evolved genetic and cellular systems," *Archives of …*, vol. 2022, Springer. ntu.ac.uk

15. W. P. J. Smith, B. R. Wucher, C. D. Nadell, et al., "Bacterial defences: mechanisms, evolution and antimicrobial resistance," Nature Reviews, 2023. google.com
16. L. Kumar, S. K. S. Patel, K. Kharga, R. Kumar, P. Kumar, "Molecular mechanisms and applications of N-acyl homoserine lactone-mediated quorum sensing in bacteria," Molecules, vol. 2022. mdpi.com
17. N. Klitgord and D. Segrè, "Environments that Induce Synthetic Microbial Ecosystems," 2010. ncbi.nlm.nih.gov
18. C. Simon and R. Daniel, "Achievements and new knowledge unraveled by metagenomic approaches," 2009. ncbi.nlm.nih.gov
19. F. Warnecke and P. Hugenholtz, "Building on basic metagenomics with complementary technologies," 2007. ncbi.nlm.nih.gov
20. D. McDonald, Y. Vázquez-Baeza, W. A. Walters, J. Gregory Caporaso et al., "From molecules to dynamic biological communities," 2013. ncbi.nlm.nih.gov
21. H. Satam, K. Joshi, U. Mangrolia, S. Waghoo, and G. Zaidi, "Next-generation sequencing technology: current trends and advancements," Biology, vol. 2023. mdpi.com
22. X. Dai and L. Shen, "Advances and trends in omics technology development," Frontiers in Medicine, 2022. frontiersin.org
23. S. Jozefczuk, S. Klie, G. Catchpole, J. Szymanski et al., "Metabolomic and transcriptomic stress response of Escherichia coli," 2010. [PDF]
24. A. Siggins, E. Gunnigle, and F. Abram, "Exploring mixed microbial community functioning: recent advances in metaproteomics," 2012. ncbi.nlm.nih.gov
25. W. Reineke and M. Schlömann, "Microorganisms at different sites: Living conditions and adaptation strategies," Environmental Microbiology, 2023. [HTML]
26. K. Timmis and J. L. Ramos, "The soil crisis: the need to treat as a global health problem and the pivotal role of microbes in prophylaxis and therapy," Microbial Biotechnology, 2021. wiley.com
27. J. Chong and J. Xia, "Computational Approaches for Integrative Analysis of the Metabolome and Microbiome," 2017. ncbi.nlm.nih.gov
28. T. Reid and J. Bergsveinson, "How Do the Players Play? A Post-Genomic Analysis Paradigm to Understand Aquatic Ecosystem Processes," 2021. ncbi.nlm.nih.gov
29. G. Fongaro, G. Augusto Maia, P. Rogovski, R. Dorighello Cadamuro et al., "Extremophile Microbial Communities and Enzymes for Bioenergetic Application Based on Multi-Omics Tools," 2020. ncbi.nlm.nih.gov
30. L. Shen, Y. Liu, L. Chen, T. Lei et al., "Genomic basis of environmental adaptation in the widespread poly-extremophilic Exiguobacterium group," 2024. ncbi.nlm.nih.gov
31. A. M. Savage, J. Hills, K. Driscoll, D. J. Fergus et al., "Microbial diversity of extreme habitats in human homes," 2016. ncbi.nlm.nih.gov
32. N. Weiland-Bräuer, "Friends or Foes—Microbial Interactions in Nature," 2021. ncbi.nlm.nih.gov
33. R. Mesquita Braga, M. Nóbrega Dourado, and W. Luiz Araújo, "Microbial interactions: ecology in a molecular perspective," 2016. ncbi.nlm.nih.gov
34. Y. Huang, H. Hu, T. Zhang, W. Wang et al., "Meta-omics assisted microbial gene and strain resources mining in contaminant environment," 2023. ncbi.nlm.nih.gov

35. L. Lange, G. Berg, T. Cernava, M. C. Champomier-Vergès et al., "Microbiome ethics, guiding principles for microbiome research, use and knowledge management," 2022. ncbi.nlm.nih.gov